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REVIEW

Laparoendoscopic single site, laparoscopic or open surgery for adrenal tumors: Selecting the optimal approach

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Abstract

Numerous surgical modalities are available to treat adrenal lesions. Minimally-invasive approaches for adrenalectomy are indicated in most circumstances, and new evidence continues to be accumulated. In this context, current indications for open surgical adrenalectomy (OS-A), minimally-invasive adrenalectomy (MI-A), and laparoendoscopic single-site adrenalectomy (LESS-A) remain unclear. A comprehensive English-language literature review was performed using MEDLINE/PubMED to identify articles and guidelines pertinent to the surgical management of adrenal tumors. A comprehensive chart review was performed for three illustrative cases. Clinical recommendations were generated based on relevant literature and the expertise of the investigator group. MI-A offers advantages over OS-A in properly selected patients, who experience fewer complications, lower blood loss, and shorter hospital stays. Robot-assisted laparoscopic and retroperitoneoscopic adrenalectomy may offer advantages over transperitoneal surgery, and LESS-A may be an even less-invasive option that will require further evaluation. MI-A remains the surgical treatment of choice for most adrenal lesions. Tumor size and stage are the primary indications for selecting alternative treatment modalities. OS-A remains the gold standard for large tumors (> 10 cm) and suspected or known advanced stage malignancy. LESS-A appears to be an appropriate initial approach for small tumors (< 4-5 cm), including pheochromocytoma and isolated adrenal metastases.

Key words: Adrenal masses; Surgical approach; Indications; Open adrenalectomy; Laparoscopic adrenalectomy; Laparoendoscopic single-site adrenalectomy

Core tip: Minimally-invasive adrenalectomy remains the surgical treatment of choice for most adrenal lesions. Tumor size and stage are the primary indications for selecting alternative treatment modalities. Open surgical adrenalectomy remains the gold standard for large tumors (> 10 cm) and suspected or known advanced stage malignancy. laparoendoscopic single-site adrenalectomy appears to be an appropriate initial approach for small tumors (< 4-5 cm), including pheochromocytoma and isolated adrenal metastases.

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INTRODUCTION

Neoplasms of the adrenal gland consist of a broad spectrum of pathologies, ranging from benign non-functioning cortical adenomas (or "incidentalomas") to locally-invasive malignancies, such as adrenocortical carcinoma or metastasis from a distant site. Adrenalectomy

Table 1 Indications and relative contraindications for each surgical approach

Indications	Relative contraindications
OS-A	
Adrenocortical carcinoma with radiographic evidence of extra-adrenal extension (stage Ⅲ-Ⅳ)	Amenable to minimally-invasive approach
Extension of adrenal vein tumor thrombus into IVC	
Size larger than 10-12 cm	Size < 10 cm
Concomitant open procedure	
Paraganglioma	
Multiple prior abdominal surgeries	
MI-A	
Non-functioning and functioning adrenal tumors, including pheochromocytoma	Locally-advanced tumors (stage Ⅲ-Ⅳ)
Isolated adrenal metastases	Known, relatively large adrenocortical carcinoma (complete resection is essential for cure)
Size < 10 cm	Size larger than 10-12 cm
Adrenocortical carcinoma, consider only if stage Ⅰ-Ⅱ and < 10 cm	Multiple prior abdominal surgeries (or discussion of possibility of conversion)
BMI ⩾ 30 kg/m^2	
LESS-A	
For patients who consider cosmesis to be of great importance	Surgeon inexperience with LESS-A
Size < 4-5 cm	Size > 5 cm
Non-functioning and functioning adrenal tumors, including pheochromocytoma	Adrenocortical carcinoma
Isolated adrenal metastases	Multiple prior abdominal surgeries
BMI < 30 kg/m^2	

OS-A: Open surgical adrenalectomy; MI-A: Minimally-invasive adrenalectomy; LESS-A: Laparoendoscopic single-site adrenalectomy; BMI: Body mass index.

is the definitive therapy for these tumors and is indicated when either hormone secretion and/or increased risk for malignancy is present[1]. Risk factors for adrenal malignancy include evidence of a functional adrenal tumor, large tumor size, and concerning radiographic findings such as hyperdensity or heterogeneity, irregular tumor margins, local invasion, lymphadenopathy, or distant metastases[2-4].

Open surgical adrenalectomy (OS-A) using a dorsal lumbotomy, midline, subcostal, or thoracoabdominal incision is the historical gold standard, but has largely been replaced by minimally-invasive alternatives. Minimally-invasive adrenalectomy (MI-A) using a transperitoneal laparoscopic approach was first described in 1992[5] and has since replaced OS-A as the operation of choice for resecting most adrenal lesions[6]. Two notable exceptions to this include known adrenal malignancy and tumors that are excessively large or difficult to approach laparoscopically. MI-A is known to offer improved analgesia, hospital stay, blood loss, and complication rate compared to OS-A[7]. Several different techniques, including robot-assisted approaches, have been proposed to address various clinical scenarios where the traditional transperitoneal approach is technically difficult. Even more recently, laparoendoscopic single-site adrenalectomy (LESS-A) has emerged as an appropriate initial surgical approach for tumors smaller than 4-5 cm[8].

Some might suggest that the least invasive approach should be used in all circumstances, but our clinical experience would suggest that the optimal approach in a given scenario may depend on patient and tumor features, such as specific adrenal pathology, prior history of abdominal surgeries, tumor size, patient body habitus, and experience of the operating surgeon (Table 1)[9]. This report details a contemporary approach to surgical decision-making for adrenal tumors, with a review of the literature regarding OS-A, MI-A, and LESS-A.

LITERATURE REVIEW

A comprehensive English-language literature review was performed using MEDLINE/PubMed to identify articles and guidelines pertinent to the indications, techniques, perioperative results, and oncologic outcomes for various treatment modalities for adrenal tumors. Combinations of MeSH search terms adrenal tumor, pheochromocytoma, adrenal cortical carcinoma, metastasis, adrenalectomy, indications, laparoscopic, open, laparoendoscopic single site, transperitoneal, retroperitoneal, and robotic were used. A comprehensive chart review was conducted for the three patients in order to detail the factors determining the elected surgical approach. Approval was obtained from the institutional IRB. Clinical implications presented incorporate relevant literature and the expertise of the investigator group.

OS-A

Case 1: A 54-year-old female presented with worsening left lower abdominal pain that radiated to the left flank. Computed tomography with Ⅳ contrast revealed a large complex solid and cystic mass in the left retroperitoneal space measuring 13 cm and displacing the left kidney laterally and inferiorly. No retroperitoneal lymph nodes or visceral metastases were identified and subsequent chest X-ray was normal. She reported no history suspicious for a functional adrenal tumor, denying uncontrolled

Table 2 Outcomes from select studies comparing minimally-invasive adrenalectomy to open surgical adrenalectomy

Ref.	*N*	Mean tumor size (cm)	ACC or MET (%)[1]	OT (min)	EBL (mL)	LOS (d)	Conversion (%)	Complications (%)
Assalia *et al*[116]	581 MI-A	2.8	0.9	184	154	2.9	-	10.9
	753 OS-A	4.1	5.8	162	309	7.2	-	35.8
Lee *et al*[59]	358 MI-A	-	13.6	174	-	4.1	-	3.6
	311 OS-A	-	44.5	234	-	9.4	-	17.4
Eichhorn-Wharry *et al*[119]	1980 MI-A	-	4.1	146	-	2.8	-	1.8
	592 OS-A	-	18.4	186	-	6.7	-	7.6
Lombardi *et al*[120]	30 MI-A	7.7	100	135	-	5.3	0	3.4
	126 OS-A	9	100	129	-	9.3	-	5.6
Mir *et al*[121]	18 MI-A	7	100	298	1500	4	24	5.0
	26 OS-A	13	100	273	1100	6	-	20.0
Donatini *et al*[79]	13 MI-A	5.5	100	-	-	7	0	8.0
	21 OS-A	6.8	100	-	-	9	-	14.0
Bittner *et al*[122]	356 MI-A	3.2	5.9	159	-	2.5	6.2	11.0
	46 OS-A	8.5	28.2	197	-	9.1	-	50.0

[1]Preoperative indication of ACC or isolated adrenal metastasis. ACC: Adrenocortical carcinoma; MET: Metastasis to adrenal gland; OT: Operative time; EBL: Estimated blood loss; LOS: Length of hospital stay; OS-A: Open surgical adrenalectomy; MI-A: Minimally-invasive adrenalectomy.

blood pressure, new hair growth or other features of Cushing's syndrome. Functional work-up was pursued with serum potassium, urinary cortisol, and plasma and urinary metanephrines. After this negative functional evaluation was completed, the patient underwent OS-A *via* an anterior subcostal incision. The patient was discharged after an uneventful 5 d hospital stay and has remained without evidence of disease recurrence more than 3.5 year since surgery.

With expansion of the indications for MI-A, fewer OS-A are being performed worldwide. Nevertheless, there remain situations in which OS-A remains the best option, such as the case just described. Tumors larger than 10-12 cm should be resected *via* OS-A, given the increased likelihood of these lesions being malignant and the technical difficulties associated with laparoscopic removal of large tumors. OS-A remains the standard treatment for all patients with preoperative radiographic evidence of extra-adrenal tumor invasion. Tumors with associated adrenal and renal vein thrombus, which can extend into the inferior vena cava and right atrium, should also be resected *via* OS-A (Table 1). All these scenarios require maximal exposure to complete the procedure safely and extract the tumor intact. A summary of the evolution of indications for and outcomes of OS-A compared to MI-A are included in (Table 2).

MI-A

Case 2: The patient is a 54-year-old female who presents with a 7 cm × 5 cm × 4 cm right adrenal mass on follow up imaging obtained 12 mo after open right radical nephrectomy for pT2N0M0 clear cell renal cell carcinoma with negative lymph nodes. The patient has a relevant past medical history of renal cell carcinoma, rectal cancer s/p low anterior resection, and morbid obesity with a body mass index (BMI) of 61. Physical examination was unremarkable except for her large abdominal girth and prior incisions. Metastatic workup was negative for additional lesions. Biopsy of the adrenal mass was positive for metastatic renal cell carcinoma. After a discussion of risks and benefits of laparoscopic adrenalectomy and the possibility of open conversion, the patient underwent a transperitoneal laparoscopic adrenalectomy (TL-A). The procedure was successful, without any significant complications other than some delayed return of bowel function, and the patient was discharged home after a 5-d hospital stay.

Three aspects of this case indicate MI-A to be the appropriate surgical approach. MI-A is the procedure of choice for isolated adrenal metastases and for adrenal masses < 10 cm. MI-A is indicated for obese patients, as reduced morbidity and improved outcomes have been demonstrated when compared to OS-A[10]. An additional consideration in this case is the patient's past surgical history. This patient is likely to have adhesions, which may complicate laparoscopic surgery, but MI-A has been shown to be a feasible and safe initial approach to patients with previous abdominal surgery[11,12]. MI-A is an appropriate initial approach, with conversion to OS-A for failure-to-progress when adhesions make laparoscopic surgery excessively difficult.

Laparoscopic adrenalectomy: Transperitoneal vs retroperitoneal

TL-A is the most widely performed MI-A. TL-A offers a large working space, familiar anatomical exposure, excellent visibility, and the ability to perform simultaneous transperitoneal procedures[13]. This approach is dependent upon the ability to retract and mobilize the abdominal organs required for adequate exposure, and may require additional instruments to accomplish this prerequisite step. Adhesions from prior abdominal surgery can complicate port placement and lysis of adhesions may lengthen operative times and increase intra-operative risk of bowel injury, and should be considered carefully when deciding upon the surgical approach in any given scenario.

Retroperitoneal laparoscopic adrenalectomy (RL-A)

Table 3 Summary of the most recent meta-analyses comparing laparoscopic techniques for adrenalectomy

Ref.	RAL-A *vs* MI-A Brandao *et al*[30]	TL-A *vs* RL-A Nigri *et al*[27]	LESS-A *vs* MI-A Wang *et al*[41]
n	600	1205	443
Mean tumor size (cm)	3.86, 3.78	4.0, 3.3	2.7, 3.43
(Odds ratio, CI)	NA	0.48 (-0.21-1.18)	- 0.69 (-1.11--0.26)
P-value	NS	0.17	0.002
Mean operating time (min)	175, 148	132, 136	113.1, 92.7
(Estimate, CI)	5.88 (-6.02-17.79)	-11.07 (-41.38-19.24)	14.97 (4.69-25.24)
P-value	0.33	0.47	0.004
Mean EBL (mL)	44, 69	115, 85	74.2, 79.7
(Estimate, CI)	-18.21 (-29.11--7.32)	29.7 (-10.32-69.72)	-1.4 (-9.72-6.91)
P-value	< 0.0001	0.15	0.74
Mean LOS	3.78, 3.17	6.4, 5.5	3.82, 4.38
(Estimate, CI)	-0.43 (-0.56--0.30)	0.66 (-0.11-1.43)	-0.5(-1.02-0.02)
P-value	< 0.0001	0.09	0.06
Mean % conversion rate	4.4, 7.1	7.23, 7.74	7.8, 1.2
(Odds ratio, CI)	0.82 (0.39-1.75)	NA	4.66 (0.88-24.64)
P-value	0.61	NA	0.07
Mean % complication rate	3.6, 6.8	8, 6	14.2, 10.1
(Odds ratio, CI)	-0.04 (-0.07--0.00)	0.923 (0.58-1.46)	1.83 (0.88-3.81)
P-value	0.05	0.73	0.1

RAL-A: Robot-assisted laparoscopic adrenalectomy; MI-A: Minimally-invasive adrenalectomy; TL-A: Transpeitoneal laparoscopic adrenalectomy; RL-A: Retroperitoneal laparoscopic adrenalectomy; LESS-A: Laparoendoscopic single-site adrenalectomy; EBL: Estimated blood loss; LOS: Length of hospital stay.

allows for direct access to the adrenal gland without bowel mobilization or interference from intraperitoneal organs or adhesions. The disadvantages of RL-A, compared with TL-A, are the smaller operating space and relative absence of anatomic landmarks. This led some authors to conclude that RL-A should be reserved for surgeons with considerable experience with retroperitoneal surgery and tumors smaller than 5-7 cm[13,14]. Recent studies have demonstrated the safety and feasibility of treating tumors up to and exceeding 10 cm[15,16]. BMI > 35 kg/m^2 has also been cited as a contraindication to RL-A because of the difficulty in establishing and maintaining this potential space, resulting in limited exposure[14,15,17].

Numerous studies have been published comparing these two approaches[13,17-24]. These generally favor the retroperitoneal approach. Meta-analyses of these studies have demonstrated that operative time, blood loss, duration of hospitalization, time to oral intake, overall and major morbidity, and mortality are equivalent or superior for RL-A compared to TL-A[25-27]. A summary of the meta-analysis performed by Nigri *et al*[27] is included in (Table 3). RL-A may be preferred to TL-A for a few select scenarios, such as patients with suspected adhesions from previous transperitoneal abdominal surgery. However, it should be noted that the retroperitoneal space is typically obliterated after nephrectomy, preventing insufflation of this space independent of the peritoneal cavity. Some surgeons use the prone position for RL-A, allowing bilateral procedures to be performed without repositioning the patient. For bilateral RL-A (or TL-A) in full or modified lateral decubitus position, repositioning is a necessity in all but the thinnest of patients. With RL-A, some have reported success with two surgical teams operating simultaneously to reduce operative time and surgical stress[28,29].

Robot-assisted laparoscopic adrenalectomy

Robot-assisted laparoscopic adrenalectomy (RAL-A) offers improved blood loss and hospital stay, and similar operative time, conversion rate, and postoperative complications when compared to traditional MI-A[30]. A summary of Brandao *et al*[30] meta-analysis comparing RAL-A to MI-A is included in (Table 3). This procedure may offer advantages in morbidly obese patients (> 30-35 kg/m^3) and those with larger tumors, by improving retraction and exposure[31-34]. The main disadvantages cited by most authors are the learning curve of the entire surgical team, particularly for those not regularly performing other robotic surgeries, and the potential added cost of robot-assisted surgery. Overall, this approach shows promise to enable a wider range of tumors to be addressed with MI-A, and is likely to become more commonly utilized in the future.

LESS-A

Case 3: The patient is a 72-year-old male presenting with an incidentally-detected adrenal mass found during staging evaluation of a suspicious lung lesion. The patient was not found to have signs or symptoms of a functioning adrenal tumor upon investigation. Relevant past medical history includes hypertension, coronary artery disease and hyperlipidemia, with no prior surgical history. The patient has a 58 pack-year smoking history and physical examination was unremarkable. The patient was referred to a multi-disciplinary clinic for evaluation of a

Table 4 Laparoendoscopic single-site adrenalectomy *vs* minimally-invasive adrenalectomy: Overview of the outcomes from available comparative studies (adapted from Rane *et al*[8] and Wang *et al*[41])

Ref.	*N*	Mean tumor size (cm)	ACC or MET (%)[1]	OT (min)	EBL (mL)	LOS (d)	Conversion (%)	Complications (%)
Jeong *et al*[36]	9 TLESS-A	2.9	0	169	178	3.2	11 (1 to MI-A)	11
	17 MI-A	4.3	0	145	205	3.5	5.8 (1 to OS-A)	5.8
Walz *et al*[40]	47 RLESS-A	2.3	2.1	56	< 10	2.4	8.5 (to TLESS-A)	8.5
	47 TLESS-A	2.6	0	40	< 10	3.1	0	6.4
Ishida *et al*[39]	10 TLESS-A	2.8	0	125	12	5.2	0	0
	10 MI-A	4.5	0	120	15	6.9	0	0
Shi *et al*[38]	19 RLESS-A	2.1	0	55	30	6	0	11
	38 MI-A	2.4	0	42	18	6	0	7.9
Kwak *et al*[123]	10 TLESS-A	3.3	0	127	-	4.5	0	10
	12 MI-A	3	8	113	-	4.1	0	-
Vidal *et al*[124]	20 TLESS-A	3	0	95	Min	3.0	0	0
	20 TL-A	3	0	80	Min	2.5	0	0
Wang *et al*[125]	13 TLESS-A	2	7.7	149	79	5.2	0	31
	26 TL-A	2.4	0	113	93	6.3	0	12
Tunca *et al*[126]	22 TLESS-A	3.3	0	64	48	2.45	-	0
	74 TL-A	4.7	4.1	68	38	3	-	0
Lin *et al*[127]	21 RLESS-A	-	0	145	Min	2	0	0
	28 MI-A	-	0	95	50	4	0	3.6

[1]Preoperative indication of ACC or isolated adrenal metastasis. ACC: Adrenocortical carcinoma; MET: Metastasis to adrenal gland; OT: Operative time; EBL: Estimated blood loss; LOS: Length of hospital stay; TLESS-A: Transperitoneal LESS-A; RLESS-A: Retroperitoneal LESS-A.

