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REVIEW

Non-pharmacologic options for the management of voiding dysfunction in multiple sclerosis

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Abstract

Multiple sclerosis is a neuroinflammatory condition that can cause significant bladder dysfunction manifesting either as overactive bladder or impaired bladder emptying. Patients will often complain of urgency, frequency, nocturia, urgency incontinence, hesitancy, straining to void, and incomplete bladder emptying. While these symptoms can be treated with pharmacologic agents, often patients will require more significant treatments. Patients should first be evaluated with urodynamics in order to adequately diagnose the pathologic condition causing their symptoms. These interventions include catheter use, injection of botulinum toxin, neuromodulation, urethral stenting, sphincterotomy, suprapubic catheter with bladder neck closure, bladder augmentation and urinary diversion. The purpose of this review is to examine the evidence supporting each of these treatment options so urologic providers can better provide for this unique and complex patient population.

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Key words: Multiple sclerosis; Neurogenic detrusor overactivity; Detrusor sphincter dyssynergia; Botulinum toxin; Sacral neuromodulation

Core tip: Patients with multiple sclerosis can present with a variety of different urologic symptoms. While they can be treated with multiple pharmacologic agents, at times they will require manipulation or surgical intervention. This article reviews the scientific evidence behind each treatment modality so providers may be more informed as they council patients.

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INTRODUCTION

Multiple sclerosis (MS) is the most common neuroinflammatory disease, affecting approximately 85 people in every 100000[1]. The disease causes demyelination plaques in central nervous system white matter. One of the most common manifestations of MS is bladder dysfunction. Bladder dysfunction results from interruption of neural pathways between the pontine micturition center and the sacral spinal cord by demyelinating plaques[2]. The clinical manifestations of neurogenic bladder dysfunction in MS include overactive bladder syndrome and impaired bladder emptying. Overactive bladder is defined by the International Continence Society as urinary urgency, with or without urgency incontinence, but usually with frequency and nocturia and can be neurogenic or idiopathic in origin[3]. Urodynamic findings of detrusor overactivity provide objective evidence of involuntary contractions of the bladder muscle, the presumed etiology of overactive bladder symptoms. Impaired bladder emptying in MS may range from symptoms of hesitancy and slow urinary stream to complete retention of urine requiring catheterization. Poor relaxation of the external urethral sphincter

is the culprit and can be demonstrated on urodynamic testing. During voiding, synergy of the bladder and urethra should occur allowing the bladder muscle to contract and the urethral muscle to relax, resulting in complete bladder emptying. In patients with MS, dyssynergy occurs such that the urethra does not relax during voiding resulting in high bladder pressure with poor or low flow through the urethra, this is termed detrusor sphincter dyssynergia (DSD).

Bladder dysfunction can be treated with pharmacologic agents and generally these are instituted prior to consideration of more invasive treatment options. Theoretically, pharmacologic agents for overactive bladder symptoms may worsen symptoms of emptying dysfunction in patients with MS and agents aimed at improving bladder emptying may worsen incontinence. In practice there are mixed results with these medications. Additional treatment options may be considered as the result of patient bother from bladder symptoms or poor bladder compliance with concern over upper urinary tract deterioration. Urodynamics can be useful in documenting the components of bladder dysfunction and monitoring bladder changes over time. For neurogenic detrusor overactivity non-pharmacologic treatment options range from simple measures such as condom catheters in men or incontinence pads in women to more invasive options such as Botulinum toxin injection or neuromodulation. For DSD with poor bladder emptying, treatment options range from urethral catheterization to sphincterotomy or intrasphincteric Botulinum toxin injection. Some patients may even require bladder augmentation or urinary diversion.

Patients with refractory bladder dysfunction as a result of MS have several therapeutic options that can be used as an adjunct to pharmacologic therapy or can be used as primary treatment when pharmacologic agents have failed. These therapies are described below.

URODYNAMICS

Many patients with MS will present with specific urinary complaints; however, it is important to evaluate these patients objectively prior to treatment, as some neurogenic bladder dysfunction may be present and may progress to renal decompensation in the absence of symptoms. Typical complaints are include urgency, frequency, nocturia, urgency incontinence, hesitancy, straining to void, and incomplete bladder emptying[4]. The best way to assess the pathologic condition causing these symptoms is to perform urodynamics. Urodynamics is a broad term that includes studies that assess bladder function including uroflometry, post void residual measures, voiding cystometry, urethral pressure profilometry, and electromyography. Having a patient maintain a bladder diary prior to urodynamics will provide further information when the urodynamic study is being interpreted. These studies may include concomitant fluoroscopic imaging to assess anatomy during voiding; this is called video-urodynamics. Common urodynamic findings in MS patients include bladder hypersensitivity, detrusor overactivity, low urinary flow rate, elevated post void residual urine volume, and DSD. Patients may also have evidence of impaired compliance and high pressure voiding suggesting obstruction. In a study by Gallien *et al*[4] in 1998, 149 patients with MS who underwent standardized urodynamic studies DSD was found in nearly 60% of the population. Approximately half of these patients also had detrusor overactivity. They did not, however, find any significant correlation between the urodynamic findings and symptom presentation. A higher post void residual was the main risk factor for developing upper tract urinary infections in this population.

Having urodynamic data can be helpful in the treatment of neurogenic bladder dysfunction as treatment options vary widely and depend primarily on the underlying dysfunction found and patient bother. While it is not a common occurrence, prevention of upper urinary tract deterioration as a result of impaired bladder compliance is critical and must be address in affected patients.

TREATMENT OF NEUROGENIC DETRUSOR OVERACTIVITY

Neurogenic detrusor overactivity (NDO) secondary to the demyelination of the dorsal columns is the most prevalent urodynamic finding in patients with MS, with anywhere from 34%-99% of patients displaying this abnormality[2]. In a prospective study comparing the urodynamic tracings of women with MS to those with idiopathic detrusor overactivity, Lemack *et al*[5] found that the initial detrusor contractions in the MS subset were of higher amplitude and suggested that neurogenic detrusor overactivity may be harder to control[5]. In fact, it has been noted that patients with NDO require higher doses of anticholinergics for effective treatment[6]. Given the not insignificant side effect profile for these drugs, in addition to the potential lack of efficacy, it is logical that patients would seek out alternative forms of treatment such as condom catheters, sacral nerve stimulation, dorsal penile or clitoral nerve stimulation, percutaneous tibial nerve stimulation and injection of Botulinum A neurotoxin.

Catheters

In patients with neurogenic injuries to the bladder causing detrusor overactivity one of the least invasive non-pharmacologic treatments is either an indwelling or condom catheter. The biggest concerns for these patients are the increased risk of urinary tract infections and local irritation and infection of the genital tissues. Additionally, with indwelling catheters there is a risk of urethral erosion that may result in urinary leakage around the catheter, urethral tissue loss, and splitting of the glans and shaft of the penis in men. With condom catheters, approximately 40% of patients will develop urinary tract

infections (UTIs) with long-term use. An additional 15% of users are at risk of ulceration, necrosis and gangrene[7]. Suprapubic catheters are also an option to avoid genital ulceration, but the patient is still at increased risk for urinary tract infection and may continue to leak per urethra from involuntary bladder contractions. In general, catheters are used as a treatment of last resort.

Neuromodulation

Stimulation of the bladder nerves has been shown to inhibit detrusor overactivity in patients with NDO. Neuromodulation is felt to work *via* somatic inhibition of hyperstimulation and overactivity of the sympathetic and sacral motor neurons however, the exact mechanism of action remains unknown[8]. Bladder neuromodulation can be accomplished by stimulating the S3 sacral roots, the tibial nerve, the pudendal nerve, or one of the pudendal nerve branches which include the dorsal nerve of the penis or clitoris. It has also been shown that the posterior tibial nerve, which contains fibers originating from L4 to S3 can have similar effects when stimulated by depolarizing somatic lumbar and sacral nerves to the bladder[2].

In 2005, sacral neuromodulation was recommended by the International Consultation on Incontinence as a second-line therapy for NDO. In the United States, the Food and Drug Administration (FDA) has approved the InterStim Device® (Medtronic, Inc., Minneapolis, MN), a subcutaneous implantable pulse generator attached to a lead that stimulates the S3 nerve root, for the treatment of idiopathic frequency, urgency, urgency incontinence and non-obstructive urinary retention. It has been studied in patients with neurologic disease and found that 66% of patient with NDO can experience significant improvements in their lower urinary tract symptoms after placement and 75% of these patients will have continued success at mean follow-up of four years[9]. In one study of 25 patients with MS, 15 patients showed improvements in their symptoms that were sustained six months after implantation of the InterStim device. Of the six who had detrusor overactivity, there was a significant reduction in frequency episodes from a mean of 18 to 9 (episodes per day), post-void residuals from a mean of 127 to 33 mL, and incontinence episodes from a mean of 13 to 3 per day. Additionally, voided volumes increased from a mean of 83 to 160 mL[10]. Patients appear to have more success if their neurologic conditions are localized[11] and, while patients may have good clinical outcomes, they still may display overactivity on urodynamic testing[12].

In spite of the potential for significant benefit, sacral neuromodulation has not been adopted as a standard treatment for two reasons. First, in the small case studies assessing efficacy it has been noted that when some patients undergo a relapse or experience MS disease progression stimulation parameters could not be reprogrammed to achieve continued clinical improvement in voiding dysfunction[9]. Additionally, patients with an implanted neurostimulator cannot undergo magnetic resonance imaging (MRI) evaluation (with the exception of the head) due to the presence of metal in their body, which is the imaging modality of choice for evaluation of MS patients.

For dorsal penile or clitoral nerve stimulation electrodes are placed on dorsum of the penis or the clitoris and labia majora. In a 2006 study of eight patients with MS and detrusor overactivity, Fjorback *et al*[8] used simultaneous dorsal nerve stimulation and urodynamics to show suppression of bladder contractions and concomitant leakage in seven patients. These patients on average had fifteen minutes between the sensation of urgency and leakage with dorsal nerve stimulation, which the authors suggested was enough time to electively void. There was an increase in bladder capacity of up to 94% on average over this time period[8]. Additional studies have shown capacity to be increased by 55%[13] and conditional suppression of detrusor overactivity allowing for a delay of incontinence[14]. Although these results are promising, dorsal nerve stimulation is not available except at specialized centers and there is considerable hygienic concern with the chronic use of electrodes in the genital area.

An alternative form of neural stimulation that is gaining popularity due to its minimally invasive nature is posterior tibial nerve stimulation (PTNS). The posterior tibial nerve is a terminal branch of the sciatic nerve, which is derived from the L4 to S3 spinal nerves. The common origin of nerve fibers seems to allow for a simultaneous effect on the nerves to the bladder when the posterior tibial nerve is stimulated. In a 2008 study on acute urodynamic effects in patients with MS and overactivity treated with PTNS, Kabay *et al*[15] showed significant increases in volume at first involuntary contraction and maximum cystometric capacity (MCC) by performing urodynamics without stimulation followed by urodynamics with stimulation. Eighteen of the twenty-nine patients had a 50% increase in the bladder volume at first contraction and thirteen had a volume increase of at least 100 cc at first contraction. There was a 50% increase in MCC in 14 of 29 and 17 patients had a 100 cc increase in MCC Kabay *et al*[15]. A criticism of this evidence has been that there could be a conditioning effect of the bladder with the first filling allowing for delayed contraction even in the absence of tibial stimulation; a control group would have been helpful in this study[16].

Kabay *et al*[17] also published a study evaluating the effect of 12 wk of PTNS on 19 patients with MS and NDO utilizing repeat urodynamics and bladder diaries. They found complete clinical responses, which they defined as a 50% decrease below baseline findings, for urgency episodes in 33%, urinary incontinence episodes in 40%, daytime frequency episodes in 57%, nocturia in 75% and pad test in 90%. They also showed significant improvements in mean volume at first detrusor contraction and MCC, as well as decreases in detrusor pressure at first contraction and MCC, increase in max flow and decrease in post void residual (PVR)[17].

Gobbi *et al*[18] evaluated 21 patients with MS and overactivity that had failed anticholinergic therapy who were then treated with PTNS for 12 wk. They found significant reduction in daytime frequency, from 9 to 6

episodes, decreased PVR and increased mean voided volume. Eighty-nine percent of these patients reported a treatment satisfaction of 70%[18].

In a multicenter study, 70 patients with MS and NDO underwent 3 mo of daily 20-min sessions of PTNS. Primary outcomes were the effect on urgency and frequency and secondary outcomes were continence, quality of life, urodynamic changes and tolerance. At the end of 90 d, 83% of the patients had significant improvements in urgency and frequency. They also found a significant decrease in overactivity on urodynamics and a trend toward increased MCC and volume at first involuntary contraction. The treatment was well-tolerated and 70% of the patients wished to continue therapy at the end of the trial[19].

Finally, in 2013, Zecca *et al*[20] published a prospective study on 83 patients with MS who underwent 12 wk of PTNS and responders were then kept on maintenance therapy and followed for a total of 24 mo. 89% of the original treatment group were classified as responders as they had a greater than 50% improvement in their symptoms as measured by the perception of bladder condition questionnaire. Maintenance therapy was one day every four, three or two weeks, depending on patient response. Most patients required treatment every two weeks. 96% of the patients who underwent maintenance therapy were still classified as responders at the end of two years with a mean reduction of frequency episodes to 7 per day from 10, nocturia to 2 times per night from 4, increase in voided volume to 268 from 171 cc, decreased PVR to 52 from 101 cc and an increase in max flow to 25 from 15[20]. It seems that PTNS has the potential to be a lasting and well-tolerated treatment for patients with MS, however there is debate throughout the urologic community as to how real these effects are. Further investigation with randomized, blinded studies of PTNS *vs* sham stimulation is necessary before incorporating this treatment into a standardized algorithm.

Botulinum toxin injections

Botulinum Toxin injections have been adopted by the urologic community and approved by the FDA as a treatment for both idiopathic and neurogenic urinary incontinence secondary to detrusor overactivity. Additionally, it can be used in the MS population for detrusor-sphincter dyssynergia, which will be discussed in more detail later. For NDO in MS patients, *Botox* has been shown to effectively reduce daytime frequency, nocturia, pad use as well as improve urodynamic parameters. In 2006, in a trial in Berlin, 16 patients underwent injection of 300 U of *Botox* into 40 sites, including the bladder base and trigone. Fourteen of these patients also had 50-100 U of the original 300 injected into the external sphincter. At three months, day time frequency decreased from 12 to 6.75, nocturia from 2.16 to 0.61 and pad use from 1.75 to 0.63 daily. Additional significant changes were noted in volume at first contraction and MCC on urodynamics. The effects in this study lasted 3-6 mo and then patients' symptoms returned to baseline[21].

Using 300 U of *Botox*, while effective, does put patients at greater risk of urinary retention requiring clean intermittent catheterization. In 2011, a pilot study in 12 patients with MS and NDO was performed, where each patient was only injected with 100 U in 10 injection sites. All 12 had significant increases in MCC and volume at first desire to void. Voiding diaries showed significant decreases in frequency, urgency episodes and pad usage with a trend to toward a decrease in mean incontinence episodes. Mean maximum flow and voided volume decreased, but only two patients required intermittent catheterization. At 12 wk, patients were beginning to experience a loss of efficacy, but average time to request for re-injection was 8 mo[22].

In 2011, Cruz *et al*[23] published a randomized, double blinded, placebo trial in 154 patients with MS and 121 with spinal cord injuries and NDO. Patients were randomized to placebo, 200 U or 300 U of *Botox*, and 30 injections were administered. The primary endpoint was change in urge incontinence episodes six weeks after treatment, with secondary endpoints of change in MCC, maximal detrusor pressure during first involuntary contraction, and Incontinence Quality of Life score. They also assessed volume at first involuntary contraction, detrusor compliance and volume per void. At week six there were significant changes in weekly urge incontinence episodes in both treatment groups compared to placebo, but no difference between the treatment arms. MCC, volume at first involuntary contraction, and detrusor compliance were all significantly increased with treatment and pressure at first involuntary contraction was significantly decreased, with no differences noted between 200 or 300 U. Median duration of effect was 42 wk for both groups. Of note, there was a higher risk of UTIs in MS patients that received *Botox* treatment. Additionally, 30% of the patients who received 200 U and 42% of the patients who received 300 U began intermittent catheterization for residuals greater than 200 cc[23].

Botox injections can be another form of treatment of detrusor overactivity and urge incontinence in patients with MS. It significant reduces the lower urinary tract symptoms that plague this population, but it is not a perfect solution. First, it is only effective for a limited time, requiring readministration two to four times yearly. Second, it is not without risks, mostly of having to begin intermittent catheterization, but also an increased risk of urinary tract infections. However, for many patients, the potential for improvement in quality of life will likely outweigh the inconvenience of repeat procedures and the risk of catheterization.

TREATMENT OF INCOMPLETE BLADDER EMPTYING AND URINARY RETENTION

DSD is a common manifestation of disease in patients with MS[4]. In DSD the patient is unable to completely relax the detrusor sphincter in correct timing with contracting the bladder to allow normal voiding. This is one

of the most common urodynamic findings in MS. There are multiple treatment options available to patients for DSD. Pharmacologic options include antispasmodics and alpha blockers, but these come with their own side effects and therefore surgical options have also become available for these patients. The ideal solution would be a method of voiding that allows low pressure bladder emptying[24].

When behavioral modifications, bladder training, and alpha blocker medications have not helped the following treatments may be implemented. The first of these is the use of clean intermittent catheterization (CIC). This allows the patient or caregiver to use a disposable catheter to empty the bladder on a regular schedule[2]. There are convenient packs that contain the catheter lubricant and drainage bag all in one, this facilitates things for those that are wheelchair bound or have limited dexterity. It is the treatment of choice because it allows the bladder to fill and empty completely. Patients rarely have issues with urgency or incontinence using CIC because the bladder is emptied to completion each time. Most patients can empty with CIC every 4-6 h, if patients are finding that it needs to be done more often that should prompt urodynamic evaluation to determine the bladder capacity. This will determine a safe CIC frequency. The risk of infection is lowest with CIC because there is no indwelling catheter.

In patients where CIC isn't possible patients require indwelling catheters. Urethral foleys have significant complications including urethral erosion and chronic urinary tract infections. In these cases suprapubic catheters are the next best option. Suprapubic catheters allow constant bladder drainage without urethral damage. They also decrease urinary tract infections[2].

Urethral stenting

One surgical option available to patients with DSD is a urethral stent, temporary or permanent. This is rarely used today due to the permanent nature and potential for significant complication. There are several stents available; Urolume, Memotherm and Ultraflex. All of these can be temporary but the longer they stay in the more difficult it becomes to remove them[25]. This was first used as an option in 1990, and most studies have looked at permanent urethral stents[26]. Shaw *et al*[26] investigated this option in 9 patients with DSD to allow patients to easily pass a catheter for clean intermittent catheterization or wear a condom catheter. At the time this was a new technique that allowed the bladder to empty completely relieving some of the upper tract complications that can occur in patients with DSD. There are many risks associated with temporary or permanent stents including encrustation and migration[27]. Gamé *et al*[24] in 2008 presents the outcomes of temporary stents in 147 men who underwent the procedure for neurogenic DSD. In his study the patients that underwent temporary stenting were unable to self-catheterize. Over the long term the efficacy of stenting has not been shown to be satisfactory[28].

Sphincterotomy

Another option for these patients is a sphincterotomy or bladder neck incision[27]. The bladder neck incision technique was the first treatment used for patients with DSD, beginning in the 1940s. Emmet started with transurethral resection of the bladder neck, he was not very successful but it brought up the idea of having a more permanent lasting effect by doing an external sphincterotomy[29]. An external sphincterotomy became the mainstay of treatment of DSD, the goals of treatment were the resolution of hydronephrosis, reduction in frequency of or complete resolution of UTIs, reduction in frequency of autonomic dysreflexia, reduction in post-void residual urine volume, reduction in voiding pressure, reduction in leak point pressure[27]. A conventional sphincterotomy can result in significant blood loss, this was resolved by the ability to use a laser to perform the sphincterotomy resulting in almost immediate hemostasis[30]. This is considered a permanent procedure causing urethral incontinence; however, in some situations the incision needs to be performed repeatedly due to the development of scar tissue.

Botulinum toxin for DSD

More recently the most commonly used surgical technique for treatment of DSD is intrasphincteric injection of botulinum toxin A. Interestingly, the first use of Botulinum toxin in the urologic patient was for DSD. The injection acts on the sphincter as it does on the detrusor muscle in overactive bladder allowing the sphincter to relax. It does result in urethral incontinence, which limits its use. This is a viable option in patients with MS, there are no permanent changes made and the effects of the Botulinum injection wear off. Patients with MS have a constantly changing spectrum of urologic manifestations and systemic manifestations; a reversible agent is of interest to some patients at the time of MS flare.

In a French study by Gallien *et al*[31] 86 patients were randomized to receive an injection of Botulinum Toxin A (100 U) or a placebo (normal saline). He found no significant difference in the post void residual volume in patients with MS, but the procedure was well tolerated[31]. In 2005 Smith *et al*[32] investigated the reduction in post void residual volume with urethral injections of Botulinum toxin. Patients were treated with 100 to 200 U of Botulinum Toxin-A (BTX-A) in 4 mL divided in equal doses into the four quadrants of the external sphincter or by injection into the bladder base using 100 to 300 U of BTX-A diluted in approximately 10 to 30 mL of sterile saline. Sixty eight patients, thirty two of whom have MS, underwent the procedure, it was found that there was a statistically significant difference in the post void residual and the peak voiding pressure. Both values improved after the urethral injection[32]. The study also showed that the patients overall well-being improved and they had less urinary tract infections. In another study out of Germany it was found that the injection of botulinum toxin into the sphincter for patients with

MS decreases the incidence of urinary retention after intradetrusor Botulinum toxin injection. The authors of the study feel that this is due to a degree of DSD that is found in many patients with MS[21]. This study also found that with an injection of 300 U of *Botox-A* into the bladder and sphincter the incontinence rate decreased. They found that daytime frequency was reduced by 30% at 6 mo and the use of pads was reduced by 64% after 3 mo[21].

Neuromodulation for bladder emptying

There is only one study that reports neuromodulation for use in DSD. Hohenfellner *et al*[33] used sacral nerve stimulation on 11 patients with difficulty emptying. In 8 of the 11 patients symptoms of lower urinary tract dysfunction were decreased by 50%. After the time period of 56 mo all but one of the neurostimulators became ineffective[33].

Despite the success of these few patients it is still important to remind patients that CIC is still the best option and neuromodulation has a marginal place at best in urinary retention neurogenic bladder.

URINARY DIVERSION AND BLADDER AUGMENTATION

Urinary diversion is an option available to patients with MS who have failed all other therapies and remain symptomatic with either overactive bladder symptoms, emptying symptoms, or impaired compliance. When patients experience symptoms refractory to pharmacologic agents and conservative alternatives mentioned above, major surgery becomes the next option. There are several surgical options for patients with MS including bladder augmentation, continence or incontinent urinary diversion, and catheterizable channels. The exact procedure or combination of procedures depends on the underlying bladder dysfunction that needs to be addressed. When patients are unwilling or unable to catheterize an incontinence ileal loop urinary diversion is preferred. For patients willing to catheterize an augmentation cystoplasty is the simplest procedure to increase bladder capacity, improve compliance and improve overactive bladder symptoms. For patients who have an incompetent urethral sphincter or have trouble catheterizing through their urethra and they desire continence, bladder neck closure with creation of a catheterizable channel is possible. This generally utilizes a segment of small intestine and is brought up to the skin in the right lower quadrant or at the umbilicus. A concomitant bladder augmentation is generally performed. Urethral closure should accompany these procedures only if the patient has an incompetent sphincter. As with any surgery these procedures come with their own set of morbidities and these should be weighed carefully with the patient before proceeding.

Patients who undergo these operations are those that have run out of other options. They have usually tried multiple other modalities. In a study by Gudziak the average time from development of neurogenic bladder to ileovesicostomy was 147 mo. This is more than 10 years of suffering with urgency, incontinence, UTIs and multiple other problems that go along with neurogenic bladder[34]. The creation of an ileovesicostomy is a major surgery and there are multiple complications that can arise. Tan *et al*[35] looked at the adverse events of 50 patients who had undergone ileovesicostomy for neurogenic bladder, 19 of those 50 patients had MS. Complications included stomal complications, occurring in 38% of patients, mechanical complications occurring in 22% of patients and wound/bowel complications occurring in 54% of patients. Although the post-operative complication rate was high the urinary tract problems that these patients had preoperatively markedly decreased making ileovesicostomy a viable option for these patients[35].

Ileovesicostomy can also be done robotically, this has been shown to be equally effective in decreasing detrusor pressure while also being safe and effective with decreased hospital stays and less blood loss intraoperatively in one study[36]. In another study by Vanni *et al*[37] the only statistically significant difference between open and robotic ileovesicostomy was in the OR supply costs.

Bladder neck closure with suprapubic catheter placement is an option in patients who have urethral incontinence, decent bladder capacity and are poor surgical candidates. This procedure is simple and can be done without entering the peritoneum[38].

In a study of 9 patients with MS who underwent augmentation cystoplasty for their symptoms it was found that this procedure increases detrusor capacity and decreases intravesical pressure[39]. This is an ideal option in patients where the main problem of their voiding dysfunction is low bladder capacity, impaired compliance or detrusor overactivity with leakage. The patients who underwent the procedure all had preserved or improved renal function. An augmentation cystoplasty with a catheterizable stoma is another option that allows the patient to retain their own bladder while increasing its capacity and decreasing pressure. This is an ideal option in patients who have problems with bladder capacity as well as bladder emptying and prefer catheterization *via* a stoma. In a study by Khavari *et al*[40], 34 patients underwent the Indiana augmentation cystoplasty, 12 patients had MS as their cause for bladder dysfunction. At their final follow up all patients were continent. The patients were found to have increased bladder capacity from an average of 239.7 mL to 444.4 mL, this increase allows the patients to have more time between catheterizations, which leads to an improvement in quality of life. The rate of complications from the surgery was still high; 17% of patients had early post-operative complications including ileus, 44.1% had long-term complications including pyelonephritis and reoperations for stomal revisions[40].

The final option is an ileal conduit urinary diversion. This allows the bladder to be removed in its entirety, and diverting the ureters to a conduit made of small bowel. This is an option for MS patients with refractory neuro-

Table 1 Comparison of studies of urinary diversion for neurogenic bladder in patients with multiple sclerosis

Study	Year	Location	Patient	Outcome
Urinary diversion/reconstruction for cases of catheter intolerant secondary progressive multiple sclerosis with refractory urinary symptoms[43]	2011	Lahey Clinic	26 MS patients (22 female) 15 ileovesicostomy, 7 enterocystoplasty and 4 ileal loop procedures	Improved continence and fewer UTIs
Management of neurogenic bladder dysfunction with incontinent ileovesicostomy[34]	1999	Wayne State	Thirteen patients incontinent ileovesicostomy 8 SCI, 4 MS, 1 TB Meningitis	Safe and effective at providing low pressure urinary drainage
Augmentation cystoplasty in patients with multiple sclerosis[38]	2003	Czech Republic	9 MS patients (7 females, 2 males)	Increased detrusor capacity and decreased pressure
Bladder neck closure and suprapubic catheter placement as definitive management of neurogenic bladder[39]	2011	Tulane	35 patients, 11 male 24 female 27 SCI, 5 MS, 4 other	97% continent, 8 requiring reprocedure to achieve continence
Functional outcomes after management of end-stage neurological bladder dysfunction with ileal conduit in a multiple sclerosis population: A monocentric experience[42]	2011	France	53 MS patients, 6 men 47 women	Statistically significant improved QOL
Robotic-assisted ileovesicostomy: Initial results[36]	2009	Lahey	8 MS patients	Safe and effective with minimal blood loss and shorter LOS
Prospective evaluation of laparoscopic assisted cystectomy and ileal conduit in advanced multiple sclerosis[41]	2012	France	44 MS patients, 34 women 10 men	Decrease in limitations and constraint scores and an increase in autonomy scores
A modification to augmentation cystoplasty with catheterizable stoma for neurogenic patients: Technique and long-term results[40]	2012	Methodist Hospital	12 MS patients	Safe and effective, no ureteral reimplants, no need for cystectomy

MS: Multiple sclerosis; UTI: Urinary tract infection; SCI: Spinal cord injry; TB: Tuberculosis; QOL: Quality of life; LOS: Length of stay.

genic bladder symptoms and poor functional status and dexterity. The indications for an ileal conduit are recurrent febrile urinary tract infections in the setting of poor bladder emptying, chronic retention or an indwelling catheter, urinary incontinence refractory to conservative treatment affecting patients' quality of life (QOL), chronic renal failure secondary to poor bladder compliance, and recurrent urethral bleeding or erosion due to urethral trauma in patients with indwelling catheters[41]. One key aspect of this procedure is the necessity to do a cystectomy at the same time as the diversion due to the risk of pyocystitis[42]. A study was done to determine the potential benefit of laparoscopic-assisted surgery on the cystectomy and ileal conduit procedure. The prospective study by Guillotreau showed that patients had improved QOL scores by decrease in limitations and constraint scores and an increase in autonomy scores. The study was also important in showing that the morbidities were similar to open cystectomy in terms of long term complications but immediate complications were less, *i.e.*, less blood transfusions, shorter hospital stay. In addition, a finding of the study was that patients the longest length of disease duration had significantly more complications, therefore it may be important to consider ileal conduit surgery earlier on in the management of these patients. These studies are summarized in Table 1[41].

There are other surgical options that are available depending on the patients' age and symptoms but these are much less common, including ileal chimney, appendicovesicostomy, and Indiana Pouch. There is very little data to support the use of these options in MS. Augmentation has the benefit of preserving the native ureteral insertion over ileal loop diversion. The ureteral stricture rate is approximately 10%, making this a large consideration[2].

CONCLUSION

MS is a debilitating disease, which can have significant effects on patients' quality of life. Bladder dysfunction is a common symptom experienced by MS patients. There are multiple treatments available if pharmacologic treatments fail. They key is understanding what the underlying bladder dysfunction is, how it may impact the kidneys over time, and what the patients treatment goals are. Minimally invasive options such as clean intermittent catheterization, external condom catheters in men. Neuromodulation, botulinum toxin, and major bladder surgery are available to address patient symptomatology. Sacral nerve stimulation has become a proven technique to treat bladder overactivity in the setting of MS with the possibility of also improving emptying function; however, it requires implantation of metal precluding future MRI. *Botox* was FDA approved to treat neurogenic detrusor overactivity in 2011 and has had a major impact on the treatment algorithm of these patients; however, intermittent catheterization is generally needed for adequate bladder emptying. Major surgery is a final option for patients suffering from bladder dysfunction due to MS. It is usually reserved for patients with deteriorating upper urinary tract function due to impaired bladder compliance and those with intolerable incontinence.

With the many options available it is important to review all of the options with the patient so they are well prepared for the option they have chosen and understand all the risks and benefits.

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REVIEW

Acute management of symptomatic nephrolithiasis

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Abstract

Over half a million patients present to emergency departments and nearly 3 million patients visit healthcare providers annually due to problems associated with urolithiasis. Despite updated guidelines from the American Urological Association and European Association of Urology for the evaluation and management of nephrolithiasis, considerable variability still exists regarding treatment for acute symptomatic upper urinary tract stones. Therefore, this article will review the current evaluation and management of acute symptomatic nephrolithiasis. Initial management includes analgesia and antiemetics. Additionally, a urinalysis and creatinine are required laboratory evaluations. Acute imaging with a non-contrast computed tomography (CT) scan is the diagnostic imaging modality of choice. However, concerns over radiation exposure have led towards low-dose and even ultra-low-dose protocols for the detection of urinary calculi. Low-dose non-contrast CT scans are now standard of care for the initial diagnosis of renal colic in patients with a body mass index $\leqslant$ 30. Medical expulsive therapy is recommended for patients with a ureteral calculus < 10 mm and no signs of infection. Emergency urinary decompression is mandatory for a specific subset of patients, especially those with infection. Although limited data exists, emergency ureteroscopy or even shock wave lithotripsy may also be therapeutic options.

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Key words: Nephrolithiasis; Low-dose computed tomography scan; Medical expulsive therapy; Ureteroscopy; Extracorporeal shockwave lithotripsy

Core tip: Despite updated guidelines from the American Urological Association and European Association of Urology for the evaluation and management of nephrolithiasis, considerable variability still exists regarding treatment for acute symptomatic upper urinary tract stones, especially in regards to imaging modalities used in the emergency department. Acute imaging with a non-contrast computed tomography scan is the diagnostic imaging modality of choice. However, concerns over radiation exposure have led towards low-dose and even ultra-low-dose protocols for the detection of urinary calculi. Low-dose non-contrast computed tomography scans are now standard of care for the initial diagnosis of renal colic in patients with a body mass index $\leqslant$ 30.

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INTRODUCTION

Over half a million patients present to emergency departments (ED) and nearly 3 million patients visit healthcare providers annually due to problems associated with urolithiasis[1]. This has lead to nearly $5 billion spent annually in the United States for hospitalizations, procedures, and time lost from work associated with renal/ureteral stone disease[2]. Despite updated guidelines from the American Urological Association (AUA) and European Associa-

tion of Urology (EAU) for the management of ureteral calculi, considerable variability exists among practitioners. In this review, the acute management of nephrolithiasis will be discussed with a particular emphasis on diagnostic imaging choice, initial medical therapy, and acute surgical interventions.

PRESENTATION

Patients with nephrolithiasis typically present with acute flank pain with or without radiation to the groin. This is referred to as renal colic. The pain is described as colicky in nature because it is intermittent and associated with restlessness, differing from peritonitic pain where patients often remain still. The pain is thought to arise due to obstruction of the ureter with continued peristalsis or spasms of the ureter around the stone. Additionally, obstruction can lead to hydronephrosis and/or hydroureter with pain arising due to distention of the collecting system and renal capsule[3]. The level of the pain can often give a hint as to its location in the collecting system. For example, stones in the proximal ureter present with classic isolated flank pain, while ureterovesical junction (UVJ) stones often present with groin pain associated with frequency, urgency, and dysuria due to irritation of the bladder[3]. In addition to renal colic, patients often present with nausea and vomiting. Furthermore, microscopic or gross hematuria can be a presenting sign of nephrolithiasis due to irritation of the mucosa by the stone or a coexisting urinary tract infection (UTI). Close attention needs to be paid to patients presenting with suspected nephrolithiasis and signs and symptoms of a UTI or sepsis as these patients may require emergency surgical intervention.

INITIAL DIAGNOSTIC IMAGING

A thorough history and physical examination is the first step in the evaluation of suspected renal colic. This is particularly important given the often non-specific flank or groin pain associated with nephrolithiasis. Once renal colic is suspected, diagnostic imaging should be performed with the choice of modality selected based on patient type. In the adult, non-pregnant patient, the preferred initial imaging modality of choice is a non-contrast computed tomography (NCCT) due to its high sensitivity (Median 98%) and high specificity (Median 97%) for identifying urinary calculi[4]. This sensitivity and specificity compares favorably to other imaging modalities (Table 1). NCCT scans not only accurately report the presence of stones, but also the size, location, density *via* Hounsfield units, evidence of obstruction, and skin to stone distance, which all help to determine the need for surgical intervention[5]. Additionally, they also provide information on alternative diagnoses such as appendicitis and diverticulitis.

Although NCCT are valuable in diagnosing urinary calculi, one disadvantage is the delivery of ionizing radiation. Recent investigations have shown an increased risk of a secondary malignancy after just 2-3 computed tomography (CT) scans in a single year, with an estimated 1.5%-2.0% of all cancers in the United States being attributed to the radiation from CT scans[5-8]. Additionally, nearly 50% of all radiation received by the United States population is a direct result of medical imaging, much of which is related to CT usage[9]. Furthermore, it has been estimated that the risk of cancer is 1 in 200 for every 100 mSv of radiation received[10]. This information is critical given the effective dose of radiation for a standard dose NCCT scan is 10 mSv[11,12].

Table 1 Median sensitivity and specificity in detecting nephrolithiasis for various imaging modalities

Imaging modality	Sensitivity[1]	Specificity[1]
Non-contrast CT	98%	97%
Abdominal X-ray	57%	76%
Intravenous pyelogram	70%	95%
Renal/bladder ultrasound	61%	97%
MRI	82%	98%

[1]Information from this table obtained from Ref. [4]. MRI: Magnetic resonance imaging; CT: Computed tomography.

As a response to the risk of ionizing radiation delivered by standard dose NCCT, low-dose NCCT (LD-NCCT) protocols were developed. This imaging has an effective dose of radiation of 4 mSv[13] or less. A study by Poletti *et al*[14] assessed 125 consecutive patients admitted to the emergency department for renal colic with both a standard dose and LD-NCCT scan. LD-NCCT scans had a 97% sensitivity and 96% specificity for the diagnosing of renal colic based on either direct identification of a calculi or *via* indirect signs of a calculi (*i.e.*, hydroureteronephrosis, perinephric stranding, *etc.*)[14].

When stratified by stone size, a LD-NCCT scan was equivalent to a standard dose NCCT scan for stones $\leqslant$ 3 mm in patients with a body mass index (BMI) < 30[14]. In patients with stones < 3 mm, a LD-NCCT scan performed worse with a sensitivity of 83%[14]. Additionally, LD-NCCT scans resulted in ± 20% size variation as compared to standard dose NCCT scan[14]. Despite these limitations, the authors noted no change in their clinical decision-making[14]. Furthermore, the majority of stones < 3 mm rarely require urgent urologic procedures and often will pass spontaneously[14].

When examining the effectiveness of LD-NCCT, BMI also is important. For patients with a BMI $\geqslant$ 30, a standard dose NCCT is still preferred. Poletti *et al*[14] reported only 50% sensitivity and 89% specificity in patients with BMI $\geqslant$ 30 as opposed to 95% sensitivity and 97% specificity for patients with a BMI < 30. Similar investigations have demonstrated equivalent results[15,16].

Additional studies have suggested that ultra LD-NCCT scans can be used for patients with a BMI < 30 without significant loss of sensitivity or specificity[17]. Udayasankar *et al*[18] investigated the use of ultra-low dose CT scans (mean effective radiation dose of 2.10 mSv)

in 163 patients presenting to the ED with abdominal pain and found a high sensitivity (100%) and specificity (98.5%) for detection of free air, stones, and intestinal obstruction[18]. Additionally, overall there was a high sensitivity (86%) and specificity (96%) for identifying other sources of abdominal pain with the conclusion that ultra-low dose NCCT scans provide accurate diagnostic information and very low radiation doses[18] in patients presenting with acute abdominal pain.

The AUA guidelines recommend the use of a LD-NCCT as the preferred initial imaging modality for patients with a BMI $\leqslant$ 30 who are presenting with symptoms of renal colic or in those with a prior history of urinary stones. However, in those with a BMI $\geqslant$ 30, LD-NCCT may be used, although the preferred imaging modality would be a standard dose NCCT. At this time, there is not enough data available to recommend an ultra LD-NCCT scan.

FOLLOW-UP DIAGNOSTIC IMAGING

A recent review of the National Hospital Ambulatory Medical Care Survey estimated that approximately 5%-10% of visits to the ED for nephrolithiasis were return visits[19]. While LD-NCCT is the imaging modality of choice during the initial presentation, Goldstone and Bushnell reported that repeat CT imaging of known nephrolithiasis changed the diagnosis in only a small percentage of patients[11]. Therefore, the AUA recommends that initial imaging should include a renal ultrasound (RUS) and KUB in patients presenting with a known radio-opaque ureteral/kidney stone and persistent symptoms[4]. If no hydronephrosis or stone is identified on KUB or RUS and the patient is still symptomatic, then a LD-NCCT scan is recommended[4]. In those with radiolucent stones and persistent symptoms, RUS can be used to assess for hydronephrosis with a clinical decision made whether to repeat a NCCT based on the RUS results[4].

INITIAL MANAGEMENT

The initial management for renal colic is supportive care with analgesia and anti-emetics. The mainstay of pain control for renal colic includes non-steroidal anti-inflammation drugs (NSAIDs) and narcotic medications. NSAIDs have been shown to provide improved pain relief *vs* narcotics without the added side effects of nausea or vomiting[3]. Therefore, an oral or intravenous NSAID is first line therapy[3]. Narcotic medications can be added for additional relief. Furthermore, antiemetic medication can be utilized as needed for nausea and/or vomiting often associated with renal colic. There is no evidence supporting increased fluid intake for acutely symptomatic stones to help with spontaneous passage; however, increased fluid intake may help prevent future stones. Patients being treated conservatively should strain their urine for confirmation of passage and for analysis[3].

INITIAL LABORATORY AND URINE EVALUATION

According to the EAU guidelines, all patients presenting with acute symptomatic nephrolithiasis should have a urine dipstick to assess for blood in the urine, leukocytes for signs of inflammation, and nitrite to assess for specific bacteria and thus infected urine[20]. If the urine dipstick is suspicious for infection, a urine culture should be sent[20]. Additionally, all patients should have a creatinine level to assess for acute kidney injury and the possibility of an obstructive process[20]. In patients with a fever, evaluation should also include a complete blood count [for analysis of a patient's white blood cell (WBC) count for evidence of inflammation or infection] and C-reactive protein[20]. Additional studies can include a basic metabolic panel for analysis of sodium and potassium levels in those with nausea and vomiting[20].

INDICATIONS FOR CONSULTATION

Many patients with an acute episode of nephrolithiasis initially present to their primary care physician or the ED. At our institution we recommend consulting urology if pain is intractable, the patient is unable to tolerate an oral diet due to persistent nausea or vomiting, there is evidence of obstructive uropathy, concurrent UTI is suspected, or in any patient with a solitary or transplant kidney. If the patient is to be discharged from a primary care physician or ED, we generally recommend urology outpatient follow up in 1-2 wk in all cases of nephrolithiasis.

EMERGENCY DECOMPRESSION

The majority of patients (83%) presenting with nephrolithiasis will pass their stone without any need for intervention[21]. Furthermore, 95% of these patients will pass their stone within 6 wk[21]. While most patients will eventually pass small ureteral stones, clear indications for decompression in the acute management of ureteral stones includes the presence of infection, intractable pain or vomiting, obstruction in a solitary or transplant kidney, bilateral obstructing stones, or relief of ureteral calculi obstruction in pregnant females pending definitive management post-partum[20]. Randomized controlled trials (RCTs) have shown ureteral stenting and percutaneous nephrostomy (PCN) tubes are equally effective for emergency decompression of the urinary system[22]. A small RCT of 42 patients by Pearle *et al*[22] investigated ureteral stent *vs* PCN tube for obstructive ureteral stones and signs of infection, reporting equal times to normalization of fever and WBC count with a trend towards longer hospital stays in those following PCN placement. Another small trial assessed 40 patients with a ureteral stone and hydronephrosis, with or without signs of infection, and did not demonstrate a difference in outcomes between ureteral stent and PCN tube placement[23]. A recent retrospective study by Goldsmith

et al[24] investigated patients with obstructive stones identified on CT scan and systemic inflammatory response syndrome at the time of diagnosis to determine differences in outcomes between ureteral stent and PCN tube placement. A total of 130 patients met inclusion criteria. Patients selected for PCN tube placement had larger stones (10 mm *vs* 7 mm), were more ill based on their APACHE score, and had a higher proportion of surgically altered urinary tract anatomy[24]. After resolution of the patient's sepsis, those undergoing ureteral stent were more likely to be treated with ureteroscopy (65% *vs* 40%, $P = 0.004$) and those undergoing PCN tube placement were more likely to be treated with percutaneous nephrolithotomy (38% *vs* 6%, $P = 0.001$)[24]. Time from initial septic event to definitive treatment and rate of spontaneous stone passage was similar between the PCN tube and ureteral stent group[24]. Intensive care unit admission rates were higher for the PCN tube group (42% *vs* 20%, $P = 0.006$), likely due to more ill patients being selected for PCN tube placement[24].

In summary, indications for emergency urinary tract decompression include intractable pain, nausea/vomiting, evidence of obstructive uropathy, symptoms or signs of infection, and calculi in a solitary or transplant kidney. The preferred method of decompression (ureteral stent or PCN) is likely equivalent, and should therefore be based on stone size, stability of the patient, available hospital resources, and anticipated future method of definitive treatment.

URGENT URETEROSCOPY

In the acute management of stones, patients are typically discharged without the need for a procedure. If, however, a procedure is indicated, then palliation with a ureteral stent or PCN is often performed. Despite this common practice, recent investigations have assessed emergency ureteroscopy. Proponents of this practice cite that immediate stone removal can relieve pain, and prevent multiple trips to the operating or emergency room. Sarica *et al*[25] published a prospective study on 145 patients presenting to the ED with obstructing ureteral stones. Stones were located in the distal ureter in 67.6% and proximal ureter in 32%[25]. Patients were split into either ureteroscopy within 24 h of first colic attack or medical expulsive therapy (MET) for > 7 d followed by ureteroscopy within 7-21 d[25]. There was no difference in intraoperative complications or stone location[25]. Ureteral stents were placed in 24.6% of those on MET *vs* 0% in those undergoing immediately ureteroscopy ($P = 0.001$)[25]. There was no difference in the need for additional procedures[25]. Stone free rate was 87.9% in the MET first group and 90.8% in the emergency ureteroscopy group[25]. Readmission rates were higher in the MET first group, with 3.03 mean readmission to the ED[25].

Al-Ghazo *et al*[26] examined 244 patients treated with emergency ureteroscopy (within 24 h of admission) for acutely symptomatic ureteral stones. Overall success rate, defined as complete absence of stone fragments at 4 wk post-operatively, was 90.6%[26]. Proximal ureter, mid ureter, and distal ureter stones had 69.4%, 94.8%, and 96.6% success rates, respectively ($P < 0.001$)[26]. Overall complication rate was 13.1%, decreasing to 2.5% when excluding stones 10 mm or greater, consistent with prior studies[27-30]. The success rate of ureteroscopy is due in part to improving optics and advances in intracorporeal lithotripters such as the holmium yttrium-aluminum-garnet laser, allowing for safe and effective lithotripsy and stone removal[31]. Another advantage to ureteroscopy is that it does not require thromboprophylaxis following surgery, except in high risk patients[32].

Although limited data exists on this topic, ureteroscopy within 24 h of initial presentation may be a viable option, especially for patients with a symptomatic, obstructing mid to distal ureteral stone without evidence of infection. However, further investigation is necessary prior to widespread adoption.

EMERGENCY EXTRACORPOREAL SHOCKWAVE LITHOTRIPSY

Since its introduction in the 1980s, emergency extracorporeal shockwave lithotripsy (ESWL) is a minimally invasive method to treat both kidney and ureteral stones. According to the EAU guidelines, ESWL and ureteroscopy are both first line treatments for proximal ureteral stones[20]. A recent meta-analysis by Picozzi *et al*[33] assessed 7 studies with a total of 570 patients who underwent urgent ESWL for the treatment of a symptomatic stone. Stone free rates and complication rates did not differ statistically from those reported in the most recent AUA or EAU guidelines for elective ESWL; however, subsequent surgery was required in 15.8% of patients to completely remove the stone[33]. ESWL is thus an option to emergently treat stones, although further investigation is needed. One must be careful in performing ESWL in patients with a known bleeding diathesis or on blood thinning medications. Appropriate bridging therapy should be utilized in patients on warfarin and patients on antiplatelet therapy should discontinue these medications prior to ESWL as severe complications have been reported[32,34,35]. These patient's tend to undergo ureteroscopy as it is safer from a bleeding standpoint. This should be taken into account when deciding the best treatment method, especially if emergency surgery is being considered.

MEDICAL EXPULSIVE THERAPY

The majority of patients (83%) presenting with nephrolithiasis will pass their stone without any need for intervention[21]. Therefore, the EAU guidelines recommend for ureteral stones < 10 mm with minimal to moderate hydronephrosis and no evidence of renal damage, observation with or without MET is standard of care[20]. MET

has been shown to improve the rate of stone passage[20]. Calcium channel blockers, steroids, and alpha-blockers have all demonstrated improved stone passage rates[20]. Steroids are usually avoided because of the numerous systemic effects. In a meta-analysis of available RCTs comparing MET to placebo, calcium channel blockers showed an absolute increase in stone passage of 9% and alpha-blockers shown an absolute increase in stone passage of 29%[20]. Therefore, alpha-blockers are the preferred agent for MET.

Tamsulosin is the most widely studied alpha-blocker used for MET. Fan *et al*[36] performed a meta-analysis of 20 RCTs across 10 countries including 799 patients in the tamsulosin arm and 794 patients in the control arm. Expulsion rates for lower and upper ureteral stones were significantly higher in the tamsulosin arm (lower ureteral stones: RR = 1.55, $P < 0.00001$; upper ureteral stones: RR = 1.28; $P = 0.02$)[36]. Additionally, expulsion time was improved in the tamsulosin group by an average of 2.63 d[36]. These patients also had fewer colic episodes and underwent fewer auxiliary procedures. In a RCT, Al-Ansari *et al*[37] studied 100 patients with lower ureteral stones and compared placebo to tamsulosin and found spontaneous passage rate to be 82% in the tamsulosin group *vs* 61% in the placebo group. Expulsion time was also shorter[37]. Yencilek *et al*[38] showed improved passage rates in those receiving tamsulosin *vs* placebo for ureteral stones < 5 mm (passage rate 71.4% *vs* 50%).

In summary, for ureteral calculi < 10 mm without signs of infection or acute renal failure, a trial of MET should be initiated. Alpha-blockers are considered first line for MET due to the familiarity with the drugs, improved rates of spontaneous passage, decreased time to stone passage, and fewer colic episodes. While tamsulosin is the most studied medication for MET, other alpha-blockers should have similar outcomes. For those with documented spontaneous passage of their stone, repeat imaging is not necessary. If the patient is persistently symptomatic after passage, Fulgham *et al*[4] recommend a follow up RUS with a NCCT if the patient has hydronephrosis. While the optimal length of time of MET before intervention is controversial, common practice is for 4-6 wk[39].

PREGNANT PATIENTS

Nephrolithiasis affects about 1 in 500 pregnancies[40-42] and often becomes symptomatic in the second or third trimester[43-45]. Fortunately 70%-80% of these patients will pass their stone spontaneously with conservative management[45]. A RUS is universally accepted as the first line study in pregnant patients presenting with suspected nephrolithiasis with a sensitivity of 34% and specificity of 86%[46]. If a RUS fails to identify nephrolithiasis or alternative diagnoses, the EAU recommends either a transvaginal ultrasound to assess for UVJ or bladder stones or an MR Urography (MRU), which avoids ionizing radiation[20]. MRU has limited capacity to identify small calculi, is costly, and is often unavailable; however, it avoids ionizing radiation, which may increase the risk of secondary malignancies[47-49]. Additionally, MRU should not be used in the first trimester due to unknown risks to the developing fetus[47,50]. Some have advocated the use of LD-NCCT scans in complicated cases where no other diagnosis has been identified, but this requires ionizing radiation and patients must be counseled extensively about the risks and benefits to the mother and fetus. Most notably, a single pelvic CT may increase the risk of childhood cancer in the exposed fetus by 2 times; however, due to the low absolute risk of childhood cancer (1 in 2000), the increase in absolute risk is extremely low[51]. The American Congress of Obstetricians and Gynecologists guidelines for diagnostic imaging during pregnancy report that exposure to less than 5 rad (which is the case for a NCCT of the abdomen and pelvis) has not been associated with an increase in fetal anomalies or pregnancy loss and that a single diagnostic X-ray procedure does not result in harmful fetal effects[52].

Pregnant patients should be treated similarly to non-pregnant patients with fluids and analgesia[20]. Additionally, urinary diversion with a ureteral stent or PCN may be required in the emergent setting when meeting the same criteria as the non-pregnant patient. These should be placed using ultrasound guidance or with limited fluoroscopic radiation as possible. Ureteral stents or PCN tubes need to be exchanged every 4-8 wk during pregnancy *vs* every 3 mo in non-pregnant patients due to an increased risk of encrustation[20]. Ureteroscopy, using laser lithotripsy, is increasing being employed in this population as experience has increased[20].

CONCLUSION

Nephrolithiasis is common and is often treated by urologist and non-urologists alike. While the AUA and EAU currently have guidelines for the evaluation and management of nephrolithiasis, these are directed at urologists. To our knowledge no national or universal guidelines exist for the acute management of stone disease in the ED. Therefore, we hope that this review will assist physicians to evaluate and manage nephrolithiasis in the acute care setting.

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REVIEW

Metabolic syndrome in the development and progression of prostate cancer

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Abstract

Prostate cancer (PCa) is the most common noncutaneous malignancy and second leading cause of cancer-specific mortality for men in the United States. There is a wide spectrum of aggressiveness ranging from biologically significant to indolent disease, which has led to an interest in the identification of risk factors for its development and progression. Emerging evidence has suggested an association between metabolic syndrome (MetS) and PCa. MetS represents a cluster of metabolic derangements that confer an increased risk of cardiovascular disease and type 2 diabetes mellitus. Its individual components include obesity, dyslipidemias, high blood pressure, and high fasting glucose levels. MetS has become pervasive and is currently associated with a high socioeconomic cost in both industrialized and developing countries throughout the world. The relationship between MetS and PCa is complex and yet to be fully defined. A better understanding of this relationship will facilitate the development of novel therapeutic targets for the prevention of PCa and improvement of outcomes among diagnosed men in the future. In this review, we evaluate the current evidence on the role of MetS in the development and progression of PCa. We also discuss the clinical implications on the management of PCa and consider the future direction of this subject.

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Key words: Diabetes mellitus; Dyslipidemias; Humans; Hyperglycemia; Hypertension; Insulin resistance; Male; Metabolic syndrome X; Obesity; Prostatic neoplasms

Core tip: The current literature is conflicted on the association between metabolic syndrome (MetS) and prostate cancer (PCa), although several studies have demonstrated that men with MetS or its individual components may have an increased risk of more aggressive disease and mortality as well as a poorer outcome after their treatment for PCa. These men may benefit from weight loss, physical activity, and the addition of medications like statins for preventing PCa and improving their outcomes after treatment. A majority of the existing evidence is retrospective or observational in nature, which underscores the need for more randomized controlled trials in the future.

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INTRODUCTION

Prostate cancer (PCa) has been the most common noncutaneous malignancy diagnosed in men since 1984 and currently accounts for almost 30% of new cancer cases in the United States. Concurrent with the introduction of prostate-specific antigen (PSA)-based screening and development of effective treatments for PCa, a steady decline in its mortality rate has been observed since 1991.

Table 1 Joint criteria for clinical diagnosis of the metabolic syndrome[1]

Component	Threshold
Abdominal obesity	Sex- and population-specific waist circumference based on definitions established by IDF and AHA/NHLBI[8,9]
Dyslipidemias (or pharmacologic treatment)	
High triglycerides and/or	⩾ 150 mg/dL
Low high-density lipoprotein cholesterol	< 40 mg/dL in males, < 50 mg/dL in females
High blood pressure (or pharmacologic treatment)	Systolic ⩾ 130 mm Hg and/or Diastolic ⩾ 85 mm Hg
High fasting glucose (or pharmacologic treatment)	⩾ 100 mg/dL

[1]Must have 3 of the following 5 components. IDF: International Diabetes Federation; AHA/NHLBI: American Heart Association/National Heart, Lung, and Blood Institute.

Currently, only 16% of men diagnosed with PCa succumb to their disease[1]. This marked disparity between the incidence and mortality rates of PCa reflects a wide spectrum of aggressiveness. Differentiating between biologically significant and indolent disease, however, has proven to be difficult and led to an interest in the identification of risk factors for its development and progression.

Although the pathogenesis of PCa remains largely unknown, both genetic and environmental factors are thought to contribute to its development and progression. Significant geographic variations in the incidence and mortality rates of PCa indicate a possible role for dietary, lifestyle-related, and other environmental factors. Epidemiologic studies, for instance, have reported a 10- to 15-fold increased incidence of PCa in western compared to Asian countries and a rapidly rising incidence in Asian countries with the adoption of a more westernized lifestyle[2-4]. Migrant studies have also revealed that Asian men living in the United States have an increased risk of PCa compared to their counterparts living in their native countries[2,5]. However, it is unclear whether this increased incidence is related to the routine use of PSA-based screening in the United States.

The influence of westernization on the risk of PCa may be related to the pervasiveness of obesity and a sedentary lifestyle. A growing body of evidence has specifically identified an association between metabolic syndrome (MetS) and PCa. Their relationship is complex and yet to be fully defined. Developing a better understanding of this relationship may provide an opportunity for the prevention of PCa and improvement of outcomes among diagnosed men in the future. In this review, we evaluate the current evidence on the role of MetS in the development and progression of PCa. We also discuss the clinical implications on the management of PCa and consider the future direction of this subject.

REVIEW OF LITERATURE

A PubMed search was performed for relevant articles between 1966 and 2014. Terms for the search included MetS, obesity, dyslipidemias, hypertension, diabetes mellitus, hyperglycemia, and insulin resistance combined with PCa. Only articles published in the English language and limited to humans were considered. All titles and abstracts were reviewed for their relevance, after which the full texts of selected articles were reviewed. The full texts of additional articles were also reviewed based on the references of selected articles.

DEFINING THE METS

MetS represents a cluster of metabolic derangements that confer an increased risk of cardiovascular disease and type 2 diabetes mellitus (DM). Its individual components include obesity, dyslipidemias, high blood pressure (BP), and high fasting glucose levels. There often is an associated proinflammatory state and insulin resistance, both of which have been implicated in the pathophysiology of MetS.

Since the introduction of syndrome X by Gerald Reavan in 1988, a considerable amount of disagreement has emerged over the terminology and diagnostic criteria related to MetS. Various definitions have been proposed by multiple groups and international organizations over the past 15 years, beginning with the original definition by the World Health Organization in 1998[6-9]. The most recent recommendations by the International Diabetes Federation and American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) still differed on the importance of abdominal obesity and its definition being based upon waist circumference[8,9]. However, an attempt has currently been made to reconcile these differences and agree upon common criteria for the clinical diagnosis of MetS (Table 1)[10].

MetS has become a global epidemic and public health-related issue with a high socioeconomic cost. Based on the National Health and Nutrition Examination Survey from 2003 to 2006, approximately 34% of adults in the United States met the National Cholesterol Education Program adult treatment panel (ATP) Ⅲ criteria for MetS. The prevalence of MetS increased with an advancing age and obesity as measured by body mass index (BMI) in this population. It also varied by race, ethnicity, and gender[11]. Similarly, MetS is prevalent in other industrialized and developing countries throughout the world. Based on a meta-analysis, the prevalence of MetS varied from 10% (France) to 36.4% (India) for men in various populations aged from 20 to 25 years and older, as defined by ATP Ⅲ criteria[12].

THE METS AND RISK OF PCA

It is well-established that the development and progression of PCa is potentiated through the dysregulated stimulation of androgen receptor-mediated pathways in prostatic cells. Due to the identification of common putative pathways involving androgen synthesis and MetS, an increasing number of authors have investigated the association between MetS and development of PCa, but their findings have been equivocal and difficult to compare (Table 2). In 2004, Hammarsten *et al*[13] retrospectively analyzed 299 men diagnosed with PCa in Sweden and demonstrated that men with higher clinical stage and grade disease were more likely to have various components of MetS than men with lower clinical stage and grade disease. These findings were later supported by 3 cohort studies from Scandinavia, in which men with MetS or various components had an increased risk of developing PCa[14-16]. It should be noted that a majority of men did not participate in PSA-based screening in these studies.

Conversely, a large cohort study of a more diverse population from the United States observed an inverse association between MetS and development of PCa. Its authors reported a decreased risk of 23% (95%CI: 0.6-0.98) in men with ⩾ 3 components of MetS, which remained significant after excluding diabetic men (RR = 0.71; 95%CI: 0.54-0.94). Interestingly, non-diabetic men with 2 components had an increased risk of 37% (95%CI: 1.01-1.87), suggesting that the extent and duration of MetS as well as the presence of DM may affect the risk of PCa[17].

Other large cohort studies have also failed to demonstrate an association between MetS and development of PCa. In a cohort of 29364 men from Norway, Martin *et al*[18] observed that MetS and its individual components were not associated with the development of incident or fatal PCa, except for an increased risk of 8% (95%CI: 1%-17%) for each 12 mmHg increase in diastolic BP[18]. Another study followed 2445 men between 40 and 79 years of age participating in the Olmsted County Study over a period of 15 years and reported that multiple components of MetS each had a distinct association with the risk of developing PCa when considered individually and in various combinations[19]. Similarly, Häggström *et al*[20] demonstrated that MetS and its individual components were differently associated with the development of incident PCa in a cohort of 289866 men from Austria, Norway, and Sweden. These authors, however, observed an increased risk of PCa-specific mortality for men in the top quintile for BMI (RR = 1.36; 95%CI: 1.08-1.71) and systolic BP (RR = 1.62; 95%CI: 1.07-2.45) as well as for each 1-unit increase in the composite z score of all metabolic factors (RR = 1.13; 95%CI: 1.03-1.25)[20]. These are the only studies to consider the effect of MetS and its individual components on the risk of PCa. The individual components of MetS were differently associated with PCa in each study, emphasizing the importance of considering their separate and combined effects.

Given other evidence suggesting an association between vitamin D levels and PCa as well as the identification of common putative pathways involving vitamin D and lipid metabolism, Tuohimaa *et al*[21] investigated the combined influence of MetS and vitamin D levels on the development of PCa in a cohort of 588 men between 40 and 58 years of age participating in Helsinki Heart Study. Vitamin D levels were defined as low if <40 nmol/L, normal if 40-59 nmol/L, and high if ⩾ 60 nmol/L. These authors demonstrated an increased risk for men in the highest quartile for BMI (OR = 2.28; 95%CI: 1.22-4.25), systolic BP (OR = 3.33; 95%CI: 1.72-6.44), and diastolic BP (OR = 2.47; 95%CI: 1.3-4.69) only when they had low vitamin D levels as well. An increased risk of PCa was also observed when low vitamin D levels were simultaneously present with a high BMI and systolic BP (OR = 3.85; 95%CI: 1.57-9.41) as well as a high BMI, systolic BP, and low high-density lipoprotein (HDL) cholesterol levels (OR = 8.03; 95%CI: 1.89-34.09) but not when considered with normal or high vitamin D levels[21].

Several studies have been conducted outside of the United States or Scandinavian countries with conflicting results. In a case-control study of 2745 men less than 75 years of age from Italy, Pelucchi *et al*[22] reported an increased risk of PCa in those with MetS (OR = 1.66; 95%CI: 1.26-1.89). There was a dose-response relationship, with men having an increased risk of 12% (95%CI: 0.89-1.42) for any 2 components of MetS, 65% (95%CI: 1.15-2.36) for any 3 components, and 299% for any 4 components (95%CI: 1.03-15.4)[22]. Conversely, Russo *et al*[23] failed to demonstrate an increased incidence of PCa in a cohort of 16677 men greater than 40 years of age simultaneously prescribed with medications for hypertension, dyslipidemias, and DM in Italy[23]. Two large cohort studies from Japan have also failed to observe an association between MetS and development of PCa[24,25].

With the exception of the study by Tande *et al*[17], all of the previously discussed studies have primarily included Caucasian men. Due to the known increased risk of MetS and PCa in African-American men, Beebe-Dimmer *et al*[26] investigated the association between MetS and development of PCa in 498 African-American men between 40 and 79 years of age participating in the Flint Men's Health Study. These authors reported an increased risk of PCa in men with hypertension (OR = 2.4; 95%CI: 1.5-3.7) and a waist circumference > 102 cm (OR = 1.8; 95%CI: 1.2-2.9) individually. There was also an increased risk of PCa in men with any 2 components of MetS (OR = 1.76; 95%CI: 1.1-2.83)[26]. A subsequent study followed a diverse population of 881 men less than 75 years of age participating in the Genes Environment and PCa Study and sought to determine any racial differences in the association between MetS and development of PCa. Its authors demonstrated a marginal association between MetS and development of PCa among African-American men (OR = 1.71; 95%CI: 0.97-3.01) but not among Caucasian men (OR = 1.02; 95%CI: 0.64-1.62). MetS was further

Table 2 Summary of studies on the association between the metabolic syndrome and prostate cancer

Ref.	Design	Country	Population	Time period	Size of cohort	Number of PCa cases	Criteria for MetS	Findings	Association
Hammarsten *et al*[13]	Cross-sectional	Sweden	Referrals with PCa	1995-2002	299	299	N/A	Increased risk of clinical stage T3 and high-grade disease with various components	Positive
Laukkanen *et al*[14]	Longitudinal population-based cohort	Finland	Kuopio communities	1984-2001	1880	56	Modified WHO	Increased risk (RR = 1.9; 95%CI: 1.1-3.5)	Positive
Lund Håheim *et al*[15]	Longitudinal population-based cohort	Norway	Oslo study	1972-1998	15933	507	Modified ATP Ⅲ	Increased risk (RR = 1.56; 95%CI: 1.21-2.0)	Positive
Tande *et al*[17]	Longitudinal population-based cohort	United States	ARIC study	1987-2000	6429	385	ATP Ⅲ	Decreased risk (RR = 0.77; 95%CI: 0.6-0.98)	Inverse
Tuohimaa *et al*[21]	Longitudinal nested case-control	Finland	Helsinki heart study	1981-1997	588	132	N/A	Increased risk with high BMI, SBP, low HDL-C, vitamin D (OR = 8.03; 95%CI: 1.89-34.09)	Positive
Beebe-Dimmer *et al*[26]	Longitudinal case-control	United States	Flint Men's Health Study	1996-2002	498	139	Modified ATP Ⅲ	Increased risk in AA men with 2 components (OR = 1.76; 95%CI: 1.1-2.83)	Positive
Russo *et al*[23]	Longitudinal population-based cohort	Italy	Men treated for PCa	1999-2005	16677	94	Treated for MetS	No association (RR = 0.93; 95%CI: 0.75-1.14)	Null
Inoue *et al*[24]	Longitudinal population-based cohort	Japan	Japan Public Health Center-based Prospective Study	1993-2004	9548	119	Modified IDF	No association (HR = 0.76; 95%CI: 0.47-1.22)	Null
Beebe-Dimmer *et al*[27]	Longitudinal case-control	United States	GECAP study	2001-2004	881	637	Modified ATP Ⅲ	Increased risk of organ-confined disease in AA men (OR = 1.82; 95%CI: 1.02-3.23)	Positive
Martin *et al*[18]	Longitudinal population-based cohort	Norway	2nd Nord Trøndelag Health Study	1995-2005	29364	687	Modified ATP Ⅲ	No association (HR = 0.91; 95%CI: 0.77-1.09)	Null
Grundmark *et al*[16]	Longitudinal population-based cohort	Sweden	Uppsala Longitudinal Study of Adult Men	1970-2003	2322	237	ATP Ⅲ, modified IDF	Increased risk only under competing risk analysis	Positive
De Nunzio *et al*[28]	Cross-sectional	Italy	Men with PSA ≥ 4 or abnormal DRE	2009-2010	195	83	ATP Ⅲ	Increased risk of high-grade disease (OR = 3.82; 95%CI: 1.33-10.9)	Positive
Wallner *et al*[19]	Cross-sectional	United States	Olmsted county study	1990-2005	2445	206	Modified WHO	No association (HR = 0.81; 95%CI: 0.2-3.3)	Null
Pelucchi *et al*[22]	Longitudinal case-control	Italy	Men admitted to participating hospitals	1991-2002	289866	6673	Joint criteria	Increased risk (OR = 1.66; 95%CI: 1.22-2.28)	Positive
Osaki *et al*[25]	Longitudinal population-based cohort	Japan	General health examinees in Tottori Prefecture	1992-2007	8239	152	Modified WHO, ATP Ⅲ, IDF	No association based on any criteria	Null
Jeon *et al*[30]	Cross-sectional	South Korea	Men with PSA ≥ 4 or abnormal DRE	2003-2011	354	90	ATP Ⅲ	Increased risk of high-grade disease (OR = 0.101; 95%CI: 0.022-0.473)	Positive
Häggström *et al*[20]	Longitudinal population-based cohort	Norway, Sweden, Austria	Metabolic Syndrome and Cancer Project	1972-2006	289866	6673	Modified ATP Ⅲ	Increased risk of PCa-specific mortality with increased composite metabolic factors (RR = 1.13; 95%CI: 1.03-1.25)	Positive

Morote *et al*[29]	Cross-sectional	Spain	Men with PSA ⩾ 4 or abnormal DRE	2006-2010	2408	848	ATP Ⅲ	Increased risk of high-grade disease (OR = 1.75; 95%CI: 1.26-2.41)	Positive
Cicione *et al*[31]	Cross-sectional	Italy	Men with HGPIN	2004-2011	161	42	ATP Ⅲ	Increased risk with widespread HG-PIN (57.4% *vs* 23.5%)	Positive

PCa: Prostate cancer; MetS: Metabolic syndrome; N/A: Not applicable; WHO: World Health Organization; ATP: Adult treatment panel; ARIC: Atherosclerosis risk in communities; AA: African-American; GECAP: Genes environment and prostate cancer; IDF: International diabetes federation; PSA: Prostate-specific antigen; HGPIN: High-grade prostatic intraepithelial neoplasia.

associated with organ-confined disease (OR = 1.82; 95%CI: 1.02-3.23) but not advanced disease (OR = 0.93; 95%CI: 0.31-2.77) among African-American men. Interestingly, obese Caucasian men had a decreased risk of PCa (OR = 0.51; 95%CI: 0.33-0.8) and high-grade disease (OR = 0.30; 95%CI: 0.15-0.59), neither of which was observed among obese African-American men[27]. Whether this increased incidence is related to more aggressive screening practices in African-American men in the United States is unclear.

Several studies have investigated the association between MetS and development of PCa in a population at risk rather than the general population. In a cohort of 195 men with a median age of 69 years undergoing transrectal ultrasound-guided biopsies for PSA ⩾ 4 or an abnormal digital rectal exam (DRE), De Nunzio *et al*[28] reported an increased risk of high-grade disease in those with MetS (OR = 3.82; 95%CI: 1.33-10.9)[28]. A similar association between MetS and high-grade disease was demonstrated in another study of 2408 men with a median age of 68 years undergoing biopsies (OR = 1.75; 95%CI: 1.26-2.41)[29]. Conversely, Jeon *et al*[30] observed a decreased risk of Gleason grade ⩾ 7 (OR = 0.101; 95%CI: 0.022-0.473) as well as a lower Gleason grade of 6.63 ± 1.92 in men with MetS compared to 7.54 ± 1.71 in men without MetS[30]. Cicione *et al*[31] also reported that men with MetS and widespread high-grade prostatic intraepithelial neoplasia in ⩾ 4 cores had an increased risk of PCa on repeat biopsy in 6 mo (57.4% *vs* 23.5%). However, there is a potential for selection bias in these studies, as primary care providers and urologists are more likely to have a higher threshold for referral and biopsy, respectively, in men with multiple medical comorbidities.

A recent pooled analysis of studies between 2004 and 2007 demonstrated a 54% increased risk (95%CI: 1.23-1.94) of developing PCa in men with any 3 components of MetS[32]. A meta-analysis of 19 studies, though, did not confirm an association between MetS and overall risk of PCa (RR = 0.96; 95%CI: 0.85-1.09) but observed an increased risk of high-grade (RR = 1.44; 95%CI: 1.2-1.72) and advanced (RR = 1.37; 95%CI: 1.12-1.68) disease[33]. However, the findings of these studies are difficult to compare due to their different designs, particularly concerning the dissimilar populations with different rates of PCa, variable screening practices, use of various and modified criteria for MetS, and exclusion of certain risk factors or diabetic men. All of these studies are also retrospective or observational with inconsistent consideration of certain confounding variables. Lastly, only a few studies consider both MetS and its individual components, which appear to be differently associated with PCa. It is therefore difficult to conclude an association between MetS and development of PCa with any certainty.

COMPONENTS OF THE METS AND RISK OF PCA

Several authors have suggested that it may not be adequate to consider MetS as an individual entity but rather as a product of the separate and combined effects of its components[19].

Obesity

Several studies have reported an increased risk of PCa in obese men, while others have demonstrated either a null or even an inverse association between obesity and development of PCa. Due to these equivocal findings, MacInnis and English performed a meta-analysis of 31 cohort and 25 case-control studies and observed an increased risk of 5% (95%CI: 1.01-1.08) for each 5 kg/m^2 increase in BMI. A sub-group analysis of only studies reporting the stage of disease demonstrated a stronger association for advanced disease (RR = 1.12 per 5 kg/m^2 increment; 95%CI: 1.01-1.23) compared to localized disease (RR = 0.96 per 5 kg/m^2 increment; 95%CI: 0.89-1.03)[34]. Several large cohort studies and a more recent meta-analysis have subsequently confirmed these findings by observing an increased risk of high-grade and advanced disease as well as a decreased risk of low-grade and localized disease in obese men[35-38].

Various theories have been proposed for the differential influence of obesity on the risk of PCa. Some authors have suggested an inherent difference in the aggressiveness

of PCa due to lower testosterone levels in obese men[39]. Others have argued that a bias against the detection of PCa leads to its delayed diagnosis due to difficulty with DRE, lower serum PSA levels, and a larger prostatic size in obese men. Despite the anecdotal reports of difficulty with DRE in obese men, Price *et al*[40] failed to demonstrate any association between BMI and findings on DRE in men being screened for PCa. Alternatively, a number of studies have observed an inverse relationship between BMI and PSA, which may lead to delayed biopsies due to lower serum PSA levels in obese men[39]. This relationship is thought to be related to lower testosterone levels and/or a hemodilution effect on serum PSA concentrations from greater plasma volumes in obese men[41,42]. Two studies have also reported a larger prostatic size in obese men, which may decrease the likelihood of detection due to sampling error[43,44].

Dyslipidemias

Many studies have investigated the association between dyslipidemias and PCa since the initial finding that a cholesterol-lowering diet may increase the risk of various cancers and cancer-specific mortality in 1971[45]. The findings of earlier studies have largely been equivocal and unable to differentiate whether low cholesterol levels are the cause or effect of PCa. More recent evidence seems to favor an increased risk of PCa in men with various derangements of lipid metabolism[46].

In a nested case-control study of 698 men between 40 and 75 years of age participating in the Health Professionals Follow-up Study, Platz *et al*[47] demonstrated a decreased risk of high-grade PCa for men in the bottom quartile for total cholesterol (TC) level (OR = 0.61; 95%CI: 0.39-0.98). Several large cohort studies have subsequently confirmed these findings and further observed an increased risk of high-grade disease, advanced disease, and cancer-specific mortality in men with high TC levels[48-52]. Conversely, Van Hemelrijck *et al*[53] failed to report an association between TC levels and development of PCa in a cohort of 200660 men participating in the Swedish Apolipoprotein Mortality Risk Study. These authors noted that the associations between various components of MetS and PCa may be altered by non-cancer-related mortality due to the competing risk of premature cardiovascular death before the development of PCa. They suggested that the association between high glucose levels and decreased incidence of PCa may be overestimated, while the increased incidence of PCa in diabetic men with high triglyceride (TG) levels may be underestimated under a competing risk analysis[53].

Few studies have investigated the separate influence of HDL and low-density lipoprotein (LDL) cholesterol levels on the development of PCa. Van Hemelrijck *et al*[54] conducted a follow-up of their initial study and investigated the association between the individual components of the lipid profile and development of PCa. These authors demonstrated an increased risk for men in the lower quartile for HDL cholesterol and apolipoprotein A- I levels but no association between LDL cholesterol or apolipoprotein B levels and development of PCa[54]. In a cohort of 29093 men between 50 and 69 years of age participating in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study, Mondul *et al*[51] observed a similar trend toward a decreased risk of PCa in men with higher HDL cholesterol levels, which persisted across all grades and stages[51]. A cohort study of 2842 Dutch men also reported an association between higher HDL cholesterol levels and non-aggressive PCa (HR = 4.28; 95%CI: 1.17-15.67) as well as an increased risk of PCa in men with higher LDL cholesterol levels (HR = 1.42; 95%CI: 1-2.02)[55]. Conversely, Jacobs *et al*[56] failed to demonstrate an association between HDL or LDL cholesterol levels and risk of aggressive PCa in a cohort of 14241 men between 50 and 79 years of age within the Cancer Prevention Study Ⅱ Nutrition Cohort. Only 6% of these men, however, met the ATP Ⅲ criteria for high LDL cholesterol levels. This proportion of men with high LDL cholesterol levels was much lower than in other studies, which may account for its conflicting results[56].

As previously discussed, Van Hemelrijck *et al*[53] reported that high TG levels were associated with an increased risk of PCa, but only in men with high glucose levels. Hayashi *et al*[57] also demonstrated an association between TG levels and development of PCa in a cohort of 905 men undergoing biopsies. This association was strengthened in men between 60-69 years of age (OR = 2.1; 95%CI: 1.31-3.37) and ≥ 70 years of age (OR = 1.91, 95%CI: 1.03-3.53), both of whom also had an increased risk of high-grade disease[57]. Several studies have supported these findings, while others have observed either a null or even an inverse association between TG levels and development of PCa but generally included a younger population of men ≤ 60 years of age[15,17,18,58-60]. In addition to age, the frequent co-occurrence of high TG levels and DM is thought to be a confounding factor that accounts for these conflicting results[17,32].

High blood pressure

The association between hypertension and development of PCa has not been as thoroughly investigated as other components of MetS but is thought to be related to the effect of sympathetic nervous activity on the androgen-mediated growth of prostatic tissue[61]. As previously discussed, several cohort studies have reported an increased risk of PCa in men with hypertension, including the 2nd Nord Trøndelag Health, Olmsted County, and Flint Men's Health Studies[18,19,26]. The latter 2 studies actually demonstrated that hypertension was the only component of MetS associated with an increased risk[18,19]. However, the remaining evidence is limited and warrants further investigation.

High fasting glucose

There is a large body of evidence supporting an inverse association between DM and development of PCa. Several meta-analyses have observed a decreased risk of

PCa in diabetic men with pooled RRs of 0.91 (95%CI: 0.86-0.96), 0.84 (95%CI: 0.76-0.93), and 0.86 (95%CI: 0.8-0.92)[62-64]. A more recent meta-analysis of 25 cohort and 12 case-control studies reported a similar association through a subgroup analysis of population-based studies (RR = 0.72; 95%CI: 0.64-0.81), cohort studies from the United States (RR = 0.79; 95%CI: 0.73-0.86), and studies with follow-up of greater than 5 years. Diabetic men on insulin were also noted to have a decreased risk of PCa in all studies included in this meta-analysis[65]. These findings suggest that the inverse relationship between DM and development of PCa is strengthened over time. There is a corresponding natural history for DM that begins with a rise in glucose and insulin levels followed by the development of insulin resistance and decline in insulin levels due to damaged pancreatic beta cells.

Various theories have been proposed for the inverse association between DM and development of PCa. Some authors have suggested a causal effect from the decreasing levels of hormones and other cancer-related mitogens such as insulin-like growth factor-1. Others have argued for a bias against the detection of PCa due to less health-care seeking behavior, lower serum PSA levels, and a larger prostatic size in diabetic men[66]. As previously discussed, a delayed diagnosis of PCa may lead to an increased risk of more aggressive disease in obese men. Several studies have indeed demonstrated an increased risk of high-grade and advanced disease in diabetic men undergoing biopsies and radical prostatectomy (RP)[67-73]. Interestingly, the association between DM and high-grade disease was only observed in obese Caucasian men in one of these studies and was strengthened in this population in another study[67,69].

THE METS AND PATTERNS OF TREATMENT FOR PCA

Men with MetS are often perceived as poor surgical candidates due to a concern for an increased risk of perioperative complications, increased technical difficulty with surgery, and poorer outcomes. These concerns may affect the counseling of these men and their resulting treatment, regardless of whether they are well-founded. There is only limited evidence on the influence of obesity on the patterns of treatment for PCa. When investigating the choice of treatment in men newly diagnosed with PCa, Davies *et al*[74] reported that obese men were more likely to receive a non-surgical therapy, such as active surveillance (AS), external-beam radiation therapy (EBRT), brachytherapy, or androgen deprivation therapy (ADT). Men with BMI $\geqslant$ 35 k/m^2, in particular, were more likely to receive brachytherapy (OR = 1.59; 95%CI: 1.01-2.52) or ADT (OR = 1.77; 95%CI: 1.12-2.81) alone[74].

THE METS AND ONCOLOGIC OUTCOMES FOR PCA

Emerging evidence has suggested that men with MetS or its individual components may have a poorer oncologic outcome after their treatment for PCa.

Radical prostatectomy

In a study of over 4000 men with a median age of 61 years undergoing robot-assisted laparoscopic radical prostatectomy (RALP), Kheterpal *et al*[75] demonstrated a higher pathologic Gleason grade and stage as well as a greater upgrading of Gleason grade 6 disease in men with MetS compared to best-matched controls. However, the prostatic volumes were not included in this study, and a larger prostatic size in obese men may account for these findings due to an increased sampling error at biopsy[75]. Another study of 261 men with a mean age of 64.5 years undergoing RP observed an increased tumor volume in those with MetS (6.6 ± 5.5 mL *vs* 5 ± 4.5 mL) but no differences in any other histopathologic features[76]. Castillejos-Molina *et al*[77] further reported that MetS was associated with an increased risk of biochemical recurrence (BCR) in men with a median age of 64.8 years undergoing RP. MetS was the strongest predictor of BCR on multivariate analysis (OR = 2.73; 95%CI: 1.65-4.5), although men with MetS had a significantly higher proportion with Gleason grade > 7 on biopsy and pathologic stage T3a-b. Therefore, the increased risk of BCR in men with MetS may have been due to selection bias with only those with high-risk disease undergoing RP. When confining their analysis to men with organ-confined disease, the 5- and 10-year BCR-free survival was 55% and 48% for those with MetS compared to 80% and 73% for those without MetS, respectively. There was still a strong association between MetS and BCR in this subgroup (OR = 3.42; 95%CI: 1.68-7.01)[77]. Post *et al*[78] also demonstrated a 50% increase in the rate of BCR after RP in men with MetS. This finding was primarily influenced by the effect of hypertension, which conferred an approximately 2-fold increased risk of BCR and was the only consistent association among all components of MetS[78]. A similar association for hypertension was observed in another study of 1428 men with a mean age of 59.1 years undergoing RP[79]. Most recently, Kwon *et al*[80] failed to report any differences in the operative parameters, histopathologic features, or functional outcomes of men with MetS undergoing RALP, except for an increased blood loss (OR = 1.592; 95%CI: 1.15-2.21)[80].

The oncologic outcomes after RP have been most thoroughly investigated in obese men. Several studies have demonstrated an increased risk of BCR after RP independent of adverse clinicopathologic features in obese men. Two recent meta-analyses confirmed these findings by observing a 25% (95%CI: 1.12-1.4) and 16% (95%CI: 1.08-1.24) increased risk of BCR for each 5 kg/m^2 increase in BMI[81,82]. Additional studies have also suggested an increased technical difficulty for all techniques of RP in obese men with increased operative times, estimated blood loss, complications, and positive surgical margins; while others have reported no differences in these operative parameters and demonstrated an increased risk of BCR independent of surgical margin status and in men

with organ-confined disease. It therefore remains unclear whether the increased risk of BCR after RP is related to an increased technical difficulty, inherently more aggressive disease, or both in obese men[39].

The oncologic outcomes after RP have also been investigated in diabetic men. Two studies have failed to observe an association between DM and risk of BCR after RP[67,83]. Interestingly, the latter study reported an increased risk of BCR after RP (HR = 2.52; 95%CI: 1.4-4.54) only in obese, Caucasian men with DM[67]. This group subsequently performed a study of 2083 United States veterans with a median age of 61 years and again demonstrated that DM was only associated with the development of metastatic disease after RP (HR = 2.8; 95%CI: 1.29-6.09) in obese men, despite receiving a more aggressive secondary treatment[84].

Radiation therapy

As with RP, a number of studies have observed that BMI is an independent predictor of BCR after EBRT and associated with a decreased PCa-specific survival. These findings are thought to be related to the greater daily variation in the location of the prostate and resulting loss of precision in the designated field of radiation in obese men[85-87]. One study also reported an increased risk of BCR in obese men with a median age of 61 years undergoing salvage EBRT therapy after RP[88]. In the absence of surgical pathology to confirm the grade and stage of disease, it is unclear whether a more aggressive disease accounts for these poorer outcomes in obese men undergoing EBRT. Furthermore, the demonstrated hemodilution of PSA in obese men may create the potential for these men being under risk-stratified and undergoing a less aggressive primary treatment with a shorter or absent regimen of ADT.

Conversely, brachytherapy appears to be feasible and effective in obese and diabetic men based on limited evidence. Several studies have failed to observe an association between BMI or DM and risk of BCR after brachytherapy[89-92].

Androgen deprivation therapy

While the development and exacerbation of MetS in men on ADT has been thoroughly investigated, the association between MetS and oncologic outcomes on ADT has not. Two studies have reported that obesity is an independent predictor of BCR and PCa-specific mortality after combined EBRT and ADT, while Keto demonstrated an increased risk of metastatic disease as well as a trend toward an increased risk of progression to castration-resistant disease and decreased cancer-specific survival in men undergoing ADT after RP[93-95]. Only 1 study has investigated the oncologic outcomes of men on primary ADT alone. Flanagan *et al*[96] observed a shorter time to PSA progression in men with MetS, who were treated with luteinizing hormone-releasing hormone agonists for BCR after a definitive local therapy or newly diagnosed metastatic PCa (16 mo *vs* 36 mo). These authors also reported a shorter time to PSA progression for each component of MetS except for TG levels as well as a decreased overall survival in men with hypertension[96]. These studies suggest that men with MetS may have a poorer response to ADT. The mechanism behind this relationship has not been elucidated but may be related to an excess level of estrogens, particularly in obese men. Estrone, estradiol, and free estradiol levels have all demonstrated a direct relationship with BMI, while lower testosterone levels have been observed in obese men[97]. Men with MetS may therefore be androgen-deprived at baseline and have an increased risk of progression to castration-resistant disease. Whether the levels of these hormones affect the progression to castration-resistant disease is unclear.

THE METS AND POST-TREATMENT QUALITY OF LIFE

Men with various components of MetS may have a worse quality of life (QoL) after their treatment for PCa.