2.5 cm, spiculated right upper lobe lung mass. A recommendation was made for biopsy of the lung lesion and this revealed moderately differentiated adenocarcinoma. A staging evaluation with PET/CT imaging revealed only a single lesion suspicious for metastatic disease: a 1.2 cm, solid left adrenal mass. Initial recommendation was for percutaneous biopsy by interventional radiology, but based on the small size and location of the lesion adjacent to the aorta, the patient was advised that surgical excision of the lesion was the recommended course. After discussion of risks and benefits with the patient, including the possibility of conversion to multi-port TL-A or OS-A, the patient was scheduled for transperitoneal LESS-A. The patient was discharged home after an uneventful 1 d hospital course. He subsequently underwent thoracotomy and wedge excision of the lung mass and remained without significant disease progression until brain metastasis was detected 2.5 year later.

The first case of LESS-A was reported in 2008[35]. Since then, several studies have compared this emerging technique with MI-A, demonstrating reduced postoperative pain, shorter hospital stay, improved cosmesis, comparable blood loss and complication rate, but with longer operative times[36-40]. A summary of all studies comparing LESS-A to MI-A is included in (Table 4). Rane *et al*[8] published a meta-analysis for LESS-A in 2012. Cumulatively, they found that LESS-A was performed for 59 functioning adenomas (Cushing's syndrome or Conn's Syndrome), 28 pheochromocytomas, and 15 non-functioning masses (adenoma, adrenal metastasis, others). They proposed early and advanced indications for LESS-A based on surgeon experience. Accepted indications for surgeons early in their operative experience include adrenal tumors up to 4 cm in size, functioning or non-functioning, that are localized, and suspected to be benign, in non-obese patients without previous abdominal surgery. Indications for surgeons with advanced LESS experience might include any adrenal neoplasm up to 10 cm in size, with consideration of moderately obese patients and those with limited prior abdominal surgery. Wang *et al*[41] published an updated meta-analysis comparing LESS-A to MI-A in 2013. A summary of this article's findings is included in (Table 4).

Unfortunately, the data on this procedure is limited at present and long-term oncologic outcomes are not yet available. Four of the nine studies included by Wang *et al*[41] had less than 15 patients. Though a learning curve for LESS-A has not been formally demonstrated, extrapolation from other LESS procedures suggests that this is likely within the surgeon's learning curve. Additionally, the patients in these studies have been carefully selected, as demonstrated by the smaller tumor size compared to MI-A. These limitations notwithstanding, LESS-A appears to be an appropriate initial approach when cosmesis is of the utmost importance in the setting of small (< 4-5 cm) adrenal tumors or metastases, that can readily be converted to MI-A with the placement of one or more accessory ports to aid with retraction and exposure. These indications are subject to change as new literature arises, better informing the optimal utilization of this emerging technology.

DISCUSSION

Pathology-benign

MI-A is the surgical approach of choice for almost all benign adrenal tumors. Functional adrenalomas, including aldosteronomas, pheochromocytomas, and cortisol-secreting adenomas, have been demonstrated to be amenable to MI-A[42-49]. For pheochromocytomas,

recurrence rates following MI-A are low (6%-8%) and not significantly different from OS-A in reports that are somewhat limited by short follow-up durations (21-102 mo)[50-54]. Most experts believe that size and tumor biology, rather than surgical approach, are more likely to determine the chance of cure in this disease[55,56]. Based on this literature, many authors and guideline-producing societies have concluded that MI-A is an appropriate initial approach to pheochromocytomas[50-57]. Recurrence of other benign adrenal tumors is extremely rare and is generally limited to tumors that are later discovered to be malignant based on metastasis after initially benign or indeterminate pathology. Advantages of this procedure compared to OS-A are well documented and include lower or equivalent blood loss, improved postoperative pain control, shorter hospitalization, improved perioperative convalescence, and excellent cosmesis[7,58-60]. Results concerning operative time for MI-A compared to OS-A have been mixed, with some series reporting longer times for MI-A[61,62], others reporting comparable durations[63,64], and still others shorter times[59,65]. Operative mortality is very low for adrenalectomy, and has not been shown to be significantly different between the two procedures[60]. At this time, selecting amongst different MI-A approaches should be determined by surgeon familiarity and experience, as there is inadequate evidence to demonstrate superiority of any one MI-A approach for a specific benign adrenal pathology.

Pathology-malignant

Adrenocortical carcinoma (ACC) is a rare disease with an annual incidence of approximately 1 per million population[66]. Overall 5 years survival is approximately 38% to 46%[66,67]. Unfortunately, only 30% of cases are detected prior to extension outside the adrenal gland or metastasis[67]. Cure of ACC is dependent upon complete surgical resection, including regional lymph nodes and involved adjacent organs[68-71]. OS-A has been the gold standard for ACC for decades[3,4]. This status has been challenged in recent years, by reports of favorable perioperative and oncologic outcomes with MI-A for ACC[72-79]. However, the use of MI-A for known ACC remains controversial, in large part based on the poor outcomes reported during initial experiences with MI-A, which included intraoperative tumor fragmentation, port-site and local recurrences, and peritoneal carcinomatosis[80-83]. Two recent studies reported that MI-A was associated with increased frequency of positive margins and intraoperative tumor spillage, shorter time to recurrence, and worse overall survival for stage Ⅱ ACC compared to OA[84,85]. These authors concluded that MI-A is inappropriate in known or suspected ACC. In contrast, numerous recent reports have described improved perioperative and equivalent or even superior oncologic outcomes for MI-A performed with contemporary techniques[74,77-79,86]. These advocates argue that so long as standard oncologic principles are strictly adhered to, comparable outcomes can be achieved for stage Ⅰ and Ⅱ ACC tumors by those with the requisite experience and expertise. Current guidelines remain equivocal, with some suggesting that MI-A is acceptable as an initial surgical approach for stage Ⅰ and Ⅱ ACC < 10 cm and others recommending OS-A for all suspected or known ACC. All guidelines currently recommend conversion to OS-A when extensive tumor adhesion, invasion, or lymphadenopathy is identified intraoperatively[50,87-89].

Pathology-metastasis

Metastasis to the adrenal gland is commonly seen in cancer of the breast, lung, colon, melanoma and lymphoma. Adrenal metastasectomy has been shown to improve survival in patients with limited metastatic burden, with average survival rates of between 20 and 30 mo after surgery compared with 6 to 8 mo without resection. Nevertheless, this heterogeneous population of patients has approximately 25% 5 year survival[3,90]. Despite poor durable oncologic outcomes post-adrenalectomy, there is also a role for this procedure as a palliative measure[91]. For the treatment of isolated adrenal metastatic disease, MI-A was initially controversial, but has since become the standard approach. Published series have demonstrated that this is a safe procedure with very low morbidity and similar long-term outcomes to OS-A[90,92-95]. In the largest and most compelling comparison of MI-A and OS-A for adrenal metastases, Strong *et al*[94] reviewed 94 adrenalectomies (31 MI-A *vs* 63 OS-A) and found no differences in margin status, local recurrence, disease-free survival, or overall survival. They also demonstrated that MI-A provided significantly shorter operative time, lower estimated blood loss, shorter length of hospital stay, and fewer total complications. These authors concluded that MI-A should be recommended as an appropriate initial approach to isolated adrenal metastases. Several additional studies comparing MI-A to OS-A for adrenal metastases reported similar results[91,93,96]. In accordance with these findings, guidelines have recommended that MI-A is appropriate for solitary metastases to the adrenal gland, given that local invasion is not present[50,87].

Prior abdominal surgery

Previous abdominal surgery is a known risk factor for laparoscopic procedures[97]. Some authors have considered this to be a relative contraindication to transperitoneal MI-A and recommend a retroperitoneoscopic approach[14,98-100]. Morris *et al*[11] analyzed 92 patients with and 154 patients without previous abdominal surgeries undergoing TL-A. Operating time, blood loss, and perioperative complications were not significantly different between the two groups. They concluded that TL-A was safe in patients with previous abdominal surgeries, conversion to OS-A occurs infrequently, and is rarely attributed to adhesions, and that surgeons should perform the surgical method they are most comfortable with. The laparoscopic approach can be tailored to accommodate patients with previous transabdominal surgery

with modification of port placement or addition of accessory ports, and should not be contraindicated in these patients. Maintaining a low threshold for conversion to OS-A for failure-to-progress can maintain an equivalent level of safety with MI-A to that obtained with an initial plan for OS-A.

Tumor size

Historically, tumor size > 5 cm was considered to be a relative contraindication to MI-A given the increased risk of treating incidentally-found ACC, along with greater complexity of procedure, longer operative time and increased blood loss[101-104]. In recent years, numerous studies have demonstrated that MI-A is safe and feasible for large (> 5 cm) adrenal masses, offering favorable outcomes, reduced convalescence, and decreased morbidity compared to OS-A[105-112]. Boylu *et al*[111] compared MI-A for adrenal tumors < 8 cm and ≥ 8 cm. They found that operative time and blood loss were significantly higher for tumors ≥ 8 cm, but noted that results were comparable between the two groups concerning transfusion rates, length of hospital stay, and conversion to open surgery. They concluded that MI-A achieved favorable morbidity and surgical outcomes for larger lesions, despite being technically difficult operations. Other studies have demonstrated mixed results regarding the impact larger tumor size has on operative time, blood loss, hospital stay, and conversion to OS-A[110,113-115]. Hemal *et al*[109] recommended TL-A over RL-A for larger masses, given the larger working space it provides, and concluded that size alone should not eliminate MI-A as an option. Assalia and Gagner[116] performed a meta-analysis of 20 case-control studies examining MI-A versus OS-A. They noted that a few studies reported MI-A for lesions up to 14-15 cm, but found that most authors cited 10-12 cm as the maximum acceptable for MI-A. Overall, we feel that MI-A is appropriate for adrenal tumors up to 10-12 cm, in the absence of pre-operative imaging suggesting peri-adrenal infiltration or venous invasion or biopsy-proven ACC.

Body habitus

The body habitus of the patient, including both BMI and abdominal girth, must be accounted for when determining the approach for adrenalectomy. Laparoscopic procedures may be preferable in obese patients. Fazeli-Martin *et al*[10] compared open and laparoscopic approaches for adrenal and renal procedures in obese patients, demonstrating that patients undergoing laparoscopic procedures had decreased blood loss, less narcotic use, shorter hospital stay, and fewer complications, compared to open procedures. It is however, important to note that obesity has been independently associated with increased operative time and postoperative complications compared to non-obese patients for MI-A[117,118]. Two studies have been published comparing different MI-A approaches in this patient subgroup. Epelboym *et al*[23] analyzed 81 RL-A and 130 TL-A procedures in obese patients. They found that operative time (90 min *vs* 130 min; $P < 0.001$) and estimated blood loss (0 mL *vs* 50 mL; $P < 0.001$) were significantly less for RL-A, but failed to demonstrate significant differences for length of stay, overall mortality, incidence and severity of postoperative complications, and rates of readmission. Aksoy *et al*[32] compared 42 retroperitoneal RAL-A and 57 RL-A procedures in obese patients. They found no difference in perioperative outcomes between these two approaches. Given the paucity of data comparing MI-A approaches in obese patients, we cannot provide specific recommendations at this time as to which approach is best. Patient body habitus should be included in patient selection and determination of operative approach, as MI-A may be beneficial in these patients despite the increased procedural difficulty and associated morbidity.

CONCLUSION

While the indications for adrenalectomy have remained reasonably stable over the last two decades, the surgical approaches have become less and less invasive. Despite these technological advances, the least-invasive procedure (LESS-A) is not always the most appropriate. Various approaches to MI-A remain the preferred surgery for many situations, with LESS-A appearing to be a viable alternative for small tumors in relatively uncomplicated scenarios, though further validation is needed for this emerging technique. There remains a clearly defined role for OS-A for large and locally-advanced malignancies. Most importantly, the surgeon should engage each patient in the medical-decision making process for each adrenal tumor encountered.

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REVIEW

Employing extracellular vesicles for non-invasive renal monitoring: A captivating prospect

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Abstract

Extracellular vesicles (EVs) are fascinating nano-sized subjects extensively studied over the recent years across several disparate disciplines. EVs are endlessly secreted into the extracellular microenvironment by most cell types under physiological and pathological conditions. EVs encompass a variety of molecular constituents from their cell of origin, such as lipids, cell specific proteins and RNAs, thus constituting an informative resource for studying molecular events at the cellular level. There are three main classes of EVs classified based on their size, content, biogenesis and biological functions: exosomes, shedding microvesicles and apoptotic bodies. Besides cell culture supernatants, biological fluids have also been shown to contain different types of EVs. Amongst the various body fluids, the study of urinary extracellular vesicles (uEVs) as a source of candidate biomarkers gained much attention, since: (1) urine can be non-invasively collected in large amounts; and (2) the isolated uEVs are stable for a relatively long period of time. Here, we review the important aspects of urinary extracellular vesicles which are fast gaining attention as a promising future tool for the non-invasive monitoring of urinary tract. Recent advancements in the purification and analysis of uEVs and collection of their constituents in rapidly developing public databases, allow their better exploitation in molecular diagnostics. As a result, a growing number of studies have shown that changes in expression profile at the RNA and/or protein levels of uEVs reveal the molecular architectures of underlying key pathophysiological events of different clinically important diseases with kidney involvement.

Key words: Urine analysis; Extracellular vesicles; Biomarkers; Kidney disease; Quantitative proteomics; Exosomes

Core tip: Urinary extracellular vesicles research is a fast growing field of biomarker discovery providing new attracting prospective for monitoring tissue alteration in easily accessible clinical samples. Over the past ten years intense research has identified the various urinary vesicular cargo molecules (*i.e.*, RNAs, proteins and lipids) and detected their alterations upon a number of renal diseases. With the number of diseases relating to kidney increasing it is essential to effectively utilize this invaluable tool for the early diagnosis. Here we provide a comprehensive overview of uEVs nicely

setting the stage for their utility in future clinical diagnostics.

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EXTRACELLULAR VESICLES: WHY ARE THEY FASCINATING?

Extracellular vesicles (EVs) are membrane-bound secretory vesicles which exhibit an array of proteins, bioactive lipids, nucleic acids and metabolites[1-4]. EVs are considered to play an important role in intercellular communication[5-8], regulating immune response[9-12], antigen presentation[13-15], transport and propagation of infectious cargo such as prions and retroviruses[2,16-18]. Based on the size and origin, EVs are classified into three main types: (1) "exosomes" (40-100 nm) vesicles derived from the endosomal compartment and released *via* fusion of the multivesicular bodies with the plasma membrane; (2) "microvesicles" (50-1000 nm) vesicles that result from the direct budding from the plasma membrane; and (3) "apoptotic bodies" (800-5000 nm) vesicles released by cells undergoing programmed cell death[19,20]. Despite some distinct features, numerous similarities exist among the different classes of EVs with respect to their physical characteristics and biochemical composition, which make the separation of different subsets challenging[21].

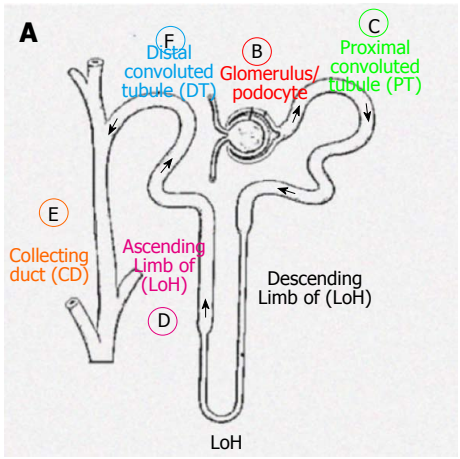
Nano- to micron-sized EVs have been isolated from many body fluids including urine[16,22-25], saliva[26,27], breast milk[9,27], cerebrospinal fluid[28], semen, pleural effusions and plasma[27,29]. Urinary extracellular vesicles (uEVs) are released from the renal epithelial cells, including glomerular podocytes, renal tubule cells, and the cells lining the drainage system[30]. They are promising starting material for biomarker discovery and also a great asset for non-invasive renal monitoring as they provide a full representation of the entire urinary system[3,16]. Recently developed quantitative workflows for uEVs transcriptomics, proteomics and lipidomics[31-34] including system biology approaches[35] enable researchers to study the expression profiles of the main bioactive constituents of uEVs in detail and with an increased rate and reproducibility than before. Research performed in the last 10 years highlighted that uEVs harbor 1%-3% of the total urinary proteins with a reduced dynamic range of protein concentration respect to the whole urine proteome. In this sense, the proteome of uEVs which today counts more than four thousand proteins can be considered as the urinary subproteome enclosed in double-layered vesicles. Despite the potential benefits of uEV analysis, there are barriers and limitations that must be dealt with. Foremost among these challenges is the reproducible isolation of pure vesicles suitable for downstream analysis[36]. In addition to proteins, uEVs encapsulate different RNA species amongst which messenger RNAs (mRNAs) and micro-RNAs (miRNAs) are the subjects of intense studies as possible biomarkers of renal diseases[3,37]. RNAs incorporated in uEVs are resistant to nuclease digestion and, similarly to proteins they originated from the different nephron regions[38]. For example, podocyte related mRNA expression of CD2-associated protein in uEVS was shown to correlate with renal function and level of proteinuria[39]. Moreover, miRNA-29c level in uEVs was shown to be a potential biomarker of renal fibrosis[40]. The relative abundance of different mRNAs do not necessarily correlate with cellular mRNA, suggesting a selective process of mRNA entry into vesicles[1]. As vesicle-enclosed mRNAs travel along the nephron, modification of nucleic acid cargo could permit targeted delivery of RNA to the kidney, a hypothesis which open a new area for the treatment of renal tubular disorders[41].

Here, we will focus on summarizing the current knowledge about uEVs, starting from the critical review of their isolation, biochemical characterization till their potential application in biomarker discovery and non-invasive renal monitoring.

URINARY SYSTEM ORIGIN OF uEVS

Exosomes are cell-derived secretory vesicles shed by proliferating cells through exocytosis[6,13,29]. During endosomal maturation, the formation of intraluminal vesicles (ILVs), ranging from 30-100 nm in diameter, inside the lumen of the endosome can be observed. The ILVs are generated by inward invagination of the endosomal membrane, and scission of vesicles from the limiting membrane into the endosomal lumen. The size of a fully matured late endosome, also called multivesicular body (MVB) is approximately 500 nm in diameter and, it contains several ILVs. The fate of MVBs may vary. They could fuse with lysosome and get degraded or fuse with the plasma membrane releasing the vesicles into the extracellular space. During the later process, the second inward budding of the endosome membrane results in a positive orientation of the ILVs lipid membrane. While the endosomal sorting complexes required for transport (ESCRT) machinery involved in sorting the cargo to lysosomes *via* MVBs within the endocytic system is well characterized (ESCRT-dependent endolysosomal pathway), protein sorting into exosomes until recently was less understood[6]. The involvement of ESCRT protein complexes and protein ubiquitination has been shown by different groups. Recently, Alvavez *et al*[3] revealed that syndecan-syntenin-*ALIX* might be a key regulator of the biogenesis of syntenin expressing exosomes. In particular, the interaction between syndecan and exosomes might thus support a novel role for proteoglycans in vesicular trafficking and cellular signaling. On the other hand, EVs displaying similar physicochemical characteristics like exosomes (density, size-distribution, presence of protein markers) have also been described to bud

A



B

UniProt Acc.Number	Protein name	Ref.
PODO_HUMAN	Podocin	48
PODXL_HUMAN	Podocalyxin	48
NPHN_HUMAN	Nephrin	48
CR1_HUMAN	Complement receptor type 1	48
KIRR2_HUMAN	Kin of IRRE-like protein 1	48
NEST_HUMAN	Nestin	98
VTDB_HUMAN	Vitamin D-binding protein	49
APT_HUMAN	Adenine phosphoribosyltransferase	62
LRRK2_HUMAN	Leucine-rich repeat kinase 2	50

C

UniProt Acc.Number	Protein name	Ref.
TR11B_HUMAN	Osteoprotegerin	49
LRP2_HUMAN	Megalin	44
CUBN_HUMAN	Cubilin	44
AMPN_HUMAN	Aminopeptidase N	44
AQP1_HUMAN	Aquaporin-1	44
CAH4_HUMAN	Type IV carbonic anhydrase	44
GGT1_HUMAN	γ-glutamyltransferase	44
SL9A3_HUMAN	Sodium/hydrogen exchanger 3	45
CD24_HUMAN	Signal transducer CD24	55
ANXA5_HUMAN	Annexin V	58
SCNNA_HUMAN	ENaC (epithelial sodium channel)	45
CHRDL1_HUMAN	Chordin-like protein 1	49
CYR61_HUMAN	Protein CYR61	49
ATF3_HUMAN	Activating transcription factor 3	22
LRG1_HUMAN	Leucine-rich alpha-2-glycoprotein-1	71
PKD1_HUMAN	Polycystin-1	45,46,54,62
MLL3_HUMAN	Isoform 1 of histone-lysine N-methyltransferase	16
GPC5B_HUMAN	G protein coupled receptor required for tubulogenesis	94
MMP9_HUMAN	Matrix metalloproteinase 9	77
CAH9_HUMAN	Carbonic Anhydrase 9	77
NGAL_HUMAN	Neutrophil gelatinase-associated lipocalin	91

D

UniProt Acc.Number	Protein name	Ref.
CD9_HUMAN	CD9 antigen	44
S12A1_HUMAN	Type 2 Na-K-2Cl cotransporter	44

E

UniProt Acc.Number	Protein name	Ref.
AQP2_HUMAN	Aquaporin-2	9
MUC1_HUMAN	Mucin-1	9
RHAG_HUMAN	Rh type C glycoprotein	9
TS101_HUMAN	Tumor susceptibility gene 101 protein	10-12
FLOT1_HUMAN	Flotillin	11,12

F

UniProt Acc.Number	Protein name	Ref.
S12A3_HUMAN	Thiazide-sensitive Na-Cl co-transporter	44

Figure 1 Schematics of Nephron showing the urinary extracellular vesicle proteins identified in the different segments. A: Core; B: Glomerulus/podocyte; C: Proximal convoluted tubule (PT); D: Ascending Limb of (LoH); E: Collecting duct (CD); F: Distal convoluted tubule (DT). UniProt Acc.Number: UniProt Database Accession Number. LoH: Loop of Henle.

from the plasma membrane. For example, podocyte-positive membrane vesicles in urine has been shown to originate from tip vesiculation of podocyte microvilli[42]. These vesicles however cannot be easily distinguished from endosome-derived exosomes and therefore their separate analysis remains a problem.

Exosomes are released from cells of different tissues or organs and while they share a common group of proteins related to their biogenesis they also harbor tissue specific proteins that reflect the origin and biological functions of their parental cells[43]. In fact, EVs isolated from biological fluids have organ and tissue specific protein and RNA profiles. The urinary system consists of the two kidneys, ureters, the bladder, and the urethra. Nephrons are the kidney functional and structural units with the main role to regulate water balance and inorganic ions by filtering the blood. More than a decade ago, the key finding of the first proteomic analyses performed on isolated uEVs was the presence of proteins known to be highly and differentially expressed in various kidney tissues from the glomerular podocyte to the epithelial cells lining the various nephron segments[44,45]. Later on proteins from the transitional epithelium of the urinary bladder were also identified, confirming uEVs may be shed from cells throughout the renal[46]. Targeted proteomics applied recently to perfused isolated rat kidney model to identified kidney originated proteins in human urine[47]. Out of the 990 kidney originated proteins with human analogues 74 were present only in plasma and 240 only in urine (including uEVs) but not in plasma. Screening the 240 kidney originated urinary proteins we have found that the major part (206) is listed in the human urinary dataset of EVpedia 2.0 database. This could be a core set of kidney originated vesicle proteins with high renal pathophysiology relevance for further studies.

Figure 1 shows a schematic diagram of a nephron

indicating sets of uEVs proteins highly expressed in the epithelial cells of glomerular podocyte[48] and to the different segments of renal tubule[44,45,49]. *Podocalyxin* (PODXL) specific for glomerular epithelial cells, aminopeptidase (AMPN) specific for proximal tubule cells, AQP2 specific for distal tubule, programmed cell death 6 interacting protein (PDCD6IP or ALIX) and *uroplakin-1* and *uroplakin-2* (UPK1 and UPK2) specific for bladder are frequently used as protein markers of human uEVs. Basal epithelial cells of collecting duct are hypothesized to be the major contributors toward the urinary pool of EVs including the expression of *leucine-rich repeat kinase-2* (LRRK2)[50]. More recently, Benito-Martin *et al*[49] showed that cultured human proximal tubular cell secrete exosomes containing osteoprotegerin (OPG) and this specific sub-group of exosomes can also be found in urine. Since, most of these have kidney relevant function, proteomic analysis of uEVs hold the promise to provide an insight into the physiological or pathophysiological processes in the various cell types facing the urinary space[44].

VESICLE ISOLATION: AN OBSTACLE TO OVERCOME

Isolation of EVs is still a major challenge in this rapidly growing field of research. The complex nature of body fluids and lack of standardized protocols makes isolation and characterization extremely difficult[34]. The research on EVs can be broadly divided into three categories depending on the end use of the vesicles: (1) discovery; (2) diagnostic; and (3) preparative, each of which demands a different level of purity, quality control and operating procedures. In most studies, ultracentrifugation is the commonly used technique for isolation of vesicles. Isolation of membrane vesicles by sequential differential centrifugations is complicated by the possibility of overlapping size distributions with microvesicles or macromolecular complexes. Recently, numerous alternate procedures were introduced, including immunoaffinity separation, filtration, microfluidic devices aided separation, and reagent based separation[20]. The choice of isolation procedure greatly depends on the source material and the goal of the EV research project.

Isolation of extracellular vesicles from urine has proven to be extremely difficult because of the presence of Tamm-Horsfall protein (THP), also known as uromodulin[36], and also due to very low vesicle load in this biofluid. Differential centrifugation is the most widely used technique for the isolation of uEVs. It includes several low-velocity centrifugation and ultracentrifugation steps with increasing centrifugal force; from 200 to 1500 g to remove cells and cellular debris, from 10000 to 20000 g to pellet microvesicles, and from 100000 to 200000 g to pellet nanometer-sized vesicles[50]. The efficiency to isolate EV depends not only on the size, shape and density of vesicles but on the volume, viscosity and temperature of the fluid in which the vesicles are present. Centrifugation time and the type of rotor used (fixed angle or swinging bucket) also influence the final yield and the purity of vesicles. Addition of dithiothreitol (DTT)[23,36,49,51] and 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS)[52] to the low-speed pellet has been shown to be efficient to disrupt the polymeric network formed by THP protein filaments, but do not solve the problem entirely[33,53]. Urinary EVs can be separated from non-membranous particles, such as protein aggregates by using their relatively low buoyant density, and differences in floatation velocity to separate differently sized classes of EV. Based on this, the crude uEVs containing pellet obtained by differential centrifugation can be further separated/purified using a sucrose density gradient[54,55] or sucrose cushion ultracentrifugation step[33,56,57]. It has been shown that both methods are efficient not only to separate exosomes from microvesicles but also to eliminate the interfering THP impurity[33,54-56]. The high sucrose concentration used in sucrose gradient centrifugation, however may negatively affect the biological function of EV. This can be avoided by layering the samples on top of the sucrose gradient or cushion in the tube subjected for centrifugation. Filtration through molecular filters (0.22-μm or 0.1-μm filters), which remove solvent and small molecule analytes while retaining and concentrating vesicles smaller than the pore size can also be used alone or in combination with centrifugation or ultrafiltration (MWCO 100 kDa) to isolate uEVs[25,58-60]. Among the primary advantages of the filtration method are the simplicity and easy scale-up. Though co-purification of abundant soluble urinary proteins and THP often compromises sample purity and the applied increasing forces with decreasing pore size has also been reported to result in artifacts[50]. The presence of characteristic surface proteins (CD9, CD63, CD81, Rab-5b, TSG101, Alix and A33) on certain EV classes is the basis for immunoaffinity isolation[57,61]. Immunoaffinity-based techniques employ antibodies thus have the potential for high specificity, an important consideration in the characterization of specific EV populations[57]. Because of the increasing interest in exosomes and other extracellular vesicles and their potential use in therapeutics or as biomarkers for disease, kits that allow for "easy isolation procedures" are being developed[16,62]. Commercial tests based on centrifugation (Total Exosome Isolation Reagent, Invitrogen™), filtration (Exomir ™ and Exo-Spin™), affinity capturing (Exotest™ and Exosome Dynabeads®) and proprietary exosome precipitation technologies (miRCURY™, ExoQuick-TC™ and DiagExo®) are already available in the market (Table 1). Such approaches for rapid purification are welcomed but they should be only considered as for bulk isolations because they often fail to pass quality test and fail to distinguish between differently sized EV and membrane-free macromolecular aggregates[50]. A widely accepted unique standardized protocol for the reproducible isolation and purification of uEVs suitable for the downstream analysis of the various RNA and protein constituents is still awaited.

Table 1 Methods used for the isolation of urinary extracellular vesicles

Technique	Type	Advantage(s)	Disadvantage (s)	Purity	Ref.
Centrifugation	Differential	Easy to perform	Co-purification of protein aggregates (THP) Inability to separate exosomes from microvesicles Lengthy Impractical for large-scale studies	++	[37,44,50,52,69,92]
	Sucrose gradient	No mechanical stress and hence allows the collection of morphologically intact particles Removes THP	High sucrose concentration may affect the biological functions of exosomes Inability to separate exosomes from particles with similar density and size Difficult and lengthy Impractical for large-scale studies	+++++	[23,50,54,55]
	Sucrose cushion	No mechanical stress and hence allows the collection of morphologically intact particles	Inability to separate exosomes from particles with similar density and size Difficult and lengthy Impractical for large-scale studies	+++++	[33,56,57,78,80]
Filtration	Nanofiltration	Easy to perform	Low purity grade Co-purification of protein aggregates (THP) Low exosome yield due to their lost on the surface of the nano-membrane	+++	[94]
	Microfiltration	Easy to perform Rapid Maintain vesicle structure	Co-purification of protein aggregates (THP) Inability to separate exosomes from microvesicles	+++	[59]
Immunoaffinity separation	Immunobeads	Allow rapid semi quantitative characterization of the surface phenotype can be tissue-specific	Not suited for large sample volumes Captured extracellular vesicles may not retain biological functionality even if successfully eluted from bead surface Co-purification of protein aggregates (THP) Low yield	++	[57,61,62]
Commercial kits	Total Exosome Isolation Reagent-Invitrogen™ Exomir™ Exo-Spin™ Exotest™ Exosome Dynabeads® miRCURY™ ExoQuick-TC™ and DiagExo®	Rapid and requires low sample volume Practical	Impractical for large-scale studies Reproducibility, yield and sample quality should be checked case by case Co-purification of protein aggregates (THP)	++	

THP: Tamm-Horsfall protein.

PHYSICOCHEMICAL AND ANALYTICAL CHARACTERIZATION OF URINARY EXTRACELLULAR VESICLES

Isolated uEVs are a heterogeneous vesicle population. Exosomes have a lipid bilayer membrane and a characteristic buoyant density ranging from 1.10 to 1.19 g/mL[55]. Determination of physicochemical properties, like integrity, morphology, size and concentration is generally the first step in the characterization of uEVs. Transmission electron microscopy (TEM)[16,23,33,52,58,59,63-65], dynamic light scattering (DLS)[66], nanoparticle tracking analysis (NTA)[67,68] and flow cytometry (FC)[69] are the methods most frequently used in this process. TEM imaging of numerous uEV samples has revealed intact vesicles with round morphology[16,23,33,52,58,59,63-65]. Depending on the isolation/purification method used, uEV samples typically show a heterogeneity in size ranging from approximately 30-100 nm in diameter (Figure 2A). Besides the determination of particle concentration and size distribution, the main advantage of using transmission electron microscopy (TEM) for uEV analysis is its ability to reveal the presence of known urinary contamination, like the long polymers of THP[36,52]. TEM[16,23,33,52,58,59,63-65] and cryo-EM[68,70] based morphological characterization, including observation of vesicle heterogeneity, therefore is a great support for the quality assessment of sample preparation[64]. Orthogonal techniques, like Dynamic Light Scattering (DLS) and nanoparticle Tracking Analysis (NTA) on the other hand, measures the Brownian motion of the particles in solution and calculate the hydrodynamic radii of the particles. Owning to its rapidity and simplicity, NTA is a quickly expanding in the field of exosome research. The mean diameter of human uEVs isolated by ultracentrifugation was measured by NTA

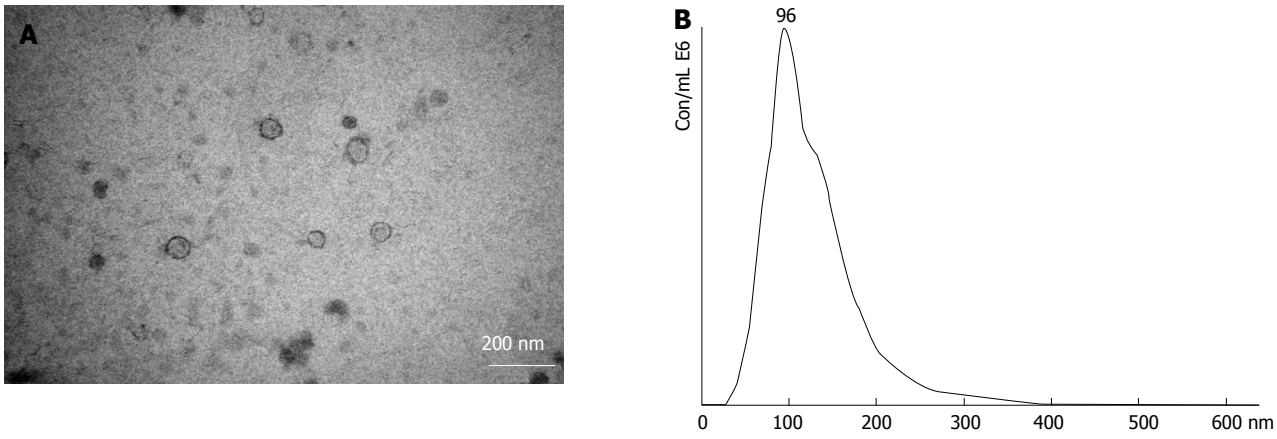


Figure 2 (A) Transmission electron microscopy and (B) nanoparticle tracking analysis images of urinary extracellular vesicles isolated by the sucrose cushion ultracentrifugation method.

around 150 nm ± 50 nm and concentration 10E9-10E11 particles/mL (Figure 2B)[68]. It should be noted that the hydrodynamic size of nanoparticles is expected to be higher to the core size measured with TEM, however the relationship between the two still need to be determined. Subpopulation of EVs can be studied by immunoelectron microscopy using antibodies to target membrane proteins, like aquaporin-2 (AQP2), aminopeptidase-N (AMPN), podocalixin (PODXL) and CD9[37,42,65,71]. Oosthuyzen *et al*[66] most recently demonstrated that NTA can also monitor specific subgroups of uEVs particles using fluorescent antibodies against specific surface proteins present on uEVs.

Analytical characterization of the components of urinary nano-vesicles (*i.e.,* proteins, mRNAs, mi-RNAs, lipids and small molecules) can be performed by transcriptomic[31,37,40,46,63,72,73], proteomic[16,19,23,25,31,33,35,44,47,48,50,52,59,60,62,63,65,66,71,74-79], metabolomic and lipidomic[2,31,46,68,75,80] tools[34]. One of the most exciting findings is that RNA transcripts exist in exosomes and maintain their function when transferred to other cells. Vesicle mediated route for cell-cell communication within the urinary tract opens new perspectives, yet its role in kidney development, function and pathogenesis needs to be elucidated[46]. Typically, commercially available extraction kits are used for total RNA isolation. Abundance of most RNAs is low in the in uEV which makes isolation and downstream RNA analysis challenging[37]. Most of the studies so far have used microarrays[72,81] or real time quantitative PCR (qPCR) assays[39,48,72] to examine exosomal RNAs, with a focus on miRNAs. Because of the inherent limitations of these methods, unknown miRNAs or other RNA species are often overlooked. To characterize RNA profiles systemically, Cheng *et al*[73] performed next-generation deep sequencing providing the base to identify miRNA biomarkers in urinary exosomes.

Since the first proteomic profiling reported by Pisitkun *et al*[44], protein content of urinary EVs has been extensively studied[16,19,23,25,31,33,35,41,45,47-50,52-54,59,60,62,63,65,66,70,71,74-80] both under healthy and numerous disease conditions. These studies let to comprehensive datasets of the identified proteins collected in the different exosome related public databases. It should be noted, that for the generation of many of the large datasets, uEV samples isolated by differential centrifugation (crude exosome preparation) was used[23,45]. As a consequence, public databases contain a relatively high percentage of urinary and other impurities related proteins. The protein content of urinary exosomes is about 1%-3% of that of total urine. The proteomic workflow generally starts with protein concentration assay (Bradford or BCA) followed by protein profile analysis which is usually performed by visualization of proteins separated by SDS-PAGE. The band at about 90 kDa corresponds to the highly glycosylated THP monomer[36] and its relative abundance is used to assess sample quality. Western blots are performed by using a set of urinary exosomal markers, such as Alix, CD9, TSG101, PODXL, AQP2, NES and Annexin V. Chemiluminescent western blotting gives valuable information for the quality of the preparation, but its use as a quantitative method for measuring the relative expression of the target proteins in the absence of appropriate normalization method is still debatable. To measure differences in protein expression between samples, quantitative proteomics is the method of choice. Both label-free quantitation[16,25,76] and stable isotope labeling by iTRAQ[33,78] and TMT reagents[50] in combination with SCX/C18 Multidimensional Protein Identification Technology (MudPIT) have been shown to be useful in the comparative analysis of uEVs. After appropriate enrichment steps, post translational modifications (PTMs), phosphorylation[45,50,79] and glycosylation[64,69] have also been studied by mass spectrometry-based proteomics in urinary exosomes. Since PTMs may affect important physiological process and its alterations may directly reflect early pathogenic events, further studies of PTMs alteration in uEV in various disease states are expected to come.