The MetS

There is no evidence on the association between MetS and post-treatment QoL for men with PCa. However, 2 recent studies have investigated the combined influence of vascular risk factors on recovery of erectile function after RP, EBRT, and brachytherapy. In a study of 984 men with a mean age of 59.6 years undergoing RP, Teloken *et al*[98] investigated the effect of vascular risk factors (hypertension, hypercholesterolemia, DM, coronary artery disease, and history of smoking) on recovery of erectile function after RP. These authors reported a worse recovery in men with $\geqslant$ 3 compared to 1 or 2 vascular risk factors at 24 mo postoperatively (P = 0.02) independent of age, erectile function before RP, and nerve-sparing status[98]. Wang *et al*[99] conducted a study of 732 men with a mean age of 65.3 years undergoing EBRT and/or brachytherapy with or without ADT over 4 years and similarly demonstrated an increasing incidence of ED with an increasing number of vascular comorbidities (hypertension, DM, hyperlipidemia)[99]. Although men with MetS have many of the same risk factors, it is unclear whether the findings of these studies may be extrapolated to this population.

Obesity

The current evidence on the QoL for obese men after their treatment for PCa is equivocal. Two studies have observed a delayed return of bowel function and increased bother after RP as well as a worse hormonal function after RP and radiation therapy in obese men[100,101]. Others have failed to report a consistent difference between obese and non-obese men in any domains of QoL after RP[102,103]. Several recent studies have specifically addressed the recovery of urinary incontinence and erectile function after RP in obese men. Their conflicting results have been difficult to compare due to the different designs of these studies and use of variable surgical ap-

proaches. There was also an inconsistent use of validated questionnaires and lack of consideration for certain confounding variables[104-117]. These studies collectively suggest that obese men may have a slightly worse perioperative QoL. However, the major predictor of post-treatment QoL remains their QoL prior to the initiation of therapy. The current evidence is insufficient to recommend any particular treatment in obese men based on their post-treatment QoL.

High fasting glucose

The current evidence on the QoL for diabetic men after their treatment for PCa is much more robust. Latini *et al*[118] demonstrated a worse urinary function after RP in both diabetic and obese men. These authors further observed that the combined influence of DM and obesity may be greater than either alone[118]. Thong *et al*[119] also reported a worse urinary and sexual function as well as a worse general health-related QoL after all types of treatment in men with pre-existing DM compared to those with incident DM diagnosed after PCa and without DM[119]. Additional studies have demonstrated an association between DM and the development of ED after both EBRT and brachytherapy[120-122]. Most importantly, a growing body of evidence has established DM as a risk factor for the development of complications after radiation therapy. Several studies have observed an increased risk of $\geqslant$ grade 2 late gastrointestinal and genitourinary complications after EBRT in diabetic men based on the Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer scale[123-127]. As with obesity, the current evidence is insufficient to recommend any particular treatment in diabetic men based on their post-treatment QoL. However, it is recommended that diabetic men be counseled about a potentially increased risk of complications after radiation therapy and be considered for the modification of its planning and delivery.

THE METS AND PCA-SPECIFIC MORTALITY

Emerging evidence has suggested an association between MetS and PCa-specific mortality. A recent meta-analysis pooled the findings of 3 cohort studies and reported an increased risk of PCa-specific mortality in men with MetS (RR = 1.12; 95%CI: 1.02-1.23)[33].

The risk of PCa-specific mortality has been most thoroughly investigated in obese men. Several studies have demonstrated that BMI is an independent predictor of PCa-specific mortality among obese men in a population-based cohort and those diagnosed with PCa. A recent meta-analysis of 12 studies confirmed these findings by observing a 16% increased risk of PCa-specific mortality in cohort studies (95%CI: 1.06-1.25) and a 20% increased risk in studies investigating the post-diagnosis survival (95%CI: 0.99-1.46) for each 5 kg/m^2 increase in BMI[81]. Although a bias against the detection of PCa may account for the increased risk of PCa-specific mortality in obese men, 2 studies reported a similar association before the introduction of PSA-based screening[128,129].

There is only limited evidence that men with other components of MetS have an increased risk of PCa-specific mortality. In a cohort of 17934 men between 40 and 69 years of age participating in the Whitehall study, Batty *et al*[50] demonstrated an increased risk of PCa-specific mortality for men in the upper tertile for TC levels over a period of 4 decades (HR = 1.35; 95%CI: 1.11-1.65)[50]. Only 1 study has similarly observed an increased risk of PCa-specific mortality in men with hypertension[20]. A recent meta-analysis also identified 4 studies investigating the association between DM and PCa-specific mortality, only 1 of which reported an increased risk. There was insufficient evidence to perform a formal meta-analysis of these studies. However, the authors identified 7 additional studies investigating the non-PCa or long-term, overall mortality and conducted a preliminary meta-analysis from 4 of these studies, which demonstrated an increased risk of overall mortality in diabetic men (HR = 1.57; 95%CI: 1.12-2.2)[130].

THE METS AND PREVENTION OF PCA

With the growing body of evidence on the association between MetS and PCa, the management of MetS has become a potential target for the prevention of PCa and improvement of outcomes among diagnosed men. Based on recommendations from the AHA and NHLBI, the primary emphasis on the management of MetS is to mitigate the modifiable risk factors of obesity and physical inactivity through dietary and lifestyle-related changes. The addition of pharmacologic treatment is a secondary consideration for patient at particularly high risk of cardiovascular disease and DM[9].

Weight loss

A majority of the evidence on the effect of weight loss and other dietary interventions on the risk of PCa is derived from animal studies. Several studies have observed a decreased risk in the development and progression of PCa in animals on a caloric restricted diet that is low in fats or carbohydrates[131].

Several studies have investigated the influence of weight change on the development and progression of PCa in humans, only a few of which focused on weight loss. The most intriguing of these studies is the PCa Lifestyle Trial, which is a randomized control trial (RCT) of men with PCa on AS. These men had a Gleason grade < 7 on biopsy, PSA between 4 and 10 ng/mL, and clinical stage T1-2 disease. They were randomly assigned to either a program that included a vegan diet, several nutritional supplements, moderate aerobic exercise (30 min of walking on 6 d per week), and various techniques for stress management or no intervention. Those in the experimental group reduced their weight by 4.5 kg and had a 4% decrease in serum PSA levels compared to a 6% increase

in the control group (P = 0.016) after 1 year. The growth of LNCaP cells was also inhibited by serum from the experimental group by almost 8-fold more than the control group[132]. At 2 years, a significantly fewer number of men pursued a conventional treatment for PCa in the experimental compared to the control groups (5% *vs* 27%)[133].

Several other trials have investigated the effect of various dietary and lifestyle-related interventions on a variety of biomarkers associated with PCa and its prevention. Freedland *et al*[131] recently published an excellent review on this subject. These trials generally reported that a low-fat and/or carbohydrate diet accompanied by weight loss may alter the tumor biology of PCa[131]. Larger studies with longer follow-up and assessment of clinical outcomes are necessary to determine the significance of these findings.

Physical activity

Physical activity has been increasingly recognized as a modifiable risk factor that may play a role in the prevention of many cancer, including PCa[134]. The mechanism behind this relationship remains unknown but is thought to be related to enhancing the immune system and altering the levels of various endogenous hormones associated with PCa, including androgens, insulin, insulin-like growth factors, and testosterone. Physical activity also assists in weight control and prevention of MetS, which may be associated with an increased risk of PCa. The findings of studies investigating the influence of physical activity on the risk of PCa have been equivocal. A recent meta-analysis of 19 cohort and 24 case-control studies demonstrated that total physical activity was associated with a small but significantly decreased risk of PCa (pooled RR = 0.9; 95%CI: 0.84-0.95). A sub-group analysis based on the type of physical activity observed a decreased risk of 19% (95%CI: 0.89-0.97) for occupational and 5% (95%CI: 0.89-1) for recreational physical activity. The risk reduction for total physical activity was reported in men between 20 and 45 years of age (pooled RR = 0.93; 95%CI: 0.89-0.97) as well as between 45 and 65 years of age (pooled RR = 0.91; 95%CI 0.86-0.97)[135]. The use of various methods to quantify the level of physical activity in these studies precluded the identification of a dose-response relationship or threshold of physical activity required for preventing PCa. There was also not any available data on the levels of various endogenous hormones associated with PCa.

Chemoprevention

The most thoroughly investigated and promising medication has been 3-hydroxyl-3-methylglutaryl-Coenzyme A reductase inhibitors (also known as statins). Our group recently published a review on this subject[136]. While there does not appear to be an association with the overall risk of PCa, several cohort studies and a meta-analysis have demonstrated a decreased risk of advanced disease in men taking statins[137-140]. Additional studies have investigated the oncologic outcomes of men taking statins after their treatment for PCa with conflicting results. Several recent meta-analyses have failed to observe an association between the use of statins and risk of BCR after RP with pooled RRs of 1.02 (95%CI: 0.8-1.29), 1 (95%CI: 0.8-1.19), and 1.05 (95%CI: 0.9-1.240)[141-143]. Studies of men undergoing EBRT were also included in the latter 2 meta-analyses. Scosyrev *et al*[142] failed to report an association with BCR after EBRT or any definitive local therapy, while Park *et al*[143] demonstrated an improved recurrence-free survival in their sub-group analysis of men undergoing EBRT (pooled HR = 0.68; 95%CI: 0.49-0.93). These findings may support the radiosensitizing effect of statins that has been observed in both *in vitro* and *in vivo* models[142,143].

Many other medications and dietary supplements targeting various components of MetS have been investigated. One particularly noteworthy medication is metformin, an oral biguanide medication used as a first-line treatment for type 2 DM. It is inexpensive, widely available, and thought to have an antineoplastic effect for various cancers. However, the current evidence on its association with the development and progression of PCa is equivocal. Several studies have reported a decreased risk of PCa and high-grade disease in diabetic men taking metformin as well as a decreased risk of progression, overall mortality, and PCa-specific mortality in those diagnosed with PCa[144-147]. In a study of 2901 men with a median age of 69 years undergoing EBRT, Spratt *et al*[148] also demonstrated a decreased risk of developing castration-resistant disease as well as an improved overall, BCR-free, distant metastases-free, and PCa-specific survival in diabetic men taking metformin[148]. Other studies have failed to observe an association between the use of metformin and development of PCa or BCR after RP in diabetic men[149-154]. Most recently, Rothermundt *et al*[155] performed a prospective clinical trial of 44 men with metastatic castration-resistant PCa on metformin and reported a stabilization of disease in 36% of men at 12 wk and 9.1% at 24 wk. These authors also demonstrated a prolongation of PSA doubling time in 52.3% of men after starting metformin[155].

Recommendations

The current evidence on the benefits of weight loss, physical activity, and medications like statins and metformin is encouraging but preliminary and requires further investigation before providing an specific recommendations. Nevertheless, it is important to recommend maintaining a desirable weight, engaging in regular exercise, and consuming a cardiovascular healthy diet to all patients. These interventions will improve their overall health and reduce their risk of cardiovascular disease, which is the primary cause of mortality among men in the United States[156].

CONCLUSION

Emerging evidence has suggested an association between

MetS and PCa. Many studies have observed that men with MetS or its individual components have an increased risk of more aggressive disease and mortality as well as a poorer outcome after their treatment for PCa, while others have not. These men may benefit from weight loss, physical activity, and the addition of medications like statins for preventing PCa as well as improving their oncologic outcomes and QoL after treatment. There is a paucity of RCTs with a majority of the existing evidence being retrospective or observational in nature. Potential biases such as screening practices, serum PSA level, or choice of treatment may therefore account for the findings of these studies. Lastly, only a few studies consider both MetS and its individual components. MetS is a complex disease with a poorly understood interplay among its individual components, which appear to be differently associated with PCa.

The association between MetS and PCa is a particularly attractive and fruitful area of research, given the increasingly aging population and epidemic proportions of both diseases. There is also a need for the identification of risk factors for the development and progression of PCa. Further research is necessary to corroborate the findings of earlier studies and to better define the separate and combined influence of the individual components of MetS on PCa. A majority of the existing evidence is retrospective or observational, which is subject to bias. More RCTs are needed to investigate the effect of dietary and lifestyle-related changes as well as chemopreventive medications on the risk of PCa and oncologic outcomes after treatment. Further research should also focus on the molecular pathways involved in MetS as well as the development and progression of PCa. A better understanding of these pathways will facilitate the development of novel therapeutic targets for the prevention and treatment of PCa.

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REVIEW

Preclinical therapy of benign prostatic hyperplasia with neuropeptide hormone antagonists

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Abstract

Benign prostatic hyperplasia (BPH) is a pathologic condition of the prostate described as a substantial increase in its number of epithelial and stromal cells. BPH may significantly reduce the quality of life due to the initiation of bladder outlet obstruction and lower urinary tract syndromes. Current medical therapies mostly consist of inhibitors of 5α-reductase or α1-adrenergic blockers; their efficacy is often insufficient. Antagonistic analogs of neuropeptide hormones are novel candidates for the management of BPH. At first, antagonists of luteinizing hormone-releasing hormone (LHRH) have been introduced to the therapy aimed to reduce serum testosterone levels. However, they have also been found to produce an inhibitory activity on local LHRH receptors in the prostate as well as impotence and other related side effects. Since then, several preclinical and clinical studies reported the favorable effects of LHRH antagonists in BPH. In contrast, antagonists of growth hormone-releasing hormone (GHRH) and gastrin-releasing peptide (GRP) have been tested only in preclinical settings and produce significant reduction in prostate size in experimental models of BPH. They act at least in part, by blocking the action of respective ligands produced locally on prostates through their respective receptors in the prostate, and by inhibition of autocrine insulin-like growth factors-Ⅰ/Ⅱ and epidermal growth factor production. GHRH and LHRH antagonists were also tested in combination resulting in a cumulative effect that was greater than that of each alone. This article will review the numerous studies that demonstrate the beneficial effects of antagonistic analogs of LHRH, GHRH and GRP in BPH, as well as suggesting a potential role for somatostatin analogs in experimental therapies.

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Key words: Benign prostatic hyperplasia; Luteinizing

hormone-releasing hormone; Growth hormone-releasing hormone; Gastrin-releasing peptide; Somatostatin; Targeted therapy

Core tip: A new, effective treatment for benign prostatic hyperplasia (BPH) is critically needed. Present side effects of therapy include impotence, decreased libido, abnormal ejaculation, dizziness, weakness, blurred vision and insomnia. Preclinical data suggest that antagonists of neuropeptides growth hormone-releasing hormone, luteinizing hormone-releasing hormone and gastrin-releasing peptide are effective in shrinking prostates in part by suppressing growth factors and inflammatory cytokines. Their effect is exerted through a decrease in levels of circulating hormones and also on a direct action on their respective prostatic receptors. These analogs seem to have the same clinical effects as the currently available BPH medical therapies but possess greater efficacy and have fewer or no side effects.

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INTRODUCTION

Benign prostatic hyperplasia (BPH) is an age-dependent condition which may start as early as 40 years of age and its prevalence increases to 50%-60% in men in their 60's[1,2]. The BPH-associated growth in prostatic volume arises from the increase in epithelial and stromal cell number occurring mainly in the transition zone of the prostate[3]. In some cases, histologic BPH remains asymptomatic and the patient does not require clinical treatment. However, the prostate gland frequently becomes substantially enlarged, resulting in compression of the diameter of the urethra thus leading to bladder outlet obstruction[1]. Lower urinary tract symptoms (LUTS) that are often associated with BPH are developed in response to the increased resistance of the urethra and the consequently elevated pressure in the bladder[4,5]. Unfortunately, the current medical modalities aimed at treating BPH are not completely effective[6]. These include therapies targeting 5α-reductase activity to inhibit the production of dihydrotestosterone as well as compounds that reduce the adrenergic tone at the bladder outlet, these collectively known as α_1-adrenergic blockers[7-9]. When an intervention is required, either a minimally invasive technique (such as transurethral needle ablation or microwave thermotherapy) or surgery (transurethral resection of the prostate or "open prostatectomy") is performed to reduce the volume of the prostate and its restriction in outlet flow[10-12].

The pathogenesis of BPH is not completely understood although it has been suggested that a decrease in the rate of cell death is more critical for the hyperplastic behavior than a rise in cell proliferation[13]. Various factors, such as a discrepancy in androgen and estrogen levels[14-17], altered autocrine regulation by growth factors [most importantly fibroblast growth factors-2 (FGF-2) and FGF-7][18] or cytokines released by infiltrated inflammatory cells[19] have been found to contribute to the development of BPH. It has also emerged that mesenchymal transition of epithelial and endothelial cells directed by the transforming growth factor (TGF)-β/Smad pathway may play a key role in the pathogenesis of BPH[20]. Most recently, neuropeptide hormones were also found to play a major role in this process, not only by indirectly controlling their classical hormonal targets but also as local regulators in the prostate[21-25]. Consequently, their receptors became potential targets for the development of new treatment strategies for BPH. These include the potential therapeutic utilization of antagonistic analogs of luteinizing hormone-releasing hormone (LHRH), growth hormone-releasing hormone (GHRH) and gastrin-releasing peptide (GRP). The utilization of these analogs in experimental BPH also improved our knowledge on the physiological role of neuropeptides and their receptors in the pathogenesis of BPH. The blockade of these receptors by specific antagonists inhibits the proliferation of stromal and epithelial cells and reduces the release of cytokines and growth factors[6,20,22,24,25] indicating the participation of the native neuropeptides in these processes. As new antagonistic analogs of neuropeptides have recently become available for clinical practice as well others are currently being developed for human trials, we felt that a review of recent findings related to their use in BPH is timely. This review therefore focuses exclusively on preclinical and clinical studies where neuropeptide antagonists were tested against BPH. Additionally, the use of somatostatin agonists is also suggested based on previous findings in prostate cancer with the hope it will facilitate their experimental and clinical testing.

ANTAGONISTS OF LHRH

Initially, LHRH antagonists were developed for the purpose of contraception using reduction of the mid-cycle pituitary follicule-stimulating hormone and luteinizing hormone (LH) release thus preventing ovulation[26,27]. Early antagonistic analogs of LHRH demonstrated low potency and significant side effects due to a substantial histamine release[28]. Since those first attempts, many antagonistic analogs of LHRH have been synthetized with higher potency and greatly decreased histamine-releasing activity[29,30]. Cetrorelix[29] was the first antagonistic analog of LHRH that was approved for use in clinical practice as part of the hormonal therapy of *in vitro* fertilization used to prevent premature LH surges[31]. Numerous clinical trials have been conducted with Cetrotide brand of cetrorelix for the treatment of ovarian cancer, endometriosis, ovarian hyperstimulation syndrome and uterine

leiyomyoma[32-35]. Cetrorelix was also tested in patients with prostate cancer[36,37]. The most advanced LHRH antagonist, degarelix, that has been approved for patients with advanced prostate cancer has an improved formula that allows the slow tonic release of the peptide, and moreover, has the lowest histamine-releasing activity among the LHRH antagonists[38,39].

The utilization of LHRH antagonists in the treatment of BPH is suggested by several previous findings. Hormonal therapy with the 5-α reductase inhibitors has long been used to treat BPH and has been shown to shrink prostate volume and improve urinary outflow[16]. This suggests a dihydrotestosterone-dependent pathology of the disease. However, only 30%-50% of patients respond to this treatment[40] highlighting the need for the development of a more effective intervention, such as a systematic suppression of testosterone levels. Cetrorelix (300 μg) was able to reduce serum testosterone levels by 80% at 12 h after administration in men with a mean age of 24[41]. This finding encouraged the clinical testing of cetrorelix in BPH.

In a study by Gonzalez-Barcena *et al*[36], 11 patients were recruited with symptomatic BPH. Subjects were treated with 500 μg cetrorelix every 12 h for 4 wk in an open label study. Improvements were seen in urinary flow just after the first week of treatment and it became normal after 4 wk. Also, the level of serum acid phosphatases reached normal levels at the end of treatment. Free testosterone levels either dropped immediately after the first cetrorelix injection or decreased gradually throughout the 4 wk, however, in 4 patients it remained similar to pretreatment values. In all cases, prostatic volume decreased significantly which suggests a testosterone-independent action of cetrorelix on the prostate in patients where testosterone level had not been reduced significantly[36]. In a subsequent Phase Ⅰ/Ⅱ clinical trial, 13 patients with moderate to severe BPH were treated with a loading dose of 5 mg cetrorelix twice daily for 2 d and then with 1 mg daily for two months[42]. In this study, testosterone fell to castrate levels during the initial high dose therapy and increased to approximately 30% of the normal serum level during the 2 mo of maintenance therapy. On week 8, the International Prostate Symptom Score (IPSS) was significantly reduced and there was a 27% decline in prostate volume.

A decade after these pilot studies, a placebo-controlled phase II trial explored the effects of a 4-wk treatment at 3 different dose levels of cetrorelix in 140 patients with symptomatic BPH[43]. LUTS were significantly improved in all treatment groups compared to placebo which effect occurred rapidly, by week 4 (time point of the first evaluation). Prostate size was also significantly reduced in two of the treatment groups and the overall reduction of symptoms lasted 16 wk after the termination of the treatment (time point of last evaluation). In a further study, cetrorelix pamoate was administered as a 60 mg sustained release formulation, in a double-blind, randomized, multicenter study[44]. One subsequent administration of cetrorelix (30 mg, sustained release) resulted in a 4-point improvement in IPSS and the significant advancement was sustained for 26 wk after the last dose was given. In these latter studies it was shown that the suppression of testosterone levels by cetrorelix was moderate and transient[43,44].

Despite the success of these studies, the phase III clinical trials conducted in the United States and in Europe by AEterna Zentaris[45,46] failed to confirm a significant improvement in IPSS in response to cetrorelix treatment compared to the placebo group. In the United States study, there were no significant changes after either 3 or 4 doses of cetrorelix administered during an 18-wk period, however, cetrorelix was beneficial in a subgroup of patients with substantially enlarged prostates[47]. Although the phase Ⅲ trial failed, all previous attempts were successful which encouraged the initiation of new clinical testing with the more potent LHRH antagonist, degarelix. This compound has greatly reduced histamine-releasing activity and upon subcutaneous administration it aggregates into a slow-release complex[38,39]. A Phase-Ⅱ study has been completed with this compound but results have not yet been released[48].

Initially, the concept of the management of prostate cancer and BPH by LHRH antagonists was based on their action on pituitary LHRH receptors (LHRHR) leading to suppression of gonadal testosterone production, however, there is a growing body of evidence that they also act directly in the prostate. This idea is supported by a number of studies showing the presence of LHRH receptor in the prostate[49-51]. In an early study by Kadar *et al*[49], a high affinity low capacity binding site for D-TRP-6-LHRH in prostate samples from patients with BPH and prostate cancer was found. A similar binding site and one with low affinity high capacity were also detected in Dunning prostate tumors[50]. In a more recent study, LHRHR was detected by reverse transcription polymerase chain reaction in 60% of patients with BPH[51].

A second line of evidence for the local action of LHRH antagonists in the prostate is derived from a number of studies where cetrorelix was tested *in vitro* on human BPH cell lines expressing LHRHR. Siejka *et al*[21] showed that cetrorelix inhibits proliferation of the immortalized human BPH cell line (BPH-1) and reduces the protein expression of proliferating cell nuclear antigen (PCNA), epidermal growth factor (EGF), EGF receptor, most abundant adrenergic receptor in the prostate (α_{1A}AR) and LHRHR in a concentration-dependent manner[21]. Proliferation was also inhibited by cetrorelix after cells were stimulated with growth factors insulin-like growth factors (IGF)-Ⅰ, IGF-Ⅱ or FGF-2. Additionally, the activation of signal transducer and activator of transcription 3 (STAT3) by phosphorylation, an event associated with increased proliferation in many cells[52], was suppressed by cetrorelix[21]. The downregulation of $_{\alpha 1A}$AR by cetrorelix might be of particular interest since an increase in $_{\alpha 1A}$AR expression induced by prolonged administration of α1A-adrenergic blockers might be responsible for development

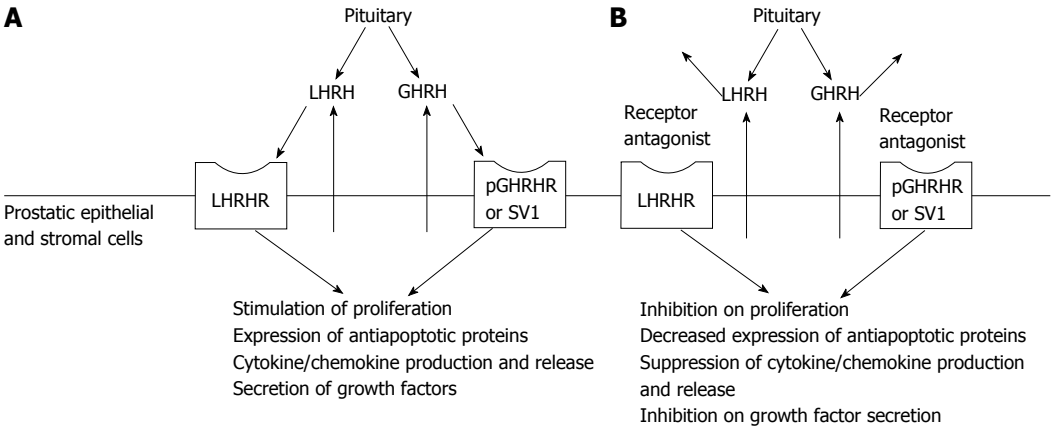

Figure 1 Local autoregulatory loop of luteinizing hormone-releasing hormone and growth hormone-releasing hormone in prostate cells and their blockade with antagonistic analogs: A novel strategy for the treatment of benign prostatic hyperplasia. A: Luteinizing hormone-releasing hormone (LHRH) secreted by pituitary somatotropes or by prostate cells activates local receptors (LHRHR). Similarly, pituitary-derived or paracrine/autocrine growth hormone-releasing hormone (GHRH) binds to pituitary type receptor (GHRHR) or its splice variant (SV1). These events lead to the changes in cellular homeostasis implicated in benign prostatic hyperplasia (BPH) pathophysiology; B: Disruption of the autoregulatory feedback loops by antagonists of LHRH and GHRH improves the condition of BPH by acting on these various cellular processes.

of the therapeutic tolerance to this treatment seen in clinical practice[53]. Rick *et al*[22] utilized a rat model of BPH in which the growth of prostate was induced by repeated administration of testosterone[54]. In this study, prostate size was reduced by cetrorelix in a dose-dependent manner compared to controls treated by testosterone only. In addition, the expression of various proinflammatory cytokines and growth factors that have been implicated in the pathogenesis of BPH were found to be reduced following cetrorelix treatment[22]. A significant reduction in serum levels of dihydrotestosterone and LH was also observed. Interestingly, cetrorelix treatment reversed testosterone-induced morphological changes to resemble the histology of the normal prostate, including a decrease in epithelial height[22]. In addition, AR and 5α-reductase levels were reduced by cetrorelix[22]. Unfortunately, the testosterone-induced BPH model has its limitations due to the complexity of the pathogenesis of BPH. Testosterone-induced hyperplasia selectively appears in the ventral prostate lobe in rats that might be the result of the distinct anatomy of this model from humans[55]. Also, the efficacy of testosterone to induce prostatic hyperplasia varies among different rat strains[56]. In addition to the noted disadvantages of the model, only the proliferation of epithelial cells is triggered by the addition of testosterone[56], whereas stromal-epithelial interactions are believed to be crucial in the pathogenesis of BPH[57,58]. Siejka *et al*[59] further examined this interaction using the BPH-1 cells and an immortalized stromal myofibroblast cell line, WPMY-1. Growth medium collected from either of the cell lines stimulated the growth of the other. This strongly supports the crucial role of epithelial-stromal cross-talk in the proliferative activity of these cells. This effect seemed to be directed through the mitogen-activated protein kinase (phosphorylation of ERK1/2) and STAT pathways. Cetrorelix inhibited the proliferation of both cell lines on its own and also after the BPH cells were stimulated with WPMY-1-conditioned medium[59].

The above mentioned studies shed light on the existence of a local autocrine/paracrine LHRH feedback in the prostate that might contribute to the pathogenesis of BPH (Figure 1). Since both LHRH ligand and its receptor are expressed in BPH[22] and the LHRH antagonist, cetrorelix, is able to decrease the proliferative activity of BPH cells *in vitro*[22], this feedback loop might act as a local stimulatory signal for cell proliferation or survival. It is also known that hormone-refractory prostate carcinomas express higher levels of LHRHR than BPH and hormone-dependent prostate cancer[60]. Consequently, LHRHR levels might gain prognostic value in the future.

GHRH ANTAGONISTS AND THEIR COMBINATION WITH LHRH ANALOGS

Antagonistic analogs of GHRH have been found to reduce the growth of various tumors[61-69] including prostate cancer in xenograft models of nude mice[70-74]. IGF-1 is a well-known growth-promoting factor for various tumors[70,75]. By blocking GHRH receptors on pituitary somatotropes, these antagonists suppress the production and secretion of growth hormone (GH) thereby decreasing circulating levels of IGF1. The full length pituitary type receptor (pGHRHR) and its main splice variant, splice variant 1, are expressed in various extrapituitary sites of normal and malignant tissues, including prostate[71,76,77]. GHRH is also secreted locally in normal and malignant prostate tissue, suggesting that it serves as an autocrine/paracrine regulator which process might be involved in the pathogenesis as well as the progression of prostate cancer[23,78,79]. Both *in vivo* tumor growth and *in vitro* cell proliferation are inhibited by GHRH antagonists in experimental androgen-dependent and-independent prostate cancers further indicating that, apart from their action in the pituitary, these peptides also function directly in the prostate[80].

Two studies have investigated the effect of GHRH antagonist monotherapy in experimental BPH models. They confirmed that both pGHRHR and GHRH are present in BPH-1 cells and in rat prostates[23,24]. Rick *et al*[23] reported also that the levels of pGHRHR and GHRH were increased following the induction of prostate growth by testosterone, indicating the importance of this autocrine/paracrine circuit in the pathogenesis of BPH (Figure 1). In the same study, GHRH antagonists were found to significantly reduce relative prostate weights better than finasteride, an 5-α reductase inhibitor. GHRH antagonists downregulated the mRNA and protein levels of various cytokines and growth factors that were elevated after testosterone treatment and also decreased proliferation and increased apoptosis in the prostate. GHRH antagonists decreased the transcriptional expression of growth hormones such as IGF-2, TGF-α, TGF-β1 and -β2, EGF, FGF-2, vascular endothelial growth factor (VEGF)-A, that have been found to contribute to the pathogenesis of BPH[81]. Cytokines interleukin (IL)-1α, IL-1β, IL-13, IL-15, and IL-17β, that have been downregulated by GHRH antagonists, otherwise promote T-lymphocyte infiltration and inflammation in BPH[82]. Interestingly, in this study, serum GH and IGF-1 levels were not affected significantly by the GHRH antagonist treatment, that might indicate the crucial role of their direct action in the prostate rather than through the pituitary axis. Intriguingly, prostates of testosterone-treated rats contained increased levels of the antiapoptotic molecule, B-cell lymphoma 2 (BCL-2), a process may explain the increased survival of cells implicated in the development of BPH[20]. Additionally, GHRH antagonist significantly dowregulated BCL-2 levels and simultaneously elevated the expression of the proapoptotic factor, BCL-2-associated X protein (BAX), and the tumor suppressor, p53, which events may underlie the strong apoptotic effect of these peptides.

In the study by Siejka *et al*[21] GHRH antagonists inhibited the proliferation of BPH-1 cells *in vitro*. The existence of the local GHRH/GHRHR loop was further supported by this study; incubation of the cells with GHRH resulted in an increased rate of proliferation which was then inhibited by the simultaneous addition of GHRH antagonist. Their study also revealed that GHRH triggers the phosphorylation of ERK 1/2, Janus kinase 2 (JAK2) and STAT3, signaling molecules that are known to be involved in the pathogenesis of BPH[83,84].

Since the existence of autocrine/paracrine systems of regulation by both LHRH and GHRH are strongly suggested in BPH, the simultaneous blockade of their receptors would be expected to result in a more effective therapy. Rick *et al*[85] studied the combination of cetrorelix plus a highly potent GHRH antagonist, JMR-132, in the testosterone-induced rat BPH model. They found that combination of LHRH and GHRH antagonists resulted in a greater decrease in prostate-specific antigen (PSA) and prostatic STEAP (six-transmembrane epithelial antigen of the prostate) protein levels than either of the peptides alone. Relative prostate weights were reduced to the control level by the combination therapy. Antagonists of GHRH and LHRH administered together were also more effective in inducing apoptosis as measured by changes in the levels of BCL-2, BAX, p53, nuclear factor (NF)-κB and cyclooxygenase-2 (COX-2). The combination therapy therefore has a great prospect in reducing hyperplastic prostate volume by triggering apoptotic cell death. In addition, chronic inflammation has been linked to the development and worsening of BPH; COX-2 has been proposed to play a key role in this process[86]. Hence, coadministration of GHRH and LHRH antagonists may also improve clinical outcome by reducing the expression of inflammation-related proteins such as NF-κB and COX-2[87]. In a subsequent study[88], the cumulative effect of cetrorelix plus JMR-132 was also superior to their individual inhibition on the proliferation of BPH-1 and WPMY-1 cells *in vitro*. Only the combination of JMR-132 and cetrorelix increased the proportion of cells in the S-phase significantly with a simultaneous decrease in the number of cells in G0/G1 and G2/M phases in BPH-1 cells. A decrease in the expression of several genes was detected in response to the combination treatment in the rat testosterone-induced BPH model; these included growth factors (EGF, FGF-1, -2, -7, -8 and -14, IGF-1 and-2, BMP5 and -7, VEGF-A, *etc*.), genes implicated in inflammatory response (chemokines, chemokine receptors, cytokines and cytokine receptors), and members of the Wnt, Hedgehog, PI3-kinase/AKT, JAK-STAT, Phospholipase C and low-density lipoprotein (LDL) pathways. According to the authors, among these changes, the downregulation of IGF-1 is of particular interest, since it has been linked to the development of BPH in diabetic men[89]. Also, inflammation-related chemokine/cytokine release has been shown to trigger the production of growth factors leading to the hyperplastic behavior of prostatic cells[90]. We therefore believe that combination therapy with antagonists of GHRH and LHRH might provide a highly beneficial approach to the management of BPH.

GRP

GRP is a bombesin-related hormone first isolated from porcine stomach and named for its ability to trigger the secretion of gastrin[91,92]. Among the three receptor subtypes that had been described for bombesin-like peptides, GRP binds to the first type (GRPR) with high affinity, and to the second type (neuromedin-B receptor) with a relatively low activity[93]. GRPR expression has been found in a variety of tissues where it regulates the secretion of gastric acid and stimulates exocrine function of the pancreas as well as triggering smooth muscle contraction in the stomach, gall bladder and urinary bladder[94,95]. In the prostate, GRP and bombesin have been shown to display mitogenic activity, affect cell migration and induce contraction in bladder and left ventral prostate[95,96]. In addition, GRPR has been implicated in the neurophysiology

of memory and fear-related behavior, and the processing of pruritus and penile reflexes[97]. In small-cell lung carcinoma xenografted into nude mice, an antibody against the GRPR receptor significantly inhibited tumor growth suggesting the crucial role of a GRP/GRPR autocrine/paracrine loop[98]. Soon after, the existence of this feedback regulation was demonstrated in various tumors, such as glioblastoma, colon cancer, hepatic cancer, prostate and gynecologic cancers[99-104]. Several antagonistic analogs that target GRPR have been synthetized by our group; among these RC-3940-Ⅱ possesses the highest affinity for GRPR combined with an increased antitumor efficacy[105].

GRPR is expressed in prostates from healthy patients as well as in those diagnosed with BPH and malignant prostate[106,107]. A study by Rick *et al*[25] using the testosterone-induced rat model, investigated the role of GRP/GRPR in BPH in greater depth. They demonstrated that GRPR and its ligand are expressed in prostates of normal as well as testosterone-induced rats and also in the human BPH-1 and WPMY-1 cell lines. A single high-affinity binding site was also identified, in control rat prostates and human cell lines, with a radioligand binding assay using ^{125}I-labeled [Tyr4]bombesin. In this study, the GRP antagonist, RC-3940-Ⅱ, inhibited the proliferation of BPH-1 and WPMY-1 cells *in vitro*. It also significantly decreased cell volume and triggered S-phase cell cycle arrest in these cells. The GRP antagonist dose-dependently decreased prostate size *in vivo* in testosterone-treated rats. The proteomic analysis of rat prostates revealed that treatment with RC-3940-Ⅱ reversed the testosterone-induced elevation in NF-κB phosphorylation and expression of androgen receptor and PCNA. Also, it decreased the mean epithelial area and induced apoptosis in testosterone-treated prostates. Analysis of the transcriptional changes in the different treatment groups identified several genes responsible for the beneficial effects of RC-3940-Ⅱ. Changes were found in the levels of growth factors, inflammatory chemokines, cytokines and their receptors; attempts to identify key signaling pathways for this process resulted in the implication of the Wnt, Hedgehog, TGF-β, NF-κB, JAK-STAT and LDL pathways. Accordingly, GRP antagonists may represent an important tool for the management of BPH, either alone or in combination with LHRH and/or GHRH antagonists.

POTENTIAL USE OF SOMATOSTATIN ANALOGS

Somatostatin inhibits the release of GH from the pituitary and also possesses inhibitory action in the gastrointestinal-tract and pancreas as shown by suppression of secretion of gastrin and glucagon, respectively[108,109]. There is much evidence that analogs of somatostatin can inhibit growth of various experimental tumors including prostate cancer[110]. Kadar *et al*[111] identified a single binding site for somatostatin using somatostatin analog RC-160 in rat prostate adenocarcinoma. In normal and pathologic prostate, findings deciphering the expression pattern of somatostatin receptors are contradictory. According to Dizeyi *et al*[112], among the five somatostatin subtypes (SSTRs), SSTR1-3 is expressed in the epithelium of normal and malignant prostate cancer, whereas SSTR4 was found only in epithelial cells. Specific neuroendocrine cells expressing SSTRs have also been identified. In a study by Tatoud *et al*[113], SSTR1 was found in most of the epithelial and stromal cell lines tested whereas SSTR2 was only detected in one BPH stromal cell line. By using fluorescent *in situ* hybridization techniques, SSTR4 mRNA expression was found only in the epithelium whereas SSTR2 was mainly detected in stromal cells of BPH and carcinoma[114]. Nevertheless, the expression of SSTRs in the prostate suggested that the use of somatostatin analogs in pathologic conditions of the prostate by inhibiting the autoregulatory loop of GHRH/GHRHR might be beneficial. In accord with this hypothesis, somatostatin analogs were shown to decrease the proliferation of androgen sensitive and androgen independent prostate cancer cells by elevating p27 and p21 protein levels, decreasing cyclin E expression and ERK1/2 phosphorylation and the secretion of IGF-1 and IGF-2[113,115,116]. The inhibitory activity of somatostatin analog on the production of growth factors, IGF-1 and IGF-2, is of particular interest since these powerful octapeptides have been linked to the pathogenesis of BPH[90].

Somatostatin analogs have also been tested clinically in patients with androgen-independent prostate cancer. A study by Maulard *et al*[117] showed improvement in PSA levels and achieved a reduction in bone pain. A Phase-I study demonstrated the favorable toxicity profile of somatostatin analog lanreotide, and showed its inhibitory effect on plasma IGF-1 levels. In contrast, no clinical improvement has been noted with this analog in advanced metastatic androgen-independent prostate cancer[118]. In a study by Berruti *et al*[119], lanreotide was also able to decrease plasma levels of IGF-1 and of the prognostic marker, chromogranin-A, but had no effect on serum PSA levels in patients with advanced prostate cancer. The poor or no inhibition of tumor growth to somatostatin analogs found in these clinical trials is thought to be due to differences in the receptor subtype-specific binding of the analogs. Consequently, the utilization of a non-receptor selective somatostatin analog has been suggested[120]. According to Cariaga-Martinez *et al*[121], whereas SSTR2 is expressed in benign prostatic hyperplasia, in most cases, it is repressed or absent in malignant prostate tissue. Conversely, the profound expression of somatostatin receptors in non-malignant prostate tissue indicates the need for preclinical and clinical testing of its analogs in BPH. This suggests that monotherapy with a somatostatin analog or a combination treatment with antagonists of GHRH and/or LHRH might represent a promising strategy for the treatment of BPH which should be investigated in the future.

CONCLUSION

The development of novel therapies for BPH is undoubtedly required. Whereas the beneficial effects of LHRH antagonists in pathological conditions of the prostate are already confirmed in clinical setting, other peptide analogs (antagonists of GHRH and GRP) have only been tested in experimental BPH models. We hope that the present review of findings on this topic will accelerate the further experimental and clinical investigation of these compounds. It appears that the local actions of various analogs in the prostate are more crucial for their beneficial influence on BPH than are their systemic effects on hormonal levels. By affecting the activation of multiple signaling pathways, LHRH, GHRH and GRP regulate cell cycle, apoptosis, cytokine and chemokine release as well as local immune response. Monotherapy or combination therapy with antagonists of LHRH, GHRH and GRP are suggested to represent an improved treatment compared to the currently available medical modalities.

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REVIEW

From the battlefield to the bladder: The development of thioTEPA

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Abstract

Effective medications for the treatment of cancer were nonexistent in the early twentieth century. Ironically the widespread use of toxic chemical weapons, chlorine and sulfur mustard gas, during the "Great War" led to the first successful chemotherapeutic treatment of cancer patients. Soon after the introduction of poisonous gas on the battlefield, reports of the resulting pancytopenia in exposed combatants appeared in the medical literature. The biologic effect of chemical weaponry on rapidly dividing cells eventually was recognized for its salutary potential in the treatment of cancer. Once this potential was appreciated, hundreds of similar compounds were synthesized and evaluated as chemotherapeutic agents. One such compound, thioTEPA, would eventually open the era of intravesical treatment of urothelial cancer.

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Key words: ThioTEPA; Bladder cancer; Urothelial cancer; Intravesical chemotherapy; Mustard gas; Mitomycin C

Core tip: We attempt to outline the lineage of intravesical chemotherapy. Specifically, we look at its relationship to poisonous gas used in wartime and chronicle its journey to the bedside.

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GAS! GAS! GAS!

While both the Allies and the Central Powers engaged in chemical warfare in World War I , it was the Germans who first developed an effective chemical weapon of mass destruction. The French tried tear gas, originally intended for riot control, but found it ineffective[1]. The Germans also deployed tear gas and additionally experimented with sneezing powder. These early forays in gas warfare had little strategic effect[2]. However, rumors of Allied research into more potent chemical weapons (subsequently proved untrue) spurred the Germans to develop more sinister chemical agents. Nobel laureate Fritz Haber would mobilize Germany's scientific community and spearhead the development of new chemical weapons for the Central Powers[3]. Of note, most of the belligerents in World War I had signed the Hague Peace Conference of 1899 that forbade "use of projectiles the sole object of which is the diffusion of asphyxiating or deleterious gases[4]". The Germans circumvented this clause by developing and then deploying chlorine gas batteries that were installed in front of their trenches, to be opened when a favorable wind could carry the gas towards the enemy. This system did not rely on "projectiles" and thus was not a violation of the Peace Conference[2]. Such chlorine gas batteries were installed near the Belgian

Figure 1 A group of British soldiers blinded by a gas attack on April, 10 1918-From the collections of the Imperial War Museum in London, England.

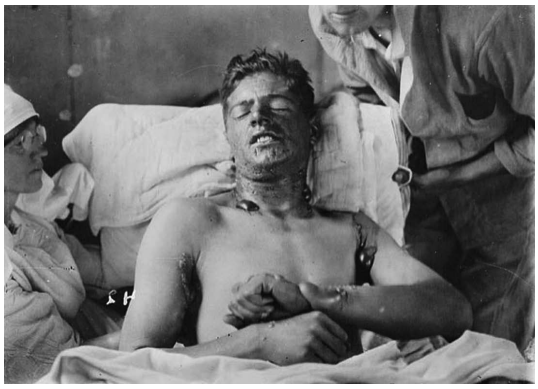

Figure 2 A Canadian soldier after exposure to mustard gas. Large bullae are present, especially where the fabric of his uniform doubled back on itself, allowing increased soaking of the compound into his clothing (at the armpit, shirt collar)-Obtained from Library and Archives Canada.

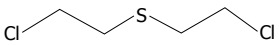

Figure 3 Bis(2-chloroethyl)sulfide, Sulfur Mustard.

town of Ypres and were "fired" on April 22, 1915[1-3,5]. The weapon system's code name was "Disinfection" and its use proved effective as it tore holes in the allied lines threatening total collapse. Within days of the attack, however, allied forces distributed gas masks neutralizing their toxic effects[4].

Gas masks proved insufficient protection against sulfur mustard gas (Figure 1), first used against British soldiers in July of 1917, as exposure damaged more than the respiratory tract[5]. Mustard gas needed only to penetrate the combatant's clothing for once in contact with the skin, it created chemical burns which blistered, ulcerated, and were slow to heal (Figures 1 and 2). Inhalation led to sloughing of the respiratory epithelia and pseudomembranous ulceration. Exposure often led to months of hospitalization[6]. To this day, no widely effective antidote exists.

Sulfur mustard gas, bis (2-chloroethyl)sulfide (Figure 3), was originally synthesized in the mid 1800's by the French chemists Cesar-Mansuete Despretz and Alfred Riche. In 1860, the German chemist Alfred Nielmann first described its toxicity and in the same year the British investigator Frederick Guthrie remarked that "its smell...resembling that of the oil of mustard…" resulting in its eponym. In 1886, the German chemist Victor Meyer described a more effective synthesis. Meyer noted that "the resulting oil is intensely poisonous, producing wounds that heal with great difficulty." In 1913, British chemist Hans T Clarke improved upon Meyer's synthesis. In 1915, while working in the laboratory of famed German chemist Emil Fischer, a flask of the compound inadvertently shattered resulting in Clarke sustaining chemical burns. The injury required two months of inpatient treatment to heal. Emil Fischer would subsequently propose sulfur mustard as a possible agent of warfare to Fritz Haber, a fact which Clarke attributed to his horrific accident[7].

Soon after chlorine and mustard gas' tactical introduction, military physicians began publishing reports on the injuries resulting from exposure. Combatants exposed to both gases often developed leukocytosis early in their clinical course[8-10]. The leukocytosis of sulfur mustard victims was characterized by immature white blood cell precursors and eventually developed into leukopenia[10,11]. The degree of leukopenia seemed to correlate with the severity of gas exposure and subsequent morbidity and mortality. Anemia developed concurrently with the leukopenia. Autopsies showed bone marrow depleted of white and red cell precursors[11,12]. Increased coagulation times indicated thrombocytopenia. It was clear from these findings that the bone marrow was particularly vulnerable[11].

A generation later, in 1941, through their studies on chemically induced gene mutations in fruit flies, British geneticists Charlotte Auerbach and John Michael Robson demonstrated that mustard gas was directly mutagenic[13]. Medical research into mustard gas was also underway in the United States during World War Ⅱ. In 1946, Alfred Gilman and Frederick S Philips published a review article exploring the physiological effects of mustard exposure. In their review, the pancytopenia sustained by those suffering exposure was highlighted. The authors noted the chemical's affinity for more mitotically active tissues[14]. This paper was a preamble to another paper Gilman was soon to publish with colleague Louis Goodman. This subsequent study was to detail the first clinical use of the alkylating agents in the treatment of human cancer.

As the name suggests, alkylating agents donate alkyl groups to other molecules, changing their structure and in the case of certain biologic molecules potentially disrupting their function. While sulfur mustard was the chemical weapon widely used in World War Ⅰ, it would be nitrogen-based mustards (Figures 4 and 5) that would see clinical use. By covalently binding to DNA, the sulfur/nitrogen mustards are cytotoxic. Rapidly dividing cells are especially vulnerable[15,16]. Nitrogen mustard would also be shown to induce metabolic derangements at the cellular level, inducing lymphocytes to convert from aerobic to anaerobic metabolism[17].

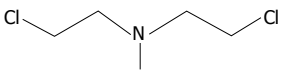

Figure 4 Bis(2-chloroethyl)methylamine, HN2.

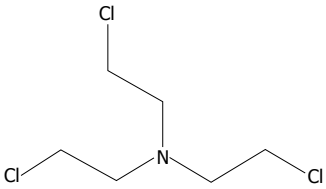

Figure 5 Bris(2-chloroethyl)amine, HN3.

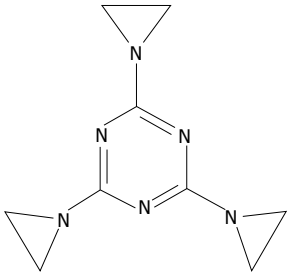

Figure 6 Triethylenemelamine, 2,4,6-tris(aziridin-1-yl)-1,3,5-triazine, triethylenemelamine.

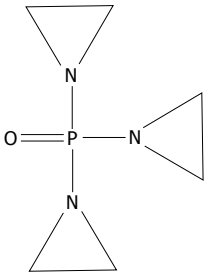

Figure 7 Triethylene phosphoramide, 1-[bis(aziridin-1-yl)phosphoryl]-aziridine, triethylene phosphoramide .

Translating use of nitrogen mustard to a clinical trial was the result of an interdisciplinary effort of the faculty at Yale in the early 1940s. Members of the departments of biochemistry, anatomy, zoology, and medicine all participated in the historic undertaking. The group had been commissioned by the federal government to find an antidote to mustard gas, research efforts for which provided a segue way to the use of nitrogen mustard for chemotherapy[18]. The collaborative report was released in 1946 and detailed the first successful application of anticancer chemotherapy. Prior to this landmark study, endeavors in the field of cancer chemotherapeutics were best characterized by the sign hanging in chemist Paul Ehrlich's lab "Give up all hope oh ye who enter[19]." In 1963, Gilman reminisced "in the minds of most physicians the administration of drugs, other than analgesic, in the treatment of malignant disease was the act of a charlatan[20]."

The specific mustards tested were bis(2-chloroethyl)-methylamine (HN2), and tris(2-chloroethyl)amine (HN3). Both are hydrophilic and were dissolved in water for intravenous injection. Because the trial was conducted during wartime, the clandestine nature of the "medicine" resulted in designating the treatment agents as "compound X". Patients had no understanding of the chemicals they were receiving. They were, however, desperate for treatment as their disease was refractory to the only treatment at the time, radiation. Yale surgeon Gustav Lindskog injected the first human with mustard chemotherapy in the early winter of 1942. In 1943 there followed a trial in which an additional 76 patients were entered.

After the drug's administration, patient's symptoms resembled those of the exposed doughboys. They developed debilitating nausea and vomiting. Thrombophlebitis occurred at the drug injection sites. All experienced profound bone marrow suppression and several developed life-threatening pancytopenia, this occurring in a dose-dependent fashion. As the drugs suppressed the marrow, they also affected the malignancies that resided within. Patients with Hodgkin's lymphoma, non-Hodgkin's lymphoma, and assorted leukemias saw their tumors recede dramatically. Unfortunately malignancies recurred with resistance to the mustard alkylating agents. This trial's success resulted in the establishment of chemotherapy as a viable treatment for human malignancy. However it was clear that toxicity and recurrence limited efficacy[21].

ROAD TO THIOTEPA

Goodman and Gilman's publication led to a flurry of research into HN2 and HN3 and stimulated a search for new alkylating agents. Molecules that bore structural resemblance to the mustards were explored. Triethylenemelamine (TEM) (Figure 6) was one such compound. It contains aziridine groups, structures similar to the 3 membered rings that HN2 and HN3 display when they react. Two or more aziridine moieties were necessary for a drug to be biologically active[22,23]. Possessing three of these moieties, TEM was found to be efficacious against various mouse cancers[24-26]. TEM was shown to induce less nausea and vomiting than HN2 and patients tolerated higher doses[27]. Like HN2 and HN3 before it, TEM went to human trial. Unlike HN2/HN3, intravenous administration of TEM caused no thrombophlebitis. TEM was also effective orally, making it possible to treat on an outpatient basis. Like HN2 and HN3 however, TEM was limited by its toxic effect on the bone marrow[26].

Other alkylating agents were examined, including triethylene phosphoramide (TEPA) (Figure 7). Like TEM before it, it was first found to be effective in treating cancer in various animal models[28-30]. Also like its cousin TEM, TEPA was tested in humans. Administration was possible intramuscularly[31]. However, its clinical efficacy did not exceed that of HN2 or TEM[31,32].

THIOTEPA

1,1',1"-phosphorothioyltriaziridine (thioTEPA) (Figure 8),

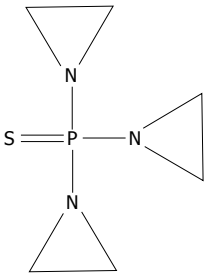

Figure 8 1,1',1''-phosphorothioyltriaziridine, thioTEPA.

and its synthesis, were patented in 1952 by the American Cyanamid Company. It was intended for use in the textile industry and in the production of plastics[33]. However, it went on to see heavy use in the medical field. It disrupts the functionality of nucleic acids[34] like HN2 and HN3 before it. Similar to TEM and TEPA, thioTEPA contains aziridine moieties.

ThioTEPA was entered into human trials in 1953 and was found effective against acute myeloid leukemia, chronic myelogenous leukemia (CML), and Hodgkin's lymphoma, much like HN2, HN3, and TEM. Additional effectiveness was found in the treatment of HN2/TEM-refractory CML. Furthermore, it showed promising results in its use in two cases of adenocarcinoma of the breast. ThioTEPA produced no nausea or vomiting and was not associated with thrombophlebitis when injected intravenously. It was efficacious when injected intramuscularly and yielded no pain at the injection site. The first clinical trial noted a "reasonable margin for safety" between the apparent effective dose and undesired bone marrow suppression[35].

Following the 1953 clinical trial of thioTEPA, Jeanne Bateman explored other routes of administration. He injected the drug into the various cavities of the body with the goal of concentrating it at disease sites. He noted improvement in patients suffering from breast and ovarian cancer. ThioTEPA proved useful in managing the masses, lesions, and effusions associated with these disease processes. Bateman concluded that thioTEPA could be useful in a palliative role for these patients, for which it is still used today[36-39]. All his patients were treated on an outpatient basis and he wrote that "clinical side effects after administration of thioTEPA were rare[40]."

Bateman's intracavitary instillation of thioTEPA led to further studies using this technique. In 1957, HN2 was instilled into the urinary bladder as an adjuvant to surgical urothelial tumor removal[41]. Another study would show that thioTEPA had little effect on surgical site healing[42]. Since healing seemed to be unaffected, thioTEPA was thought to be ideal as an adjuvant to endoscopic bladder tumor removal.

This tactic was first described in an article published in 1961 by HC Jones and John Swinney. At that time, these cases were typically managed solely endoscopically. The investigators described intravesical thioTEPA's efficacy in treating smaller papillary tumors. In cases where multiple tumors would have precluded meaningful surgical management, lesion regression was sufficient such that endoscopic management of the disease became possible[43]. Jones would publish another trial in 1963 that showed similar results. He would note that there were few side effects of intravesical thioTEPA treatment[44].

Clinical use by Veenema demonstrated that bladder carcinomas of lower grade responded better to intravesical thioTEPA and that patients could be treated on an outpatient basis[45]. He too coupled thioTEPA to surgical therapy and concluded that thioTEPA was especially useful perioperatively in cases in which the tumor hadn't grown into the muscular layers of the bladder[46]. He would later state that "The only real indication for topical agents is in the treatment of multiple superficial papillary tumors and for an attempt at prevention of recurrences[47]." Randomized control trials would confirm Veenema's findings. ThioTEPA was clinically beneficial in reducing short-term recurrence in patients with stage I bladder carcinoma that were managed endoscopically[48-50].

CARCINOMA OF THE BLADDER TODAY

Other intravesical agents are available today of which mitomycin C, an intercalating compound, is widely utilized. It was tested in Japan in the mid-70s and showed efficacy similar to that of intravesical thioTEPA[51]. Following initial trials, some reports suggested that mitomycin C was more effective than thioTEPA[52,53]. But, the proposed increased efficacy would turn out to be minimal[54]. Much of the later, definitive research showed the two to be generally equally efficacious in preventing short term recurrence[55]. Combined with its increased cost, the Urologic community had little reason to switch to mitomycin C in the early years[56]. It was, however, useful in treating patients with carcinoma refractory to thioTEPA[57].

Today, mitomycin C is often used instead of thioTEPA because it undergoes less systemic absorption from the bladder. thioTEPA's smaller size allows for potential, significant leukopenia following its introduction. Because of these concerns, Mitomycin C has by-and-large replaced thioTEPA as the preferred intravesical chemotherapeutic[58].

Of all the available intravesical chemotherapeutics, no single agent has clearly demonstrated increased efficacy[58-60]. These agents are primarily used in cases of low-grade disease in conjunction with endoscopic ablation because all have been found to be ineffective in preventing long-term recurrence[61] and are thus most appropriate in treating lesions that are at low risk of progressing[59].

Intravesical immunotherapy followed the chemotherapeutics. The anti-tuberculosis vaccine agent Bacillus Calmette-Guérin (BCG) has been found to be the most effective of these agents. Its mechanism of action is believed to work by inducing a T1 cellular response in the bladder wall, in which recruited immune cells target the neoplastic tissue[60]. Maintenance BCG has been demonstrated to prevent long-term disease recurrence and additionally is effective against higher grade disease. Because it is an attenuated mycobacterium, it can be life-threaten-

ing in immunocompromised patients. It can induce intense hypersensitivity responses in the immunocompetent patient. Currently, intravesical chemotherapeutic agents maintain their role in the management of carcinoma of the bladder, specifically in treating low-grade disease that has little risk of progression[59,60].

Since their introduction as weapons of mass destruction, alkylating agents have transformed the prognosis for patients suffering from a number of malignancies including bladder cancer. In particular they led to the clinical use of chemical agents for intravesical chemotherapy. The history of their development from poisons to potent antineoplastic agents fulfills the biblical dictum "...and they shall beat their swords into plowshares and their spears into pruning hooks....".

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REVIEW

Spontaneous regression of renal cell carcinoma: Reality or myth?

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Abstract

Spontaneous regression of a malignant tumor is a very rare phenomenon. Renal cell carcinoma (RCC) is an aggressive malignancy with an often unpredictable behaviour. The incidence of spontaneous regression in metastatic RCC has been estimated to lie between < 1% and 7%. The spontaneous regression of a primary RCC has been reported much less commonly. Our literature review assesses the published literature concerning spontaneous regression of either primary or metastatic RCC. In order to examine this phenomenon in more detail we performed a literature search in the PubMed Database using the Keywords "renal cell carcinoma", "metastatic disease", and "spontaneous regression" and included reports from the last 100 years. The incidence of spontaneous regressions in RCC has always been considered a special feature of RCC compared to other solid malignancies. The majority of case reports of spontaneously regressed RCC describe the regression of metastases after nephrectomy rather than the spontaneous regression of a primary tumor. In cases of reported regression of metastatic RCC, this mostly applied to pulmonary lesions. As possible reasons for spontaneous regressions host immune defense mechanisms against metastatic RCC tissue following nephrectomy are discussed as important factor. RCC is known to be highly immunogenic and the possible existence of cytotoxic serum factors and tumor-specific surface antigens may trigger a cell-mediated cytotoxicity as an immunological basis for regression. Histological verification of supposed regression of a primary tumor may cause diagnostic difficulties, since large central areas of necrosis and cystic lesions of the tumor can occur simultaneously. The well-known phenomenon of necrosis in a fast growing RCC at the time of nephrectomy must not be confused with true spontaneous regression. Therefore, in our opinion such reported cases of supposed partial spontaneous regressions of primary RCCs are highly questionable. Most cases of spontaneous regression of RCC metastases have been reported after nephrectomy as the only treatment. Debulking by tumor nephrectomy then gives the immune system the chance to cope effectively with the remaining much lower quantity of tumour antigens. However, the mechanisms leading to spontaneous regression of metastatic lesions after cytoreductive nephrectomy are still poorly understood.

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Key words: Renal cell carcinoma; Spontaneous regression; Primary renal cell carcinoma; Metastatic renal cell carcinoma lesions; Cytoreductive nephrectomy

Core tip: Renal cell carcinoma (RCC) is an aggressive malignancy, which, from an immunological point of view, is highly variable. In the era of immunotherapy for metastatic RCC with interferon or interleukin it was always emphasized that spontaneous remissions of RCC, although comparatively rare, do occur and support the use of immunological therapies in metastatic disease. However, we suspected that this frequently cited occurrence of spontaneous remissions is more

legend than reality. We therefore undertook an extensive literature search and included reports from the last 100 years in order to evaluate the scientific evidence describing spontaneous regressions of primary or metastatic RCC.

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INTRODUCTION

Spontaneous regression of a malignant tumor must by necessity be considered a very rare phenomenon. One of the first reported cases was that of a soft tissue sarcoma described by Coley[1] in 1893. In a historic study from 1918, 302 cases of spontaneous tumor regressions were described with only one case of a renal tumour among them[2].

Renal cell carcinoma (RCC) is an aggressive malignancy with an often unpredictable behaviour. At the time of diagnosis, about one third of all patients will already have metastases and another third will develop metachronous metastatic disease after surgery[3].

As early as 1928, Bumpus[4] reported the first case of a spontaneous regression of metastatic RCC. Further reports of spontaneous regressions of metastatic RCC have mostly been those of pulmonary RCC metastases, which supposedly regressed after radical nephrectomy. The incidence of spontaneous regression in metastatic RCC has been estimated to lie between < 1% and 7%[5,6]. However, the spontaneous regression of metastatic RCC lesions has also been reported for brain, bone, adrenal and liver metastases[7].

The spontaneous regression of a primary RCC has been reported much less commonly. However, underlying hypothetical mechanisms for spontaneous regression, which have been mentioned in the literature include humoral, immunological and vascular factors, *e.g.*, autoinfarction[8,9].

In the reported cases of spontaneous regression, the duration of disease remission is either relatively short or has not been reported at all. The longest reliably observed durations of regression of metastatic RCC lasted for 10 years[10] and for over 20 years[5]. Our literature review assesses the published literature concerning spontaneous regression of either primary or metastatic RCC.

LITERATURE SEARCH

A literature search was performed in the PubMed Database using the Keywords "renal cell carcinoma", "metastatic disease", and "spontaneous regression" and included reports from the last 100 years.

RESEARCH

Spontaneous regression of a malignant tumor or its metastases has been defined as a partial or total disappearance of disease without any treatment or induced by local treatments or interventions like embolisation of the primary tumor. The incidence of spontaneous regressions in RCC has usually been reported to be about 1% and this has always been considered a special feature of RCC compared to other solid malignancies. However, the natural course of RCC is not always predictable and includes spontaneous regression of pulmonary metastases following nephrectomy, prolonged survival and stable disease, late relapse after nephrectomy and poor long term outcome despite spontaneous regression[11]. Spontaneous regression is not synonymous with cure, as later recurrences have been reported[12,13]. Thus, a patient cannot be considered cured even if spontaneous regression is suspected if an RCC has been diagnosed[14].

Possible causes of spontaneous regression

Following nephrectomy, it is conceivable that host immune defense mechanisms against metastatic RCC tissue may be activated. Everson *et al*[12] in 1966 postulated such an immune mechanism as the most important factor for spontaneous regression of cancer.

Clinical observations seem to support this hypothesis and several examples for the relationship between neoplastic disease and the function of the immune system exist such as the incidence of lymphomas in patients with AIDS or after organ transplantation, the regression of Kaposi's sarcoma after withdrawal of immunosuppressive therapy[15-18] and the generally increased risk of cancer development with immunosuppression after organ transplantation[19]. As a possible reason some authors postulated a lack of immuno-surveillance of virus-transformed cells by strong immunosuppression. That may lead to an increased frequency of viral infections and/or virus-induced malignancies. Nevertheless, other types of malignant tumors, which are not associated with viral infections, are frequently increased in transplant recipients, too, in dependence of the duration of exposure to immunosuppression[19].

RCC is known to be highly immunogenic and the possible existence of cytotoxic serum factors and tumor-specific surface antigens may trigger a cell mediated cytotoxicity as an immunological basis for regression[20,21]. The majority of case reports of spontaneously regressed RCC describe the regression of metastases after nephrectomy rather than the spontaneous regression of a primary tumor. In malignant melanoma, studies analyzing the T-cell response in regressive primary melanoma in comparison to the metastatic lesions have found a major difference in the number of T-cells in the regressed primary and in metastatic lesions[22].

In cases of reported regression of metastatic RCC, this mostly applied to pulmonary lesions. The constant antigenic stimulation to which the lungs are exposed and

the high quantities of macrophages, lymphocytes and immunoglobulin IgA present in pulmonary tissue have been discussed as possible factors explaining such a phenomenon[23-25]. In contrast, the spontaneous regression of brain metastases has rarely been reported and this has been explained with the blood-brain-barrier limiting an immune response because of a lack of lymphocytes infiltrating the brain tissues compared to other organs and tissues[26]. Thus, hypotheses explaining observed immunological responses against malignant lesions in different sites are available.

This theory of an underlying immune mechanism has been proposed by several authors[27,28]. A remarkable report is that by Horn *et al*[26] (1971) about the induction of an RCC regression in a patient with metastatic disease after the transfusion plasma from another patient of the same family who had experienced spontaneous regression. The authors suggested "some sort of host resistance" in this case, mainly a plasma-related transfer factor, an interferon-like agent or a kind of cytotoxic antibody or a substance mediating cellular immunity[27].

In contrast, the generally poor response of metastatic RCC to immunotherapy is perhaps an argument not supporting the general importance of immunological mechanisms.

From reported experience with other malignancies which have undergone spontaneous regression (neuroblastoma, malignant melanoma, malignant lymphoma and leukemias), several other factors have been proposed as underlying mechanisms such as growth factors and/or cytokines, the induction of differentiation, endocrine mechanisms, the elimination of a carcinogen, tumor necrosis, apoptosis and/or the inhibition of angiogenesis and epigenetic mechanisms[29]. This number of proposed mechanisms just underscores the fact very little is actually known about spontaneous regression and/or that different mechanisms may be of importance in different cases[10,29,30]. For example, cytokine production by the tumor itself or by host tissue has been postulated to be involved in regressions of RCC because in one reported case of regressed intrathoracic metastases elevated serum levels of interleukin 2 receptor were reported[31].

Necrosis and apoptosis both occur in RCC and result in cell death. Gross central tumour necrosis is often clinically and pathologically seen in large and rapidly growing RCCs. This is usually considered as indicating that the rapid growth outgrowths the tumour's blood supply. Interestingly, synchronous necrosis within the primary tumor and in the metastatic lesions of RCC seems to be very rare. Boasberg *et al*[25] (1996) reported such a case of RCC with a caval thrombus and the spontaneous regression of pulmonary metastases. After resection of the primary tumor and the thrombus, histological examination verified necrosis at both sites.

Apoptosis, programmed cell death, has also been suggested to be an underlying mechanism of spontaneous regression in RCC. Pansera[31] (1992) postulated spontaneous RCC regression to be a re-expression of cell death programs typical for renal tissues since pronephros and mesonephros undergo complete regression during embryogenic renal development. Such an embryological cell death program could hypothetically be reactivated in immature RCC tissue. Indeed, the manifestation of embryological cell characteristics does occur in many neoplasms[32]. This phenomenon of morphological similarities between growing tissues, like embryological and cancer cells has its reasoning in a common origin from a precursor stem cell. Thus, spontaneous regression of RCC may be explained as a kind of re-expression of embryonic features by adult carcinoma[32].

Histological verification of regression

In a primary RCC: Histological verification of supposed regression of a primary tumor may cause diagnostic difficulties, since large central areas of necrosis and cystic lesions of the tumor can occur simultaneously. Therefore, the differential diagnosis of a spontaneously regressed RCC should include inflammatory lesions of the kidney, *e.g.*, xanthogranulomatous pyelonephritis, sinus histiocytosis and tuberculosis or malakoplakia. This requires an extensive tissue sampling by the pathologist to confirm or refute the diagnosis of spontaneous regression of an RCC[33].

In our review of the literature we found only 7 reported cases of partial or total spontaneous regression of primary RCCs (Table 1). However, in most of these seven cases the regressions were not unequivocally confirmed.

The first documented case of a total spontaneous regression of a primary RCC was reported by Choi *et al*[33] in 1986. The authors diagnosed a left sided renal tumor and performed a nephrectomy. Histologic examination revealed a cyst-like capsule with coagulated blood, necrotic tissue, calcifications and a cluster of tumor cells, which were classified as a spontaneously regressed primary RCC.

Hamid *et al*[32] in 1998 described two similar cases, one with a cystic cavity of the kidney "containing necrotic debris and brown fluid occupying virtually the whole of the specimen" and "occasional foci of viable renal cell carcinoma …seen in the capsular area". These findings were deemed to represent spontaneous regression by the authors. However, the differential diagnosis must include a developing RCC within a cystic renal lesion with previous hemorrhage. The second case reported by Hamid *et al*[32] should also be viewed critically. There they found "an extensively involuted/hyalinised lesion with extensive metaplastic ossification and also foci of dystrophic calcification" and, again, "occasional foci of cells with clear cytoplasm". Neither of these two case reports included any follow-up information at all[33].

More stringently, spontaneous regression of an RCC or its metastases should be defined as a partial or complete regression of a renal neoplasm which has been histologically confirmed first and then regressed either without treatment or sometimes following an intervention, *e.g.*, cytoreductive nephrectomy. It is important to note that it

Table 1 Regression of primary renal cell carcinoma

Case	Year	Type of regression	Histology documented	Follow up (interval)
Hall[53]	1908	Total regression (?)	Entirely necrotic tumor	Not given
Choi *et al*[33]	1986	Total regression (?)	Cyst-like capsule with necrotic tissue Calcifications and a cluster of tumor cells…	Not given
Edwards *et al*[34]	1996	Partial regression	Residual RCC with marked fibrosis And calcification	36 mo
Hamid *et al*[32]	1998	"Extensive regression"	Extensively hyalinised lesion, also foci Of cells with clear cytoplasm	Not given
			Cystic cavity containing necrotic Debris, occasional foci of viable RCC	Not given
Lacquaniti *et al*[35]	1999	Partial regression	Fibrotic involution….. With few central areas of RCC	7 mo
Kobayashi *et al*[8]	2002	Partial regression of primary RCC with inferior V. cava Tumor thrombus	No	2 yr

RCC: Renal cell carcinoma.

is possible to find necrosis in a fast growing RCC at the time of nephrectomy and this well-known phenomenon must not be confused with true spontaneous regression. However, some authors do consider such a necrosis as a partial regression[34-36]. Thus, in our opinion such reported cases of supposed spontaneous regressions of primary RCCs are highly questionable (Table 1).

In metastatic RCC lesions: In all, we found 94 reported cases of spontaneous regression of metastatic lesions in patients with an RCC (Table 2). Most of these reports concern pulmonary metastases (75 cases) and only a few other sites: pleura and mediastinum (3), liver (4), pancreas (1), brain (3), bone (5), eyes (2) and skin (1) (Table 2). Most of these case reports give no histological verification of the supposedly metastatic lesions. Thus, the diagnosis of spontaneous regression was based on changes in size on imaging which therefore cannot be considered as a proof beyond doubt.

Kavoussi *et al*[36] reported a rate of regression of 20% based on diagnosis by cytology. However, Davis *et al*[37] found a regression of pulmonary RCC metastases in only 3/14 documented cases, *i.e.*, unrelated to any kind of treatment including nephrectomy[38].

In evaluating spontaneous regression of a primary tumor as well as metastatic lesions, histological verification can be a diagnostic challenge. Patients with advanced metastatic disease are often not in good general condition for any surgical or interventional procedures. Edwards *et al*[34] (1996) pointed out that fine needle biopsy carries a risk of gross bleeding due to blood coagulation potentially affected by paraneoplastic mechanisms. Furthermore, computed tomography (CT)-guided biopsies may be unsuccessful in rendering good histologic reports because of insufficient sample size.

In case of eye or brain lesions, cytological or histological verification is even more difficult and dangerous. Thus, in clinical practice such procedures are usually avoided when the primary tumor has been histologically confirmed as an RCC[39-41].

Thus, in cases without convincing histological evidence of pulmonary RCC metastases, several other benign conditions must be considered in the differential diagnosis, such as fungal or mycobacterial infections, sarcoidosis, Wegener granulomatosis and vasculitic lesions which can all appear as pulmonary lesions and regress later[38]. In the pre-CT era, the misinterpretation of radiological findings in conventional chest X-ray studies may have been more common than appreciated at the time. Thus, cases diagnosed as spontaneous regression of RCC metastases in the lungs by chest x-ray only should also be regarded with caution. Furthermore, even cases of pulmonary RCC lesions by cytology or even histology which then had spontaneous regression diagnosed by chest X-ray only must be questioned in retrospect[42]. Embolisation of the lung or a pulmonary segment by tumor thrombi from the renal vein may cause regional pulmonary infarction, which may have the radiological appearance of metastatic lesions. The disappearance of these findings after improvement of inflammatory lesions close to such an embolus could also be misinterpreted as a spontaneous regression[43,44].

Cytoreductive nephrectomy

More than 40 years ago, Markewitz *et al*[44] advocated a palliative nephrectomy only to be considered in individually selected cases with careful evaluation of the potential benefit. However, since then a markedly longer survival has been shown in patients with RCC after nephrectomy and metastasectomy[45,46]. Therefore, the concept of cytoreductive nephrectomy should today be taken into consideration in all patients with metastatic RCC when the short-term outcome of the surgical procedure can be predicted to be acceptable[47].

Indeed, most cases of spontaneous regression of RCC metastases have been reported after nephrectomy as the only treatment. Two possible hypotheses have been put forward as explanations for this phenomenon:

First, a dissemination of tumor cells into the systemic circulation and the lymphatic system induced by the surgical procedure results in a large and ubiquitous presentation of tumor antigen and this may initiate a strong anti-

Table 2 Regression of metastases from renal cell carcinoma

Site of metastases	Source	Number of cases	Histological documentation	Follow up (interval)
Lung	Meinders[54]	1	No	3 yr
	Boasberg *et al*[25]	1	No	2.5 yr
	Cited by Freed *et al*[23]	45 (from 1928-1976)	13/45	
	Vizel *et al*[55]	1	No	11 mo
	Mohr *et al*[56]	1	No	22 mo
	Snow *et al*[5]	1	Yes	6.5 yr
	Nakano *et al*[57]	1	No	8 yr
	Barré *et al*[58]	2	Yes	5 yr
	Kavoussi *et al*[36]	1	Yes (cytologically)	6 yr
	Eissler[59]	1	Yes	7 yr
	Omland *et al*[60]	1	No	14 mo
	Davis *et al*[37]	1	Cytologically	18 mo
	de Riese *et al*[14]	2	No	5.5-11 yr
	Vogelzang *et al*[61]	1	Yes (cytologically)	5 yr
	Palmer *et al*[62]	1	No	15 mo
	Garcia-Del-Muro *et al*[63]	1	No	1 yr
	Czaplicki *et al*[64]	1	Not given	16 yr
	Marcus *et al*[65]	4	No	1-4.5 yr
	MacManus *et al*[30]	1	Yes	9 mo
	Bos *et al*[24]	1	No	1 yr
	Edwards *et al*[34]	1	No	36 mo
	Lokich[7]	1	No	2 yr
	Rauh *et al*[66]	1	Yes	8 mo
	Chang *et al*[11]	1	Yes	16 mo
	Sánchez-Ortiz *et al*[67]	1	Yes	10 mo
	Lekanidi *et al*[29]	1	No	5 mo
Bone	Mims *et al*[68]	1	Yes	1 yr
	Doolittle[69]	1	Yes	4 yr
	Freed *et al*[23]	1	Yes	10 yr
	Kerbl *et al*[70]	1	Yes	13 mo
	Nakajima *et al*[71]	1	Yes	8 mo
Pleural/mediastinal	Kallmeyer *et al*[72]	1	Yes	3 mo
	Abubakr *et al*[27]	1	Yes	1.5 yr
	Thoroddsen *et al*[49]	1	Yes	9 yr
Liver	Deweerd *et al*[73]	1	Yes	6 mo
	Ritchie *et al*[74]	1	Yes	9 mo
	Wyczólkowski *et al*[75]	1	Yes	12 mo
	Christophersen *et al*[10]	1	Yes	5 yr
Pancreatic choroidal	Altschuler *et al*[76]	1	Yes	2.5 yr
	Langmann *et al*[38]	1	No	6 mo
	Hammad *et al*[40]	1	No	
Brain	Omland *et al*[60]	1	No	14 mo
	Guthbjartsson *et al*[39]	1	No	9 yr
	Hensiek *et al*[77]	1	No	4 yr
Skin	Chang *et al*[11]	1	No	16 mo

tumoral immune response by the host. Secondly, because of the mass of tumour antigen is located in the primary tumor, debulking by tumor nephrectomy then gives the immune system the chance to cope effectively with the remaining much lower quantity of tumour antigens and thus to mount an effective antineoplastic response[13,14].

Although the morbidity and mortality of nephrectomy should always be taken into consideration, a palliative cytoreductive nephrectomy in metastatic RCC may also be beneficial for other reasons: in terms of the prevention of tumor toxicity, for the correction of hypercalcaemia and for the improvement of local symptoms, such as pain or hematuria[40,48-53].

Regression has also been described to occur after other local treatments such as radiotherapy or embolisation of the primary tumor[7,37,38,48-50].

CONCLUSION

Spontaneous regression in renal cell carcinoma is very rare and there probably has been an overreporting in the literature. However, it has been described plausibly in metastatic RCC sites-mostly pulmonary-and then mostly after nephrectomy, thus supporting the concept of cytoreductive nephrectomy. Despite several plausible hypotheses, the mechanisms leading to spontaneous regression of metastatic lesions after cytoreductive nephrectomy are still poorly understood.

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REVIEW

Injectable treatments for female stress urinary incontinence

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Core tip: While there are different types of injection materials available, it is unknown which one is superior as few head to head studies have been performed between the newer agents. It is important to inform patients that treatment with injectable agents is not as effective as surgical treatment, and that such agents might necessitate additional and repeated administrations in order to achieve the desired therapeutic effect.

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Abstract

The use of injectable agents for the treatment of stress urinary incontinence (SUI) is an option for female patients who are unwilling to undergo surgery, or have concurrent conditions or diseases that render surgical treatment unsuitable. To be effective for SUI, an injectable agent must be nonimmunogenic, hypoallergenic, biocompatible, permanent, nonerosive, nonmigratory and painless. It must also heal with minimal fibrosis, possess a long-term bulking effect, and be easily stored and handled. Glutaraldehyde cross-linked bovine collagen (Contigen), silicone polymers (Macroplastique), Durasphere, calcium hydroxyapatite (Coaptite), polyacrylamide hydrogel (Aquamid, Bulkamid), Permacol, and stem cell therapy have been used as injectable agents. Patients must be informed that treatment with injectable agents is not as effective as surgical treatment, and that such agents might necessitate additional and repeated administrations in order to achieve the desired therapeutic effect.

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Key words: Stress urinary incontinence; Injectable treatment; Bulking agent; Outcomes; Adverse events

INTRODUCTION

Injection treatment for female stress urinary incontinence (SUI) is not new. Murless[1] reported the first results in 1938, followed by Quackles[2] in 1955 and by Sachse[3] in 1963. In the 1970s and 1980s, polytetrafluoroethylene (Teflon) was used extensively. However, safety concerns that included distant particulate migration, foreign body reaction, severe granulomatosis reaction and possibly carcinogenic effects inhibited its usage[4,5]. In 2001, autologous fat was studied in women with urethral hypermobility but serious adverse events including systemic embolization and death were reported[6-8]. Dextranomer/hyaluronic acid copolymer (Deflux) was another agent used as a bulking agent, and while short term results were satisfactory, long term durability was poor[9,10]. Ethylene vinyl alcohol copolymer (Tegress), a permanent, hypoallergenic, nonimmunogenic implant demonstrated equivalence in outcomes with collagen and was approved by the Food and Drug Administration (FDA) in 2004[11,12]. However, a high rate of urethral erosion was noted and as a result, the manufacturer voluntarily withdrew Tegress from the market in 2007[13].

After glutaraldehyde cross-linked bovine collagen

(GAX-collagen) was introduced in 1993, it was reported as a safe and reliable endoscopic treatment option of SUI. Currently, collagen is the gold standard method in SUI treatment modalities[14]. To be effective against SUI, an injectable agent must be nonimmunogenic, hypoallergenic, biocompatible, permanent, nonerosive, nonmigratory and painless. It must also heal with minimal fibrosis, possess a long-term bulking effect, and be easily stored and handled[15-17]. It is important that the components of these agents remain structurally stable following administration. In addition, to preclude any significant risk of migration, the microcrystalline or micro polymer particles of such agents must have nearly spherical shapes, with diameters greater than 110 micrometers[15]. No agent to date has satisfied all these requirements and research continues on additional bulking materials and modes of delivery.

RESEARCH

We searched the Medline database for published articles, reviews and case reports between 2000 and 2013. The key words "stress urinary incontinence", "injectable treatment" and "bulking agent" were used for literature research. Three hundred and ninety nine abstracts were viewed, and many of these articles were chosen for review and inclusion.

MECHANISM OF ACTION

Although the exact mechanism is not understood, it is thought to improve the urethral sphincter function. Urethral mucosal surface expands with injection, augments urethral coaptation and consequently post injection abdominal leak point pressure (ALPP) increases[17]. Though originally thought to be primarily an obstructive effect, Monga *et al*[18] showed that the mechanism of action might be related to an expanded urethral area and better pressure passage on the proximal urethra. The bladder neck opening during stress maneuvers could be prevented by injection of the proximal urethra and bladder neck[19]. Klarskov *et al*[20] introduced urethral pressure reflectometry, a technique for measuring pressure and cross sectional area in the urethra, which provides parameters useful for studying the mechanism of action of urethral injection therapy. The authors found that the group who showed subjective improvement after injection therapy had significantly higher squeezing opening pressure compared with the group that didn't improve. They concluded the bulking material might function as additional central filler volume which increase the length of the muscle fiber and thereby the power of the sphincter[20].

PATIENT SELECTION CRITERIA

Patients normally considered for intraurethral bulking include females with SUI attributed to intrinsic sphincter deficiency (ISD) and a urethral mobility below 30 degrees. However, various studies have demonstrated that intraurethral bulking is also effective for patients affected by urethral hypermobility[21]. Herschorn *et al*[22] previously demonstrated the absence of any significance difference between patients with urethral hypermobility and patients without urethral hypermobility. Overall, the dry rate at 1 year, 2 years and 3 years were 72%, 57% and 45%, respectively. Others have demonstrated similar results[23-25].

Intraurethral bulking agents are a good choice for patients who do not desire to undergo more invasive surgery and who comprehend that efficacy and durability are inferior to surgery[26]. Bulking agents should not be offered to patients who are seeking a permanent therapy for SUI[27]. Potential candidates for bulking agents are: the elderly; those who are a high anesthetic risk; those who are at an increased risk of urinary retention after a sling operation; those who are on anticoagulation therapy; those who desire to have children; those who have mild persistent SUI after an incontinence surgery; those who have SUI and insufficient bladder emptying; those who have mild SUI after exercise; those who don't want more invasive procedures; and those who are content with improvement instead of a cure[16].

For intraurethral bulking, exclusion criteria include urinary tract infections, hypersensitivity reactions to the bulking agent, and urinary incontinence caused by abnormal detrusor contractions[28].

INJECTION TECHNIQUES

Bulking agents are generally administered with cystoscopic assistance under local anesthesia. The implant can be administered *via* the urethral submucosa or lamina propria by transvaginal, transurethral or periurethral approaches. Currently the transurethral approach is the most preferred technique[28]. During implantation, it is very important to place the bulking material into the proximal urethral wall near the bladder neck. The amount of material needed for injection is the volume that achieves complete coaptation.

Before commencing the injection procedure, the patients are positioned in dorsal lithotomy, and prepared in accordance to the applicable sterile procedures. To prevent any infections, antibiotics are usually administered both before and after the procedure. During the procedure, a 20% benzocaine ointment can be applied to the vulvar vestibule as a local anesthetic. A 2% lidocaine jelly can also be administered intraurethrally. Perimeatal blebs are raised with 1% or 2% lidocaine at the 3- and 9-o'clock or 4- and 8-o'clock positions, 3 to 4 mm lateral to the urethral meatus using a 25-gauge needle, followed by 4 mL of 1% or 2% lidocaine injected periurethrally. The cystourethroscope is inserted and the bladder is inspected. After the bladder is emptied, the endoscope is backed to the mid-urethra with an irrigation rate just enough for visibility. Generally the 3- and 9-o'clock or 4- and 8-o'clock positions are preferred area for injection[29]. For the initial injection, the 6-o'clock position is often an ideal starting

Table 1 Level of evidence and grade of recommendation for injectable agents (b)

Evidence summary	LE
Periurethral injection of bulking agent may provide short-term improvement in symptoms (3 mo), but not cure, in women with SUI	2a
Repeat injections to achieve therapeutic effect are often required	2a
Bulking agents are less effective than colposuspension or autologous sling for cure of SUI	2a
Adverse effect rates are lower compared to open surgery	2a
There is no evidence that one type of bulking agent is better than another type	1b
Transperineal route of injection may be associated with a higher risk of urinary retention compared to the transurethral route	2b
Recommendations	GR
Do not offer bulking agents to women who are seeking a permanent cure for stress urinary incontinence	A

LE: Level of evidence; GR: Grade of recommendation; SUI: Stress urinary incontinence.

point[28]. The needle diameter is dependent on the viscosity of the injected material. The patient can be instructed to perform a valsalva maneuver after injection and if urine leakage is still present, additional material may be injected[28-30].

Faerber *et al*[31] previously performed a review of the transurethral and periurethral techniques for ISD treatment and found both techniques were similar with respect to treatment outcome and the occurrence of adverse events. It was noted, however, that the transurethral technique involved the injection of smaller amounts of material[31]. Likewise, in a prospective and randomized study performed by Schulz *et al*[32], it was observed that the transurethral and periurethral techniques were similar with respect to efficacy however, it was also observed that the periurethral group required the injection of larger volumes of material as well as exhibited a higher rate of urinary retention[32]. Thus, the periurethral technique appears to require higher volumes of material than the transurethral technique, while also requiring more time to be learned effectively. This is important as higher volumes are associated with higher costs, and may increase the potential for postoperative complications.

INJECTABLE AGENTS

Many different agents have been studied for use as bulking material with varying success and adverse events. Patients should be informed that surgery is associated with greater improvement of symptoms, but at the expense of higher risks. Patients should also be informed that treatment with injectable agents is less durable and repeated injections will likely be needed to maintain effect. Cochrane systemic review did not show significant differences in clinical outcomes and complications between different injectable agents[33,34]. Table 1 list the level of evidence and grade of recommendation for injectable therapies for SUI in women by European Association of Urology guidelines[27].