The bioactivity of exosomes is associated not only to their protein and RNA contents but also to their lipids. Compared to their cells, exosomes have been shown to be enriched in *Cholesterol* and *Sphingomyelin*. Total

exosomal lipids can be extracted by organic solvents according to Bligh and Dyer[82] or, alternatively using THF: H_2O (4:1)[2]. Different lipid classes (phospholipids and glycosphingolipids) can be purified and analyzed by thin layer chromatography (TLC), gas chromatography-mass spectrometry GC-MS and liquid chromatography-mass spectrometry (LC-MS). Recent progresses in electrospray ionization-tandem mass spectrometry-(ESI-MS/MS) based high-throughput lipidomics allowed the first comparative lipid analysis uEVs isolated from healthy individuals and renal cell carcinoma patient[2].

DATABASES FOR URINARY EXTRACELLULAR VESICLES RESEARCH

Recent studies have ignited significant interest on uEVs as possible players in kidney physiology and also as potential reservoirs of biomarkers. With such a huge interest, the amount of data accumulated has increased over time. High throughput proteomics study routinely identifies more than thousand proteins in human uEVs[23,45,50] and necessitates systematic classification of the data acquired[83]. Currently, there are two integrated manually curated web-based databases publicly available: Vesiclepedia[84] and EVpedia[85]. Both dedicate separate sections for the constituents of human urinary vesicles.

Vesiclepedia 2.1 (http://www.microvesicles.org) catalogs information from published non-mammalian eukaryotic and mammalian extracellular vesicles. Based on the 15 studies published on human uEVs, the current version contains 1162 unique proteins, 20 unique mRNAs and 690 unique miRNAs. To aid biomedical scientists in assessing the quality of the preparation Vesiclepedia also contains information on the methods used for the purification as well on the biophysical and molecular characterization of EVs.

EVpedia 2.0 (http://evpedia.info) provides a comprehensive lists of proteins, mRNAs, miRNAs, and lipids identified in EVs of both eukaryotic and prokaryotic (bacteria and archaea) origin, including non-mammalian eukaryotic extracellular vesicles[31,85]. Currently, out of the 263 studies included in this database 16 deal with urinary EVs. EVpedia 2.0 is cataloging a total of 12869 proteins isolated from human uEVs out of which 6275 are unique. 10 studies are related to the study of uEVs isolated from healthy donors and the number of unique proteins in this set is 4536. The most frequently identified 100 proteins are also listed in the database. Sets of proteins identified in different disease related samples (312 from early IgA nephropathy, 552 from autosomal recessive polycystic kidney disease, 621 from basement membrane nephropathy, 689 from bladder cancer, 480 from hernia, 184 from renal cell carcinoma) are particularly valuable components of this database. In the present 2.0 release of EVpedia there are no mRNAs, miRNAs and metabolites in the human urinary EVs subset reported. Regarding lipids, 28 different lipids were identified in human uEVs from healthy donors and 26 lipids were found in patients with renal cell carcinoma. Moreover, EVpedia offers a range of analytical tools: (1) search for and browse vesicular components; (2) Gene Ontology enrichment analysis of vesicular components; (3) network analysis of vesicular components; and (4) set analysis: a comparison of vesicular datasets by ortholog identification. Detailed methods for the isolation of extracellular vesicles and publications on extracellular vesicles are also listed in this database. An overall comparison of EVpedia with Vesiclepedia is beyond the scope of this paper and has recently been published[31,85]. Regarding urinary EVs, the current release of EVpedia contains more data on proteins and lipids while miRNAs and mRNAs are more represented in Vesiclepedia. There is a concern that a relatively high percentage of the entries in the single datasets included in the databases are not native constituents of uEVs but matrix impurities related to isolation/purification[83,86]. Complete workflow solution, starting from fast and efficient purification of EVs from urine without contamination by non-vesicular components (including abundant urinary proteins, protein aggregates), is a critical prerequisite for future high-throughput analyses. Important prerequisites for the efficient use of the databases are: (1) the definition of a reliable core set of uEVs' constituents; and (2) handling data redundancy of current databases, are still awaited.

EMPLOYING URINARY EXTRACELLULAR VESICLES IN BIOMARKER DISCOVERY

Biomarkers are defined as substances or characteristics that are objectively measured and evaluated as an indicator of normal or pathogenic processes, or pharmacologic responses to a therapeutic intervention. Amongst the different body fluid, urine is one of the most important sources of biomarkers for both urologic and non-urologic diseases. High throughput omics studies have resulted in a great number of potential urinary biomarkers which are publicly available[87]. While the characterization of the normal urinary proteome is steadily progressing, there are three major obstacles in the classical urine-based protein biomarker discovery: (1) sample instability; (2) high dynamic protein concentration range; and (3) the relatively high inter-individual and inter-gender variations of urinary proteome. Urinary EVs, on the other hand, possess some characteristics that make them particularly attractive for biomarker research, like increased stability, reduced complexity, lower dynamic range, and composition which is closely related to the cell of origin. Biomolecules enclosed in the extracellular vesicle are surrounded by a lipid bilayer which protects them against degradation by proteases and nucleases. Therefore, uEVs have been shown to be particularly stable over time[37,88]. In addition, extracellular vesicles exhibit a reduced complexity comparing that to the whole urine composition. The dynamic concentration range of proteins and RNAs was shown to be lower in uEVs when compared to whole urine[33,78]. Moreover, the content of EVs reflects

Table 2 Putative disease-associated protein and RNA biomarkers identified in urinary extracellular vesicles

Name of the putative marker	UniProt ID/RNA name	Molecular and biological function	Associated Kidney disease(s)	Disease/model	Ref.
Adenine phosphoribosyltransferase	APT_HUMAN	Enzyme involved in the purine nucleotide salvage pathway. Its deficiency can lead to urolithiasis and renal failure	APRT deficiency	Healthy	[62]
Low-density lipoprotein receptor-related protein 2 mRNA	LRP2_HUMAN (megalin), LRP2	Multi-ligand binding receptor; mediates endocytic uptake of complexes between the steroid 25(OH) vitamin D3 and vitamin D-binding protein in kidney proximal tubules	Donnai-Barrow syndrome; Renal aminoglycoside accumulation; Nephrotoxicity	Healthy	[38,78]
Polycystin-1, Polycystin-2	PKD1_HUMAN PKD2_HUMAN	Integral membrane glycoproteins, associated with structural and/or functional defects in the primary apical cilium of epithelia and polycystic kidney disease	ADPKD	Healthy	[45,46,54,62]
Solute carrier family 12 member 2 (Sodium potassium chloride cotransporter-2)	S12A2_HUMAN	Membrane transporter: aids in the active transport of sodium, potassium, and chloride into and out of cells; NKCC2 mutations lead to type I Bartter syndrome	Antenatal Bartter syndrome type 1	Healthy	[33,41,62]
Myosin-9	MYH9_HUMAN	MYH9 polymorphisms have been shown to associate with glomerulosclerosis and non-diabetic end stage renal disease	Fechtner syndrome and Epstein syndrome	Healthy	[62]
Aquaporin-2 mRNA	AQP2_HUMAN AQP2	Molecular water channel in the basolateral and apical plasma membranes of the collecting duct	Autosomal dominant and autosomal recessive nephrogenic diabetes insipidus	Healthy; Various kidney diseases	[24,33,38,39,44,54,58,62,95]
Neprilysin (CD10)	NEP_HUMAN	Surface metallo-endopeptidase highly expressed in the kidney brush-border membranes	Membranous glomerulonephritis	Healthy; Diabetic nephropathy, Renal cell carcinoma	[25,33,77,95]
Osteoprotegerin	TR11B_HUMAN	Decoy receptor of proximal tubular cells	chronic kidney disease-mineral and bone disorder	Healthy; ADPKD CKDD patients	[49]
Podocalyxin	PODXL_HUMAN	he major sialoprotein of kidney glomerulus; Involved in the regulation of cell adhesion, cell morphology and cancer progression	Autosomal recessive steroid–resistant nephrotic syndrome	Healthy; Glomerular disease; Renal cell carcinoma	[42,58,62,77,95]
Solute carrier family 12 member 3 (Thiazide-sensitive Na-Cl cotransporter; NCC)	S12A3_HUMAN	Membrane transporter highly expressed in the kidney: aids reabsorbing sodium and chloride ions from the tubular fluid into the cells of the distal convoluted tubule of the nephron	Gitelman syndrome; Aldosteronism	Healthy; High blood pressure; Aldosteronism	[41,62,79]
Protein AMBP (alpha-1-microglobulin; bikunin precursor)	AMBP_HUMAN	Membrane glycoprotein with serine protease inhibitor activity	Diabetic nephropathy	Healthy; DN patients in advanced disease stages	[16,45]
Leucine-rich alpha-2-glycoprotein-1	LRG1_HUMAN	Involved in protein-protein interaction, signal transduction, and cell adhesion and development; Expressed during granulocyte differentiation	NSCLC	Healthy; NSCLC	[71]
Matrix metalloproteinase 9	MMP9_HUMAN	Involved in extracellular matrix remodeling	Renal cell carcinoma	Healthy; Renal cell carcinoma	[77]
Basigin , Extracellular Matrix Metalloproteinase Inducer	BASI_HUMAN	Involved in extracellular matrix remodeling	Renal cell carcinoma	Healthy; Renal cell carcinoma	[77]
Ceruloplasmin	CERU_HUMAN	A ferroxidase enzyme in serum	Renal cell carcinoma	Healthy; Renal cell carcinoma	[77]
Dickkopf related protein 4	DKK4_HUMAN	Involved in extracellular matrix remodeling	Renal cell carcinoma	Healthy; Renal cell carcinoma	[77]
Carbonic Anhydrase 9	CAH9_HUMAN	A metalloenzyme that catalyzes the reversible hydration of carbon dioxide	Renal cell carcinoma	Healthy; Renal cell carcinoma	[77]
Dipeptidase 1	DPEP1_HUMAN	A kidney membrane enzyme that hydrolyzes a variety of dipeptides and is implicated in renal metabolism of glutathione and its conjugates	Renal cell carcinoma	Healthy; Renal cell carcinoma	[77]
Syntenin-1	SDCB1_HUMAN	Scaffold protein Pbp1 Syndecan-binding protein	Renal cell carcinoma	Healthy; Renal cell carcinoma	[77]

Leucine-rich repeatkinase2	LRRK2	A member of the leucine-rich repeat kinase family; Associated to risks to inflammation-linked diseases that include Crohn's disease and mycobacterium infection	Parkinson's disease	Healthy; Parkinson's disease	[50]
Activating transcription factor 3	ATF3_HUMAN miRNA494	Transcription factor with protective role in renal ischemia-reperfusion injury	AKI	AKI	[22]
Fetuin-A	FETUA_HUMAN	Plasma binding glycoprotein; inhibitor of calcification, regulator of cell-dependent process of osteogenesis	AKI	AKI	[46,96]
Wilm's tumor protein	WT1_HUMAN	Zinc finger protein with essential role in the normal development of the urogenital system	Early podocyte injury in diabetic nephropathy; steroid responsiveness or renal pathological conditions in patients with idiopathic nephrotic syndrome	FSGS patients; Diabetic Patients with or without Proteinuria; idiopathic nephrotic syndrome	[22,30,92]
Isoform 1 of histone-lysine N-methyltransferase	MLL3_HUMAN	Histonemethyltransferase which methylates "Lys-4" of histone H3, a specific tag for epigenetic transcriptional activation	Diabetic nephropathy	DN patients in advanced disease stages	[16]
Voltage-dependent anion-selective channel protein 1	VDAC1_HUMAN	Porin ion channel of the outer mitochondrial membrane	Diabetic nephropathy	DN patients in advanced disease stages	[16]
GPRC5B	GPC5B_HUMAN	G protein coupled receptor required for tubulogenesis	Acute Kidney Disease	AKD	[94]
Aquaporin-1 mRNA	AQP1_HUMAN AQP1	Molecular water channel in the basolateral and apical plasma membranes of the proximal tubules.	Renal ischaemia reperfusion injury	Renal transplantation; Renal cell carcinoma	[38,77,97]
Neutrophil gelatinase-associated lipocalin	NGAL_HUMAN	Iron-trafficking protein involved in multiple processes such as apoptosis, innate immunity and renal development. In the kidney it is produced by distal nephron	kidney transplantation	kidney allograft recipients	[91]
Immunoglobulin light chain	IGLL1_HUMAN IGKC_HUMAN	Immunoglobulin light chain is the small polypeptide subunit of an antibody	Light Chain Amyloidosis	Light chain amyloidosis; multiple myeloma; monoclonal gammopathy; non-paraproteinemia related kidney disease	[65]
Panel of 24 proteins _HUMAN	SERPINA1, A2M, APOA1, APOA2, CA1, CD5L, DPEP1, FGA, FGB, FGG, FN1, GSTT1, SLC2A1, HBA1, HBB, SERPIND1, HP, HPR, ITIH1, PON1, RAB27B, S100A8, S100A9, TACSTD2	-	Bladder cancer	Bladder cancer; Hernia	[77]
PSA	KLK3_HUMAN	Serine protease with diverse physiological functions; implicated in carcinogenesis	Prostate cancer	Prostate cancer	[56]
Glutamate carboxypeptidase 2 (Prostate-specific membrane antigen-PSMA)	FOLH1_HUMAN	Folate hydrolase and N-acetylated-alpha-linked-acidic dipeptidase activity; Involved in prostate tumor progression	Prostate cancer	Prostate cancer	[56]
microRNA	MicroRNA-29c	-	Renal fibrosis	Healthy; CKD	[40]
microRNA	miR-130A	-	Type 1 diabetes	type 1 diabetic patients with and without incipient diabetic nephropathy	[81]

microRNA	miR-145	-	Type 1 diabetes	type 1 diabetic patients with and without incipient diabetic nephropathy	[81]
microRNA	miR-155	-	Type 1 diabetes	type 1 diabetic patients with and without incipient diabetic nephropathy	[81]
microRNA	miR-424	-	Type 1 diabetes	type 1 diabetic patients with and without incipient diabetic nephropathy	[81]
mRNA (CD2-associated protein)	CD2AP	-	Podocyte injury	Healthy; diabetic nephropathy; focal segmental glomerulosclerosis; IgA nephropathy;membranous nephropathy	[39]
mRNA (Prostate cancer antigen 3)	PCA-3	-	Prostate cancer	Healthy; Prostate cancer	[32]
mRNA (TMPRSS2/ERGa fusion transcript)	TMPRSS2:ERG	-	Prostate cancer	Healthy; Prostate cancer	[32]

CKD: Chronic kidney disease; AKI: Acute kidney injury; PSA: Prostate specific antigen; NSCLC: Non-small cell lung cancer; APRT: Adenine phosphoribosyltransferase deficiency; CD2AP: CD2-associated protein; PCA-3: Prostate cancer antigen 3; ADPKD: Autosomal dominant polycystic kidney disease.

the cell of urinary tract they shed from. Therefore, it was expected that biomolecules of uEVs could provide clinically more specific information for both early diagnosis of disease and also for monitoring drug responsiveness than that of urine[89]. Since the first publication of uEVs[44], the majority of the works in this field is focused on exploring the potential use of uEVs in preclinical and clinical studies[2,6,24,25,33,35,37,39-41,71,74-78,81,89-92]. Since plasma derived EVs are too large to pass through the glomerular filtration barrier, urinary tract originated vesicles (Table 2)[41,44] carry cell specific markers from the specific regions of the kidney with relevant physiological and pathophysiological information[41].

Proteomic analysis has revealed proteins and RNAs that have been isolated from healthy individuals but are associated with different human diseases (Table 2): Adenine phosphoribosyltransferase in APRT deficiency[62]); Low-density lipoprotein receptor-related protein 2 in Donnai-Barrow syndrome, renal aminoglycoside accumulation and Nephrotoxicity; Polycystin-1 and Polycystin-2 in ADPKD[54]; Neprilysin in Membranous glomerulonephritis[41]; Non-muscle myosin heavy chain IIA in Fechtner syndrome and Epstein syndrome[62]; Sodium potassium chloride cotransporter-2 and Thiazide-sensitive Na-Cl cotransporter in Antenatal Bartter syndrome type 1 and Gitelman syndrome, respectively[41,62]. The presence of theses disease associated analytes in uEVs is promising but their expression levels in well-defined disease cohort and conditions still need to be determined.

In disease cohorts including bladder[57,74,93], prostate[32,56,75] and renal cell carcinoma[2,77] altered expressions of vesicle derived proteins, RNAs and lipids have been demonstrated (Table 2) and panels of putative protein biomarkers have been set up for validation. Vesicle expressed aquaporin water channel proteins (AQP1 and AQP2) have been found to be differentially expressed in a number of different renal diseases too. Other putative biomarkers related to urinary system diseases, like activating transcription factor 3 and fetuin-A in acute kidney injury[22,46]; podocalyxin in autosomal recessive steroid-resistant nephrotic syndrome[62]; and Wilm's tumor-1 in early podocyte injury[22,92] have also been described. There are only a few studies though which report putative biomarkers related to treatment, like the effect of low sodium diet and infusion of aldosterone on the phosphorylation of Thiazide-sensitive Na-Cl cotransporter and prostatin in uEVS[79], or renal transplantation[91].

The reservoir for biomarker discovery could however extend beyond diseases of the urinary tract[41]. Conde-Li *et al*[70] investigated a rat model of liver injury induced by galactosamine and reported a change in urinary exosomes that coincided with liver injury. Gildea *et al*[71] reported that leucine-rich a-2-glycoprotein (LRG-1) was increased in both human urinary exosomes from patients with lung cancer and the lung cancer tissue. High-level of LRRK2 was found in uEVs of patients affected by Parkinson's disease[50]. These observations open a novel

scenario towards the future application of uEVs also in non-renal diseases.

CONCLUSION

Chronic kidney disease (CKD) is increasingly recognized as worldwide public health problem. CKD increases the risk for many adverse health outcomes, including cardiovascular disease, end-stage renal disease, and mortality. Because CKD usually progresses asymptomatically until its advanced stages, detection of early-stage CKD requires laboratory testing. The two key markers used for the definition, classification, and monitoring of kidney function are urine albumin and estimated glomerular filtration rate (eGFR). Kidney dysfunction is indicated by eGFR of less than 60 mL/min per 1.73 m^2, while kidney damage most frequently is manifested as increased urinary albumin excretion. When less invasive blood and urine tests are insufficient fine-needle aspiration (FNA) or renal mass biopsy (RMB) is performed especially to substantiate the diagnosis in renal masses. While these traditional markers are certainly of great utility they also present several limitations. Protein (albumin) concentration in urine is not very specific. Levels may rise with use of certain non-steroidal anti-inflammatory drugs, rheumatoid arthritis, lupus and cancers etc. Creatinine level used to estimate GFR, on the other hand, provides little information about the underlying cause of kidney injuries, and lacks specificity in case of low muscle mass and unusual diets.

Because of the above limitations of currently used kidney function markers intensive research is going on to find more accurate ones. Many genes have been shown to be differentially expressed in the kidney upon glomerular injury, endothelial dysfunction, inflammation, fibrosis, cardiovascular dysfunction, metabolic disorders and cancer with the corresponding protein products appearing in plasma and urine. In urine, a unique biomarker source with great potential, a number of new candidate protein biomarkers have already been proposed: cystatin, N-acetyl-β-o-glucosaminidase (NAG) and liver-type fatty acid-binding protein (L-FABP), neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule 1 (KIM-1), just to mention a few, are potential biomarkers awaiting administration (FDA) approval. NGAL is suggested to be used as a urinary biomarker of delayed graft function (DGF), a frequent complication after kidney transplantation too. Recently Alvarez *et al*[90] have demonstrated that NGAL is mainly secreted into urinary vesicles and that the expression level of NGAL in uEVs is a sensitive measure of DGF, findings which might support the clinical management of patients undergoing kidney transplantation.