Biomaterials

GAX-collagen: GAX-collagen is produced by cross-linking bovine collagen with glutaraldehyde. The resulting substance is a highly stable collagen complex with a fibrillar structure. This structure confers GAX-collagen resistance to enzymatic breakdown by collagenases, thus increasing the durability of the implant[35]. Contigen is composed of 1%-5% collagen type Ⅲ, and approximately 95% of collagen type Ⅰ [36]. It was available as pre-filled syringes containing 2.5 mL of agent and which needed storage in the refrigerator. Injection was performed with 23 G injection needle through the cystoscope. A volume of 30 mL injection material may be necessary to ensure enough urethral coaptation during the operation[29]. Since GAX-collagen is both biocompatible and biodegradable, only minimal inflammatory changes occur[37]. It is not known to migrate. Although the collagen in GAX-collagen begins to denature by the 12th week of application, it can persist for up to 19 mo[38].

GAX-collagen is a widely used injectable agent and there are numerous studies pertaining to its efficacy and safety[39,40]. It has been considered the gold standard of urethral bulking material such that the FDA required direct comparison to collagen for any new bulking agents in clinical trials[14]. In the North American study group, 382 patients were followed for 2 years, with improvement and cure rates determined as 45% and 33% respectively. The dryness rate at the end of the first-year was 52% but dropped to 38% in the second year[41]. Many patients required repeat injections to maintain efficacy[42]. In a study by Winters *et al*[17], it was observed that 45% of the treated elderly women showed discernible improvement up to 24.4 mo post treatment; however, more injections were required to maintain efficacy in 40% of the patients after an average of 7.9 mo. Corcos *et al*[43] reported that GAX-collagen cured approximately 30% of the women, while improving the condition of 40% of the women in a study group of 40 women at 50 mo of follow up. However, they also reported that four of the women who were cured and five of the women who showed improvement later required "maintenance" injections.

Only a limited number of side effects are associated with GAX-collagen treatment[44]. Clinical trials in the United States have reported transient urinary retention among 15% of the patients, urinary tract infections (UTIs) among 5% of the patients, and irritative voiding syndrome among 1% of the patients[45]. *De novo* urgency as high as 10% has also been reported in certain studies[43]. Due to the minute amounts of GAX-collagen injected to the patients during treatment, the substance is not associated with any signifi-

cant immunoreactivity and cytotoxicity[46]. For this reason, no previous cases of migration or foreign-body responses have been reported with the GAX-collagen.

Importantly, GAX-collagen has the potential to trigger an allergic reaction to bovine protein in patients. For this reason, all patients are required to undergo an allergy skin test 30 d prior to treatment. In general, 3% of the patients are expected to exhibit a positive allergy result. Nearly 70% of such patients will display an allergic reaction within 3 d following the test, which indicates that their allergy to bovine collagen is pre-existing through dietary exposure. The other 30% of the patients will take longer to display any reaction, which may take up to 4 wk. Patients who display a positive allergy test result will not be able to receive GAX-collagen treatment. However, it is still possibly for patients with a negative allergy test result to later display an allergic reaction during and after GAX-collagen administration[47].

Owing to its lack of migration, its degradability and its limited allergenic potential, GAX-collagen is the most popular and widely used intraurethral bulking agent for the treatment of urinary incontinence[14]. However, despite its long track record, although with modest results, the manufacturer ceased production in 2011 and the implant is no longer available.

Synthetic materials

Silicone polymers (macroplastique): Macroplastique (Uroplasty Inc, Minneapolis, MN, United States) is composed of vulcanized polydimethylsiloxane macroparticles suspended in a hydrogel of polyvinylpyrrolidone (povidone). This hydrogel also functions as a lubricant during injection[48]. It has been in use in Europe for SUI since 1996. The material consists of particulates of various shapes and sizes with marked variability but 99% of the particles are greater than 100 μm. However, the potential for migration is present, though remote. Henly *et al*[49] identified small macroplastique particles within the lymph nodes, kidneys, lungs and brain of dogs within 4 mo following administration. Large particles, on the other hand, were identified in the lungs of only one case but without associated reaction[49].

Marcoplastique can be injected with standard cystoscopic equipment but since the substance is fairly viscous, it requires a high pressure 18-gauge Uroplasty needle gun for administration. Alternatively, non-endoscopic transurethral injection device, the Macroplastique Implantation System (MIS) can be used. The MIS consists of a multichannel needle positioning device that is oriented at the 2-, 6-, and 10-o'clock positions for injection. Henalla *et al*[50] noted successful results (74.3%) at the three months follow-up. Tamanini *et al*[51] published the results of 21 patients treated with MIS and at the 12 mo follow-up, an improvement was reported in 57% of the patients, but 23% of patients were deemed failures. ter Meulen *et al*[52] analyzed the efficacy of MIS in women with SUI and urethral hypermobility after an unsuccessful conservative treatment. Twenty-four women received Macroplastique *via* MIS compared with 21 patients who underwent a pelvic floor muscle exercise program. Decreased pad usage and improved questionnaire scores were seen in the MIS group ($P = 0.017$, $P = 0.015$, respectively)[52].

A recent North American multicenter trial randomized 247 patients with ISD to transurethral injection of either Macroplastique or Contigen. After 12 mo, there were significant clinical improvement and dry/cure rates in 61.5% and 36.9% of patients treated with macroplastique *vs* 48% and 24.8% of patients treated with Contigen, respectively. This indicated that macroplastique was non-inferior to Contigen ($P < 0.001$)[53]. In a randomized study by Maher *et al*[54], macroplastique was compared with pubovaginal sling. At the 12 mo follow-up, subjective success rates were 90% in the sling group and 77% in Macroplastique group ($P = 0.41$), and there was no differences between the two groups in patient satisfaction. The authors emphasized the obvious advantages of macroplastique in terms of shorter operative time, less blood loss and shorter hospitalization time.

Complications associated with macroplastique use include urinary retention (5.9%-17.5% of cases), urinary frequency (0%-72.4%, of cases), dysuria (0%-100% of cases), and UTI (0%-6.25% of cases)[55].

Durasphere

Durasphere consists of nonabsorbable zirconium oxide beads coated with pyrolytic carbon, and suspended in a water-gel with 2.8% beta-glucan. It gained FDA approval for use in patients with ISD in 1999[28]. During administration, the zirconium oxide beads are encapsulated by the periurethral tissues[56], which allows it to have a long-lasting bulking effect. In contrast to GAX-collagen, Durasphere is nonimmunogenic and inert. Thus, skin testing with this agent is not necessary prior to administration. Durasphere was designed with larger caliber particles (> 80 μm) to prevent migration. The size of the beads varies between 212-500 μm which contributes to its higher viscosity. As a result, the injection of Durasphere is often more difficult. To overcome this difficulty, an alternative injection method was developed for Durasphere, which involves the injection of local anesthetic to raise a circumferential bleb into which agent was injected[57]. Additionally, as a way to remedy this, the manufacturer introduced Durasphere EXP (Injectable Bulking Agent from Carbon Medical Technologies Inc.) in 2006, whose smaller bead size (range 90 to 212 μm), facilitated injection. However, the size of these particles is still greater than the threshold for migration. As of late 2013, no clinical results have been published with this new formulation.

In a multicenter, randomized, controlled, double-blind study consisting of 355 patients, Lightner *et al*[56] reported that Durasphere was as effective as GAX-collagen. The authors emphasized that the Durasphere group required significantly less volume of injected material to obtain comparable clinical results (4.83 mL *vs* 6.23 mL, $P < 0.001$), and that the probability of achieving successful treatment

with a single injection was higher for the Durasphere group. At the one year follow-up, the ratio of patients that demonstrated an improvement of one Stamey grade or more in the Durasphere and the GAX-collagen groups were 80% and 69%, respectively[56]. Long-term data have been reported by Chrouser *et al*[58], and they observed a decrease in clinical success over time. For the Durasphere group, the success rate for treatment was 63% at one year, 33% in the second year, and 21% in the third year follow-up. For the collagen group, the success rate was 19% in the second year, and 9% in the third year follow-up[58].

In the clinical studies that served as the basis for the FDA approval, the most commonly observed adverse effects included acute retention for ≤ 7 (13% of subjects), dysuria (12% of subjects), UTI (9% of subjects), hematuria (6% of subjects), and retention for > 7 d (6% of subjects)[59]. In a randomized multicentre clinical study comparing Durasphere and GAX-collagen, adverse events were similar between the two groups, except for a higher incidence of urgency and acute retention in the Durasphere group (24.7% and 16.9%, respectively) than in the collagen group (11.9% and 3.4%, respectively)[56]. There have also been case reports of pseudo abscess formation and urethral prolapse[60,61]. Distant particle migration has been reported with the original Durasphere formulation, which raised concern since its bead sizes ranged from 212 to 500 μm, higher than the threshold thought to be critical for migration. With its smaller bead sizes (range 90 to 212 μm), it is unknown whether the risk for migration is increased with Durasphere EXP[62].

Calcium hydroxyapatite (Coaptite)

Calcium hydroxyapatite received its first approval for the treatment of SUI in women in 2005. Calcium hydroxyapatite is a synthetic substance composed of glycerin and carboxymethylcellulose, and which structurally forms an aqueous gel. The spherical calcium hydroxyapatite particles have an average diameter of 100 μm (75-125 μm), which is above the threshold for migration[28]. The gel facilitates injection and provides the initial bulking effect but is designed to degrade over time, and allowing for ingrowth of tissue around the particles. Calcium hydroxyapatite is a natural component of teeth and bones; it has hence been used in orthopedic and dental applications, and also in the ureteral orifice for vesicoureteral reflux, with excellent biocompatibility[63,64]. It is not antigenic, immunogenic or toxigenic and thus a pre-procedure skin test for hypersensitivity is not needed. Refrigeration or special handling are not required[63,64]. Because the material is not viscous, it can be performed with a 21 G injection needle. Since calcium hydroxyapatite is radiopaque, it can be seen on ultrasonography or fluoroscopy, which may aid in accurate localization and placement[14].

In the first pilot study with calcium hydroxyapatite, Mayer *et al*[65] reported a reduction in average pad weight, a reduction of 45% in pad usage, and an increase in average ALPP from 39 to 46 cm H_2O at the 12 mo follow-up. Seven patients needed a second injection after 8.4 mo[65]. In the data leading up to FDA approval, there was no significant difference in quality of life, pad weight or Stamey grade change between coaptite and collagen at a mean follow-up of 11.2 mo[66]. In a large multicenter randomized clinical study, Mayer *et al*[65] compared Coaptite *vs* collagen in 296 women with ISD and demonstrated, at the 12 mo follow-up, an improvement of one or more Stamey grade in 63.4% of the Coaptite group and 57% of the collagen group (P = 0.34). There was no difference in cure rates (39% Coaptite *vs* 37% collagen). More patients in the Coaptite group required only one injection (38%) compared with the collagen group (26.1%) (P = 0.03) and the mean injected volume was lower (4.0 mL *vs* 6.6 mL, $P < 0.0001$)[67].

Common adverse associated with calcium hydroxyapatite are urinary retention (41% of patients), hematuria (19.6% of patients), dysuria (15.2% of patients), UTI (8.3% of patients), urinary urgency (7.6% of patients), urinary frequency (7.0% of patients), and urge incontinence (5.7% of patients). The overall erosion rate was 1.3%[49]. Palma *et al*[68] published a case with urethral prolapse 3 mo after calcium hydroxyapatite injection therapy. The prolapsed nodule was surgically extracted and the resulting pathology reported lymphocytic infiltration, giant cells and granulomatous reaction characterized with macrophages[68].

Polyacrylamide hydrogel (Aquamid, Bulkamid)

Aquamid and Bulkamid are polyacrylamide hydrogels composed of 2.5% polyacrylamide that are nontoxigenic. They possess a homogeneous composition and texture, presenting elasticity and viscosity properties similar to tissue. They contain no solid particles, thereby eliminating any risk of particle migration[28]. Aquamid is used clinically as soft tissue filler in plastic surgery and reconstructive procedures[69]. Bulkamid is the corresponding product for the SUI indication. It does not need refrigeration or any special handling[16]. Bulkamid and Aquamid are currently not approved for use in the United States; however, randomized clinical studies involving numerous centers are currently being conducted in both Europe and North America. Toozs-Hobson *et al*[70] reported a noncomparative, multicenter case series of 135 women in 2010. Half the patients had mixed urinary incontinence. At 12 mo, two-thirds of the women reported being cured or improved, and there were significant reductions in incontinence episodes per 24 h and pad weight testing. Efficacy was similar between patients with pure SUI and those with mixed incontinence. There was a 35% reinjection rate. Minor adverse events were noted and included UTI (5%), transient urinary retention (3%), hematuria (1%), transient *de novo* urgency and urge incontinence (1%)[65]. In the 2 years follow up analysis of this cohort, there was durability of success with 64% of women cure/improved, which was not significantly different compared with the 12 mo data[70].

In a recent prospective, multicenter study Sokol *et al*[71] randomized 345 women with SUI or stress predominate

mixed incontinence to Bulkamid or Contigen and a > 50% reduction in leakage and incontinence episodes was seen in 53.2% of the Bulkamid group *vs* 55.4% of the Contigen group at 12 mo. Additionally, at 12 mo, 47.2% of the Bulkamid patients and 50% of the Contigen patients reported no SUI episodes. Seventy seven percent of the Bulkamid patients and 70% of the Collagen patients considered themselves cured or improved. The authors concluded that Bulkamid is not inferior to Contigen and is a promising new simple office based bulking agent for women with SUI, particularly since Contigen is no longer commercially available[71].

Permacol

Permacol is a dermal implant made from non-reconstituted collagen obtained from porcine skin. Until now, it has been mainly used for pelvic reconstruction and hernia repair. Non-collagenous material, except elastin, is removed and a cross linking process is performed. It maintains its 3-dimensional structure and when implanted, it allows for in-growth of new tissue, which can potentially be permanent. Permacol is non-allergenic, obviating the need for skin hypersensitivity testing[72].

Bano *et al*[73] compared Permacol and Macroplastique in a randomized controlled trial that included 50 women with urodynamic SUI. A great majority of the injections (84%) were performed peri-urethrally in the Permacol group. At 6 mo, 62.5% in the Permacol group were dry *vs* 37.5% in the Macroplastique group but no statistical analysis was reported. Rates of adverse events were similar between both groups[73].

Future directions

Stem cell therapy: Recently, within the context of tissue engineering strategies, stem cell therapy was evaluated for the experimental treatment of SUI[74,75]. The first report evaluating the safety and efficacy of cell therapy in humans yielded an 81.3% cure/improved rate at 12 mo[76]. Multiple small series have been published since but none generated as much excitement as the study by Strasser *et al*[77], which demonstrated very impressive outcomes from sphincteric injection of autologous myoblasts compared with collagen in a randomized trial[77]. This article was later retracted due to ethical violations and investigators question whether this trial as described ever existed. Most recently, Stangel-Wojcikiewicz *et al*[78] reported a case series of 16 women who had sphincteric injection of muscle derived stem cells with a cure/improved rate of 75% at 2 years of follow-up. There were no adverse events from either the deltoid muscle biopsy or from the injection[78]. Cook MyoSite, a part of Cook Medical, has two active clinical trials underway with intrasphincteric injections of autologous muscle-derived cells for the treatment of SUI. While still considered experimental, stem cell therapy holds great promise and in the near future may very well be an additional option in therapy for the treatment of SUI[79]. The clinical trials on injectable treatments for female SUI is shown on Table 2.

POSTOPERATIVE PERIOD

It is rare to observe postoperative complications immediately following the administration of injectable treatments. Once the injection procedure is completed, patients must be capable of voiding without difficulty. In the unlikely case of post operative urinary retention, a Foley catheter should be avoided, as is possible for the bulking agent to become molded around the catheter, and to thus lose its effectiveness. However, it should be noted that there is currently no data or findings indicating that short-term use of catheters might adversely affect intraurethral bulking. For patients that require catheterization for longer periods, it is preferable to use suprapubic catheters in order to prevent the bulking agent's position or efficacy from being affected.

For most patients, multiple treatments with injectable agents will be necessary before achieving the desired therapeutic effect. Each bulking agent requires different time intervals between successive treatments. For instance, GAX-collagen can be administered once every 7 d (although a 4 wk time interval was used in the initial clinical studies with GAX-collagen); however, in order to better evaluate the patient response to the injections, many physicians prefer to wait for 4 or more weeks before continuing with the next injection[23]. Teflon requires a 4-mo waiting period, while Macroplastique injections can be performed at 12-wk intervals. Durasphere injections require a minimum time interval of 7 d, while Coaptite can be injected once every month. In case erosion is observed within the bladder during repeat injections, the eroded side should not be reused for injection purposes until the epithelium recovers[23].

CONCLUSION

Injectable agents represent an effective and safe approach for treating SUI in women. They should especially be considered for female patients who are unwilling to undergo surgery, or have concurrent conditions or diseases that render surgical treatment unsuitable. It must be remembered that injection treatments will mainly serve to improve the patients' symptoms, and that they will not provide a definite cure for SUI. It is also important to inform patients that treatment with injectable agents is not as effective as surgical treatment, and that such agents might necessitate additional and repeated administrations in order to achieve the desired therapeutic effect. Although there are different types of injection materials with different properties, the superiority of one agent over another is not known. There is thus a continuous search for effective, inert, nonmigratory and nonimmunogenic materials that can be readily injected and incorporated into the patient's tissues, and that can maintain their structure for extended periods of time once injected.

Table 2 The clinical trials on injectable treatments for female stress urinary incontinence

Ref.	Bulking agents	Number of patients	Assessment methods	Outcomes
Lightner *et al*[56]	Durasphere *vs* Collagen	*n* = 61 (Durasphere) *n* = 68 (Collagen)	SUIS Standardized pad test	At the one year follow up, the Durasphere group achieved improvement in one Stamey grade or more in 80.3% of patients compared to 69.1% of patients in the Collagen group (P = 0.162)
Chrouser *et al*[58]	Durasphere *vs* Contigen	*n* = 43 (Durasphere) *n* = 43 (Contigen)	Patient satisfaction and continence were subjectively evaluated *via* telephone interview	Success rateswere reported in 33% of Durasphere group and 19% in Contigen at 24 mo; at 36 mo, 21% in Durasphere, 9% in Contigen No significant difference was observed in time to failure between the injection groups (P = 0.25)
Bano *et al*[73]	Permacol *vs* Macroplastique	*n* = 25 (Permacol) *n* = 25 (Macroplastique)	1-h pad test SUIS KCQ	At 6 mo, 62.5% in the Permacol group were dry *vs* 37.5% in the Macroplastique group but no statistical analysis was reported
Hurtado *et al*[13]	Tegress	*n* = 19	Physical exam Urodynamic findings Complications	A 58% of the patients had a complication related to the procedure with 37% experiencing urethral erosion 10.5% of the patients reported at least a 50% subjective improvement
Mayer *et al*[67]	Coaptite *vs* Collagen	*n* = 131 (Coaptite) *n* = 100 (Collagen)	SUIS	Improvement of one or more Stamey grade was showed 63.4% in Coaptite group and 57% in the Collagen group, at 12 mo follow-up (P = 0.34) More patients in the Coaptite group required only one injection (38%) compared with the Collagen group (26.1%) (P = 0.03)
Ghoniem *et al*[53]	Macroplastique *vs* Contigen	*n* = 122 (Macroplastique) *n* = 125 (Contigen)	SUIS 1-h pad test Urinary Incontinence QoL Scale scores	After 12 mo, improved 1 or more Stamey grade and dry/cure rates were determined in 61.5% and 36.9% of patients treated with Macroplastique, *vs* 48% and 24.8% of patients treated with Contigen, respectively (P < 0.05)
Toozs-Hobson *et al*[70]	Bulkamid	*n* = 135	24-h pad weighting test 3-d micturition diary ICIQ score QoL score VAS score	There was durability of success with 64% of women cure/improved, which was not significantly different compared with the 12 mo data
Sokol *et al*[71]	Bulkamid *vs* Contigen	*n* = 229 (Bulkamid) *n* = 116 (Contigen)	Bladder diaries QoL questionnaire Pad weight testing VLPP	47.2% of Bulkamid patients and 50% of Contigen patients reported no SUI episodes 77.1% of Bulkamid patients and 70% of Collagen patients reported improvement or cure

SUIS: Stamey urinary incontinence scale; KCQ: Kings college hospital quality of health questionnaire; QoL: Quality of life; SUI: Stress urinary incontinence; ICIQ: International consultation on incontinence questionnaire; VAS: Visual analogue scale; VLPP: Valsalva leak point pressure.

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REVIEW

Adenine phosphoribosyltransferase deficiency: Leave no stone unturned

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Abstract

Adenine phosphoribosyltransferase (APRT) deficiency is a rare autosomal recessive disease leading to generation of large amounts of 2,8-dihydroxyadenine (DHA). DHA is excreted in urine, where it precipitates into crystals due to its low solubility. DHA crystals can aggregate into stones or cause injury to the renal parenchyma (DHA nephropathy). Recurrent urolithiasis and DHA nephropathy are the two clinical manifestations of APRT deficiency. Diagnosis of APRT deficiency can be made during childhood as well as adulthood. Diagnosis mainly relies on the recognition of DHA in stones or urine crystals. Measurement of APRT activity and genetic testing are useful for confirmation of diagnosis, for family screening and should be considered in difficult cases of urolithiasis or crystalline nephropathy. Allopurinol therapy is the cornerstone of treatment and is highly effective in preventing recurrence of stones and kidney disease. High fluid intake and dietary modifications are also recommended. Early diagnosis and treatment are of paramount importance to prevent renal damage. Unfortunately, diagnosis of APRT deficiency is often overlooked and irreversible renal failure still occurs in a substantial proportion of patients. Clinicians must be alert to the possibility of APRT deficiency and consider the appropriate diagnostic tests in certain cases. This review discusses the genetic and biochemical mechanisms of APRT deficiency, and the issues of diagnosis and management.

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Key words: Adenine phosphoribosyltransferase; Dihydroxyadenine; Urolithiasis; Crystalline nephropathy; 2,8-dihydroxyadenine nephropathy

Core tip: Adenine phosphoribosyltransferase (APRT) deficiency is a rare but underrecognized genetic disease causing recurrent dihydroxyadenine urolithiasis and crystalline nephropathy. Clinical presentation is variable and diagnosis can be made at any age. Treatment with a xanthine dehydrogenase inhibitor is highly effective in preventing recurrence of stones and kidney disease. Unfortunately, diagnosis of APRT deficiency is often overlooked and irreversible renal failure still occurs in a substantial proportion of affected individuals. Early diagnosis is of paramount importance to prevent long term complications.

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INTRODUCTION

In 1968, Kelley *et al*[1] identified partial adenine phosphoribosyltransferase (APRT) deficiency of autosomal inheritance in otherwise healthy individuals and reported mutant forms of the enzyme. In 1974, Cartier *et al*[2] described one child with 2,8-dihydroxyadenine (DHA) urolithiasis secondary to complete APRT deficiency inherited in an autosomal recessive manner. In 1977, Van Acker *et al*[3] described one family with APRT deficiency. Later, most cases were reported in Japan, where the prevalence of DHA stones appeared to be especially important[4]. Clinical presentation of APRT deficiency is not restricted to urolithiasis. DHA can cause renal failure by precipitating in the renal tubules and interstitium[5-7]. We previously proposed the denomination "DHA nephropathy" for the kidney disease caused by the precipitation of DHA crystals into the renal parenchyma[8]. Unfortunately, APRT deficiency is often recognized late after recurrent stone episodes or once irreversible renal insufficiency has occurred[7,9-11]. Given that the disease is easily treatable, clinicians must be alert to the possibility of APRT deficiency and consider ordering the diagnostic tests in appropriate cases. This review discusses the genetic and biochemical mechanisms of APRT deficiency, and the issues of diagnosis and management. Unless otherwise indicated, the term "APRT deficiency" refers to complete APRT deficiency in this review.

MECHANISMS OF THE DISEASE

APRT is an ubiquitously expressed enzyme that catalyzes the reaction in which 5'-adenosine monophosphate and inorganic pyrophosphate are synthesized from adenine and phosphoribosyl pyrophosphate. APRT provides the only pathway for the metabolism of adenine[12]. As a result of APRT activity, adenine is present only at low levels in blood and urine[13]. In the absence of functional APRT, adenine is metabolized to 8-hydroxyadenine, which is then converted to 2,8-dihydroxyadenine (DHA) by the xanthine dehydrogenase enzyme (XDH)[14]. DHA is eliminated in urine through and tubular secretion[15]. APRT deficiency thereby leads to excretion of large amounts of DHA in urine[3]. Due to the very low solubility of DHA, this causes the formation of DHA crystals, which can aggregate into stones[3,16], or precipitate into tubular lumens, inside renal epithelial cells, and in the interstitium, thereby causing crystalline DHA nephropathy (Figure 1)[4,7,9,11,17-19].

Two types of APRT deficiency are recognized based on the level of enzyme activity in cell extracts[13]. APRT activity is null in type Ⅰ, whereas it is about 15% to 30% of the normal activity in type Ⅱ[4]. It has to be stressed that this classification has no relevance *in vivo* or in intact cells, where enzyme activity is less than 1% in types Ⅰ and Ⅱ[20,21]. The clinical presentation is similar in both types of deficiency[4,7,10,22]. However, it is still important for clinicians to be aware of this classification when it comes to interpreting the results of APRT activity measurement (see below Diagnostic tests). Type Ⅰ APRT deficiency has been mostly reported in Caucasian individuals, but also in diverse ethnic groups[7,10,23]. Type Ⅱ has been almost exclusively described in Japanese patients, where it accounts for 70% of cases of APRT deficiency[4].

PREVALENCE OF APRT DEFICIENCY

Although APRT deficiency is often viewed as a very rare condition, its prevalence worldwide remains unknown and the number of reported cases are increasing each year. The vast majority of cases and studies published came from Japan, France and Iceland[4,7,10]. One explanation to this may be that certain mutations are frequent in these countries (Met136Thr in Japan, c.400 + 2dup in France and Asp65Val in Iceland). However, the variability in number of cases identified and reported among different countries may also reflect variability in awareness of APRT deficiency and availability of diagnostic tests. In our series, several affected families originated from places outside Europe, including African countries, Turkey, Martinique, Lebanon and Canada[7]. This suggests that APRT deficiency is a ubiquitous disease. The increase in the number of identified cases in some countries, like France, probably reflects improved recognition and diagnosis of the disease[8].

The prevalence of complete APRT deficiency was estimated to be 1/27000 in the Japanese population, corresponding to a heterozygote frequency of 1.2%[4]. The heterozygote frequency in caucasian populations, estimated from measurements of enzyme activity in healthy subjects, ranges from 0.4% to 1.2%, suggesting that the prevalence of homozygosity is higher than 1/100000[24,25]. If this holds true, more than 60000-80000 individuals may be affected worldwide. The limited number of cases recorded in most countries suggests that many individuals with APRT deficiency are currently unrecognized[26]. APRT deficiency may be a seriously underestimated cause of urolithiasis and chronic kidney disease, progressing over time to end stage renal disease (ESRD) in a non-negligible proportion of cases when left untreated[7,10].

CLINICAL PRESENTATION AND NATURAL HISTORY

The age at diagnosis of APRT deficiency varies widely, ranging from infancy to more than 70 years of age[4,7,10,22]. In our series, diagnosis was made before the age of 16 in only 37% of patients[7]. In an Icelandic series, 47% of affected individuals were diagnosed before the age of 18[10]. In some instances, APRT deficiency is diagnosed late because some patients present with symptoms late in their adulthood, while in other instances, despite early onset symptoms, recurrent urolithiasis and kidney disease, diagnosis is often delayed due to low index of suspicion[7,8,10,22]. Urolithiasis is the most common manifestation of APRT deficiency in both children and adults[10,27]. The first urolithiasis episode may occur during infancy as

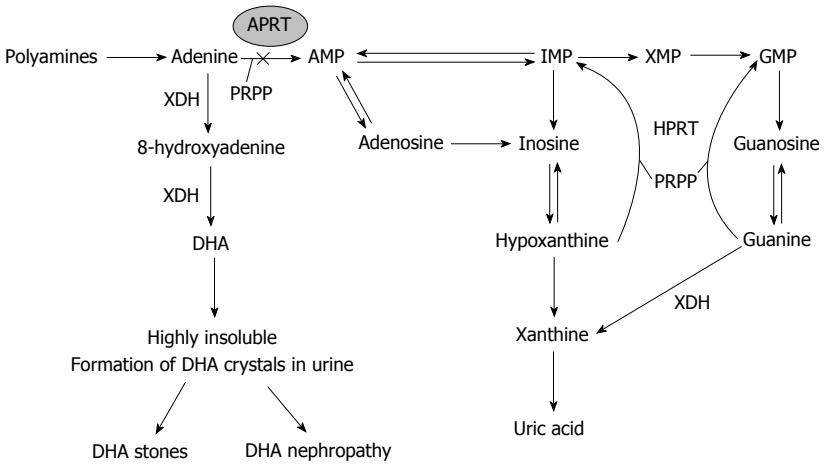

Figure 1 Biochemical pathway of purine metabolism and mechanisms of adenine phosphoribosyltransferase deficiency. DHA: 2,8-dihydroxyadenine; APRT: Adenine phosphoribosyltransferase; HPRT: Hypoxanthine phosphoribosyltransferase; PRPP: Phosphoribosyl-pyrophosphate; IMP: Inosine monophosphate; XDH: Xanthine dehydrogenase enzyme; AMP: Adenosine monophosphate; GMP: Guanosine monophosphate; XMP: Xanthosine monophosphate.

well as later than age 40[4,7,8,22]. In affected infants, reddish-brown diaper stains related to DHA crystalluria can be observed[10]. In some instances, bilateral DHA stones can cause urinary tract obstruction and acute renal failure, especially in children[10,28,29].

DHA stones are usually radiolucent and thus can be detected only by imaging techniques able to detect radiolucent stones, such as ultrasonograpy or computed tomography. However, DHA stones may sometimes appear as radiopaque when containing calcium salts[4,30]. Due to their radiolucent character, DHA stones are often mistaken for uric acid stones. Differential diagnosis for radiolucent stones also includes cystine and xanthine.

DHA nephropathy represents the second manifestation of APRT deficiency. It is commonly observed in adults but rarely in children[10,27]. DHA nephropathy typically occurs in patients who have remained undiagnosed and untreated despite a history of recurrent urolithiasis. However, it should be emphasized that DHA nephropathy can also occur in patients who experienced just a few episodes of stones or even in patients with no history of urolithiasis[31]. Imaging studies demonstrating the absence of stone do not definitively rule out the possibility of DHA nephropathy. DHA nephropathy usually develops insidiously and cause chronic kidney disease progressing over a period of years[31]. Less commonly, the presentation can be acute or subacute. Massive precipitation of DHA into the kidney can sometimes be triggered by urine concentration and supersaturation of DHA in the context of dehydration. In Japanese and European studies, nearly 30% of patients had decreased renal function and 10% had ESRD when APRT deficiency was diagnosed[4,7,10]. In our French cohort, among patients who were diagnosed and treated later than 40 years of age (n = 14), 6 patients (42.8%) had glomerular filtration rate (GFR) > 60 mL/min per 1.73 m^2, 3 patients (21.4%) had GFR of 30 to 60 mL/min per 1.73 m^2 and 5 patients (35.8%) had GFR < 15 mL/min per 1.73 m^2[7].

In some patients, APRT deficiency is not diagnosed until after kidney transplantation, which can have disastrous consequences. These patients are at high risk of losing their transplant in the absence of appropriate therapy. Several cases of DHA nephropathy recurring after kidney transplantation and rapidly leading to transplant failure have been reported[9,11,18,19,32,33]. In most tragic cases, several kidney transplantations failed before APRT deficiency was properly recognized and treated[19,32].

Nearly 15% of individuals with APRT deficiency may be asymptomatic[7,10], but are at risk of developing complications if left untreated. The factors underlying the variability of clinical presentation are unknown. There is no phenotype-genotype correlation, which is explained by the fact that biallelic mutations in *APRT* lead to null enzyme activity in all cases, whatever the mutations maybe. Inter-individual differences in water intake and consumption of foods high in purines may be involved in the variability of the clinical presentation. The potential influence of modifying genes has not been reported in humans. It is unknown whether osteopontin is a modifier of APRT deficiency severity in humans, as demonstrated in mice[34].

APRT deficiency is not known to cause extrarenal manifestation. Although clinical observations in some heterozygotes suggested that APRT deficiency may contribute to hyperuricemia and gout[35-37], patients with APRT deficiency usually show serum uric acid within the normal range. Rare cases of eye discomfort and corneal involvement were reported in APRT deficiency, but the significance of this association remains undetermined[38,39]. It is unknown whether long-term exposure to high systemic levels of DHA could have deleterious consequences. This issue may be of particular relevance for patients with APRT deficiency and ESRD, who might be subjected to constant DHA exposure due to the loss of renal clearance of DHA.

Heterozygotes are asymptomatic and usually have normal excretion of DHA and no DHA crystals in their urine, despite the fact that they have partial APRT defi-

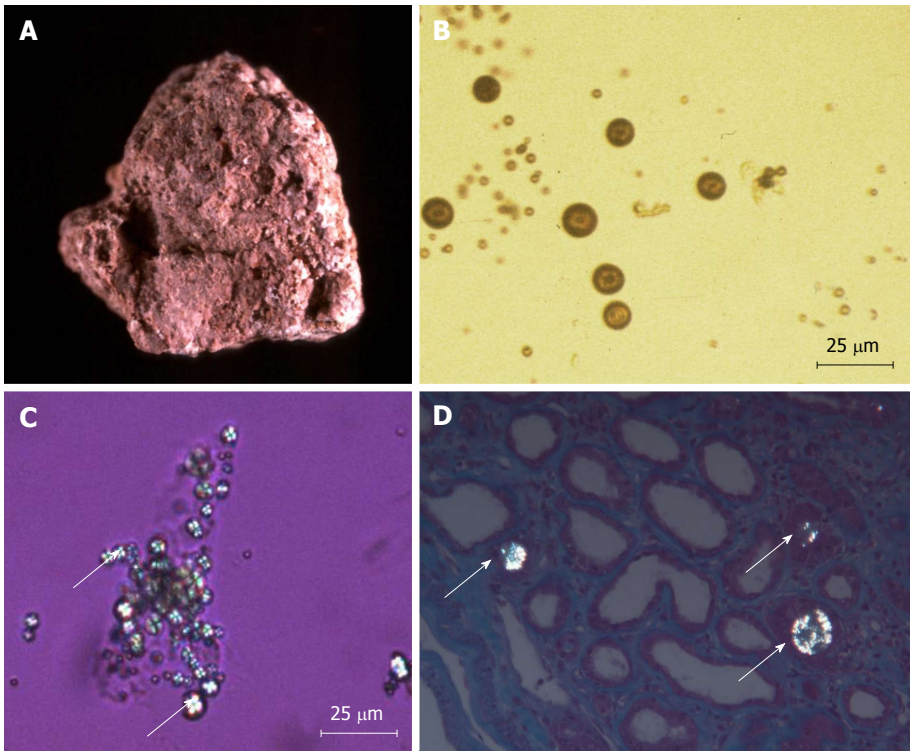

Figure 2 Stones and crystals of 2,8-dihydroxyadenine. A: Typical aspect of a 2,8-dihydroxyadenine (DHA) stone, showing a rough and humpy surface and a reddish-brown color that turns grey when drying. The stone is friable and sections show porosities and beige to brown color; B: Light microscopy aspect of DHA crystals in urine (non-polarized image), showing a round shape and a reddish-brown color; C: Urine DHA crystals in polarized light, showing typical central Maltese cross pattern (arrows); D: Periodic acid-Schiff of kidney biopsy under polarized light in a patient with adenine phosphoribosyltransferase deficiency, showing crystals within the renal tubules (arrows) (× 400). Fourier transform infrared microscopy study of the biopsy demonstrated that crystals were composed of DHA.

ciency[3,38]. However, one case of DHA urolithiasis was reported in an individual with a mutation (*APRT*J*) in only one of the two alleles[40]. To the best of our knowledge, no other case of symptomatic heterozygote has been reported to date and the mechanisms that might cause urolithiasis in some heterozygotes remain unknown.

GENETICS

APRT deficiency is caused by biallelic mutations (homozygous or compound heterozygous) in *APRT* gene, which is over 2.8 kb long. The gene contains five exons and encodes a 540 bp mRNA[5]. Over 40 mutations in the coding region of *APRT* gene have been identified in more than 300 affected individuals worldwide, with the majority arising from Japan[38]. Mutations causing type I APRT deficiency are referred to as *APRT*Q0*, which includes a wide variety of mutations in the coding region, including missense[5,41-43], non-sense[5-7], insertion and deletion[5,7,44,45], and splice-site mutations[5,7,44,46]. Type I deficiency is caused by homozygous or compound heterozygous *APRT*Q0* mutations. Certain mutations are more common in some populations. The c.400 + 2dup mutation (previously named IVS4 + 2insT)[44], appears to be the most frequent mutation in the European population[5,13,44,47,48]. and account for 40% of mutations in France[7]. Another mutation, Asp65Val , is highly prevalent in Iceland[10] and has been also reported in British and Spanish families[7,41,47].

Type Ⅱ APRT deficiency has been observed almost exclusively in Japan and is due to a missense mutation (*Met136Thr*) called APRT*J[4,42]. Patients with type Ⅱ deficiency have two APRT*J alleles, or less frequently one APRT*J and one APRT*Q0 allele[45]. The only known exception to this is a peculiar mutation (*V150F*) that was shown to cause type Ⅱ deficiency in a Polish patient[49].

DIAGNOSTIC TESTS

The identification of DHA in stone or urine is pathognomonic of APRT deficiency. Tests available for diagnosis of APRT deficiency include stone analysis, urine microscopy, renal biopsy, APRT activity and molecular genetic testing.

Stone analysis

Stone analysis should combine morphological examination by stereomicroscopy (Figure 2A) and analysis using infrared spectroscopy or X-ray crystallography, which will unambiguously demonstrate the DHA nature of the stone[50-52]. Standard biochemical methods to evaluate the composition of urinary stones can mistake DHA for uric acid and other purines, and are no longer recommended.

Urine microscopy

Urine examination by light and polarized microscopy can

detect DHA crystals, which have a characteristic appearance (Figure 2B and C)[50,51]. In general, the first morning urine specimen is particularly valuable for crystalluria study, because it is more concentrated. The amount of crystals is high in the urine of untreated affected individuals[7]. Infrared spectrophotometry (IRS) provides characterization of the composition of crystals and confirms diagnosis. In our experience, urine microscopy has an excellent sensitivity and DHA crystals can be detected in the urine of nearly all affected individuals. Rarely, false-negative may be observed[10].

Renal biopsy

Renal biopsy is, at least in theory, not necessary for diagnosis, given that DHA crystals can be identified in urine. In some instances where crystalline nephropathy was not expected, however, renal biopsy can demonstrate the presence of DHA crystals into the renal parenchyma and lead to diagnosis of APRT deficiency (Figure 2D). DHA crystals are mainly observed within tubules and in the renal interstitium. It must be emphasized that DHA crystals in renal biopsy often lack the characteristic morphology of crystals that can be observed in urine. We strongly recommend that crystals seen in renal biopsy specimen be fully characterized in order to avoid confusion with other crystalline deposits, especially uric acid and calcium oxalate. Whenever available, the combination of polarizing microscopy and Fourier transform infrared microscopy (FTIRM) is a reliable method for characterizing crystals in renal biopsy[53,54].

APRT activity

Measurement of enzyme activity in cell lysates is a useful tool for diagnosis of APRT deficiency[55,56]. Unfortunately, the availability of this test is limited in most countries. As discussed above, APRT activity is null in almost all non-Japanese patients with APRT deficiency (type I APRT deficiency). The only exception known to this is a patient of Polish origin with type Ⅱ deficiency related to a peculiar mutation[49]. Complex *in vivo* assays, such as uptake of adenine by intact cells, may rarely be used to assess the functional significance of such new mutations associated with residual activity in cell extracts. In type Ⅱ APRT deficiency, which is observed in patients of Japanese origin, APRT activity is usually less than 30% of normal level[4]. Therefore, a detectable APRT activity in cell extracts does not rule out the possibility of APRT deficiency, although this possibility is an exception in non-Japanese patients.

In heterozygous individuals with one APRT*Q0 and one non-mutated allele, APRT activity is decreased but still detectable (in our experience 5% to 60% of normal value)[8]. In heterozygotes carrying the APRT*J allele, the enzyme activity is usually higher than 50%, which tends to overlap with the values observed in normal individuals[21,22]. To put it in a nutshell, APRT activity assay demonstrates abnormal values in virtually all individuals with APRT deficiency (0% in type I and less than 30% in type Ⅱ) but is not a reliable technique to identify heterozygotes.

Molecular genetic testing

Mutation screening of the *APRT* gene can be relatively easily performed by sequencing of exons and flanking intronic sequences[5]. The diagnosis is confirmed if genetic testing shows functionally significant mutations in both alleles (see Genetics). In our experience, approximately 10% of mutations are not unidentified by *APRT* sequencing[7]. This may be due to large allelic deletions or mutations in promoter region.

Others

Measurement of purine metabolites in urine, as performed by certain laboratories, may reveal increased levels of adenine, suggesting a diagnosis of APRT deficiency. An assay for measurement of DHA would be more desirable but is not currently available. Developing a urinary DHA assay for screening and monitoring of treatment in clinical laboratories is one of the objectives of the APRT Deficiency Research Program, which is a part of the international Rare Kidney Stone Consortium (rarekidneystones.org)[38].

TESTING STRATEGY

Testing strategy may vary depending on the local availability of diagnostic tests. It may also depend on whether the aim is to establish diagnosis in a proband (individual without a family history of APRT deficiency) or to screen relatives of an affected individual. An algorithm for the diagnosis and treatment of APRT deficiency is provided in Figure 3. Key points are also summarized in Table 1.

Strategy for diagnosis in a proband

We recommend screening for APRT deficiency in all cases of urinary stones in children, recurrent urinary stones (especially if stones are radiolucent) and history of urinary stones associated with acute or chronic kidney disease of uncertain cause (including ESRD patients and renal transplant recipients).

Diagnosis primarily relies on the recognition of DHA in stones or crystals. Whenever a stone is available, it should be analyzed, even if it was passed a long time ago. Urine microscopy examination should be systematically done. IRS analysis of crystalluria is recommended when DHA is suspected or, more broadly, when crystals of uncertain composition are observed.

As discussed above, renal biopsy is not necessary for the diagnosis of DHA nephropathy, given that DHA crystals can be detected in urine in almost all affected individuals. Whenever histopathological findings consistent with crystalline nephropathy are observed, full characterization of the nature of crystals is mandatory. Such findings should prompt clinicians to search for crystals in urine. Crystals can be characterized in renal biopsy specimen using FTIRM. However, this technique is restricted to a few laboratories and crystalluria study is often an

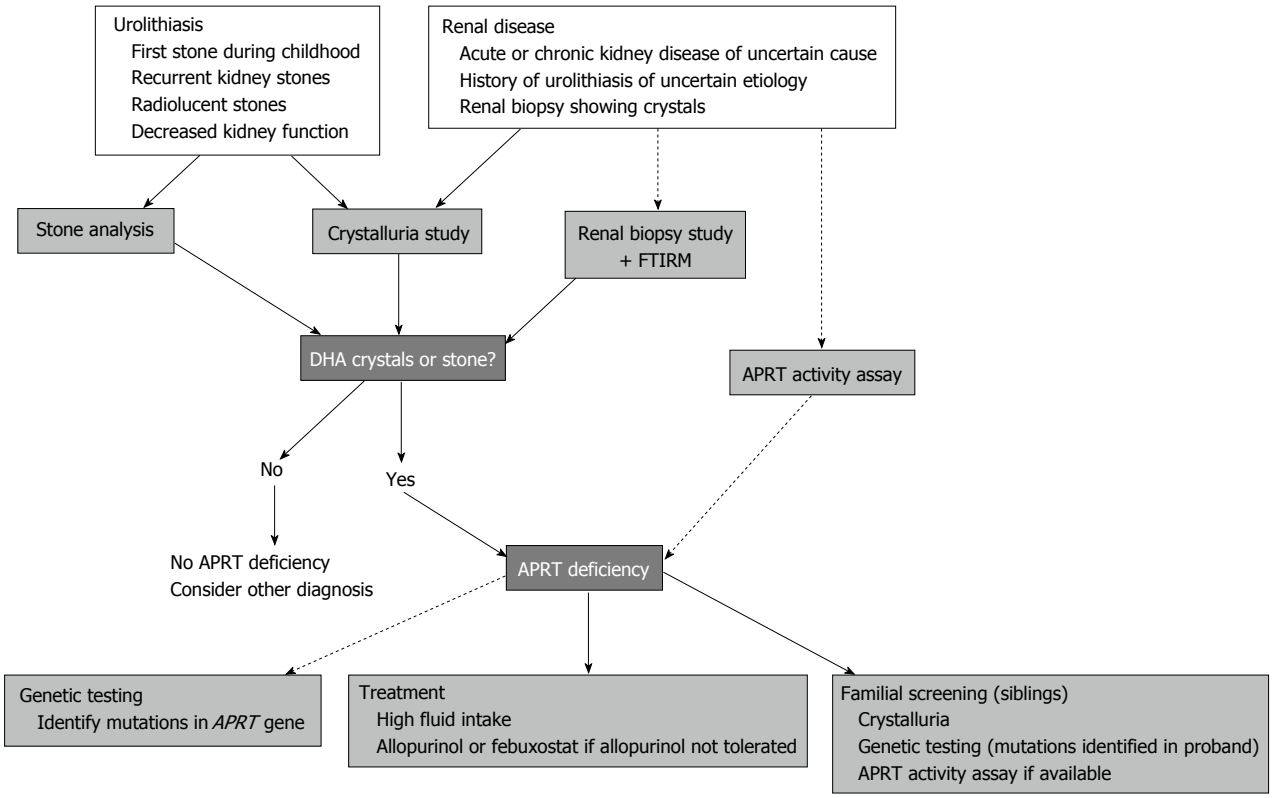

Figure 3 Algorithm for diagnosis and management of adenine phosphoribosyltransferase deficiency. This clinical algorithm summarizes situations where adenine phosphoribosyltransferase (APRT) deficiency should be suspected, testing strategies and management of the disease. FTIRM: Fourier transform infrared microscopy; DHA: 2,8-dihydroxyadenine.

Table 1 Key points about the diagnosis and treatment of adenine phosphoribosyltransferase deficiency

Key points
APRT deficiency is a rare but underrecognized genetic disease
Recurrent urolithiasis and DHA nephropathy are the two clinical manifestations of APRT deficiency and diagnosis can be made at any age
DHA nephropathy can relapse after renal transplantation
In most cases, urine microscopy and stone analysis will lead to diagnosis
APRT activity assay and genetic testing are useful for confirmation of diagnosis, for family screening and in difficult cases of urolithias or crystalline nephropathy
Allopurinol is the cornerstone of preventing recurrence of kidney stones and DHA nephropathy

APRT: Adenine phosphoribosyltransferase; DHA: 2,8-dihydroxyadenine.

easier way to identify DHA crystals.

If adequate stone and urine analysis exclude the presence of DHA, APRT deficiency is very unlikely and no other tests are usually needed. However, an exception can be made when the index of suspicion is high, especially in cases of crystalline nephropathy of uncertain cause.

Although the presence of DHA in crystals or stones is pathognomonic of APRT deficiency, APRT activity assessment and/or genetic testing are recommended to confirm diagnosis[38]. Measurement of enzyme activity is particularly helpful when stone or urine analysis is not feasible (*e.g.*, anuric patient). We strongly recommend APRT activity measurement in ESRD patients awaiting a kidney transplant who have a history of urolithiasis, when the cause of the kidney disease and the composition of the stones are uncertain.

Genetic testing is useful as a confirmatory test but is not intended as a primary screening procedure. Identifying the disease-causing mutations is of great relevance for familial screening once diagnosis has been confirmed in the proband.

Strategy for familial screening

Each sibling of a proband with APRT deficiency has a 25% chance of carrying two mutations and being affected. It is important to keep in mind that affected individuals may be asymptomatic but is still at risk for developing complications if the disease remains undiagnosed and untreated. All siblings, symptomatic or not, should therefore be investigated for APRT deficiency.

Once the mutations causing APRT deficiency have been identified in the proband, it is recommended that

the siblings undergo genetic testing. APRT activity measurement may also be useful, especially in the case where causative mutations could not be found in the proband. Urine microscopy examination should also be performed. Considering the risk, although very small, of false negative[10], urine microscopy should not be solely used to screen at-risk relatives. Further investigations, including assessment of renal function and imaging studies, are warranted in individuals with biallelic mutations, decreased APRT activity, or DHA crystals in urine. As discussed above, DHA crystals are usually absent in the urine of heterozygotes. APRT activity may be decreased but not null in heterozygotes.

TREATMENT AND SURVEILLANCE OF APRT DEFICIENCY

Treatment

No treatment is known to increase APRT activity. However, the disease can be efficiently treated with allopurinol, which inhibits XDH, thereby blocking the formation of DHA from adenine. Allopurinol is the cornerstone of treatment for APRT deficiency. Allopurinol therapy usually leads to a rapid reduction of DHA crystalluria and stone formation[7,27]. Allopurinol efficiently prevents the occurrence or progression of DHA nephropathy in most patients[7,10]. However, kidney disease can be irreversible, especially if tubulointerstitial lesions are advanced. The usual daily dose of allopurinol is 300 to 600 mg (maximum dose 800 mg) in adults and 5 to 10 mg/kg in children. In adults, we recommend initiating allopurinol therapy at a dose of 300 mg/d, which is sufficient to achieve good control of the disease in most patients. The dose should be increased in patients with persistent crystalluria and must be adapted when renal function is impaired. Allopurinol is well-tolerated by most patients, including children[27]. Febuxostat, a specific inhibitor of XDH[57], may be used in patients who do not tolerate allopurinol. However, the benefit and safety of febuxostat in APRT deficiency patients has not been evaluated. All patients with APRT deficiency, symptomatic or not, must receive life-long therapy with a XDH inhibitor. Patients and their families should be educated on the importance of life-long therapy and the risk of developing urolithiasis and DHA nephropathy if the treatment is stopped. Treatment with allopurinol is of paramount importance in patients undergoing kidney transplantation in order to prevent recurrence of DHA nephropathy, which can lead to transplant failure[9,11,18,19,32,33].

Whether patients with APRT deficiency on dialysis benefit from allopurinol is unknown. One may be concerned about the long-term impact of chronic exposure to high systemic levels of DHA. Although no deleterious effects have been reported to date, existing data on APRT deficiency patients undergoing chronic dialysis are very limited. In dialysis patients awaiting a kidney transplant, it seems preferable to initiate allopurinol therapy and achieve stable metabolic control prior to transplantation rather than initiate treatment only after a new kidney has been implanted.

Along with XDH-inhibiting drugs, high fluid intake achieving a urine volume of 2.5 liters daily (in adults) should be advised. It is usually recommended to avoid foods high in purines, although the impact of this diet on DHA excretion has not been established. Urinary alkalinization is not recommended, as DHA has very low solubility at pH values lower than 8.5[13].

Available data are limited regarding urological management of DHA stones. In our experience, patients can benefit from various procedures, including extracorporeal shock-wave lithotripsy, endoscopy and surgery, as for the treatment of other types of stones.

Surveillance

For the surveillance of patients with APRT deficiency, we recommend monitoring their renal function, performing quantitative analysis of crystalluria, and renal ultrasound every 6 to 12 mo in stable patients. The treatment usually leads to the disappearance or at least a drastic reduction of the number of DHA crystals[7,27]. A minority of patients treated experience stone recurrence[7,27]. Non-compliance or an insufficient dose of XDH inhibitor should be suspected in these patients or if there is no marked reduction in crystalluria.

CONCLUSION

APRT deficiency is a potentially severe condition that tends to be overlooked, especially in adults. A high index of suspicion for APRT deficiency and performing the appropriate investigations are mandatory in patients with recurrent urolithiasis and decreased renal function. There are few examples of diseases that can lead to complications as severe as irreversible renal failure but that can be efficiently treated with one pill a day. No stone should be left unturned in the effort to better recognize APRT deficiency and thereby enabling early and effective therapeutic intervention.

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REVIEW

Functional and metabolic complications of androgen deprivation therapy

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Abstract

Prostate cancer is the most common non-cutaneous cancer in men worldwide. Several different treatment strategies are available including minimally invasive procedures for localized tumors such as radical prostatectomy, radiotherapy, and androgen deprivation therapy, among others. All these strategies can be given as mono-therapy or as combination therapy. For this review, we will focus on the side effects of androgen deprivation therapy, independent of the other treatment modalities. Some of the most common affections are loss of bone mineral density, weight gain and obesity, myocardial infarction and sudden death, metabolic syndrome and insulin resistance, dyslipidemia, loss of libido and erectile dysfunction, fatigue, cognitive decline, vasomotor flushing, to mention a few. All these alterations can have an impact on quality of life and even lead to more serious complications such as fractures and cardiovascular complications. We present recommendations for prevention, early recognition and treatment. The different modalities for androgen deprivation therapy have particular side-effects profiles and indications should be made in an individualized manner. Androgen deprivation therapy is a useful tool for some patients with prostate cancer but every effort should be made to avoid related complications. The use of guidelines and educational programs for both, patients and urologists, are extremely useful strategies.

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Key words: Prostate cancer; Metabolic syndrome; Frailty syndrome; Complications; Sarcopeny; Androgen deprivation therapy

Core tip: The article will review the most common complications related to the androgen deprivation therapy. It includes the most relevant and up-to-date information, aiming to provide a reliable and concise review of the side effects of such therapy. Recommendations are made for the prevention, early detection and early treatment of patients.

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INTRODUCTION

Overall, the highest incidence of tumors is from the genital system (almost 338450 new cases expect to be diagnosed in 2013). Out of these, prostate cancer (PCa) is the most common accounting for more than 233000 new cases[1]. Anyhow, incidence of mortality from prostate cancer remains lower than that of lung cancer[1].

Patient prognosis depends widely on the risk classification[2]. With radical prostatectomy as the standard treatment for localized PCa, 5-year recurrence rates go from 6%-45%. Death rates from PCa also vary, going

from 65% survival at 5 years to a mortality of 1%-8% by year 10[3]. Considering these facts PCa can be considered a chronic disease, with chronic complications.

With an increasing survival expectancy, quality of life and functionality become a more relevant problem. In the general population, by age 70 about 20%-30% of patients present some form of disability [mobility, Instrumental or in activities of daily living (ADL)][4]. In patients with PCa, good oncologic outcome can be achieved with several treatment options. Each treatment modality has its own set of side effects that can interfere with patients' performance.

The following review will focus on the functional and metabolic complications that PCa patients develop during the follow-up of the disease.

EVIDENCE ACQUISITION

Original and review articles addressing complications of androgen deprivation therapy were obtained by a systematic search of PubMed. Keywords included "prostate cancer", "androgen deprivation therapy", "hormonal therapy", "frailty syndrome", "sarcopeny", "obesity", "osteoporosis", "metabolic syndrome", "arterial stiffness", "cardiovascular", "fatigue", "cognitive impairment", "loss of libido", "erectile dysfunction", "adverse effects" and "complications". We selected the most recent articles with a good level of evidence to be examined and included in our review. Prospective, randomized trials were prioritized. The review will focus on androgen deprivation therapy complications, in terms of related metabolic and functional impairment.

INTRODUCTION-ANDROGEN DEPRIVATION THERAPY

After the description of Huggins *et al*[5] about the hormonal dependency of PCa, androgen deprivation therapy (ADT) has been widely used for its treatment. There are several indications for ADT including metastatic and locally advanced tumors, concomitant administration with radiotherapy, recurrence treatment, primary treatment for localized tumors. It has also been proposed for all high-risk patients after radical prostatectomy[6,7]. Average survival of patients using ADT, with recurrence after primary treatment is 84 mo[8].

Physicians must be aware of the potential benefits and side effects associated to ADT. Such therapy should be used exclusively when there is a known benefit. The benefit must outweigh the affection in Quality of Life (QoL). Once the decision to start ADT is made, actions must be taken to reduce adverse side effects. Patients should start an exercise program[9]; early detection strategies and adequate treatments could improve outcomes.

In an interesting prospective randomized trial by Salonen *et al*[10], they treated patients with advanced PCa for 6 mo with goserelin acetate. After the initial 6 mo, patients were randomized to receive either continuous or intermittent ADT (given for at least 6 mo when PSA reached levels > 20 ng/mL). Adverse effects (cardiovascular complications and cardiovascular deaths, bone fractures and hot flushes) were similar among groups. Nevertheless, the primary outcome was QoL (activity limitation, physical activity and sexual function), which was significantly better in the group of intermittent therapy. Erectile dysfunction (ED) (15.7% *vs* 7.9%) and depressed mood (2.2% *vs* 0%) were more frequent in the intermitted ADT group. Hot flushes were the most frequent adverse events (47.1% *vs* 50.4%)[10]. Other studies have also shown a benefit of intermittent treatment in terms of QoL, fatigue, hot flashes, sexual desire and urinary symptoms in patients with biochemical recurrence after radiotherapy[11].

In a recent systematic review by Botrel *et al*[12] including 13 trials and 6419 patients under continuous or intermittent ADT, there was no benefit in overall QoL. Except for the presence of hot flushes and sexuality scores, there were no differences between the two treatment strategies. A benefit in survival could not be demonstrated[12]. A Cochrane Database Systematic Review obtained similar conclusions[13].

ADT can have several side effects including new onset diabetes mellitus, osteoporosis, decreased libido, ED, hot flushes, acute kidney injury and cognitive decline, among others[14]. These side effects can occur both with short- and long-term ADT[7]. For these reasons, urologists and oncologists should discuss the possible treatment strategies with the patients. The patient with PCa must be well aware of the possible complications before initializing either therapeutic option. They must know that even though intermittent ADT might have a slight benefit in terms of QoL or sexual function, such effects do not seem to last long, and there is no proven benefit in terms of survival[15,16]. Particularly concerning ADT, the patient must be aware that even though ADT may be regarded as a "non-invasive treatment" the side effects can be very deleterious and have a significant impact in QoL, or even life expectancy due to cardiovascular implications[17]. Urologists should consider the evidence based benefits and side effects before initiation of therapy[7].

The following review will focus on the adverse effects of ADT, as well as the management strategies of these complications. True benefits and complications should be analyzed before indicating ADT.

ANDROGEN DEFICIENCY IN THE AGING MALE

Testosterone deficiency is a common condition in aging men, with a decline rate of 0.8%/year in total testosterone levels. Such decline is greater after the age of 60 and in patients with chronic illness, including obesity[18]. Symptoms are non-specific and frequently go unrecognized. Several terms have been used for the condition encompassing a low testosterone level and compatible symptoms in a male patient, usually older than 40 years

old. The most accurate term is "Androgen Deficiency in the Aging Male" (ADAM)[19].

ADAM symptoms are divided in physical (decreased bone mineral density, decreased muscle mass and strength, increased body fat and body mass index, gynecomastia, anemia and fatigue), psychological (depressed mood, diminished energy, diminished sense of vitality or well-being, impaired cognition and memory) and sexual (diminished libido, ED, difficulty achieving orgasm, decreased morning erections, decreased performance)[20].

Testosterone replacement therapy (TRT) can improve many of the associated symptoms (mostly low libido, energy, mood, low muscle mass, osteoporosis and hot flushes). TRT is only indicated when the patient has symptoms and a corroborated low testosterone level[19].

In patients with PCa or at risk of PCa [first degree relatives with PCa, prostate specific antigen (PSA) > 4 ng/mL, palpable prostate nodules, PSA dynamics], treatment is controversial and assessment by an urologist is recommended[21]. Several studies have questioned the possible deleterious effects of TRT in patients with PCa. Recently, a meta-analysis showed no statistically significant differences in terms of progression or development of PCa, particularly with short term use (less than 12 mo)[22]. Also, in a study using the Surveillance, Epidemiology and End Results Program (SEER) database, Kaplan *et al*[23] showed that 0.79% of the patients who were diagnosed with PCa from 1992-2007 received TRT. In their analysis, they found a statistically significant higher overall and cancer specific mortality in patients not receiving TRT in comparison to those who did receive TRT. The need of salvage ADT was not different in both groups, reflecting similar cancer control.

Testosterone replacement in patients with diagnosis or high risk factors for PCa is still very controversial and no formal recommendations can be made in favor of such therapy.

OSTEOPOROSIS

After age 45, about 25% of male patients suffer some degree of osteoporosis. Risk factors are hypogonadism, alcohol abuse, smoking, sedentary lifestyle and calcium and vitamin D deficiency[7]. Osteopenia is defined as a T score between -1.5 and -2.4, and osteoporosis as a T score greater than -2.5, according to the World Health Organization[24]. It is recommended that all men over 50 years of age take calcium (1200 mg/d) and vitamin D supplements (800-1200 IU/d) along with exercise, quit smoking and limit alcohol drinking, regardless of ADT[25]. In men with prostate cancer, dose of supplementation has not been well established[26]. It is recommended that patients should be evaluated with a dual-energy X-ray absorptiometry (DXA) before initiation of ADT[27]. Further DXA should be done according to the results of the basal exam. Osteoporotic patients should have a DXA done every 6 mo, osteopenic patients every year and patients with a normal DXA can do the next exam up to 24 mo after the previous, provided they do not have high risk factors[28].

ADT has been proven to cause a decreased bone mineral density (BMD), independent of the modality used (either pharmacological or surgical blockade)[24]. During ADT, bone remodeling takes place, osteoclast activity seems to be increased whereas osteoblast repair is insufficient[29]. Such phenomenon causes decreased BMD and increases bone fracture risk and clinical fractures[30,31]. In a study by Shahinian *et al*[32] using the SEER database, the fracture rate for patients using gonadotropin releasing hormone (GNRH) agonists was 19.4% *vs* 12.6% in the non-ADT group. The risk was higher for patients receiving ≥ 9 doses of medication and orchiectomized[32]. The rate of decrease in BMD is 3%-5.6% during the first year of ADT and 1.1%-2.3% a year from the second year on[33]. The longer the time of ADT, the patients have a greater risk of having clinically significant fractures[34]. Also, in patients with osteoporosis the standardized mortality ratio after fractures increased according to the site of fracture (1.45 for minor fractures and 3.17 for proximal femur fractures)[35]. The use of calcium and vitamin D supplements is a possible treatment. Nevertheless, calcium supplementation has been associated with aggressive prostate tumors and increased cardiovascular disease[26].

It has been suggested that ADT should be avoided in patients with a high fracture-risk (age ≥ 80 years old, diabetes mellitus, alcoholism, cigarette smoking, rheumatoid disease, moderate-severe liver disease, paralysis and/or history of osteoporosis and fractures)[36]. The use of estrogens [diethylstilbestrol 1 mg/d orally (PO) or polyestradiol phosphate] for ADT may be the better option for high-risk patients. The cardiovascular effects of the latter medication have been minimized according to recent studies, and the repercussion on skeletal-related events seems less than using other ADT strategies[37].

Patients can be initially treated in a conservative fashion with smoking[38] cessation, controlled exercise, adequate calcium (1200 mg/d) and vitamin D (400-800 IU/d) intake as the first line of treatment. The initiation of exercise within 10 d from the first dose of ADT improves BMD compared to usual care. For more advanced cases the use of bisphosphonates is advised. The above mentioned therapies have shown benefits in prostate cancer patients receiving ADT[39], or even before initiation of ADT[27]. The use of alendronate (70 mg PO weekly) has showed benefits in patients with prostate cancer and osteoporosis or severe osteopenia[40,41]. For patients with non-metastatic disease, bisphosphonates can keep the BMD stable[42].

Denosumab, a human monoclonal antibody against the receptor of the nuclear factor-κβ ligand, has proven better in terms of risk of fracture and BMD increase on patients with ADT, compared to placebo[43,44]. Also, in a randomized study with 1904 patients with metastasis and castration resistant disease, denosumab 120 mg subcutaneously was compared against zoledronic acid 4 mg IV.

Denosumab was better for prevention of skeletal-events; adverse events were similar for both groups[45]. Both, denosumab and zolendronic acid can cause osteonecrosis of the jaw and hypocalcemia[45]. Bisphosphonates can cause nephrotoxicity, particularly in patients with chronic kidney failure and also a flu-like condition during the first doses[28].

Even though several studies have pointed to the benefits of exercise in different aspects of PCa and ADT related adverse effects, such strategy is still poorly applied. Osteoporosis and fracture prevention strategies are unknown to many patients and therefore underutilized[46].

OBESITY

Other side effects of ADT are a loss of lean muscle mass and a gain in body fat[47], particularly subcutaneous fat[48]. It causes a weight gain of about 1.8%-2.4% and an increase in fat body mass by 9.4%-11%[48,49]. The term used to describe the increased body fat along with decreased lean muscle mass that characterizes ADT-induced obesity is "Sarcopenic Obesity"[50]. Sarcopenia is reported in about 20% of patients with an average lean muscle mass loss of 2.8%[51].

Obesity and increased insulin secretion have been related to an increased incidence and more aggressive PCa[52,53]. Obese patients have increased progression to castration-resistant disease; increased rate of metastasis development and some authors have proposed a relationship between obesity and larger cancer specific mortality[54]. Obese patients have an increased oxidative stress, predisposing them to the development of several cancers such as endometrial, bladder, breast and prostate[55].

A structured exercise program, including both resistance and aerobic exercises helps against the metabolic complications of ADT. In a randomized trial by Cormie *et al*[38] the initiation of such exercise program at the beginning of ADT has improved outcomes in terms of lean muscle mass and less fat body mass. Such improvement is evident within the first 3 mo of treatment[38].

CARDIOVASCULAR COMPLICATIONS

These have been described in about 30% of patients undergoing ADT (intermittent and continuous). Also, cardiovascular death events happen in around 8% of such patients[10]. In a large study, comparing PCa patients without ADT with patients using GNRH agonists and surgically castrated patients, increased coronary heart disease (CHD), myocardial infarctions (MI) and sudden cardiac death (SCD) were seen in both ADT modalities[17]. Nevertheless, other large studies have not shown such findings in orchidectomized patients, only showing them in the group receiving GNRH agonists[56]. Estimated increased risk using ADT is 16% for CHD, 11% for MI and 16% for SCD[50]. In a comparative study of abiraterone *vs* placebo in patients with metastatic PCa, cardiovascular events occurred in 13% of patients in the abiraterone group *vs* 11% in the placebo group[57]. Recently, an observational study found that risk factors for developing cardiovascular diseases using ADT are the same than those for patients not receiving such therapy[58]. Screening strategies for cardiovascular disease should not change for patients whether or not they receive ADT.

In a comparative analysis of patients receiving GNRH agonists *vs* antagonists, interesting differences were found in terms of cardiovascular complications. There were no differences among men without preexisting cardiovascular disease. On the other hand, when analyzing patients with a previous history of cardiovascular disease, there was a 56% less chance of cardiovascular events or death within the first year of treatment in the GNRH antagonist group compared to the group receiving GNRH agonists (6.5% *vs* 14.7%)[59].

In a multicenter study by Galvão *et al*[60], they compared the benefit of 6 mo of supervised exercise training (resistance and aerobic training) followed by 6 mo of home training routines *vs* 12 mo of handouts of printed educational material of physical activity. Self-reported physical functioning, and objective measurements of muscle strength were better for the supervised group at 6 and 12 mo. Blood values showed little change between groups[60]. This should be taken into account when recommending exercise to patients; supervised training should be encouraged.

In conclusion, there is no definitive evidence about an increased cardiovascular death in patients using ADT, but they do seem to have more cardiovascular events. This is something physicians must keep in mind and make patients aware of when starting ADT. In selected patients with known cardiovascular disease, revascularization previous to starting ADT can improve survival[61].

METABOLIC SYNDROME

An increased incidence of metabolic syndrome (MetS) has been described, particularly in the group of PCa patients receiving ADT (50% *vs* 20% of naive patients)[62]. When patients with MetS are analyzed, they appear to have an increased risk for PCa (particularly clinically significant PCa, intermediate/high-risk tumors, progression and upgrading after surgery)[63-66]. The more elements of MetS they have, the greater the risk of PCa[67]. Specifically, high blood pressure and high body mass index are the two most relevant factors in terms of PCa death[68]. The possible explanations for this increased aggressiveness are the state of chronic inflammatory, high insulin levels, increased leptin and low adiponectin, and increased estrogen levels[63].

Saylor *et al*[50] recently reviewed the effect of ADT on the different MetS components. Patients showed a weight gain of 2% in the first year, 4%-8% fat body weight increase by the third month and 10% after 12 mo, lean muscle mass decrease of 3% by 12 mo, 26% increase in triglycerides by 6 mo, 8%-20% HDL increase by a year, 7% LDL increase by the third month, 26%-65% increase

in fasting plasma insulin and a 13% decrease in insulin sensitivity index. ADT confers a 44% risk of developing diabetes mellitus.

It is important to mention that unlike the "regular MetS", when patients receive ADT they gain mostly subcutaneous fat, not visceral fat; HDL increases instead of decreasing; blood pressure, waist-hip ratio and inflammatory markers such as C-reactive protein remain unchanged[49,69]. Also, patients on ADT do not develop non-alcoholic steatohepatitis[70]. These different features of the "ADT-related" MetS, perhaps, should be considered and treated independently as it is not a systemic inflammatory condition as opposed to the "regular MetS".

A supervised exercise program twice a week including aerobic and resistance training at the initiation of ADT offers benefits by reducing changes in body composition, physical function, lipid profile, sexual function and psychological distress[38]. The use of metformin along with an exercise program have shown improved outcomes in terms of blood pressure and body weight[62]. Metformin can control MetS, but also has an anti-proliferative effect by inhibiting the anabolic stimulation of insulin and activating the 5'-adenosine monophosphate-activation protein kinase[70].

Finally, the relation between MetS and PCa appears to be bidirectional. MetS could increase the risk of PCa, specifically aggressive and clinically significant tumors. On the other hand, patients with PCa and ADT have a high incidence of a "MetS-like" condition. Metformin, exercise, and other alternatives like statins and orlistat, can play an important role in treatment of patients with ADT and MetS.

INSULIN RESISTANCE AND LIPID ALTERATIONS

An increase in triglycerides and cholesterol levels is seen after ADT[49]. These side effects are more significant within the first 12 wk of therapy[71].

The incidence of diabetes mellitus increases with both, pharmacological and surgical ADT[17]. In only 12 wk, glycosylated hemoglobin and fasting plasma insulin levels increase significantly. By the same time, insulin sensitivity decreases[71]. There is an increase in glycosylated hemoglobin (HbA1c) of 0.13% after two years of treatment in non-previously diabetic patients[42].

It has been proposed, that the insulin increase in patients after ADT causes an increase in insulin-like growth factor (IGF-1). While this last situation could control the metabolic effects of ADT, it might also favor the development of castrate-resistant prostate cancer (CRPC). The insulin increase appears to stimulate intra-tumoral androgen synthesis, leading to CRPC development[72]. It seems that IGF-1 proteins can be involved in the change from benign cells to malignant prostate cancer cells. This is a hypothesis that explains why diabetics, obese individuals and patients with insulin resistance develop more PCa and more aggressive variants of PCa. Specifically, these patients have a shorter time between biochemical recurrence to the development of CRPC. Perhaps, diabetes treatment in patients with ADT is beneficial not only against the hyperglycemic state, but also to control IGF-1 and progression to CRPC[73].

Interestingly, some diabetic and weight control medication such as metformin, orlistat, statins and thiazolidinediones, could control cancer progression promoting apoptosis, decreasing cell mitosis and increasing sensitivity to chemotherapeutic agents[55]. Among these, metformin is the most studied medication; with a good safety profile it improves the lipid results, normalizes insulin levels and does not cause dysglycemia in non-diabetic patients.

As ADT increases insulin and causes insulin resistance, the development of diabetes is not the only complication we should worry about. The relationship between insulin rises and disease progression to CRPC is something we should be aware of. Perhaps, metformin should become an imperative companion to ADT. We recommend performing a metabolic assessment including fasting plasma glucose, HbA1c and a lipid profile every six months during the first year of initiating ADT, followed by yearly assessment thereafter; even in patients on intermittent ADT.

FRAILTY SYNDROME

With the growing age of the population and the survival rate of patients with PCa, disability has become a growing issue that should be taken into consideration by the treating physician. Disability, referred to as the dependency of another person to perform ADL, can happen due to several causes including weakness, comorbidities and aging[4]. Similarly, inflammatory markers such as IGF-1 (which is frequently elevated during ADT), predispose patients to the development of frailty syndrome[74,75].

In a large prospective cohort, Rockwood *et al*[76] developed a scale known as "Frailty Index". After a 5-year follow-up, such scale correlated with the risk of death and the risk of entry to an institution. This is a simple tool that can be applied to patients with PCa. In general, Frailty Syndrome (FS) is characterized by the presence of $\geqslant$ 3 of the following conditions: weight loss, weakness, fatigue, low activity and slow motion performance with balance and gait abnormalities[77,78]. Also, a "Pre-Frailty" condition has been described in which patients can either develop a full-blown FS or recover[4,77]. Early detection is a major intervention for such patients. Pre-Frailty patients must be advised of the increased risk of a worsening condition.

ADT can cause FS. Sarcopenia in patients with ADT can have the same impact as weight loss[77]. The "weight loss" definition for FS is recognized as an unintentional loss of > 10 pounds (4.5 kg) in the last year. Furthermore, obesity is recognized as a risk factor for the development of FS[79]. Weakness is defined as low grip strength measured with a hand-held dynamometer. Weakness can also

be caused by ADT[80]. A 15 feet (4.6 m) walk (speed less than 0.8-1 m/s) evaluates motion performance, which can be affected by a hypogonadal state[74,81]. Fatigue, is a well known side effect of ADT that will be discussed later in this review. Low physical activity can be caused by sarcopenia as well. Finally, as most patients with PCa are older than 65 years old, this is also a contributing factor for the development of FS[82].

FS has been associated with increased mortality, hospitalizations and worsening daily functions. Risk of falls and dependency are also increased in patients receiving ADT[77,83].

We advise physicians to perform scrutiny of FS before starting ADT. Patients with Pre-Frailty should be recognized and conditions optimized (weight loss, exercise routine, dietary advice) before ADT initiation.

LOSS OF LIBIDO AND ERECTILE DYSFUNCTION

Loss of libido is quite frequent in patients with ADT. Within the first year of ADT, 80% of patients without previous erectile dysfunction refer to having impotence[84]. The reason is a lack of testosterone stimuli. Patients often stop having sexual impulse and difficulty achieving an erection good enough for sexual intercourse. This leads to and avoidance of sexual contact because they might feel ashamed. Evaluation consists on interrogation of the patient and partner. Questionnaires such as the International Index of Erectile Function can be used to standardize results.

First line of treatment is the use of phosphodiesterase inhibitors, although the benefit is not always as good as with patients without ADT. Contraindications such as severe coronary artery disease, severe liver failure, nitrate therapy, *etc*. should be considered before initiation of therapy. Other options are penile prosthesis, vacuum devices and intracavernosal injections of prostaglandins[28].

FATIGUE

Fatigue in prostate cancer may be related to the loss of lean muscle mass, gain of body fat, and/or emotional distress in patients undergoing ADT[85,86]. Fatigue is described in about 40% of these patients[87]. With the use of enzalutamide fatigue was reported in 34% of patients (*vs* 29% in the placebo group)[88], and with abiraterone 44% (*vs* 43% in the placebo group)[57].

Cancer-related fatigue can be improved by aerobic exercise[89]. Better outcomes have been shown in group-exercise programs and in such programs including resistance training. Exercise should be recommended under supervision whenever it is possible[90,91]. Increased muscle mass and muscle strength can be obtained along with improved quality of life and fatigue[91,92].

In conclusion, supervised resistance and aerobic exercise is recommended two to three times a day in order to improve muscle mass, strength and fatigue.

COGNITIVE DECLINE

Even though there is not much evidence regarding cognitive decline associated with ADT, both transdermal estrogen therapy and exercise programs can improve this possible side effect[87]. Cognitive decline happens in up to 48% of patients on ADT[93]. Most studies assessing cognitive affection include a small number of patients. The most affected areas of cognition are executive, verbal and spatial functioning[28]. Mini-Mental Exam can be used as a standardized evaluation.

The reason for the mental decline during ADT might be an affection of the sex-steroid receptors in prefrontal cortex and hypothalamus. Gonadectomy in animals causes a decrease in 40% of synaptic unions that can be restored by androgen replacement. Transdermal estradiol (0.6 mg/24 h) applied every 7 d can improve memory loss[94].

Because of the safety profile, the most recommended strategy is a resistance and aerobic exercise program. Mental health and psychological distress benefits are evident after 3 mo of supervised exercise[38].

In a study including patients receiving ADT for causes other than PCa, ADT receiving patients had better scores of "agreeableness" (kind, cooperative and considerate personality) compared to patients not on ADT. This was the only significantly affected element of the "Big Five Personality Traits"[95].

Depression diagnosis can be made in a quarter of patients. Nevertheless, such diagnosis cannot be completely attributed to ADT[51]. According to a SEER study, ADT by itself does not increase the possibilities of developing depression[96]. Either way, PCa patients have a raised incidence of depression (seems to be multifactorial). Periodic evaluation of depressive symptoms and psychiatric attention to those with positive results should be part of the multidisciplinary approach. Interrogation of the patient's partner can be useful for gathering information.

VASOMOTOR FLUSHING

This is the most frequently found side effect of ADT (about 75% of patients with ADT). Interestingly, with the use of enzalutamide the reported incidence is much lower (20%)[88]. It is referred to as sweating, flushing of the upper body and a feeling of anxiety that lasts for about 3-10 min. Mean time of appearance is 2.7 mo after the start of ADT[97]. The physiopathologic explanation for such phenomenon seems to be an affection of the hypothalamic thermoregulatory center due to sex hormones, affecting serotonin and norepinephrine amounts at this level[98]. The psychological affection in these patients is quite relevant, feeling an impact in masculinity, powerlessness against such symptoms and social embarrassment[99]. A classification of the severity of hot flashes is used frequently[100].

Behavioral modifications oriented to keeping a low body temperature (dressing with cool cloth, drinking cold beverages, using a fan, avoiding spicy food, *etc*.) are

the first line of therapy when facing a patient with hot flushes[101,102].

Several strategies have been attempted in order to improve hot flushes. Estrogen and progesterone supplements are effective in about 85%-91% of patients (medroxyprogesterone acetate 5 mg twice daily[11] PO or 400 mg intramuscular, and megestrol acetate 20-40 mg bid PO)[97,103]. This seems to be the most effective therapy[98]. One should be aware of case-reports about a decrease in PSA levels when megestrol acetate is discontinued, considering it a possible tumor growth-stimulant when patients' PSA levels increase under such treatment. This last phenomenon may require suspension of megestrol acetate[104].

One-milligram diethylstilbestrol has shown effectiveness in 70% of patients, with no increase in thromboembolic or cardiovascular complications. There is concern about the increased risk of breast cancer in women, although it has not been well studied in men with ADT.

Gabapentin has been used in both men and women experiencing hot flushes. In men, it has an efficacy of up to 49% with a 300-900 mg/d dose. Side effects include nausea, loss of appetite, vomiting, dizziness and somnolence[105,106].

Anti-depressive medications such as Venlafaxine (serotonin and norepinephrine reuptake inhibitor) and paroxetine have achieved symptomatic reduction (about 55% with Venlafaxine) with few side effects (dry mouth, weight gain, nausea, headache and decreased appetite[87]) and improvement in fatigue, diaphoresis and sleeping trouble[107]. Unfortunately, these are mostly from small trials as well as evidence derived from menopausal women[108]. Caution should be exercised when using abiraterone acetate since this medication could narrow therapeutic index[104].

A large randomized trial, including 919 patients comparing vanlafaxine 75 mg/d, cyproterone acetate and medroxyprogesterone acetate 20 mg/d proved a significant benefit from all therapies (-47.2%, -94.5% and -83.7% after 4 wk, and -56.7%, -100% and -97.3% after 8 wk from randomization, respectively). No statistically significant difference was found in cyproterone and medroxyprogesterone groups[109].

Some medications such as vitamin E and Clonidine have been used in women, although benefit has not been proven in men and they have significant side effects, therefore are not recommended. Acupuncture electrostimulated and traditional[110], has also been studied in small trials. One systematic review assessed the utility of acupuncture in patients with PCa and was unable to recommend such therapy[111].

With such high incidence, vasomotor flushing risk should be commented with the patients and behavioral recommendations should be given to every patient from the beginning. If conservative treatment is not enough, the other available strategies can be considered according to the benefits and specific side effects.

OTHER COMPLICATIONS

Other complications such as gynecomastia can develop with ADT, interfering with patients social performance in up to 28% of cases[51]. Penile length decrease of > 1 cm was reported by 93% of patients in one study[112]. A hemoglobin decline of -1.11 g/dL (normocytic normochromic) has been reported, and most patients are asymptomatic[113]. We recommend an assessment prior to initiation of ADT and a complete evaluation if positive for anemia. Further testing every 3-6 mo should be done depending on hemoglobin values.

CONCLUSION

Prostate cancer and ADT is nowadays a common combination. ADT represents one of the most utilized therapies for PCa and can be implemented in nearly every stage of the disease. Side effects are frequent and can have serious implications in quality of life or even mortality. All types of ADT are prone to present side effects, with certain differences among the different modalities. Adherence to guidelines has great implications on patients. Individual patient selection, surveillance of complications and educational strategies (for both patient and urologists) are important cues in treatment.

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REVIEW

MiRNA in bladder carcinogenesis: A review

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Abstract

Bladder cancer (BC) is the second urological malignancy in incidence, currently being one of the most neoplasms studied with profile and biology poorly defined. In the world, BC is responsible by about 386000 new cases and 150000 deaths annually with considerable economic impact and high costs for health systems. After its discovery more than 20 years, micro RNAs (miRNAs) have been recognized as molecules that work specifically in post-transcriptional control in majority of eukaryote genomes. MiRNAs are a family of small non-coding RNAs of 19-25 nucleotides in length, expressed in a wide variety of organisms, comprising plants, worms and mammals, including humans. They have a fundamental role in physiological and pathological processes in organs and tissues in a context-dependent manner. This review brings new roles of protective and oncogenic miRNAs linked to carcinogenesis of urothelial carcinoma of the bladder, and associated with behavior of disease. Many studies have demonstrated promising roles of miRNAs working as diagnostic and prognostic biomarkers or involved in target therapies, consolidating miRNAs as crucial players in human cancer. This review allowed a reflection about the true functions of miRNAs in bladder carcinogenesis. Not only by their wide capacities of action, but also by abilities in define the cell date. The future of anti-tumor target therapies will be based not in one, but in groups of miRNAs working together in several steps of carcinogenic process, being able to identify the disease, predicting behavior and effectively treat bladder cancer.

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Key words: Bladder cancer; Urothelial carcinoma; MiRNA; Biomarkers

Core tip: Bladder cancer is the second urological malignancy in incidence, currently being one of the most neoplasms studied with profile and biology poorly defined. Micro RNAs (miRNAs) are a class of small non-coding RNAs that play roles in many physiological and pathological processes, including cancer. This review brings new roles of protective and oncogenic miRNAs linked to carcinogenesis of urothelial carcinoma of the bladder, and associated with behavior of disease. Most importantly, we provided a reflection about the true functions of miRNAs in bladder carcinogenesis, not only by their wide capacities of action, but also by abilities in define the cell date.

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INTRODUCTION

Bladder cancer (BC) is the second urological malignancy in incidence, currently being one of the most neoplasms studied with profile and biology poorly defined. BC development is related to environmental exposures that by genetic and epigenetic mechanisms can modify the cellular machinery and trigger the carcinogenic process. In the world, BC is responsible by about 386000 new cases and 150000 deaths annually[1] with considerable economic impact and high costs for health systems[2].

Conventional clinical and pathological parameters are used for BC histological graduation and stage and they are still the only tools now available employed to predict the prognosis of disease. However, this ability is limited and lacking data allowing a prospective analysis of risk of progression and behavior of BC.

Scientific evidences support the concept that BC is a many phases disease, and several alterations are needed until clinical presentation of BC. Thus, BC has been used as a main information source about mutational events that trigger carcinogenic pathways of solid human tumors[3,4]. Although genetic and molecular pathways are relatively well demonstrated and some biomarkers established, there are many questions to be answered about biological behavior or BC and novel methods that more specifically predict BC behavior are necessary.

The most common histological type of BC is bladder urothelial carcinoma (UC), occurring in 80%-90% of cases. UC can present in some different forms, from a small low-grade non-invasive tumor to advanced disease invading bladder wall and adjacent organs, with grade and stage established as main prognostic factors in bladder UC[4]. At diagnosis, 70% are low-grade non-invasive tumors evolving with optimal survival rates, while 10%-20% is high-grade invasive and aggressive disease with poor prognosis and increased index of mortality.

MOLECULAR BIOLOGY OF UC

A wide number of genetic events are involved in etiology, progression and treatment responses of UC[5]. The light of the molecular pathways related to UC carcinogenic process is crucial to know its etiopathogenesis and behavior. Biological variations such as both carcinogens conversion and detoxification and DNA repair can modify the expression and action of related genes in different phases of UC carcinogenesis.

Genetic events in non-invasive UC

Carcinogenic pathways that trigger low-grade non-invasive and high-grade invasive tumors are specific and mutually exclusives, and are showed in Figure 1[6-8]. The most of bladder malignancies is non-muscle invasive in initial presentation and its main tumorigenic route is mediated by fibroblastic growth factor receptor 3 (*FGFR3*) gene. Nevertheless, less common mutations in *RAS* gene have been described.

FGFR3 has 18 exons, it is located in 4p16.3 chromosomal region and belongs to tyrosine kinase growth factors receptors family, involved in functions related to embryogenesis and tissue homeostasis[8,9], regulating several biological processes, including proliferation, differentiation, migration and apoptosis[10]. Point mutations or other alterations which lead to *FGFR3* over-activity can alter cellular proliferation and trigger low-grade well differentiated UC, having little effect in cellular differentiation and apoptosis. These influences propitiate advantage to cell proliferation, but they do not change the genomic stability. *FGFR3* point mutations were described for the first time by Cappellen *et al*[11] (1999) that identified mutations in 35% of tumors. Point mutations in codons 248, 249 and 375 comprise more than 95% of *FGFR3* mutations.

Mutations in *RAS* oncogene has also been associated with UC non-invasive tumors and can be responsible by 30% of human cancers[12]. RAS works in regulation of cellular functions as proliferation, differentiation, motility and apoptosis in response to extracellular signals. In bladder UC, RAS seems to act through both mitogenic-activated protein kinase (MAPK) and AKT/STAT pathways[13]. Interestingly, *RAS* and *FGFR3* mutations do not occur at the same time, being considered mutually exclusive events indicating biological equivalence between these two types of point mutations[13].

Genetic events in invasive UC

The most of known genetic events in bladder UC is described in high-grade invasive tumors and many of them, as mutations and loss of function in the protective genes *p53*, retinoblastoma (RB1) and phosphatase and tensin homolog gene (*PTEN*) are associated with poor prognosis and high genetic instability[3].

p53 gene product is a tumor suppressor protein that is activated in response to signals of cellular stress, promoting transcriptional regulation of genes that induce cell cycle arrest, apoptosis, senescence, DNA repair and alterations in metabolism of the cell. Somatic mutations in *p53* are described in more than 50% of human tumors and germinal mutations can promote the tumor development in some hereditary syndromes. Unlike FGFR3, loss of function of p53 lead to important genomic instability associated with high-grade and stage tumors (Figure 1)[14-16].

RB1 suscetibility, a prototype of suppressor tumor gene, has been associated with UC progression and development. This phosphoprotein is a negative regulator of cell cycle and promotes chromatin stabilization allowing maintenance of its structure. RB1 mutations are strongly related to infant retinoblastoma, osteogenic sarcoma and bladder cancer (www.ncbi.nlm.nih.gov/gene). RB1 inac-

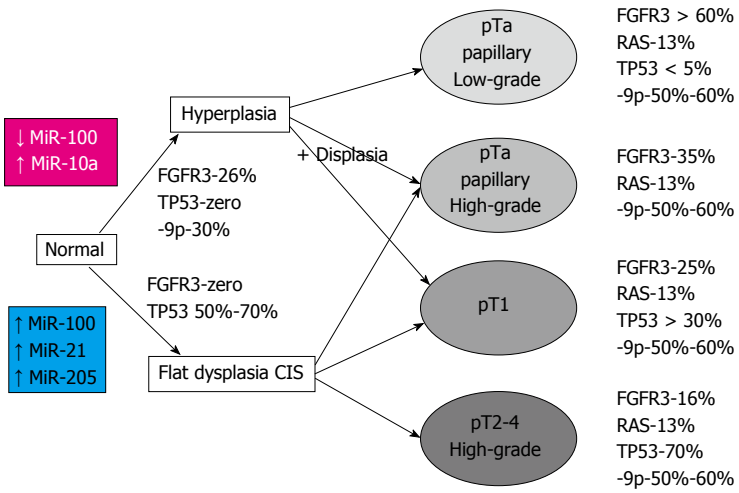

Figure 1 Genetic carcinogenic pathways of bladder urothelial carcinomas incorporating micro RNA. FGFR3: Fibroblastic growth factor receptor 3.

tivation is linked to UC, more specifically to invasive and aggressive disease[17,18].

PTEN is located in chromosome 10 (10q23) and works like a traditional tumor suppressor, acting in proliferation control, migration and cellular invasion by PI3K/AKT/mTOR pathway[4,19]. Despite its influence in non-invasive bladder UC, PTEN is more associated with invasive carcinogenic pathways. Although there are evidences that show its role in initiation and neoplastic progression, PTEN is not able to trigger them alone[20]. Recent data show that, when loss of function of PTEN is associated with *p53* mutations, invasive UC progresses more quickly, demonstrating worst prognosis and lower survival rates[21].

Associations of genetic alterations into UC high-grade invasive carcinogenic pathway seem to be the key event leading to initiation and progression of bladder urothelial carcinomas.

Epigenetic events in UC

Genetic alterations only are not able to explain cancer molecular diversity. Other mechanisms can also affect gene expression and signal pathways. Epigenetic changes, such as DNA methylation and histones deacetylation, can occur without changing DNA structure and seem contribute to malignant transformation and UC progression[22,23]. They can be promoted by external agents, including smoke, diet and carcinogens exposure.

A wide variety of important genes in several cellular processes could present DNA methylation oscillating from 1% to 98%, and appear in initial stages of disease[24-30]. Another epigenetic mechanism is the transcription repression through interaction between micro RNA (miRNA) and specific sequences in messenger RNA (mRNA), as discussed below.

MiRNA

After its discovery more than 20 years[31], miRNAs have been recognized as molecules that work specifically in post-transcriptional control in majority of eukaryote genomes. miRNAs are a family of small non-coding RNAs of 19-25 nucleotides in length, expressed in a wide variety of organisms, comprising plants, worms and mammals, including humans[32]. They have a fundamental role in physiological and pathological processes in organs and tissues in a context-dependent manner. Many miRNAs are highly conserved between species and the machinery of its biogenesis can be found in archaebacteria and eubacterias, establishing its ancestral characteristic. Currently, there are more than 2500 miRNAs with specific biogenesis (Figure 2) and related to control of more than 30% of human genes (www.mirnabodymap.org)[33] involved in multiple processes of development and cell differentiation, apoptosis, homeostasis and metabolic pathways[34-36]. In oncologic research, miRNAs work tumor suppressors or oncogenic (oncomiR), showing specific profiles that could characterize different types of cancer[36,37]. Albeit there are studies exploring miRNA expression profile in bladder UC, data are still scarce and biological field so vast[38-41].

MiRNA in bladder UC

MiR-100: MiR-100 is a protective miRNA in human cells[42], acting in a context-dependent manner[43]. Under-expression profiles has been found in non-invasive bladder UC[44,45], ovarian carcinoma[46], oral cavity carcinoma[47], osteosarcoma[48], vulvar carcinoma[49], lymphoblastic leukaemia[50], gastric cancer[51] and several other types of human cancer.

In non-invasive bladder UC, miR-100 has as main target the *FGFR3* gene, whose mutation and over-activity is related to this neoplasm[44]. In physiological conditions, miR-100 exerts negative control over FGFR3 decreasing their post-transcriptional expression levels (Figure 3). As Blick *et al*[52], we also suggest that there might be an alternative pathway triggering UC non-invasive carcinogenesis not associated with *FGFR3* activating point mutations[53]. Under-expression of miR-100 could be responsible by lack of negative control and FGFR3 over-expression, promoting non-invasive UC

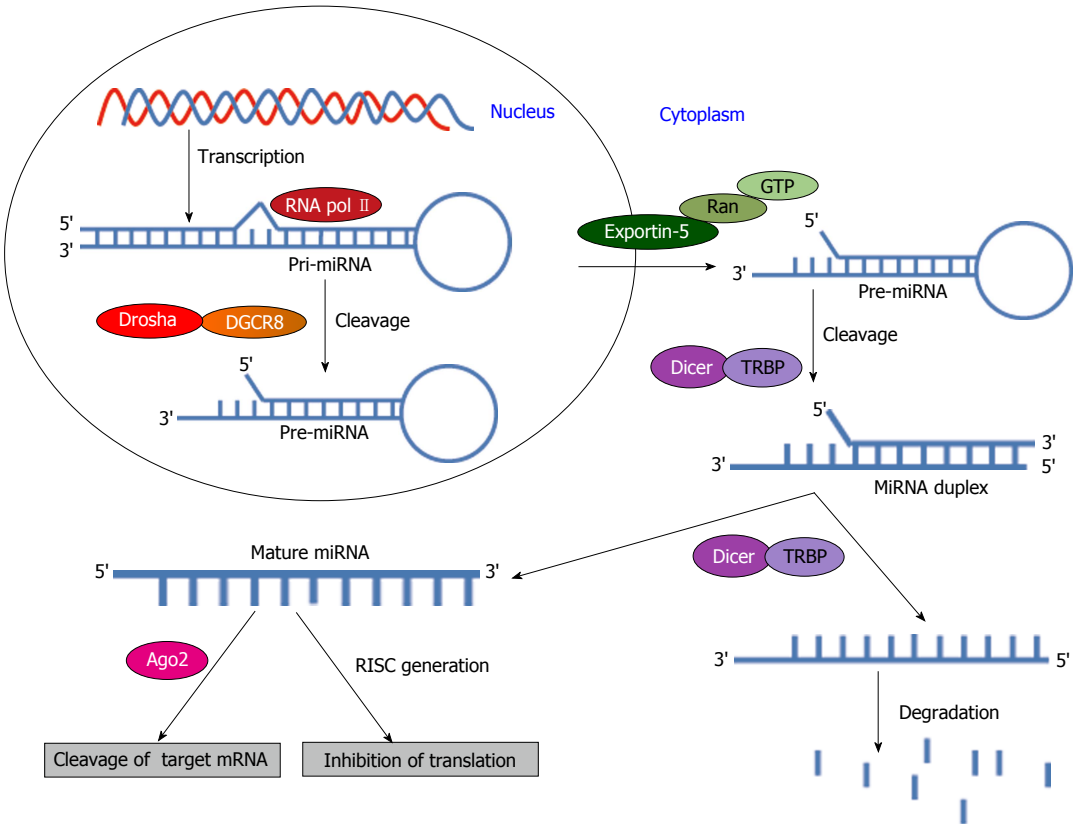

Figure 2 Micro RNA biogenesis in human cells. After transcription by RNA polymerase Ⅱ, the primary micro RNA (miRNA) precursor (Pri-miRNA) is cleaved by Drosha microprocessor complex and converted in Pre-miRNA, a 60-70 nt double-strand molecule. The Pre-miRNA is transported from nucleus to the cytoplasm by Exportin-5 and then it is cleaved by Dicer to generate the miRNA duplex. Again, Dicer enzyme acts over miRNA duplex and produces single-strand mature miRNA that, in turn, is incorporated into RNA-Induced Silencing Complex (RISC). RISC drivers mature miRNA to the target messenger RNA (mRNA), triggering mRNA cleavage (Slicer activity) or inhibition of translation by complete or incomplete complementarity, respectively.

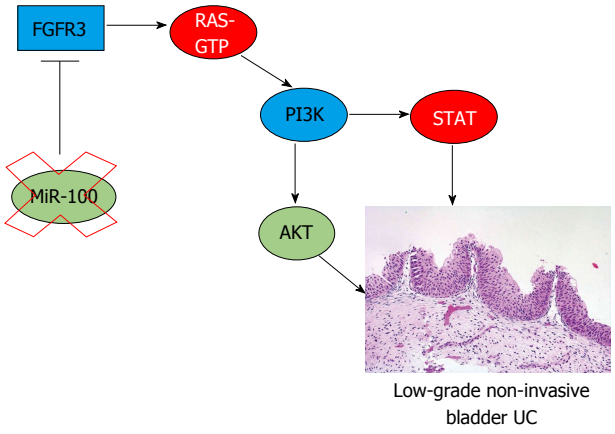

Figure 3 MiR-100 in low-grade non-invasive urothelial carcinoma carcinogenesis. Under-expression of miR-100 leads to *FGFR3* gene over-expression, stimulus to PI3K/AKT/STAT pathway and low-grade non-invasive tumor development. Adapted by Wu[19], 2009. FGFR3: Fibroblastic growth factor receptor 3; UC: Urothelial carcinoma.

carcinogenesis and low-grade tumor development (Figure 3). Catto *et al*[44] found an inverse ratio between miR-100 and *FGFR3*, where the under-expression of miR-100 led to increased gene activity before the occurrence of point mutation, suggesting that increased levels of *FGFR3* could facilitate the mutational event through increased cellular turnover or natural selection of mutant cells. Maybe, miR-100 loss of expression can be the first trigger event of disease and could occur before clinical presentation of the tumor. This fact is important because this molecular characteristic could be used for initial diagnostic and predicts disease behavior, allowing a conservative treatment due to rare chance of progression and excellent survival. We speculate that miR-100 will be used in clinical practice as a diagnostic and prognostic biomarker and employed in target therapies.

On the other hand, we showed an over-expression of miR-100 in high-grade invasive UC[45]. We suggest that miR-100 acts as a negative controller of *THAP-2* gene, directly involved in proliferation control through modulation of proteins that control cell cycle such as pRB and E2F[54]. Loss of function of RB1, p53 and PTEN is involved in carcinogenic route of invasive UC, promoting genomic instability and facilitating tumor progression. MiR-100 over-expression could trigger THAP-2 silencing and, consequently, RB1 inactivity (Figure 4). *BAZ2A* and *SMARCA5* genes are also targets of miR-100 and are associated with DNA transcription repression and chromosomal instability[55,56]. Recently we demonstrated in cell cultures of BC that miR-100 has a role over BAZ2A and SMARCA5 activity (data submitted for publication), but better investigation are needed to establish the role of

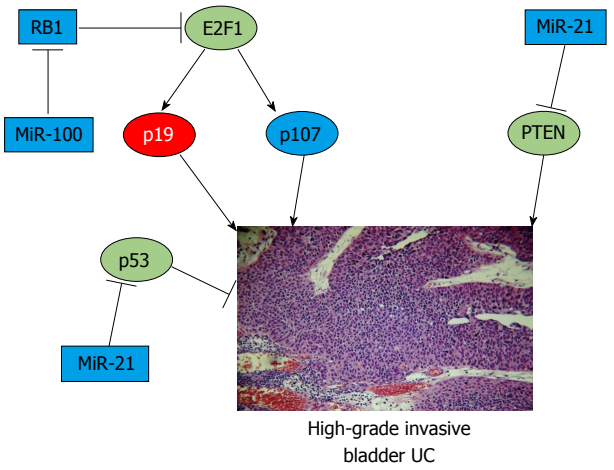

Figure 4 MiR-100 and miR-21 in high-grade invasive urothelial carcinoma carcinogenesis. High levels of miR-100 inhibit retinoblastoma (RB1) and over-expression of miR-21 suppresses p53 and phosphatase and tensin homolog gene (*PTEN*), three crucial protective genes. Inactivity of RB1 associated with loss of function of p53 and *PTEN* trigger high-grade urothelial carcinoma (UC) carcinogenesis. Adapted by Wu[19], 2009.

miR-100 in invasive UC carcinogenesis.

MiR-10a: MiR-10a comprises 23 nitrogenous bases and is located in chromosome 17q21.23, within *HOXB* gene cluster, upstream of *HOXB4* (www.mirbase.org). A number of *HOX* genes have been found to be regulated by miR-10. These genes encode mainly transcription factors which have crucial roles in embryonic development and cell differentiation. In humans, miR-10a exerts a negative control over *HOXA1* and *HOXA3* genes supporting that this miRNA can play fundamental roles in physiological activities of the cell[57,58].

MiR-10a has two different mechanisms of action over control of gene expression. The first one, extensively demonstrated by literature, is the canonical inhibition of protein product by miR-10a binding in target 3'UTR mRNA. On the other hand, Ørom *et al*[59] have described the second mechanism of action, and demonstrated a positive effect of miR-10a through its complementary interaction with mRNA 5´UTR, allowing a translational stimulus of proteins associated to the ribosomal machinery, increasing global cellular activities[59]. Although miR-10a is linked to carcinogenesis of several human tumors, it is believed that its functions are involved in physiological situations of the cell. Nowadays it is well established the crucial role of miR-10a in physiological process of cellular differentiation. Even being able to act through mechanisms described above, translational inhibition by negative control of miR-10a on its target genes highlights as the most active mechanism in the differentiation process.

Recently, we have demonstrated a miRNA expression profile in bladder urothelial carcinoma and we found that miR-10a over-expression was one of the most evident changes. MiR-10a is able to effectively separate two genetically distinct tumor groups, which are, low-grade non-invasive pTa from high-grade invasive pT2-3 UC, with over-expression in first and under-expression in the second tumor group[45]. Moreover, miR-10a expression profile was associated with disease-free and cancer-specific survivals between groups[45].

Regarding low-grade non-invasive pathway, FGFR3 over-activity triggered by down-regulation of miR-100 could be corroborated through up-regulation of miR-10a, both promoting higher cell proliferation rates. Maybe this mechanism miR-10a-mediated is constituted in a negative control of physiological inhibitor of FGFR3 through its canonical activity, increasing cellular proliferation without modify the genetic stability of the neoplastic cell. About high-grade invasive tumors, sharing close homology with miR-100, miR-10a maybe could be continuously blocking *HOX* genes, promoting poor differentiation, enhanced aggressiveness and worse tumor behavior.

MiR-21: Corroborating with findings published by Neely *et al*[39], we recently have demonstrated that miR-21 presented strong over-expression (17-fold higher) in high-grade invasive UC[39,45]. Recent evidences show that miR-21 is a truly oncogenic miRNA, presenting over-expressed in wide majority of human tumors. miR-21 can promote tumorigenesis by inducing cell proliferation and blocking of apoptotic control mechanisms[60], thus triggering more aggressive disease and poor responses to treatments[61,62].

p53 is considered the most important gene involved in invasive UC carcinogenesis[14]. p53 is responsible for control of global activities of the cell by cell cycle arrest, stimulus of apoptosis and DNA repair. Catto *et al*[44] have observed miR-21 over-expression associated with p53 inactivation, invasiveness and tumor progression. Another important protective gene related to UC carcinogenesis is PTEN, also being target of miR-21[4,19,63]. PTEN is a lipid phosphatase that inhibits PI3K/AKT pathway, blocking cell proliferation. In an actual review about genetic and molecular mechanisms involved in initiation and progression of UC, McConkey *et al*[4] have suggested that loss of function of PTEN is much more common in invasive disease related to PTEN/PI3K/AKT/mTOR characterizing a worse prognostic factor. Figure 4 shows a schematic flowchart regarding miR-21 roles in high-grade invasive bladder UC.

We showed that miR-21 under-expression was associated with better disease-free survival in non-invasive UC, consolidating oncogenic behavior for miR-21[45].

MiR-205: MiR-205 has been defined as a tumor suppressor miRNA involved in epithelial-to-mesenchymal transition (EMT), a process provided by malignancies to perform a fundamental step to tumor progression and systemic dissemination. Like miR-200 family, miR-205 acts negatively over ZEB-1 and ZEB-2 that, in turn, suppress E-cadherin, an adhesion molecule responsible for physiological conditions of bladder epithelia[64,65]. Under-expression of miR-205 leads to ZEB-1 and ZEB-2 over-

activity and sequential E-cadherin inhibition, facilitating the metastasizing process. EMT is crucial to success and maintenance of bladder carcinogenesis, being directly associated with worse behavior, high tumor aggressiveness, poor prognosis and shorter survival. The role of miR-205 is similar in several human tumor types, blocking tumor progression and dissemination. Some authors have already demonstrated a miR-205 under-expression in lung, breast and prostate cancer[66-68].

In bladder UC, evidences has been suggested aberrant methylation in chromosome 1q32.2 where is located the *miR-205* gene[69]. Neely *et al*[39], in 2008, established that miR-21:miR-205 ratio is progressively increased according tumor progression. Interesting data are shown by Brabletz *et al*[70], demonstrating that ZEB/miR-200 feedback loop is crucial to define cell status. Thus, an invasive and progressive tumor status occurs when the loop is favoring ZEB, leading tumor dissemination beyond epithelial barriers. On the other hand, a proliferative environment predominates when the loop tend to miR-200 family over-expression, allowing tumor growth. Second authors, both states are needed to carcinogenic process, a first early proliferative promoting tumor growth and a second late progressive triggering tumor dissemination[70]. A very interesting fact is that miR-200 over-expression could favor the metastasizing process but, after metastasis sites are defined, the proliferation occur again, being necessary the re-expression of miR-200. In 2012, we published data supporting this idea, where miR-205 was under-expressed in 100% of low-grade non-invasive pTa UC, according with early carcinogenic state, while there was a re-expression of miR-205 in a third of cases of high-grade invasive UC[45].

MiR-let7c: The literature consolidates the suppressor role of miR-let7c in almost all human cancers, reflecting its important suppressive action against malignant events. MiR-let7c targets RAS and c-MYC oncogenes and under-expression of this miRNA is related to neoplasm development[71,72]. RAS is the second most important oncogene in low-grade non-invasive tumorigenesis[73]. Point mutations can lead to genetic alterations that, in turn, promote a strong stimulus of AKT and STAT pathways, triggering high rates of cellular proliferation. c-MYC is a prototype of oncogene promoting RB1 inhibition in high-grade invasive UC[71]. c-MYC is able to stimulate cyclins CDK4, CDK6 and D1 leading to phosphorylation and inactivation of RB1 and increased mitogenic and proliferative processes. Otherwise, c-MYC can stimulate p53 over-activity *via* MDM2 inhibition, favoring cell cycle arrest and apoptosis[3]. Moreover, p53 can enhance expression levels of miR-let7 promoting a cumulative protective effect against carcinogenesis[74].

MiR-125b: Evidences are controversial regarding role of miR-125b in cancer. While some studies define miR-125b as a tumor suppressor, other suggest its oncogenic functions[40,75-78]. However, miR-125b seems to have a protective role in bladder UC. Four main authors demonstrate the suppressive role of miR-125b in bladder cancer, acting over transcription factors, oncogenes and metallopeptidases[40,79-81]. In agreement to these authors, we have also verified an under-expression profile for miR-125b in almost all cases of low-grade non-invasive and high-grade invasive UC, suggesting its suppressive function in bladder UC[45].

MiR-143 and miR-145: MiR-143 and miR-145 are closely located in 5q32 chromosome and share similar suppressive tumor functions, including bladder cancer[45,82-85]. Evidences demonstrate RAS as a target gene of miR-143. Mutations in RAS lead to stimulus of MAPK/AKT/STAT pathway, triggering low-grade non-invasive UC[19], but RAS may also be involved in high-grade tumors[4]. Lin *et al*[86] showed by studies in malignant tissues and cell cultures that under-expression of miR-143 is the rule in UC. In tumor tissues, miR-143 presented 13.7 fold-changes down-regulated in comparison to normal bladder tissues, while in EJ and T24 cell lines it was not identified. When transfected in cell lines, miR-143 significantly inhibited cellular proliferation[86]. Noguchi *et al*[87] also showed the suppressive role of miR-143 in bladder cancer cell lines, and replacement treatment with miR-143 and miR-145 induced synergistic inhibition of tumor by regulating PI3K/AKT/MAPK signaling pathways. Moreover, miR-143 can regulate other target genes involved in UC carcinogenesis. For example, Song *et al*[88] established an inverse correlation between miR-143 and COX-2, an oncogene associated with grade, prognosis and recurrence of bladder UC. Furthermore, the authors verified in T24 cell line that restoration of miR-143 by transfection decreased COX-2 expression and reduced proliferation and motility of tumor cells.

Many studies have validated miR-145 as an inhibitor of cell cycle and tumor growth, promoting induction of apoptosis and lower progression of disease. MiR-145 is a protective miRNA presenting under-expressed in many human tumors, such as colorectal, lung, breast, prostate and renal cancer, and in non-malignant disease such as benign prostatic hyperplasia[89-93]. The first report of miR-145 under-expression was performed by Michael *et al*[94] in a study suggesting that these alterations could be involved in the initiation of colorectal cancer. These findings were confirmed by Shi *et al*[95] who showed that miR-145 under-expression was associated with malignant tumors. Sachdeva *et al*[96] showed in breast cancer cell lines that *MUC-1* gene was associated with tumor onset, invasion and dissemination, where miR-145 was able to inhibit these factors controlling tumor development[96]. Another target of miR-145 is *c-MYC* gene, an oncogene implicated in carcinogenic process of high-grade invasive UC[3,91]. Furthermore, miR-145 is induced by p53 activity, being directly related to invasive UC tumorigenesis. In 2009, Sachdeva *et al*[91] observed that, in physiological conditions, cellular stress promoted higher levels of p53 that, in turn, increased concentrations of miR-145 through

p53 response element. Higher levels of miR-145 inhibit c-MYC activity, allowing normal function of p21 and cell cycle arrest[91]. Under-expression of miR-145 could promote fail in c-MYC control, decreasing p21 levels and stimulates cell proliferation. These complex mechanisms could lead, or at least initiate, UC carcinogenesis[97]. Following the same idea, Spizzo *et al*[98] analyzing breast cancer cell lines demonstrated the crucial suppressor role of miR-145, and its transfection inhibited growth and cell proliferation, inducing p53-mediated apoptosis. Chiyomaru *et al*[99] demonstrated an association between FSCN-1 oncogene and miR-145 under-expression in UC, suggesting that loss of expression of miR-145 and consequent FSCN-1 up-regulation may be associated with bladder tumors in all stages, promoting more aggressive and invasive tumors. Last year we published a work demonstrating that miR-145 is a well-characterized tumor suppressor miRNA in UC. We hypothesize that lack of protector role promoted by miR-145 over probable target genes PI3K/AKT, FSCN-1, MDM-2, c-MYC and MUC-1 could be involved in carcinogenic process of low-grade, non-invasive and high-grade invasive urothelial carcinomas, being an interesting candidate diagnostic biomarker[100].

MiR-199a: Low levels of miR-199a have already found in ovarian and endometrial cancer, testicular tumors, hepatocarcinoma (HCC) and osteosarcoma[101-105] and it seems to be directly involved in tumor progression and worse prognosis. Fornari *et al*[102] studying HCC suggest that miR-199a targets mTOR, demonstrating an inverse relationship between them. Similarly to HCC, PI3K/AKT/mTOR pathway is also involved in high-grade UC carcinogenesis and miR-199a under-expression could explain mTOR over-activity and, at least partially, the tumor onset and progression of disease.

In bladder UC, even though we have shown miR-199a under-expression in most of both low-grade non-invasive and high-grade invasive tumors, statistical differences were not observed[45]. On the other hand, Ratert *et al*[106] found miR-199a down-regulated in malignant bladder samples compared to healthy tissue. Also, the authors demonstrated that miR-199a was differentially expressed between non-invasive and invasive bladder cancer, underling together miR-205 and miR-141, the potential to work as biomarkers of diagnosis and prognosis in bladder UC[106].

MiR-452: There are few and controversial studies concerning miR-452 in human cancer. Some works demonstrate an under-expression of miR-452 in squamous lung tumors, gliomas, prostate and breast cancer[107-110], suggesting a protective role of miR-452 in these assessed tumors.

In bladder cancer, Veerla *et al*[41] and Puerta-Gil *et al*[111] showed that higher levels of miR-452 are the rule in UC and associated with high-grade and invasive disease, poor behavior and metastatic process of disease. In disagreement with the former two researchers, but in concordance with other several studies in human cancer, we verified a strong under-expression profile for miR-452 in both low-grade non-invasive and high-grade invasive UC, suggesting a tumor suppressor role for this miRNA[45]. Moreover, we observed increased under-expression levels according higher grade and stage, reinforcing the protective function of miR-452. Human miR-452 has approximately 220 target genes and we speculate that loss of control over genes involved in cell growth and proliferation as E2F3 and MEF2C could explain the involvement of miR-452 in bladder carcinogenesis. However, complementary mechanistic studies are needed to consolidate this hypothesis.

Final considerations

Many studies have demonstrated promising roles of miRNAs working as diagnostic and prognostic biomarkers or involved in target therapies, consolidating miRNAs as crucial players in human cancer. This review allows a reflection about the true functions of miRNAs in bladder carcinogenesis, not only by their wide capacities of action, but also by abilities in define the cell date. MiRNAs characterization in plasma and urine, representing tissue levels, are potential non-invasive methods that could assist diagnosis, treatment and evaluation of bladder cancer improving management of this prevalent disease.

Finally, the future of anti-tumor target therapies will be based not in one, but in groups of miRNAs working together in several steps of carcinogenic process, being able to identify the disease, predicting behavior and effectively treat bladder cancer.

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REVIEW

Phosphodiesterase inhibitors for treatment of voiding dysfunction: An overview of experimental and clinical evidence

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Abstract

Recently, the focus of the origin of lower urinary tract symptoms (LUTS) has change from the prostate to the bladder. Regardless of the underlying mechanism associated with the origin of LUTS, alpha-blockers continue to be the most common medicine prescribed to treat LUTS due to benign prostatic obstruction (BPO). The newest class of drug introduced to treat LUTS/BPO is phosphodiesterase inhibitors (PDEi) and the aim of this study was to review the role of PDEi in the treatment of LUTS/BPO. In this review, the first evidence was evaluated based on epidemiological studies followed by randomized clinical trials which provide evidence on the administration of PDEi in patients with LUTS/BPO. Experimental studies were also assessed to tentatively elucidate the association between LUTS and erectile dysfunction, and to elucidate the underlying mechanism. There is still controversy regarding the administration of PDEi due to the fear of detrusor impairment, response to acute administration, and the effects of PDEi combined with alpha-blockers. Following this review, we conclude that treatment of BPO/LUTS with PDEi is beneficial, based on experimental studies, strong evidence and the large number of randomized clinical trials confirming their efficiency.

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Key words: Benign prostatic hyperplasia; Lower urinary tract symptoms; Phosphodiesterase inhibitor; Mechanism of action; Urodynamics

Core tip: In this study, an extensive review was performed on the use of phosphodiesterase inhibitors to treat lower urinary symptoms due to benign prostatic obstruction. This study explored experimental and recent clinical evidence in order to assist in the decision-making process in daily practice.

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INTRODUCTION

The population is ageing worldwide and consequently the prevalence of lower urinary tract symptoms (LUTS) is increasing and becoming a public health problem. As men grow older the prevalence of histologic benign prostatic hyperplasia (BPH) also increases. BPH is observed in approximately 8% of men aged 31-40 years, 42% of men aged 51-60 years, 71% of men aged 61-70

Table 1 Initial evidence based in epidemiological studies of a common pathophysiology between lower urinary tract symptoms and erectile dysfunction

Ref.	Number of participants	Major conclusion
Cologne Male Survey Braun *et al*[6]	4000	72.2% of patients with ED had concomitant LUTS Only 37.7% had LUTS without ED
Population-based cohort study in Brazil Moreira *et al*[7]	602	Incidence of ED was 65.5 cases per 1000 person-years Relative risk of ED was 1.8-7.5 in patients with LUTS
Sexual dysfunction in European men Vallancien *et al*[8]	1274	Prevalence ED-Mild (55%), severe (70%) LUTS Prevalence of ED was 55% in men with mild LUTS and increased to 70% in severe LUTS
Association of LUTS in Japanese men with erectile dysfunction Terai *et al*[9]	3189	Severity of ED was significantly associated with moderate to severe IPSS, RR = 1.5 which persisted after adjustment for age
Boston Area Community Health survey Brookes *et al*[10]	2301	Strong association was observed between the AUA-SI associated to ED and ED after adjusting for age

ED: Erectile dysfunction; LUTS: Lower urinary tract symptoms; RR: Relative risk; IPSS: International Prostatic Score Symptoms; AUA-SI: American Urological Association Symptom Index.

years, and 88% of men aged 81 years and older[1]. BPH may result in enlargement of the prostate, also defined as benign prostatic enlargement and may be associated with bladder outlet obstruction (BOO). BOO in this case is defined as a benign prostatic obstruction (BPO). Permanent BPO may result in adaptive changes of the detrusor muscle causing storage LUTS, if BPO progresses and persists this may lead to a failure of the detrusor resulting in emptying LUTS. Due to these observations the focus of the origins of LUTS has changed from the prostate to the bladder. As the pathophysiology of LUTS is not totally understood it has been hypothesized that possible LUTS/BPO arises due to local alterations in detrusor smooth muscle cells, local receptors, neural signalization, blood flow and changes in the extracellular matrix. Regardless of the underlying mechanism associated with the origin of LUTS, alpha-blockers are the most common medicine prescribed to treat LUTS/BPO[2]. Alpha-blockers decrease urethral resistance and improve the urinary flow by relaxation of smooth muscle of the prostate and bladder neck.

Other drugs have been used to treat LUTS related to BPO such as 5-alpha-reductase inhibitors. These are taken alone or with alpha-blockers to decrease progression of the disease or to avoid urinary retention[3,4]. Anticholinergics have also been administered to patients with predominant storage LUTS/BPO with a low risk of urinary retention regardless of obstruction[5]. The newest class of drug introduced to treat BPO is phosphodiesterase inhibitors (PDEi) and the aim of this study was to review the role of PDEi in the treatment of LUTS/BPO.

INITIAL EVIDENCE

The use of PDEi in patients with LUTS/BPO was proposed initially based on observational epidemiological studies specially designed to evaluate erectile dysfunction (ED). It was observed in these studies that demographic data showed a similar prevalence of ED and LUTS/BPO in men as they aged, raising the possibility of a common underlying mechanism contributing to both conditions.

The pioneering work carried out in 2000 to study the prevalence of ED in Germany in the Cologne Male Survey evaluated 4000 patients[6]. LUTS/BPO was present in 72.2% of patients with ED, however, only 37.7% had LUTS/BPO without ED. It was also observed in a Brazilian Cohort Study that an epidemiological association existed between LUTS/BPO and ED. In this particular study, the relative risk of ED was 1.8-7.5 in patients complaining of LUTS and this risk was greater than smoking or cardiac symptoms[7]. In Europe a demographic study evaluating 1274 European men showed that 55% of patients with mild LUTS/BPO had ED, however, the prevalence of ED increased to 70% in patients with severe LUTS/BPO[8]. In a Japanese Cross-Sectional Survey, a correlation between ED and LUTS/BPO was observed and the relative risk was 1.5 which persisted after adjustment for age[9]. In the United States of America, multivariate regression of the Boston Area Community Health Survey data found an association between the American Urological Association Symptom Index and ED without differences in race or ethnicity[10]. Therefore, in different parts of the world several studies showed an epidemiological correlation between ED and BPO/LUTS (Table 1).

Clinical use of phosphodiesterase inhibitors to treat BPO/LUTS

Following and during these observational studies, a proof-of-concept clinical study to evaluate improvement in BPO/LUTS in men taking sildenafil for ED was performed in 2002[11]. Patients taking sildenafil were evaluated using the International Index of Erectile Function (IIEF) and International Prostate Symptoms Score (IPSS) instruments at baseline, one and three months. During

the treatment period, an improvement in the IPSS and quality of life (QoL) was observed. An inverse relationship between IPSS and IIEF during treatment with sildenafil was also noted. The major limitations of this study were its open label and uncontrolled design. In other uncontrolled studies, a similar impact of sildenafil in BPO/LUTS and ED was observed[12,13]. Different from the uncontrolled design of the papers reported above, the next generation of studies included randomized and placebo-controlled trials.

In 2007, the first multicenter, randomized, placebo-controlled, double-blind trial was reported[14]. The end point was defined as change from baseline of erectile function assessed with the IIEF instrument. Secondary end points were changes in LUTS from baseline evaluated with the IPSS, QoL question of the IPSS, Benign Prostatic Hyperplasia Impact Index (BPHII), peak flow rate (Qmax), Self-Esteem And Relationship (SEAR) scores and end of treatment satisfaction using Erectile Dysfunction Inventory of Treatment Satisfaction Index Score. Compared with placebo, sildenafil significantly improved the IIEF, IPSS, BPHII, IPSS QoL and SEAR score. Significant improvement in Qmax was not observed in the sildenafil group compared with placebo. The limitations of this study were lack of a placebo run-in period and determination of correlations between LUTS and ED improvements.

In 2007, another multicenter, randomized, placebo-controlled, double-blind trial assessed the efficacy of tadalafil once daily for BPO/LUTS[15]. Inclusion criteria were age greater than 45 years and IPSS > 12 for at least six months. Exclusion criteria were elevated prostatic score antigen (PSA), recent use of 5∝-reductase inhibitors, use of BPH medication during study, history of pelvic surgery, liver failure, other causes of LUTS, uncontrolled diabetes, and nitrate use or chemotherapy. Different from previous studies, a placebo run-in period was included in the study design. After a four-week placebo run-in period, 281 men with BPO/LUTS were randomized to 5 mg tadalafil daily for six weeks, followed by dose escalation to 20 mg for six weeks or placebo for a total of 12 wk. Tadalafil significantly improved the mean change from baseline IPSS compared with placebo. Improvement was also seen in the IPSS QoL, BPHII and IIEF. No significant change was observed in Qmax. Based on these results, the authors concluded that daily tadalafil caused a significant improvement in BPO/LUTS and ED.

In 2008, in an 8-wk randomized, double-blind, placebo-controlled study, vardenafil 10 mg was administered to 222 men with BPO/LUTS with or without ED. Inclusion criteria were age 45-64 years and IPSS score ⩾ 12[16]. Exclusion criteria were vardenafil contraindications, spinal cord injury, prostatitis, urethral stricture, urinary retention, bladder or prostate cancer, past cancer with low life expectancy, use of androgens, anticoagulants, ED treatments, or alpha-blockers during the treatment period. The IPSS score, Qmax, postvoid residual urine volume (PVR), and the erectile dysfunction domain of the IIEF were assessed. Vardenafil significantly improved the mean change in the IPSS from baseline compared with placebo. It also improved ED and QoL. However, no changes in Qmax or PVR were noted. A weak point of this study was the lack of a placebo run-in phase.

A dose-finding study was reported in 2008[17]. In this study, after a 4-wk placebo run-in period, 1058 men with BPO/LUTS were randomized to receive daily tadalafil (2.5, 5, 10 or 20 mg) or placebo. Inclusion criteria were age greater than 45 years, IPSS score ⩾ 12 for at least 6 mo, and Qmax between 4 and 15 mL/s. Exclusion criteria were elevated PSA, recent use of 5∝-reductase inhibitors, use of BPH medication during study, history of pelvic surgery, liver failure, other causes of LUTS, uncontrolled diabetes, and nitrate use or chemotherapy. IPSS change from baseline to endpoint was improved with all tadalafil doses compared with placebo. In the Global Assessment Questionnaire, LUTS also improved at all doses, however, doses greater than 5 mg had minimal improvement with more side effects. As a consequence, this improvement demonstrated a dose-response relationship and 5 mg tadalafil once daily had a positive risk-benefit profile. No significant change in Qmax was observed.

It is possible to conclude with a high level of evidence that PDEi clearly improves BPO/LUTS based on the results presented in these four clinical trials.

Following these randomized controlled trials (RCTs), more recent systematic reviews and meta-analyses have emerged[18-20].

In 2013, a study that aimed to evaluate the efficacy and safety of tadalafil 5 mg once daily compared to placebo over 12 wk for the treatment of both LUTS/BPO and ED in sexually active men was performed. The data were pooled from four multinational, randomized studies of men ⩾ 45 years with LUTS/BPO. The randomization and placebo run-in period were strong points in this study. Principal end-points were change in the IPSS, QoL, BPHII, and IIEF. Tadalafil (n = 505) significantly improved total IPSS *vs* placebo (n = 521); mean changes from baseline were -6.0 and -3.6, respectively ($P < 0.001$). Improvements in the IIEF Domain score (tadalafil, 6.4; placebo, 1.4) were also significant *vs* placebo, as were the IPSS, IPSS QoL, and BPHII (all $P < 0.001$). The authors concluded that tadalafil was efficacious and well tolerated in the treatment of ED and LUTS/BPO[20].

In one of these meta-analyses, the use of PDEi alone or in combination with alpha-blockers was summarized to identify the best candidates for this treatment based on clinical features and LUTS severity[18]. Trials included in this review were selected using the following inclusion criteria: (1) They were RCTs; (2) The subject of the study was a PDEi for LUTS/BPO; (3) Control groups received placebo for PDEi alone or alpha-blockers alone and PDEi plus alpha-blockers; and (4) The primary outcomes were the IPSS, IIEF, and Qmax. Of 508 retrieved studies, 497 articles were excluded; leaving only 11 studies. More than 6000 men evaluated in these 11 studies were includ-

Table 2 Randomized clinical trials and meta-analyses with strong evidence for the use of phosphodiesterase inhibitors in patients with lower urinary tract symptoms due to benign prostatic obstruction

Ref.	Design of study	Placebo run-in	Participant/inclusion criteria	End point	Major conclusion
Sairam *et al*[11]	Not RCT	No	112 male patients All taking sildenafil Inclusion criteria was presence ED	Assess relationship between ED and LUTS; if sildenafil influences LUTS in patients with ED	No relation between ED score and LUTS before treat ED Sildenafil improves ED and LUTS
McVary *et al*[14]	Open-label, randomized, double-blind, placebo-controlled	No	369 patients were randomized to sildenafil 100 mg (n = 189) or placebo (n = 180) during 12 wk/Men with ED and LUTS	Change IPSS, QoL,BPHII, Qmax, SEAR, and EDITS	Sildenafil improve IIEF, IPSS, BPHII, IPSS QoL and SEAR score Qmax not altered
McVary *et al*[15]	Randomized, double-blind, placebo-controlled	Yes	281 men randomized to tadalafil 5 mg daily, followed by dose escalation to 20 mg/Men aged 45 yr or higher and IPSS > 12	Change IPSS, QoL, BPHII, Qmax, and IIEF	Tadalafil improve IPSS, QoL, BPHII, and IIEF Qmax not altered
Stief *et al*[16]	Randomized, double-blind, placebo-controlled	No	222 men were randomized to vardenafil 10 mg twice daily or placebo/age 45-64 yr, IPSS ⩾ 12, with or without ED	Change in IPSS, Qmac, PVR, and IIEF	Vardenafil improve IPSS, IIEF, and QoL Qmax and PVR not altered
Roehrborn *et al*[17]	Randomized, double-blind, placebo-controlled	Yes	1058 men were randomized to receive daily tadalafil 2.5, 5, 10 or 20 mg/age greater than 45 yr, IPSS ⩾ 12, and Qmax between 4-15 mL/s	Change in IPSS, IIEF, QoL, BPHII, GAQ, and Qmax	Tadalafil improve IPSS and GAQ in all doses But, dose higher than 5 mg had minimal improvement with higher side effects Qmax not altered
Porst *et al*[20]	Meta-analysis		1026 men, tadalafil (n = 505) compared to placebo (n = 521). Data pooled from four multinational study/age ⩾ 45 yr, presence of LUTS/BPO	Change in IPSS, QoL, BPHII, and IIEF	Tadalafil improve IPSS, QoL, BPHII, and IIEF compared with placebo
Gacci *et al*[18]	Meta-analysis		Twelve studies, been seven studies (n = 3214) comparing PDEi *vs* placebo, and five (n = 216) on the combination of PDEi with ∝-blockers *vs* ∝-blockers alone/Men with LUTS/BPO	Change in IPSS, IIEF, and Qmax Identify best candidates for treatment with PDEi based on clinical features	PDEi alone improve IPSS, IIEF, but not Qmax Association of PDEi with ∝-blockers improve IPSS, IIEF, and Qmax
Yan *et al*[19]	Meta-analysis		515 patients (seven studies)/ patients with LUTS/BPO and ED	Compare combination of PDEi with ∝-blockers *vs* ∝-blockers alone. Change IPSS, QoL, BPHII, Qmax, and IIEF	Combination of PDEi with ∝-blockers has additive favorable effects compared with PDEi monotherapy

RCT: Randomized control trial; ED: Erectile dysfunction; LUTS: Lower urinary tract symptoms; IPSS: International prostatic symptoms score; QoL: Quality of life; BPHII: Benign Prostatic Hyperplasia Impact Index; Qmax: Peak flow rate; SEAR: Self-esteem and relationship; EDITS: Erectile Dysfunction Inventory of Treatment Satisfaction Index Score; IIEF: International Index of Erectile Function; PVR: Postvoid residual urine volume; GAQ: Global Assessment Question; BPO: Benign prostatic obstruction; PDEi: Phosphodiesterase inhibitors.

ed in this meta-analysis, with seven evaluating PDEi *vs* placebo in 3214 men, and five evaluating the combination of PDEi with alpha-blockers *vs* alpha-blockers alone in 216 men. Median follow-up in all RCTs was 12 wk. The IIEF score (5.5; $P < 0.0001$) and IPSS (-2.8; $P < 0.0001$) were significantly different, but not the Qmax (-0.00; P = not significant) at the end of the study as compared with placebo. The association of PDEi and alpha-adrenergic blockers improved the IIEF score (3.6; P <0.0001), IPSS score (-1.8; $P = 0.05$), and Qmax (1.5; $P < 0.0001$) at the end of the study as compared with a-blockers alone. Therefore, the meta-analysis suggested that PDEi can significantly improve LUTS and EF in men with BPO.

A recent meta-analysis was carried out to evaluate the efficacy of PDEi alone or in combination with alpha-blockers for the treatment of ED and LUTS/BPO. The databases MEDLINE, EMBASE, PubMed, the Cochrane Controlled Trial Register of Controlled Trials, and the Chinese Biological Medical Database were searched to identify RCTs that referred to the use of a combination of PDE5 inhibitors and alpha-blockers for the treatment of ED and LUTS associated with BPH. The principal objectives were to evaluate the IPSS, Qmax, and IIEF. Seven publications involving 515 patients were included in the meta-analysis. PDE5 inhibitors and alpha-blockers significantly improved the IIEF, IPSS, and Qmax values compared with PDE5 inhibitors alone ($P = 0.04$, 0.004, 0.007, respectively). The major conclusion was that the combined use of PDEi and alpha-blockers results in additive favorable effects in men with ED and LUTS/BPO compared with PDEi monotherapy[19] (Table 2).

It is important to note that although many of these studies evaluated men with ED and LUTS/BPO, some studies reported an improvement in LUTS/BPO inde-

pendently of ED[21,22].

Experimental studies

Due to the particular course presented above, the clinical use of PDEi to treat BPO/LUTS began before the mechanism of action of these drugs was known. Therefore, because the mechanism of action was not understood, a common pathophysiological link between ED and BPH was investigated, and an increasing number of experimental studies have emerged in subsequent years[23,24].

To explain the mechanism involved, several theories have been proposed. The four principal hypotheses are: ischemia due to pelvic atherosclerosis, autonomic hyperactivity, a calcium-independent Rho-kinase activation pathway, and reduced nitric oxide (NO) levels[25,26].

The ischemia hypothesis is based on blood flow to the lower urinary tract (LUT) being affected by smooth muscle cell (SMC) contraction, thus decreasing oxygenation leading to chronic ischemia of LUT tissue and contributing to LUTS. Atherosclerosis is associated with remodeling of SMCs in the pelvic vasculature[27,28], penis[29,30], and bladder[28] also associated with LUTS. Therefore PDEi may act by increasing perfusion of the bladder through relaxation of SMCs resulting in increased oxygenation.

It has also been postulated in experimental studies that overactivity of terminal afferent nerves (autonomic) within LUT may be associated with contraction of SMCs[31-33]. Again PDEi might be associated with relaxation of SMCs thus improving LUTS.

Rho-kinase/RhoA activation has been shown to mediate detumescence and maintain flaccidity. Rho kinase inhibits the regulatory subunit of myosin phosphatase within SMCs and maintains contractile tone under low-cytosolic calcium concentration. Upregulated Rho-kinase activity has been reported in ED, as a consequence Rho-kinase inhibitors have been examined to treat ED[34].

Despite the candidate mechanisms mentioned above, it is likely that there is an overlap between the roles of these mechanisms. The reduced NO hypothesis seems to be the best one.

The cornerstone of the process seems to be cyclic nucleotide monophosphate, cyclic adenosine monophosphate and cyclic guanosine monophosphate (cGMP). Cyclic nucleotides are synthesized from the corresponding nucleoside triphosphates by the activity of adenylyl and guanylyl cyclases. Soluble guanylyl cyclase is a widely distributed signal transduction enzyme that, under activation by NO, converts GTP into the second messenger, cGMP, which exerts its effect by activating cyclic guanylyl kinase I (cGK Ⅰ) and cGK Ⅱ, cGMP-gated ion channels, and/or cGMP-regulated phosphodiesterases (PDE). The accumulation of intracellular cGMP triggers a cascade, leading to decreased intracellular calcium level and subsequent relaxation of SMCs[35,36]. The amount of cGMP results from the balance between production (NO) and degradation due to PDE isoenzymes which can hydrolyze and inactivate cyclic nucleotides[24]. Therefore, increased smooth muscle tension may play a central role in the pathophysiology of LUTS.

An *in vitro* study revealed that 4 wk of treatment with the NO synthase (NOS) blocker, N^{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME), caused *in vitro* detrusor muscle supersensitivity to muscarinic agonists *via* increases in the levels of [H^3]-inositol-phosphate[23]. This finding was corroborated by in vivo experimental studies which showed that administration of L-NAME resulted in a significant increase in non-voiding contractions (NVC) in rats[24].

Based on experimental studies which have shown that rat PDE expression is highest in the bladder, approximately 10-fold higher than in rat corpora cavernosa followed in decreasing prevalence by vas deferens, prostate, kidney, testis, and epididymis[37], further experimental studies evaluated the action of PDEi on LUT. One of these studies demonstrated that administration of sildenafil in rats improved detrusor overactivity and bladder outlet obstruction (lack of urethral relaxation) caused by the NOS inhibitor, L-NAME, in a urodynamic study (UDS)[24]. In another study, with similar methodology, it was also demonstrated that tadalafil decreased NVC and frequency of micturition (FM) in rats in a UDS[38].

As a consequence of these experimental studies, there is good support for the use of PDEi in the treatment of BPO/LUTS.

CONTROVERSIES IN ADMINISTRATION OF PHOSPHODIESTERASE INHIBITORS TO TREAT BPO/LUTS

Impairment detrusor

It has been observed in several clinical trials that PDEi improved the IPSS without changing Qmax in uroflowmetry[14,16,17,39]. If a PDEi caused relaxation of the bladder neck, urethra, and prostatic relaxation in human and animals[24,37,40], it was expected to increase Qmax. Thus, these findings have raised the theoretical possibility that administration of PDEi cause impairment in detrusor function with unknown long-term effects[41].

As a consequence, an experimental study was performed with the endpoint of determining whether tadalafil caused detrusor muscle impairment. In this study, it was reported that chronic depletion of NO caused an increase in NVC, volume threshold (VT) and FM in rats and treatment with tadalafil reduced VT and FM. However, tadalafil did not decrease threshold pressure or peak pressure (PP) in rats with chronic NO deficiency. Tadalafil which increased cGMP probably explains the reduction in VT (decrease in urethral resistance) and MC (relaxation of detrusor) observed in this study. As tadalafil did not decrease detrusor pressure (threshold pressure or PP) it is evident that PDE5i do not cause impairment in detrusor muscle[38].

A clinical trial assessed the impact of tadalafil treatment (20 mg once daily) compared to placebo on detru-

sor pressure and maximum flow (pdetQmax) in men with BPO/LUTS with or without bladder outlet obstruction at baseline. In this study, tadalafil was not associated with a negative impact on detrusor function as the change of pdetQmax was not significant compared with the placebo arm[42].

Acute effects of phosphodiesterase inhibitors on BPO/LUTS

There are few reports of the acute effects of PDEi on BPO/LUTS. The vast majority of studies have evaluated chronic administration of PDEi.

In one clinical trial, 68 patients were randomized to the placebo (n = 32) or sildenafil arm (n = 36). All patients were evaluated at baseline with free uroflowmetry. Uroflowmetry was repeated two hours after administration of placebo or sildenafil. The authors concluded that sildenafil caused a significant improvement in Qmax 15.6 ± 6.8 mL/s from baseline to end point 19.3 ± 7.2 mL/s (P < 0.001), and compared with the placebo arm 15.8 mL/s (P < 0.0001). The increase in Qmax was attributed to urethral relaxation[43].

An experimental study was performed to observe the effect of acute infusion of sildenafil in rats with detrusor overactivity. It was observed that sildenafil decreased the number of micturition cycles from baseline to end point (-0.93 ± 0.34, P = 0.031)[24].

Combination of phosphodiesterase inhibitors with alpha-blockers

According to the American Urological Association guidelines, a1-adrenergic blockers are considered to be the most effective monotherapy for the treatment of LUTS secondary to BPH[2]. PDE5 inhibitors are the first-line treatment for erectile ED. Due to the strong association between BPO/LUTS and ED, the coprescription of PDE5 inhibitors and a1-adrenergic blockers is likely to increase. Thus, one of the most frequently asked questions by physicians is whether to combine or replace alpha-blockers with PDEi in patients with BPO/LUTS and ED.

To address this issue a randomized, double-blind, placebo- and active-controlled (tamsulosin 0.4 mg), and parallel design trial was carried out to compare the effects of daily tadalafil 5 mg in patients with LUTS/BPO. The IPSS, BPH Impact Index, and IIEF-Erectile Function Domain (IIEF-EF) were assessed at baseline and end point (12 wk or end of therapy). The Patient and Clinician Global Impression of Improvement (PGI-I and CGI-I, respectively) instruments and the subject-rated Treatment Satisfaction Scale-BPH (TSS-BPH), evaluated from 0% (greater) to 100% (lower) satisfaction, were administered at end point. Uroflowmetry and postvoid residual were also assessed at screening, baseline, and end of visits. Tadalafil and tamsulosin caused an improvement in the IPSS from baseline to endpoint. However, for the IPSS QoL a significant improvement compared with placebo was only reported in the tadalafil arm, but not the tamsulosin arm. The TSS-BPH overall satisfaction score at endpoint was significantly lower (indicating higher satisfaction) in the tadalafil group compared with placebo, driven by greater satisfaction with efficacy. There was no significant difference between tamsulosin and placebo in TSS-BPH overall satisfaction or satisfaction with efficacy. Tadalafil resulted in an improvement in IIEF-EF, but tamsulosin did not change this index. Tadalafil and tamsulosin caused a significant increase in Qmax. For PVR, both active treatments caused reductions, but these were not statistically significant. The strong point of this study was the wash-out and placebo run-in periods. The principal limitation was that it was not powered to assess noninferiority or superiority between tadalafil and tamsulosin. The author concluded that tadalafil or tamsulosin resulted in significant improvements in IPSS and Qmax related to BPO/LUTS. However, only tadalafil had a significant impact on the IPSS QoL and erectile function[44].

A randomized, double-blind, placebo-controlled study was performed to compare the effect of tamsulosin 0.4 mg/daily and tadalafil 5 mg daily with tamsulosin 0.4 mg/placebo on the lower urinary tract in a UDS. All patients underwent a baseline UDS before randomization to tamsulosin 0.4 mg/tadalafil 5 mg or tamsulosin 0.4 mg/placebo once daily for 30 d. An end of study UDS was performed on completion of treatment. The UDS assessed pdetQmax, Qmax during voiding, bladder outlet obstruction index calculated as pdetQmax-2Qmax, and detrusor overactivity (assessed as incidence). The primary end points were a change in urodynamic variables in the voiding phase, pdetQmax and Qmax, from baseline to week four. The secondary endpoint of this study was improvement in the IPSS. A total of 40 men were randomized to receive tamsulosin 0.4 mg/tadalafil 5 mg (n = 20) or tamsulosin 0.4 mg/placebo (n = 20) once daily for four weeks. When the groups were compared, pdetQmax decreased significantly in the tamsulosin/tadalafil group compared with the tamsulosin/placebo group. In both groups, Qmax increased from baseline to endpoint, however, the difference in Qmax at endpoint was not significant between the groups. Significant improvements were observed in total IPSS, IPSS filling and voiding subscore in the tamsulosin/tadalafil group compared with the tamsulosin/placebo group. The limitations of this study were lack of placebo run-in period and the small number of participants. A strong point was that this was the first study to define the action of tadalafil in LUT using a computerized UDS. The principal conclusion of this study was that only the combination of tamsulosin/tadalafil decreased after-load (pdetQmax) and had the potential to protect detrusor smooth muscle. In addition, the combination significantly improved the IPSS compared with tamsulosin/placebo[45].

In another clinical trial similar to the above study, tadalafil 20 mg in combination with tamsulosin 0.4 mg was compared to tamsulosin 0.4 mg in patients with LUTS. Improvement in the IPSS was greater with the combination treatment. No difference was observed in

Table 3 Studies evaluating the combination of phosphodiesterase inhibitors and alpha-blockers in patients with lower urinary tract symptoms

Ref.	Design of study	Placebo run-in	Participant /Inclusion criteria	End point	Major conclusion
Oelke *et al*[44]	Randomized, multicentric, placebo controlled, and parallel-group	Yes	Men ⩾ 45 yr of age with BPO/LUTS, IPSS ⩾ 13, and Qmax between 4-15 mL/s 512 men were randomized to placebo (n = 172), tadalafil 5 mg (n = 171) or tamsulosin 0.4 mg (n = 168); Men ⩾ 45 yr of age with BPO/LUTS, IPSS ⩾ 13, and Qmax between 4-15 mL/s	Compare effect of tadalafil 5 mg once daily with placebo on BPO/LUTS	Tadalafil 5 mg and tamsulosin 0.4 mg had similar improvement in BPO/LUTS and Qmax compared with placebo However, only tadalafil caused a significant improvement in QoL, treatment satisfaction, and erectile function
Regadas *et al*[45]	Randomized, double-blind, and placebo controlled	No	Men ⩾ 45 yr of age, BOOI ⩾ 20 and IPSS ⩾ 14 A total of 40 men were randomized to tadalafil 5 mg/tamsulosin 0.4 mg (n = 20) or tamsulosin 0.4 mg /placebo (n = 20) Men ⩾ 45 yr of age, BOOI ⩾ 20 and IPSS ⩾ 14	Observe changes in urodynamic variables (Qmax and PdetQmax)	Combination of tamsulosin/tadalafil decrease after-load (pdetQmax) and has potential to protect detrusor smooth muscle Additionally, the combination resulted in a significant improvement in IPSS compared with tamsulosin/placebo
Bechara *et al*[46]	Randomized, double-blind, and crossover study	No	History of LUTS/BPO of at least six months Thirty men were randomized to tamsulosin 0.4 mg or tamsulosin 0.4 mg/tadalafil 20 mg daily History of LUTS/BPO of at least six months	Acess efficacy and safety of combination of tamsulosin with tadalafil compared with tamsulosin alone	Combination therapy had more significative impact in IPSS and ED compared with tamsulosin alone
Kaplan *et al*[39]	Randomized, double-blind study	No	Men aged 50-76 yr with untreated LUTS and ED Sixty two patients were randomized to receive alfuzosin 10 mg (n = 20), sildenafil 25 mg (n = 21), or a combination of both (n = 20) Men aged 50-76 yr with untreated LUTS and ED	Acess efficacy and safety of combination of alfuzosin with sildenafil	Only sildenafil or combination improve ED Improvement in IPSS was observed with three treatments
Gacci *et al*[47]	Randomized, double-blind placebo-controlled trial	Yes	Men with persistent storage LUTS Sixty men were randomized to tamsulosin 0.4 mg or tamsulosin 0.4 mg/vardenafil 10 mg Men with persistent storage LUTS	Acess efficacy and safety of combination of tamsulosin with vardenafil	Combination therapy had more significative impact in IPSS and ED compared with tamsulosin alone

BPO/LUTS: Benign prostatic hyperplasia/lower urinary tract symptoms; Qmax: Peak flow rate; QoL: Quality of life; IPSS: International Prostatic Symptoms Score; PdetQmax: Detrusor pressure at maximum flow; ED: Erectile dysfunction; BOOI: Bladder outlet obstruction index.

Qmax or PVR between the groups. The IIEF improved in the combination group, but not in the tamsulosin group. The authors concluded that tamsulosin with tadalafil was more effective than tamsulosin alone in improving LUTS and ED. Small sample size was the principal limitation of this study[46].

In 2007, a pilot study was carried out to assess the efficacy of alfuzosin 10 mg, sildenafil 25 mg daily, and the combination of both on LUTS and ED. Improvements in the IPSS and IIEF were significant with the three treatments, but were greatest with the combination of alfuzosin and sildenafil. An improvement in the IIEF was only observed in the sildenafil and combination groups. The authors concluded that the combination of sildenafil and alfuzosin was more effective than monotherapy in improving both voiding and sexual dysfunction in men. Weak points of this study were no placebo arm in the study design, small sample size, and the experimental dose of sildenafil (25 mg once daily)[39].

Tamsulosin 0.4 mg/d was compared with the combination of tamsulosin 0.4 mg/d plus vardenafil 10 mg/d. After a 2-wk run-in period, tamsulosin patients were randomized to a 12-wk treatment period. The IPSS, IPSS-bother, IIEF-5 and Over Active Bladder questionnaire scores, uroflowmetry data (Qmax, Qave), and postvoiding residual urine were recorded after run-in (baseline), and 2 and 12 wk after treatment. Significant differences from baseline to 12 wk were observed in the following: Qmax (placebo: 0.07, vardenafil: 2.56, P = 0.034); Qave (placebo: -0.15, vardenafil: 1.02, P = 0.031); filling-IPSS subscores (placebo: -1.67, vardenafil: -3.11, P = 0.039); and IIEF (placebo: 0.06, vardenafil: 2.61, P = 0.030). The authors concluded that the combination of tamsulosin and vardenafil was more effective in improving both LUTS and erectile function, as compared with tamsulosin alone[47] (Table 3).

CONCLUSION

Treatment of BPO/LUTS with phosphodiesterase inhibitors is beneficial, based on experimental studies, strong evidence and a large number of randomized clinical trials confirming their efficiency.

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REVIEW

Benefits and risks of erythrocyte-stimulating agents

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Core tip: Renal anemia is a common clinical problem in patients with severe chronic kidney disease. To overcome the shortage of endogenous erythropoietin (EPO), administration of exogenous EPO is an effective treatment. The advent of recombinant human erythropoietin (rHu-EPO) products has dramatically changed the therapeutic strategy and has shown outstanding effectiveness in patients with renal anemia. Here we discuss the treatment of renal anemia and the adverse effects of rHu-EPO.

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Abstract

Chronic kidney disease (CKD) is a common and serious clinical problem. Anemia in patients with advanced CKD, frequently called renal anemia, causes disabling fatigue and diminishes patients' quality of life. Frequent and excess transfusions or iron supplementation are potentially hazardous. Although it remains unclear whether the main factor in the development of renal anemia is the failure of erythropoietin (EPO) production in the kidney or a dysfunction in oxygen sensing exogenous EPO administration is considered a rational treatment. The advent of recombinant human erythropoietin (rHu-EPO) products has dramatically changed the therapeutic strategy for renal anemia. Although rHu-EPO therapy has improved patients' quality of life and decreased the need for blood transfusions, some potential adverse effects have been reported till date. This brief review discusses the treatment of renal anemia with regard to the following: (1) historical background; (2) effectiveness of rHu-EPO; (3) some topics regarding the treatment of anemia, including EPO resistance, hemoglobin (Hb) cycling, and adequate Hb levels; (4) major adverse effects of rHu-EPO, including hypertension, thrombotic complications, and pure red cell aplasia; and (5) future problems to be resolved.

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HISTORICAL BACKGROUND

Serious anemia requiring transfusion and/or iron supplementation is one of the frequent complications in patients with progressed chronic kidney disease (CKD)[1]. This type of anemia is also known as renal anemia, and it causes disabling fatigue and diminishes the quality of life (QOL) in patients with CKD. Excessive blood transfusions and/or iron supplementation are potentially hazardous. Rare but serious risks associated with transfusion include procedural complications, pulmonary congestion, electrolyte imbalance, metabolic alkalosis, hypocalcemia, and severe lung injury (Table 1)[1]. Moreover, the great risk of transfusion-related infections, such as hepatitis virus, West Nile virus, and human immunodeficiency virus infections, cannot be ignored. The other important and common therapy for renal anemia is iron supplementation. Iron deficiency is a serious clinical problem in patients with chronic heart failure[2,3]. However, iron overload has potentially adverse outcomes, including hy-

Table 1 Risks associated with blood transfusions[11]

Adverse effects
Fever/allergic reactions
Hemolytic reaction
TRALI
Anaphylaxis
Fatal hemolysis
GVHD
Thrombotic complications
Mistransfusion

TRALI: Transfusion-related acute lung injury; GVHD: Graft-versus-host disease.

potension and dyspnea[4,5], transfusional hemosiderosis, and increased risk of infection[6-8].

The advent of recombinant human erythropoietin (rHu-EPO) was a major breakthrough in the treatment of renal anemia. Although it remains unclear whether the leading factor in the development of renal anemia is the failure to produce EPO in the kidney or a dysfunction in oxygen sensing[9], exogenous EPO administration is considered a rational method to treat anemia in patients with CKD. Human EPO, which is derived from the urine of patients with aplastic anemia (AA), is purified to apparent homogeneity. In 1977, Miyake *et al*[10] first purified human EPO in an amount sufficient for chemical characterization. Recombinant human EPO was subsequently produced and became available for clinical use more than two decades ago. Consequently, it completely altered the medical management of renal anemia.

THE EFFECTIVENESS OF ERYTHROCYTE-STIMULATING AGENTS

The introduction of exogenous rHu-EPO into clinical practice dramatically altered the treatment of renal anemia in patients with CKD[11]. Some of the beneficial effects of rHu-EPO therapy include elevated hemoglobin (Hb) levels, improved QOL and cognitive function[12-14], and decreased left ventricular mass, among others[15]. In 1990, Evans *et al*[16] reported that rHu-EPO therapy led to significant improvements in personal activity levels, functional ability, appetite, sleeping hours, condition or satisfaction with health, and happiness. However, no significant differences were observed in patients' working capacity or job status[16]. Overall, the spread of exogenous rHu-EPO treatment diminished the need for transfusion[17] and the risk of transfusion-related complications. Roth *et al*[18] reported that rHu-EPO therapy improves anemia in patients with stage 3 or 4 CKD [glomerular filtration rate (GFR) = 15-59 mL/min per 1.73 m^2] and does not increase the severity of CKD[18]. The administration of rHu-EPO is definitely regarded as an effective therapy for transfusion-dependent long-term dialysis patients (GFR < 15 mL/min per 1.73 m^2 and use of maintenance renal replacement therapy; CKD 5D) with extremely low Hb levels.

EPO-RELATED TOPICS

EPO resistance

Erythrocyte-stimulating agents (ESA) resistance is a serious clinical problem that physicians often encounter in patients with renal anemia. It has been reported as the most predominant predictor of cardiovascular events and fatality[19]. The Kidney Disease Improving Global Outcomes (KDIGO) guidelines describe the evaluation and management of ESA resistance (Table 2)[20]. Table 3 presents a clinical approach to manage ESA resistance. Among many factors associated with ESA resistance, including hyperparathyroidism, inflammation, and underdialysis, iron deficiency is the leading cause. Iron supplementation often results in the improvement of anemia[1].

Hb cycling

Fishbane *et al*[21] previously defined Hb cycling as cycles with > 1.5 g/dL and > 8 wk and excursions as half of a cycle. In their report, more than 90% hemodialysis (HD) patients were reported to experience Hb cycling. The mean duration of Hb excursions was 10.3 ± 5.1 wk. An increase in the rHu-EPO dose, intravenous iron treatment, and post-hospital discharge were the factors associated with upward excursions[21]. Yang *et al*[22] reported that Hb variability is related to severe mortality in CKD 5D patients in the United States. However, this trend was negated in a subsequent study conducted in Europe[23] and remains to be verified in future research.

Adequate Hb levels

According to recent major randomized controlled trials (RCTs), more harm than benefit may be caused by higher Hb levels. Therefore, setting the Hb level at < 11.5 g/dL in adult patients with CKD is suggested by the KDIGO 2012 guidelines[20]. In the Canadian Cardiac Randomized Evaluation of Antidepressant and Psychotherapy Efficacy trial[24], 603 CKD 3-5 patients (GFR < 59 mL/min per 1.73 m^2) treated with rHu-EPO were evaluated. The number of patients who required HD was greater in the high Hb group (13.0-15.0 g/dL) than in the low Hb group (10.5-11.5 g/dL). The rate of decrease in GFR between the two groups was similar. On the other hand, 1432 CKD patients (GFR, 15-59 mL/min per 1.73 m^2) were studied in the Correction of Hemoglobin and Outcomes in Renal Insufficiency trial[25]. Patients receiving strong treatment for anemia (aiming at 13.5 g/dL) experienced a greater incidence of combined cardiovascular adverse events compared with those receiving standard treatment (11.3 g/dL). Considering these findings, the adequate and appropriate range of Hb levels that should be achieved with rHu-EPO therapy remains to be determined. Intentionally increasing Hb levels to > 13 g/dL using erythrocyte-stimulating agents (ESAs) is not recommended for all adult patients according to the KDIGO guidelines[1,20]. In addition, an excessively high dose of ESA is reported to be potentially harmful for patients according to the results of post-hoc analysis of RCTs[26,27],

Table 2 Erythrocyte-stimulating agents hyposensitivy (Kidney Disease Improving Global Outcomes Guideline 2012)[20]

Initial ESA hyporesponsiveness
Classify patients as having ESA hyporesponsiveness if they have no increase in Hb concentration from baseline after the first month of ESA treatment on appropriate weight-based dosing
In patients with ESA hyporesponsiveness, avoid repeated escalations of the ESA dose beyond double the initial weight-based dose
Subsequent ESA hyporesponsiveness
Classify patients as having acquired ESA hyporesponsiveness if after treatment with stable doses of ESA, they require two increases in ESA doses up to 50% beyond the dose at which they had been stable in an effort to maintain a stable Hb concentration
In patients with acquired ESA hyporesponsiveness, avoid repeated escalations in ESA dose beyond double the dose at which they had been stable
Management of poor ESA responsiveness
Evaluate patients with either initial or acquired ESA hyporesponsiveness and treat for specific causes of poor ESA response
For patients who remain hyporesponsive despite the correction of treatable causes, accounting for relative risks and benefits: decline in Hb concentration; continuing ESA if needed to maintain Hb concentration, with due consideration of the doses required; blood transfusions

ESA: Erythrocyte-stimulating agent; Hb: Hemoglobin.

Table 3 Practical approach in the presence of erythrocyte-stimulating agents resintance (Kidney Disease Improving Global Outcomes Guideline 2012)[20]

Tests	Finding and action
Check adherence	If poor, attempt to improve (if self-injection)
Reticulocyte count	If > 130000/μL, look for blood loss or hemolysis: endoscopy, colonoscopy, hemolysis screen
Serum vitamin B_{12}, folate	If low, replenish
Iron status	If low, replenish iron
Serum PTH	If elevated, manage hyperparathyroidism
Serum CRP	If elevated, check for and treat infection or inflammation
Underdialysis	If underdialyzed, improve dialysis efficiency
ACEi/ARB use	If yes, consider reducing dose or discontinuing drug
Bone marrow biopsy	Manage condition diagnosed, *e.g.*, dyscrasia, infiltration, fibrosis

ACEi: Angiotensin-converting enzyme inhibitor; ARB: Angiotensin-receptor blocker; CRP: C-reactive protein; PTH: Parathyroid hormone.

Table 4 Nonhematologic complications associated with erythropoietin therapy[11]

Adverse effects
Hypertension
Injection site pain
Seizure
Pure red cell aplasia
Liver dysfunction
Shock, anaphylaxis
Thrombotic complications

notably in concurrence with high Hb levels[28].

ADVERSE EFFECTS OF ESA

Table 4 shows the major complications associated with EPO therapy[11].

Hypertension

Hypertension has been considered a common complication of ESA therapy, particularly in the early phase of indication for rHu-EPO therapy[29]. Previous studies have noted that rHu-EPO is the leading factor in approximately 20% patients with clinically important increases in blood pressure during amelioration of anemia. Hb levels, history of hypertension, and previous antihypertensive drug use were not confirmed as risks[30,31]. Similar to the observation in essential hypertension and CKD-associated hypertension, in which an increase in peripheral vascular resistance is the main cause, rHu-EPO-induced hypertension manifests hemodynamic changes[11]. An approximately 30% increase in systemic vascular resistance has been reported[32,33]. Management of this adverse event includes the adjustment of dry weight or limiting the rate of Hb increase and prescription of antihypertensive medication.

Thrombotic complications

The increased incidence of vascular access thrombosis or serious cardiovascular events associated with rHu-EPO therapy is multifactorial and controversial[34,35]. Churchill *et al*[36] reported an insignificant difference between patients treated with rHu-EPO and comparison groups in the time to development of the first thrombosis of fistula. However, rHu-EPO treatment increased the frequency of graft thrombosis[36]. Tang *et al*[37] confirmed that the occurrence of thrombosis in rHu-EPO-treated patients was not related to patients' hematological responses to the drug; rather, it depends on the integrity of patients' vasculature and the type of vascular access used.

Pure red cell aplasia

Normocytic anemia with decreased reticulocytes and

Table 5 Evaluation for pure red cell aplasia (Kidney Disease Improving Global Outcomes Guideline 2012)[20]

Investigate for possible antibody-mediated PRCA when a patient receiving ESA therapy for more than 8 wk develops the following:
Sudden rapid decrease in Hb concentration at the rate of 0.5 to 1.0 g/dL (5 to 10 g/L) per week OR requirement of transfusions at the rate of approximately 1 to 2 per week
Normal platelet and white cell counts
Absolute reticulocyte count less than 10000/mL
ESA therapy should be stopped in patients who develop antibody-mediated PRCA
Peginesatide should be used to treat patients with antibody-mediated PRCA

PRCA: Pure red cell aplasia; ESA: Erythrocyte-stimulating agents; Hb: Hemoglobin.

absence of erythroblasts is the main feature of Pure red cell aplasia (PRCA). The onset of secondary PRCA may follow parvovirus infection, leukemia, lymphoma, collagen disease, or rHu-EPO treatment. In Europe, the frequency of rHu-EPO-associated PRCA reached a peak in 2001-2002, in connection with Eprex (Johnson and Johnson, New Brunswick, NJ, United States)[38]. It was suggested that subepidermal immune reactions play an essential role in PRCA induced by rHu-EPO, as the condition was mostly induced by subcutaneous administration[39]. To guide the close examination and therapy of patients with a strong possibility of developing antibody-associated PRCA, recommendations based on expert opinions have been published[40,41]. The two major clinical features of antibody-mediated PRCA are as follows: (1) An associated decline in blood Hb levels of 4 g/dL per month; and (2) A decrease in the number of reticulocytes to < 10000/mL (Table 5)[20].

Currently, the incidence rate of PRCA is very low. However, PRCA may still occur even though rHu-EPO is thought to be well preserved under good storage conditions. This is because rHu-EPO has been more popular for the treatment of renal anemia[42,43].

We previously reported an elderly patient with PRCA who was positive for anti-erythropoietin (anti-rHu-EPO) antibodies[43]. Transfusions for symptomatic anemia and discontinuation of rHu-EPO treatment are important for the initial management of anti-EPO antibody-mediated PRCA[44]. Subsequent immunosuppressive therapy should be considered because PRCA in this setting is immune-mediated and because spontaneous remission is rare. In a previous study related to anti-EPO antibody-mediated PRCA (N = 47), 78% (N = 29/37) patients who received immunosuppressive therapy recovered[45], whereas all patients without immunosuppressive drug therapy (N = 9) did not recover from PRCA. According to another report of 62 PRCA patients who did not receive immunosuppressive therapy, only one patient showed spontaneous recovery[46].

Fisch *et al*[47] reviewed serum antibodies and natural killer cells or T-lymphocyte associated mechanisms of erythropoiesis. Rituximab (an anti-CD20 monoclonal antibody)[48] and alemtuzumab (an anti-CD52 monoclonal antibody)[49] are expected to represent an alternative therapeutic strategy for patients with refractory PRCA. However, the patient in our presented case report refused immunosuppressive treatment, and successful remission was achieved with cessation of rHu-EPO treatment alone. The patient's severe anemia gradually ameliorated along with a decrease in antibody titer[43]. In 1997, Prabhakar *et al*[50] reported the first case of a patient with PRCA caused by rHu-EPO who recovered after the discontinuation of ESA therapy. In 1996, Casadevall *et al*[51] reported a patients with PRCA and spontaneously decreasing anti-EPO antibodies. It should be emphasized that spontaneous PRCA remission following the cessation of rHu-EPO therapy is extremely unlikely. Early recognition as well as appropriate and prompt management is important for managing the consequences of this antibody-related PRCA.

FUTURE PROBLEMS TO RESOLVE

Peginesatide is an rHu-EPO receptor agonist without cross-reactivity with anti-EPO antibodies[52]. Peginesatide is expected to be a potential alternative therapy for managing patients with anti-EPO antibody-associated PRCA[53]. In February 2013, the drug industry recalled peginesatide because the Food and Drug Administration received reports of anaphylactic reactions following peginesatide administration, some of which resulted in death[54].

As an alternative to the administration of exogenous rHu-EPO or its mimetics, induction of endogenous EPO production by several mechanisms, including prolyl hydroxylase domain protein inhibitors and GATA-binding protein inhibitors, is considered to have potential advantages of better availability and lower immunogenicity[55].

Further research in this field is required to answer the following clinical questions[20]: (1) Is there any difference in outcomes between intravenous and subcutaneous ESA administration? (2) Can any vascular complications occur in association with normalization of Hb levels using ESA therapy? and (3) Are there any potential risks associated with ESA use in patients with a history of cancer?

CONCLUSION

rHu-EPO therapy is undoubtedly an effective strategy for renal anemia. However, clinicians must balance the benefits, such as a decrease in the requirement of transfusions and amelioration of anemia-related symptoms, with the potential side effects of rHu-EPO administration. Further research is expected to answer some interesting

clinical questions related to the use of rHu-EPO therapy for renal anemia.

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REVIEW

Review of novel therapeutic medicines targeting androgen signaling in castration-resistant prostate cancer

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Abstract

Prostate cancer is the most common male malignant neoplasm. Androgens and the androgen receptor (AR) play a key role in the onset and progression of prostate cancer. The expression of the AR is still preserved in the majority of patients with castration-resistant prostate cancer (CRPC). CRPC is considered to be induced by the following mechanisms: (1) sustained AR activation by enhancing intracellular conversion of adrenal androgens to dehydrotestosterone *via* a *de novo* route; (2) AR hypersensitivity; (3) promiscuous activation of AR signaling; and (4) outlaw pathways. Recent advances in the treatment of CRPC include novel medicines targeting AR signaling pathways. In addition, functional molecular studies have shown that some of the AR-regulated genes and AR coregulators are prognostic markers and potential therapeutic targets for prostate cancer, particularly in the castration-resistant state. Therefore, identification of the AR signaling pathways responsible for establishment of CRPC is critical for developing new strategies for the treatment of CRPC.

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Key words: Androgen receptor; Prostate cancer; Pyrrole-imidazole polyamide

Core tip: Prostate cancer is the most common male malignant neoplasm. Androgens and the androgen receptor (AR) play a key role in the onset and progression of prostate cancer. The expression of the AR is still preserved in the majority of patients with castration-resistant prostate cancer (CRPC). Therefore, identification of the AR signaling pathways responsible for establishment of CRPC is critical for developing new strategies for the treatment of CRPC.

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INTRODUCTION

Prostate cancer has been the most common male malignant neoplasm for more than 30 years and is the second leading cause of cancer-related death of men in the United States[1]. In Japan, partially because the diet seems to

be becoming Westernized, the incidence of prostate cancer has been increasing. The population of older males is also becoming larger and may also be a contributor.

Androgens and the androgen receptor (AR) play a key role in the onset and progression of prostate cancer. Functional ARs are expressed during various stages of prostate carcinogenesis, from prostate intraepithelial neoplasia to locally advanced primary tumors. Approximately 80%-90% of prostate cancers are androgen-dependent at the time of diagnosis[2-4].

Since the discovery that the progression of prostate cancer could be inhibited by castration in the 1940s[5,6], hormonal therapy that specifically inhibits AR activity by using a luteinizing hormone-releasing hormone analog/antagonist, with/without anti-androgens [androgen deprivation therapy (ADT)] has become the most effective and widely used palliative method for advanced and/or metastatic prostate cancer[7-9]. In the majority of patients, although ADT leads to a biochemical response for up to 3 years, prostate cancer eventually continues to progress through cell transformation. Previously, these conditions were known by various names over the years, including hormone-resistant prostate cancer and androgen-insensitive prostate cancer. However, most recent reports indicate that AR is still expressed in the majority of ADT resistant cases, and expression of AR target genes, such as prostate-specific antigen (PSA), remains persistently high despite serum testosterone in the castrated range after surgical castration or ADT[9-11]. This condition is called castration-resistant prostate cancer (CRPC)[12]. Patients with CRPC demonstrate poor prognosis associated with a deterioration in the quality of life, and few therapeutic options are currently available[13]. Therefore, it is important to understand the AR signaling pathway to develop an effective treatment for CRPC. In this review, we summarize the roles of the AR signaling pathway and novel therapeutic medicines that target this pathway in prostate cancer. We focus in particular on functional analyses of AR targets and indicate future directions for their therapeutic use.

AR STRUCTURE

The *AR* gene is a member of the steroid hormone receptor superfamily, which includes genes encoding receptors for estrogen, progesterone, glucocorticoids, mineralocorticoids, vitamin D, retinoic acid, and the retinoid X receptor. Similar to many other steroid receptors, the AR is characterized by a modular structure consisting of distinct functional domains: a poorly conserved N-terminal domain (NTD; 555 amino acids coded by exon 1), a highly conserved DNA-binding domain (DBD; 68-amino acid coded by exon 2 and 3), a hinge region, and a moderately conserved ligand binding domain (LBD; 295 amino acids coded by exons 4-8)[14]. The AR NTD contains the major transactivation function of the AR, which is known as activation function (AF)-1, and consists of two transcriptional activation units (TAU): TAU-1 and TAU-5[15]. AF-1 interacts with the LBD, the basal transcription factors transcription factor ⅡF (TFⅡF) and TFⅡH, members of the p160 family of nuclear receptor coactivator proteins and the general coactivator cAMP response-binding protein-binding protein[16-28]. These reports also indicate that AF-1 is one of the major domains responsible for mediating AR transcriptional activity.

The DBD has important roles in mediating AR nuclear localization, homodimer formation, and specific DNA binding. The activated AR binds as a homodimer to specific DNA sequences called androgen response elements (AREs) located around the target genes[29]. These AREs can be classified into two types: canonical and non-canonical AREs[29]. Canonical AREs consist of an inverted repeat of hexameric half-sites (5'-TGTTCT-3') with a 3-bp spacer[30]. The non-canonical AREs have an atypical motif, and their binding specificity to steroid receptors is relatively weak, resulting in their need for coregulators[31,32]. Data indicate that some important androgen-regulated genes in prostate cancer are regulated by atypical AREs. The DBD contains two zinc finger domains. The first zinc finger contains a conserved P-box motif that binds the half site of the classical ARE. The second zinc finger contains a conserved D-box motif that functions to stabilize the DNA-receptor complex and to produce a receptor homodimer[33].

The LBD is located in the C-terminal region and comprises 12 helices. The LBD assists in the binding of dihydrotestosterone (DHT), which is the first step in the androgen signaling pathway. Like the AF-1 region in the NTD, the LBD contains an AF2 that interacts with coregulators [the steroid receptor coactivator (SRC)/p160 family] to bind to the NTD[34,35]. Because AF-2 shows a higher affinity for the NTD than the coactivator, interaction with the NTD is the primary role for AF-2 rather than direct transcriptional activation. The LBD plays a key role in current ADT for prostate cancer. Although anti-androgens used in ADT, such as bicalutamide, block the activity of AF-2 by binding to the LBD[36], point mutations in the AR in prostate cancer primarily occur in the regions of the LBD that include amino acids 670-676, 701-730, and 874-919, causing resistance to anti-androgens binding and proliferation in the presence of the anti-androgens[37-39].

THE AR SIGNALING PATHWAY

Androgens, the male sex steroids, regulate numerous physiological responses ranging from male sexual differentiation to the development of bone and muscle. The biological action of androgens is mediated through the AR. In prostate tissue, DHT is the primary ligand for the AR and is synthesized from testosterone by 5-reductase. The ligand-unbound AR is present primarily in the cytoplasm, where it interacts with heat shock proteins (Hsp)-90, -70, -56, cytoskeletal proteins, and other co-chaperones[40]. The AR-Hsp90 interaction is necessary to maintain the AR in a high-affinity ligand-binding confor-

mation, suggesting that Hsp90 plays a key role in the activation of agonist-bound AR regulation of nuclear transfer, nuclear matrix binding, and transcriptional activity. Following DHT binding to the AR, the AR translocates into the nucleus, and binds to the ARE in the promoter and enhancer regions of target genes. For example, PSA is a typical product of the AR-dependent gene and an important biomarker for prostate cancer.

The AR transcriptional complex is completed by recruitment of coregulators, which ultimately results in regulation of gene expression[36]. After the discovery of SRC-1, more than 200 nuclear receptor coregulators have been identified[41,42]. Coregulators were previously classified as either enhancing (coactivators) or repressing (corepressors) AR activity, and the requirements for coregulators vary among genes[43].

The AR is also regulated by post-translational modifications generated by signal transduction pathways. These modifications can be further divided into two categories: (1) reversible modifications of specific amino acid residues of target proteins (phosphorylation and acetylation); and (2) modifications involving addition of other proteins or polypeptides (ubiquitination and sumoylation). These changes have the potential to affect AR stability, subcellular localization, and interaction with other proteins, including coregulators.

MECHANISMS OF CASTRATION RESISTANCE

Castration resistance has been reported to be induced by: (1) sustained AR activation by enhancing intracellular conversion of adrenal androgens to DHT *via* a *de novo* route[44]; (2) AR hypersensitivity[45]; (3) promiscuous activation of AR signaling; and (4) outlaw pathways[9]. AR hypersensitivity results in the facilitation of a susceptibility to androgen by variation of a coregulator or cytokine activity and overexpression of the AR. In addition, Cai *et al*[46] reported that prostate cancer cells incubated with levels of androgen comparable to those seen in castration decreased AR-induced lysine-specific demethylase 1 levels, which negatively regulates AR signaling, resulting in an increase in the expression of AR and of multiple genes that contribute to increased androgen synthesis and sensitivity in CRPC[46].

Promiscuous activation of the AR signaling pathway occurs in cases of AR structural change and when the AR combines with ligands other than androgen. This phenomenon induces the anti-androgen withdrawal syndrome, *i.e.*, anti-androgen itself serves as an accelerator of progression. Outlaw pathways include AR structural changes when androgen is absent or when cytokines other than androgen bind to the AR to activate a signaling pathway, resulting in a facilitation of an androgenic response in gene expression. The extragonadal androgen synthesized in adrenal or prostate cancer cells plays a key role in the occurrence of sustained AR activation. Androgen is a metabolite of cholesterol in the testis and adrenal gland. Most of its synthetases belong to the cytochrome P450 (CYP) family. CYP17 in particular has both 17-hydroxylase and 17,20-lyase activity that plays an important role in the synthesis of adrenal androgen. Castration does not influence the synthesis of adrenal androgen. Adrenal androgen is converted into DHT by 5-reductase in prostate cancer cells. The affinity of DHT for the ligand-binding domain on the AR is higher than testosterone as a main ligand in prostate cells. Furthermore, the CRPC cells contain increased CYP17 activity with a new metabolic pathway that converts cholesterol to androgen. Thus, the expression of androgen-dependent genes is achieved by a very small amount of testosterone under castration[47].