To become a clinically approved, a putative biomarker should be validated and implemented into clinical tests. Translating a novel discovery into clinical practice is however extremely challenging, consequently there are only a few urine-based protein biomarker assays which have been developed and approved so far (*i.e.*, BTA and NMP-22 for the diagnosis of bladder cancer and PCA3 for the diagnosis of prostate cancer). Rapidly expanding urinary extracellular vesicle research represents an interesting field relevant to the development of disease specific, non-invasive methods for clinical diagnostics as well as to the development of new therapeutic approaches. Especially, growing evidence suggest that EV-imprinted genetic and proteomic information may well reflect the state of their parental cells. In this sense, expression analysis of proteins and RNAs in circulating urinary vesicles could provide specific information about the change in the state of specific nephron segment(s) and/or of the epithelial cells of urogenital tract. Urinary EV-mediated cell-cell communication within the nephron is another interesting aspects which can have major impact in our current understanding in renal physiology. However, how to translate these captivating ideas to a non-invasive renal monitoring system, there is still a lot more to understand.

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REVIEW

Kidney stones over 2 cm in diameter-between guidelines and individual approach

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Abstract

The prevalence of urolithiasis has been observed to increase during last decades. Kidney stones over 2 cm in diameter are the common urologic problem. European and American Associations of Urology has published guidelines on Urolithiasis and presented the most effective tools to treat large stones. On the other hand many experienced endourologic centres choose other modalities from their armamentarium. All treatment methods are characterized by their efficacy and safety which are usually inversely proportional. It is crucial for patients and physicians to find a golden mean. Percutaneous lithotripsy is still considered treatment of choice with more than 95% efficacy. Less invasive retrograde intrarenal surgery is also less effective, but burdened with lower complication rate. Extracorporeal shockwave lithotripsy is feasible in paediatric patients with acceptable stone free rates. Open surgery (pylolithotomy and anatrophic nephrolithotomy) are almost obsolete techniques. All methods have their pros and cons. Physicians should share decisions regarding treatment modalities with patients.

Key words: Kidney stones; Percutaneous lithotripsy; Shockwave lithotripsy; Retrograde intrarenal surgery; Pylolithotomy; Anatrophic lithotomy

Core tip: There are various modalities for treatment of kidney stones over 2 cm in diameter. Guidelines indicate the most appropriate methods. Percutaneous lithotripsy is considered first line treatment while retrograde intrarenal surgery or shockwave lithotripsy are optional approaches. Apart from guidelines physicians should share decisions regarding optimal treatment with patients.

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INTRODUCTION

Nephrolithiasis is one of the most common diseases afflicting mankind. It has been reported in various medical writings since antiquity. In United States its prevalence has doubled since the sixties being now between 2% and 7%. Similarly in countries of Western Europe like Germany, Spain and Italy its prevalence has also been rising[1,2]. It has recently been shown that the real prevalence might even be higher reaching 8.4%. Men are afflicted more frequently than women (10.6% *vs* 7.1%). Racial differences are evident. The most commonly afflicted are white males. African-American females develop least likely urinary stones, while other races are in-

between[3].

Urolithiasis manifest itself clinically mostly between 30 and 50 years of patients' age. The risk of recurrent renal colic after the first stone episode is roughly 15% during the first 3 years and grows up to 50% for the next 7 years[4,5]. In patients with more than one stone diagnosed during their first renal colic this ratio might increase to 75%. After every urolithiasis treatment, the patients should be stratified and accordingly assign to low or high risk group of stone recurrence. Urolithiasis promoting factors as patients' age, recurrent stone formers, familial urolithiasis, calcium hydrogenphosphate (brushite), uric acid, cystine, and so called infection stones have to be analysed and appropriately considered for the further management. This group requires thorough metabolic evaluation and a close follow-up. However, only in 20% of the patients a systemic disease predisposing to stone formation can be identified[6].

Over the last centuries a significant shift in stone location has been observed from the lower to the upper urinary tract. A disease considered previously as male ailment is now gender blind. Metabolic diseases such as obesity and diabetes are strongly associated with urolithiasis. It seems that diet and lifestyle play an important role in disease development[7]. Nephrolithiasis might be an effect of other systemic diseases such as: inherited and acquired renal tubular acidosis, primary and secondary hyperparathyroidism, gout, various neoplasms, primary hyperoxaluria, gastrointestinal diseases, sarcoidosis, recurrent/persistent urinary tract infection, metabolic syndrome and cystinuria. Some anatomical abnormalities of the urinary tract are also associated with lithogenesis and comprise: horseshoe kidney, ureteropelvic junction obstruction, medullary sponge kidney, calyceal diverticulum and vesicoureteral reflux[8]. Urinary tract infections play an important role in stone formation and thus need a special clinical attention and management. As it can be seen in the example of staghorn calculi, most of them have an infectious origin and consist of magnesium ammonium phosphate-struvite or carbonate apatite- dahlite or ammonium urate[9,10].

The aim of our study was to present and compare several treatment methods that can be offered for patients with renal stones over 2 cm in diameter.

Medline was search for articles published between 1977 and 2013. The following keywords were entered: "kidney stone", "Percutaneous nephrolithotripsy", "extracorporeal shockwave lithotripsy" and "retrograde intrarenal surgery". Only English written papers were included. Only papers most relevant for the purpose of this review were included.

DO WE HAVE TO TREAT KIDNEY STONES?

Until now this crucial question remains at least partially unanswered. What is the appropriate clinical management for small asymptomatic calyceal stones, that do not grow? For all other renal stones active treatment is recommended. Referring to this, Guidelines on Urolithiasis of the European Association of Urology changed in 2011. Previously, active stone removal had only been recommended for calculi > 6 mm. In accordance to the literature, a rate of spontaneous passage was estimated to be 1% in comparison to smaller stones (*i.e.*, < 3 mm) when almost all stones can be expelled[11]. Currently guidelines state that all stones over 15 mm in diameter should be removed. This recommendation is based on a trial revealing, that there are no differences between active and conservative approach in asymptomatic calyceal kidney stones < 15 mm in diameter in terms of stone free rate, symptoms, quality of life and renal function[12]. Therefore patients who elect observation instead of active treatment of their kidney stones should be informed about the possible course of the disease. In that situation, in three years 77% of asymptomatic patients with kidney calculi will progress and 26% will require active treatment. Moreover, lower pole stones grow more frequently than middle and upper pole stones (61% *vs* 47%). The rate of growth is positively correlated with uric acid concentration in serum and urine[13]. Therefore individual approach for each patient is advised with abovementioned consideration and taking into account other clinical information obtained from patients history (*e.g.*, occupation, *etc.*). For that reason, even small asymptomatic calyceal stones should be actively treated in jet pilots, travelers, *etc.*

There is no such question in terms of larger stones. Staghorn calculi inevitably lead to unresolved/persistent urinary tract infections with loss of renal parenchyma, chronic pylonephritis and eventually loss of kidney function[9,14-17]. Untreated large stones may also cause life-threatening urosepsis which in some circumstances requires intensive care management or even nephrectomy.

METHODS OF TREATMENT

Renal stone treatment has gone through significant changes over last decades from mainly open surgeries to minimally invasive ones. The treatment modality depends mainly on stone size, hardness and position within the kidney. The last Guidelines on Urolithiasis of the European Association of Urology (EAU) recommend endourology as a treatment option for renal calculi over 20 mm in diameter stating simultaneously superiority of percutaneous lithotripsy (PNL)[8]. However, optional approaches in large stones are feasible and comprise retrograde intrarenal surgery (RIRS) with flexible(fRIRS) or semirigid ureterorenoscopes (rRIRS), endoscopic combined intrarenal surgery (ECIRS), shockwave lithotripsy (SWL) and exceptionally open surgery (pylolithotomy and anatrophic nephrolithotomy).

PNL and open surgery

Until the last year, PNL had been the gold standard in the treatment of renal stones over 2 cm in diameter. Still

this technique is being chosen among other methods as first line therapy for large renal calculi. It remains also an alternative for smaller stones formed by cystine, brushite and whewellite which are usually very hard and associated with lower stone free rates when treated with different modalities. It was shown that stone density over 970 Hounsfield units on non-contrast computed tomography are efficiently destructed by SWL in 38% in comparison with softer stones where such ratio reached 96%[18]. Usually 3-5 ineffective sessions with SWL also should prompt physician to offer more invasive methods to the patient.

There are many different PNL techniques. None appears to be more efficient than the others. The procedure can be conducted in prone and supine position. Originally PNL was described in prone position with specially invented metal dilators[19]. This kind of patient positioning offers an unlimited access to the kidney even in terms of a multi-track approach. Subsequently supine position was proposed by Valdivia Uría *et al*[20] to improve direct anesthesiological access to the patient's chest and to minimize the vena cava-syndrome. A further miniaturization of the equipment allowed to perform PNLs in children[21,22]. As standard PNL procedures are performed with 28-Fr or 30-Fr channel mini PNL offers smaller sheaths between 12-Fr and 20-Fr. Unfortunately ultrasonic disintegration is technically unfeasible in these systems. The next step of miniaturization called ultra-mini-PNL (UMP) has been shortly presented[23]. The procedure is carried out using a 3.5-F telescope and special inner and outer sheaths. After puncturing the kidney, tract dilatation up to 13-F is performed. Stones are disintegrated with a 365-μ holmium laser fibre and actively evacuated by creating an eddy current of saline in the instrument shaft. Further miniaturization has allowed to disintegrate stones with the so called "all-seeing needle" (4.8-Fr). Using this, micro PNL device, renal stones can be disintegrated but neither actively extracted nor washed out. Concerning the size of the instrument, it can be excellently used in paediatric urology[24,25].

To make PNL more convenient for patients tubeless (without nephrostomy) and totally tubeless (without nephrostomy and ureteral catheter) variations of procedure were proposed. Conventional PNL comprise insertion of nephrostomy tube after completion of surgery. This allows free drainage of clots and remnant stones as well acts as hemostat when closed for a short period after procedure. However, hospitalization and operation times are significantly longer in comparison with tubeless procedures[26-29].

Over the last three decades PNL has supplanted pylolithotomy and anatrophic nephrolithotomy in treatment of larger stones mostly due to its significantly decreased invasiveness with only marginally worse efficacy. The last comparison between open stone surgery and percutaneous nephrolithotomy was performed in the late 90's and showed that pyelolithotomy or nephrolitotomy was superior in terms of SFRs[30,31]. Despite inferior SFRs, PNL replaced open surgery in treatment of large kidney calculi. The reason for that might have been the acceptance of SFRs in favour of lower complication rate during PNL compared to the open approach. Due to continuous increase of expertise in PNL, both methods seem to have similar efficacy (see Table 1). Another endoscopic alternative in treatment of large renal stones might be a laparoscopic approach. As a minimally invasive procedure, PNL similar SFRs burdened with longer operative times were documented[32]. It is worth emphasizing that PNL is not free from complications. The most common are infections occurring in up to 35% of patients. Significant bleeding at 7.8% and mortality rates at up to 0.5% were estimated[33,34].

Shockwave lithotripsy for large renal stones

In 1984 first SWL machine was introduced for the treatment of kidney stones. Dornier Human Model 1 was a prototype while model number 3 was the first generation lithotripter that was widely used in the clinic[35,36]. First interventions were performed under general anaesthesia. The patient was positioned in a large basin filled with degassed fluid. Until now HM-3 Dornier has had the highest known efficacy throughout all shockwave lithotripters.

The mechanism of stone fragmentation is based on a rule that focused ultrasound waves can cause hard object disintegration through tear forces, spalliation, cavitation and squeezing[35,37-41].

With only 45%-60% stone free rate SWL efficacy in kidney stones over 2 cm in diameter may be consider as disappointing[42,43]. In older studies SFRs up to 70% with low complications rate were reported[44]. Fortunately, significantly higher SFRs reaching 85% can be achieved in paediatric patients[45-47]. In comparison PNL in children shows the same treatment efficacy as in adults. PNL stone free rates in this group ranges from 68% to 100%[48,49].

In conclusion SWL in patients with stones over 20 mm in diameter is inappropriate except paediatric patients, where an individual approach should always be aimed.

RIRS

Continuously mastered lithotripsy through natural body orifices (as ureteroscopy) has nowadays allowed to achieve satisfactory results with low complication rates. Although lower SFRs in comparison to PNL, natural orifice transluminal endoscopic surgery (NOTES) is characterized by low morbidity rates, pain and hospitalization times[50]. RIRS may be done using flexible (fRIRS) and semirigid instruments (rRIRS). Flexible ureterorenoscopy is characterized by a small shaft calibre, usually less than 10-F. In stones over 2 cm prolonged operation times can be observed (mean 82.5 max up to 215 min). Excellent SFRs above 90% are reported in centres with profound experience in urinary stone management and high case load. It was shown that 1.6 procedures per patient are needed to

Table 1 The summary of procedures feasible in treatment of renal stones over 2 cm in diameter

	Stone free rate[2]	Complications rate[3]	Ancillary procedures	Operating room time, min
Open surgery[30,31][1]	71%-84%	46%	-	130
PNL[26-29,32,34]	75%-98%	0%-33%	9%-33%	52
ESWL[42-47]	45%-60%–adults 85%–children	6%	18%	50-70
fRIRS[50,51-56]	90%	8%-10%	3%-13%	82-94
rRIRS[50,54]	Aug-81%	8%-15%	12%-5%	85-98

[1]Data from 1986; [2]Including insignificant small stone fragments; [3]Including minor and major complications. PNL: Percutaneous lithotripsy; ESWL: extracorporeal shockwave lithotripsy; RIRS: Retrograde intrarenal surgery.

achieve superior results in terms of RIRS treatment[51,52]. Complication rates were calculated to occur in 10% of patients while major complications contribute approximately to half of them[53]. Instrument costs for flexible ureterorenoscopy are high and appear to be a limiting factor. Due to a very fragile laser fibres which is frequently bent within the working channel of the instrument, its breakage and a consecutive damage of the scope might occur. On contrary, the latest comparison of costs between PNL and fRIRS revealed a vast economic advantage towards ureteroscopy ($ 19845 *vs* $ 6675) at least in the United States health care system[53].

The main disadvantages of NOTES-based techniques using semirigid ureterorenoscopes in comparison to fRIRS is their inability to disintegrate stones in lower and middle calyces, potentially high renal fluid pressure, limited intraoperative manoeuvrability and occasional inability to pass the scope through a tight ureter. The main advantage is the ability to pass stone extraction devices through wide working channels and high irrigation flow significantly improving visibility. In the last years we observe many efforts to increase the disintegration rate while lowering the morbidity[54,55]. However, even with this improvements reported SFRs are lower in comparison to fRIRS (90% *vs* 81.8%)[50,51,53,54,56]. On the other hand the number of ancillary procedures is inferior for rRIRS (see Table 1)[51]. The costs for rRIRS are lower than for fRIRS strongly depending on scope damages during procedure.

One of the most important questions regarding urolithiasis therapy has still to be answered. "Can we achieve high stone free rates with low morbidity only in experienced institutions specialized in urinary stone treatment or is it also feasible for all centres".

HOW TO ASSESS TREATMENT SUCESS?

The answer seems to be simple at first sight–lack of stones after the procedure. In most cases stone free status is estimated on the basis of ultrasound and X-ray, rarely on computed tomography (CT). It was shown that results documented by CT and ultrasound + X-ray may tremendously differ (62.3% *vs* 20.8%) in the same treatment group[57]. Noncontrast enhanced computer tomography (NCCT) has become a new diagnostic standard for evaluation of acute flank pain. Its sensitivity for identifying urinary stones was estimated by 96%[58]. Sensitivity of ultrasound for identifying renal stones over 5 mm is also 96%[59]. X-ray is used mainly due to its high specificity (80%-87%) in detection of urolithiasis[60]. Taking into consideration the abovementioned facts one may think that X-ray and ultrasound could be equal to CT in identifying significant residual stones (> 4 mm). Indeed this is not true. Park *et al*[57] in their study shown that almost 50% of stones over 4 mm in diameter are visible on NCCT and are not visualized on X-ray (mean size 7.4 mm). These facts strongly support the need for performing NCCT to assess residual stones after lithotripsy. It is also very difficult to compare the results of studies where other than NCCT diagnostic methods of efficacy were applied.

The definitions of stone SFRs are various. Some authors conservatively consider a stone free status as a renal pelvis free of any remaining fragments. Some are more liberal and treat insignificant stones as no stones at all. That concept of insignificant stone is based on statistics which states that almost all stones < 3 mm are freely expellable. On the other hand, some date suggest, that even small persistent calculi might accelerate stone formation and significantly shorten recurrence free intervals.

At last, appropriate scheduling for postoperative evaluation and imaging is crucial. It was shown that up to 25% of patients may become stone free when assessed 1 mo postoperatively in comparison to a group examined one day after a rRIRS intervention[50].

WHICH TECHNIQUE SHOULD BE CHOSEN FOR KIDNEY STONES OVER 2 CM?

The last EAU guidelines on urolithiasis recommend endourology for the treatment of > 2 cm renal calculi[8,9]. Nowadays, a wide spectrum of procedures and therapeutic modalities is available and allows the surgeon to offer an individualized treatment strategy to the patients taking into account all relevant clinical and patient-related parameters. The patient should also be well informed about advantages and disadvantages of each option and be involved in the decision making process. While many

patients choose PNL as widely established standard for treatment of a > 2 cm kidney stone, others may benefit from less invasive procedures accepting lower efficacy and necessity for ancillary procedures. The summary of abovementioned procedures is given in Table 1.

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REVIEW

Mesenchymal stem cells for kidney transplantation

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Abstract

The long term consequence of immunosuppressive therapy in kidney transplantation has prompted investigation of alternative means to modify the immune response to the allograft. Cell based therapies are potentially attractive as they may provide a long lasting immunomodulatory effect, may repair tissues and reduce the necessity to take immunosuppressive drug therapy. Of the current cell therapies, mesenchymal stem cells have now been trialled in small numbers of human kidney transplantation with apparent safety and potential efficacy. Many issues however need to be resolved before these cells will become mainstays of transplant immunosuppression including *ex vivo* modification to enhance immunomodulatory properties, cell number, route and frequency of administration as well as cellular source of origin.

Key words: Mesenchymal stem cells; Kidney transplantation; Immunosuppression; Solid organ transplantation; Cellular therapies

Core tip: This review summaries several of the most prominent cellular therapies currently being examined for use in immunosuppression. From the current evidence the reviewers make the argument that mesenchymal stem cells offer the best chance of a useful and functional cellular therapy for solid organ transplantation.

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INTRODUCTION

Kidney transplantation remains the optimal treatment for end stage renal disease (ESRD) providing excellent short term outcome with greater quality of life than that provided by dialysis[1]. Whilst short term graft survival is improving and acute rejection rates are dropping long term graft survival rates remain a major focus for clinical improvement. There are many factors that can impact the prognosis of a kidney transplant, from graft or donor considerations[2,3], factors involving the immunosuppressant regime[4,5], and issues concerning the recipient[6,7].