NEW THERAPEUTIC DRUGS TARGETING THE AR SIGNALING PATHWAY

Standard therapeutic strategies for prostate cancers have recently been changed to utilize newly developed medicines for CRPCs. As described above, because androgens and the ARs remaining in CRPC cells are important for cancer progression, novel medicines should be designed to target the androgen synthesis pathway or the AR signaling pathway (Figure 1). Here, we introduce these new medicines, including compounds currently being developed in Japan.

Abiraterone

Abiraterone is a dual inhibitor of the 17-hydroxylase and 17,20-lyase expressed in testicular, adrenal, and prostatic tumor tissues. Ketoconazole, an azole antifungal medicine that has a similar target as abiraterone, initially showed a preferable outcome in patients with CRPC[48]. Ketoconazole inhibits both testicular and adrenal androgen biosynthesis by targeting cytochrome P450 isozyme 3A4 and 17,20-lyase. However ketoconazole results in severe toxicities because it has low specificity for the CYP17 family. Based on these pieces of evidence, abiraterone was selected for development at the Institute of Cancer Research in the UK as a selective inhibitor of CYP17. The multicenter phase Ⅲ randomized placebo-controlled trial COU-AA-301 evaluated the efficacy of abiraterone compared with docetaxel for men with progressive CRPC[49]. The overall survival in the abiraterone arm was significantly longer than in the docetaxel arm (14.8 *vs* 10.9 mo). However, abiraterone induces hyperaldosteronism by reduction of glucocorticoid following secondary adrenocorticotropic hormone overexpression. Although steroids are useful to protect against such adverse effects, the combination of steroids with abiraterone could limit its use for patients with early-stage disease and longer life expectancies.

TAK-700

TAK-700 is a next-generation CYP17 inhibitor that requires no steroid administration. Because TAK-700 selectively targets 17,20-lyase, and inhibition of 17α-hydroxylase

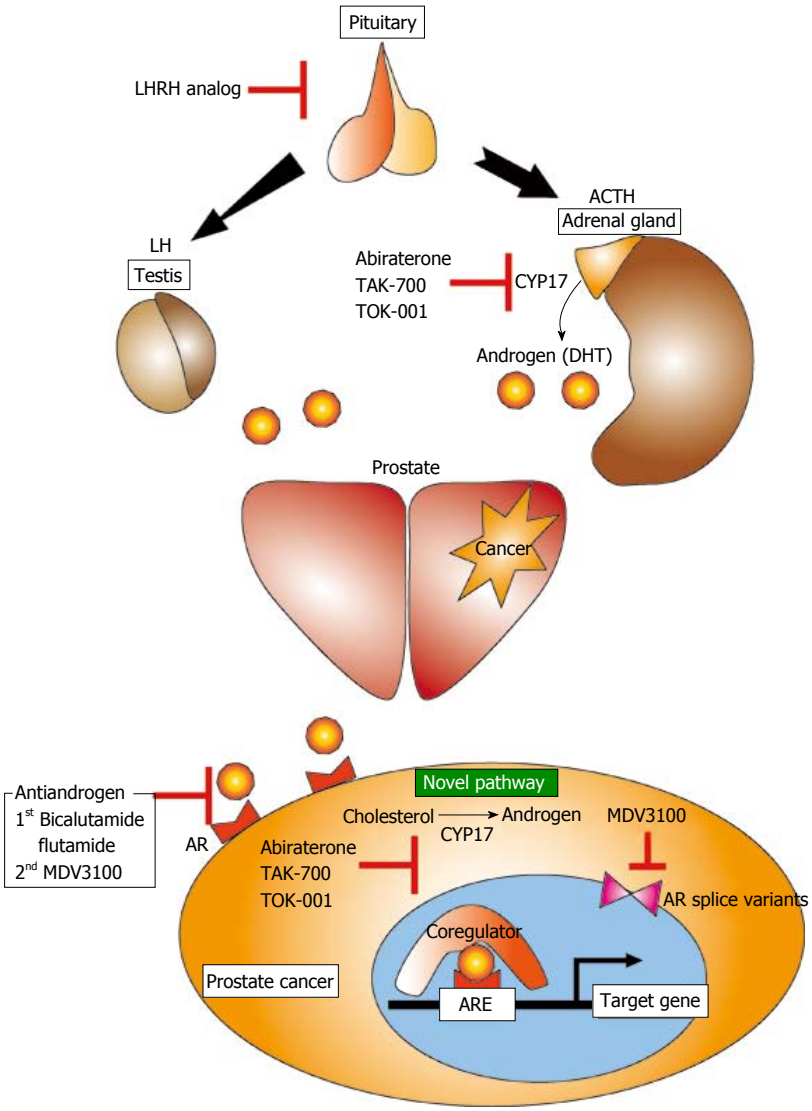

Figure 1 Schematic view of the therapeutic medicines targeting androgen receptor signaling pathways. ACTH: Adrenocorticotropic hormone; AR: Androgen receptor; ARE: Androgen response element; LH: Luteinizing hormone; LHRH: Luteinizing hormone-releasing hormone; DHT: Dihydrotestosterone; CYP: Cytochrome P450.

and reduction of glucocorticoids were smaller than those seen in patients treated with abiraterone[47,50]. A phase Ⅲ clinical trial of TAK-700 for metastatic CRPC (Evaluation of the Lyase inhibitor orteronel in Metastatic Prostate Cancer 5) is ongoing. The results of the interim analysis show that although overall survival was not significantly improved as a primary endpoint, progression-free survival was improved in the TAK-700 arm compared with the placebo arm (HR = 0.755).

TOK-001

The chemical composition of TOK-001 is similar to that of abiraterone. In addition to 17-hydroxylase inhibitory activity, TOK-001 also has AR antagonistic action[51-53]. Phase Ⅰ / Ⅱ clinical trials for CRPC are ongoing.

MDV3100

MDV3100 binds to the AR directly and targets multiple steps in the AR signaling pathway, including translocation to the nucleus and binding of the AR to the ARE and coregulators. Because MDV3100 differs from an agonistic anti-androgen, this compound does not induce anti-androgen withdrawal syndrome. MDV3100 induces the same action as the structural variant AR, which causes castration resistance; therefore, it is considered to be a second generation anti-androgenic agent[54]. In a phase Ⅲ double-blind, placebo-controlled trial (AFFIRM Clinical Trials), MDV3100 was superior in the proportion of patients with a reduction in PSA level by 50% or more, the quality-of-life response rate, progression-free survival, and overall survival of men with metastatic CRPC after chemotherapy[55]. Based on these results, MDV3100 obtained approval for treatment of metastatic prostate cancer in Europe. In addition, recent clinical trial (PREVAIL Clinical Trials) showed that MDV3100 significantly decreased the risk of radiographic progression and prolonged overall survival in men with metastatic CRPC who have not received chemotherapy[56].

DEVELOPMENT OF THERAPEUTIC DRUGS THAT TARGET THE ANDROGEN RESPONSIVE GENES

The goal of the AR signaling pathway is the transcriptional activation of target genes (androgen-responsive genes). The overexpression of the androgen-responsive genes is the main cause of cancer progression regardless of the presence or absence of castration resistance. We have reported the results of the functional analysis of novel androgen responsive genes and AR coregulators that influence the progression of prostate cancer[57-62]. Here, we introduce a part of these studies, including therapeutic medicines.

ADP ribosylation factor GTPase-activating protein 3

ADP ribosylation factor GTPase-activating protein 3 (*ARFGAP3*) is a novel androgen-regulated gene that is considered to be associated with regulation of the vesicular transport of the Golgi apparatus. In androgen-sensitive prostate cancer LNCaP cells, we observed induction of ARFGAP3 expression at the mRNA and protein levels in response to stimulation with 100 nmol/L DHT[59]. In functional analyses using LNCaP cells, the increased expression of ARFGAP3 was associated with cell growth, the G1/S cell cycle progression and cell migration. In addition, we found that ARFGAP3 interacted with paxillin as an AR coactivator, and enhanced migration activity and AR activity in LNCaP cells[59]. These findings suggest that *ARFGAP3* is a novel androgen-regulated gene that can promote prostate cancer cell proliferation and migration in collaboration with paxillin.

Octamer transcription factor 1

Octamer transcription factor 1 (Oct1) is a ubiquitous member of the Pit-Oct-Unc-homeodomain family. Although Oct1 does not demonstrate androgenic responsiveness, it works as a coregulator of the AR, binding to neighbors of the ARE and regulating AR activity[29]. We found that Oct1 is expressed in the nuclei of LNCaP cells using immunocytochemistry. SiRNA silencing of Oct1 inhibited the proliferation in LNCaP cells[60]. In addition, using surgical specimens, we found a positive correlation between Oct1 immunoreactivity in samples with a high Gleason score and AR immunoreactivity. Moreover, patients with high immunoreactivities of both Oct1 and AR exhibited poorer cancer-specific survival[60]. These results demonstrate that Oct1 may be a prognostic factor for prostate cancer and a contributing factor for increased AR sensitivity and castration resistance.

Pyrrole-imidazole polyamides

Pyrrole-imidazole (PI) polyamides are small synthetic molecules that recognize and attach to the minor groove of DNA to inhibit DNA-transcription factor interactions with high affinity and sequence specificity[63-65]. DNA recognition by PI polyamides depends on a code of side-by-side pairing of pyrrole and imidazole in the hairpin structure. Various types of sequence-specific PI polyamides have recently been developed to control gene expression[66-69]. One of the most important advantages of PI polyamide is their resistance to biological degradation by nucleases and proteases compared to nucleic acid medicines, including siRNA. In addition, PI polyamides could be efficiently delivered to cell nuclei without any specific drug delivery system. Another important advantage is the safety of PI polyamides when they are injected intravenously or *via* peritoneal to mice or rats[66,67]. PI polyamide that recognizes AREs suppresses DHT-dependent gene expression in LNCaP cells[70]. In addition, this polyamide was reported to inhibit the binding of RNA polymerase Ⅱ to the transcription start site of AR-driving genes[71]. These reports indicate that PI polyamides could be a powerful tool in the development of molecularly targeted therapeutics for androgen responsive genes/AR coregulators in prostate cancer.

CONCLUSION

The clinical challenges in prostate cancer are currently focused on controlling the action of the AR, which plays an important role in the development of hormone therapy naïve prostate cancer and also CRPC. Recent evidence shows that CRPC cells are still dependent on AR activity after ADT. Selective inhibition of the AR signaling pathway by typical ADT induced a bypass mechanism to activate the AR in low dose or in the absence of DHT, and thereby restore AR-dependent cellular proliferation. Thus, blocking the AR with second-generation AR antagonists has the potential to treat CRPC because of stronger and more durable inhibition of transcriptional activity than previous compounds. Various functional studies, including our reports, have cited androgen-regulated genes and AR collaborating factors, such as Oct1, as preferable candidates for biomarkers and therapeutic targets for CRPC. We believe future investigations of the AR signaling pathway and novel therapeutics targeting this pathway are mainstays for considering new strategies to treat CRPC.

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REVIEW

Stem cell therapy for erectile dysfunction

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Abstract

Erectile dysfunction (ED) is an important health problem that has commonly been clinically treated using phosphodiesterase type 5 inhibitors (PDE5Is). However, PDE5Is are less effective when the structure of the cavernous body has been severely injured, and thus regeneration is required. Stem cell therapy has been investigated as a possible means for regenerating the injured cavernous body. Stem cells are classified into embryonic stem cells and adult stem cells (ASCs), and the intracavernous injection of ASCs has been explored as a therapy in animal ED models. Bone marrow-derived mesenchymal stem cells and adipose tissue-derived stem cells are major sources of ASCs used for the treatment of ED, and accumulated evidence now suggests that ASCs are useful in the restoration of erectile function and the regeneration of the cavernous body. However, the mechanisms by which ASCs recover erectile function remain controversial. Some studies indicated that ASCs were differentiated into the vascular endothelial cells, vascular smooth muscle cells, and nerve cells that originally resided in the cavernous body, whereas other studies have suggested that ASCs improved erectile function *via* the secretion of anti-apoptotic and/or proangiogenic cytokines rather than differentiation into other cell types. In this paper, we reviewed the characteristics of stem cells used for the treatment of ED, and the possible mechanisms by which these cells exert their effects. We also discussed the problems to be solved before implementation in the clinical setting.

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Key words: Erectile dysfunction; Stem cell therapy; Bone marrow-derived mesenchymal stem cells; Adipose tissue-derived stem cells; Endothelial progenitor cells; Adrenomedullin; Angiopoietin-1

Core tip: Adult stem cells (ASCs) have been used for the treatment of erectile dysfunction. Although previous studies reported that ASCs differentiated into cells that originally resided in the cavernous body, recent studies indicate that the major, if not all, effects of ASCs on erectile function are achieved through the secretion of paracrine factors rather than their direct differentiation into the cells in the cavernous body. Among various cytokines that ASCs produce, we have recently identified adrenomedullin as a candidate peptide that is implicated in the restoration of erectile function. We introduced these data in this review.

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INTRODUCTION

Erectile dysfunction (ED) is a worldwide health problem. Although psychogenic factors are a major cause of ED, other factors such as age, diabetes, total prostatectomy and radiation in the pelvis also contribute to the occurrence of ED. These factors cause structural changes as well as functional abnormalities in the cavernous body,

and therefore selective phosphodiesterase type 5 inhibitors (PDE5Is) are not so effective in the treatment of these diseases. For the recovery of erectile function in these patients, the regeneration of the cavernous body is necessary. In this regard, much attention has recently been placed on gene therapy and stem cell therapy.

Stem cells are defined as being capable of self-renewal and of differentiation into a variety of phenotypes[1]. There are two categories of stem cells: embryonic stem cells (ESCs) and adult stem cells (ASCs). ESCs were originally isolated from the inner cell mass of blastocysts[2]. ESCs are pluripotent stem cells that can give rise to the three germ layers. However, harvesting ESCs requires the destruction of human embryos and has therefore raised ethical concerns. To overcome this limitation, induced pluripotent stem (iPS) cells have been produced. Adult fibroblasts were reprogrammed by introducing four factors, Oct3/4, Sox2, c-Myc and Klf4 under ESCs culture conditions[3,4]. iPS cells are pluripotent stem cells with very similar characteristics to ESCs. Furthermore, many groups have now succeeded in reprograming somatic cells to create iPS cells by overexpression of variable sets of several transcription factors in cells without employing viruses or vectors[5-7]. Therefore, iPS cells are a promising option for regenerative medicine in the near future. A further option for avoiding the ethical problems of ESCs is the use of ASCs, which are basically multipotent stem cells that reside in various tissues, including the brain, skeletal muscle, bone marrow, adipose tissue, and dental pulp[8-11]. Besides having the potential to differentiate into various cell types, ASCs produce a broad range of cytokines that exert their effects in a paracrine and/or autocrine manner. Among ASCs, bone marrow-derived mesenchymal stem cells (BMMSCs) are the most commonly studied. BMMSCs reportedly have a potential to differentiate into various cell types including bone, cartilage, cardiac muscle, skeletal muscle, vascular endothelial cells (VECs) and vascular smooth muscle cells (VSMCs)[12,13]. Recently, adipose tissue-derived stem cells (ADSCs) have gained much attention because of the simplicity of harvesting hundreds of grams of subcutaneous adipose tissue without using invasive procedures, whereas painful bone marrow aspiration is required to collect just grams of bone marrow. Similar to BMMSCs, ADSCs have the potential to differentiate into various cell types[14]. ADSCs strongly resemble BMMSCs in that they share similar expression patterns of cell surface markers and similar gene expression profiles[15,16].

TREATMENT OF ED USING ASCs

BMMSCs

Many studies have demonstrated the efficacy of BMMSCs in the treatment of ED, and their efficacy seems to be reliable[17-27]. However, the mechanism by which BMMSCs restore erectile function remains controversial. BMMSCs were originally believed to home in on damaged tissues efficiently and differentiate into various cells at the target site. Genetically manipulated BMMSCs were also used expecting that these cells remain in the tissues and express a specific gene over a long period. Bivalacqua *et al*[17] infected BMMSCs with an adenovirus expressing endothelial nitric oxide synthase (eNOS) and injected these cells into the penis of aged rats. The eNOS-modified BMMSCs restored erectile function, with the recovery being associated with increased eNOS protein expression, NOS activity, and cyclic guanosine monophosphate levels. Furthermore, the authors demonstrated that the injected BMMSCs survived for at least 21 d in the cavernous body and expressed markers for VECs and VSMCs. Song *et al*[18] used immortalized human BMMSCs and injected them into rat penises, where the cells expressed markers for VECs and VSMCs in the cavernous body, although the authors did not examine whether erectile function was restored or not. Qiu *et al*[21] injected BMMSCs into the penis of streptozotocin (STZ)-induced diabetic rats and found that the BMMSCs injection restored erectile function. The authors also demonstrated that injected cells remained in the cavernous body for at least 4 wk and some expressed markers for VECs and VSMCs. Although the injection of BMMSCs restored erectile function in these studies, there are several problems. First, these studies did not clearly calculate the percentage of injected BMMSCs that remained in the cavernous body and obtained markers for VECs and VSMCs, or the percentage mentioned in these studies was not sufficient to explain the recovery of erectile function. Second, there was a possibility that the injected BMMSCs fused with the residing cells in the cavernous body and acquired the phenotype of the residing cells. Terada *et al*[28] used BMMSCs obtained from female transgenic mice expressing green fluorescent protein (GFP) and puromycin resistance gene. The BMMSCs were cocultured with a male embryonic stem cell line and then puromycin was added to remove the embryonic stem cells. The remaining cells were GFP positive and puromycin resistant, and morphologically similar to embryonic stem cells. These cells could be induced to differentiate into cardiac myocytes and neuronal cells, suggesting that embryonic stem cell-like pluripotent stem cells were established from BMMSCs. However, following DNA ploidy (the number of DNA copies) analysis using fluorescence-activated cell sorting, the cells were found to be tetraploid (4n) or hexaploid (6n), suggesting that they had developed from spontaneous fusion between the BMMSCs and the embryonic stem cells. The possibility of cell fusion *in vivo* was also reported. Alvarez-Dolado *et al*[29] used transgenic mice that contain the *lacZ* reporter gene downstream of a stop codon flanked by loxP sites (floxed). Therefore, the *lacZ* reporter gene was only expressed when the loxP-flanked stop codon was excised by Cre recombinase. The authors lethally irradiated these mice and intraperitoneally injected BMMSCs from mice that ubiquitously express Cre recombinase and GFP. If cells from the donor and recipient fused, the Cre enzyme would excise the Lox P-flanked stop codon, thereby allowing expression of

the *lacZ* gene. Results revealed that β-gal+ (fused) and GFP+ cells were found in the brain, heart, and liver of recipients, 2 and 4 mo post-transplantation[29]. Thus, BMMSCs potentially fuse with other cell types *in vivo* and it appears that BMMSCs are differentiated into other cell types because of this phenomenon. In contrast, the paracrine effects of BMMSCs have been reported. Kendirci *et al*[20] isolated BMMSCs positive for p75 low-affinity nerve growth factor receptor using magnetic-activated cell sorting, and injected these cells into the penis of rats following bilateral cavernous nerve crush injury. The injection of these cells restored erectile function[20]. The engraftment of these cells in the cavernous tissue occurred very rarely, and the engrafted cells appeared fibroblastic. Furthermore, these cells secreted fibroblast growth factor 2 (FGF2), which suggested that FGF2 might protect the cavernous nerve after crush injury. More direct evidence of the paracrine effects of BMMSCs was reported by Yeghiazarians *et al*[30] who injected BMMSCs extracts into infarcted hearts and found that the procedure effectively improved cardiac function, suggesting that BMMSCs *per se* were not required for their tissue protective effects. Although this scenario is attractive, no cytokines that are implicated in the recovery of erectile function have been specifically identified.

ADSCs

The efficacy of ADSCs in the treatment of ED seems to be reliable from the results of many previous reports[31-45]. However, the mechanisms by which ADSCs restore erectile function remain controversial. Ning *et al*[46] reported that ADSCs could differentiate into VECs. ADSCs injected into the penis obtained a marker for VECs. FGF2 appeared to be necessary for the differentiation of ADSCs into VECs *in vitro*, although the functional role of FGF2 in the differentiation of ADSCs into VECs *in vivo* was not studied[46]. Ryu *et al*[40] demonstrated that the injection of ADSCs into the penis of STZ-induced diabetic mice restored erectile function. They also found that some injected ADSCs became CD31 positive, suggesting that these cells differentiated into VECs[40]. However, ADSCs injected into the penis disappeared within 14 d. Kim *et al*[43] applied human ADSCs and nerve growth factor-incorporated hyaluronic acid-based hydrogel to the cavernous nerve of rats following bilateral cavernous nerve crush injury. The authors demonstrated that this treatment restored erectile function. They also showed that some ADSCs were engrafted into the cavernous nerve 4 wk after treatment, suggesting that ADSCs could differentiate into nerve tissue[43]. Although these studies showed that ADSCs have the ability to differentiate into the cells located in the cavernous body, the possibility of cell-cell fusion was not excluded in these studies. Alternatively, paracrine effects of ADSCs have been suggested. Several reports suggested the possibility of paracrine effects of ADSCs because ADSCs did not remain in the cavernous body for a long period[32,33]. Albersen *et al*[31] injected both ADSCs and the lysate of ADSCs into the penis of rats that were subjected to bilateral cavernous nerve crush injury. They demonstrated that the injection of ADSCs lysate significantly restored erectile function[31]. These results suggested that most, if not all, of the effects of ADSCs were mediated through their production of cytokines and/or immune modulators, although the authors did not identify any molecules that are functionally implicated in the restoration of erectile function. Zhang *et al*[47] reported that ADSCs produced chemokine (C-X-C motif) ligand 5 (CXCL5) and that CXCL5 was implicated in the neurotrophic effects of ADSCs *in vitro*, although they did not confirm this finding *in vivo*. We recently reported that adrenomedullin (AM) is implicated in ADSCs-induced restoration of erectile function in diabetic rats[36]. AM was originally isolated from human pheochromocytoma tissue, and has potent vasorelaxant and diuretic effects[48]. In addition, AM is also produced by VECs, VSMCs and macrophages[49-51] and has the ability to stimulate angiogenesis[52-54]. When rat ADSCs were cultured in a medium containing growth factors for VECs, ADSCs produced significant amounts of AM (Figure 1A). The injection of ADSCs into the penis of diabetic rats restored erectile function (Figure1B), the morphology of the cavernous body (Figure 1C), and the expression of vascular endothelial (VE)-cadherin, a marker for VECs. However, when the expression of AM was knocked down using a small interfering RNA for AM, the favorable effects of ADSCs disappeared (Figure 1B and C). Furthermore, when AM was overexpressed in the penis using an adenovirus expressing AM, erectile function and the morphology of the cavernous body were restored in diabetic rats (Figure 1B and C). We also demonstrated that ADSCs produce angiopoietin-1 (Ang-1) and Ang-1 secreted from ADSCs are implicated in ADSCs-induced suppression of neointimal formation and stimulation of reendothelialization in a wire injury model of the rat femoral artery[55]. Furthermore, we reported that overexpression of both AM and Ang-1 using adenoviruses expressing those proteins restored erectile function in diabetic rats to the same level as that observed in age-matched positive control rats (Figure 2)[44]. Therefore, it seems obvious that ADSCs produce various cytokines that potentially restore erectile function. The limitation is that ADSCs do not remain in the cavernous body for a long period, usually disappearing within a month[32,33,36]. Where do they go? Do they die or migrate to other tissues? Several interesting papers have been published regarding these points. Lin *et al*[56] injected rat ADSCs and traced their locations after 2 and 7 d. ADSCs remained not only in the penis but also in the major pelvic ganglia (MPG), spleen and bone marrow. ADSCs preferentially remained in the bone marrow and the number of ADSCs remaining in the bone marrow was much larger than that remaining in the penis. Because ADSCs secrete various cytokines, this result suggests that ADSCs remaining outside the penis may affect the restoration of erectile function, although the survival of ADSCs for long periods was not examined in the study. Several

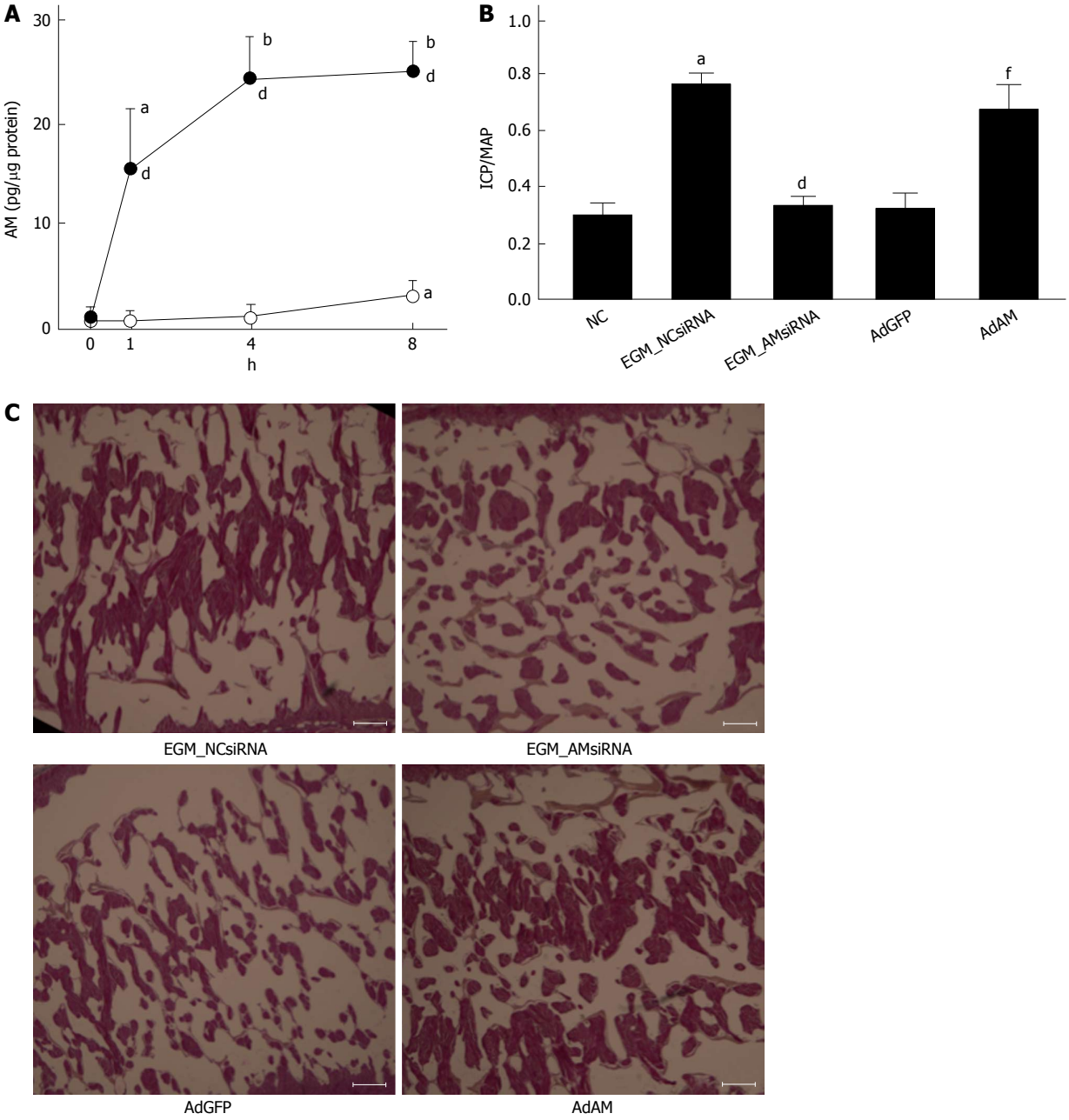

Figure 1 Production of adrenomedullin by adipose tissue-derived stem cells, and effects of knockdown and overexpression of adrenomedullin on the function and histology of the penis. A: Adipose tissue-derived stem cells produce adrenomedullin especially when they were cultured in a medium containing growth factors for vascular endothelial cells (VECs); adipose tissue-derived stem cells (ADSCs) were cultured in endothelial basal medium (EBM: white circles) or endothelial growth medium (EGM: black circles) that contains growth factors for VECs. Medium was replaced with serum-free medium and incubated for the indicated periods. Adrenomedullin (AM) accumulated in the medium was measured. $^{a}P < 0.05$ *vs* 0 h, $^{b}P < 0.01$ *vs* 0 h and $^{d}P < 0.01$ *vs* EBM culture at each time point ($n = 6$ per group); B: Effect of knockdown and overexpression of AM on erectile function. ADSCs were infected with lentivirus expressing negative control siRNA (LV_NCsiRNA) that is predicted not to target any known vertebrate gene or lentivirus expressing AM siRNA (LV_AMsiRNA). ADSCs were cultured in EGM for 1 wk, and those LV_NCsiRNA-infected ADSCs (EGM_NCsiRNA) and LV_AMsiRNA-infected ADSCs (EGM_AMsiRNA) were injected in the cavernous body of STZ-induced diabetic rats. ICP was measured 4 wk after the ADSCs injection. An adenovirus expressing AM (AdAM) or adenovirus expressing green fluorescent protein (AdGFP) was also injected into the cavernous body of STZ-induced diabetic rats, and ICP was measured 4 wk after the infection. Nontreated STZ-injected diabetic rats were used as the negative control (NC). Bar graphs show ICP/MAP ($n = 5$ per group). $^{a}P < 0.001$ *vs* NC, $^{d}P < 0.001$ *vs* EGM_NCsiRNA injection and $^{f}P < 0.001$ *vs* AdGFP infection; C: Effect of knockdown and overexpression of AM on the morphology of the cavernous body. Experiments were performed in the same way as described in the legend for Figure 1B. The cavernous body was stained by the Elastica van Gieson method. The histology of the root portion of the penis (longitudinal section) is shown. Bars are 200 μm. Note that the size of trabeculae of the cavernous body is smaller when EGM_AMsiRNA or AdGFP was injected into the penis of diabetic rats, compared with when EGM_NCsiRNA or AdAM was injected into the penis. ICP: Intracavernous pressure; STZ: Streptozotocin; MAP: Mean arterial pressure.

studies have reported that ADSCs injected into the penis or placed around the prostate gland migrated to the MPG[35,39,45]. Fandel *et al*[35] demonstrated that ADSCs injected into the penis migrated to the MPG, although ADSCs were not engrafted in the nerve tissue. Interestingly, the expression of stromal cell derived factor-1 (SDF-1)

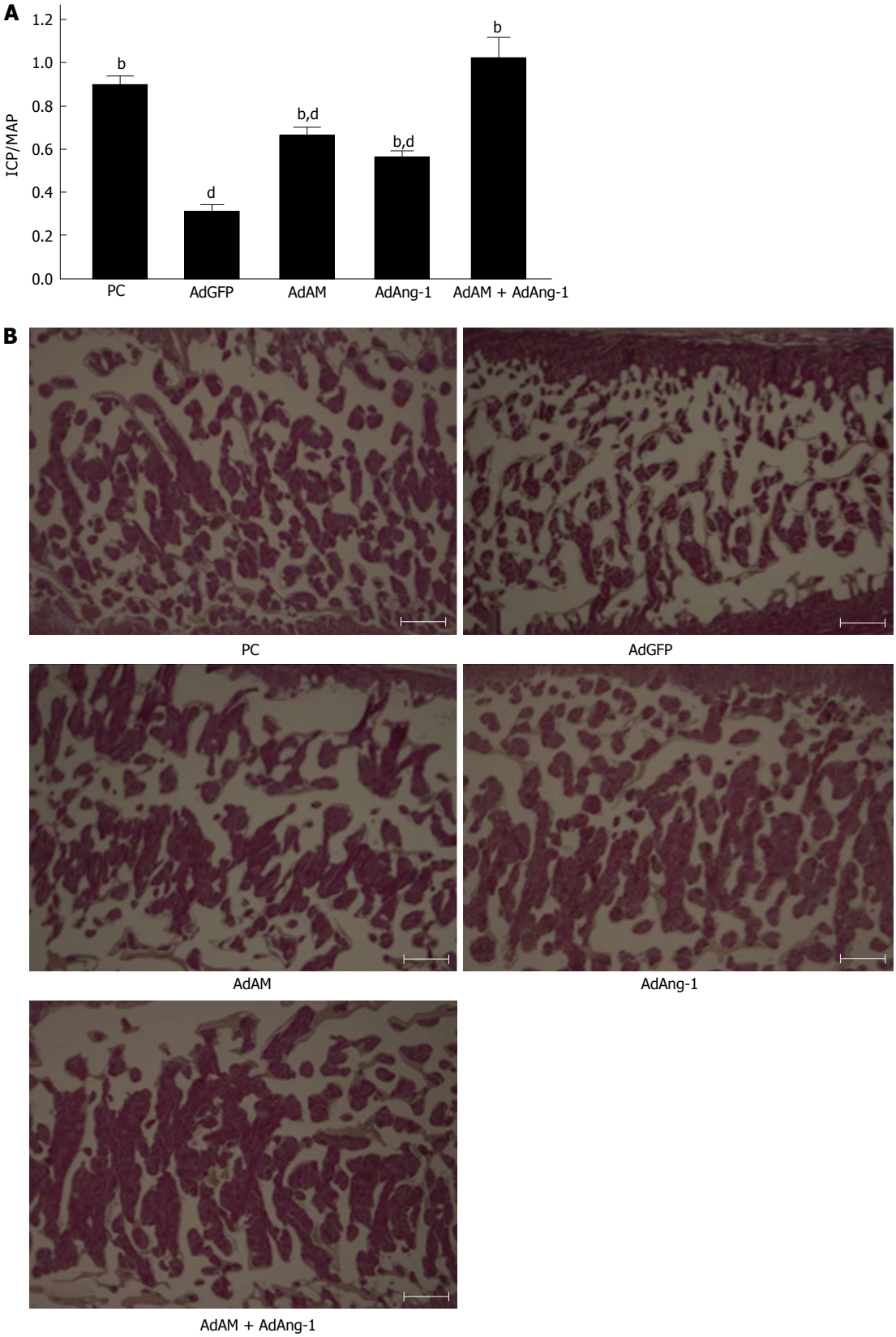

Figure 2 Effects of adrenomedullin and angiopoietin-1 on the function and morphology of the penis. A: Effects of adenovirus expressing adrenomedullin (AdAM), adenovirus expressing angiopoietin-1 (AdAng-1) and AdAM plus AdAng-1 infection on erectile function. These adenoviruses were infected into the cavernous body of STZ-induced diabetic rats. Adenovirus expressing green fluorescent protein (AdGFP) was also infected as the negative control. Age-matched Wistar rats were used as the positive control (PC). Bar graphs demonstrate ICP/MAP (n = 6 per group). $^{b}P < 0.001$ *vs* AdGFP infection and $^{d}P < 0.001$ *vs* PC; B: Histological analysis of the cavernous body after adenoviral infection. Elastica van Gieson staining of the cavernous body isolated from age-matched Wistar rats (PC), and STZ-induced diabetic rats infected with AdGFP, AdAM, AdAng-1, and AdAM plus AdAng-1. The histology of the root portion of the penis (longitudinal section) is shown. Bars are 300 μm. Note that the size of trabeculae of the cavernous body is small when AdGFP is injected into the penis of diabetic rats, and that the size is restored to a similar level as observed in the age-matched control group when AdAM and/or AdAng-1 are injected. ICP: Intracavernous pressure; STZ: Streptozotocin; MAP: Mean arterial pressure.

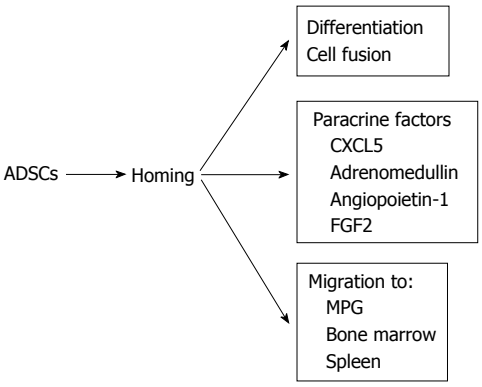

Figure 3 Possible mechanisms by which adipose tissue-derived stem cells stimulate the recovery of erectile function. FGF2: Fibroblast growth factor 2; MPG: Major pelvic ganglia; ADSCs: Adipose tissue-derived stem cells; CXCL5: Chemokine (C-X-C motif) ligand 5.

increased in MPG, suggesting that ADSCs preferentially migrated to the site of SDF-1 production. Qiu *et al*[39] also reported that intracavernously injected ADSCs migrated to the MPG, where they remained 17 wk after injection, although the number of ADSCs remaining in the MPG was quite low. You *et al*[45] showed that periprostatic implantation of ADSCs, but not their intracavernous injection, resulted in the migration of ADSCs to MPG. In summary, some ADSCs injected into the penis migrated to the tissues such as the bone marrow and MPG, and these cells may be implicated in the restoration of erectile function. The possible mechanisms by which ADSCs restore erectile function are summarized in Figure 3.

Endothelial progenitor cells

Endothelial progenitor cells (EPCs) were originally isolated from human peripheral blood by Asahara *et al*[57]. They isolated CD34-positive mononuclear blood cells and demonstrated that these cells obtained the characteristics of VECs when cultured on fibronectin-coated dishes. They also demonstrated that these cells were incorporated in ischemic tissues *in vivo* and expressed markers for VECs such as CD31 when introduced into the circulation using a hindlimb ischemia model. Furthermore, the authors showed that Flk-1-positive mononuclear blood cells were also integrated in the capillaries and small arteries when the hindlimb ischemia model was used. These cells were designated as EPCs. EPCs are progenitor cells whose differentiation potential is restricted to one lineage (VECs); therefore, they are not multipotent stem cells. Results of subsequent studies revealed that EPCs express three cell surface markers; CD133 (termed originally AC133), CD34, and Flk-1[58-60]. Premature EPCs either in the bone marrow or immediately after entering into the systemic circulation are positive for CD133/CD34/Flk-1. However, when EPCs become more mature, they lose the expression of CD133 and begin to express CD31 and VE-cadherin. An attempt to use EPCs for the treatment of ED has been reported by Gou *et al*[61]. They transfected EPCs with the vascular endothelial growth factor gene and injected the transfected cells into the penis of diabetic rats. The authors found that this treatment restored erectile function and the injected cells were integrated into the sites of neovascularization in the cavernous body[61]. However, most researchers in this field regard EPCs as a marker for ED rather than a therapeutic tool for ED. Factors affecting the number of circulating EPCs or their functions have been reported. The number of circulating EPCs and their migratory activity are reportedly reduced in patients with coronary risk factors[62,63]. EPCs isolated from type 2 diabetes patients have a decreased capacity for proliferation and the formation of capillary tubes *in vitro*[64]. In contrast, the number of circulating EPCs rapidly increases after limb ischemia and acute myocardial infarction[65,66]. Because atherosclerosis and ED share a common feature, *i.e.*, endothelial dysfunction, it has been speculated that the dynamics of EPCs might change in ED. Indeed, several studies have reported that the number of circulating EPCs decreased in ED patients[67-69], suggesting that the decrease in the number of circulating EPCs can predict the presence of ED as well as cardiovascular diseases. Interestingly, the number of circulating EPCs increased when patients were administered statins or PDE5Is[70-73] probably *via* the mobilization of EPCs from the bone marrow. Therefore, these drugs may improve erectile function *via* the mobilization of EPCs to the cavernous body.

Muscle-derived stem cells

Muscle-derived stem cells (MDSCs) are ASCs that exist in skeletal muscle. MDSCs can be obtained from autologous muscle biopsies and have been used for the treatment of ED in several studies. Nolazco *et al*[74] injected MDSCs into the penis of aged rats and found that MDSCs differentiated into VSMCs in the cavernous body, resulting in the recovery of erectile function. Woo *et al*[75] used a bilateral cavernous nerve injury model in rats and examined the effects of the injection of MDSCs into the penis on erectile function. They demonstrated that MDSCs remained in the cavernous body 4 wk after injection, and erectile function was significantly restored. Kovanecz *et al*[76] used a bilateral cavernosal nerve resection model of rats and, following the injection of MDSCs into the cavernous body, found that erectile function was restored and α-smooth muscle actin expression was increased. The injection of MDSCs also increased the expression of neural nitric oxide synthase and brain-derived neurotrophic factor[76]. In summary, MDSCs seem to be useful for the treatment of ED. However, because harvesting MDSCs from the skeletal muscle is relatively more invasive than the collection of ADSCs, the beneficial characteristics of MDSCs compared with ADSCs should be clarified before introducing them to clinical application.

Umbilical cord blood stem cells

Umbilical cord blood stem cells (UCBSCs) are an attractive type of stem cells in that they are youngest stem cells among a variety of ASCs. Because they are young, they have less possibility to have DNA damage than other ASCs[77,78]. Bahk *et al*[79] used human UCBSCs to treat

diabetic patients with ED, and demonstrated that erectile function was restored and that blood glucose levels decreased in these patients, although the mechanisms remain unknown.

Brain-derived stem cells

Brain-derived stem cells (BDSCs) reportedly have capacity to differentiate into VSMCs[80]. Song *et al*[81] isolated fetal BDSCs from embryonal 12-d rats and injected them into the penis. They demonstrated that injected BDSCs obtained characteristics of VSMCs *in vivo* 6 wk after injection[81], although they did not examine their effect on erectile function. Considering the source of BDSCs, it will be difficult to use them in clinical settings.

Neural crest stem cells

Neural crest stem cells (NCSCs) are the progenitor cells of several cell types that constitute the peripheral nervous system, including neurons, Schwann cells, adrenal chromaffin cells and smooth muscle cells. Transplantation of NCSCs could reportedly induce the regeneration of connective tissues, VSMCs, skeletal muscle and VECs[82,83]. Song *et al*[84] injected NCSCs into the penis of rats and demonstrated that they obtained markers for VECs and VSMCs 2 wk after injection, although their effects on erectile function was not analyzed[84]. Clinical application of NCSCs may also be difficult considering the source of these cells.

Treatment of ED using ESCs and iPS cells

Because iPS cells have been created, clinical application of pluripotent stem cells will be intensively explored in the future. To our knowledge, no studies have been published in which iPS cells or iPS cells-derived cells were used to treat ED. Bochinski *et al*[85] used ESCs that had differentiated into the neural cell line, and injected them into the MPG or cavernous body using a bilateral cavernous nerve injury model. They found that the injection into the both MPG and cavernous body restored erectile function. They also found that neurofilament staining was recovered in the ESCs-injected group[85]. Therefore, ESCs and iPS cells may be useful for treatment of ED. However, these cells may not efficiently home and survive for a long period under persistent inflammation. For example, in diabetic conditions hyperglycemia and adipocytokines induce persistent inflammation in the tissues. Implanted cells may not home and survive under these conditions unless such an inflammation is sufficiently controlled.

FUTURE DIRECTIONS

As mentioned above, stem cell therapy for ED appears to be a promising strategy. However, several problems should be solved before moving to clinical application.

Tumorigenesis

It is well known that ESCs and iPS cells easily form tumors, because these cells are pluripotent. Although ASCs seem to be less prone to forming tumors, ASCs can form malignant tumors when transplanted *in vivo*[86]. Jeong *et al*[86] injected BMMSCs into the peri-infarct area of myocardial infaction (MI) model of mice and hindlimb muscle of mice with diabetic neuropathy. They found sarcoma formation in 30% of hearts in the MI model and in 46% of hindlimbs in the diabetic neuropathy model[86]. Therefore, it will be necessary to sufficiently investigate the malignant potential of stem cells prior to their clinical use and establish methodology to select "healthy" stem cells that will not form tumors.

Fate of injected cells

As described above, some (most) of stem cells injected into the penis do not remain in the penis and migrate to other tissues such as the bone marrow and spleen. Little is known about the fate of these cells that have migrated to non-diseased organs. Detailed examinations will be necessary to detect the fate of these cells before moving to clinical applications.

How to improve homing and survival of stem cells

Most studies suggested that injected stem cells disappeared from the penis in one month. It is crucially important to explore methods to improve homing and survival of stem cells. It was reported that expression of SDF-1 was increased in the MPG and SDF-1 might stimulate migration of ADSCs to the MPG[35]. Therefore, SDF-1 is a candidate that stimulates migration and homing of stem cells into injured sites. Intensive studies will be necessary to identify molecules that are implicated in migration, homing and survival of stem cells.

Activation and mobilization of endogenous stem cells

It is suggested from EPC study that statins or PDE5Is can stimulate mobilization of EPCs from the bone marrow[70-73]. Therefore, it may be possible to activate and/or mobilize tissue-residual endogenous stem cells by some drugs. If endogenous stem cells residing in the penis can be efficiently activated, it will help to regenerate the cavernous body. This possibility should be examined in the future.

Identification of paracrine factors

ASCs produce a variety of paracrine factors that potentially regenerate the cavernous body. However, paracrine factors that are implicated in the regeneration of the cavernous body have not been sufficiently identified. Furthermore, it remains unknown what combinations of these paracrine factors are most suitable to stimulate the regeneration of the cavernous body. If these problems are solved, administration of cytokines cocktail may be more effective to treat ED than ASCs injection.

CONCLUSION

Stem cells especially ASCs have been used in the treatment of ED, and stem cell therapy seems to be effective

at least in animal modes. Major, if not all, effects of ASCs on erectile function appear to be achieved by secretion of paracrine factors rather than their direct differentiation into cells residing in the cavernous body. Before moving to clinical application, malignant potential of stem cells should be carefully considered. It is also necessary to explore methods to improve homing and survival of stem cells.

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REVIEW

Postnatal management of antenatally detected hydronephrosis

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Abstract

With the increasing use of ultrasonography, congenital anomalies are often picked *in utero*. Antenatally detected hydronephrosis is amongst the most commonly detected abnormality. The management of this condition has raised considerable debate amongst clinicians dealing with it. This article is written with an idea to provide comprehensive information regarding the postnatal management of antenatally detected hydronephrosis. A detailed review of the current literature on this topic is provided. Also, guidelines have been given to facilitate the management of this condition.

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Key words: Antenatal hydronephrosis; Ultrasonography; Pelvi ureteric junction obstruction; Megaureter; Hydronephrosis; Multicystic dysplastic kidney

Core tip: This article provides practical guidelines for the postnatal management of antenatally detected hydronephrosis.

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INTRODUCTION

The detection of renal abnormalities during prenatal ultrasonography was first reported by Garrett *et al*[1] in 1970. Since then routine use of ultrasonography for detection of congenital anomalies has become a part of routine care during the antenatal period. Currently it is estimated that genitourinary anomalies comprise nearly 20% of all prenatally detected fetal anomalies[2]. Amongst these hydronephrosis is one of the most commonly detected anomalies seen in approximately 1% to 5% of all pregnancies and it occurs due to various causes[3] (Table 1). Thus we have an increasing number of patients who are presenting to the clinician with a presumptive diagnosis rather than a symptom and that too before they are born[4]. Logic dictates that this early detection should help in improving post natal outcomes and help in better preservation of the renal function. Lee *et al*[5] in their meta-analysis found that 12%-88% of these children will have demonstrable pathology depending on the degree of prenatally detected hydronephrosis. Hence a thorough postnatal evaluation of the upper and lower tracts is mandatory postnatally. But this also means that 88%-12% of these children will have no demonstrable pathology postnatally. This is borne out by various studies showing that the most common cause of antenatally detected hydronephrosis is transient or non obstructive dilatation of the pelvicalyceal system[6,7]. Thus, postnatally, the clinician is faced with dilemma to differentiate the hydronephrosis which will resolve spontaneously from the one which will become clinically significant and would need

Table 1 Differential diagnosis of prenatal hydronephrosis

Etiology	Incidence
Transient/physiologic	50%-70%
PUJ obstruction	10%-30%
Vesicoureteral reflux	10%-40%
Ureterovesical junction obstruction	5%-15%
Multicystic dysplastic kidney	2%-5%
Posterior urethral valves	1%-5%
Ureterocele	1%-5%
Others like ectopic ureter, *etc.*	< 1%

PUJ: Pelviureteric junction.

Table 2 Descriptive definition of hydronephrosis by Antero Posterior Diameter

Classification of hydronephrosis	Second trimester APD in mm	Third trimester APD in mm
Mild	4-7	7-9
Moderate	7-10	9-15
Severe	> 10	> 15

APD: Antero posterior diameter.

Table 3 Society of fetal urology grading of hydronephrosis

Grade 1	Urine barely splits the sinus
Grade 2	Moderate renal pelvis splitting confined to renal border with dilated major calyces
Grade 3	Pelvis distended outside the renal border, major and minor calyces are dilated; the parenchyma is spared
Grade 4	Parenchyma is thinned

surgery. This differentiation needs to be done by utilizing appropriate investigations using the lowest radiation and least invasive techniques so that timely surgical intervention can be done, in those who need it, to prevent renal function deterioration[4]. This article reviews the primary literature and consensus statements pertaining to antenatally detected hydronephrosis and sets forth our own recommendations regarding management of infants with this finding.

DIAGNOSING HYDRONEPHROSIS ANTENATALLY

The diagnosis of fetal pelvis dilatation and its natural history postnatally is best understood if we understand that the definition of hydronephrosis has undergone a sea change. Traditionally hydronephrosis was defined as dilatation of the pelvicalyceal system due to partial or complete obstruction. Now clinicians understand that hydronephrosis is aseptic dilatation of the collecting system and it may or may not be associated with obstruction. Investigators have proposed that the term pyelectasis be used to describe dilatation of renal pelvis whereas pyelocaliectasis and hydronephrosis include dilatation of calyces. However, all these three terms are used interchangeably and are used to describe a dilated pelvicalyceal system regardless of its etiology[8].

The antenatal ultrasound screening is most commonly performed at 18-20 wk of gestation. This is the time when the renal architecture becomes visibly distinct. Normally the renal pelvis and calyces are not seen, if seen then it indicates hydronephrosis. The sonologist should be vigilant in the antenatal period to differentiate a dilated collecting system from the hypoechoic sonolucent pyramids which may mimic hydronephrosis. Once the diagnosis of a dilated collecting system is made, it should be objectively described using one of the various classification systems. The majority of authors use either the Antero posterior diameter (APD) system or the Society of Fetal Urology (SFU) classification. With the sophisticated ultrasound machines with better resolution detecting smaller dilatations of the renal pelvis, the cut off value of the renal pelvis dilatation necessitating cognizance and achieving clinical significance has been a matter of debate. In the early 80's a threshold value of 10 mm indicated the need for further investigations in the post natal period[8]. In 1990, Mandell proposed a classification system based on APD and gestational age that helps to categorize antenatal hydronephrosis in the mild, moderate and severe variety[9]. This was further substantiated by the work of Corteville *et al*[10] and should now be taken as a standard classification of antenatal hydronephrosis based on APD[11,12]. A number of other studies have noted persistent postnatal uropathy when the APD measures > 6 mm at < 20 wk, > 8 mm at 20-30 wk and > 10 mm at > 30 wk gestation[13,14]. Recently cut off of 6 mm at 20 wk and 10 mm at 30 wk have been suggested for pyelectasis and an APD cut off of 10 mm at 20 wk and 12 mm at 30 wk for hydronephrosis[15]. An interesting feature of this study has been the effort to separate pyelectasis from hydronephrosis so that postnatal ultrasound can be avoided in a number of patients. However given the subjective nature of sonography and the factors like maternal hydration affecting the measurements, this issue has not been resolved as of today and hence clinicians should follow a standard classification and grade the dilatation as mild, moderate and severe as suggested in Table 2.

Another classification used to describe hydronephrosis is the Society of Fetal Urology classification which was first described in 1993[16]. This system describes the renal pelvis dilatation along with the dilatation of the calyces and hence its effect on the parenchyma (Table 3). However this grading system is not universally followed and due to the ambiguity in inter and intra observer agreement especially in grade 3 and grade 4 hydronephrosis, modifications have been proposed[17,18]. Even these modifications have not gained universal acceptance.

Given these discrepancies, it is imperative that worldwide a uniform system of classifying and grading hydronephrosis should be followed. In order to overcome these variations and negate the effect of hydration and full bladder, group from Hong Kong has proposed a Hy-

dronephrosis Index and have given nomograms to help clinicians judge the degree of renal pelvic dilatation based on the gestational age[19]. The hydronephrosis index is defined by the APD of the fetal kidney divided by the urinary bladder volume. It is an interesting index but its clinical usefulness and specificity in fetuses with abnormally dilated renal pelvis or gross hydronephrosis is not established.

Till a reliable method is described, which overcomes the variables of maternal hydration, bladder fullness of fetuses and the operator dependency, clinicians should mention the method used to diagnose antenatal hydronephrosis and grade its findings. If the APD is used than the presence or absence of associated calyceal dilatation should also be mentioned.

OTHER FINDINGS

Besides the diagnosis of hydronephrosis, the antenatal ultrasonography should document the amniotic fluid level, degree of urinary bladder distension; it's emptying and wall thickness visualization of ureter, presence of normal or any abnormality in the opposite kidney and the echogenicity of the kidneys. These additional findings often contribute to establishing the postnatal diagnosis[20]. In cases of posterior urethral valves the level of amniotic fluid is a significant predictor of renal function and clinical outcome[21].

TIMING AND FREQUENCY OF ANTENATAL ULTRASOUND

Currently, there is no agreed upon protocol for the antenatal evaluation and its follow up. The first anomaly scan is done usually between 18-20 wk, this should reliably diagnose antenatal hydronephrosis. The subsequent frequency of follow up ultrasound is often based on the severity of findings and the pathology suspected. There is usually no added advantage of doing very frequent ultrasound examinations for it adds very little to the diagnosis and subsequent management and only aggravates parental anxiety. Once the diagnosis is made then the next ultrasonography can be done in the third trimester between 28-32 wk. However more frequent ultrasounds, every 4-6 wk, will be needed in cases having bilateral hydronephrosis, posterior urethral valves, prune belly syndrome and severe hydronephrosis in a solitary kidney. Ultrasound findings in these clinical scenarios have an important bearing on the decision making in deciding the obstetric course of the patient.

ANTENATAL COUNSELLING

Once the diagnosis of ante natal hydronephrosis (ANH) is made, the parents are engulfed by a myriad of emotions. The two important things that the clinician is often asked to answer is-Should antenatal intervention be done and what happens postnatally. Addressing the parental anxiety and concerns is as important as the clinical management of the child.

Prenatal intervention

Studies have shown that urinary obstruction can cause renal dysplasia and relief of that obstruction can prevent dysplasia if performed early enough[22]. The goal of fetal intervention would be to relieve this obstruction and allow for normal renal development. This in turn would maintain the amniotic fluid levels to allow for normal lung development. Currently, fetal intervention is recommended for those with documented lower tract obstruction, the commonest and most widely studied being posterior urethral valves, where intervention would significantly benefit the overall fetal (and its renal function) prognosis. Open fetal surgery, vesico-amniotic shunt, renal pelvis aspiration, vesicocentesis, fetoscopic fulguration of posterior urethral valves etc have been tried. Though this sounds fascinating, its attendant problems and risks cannot be overlooked. Also does it alter the prognosis significantly and does the benefit outweigh the risks should be evaluated diligently.

At present fetal intervention is indicated in cases where the life of the fetus is at risk, typically a second trimester fetus with significant oligohydramnios, suspected good renal function and absence of other life threatening anomalies[21]. However, this is often too late to prevent renal dysplasia. The procedure is associated with significant risk of infection and also significant fetal and maternal morbidity and fetal mortality[23,24]. Thus, except in a select few cases fetal intervention should not be done and even these cases should be done in centres where the necessary expertise and experience is available.

What happens postnatally?

When the diagnosis of ANH is made the parents often have apprehension that the child will need surgery postnatally[25]. They need to be assured and often a session of counseling with the pediatric urologist who will be taking care of the child postnatally goes a long way to allay the apprehensions of the parents. What should be emphasized that though there is no cause for alarm in majority of cases, a proper and rigorous follow up is a norm in majority of the cases.

POST NATAL MANAGEMENT

"A perfection of means but a confusion of conclusion seems to be our problem-Albert Einstein". There is no ambiguity regarding that all antenatally detected hydronephrosis should be evaluated by an ultrasound postnatally[5] (Level I evidence Grade A recommendation). Since infants are relatively dehydrated at birth, the initial postnatal ultrasonography should be performed after 48 h of birth. Day two of life is preferred to enable adequate hydration after delivery but circumstances pertaining to early discharge following delivery may not allow this. Also breast fed neonates may not be adequately

Table 4 Measures to be taken within first 48 h after birth in infants diagnosed with antenatal hydronephrosis

USG	Suspected lower tract obstruction, *e.g.*, Posterior urethral valves, prune belly syndrome Bilateral hydronephrosis with or without hydroureter Solitary kidney with APD > 15 mm or SFU grade 2 or more
Antibiotic prophylaxis	Suspected lower tract obstruction APD > 10 mm or SFU grade 2 or more in the third trimester Solitary kidney with hydronephrosis of any grade Bilateral hydronephrosis
VCUG	Suspected posterior urethral valves antenatally
Catheterization	Suspected lower tract obstruction-posterior urethral valve or prune belly syndrome

VCUG: Voiding cysto urethrogram; APD: Antero Posterior Diameter; SFU: Society of Fetal Urology; USG: Ultrasonography.

hydrated until a steady milk flow is established. Hence the first postnatal ultrasound is preferably done between 5-7 d after birth[12,26,27]. The exceptions to this caveat are: (1) Suspected lower tract obstruction *e.g.*, Posterior urethral valves; (2) Severe bilateral hydronephrosis with or without hydroureter; and (3) Solitary kidney with hydronephrosis especially if the APD is > 15 mm or it is SFU grade 2 or more in the third trimester. Early sonography in these situations has obvious bearing on further management.

SHOULD CHEMOPROPHYLAXIS BE STARTED IMMEDIATE POSTNATALLY?

Whenever there is hydronephrosis the treating clinician is worried about two things-obstruction and infection. The obstruction needs to be established in most cases with ANH. However the clinician is worried about the possibility of infection in a dilated system with stasis of urine. So, should neonates and infants with ANH be put on antibiotic prophylaxis? Till date there are no prospective studies providing level I evidence to support the use of prophylaxis. The available literature is conflicting. Studies have shown that the risk of infection increases with the degree of hydronephrosis[28-31]. Coelho *et al*[28] found the incidence of urinary tract infection (UTI) to be 10% for those with mild hydronephrosis, 20% for those with moderate and 40% for those with severe hydronephrosis. Girls appear to be at greater risk than boys[29]. However these studies are observational in nature and not standardized as regards, the method of urine collection, definition of infection, selection of patients for voiding cystourethrogram and use of prophylactic antibiotics.

More and more data is coming regarding the limited usefulness of prophylactic antibiotics and with the varying practice patterns due to variations in geographic location, clinician experience and above all variable health care practices in developing countries, as of yet, no standardized uniform guidelines have been proposed. However undeniably patients with mild hydronephrosis are at much less risk of infection as compared to those with moderate to severe hydronephrosis[12,32-36]. If prophylaxis is started than the choice of antibiotics are Amoxicillin (15 mg/kg) or Cephalexin (2 mg/kg). Based on the available evidence we propose the following to be done within the first 48 h after birth in neonates born with antenatally diagnosed hydronephrosis (Table 4).

ULTRASOUND AT 5-7 D AFTER BIRTH

All infants detected to have ANH should be evaluated by a postnatal ultrasound, which is usually done at 5-7 d after birth[11,37] (for the reasons described above). The following should be the aim of doing this evaluation using a tool which is easily available, provides good anatomical information, is non invasive and is not associated with any radiation: (1) Confirm the presence of hydronephrosis; (2) Grade the degree of hydronephrosis; (3) Plan further tests and evaluation and management strategies based on the ultrasound findings; and (4) Decide the need for antibiotic prophylaxis.

The ultrasonography should be done with the baby being well fed. It is the practice of one of the authors (Sharma A) to start the examination of these babies by scanning the bladder first. If the bladder is full, usually the baby voids and the degree of bladder emptying is known immediately giving a fair idea regarding the absence of outflow obstruction. Also once the bladder is empty, the effect of a distended bladder on the filling and emptying of the collecting system resulting in fallacious diagnosis of pyelectasis is avoided. The mechanism by which a full bladder causes dilatation of the renal pelvis and the maximal degree of normal dilatation is not known. However it is accepted that when urinary bladder is distended than false positive cases may occur[19]. Hence if a sonologist sees mild degree of hydronephrosis than whether it persists or disappears after bladder emptying should be looked for and mentioned in the report.

Ultrasonography at 5-7 d would show one of the following scenarios: (1) No hydronephrosis-Normal pelvicalyceal system; (2) Unilateral hydronephrosis; (3) Bilateral hydronephrosis; (4) Unilateral Hydronephrosis with hydroureter; and (5) Bilateral hydronephrosis with bilateral hydroureter. Let us see each scenario and discuss its management.

NO HYDRONEPHROSIS-NORMAL KIDNEYS

Postnatal ultrasound will be normal in 41%-88% of cases diagnosed to have hydronephrosis antenatally[10,11,38]. Why this happens is a matter of speculation. Constantinou[39] suggested that a pacemaker in the renal pelvis activates the smooth muscle of the renal pelvis to initiate peristaltic contractions. The direction of the peristalsis is from the renal calyces and pelvis towards the urinary bladder. Any immaturity of the pacemaker in the renal

Table 5 Management recommendations in neonates with antenatal hydronephrosis but Normal Post natal ultrasound

USG	At 1 mo and at 3-6 mo
VCUG	Not recommended if two USG are normal
Antibiotic prophylaxis	Not recommended routinely Would be prudent to be started if the follow up is not reliable For those not getting prophylaxis, parents should be told to get a urine routine if the neonate shows any signs of not being well

VCUG: Voiding cysto urethrogram; USG: Ultrasonography.

pelvis might lead to poor co-ordination of the peristaltic activity[40-42]. Thus there is impediment of the emptying of the renal pelvis resulting in urinary stasis in the renal pelvis. Also, disco-ordination of muscle cell excitation can spread in any direction so that retrograde peristalsis can occur[43]. It has been speculated by Leung *et al*[19] that the pacemaker in the renal pelvis does not mature at an early gestational age. Maturation of this pacemaker and ureteral peristalsis starts around 28 wk of gestation, after which equilibrium is gradually established between pelvicalyceal filling and bladder filling/emptying in the fetus. This probably explains the disappearing fetal hydronephrosis postnatally when the physiological function of the urinary tract becomes more mature.

Even if the first postnatal scan does not show hydronephrosis a repeat scan at 3-6 mo is mandatory. If the scans, on both occasions, do not show hydronephrosis, than a diagnosis of transient hydronephrosis can be safely and surely made. Emphasizing the need for a second scan is of paramount importance as late worsening or recurrent hydronephrosis is seen in nearly 15% of infants[44-46].

These infants have a 25% incidence of associated vesico ureteral reflux (VUR)[47]. Hence some investigators have proposed antibiotic prophylaxis and a Voiding Cysto Urethrogram (VCUG) study in these patients[48-51]. The objections to VCUG being performed in all cases has been based on the feeling that it is not an entirely benign procedure due to its invasive nature, radiation exposure, expense and up to 15% rate of post procedure urinary tract infection[52]. Also majority of the VUR in this category of patients would be low grade with a high chance of resolution of spontaneously. Ismaili *et al*[53] found that if two successive ultrasonography were normal than VCUG was not justified. Two recent studies have also shown that routine VCUG and antibiotic prophylaxis are not to be recommended in these patients who are at low risk of infection[6,54,55]. But as those cases where the follow up is unlikely to be very rigorous and methodical as occurs in the low socio economic group especially in developing countries, advising prophylactic antibiotics would be a natural extension of the logic to make attempts to prevent renal damage.

We propose the following recommendations based on the presently available literature in this category of patients (Table 5).

UNILATERAL HYDRONEPHROSIS BUT NO HYDROURETER

This constitutes the largest category of patients with prenatally detected hydronephrosis. 50%-70% of these would have transient or physiologic hydronephrosis which regresses over a period of time and has no clinical implications; pelviureteric Junction (PUJ) obstruction accounts for the remaining 30%-50% of cases[56,57].

The following questions need to be addressed when these patients are being evaluated: (1) When and how to evaluate them initially? (2) How to do follow up? (3) When to do a functional study? (4) How to differentiate non obstructed from obstructed systems? (5) How long to follow them? and (6) When to Intervene?

When and how to evaluate Initially?

There is no ambiguity regarding the fact the first evaluation should be on the 5th to 7th day after birth and is by ultrasound. However the agreement ends here. How to grade hydronephrosis has been a matter of much debate. The landmark study by Dhillon *et al*[58] in 1998, proposed the measurement of APD of the renal pelvis as a means of judging the severity of hydronephrosis and predicting the need for surgery. While the APD measurement provides an objective means of predicting pathology, many felt that other features are also important in determining the severity of hydronephrosis. Therefore, features such as calyceal dilatation and parenchymal thinning should also be considered in grading the severity of hydronephrosis. These factors were taken into account by the Society of Fetal Urology and a grading system for hydronephrosis was proposed[3]. This is a five point severity stratification system which also helps in predicting the need for surgical intervention[59]. Although SFU is a useful system, two alternative grading systems have been proposed. Sibai *et al*[60] proposed sub classifying SFU grade 4 into Grade 4 A-with segmental cortical thinning and Grade 4 B-diffuse cortical thinning. Onen[61] proposed subcategorizing patients with SFU grade 4 into those kidneys with mild to moderate *vs* severe parenchymal compromise to account for the underestimation of disease severity in patients with intra renal pelvic configuration.

Whatever system is followed, after the first postnatal ultrasound, the clinician should be able to categorize these patients in the mild, moderate and severe hydronephrosis categories so that further management can be decided[12]. The categorization of this category of patients in Mild, Moderate and Severe types, based on APD and SFU grading is given in Table 6. After the Initial Ultrasound at 5-7 d after birth the next follow up ultrasound should be done at 4 wk.

How to follow up?

The important questions to be answered during follow up of these infants are: (1) Do they need prophylactic antibiotics; (2) Do they need VCUG; and (3) When to

Table 6 Categorization of patients with unilateral hydronephrosis with no hydroureter into mild, moderate and severe hydronephrosis based on Antero Posterior Diameter /Society of Fetal Urology Grading

	Mild	Moderate	Severe
APD	< 20 mm	20-30 mm	> 30 mm
SFU	Grade 1 and 2	Grade 3	Grade 4

APD: Antero posterior diameter; SFU: Society of Fetal Urology.

repeat ultrasound?

Do they need prophylactic antibiotics

Regardless of gender prophylactic antibiotics are not recommended for patients with mild degree of hydronephrosis because of the low risk of developing a urinary tract infection or need for subsequent surgery[6,28,35]. But chemoprophylaxis is indicated in those with moderate or severe degree of hydronephrosis till VCUG is done.

Do they need VCUG

Patients with mild degree of hydronephrosis do not need VCUG. Though a small subset will have associated VUR, majority of the times it is a low grade VUR which subsides on its own[47]. However those with moderate to severe hydronephrosis need a VCUG. VUR would be diagnosed in about 20% of these patients[62,63].

The timing of VCUG in this group of patients should be at 4-6 wk. These patients are on prophylactic antibiotics, hence to diagnose or rule out VUR, it would be prudent to wait till the neonate is old enough. Whether it should be a conventional VCUG or a radionuclide cystogram is a matter of personal preference and debate. A conventional VCUG would not only diagnose lower grade of VUR but would also exclude the possibility of posterior urethral valve, which can present indolently[64].

It is recommended that if no reflux is seen then chemoprophylaxis can be stopped unless it is a solitary kidney (to avoid the slightest chance of infection affecting a solitary renal unit). In those with VUR chemoprophylaxis should be continued[12,65].

When to repeat ultrasound?

Irrespective of the grade of hydronephrosis a repeat ultrasound is warranted at 4 wk after birth. It confirms the severity of hydronephrosis and also gives an insight into progression/regression of hydronephrosis. Also the hydronephrosis can be categorized into mild, moderate and severe type again. Changes in the severity of hydronephrosis can occur as kidneys mature and/or signs of obstruction manifest[12].

If there is mild hydronephrosis (APD < 20 mm or SFU Grade 1 or 2) and for moderate hydronephrosis (APD 20-30 mm or SFU Grade 3)-confirmed at 1 mo a repeat ultrasound is indicated at 3 mo and then six monthly till the age of 3 years and then yearly till the age of six years. Whenever the sonography shows resolution of hydronephrosis a repeat ultrasonography at 3-6 mo is warranted to confirm the finding as recurrence is noted in previously resolved hydronephrosis[34].

For severe grade of hydronephrosis (APD > 30 mm or SFU Grade 4)-ultrasound at 1 mo confirms the findings and then further sonography is done based on the need for intervention. If conservative management is opted (in cases with differential function > 40%) then ultrasonography should be done at monthly intervals for 3 mo, then bimonthly till the age of 1 year. Any sign of increasing hydronephrosis would warrant intervention or a further radionuclide study to determine the need for intervention.

When to do a functional study?

A diuretic renogram is indicated in those with severe degree of hydronephrosis at 4 wk after birth. All other patients can be followed with ultrasound as mentioned above, with a radionuclide study done when there are signs of increasing hydronephrosis. The functional evaluation should be by mercapto acetyl triglycine (MAG3) or ethyl cysteine (EC) Renogram using a F-15 or F0 protocol. Due to lack of maturity of the kidneys and a very high background activity resulting in erroneous calculation of differential function a DTPA renogram should be avoided in the first 6 mo of life[66-68].

How long to follow them?

If there is no increasing hydronephrosis on serial ultrasounds then also the child needs to be followed up till the age of 6 years. A stable dilated system at 6 years would not warrant further study except around puberty when it would be worthwhile having a look at the kidneys by ultrasound to rule out any deterioration of hydronephrosis with the spurt in growth that occurs. This area, where the dilatation has stabilized in the early childhood, has hitherto not been investigated thoroughly and further studies are awaited to look into the fate of those kidneys later in life. A radionuclide study at 6 years, before stopping follow up, would be useful to confirm the good functional status of the kidney and establish a baseline value for further comparison in future.

When to intervene?

Patients with unilateral hydronephrosis is the category where the clinician faces the biggest dilemma of differentiating a non obstructed dilated system, where hydronephrosis will regress spontaneously over a period of time (or remain stable) from a dilated but obstructed system. This needs to be diagnosed as early as possible so that intervention can be done before renal damage occurs. The dilemma gains much significance as the clinician has to choose between conservative approach *vs* surgery. Till an answer to this dilemma is obtained, there is much parental anxiety, as the need for surgery hangs like a sword of Damocles on the head of the patient.

Two modalities have been extensively studied to provide an answer to this vexing question-Ultrasonography

and Radionuclide studies. Though a diuretic renogram has been considered the gold standard to diagnose obstruction, numbers of studies have questioned its ability, especially in the way it is done at present, to diagnose obstruction and more importantly diagnose cases which will need surgical intervention. Hafez *et al*[69] in 2002 had shown that drainage curves from the initial renogram are not always predictive of cases which need surgical intervention. For many years the output function (drainage) has been empirically estimated on the basis of the slope of the frusemide curve, a T half > 15-20 min reflecting an obstruction and a short T half excluding obstruction[70]. This method is still largely used many urologists. Of course a good renal emptying practically excludes any significant risk factor related to obstruction; but what should be the conclusion when the T half is high? If the function has been compromised then the diagnosis of obstruction is beyond doubt. But can the diagnosis of obstruction be made based on the curve pattern in the presence of well preserved renal function, the answer is probably not. The major pitfall in this interpretation is what has been called the "reservoir function". When there is a dilated system, the tracer, even under the influence of frusemide has to fill the renal pelvis before leaving the kidney, even if there is no significant restriction to urinary flow. Thus, despite all technical precautions, one can end up the test with no or limited renal pelvis emptying, simply due to this reservoir effect[66-68]. It is therefore not acceptable to conclude that the kidney is obstructed simply because of poor drainage. It has been shown during longitudinal conservative follow up of these children that the drainage might improve considerably and spontaneously, sometimes after several years[71]. Newer parameters like output efficiency and Normalized Residual Activity have been evaluated and are found to be more reliable[66-68]. However they have not gained universal acceptance and also there values have not been standardized. Two new parameters which have shown promise in differentiating an obstructed from a non obstructed system are-Post micturition and post erect images acquired 1 h after tracer injection[66-68] and the cortical transit time[72,73]. The post micturition post erect images taken at 60 min showing retained tracer are more indicative of poor drainage and obstruction then the post frusemide curves. They can be taken easily and even an infant can be held in the arms of the parent to obtain the post erect images. However this also has not been incorporated in routine diuretic renogram all over the world.

Cortical Transit time has shown promise to identify those renal units which are at risk of deterioration of renal function due to obstruction. It is the passage of the tracer from the outer cortex to the inner structures *i.e.*, the medulla and collecting system. In a normal kidney one expects a rapid transit with more or less homogenous kidney filling in about 2 min. A delay in this suggests obstruction. It has also been found that kidneys with delayed cortical transit times are not only at high risk of deterioration of renal function, but also show good post operative improvement in 80% of cases[72,73]. However, 20%-30% of these kidneys would not show good recovery of function. Also a large prospective study is needed to confirm that cortical transit time is the best predictor of which children should be operated upon[67,68]. Till one of these parameters are universally accepted, the current practice of getting a well tempered renogram, lacking sensitivity and specificity, serves only to get the differential renal function and is a poor man's Di Mercapto Succinic Acid Scan[36,74]. The decision to operate is simple when the differential renal function is < 40%. But the dilemma persists in kidneys with function > 40%.

It is imperative to make this differentiation between a dilated but non obstructed system form an obstructed system at an optimum period of time as delay in his can lead to irreversible damage to the obstructed kidneys and intervention later may not lead to partial/complete recovery of the lost function. When the differential function is > 40%, the infant is under observation. Ultrasound is the most universally accepted, non invasive and non ionizing tool used to evaluate these children during this period of observation. Investigators have studied whether ultrasonography can be used to predict the need for surgery. Dhillon[58], from the Great Ormond street group was the first to describe the predictive value of anteroposterior diameter of the renal pelvis for determining the need for pyeloplasty. This landmark study found that amongst those with an APD > 40 mm, 80% needed surgery; while those with an APD between 30-40 mm; 55% needed surgery and no intervention was needed in those with an APD < 12 mm. However, mere APD cannot definitively predict the need for surgery as a good number of patients with an APD between 20-40 mm did not need surgery. Burgu *et al*[75] also found that an APD of, 20 mm correlated with persistence of differential renal function and that stable or decreased APD on serial imaging was also predictive of retained or improved function. Other investigators have used renal parenchymal area, calyx to parenchymal ratio and pelvis cortex ratio and hydronephrosis index to evaluate the hydronephrosis postnatally and predict the need for surgery[76,77]. However these parameters have not found widespread acceptance.

Recently, an interesting observation has been published by Sharma *et al*[78], demonstrating the utility of comparing APD measurements in patients with unilateral hydronephrosis in supine and prone positions. They found that in those cases where the APD decreases in prone position by > 10% as compared to supine position, the hydronephrosis decreases over a period of time or does not increase, resulting in preserved differential function. These cases did not need surgery. In contrast, if the APD does not change in prone position or increases in prone position then these cases needed surgical intervention as their differential function showed a substantial drop[78]. This small study of 39 patients from a single center is based on the simple principle that the pelvicalyceal system drains better in prone position, hence the obstructed systems would not show better drainage and

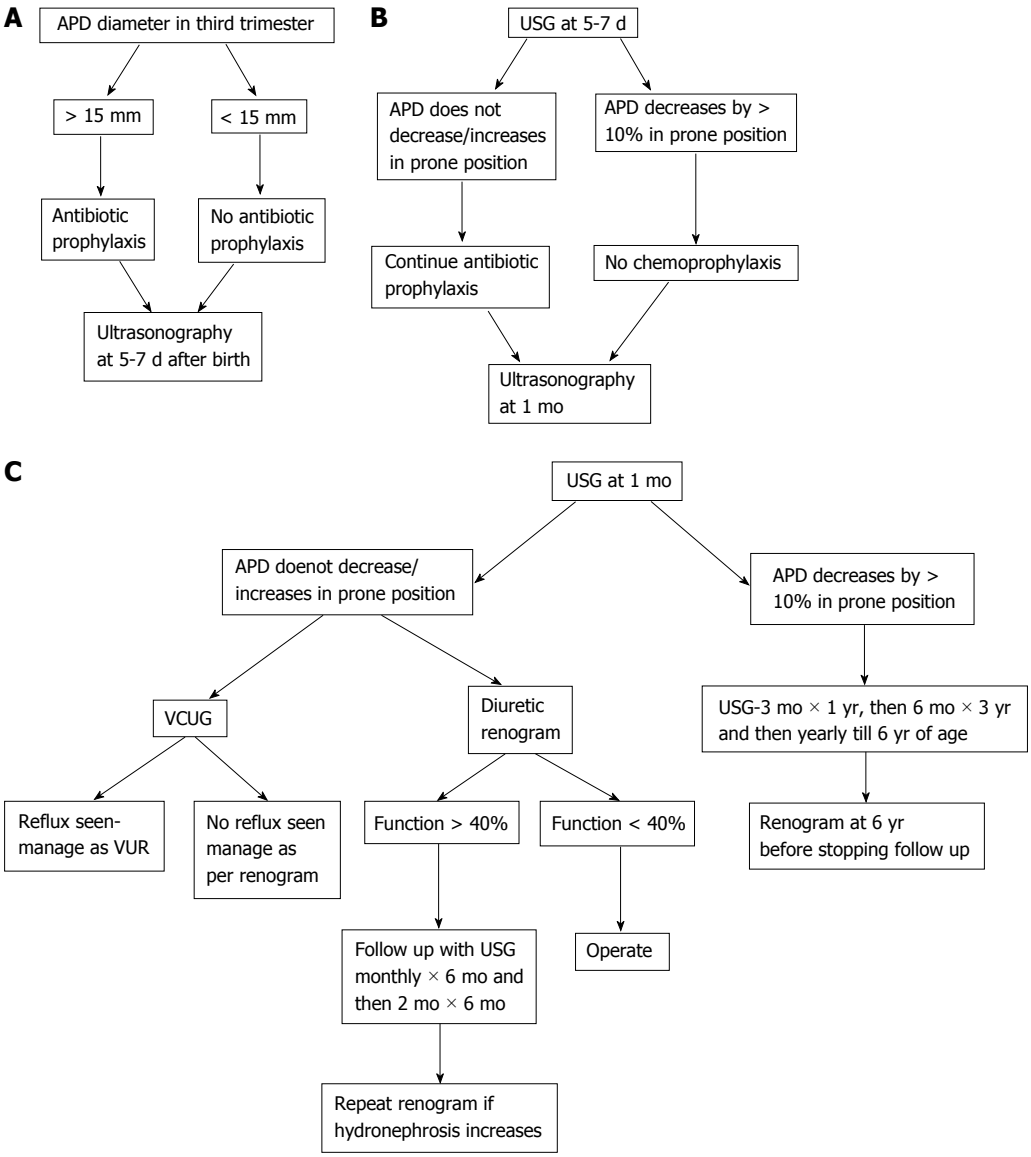

Figure 1 Proposed management plan for patients with Unilateral Hydronephrosis with no hydroureter (A-C). APD: Antero Posterior Diameter; VCUG: Voiding cysto urethrogram; USG: Ultrasonography; VUR: Vesico ureteral reflux.

the APD would remain the same or increase in prone position as the urine from the different calyces pools in the pelvis. If it is a dilated but non obstructed system that in the dependent prone position there would be better drainage and the APD would decrease in prone position as compared to supine position. These measurements of course would have to be done with an empty bladder as a full bladder interferes with the drainage form the pelvicalyceal system. At present, this seems to be the simplest way of differentiating a dilated but non obstructed system from a dilated and obstructed system. Also, variables like the degree of hydration would not affect the conclusion drawn.

The aim of evaluation by noninvasive and inexpensive modality like ultrasonography is to diagnose those patients at risk of deterioration of renal function, differentiate them from those who would do well in the long run and help in judiciously utilizing renogram to intervene at the optimum moment before renal function is affected. We propose the following algorithm to manage these patients with unilateral hydronephrosis (Figure 1).

BILATERAL HYDRONEPHROSIS

Infants with bilateral hydronephrosis are at an increased risk of infection compared to children with unilateral hydronephrosis. The risk of renal function deterioration is high in this group[36]. In this group of patients difference in differential function on renogram is not a reliable way of predicting the need for surgery as both the renal units may have deterioration of function which would not be reflected in the percentage difference in function. Literature is sparse in providing guidelines to manage this group of patients. We propose the following algorithm to manage these patients (Figure 2). This logical proposal is based on the literature available for unilateral hydrone-

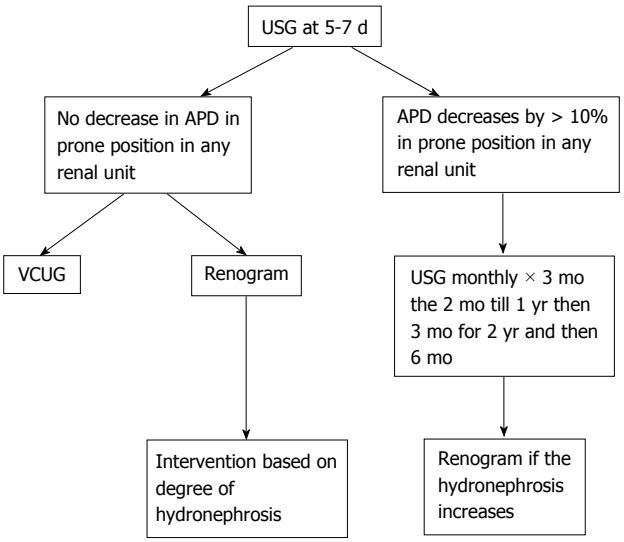

Figure 2 Management of patients with bilateral hydronephrosis with no hydroureter. APD: Antero Posterior Diameter; VCUG: Voiding cysto urethrogram; USG: Ultrasonography.

phrosis and needs to be substantiated by a larger multicenter study.

UNILATERAL HYDRONEPHROSIS WITH UNILATERAL HYDROURETER

This is the group of patients who has a megaureter, the nature of which needs to be ascertained. Literature in this group of patients is quite clear regarding the following points in their management: (1) Definition of megaureter-retrovesical ureteric diameter > 7 mm from 30 wk gestation onwards is taken as megaureter[79]; (2) Antibiotic prophylaxis-is recommended for the first 6-12 mo of life[80,81] as the risk of UTI is higher with uretero vesical junction obstruction than with PUJ obstruction; (3) VCUG-an early VCUG is recommended as 14% of these patients may have an associated posterior urethral valves[82]. VCUG not only would rule out bladder outflow obstruction but also would confirm or rule our reflux and thus define further course of management; (4) Renogram-based on the data from Great Ormond Street a Diuretic renogram is indicated using MAG3 or EC in patients with ureteric dilatation > 10 mm[83]; and (5) Defining obstruction-Interpretation of renogram in the presence of a dilated ureter may be difficult, as delayed transit may be caused by an increased capacity of the dilated ureter and pelvis. Poor drainage is also apparent because the bladder is full and the effect of gravity is incomplete[84]. For these reasons interpretation of the wash out curves should be made in the light of differential renal function and the degree of renal pelvis dilatation. An initial differential renal function of < 40% or a drop in function by > 5% on serial scans is taken as significant. On the other hand delayed transit on diuretic renogram in the presence of stable or improving dilatation and a differential function above 40% in an asymptomatic patient are not strong indicators of obstruction. A close follow up with serial ultrasounds is recommended in this group[85].

BILATERAL HYDRONEPHROSIS WITH BILATERAL HYDROURETER

Most of these cases are associated with Bladder outflow obstruction and/or bilateral reflux. The following recommendations are just an extension of the rationale thinking based on the now standardized protocol in the management of unilateral hydronephrosis with hydroureter: (1) Antibiotic prophylaxis-recommended; (2) VCUG-to be done at the earliest under antibiotic cover to confirm or rule out posterior urethral valves; (3) Renogram-to be done using MAG3 or EC within the first 4 wk of life, in cases of bilateral megaureter(not refluxing and not associated with posterior urethral valves); and (4) Definition of obstruction-Differential renal function should be interpreted in clinical context, since values within normal range will be seen when there is bilateral renal damage and/or in the presence of chronic renal failure.

CONCLUSION

Postnatal management of prenatally detected hydronephrosis is a topic which has evoked widespread interest. The issue which remains ambiguous at present and is the area of much study and research is how to differentiate a dilated but non obstructed system from a dilated and obstructed system. The utilization of sonography and the acceptance of parameters like output efficiency normalized residual activity and cortical transit time on renogram would be able to provide definitive answers to this dilemma in the near future.

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REVIEW

Periodontitis: Tip of the iceberg in chronic kidney disease

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Abstract

The prevalence of chronic kidney disease (CKD) is constantly escalating not only in industrialized countries but throughout the world. It is of major significance because of to its high morbidity and mortality. Strategies to tackle this worldwide health problem include identification of its associated risk factors, comorbidities, and complications as well as proper management to handle all the pertinent issues. Periodontal disease, a treatable infectious state of the dental supporting tissues, is common in CKD patients. Its association with CKD is believed to be in a reciprocal or bidirectional fashion and has been massively studied. This paper, therefore, aims to review the recent evidence pertaining to the association between periodontal disease and a variety of renal illnesses. Most of the current evidence was collected from cross-sectional studies and clinical trials. There is substantial evidence indicating that periodontal disease contributes markedly to the chronic systemic inflammatory burden, leading to cardiovascular and cerebrovascular complications, the principal causes of death among chronic renal disease patients. Furthermore, several studies demonstrated that proper periodontal intervention could help improve systemic inflammation and even nutritional status among CKD patients, resulting in a better quality of life. Suggestions have been made that periodontal disease should be diagnosed early, and managed and controlled to, at least, eradicate a source of inflammation in this population. Awareness of such an important issue should be increased in the relevant medical personnel.

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Key words: Periodontitis; Chronic kidney disease; Dialysis; Kidney transplantation; Inflammation

Core tip: Periodontitis is gaining extensive public recognition due to its devastating impact on systemic diseases. Its association with chronic kidney disease is believed to be in a reciprocal or bidirectional fashion and has been extensively studied. In this review article, careful selection of involved studies was performed. This paper, thus, illustrates both the supporting and conflicting results of current publications pertaining to the association of periodontal disease and a variety of renal illnesses including glomerular diseases, and dialysis and kidney transplant populations.

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INTRODUCTION

Chronic kidney disease (CKD) is recognized as a significant global public health issue[1,2] because it leads to high morbidity and mortality[3,4]. The prevalence of CKD is progressively increasing[5] across the world[6,7]. Globally, CKD affects more than 50 million people[8] and more than 1 million of these undergo renal replacement therapy (RRT)[9]. CKD poses extensive burdens not only on the inflicted individuals particularly in terms of quality of life, but also on society as a whole in terms of medical care and subsequent costs. Many developed nations spend more than 2%-3% of their annual health-care budget to

provide treatment for end-stage renal disease (ESRD)[10]. For instance, a study in the United States revealed that the total management cost of CKD alone was $8000 per patient. In addition, if CKD-related comorbidities existed, the cost of care increased to $14000[11]. In 2007, the United States Medicare expenditures on CKD patients exceeded $60 billion, representing 27% of the total Medicare budget[10]. Likewise, in England, the National Health Service (NHS) disclosed that the annual cost of medical care for kidney disease was about the same as those for American patients. The estimated cost of English CKD patients was about one-third of all NHS expenditure and more than a half of the sum was spent on RRT[12].

As a result, it is important that all modifiable risk factors for CKD be identified and controlled such as blood pressure, blood glucose level, and calcium or phosphorus level[3]. Moreover, there is a significant non-traditional risk factor, chronic systemic inflammation, and its source should be identified and treated.

Periodontal disease (PD), a treatable infection of the oral cavity, is gaining more attention nowadays. PD *per se* serves as a "source of social inequality, reduced quality of life, reduced chewing function, esthetic impairment, tooth loss and disability"[13]. In relation to other diseases, a large number of studies have been carried out to demonstrate its association with numerous systemic diseases such as cardiovascular disease[13], CKD[5,14,15], ESRD[16], glomerulonephritis (GN)[17], diabetes mellitus (DM)[18], rheumatoid arthritis[15,19], chronic obstructive pulmonary disease, cognitive impairment, obesity and cancer[15].

In relation to CKD, studies have shown an increased rate of PD in this population[20], which probably results from the state of inflammation and malnutrition. The observed prevalence of PD in CKD is globally elevated and is noted as the fourth most costly disease to manage in developed countries[21]. Several studies demonstrated that CKD patients suffered more from PD than healthy subjects. For instance, one study revealed a 100% prevalence of mild to moderate gingivitis in CKD subjects compared with 85% in the general population[22]. Similarly, an investigation in the United States by the Third National Health and Nutrition Survey Ⅲ disclosed an increased prevalence of moderate periodontitis (14.6%) among CKD individuals in comparison with the control group (8.7%)[22,23].

A number of retrospective and prospective studies have also shown a marked association between periodontitis and mortality in CKD subjects, even after adjustment for various confounders such as age and smoking. The studies revealed a notable increase in mortality rate of CKD patients with periodontitis of particularly moderate-to-severe degree[20,24]. Because of the above-mentioned significance of PD, this paper aims to review the recent evidences pertaining to the close association between PD and CKD.

Definitions

CKD is defined as a condition where there is persistent kidney damage along with progressive and irreversible renal function loss[25-27] through such mechanisms as "renal hyperfiltration, increased intra-glomerular pressure, arterial hypertension, renin-angiotensin system activation, proteinuria and renal ischemia"[26]. Diagnostic criteria for CKD involve either the presence of reduced glomerular filtration rate (GFR) or markers of renal damage such as albuminuria, abnormal urine sediment, electrolytes or structure and histology for 3 or more months, and a history of kidney transplantation (KT)[28].

The National Kidney Foundation's Kidney Disease Outcome Quality Initiative classified CKD into 5 stages according to the severity of the condition. Thus, microalbuminuria represents the degree of renal damage and the measured GFR signifies the reduced renal function[29]. Simply put, CKD is classified based on Cause, GFR category, and Albuminuria category (referred to as CGA)[28].

PD is a group of infectious diseases affecting the dental supporting tissues[29-31], and is characterized by progressive destruction of the tooth supporting apparatus[13,15,32]. Commonly noted signs of PD comprise "gum tenderness, gum bleeding, gum recession, alveolar bone loss, tooth mobility, and tooth loss"[33].

PDs are derived from the microflora formed in the biofilm or dental plaque, adhering to the teeth. Significantly, periodontal pathogens are capable of invading the dental superficial tissue (gingiva) to the deeper structures (bone and ligaments)[16,30,31]. They contribute to the chronic systemic inflammatory burden by causing both local infection and disseminating into the bloodstream, leading to bacteremia[13,29]. An inflammatory cascade is then activated, as clearly seen by an elevation of inflammatory mediators, particularly C-reactive protein (CRP) and interleukin-6 (IL-6)[5,18,29], which adhere to and proliferate in the coronary endothelial cells, resulting in formation, maturation and exacerbation of atheroma, platelet aggregation and impairment of vascular relaxation[13,14,34]. These mechanisms eventually result in atherosclerotic complications, the primary cause of high morbidity and mortality among the CKD population[5,33].

Classification of PD encompasses plaque-induced gingivitis, and chronic and aggressive periodontitis. Plaque-induced gingivitis involves only tissue of the gingivae, characterized by gingival erythema, edema, hemorrhage and tissue enlargement. It is treatable and reversible, in contrast to the other 2 conditions. The pathogenesis of periodontitis includes reduced salivary production, as well as increased salivary pH and salivary urea concentration, a shift of normal oral flora from Gram-positive to Gram-negative, and secondary hyperparathyroidism, the role of which remains to be elucidated[11].

ASSOCIATION BETWEEN PERIODONTITIS AND KIDNEY DISEASES

CKD and periodontitis

Several studies have established the relationship between

Table 1 Common orofacial problems associated with chronic kidney disease itself or caused by therapy[35]

Organs	Manifestations	Organs	Manifestations
Oral mucosal lesions	Ulceration	Mouth	Poor oral hygiene
	Uremic stomatitis		Uremic odor, bad odor/halitosis
	Mucosal petechia/ecchymosis		Uremic frost
	Metastatic soft tissue calcifications		
	Macules, nodules		
	Papillomas		
	Pyogenic granuloma	Salivary glands	Acute suppurative sialadenitis
	Fibro-epithelial polyps		
	White patch, erythematous patch		
	Angular cheilitis/candidiasis	Tongue	Tongue coating
	Oral hairy leukoplakia		Geographic tongue
	Lichen planus-like disease		Black hairy tongue
	Epstein-Barr virus like lesions		
	Non-Hodgkin's lymphoma		
	Kaposi's sarcoma		
Bone	Decreased thickness of cortical bone	Periodontium	Gingival overgrowth
	Radiolucent lesions		Increased deposits of calculus
	Abnormal bone healing after extraction		Severe periodontal destruction
	Osteolytic areas		Increased tooth mobility
	Premature bone loss in the jaw		
	Decreased trabeculation	Teeth	Premature tooth loss
	Bone demineralisation		Malocclusion
	Brown tumour of the maxilla		Erosion of lingual tooth surface
	Pulp calcification		
	Pulp narrowing		
	Delayed eruption		
	Necrotic teeth		

systemic diseases, including CKD, and oral disease as an association rather than causal relationship[14,16,33,35,36]. Many described that the link between the 2 entities was bidirectional[29,30] or reciprocal[5,27]. That is, CKD and RRT, in any form, can exert an effect on oral tissues as well as dental management among these individuals. Periodontitis, likewise, adds greatly to the overall systemic inflammatory burden and the management of ESRD patients[5].

A large survey of 11200 adults demonstrated the bidirectional relationship between PD and CKD. Multivariable logistic regression models were used to evaluate the direct effect of PD on CKD, while simultaneously controlling for a direct effect of many other factors such as DM, hypertension, and socioeconomic status. Adults with PD and edentulous adults were approximately twice as likely to have CKD (OR = 1.62 and 1.83, respectively) after adjusting for 14 other potential risk factors. Moreover, the PD score was statistically important, such that for every one unit increase in the continuous PD score, the risk of having CKD increased by 1% when adjusting for other factors[34].

Impact of chronic renal disease on periodontal tissues

Numerous studies have agreed on the fact that ESRD patients neglect or pay less attention to dental care, and access and utilize dental resources and procedures less often, hence, are more likely to present with poor oral hygiene. This was attributed largely to the immense physical and psychological burden and time-consuming RRT sessions. Furthermore, confounding factors, for instance, DM, smoking, dialysis period, age, degree of medical management of renal failure complications, and demographic variables, may potentially impact on the seeking of dental care[5]. The impact of CKD and RRT was identified in oral tissues, such as xerostomia, delayed tooth eruption, enamel hypoplasia and altered salivary pH[5,30,33,34]. The tongue and salivary glands are also affected (Table 1)[35].

A frequent oral problem is cyclosporine-induced gingival hyperplasia among RRT patients particularly those with a renal transplant[5,31,34]. In ESRD patients on hemodialysis (HD), there were elevated levels of plaque and calculus, gingival inflammation, and increased rates and severity of PD. The uremic state was considered an important underlying cause, along with immune system alteration and disturbances, and hence, a reduction in the host response[2,5,31]. In the pre-dialysis population, a study in Sweden was performed in 51 subjects presenting with a variety of CKDs, and close to dialysis commencement. These participants were given a comprehensive dental inspection and the results undoubtedly revealed poor oral health in these ESRD individuals[37].

Nevertheless, not all studies reported similar findings with regard to the extent and severity of periodontitis among the CKD population. In recent literature, conflicting study results were reported. Many studies published in some Asian, and North and South American countries (Taiwan, Canada, United States and Brazil) demonstrated a higher prevalence of periodontitis in CKD and a higher frequency of chronic severe periodontitis among HD patients. In contrast, both cross-sectional and clinical trial studies from some European countries such as Spain and

the Netherlands found no statistically significant association between periodontitis and HD patients, compared with healthy control group[35].

In the same way, investigation of the association of CKD and PDs did not show uniform findings even though performed in various ethnic groups. In a cross-sectional study among non-Hispanic blacks and whites, non-Hispanic whites and those with a low income and lower educational level showed the strongest association between PD and CKD after adjustment of such variables as age, race or ethnicity, diabetes and dental care use[14]. In contrast, other studies done in the same ethnic groups reported that such an association in non-Hispanic whites was unremarkable compared with Mexican-American and non-Hispanic black CKD patients[2].

Impact of periodontal diseases on CKD

The appreciable influence of PD on CKD is amplified by the systemic inflammatory burden[5,31,33-35]. Periodontal pathogens are able to not only cause a local inflammatory reaction but also invade into the bloodstream and cause bacteremia[33,34]. The inflammatory process involves induction of several acute-phase mediators, such as elevated CRP, blood sugar and low-density lipoprotein level as well as a reduction in high-density lipoprotein level and peripheral neutrophil counts and function. This then activates the inflammatory cascade. Activation of the complement system, accumulation of pathogens in the vascular endothelium, atheroma formation and impaired vascular relaxation are all involved in the pathogenesis of atherosclerosis[13,14,35]. Subsequently, this leads to the final events of myocardial infarction and a cerebrovascular accident, the primary causes of death in CKD patients[5,33].

It is often mentioned that a greater number of pathogens are present in HD patients. *Porphyromonas gingivalis* (*P. Gingivalis*) is one of those species playing vital roles in bringing about serious outcomes in these populations. A further study regarding major periodontal pathogens and their severity among CKD patients as compared to systemically healthy controls was performed in Brazil. Sixty-six eligible chronic periodontitis patients comprised 19 healthy subjects, 25 pre-dialysis CKD patients, and 22 ESRD patients receiving RRT treatment. These patients underwent periodontal assessment in terms of periodontal pocket depth (PPD), gingival recession, and clinical attachment loss (CAL). Subgingival plaque was collected and then analyzed by polymerase chain reaction. The findings suggested a higher severity of periodontitis in CKD patients compared with their counterparts. *Eikenella corrodens* was the most prevalent periodontal pathogen found in parallel between the control and pre-dialysis groups. Conversely, the RRT group had greater numbers of *P. Gingivalis* and *Candida albicans* which are of importance in the CKD population as they often predispose these patients to opportunistic infections, signifying that administration of an antifungal agent is crucial when chronic periodontitis occurs[26].

Aside from being the source of inflammation and infection, poor oral health may act as a contributor to protein-energy wasting in the CKD population. It presents as anorexia, muscle atrophy, low anabolic hormones, insulin resistance and raised energy expenditure through a number of pathways. A variety of medications taken by CKD patients produce xerostomia which, in turn, causes deglutition problems. A metallic taste is also reported in one-third of advanced CKD individuals, affecting the flavor of food and leading to diminished nutrient intake. Poor oral hygiene, likewise, acts as a contributor to cardiovascular complications in CKD as a byproduct of the inflammatory cascade[35].

Recently, a systematic review evaluating the association between periodontitis and CKD as well as the effect of periodontal treatment on the estimated GFR was published. Four cross-sectional studies, one retrospective and 3 interventional, were included. The correlation between periodontitis and CKD was demonstrated, with an odds ratio of 1.95 (95%CI: 1.35-2.01) by pooled estimates. Interestingly, all interventional studies reported a positive effect of periodontal therapy on estimated GFR[38]. Unfortunately, the reviewer considered that the report was not a well conducted systematic review[39].

Glomerular diseases and periodontitis

In contrast to frequent studies on the association between periodontitis and CKD, limited studies in GN have been mentioned. Ardalan *et al*[17] reported a preliminary study of the association, recruiting 10 subjects with unknown primary GN (7 mesangioproliferative GN, 2 membranoproliferative GN and 1 of unknown origin). The severity of PD was determined by plaque index (PI), gingival index (GI) and PPD. All received appropriate dental treatment after initial examination. After therapy, median urine protein excretion was reduced significantly from 3100 to 900 mg/d ($P = 0.008$), and 40% of the patients were found to have decreased CRP levels. The study reported a high rate of PD through such mechanisms as direct glomerular invasion by periodontal pathogens and an indirect systemic inflammatory burden of CRP. The authors, therefore, concluded that the association between periodontitis and primary GN was plausible[17]. In addition, another comprehensive study analyzed tonsil flora in immunoglobulin A nephropathy (IgAN) patients, the most common primary glomerular disease. Sixty-eight IgAN patients and 28 controls were enrolled. *Treponema* spp. or *Campylobacter rectus*, anaerobic bacteria reported to be causative agents of PDs, played a marked role in the remission of proteinuria (HR = 2.35, $P = 0.019$), by which the IgAN subjects were found to have a higher incidence than those without these organisms[40].

The most common secondary glomerular disease is diabetic nephropathy, a complication of longstanding DM. DM is an unequivocally major risk factor of periodontitis. Commonly, the risk of periodontitis is increased about 3-fold in diabetic compared with non-diabetic populations. The level of glycemic control was determined as a key risk indicator[41]. The poorer the HbA_{1C} level among diabetic patients, the greater the risk of developing periodontitis. This, in turn, may predict the

development and progression of ESRD itself. Diabetic nephropathy patients possessed worse dental health, in terms of more dental caries and deep periodontal pockets, as well as lower salivary secretion. These patients also tended to have higher observed yeast counts, predisposing to oral candidiasis[6].

A study in the Gila River Indian community of type 2 DM patients investigated the effect of periodontitis on development of macroalbuminuria or ESRD. Sixty percent of the subjects had moderate to severe periodontitis and 20% were edentulous at baseline and during follow-up. Thirty-six percent developed macroalbuminuria and about 13% progressed to ESRD. Interestingly, the incidence of macroalbuminuria and ESRD was increased as the degree of periodontitis progressed; thus, it predicted the development of overt diabetic nephropathy and ESRD in a "dose-dependent" manner[18]. A similar study in Pima Indians with type 2 DM was performed. The relationship between number of deaths per 1000 persons and years of follow-up increased with the continuum from none/mild, moderate to severe periodontitis. After adjustment for age, sex, duration of DM, HbA1c, macroalbuminuria, magnetic resonance imaging (BMI), serum cholesterol, hypertension, electrocardiographic abnormalities and current smoking status, patients with severe periodontitis a 3.2-fold risk of cardio-renal mortality (ischemic heart disease and diabetic nephropathy combined) compared with the controls[42].

Dialysis and periodontitis

Major changes in the oral condition usually accompany the initiation of dialysis. Studies have illustrated higher rates of oral pathology in dialysis patients, presenting with one or more oral manifestations. Oral features include uremic odor, dry mouth, taste disturbance, tongue coating, mucosal petechiae and ecchymosis, reduced salivary flow, mucosal inflammation and oral ulceration[5,16,35]. Progression of PD has been reported as severity increases along the continuum from pre-dialysis, peritoneal dialysis to HD. However, studies on the prevalence of these symptoms in peritoneal dialysis and HD patients are still sparse[35].

Peritoneal dialysis

A study in Poland compared the periodontal status between 3 adult CKD groups, those on maintenance HD, continuous ambulatory peritoneal dialysis (CAPD), and pre-dialysis treatment. The study involved 202 dialysis patients (141 on HD and 61 on CAPD), and 160 CKD patients (35 on HD, 33 on CAPD and 38 pre-dialysis). Two control groups were allocated: a group of 26 healthy individuals with advanced periodontitis and 30 individuals from the general population. The severity of PD was clinically measured by the GI, papillary bleeding index, PI, CAL and community periodontal index of treatment needs (CPITN). HD patients showed the most concerning values in all parameters of PD severity[43]. A study in Brazil in 3 similar groups as above revealed that pre-dialysis patients, compared with CAPD and HD subjects, showed greater bleeding on probing, and frequency of generalized chronic periodontitis, which was enhanced by smoking. Pre-dialysis and HD groups paralleled such findings in having higher frequency of severe chronic periodontitis and percentage of sites with CAL > 6 mm. Due to the heterogeneity of the data, PD seems to have a higher prevalence of PD than the general healthy population, but lower than in the HD population. Conversely, CAPD patients and systemically healthy individuals appeared to share similarities in the periodontal conditions[44].

In Turkey, many studies were performed. One study revealed that GI, PI and calculus surface index were significantly higher in PD and HD groups than the healthy controls[45]. In addition, PD patients presented with higher salivary flow rate, salivary pH, salivary buffering capacity, decayed, missing, and filled teeth index and numbers of filled teeth than the HD counterparts[46]. Another study on the relationship between periodontitis and risk of atherosclerosis was published. The authors evaluated periodontal status of 110 eligible PD patients by using PI, GI and PD index (PDI). Atherosclerotic risk and nutritional and inflammatory markers were also assessed. There was an association between poor periodontal status and parameters of malnutrition, inflammation and atherosclerotic risk. Multiple regression analysis demonstrated that age, albumin level and duration of dialysis were independently associated with the severity of periodontitis. This study consequently confirmed the relationship between both periodontitis and non-surgical periodontal treatment and chronic systemic inflammation[47].

Recently, in a study conducted in Thailand, clinical periodontal status was evaluated in 32 stable PD patients by PI and PDI. At baseline, high sensitivity CRP positively correlated with clinical periodontal status (PI; $r = 0.57$, $P < 0.01$ and PDI; $r = 0.56$, $P < 0.01$). After completion of periodontal therapy, clinical periodontal indexes were significantly lower and CRP significantly decreased from 2.93 to 2.21 mg/dL. In addition, increased blood urea nitrogen (BUN) from 47.33 to 51.8 mg/dL, reflected nutritional status improvement. Erythropoietin dosage requirement decreased from 8000 to 6000 units/wk while the hemoglobin level remained stable. The authors, hence, concluded that periodontitis is a potential treatable source of systemic inflammation in PD patients and periodontal treatment can improve systemic inflammation, nutritional status and erythropoietin responsiveness among these patients[48].

Hemodialysis

Among the 3 modalities of RRT, HD, peritoneal dialysis and KT, HD is the most common among ESRD adult patients[49]. ESRD patients, particularly those on maintenance HD, are at great risk of cardiovascular complications, resulting in high morbidity and mortality[5,49]. In the United States, atherosclerotic events were reported as the

leading cause of death, accounting for 44% of all deaths among ESRD individuals, followed by infection.

To support this, a prospective observational study was performed in Taiwan, aiming to investigate the relationship between periodontitis and cardiovascular disease (CVD) mortality in HD patients. The dental health status of 253 included HD patients was indicated by 3 methods of examination, namely, PI, GI and PDI. After a 6-year follow-up period, the subjects with moderate-to-severe periodontitis were found at greater risk of having increased CVD-related mortality (1.83-fold), even after adjustment for various confounders such as age, smoking and educational level[24]. Similarly, with the same objective as above, a retrospective analysis was carried out in the United States One hundred and sixty-eight adults were recruited and given a dental examination to determine PD. After 18 mo of follow-up, the study concluded that HD subjects with moderate-to-severe periodontitis had a 5-fold increase in CVD mortality. Adjustment for other covariables did not significantly affect such a strong association between the 2 conditions[20].

In another study, the 253 Taiwanese HD subjects were included in a study to investigate the adverse effect of periodontitis on inflammation and malnutrition status. Three methods of assessment included PI, GI and PDI. Data on demography such as age and sex, biochemistry such as BUN, Cr, CRP, albumin and ferritin, and hematology were collected for analysis. The results conclusively revealed a significant prevalence of periodontitis among the studied patients. Not only did these individuals face an increased risk of CVD, they were also more likely to have protein-energy malnutrition. An increased degree of inflammation was observed from elevated levels of serum ferritin and CRP. The authors strongly believed that an association between clinical periodontal status and systemic inflammation existed[32]. Accordingly, effective periodontal management could result in CRP reduction and might imply a lower level of risk in developing systemic complications in this population[27], although a reduction in cardiovascular complications remain to be explored[5].

Most studies on the effect of periodontal treatment in the HD population showed a positive effect on inflammation[27,50-53]. In addition, the effect of periodontal therapy in 30 stable HD patients demonstrated a significant improvement in clinical periodontal status (both PI and PDI), nutritional markers (pre-dialysis BUN and serum albumin) and erythropoietin responsiveness after completion of treatment[50]. Nevertheless, some studies failed to show this effect. An example was a randomized controlled trial investigating an effect of intensive periodontal therapy on metabolic and inflammatory markers in 342 HD patients. The study reported an insignificant difference between the treated and control groups for serum albumin or IL-6 level at any time when adjusted for BMI, diabetes and PI. The limitation of the study was the small sample size, only 53 randomly assigned patients, the relatively healthy subjects, and the imbalance in numbers with diabetes[54].

Kidney transplantation and periodontitis

Kidney transplantation (KT) is the most efficient and preferred choice of long-term RRT despite disadvantages such as risk of opportunistic infection, graft-*vs*-host rejection, hypertension, and the tendency for reduced renal function with increased age[5,55].

Gingival hyperplasia is a notable condition observed among renal transplant patients, secondary to post-transplantation immunosuppressive agents such as cyclosporine and prednisolone. Primarily, cyclosporine was said to be the focal cause of gingival hyperplasia in a dose-dependent manner. Additional use of calcium channel blockers such as nifedipine may enhance the incidence and accelerate the severity of gum overgrowth. This can be successfully reduced by substituting cyclosporine with tacrolimus and by surgical intervention[5]. An attempt to evaluate the link between KT and oral lesions was carried out in a study including 33 renal transplant patients, 46 dialyzed patients and 37 controls. All subjects were intra-orally examined and oral disorders were identified and treated. Gingival overgrowth was the most prevalent finding among KT patients, resulting from cyclosporine administration either alone or in combination with calcium channel blockers. Other frequent findings comprised of xerostomia, geographic tongue, and oral candidiasis. A metallic taste was observed more in dialysis patients. In summary, the prevalence of oral lesions was higher in renal disease patients[55].

Investigation of the association between periodontal status and renal allograft function, estimated by GFR, was carried out in the United States. KT patients were categorized into 2 groups: deterioration or stable/improvement of renal function between 2 time points at least 6 mo apart. Chronic periodontitis, defined by $\geqslant 6$ sites with probing depth $\geqslant 5$ mm, or clinical attachment level $\geqslant 4$ mm in at least 6 proximal sites, was significantly more prevalent in patients with reduced renal function ($P = 0.04$). It acted as a statistically significant predictor of improved renal function over time ($P = 0.04$)[56]. Interestingly, the impact of chronic periodontitis on other cardiovascular conditions among these individuals was further studied. A study from Poland illustrated this when it aimed to assess whether periodontitis may contribute to left ventricular hypertrophy (LVH) in KT patients. Ninety-nine patients were classified according to CPITN score into patients with advanced disease (CPITN 3-4) and none/moderate lesions (CPITN 0-2). The advanced lesion group had higher plasma high-sensitivity CRP concentration ($P < 0.05$) and left ventricular mass index (LVMI) ($P < 0.001$), compared with the other group. Besides, LVMI was dependent on CPITN ($P < 0.001$), high-sensitivity CRP ($P < 0.05$), serum cholesterol ($P < 0.05$) and Cr levels ($P < 0.05$). The authors concluded that chronic periodontitis and a concomitant systemic inflammatory reaction in KT patients may be associated with LVH[57].

Finally, inflammation is recognized as playing a pivotal role in transplant rejection. CRP and IL-6, significant

inflammatory markers, are documented as surrogate markers to identify patients who are at greater risk of rejection. In light of this, a hypothesis was proposed that periodontitis may increase the risk of organ rejection by contributing to the systemic inflammatory load in organ transplant patients. A study about the link between periodontitis and serum IL-6 was performed in 47 transplant patients and 18 healthy age-matched controls. The result demonstrated a significantly higher level of serum IL-6 and increased mean probing depth in transplant patients. Multivariable linear regression analysis, after adjustment for sex, diabetes, smoking and immunosuppressant dose, showed that the mean probing depth, number of missing teeth and mean percentage of sites with at least 4 mm of attachment loss were independent predictors for elevated serum IL-6 levels[58]. Besides, a study with a larger group of subjects (90 transplant patients compared with 72 age-matched controls) reported a higher level of IL-6 and CRP in transplant individuals, compared with the control group[59].

CONCLUSION

The current literature strongly supports an association between PD and CKD. They affect each other reciprocally. PD, a treatable infective dental condition, potentially places a devastating chronic systemic inflammatory burden on the CKD population, resulting in significant atherosclerotic complications and death. In the same way, chronic renal disease impacts on oral health in this population with gingival overgrowth as the most prevalent finding due to a cyclosporine-mediated mechanism. Several studies concluded that proper periodontal intervention rendered a promising outcome in the systemic improvement of CKD subjects although its impact on cardiovascular complications remained to be further explored. Thus, PD diagnosis and management deserve better awareness. Further investigation, especially prospective randomized controlled trials, with intervention should be conducted to allow more accurate evaluation. Also, a larger number of participants and ethnic subgroups are required to be recruited in the research to provide more data to make firmer conclusions.

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MINIREVIEWS

Stress urinary incontinence in women and cell therapy: What can we expect from the future?

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Key words: Stem cell; Stress urinary incontinence; Women; Basic science; Animal model

Core tip: This is a review of the current literature regarding the use of stem cell for stress urinary incontinence. It has been focused on cell sources, animal model creation and the possibility of translating this therapy for humans.

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Abstract

Stress urinary incontinence (SUI) is a common disorder that affects a large number of women and their quality of life. The aim of SUI therapy is to restore the existing urethral function *via* physical therapy, biofeedback, pelvic floor rehabilitation, pharmacological therapy, bulking agents and surgical approaches. Currently, the gold standard for the management of SUI is the tension-free vaginal sling, which provides structural support to the female urethra. However, even minimally invasive surgical procedure such as "slings" carries risks for the patients, lost efficacy over the time and has long-term complications. For this reason, new therapeutic modalities are needed. Cell therapy has been emerged as an alternative to be used on the treatment of different diseases. The use of stem cells as a therapeutic option for SUI is an attractive alternative because, theoretically, injected cells could restore functional muscle cells and aid in sphincter closure in women with sphincter-associated incontinence. This study aims to review the current literature regarding evidences for using stem cell therapy on stress urinary incontinence in women.

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INTRODUCTION

Stress urinary incontinence (SUI) is a common disorder that affects a large number of women and their quality of life. According to the National Center for Health Statistics 16% of adult United States women suffer from moderate to severe urinary incontinence, which has a significant socioeconomic impact with an estimated treatment cost of up to $16 billion annually in the United States[1-4].

The pathophysiology of SUI is multifactorial and poorly understood. It appears to be due to the dysfunction of the sphincteric mechanism of the urethra, changes in connective tissues, poor blood perfusion in the periurethral and submucosal areas, and neuronal dysfunction[5,6].

The aim of SUI therapy is to restore the existing urethral function *via* physical therapy, biofeedback, pelvic floor rehabilitation, pharmacological therapy, bulking agents and surgical approaches. Currently, the gold standard treatment for the management of SUI is the tension free vaginal sling, which provides structural support to the female urethra through a minimally invasive surgical procedure. However, even minimally invasive surgical

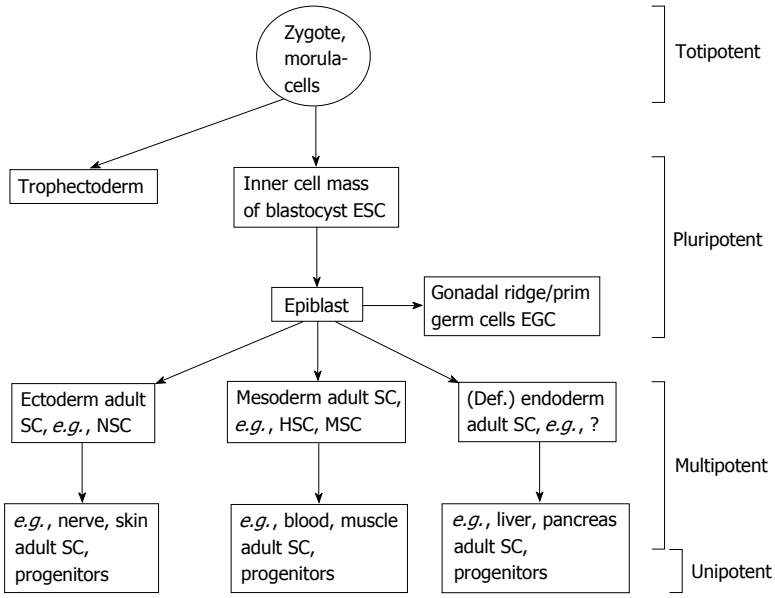

Figure 1 Stem cells and its potential for differentiation. Adapted from Ref. [12]. ESC: Embryonic stem cells; EGC: Embryonic germ cells; SC: Stem cells; HSC: Hematopoietic stem cells; MSC: Mesenchymal stem cells.

procedure carries risks for the patients, lost efficacy over the time and has long-term complications such as voiding dysfunction and "*de novo* urgency". For this reason, new therapeutic modalities are needed[7-9].

Cell therapy has been emerged as an alternative to be used on the treatment of different diseases. Despite being described for more than 40 years in hematopoietic system regeneration, their therapeutic potential has been started only in 1999, when the cover of Science, one of the most respected scientific journal, announced "Breakthrough of the year: stem cells show their potential"[10].

The use of stem cells as a therapeutic option for SUI is an attractive alternative because, theoretically, injected cells could restore functional muscle cells and improve the urethral sphincter activity in women with sphincter-associated incontinence. Cell types considered for this therapeutic purpose include bone marrow stem cells (BMSC), adult mesenchymal stem cells (MSC), human cord blood stem cells, human amniotic stem cells (HASC), induced pluripotent stem cells (iPSC)[11]. This study aims to review the current literature regarding evidences for using stem cell therapy on stress urinary incontinence in women.

STEM CELLS AND ITS POTENTIAL FOR DIFFERENTIATION

Stem cells are undifferentiated cells that are defined by their abilities of self-renewal and differentiation capacity[12,13]. There is a lack of defined morphologic and molecular characteristics of stem cells. Therefore, they are classified according to their potency. The hierarchic order for stem cells ranges from totipotency to pluri and multipotency to unipotency[14]. Totipotent cells are capable of forming cells of the ectoderm, mesoderm, endoderm, and trophoblast. Pluripotent cells can give rise to cells of the three germ layers, but not extra-embryonic tissues. Multipotent cells can be isolated from the developing germ layers or descended adult organs and are capable of self-renewal and differentiate into multiple cell types. Ultimately, the unipotent cells can differentiate into only one cell type[15-18] (Figure 1).

EXPERIMENTAL ANIMAL MODEL: AN ESSENTIAL STEP TO DEMONSTRATE THE EFFECTIVENESS OF CELL-BASED THERAPY FOR STRESS URINARY INCONTINENCE

The development of a reliable experimental animal model is the first step to demonstrate the effectiveness of cell-based therapy for stress urinary incontinence. However, once the physiopathology of SUI has not been well established a reliable animal model for urinary incontinence remains a big challenge[4,11].

Mice, rats, rabbits, pigs, and more recently monkeys have been used in experimental studies. There are different ways to induce urinary incontinence in animals such as vaginal balloon dilation, urethrolysis, urethrolysis and cardiotoxin injection, electrocauterization of pudendal branches, and bilateral pudendal nerve transection. Despite some studies have shown promising outcomes, the main question that should be addressed is: Any of these current methods reproduce the pathophysiology stress urinary incontinence in women[19-25]?

Badra *et al*[4] published the first study performed in monkeys demonstrating a long-term animal model of stable urethral deficiency. They emphasize that nonhu-

man primates have a natural menarche, a 28-d menstrual cycle, natural menopause as well as age associated health risks that closely mirror those of women.

The objectives of creating a reliable animal model are to avoid methodological bias and outcomes misinterpretation. In the literature, most of the studies, which demonstrate promising outcomes have a short follow up and were performed in small young animals. Therefore, the translation of this therapy for humans needs further and more detailed investigation[9,11].

STEM CELLS AND STRESS URINARY INCONTINENCE

There are two different mechanisms of actions for cell therapy. First, the cells can differentiate, integrate into the site of injury and then, replace the damaged tissue. Second, cells can release cytokines, chemokines, and growth factors, which act in paracrine or endocrine manner. Ideally, autologous cells are always recommended to decrease the risk of immune response and rejection[26].

Embryonic stem cells

Embryonic stem cells (ESC) are pluripotent cells derived from blastocysts. These cells propagate readily and remain undifferentiated when cultured with leukemia inhibitory factor (LIF). When LIF is withdrawn, ESCs form aggregates called embryoid bodies that generate a variety of specialized cell types. However, the extraction of these cells involves the destruction of embryos, therefore their use is associated with controversial ethical dilemmas. Despite the self-renewing potential and the capability of differentiation into tissues derived from the three germ layers, embryonic stem cells are associated with uncontrolled growth and teratoma formation. For this reason, embryonic stem cells have never been tested for stress urinary incontinence[27].

Adult stem cells

The regenerative capacity of a tissue is determined in part by whether it contains endogenous stem cells. These stem cells remain quiescent in the niche for long periods until they are activated by the requirement of new cells to maintain the tissue or because of the tissue damage. Despite the limited potential of differentiation and self-renewal, adult stem cells are an attractive source of cells for urinary sphincter regeneration. Most of the studies regarding cell-based therapy and SUI were performed using adult stem cells derived from adipose tissue, skeletal muscle, bone marrow, human cord blood, and amniotic fluid[27].

Bone marrow-derived stem cells: The bone marrow contains two major populations of stem cells: hematopoietic stem cells (HSCs) and MSC, which provide stromal support for HSCs. It has been demonstrated that bone marrow derived stem cells have *in vitro* myogenic differentiation potential, and when placed in culture they expressed myosin heavy chain and desmin[8,9,12].

There is a lack of studies correlating SUI and BMSC. Kinebuchi *et al*[28] transplanted BMSC into rat's injured urethral sphincter. After 13 wk, cell-treated rats did not have improvements on leak point pressure whether compared to controls. On the other hand, Kim *et al*[29] demonstrated that the periurethral injection of BMSC increased the leak point pressure and urethral closure pressure at one-month follow up.

The outcomes regarding bone marrow-derived stem cells and SUI are controversial. Some studies have shown histological and functional recovery, whereas other studies have shown no improvements. These conflicting results may be related to different methodologies, animal models, route of administration, and amount of injected cells. The current literature does not have strong evidences that support the use of BMSC for SUI.

Adipose derived stem cells: The adipose tissue is derived from mesoderm, is easily accessible and overwhelmingly abundant. It has been demonstrated that under specific conditions the adipose tissue has stem cells, which can differentiate into myogenic, adipogenic, osteogenic, chondrogenic, and neurogenic tissue[30].

Shi *et al*[31] injected a tissue engineering bulking agent composed of adipose derived stem cells (ADSC) and silk fibrin microspheres in 4 different points around the urethra of rats with urinary incontinence. In this study, urinary incontinence was established by bilateral pudendal nerve transection and confirmed by decreasing leak-point pressure (LPP). Injection of silk fibroin microspheres without cells could recover LPP and lumen area at 4 wk but its efficacy disappears at 8 and 12 wk follow up. The injection of microspheres with ADSC brought long-term efficacy at 8 and 12 wk post-injection and improved the urethral sphincter regeneration[31].

Li *et al*[32] developed a model of postpartum urinary incontinence in rats through the placement of vaginal balloon dilator for four hours followed by bilateral ovariectomy. They labeled isolated ADSC's from the peri-ovarian fat with thymidine analog 5-ethynyl-2-deoxyuridine. After four weeks leak-point pressure and bladder capacity were significantly higher in ADSC-treated rats compared to the balloon-injured ovariectomized rats. Histological analysis of ADSCs treated group demonstrated a higher density of peri-urethral blood vessel and the preservation of the urethral fibromuscular structure[32].

Jack *et al*[30] isolated pluripotent adipose derived stem cells from human lipoaspirate. These cells were stained with Vybrant Red and injected around the urethra of athymic rats. Eight weeks later, injected cells remained viable and randomly distributed in the local submucosa and lamina propria[30].

Muscle derived stem cells

There are different cell populations that can be isolated form skeletal muscle biopsies. These cells include myoblasts, satellite cells, muscle progenitor cells, and muscle derived stem cells (MDSC). As well as adipose derived

stem cells, it has been demonstrated that adult stem cells isolated from skeletal muscle also have multilineage differentiation capacity[33].

Xu *et al*[34] isolated MDSCs from the hind gastrocnemius of 4-wk-old Wistar rats and infected them with lentivirus encoding green fluorescent protein. In this study, urinary incontinence was established by bilateral pudendal nerve transection and confirmed by LPP decrease. MDSCs were injected at proximal urethra and the animals were sacrificed at four weeks. MDSC-treated rats had higher leak-point pressure and sphincter muscle thickness than controls[34].

Badra *et al*[4] developed a model of urinary sphincter deficiency in adult premenopausal female cynomolgus monkeys cauterizing and then bilaterally transecting the pudendal nerve. Autologous muscle progenitor cells were injected at four locations (12, 3, 6 and 9 o'clock positions) around the sphincter area. At 12 mo follow up, cell-treated monkeys had an improvement in resting, somatic and adrenergic nerve stimulated maximal urethral closure pressure, and a greater percentage of sphincter area occupied by muscle. The GFP labeled precursors cells were found in the skeletal muscle layer and expressed desmin and connexin-43. According to the authors it seems reasonable to assume that their outcomes have a high translational values[4].

Cord blood stem cells

Cord blood is a rich source of stem cells, including hematopoietic and mesenchymal stem cells, which have been used in the treatment of many diseases, including leukemia, lymphoma and anemia[35].

There are few experimental studies that use stem cells derived from cord blood for SUI therapy[35-37]. Lim *et al*[35] reported the short-term effects of human umbilical cord blood mononuclear transplantation in incontinent rats. Urinary incontinence was created through the electrocauterization of periurethral soft tissue and confirmed by leak point pressure measurement. At four weeks, cell-treated rats had significant higher leak-point pressure than controls; however, the injected cells were not found in the urethral tissue[35].

Human amniotic fluid stem cells

Amniotic fluid, due to its contact with the fetus, has been considered an interesting source for undifferentiated or partially differentiated cells. HASC express surface markers and transcription factors distinctive of embryonic stem cells. These include octamer-binding transcription factor 4 and stage specific embryonic antigen-4. HASCs have high replicative self-renewal potential and multilineage differentiation capacity[38-41].

Chun *et al*[42] injected three different lineages of early differentiation human amniotic fluid-derived cell to restore urinary sphincter function in mice with urinary incontinence. In this study, urinary incontinence was created through bilateral pudendal nerve transection and the follow up was two and four weeks. HASC's were early differentiated into muscle, neuron, and endothelial progenitor cells, and then injected into the urethral sphincter as a single, double, or triple combination. Urodynamic study showed that mice treated with the three cell populations had better outcomes than the other ones. These functional results were confirmed by histological and immunohistochemical analysis, as evidenced by the formation of new striated muscle fibers and neuromuscular junctions at the cell injection site[42].

iPSC

iPSC were first described by Takahashi *et al*[43] in 2006 when they reprogrammed human fibroblasts to become pluripotent stem cells by the addition of four different genes: Oct3/4, Sox2, c-Myc, and Klf4. Despite being a good source of cells, not all adult stem cells can be reprogrammed using the same method, which means that each cell type may have critical factors. Unlike embryonic stem cells, iPSC have no ethical issues and no immune rejection. On the other hand, these cells are reprogrammed through the addition of oncogenes, which increase the risk of uncontrolled growth[44]. These cells have never been tested to treat stress urinary incontinence.

REGENERATIVE MEDICINE OF THE CONNECTIVE TISSUES-PELVIC PROLAPSE AND LIGAMENT REINFORCEMENT

The current surgical approaches for pelvic organ prolapse treatment are associated with high rates of long-term complications such as erosions, infections, pain, and vagina shrinkage. There is a lack of studies in the literature with regards to cell-therapy and pelvic organ prolapse[45].

Boennelycke *et al*[46] used fresh muscle fiber fragments seeded on synthetic biodegradable methoxy polyethylene glycol-polylactide-co-glycolic acid scaffolds implanted subcutaneously on the abdomen of rats. After 8 wk, new striated muscle was created and the scaffolds had disappeared[46]. Petros *et al*[47] implanted mersilene tapes between rectus abdominis and midurerhra in 13 canines for periods of 6-12 wk. They observed a linear deposition of collagen around all implanted tapes and some interstitial macrophages. In this study, the main goal was to reinforce damaged pubourethral ligaments using an autogenic collagenous ligament[47].

Regarding prolapse and regenerative medicine approaches, the current literature does not have sufficient data to support cell-based therapy. Further studies using a reliable animal model, different cell sources and scaffolds should be addressed before clinical use.

STEM CELL THERAPY AND STRESS URINARY INCONTINENCE-CLINICAL OUTCOMES

The translation of basic science to humans is the main

goal of scientists and researchers. When a new therapy reaches acceptable outcomes in animal models and has the potential to be applied in humans, some issues should be considered before moving forward. Despite expectations inherent to new breakthroughs, a carefully analysis of pros and cons regarding the procedure should be considered to avoid potential avoidable mistakes.

Aref-Adib *et al*[11] published a systematic review addressing the use of stem cell therapy for stress urinary incontinence in humans. They assessed different outcomes such as adverse effects, incontinence, quality of life, urodynamic, transurethral ultrasound and urethral EMG. Out of 89 studies, only eight met the inclusion criteria, which was a quality score tool designed for use in systematic reviews. According to the authors, the outcomes from the studies published to date are promising. Stem cell therapy may improve patients' quality of life as well as objective measures of urinary incontinence. Furthermore, the procedure is safe with minimal adverse effects. However, despite promising results, there is no long-term follow up data and many patients lost to follow up without any explanation and withdrew from the studies[11].

Shirvan *et al*[48] described the safety assessment of urethra injections of autologous nucleated cells harvested from peripheral blood along with platelets in 9 women with stress urinary incontinence. At 6 mo follow up all the patients considered themselves completely cures with 8 women completely continent and one marked improvement. The idea is that peripheral blood has multipotential cell, which can improve the tissue regeneration[48].

FUTURE PERSPECTIVES AND DIRECTIONS

The stress urinary incontinence is a benign condition that has other effective, simple, cheap and safe treatment alternatives. I believe that stem cell therapy may be an alternative to treat those patients who has urethral sphincter deficiency and did not respond to well-established procedures. Historically, any new therapeutic modality has an initial enthusiasm; however, as well demonstrated in this paper, there are still few evidences, which support cell-based therapy for SUI. The enthusiasm surrounding the new therapeutic possibility should be replaced by moderation, reflection, and careful analysis of the available data associated with consistent experimental studies. The indiscriminate use without sufficient basic evidence could lead to discredit and retrocession.

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MINIREVIEWS

Innovative microsurgical treatment of male external genitals lymphedema

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Abstract

Secondary lymphedema of male external genital organs, characterized by increase in genital organs volume, tissue fibrosis, erysipelas, and objective difficulties in the normal use of lower limbs and the penis, is a very common and impairing consequence of invasive surgery, radical lymphadenectomy and radiotherapy of the pelvic-inguinal area. Standard surgical approach to lymphedema are either very invasive and/or at high risk of lymphedema recurrence and do not guarantee an efficient long-term treatment. Alternatively, we developed a microsurgical technique to perform direct anastomoses between the lymphatic collectors of the spermatic funiculum afferent to the external iliac chains and the vessels tributary to the spermatic vein. This innovative approach, although surgically demanding, provided a long term successful treatment of external genitals with no clinical complications, low invasivity, rapid post-surgical recovery, minor tissue demolition and satisfactory post-surgical functional and esthetic results. In addition, lympho-venous microsurgery seems to trigger the local development of new lymphatic vessels that not only canalize along new collecting channels, but also form complex meshes in proximity to the anastomosis area, thus improving lymphedema also in adjacent tissues like lower limbs, supplied by lymphatics emptying into common developed lymphatic shunt.

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Key words: Microsurgery; Lymphatic meshes; Secondary lymphedema; Lympho-venous anastomosis; Lymphangiogenesis

Core tip: Treatment of secondary lymphedema of male external genital organs through invasive standard surgical techniques may be complicated by impairing consequences for the patient and often do not guarantee an efficient long-term outcome. Alternatively, microsurgical suture of lympho-venous anastomoses between lymphatic collectors of the spermatic funiculum efferent to the external iliac chains and the pampiniform plexus tributary to the spermatic vein provides a successful long term treatment of genitals lymphedema and triggers the development of new lymphatic meshes in proximity to the anastomosis area, thus improving lymphedema also in tissues, like lower limbs, supplied by lymphatics emptying into common developed shunts.

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INTRODUCTION

The lymphatic system is a closed vascular network, whose smallest vessels (initial lymphatics) originate as

a dead-end tubes directly from the extracellular tissue space[1] to subsequently confluence in progressive larger collecting lymphatics that paralleling main artery and veins, propel the lymph unidirectionally. At variance with what observed in initial lymphatics, the wall of collecting lymphatics is supplied with one or more layers of smooth muscle cells arranged in the media externally to the endothelial cells layer and interspersed with collagen and elastic fibres. Fluid flux along the lymphatic conduits is maintained unidirectional by the presence of primary valves in the wall of initial lymphatics[2,3] and secondary or intraluminar valves all along the lymphatic network. Intraluminar valves are formed by two endothelial leaflet attached at opposite sites to the lymph channel wall, connected with zonulae and sustained by a basement membrane composed of collagen, fibrillar material and scanty fibroblasts[4]. Two consecutive valves, arranged at a distance ranging between approximately 1 μm and approximately 15-20 μm delimit the "lymphangion"[1] a sort of primitive heart often considered the "functional unit" of the lymphatic vascular system.

In its flow along the main lymphatic ducts, the lymph is conveyed through secondary lymphoid organs, the lymph nodes. The lymph enters the node through afferent lymphatic vessels, percolates through the node's inner structure dense of lymphocytes and emerge through efferent vessels to be eventually returned into the venous blood stream.

The upper and lower extremities are drained by a superficial and a deep lymphatic network, both equipped with intraluminar valves and smooth muscle cells. Lymph from the skin and superficial connective tissues are carried by superficial ducts, while the muscles and the periosteum drain into the deeper network which accompanies the main vascular fascia. The superficial and deep networks connect only at the level of the popliteal nodes, the adductor canal of the lower leg and the supratrocheal node of the arm. Collecting ducts from the upper limbs drain into the axillary lymph nodes while those of the lower extremities drain into inguinal lymph nodes. The latter also receive lymph from the gluteal area, the uterus, the anterior abdominal wall, the external genital organs and the lower anal canal.

Lymphatic drainage of the external male genitals occurs through two main pathways. A superficial diffuse lymphatic mesh which supplies the scrotal tissues, empties into collecting ducts running along the scrotal raphe and reaches the inguinal lymph nodal chains. A deeper lymphatic network which supplies the gonads is basically organized in two plexuses: (1) one from the testis parenchyma which run proximally along the testicular artery and discharges into the para- and pre-aortic lymph nodal stations; and (2) the other, from the vaginal tunica, which runs in the spermatic funiculum and empties into the external iliac lymphnodes.

The lymphatic vessels efferent from the external, internal iliac and obturator nodes merge into the common iliac lymphatic duct and travel through the corresponding lymph nodes which also receive lymph from the perineum, the buttock, thigh and the pelvic viscera. Efferent lymphatics from iliac nodes drain into para-aortic lymph nodes plexus; the latter, which also receive from all abdominal organs, discharge into the cysterna chyli, a dilated saccular structure located along the abdominal aorta. The larger and main lymphatic collector, the thoracic duct, departs from the cysterna chyli, runs dorsally to the aorta and eventually empties into the venous stream at the junction between the left internal jugular and subclavian veins. The right lymphatic duct collects instead the lymph from the right arm, the right and most of the left lung, the heart, the right side of head, neck and the right anterior chest to empty into the right brachiocephalic vein at the confluence of the right internal jugular and subclavian veins.

The connection between the large collecting ducts and the venous system is usually controlled by unidirectional valves that prevent backflow of blood into the lymphatic vessel. Additional communications between smaller collecting lymphatic and veins may be found along the iliac, renal, subclavian, azygos, and portal veins. However, functional study have revealed that these lymphaticovenous communications are not recruited under normal conditions and may become functional only in pathological conditions[5,6]. No direct lympho-venous anastomosis have instead been detected in lymph nodes.

Because of its peculiar anatomical arrangement the lymphatic system fulfils several important functions in the body: (1) by removing fluid from the interstitial tissue and returning it to the blood stream, it maintains the normal tissue hydration and plasma volume; (2) it returns proteins and high molecular weight soluble molecules from the interstitial tissue to the blood; (3) it returns leukocytes, cell debris and tumoral cells to the blood stream; and (4) the dissemination of lymph nodes along the lymphatic network provides an important contribution to host immune defence by presenting antigens and antigen-presenting cells to the B and T lymphocytes in the lymph node hylum.

SECONDARY LYMPHEDEMA: A COMPLEX IMPAIRMENT OF TISSUE FLUID HOMEOSTASIS

Transport of fluid and solute from the interstitial space to the lymphatic lumen occurs in initial lymphatics by bulk flow through the highly permeable interendothelial junctions[7] that allow free passage of fluid, interstitial hydrophilic molecules of any size, cells, viruses and bacteria. Since the initial lymphatic endothelium offers no effective sieving to large macromolecules, the protein concentration and the colloid osmotic pressure of lymph in initial lymphatics equals that of the surrounding interstitium.

Therefore, the requirement necessary for the formation of the lymph is that the hydraulic pressure in the

interstitium be higher than that existing in the initial lymphatics, while propulsion of the lymph along the lymphatic conduits is sustained by intraluminar pressure gradients developing between adjacent lymphangions. The maintenance of the pressure gradients which sustain lymph formation and propulsion is supported by both local tissue movements such as cardiogenic oscillations, heart activity[8] and respiratory muscle contraction[9], as well as by the action of synchronous contraction of the smooth muscle wall of the collecting lymphatic vessels[1]. Through these mechanisms, lymph flow may increase up to 20-30 folds its physiological value, to counteract an increased interstitial fluid volume depending, for example, upon an increased fluid filtration across the blood microvasculature wall. Such a modulation allows an efficient control of tissue fluid volume only until attainment of a saturation threshold, beyond which the filtration rate through the wall of blood capillaries overwhelms the maximal flow capability of the normally performing lymphatic network, leading to a progressive development of tissue edema. Expansion of tissue fluid is instead defined lymphedema when it depends not only from an increased capillary filtration rate, but rather to an absent or greatly impaired lymphatic drainage. Depending on the underlying cause lymphedema can be distinguished in: (1) primary lymphedema, caused by absence or deficient development of the lymphatic vasculature and/or lymph nodes. In its more severe forms, the primary lymphedema appears at birth (congenital primary lymphedema), and is accompanied by functional impairment of most organs, peritoneal and pleural effusions, pulmonary edema, *etc*. Congenital lymphedema is likely due to genetic faults or abnormalities in the formation of lymph nodes and/or lymphatic vessels due to a defect of the lymphangiogenic vascular endothelial growth factor receptor (*VEGFR-3*)-gene or of the *FOXC-2* (fork head transcription factor for the formation of collecting vessels) gene, both involved in the development and control of lymphangiogenesis. The primary idiopathic lymphedema occurs instead in adolescence or within the 35th year of age and typically affects the upper and lower limbs and/or the external genitalia[10]. The causes of this pathology are still unclear although phenomena like abnormalities of lymph nodes and/or lymphatic vessels, hypoplasia of the lymphatic structures or extracellular matrix degradation might all interplay to determine the primary lymphatic drainage impairment. Due to its complexity, congenital and idiopatic lymphedema represents a challenging, often unsolvable clinical and, more so, surgical problem which, at the moment, goes beyond the focus of the present Review.

Instead, we will focus on a recent application of microsurgery in the treatment of secondary lymphedema, a more localized lymphatic insufficiency caused by excision, lesion or radiation of the lymphatic vasculature and of the lymph nodes secondary, as an example, to major surgery and/or oncological therapies. Impairment of the lymphatic fluid and solute drainage, such as that observed in secondary lymphedema, causes uncontrolled increase of tissue fluid volume, as well changes in tissue matrix composition, thus deeply compromising tissue function.

Secondary lymphedema of the external genital organs is a very common consequence of invasive surgery of the pelvic[11] and inguinal area[12] with associated radical lymphadenectomy and radiotherapy[13]. In particular, lymphedema of the male genital organs and of the hypogastric area is a very impairing condition characterized by an important increase in the volume of the external genital organs, tissue fibrosis, erysipelas, and objective difficulties in the normal use of lower limbs and the penis. Hence, although pelvic lymph node dissection (PLND) is an essential staging approach to cancer patients, the secondary lymphedema that can develop as a complication of this procedure is very impairing to the patient's normal quality of life, even from a psychological standpoint.

TREATMENT OF THE SECONDARY LYMPHEDEMA OF THE MALE EXTERNAL GENITALS: STATE OF THE ART

In the upper and lower limbs, the first treatment of secondary lymphedema consists in local physical therapy, massage, bandage and external compression, which improves the lymphatic drainage by slowly reducing tissue fluid imbibition, increasing local tissue pressure and/or muscular tone and, eventually, shunts development. However, when the lymphatic drainage pathways have been excised and/or interrupted by fibrous scars developed after surgery and/or radiotherapy, medical treatment of lymphedema very seldom provides satisfactory results[14]. This is particularly true in patients affected by secondary lymphedema of the external male and female genital organs in which effective physical therapy cannot be completely performed and that can therefore be treated only by surgery. Various surgical approaches has been utilized: (1) lymphangiectomy associated to adipectomy, the excision of involved subcutaneous tissue, remains the most common approach to the treatment of chronic male external genital organs lymphedema[15]; and (2) more recently, lymphangenctomy/adipectomy has been improved by a plastic surgery procedure consisting in resection of scrotal and penile lymphedematous tissue, reconstruction with a posterior fascio-cutaneous flap from the scrotum itself and penile reconstruction with a skin graft[16,17].

These classical surgical approaches are usually very invasive and imply important demolition of a large part of the scrotal and penile tissues with high risk of lymphedema recurrence. Therefore, in the last decade, with the development of more sophisticated microsurgical techniques and aiming at favoring new lymphangiogenesis in the lymphedematous tissues, the homologous lymph nodes contained in vascularized tissue flap excised from the inguinal, crural or axillary districts of the same patient have been transplanted in lymphedematous external male genitals or limbs[18]. An alternative microsurgical de-

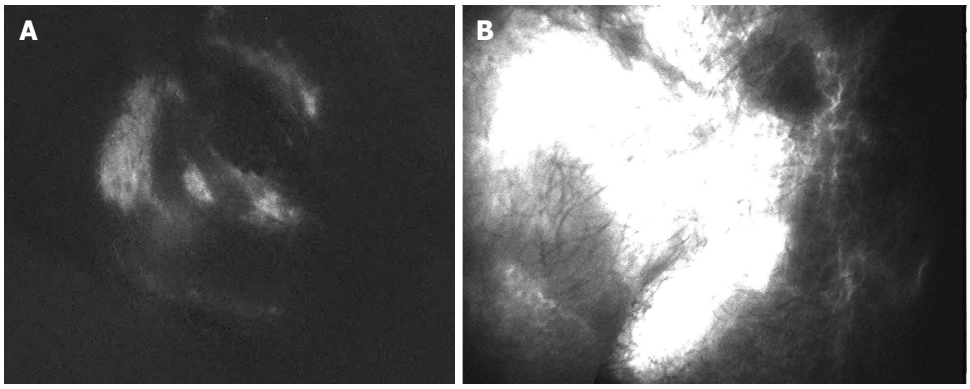

Figure 1 Photodynamic eye lymphography. A: Photodynamic eye (PDE) lymphography of normal testis. The injected indocyanine is canalized into the local lymphatic network and is rapidly drained out of the subcutaneous scrotal tissue; B: PDE lymphography of lymphedematous testis. Indocyanine diffusively accumulates in the scrotal subcutaneous tissue as a consequence of the impaired lymphatic drainage.

rivative procedure utilizes only the subcutaneous scrotal lymphatic vessels, ignoring the alternative draining pathways[19]. In spite of the great technical improvement offered by these pioneering microsurgical approaches, they do not seem yet to offer enough guarantees to ensure an efficient long-term treatment of the extended, severe, secondary lymphedema of the external genitals organs.

A NEW MICROSURGICAL APPROACH IN THE TREATMENT OF MALE EXTERNAL GENITALS SECONDARY LYMPHEDEMA: BILATERAL LYMPHO-VENOUS SHUNT OF THE SPERMATIC CORD

The development of microsurgical techniques has allowed a minimally invasive approach to the treatment of secondary lymphedema of lower or upper extremities and more rarely of other body districts. The present Review describes the application of a new microsurgical lymphovenous derivation technique for the treatment of male external genital organs secondary lymphedema. Indeed, following a careful autoptic observation of the lymph node-chain tributaries of the collecting lymphatic ducts draining the testis during previous external iliac-chain lymphadenectomy, we developed a technique that allowed us to perform direct anastomosis between the lymphatic collectors running within the spermatic cord afferent to the external iliac chains and the vessels tributary to the spermatic veins[14,20]. The idea of developing new drainage pathways of testicular lymphatic collectors in spermatic cord originated from the consideration that excision of lymph node-chain tributaries of lymphatic ducts draining the testis could determine lymphedema of external genitals as a consequence of the insufficient drainage through the superficial external genitals lymphatics.

On the other hand, locoregional PLND with excision of the pelvic fibrous-fatty tissue using as margins the external iliac vein and the posterolateral aspect of obturator fossa, is required in prostate cancer to attain an accurate diagnosis of occult micrometastases and to stratify patients who might benefit from adjuvant therapeutic measures.

From January 2006 to November 2013, 18 patients suffering from lymphedema of male external genitals or male genital and/ or lower limbs lymphedema were screened for lymphedema treatment. Out of these patients, 10 were candidate to microsurgery according to the following inclusion criteria: (1) patients from 25 to 75 years old; (2) at least 3rd degree lymphedema, with no satisfactory and/or durable results after physical and/or pharmacological therapy; (3) free of tumor for at least two years; (4) at least one year since the last post-surgical adjuvant oncological treatment; and (5) less than 15 years since the primary oncological surgery lymphedema development. Patients with co-morbidity as cardiopathy, respiratory diseases, renal failure or severe bilateral lower lymphedema were oriented towards medical and physical therapy.

The pre-operative screening included: (1) lower abdomen and genitals echography, lymphoscintigraphy of lower limbs and inguinal area. Indeed, since there are currently no studies documenting the use of lymphoscintigraphy for the identification of genital organs pathologies, echography was the only diagnostic instrument available for this purpose. Subsequently, the green indocyanine lymphography, Photodynamic eye (PDE, Hamamatsu Photonic K.K., Tokyo, Japan) imaging[21] was utilized to investigate the patency of the genitalia lymphatic network (Figure 1). Briefly, 0.2 to 1 mL green indocyanine were injected bilaterally at the base of the scrotum through an insulin needle. After few minutes of massage, the fluorescent dye in subcutaneous lymphatic vessels at a maximum depth of 2-3 cm were visualized through a portable near-infrared camera system. Images were conveyed on a PC monitor and stored. The Echo-color doppler of lower limbs was also performed to exclude vascular disease such as deep veins thrombosis.

In the first two candidate patients, lymphedema affected both the external genitals and the lower limb: therefore, a preliminary lympho-venous shunt of lower limb was performed followed, after six months, by the

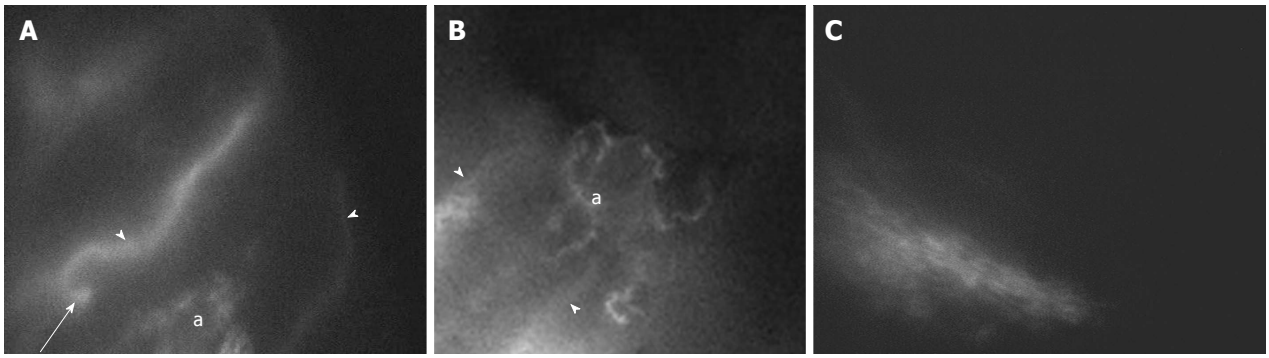

Figure 2 Photodynamic eye lymphography of lymphatic meshes. A and B: Photodynamic eye (PDE) lymphographies of the complex mesh of small lymphatic vessels (a) developed between lymphatic collectors (arrowheads) at 3 mo from surgery in responder patients. Arrow: lympho-venous anastomosis; C: PDE lymphography showing, in a non responder patient, the complete absence of lymphatic network at 3 mo from surgery.

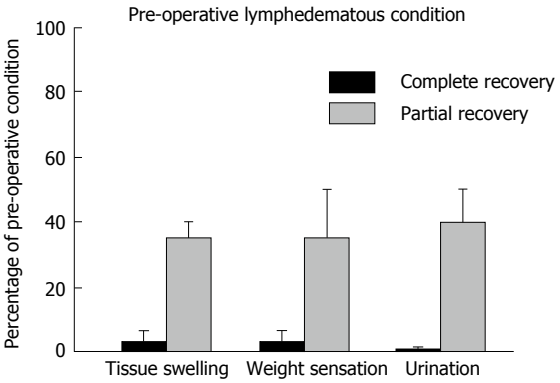

Figure 3 Post surgical self-evaluation of tissue swelling, weight sensation and urination expressed as percentage of the pre-operative lymphedematous condition in patients showing a complete or only partial recovery at three months after surgery. Based on patients evaluation, even partial recovery represented a significant improvement with respect to pre-operative condition. In responding patients the objective clinical outcome was accompanied by a subjective evaluation of almost complete recovery of the normal tissue swelling, weight sensation and urination. Zero percentage on the Y axis corresponds to the normal condition. Histograms presents mean values ± SD of the mean.

planned lympho-venous shunt of spermatic cord. Under general anaesthesia, bilateral funiculum lymphatic collectors (diameter approximately 500 μm) were visualized under stereomicroscopic magnification (40 ×) after cutaneous inguino-scrotal, cremaster and vaginal tunica incision. Identified lymphatic collectors were used to perform (with interrupted stitches, Vicryl 10-0 or 9-0) the lympho-venous termino-lateral or termino-terminal anastomosis, depending upon the proximity and the pathway followed by either the veins of the pampiniforms plexus or the spermatic vein previously prepared through adventixectomy. The number of anastomosis (up to four) depended upon the number of lymphatic collectors found, the higher the anastomosis number the higher the chances of a more effective drainage.

The microsurgical procedure lasted between five and seven hours, depending upon the extent of tissue fibrosis and surgery complexity: indeed, the microsurgical approach is highly demanding in terms of intra-operative technical skill, surgical and laboratory training and on-site imaging equipment. On the other hand, the described microsurgical effort is worth, as it offers several major advantages with respect to the standard approach, such as: (1) absence of clinical complications, (2) low invasivity and more rapid post-surgical recovery; and (3) minor tissue demolition and more satisfactory post-surgical functional and esthetic results.

Since low-weight molecular heparin has been used during the surgery, incidence of thrombosis into the lymphatic channels or in the anastomosis was minimized. Antibiotic was maintained for seven days, while heparin therapy continued for at least 30 d. Neither warfarin nor thrombin inhibitors were necessary.

LONG TERM CLINICAL OUTCOME OF THE MICROSURGERY

At three months after surgery, the patency of lympho-venous anastomosis was assessed by PDE: of the 10 patients who underwent microsurgery of spermatic cord, 8 showed early improvement, while an almost complete (95%-100% of scrotal swelling) remission was observed at six months after surgery. The clearest observation was the correspondence between the clinical outcome, the restored motility of lymphatic collectors observable during PDE lymphography[22], and the anastomosis patency. Indeed in responding patients, the distribution and canalization of the injected fluorescent dye improved over time with clearly visible patent anastomosis (Figure 2A and B). Clear identification of patent scrotal subcutaneous and spermatic lymphatic vessels in responding patients is accompanied by a significant reduction of tissue fluid volume and of the hypogastric abdominal wall thickness. Viceversa, only two patients (not-responders) showed much slower improvement, with only partial recovery (approximately 60%) at six months. In these non-responding patients, PDE images obtained at three months after microsurgery (Figure 2C) highlight the disperse distribution of fluorescent dye in the subcutaneous with difficult localization of the upstream lymphatic collectors and anastomosis.

The post-surgical patient self-evaluation demonstrated (Figure 3): (1) improvement of scrotal tissue softness

and scrotal skin normochromic aspect; (2) pain absence, (3) disappearance of the edematous volume with evident reduction of scrotal and penile dimensions with normal palpability of the testis and decreased weight sensation, (4) decreased urination and (5) the disappearance of episodic erysipelas with respect to the pre-operative condition. The postsurgical improvement assessed at three months after surgery by PDE and self evaluation was maintained over several years in all responding patients. The restoration of the preoperative scrotal size may sometime require an additional plastic surgery to remove the excessive scrotal skin.

COMBINED SWELLING OF EXTERNAL GENITALS AND LOWER LIMBS

The lower limb is supplied by: (1) a superficial lymphatic network, merging into lymphatic collectors directed to the inguinal lymph nodes from which the lymph is carried through the iliac collectors into the cisterna chili and thereafter to the thoracic duct and, eventually, into the subclavian veins; and (2) a deeper lymphatic network, whose collectors follow the sub-fascial limb neuro-vascular plexus to reach the peri-aortic lymph node chains and the thoracic duct. As a consequence, patients undergoing to inguinal and/or pelvic lymphadenectomy may develop a secondary lymphedema of the lower limb. We chose to micro-surgically treat the secondary lymphedema of the lower limb by performing a lympho-venous termino-terminal anastomosis between inguinal pre-nodal lymphatic collectors of the limb and collateral branches of the saphena or, when collaterals were not available, a termino-lateral anastomosis between inguinal pre-nodal lymphatic collectors and the saphena itself[14,20]. In the lower limb, the lympho-venous anastomosis is preferable compared to the lymphatic grafting technique usually performed in the treatment of upper and/or lower limb lymphedema[23]: (1) to more efficiently exploit the strong muscular contraction of the thigh musculature; and (2) to avoid the risk of inducing lymphedema in the previously healthy controlateral leg.

A common clinical observation is that lymphedema of the external genitals is often combined with either unilateral or bilateral chronic swelling of the lower limbs. In the first two cases coming to our observation, a two-stage treatment was performed: first, the microsurgical lympho-venous shunt of the lower limb and then, after six months, the derivative lympho-venous anastomosis of genital organs. The results of this approach clearly indicated that surgical treatment lymphatic collectors of the thigh draining lower limb edema may result in an improvement of the genitalia edema (Figure 4); in turn, shunting of the spermatic cord in treating of genital organ edema often results in recovery from lower limb edema so that lower limb surgery was deemed unnecessary. This could be explained by the fact that, after excision of inguinal lymph nodes, lymph from lower limbs is shunted through the scrotal and testicular pathways. Similarly, when testicular drainage is restored, edema of the scrotum and penis is also significantly improved, possibly because of lymphatic connections between the testicular and scrotal lymphatics networks. These results point to the existence of complex shunt network connecting the local lymphatic drainage pathways. It is worth noting that the target of physical therapy is to improve lymph-edematous tissue drainage bypassing the obstruction by recruiting shunted pre-existing lymphatic vessels usually supplying adjacent tissue districts.

POST-SURGICAL RESPONSE OF THE LYMPHATIC VASCULATURE

In addition to recruitment of patent superficial and spermatic cord lymphatic vessels, the development of peri-anastomotic lymphatic meshes in different tissues such as the spermatic cord, the inguino-crural and brachial regions was observed and documented for the first time in several patients who positively responded to microsurgery. Meshes were instead never encountered by PDE in non-responding patients or in normal tissues. Lymphatic meshes (Figure 2) consist of several lymphatic vessels sprouting from the anastomosis and/or between afferent lymphatic collectors and merging into well-canalized and complex networks. Subcutaneous meshes could be detected as early as three months after surgery and their complexity and extension seemed to improve over time, as shown by PDE images at 6 mo.

Lymphatic meshes were observed not only in patients who underwent spermatic funicular microsurgery, but also in patients who positively responded to the treatment of the lower limb swelling (Figure 5). On the contrary, no canalized lymphatics were evidenced at the same post-surgical follow-up in non-responding patients, who showed no significant improvement when compared to the pre-surgical PDE.

The development of this network might well explain the successful outcome of the microsurgical procedure in responding *vs* non-responding patients with little or no improvement. Indeed, it seems difficult to attribute the positive results of the surgical treatment to improvement of only one single lymphatic draining district. It is at present not known whether lymphatic meshes of the type observed in this study after microsurgical procedure may develop also in conservative physical therapy.

The post-surgical development of peri-anastomotic lymphatic meshes might serve as: (1) capacitance reservoirs to improve the draining capability of the existing lymphatics and maximize the drainage capacity of the postsurgically-canalized lymphatic vessels; and (2) intermediate compartments placed in series between the smallest initial lymphatics taking place from the edematous tissue and the larger collecting vessels transporting the lymph out of the tissue. At present, we are unable to precisely establish whether these lymphatic meshes reflect recruitment of previously existing but obliterated networks or, rather, new lymphangiogenesis. The fact that

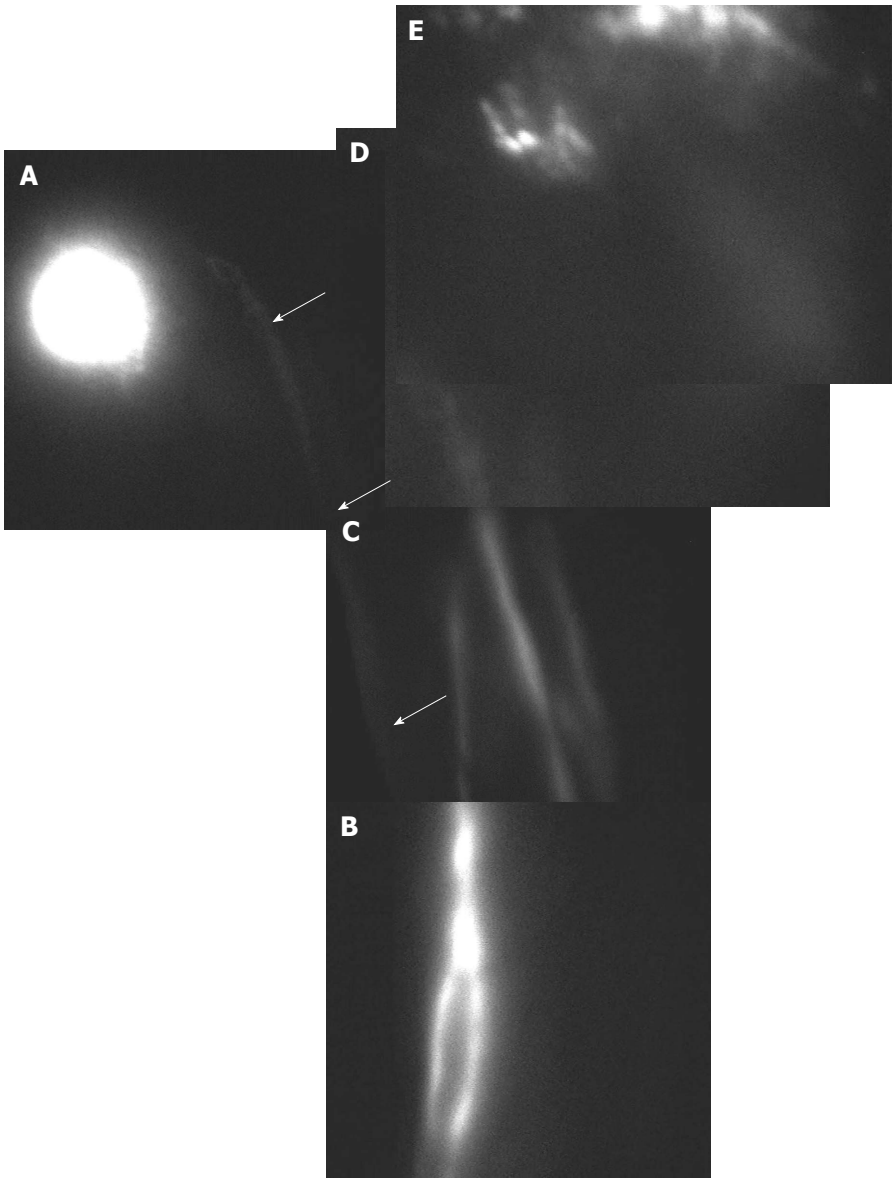

Figure 4 Photodynamic eye lymphography performed at three months after lympho-venous shunt of the the spermatic cord in a patient affected by lymph-edema of the lower limb and of the external genitals secondary to radical prostatectomy and lymphadenectomy of the pelvic-inguinal lymph-nodes chain. After injection (bright spot, A) in the scrotum, fluorescent indocyanine travels along a lymphatic vessel (arrows) in the thigh to connect with the lymphatic network of the lower limb (B). After injection in the foot, indocyanine flows along the lower limb lymphatics vessels (B and C) to be shunted directly into the external genitals lymphatic plexus (D and E) bypassing the excised pelvic-inguinal lymph-nodes chain.

meshes were never observed in PDE lymphography of normal healthy tissues, but only during the post-surgical follow up in previously oedematous tissues of patients which positively respond to microsurgery might support the hypothesis of local lymphangiogenesis triggered by the minimally invasive microsurgical procedure within a process of extracellular matrix and lymphatic repair. In particular, the visual observation that the sprouting was particular intense in proximity of the patent anastomosis, is in line with the experimental observation[24] that, in the rat tail in presence of interstitial fluid flow, such as that induced by the new patent lympho-venous anastomosis, lymphatics may bypass a tissue lesion, reconstructing the original lymphatic pathway.

Although the lymphatic system still represents the least known compartment, particularly in humans, of the cardiovascular-extracellular tissue system, a growing amount of information is available on lymphatic cell biology and on the mechanical and molecular factors promoting functional lymphangiogenesis[25-32]. During embryogenesis the endothelial cells of the cardinal vein express the lymphatic endothelium-specific hyaluronan receptor (LYVE-1) and the VEGFR-3, which serves as a signalling receptor for the lymphatic-specific growth factors C (VEGF-C) and VEGF-D. Activation of VEGFR-3 signal is sufficient to induce lymphangiogenesis[30]. Later in adult life both LYVE-1 and VEGFR-3 are expressed by the lymphatic, but not by the blood endothelial cells. Subsequently, a still undefined mesenchymal factor induces, in specific endothelial cells of the cardinal

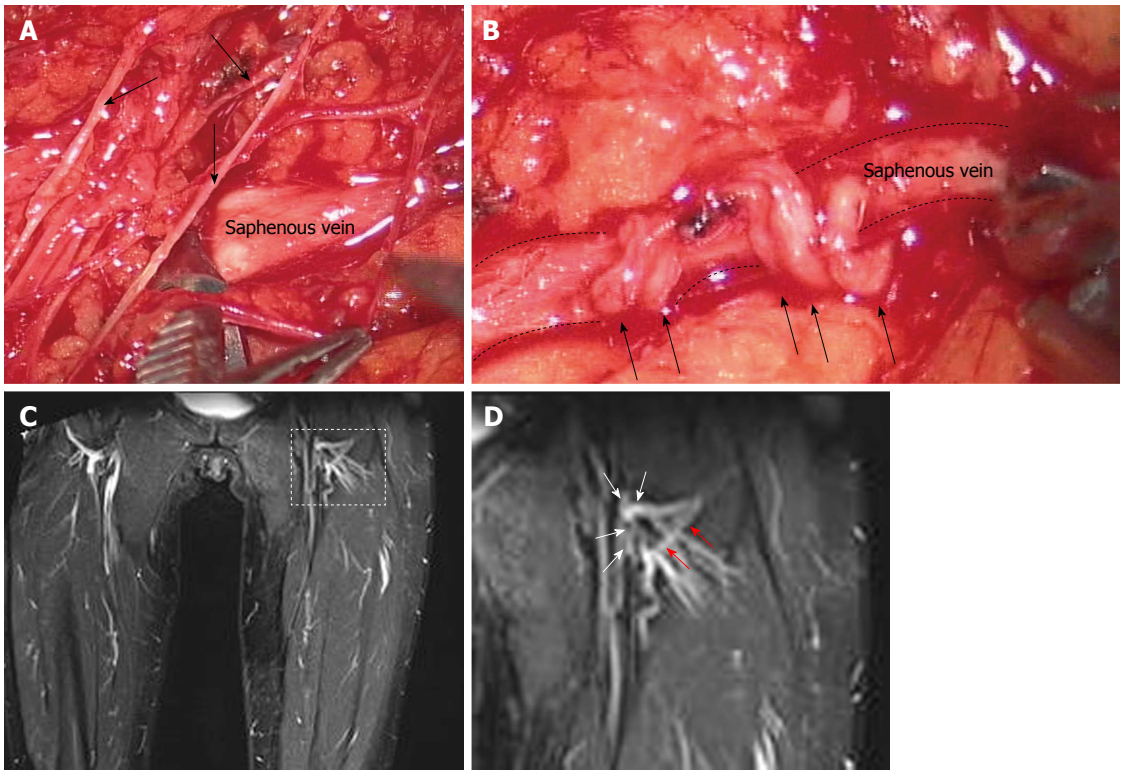

Figure 5 Lymphatic meshes were observed not only in patients who underwent spermatic funicular microsurgery, but also in patients who positively responded to the treatment of the lower limb swelling. A: Microsurgical identification of the saphenous vein and of lymphatic vessels (arrows) in the crural area; B: Latero-terminal lympho-venous anastomosis, confectioned with interrupted stitches, between lymphatic vessels (arrows) and the saphenous vein (dashed line) in proximity of the femoral-saphenous cross; C: Magnetic resonance image of the lower limb obtained in the same patient of A, B at 8 years after microsurgery. Details of the left limb crural area (delimited by the dashed line) are presented in D; D: White arrows identify the sites were latero-terminal lympho-venous anastomosis were confectioned, while red arrows focus on a new developed lymphatic collector.

veins the expression of the protein Prox1[31,32] which in the adult, is expressed by the lymphatic endothelium and by several non endothelial cells like lens, retina, central nervous system, heart, liver and pancreas[33-35]. Prox1-positive cells emerge from the original vessels to form new lymphatic sprouts. Prox1 is necessary to the development and differentiation of lymphatic endothelial phenotype[31,32], a phenomenon which implies induction of lymphatic specific genes such as VEGFR-3, podoplanin and desmoplakin-1[25,28] and simultaneous repression of blood vascular specific genes like collagens, laminin, VEGF-C, E-selectin, neuropilin-1 and interleukin-8. The transmembrane mucin-type glycoprotein podoplanin, expressed during embryogenesis by all cardinal vein and Prox1-sensitive cells seems to play an important role in lymphatic endothelial cells migration, adhesion and vessel formation[29].

At present, no information are available concerning the expression of pro-lymphangiogenetic endothelial and tissue molecules in human lymphedematous compared to normal tissue in murine models of primary lymphedema[33], dermal tissue swelling, interstitial fluid and solute transport critically depend on collagen and lipid dermal composition, suggesting that lymphatic function may be modulated by extracellular structure and macromolecular content. However, the behavior of normal lymphatic vessels exposed to either radiation or trauma, as in secondary lymphedema patients, may be quite different from those induced by primary genetic mutation.

CONCLUSION

The choice of the microsurgical lympho-venous derivation in the spermatic cord is highly recommended in the treatment of secondary severe chronic lymphedema of the external male genitals. Indeed, in addition to its direct significant advantages with respect to the standard approaches, such as: (1) minor tissue demolition and more satisfactory post-surgical functional and esthetic results; (2) absence of clinical complications; and (3) low invasivity and more rapid post-surgical recovery, the microsurgical lympho-venous derivation offers the unique possibility of improving lymphedema in adjacent tissues which are supplied by lymphatic collectors emptying into common lymphatic shunt.

Last but not least, lympho-venous microsurgery triggers the development of a complex network of new lymphatic vessels in proximity to the anastomosis area. This phenomenon, that we documented for the first time in human patients, seems to play a crucial favorable role in guaranteeing the long term recovery from chronic lymphedema after microsurgical treatment.

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MINIREVIEWS

Is periprostatic adipose tissue associated with aggressive tumor biology in prostate cancer?

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Abstract

The prevalence of overweight and obesity and their health-related problems have been increasing. Obesity is increasingly recognized as a risk factor in different types of cancer in humans. The mechanisms supporting the link between obesity and cancer development have not been fully understood. Leptin, a circulating cytokine produced by adipocytes, may influence prostate cancer (PCa) progression in different ways. Body mass index seems to be an unreliable predictor for the development of PCa, but its influence on progression and poor oncological outcomes seems to be clear. Given the fact that abdominal fat is the most metabolically active fat, with different metabolic and paracrine effects, related anthropometric measurements may lead to a better estimation of PCa risk. Metabolically active periprostatic abdominal fat may also play an important role in releasing cytokines and growth factors that may promote tumor cell proliferation or even create a favorable environment for aggressive tumor biology. Different imaging measurements, *e.g.*, periprostatic adipose tissue (PPAT) thickness, may be significant predictors of PCa. Several genes in the PPAT of obese men have been identified to contribute to chronic immuno-inflammatory responses which eventually lead to cell cycle alteration with oncological potential. *In vitro* studies showed the importance of PCa and its interaction with its microenvironment particularly in patients with aggressive PCa. Different types of cytokines, such as interleukin-6, may promote a tumorigenic microenvironment. This article endeavors to review the current literature on the association of PPAT with aggressive tumor biology in PCa.

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Key words: Prostate cancer; Obesity; Periprostatic fat

Core tip: Globally, prostate cancer (PCa) is highly prevalent. Although the prevalence of PCa is similar across different populations, major differences in PCa incidence and mortality are seen worldwide. A contribution of environmental factors, such as obesity, may play an important role. Most studies used body mass index as a factor of obesity. However, only visceral fat is metabolically active. In a study by van Roermund *et al*, periprostatic fat measured on a computed tomography scan correlated with tumor aggressiveness. In this review, we aim to give more insight into the relationship between periprostatic fat and Pca aggressiveness by reviewing the recent literature.

Den Hollander PP, Rademakers KLJ, van Roermund JGH. Is periprostatic adipose tissue associated with aggressive tumor biology in prostate cancer? *World J Clin Urol* 2014; 3(3): 320-324 Available from: URL: http://www.wjgnet.com/2219-2816/full/v3/i3/320.htm DOI: http://dx.doi.org/10.5410/wjcu.v3.i3.320

INTRODUCTION

Over the past few decades, the prevalence of overweight and obesity have been increasing in developed countries[1]. Obesity is currently seen as a serious health problem in particularly well-developed populations. More

recently, it has been hypothesized that obesity could potentially influence the risk of developing cancer. Several studies already showed a correlation between obesity and different types of cancer[2-4]. However, the relationship between obesity and the risk of developing prostate cancer (PCa) is still unclear, as studies are inconsistent in their outcome[5,6]. The incidence of clinically significant PCa is highest in well-developed countries, and in Europe, PCa is the second most common cause of cancer-related death in men[7].

Although many risk factors for PCa are well known, including age, ethnic background, and family history, the mechanisms of the interactions between lipids and carcinogenesis are not fully understood. Multiple studies suggest an important role for metabolic active periprostatic adipose tissue (PPAT) in the risk of developing PCa[8-12]. This review specifically considers the relationship between PPAT and the risk of developing aggressive PCa, and discusses the underlying mechanisms and clinical consequences.

RESEARCH

For this review a literature search in PubMed was performed using the MeSHsearch terms; "periprostatic fat", "obesity" linked with "prostate cancer". Studies relating to periprostatic adipose tissue and PCa were selected, and limited to articles in the English language. Studies identified in references and considered relevant were also included.

OBESITY AND PROSTATE CANCER

Obesity is considered as one of several factors potentially related to the development of clinically significant PCa. Earlier postmortem research showed a high prevalence of PCa, occurring in approximately 80% of men > 70 years of age who died from other causes[13,14]. The high prevalence of PCa in elderly males supports a hypothesis that progression of latent PCa to clinically significant PCa might be associated with environmental and lifestyle factors. In particular, abdominal obesity may be an important factor influencing progression of latent PCa to clinically significant PCa, rather than development of latent disease.