Tissue typing and stringent exclusion criteria are implemented pre-transplant to reduce the risk of donor related problems[3]. Issues with the recipient such as non-compliance and co-morbidity are much more difficult to manage and are often beyond a clinician's power to control[6,7].

When a suitable kidney donor is found, it is then important to make sure that the graft does not reject by suppressing the recipient's immune system. Current immunosuppressive drugs may be classified into five groups based on their mechanism of action: (1) regulators of gene expression; (2) alkylating agents; (3) inhibitors of de novo purine synthesis; (4) inhibitors of de novo pyrimidine synthesis; and (5) inhibitors of kinases and phosphatases[5]. Targeting each of these mecha-

nisms has its benefits and disadvantages and tailoring a drug schedule has the potential to impact long term graft function and the quality of life of the recipient. However all current drugs are associated with a range of adverse effects including renal toxicity, opportunistic infections, development of malignancy and metabolic complications[5]. A common trait among all these drug classes is the targeting of T cell function[5,8-10]. T cells play an important role in rejection via alloantigen recognition and the direction of an effector response that results in graft damage and dysfunction[11].

Of these issues it is the modification of immunosuppression that is an obvious place to try and improve patient outcomes, as more options will allow for customised treatment programs unique to each patients needs. Towards this end, there has been a recent increase in the development of alternative means of immunosuppression for organ transplantation. Utilizing cell-based therapies for immunosuppression is an alternative approach to traditional pharmacological methods and represents a change in paradigm for transplantation therapies.

CELL THERAPIES FOR ORGAN TRANSPLANTATION

The basic concept of cell therapy is to implant cells with desired properties into a patient in an attempt to treat or cure. Although this idea has been around since the 19th century, it was not until 1968 that it became a viable treatment with the first bone marrow transplant[12]. Since then, there has been a steady expansion in the type of cells transplanted and the conditions that can be treated. The purpose of this review is to examine the state of several cell types that are being evaluated for preclinical or early clinical trials in solid organ transplantation (SOT), including; T regulatory cells (Tregs), dendritic cells (DCs), and with a particular emphasis on mesenchymal stem cells (MSCs) which have shown the greatest progress and potential as a cellular therapy.

RGULATORY T CELLS

Tregs are naturally occurring T cells which express the cell surface markers $CD4^+CD25^+$ $FoxP3^+$ and a variety of differing cell surface markers (CD127, Helios)[13,14]. Tregs are concerned with the maintenance of immunological self-tolerance by suppressing self-reactive lymphocytes that escape clonal deletion[14]. Naturally occurring Tregs are formed from naive T cells in the thymus. However these naive T cells can be converted to Tregs *in vitro* using TGF-β induction of FoxP3[15], providing a second source of Tregs for cell therapy.

Tregs are able to suppress the immune system on many levels, combining inhibitory cytokine secretion(*e.g.*, *via* TGF-β, IL-10)[16,17], cytotoxicity and inhibition of NK cells[18,19], and direct modulation of antigen presenting cells[20-22]. This multifaceted approach to immunosuppression makes Tregs a promising therapy to facilitate long term graft survival. Recently there have been advances in the methods for Treg isolation and expansion, with large scale expansion from peripheral blood (PB), umbilical cord blood (UBC), and induced Tregs from naive peripheral blood precursors[23]. There have also been positive results from experimental animal models[24]. Of greatest interest are the clinical trials that have used Tregs as a cellular therapy in graft-*vs*-host disease (GVHD), a major and potentially lethal transplant complication that is particularly prevalent in patients who have undergone a hematopoietic stem cell transplant (HSTC)[25,26]. With generally positive outcomes from the GVHD trials[26], it is likely that we will see Tregs initially deployed as an adjunctive therapy in SOT before being used in patients who have a high risk of rejection or who have already experienced adverse effects from standard immunosuppression. This would allow for the efficacy of Tregs to be determined in a way that would be ethical and pose a minimal risk of complications.

In addition to their safety, there are several other important issues that need to be addressed in the pursuit of an effective Treg based therapy. As mentioned above, there have been advances in the isolation and expansion of Tregs. These advances go some way to addressing the large number of cells that would be required for an effective therapy, with some estimates placing the required number at 11×10^8 cells/kg[27]. Another concern is the source of the Tregs. Currently, the most appropriate source for therapy is unknown, with uncertainty focused on whether alloantigen or antibody mediated expansion is the safest and most effective method[23]. The stability of Tregs *in vivo* has also been found to be problematic with studies finding that Tregs can lose FoxP3 expression and develop an effector cell phenotype, becoming pathogenic[28]. Of relevance to the previous point about the source of Tregs is evidence suggesting that induced Tregs lose FoxP3 expression at a much higher rate than natural Tregs[29,30]. These are just a few of the issues surrounding the use of Tregs for SOT that the ONE study (www.theonestudy.org) hopes to address. Currently the ONE study is examining the use of polyclonally expanded Tregs and alloantigen driven Tregs in kidney transplantation at doses of 1, 3, 6 and 10 $\times 10^6$ Tregs/kg. As of writing this no results have been published[23].

DENDRITIC CELLS

Dendritic cells (DCs) are able to function as antigen presenting cells that drive graft rejection (immunogenic DC) or have a role in promoting graft acceptance (tolerogenic DC; TolDC) depending on their state[31]. Immunogenic DCs cause T cell activation and proliferation with the use of three signals: (1) they present antigens on MHC molecules; (2) They provide co-stimulatory molecules; and (3) they secrete pro-inflammatory molecules. Only when all three signals are present can DCs activate T cells[31]. TolDCs are also able to interact with regulatory

T cells to promote immune tolerance. The role that DCs play in immune tolerance is twofold. Firstly, they play a role in the deletion of self-reactive thymocytes in the thymus[32]. Secondly, and of relevance to transplantation, they aid in peripheral tolerance. They do this by the presentation of antigens while lacking the co-stimulatory molecules required for T cell activation[32,33]. This causes T cell unresponsiveness as well as Treg induction[33].

Two strategies for the use of TolDCs in transplantation are likely to be applied in the setting of allotransplantation. The first involves negative immunization by administering either autologous DCs that have been exposed to alloantigens or donor derived DCs, pre-transplant[34]. The second method involves the use of recipient derived DCs delivered on the day of transplantation[35]. Intravenous injection of immature DCs of either donor or recipient origin at the time of transplantation have prolonged allograft survival in SOT models[36]. There is a large amount of literature on the use of DCs in pre-clinical experimental models[36,37]. Clinical trials looking at DCs have been carried out in both type-1 diabetes[38] and rheumatoid arthritis[39]. This has shown that the use of DCs for immunomodulation is safe and effective.

Many of the issues that face Tregs are also pertinent in the consideration of DCs as a cellular therapy. Cell dose and the best method for the isolation and expansion of the cells is uncertain. The use of either recipient derived DCs or donor DCs is yet to be resolved and adding additional complexity to this issue is the question of negative immunization vs. recipient derived DCs delivered peri-transplant. Again, the ONE study aims to answer these questions and early trials of DCs in SOT are ongoing as of writing this.

MESENCHYMAL STEM CELLS

Mesenchymal stem cells (MSCs) are a multipotent cell lineage that has great potential for use in cellular therapies and is already being widely tested in clinical trials. www.clinicaltrials.gov currently lists 396 studies using MSCs in conditions such as spinal cord injury, diabetes, Alzheimer's disease, and kidney injury.

The International Society for Cellular Therapy (ISCT) has set the minimal criteria for defining MSCs as being plastic adherent, capable of differentiation into osteoblasts, adipocytes, and chondroblasts, and expressing CD105, CD73, and CD90 while lacking expression of CD45, CD34, CD14 or CD11b, CD19, and HLA-DR surface molecules[40].

MSCs are capable of being isolated from many tissues including bone, fat, and placenta. When cultured they adhere to plastic and have a fibroblast-like appearance, possessing a long, thin body and a small number of protrusions[40]. MSCs have a role in the formation and homeostasis of connective and structural tissues *via* the production of extracellular matrix, stabilization and regulation of the tissue vascularisation, and the creation of new connective tissue cells[41,42]. In addition to this, they also play a role in the immune system by inducing tolerogenic[42] properties that can be enhanced by *in vitro* treatment[43]. These roles are able to be exploited to aid in regenerative medicine and in immunosuppression. Combined with the many tissues from which they can be isolated and their ability to remain stable while being expanded *in vitro*[44] it becomes clear why so much work is now being carried out using MSCs for a large number of clinical applications.

The immunosuppressive abilities of MSCs are mediated by either nitric oxide synthase (iNOS) in mice[45,46], or indolamine 2,3-dioxygenase (IDO) in humans[46]. iNOS results in the production of nitric oxide (NO) which is an immunosuppressive agent in high concentrations[47]. Alternatively, IDO degrades the essential amino acid tryptophan thereby resulting in immunosuppression. The accumulation of the tryptophan metabolite kynurenine is also known to mediate the immunoregulatory effects of MSCs[48].

The exact mechanisms of how two pathways cause immunosuppression are not fully understood. In addition to these key factors, there are several immunosuppressive molecules secreted by MSCs. These include; PGE-2, IL-10, HO-1, PD-L1, and IL-6[49].

In reaction to stimulus from interferon-gamma (IFN-γ and proinflammatory cytokines, MSCs also secrete chemokines and adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)[45]. This results in a close proximity of immune cells allowing the local immunosuppressive environment to have a more pronounced effect[49].

A substantial amount of work has been focused on the potential for MSCs to treat GVHD. Ringdén *et al*[50] treated 8 patients, who had developed steroid-refractory GVHD, with bone marrow derived MSCs. In 6 of these patients acute GVHD ameliorated. The same group later went on to perform a phase Ⅱ trial consisting of 55 patients with acute GVHD. In this trial, 30 patients completely recovered from GVHD and a further 9 showed improvement. None of the patients developed adverse reactions due to the administration of MSCs[51]. Another phase one trial administering MSCs for GVHD was carried out by Introna *et al*[52] This multicentre study looked at 40 patients (15 children and 25 adults) with steroid resistant GVHD and gave them a median of 3 third-party derived MSCs infusions. Here it was found that the MSCs had a 67.5% T cell mediated response rate with a 27.5% complete response, 86 adverse effects were reported however most of these were of an infectious nature (72.1%) and not due to the administration of MSCs[52]. They concluded that MSCs could safely be administered in addition to conventional immunosuppression (*e.g.*, cyclosporin, steroid). Despite these positive results, there is some concern over a phase III clinical trial that failed to meet its primary clinical end point (NCT00366145)[53]. In this trial, patients received 8 infusions of 2×10^6 cells/kg over 4 wk and 4 more infusions administered weekly after 28 d. The trial did not meet its primary end point of a significant increase of

complete response of steroid resistant GVHD. Galipeau *et al*[54] provides a comprehensive failure analysis of the trial. The main conclusion of this analysis is that there are significant differences between the Martin study and studies from Europe that could account for the failure, in particular the passage number of the cells used[54]. As such, this study is not damning of MSCs but rather provides more areas that require examination before they can be used more widely.

Unlike the other cell types, there are now completed early clinical trials that have deployed MSCs as a therapy for SOT. The largest comes from Tan *et al*[55] In their trial they had 159 kidney transplant patients split into 3 groups, with 2 groups receiving autologous MSCs with either standard dose calcineurin inhibitors (CNIs) or low dose CNIs and the control group receiving standard dose CNIs and anti-IL-2 receptor antibody. The major conclusions from this study were that the MSC groups had a lower incidence of glucocorticoid-resistant rejection, a faster recovery in renal function, and significantly decreased risk of opportunistic infections than the control group[55]. This study also addresses safety concerns over the use of MSCs as there were no adverse reactions reported in either of the test groups. However this trial was not without its problems. It was noted by the authors that the number of rejection episodes in the control group was higher than what would be expected. This made it appear that the MSC groups performed better than standard immunosuppression when this may not be the case[55]. Additionally, the major differences in graft function were only noticed in the first 2 wk. It is conceivable that this was due the regenerative abilities of MSCs repairing the reperfusion injury associated with all kidney transplants. And lastly, the major difference in opportunistic infections was noted in the MSC and low dose CNI group. As there was no control low dose CNI group, we cannot be certain that the observed reduction in infection is due to MSCs or simply due to the reduced use of immunosuppressive drugs.

In addition to the work from Tan there have been several case reports looking at the use of MSCs in a small number of SOT patients. Perico *et al*[56,57] have performed two pilot studies looking at the use of MSCs in kidney transplantation in 4 patients. In their first study they administered intravenous autologous MSCs 7 d after transplantation and followed the patients for 360 d. From days 7 to 14 post transplant, serum creatinine increased in 1 of their patients, however acute graft rejection was excluded *via* biopsy. They also noted an increase in patient Tregs and a decrease in T cell expansion post-transplant. Long term, both patients showed stable graft and the authors concluded that MSC infusion in kidney transplant recipients is feasible, allows increase of Treg in the peripheral blood, and controls memory $CD8^+$ T cell function[57]. In their second trial, they dosed two living-related kidney transplant recipients with autologous MSCs one day before transplantation. The change in dosing time was an attempt to avoid the acute graft deterioration observed to be caused by intragraft localization of MSCs when dosing 7 d post-transplant. Although both patients had no side effects to the MSC infusion and both had stable graft function at 12 mo, one of their patients did have an acute rejection episode 14 d post-transplant that was resolved with corticosteroid therapy[57]. The authors attribute the rejection episode to a higher number of HLA mismatches. They concluded that pre-transplant administration of MSCs avoided the cell induced graft dysfunction associated with post-transplant MSC administration and that this method is favourable for future trials. Peng *et al*[58] examined the effect of autologous MSCs on renal transplants by giving 6 patients MSCs combined with half doses of tacrolimus and comparing acute rejection, graft function, and graft survival at 12 mo to a control group of 6 patients receiving standard dose tacrolimus. The results of this showed no toxic adverse effects associated with MSC infusion and all patients survived with stable graft function to 12 mo with only 1 acute rejection episode in the control group. The one difference they did notice was elevated B-cell counts in the MSC group at 3 mo compared to the control[58]. They concluded that MSCs may provide benefits in renal transplantation by reducing the required dose of conventional immunosuppressive drug that is required for long term graft survival. The results of these case reports are consistent with those of the Tan study, with no adverse reactions, stable graft function, reduced rejection, and the ability to lower maintenance immunosuppression (Table 1).

From these early clinical trials, summarised in Table 1, it is evident that MSCs have an acceptable safety profile and have beneficial effects for transplantation. There still remain several very important questions to be answered before MSCs can obtain mainstream clinical use. The issue of whether autologous or allogeneic MSCs are better is significant, with arguments for both being put forward. Tan *et al*[55] employed autologous MSCs because of the issues surrounding MSC isolation from deceased donors. Furthermore, the use of autologous MSCs would avoid any potential for rejection of the cells and a subsequent loss of their function. However, there is some evidence that MSCs are immuno-evasive allowing them to escape recognition by the hosts immune system[59]. If this is the case then allogeneic MSCs are promising as obtaining them will not impact the eventual recipient who may have serious health issues that could be exacerbated by the collection of MSCs or could impact the quality of the MSCs. The immuno-evasive status of MSCs also opens up the potential for third party derived MSCs. This would invalidate concerns about obtaining MSCs in the cases of deceased donors. Nevertheless, issues pertaining to the immunogenicity of allogeneic or third-party derived MSCs has not been substantially addressed *in vivo* and have not been addressed in large animal models. There are preclinical studies demonstrating that allogeneic MSC monotherapy alone failed to prevent allograft rejection[60-69]. Studies reporting on the benefits of allogeneic MSCs have also shown short term prolongation of graft

Table 1 Summary of clinical trials using mesenchymal stem cells in kidney transplantation

Ref.	Patient number	Cell number	Cell source	Adverse reactions	Graft survival
Tan *et al*[55], 2012	106	1-2 × 10^6 cells/kg	Autologous, bone marrow	None	100% at 1 yr
Perico *et al*[56], 2011	2	2 × 10^6 cells/kg	Autologous, bone marrow	Acute graft dysfunction	100% at 360 d
Perico *et al*[57], 2013	2	2 × 10^6 cells/kg	Autologous, bone marrow	HLA induced rejection	100% at 1 yr
Peng *et al*[58], 2013	6	5 × 10^6 1st dose 2 × 10^6 cells/kg 2nd dose	Donor derived, bone marrow	None	100% at 1 yr

HLA: Human leukocyte antigen.

survival[64]. More importantly, in some studies, pre-transplant allogeneic MSC monotherapy accelerated allograft rejection thereby questioning the immunoprivileged status of MSC. There is evidence that allogeneic MSCs can trigger an anti-donor immune response resulting in accelerated allograft rejection[65-67]. The co-administration of allogeneic MSC with immunosuppressive drugs however showed better outcome of the allograft compared to MSC monotherapy[63,64,70-72]. Therefore, the synergistic effects of allogeneic MSC with immunosuppressive drugs need to be taken into consideration in MSC therapy. We have previously reviewed in detail the mechanisms associated with allogeneic or third-party derived MSC immunogenicity and the synergistic effects of MSC with immunosuppressive drugs, in Sivanathan *et al*[43]. Questions around the dose rate, the timing, the route of administration, what happens to the cells and what exactly the MSCs are doing and their mechanism of action still remain unanswered. Given the state of the field it is not possible to accurately speculate on the answers to these questions. Additionally there is the potential for the modification of MSCs that further expands the possible methods of application

MODIFYING MSC FOR ENHANCED IMMUNOSUPPRESSION

The *ex vivo* manipulation of MSCs with proinflammatory cytokines, particularly IFN-γ modification of MSC enhances the immunomodulatory, reparative and homing potential of MSCs[43]. The enhancement of these MSC properties would be beneficial in a transplant setting and may hasten the translation of MSC therapy into SOT patients.

Of key benefit, the priming of MSCs with IFN-γ is critical to active MSCs immunosuppressive function[73-75]. IFN-γ primed MSC have an enhanced ability to suppress T cell responses compared to untreated MSC[76-80]. Increase suppression of T cell responses is mediated by the induction of immunosuppressive factors such as iNOS andIDO[75,81]. IDO is also well known for its roles in preventing rejection and induction tolerance at the fetal-maternal interface[82]. In addition, MSC-expressed IDO have been shown to induce tolerogenic DCs and Tregs[83], which are two other cell based therapies that have gained significant interest in SOT, as we have discussed above. The upregulation of other MSC immunomodulatory factors, the enhancement of negative T cell signalling, the inhibition of proinflammatory T cell response and the increase in Tregs further support the benefits of administering IFN-γ primed MSC therapy for SOT.

Regardless of the potential therapeutic benefits of IFN-γ primed MSC therapy, it should be noted that IFN-γ upregulate MHC class I and induces MHC class II expression on MSCs[84-86]. This may render these cells more immunogenic in MHC-mismatched recipients[43], thereby decreasing their effectiveness at suppressing inflammation as reported in some studies[87,88]. Only two studies have directly addressed IFN-γ primed MSC immunogenicity *in vivo*[88,89] and this warrants further investigation. Thus, when considering IFN-γ primed MSC therapy, then administration of autologous MSC may be more beneficial. If allogeneic or third-party IFN-γ primed MSC were to be considered, the co-administration of these cells with immunosuppressive drugs would be necessary as an attempt to control anti-donor immune response towards MSC to enable MSCs to exert their beneficiary effects *in vivo*.

CONCLUSION

In summary, there are numerous cell based therapies that have shown potential for use in the immunomodulation of SOT in pre-clinical, small, and large animal models. Tregs and DCs have shown promise *in vitro* and in animal models as well as displaying safety and efficacy in clinical trials involving GVHD, diabetes, and rheumatoid arthritis. However, only MSCs have completed large clinical trials to date. MSC have shown the most promise having been tested in GVHD and in early clinical trials for kidney transplantation. Based on the GVHD experience and the early transplant work, it appears that MSC have an acceptable safety profile and potential therapeutic effect. However, much needs to be resolved, including the issue of autologous *vs* allogeneic (third party cells), frequency of administration and mechanism of action. The optimal immunosuppressive therapy to be co-administered should also be studied. The results from these early trials are positive but have presented numerous issues that need to be addressed before MSCs gain widespread clinical use.

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REVIEW

Comprehensive urodynamics: Being devoted to clinical urologic practice

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Abstract

As a combined electrophysiological system for evaluating the lower urinary tract (LUT), comprehensive urodynamics (UDS) aims at duplicating patient's micturition process, either normal or abnormal, and further seeking for possible causative origin, either neurogenic or non-neurogenic, in order to guide treatment. Through thorough analysis, some so-called cut-off values, for example, bladder outlet obstruction (BOO) degree or dyssynergic degree between the detrusor and sphincter, could be gained; however, in most cases, their qualitative description, such as stress urinary incontinence, idiopathic detrusor underactivity (DUA), detrusor overactivity (IDO), low compliance, and idiopathic sphincter overactivity (ISO), is more preferable and important. In aged neurologically intact male patients with symptoms of the LUT (LUTS) including benign prostatic hyperplasia, a combined UDS system, which coupled BOO with compliance, was constructed. The patients may be categorized into one of the seven subgroups, including equivocal or mild BOO with sphincter synergia with or without IDO (pattern A), equivocal or mild BOO with ISO (B), classic BOO with sphincter synergia (C) or ISO (D), BOO with only low compliance (E), BOO with both DUA and low compliance (F), and potential BOO with DUA (G). This new system can be used to optimize diagnosis and treatment according to a derived guideline diagram.

Key words: Detrusor overactivity; Electromyography; Sphincter overactivity; Stress urinary incontinence; Urodynamics; Urology

Core tip: Scant progress during the last 2 decades and poor prognostic value of urodynamics (UDS) for benign prostatic hyperplasia interventional therapy may come from some technological problems, here we mean the underestimation of the role of electromyogram and some shortcomings of the UDS technology. Based on individualized UDS evaluation of more than 9000 cases, some so-called cut-off values, for example, degrees of bladder outlet obstruction and dyssynergia between the detrusor and sphincter, could be gained; however, in most cases, their qualitative description, such as stress urinary incontinence, idiopathic detrusor underactivity, detrusor overactivity, low compliance, and idiopathic sphincter overactivity, is more preferable and important.

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INTRODUCTION

Comprehensive urodynamics (UDS) performed for stress urinary incontinence (SUI) female patients has been challenged by two recent published papers[1,2]. Does it take no good for SUI or patients with symptoms of the lower urinary tract (LUTS)? This was just the same

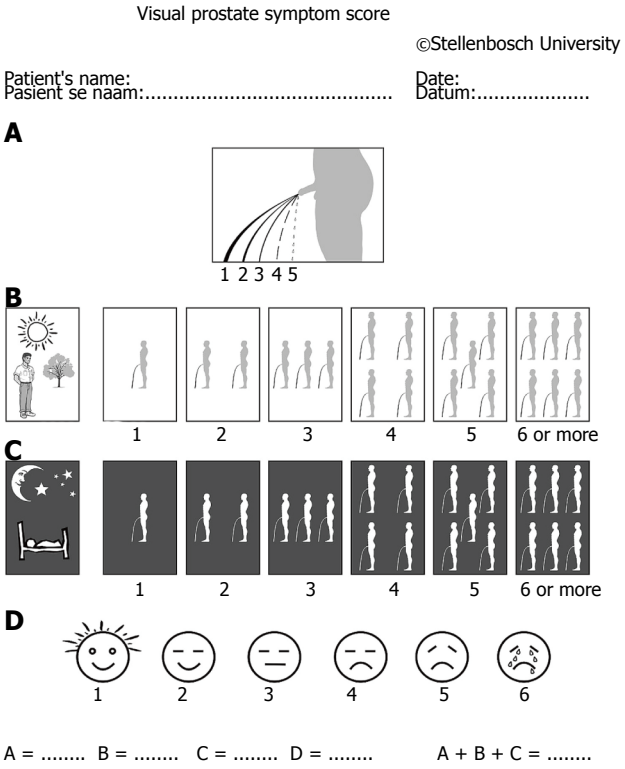

Figure 1 Visual prostate symptom score consisting of pictograms to evaluate (A) force of the urinary stream, (B) daytime frequency, (C) nocturia, and (D) quality of life[9].

as UDS for benign prostatic hyperplasia (BPH) patients in the late last century[3,4]. However, discerning experts indicated that the studies may have some common conceptual flaws, and there is a need to do more[5]. The authors therefore sighed, "scant progress has been made in the UDS recently[5]."

The NICE guidelines for the management of SUI[6] state that "the use of multichannel cystometry is not routinely recommended before surgery in women with a clearly defined clinical diagnosis of pure SUI". However, in a study of 6276 women with urinary incontinence, Agur *et al*[7] found that only 324 (5.2%) women had pure SUI. Although the symptomatic assessment had a specificity of 98%, its sensitivity was too low (11.4%).

As a combined electrophysiological system for evaluating the lower urinary tract (LUT), comprehensive UDS aims at duplicating patient's micturition process, either normal or abnormal, and further seeking for possible causative origin, either neurogenic or non-neurogenic, in order to guide treatment. Through thorough analysis, some so-called cut-off values, for example, bladder outlet obstruction (BOO) degree or dyssynergic degree between the detrusor and sphincter (TL value), could be gained; however, in most cases, their qualitative description, such as SUI, idiopathic detrusor underactivity (DUA), detrusor overactivity (IDO), low compliance, and idiopathic sphincter overactivity (ISO), is more preferable and important[8].

Whether a measuring system is inferior or non-inferior (*i.e.*, superior) is confirmed or based on large-sample population studies on one hand; however, decision on given patients should be made individually on the other hand. The important recording of electromyogram (EMG) was often absent in large sample trials and whether the patients had such finding as ISO or DUA was scant too[1,2,7]. Symptomatic analysis is usually preferable as compared with invasive measures, however, there are many uncertainties and variable factors. The most often used symptom score system IPSS (International Prostatic Score System) has been challenged by newly launched system VPSS (Visual Prostatic Score System) (Figure 1). A combination of VPSS > 8 and Qmax < 15 mL/s was used to select invasive evaluation during follow-up in men with urethral strictures[9]. Patients with bladder pain syndrome/interstitial cystitis (BPS/IC) and OAB patients had significant differences in their 3-d voiding diary records. Patients with BPS/IC had higher voiding frequencies and smaller maximal voided volume compared with OAB patients[10].

There are some differences between symptomatic and laboratory findings in clinical practice. Most clinical studies have relied on questionnaires as to the prevalence, symptoms, and treatment usage; however, the surveys must be interpreted with caution. So we should not let this prevent us from obtaining information *via* the use of laboratory tests including UDS combined with sphincter EMG. Furthermore, the symptom location is usually very factitious and clinical tests are necessary. For example, the pain caused by BPS/IC was often obscure. The study on BPS/IC prevalence could not find a single symptom-based definition of BPS/IC with ideal sensitivity and specificity to distinguish patients with BPS/IC from those with OAB, vulvodynia, or endometriosis[11]. The symptoms of irritable bowel syndrome, fibromyalgia, do overlap with those of BPS/IC[11]. The patients and doctors make every effort to reveal a sufficient description of the symptoms to prompt a rational diagnosis. The similarity of symptoms might have nosologic implications. If we want to locate the origin of symptoms and to validate the nature of the disease, necessary examinations, including UDS and EMG, had to be carried out[11].

The targets of invasive UDS test are: pursuing after completeness instead of simplicity[12] and selecting complete or appropriate UDS if feasible; clearly defining UDS entities and seeking cutoff values in order to subcategorize possible pathological process, although descriptive recording is often more preferable; and developing evidence-based or knowledge-based strategies based on UDS findings[13].

New studies should be performed using high quality UDS with possible cutoff parameters, and show treatment methods linked to UDS findings. This paper broadly reviews the fundamental concepts behind the technique, application, and interpretation of UDS testing and how they are applicable to general urologists in office settings. Some of the discussion is based on

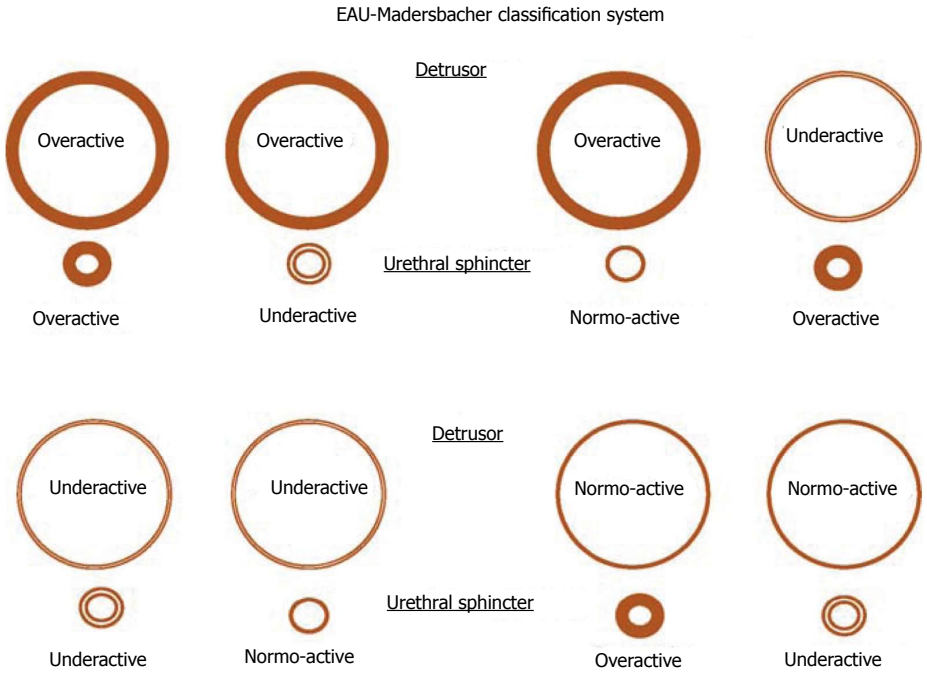

Figure 2 The European Association of Urology-Madersbacher classification system[15]. EAU: European Association of Urology.

the authors' experience and the existing literature in the field.

BASIC CONSIDERATION

Theoretical

The field of UDS has evolved significantly since its conceptual inception in the early twentieth century. UDS is a general term for a collection of techniques performed in an attempt to qualify and quantify the LUT activity during filling or storage, and emptying phases. Conceptually, normal, efficient bladder filling and storage require five components: (1) bladder compliance (distensibility); (2) bladder stability; (3) competence of ureterovesical junctions (*i.e.*, non-refluxing ureters); (4) closed vesical outlet at rest and during times of increased intra-abdominal pressure; and (5) appropriate bladder sensations. Bladder emptying requires: (1) constant detrusor contraction; (2) simultaneous relaxing of the smooth and striated sphincter; and (3) non-obstructed bladder outlet. Any abnormality of filling and storage or of emptying, regardless of causative pathophysiology, must result from a problem related to one of these factors. UDS studies can assist in categorizing and quantifying these problems[13]. Comprehensive UDS studies are a combination of noninvasive measures, such as initial uroflowmetry, and invasive measures, such as cystometrogram (CMG), pressure-flow study (PFS) with EMG, and urethral pressure profilometry (UPP). With the evolution of the personal computer, much development has been achieved in the field of UDS. The advent of smaller, less cumbersome, and less expensive machines has expanded the availability of complex UDS, including videourodynamics (VUDS) and ambulatory UDS (AUDS), to more practicing urologists[13,14].

PFS has been viewed as the urologic equivalent of cardiac catheterization[3]. These figurative words are right in some extent. There are still some differences between the two techniques: first of all, cardiac catheterization only needs patient's quiet recumbency, and not complex cooperation. UDS needs the patient's full cooperation to fulfill the process of storage and emptying. We would rather like to analog a patient as an actor or actress, and an urodynamicist as a director whose duty is to guide the patient to show his/her micturition process as actually as possible. Through their performance (complete storage and voiding behaviour, not only storage) by not only the leading role (detrusor), but also the co-star (sphincter), we could know whether the detrusor relaxes and the sphincter contracts during storage phase, and the detrusor contracts and the sphincter relaxes during empty phase or not, or vice versa. The guideline of normal detrusor and sphincter is "stretch out whenever necessary, and do not stretch out whenever unnecessary". Breach of the principle leads to dysfunction of the LUT: overactivity means "stretching out whenever unnecessary", and underactivity means "unable to stretch out whenever necessary". These demands and disability of the patients with potential lesions involving neurogenic system were exhibited in neurogenic LUTD guidelines (Madersbacher classification system)[15], in which the interrelationship or mutuality of the detrusor and sphincter was evaluated (Figure 2). In male patients, sphincter underactivity may exist clinically, however, its standard is not practical and data about its prevalence is scant. Furthermore in non-neurogenic LUTD, dysfunction of the detrusor or sphincter was presented separately, not integratedly as in neurogenic LUTD (NLUTD)[16] (Table 1).

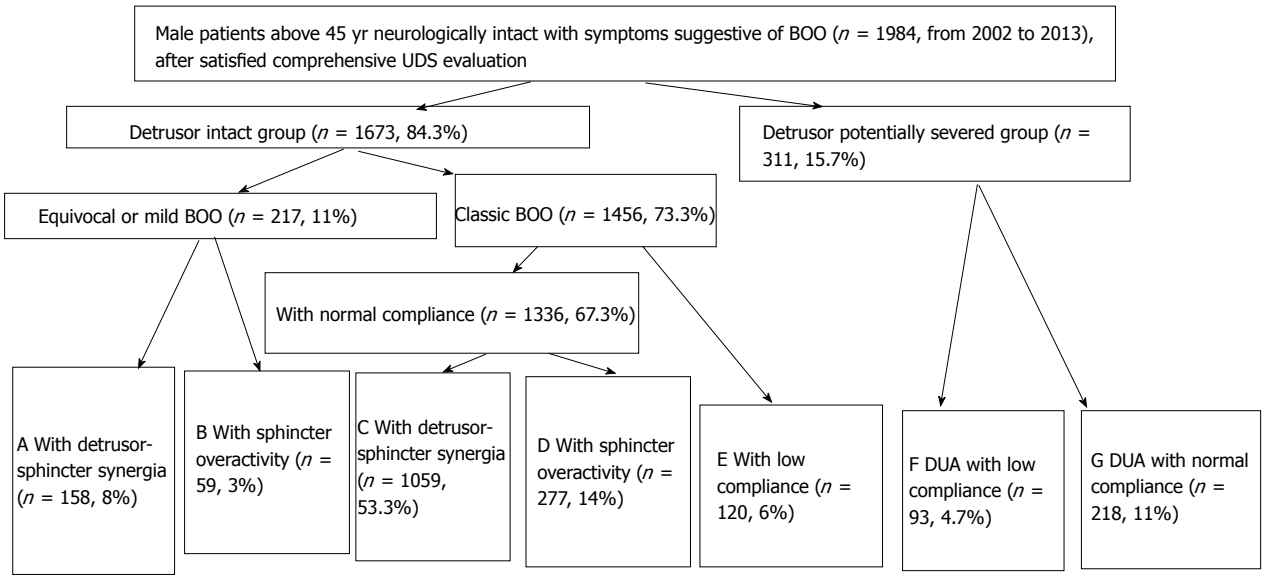

Figure 3 Study design, flow diagram and final outcome of male patients above 45 years neurologically intact with symptoms suggestive of male symptoms of lower urinary tract and bladder outlet obstruction[17]. UDS: Urodynamics; DUA: Detrusor underactivity; BOO: Bladder outlet obstruction.