Evidence that body mass index (BMI) is related to a higher incidence of PCa is still inconsistent. A prospective cohort study in 900000 adults showed a significant positive linear trend in death rates with increasing BMI for all cancers, including PCa[3]. In that study, men with a BMI of at least 35.0 appeared to have a significantly elevated relative risk of cancer-related death compared with normal weight males. In relation to these data, a systematic review of MacInnes evaluated the association between anthropometric measurements and the risk of PCa[15]. In that meta-analysis a weak positive correlation between the risk of PCa and BMI and waist-hip ratio (WHR) was found. Moreover, subgroup analysis showed a positive correlation between the risk of advanced PCa and BMI and WHR, supporting the hypothesis that obesity might influence PCa progression or tumor biology. In contrast, no direct correlation between PCa and weight was found in the study.

A large European prospective cohort study in 150000 men suggested that higher waist circumference and WHR may be associated with increased risk of advanced PCa and high-grade PCa amongst individuals with lower BMI. No association was seen in males with higher BMI levels, suggesting that abdominal adiposity mainly played an important role in influencing PCa[16]. An explanation for the inconsistency in the results can be found in the fact that BMI, or even WHR, are inadequate measures of obesity as they include adipose and non-adipose body components. Given the fact that abdominal fat is the most metabolically active fat tissue, we should explore other methods for estimation of adipose tissue, such as fat distribution, which may eventually link obesity to PCa.

Several studies have correlated obesity with higher PCa grade and poorer oncologic outcomes[17-20]. Capitanio *et al*[17] showed that BMI was independently associated with PCa volume at radical prostatectomy. These results were confirmed by Freedland *et al*[18] in over 2200 patients treated with radical prostatectomy, and showed that obese males were more likely to have high-grade tumors. In addition, more adverse pathological features and a higher risk of biochemical recurrence were seen in obese patients. Significantly higher rates of PCa recurrence over time were observed in obese patients (BMI $\geqslant$ 30) compared with their non-obese counterparts in two other studies[19,20].

Possible mechanisms linking obesity and cancer involve endocrine disturbances such as increased serum estrogen, insulin, insulin-like growth factor and leptin, and decreased free testosterone[21-25]. However, the mechanisms by which obesity leads to an increased risk of PCa are not known, and may occur at multiple levels. Recently, different *in vitro* studies on leptin have been performed. Leptin is a cytokine produced by adipocytes. Like insulin, leptin controls body weight homeostasis through food intake and energy balance[26]. Circulating serum leptin levels correlate with body fat in humans. Hoda *et al*[21] showed that a chronic systemic increase in leptin might enhance the growth of prostate or other cancer cells by activation of the mitogen-activated protein kinase pathway. Moreover, leptin stimulated growth of breast, esophagus, and prostate cancer cell lines in different studies[24,27,28]. In addition, leptin may accelerate PCa progression by promoting androgen-independent cell proliferation[25].

PERIPROSTATIC ADIPOSE TISSUE

PPAT surrounds the prostate capsule[29]. In an earlier study of Hong *et al*[30], 100 prostatectomy specimens were analyzed, and showed that adipose tissue covering the prostatic surface was present in 48% of prostates, with the least adipose tissue found posteriorly. By analyz-

ing PPAT, valuable new insights could be attributed to the role of adipose tissue in PCa pathophysiology. This particular adipose tissue is not only used for storage of triglycerides, but also plays a role in releasing cytokines and growth factors that may promote tumor cell proliferation or play a role in creating a favorable environment for aggressive tumor biology[10,11,31,32]. Finley *et al*[10] analyzed PPAT of patients who underwent a radical prostatectomy. They suggested that once cancer cells extend beyond the prostate capsule, these PPAT-secreted factors, may influence the phenotypic behavior of malignant cells *via* extracellular matrix components or direct cell-to-cell contact. This cellular mechanism and PPAT-secreted factors have been proposed in a limited number of different publications which we will discuss below.

IMAGING

Only three studies used *in vivo* imaging techniques in investigating the relationship between PPAT and PCa. van Roermund *et al*[8] retrospectively analyzed whether periprostatic fat and subcutaneous fat measured by computed tomography correlated with advanced PCa stage in a group of patients who underwent external radiotherapy or brachytherapy for localized PCa with different risk classifications according to Ash *et al*[33]. A significantly larger total periprostatic fat area and higher periprostatic fat density was seen in patients with high risk PCa, compared with low and intermediate risk patients[8]. An association was found between more periprostatic fat and higher periprostatic fat density, and prostate-specific antigen (PSA) $\geqslant$ 10 ng/mL, a T3 tumor, and a Gleason score $\geqslant$ 8. A different study, analyzing PPAT as a marker for PCa aggressiveness in patients only treated with brachytherapy, showed no correlation between PPAT and advanced PCa stage[34]. A confounding factor might be that brachytherapy is mainly used in low-risk PCa patients, which might explain the absence of a correlation in this particular study.

Bhindi *et al*[9] evaluated whether PPAT thickness on transrectal ultrasound could be a predictive factor for the detection of PCa in men with no prior diagnosis of PCa. Besides known predictors of PCa, such as older age, African ethnicity, family history of PCa, abnormal digital rectal examination (DRE) and elevated PSA level, PPAT thickness was also a significant predictor for detection of PCa or high-grade PCa. They suggested that for each millimeter increase in PPAT thickness, the odds of detecting any PCa or high-grade PCa increased by 12% and 20%, respectively. So beside other clinical parameters, including age, abnormal DRE and PSA level, PPAT may have a clinically significant role in diagnostic algorithms for PCa in the future[9].

GENETICS

The individual genome represents the starting point for transformation of prostate epithelial cells and its microenvironment. Knowledge of the PPAT genetic profile may uncover more about the relationship with PCa tumor biology. Until now, only one study reported on the gene expression of PPAT in relation to PCa. Ribeiro *et al*[35] aimed to determine the spectrum of genes expressed in PPAT to evaluate the influence of obesity on PCa and vice versa. PPAT was obtained from 18 patients, with an equal distribution of obese and lean men. Patients underwent a retropubic radical prostatectomy for PCa (organ-confined and extra-prostatic) or open prostatectomy in the case of benign prostatic hyperplasia. Results showed that several genes in the PPAT of obese men contributed to chronic immuno-inflammatory responses, *i.e.*, anti-lipolytic, lipogenic, proliferative and anti-apoptotic activities. These immuno-inflammatory responses lead to white adipose tissue overgrowth and alteration of the cell cycle to promote oncogenesis[35,36]. These findings support the importance of PPAT and the presence of a complex relationship between obesity and PCa.

MICROENVIRONMENT

Tumor cell progression depends on the tumor characteristics as well as on the surrounding microenvironment. Moreover, after highlighting the importance of the genetic profile, we should not underestimate the influence of interactions between tumor cells and their microenvironment[10,31,37-39]. The microenvironment is able to influence the proliferation, migration and metastatic behavior of tumor cells by modulating the extracellular matrix and growth factor production[40]. In breast cancer, a paracrine effect of adipose tissue on the microenvironment of the tumor is already associated with poorer oncologic outcomes[41]. Tokuda showed in an *in vitro* study that PCa cells grow differently in an adipocyte-rich environment, compared with control cultures[39]. In particular, higher cytokine expression in PCa cells was seen when cultured in an adipocyte-rich environment.

A pilot study by Venkatasubramanian *et al*[42] on PPAT from obese PCa patients aimed to explore whether PPAT from obese patients differed from that of lean patients, with specific attention to differences in the microenvironment. *Ex vivo* magnetic resonance imaging and histology were used on the collected tissue of patients who underwent a radical prostatectomy for primarily low-grade PCa. PPAT from obese patients showed significantly increased proliferation of PCa, compared with PPAT from lean patients.

Visceral adipose tissue differs from peripheral adipose and secretes different types of cytokine-like adipokines, leptin and adiponectin, numerous inflammatory mediators, interleukins, chemokines and growth factors. Finley *et al*[10] explored the association of cytokines and growth factors secreted by PPAT in patients with aggressive PCa. In this study, significantly higher levels of interleukin-6 (IL-6) were shown in PPAT of patients with higher grade tumors. Higher IL-6 levels were associated with downstream activation of signal transducer and activa-

tor of transcription 3 phosphorylation, which promotes cell cycle progression, tumor invasion and host immune system evasion. This suggests that PPAT modulates PCa aggressiveness by serving as a source of IL-6. This modulating role of PPAT is confirmed by Ribeiro *et al*[11], who showed locally increased activity of matrix metalloproteinase (MMP), a proteolytic enzyme facilitating and promoting PCa cell survival and migration. Interestingly, higher levels of MMP9 were seen in obese men with esophageal adenocarcinoma, and is associated with poor tumor differentiation in these patients[43]. In PCa, MMP activity in PPAT is significantly increased compared with that in the PPAT of patients with benign prostatic hyperplasia[31].

CONCLUSION

Though limited, studies evaluating the association between PPAT and PCa describe evidence for an interaction between PCa and its surrounding tissues. Data suggest that local adipose tissue can alter the behavior of cancer cells in different ways. Microenvironment-related release of factors such as cytokines and interleukins, play an important role in promoting survival and migration of PCa cells, and thus PCa progression. Different genes in PPAT of obese patients also contribute to a favorable environment for tumor growth. Given that PPAT thickness can be related to PCa aggressiveness, this may represent a diagnostic tool with important prognostic value, since BMI is unreliable as a prognostic factor. The findings of this review of the literature should promote future studies to further investigate the value of PPAT in PCa etiology and its relationship to the development of aggressive PCa. New therapeutic strategies could be developed, and more studies of the diagnostic value of PPAT should be pursued.

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MINIREVIEWS

Morphological and functional evaluation of chronic kidney disease using magnetic resonance imaging

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Abstract

X-ray computed tomography (CT), ultrasonography (US) and radionuclide scanning are important clinical methods for evaluating morphology of the kidney. These modalities are also applicable for estimating kidney function with time lapse analysis using proper contrast-media as may be necessary. In the case of US, it can estimate kidney function based on the measurement of blood flow using the Doppler effect. Formerly, magnetic resonance imaging (MRI) was an inappropriate diagnostic imaging technique for abdominal organs because of their respiratory displacements. However, MRI is now actively used for kidney as well as liver or other parenchymal organs, in tandem with the technological advances. Unlike unenhanced X-ray CT, "conventional" MRI can distinguish the border between cortex and medulla in T1 or T2 weighted images. It was known that the border blurred with decreasing kidney function. Moreover, several other particular imaging methods were introduced in recent years, and these could be called "functional" MRI. In this review, the following are discussed: functional MRI for chronic kidney disease, which include blood oxygenation level-dependent MRI for evaluation of hypoxia, diffusion-weighted imaging for evaluation of fibrosis, diffusion tensor imaging for evaluation of microstructure, and arterial spin labeling to evaluate the amount of organ perfusion, accompanied with several related articles. The ultimate goal of functional MRI is to provide useful *in vivo* information repeatedly for daily medical treatment non-invasively.

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Key words: Magnetic resonance imaging; Chronic kidney disease; Blood oxygenation level-dependent effect; Diffusion-weighted imaging; Diffusion tensor imaging; Arterial spin labeling; Fibrosis; Hypoxia; Functional magnetic resonance imaging

Core tip: Recent advances in diagnostic imaging technology allow us to evaluate the function of parenchymal organs such as kidney and liver. For example in the kidney, magnetic resonance imaging technique can visualize changes in the ratio of oxy-/deoxy-hemoglobin, the accumulation of extra cellular matrix, the alteration of microstructure of tubules and blood capillaries in the interstitium, and the amount of organ perfusion without using any contrast medium. Thus, with imaging technique, the nephrologist can evaluate a kidney from multiple points of view. This is an emerging modality, and it's hoped-for mission is providing significant information for daily clinical decisions. Accumulation of further knowledge is keenly anticipated.

Inoue T, Kozawa E, Okada H, Suzuki H. Morphological and functional evaluation of chronic kidney disease using magnetic resonance imaging. *World J Clin Urol* 2014; 3(3): 325-329 Available from: URL: http://www.wjgnet.com/2219-2816/full/v3/i3/325.htm DOI: http://dx.doi.org/10.5410/wjcu.v3.i3.325

INTRODUCTION

Magnetic resonance imaging (MRI) until now has had an

important role as a diagnostic imaging method for urology and nephrology. Gadolinium contrast medium was used frequently for examinations in the past. However, since nephrogenic systemic fibrosis was identified, the frequency in use of the contrast media in renal disease seems to have decreased. However, significance of the role of MRI is increasing. MRI, which is superior in contrast resolution of soft tissues to X-ray computed tomography (CT) and ultrasonography, provides important information for internal architecture of the kidney and adrenal gland, or qualitative diagnosis of tumor lesions (*e.g.*, presence of fat composition) particularly in cases of kidney dysfunction for which it is difficult to use iodine-based contrast medium. Furthermore, magnetic resonance angiography is useful for evaluation of renal artery lesions similar to ultrasonographic examinations. Even for similar tomography, MRI, unlike X-ray computed tomography, enables the evaluation of the inner structure such as the corticomedullary border in the kidney without using contrast media. Changes related to renal functions have been known to occur, such as the disappearance of the corticomedullary border as renal dysfunction worsens. Moreover, what is frequently used for clinical MRI examinations presently is nuclear magnetic resonance of the hydrogen (proton) atomic nuclei abundantly contained in human bodies as moisture, which enables various evaluations by devising imaging methods as one of the features of MRI.

ORIGIN OF FUNCTIONAL MRI

Development of origin of functional MRI (fMRI) originally started as a noninvasive imaging method for brain functions. The phenomenon that is frequently targeted in fMRI is blood oxygenation level-dependent (BOLD) effects, indicating that difference in susceptibility of oxidized hemoglobin and reduced hemoglobin exerts a particular influence on the T2* relaxation time. In an activating site of the brain, the blood flow that surpasses it increases with an increase of the oxygen consumption, the reduced hemoglobin which is paramagnetic material decreases, and prolongation of the T2* relaxation time occurs. In other words, it is a method to grasp an activating site of a brain not by electrical activity of cerebral nerve cells but by changes in accompanying hemodynamics[1]. Brain MRI is captured continually and an activating site is mapped in comparison with the resting state, while having the subject repeat specific tasks such as visual/auditory stimulation and finger motion.

fMRI IN THE KIDNEY

Hypoxia and fibrosis are important factors determining progression of chronic kidney disease

Renal functions are evaluated in tests related to the renal blood flow such as the glomerular filtration rate and renal plasma flow (RPF), which is still a gold standard. However, in imaging studies, in addition to X-ray CT with contrast medium and MRI, quantitative blood flow evaluation is realized by using carbon dioxide contrast medium and ultrasonography. It has already been shown that the renal blood flow measured by these imaging studies correlate well with estimated glomerular filtration rate (eGFR) with serum creatinine, and it can be regarded as functional image evaluation of kidney. The decrease of renal blood flow in chronic kidney disease (CKD) occurs not only in large or medium vascular diseases such as renal-artery stenosis but also accompanies degeneration of the kidney as in glomerulosclerosis and tubular atrophy/interstitial fibrosis. A number of past studies have revealed that a decrease in the renal blood flow causes hypoxia, which causes further inflammation and fibrosis and lowers the renal blood flow, with ensuing CKD[2]. From the above, it was decided that MRI for evaluating the clinical condition of CKD including blood flow and partial pressure of oxygen, as well as fibrosis of the kidney is called fMRI for kidney. It is distinguished from the conventional MRI, which focuses on forms/morphology. The renal fMRI, which is expected to be applied to future clinical practice, is described below (Figure 1).

BOLD MRI: Evaluation of hypoxia

The first report was from Prasad *et al*[3] in 1996. His group evaluated kidneys based on T2* weighted images and revealed that when energy consumption of the renal tubule is reduced with administration of furosemide and a large quantity of drinking water, T2* relaxation rate: R2* (= 1/T2*) in the medulla decreases. They concluded that the change was reflected by a decrease in reduced hemoglobin, capturing improved oxygenation in the site. Further, partial pressure of oxygen was higher in the cortex than in the medulla even before the drug administration and drinking water load and its change was still poor thereafter. Based on the past results, it was expected with more blood flow per unit weight in the cortex than in the medulla and the high partial pressure of oxygen in the tissues that much energy is consumed by the medulla for urine concentration. Then the difference in the partial pressure of oxygen in renal parenchyma and the physiological function of each tubular segment was reconfirmed by fMRI, which is a totally different method. In 2005, the relationship between the microelectrode method, which is a direct oxygen partial pressure evaluation method, and R2* values of BOLD MRI was studied using porcine kidneys. Changing inspired oxygen pressure using a respirator continually, the authors measured pO_2 by the microelectrode method and R2* values of the MRI for both medulla and cortex of kidney and a significant correlation between them was found[4]. As described above, the utility of MRI has been recognized since it is an experimental method that can examine an acute and transient oxygen pressure change in a noninvasive manner before and after medication in place of the microelectrode method. In 2006, Thoeny *et al*[5] studied 15 cases of renal-transplant recipients and healthy subjects after drinking sufficient water. They reported a significant correlation

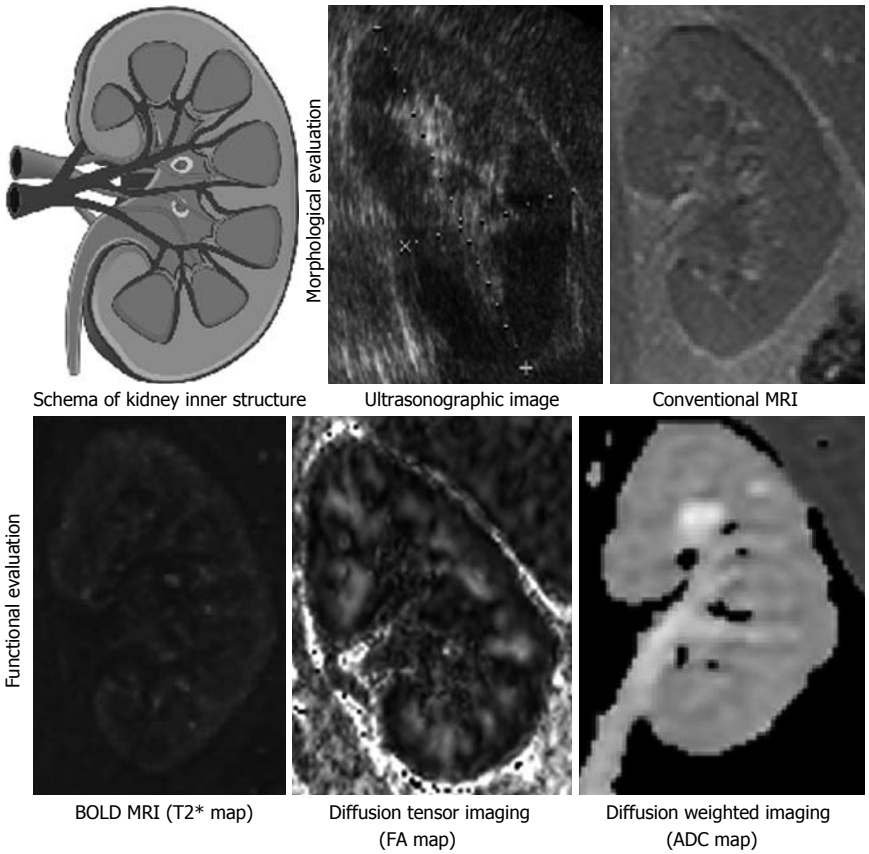

Figure 1 Various modalities for kidney. It is noteworthy that the cortex and medulla are distinguishable on the basis of function or microstructure in functional MRI. For example, in T2* map of BOLD MRI, the medulla was seen darker than the cortex because of the difference in amount of deoxy-hemoglobin; in FA map of diffusion tensor imaging, FA of medulla was higher than the cortex probably because straight microstructure of medulla including distal tubules and surrounding blood capillaries restrict irregularity of Brownian movement of protons. Contrast and angle are adjusted respectively. Each of the kidney images is of a different patient. BOLD: Blood oxygenation level-dependent; MRI: Magnetic resonance imaging; FA: Fractional anisotropy; ADC: Apparent diffusion coefficient.

of the serum creatinine level and R2* value of MRI, with the apparent diffusion coefficient (ADC) of diffusion weighted images described below[5]. It was reported for the first time that R2* values enable evaluation not only of the relative change in transient oxygenation but also oxygenation (or hypoxia) when renal function and hemodynamics are stable. It was also an important report in indicating the possibility of MRI as an evaluation method for chronic hypoxia in CKD.

Diffusion-weighted imaging: Evaluation of fibrosis

The diffusion weighted image (DWI) is one that visualizes the difference in random movements of water molecules in tissues (Brownian motion). It is thought that perfusion in capillaries and diffusion in the target tissues are more purely evaluated for small *b* values (s/mm^2) and great *b* values, respectively. Further, ADC values calculated with plural diffusion weighted images from different *b* values can be quantitatively evaluated without being affected by T2-weighted images. Namimoto *et al*[6] measured ADC values of cortex and medulla of 16 kidneys from 8 CKD cases in which renal function had deteriorated, and 8 kidneys from 16 cases of subjects with normal renal function. They showed ADC was significantly decreased in both cortex and medulla in CKD[6]. For *b* values, Thoeny *et al*[5] studied ADC for $b = 0.50, 100$ s/mm^2 (low); that for $b = 500, 750, 1000$ s/mm^2 (high); and separately for the average (avg) ADC. They found all ADC (low), ADC (high) and ADC (avg) decreased in both cortex and medulla of CKD patients as compared with those of normal kidneys from healthy volunteers although there were only 18 healthy volunteer cases and 15 CKD cases[7]. In healthy kidneys, both ADC (high) and ADC (avg) were always lower in the medulla than in the cortex whereas a difference was not seen in ADC (low) between cortex and medulla. Possibly this is because the regularly-structured renal tubules and blood capillaries, which are a characteristic of medulla, are generated by the diffusion limit of water molecules. In 2007, for 110 kidneys from 55 cases, the relation of ADC values ($b = 0$ and 500 s/mm^2) was studied with 99mTc-diethylenetriaminepentaacetic acid renal dynamic scintigrams and their positive correlations were found[8]. It has been known for a long time that the remaining renal function of CKD correlates well with degrees of tubular degeneration and fibrosis of interstitial tissue. It has been inferred that ADC values are an index of fibrosis from studies on imaging principle and cirrhosis; in 2010, Togao *et al*[9] reported that ADC values decreased renal parenchyma on the ligation side and it is the change corresponding to fibrosis that was

histologically demonstrated, using the unilateral ureteric ligation model, which is a model of postrenal failure[9].

Diffusion tensor imaging

Originally, diffusion of water molecules is random three-dimensionally, and does not have specific directionality though diffusion that has "directionality" when there is structure disturbing the diffusion occurs. This is called "anisotropic diffusion" and diffusion tensor imaging (DTI) is the method to image this anisotropic diffusion as well as evaluating it quantitatively. The myelin sheath of nerve fibers is an obstacle to the diffusion of water molecules in the brain and spinal cord and therefore courses of the nerve fibers can be visualized in DTI. Fractional anisotropy (FA) is an index that reflects diffusive anisotropy. This is an index that shows how the diffusion deviates from isotropic diffusion (fully random diffusion without diffusion restrictions), and it gets close to 0 in the case of isotropic diffusion and to 1 when the anisotropic diffusion is large. Moreover, in tractography the direction of anisotropy is tracked linearly and displayed three-dimensionally, and in particular an image of a brain seems to imitate the course of nerve fibers[10]. Since DWI has begun to be applied to kidneys, anisotropic diffusion is likely to occur in the renal structure, which has regularity in renal tubules and vascular courses. As a result of studying FA values of kidneys for 10 healthy subject cases, FA values of medullas that had regular radial structures were higher than those of cortex, indicating that DTI enables evaluation of renal microstructure. In a study on kidney transplantation, a significant positive correlation was recognized in FA values and eGFR, and it is presumed that change in the inner structure accompanied by a decrease of the renal function was captured[11]. The past studies reported a significant correlation between the renal function and FA values of medulla, indicating mainly that they reflect the degrees of disorder of the tubular structure[12].

Study results of fMRI for CKD and transplanted kidney

The preceding material described methods of renal fMRI that have been most widely reported. Hypoxia and fibrosis, two major factors that are related to the development of CKD, can be evaluated. If it can be applied to clinical practice as in a periodic examination, clinical conditions of CKD at the time of the primary diagnosis can be understood and can capture the changes in the kidneys before alterations are detected in the serum creatinine levels and proteinuria. In addition, it is useful for measurement of effect after treatment intervention, and can contribute to efficient innovative drug development according to the authors. Indeed, reports on its usability as a laboratory study have been increasing. The author measured T2* values of the BOLD MRI and ADC values of DWI for CKD of 43 diabetic nephropathy cases and 76 nondiabetic nephropathy cases, (approximately 120 cases in total) and examined their correlations with eGFR and renal major axes[13]. The results revealed that T2* values and ADC values had significant correlation with eGFR and renal major axes in nondiabetic nephropathy and that major axes of kidneys with decreasing GFR were shortened (= atrophy), and fibrosis developed in such kidneys and were hypoxic. Since renal biopsy was performed for 37 cases, deposition of collagen fibers was measured by Masson trichrome stain as evaluation of interstitial fibrosis. In the kidneys in which interstitial fibrosis had developed, the ADC value significantly decreased, which was the result expected by imaging principle and animal experiment. The degrees of atrophy of the kidneys and ADC values were correlated even for diabetic nephropathy and "fibrosis kidney = kidney atrophic" though hypoxic degrees were independent from fibrosis and eGFR. The cases indicating high hypoxia without noteworthy fibrosis nor decrease of eGFR were remarkable, because future aggravation of renal function is expected. In addition, the hypoxia is likely occurring by a reversible cause and therefore it is amenable for stricter intervention by nephrologists. Recently, results of tracking recipients from 9 renal transplant cases for 3 years have been reported[14]. ADC values and the R2* values of the transplanted kidney by cortex and medulla were compared soon after the transplantation (7 ± 3 mo) and approximately three years later (32 ± 2 mo). In the eight cases in which renal function was stable for three years, other measured values except the R2* values of cortex were almost similar and no difference was noted. On the other hand, in the one case in which renal function decreased, increase in R2* values and decrease in ADC values were recognized, suggesting aggravation of hypoxia and development of fibrosis. These results showed reproducibility that can be evaluated longitudinally for at least DWI and BOLD MRI. In addition, the interesting point is that change in measured values accompanied by the degenerating renal function was captured in the same case. Further, even when renal function is stable on the blood test, significant change in the R2* values suggest progress of hypoxia in cortex is recognized, and it is particularly worth noting that it reminds us of its relation with the immunosuppressive drug used frequently after transplantation, particularly calcineurin inhibitor.

Other imagings

There is an imaging method called arterial spin labeling (ASL), which is a method to evaluate brain perfusion. This is a method by which the spin in blood flowing into the target organ is labeled by magnetizing with radio frequency to evaluate the amount of organ perfusion with the blood itself as an endogenous tracer. A perfusion image is obtained from the difference of the images before and after the labeling. If however, the signal noise ratio (S/N) is low, it is necessary to image repeatedly and integrate them and therefore the imaging time is increased. Instruments having a magnetostatic field of 3 teslas have been introduced for clinical use. The improvement of images and shortening of imaging time are being attempted and therefore their clinical application is approaching feasibility. There still are a few reports that examined the

amount of kidney perfusion by similar methods. The relationship of kidney perfusion with clearance of para-aminohippuric acid that is RPF was studied in 2010, and a significant correlation was shown with amounts of perfusion per unit weight measured by ASL[15]. In addition, reports on kidney transplantation and reports that clarified a decrease in amount of perfusion in a CKD case in comparison with healthy subjects have come to be recognized[16]. MRI images not only protons but also other atoms. In evaluation of kidneys by Sodium-23 (^{23}Na) MRI, the behavior of Na concentration increasing progressively toward the depths of the medulla from a cortex surface layer can be obtained as an image. MRI also observed that a Na concentration gradient gradually disappears when renal ischemia is caused experimentally. It is probably the change that reflects tubular impairment due to ischemia[17].

CONCLUSION

Most of the research reports have been published within the past ten years and renal fMRI is now in the dawn of its emergence. Correlation with eGFR and RPF, which are classic indices of the renal function, is discussed in most papers. However, in kidneys for which positional information is not as important as in the brain (though there is difference between right and left), it is meaningless to introduce a large-scale device and re-evaluate the indices that are not needed because serum creatinine is sufficient for evaluation. The ultimate goal of the fMRI study was to provide useful information for daily medical treatment such as, for example, a renal biopsy diagnosis by evaluating kidneys non-invasively over time. Accumulation of further knowledge is eagerly anticipated.

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MINIREVIEWS

Metabolic syndrome and lower urinary tract symptoms

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Abstract

Recently, clinical and epidemiologic data indicating the involvement of metabolic syndrome (MetS) in the pathogenesis and progression of lower urinary tract symptom (LUTS)/benign prostatic hyperplasia (BPH) are reported. This review evaluates the reports on the influence of MetS in the development and progression of LUTS/BPH, and discusses possible clinical implications for the management and treatment of this disease. Recent studies on the epidemiological relationship between MetS and LUTS hypothesize that MetS may be associated with an overactivity of the autonomic nervous system for which hyperinsulinemia, a key element of the MetS, might be responsible. An alternative explanation is that LUTS are associated with chronic ischemia of pelvis resulting from atherosclerotic changes in blood vessels, which leads the production of reactive oxygen species, which can damage the bladder detrusor. Control of autonomic nervous system overactivity and control of chronic bladder ischemia have potential as new targets for LUTS treatment. Studies suggest an association of MetS with LUTS/BPH, although further research is needed to understand how MetS influences LUTS/BPH. MetS should be considered a new domain in basic and clinical research in patients with LUTS/BPH and as a target for treatment.

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Key words: Metabolic syndrome; Lower urinary tract symptoms; Sympathetic overactivity; Chronic bladder ischemia

Core tip: Associations between metabolic syndrome (MetS) and lower urinary tract symptom (LUTS) are noteworthy. MetS treatment may have the potential to prevent LUTS from worsening as well as to prevent the occurrence of new LUTS. Medication for LUTS patients with MetS should not only target the lower urinary tract, but should also be recognized as systemic treatment. Medications which are essentially thought to be unrelated to LUTS treatment may have potential for development as a specific medical treatment for LUTS with MetS.

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INTRODUCTION

Lower urinary tract symptoms (LUTS) are a symptom complex which decreases quality of life in both the males and females. It is known that the number of LUTS patients increases in an age-dependent manner. It has been reported that bladder outlet obstruction (BOO), which occurs in patients with benign prostatic hyperplasia (BPH), is the main factor that triggers LUTS and efforts have focused on BOO. However, recent reports have shown that various etiologies are also associated with LUTS. Metabolic syndrome (MetS) is a cluster of several metabolic abnormalities or risk factors, including visceral obesity, dyslipidemia, hypertension, insulin resistance, and glucose intolerance[1]. Recently, it has been suggested that MetS may play an integral role in LUTS/BPH etiologies. In order to improve the quality of LUTS treatment, it will be useful to understand how MetS influences LUTS.

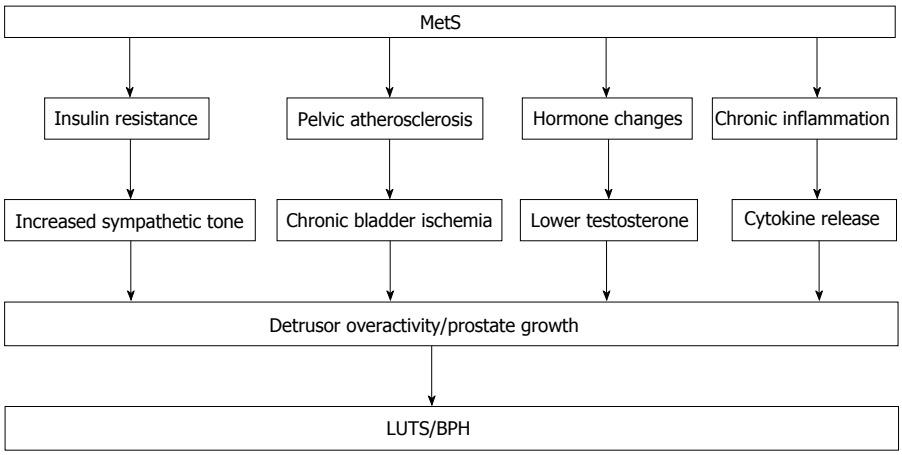

Figure 1 Potential role of metabolic syndrome on the lower urinary tract symptom. MetS: Metabolic syndrome; LUTS: Lower urinary tract symptom; BPH: Benign prostatic hyperplasia.

The present review highlights current knowledge about the influence of MetS on LUTS/BPH and about the expected effect of MetS treatment on LUTS improvement. The application of this knowledge in clinical use should contribute to improvements in the treatment quality of LUTS patients with MetS.

METS AND LUTS/BPH

Associations between LUTS/BPH and various components of MetS (obesity, hypertension, and fasting blood glucose) have been previously reported[2-4]. Moreover, cases of LUTS/BPH have also been positively associated with the number of MetS components[5].

It is known that hyperinsulinemia caused by insulin resistance, the core pathophysiology of MetS, may increase prostate smooth muscle tone[6], resulting in the increased prostate volume[7,8]. The insulinlike growth factor (IGF) pathway may contribute to the relation between insulin resistance and prostate enlargement. Insulin presents a structural similarity to IGF-1 and can bind its receptor, result in activating of a complex pathway influencing proliferation of prostatic cells. Another relevant study has demonstrated that the chronic inflammation induced by MetS may lead to the prostate enlargement[9,10]. It has been proven that insulin resistance and inflammation increase as the number of MetS components increases[11]. T-cell activity in prostate with inflammation infiltrates may result in increased stromal and epithelial cell proliferation that is sustained by an autoimmune mechanism. Repetitive tissue damage and the subsequent wound healing induced by inflammation may result in prostate enlargement[7].

Alternative hypotheses include overactivity of the sympathetic nervous system by hyperinsulinemia, which may lead to more sever LUTS independent of prostate enlargement, and pelvic atherosclerosis causes chronic ischemia of the bladder, which may lead to functional impairments clinically manifested as LUTS (Figure 1).

SYMPATHETIC OVERACTIVITY AND LUTS

Insulin resistance is thought to be the central underlying mechanism of MetS and is known to cause sympathetic overactivity[12]. Recent studies trying to show the epidemiological link between MetS and LUTS hypothesize that MetS is related with increased autonomic nervous system activity for which hyperinsulinemia, a key element of MetS, might be responsible[13,14]. Briefly, hyperinsulinemia that induces increased autonomic nervous system activity results in prostate enlargement and detrusor overactivity. McVary *et al*[14] revealed that LUTS are related with changes in blood pressure. The important role of α1-adrenoceptor (α1-AR) in the sympathetic nerve terminals of the prostate has been indicated. It has been also indicated that dynamic obstruction is mediated by stimulation of α1-AR. Previous studies indicate a relationship between overactivity of autonomic nervous system and LUTS. Spontaneously hypertensive rats (SHRs) have been reported to void more often than normotensive control rats[15], and have also been reported to have an overabundance of sympathetic fibers innervating the bladder. Urodynamic studies on SHRs have indicated spontaneous bladder contractions at low volumes, an effect that is largely ameliorated by α1-AR blocker and appears to be mediated by increased nerve factor. Thus, sympathetic overactivity has been suggested to have relation with bladder storage function. Autonomous nervous system overactivity because of MetS might contribute to detrusor overactivity. Hammarsten *et al*[16] represented an association between a more rapid enlargement of prostate and diseases related with sympathetic activation, including obesity, non-insulin-dependent diabetes mellitus and treated hypertension (HT). Furthermore, it has been proven that the over input of autonomic neural stimulation to the prostate may lead to the prostatic enlargement[17].

CHRONIC BLADDER ISCHEMIA AND LUTS

An association among smoking, vascular risk factors and LUTS has been reported[18]. Furthermore, previous study has shown decreased bladder compliance, reduced contractile force in the bladders and increased micturition frequency in pelvic ischemic model of rabbits[19]. Various researchers have shown the influence of chronic ischemia of the bladder detrusor resulting from atherosclerotic changes in blood vessels on LUTS; specifically atherosclerosis narrows the bladder vessels. Bladder filling in atherosclerotic models results in a significant decrease in blood flow of the bladder. Repeated ischemia and reperfusion produces reactive oxygen species, which damages the tissues[20,21]. Shimizu *et al*[22] found that an angiotensin receptor blocker (ARB), olmesartan, protects bladder function in a chronic bladder ischemic model by recovering blood flow and decreasing oxidative stress. Protective effects of coenzyme Q10[23] and melatonin[24] on bladder hyperactivity in a chronic bladder ischemia model due to their action as antioxidants have been indicated. Treatment targeting the bladder blood flow and protection from oxidative stress caused by chronic ischemia can be a new strategy for LUTS treatment.

MEDICINAL TREATMENT FOR LUTS

Medical treatment of voiding and/or storage symptoms is now the initial choice of therapy and α1-AR blockers remain the most widely used pharmacological agents aimed at the dynamic component of prostatic obstruction[25]. α1-ARs predominate in prostatic stroma at the mRNA and protein levels, and it is reasonable to think that they may play a role in combatting the dynamic component of obstruction[26]. Increased activity of the autonomic nervous system may result in an acceleration of the sympathetic tone of the lower urinary tract, lead to C-fibers stimulation, which in turn causes storage symptoms. By bringing these findings together, it is suggested that α1-AR-blocker exerts an suppressive effect on C-fiber urethral afferent nerves by inhibiting the muscle tone of prostate activated by HT, which may lead to improve storage symptoms. We previously showed that the effect of tamsulosin on storage symptoms was more prominent in patients with HT than in patients without it[27]. An α1-ARs subtype analysis showed that the expression levels of α1-AR mRNAs in the detrusor were equally at low densities[26], indicating that α1-AR blockers have little effect on contractility of detrusor, which in turn may have little effect on voiding symptoms. Although α1-AR-blockers improve functional obstruction in BPH, detrusor contractility may not be improved in elderly patients.

There is no special treatment for LUTS patients with MetS at the present time. Strict control of MetS may be needed.

EFFECT OF METS TREATMENT ON LUTS

Increased physical activity have been associated with a decreased risk of LUTS[28], although the mechanism of this effect is not completely understood. MetS patients may receive medications for HT, hyperlipidemia and diabetes. These medications may possibly improve LUTS as well as control MetS. As the populations of LUTS and HT both increase with aging, a great number of LUTS patients receive medications for HT. Ang-Ⅱ is a potent vasoconstrictor and plays an important role in the systemic renin-angiotensin system (RAS), which controls blood pressure by regulation of vascular tone and sodium homeostasis. Medications which inhibit the RAS, such as ARBs, are mainstays in HT treatment. In addition to its systemic effects, Ang-Ⅱ may affect cellular proliferation and function in several organ systems, including bladder and prostate, through local RAS. It has been well established that Ang-Ⅱ receptors exists in the bladder wall and Ang-Ⅱ on the detrusor muscle has contractile effects[29,30]. Because Ang-Ⅱ is the principal mediator of the RAS and strongly stimulates cell growth[31], researchers have also reported its possible role as a mediator in collagen production and smooth muscle growth in BOO[32,33]. Previous studies report that Ang-Ⅱ induces bladder muscle contractions[29,34]. Ang-Ⅱ in the bladder is known to regulate smooth muscle growth and connective tissue production[35], and has been partly associated with the pathogenesis of urinary dysfunction with subsequent outlet obstruction[36] and stress urinary incontinence in rats[37].

It has been proven that morphological changes are remarkable in the bladder smooth muscle cells, connective tissues, and innervation in the detrusor muscles in patients with chronic BOO[38]. It has been shown that repeated captopril administration, the angiotensin-converting enzyme inhibitor, in neonatal BOO rabbits significantly reduced the expression level in total DNA and decreased the amount of total collagen in the bladder[39]. It may hypothesized that these pathological variations in the BOO bladder may be caused by the activation of Ang-Ⅱ-mediated changes. It has been indicated that urethral resistance in a rat model of stress incontinence may be reduced by the blockade of Ang-Ⅱ receptors[37]. Taken together, it can be proposed that the development of BOO results in a significant change of Ang-Ⅱ receptors in the lower urinary tract. It has also been indicated that the Ang-Ⅱ antagonist telmisartan suppresses both the suppression of Ang-Ⅱ receptors in the bladder and the bladder hypertrophy in BOO rats[36]. Consequently, telmisartan is effective in attenuating the increase in bladder weight in BOO. We previously showed that ARB administration in hypertensive patients improves LUTS[40]. One possible mechanism indicating the improvement of storage symptoms by ARB administration may be the effect of stabilization of the smooth muscle activity by down regulation of the local RAS in the bladder. It is also

discussed that Ang-Ⅱ may contribute in the prostate. It has been reported that RAS has been overactivated in BPH[41]. The elevated local levels of Ang-Ⅱ in the prostate in BPH patients has also been reported[42]. Ang-II may have effect on cell proliferation and smooth muscle tone in the prostate *via* Ang-Ⅱ receptors. Nevertheless, the functional role of elevated Ang-Ⅱ in the prostate is not clearly understood. It is expected that ARB may improve LUTS by down regulating noradrenaline release in the prostate. Recent reports indicated that the effect of apoptosis induced by ARB in prostatic tissue of SHR[43]. ARB may improve voiding symptoms by minimizing the prostatic microenvironment, result in lowering urethral resistance. As ARB can also improve blood flow, as mentioned above, the possibility exists that ARB may improve LUTS.

OBJECTION TO THE ASSOCIATION OF METS AND LUTS

The role of MetS in the occurrence of LUTS remains controversial. Some reports, in particular in Asian populations, found no association between MetS and LUTS[44-46]. Moreover, a report from Korea showed favorable effects of MetS on LUTS[47], that is, the opposite result. The differences in these results could stem from several factors. First, the ages of the study participants are different among studies. Second, the criteria used to define the presence of MetS or LUTS vary among studies. And third, the prevalence of MetS and BPH are lower in Asian populations.

It is important to emphasize that most studies indicating the possible association between MetS and LUTS are derived from observational studies, which are often limited to a specific area or population. Future clinical trials are expected.

CONCLUSION

Associations between MetS and LUTS are noteworthy. MetS treatment may have the potential to prevent LUTS from worsening as well as to prevent the occurrence of new LUTS. Medication for LUTS patients with MetS should not only target the lower urinary tract, but should also be recognized as systemic treatment. Statin and ARB, which are essentially thought to be unrelated to LUTS treatment, may have potential for development as a specific medical treatment for LUTS with MetS.

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MINIREVIEWS

Percutaneous nephrolithotomy *vs* laparoscopic ureterolithotomy for large upper ureteral stone: A review article

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Key words: Upper ureteral stones; Percutaneous nephrolithotomy; Laparoscopic ureterolithotomy; Laparoscopy; Ureteral calculi

Core tip: It's an honor to write a prestigious editing board; there is a review article study to compare between percutaneous nephrolithotomy and laparoscopic ureterolithotomy for treatment of large upper ureteral stones. These two procedures are different in many aspects and it's not clearly defined that which one is preferred option.

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Abstract

To investigate the best treatment option for large upper ureteral stone, percutaneous nephrolithotomy or laparoscopic ureterolithotomy. We searched three key word of upper ureteral stone, laparoscopic ureterolithotomy, percutaneous nephrolithotomy in PubMed, Scopus and Ebsco. We found approximately twenty suitable articles about this subject since January 1980 until January 2014. All articles studies and reviewed meticulously and brief review of these articles was written and some Ideas of experts was added. In many studies, it is suggested that success rate and complications of laparoscopic ureterolithotomy and percutaneous nephrolithotomy are the same, but percutaneous nephrolithotomy has less hospital stay time, duration of surgery and it is more cost effective. Overall it seems that percutaneous nephrolithotomy for treatment of upper ureteral stones is preferable rather than laparoscopic ureterolitothomy

INTRODUCTION

Large impacted upper ureteral calculus defined as a stone that is located above the rim of pelvic Inlet bone. Upper ureteral stones may be single or multiple and may be associated with kidney stones.

The best treatment modalities for large proximal ureteral stones are not well defined[1] and include extracorporeal shock wave lithotripsy, ureterolithotripsy, percutaneous nephrolithotripsy, laparoscopic ureterolithotomy and open surgery. For large upper ureteral stones, both percutaneous and Laparoscopic ureterolithotomy are possible less invasive modalities, but how we can choose one of these two procedures, is not clearly defined.

The aim of this review article study is to compare between two main approaches to this stones that are

percutaneous nephrolithotomy (PNL) and Laparoscopic nephrolithotomy.

RESEARCH

This review was conducted on a request from Baishideng Group. For this study we searched all full text papers indexed in three major resources of medical literature (PubMed, Scopus and Ebsco) using the following key words: Upper ureteral stone, laparoscopic ureterolithotomy and percutaneous nephrolithotomy. We defined large upper ureteral stones as a stone larger than 1 cm.

All papers fulfilling these inclusion criteria were included: (1) Involving treatment of large proximal ureteral stones; (2) Published between Jan 1980 and Jan 2014; and (3) If several studies were reported by the same center and authors, the most recent publication was included in writing of this review. Commentaries and case reports were excluded. After a rapid scan of the literature, ultimately 21 articles were found to be relevant to these criteria and used for compiling this review article.

DISCUSSION

Upper ureteral stones are a prevalent problem. There are many different treatment modalities that include medical expulsive treatment, shockwave lithotripsy, ureteroscopic stone extraction; PNL and laparoscopic ureterolithotomy. In large upper ureteral stones, PNL and laparoscopic ureterolithotomy are the two main approaches reported in the literature. Indications of these two procedures are nearly same and include large upper ureteral stone that is resistant to shock wave lithotripsy and stones that are not suitable for other treatment modalities. Contraindications are also not specific and are the same general contraindications for laparoscopy and PNL. These two procedures may be compared in many different aspects such as invasiveness, cost, learning curve, complications, success rate, duration of surgery and hospital stay. Therefore the choice between two modalities for each given patient would only be prudent once these differences have been taken into account.

For the ureteral stone accompanied by complex kidney stones that need surgery, PNL is the modality of choice. In upper ureteral stones located at a reasonable distance from the uretero pelvic junction [*i.e.*, close to the ureteropelvic junction (UPJ)] and accessible from the renal pelvis too, PNL is preferred[2].

But if the stone is located far from the UPJ, the surgeon must first displace the stone from its position at the upper ureter into the pelvis, and then perform routine PNL. This approach, commonly termed push-back PNL is the alternative to laparoscopic ureterolithotomy.

In one study, Li *et al*[3] evaluated stone location based on vertebral level. They concluded that if a ureteral stone is located higher than the upper border of the fourth lumbar vertebra, it is inappropriate for ureteroscopic lithotripsy and should be treated by another treatment modality such as PNL or laparoscopy. In their study, stone free rate for PNL was 96.4%. Mean operation time and post operative hospital stay were 108.78 min and 2.49 d respectively[3].

For the upper ureteral stone accompanied by kidney stones, Xiong *et al*[4] conclude PNL to be the reasonable choice for stone treatment, In their study, they performed PNL on 108 patients with kidney and upper ureteral stones. Stone clearance rate was 99.1%, hemoglobin decrease was 4.8 ± 2.7 g/L and no blood transfusion was needed[4].

PNL is a trans-parenchymal procedure, but in laparoscopic ureterolithotomy, the kidney remains intact. This difference between the two approaches has been underlined by some authors, stating that they may theoretically expect bleeding and kidney injury to dominate in PNL compared to laparoscopic ureterolithotomy. In reality, in uncomplicated PNL, bleeding is not significant. Lang[5] reported their post PNL transfusion rate to be 0.43%.

Through the unavoidable step of tract dilation, PNL inherently causes some renal parenchymal invasion. Renal dimercapto succinic acid scans performed to assess the state of cortical function at varying intervals post op, shall reveal focal cortical defects at the point of access, but at the same time usually confirm that as result of the removal of obstructive stones, overall renal function will be preserved or even improve in the post operative scan after PNL[6].

In laparoscopic ureterolithotomy it is clear that the procedure does not involve direct parenchymal trauma. In one study Yasui *et al*[7] evaluated the impact of laparoscopic ureterolithotomy on renal function by performing renal scintigraphy using 99mTc-mercaptoacetyltriglycine (99mTc-MAG3) before and 3 mo after surgery. Ultimately they conclude that pre-operative and post-operative affected renal function images shown no significant change in MAG3 clearance[7].

One of the most important positive aspects of choosing laparoscopy for the treatment of large upper ureteral stones is its high success in achieving stone free state. This has been confirmed by several studies in which success rates were reported to stand between 96% to 100%[8-10].

Some factors may hamper the success of laparoscopic ureterolithotomy including severe fibrosis and tissue adherence surrounding the ureteral stone site. This can make dissection very difficult and occasionally impossible, and these factors are limitations of laparoscopic ureterolithotomy especially in retroperitoneal route[11].

Another complication of laparoscopic ureterolithotomy that directly impacts its success is upward displacement of the ureteral stone into the renal pelvis. Upward migration may preclude laparoscopic access to the stone. This situation can occur when a non-impacted stone is located close to the UPJ and the adjacent proximal ureter is very dilated. In such a circumstance, it's better to switch the treatment plan to push-back PNL. Several studies have reported the success rate for push-back PNL between 82.8% and 99.1%[4,12,13].

Table 1 Summary of some characteristics of two treatment modality for large upper ureteral stones: Laparoscopic ureterolithotomy, push back percutaneous nephrolithotomy

	Laparoscopic ureterolithotomy	Pushback PNL
Stone free rate range (%)	96.5-100	82-99
Operation time range (min)	45-190	30-160
Hospitalization time (h)	24-144	12-96
Transfusion rate average (%)	Rare	0.43

PNL: Percutaneous nephrolithotomy.

Regarding the approach to the kidney for percutaneous treatment of upper ureteral stones, there is essentially two ways to reach the stone. One is by creating access through a tract from superior calyx. Upper ureteral stones are readily accessible *via* this approach, provided the ureteral segment proximal to the stone is dilated enough to permit navigation by the nephroscope[13].

The second approach is push-back PNL. For this purpose the stone must first be displaced from the upper ureter into the renal pelvis by retrograde ureteroscopy, followed by obtaining a routine subcostal inferior calyx access for PNL. But at times the stone is tightly impacted. This renders the push-back strategy difficult and even perilous. Precisely such impacted ureteral stones should better be approached electively by laparoscopic ureterolithotomy, as push-back PNL is apt to fail.

In comparing the two surgical procedures, duration of anesthesia and surgery have also been evaluated in several studies. All allude to the slightly lengthier approach being laparoscopic ureterolithotomy which takes anywhere between 45 and 190 min on average[14-18]. Laparoscopic ureterolithotomy is fairly time consuming, while push-back PNL is relatively quicker. Upper ureteral stones chosen for push-back PNL were usually solitary. This might have contributed to the observation that in many small studies, push-back PNL takes between 30 to 160 min[3,4,12,19].

Duration of hospital stay is an important consideration. Most health systems favor limiting hospital stay as a strategic objective to help lower the financial burden of treatment. In PNL, the duration of hospital admission has been reported between 12 and 96 h[12,20]. For laparoscopic ureterolithotomy this period spans between 1 and 6 d[10,14,16] (Table 1).

Another aspect of any less invasive surgery is the direct cost of such procedures which generally require advanced equipment and disposable devices, in addition to trained surgeons and operating room staff. This factor has not been covered in any of the papers contributing to this article.

CONCLUSION

Both laparoscopic ureterolithotomy and push-back PNL can be used for treating large upper ureteral stones.

These two modalities are similar in many aspects such as success rate and complication rate; but differ in such areas as duration of anesthesia and hospital stay, indirectly leading to treatment cost. As result it appears that push-back PNL is slightly favorable over laparoscopic ureterolithotomy for treating large upper ureteral stones.

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MINIREVIEWS

Varicocele and infertility: Role of pressure flow dynamics

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Abstract

Varicocele is prevalent in infertile individuals as well as in normal adolescents and adults. It has an increasing trend with growing age. Infertile individuals with varicocele, develop varying degrees of sperm abnormalities that range from mild to severe semen abnormalities, even azoospermia may develop. The main proposed features of these abnormalities are incompetence of one-way valves of the draining veins of testes, that allow backflow of blood into testes. This backflow produces abnormally high intra-testicular pressure and temperature, that has been confirmed by thermography and pressure estimation in various studies. Microsurgical varicocelectomy may reverse the pathologic effects on spermatogenesis in most patients, which points towards the cause and effect relationship of varicocele with testicular damage. We propose that the prolonged effect of gravity might or may not be the initiating factor for varicocele, as in our experience, around 1/4th of hypogonadotropic hypogonadism patients who had no varicocele before treatment, developed varicocele within 3 to 6 mo of treatment with gonadotropins. Occasionally varicocele is produced by "Nutcracker phenomenon", which is compression of left renal vein between the abdominal aorta and superior mesenteric artery. The deleterious effects of varicocele may develop slowly, causing delayed secondary infertility or rapidly, leading to azoospermia or individual may be spared of damage due to unknown factors that need further research.

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Key words: Varicocele; Scrotal Doppler ultrasonography; Testicular blood flow; Pressure flow dynamics; Testes; Infertility; Oligospermia; Aesthenospermia

Core tip: Varicocele is prevalent in infertile individuals as well as in normal adolescents and adults. It has an increasing trend with growing age. Infertile individuals with varicocele, develop varying degrees of sperm abnormalities. Microsurgical varicocelectomy may reverse the pathologic effects in most patients, which points towards the cause and effect relationship of varicocele with testicular damage. The question as to how some individuals are spared of the deleterious effects of varicocele, needs further research.

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INTRODUCTION

Varicocele is a disorder of the draining veins of testes. Normally these veins have competent valves, which are required to prevent backflow of blood into testes. In individuals with varicocele, these valves become incompetent, leading to reflux/backflow of venous blood into testes[1-3]. This backflow increases intra-testicular pressure and heat, while decreasing oxygen concentration in testes. These

factors cause testicular injury through inflammatory mediators and production of free radicals[4]. As a result, testicular function, especially spermatogenesis, is affected which may cause male infertility. The consequences of varicocele are variable in different individuals, some may suffer from infertility whereas others may not[3,5].

The pathophysiology of varicocele is yet not clear although its association with male infertility is known since 1950[2]. It is suggested that not a single factor, but various factors are involved in the pathological effect of varicocele. Patient's life style and genetic factors may be among them[2]. The effects of varicocele are more prominent on the left side, due to higher hydrostatic pressure in the left internal spermatic vein. Also the blood column on the left side, after incompetence of one-way venous valves is longer (40 cm) on the left side. It has been suggested that varicocele is a bilateral disease. In a series of venographies, varicocele was found to be bilateral in 84% cases. Amongst them, collateral venous channels and retroperitoneal venous bypasses were observed in 70% cases on left side and 75% on right side[6-8].

RESEARCH

A thorough literature search was conducted at the Medline database. The controlled vocabulary of the medical subject headings included varicocele and pressure, varicocele and blood flow, varicocele and hemodynamics, varicocele and Doppler ultrasonography and varicocele and scrotal Doppler ultrasonography (CDUS). The references retrieved were reviewed and relevant references were chosen according to their relevance for the subject. The references list from each reference was further checked to identify relevant references. Only English language articles were selected.

PREVALENCE OF VARICOCELE

World Health Organization has reported that among men with normal sperm quality, 11.7% have varicocele whereas 25.4% individuals with infertility have varicocele[9]. The prevalence of varicocele in middle school boys has been found to be 16.5%. Its prevalence increases with age, around 10% per decade and reaches 75% at the eighth decade of life[10,11].

When individuals with varicocele were investigated by CDUS, it was found that 94% had grade 2 and 3 reflux on Doppler study. In individuals without clinical varicocele, 40% were found to have reflux of grade 1 and 2 in their testicular veins[12].

DIAGNOSTIC IMAGING FOR VARICOCELE

Scrotal CDUS has been used for determining the flow of varicocele veins including their backflow on valsalva maneuver[12]. If blood flow in a varicocele vein reverses its direction by raised intra-abdominal pressure, it may be considered significant for the diagnosis of varicocele[13]. In the same study, similar kind of blood flow pattern was also detected in 54% individuals without varicocele, which invites ambiguity. The backflow pattern has been graded during valsalva maneuver, from Grade 1 (reflux < 2.0 s) to Grade 3 (reflux > 2.0 s)[14]. The CDUS findings have been proposed to be clinically useful because the maximal reflux velocity and total cross-sectional area of the affected testicular veins can predict the number of internal spermatic veins requiring ligation during microsurgical subinguinal varicocelectomy[15].

PRESSURE FLOW DYNAMICS OF VARICOCELE

In normal population, testicular veins, like other veins, have low-pressure dynamics with no backflow. It has been proposed that if venous valves are incompetent, high intravenous pressure in the internal spermatic veins may be transmitted to the testes. This happens due to the intra-abdominal destination of these veins. High intra-abdominal pressure, in the absence of proper functioning venous valves, delivers backpressure into the testicular veins[16]. The reflux of blood in the internal spermatic veins of testes has been found to be similar at all levels of veins irrespective of its branching pattern. However, the velocity of blood in these veins decreases as the number of branches increase closer to the testes[15]. It has been found that varicocele veins have high hydrostatic pressure that exceeds the pressure in testicular arterial microcirculation, leading to ischemic damage[6]. It has been reported that animal studies have shown conflicting evidence of both increased as well as decreased testicular blood flow after experimentally induced varicocele[17]. This is probably due to the fact that animal models do not mimic the human disease process well, at least in its diversity of pathophysiological phenomenon.

In clinical situations, patients, with upper motor neuron lesion and spastic paralysis of the abdominal muscles, develop varicoceles whereas lower motor neuron lesion patients, with flaccid paralysis rarely, develop varicocele[18]. This can be explained by the phenomenon that spastic paralysis produces high pressure in the abdomen whereas flaccid pressure does not. This study favors the concept of high abdominal pressure as a major cause of varicocele related changes. Hydrostatic pressure in varicocele veins depends on the height of blood column rather than vein diameter[6]. The taller individuals have higher prevalence and severity of left-sided varicocele and it is probably related to the length of left internal spermatic veins and the consequent increased hydrostatic pressure in them[19]. During sclerotherapy of internal spermatic veins of varicocele patients, it was observed that most men had absent valves of the internal spermatic veins[20]. It is the destruction of one-way valves of the veins that allows transfer of pathologic hydrostatic pressure towards testicular venous microcirculatory system. In varicocele, this pressure is around five times higher than normal and exceeds arteriolar pressure, leading to hypoxia. This is also true for right-sided varicocele[16].

It has been suggested that varicocele is either of "pressure-type" or "shunt-type". The "pressure type" varicocele is due to valvular incompetence of internal spermatic vein (testicular vein) whereas "shunt type" is caused by the cremasteric vein and/or deferential vein incompetence[21]. The authors were also of the opinion that spontaneous reflux causes the shunt type varicocele, *i.e.*, the dilatation of medium-sized and large varicocele veins, whereas Valsalva-induced reflux results in "pressure type" or the stop type mechanism, associated with subclinical varicocele[22,23]. This needs further investigation for better understanding.

"Nutcracker syndrome" is another proposed, though rare explanation for varicocele in many studies. According to this view, varicocele results from compression of left renal vein between the abdominal aorta and superior mesenteric artery that may result in varicocele and left flank pain. This phenomenon has mainly been reported in the pediatric population[24-26]. Adolescent patients with the nutcracker phenomenon and left varicocele may present with symptoms of hematuria, proteinuria, scrotal discomfort, and flank pain. In 12 such patients, a shunt anastomosis of the proximal part of the spermatic vein and inferior epigastric vein was done and varicocele was treated by ligation of the left spermatic vein. The symptoms disappeared in all the patients. The diameters and peak velocities of left renal vein significantly decreased and left testicular volume significantly increased after surgery[27].

Experimental anatomic modeling suggests that in varicocele, both testicular as well as epididymal venous outflow is deranged resulting in pathological compensatory hemodynamic changes. Intra-testicular pressure increases, intra-organ vascular integrity is impaired leading to extravasation and testicular venous infarction. There is local arterial hypertension and inter-arterial shunting of the arterial blood. This triggers the mechanism of secondary arterial ischemia of testis and epididymis. The circulatory disorder is pronounced in case of testicular venous outflow blockade which is severe in combined testicular-cremasteric venous block which causes hemodynamic collapse[28].

Varicocele veins have a higher intravascular pressure compared to other veins and it leads to deterioration in blood flow of testes[29,30]. It was reported that normal individuals have 59.9 mm. Hg venous pressure on the left spermatic veins which in varicocele patients was higher by 19.7 mm. Hg at rest and 22 mm. Hg during Valsalva' s maneuver in varicocele patients[31]. It has been observed that after transplant nephrectomy varicocele develops in 23.3% male patients[32].

It has been observed that in normal individuals on valsalva test, femoral vein caliber increases by 22.6% ± 5.7%, whereas in patients with varicocele, it rises to 61.1% ± 10.0%. It has been suggested that the formation of tension chamber in femoral vein may be the cause of ilieo-spermatic reflux of varicocele[33].

In our recent publication, we have reported that hypogonadotropic hypogonadism patients, on presentation, have small size testes and barely appreciable blood flow with no evidence of varicocele. After treatment with gonadotropins, 23% patients developed varicocele within 3-6 mo of treatment[34]. We propose that as this change appeared in a short period of time, which is not enough for the gravity effect to take place, it is most likely that the varicocele veins along with incompetent valves were already there but remained undetectable due to poor arterial blood flow, which was insufficient to fill up the veins. This finding of course needs further investigation; however it favors the hypothesis that the prolonged effect of gravity may not be, at least, the initiating factor for the development of varicocele. Similarly, the effect of constipation on varicocele was studied and found that chronic constipation alone, does not cause varicocele, but it can facilitate its effects[35].

A body of evidences demonstrated that varicocele causes serious damage to testes, which can be successfully improved by microsurgical varicocelectomy, thus proving the cause-effect relationship[36]. Even in non-obstructed azoospermic patients suffering from varicocele, sperms appeared in ejaculate in 34.6% patients after microsurgical varicocelectomy[37]. Based on aforementioned evidences, it seems appropriate that varicocele produces harmful effects but what we still don't know is that how some individuals are spared of these effects.

In conclusion, varicocele is a disease that may cause deleterious effects quickly, leading to azoospermia or slowly, causing infertility due to semen abnormalities or the individual may be spared due to various unknown factors that need further research.

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MINIREVIEWS

Vitamin C supplementation in patients on maintenance dialysis

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Abstract

As one of the most important water-soluble non-enzymatic antioxidants, vitamin C consists of ascorbic acid and its oxidized form, dehydroascorbic acid. Maintenance hemodialysis (MHD) patients have a generally lower plasma vitamin C level compared with general population. Moreover, dialysis patients also exhibit a low plasma vitamin C level, which is largely related with increased inflammation, refractory anemia and oxidative stress. In this review, we described, in great detail, the vitamin C deficiency in MHD patients and its effects on anti-oxidation, anti-inflammation, pro-oxidation and secondary hyperparathyroidism. In addition, we described the possible potential value of vitamin C in anemia, and the side effects of over-doses of vitamin C supplementation in this particular population. In summary, MHD patients may benefit from vitamin C administration. However, further research should be carried out to confirm its potential beneficial effects, optimal dosage and side effects from vitamin C supplementation.

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Key words: Vitamin C; Supplementation; Maintenance dialysis; Anti-oxidation; Anti-inflammation; Anemia; Oxalosis

Core tip: In this review, we described the vitamin C deficiency in maintenance hemodialysis patients and its effects on anti-oxidation, anti-inflammation, pro-oxidation and secondary hyperparathyroidism. In addition, we described the possible potential value of vitamin C in anemia, and the side effects of over-doses of vitamin C supplementation in this particular population.

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INTRODUCTION

As one of the most important water-soluble non-enzymatic antioxidants, vitamin C consists of ascorbic acid and its oxidized form, dehydroascorbic acid. The former is easily to be oxidized into the unstable dehydroascorbic acid while exposed under chronic or acute oxidant conditions, such as in smokers and in diabetes. Humans can not synthesize ascorbate due to the lack of the gene encoding the enzyme gulonolactone oxidase, which is involved in the last step in biosynthesis of L-ascorbic acid[1]. Therefore, vitamin C can be obtained only from fresh fruits and vegetables, such as strawberry, kiwi, orange juice and broccoli.

In normal population, the plasma vitamin C level ranges from 30 to 60 μmol/L[2]. Plasma level of vitamin C in maintenance hemodialysis (MHD) patients is generally lower[3,4] compared with general population. Moreover, dialysis patients exhibit a low plasma vitamin C level, which is largely related with increased inflammation, refractory anemia, oxidative stress, and secondary hyperparathyroidism (SHPT)[5-8].

Previous investigations demonstrated that vitamin C

supplementation possesses promising effects in MHD patients, such as better improvement of oxidative stress[9,10], inflammation[11] and anemia[12,13]. Therefore, vitamin C adjuvant therapy has been highly recommended for this particular group of patients.

In this review, we described the possible contents of physiological functions of vitamin C, deficiency of vitamin C in MHD patients as well as effects of vitamin C on anti-oxidation, anti-inflammation, pro-oxidation, anemia and SHPT in MHD patients. Moreover, dosages of vitamin C supplementation and side effects of vitamin C due to its metabolite, oxalosis accumulation, were also discussed.

PHYSIOLOGICAL FUNCTIONS OF VITAMIN C

Vitamin C can act as both antioxidant and as prooxidant[9,10,14-16]. It is generally regarded as a protective antioxidant due to its direct oxyradical scavenging properties[17,18]. Recent research revealed that vitamin C plays many prominent roles, and it functions in the biosynthesis collagen, norepinephrine and carnitine as a cofactor for several enzymes[19-21]. Moreover, vitamin C also plays a key role in peptide amidation and tyrosine metabolism. In addition, vitamin C has a potential to enhance the non-heme iron absorption[22] and alter the metabolism of the iron (Fe) from inert tissue stores[12,23,24]. At the gastrointestinal tract with an alkaline pH, vitamin C provides auxiliary aids in maintaining iron in a more soluble state, which is more readily absorbed across the intestinal mucos[22,25].

Scurvy is a disease resulting from deficiency of vitamin C in diet[26]. It is preceded by certain symptoms, including increased bone resorption[27], gingival problems[28], weakness, fatigue, irritability, vague myalgias, joint pains, connective tissue disorders, mood changes and poor wound healing.

DEFICIENCY OF VITAMIN C IN MHD PATIENTS

It is well known that MHD patients have a generally lower plasma vitamin C level compared with normal population[3,4]. MHD patients exhibit remarkably low vitamin C levels in plasma, frequently < 10 μmol/L or even < 2 μmol/L[3,29]. In our previous study, a plasma vitamin C level of < 4 μg/mL (22.8 μmol/L) is presented in 64.4% dialysis patients[5]. The observed low level of plasma vitamin C might be attributed to the dietary restrictions on fruit and vegetable intake, and impaired metabolism in uremia during dialysis procedure[4,30-32], as well as increased inflammation[5], oxidative stress[6] and SHPT[8]. Previous investigations demonstrated that dialysis treatment induces a decrease in plasma vitamin C level, which is approximately reduced by 33%-50% from the baseline values[3,33] and equal to a removal of 100-300 mg vitamin C during a dialysis session[4,34]. Therefore, vitamin C deficiency might be more frequently detected in these individuals. Conventional hemodialysis (HD), on-line hemodiafiltration and high-flux hemodialysis eliminate plasma vitamin C in a similar way[5,33]. These findings could be associated with its small molecule (176.1 Da), low protein-bound and high soluble characteristic in water.

EFFECTS OF VITAMIN C ON ANTI-OXIDATION AND ANTI-INFLAMMATION IN MHD PATIENTS

Widely observed in long-term dialysis patients, oxidative stress is associated with chronic inflammation and increased mortality risk. Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species (ROS) and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage in uremic patients[35,36]. The relationship between oxidative stress and inflammation is certainly bidirectional since oxidative stress affects inflammatory status and inflammation exerts influence on the state of oxidative stress. Inflammation in uremic patients can be triggered by oxidative products during dialysis procedure, such as superoxide anion (O^{2-}) and hydrogen peroxide (H_2O_2)[37]. On the other hand, inflammation may further aggravate oxidative stress through potentiating respiratory burst activation in monocytes and neutrophils[38].

As an important anti-oxidant, vitamin C possesses beneficial effects on reducing ROS and improving inflammatory status. Acute administration of vitamin C reduces oxidant stress levels and improves NO-mediated resistance vessel dilatation in renal failure[39]. Moreover, Tarng *et al*[10] reported that in MHD patients vitamin C supplementation for 8 wk reduces the 8-OHdG level of cellular DNA, an index of oxidative DNA damage in ROS-mediated diseases[40]. Abdollahzad *et al*[9] found that malondialdehyde levels are decreased and lipid profiles are improved in MHD patients orally supplemented with 250 mg vitamin C every other day for 12 wk. In our recent crossover study, we found that the level of hypersensitive C-reactive protein (hs-CRP) in MHD patients is lowered by oral vitamin C supplementation of 200 mg/d for 3 mo, and the hs-CRP level is increased again after the vitamin C supplementation is withdrawn[11]. Considered as a co-antioxidant, vitamin C can regenerates a-tocopherol (vitamin E) from the a-tocopheroxyl radical, produced *via* scavenging of lipid-soluble radicals[41]. High doses of vitamin C administration in a low infusion rate during HD session can prevent an increase in lipid peroxidation, which might be probably associated with the enhanced rate of endogenous vitamin E regeneration[42].

However, some other studies did not show this beneficial effect in MHD patients[43-45]. For example, in Fumeron's study[45], 250 mg vitamin C is orally given to 33 MHD patients thrice weekly after each dialysis session for 2 mo, and no improved situation of oxidative/anti-oxidative stress and inflammation has been observed. Chan *et al*[44] also reported that there is no effect on markers of

oxidative stress in MHD patients after 250 mg vitamin C supplementation thrice weekly for 8 wk, either intravenously or orally. Kamgar *et al*[43] recently reported that the CRP level exhibits a decrease trend in MHD patients after an oral vitamin C supplementation of 250 mg/d for 2 mo. Similar findings have been observed by Ramos *et al*[46].

These conflicting data may be partially explained by the following reasons: (1) the inflammatory status of patients in some previous studies is altered due to daily oral vitamin C supplementation; (2) certain factors are different in the study populations, such as age, dialysis vintage, smoking status and proportion of diabetes; (3) difference in doses, duration and route of vitamin C administration; and (4) different markers of oxidative stress have been used in the above-mentioned studies.

EFFECTS OF VITAMIN C ON PRO-OXIDATION IN MHD PATIENTS

Vitamin C also acts as a pro-oxidant due to promoting Fenton chemistry, which converts the Fe^{3+} into Fe^{2+} and catalyzes the formation of ROS[47-49].

Eiselt *et al*[50] documented a pro-oxidative effect of vitamin C during intravenous iron sucrose and vitamin C administration in MHD patients. In this study, they found that intravenous administration of Fe together with vitamin C supplementation results in a greater increase in plasma thiobarbituric acid reacting substances compared with single intravenous administration of Fe. Ferretti *et al*[15] indicated that intravenous administration of vitamin C increases lipid hydroperoxides and advanced glycation end product levels and decreases paraoxonase activity in hemodialysis patients. In addition, De Vriese *et al*[16] also found similar results by oral vitamin C supplementation in MHD patients.

POTENTIAL VALUE OF VITAMIN C IN ANEMIA IN MHD PATIENTS

Although anemia management has been improved in patients with chronic kidney disease (CKD), anemia is still prevalent in these patients[51]. Approximately 35% of patients with end stage renal disease have refractory anemia[52]. This persistent anemia might be explained by relative resistance to erythropoietin (EPO) due to functional iron deficiency, which is a situation characterized by low transferrin saturation[52].

Latest study showed that the level of plasma vitamin C has a positive correlation with the hemoglobin level[53] as well as a negative correlation with the EPO resistance index[54-56], and ascorbic acid has also been used to improve response to erythropoiesis-stimulating agents. Vitamin C plays an important role in the utilization of iron from storage sites. As an electron donor, vitamin C can reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) in order to mobilize storage iron, including the portion of tissue iron as hemosiderin[57], resulting in an activated iron bioavailability and enhanced production of red blood cells.

Previous work showed that ascorbic acid administration ameliorates the hemoglobin level in MHD patients with iron-overloading[12,13]. These findings have been subsequently confirmed in anemic MHD patients receiving iron administration accompanied by vitamin C[7,54,58]. However, in patients with normal iron status, the situation of EPO needs and transferrin saturation is not improved with vitamin C supplementation[12]. A recent meta-analysis[52] of the available studies indicated that vitamin C supplementation is closely related to a decreased rHuEPO dose and an increased transferring saturation, but no apparent effect on ferritin levels has been observed.

Patients on dialysis have a shortened red blood cell half-life compared with the normal level of 120 d[59,60]. A series of factors have been demonstrated to contribute to this reduced lifespan of red blood cells, including increased oxidative stress and decreased antioxidant products[60,61], the breakdown of phospholipid asymmetry in red blood cell membranes[61,62], increased level of parathyroid hormone[63,64] as well as deficiencies of carnitine[65] and zinc[66]. In addition, although the exact mechanism remains unclear, the half-life of circulating red cells might be extended by vitamin C supplementation[67].

VITAMIN C DEFICIENCY AND SHPT

SHPT is common in chronic hemodialysis patients[68], and it develops because of the increased parathyroid hormone (PTH) synthesis, secretion and the impaired renal clearance[69]. About 50% MHD patients have increased PTH levels[68]. High level of plasma PTH has been linked to uraemic toxin[70], leading to an increased cardiovascular mortality in MHD patients[71].

The effect of vitamin C on SHPT remains unknown in MHD patients. Richter *et al*[8] showed that higher level of plasma vitamin C is correlated with lower level of bio-intact PTH in MHD patients. Vitamin C has an effect on post-receptor events in the calcium-sensing receptors on parathyroid cells, which might partially explain the inverse interaction between the vitamin C level and PTH[72]. Moreover, the cyclic adenosine monophosphate response to PTH can be also enhanced by vitamin C supplementation[73]. Sanadgol *et al*[74] reported that the mean level of serum PTH is decreased in the first 2 mo compared with baseline after a 3-mo intravenous administration of vitamin C (200 mg, thrice weekly). However, this effect is gradually diminished at month 3, which might be explained by the reduced sensitivity of calcium-sensing receptors on parathyroid gland cells with the passage of time. Interestingly, Biniaz *et al*[75] did not find the beneficial effect of vitamin C on SHPT in a double blinded, placebo-controlled study in MHD patients.

DOSAGES OF VITAMIN C SUPPLEMENTATION

In healthy subjects, a daily administration of 90 mg or

75 mg vitamin C is enough for men or women, respectively[76]. For MHD patients, the recommended dosages are more controversial. Generally speaking, vitamin C supplementation is recommended for MHD patients due to the restrictions on their dietary intake as well as losses during dialysis procedure. Many clinicians only recommend vitamin C to a conservative range of 60-100 mg/d with the consideration of oxalate accumulation in the tissues of renal failure patients. These recommendation may not be optimal, because the loss of vitamin C during a single dialysis treatment may reach several hundred milligrams of vitamin C[4,34], resulting in vitamin C deficiency common in MHD patients.

Many literatures documented that the oral doses of vitamin C in the range of 100-200 mg/d[11,77-80] are considered as the sufficient and safe dosages[79], or intravenous administration of 300 to 500 mg vitamin C thrice weekly at the end of dialysis is regarded as "guidelines for intravenous ascorbic acid adjuvant therapy"[56,81,82]. However, some other studies hold a contrary opinion[83]. This conservative recommendation is mainly due to the side effects of oxalosis following the vitamin C administration.

OXALOSIS

In the past several decades, researchers have also investigated side effects of vitamin C supplementation. Since oxalate is one of the products of vitamin C metabolism, vitamin C supplementation in MHD patients may enhance oxalate plasma levels in patients with uremia. Significant plasma oxalate levels induced by vitamin C supplementation (500-1000 mg/d for 3 or more than 3 wk) have been observed[7,84-86]. Canavese *et al*[83] reported that plasma oxalate levels are progressively increased even the dosage of vitamin C is set at 500 mg/wk. The peak level is observed after 1 year of treatment, and then it is leveled off thereafter. Therefore, the safety of this protocol in terms of oxalate metabolism should be carefully considered.

However, some studies showed that this potential hazard may be prevented by taking certain measures, such as avoiding vitamin B_6 deficiency[87] and improving dialysis technology[88,89]. Tomson *et al*[88] showed that tissue oxalate accumulation is completely absent in well-dialyzed CKD patients.

CONCLUSION

Taken together, vitamin C deficiency is common in MHD patients. Therefore, vitamin C supplementation is crucial for MHD patients. Patients can potentially benefit from vitamin C supplementation by its effects on anti-oxidative stress, anti-inflammation, SHPT and improved anemia. However, vitamin C overdose should be avoided due to its secondary oxalosis.

To date, recommendations of vitamin C therapy in MHD patients can not reach consensus because of the wide use of variety of dosages, route of administration, durations and the severe side effects of oxalosis. Oral doses of vitamin C in the range of 100-200 mg/d, or intravenous administration of 300 to 500 mg vitamin C thrice weekly at the end of dialysis are commonly regarded as adequate to prevent ascorbate deficiency in MHD patients.

In summary, MHD patients may benefit from vitamin C administration. However, further large-scale randomized-controlled clinical trials should be carried out to confirm its beneficial effects, optimal dosage and side effects from vitamin C supplementation.

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ORIGINAL ARTICLE

Programmed cell death protein 4 expression in renal cell carcinoma, penile carcinoma and testicular germ cell cancer

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Abstract

AIM: To investigate the expression of programmed cell death 4 (Pdcd4) tumor suppressor gene in tissue specimen of renal cell carcinoma (RCC), testicular germ cell cancer and penile cancer.

METHODS: Pdcd4 expression was studied using immunohistochemistry in 188 cases of RCC and 28 controls (including 9 oncocytoma); in 74 cases of penile carcinoma (including 17 metastatic tissue samples) and 26 controls; in 11 cases of seminoma, in 14 cases of non-seminoma and 5 controls.

RESULTS: Control tissues exhibited strong core and cytoplasmatic Pdcd4 staining. In contrast, core and cytoplasmatic Pdcd4 levels were significantly decreased in cancer tissues.

CONCLUSION: Our data support a role for Pdcd4 (down-) regulation in urologic tumors. Interestingly, Pdcd4 expression seem to be a potential diagnostic marker for renal or penile tumors.

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Key words: Programmed cell death 4; Seminoma; Non-seminoma; Testicular cancer; Renal cell carcinoma; Penile carcinoma; Expression; Apoptosis; Immunohistochemistry

Core tip: Programmed cell death 4 has increasingly become the focus of investigative tumor research in the last years. It has shown to be involved in many tumorous entities, some of which we present for the first time in this paper. Its involvment in apoptosis, invasion and metastasis has been proved in numerous works and showed to be a target for diagnostic and therapeutic measures. We investigate its role and cellular expression patterns in urologic tumors, especially some, that haven't been investigated to this extent or at all to this date.

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INTRODUCTION

Programmed cell death 4 (Pdcd4) expression is known

Table 1 Clinicopathological parameters for patients with seminomatous testicular germ cell cancer *n* (%)

Variables	Carcinoma (*n* = 11)	Control (*n* = 5)
Age in years mean (range)	40.58 (33-53)	30.75 (24-42)
Pathological stage		
pT1	7 (63.6)	NA
pT2	2 (18.2)	NA
pT3	2 (18.2)	NA
Lymphnode metastasis		
cNx	1 (9.1)	NA
cN0	7 (63.6)	NA
cN1	1 (9.1)	NA
cN2	2 (18.2)	NA
M-stage		
cMx	1 (9.1)	NA
cM0	10 (90.9)	NA
Tumor marker		
HCG increased	5 (45.5)	NA
LDH increased	3 (45.5)	NA
AFP increased	0 (0)	NA
Clinical tumor stage		
CS I A	5 (45.5)	NA
CS I S	3 (27.3)	NA
CS II B	1 (9.1)	NA
CS II C	2 (18.2)	NA

NA: Not applicable; HCG: Human choriogonadotropin; LDH: Lactatdehydrogenase; AFP: Alpha-fetoprotein; CS I A: Clinical stage 1A.

to be suppressed in many tumors, for example urothelial- or colorectal cancer[1,2]. As shown in a previous study, Pdcd4 expression levels may have a tumor specific expression pattern and a potential as a diagnostic marker[1]. It inhibits RNA binding of the initiations factors eIF4A and eIF4G[3,4]. The *Pdcd4* gene is located on chromosome 10q24 and encodes a 469 aminoacids long protein. A major regulator of Pdcd4 expression, microRNA 21 (miR-21) is induced by the transforming growth factor-β pathway. High miR-21 expression leads to a suppression of Pdcd4 expression and an induction of metastasis, invasion and intravasation in cell culture[5]. In Pdcd4 overexpressing cells, the subsequent carbonic anhydrase Ⅱ down-regulation shows its influence on the translational level[6]. At the transcriptional level Pdcd4 influences the *uPAR* gene promoter through phosphorylation of the Sp transcription factors in colorectal cells[7]. Interestingly, this pathway is not confirmed for breast cancer: the lack of suppression of uPAR transcription by Pdcd4 overexpression therefore shows a possible tissue specific role of Pdcd4 and its involvement in carcinogenesis[8].

Renal cell carcinoma

Renal cell carcinoma (RCC) stands for 2%-3% of all human cancers with an incidence of 5.8 and a mortality of 1.4 per 100000. Mostly men in the age of 60 to 70 are affected. Important risk factors are smoking, obesity and hypertension as well as having a first-degree relative with RCC. The different subtypes of RCC are clear cell RCC (ccRCC), papillary RCC (pRCC), chromophobe RCC (chRCC) and sarcomatoid RCC (sRCC).

Testicular cancer

This entity represents between 1%-1.5% of male cancers and about 5% of all urological tumors, with an incidence of 3-10 per 100000 males/year Peak incidence is in the third decade of life for non-seminoma and in the fourth decade for seminoma. Risk factors are cryptorchidism, undescended testes, Klinefelter's syndrome, positive familial history, a contralateral tumour or a precancerous lesion and infertility. The two subtypes include seminoma and non-seminomatous germ cell tumor (NSGCT).

Penile cancer

Penile cancer has an incidence of < 1/100000 males in western countries. It is related to race and ethnicity, most frequently affecting white hispanics (1/100000). Risk factors are social, cultural, hygienic and/or religious practices.

The role of Pdcd4 in penile- and testicular carcinoma has not been reported or investigated to this date. Recent studies to RCC stipulate a role for Pdcd4 in tumor progress and survival[9]. We thus investigated its expression pattern in human renal-, testicular-and penile tissue in the largest cohorts published to this date.

MATERIALS AND METHODS

Patients

The expression of Pdcd4 in tumorous and normal tissue was studied using a tissue microarray as described in a previous study[10]. The microarray included 188 RCC, 74 penile squamous cell carcinoma and 25 testicular cancer (11 seminoma, 14 NSGCT) samples with their complementary controls; see Supplementary tables for clinicopathological parameters. The study was approved by our institutional ethics committee (ethic vote 199/10). The clinical pathological parameters are listed in Tables 1-4.

Immunohistochemistry

The construction of the tissue microarray was reported earlier[1]. Immunohistochemical staining was performed automatically (DAKO TechMate™ 500, Danmark) for Pdcd4 [1:400, Anti-Pdcd4 (rabbit) antibody, United States] following the manufacturer´s instructions as described in a previous work[1]. Negative and positive controls were run using rabbit *ig*G-isotype in a concurrent manner. Inflammatory, stromal and normal cells expressed Pdcd4 and therefore served as internal positive control. The stained microarrays were archived with a pathology scanner (Pannoramic MIDI Scanner, 3D-HISTECH, Hungary) for subsequent analyses (Figures 1 and 2).

Pdcd4 expression was scored by one pathologist who was unaware of the patients' clinical history. According to Mudduluru *et al*[11], we evaluated the core and cytoplasmatic immunostainings. Core Pdcd4 staining was classified in four groups according to the amount (in percent) of stain-positive nuclei (core quantity: score): none: 0; ⩽ 30%: 1; 30%-70%: 2 and ⩾ 70%: 3. Cytoplasmic and core stainings were matched by the intensity of the staining results (none, weak, intermediate or strong). The sum

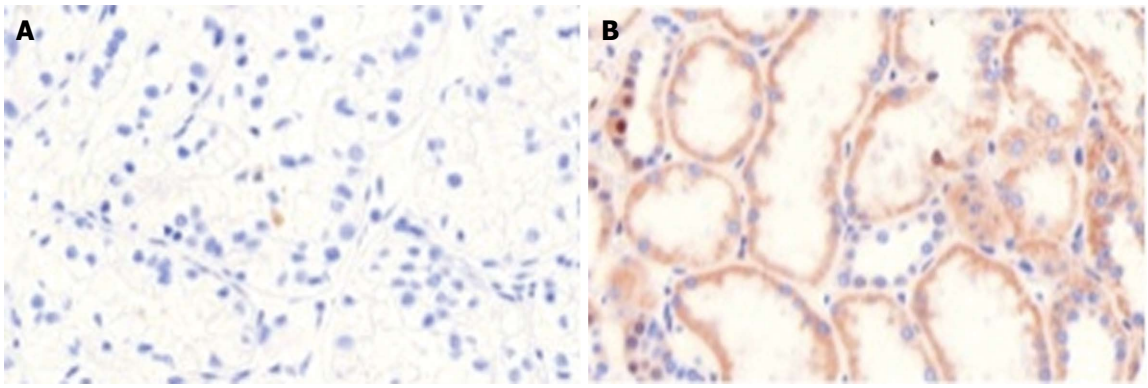

Figure 1 Histopathological staining results of malignant (pT2) renal tissue (A) and of a control sample (B) (haematoxylin and eosin staining, magnification factor: 40 ×).

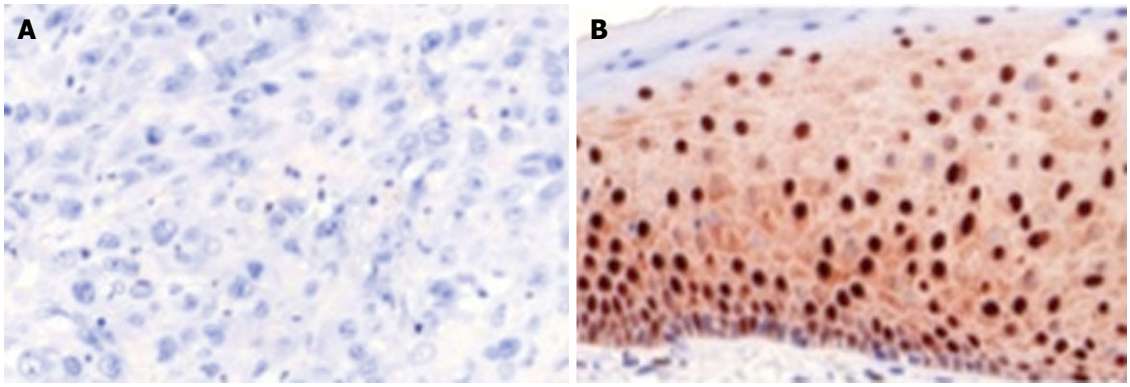

Figure 2 Histopathological staining results of malignant (pT3) penile tissue (A) and of a control sample (B) (haematoxylin and eosin staining, magnification factor: 40 ×).

Table 2 Clinicopathological parameters for patients with non-seminomatous testicular germ cell cancer *n* (%)

Variables	Carcinoma (n = 14)	Control (n = 5)
Age in years mean (range)	32.58 (16)	30.75 (18)
Pathological stage		
pT1	5 (26.3)	NA
pT2	5 (26.3)	NA
pT3	3 (15.8)	NA
pTx	1 (18.2)	NA
Lymphnode metastasis		
Nx	7 (36.8)	NA
N0	4 (21.1)	NA
N3	3 (15.8)	NA
M-stage		
Mx	4 (21.1)	NA
M0	9 (47.7)	NA
M1	1 (5.3)	NA
Tumor markers		
HCG increased	13 (68.8)	NA
LDH increased	7 (36.8)	NA
AFP increased	12 (85.7)	NA
Clinical stage		
I S	8 (42.1)	NA
II A	2 (10.5)	NA
II C	3 (15.8)	NA
NA	1 (5.3)	NA

NA: Not applicable; HCG: Human choriogonadotropin; LDH: Lactatdehydrogenase; AFP: Alpha-fetoprotein.

of these scores was assesed as well.

Statistical analysis

Clinicopathological parameters were correlated with Pdcd4 expression using the χ^2-test. Statistical analyses were performed with SPSS, version 20 (IBM Corporation, United States). Statistical significance was concluded at $P < 0.05$.

RESULTS

RCC

Cytoplasmic and core Pdcd4 levels were increased in normal renal tissue compared to RCC ($P < 0.001$). Pdcd4 expression was reduced in patients with locally advanced RCC. Furthermore, statistical analysis showed high significance levels in staining patterns between the renal tumor types (sRCC, pRCC, ccRCC and chRCC). Especially cytoplasmatic staining showed the strongest correlation levels ($P < 0.0001$). Further significant results showed in T-stage dependent stainings ($P = 0.001$) and tumor size ($P = 0.011$). Figure 3 presents tumor type dependent staining results.

Penile cancer

Both, core ($P < 0.001$) and cytoplasmic ($P = 0.047$)

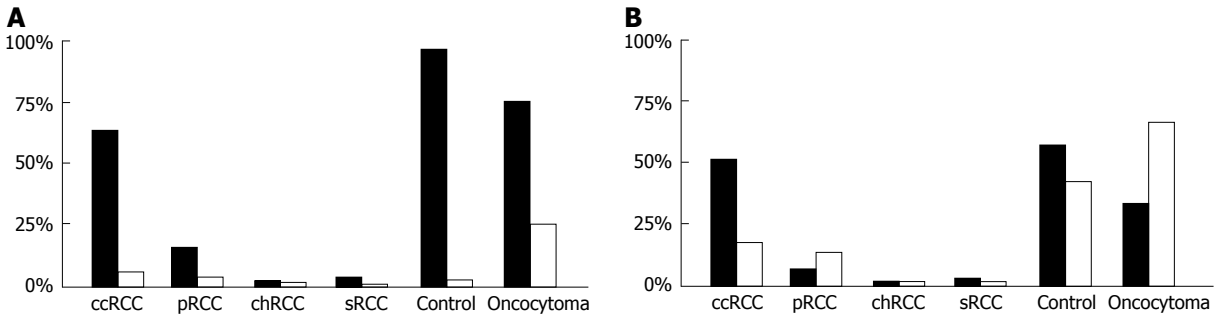

Figure 3 Percentual distribution of core intensity (A), cytoplasmatic-(B) and programmed cell death 4 staining results in correlation to renal cell carcinoma subtype and controls. Left weak/negative staining, right strong/positive staining result. RCC: Renal cell carcinoma; ccRCC: Clear cell RCC; pRCC: Papillary RCC; chRCC: Chromophobe RCC; sRCC: Sarcomatoid RCC.

Table 3 Clinicopathological parameters for patients with renal tumors *n* (%)

Variables	RCC (*n* = 188)	Normal/oncocytoma (*n* = 28)
Age in years mean (range)	60.4 (0-85)	56.86 (26-75)
Sex		
Male	128 (68.1)	12 (42.9)
Female	60 (31.9)	16 (57.1)
Pathological stage		
pT1	81 (37.5)	NA
pT2	40 (18.5)	NA
pT3	57 (26.4)	NA
pT4	3 (1.4)	NA
Missing	7 (16.2)	NA
Lymphnode metastasis		
pN0	96 (44.4)	NA
pN1	6 (2.8)	NA
pN2	9 (4.2)	NA
pNx	72 (33.3)	NA
Missing	5 (15.3)	NA
Metastasis		
M0	114 (52.8)	NA
M1	24 (11.1)	NA
M2	1 (0.5)	NA
Mx	42 (19.4)	NA
Missing	7 (16.2)	NA
Grade		
G1	56 (25.9)	NA
G2	111 (51.4)	NA
G3	12 (5.6)	NA
G4	2 (0.9)	NA
Missing	7 (16.2)	NA

NA: Not applicable; RCC: Renal cell carcinoma.

Pdcd4 levels were decreased in penile carcinoma tissue when compared to non-malignant penile skin (Figure 4). Furthermore, we observed lower Pdcd4 levels in locally advanced and less-differentiated penile carcinomas. A strong correlation in differentiating tumorous from healthy tissue was found. Especially core stainings showed the strongest significance ($P < 0.0001$) level in this group. T-Stage dependent stainings also correlate with core and cytoplasmatic Pdcd4 expression, insinuating a stage dependent expression and regulation of Pdcd4 (Figure 5). Other significant results were calculated when immunohistochemistry (IHC) results were compared to grading and tumor recurrence. There is no correlation with nodal status.

Testicular cancer

In seminoma and NSGCT, only weak correlation levels to clinicopathological parameters were calculated. Thus, Pdcd4 staining results correlate in differentiating healthy from malignant tissue in both entities ($P = 0.007$). Another notable significance is cytoplasmatic stainings to alpha-fetoprotein (AFP)-levels ($P = 0.032$) (Figure 6). This insinuates a potential for Pdcd4 as a diagnostic marker in this tumorous entity.

DISCUSSION

Our results show a decreasing Pdcd4 expression in urologic tumors (*i.e.*, RCC and penile carcinoma). As shown in a previous study, Pdcd4 expression levels may have a tumor specific expression pattern[1].

Shiota *et al*[12] showed that Pdcd4 interacts with the DNA binding domain of Twist1, through inhibition of the DNA binding ability and Y-box binding protein-1 (YB-1) expression, reducing cell growth. The immunohistochemical stainings showed an inverse correlation between core Pdcd4 and YB-1 expression.

Shi *et al*[13] could show an effect of miR-21-expression on docetaxel resistance, insinuating a more complex role of Pdcd4 in tumor aggressivity and chemoresistance. Pdcd4 overexpression in the study of Shiota *et al*[12] showed a high sensitivity to chemotherapeutics (cisplatin and paclitaxel). Thus, the data displayed there (clinicopathological parameters and follow-up) and the data of our study is not extensive enough to correlate Pdcd4 expression levels and tumor chemosensitivity. A COX regression analysis could not confirm this hypothesis of Pdcd4 as a prognostic marker in survival or progress. The weak correlation levels are presumably due to the small cohort restraining the arithmetic power of this analysis. Because of these results and the cohort size, a multivariate analysis was redundant. Another possible explanation for these results are the weak responses of metastatic disease to further therapy in advanced tumor stages.

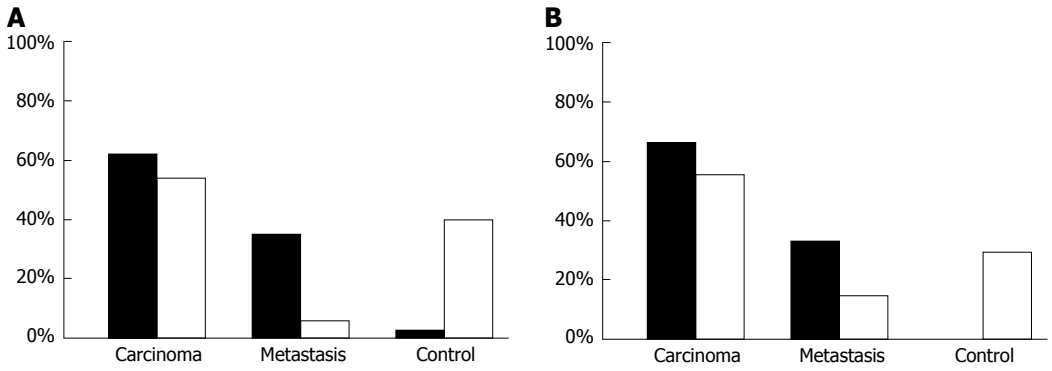

Figure 4 Percentual distribution of core (A) and cytoplasmic programmed cell death 4 (B) levels in penile carcinoma tissue compared to metastasies and controls. Left weak/negative staining, right strong/positive staining result.

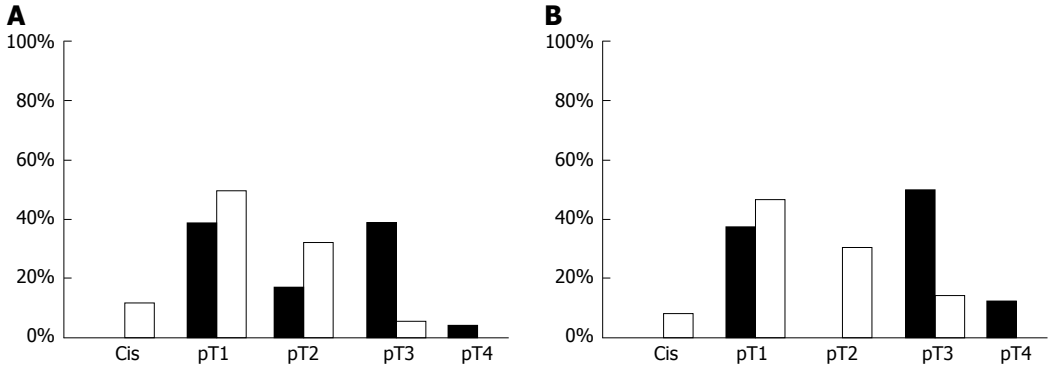

Figure 5 Percentual distribution of core (A) and cytoplasmatic (B) programmed cell death 4 staining results in correlation to T-stage of penile carcinoma and controls. Left weak/negative staining, right strong/positive staining result.

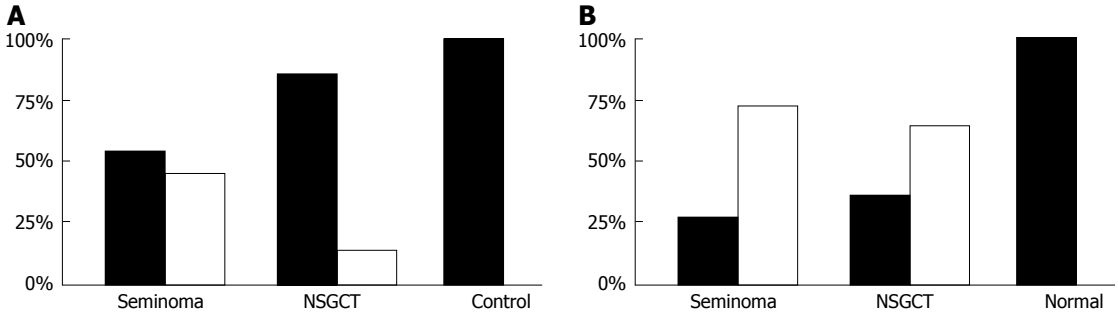

Figure 6 Percentual distribution of core (A) and cytoplasmatic (B) programmed cell death 4 staining results in correlation to Seminoma-, non-seminomatous germ cell tumor and controls. Left weak/negative staining, right strong/positive staining result. NSGCT: Non-seminomatous germ cell tumor.

A recent study by Li *et al*[9] showed a T-stage dependent expression of Pdcd4. Thus, the significant correlation between Pdcd4 expression and survival could not be confirmed in our study. A possible explanation for this is our differentiation of RCC subtypes in our study design. Our analysis shows a clear difference in Pdcd4 expression patterns within the subgroups, this being probably due to their distinct tumor biology. IHC Pdcd4 expression was positive in the core and cytoplasmatic compartment in RCC samples: The levels changed according to tumor progression (T-stage dependent). This supports the hypothesis of tumor-specific expression patterns of Pdcd4 and a possible shuttle function between the cellular compartments. Thus, Li *et al*[9] described a solely core expression; the use of a different antibody for IHC could explain these diverging results. However, this differentiation is important regarding the good discriminative potential of our IHC for differing malignant RCC tissue from the benign renal tissue and oncocytoma. Prognostic relevance such as described by Li *et al*[9] could not be verified in our study.

A high sensitivity and specificity could be confirmed for penile carcinoma as well. These data are similar to results in bladder carcinoma samples obtained in a previous study and suggerate a similar regulation in these tumorous entities[1]. To assess these similarities and to evaluate

Table 4 Clinicopathological parameters for patients with penile carcinoma *n* (%)

Variables	Carcinoma (*n* = 57)	Metastasis (*n* = 17)	Control (*n* = 26)
Age in years mean (range)	63.32 (35-93)	62.96 (11-87)	64.15 (37-97)
Pathological stage			
Cis	4 (7)	NA	
pT1	26 (45.6)	NA	
pT2	15 (26.3)	NA	
pT3	11 (19.3)	NA	
pT4	1 (1.8)	NA	
Lymph node metastasis			
cN0	50 (87.7)	NA	
pN+	7 (12.3)	NA	
M-stage			
cM0	57 (100)	NA	
Grading			
G1	9 (17)	NA	
G2	36 (67.9)	NA	
G3	8 (15.1)	NA	
Missing	4 (7)	NA	
Surgical margins			
R0	55 (79.7)	10 (58.8)	
R1	2 (2.9)	0 (0)	
R2	0 (0)	2 (11.8)	

NA: Not applicable.

the role of a shuttle function of the gene, we calculated different IHC-expression variables (core and cytoplasmatic intensity and quantity). A statistical evaluation (χ^2-test) of these IHC expression patterns with established variables (T-stage, resection status and tumor grade) support the similarities between these epithelial tumors. Thus, a validating COX regression analysis could again not confirm this hypothesis of Pdcd4 as a prognostic marker in survival or progress.

In testicular samples, expression levels correlated significantly in the malignant/healthy group, in T-stage-dependent staining results and to AFP-levels. This insinuates a potential for Pdcd4 as a diagnostic marker. However, our cohort did not have a sufficient size to evaluate clinical outcome. Accordingly, a ROC analysis revealed only weak sensitivity and specificity and a survival analysis (COX) was not possible. Another problem in elucidating the role of Pdcd4 in NSGCT is the cellular inhomogeneity of the tumor cells within each probe (chorion-, embryonal-, cystic-carcinoma and yolk sack tumor).

Our study supports the important role for Pdcd4 downregulation in renal-, testicular- and penile carcinoma. Interestingly, Pdcd4 expression seem to be a potential diagnostic marker for renal or penile tumors.

COMMENTS

Background

Programmed cell death 4 (Pdcd4) is involved in the process of apoptosis. It was first described in 1995 by Shibahara *et al*. Its upregulation has an effect on transcription, translation and many signal transduction pathways. In many tumorous entities its expression levels are suppressed, so that it was stipulated that it has a tumor-suppressive function through the initiation of cellular apoptosis. Especially translation and transcription pathways seem to be affected by the active gene. This could not be verified in all tumorous entities, so that the authors assume a cancer-specific expression pattern. Wei *et al* (2009), Matsuhashi *et al* (2007) and Mudduluru *et al* (2009) compared expression levels in healthy and tumorous tissues of ovarian-, squamous- and colorectal-carcinoma. In these probes there was a "shift" from the nuclear-in healthy probes to the cytoplasmatic compartment in malignant tissue. These results could not be verified in all tumorous entities. Studies with urologic tumors have shown diverging results concerning the expression levels or were not published at all. In a previous work, the authors could show a very significant correlation between the tumor stage and expression patterns in transitional cell carcinoma.

Research frontiers

Shi *et al* could show an effect of microRNA 21 (miR-21)-expression on docetaxel resistance, insinuating a more complex role of Pdcd4 in tumor aggressivity and chemoresistance. In this study, there was a negative correlation between low miR-21 expression levels with high docetaxel chemosensitivity and high Pdcd4 expression. The expression levels of Pdcd4 could be used to elaborate an individual therapeutic strategy and a prognostic evidence for each patient.

Innovations and breakthroughs

The studies of Woodard *et al* (2008) and Jansen *et al* (2004) demonstrated high Pdcd4 expression in clear cell renal cell carcinoma cell lines after having treated these with Fluvostatin and a higher geldamycin sensitivity in cells with high Pdcd4 expression levels. Fluvostatin being an inhibitory agent for pAkt and S6K1, inhibitors of Pdcd4. A recent study by Li *et al* (2012) showed a T-stage dependent expression of Pdcd4. This analysis shows a clear difference in Pdcd4 expression patterns within the subgroups, this being probably due to their distinct tumor biology. Immunohistochemistry (IHC) Pdcd4 expression was positive in the core and cytoplasmatic compartment in RCC samples: The levels changed according to tumor progression (T-stage dependent). This supports the hypothesis of tumor-specific expression patterns of Pdcd4 and a possible shuttle function between the cellular compartments.

Applications

This study supports the important role for Pdcd4 downregulation in renal-, testicular- and penile-carcinoma. Therapeutic and prognostic possibilities are discussed for other tumourous entities (*i.e.*, squamous carcinoma, Dou *et al* 2014). Especially chemosensitivity and therapeutic options can be evaluated for patients showing high IHC Pdcd4 expression levels.

Terminology

Pdcd4: Programmed cell death 4; NSGCT: Non-seminomatous germ cell tumor.

Peer review

The manuscript by Fisher *et al* is to investigate the Pdcd4 expression in renal cell carcinoma, penile carcinoma, and testicular germ cell cancer. The authors found that the Pdcd4 is strongly expressed in core and cytoplasm, but core and

cytoplasmic Pdcd4 levels is significantly decreased in the cancer tissues. These findings are important and may reveal a novel biomarker for renal and prostate cancers.

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RETROSPECTIVE STUDY

Single-site laparoscopic partial nephrectomy: Where are we going?

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Abstract

AIM: To review an evolution of laparoscopic surgery, there has been a growing interest in laparoendoscopic single-site surgery (LESS).

METHODS: A comprehensive electronic literature search was conducted using PubMed database to identify all publications relating to LESS-partial nephrectomy (PN). The research includes articles published from April 2008 to January 2014. We focused our attention only on articles in which were cited the single-site surgical technique (laparoscopic and robotic), tumour stage and grade, mean tumour size, intraoperative variables, blood loss and transfusion rate, length of post-operative stay and complication rates, Clavien classification, positive of surgical margins, pain assessment at discharge.

RESULTS: A total of 9 studies were collected with 221 patients included. The mean patients age was 62 years. The mean tumor size was 2.35 cm with a mean operative time of 181 min (range 111-270 min) and 58.3% were done by robot. The mean ischemia time was 23.6 min. The 25.8% of patients underwent an unclamp LESS-PN. Mean estimated blood loss was 296 mL and median length of hospital stay was 4 d. The rate of severe post-operative complications (≥ Clavien grade Ⅲ) was 5.4%. Not all surgical series of LESS-PN or Robotic-LESS-PN shows conversion in Multiport Laparoscopic or Open Surgery. Regarding oncologic outcomes, surgical margins were positive 4% of patients (9/221), no distant or port-site metastases were recorded.

CONCLUSION: LESS-PN and RLESS-PN are feasible and associated with reduced postoperative pain, shorter median hospital stay, shorter recovery time, and better cosmetic satisfaction without compromising surgical and oncological safety.

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Key words: Nephron sparing surgery; Partial nephrectomy; Laparoendoscopic single-site surgery; Single-port access surgery; Single-incision laparoscopic surgery; Robotic single-port partial nephrectomy

Core tip: In recent years, there has been a growing interest in laparoendoscopic single-site surgery (LESS). Some authors has used da Vinci surgical system for LESS surgery. Although almost every laparoscopic procedure in urology has been duplicated by using a LESS approach, only a few studies have reported problems and challenges encountered during LESS partial nephrectomy. The aim of our study is to evaluate the current literature in order to assess the efficiency, safety, and potential advantages of LESS-partial nephrectomy and Robotic-LESS PN.

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rardinelli F, Pellegrini F, Schips L. Single-site laparoscopic partial nephrectomy: Where are we going? *World J Clin Urol* 2014; 3(3): 358-363 Available from: URL: http://www.wjgnet.com/2219-2816/full/v3/i3/358.htm DOI: http://dx.doi.org/10.5410/wjcu.v3.i3.358

INTRODUCTION

Renal cell carcinoma represents 2%-3% of all cancers with an age-standardised rate incidence and mortality of 5.8 and 1.4 per 100000, respectively, in more developed areas[1].

The widespread use of modern imaging methods has resulted in a marked increase in the number of renal tumors detected incidentally in patients with non-urological symptoms[2]. These tumors are often of lower grade and stage, and nephron-sparing surgery (NSS) is a good treatment option for small (≤ 4 cm) renal lesions[2,3].

Currently available evidence suggests that localized kidney cancer is best managed by NSS whenever technically feasible[4].

Although open partial nephrectomy (PN) is considered the "gold standard" in the surgical therapy of T1 renal tumors, advances in laparoscopic surgery have led to increasing use of laparoscopic PN (LPN) for NSS[2].

LPN has also gained popularity, but is currently performed mainly in high-volume reference centres, and its diffusion has been limited by the steep learning curve[5].

Laparoscopic NSS combining the preservation of renal function and the minimal invasiveness of laparoscopy represents a robust alternative to open surgery especially because the incidence of benign lesions on final histopathology is high (nearly 30%) in small incidentally discovered renal masses[2,6].

In recent years, there has been a growing interest in laparoendoscopic single-site surgery (LESS), an evolution of laparoscopic surgery. "Single-site surgery" is the term given to various laparoscopic techniques that use a single skin incision to gain access to the abdominal cavity[7]. It has been proved to be applicable in the clinical field, being safe in hands of experienced laparoscopic surgeons in well-selected patients[8,9].

LESS tries to overcome even the rare port-related complications of laparoscopy and seems to achieve a fast and painless postoperative recovery with excellent cosmetic results[10,11].

Cindolo *et al*[12,13] were pioneers in the field of LESS-PN, and described the unclamped technique in 2009, achieving favourable early outcomes. Although almost every laparoscopic procedure in urology has been duplicated by using a LESS approach, only a few studies have reported problems and challenges encountered during LESS partial nephrectomy[14,15]. In spite the development of new specific equipment for LESS, the surgical instruments have limited range of motion, and clashing of instruments is a major disadvantage[14]. For this reason, some authors have used da Vinci surgical system for LESS surgery[16,17]. The introduction of robotic technology has provided some attractive features such as magnified 3D vision, articulating instruments, scaling of movement, tremor filtration, fourth robotic arm assistance, and a live intraoperative ultra-sound platform. Consequently, these features have reduced the crowding of instruments, enabled better precision with tumor resection and renal reconstruction. Nevertheless, there is still paucity of relevant studies of Robotic LESS-PN (R-LESS-PN) on its intermediate term clinical outcomes as it is a relatively new approach[16].

The aim of our study is to evaluate the current literature in order to assess the efficiency, safety, and potential advantages of LESS-PN and R-LESS PN.

MATERIALS AND METHODS

Bibliographic research

A comprehensive electronic literature search was conducted using PubMed database to identify all publications relating to LESS-PN. The research includes articles published from April 2008 to January 2014; it was conducted using a free-text protocol that included the following terms: nephron sparing surgery, partial nephrectomy, laparoendoscopic single-site surgery, single-port access surgery, single-incision laparoscopic surgery, robotic single-port partial nephrectomy.

The inclusion criteria for LESS were single, exophytic, cortical, small (4.0 cm) renal masses suitable for standard laparoscopic PN without ischemia. Patients with renal tumors up to stage T2 in the absence of nodal and systemic metastases were considered for the procedure, while those ones with significant cardiovascular and respiratory comorbidities or uncorrected coagulopathy were excluded. Even though patients with body mass index (BMI) < 30 were selected for this procedure during the early part of our learning curves, at present, we do not consider obesity as exclusion criteria.

Among all the articles found, we selected only those that were single-centre studies (not multicentre studies); we analysed only articles single-site surgical technique series (laparoscopic and robotic) that included information about tumour stage, tumour grade, mean tumour size, mean operative time, blood loss, transfusion rate, length of post-operative stay and complications. In addition the following data were collected: age, gender, BMI, intraoperative variables (number of additional ports), post-operative complications (Clavien classification), positive surgical margins, pain assessment at discharge [visual analogue scale (VAS) scale]; In the study incision length and patient subjective scar satisfaction were also evaluated[18]. In all the studies, all patients underwent renal ultrasonography and computed tomography scan before surgery, to give detailed information on tumour size, location, extent of parenchymal infiltration and proximity to the pelvicalyceal system.

Table 1 The surgical relevant data of all studies

Ref.	*n*	Robotic	Age (yr)	BMI	Mean tumor size (cm)	Mean operative time (min)	Mean EBL (mL)	Length of stay (d)	Mean ischemia time (min)	Transfusion rate (%)	Complication > Ⅲ grade clavien	Additional trocars % (diameter)	Positive margins	VAS	Conversion in MLS	Conversion in OS
Desai *et al*[20]	6	0	62	25	3	270	475	7.2	20	0	2	100% (6 mm × 2 mm; 1 mm × 5 mm)	0	-	-	-
White *et al*[21]	15	4	-	-	3.01	196	422	4.5	NA	26	0	13% (NA)	1	6	2	1
[2]Kaouk *et al*[16]	7	2	63.5	27.5	2.1	165	260	3.3	0 in 6 pts 16 in 1 pts	14	0	14% (NA)	1	1.35	-	-
Choi *et al*[15]	59	56	-	-	2.6	212	171	4.5	27.5	13	1	Most of the cases (12 mm)	2	-	2	2
Bazzi *et al*[22]	17	0	60.6	26.8	1.8	176.6	170.6	3.4	28.6	0	3	0	0	1.58	1	1
[2]Rais-Bahrami *et al*[23]	15 (14 pts)	0	57.9	29.3	2.3	167.3	293.3	2.7	24.6 (9 pts) 0 (6 pts)	0	2	0 (1 case converted LPN)	0	2.1	1	0
[1]Tiu *et al*[24]	67	67	52.4	23.2	2.4 (47)	178	271	4	24	10.64	0	Cases right 5	2	-	0	0
			51.9	24.7	5.4 (20)	197	408	5.3	31	10	0	mm-trocar	1	-	0	1
Schips *et al*[19]	21	0	58.4	25	1.8	111	196	4.4	0	0	2	50% (2 mm × 3 mm; 5 mm × 5 mm)	2	2.1	3	0
Springer *et al*[18]	14	0	52.5	26.2	2	120	165	4	0	0	0	76% (11 mm × 3 mm)	0	1	0	0

[1]Tiu uniforms procedures depending on the size of the neo-formations, in the first row showns formations < 4 cm in the second those > 4 cm; [2]In these cases, authors specify the number of procedures that executed without ischemia from those in which the removal executed with ischemia. BMI: Body mass index; EBL: Estimate blood loss; OS: Conversion in open surgery; LPN: Laparoscopic partial nephrectomy; NA: Not attempted; VAS: Visual analogue scale; MLS: Multiport laparoscopic surgery.

We selected studies that described both LESS-PN and R-LESS PN (with or without ischemia).

RESULTS

A PubMed search revealed 9 surgical series of LESS NSS describing 221 cases overall including the authors' updated experience[15,16,18-24]. No randomised or comparative clinical trials were found. The surgical relevant data are described in Table 1. The mean average age was 62 years with a mean BMI of 25. Overall, the mean tumour size was 2.35 cm, the mean operative time was 181 min (range 111-270). Fifty-eight point three percent of the cases were R-LESS-PN. The mean ischemia time was 23.6 min (range 31-16 min), 25.8% of the cases were done without ischemia.

Mean estimated blood loss was 296 mL (range 165-475 mL) and median hospital stay was 4 d (range 2-7.2 d).

Severe (≥ Clavien grade Ⅲ) postoperative complications occurred in 5.4% of patients (12/221).

In some surgical series of LESS-PN or R-LESS-PN conversion to multiport laparoscopic or Open Surgery (OS) was necessary. However, only 14/208 cases needed conversion.

Reviewing the literature the use of an additional trocar to either multiport laparoscopic surgery (10 cases) or OS (4 cases) is valid option during the LESS procedure. The majority of cases only one additional trocar was used.

Rha used an additional 8-mm trocar to lift the liver in procedures of the right kidney[25]. In another paper an additional 3-mm trocar placed in the majority of patients was used to better control haemostasis during the removal of the tumour[18]. Some authors however, like Bazzi *et al*[22] did not used it at all.

Regarding oncologic outcomes, surgical margins were positive in 4% (9/221). Out of these 7/9 had renal cancer cells at the level of the inked parenchymal excision surface (although in four cases the intraoperative frozen section was negative); the remaining 2/9 were benign lesions (one angiomyolipoma and one recurrent oncocytoma). In most of the articles the patients were discharged with minimal discomfort, as demonstrated by their pain assessment scores (median VAS = 1)[18,19,21-23].

DISCUSSION

During the last decade the use of high-tech devices has become more and more common in urological procedures; efforts are done in the development of minimally invasive surgery. The conventional laparoscopic surgery is now paving way to the new technologies including LESS[26].

In fact, robotic and laparoscopic LESS partial nephrectomy represents an attractive and minimally invasive treatment option for patients with small renal tumours. Although the use of an additional 5-mm subxiphoid liver retraction port for right-sided renal tumours was a deviation of the strict philosophy of LESS surgery; however, it has become an accepted practice[25].

Several studies have been performed to evaluate the efficacy and surgical feasibility of LESS in current clinical practice[24,25,27]. In the oncological arena Bensalah confirmed that LESS is safe, but revealed that the indication and tumor location, rather than margin status, were significant predictors of local recurrence with a mean follow-up of 37 mo[28].

The oncological and functional outcomes of R-LESS-PN has been published by Tiu *et al*[26]. In their work they evaluated 39 patients who underwent R-LESS-PN with a minimum of 2 years follow-up. They showed comparable results with other minimal invasive surgical options for the management of renal tumours. They concluded that were still to address the current challenge of R-LESS surgery before this technique might be considered as the standard of care[26].

The main limitations of LESS-PN are: lack of availability of dedicated tools, impossibility of triangulation of the instruments using conventional laparoscopic instruments, and limits in the range of movement, that causes inter-instrument interference both inside and outside the operative field[29].

For these reasons Stolzenburg *et al*[30] tried to improve efficiency moving on from articulating instruments to the curved (pre-bent) laparoscopic instruments. These procedures were performed using different ports and instruments proving the feasibility of the laparoscopic single-site surgery instruments with variable equipment, at least in the hands of experienced laparoscopic surgeons[30].

The introduction of robot-assisted techniques minimize some of these problems despite the current da Vinci system (Intuitive Surgical Inc, Sunnyvale, CA, United States), is not designed to be used in this way. The more common problems are instrument conflicts (internal or external), significant gas leak, and the insufficient tissue retraction due to the absence of the fourth robotic arm[31].

Nevertheless, the development of new devices for LESS (*e.g.*, prebent instruments, streamlined and flexible optics, and magnetic anchors) will surely reduce the technical difficulties that were reported when this technique was just beginning[32].

Song have shown that the recovery of renal function after partial nephrectomy is impacted by patient age, comorbidities, and baseline renal function, along with the amount and depth of parenchyma excised. Interestingly, these investigators have reported warm ischemia times to have no impact on renal function[33].

The goal of LESS should be the Trifecta achievement defined as the combination of warm ischemia time < 20 min, negative surgical margins, and no surgical complications[11].

In this respect, Buffi *et al*[34] observed a significant longer operative time and mean ischemia time in R-LESS-PN cases than with the multiport robotic partial nephrectomy procedure. They also observed that patients with increased tumour size, moderate and high Preoperative Aspects and Dimension Used for an Anatomic Classification and Radius/Exophytic/Nearness to collecting system/Anterior/Location scores, infiltration of the collecting system, and renal sinus involvement had an increased probability for trifecta failure[34].

Cost-efficacy is also an important issue that should be taken into account. Pini *et al*[35] have compared the real costs of the various procedures (PN, LPN, laparoscopic nephrectomy, N-LESS and LESS-PN). They analysed the intra and post-operative costs, concluding that the minimally invasive surgery is the most expensive in the intraoperative phase (use of materials, operating time, *etc.*), but less wasteful post-operatively (days of hospitalization, post-surgical complications). Unfortunately the German government (as Italian government) uses a prospective payment system based on diagnosis-related groups. This system calculates the cost encoding peri- and postoperative complications and hospitalization time, without any distinction of surgical approach, blood transfusions, need of perioperative Double J stenting and intraoperative frozen section. For this reason, the total cost remained almost unmodified among all groups that they analysed. In conclusion, they stated that local health systems should consider a subclassification with different reimbursement, which should incentive NSS and minimal invasiv surgery approaches[35].

The evidence available in the literature indicate that LESS-PN and RLESS-PN are feasible and associated with favourable reduced postoperative recovery. However, the prolonged learning curve with LESS is a major drawback. Continued innovation may allow single-port surgery to become more easily incorporated into standard practice.

COMMENTS

Background

During the last years it was developed a new minivasive tecHnique, laparoendosocopic single site (LESS) surgery, that is applied to small renal tumours. The purpose of this method is to be less invasive than usual one, because of its only one access point. Therefore this innovative approach should lead to a minor morbidity for patients and a more suitable cosmetic result, especially in young patients.

Innovations and breakthroughs

In this paper the authors assembled various articles, published all over the world, trying to offer a topic overview.

Applications

This technique could be used for all the small tumors. However, the prolonged learning curve with LESS is the major drawback.

Peer review

This is a reasonably well written review of LESS partial nephrectomy.

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SYSTEMATIC REVIEWS

Use of intralesional collagenase in the treatment of peyronie's disease: A review

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Abstract

AIM: To review the relevant literature in an effort to examine the body of evidence available to date.

METHODS: Ovid MEDLINE search database was queried using MeSH terms "penile induration", "peyronie's disease", "Collagenases" and "Collagenase" using various permutations. No temporal parameters were employed.

RESULTS: In all, 5 relevant clinical trials were isolated from 34 results. These trials were analyzed using the Oxford Centre for Evidence-Based Medicine criteria. They were further examined based on study design and methods; the primary and secondary outcomes were reviewed for treatment efficacy and collagenase-related side effects.

CONCLUSION: Intralesional collagenase appears to be safe and effective in the non-surgical treatment of Peyronie's disease. However, the data remains limited and further inquiries into the safety of collagenase, treatment standardization and standardized outcomes reporting remain necessary. Furthermore, studies comparing intralesional collagenase to alternative medical and surgical therapy will be important in guiding the future treatment decision process.

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Key words: Peyronie's disease; Collagenase; Reconstructive urology; Plaque; Intralesional injection

Core tip: In December of 2013, the United States Food and Drug Administration approved the use of collagenase clostridium histolyticum (CCH) for the treatment of Peyronie's disease (PD). In all, 5 relevant clinical trials were isolated from 34 results. With limited data on medical PD treatments, the studies to date appear to support CCH as a reasonably safe and well-tolerated non-surgical intervention. However, because no studies compared CCH to other medical interventions and no trials have been conducted to assess the ultimate need for surgical intervention, further comparative investigations are necessary to determine the ultimate role that the intralesional collagenase may play in the treatment of PD.

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INTRODUCTION

In December of 2013, the United States Food and Drug Administration (FDA) approved the use of collagenase clostridium histolyticum (CCH) for the treatment of Peyronie's disease (PD). This approval was based on several clinical trials, most notably the recently completed

concurrently run Investigation for Maximal Peyronie's Reduction Efficacy and Safety Studies (IMPRESS) I and Ⅱ trials. The goal of this paper is to describe the body of evidence leading to the FDA's approval of this drug in the treatment of PD.

The prevalence of PD is strikingly high with epidemiological estimates ranging from 3.2% in Europe[1] to 8.9% in the United States[2]. Yet despite its high prevalence, PD remains both underreported and difficult to treat[3,4]. Many men do not seek treatment, oftentimes due to embarrassment or lack of access to accurate information. The presentation of PD is varied and includes penile pain with erection, angulation of the penis, erectile dysfunction, and the presence of a palpable plaque, typically located on the dorsum of the penis[5-10]. Furthermore, men with PD are at a higher likelihood to have an inability to perform intercourse as curvature of 60 degrees of higher has been associated with a threefold increase in the odds of sexual disability[11]. These factors and the complicated nature of PD make it psychologically and physically detrimental to not only the patient but also his sexual partner[12,13].

Although 250 years have passed since Francois Gigot de la Peyronie described the first case series of PD, little understanding of its etiology has emerged[14,15]. The disease is believed to occur secondary to abnormal collagen deposition and scar formation from buckling trauma to the tunica albuginea of the penis. The current pathophysiological model of PD reflects the body's response to microtrauma to the tunica albuginea where a disordered healing process allows scarring to occur. This scarring restricts symmetrical expansion of the tunica albuginea during an erection, thus resulting in angulation of the penis. As with all scar formation, the major step is collagen synthesis. It is theorized that microtrauma combined with an underlying genetic predisposition allows for abnormal collagen deposition and scar formation[16].

The formation of scar is a balance between collagen formation and its degradation through collagenase-a naturally occurring enzyme that functions to break down collagen. This enzyme, produced by humans, is also formed by bacteria. Clostridium histolyticum, a gram-positive anaerobic bacterium, is well known to cause destructive, rapidly spreading infections through soft tissue planes which occurs mainly through the function of collagenase. This bacterial enzyme was first isolated in 1953[17]. It has since been purified and is now commercially available. CCH (Xiaflex, Auxillium Pharmaceuticals) is a purified mixture of two collagenases, AUX-1 and AUX-2. The first *in vitro* biochemical study of CCH, published in 1962, demonstrated collagenolytic activity on animal tissues[18]. Gelbard *et al*[19] subsequently examined the *in vitro* effects of CCH on the tunica albuginea of patients with and without PD. Their research demonstrated a significant collagenolytic effect of CCH on both normal tissue and tissue with Peyronie's plaques. Importantly, vascular smooth muscle was not digested and preservation of all vessels except small venules was observed. This led to the notion that collagenase would be a safe treatment for PD and other similar afflictions, including Dupuytren's disease (DD).

In February 2010, CCH was approved by the FDA for treatment of DD. The approval came as a result of a large double blind randomized, placebo controlled trial (DBRCT)[20] demonstrating that localized injection of CCH, in conjunction with passive joint manipulation, reduced the contracture to full extension within 30 d of last injection, compared to placebo (from 43.9 to 80.7 degrees *vs* from 45.3 to 49.5 degrees, $P < 0.001$). This observation was achieved with minimal adverse events (AEs). The pathological and epidemiological similarity between DD and PD and the promising of DD trials lead to the initiation of two DBRCTs to examine the effects of CCH on PD[21].

MATERIALS AND METHODS

Relevant articles for this review were obtained through the Ovid MEDLINE search database. Search strategy included MeSH terms "penile induration", "peyronie's disease", "Collagenases" and "Collagenase". The search was carried out as follows: ("penile induration" OR "Peyronie's disease") AND ("Collagenases" OR "collagenase"). This produced in 34 results, which were further narrowed down by clinical trials focusing on collagenase and PD yielding a total of five relevant trials. Due to the limited availability of clinical trials on this subject, no year limits were used in this search and publication dates ranged from 1985 through 2013.

Evaluation of these clinical trials was based on the Oxford Centre for Evidence-Based Medicine criteria.

RESULTS

A total of 5 clinical trials have examined the use of CCH in the treatment of PD (Tables 1-4).

The use of collagenase in the treatment of PD

The first clinical trial examined the safety of intralesional collagenase to treat PD[22]. It included 31 men, each with a mean curvature of 42 degrees, 10 of which had undergone prior non-CCH treatments for their condition. Collagenase was injected daily for three days at a dose of 470 to 620 μg/mL for the first 15 patients and 910 μg/mL for the rest. Total dose ranged between 470 to 2730 μg/mL per patient, with a mean of 2330 μg/mL. This low dosing regimen was chosen to assess both safety and efficacy during this phase 1 trial. Objective improvement was observed in 20 patients; 4 had resolution of their plaques, and 16 had penile curvature reduction by 20%-100%. These results were seen in the majority of patients within 2 wk of treatment. Pain with erection was eliminated in 13 of the 14 patients who entered this study with erectile pain. Three out of 4 men who were unable to have intercourse regained the ability after treatment. The one who failed had a pre-treatment 180-degree penile bend. AEs

Table 1 Study designs

Ref.	No. of patients	Study design	Length of study	Control group	Length of follow up
Gelbard *et al*[22]	31	Prospective	Mean of 22 mo per patient	None	9.8 mo (mean)
Gelbard *et al*[23]	49	Double blind, RCT	3 mo	Placebo	3 mo
Jordan[24]	25	Prospective	9 mo	None	None
Gelbard *et al*[25]	147	Double blind, RCT	Up to 18 wk	Placebo	None
Gelbard *et al*[26]	832	Double blind, RCT	Up to 52 wk	Placebo	None

RCT: Randomised controlled clinical trial.

Table 2 Study treatments

Ref.	Intralesional injection therapy dose and schedule
Gelbard *et al*[22]	One daily dose of 470620 μg/mL (15 patients) or 910 μg/mL (16 patients) for three consecutive days
Gelbard *et al*[23]	CCH single injection (6000-14000 Units)
Jordan[24]	Three 10000 Units injections administered over 7-10 d, repeated at 3 mo
Gelbard *et al*[25]	CCH 0.58 mg (10000 Units), 2 injections 24-72 h apart, repeated every 6 wk for up to 3 cycles
Gelbard *et al*[26]	CCH 0.58 mg (10000 Units), 2 injections 24-72 h apart, repeated every 6 wk for up to 4 cycles

CCH: Collagenase clostridium histolyticum.

Table 3 Adverse events

Ref.	Adverse effects (*n*)
Gelbard *et al*[22]	Ecchymosis (21), Pain (2), Albuginea rupture (1)
Gelbard *et al*[23]	Tenderness at injection site (Majority), tunica albuginea rupture (1)
Jordan[24]	Edema/pain/or ecchymosis (20), No serious AEs
Gelbard *et al*[25]	Bruising (96), Edema (50), Pain (58), No serious AEs
Gelbard *et al*[26]	Ecchymosis (441), Edema (30), Pain (250), Corporeal rupture (3), Penile hematoma (3)

AEs: Adverse events.

Table 4 Study outcomes

Ref.	Outcome	Oxford Centre for Evidence-Based Medicine 2011 Levels of Evidence
Gelbard *et al*[22]	Improvement seen in 20 patients (4 had plaque disappearance, 16 had curvature decrease by 20%-100%)	2
Gelbard *et al*[23]	Overall, 36% responsed to treatment. Improvement greatest in patients with lesser degree of pre-treatment curvature	2
Jordan[24]	Significant mean changes from baseline angular deviation and plaque width at 3, 6, and 9 mo	2
Gelbard *et al*[25]	Improved penile curvature (29.7% *vs* 11.0%, $P < 0.05$) and patient reported bother scores for treatment group *vs* placebo	1
Gelbard *et al*[26]	Improved penile curvature (34% *vs* 18%, $P < 0.0001$) and patient reported symtom bother score for treatment group *vs* placebo	1

were minimal and included ecchymosis (21 patients) and pain (2 patients). One patient had an albuginea rupture two weeks after treatment. This was a 23 years old who experienced pain with a popping sensation during intercourse. The penis was bandaged and he was instructed to avoid intercourse for 3 wk. After healing, the degree of penile curvature was straighter than before treatment. Mean follow up for this study was 9.8 mo.

Collagenase vs placebo in the treatment of PD: A double-blind study

Eight years later, a trial of 49 patients was conducted stratifying patients by degree of curvature. The patients were divided into three groups: group 1; 30 degrees or less and/or palpable plaque less than 2 cm, group 2; 30 to 60 degrees and/or plaque 2 to 4 cm, group 3; over 60 degrees and/or plaque greater than 4 cm[23]. Pre and post intervention deformity was measured with vacuum induced erection photography. The patients in each group were randomized to receive either treatment or placebo (saline injections). The three treatment groups received a total of 6000 units, 10000 units, 14000 units, respectively. All injections were administered into the Peyronie's plaque, and patients were instructed to avoid intercourse for 2

wk after treatment. Patients were evaluated at 1 week, 1 mo, and 3 mo after treatment. Overall, 36 percent (8 of 22 patients) of the treatment group responded to CCH, compared to 4 percent (1 of 27 patients) for placebo, $P < 0.007$. In general, patients from group 1 had a higher response rate to CCH compared to group 2 and 3 (100%, 36%, 13%, respectively). Response differences for treatment *vs* placebo in groups 1 and 3 were not statistically significant. One category 3 patient experienced a tear of the tunica albuginea during intercourse 3 wk after treatment. The tear was treated conservatively and resolved. The majority of patients experienced tenderness at the injection site, yet this AE was observed as frequently in both the treatment and placebo groups.

The use of intralesional clostridial collagenase injection therapy for PD: A prospective, single-center, non-placebo-controlled study

A 2008 prospective trial including 25 patients with PD was conducted with a more uniform treatment protocol: three injections of intralesional CCH at 10000 units/0.25 cm^3 per dose, administered over 7-10 d[24]. This process was repeated at 3 mo after first treatment. Plaque size and angle deformity was assessed at 3, 6, and 9 mo. Eighteen of the 25 patients completed the treatment and follow-up. Primary outcome was a > 25% reduction from pretreatment angular deviation, considered a successful response. A secondary end point was a patient questionnaire. This questionnaire included a subjective patient evaluation of both visual outcomes and restoration of sexual function. Results demonstrated significant decreases in mean deviation angle observed at months 3 and 6 (*P*-values at 9 mo were not reported). Positive treatment response for the primary outcome (> 25% angular reduction) peaked at month 3 yet declined by month 9, likely due to drop-out of successful patients. However, patients who experienced treatment success in month 3 continued to experience success at month 9. Additionally, significant decreases were observed in plaque width at 3, 6, and 9 mo and plaque length at months 3 and 6. The patient questionnaire revealed overall global evaluations of the patient's disease condition improved. More than 50% were considered "much improved" or "very much improved". One third responded "minimal improvement" or "no change". AEs occurred in eighty percent of patients and included edema, penile pain, or ecchymosis. No serious AEs were observed.

As stated previously, by February, CCH had been FDA approved to treat DD at a dose of 10000 Units per injection. Studies have shown that intralesional injection followed by finger extension and manipulation, intended to further break down the cord, had shown the greatest improvement in plaque breakdown[20]. These protocols were thus applied to studies regarding CCH and PD.

Phase 2b study of the clinical efficacy and safety of CCH in patients with Peyronie's disease

A phase 2b DBRCT study including 147 subjects examined the effect of CCH and "modeling," or bending of the flaccid penis in order to break up a PD plaque[25]. Subjects were divided into 4 groups to receive CCH or placebo (saline injections) (3:1 randomization) with or without penile plaque modeling (1:1 randomization). Penile plaque modeling consisted of stretching the penis for 30 s, followed by 30 s in the nonmodeled state, repeated for 3 cycles at a time. Subjects in the CCH group received 2 injections at 10000 units/0.25 cm^3 per dose, given 24 to 72 h apart, repeated for up to 3 cycles at 6-wk intervals. Outcomes included change in penile curvature based on goniometer measurement, patient reported outcomes *via* questionnaire (PD-PRO and IIEF), and AEs. There were significant differences in penile curvature in the CCH *vs* placebo groups when modeling was applied (32.5% *vs* 2.5%, $P < 0.001$). Minimal difference was observed between treatment and placebo groups in the absence of modeling. Patient questionnaires revealed that CCH treated patients has a significantly better PD symptom bother score than those on placebo ($P = 0.05$). However there was no significant difference in the other questionnaire domains (intercourse discomfort, constraint, penile pain). Common AEs in CCH groups included bruising (86.5%), edema (45%), and pain (52.3%). No serious AEs related to treatment were observed. No systemic immunologic events were reported.

Clinical efficacy, safety and tolerability of CCH for the treatment of peyronie's disease in 2 large double-blind, randomized, placebo controlled phase 3 studies

Most recently, the Investigation for Maximal Peyronie's Reduction Efficacy and Safety Studies (IMPRESS) I and Ⅱ trial, a phase 3 DBRCT with the largest cohort of 832 subjects, examined CCH *vs* placebo in patients with PD[26]. Modeling was not performed. Dosing and scheduling was identical to the prior phase 2b study, although cycles could be repeated up to 4 times. Subjects treated with CCH had a mean 34% improvement in curvature, compared with 18% in the placebo group ($P < 0.0001$). PD symptom bother score was also significantly improved in the treatment *vs* placebo groups ($P = 0.0037$). AEs in the CCH group were similar to those observed in the phase 2b trial, including ecchymosis (80%), edema (55%), and pain (45.4%). However, six serious AEs were observed. Three men experienced corporeal rupture, all of which were successfully repaired surgically. The other three experienced penile hematoma, one resolving without intervention, one resolved with aspiration, and one successfully repaired surgically. No systemic or immunologic events were observed.

DISCUSSION

The recent FDA approval of CCH for the treatment of PD marks an important step in the treatment of this widespread and often debilitating disease. CCH is the first non-surgical therapy to become FDA approved for this purpose. Though limited clinical data currently ex-

ists on intralesional collagenase-in all, 5 clinical trials were isolated-the results appear promising. Though side effects throughout the trials were relatively common, they were mild and transient. Serious AEs were rare, and included corporeal rupture and penile hematoma. These serious AEs were reported both in the trials from 1985, 1993, and the most recent IMPRESS trial.

Improvement in penile curvature, both objective and subjective, was reported in all five trials; the highest level of objectivity was reached in the IMPRESS trial through the use of goniometer. Most importantly, two thirds of the sexually active men in the IMPRESS trial cohort received a validated PD symptom questionnaire taking into account the psychological burden that PD has on the patient.

There are limitations to this area of research. Although results support CCH as safe and effective, none of these trials compared CCH to other treatment modalities, or to placebo. Furthermore, the same team of researchers authored 4 out of 5 publications, which could contribute to bias. Yet given the excellent safety results, coupled with its efficacy displayed in DD trials, the authors of this paper still recommend CCH as a safe and effective treatment option for PD.

To date, no studies comparing CCH to other medical interventions have been conducted and no assessment of the ultimate need for surgical intervention has been performed. However, given the paucity of data on medical PD treatments, the studies to date appear to support CCH as a reasonably safe and well-tolerated non-surgical intervention for PD.

COMMENTS

Background

Trials on the use of intralesional collagenase for the non-surgical treatment of Peyronie's disease (PD) first appeared in 1985. However, in the three decades leading up to the United States Food and Drug Administration's (FDA) approval of this therapy, limited studies have been conducted to measure its efficacy and safety. Furthermore, few, if any review articles are available regarding this therapy.

Research frontiers

Collagenase clostridium histolyticum (CCH) was recently FDA approved for the treatment of PD. There have been a handful of trials focusing on the use of CCH to treat PD, yet few examined the safety and efficacy of CCH. To the authors' knowledge, there have been few, if any, review articles discussing these trials in detail, including CCH background, safety, efficacy, and future directions of research.

Innovations and breakthroughs

CCH is a promising, non-surgical alternative to treat PD. This review article discusses the CCH background, summarizes clinical trial results, and comments on future research directions. The authors believe this is a novel article and one that can help provide useful information to both clinicians and researchers who aim to treat this disease.

Applications

This article provides summaries of the efficacy, safety, and future directions of intralesional collagenase. This information can be applied to clinical practice, as well as future research.

Terminology

There are no terms in this article which the authors feel cannot be understood by the majority of readers.

Peer review

This article summarize the five clinical trials published from 1985 to 2013 about safety and advantage in the use of CCH in the treatment of PD. The studies support CCH as a reasonably safe and well-tolerated non-surgical intervention for PD: rare serious adverse events included corporeal rupture and penile hematoma.

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SYSTEMATIC REVIEWS

Best surgical treatment for very large benign prostatic obstruction

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Abstract

AIM: To investigate the best surgical treatment for very large benign prostatic obstruction (BPO).

METHODS: A revision of literature was conducted in PubMed database with 167 search results. Key words for the search were benign prostatic hyperplasia, surgical treatment, large, and volume. Inclusion criteria for this study were surgical treatment of benign prostatic obstruction for prostates equal to or larger than 80 cc. Among article search results, 9 completed inclusion criterion and were revised. Each surgical technique included in those articles was compared to each other. The results were observed, and conclusions derived from this are presented. There is no statistical analysis.

RESULTS: Of the 5 techniques presented in the revised articles [open transvesical enucleation, holmium laser enucleation of the prostate (HoLEP), photoselective vaporization of the prostate using potassium titanyl phospate laser, transurethral resection with bipolar energy, and transurethral enucleation with bipolar energy], open transvesical enucleation best permits the resolution of obstructive symptoms. It presents excellent maximum flow rates, high resected tissue volume and maintenance of results over time. These characteristics explain why it has been the gold standard treatment for prostates greater than 80 cc. However, it is at the expense of greater blood loss, urethral catheter and hospital stay times. Since its initial application in 1996, the transurethral enucleation of the prostate by means of a holmium laser has become a procedure that has similar surgical outcomes with fewer complications when compared to open surgery making it an interesting alternative for very large BPO. Nonetheless, no procedure has removed open surgery as the gold standard for very large BPO.

CONCLUSION: Open surgery has proved to be the gold standard for very large BPO. HoLEP appears as a minimally invasive alternative with same benefits but less morbidity.

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Key words: Benign prostatic obstruction; Surgical treatment; Prostatectomy; Holmium laser enucleation of the prostate

Core tip: Though the gold standard for surgical treatment of very large benign prostatic obstruction has been open prostatectomy, in the last three decades there has been a notorious absence of publications showing the outcomes of this surgery. The only procedure with similar results and fewer complications seems to be the holmium laser enucleation of the prostate making it an interesting alternative when confronted with large sized prostates. New methods of treating

large prostates have an interesting challenge since both open surgery and holmium laser enucleation of the prostate present favorable results.

Sáez ID, de la Llera JF, Horn CD, López JF, Chacón RA, Figueroa PA, Vivaldi BI, Coz F. Best surgical treatment for very large benign prostatic obstruction. *World J Clin Urol* 2014; 3(3): 370-375 Available from: URL: http://www.wjgnet.com/2219-2816/full/v3/i3/370.htm DOI: http://dx.doi.org/10.5410/wjcu.v3.i3.370

INTRODUCTION

Open prostatectomy has been one of the oldest procedures practiced in urology to treat large benign prostatic obstruction (BPO) and it still remains valid today. Historical accounts divide open prostatectomies according to different techniques: perineal, suprapubic and retropubic approach[1,2].

The first open prostatectomy described was through the perineal approach by Covillard. In 1639, he became the first to remove a prostatic middle lobe through perineal approach. However, it was Goodfellow that became the first to perform open perineal prostatectomy routinely. Numerous physicians perfected the technique employed afterwards but it was Young at Johns Hopkins University in 1905 who published his operative technique[3].

Suprapubic prostatectomy techniques followed perineal approaches. In 1894, Eugene Fuller is regarded as the first man to perform a complete suprapubic removal of a prostatic adenoma. However, it was Peter Freyer in 1900 that gained most fame performing a transvesical prostatectomy. His publication of cases in the British Medical Journal popularized this procedure[4].

In terms of retropubic prostatectomy, Van Stockum is regarded as the first person to complete an enucleation of prostatic adenoma using this surgical approach in 1908. It was Millin, in 1945, who popularized this approach publishing his operative technique and results[5]. His technique has been employed then since worldwide for over 65 years with minor modifications.

The introduction of instruments such as the Stern-McCarthy resectoscope in 1926 opened a new era in urology and BPO surgery. It became one of the leading discoveries in the field of urology in the 20th century. This resectoscope was utilized worldwide as one of the first endourological approaches to BPO. It became the first minimally invasive procedure for endoscopic treatment of pathologies such as bladder neoplasms and BPO. Perhaps the most important advance made to this instrument was provided by Iglesias in 1975 who modified it to allow both irrigation and suction[6]. With the development of new lenses this enhanced vision of the operatory field and shortened operatory times.

Numerous endoscopic techniques have been described and published since Iglesias published his results with his modified resectoscope. The most important breakthrough since then has been the development of transurethral resection of the prostate (TURP). Initially developed with monopolar and posteriorly with bipolar energy, TURP became the treatment of choice of BPO, especially with prostate sizes between 30 and 80 cc[7]. Utilized to this day worldwide, TURP has fulfilled a crucial role in the treatment of prostatic obstructions symptoms with small and middle sized prostates. Nonetheless, there is little evidence that discusses the role of TURP in prostates sized over 80 cc and especially over 120 cc.

Numerous articles have been published showing that novel techniques may be the new state of the art treatment for such condition. Procedures such as transurethral incision of the prostate, transurethral needle ablation of the protate, transurethral ultrasound guided laser induced prostatectomy, Prostatron, Thermex and visual laser ablation of the prostate have been employed to treat small and midlle sized prostates. With initially positive results, most of these techniques have failed in their attempt to become the new gold standard treatment for such a disease. Most have never been an option for the treatment of very large BPO. They have been employed only in the setting of small and middle sized prostates. These instruments have quickly fallen from being strongly advertised solutions to BPO to adorning the basements of hospitals around the world.

Various other techniques have been developed to defy open prostatectomy as the treatment of choice for very large BPO. The most accepted of these has been that of the holmium laser enucleation of the prostate (HoLEP). Initially described in 1996 in New Zealand by Gilling *et al*[8], this procedure has seen to have benefits over other endoscopic procedures such as TURP for the treatment of large prostates.

MATERIALS AND METHODS

To evaluate the best surgical treatment for very large BPO we conducted a meticulous search and revision of available literature.

A systematic search was conducted at PubMed database with the following keywords: benign prostatic hyperplasia, surgical treatment, large, volume. A total of 167 articles were found (including randomized controlled trials, prospective and retrospective series). All publications were reviewed and those that fulfilled inclusion criterion were considered in our study. These criteria were the following: prostatic volume greater than 80 mL sized by transrectal ultrasound (TRUS), measurement of international prostate symptoms score symptoms, maximum urinary flow, prostatic specific antigen (PSA) values pre and post surgery and registry of surgical complications with a follow up of at least 12 mo. Studies in which authors employed resective techniques had to include measurement of enucleated or resected tissue. Those who used vaporization techniques had to include exclusively measurement of prostatic volume by TRUS before and

Table 1 Overview of the published studies

Ref.	Follow up (mo)	Patients (*n*)	Change in PSA		Change in symptoms (IPSS)		Change in Q max		Amount of tissue removed		Time urethral catheter (h)	Length of stay (h)
			ng/dL	%	Absolute	%	mL/seg	%	mL	%		
Transvesical												
Alivizatos *et al*[9], 2008	12	60	-4.3	-68.2	-13	-62	7.1	88.7	86	89.5	120	144
Ou *et al*[10], 2013	12	49	-4.4	-78.5	-19.3	-76.8	11.8	231.3	109.8	77.8	182	223
Rao *et al*[11], 2013	12	40	-3.91	-86.5	-21	-85.7	19.7	333.8	75.2	68.3	148	223
HoLEP												
Elzayat *et al*[12], 2006	24	225	-8.1	-90	-14.3	-79.4	20	250	86	68.2	30	29
Matlaga *et al*[13], 2006	12	86	-8.86	-90.2	-14.5	-73.9	15.8	173.6	140	82.3	15.1	26
Kuntz *et al*[14], 2008	60	42			-19.2	-86.4	20.5	539.4				
PVP with KTP												
Rajbabu *et al*[15], 2007	24	54	-4.6	-41.8	-16.3	-74	11	137	59	43.7	23	11
Alivizatos *et al*[9], 2008	12	65	-3.8	-61.2	-11	-55	7.4	86	38	40.8	24	48
Bipolar TURP												
Zhu *et al*[16], 2009	36	132	-4.17	-57.3	-18.5	-86.4	15.7	237.8	58.1	72.9	69	117
Enucleacion bipolar												
Rao *et al*[11], 2013	12	43	-4.22	-88.4	-21.4	-86.2	20.8	358.6	65.9	56.8	79.2	129
Ou *et al*[10], 2013	12	45	-4.3	-72.8	-17.6	-75.8	9.6	162.7	98.7	74.7	103	139

PSA: Prostatic specific antigen; IPSS: International prostate symptoms score; HoLEP: Holmium laser enucleation of the prostate; TURP: Transurethral resection of the prostate; KTP: Potassium titanyl phosphate; PVP: Photo vaporization of the prostate.

after the intervention as well.

Of the 167 articles selected for revision, only 9 fulfilled criteria for inclusion (Table 1). These investigations analyze 5 therapeutic alternatives for benign prostatic hyperplasia surgery: transvesical or transcapsular enucleation of the prostate, endoscopic resection with bipolar energy, vaporization with potassium titanyl phospate (KTP) laser, transurethral enucleation with Holmium laser and transurethral enucleation-resection with bipolar energy (mushroom technique). A detailed analysis of these articles is presented, grouped by operatory technique.

RESULTS

Simple open prostatectomy (transvesical)

Developed by Freyer in 1900 and still a valid procedure today, three contemporary articles describe this technique. Alivizatos *et al*[9], Ou *et al*[10] and Rao *et al*[11]. Together they compile 149 patients with a mean follow-up of 12 mo. With an average prostate volume of 115 cc, they achieve a mean enucleation of 78 cc of prostatic tissue. This corresponds to the greatest resected volume of all techniques analyzed. PSA values dropped 77% (from an average of 5.3 to 1.2 ng/dL). This technique owns the highest recatheterization rate with an average of 4.7% of patients (Rao *et al*[11] has a 7.5% recatheterization rate). This series also has the greatest number of reinterventions with 5.3% of patients undergoing a new surgical intervention.

This technique has the longest need of urethral catheterization and hospital stay with 150 and 196 h, respectively. This procedure also has the highest transfusion rate, with an average of 9.8% of patients transfused.

Holmium laser enucleation of the prostate

Published by Gilling *et al*[8] in 1996, this technique uses the Holmium YAG laser, allowing to transurethrally enucleate prostatic lobes in a similar fashion as when done with fin-

Table 2 Complication rates of each technique

	Reoperation (%)	Urethral stenosis (%)	Transfusion (%)
Technique			
Transvesical	5.3	7.9	9.8 (6.1-13.3)
HoLEP	1.33	0.66	0.84 (0-7.14)
PVP (KTP)	2.52 (0-3)	1.68 (0-2)	0
Bipolar TURP	4.5	4.5	0
Bipolar enucleation	2.5 (0-2.2)	2.4 (2.2-2.5)	7.5 (0-6.6)

HoLEP: Holmium laser enucleation of prostate; PVP: Photo-selective vaporization of prostate; KTP: Potassium titanyl phosphate; TURP: Transurethral resection of prostate.

ger enucleation in open techniques.

Three study groups employ this technique with a total of 353 patients and a 32 mo average follow-up (Elzayat *et al*[12], Matlaga *et al*[13], Kuntz *et al*[14]).

It is the technique that resects the second greatest amount of prostatic tissue with an average 75.2% reduction of prostate volume. It also has the greatest PSA drop with a 90.1% reduction rate (from 9.41 to 0.93 ng/dL). It provides the greatest improvement of maximum urinary flow with an average increase of 17.9 mL/seg with respect to baseline values.

Nonetheless, this technique is known to be the slowest procedure. In effect, it is the slowest of all with 121 min mean intervention time.

Only 1 patient required recatheterization and 1 required reintervention due to persistent hematuria. Transfusion rate was 0.34% (3 of 353 patients). Patients needed a total of 22.5 h of urinary catheterization, while hospital stay was 27.5 h, second shortest of techniques compared.

Photoselective vaporization of the prostate using KTP laser

This is the only non resective technique reviewed. It does not extract tissue, hence, there are no biopsy results or accurate total tissue removal volumes (% prostatic size reduction is obtained with the difference between pre and post operative transrectal echography). This does not permit adequate comparison with other techniques.

This technique is presented in 2 series (Rajbabu *et al*[15], Alivizatos *et al*[9]), with a total of 119 patients and 18 mo mean follow-up.

Reduction of prostatic volume is 42%, constituting the technique with the lowest volume drop. PSA drop and maximum urinary flow are also the lowest with an average decrease of 51.5% (from 8.6 to 4.4 ng/dL) and 9.2 mL/s improvement, respectively.

It has no transfusion rate and has a short catheter and hospital stay time (23.5 h and 29.5 h, respectively).

Bipolar energy

Two techniques utilize this method and were included for revision.

Transurethral resection with bipolar energy

Basically, it is similar to classical TURP but with the use of bipolar energy. This allows irrigation with a saline solution, consequently permitting to extend operatory times with less risk of post TURP syndrome.

There is one article that uses this procedure (Zhu *et al*[16]), with a total 132 patients and a 36 mo follow-up.

This series presents a reduction of prostatic volume of 72.9%, behind transvesical adenectomy and HoLEP. Drop of PSA was 57.3% (7.27 to 3.1 ng/dL), while improvement of flow was 15.7 mL over baseline.

This study group published a 4.5% reoperation rate (second after open surgery). Average usage of urinary catheter and hospital stay was that of 69 and 117 h, respectively.

Transurethral enucleation with bipolar energy (mushroom technique)

Two articles employed this procedure (Rao *et al*[11], Ou *et al*[10]). Both compared this intervention with transvesical open surgery (results previously mentioned).

These studies comprise a total of 88 patients and have a follow up of 12 mo. Preoperative mean volume was 124 cc with a reduction of tissue of 65.7%. This places it in fourth place, only overcoming vaporization with KTP laser. However, it has the second greatest PSA fall, behind HoLEP, with a reduction of 80.6% (5.33 to 1.07 ng/dL). Requirements of catheterization and total hospital stay were just behind open transvesical surgery, with average times of 91.1 and 134 h.

DISCUSSION

Within the spectrum of techniques employed to treat prostatic obstructive hyperplasia, TURP is considered the gold standard for small and medium sized prostates (80 mL or smaller). When confronted with larger sized prostates, studies do not compare TURP to other techniques. Also, no significant sized series have been published to show results in this large volume subgroup.

Of the 5 techniques presented, there are no doubts that open transvesical enucleation permits the resolution of obstructive symptoms. It presents excellent maximum flow rates, high resected tissue volume and maintenance of results over time. In fact, it is the treatment of choice for very large BPO according to american urological association (AUA) and European guidelines. These characteristics explain why it has been the gold standard treatment for prostates greater than 80 cc[12] but at the expense of greater recatheterization, blood loss, urethral catheter and hospital stay times (Table 2). Favoring open surgery is the fact that this procedure can be completed in basically equipped surgical wards and should be mastered by all urologists worldwide.

In the last few years HoLEP has had an important role in the discussion of the best treatment of large benign prostates. This technique has similar results when compared to open prostatectomy both in resected vol-

ume and long term studied parameters. In particular, Kuntz *et al*[14] has a 5 years follow up in which the score symptoms of the AUA and maximum flow rate have maintained inalterable over time in both the open adenectomy and HoLEP groups. This has not been reported for other techniques, converting HoLEP in the sole option that equals benefits to open surgery but with lesser transfusion and shorter catheter and hospital stay rates.

Some benefits reported for KTP laser fulguration are similar to those obtained with HoLEP. These include lower catheter requirements, hospital stay and transfusion rates. However, HoLEP reports better flow rate and symptom results in the long term.

Most urologists state that the HoLEP learning curve is slow and is estimated at a mean of 30 cases. It also requires highly sophisticated equipment and trained personnel to implement this procedure efficaciously.

Other interesting results are those achieved by the fall of PSA by enucleation with the bipolar loop (mushroom), situating itself immediately behind HoLEP, even better than open surgery. This may be due to both the enucleation of tissue as well as the fulguration of the prostatic capsule, which reduces the number of glandular cells of the prostate. It would be interesting to have a close follow up and comparison of these techniques on prostatic cancer incidence in the very long term.

In light of this review, it seems valid to state that the ideal technique for treating prostates larger than 80 cc is enucleation. While this is performed transvesically or minimally invasively, it is this condition that differences results from resective or fulgurative techniques.

In attempts to maintain this condition of enucleation, alternative techniques have appeared that combine the benefit of being less invasive and the advantages of open surgery. Small series of laparoscopic enucleation, utilizing the Millin technique or through a unique transvesical trocar were first described by Mariano *et al*[17] in 2002. Recent publications such as García-Segui *et al*[18] in 2012 compared 17 laparoscopic extraperitoneal to 18 open Millin technique patients. The laparoscopic group had a lower hemorrhage rate and lower irrigation, catheterization and hospital stay time. There are no reports in the medium or long term. The largest series employing this technique is McCullough *et al*[19], who publishes in 2009 a series of 280 cases, 96 of them laparoscopically approached, with results discretely superior to open surgery. There are no prospective randomized trials with adequate follow up that permit to conclude the potential benefits of these techniques. Hence, we do not recommend it over those analyzed in this revision.

It would be of great use to incorporate user satisfaction and hospital cost surveys to treated patients to permit a more valid conclusion on whether to incline the balance toward open surgery or HoLEP.

Open prostatectomy has been the gold standard of treatment of very large BPO for the last 65 years. It is a procedure that is practiced routinely worldwide and solves obstructive symptoms efficaciously. In the past years, new technologies have been developed to treat very large BPO. This review shows that of these procedures, HoLEP is the only one that has come to compete with open surgery side by side. However, it is still an incognito if HoLEP will resist the trial of time and become the standard of care surgery for this illness in 65 more years.

COMMENTS

Background

Management of very large benign prostatic obstruction (BPO) (80 cc or greater) remains a challenge in urological practice. For the past 65 years, open prostatectomy has been the gold standard procedure for these cases. Nowadays, in the minimally invasive era, the search for an alternative with less morbidity, shorter hospitalization stay and recovery time force the authors to evaluate and become familiar with other techniques recently developed but that remain unknown or are unavailable for the majority of urologists worldwide.

Research frontiers

Of the minimally invasive alternatives available to manage very large BPO, there is little evidence published to sustain Holmium laser enucleation of the prostate (HoLEP) as the best surgical approach. More research is needed to compare HoLEP with other minimally invasive procedures and open surgery to sustain it is a new gold standard for this morbid condition.

Innovations and breakthroughs

Open surgery remains the sole alternative for very large BPO when associated with certain comorbid conditions. Such is the case when very large BPO coexists with significant bladder stone burden and/or large bladder diverticula. No other procedure simultaneously resolves these comorbid conditions as effectively as open surgery. It is also important to note that HoLEP was described over 18 years ago. Since its introduction it has proved great effectiveness, but with limited diffusion. Most articles portraying the benefits of this technique have only been published in the last few years. A possible explanation of this phenomenon is the country of origin where it was initially described. If HoLEP had been developed in Europe or in the United States of America, would it have taken over 15 years to become a worldwide recognized technique for BPO treatment?

Applications

According to the authors' research, they consider it necessary to be familiar with minimally invasive approaches to treat BPO. Of these techniques, perhaps urologists should be specifically acquainted with HoLEP, as it has proven to be a minimally invasive and less morbid alternative for these patients.

Terminology

HoLEP: Holmium laser enucleation of the prostate. It is an endoscopic approach that allows enucleating hyperplastic tissue in a similar manner as open surgery but using a resectoscope tip as a fingertip. Laser energy fulgurates the perforating vessels in the enucleation plane. At the end of the procedure, prostate lobes resected placed in the bladder lumen are morcelated and aspirated by an endoscopic morcellator introduced through the resectoscope; PVP: Photoselective vaporization of the prostate. It consists in the ablation of prostatic tissue of the transition zone as potassium titanyl phospate laser energy is applied using a side fire fiber.

Peer review

The paper is a thoroughly insight in the surgical treatment of large (> 80 mL) and very large (> 120 mL) prostates.

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CASE REPORT

Pre-fabricated radial forearm phalloplasty with cadaveric bone graft

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Abstract

Phalloplasty is a complex set of procedures used in efforts to improve the anatomical, physiological, and aesthetic deficiencies caused by loss or absence of the penis. Methods have evolved significantly, and the use of free tissue transfer has become common amongst reconstructive surgeons. The inclusion of bone autograft, usually radius or fibula, within the neophallus has caused significant morbidity, and efforts continue to find the optimal solution. We present a novel approach using a pre-fabricated, radial forearm fasciocutaneous free flap containing cadaveric bone graft for phalloplasty following traumatic penis amputation.

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Key words: Phalloplasty; Pre-fabrication; Cadaver allograft; Radial forearm; Penile reconstruction

Core tip: While there are many options for penile reconstruction, many techniques have associated morbidity when attempting to recreate rigidity in the neophallus by using bone autograft mostly from either the radius or fibula. In our case report, we use a novel approach to addressing rigidity in the neophallus without causing associated morbidity by using a pre-fabricated, radial forearm fasciocutaneous free flap containing cadaveric bone graft for reconstructive phalloplasty.

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INTRODUCTION

Phalloplasty is a complex set of procedures that can pose significant anatomical, physiological, and aesthetic challenges, and the surgical techniques implemented have evolved significantly over the past sixty years. In the 1930s, Borgoras used bipedicled abdominal tube flaps to reconstruct the penis[1]. This method was later replaced by the use of fasciocutaneous and extended pedicle island flaps with continued suboptimal results[2,3]. In the 1980s, microsurgical advancements made the use of free flaps a better option, and the use of sensate, radial forearm fasciocutaneous or osteocutaneous flaps and fibular osteocutaneous flaps improved sensation and rigidity of the neophallus, giving a more desirable and functional outcome[4-7]. This method comes with significant donor site morbidity including extensive donor site scarring, bony fracture, and wrist and ankle instability[2,7]. Implant placement in the radial forearm free flap also has risks, including extrusion, costs, infection, and delayed placement (usually 1 year). We report a novel approach using cadaver bone graft to provide penile rigidity without sacrificing donor site morbidity in a patient undergoing phalloplasty following traumatic penis amputation.

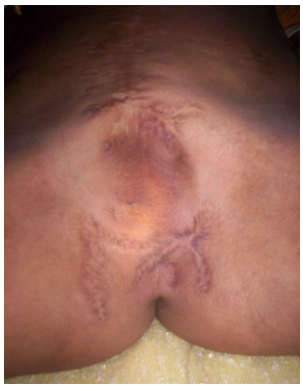

Figure 1 Preoperative view demonstrating perineal urethrostomy.

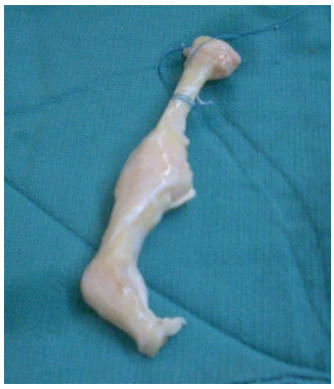

Figure 2 Cadaveric bone allograft construct, using metacarpal and proximal phalanx.

CASE REPORT

The patient is a 17-year-old male who sustained a gunshot wound to the lower abdomen and perineum at the age of nine. All penile substance and his left testicle were lost. He underwent emergent surgery with creation of a perineal urethrostomy. He has developed normally without other medical problems. The perineal urethrostomy allows him to urinate without incontinence. He has a viable right testicle within his scrotum (Figure 1). Through the work of a charitable organization, he was transferred to our institution for penile reconstruction. This is a retrospective case report and received an exempt status from our institutional review board.

The patient underwent general endotracheal anesthesia and was placed in low lithotomy position. His non-dominant left arm was chosen as the donor site for construction of the neophallus. An Allen's test was performed on the left wrist which demonstrated adequate flow to the hand *via* the ulnar artery. Two operative teams were utilized in the operation.

Dissection in the abdomen proceeded through the previous abdominal scar with an extended right Pfannenstiel incision for harvest of the inferior epigastric artery and vein recipient vessels under loupe magnification. These vessels were traced to their origin, and the ilioinguinal nerve was dissected for microscopic nerve anastomosis. The remnant scrotal tissue was elevated to isolate the perineal urethra, and the pudendal nerve and genitofemoral nerve were identified for later nerve anastomosis.

A template was used to design the neophallus on the volar surface of the forearm creating a 5.5 inch long neophallus. Using a tourniquet, the radial forearm flap was elevated in a suprafascial fashion, extending radially to the flexor carpi radialis and ulnarly to the brachioradialis, with identification of the radial artery and its venae comitantes and their inclusion within the flap. The vascular pedicle was dissected to its origin at the brachial artery. The lateral and medial antebrachial cutaneous nerves were also included within the flap.

The neophallus was created by first establishing the urethral conduit along the ulnar side of the flap. This area was de-epithelialized for a width of 0.5 cm, giving a 26 mm diameter neourethra. The ulnar aspect of the flap was rolled over an 18-French Foley catheter and secured to the radial aspect of the flap with an inner layer of interrupted 3-0 absorbable suture and an outer layer of running, locking, 3-0 absorbable suture, creating a water-tight closure of the urethra. The remainder of the radial forearm flap was rolled to create a cylindrical penile shape. To give added infrastructure to the neophallus, cadaveric phalangeal and metacarpal bones were placed in its dorsal aspect. These bones were provided by the University of Miami Tissue Bank (Miami, Florida). The cadaveric bones were stripped of periosteum and soft tissue, trimmed into a more narrow shape, and drill holes were created within the substance of the bones to allow for vascular penetration (Figure 2). The bones were anchored to each other with suture, allowing for minimal movement between them. The bone construct was then placed into the flap, dorsal to the neourethra. The metacarpal bone was placed proximal to the proximal phalanx.

The neophallus was then transferred to the perineal area. The neourethra was sutured to the urethral remnant in the perineum in two layers with absorbable suture. The bone construct within the neophallus was secured to the pubic symphysis. Microvascular anastomosis was performed between the radial artery and right inferior epigastric artery, as well as between the radial artery venae comitantes and the inferior epigastric venae comitantes. Adequate flow was established through the vasculature, but due to the small size of the veins, the patient was given a Heparin bolus and started on a drip. Microscopic neurorrhaphy was performed with anastomosis between the lateral antebrachial cutaneous nerve to the ilioinguinal nerve, and between the medial antebrachial cutaneous nerve to the branch of the genitofemoral nerve and pudendal nerve.

Drains were placed within the abdominal wall dissection and perineum. The perineum was closed with a scrotoplasty to ensure complete soft tissue coverage of the right testicle. A split-thickness skin graft was placed over the donor site on the left forearm. The glans penis was sculptured on the neophallus, using the Norfolk technique to increase its prominence. A skin graft was also

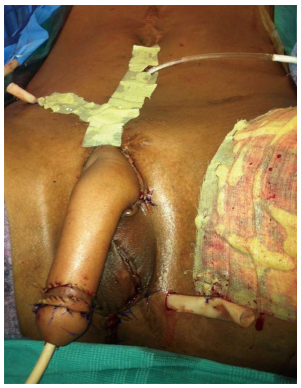

Figure 3 Immediate post-operative view.

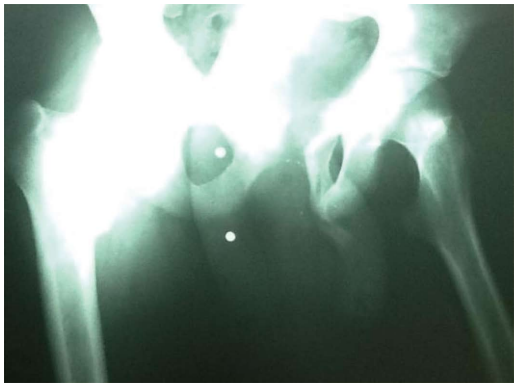

Figure 4 X-ray views demonstrating bone allograft construct within the substance of the neophallus.

placed to further create a transition zone from the shaft to the glans (Figure 3).

The patient was extubated and transferred to the Intensive Care Unit. On post-operative day 2, the patient was taken back to operative room for evacuation of a hematoma that had caused arterial insufficiency to the flap. The microvascular anastomosis was intact, with slight compromise of arterial inflow and venous outflow; however, upon evacuation of the hematoma, there was excellent vascular supply to the flap. The abdominal wall was loosely closed, with a portion of the skin left open due to tension. This area was covered with bovine collagen-dermal bilayer matrix.

The patient was discharged from the hospital and to his native country on post-operative day 28, and he was satisfied with his overall result. The Foley catheter was removed at 6 wk. The patient developed a urethral-cutaneous fistula three months post-operatively at the penoscrotal junction, but he remained continent of urine. On radiograph, the bone allograft construct is viable without evidence of osteomyelitis (Figure 4). At 6 mo, the patient has a rigid and viable neophallus (Figure 5).

DISCUSSION

As the technical innovation of phalloplasty continues to evolve, from a fasciocutaneous flap to a radial forearm fasciocutaneous or osteocutaneous flap or fibula osteocutaneous flap to a sensate, prelaminated fibula flap[8], we propose a new approach using cadaveric bone graft reinforced by free radial forearm fasciocutaneous flap for penile reconstruction. We recognize that the radial forearm osteocutaneous flap has morbidity, including donor site functional and aesthetic deformities requiring skin grafting, risk of radius fracture, and aesthetic deformity from autologous bone harvesting[2]. Additionally, the radial osteocutaneous phallus has been reported to develop a "softened phallus" from cortical degeneration. Kim described 12 softened penises out of 58 radial forearm osteocutaneous phalloplasties[9]. Cavadas described a second stage reconstruction using a free fibular osseous flap for rigidity after a radial forearm fasciocutaneous flap[10].

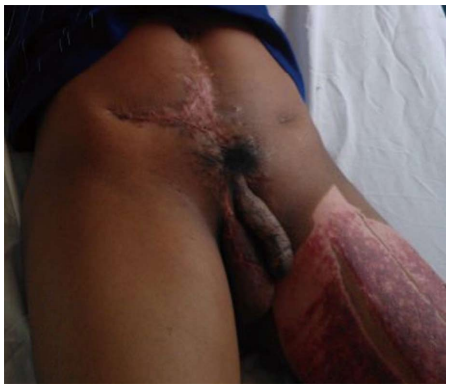

Figure 5 Six months follow-up demonstrating viable neophallus.

Employing the development and application of over five million musculoskeletal allografts and considering the significant morbidity associated with harvesting of the radius bone autograft, we elected to use a metacarpal and phalangeal allograft from the University of Miami Tissue Bank. Allografts are chemically processed with low dose radiation to prevent the transmission of infection while maintaining biological integrity. Although there is a theoretical risk of disease transmission, it is rare. By using a metacarpal and phalangeal allograft, this eliminates the need for a second operation to provide rigidity with an implant and minimizes donor site morbidity. We were able to provide adequate rigidity and support to the neophallus, as well as place a spacer for a second stage procedure in the event the implant was unsuccessful. This was particularly useful in this humanitarian case, as commonly surgeons only have one opportunity to impact the patient's care.

Penile prosthesis implantation has also been used in reconstructive phalloplasty, usually as a second stage to the procedure. This form of reconstruction has the disadvantage of significant cost and has not always been covered by insurances in the United States. There are also the added risks of extrusion, infection, and revision surgery[11]. This form of reconstruction was not available for this humanitarian case, as the patient was unable to make multiple trips to our institution for staged operations.

In our case, the patient has good overall success. He has regained his self-esteem as well as the physiological use of his neophallus, although he has not reported sexual intercourse. His complication of urethral fistula is consistent with the reported incidence of 63.6%[2]. This has been managed conservatively without further sequelae. The drawback of this report is that long term rigidity has yet to be determined, and long term photographs have not been able to be obtained due to patient limitations. In spite of this, the use of an allograft bone implant has many advantages, and further study of this technique is warranted to determine its inclusion in the ongoing evolution of phalloplasty.

COMMENTS

Case characteristics

A 17 year-old male with a traumatic penis amputation.

Clinical diagnosis

Absence of penis with the presence of a perineal urethrostomy.

Differential diagnosis

Penile amputation.

Imaging diagnosis

Absence of penile substance.

Treatment

The patient was treated with a pre-fabricated radial forearm phalloplasty with cadaveric bone graft.

Related reports

Phalloplasty consists of a complex set of procedures, each with its own morbidity, and the issue of creating and maintaining rigidity in penile reconstruction is challenging.

Term explanation

Phalloplasty is the reconstruction or creation of the penis by surgical methods. Pre-fabrication refers to the method of placing a tissue or implant into another vascularized tissue bed and then transferring this construct into a body of tissue that is desired to be reconstructed.

Experiences and lessons

This case report represents a unique method of penile reconstruction in which the authors create rigidity through the use of a cadaveric bone graft rather than utilizing a bone autograft that causes morbidity at the donor site.

Peer review

This article demonstrates a novel surgical technique of a pre-fabricated radial forearm phalloplasty with cadaveric bone graft in reconstruction of the penis following a traumatic amputation. This type of operation is intended to limit the morbidity of using bone autograft, from either the radius or fibula, as well as complete the operation in one stage, as compared to the multiple stages used with placement of a penile prosthesis implantation.

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2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

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