Table 1 Expanded functional classification[16]

Failure to restore
Because of bladder
Detrusor hyperactivity
Involuntary contractions
Neurogenic diseases, injury, or degeneration
Bladder outlet obstruction
Inflammation
Idiopathic
Decreased compliance
Neurogenic disease
Fibrosis
Idiopathic
Detrusor hypersensitivity
Inflammatory
Infectious
Neurogenic
Psycologic
Idiopathic
Because of outlet
Stress incontinence (hypermobility related)
Nonfunctional bladder neck-proximal urethra (intrinsic sphincter dysfunction)
Failure to empty
Because of bladder
Neurogenic
Myogenic
Psycogenic
Idiopathic
Because of outlet
Anatomic
Prostatic obstruction
Bladder neck contracture
Urethral stricture
Urethral compression
Functional
Smooth sphincter dyssynergia
Striated sphincter dyssynergia

We think in urology practice, dysfunction of the detrusor, sphincter, detrusor compliance, incontinence state, and BOO had better been parceled on the basis of individualization. We have attempted this work and published a paper[17], which aimed at developing a UDS pattern system for aged male patients who complained of non-neurogenic LUTS to create a reference guideline for their diagnosis and treatment by a retrospective analysis. A retrospective analysis of UDS data was carried out in 1984 male patients neurologically intact with symptoms suggestive of BOO aged older than 45 years (2002-2013). On the basis of their UDS characteristic findings, the patients were classified into 1 of 7 subgroups: equivocal or mild BOO with sphincter synergia with or without IDO (pattern A); equivocal or mild BOO with ISO (B); classic BOO with sphincter synergia (C) or ISO (D); BOO with only detrusor low compliance (E); BOO with both DUA and low compliance (F); and equivocal BOO with DUA (G). The feasibility and rationality of this system were confirmed. The distribution of 7 patterns (pattern, case number, %) was A 158, 8%; B 59, 3%; C 1059, 53.3%; D 277, 14%; E 120, 6%; F 93, 4.7%; and G 218, 11% (Figures 3 and 4). The Abram-Griffiths (A-G) numbers (PdetQmax-2Qmax) in patterns C, D and E were 103.1-141.4, higher than those in other patterns ($P < 0.001$), and functional pressure lengths (FPL) in patterns C and D were 7.0-7.2 cm, longer than those in other patterns ($P < 0.001$). At last, a practical UDS pattern system for aged male patients with LUTS suggestive of BOO was constructed, which helps us to optimize the diagnosis and treatment[17]. For those with patterns A and B, medicinal therapy with 5a reductase inhibitor, antimuscarinics, or baclofen was administered first, whereas surgical intervention was reserved as an alternative option if medicinal therapy failed or their symptoms of BOO aggravated later. Those with patterns C, D and especially with E received transurethral resection of the prostate, and those with patterns F and G received 4-6

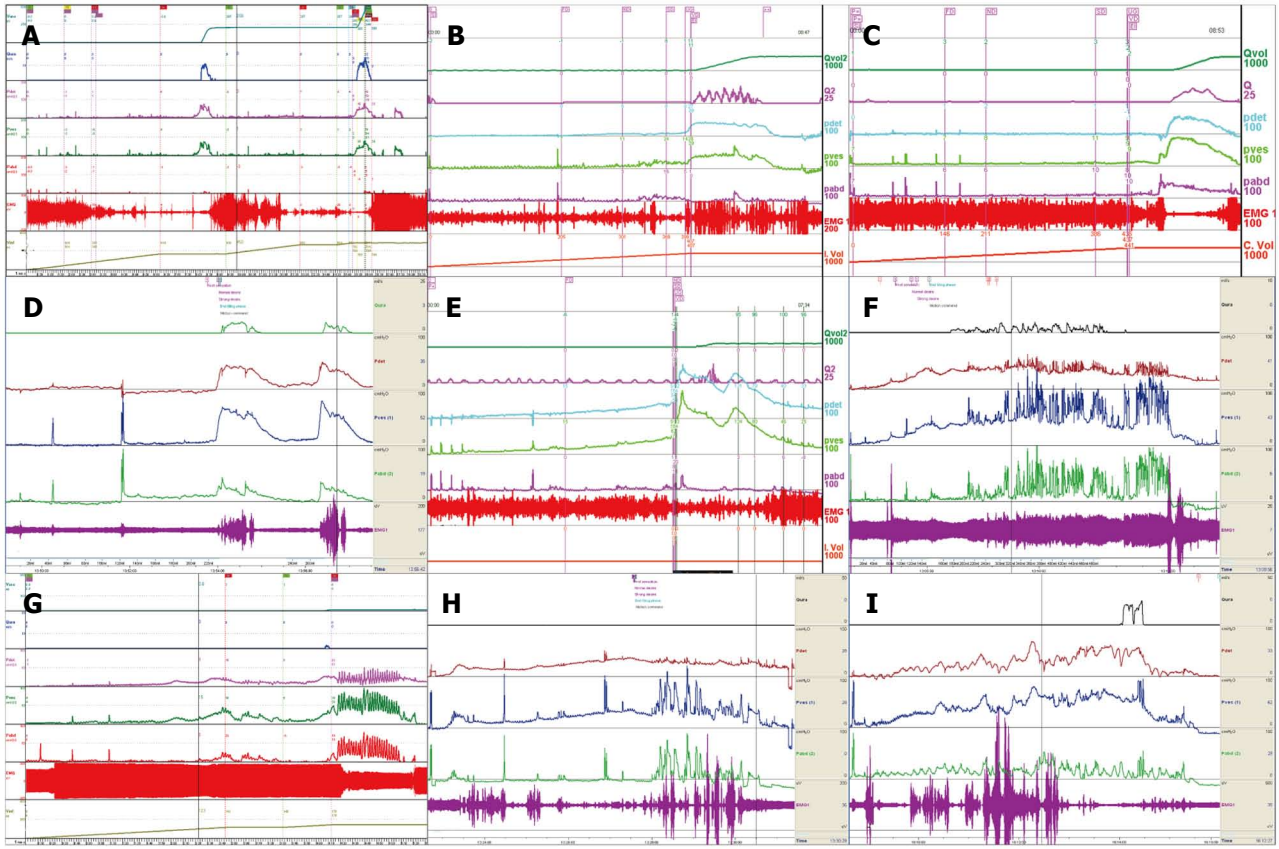

Figure 4 Urodynamic patterns of male patients older than 45 years neurologically intact with symptoms of lower urinary tract/bladder outlet obstruction[17]. A: Pattern A [equivocal or mild bladder outlet obstruction (BOO) with sphincter synergia with or without idiopathic detrusor overactivity]; B: Pattern B (equivocal or mild BOO with idiopathic sphincter overactivity); C: Pattern C (classic BOO with sphincter synergia); D: Pattern D (classic BOO with idiopathic sphincter overactivity); E: Pattern E (BOO with only detrusor low compliance); F: Pattern F (BOO with both detrusor underactivity and low compliance); G: Pattern G (equivocal BOO with detrusor underactivity); H: Urodynamic study of a patient with pattern F; I: The same patient with pattern F as in H after 4 wk urinary draining, whose detrusor contraction recovered and urodynamic study pattern was transformed into pattern E.

wk indwelled catheterization and administration of pyridostigmine bromide, baclofen, and decoction of Chinese medicinal herbs in an attempt to promote recovery of the detrusor contraction (Figure 5). These affordable principles may enrich the therapy of male LUTS using medical and/or conservative methods[18]. An integrated UDS pattern trial of 2195 female LUTS patients had been conducted, which revealed different results from those obtained from male patients[19]. At first they were divided into SUI and non-SUI groups and low compliance was seldom seen in neurologically intact women and was omitted in the calculation. At last, the distribution of UDS patterns were NA (normal detrusor-sphincter function) 50.2% (1101 cases), IDO 18.3% (401 cases), ISO 13% (286 cases), IDO + ISO 7.6% (167 cases), and DUA 10.9% (240 cases). We think the applicable principles from male population suit to female population too[19].

Technological

EMG technology: Scant progress during the last 2 decades and poor prognostic value of UDS for BPH interventional therapy may come from some technological problems, here we mean the underestimation of the role of EMG and some shortcomings of the UDS technology. Surface or patch electrode was routinely used as the standard option during UDS and the abnormal EMG findings were usually blamed as artifacts[20]. There are surface electrode, concentric needle electrode (CNE), and needle-guided wire electrode (simple as wire electrode[8]) available in practice. CNE was superior over surface electrode[21]. From our experience, needle guided wire electrodes were superior to CNE[22]. From our experience of about 9000 patients from 2002 to 2014, nearly 70%-80% so-called "artifacts" or bad recordings of EMG came from technical errors and we can record detrusor sphincter synergia or dyssynergia well using Solar from MMS (Netherlands), or Andromeda from Germany, as Janus from Life-tech (United States). We used the anal sphincter instead of the urethral sphincter to obtain EMG information[23]. Although some authors stated that anal and urethral sphincter EMG had the same significance[24], we believe that finding abnormal EMG signs in neurogenic diseases is more important than interpreting potential differences between the two routes. Anal external sphincter has a bigger mass than urethral external sphincter, less potential of pain and bleeding when needle is inserted into them, and less ar-

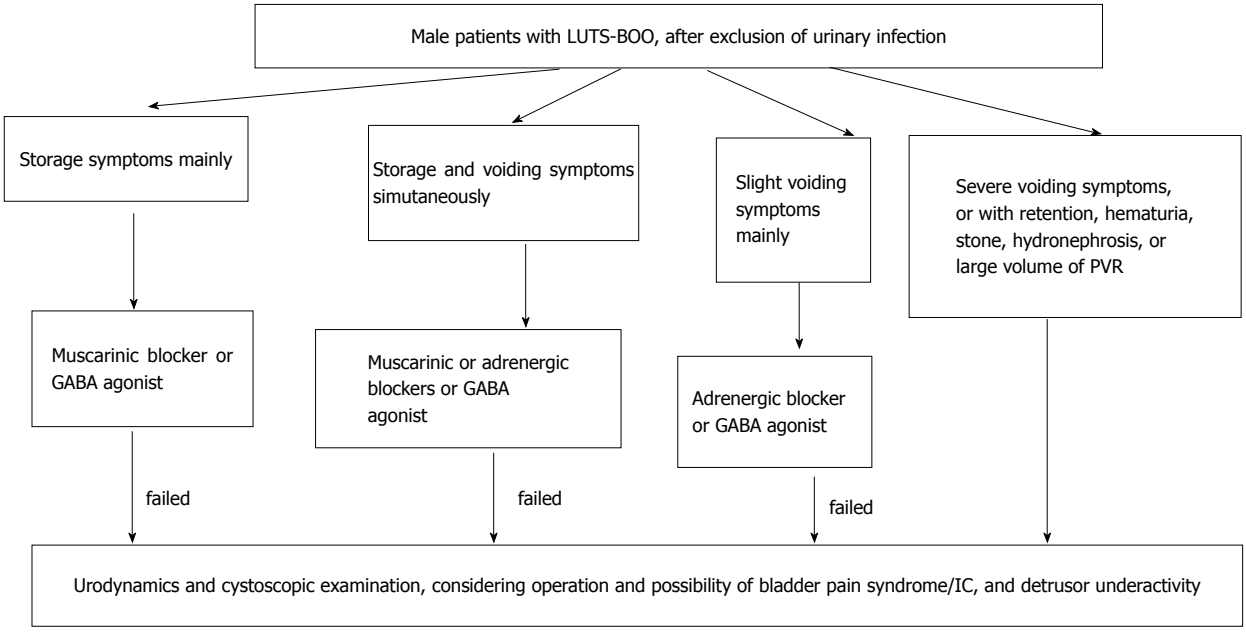

Figure 5 A proposed supplement to the guideline of male symptoms of lower urinary tract/bladder outlet obstruction[17]. BOO: Bladder outlet obstruction ; LUTS: Symptoms of the lower urinary tract.

tifact possibility. In our laboratory CNE, anal plug electrodes and urethral catheter containing concentric electrodes are abandoned because of lower accuracy. CNE has also two poles indeed: one in the core of the needle, the other in the outer coating of the needle.

Patient position during UDS procedures: The position should be as natural and physiologic as possible, and we prefer sitting for female patients, sitting or standing for male patients. For patients whose sitting or standing position is unfeasible, they may take the supine position on the UDS bed with the barrow together. If they could pass urine during PFS stage, the simultaneous imitation of urinary flow into the commode is conducted. For patients whose original position does not fit PFS, their position should be changed, *i.e.*, from sitting to squatting or standing. After position change, the same magnitude of increase of abdominal and bladder pressure produces, and their detrusor pressure is stable and no adjustment is required. The abdominal catheter may be ejected out by excessive abdominal strain during emptying phase in patients with DUA. If this happens, the abdominal pressure (*Pabd*) no longer works as a reference value for detrusor pressure, which means the difference between the vesical pressure *(Pves)* and *Pabd* (*Pdet* = *Pves-Pabd*). Measures should be taken to adjust *Pabd*, usually zeroing it.

UDS catheter and infusion medium selection: Catheters of UDS include urethral catheter and anal catheter. The former, either one, double or three-lumen, is usually afforded by appropriate companies and is disposable. Now an 8F double-lumen transurethral catheter (Xubu Medical Appliance Company, Dantu District, Jiangsu Province, China) is used for *Pves* recording and infusion of normal saline in our institution. The *Pabd* is recorded using a 12F transrectal balloon catheter (Cook Urological Incorporated, IN)[17]. The balloon covered with an envelope is lubricated and inserted into the anus and then semi-filled with saline, which assures satisfactory recording of the pressure. As far as the infusion medium is concerned, saline and air are used at the early stage, and normal saline is more suitable than air because of its physiologic property and ability to suit PFS[25].

UDS manufacturers: There are many UDS manufacturers or companies around the world, including MMS (Netherland), Life-Tech (United States), Andromeda (Germany), Leborie (Canada), Wearnes (Chengdu, China), *etc*. The design and performance are nearly the same. Our institution has the experience with products from all these companies.

CLINICAL CONSIDERATION

Early diagnosis and treatment are essential for patients with LUTS[15]. UDS findings, either as cut-off values or descriptive conclusions, may indicate possible lesions that later emerge, give preoperational forecasting of surgery for BOO, evaluate possible organic lesions other than functional origin, and main dysfunction sites, either the bladder or urethra or both.

LUTS followed by demonstrable abnormal UDS findings

Uroflowmetry curve: Uroflowmetry usually indicates whether the bladder outlet is obstructed or the detrusor is unable to contract. Here we report a special case with sustained urinary incontinence after birth and her low and smooth "uroflowmetry" curve represented infused fluid leakage. This was a 24-year-old unmarried Chinese

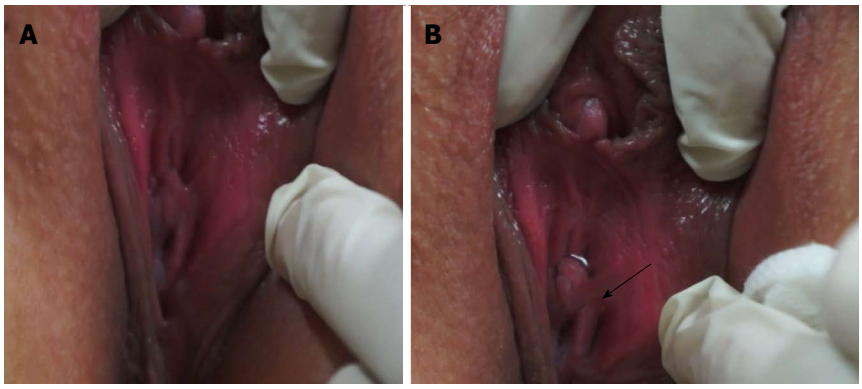

Figure 6 Perineal view of a female patient with urethral external sphincter agenesis before urodynamic evaluation. A: Urethral orifice and hymen as the urethral orifice was dried with gauze; B: Urine expelled out as solid arrow indicated.

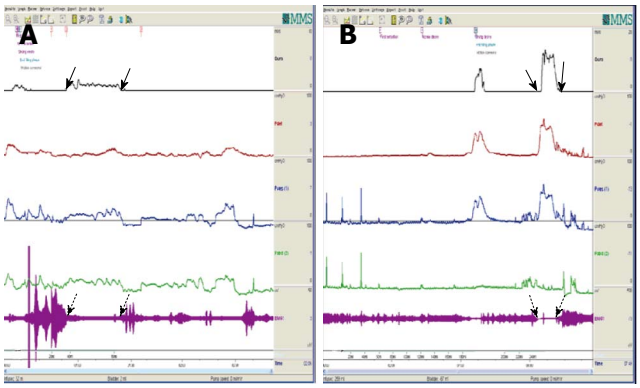

Figure 7 Comprehensive urodynamic evaluation of a patient with urethral sphincter agenesis. A: As saline was infused into the bladder, saline and her urine were expelled steadily (solid arrows) in the initial examination, however, during this phase, anal sphincter electromyogram showed synergia (as dotted arrows indicated); B: The storage and voiding function was recovered to normal and incontinence cured 4 mo after sphincteroplasty.

female referred for evaluation of congenital urinary incontinence. A transperitoneal anastomosis of both ureters to the bladder was carried out based on an assumption of ectopic ureteral orifices in the bladder neck and failed to cure her incontinence five years ago. She was neurologically normal and denied the presence of any other symptoms. She had good pelvic support and normal external genitals including the hymen. No abnormal neurologic signs were found. Ultrasonic test revealed that her kidneys, uterus, ovary and bladder were all normal. However, transrectal ultrasonography revealed that circular low echogenic zone of the peripheral region of the mid urethra, which represents the urethral striated sphincter[26], was absent. An intravenous urogram showed normal kidneys, ureters and bladder, too. There was no visible endoscopic evidence of vesicourethral lesions except for urinary leakage. When an inspection of her perineum in lithotomy position was performed, a rhythmic leakage of urine was observed, which was much like the way that urine jets ejected from ureteral orifices into the bladder being observed during cystoscopy both in rhythm and quantity (Figure 6). Each time she could only pass less than 30 mL with a maximum flow rate of 5 mL/s. UDS revealed that her bladder had no storage function at all, and we observed that more than 400 mL liquid was expelled after 360 mL normal saline had been infused at a rate of 50 mL/min. The flow or leakage rate was 1 mL/s, *i.e.*, 60 mL/min. During storage phase with virtually constant leakage, the detrusor had no contraction (Figure 7A), and as infusion ended, the anal sphincter EMG recovered to normal storage state (Figure 7A), which meant normal anal sphincter function, but not urethral sphincter function. Congenital urethral sphincter agenesis (much severe than intrinsic sphincter deficiency, ISD) was a supposed diagnosis. In order to reconstruct a functional urethral sphincter, urethral external sphincteroplasty using an autologous fascial sling which was obtained from her left fascia lata, was performed successfully. Double rolls of the sling around the urethra were formed, one fixed to the public union and the other to the sheath of the rectus. The patient achieved full continence thereafter and could pass urine ideally in an interval of 30 min to 2 h two weeks after the operation. At the follow-up 4 months after the procedure, she could pass urine with more than 250 mL in an interval of 2-3 h, and UDS showed that her detrusor could contract normally with a Qmax of 23 mL/s and her anal sphincter worked as preoperational status (Figure 7B).

Low-smooth uroflowmetry curve is also associated with constrictive BOO, for example, urethral stricture (Figure 8A). This type of BOO is different from compressive BOO, such as that due to BPH[27]. Constrictive BOO produces a decreased slope of the passive urethral resistance relation (PURR) curve and a normal minimal opening pressure (Pmuo) (Figure 8B), whereas compressive BOO produces a steep slope of the PURR curve and a higher Pmuo[27] (Figure 8C). In typical cases, low-smooth uroflowmetry curve and characteristic finding of constrictive BOO aid the diagnosis of urethral stricture, especially in female patients with a UTI history.

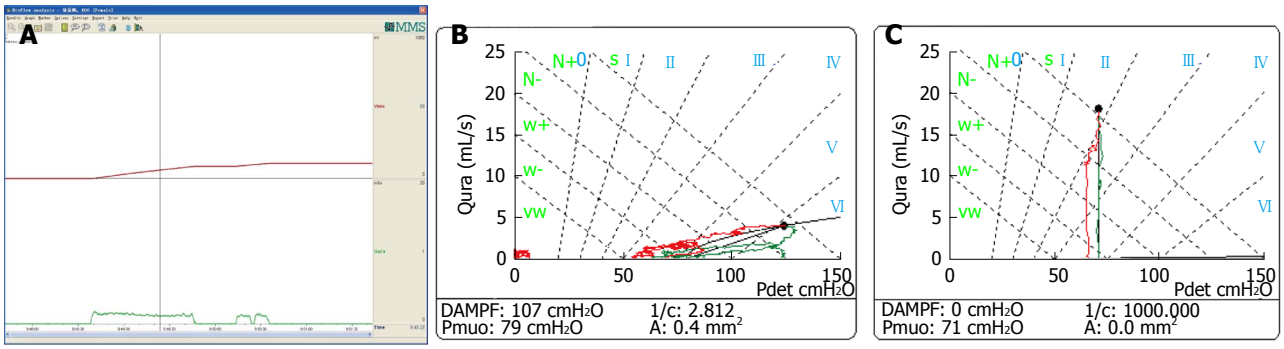

Figure 8 Low-smooth uroflowmetry curve in a female patient confirmed with urethral stricture, constrictive bladder outlet obstruction and compressive bladder outlet obstruction. A: Low-smooth uroflowmetry curve in a female patient confirmed with urethral stricture; B: Constrictive bladder outlet obstruction (BOO) produces a decreased slope of the passive urethral resistance relation (PURR) curve and a normal minimal opening pressure (Pmuo); C: Compressive BOO produces a steep slope of PURR curve and a higher Pmuo[25].

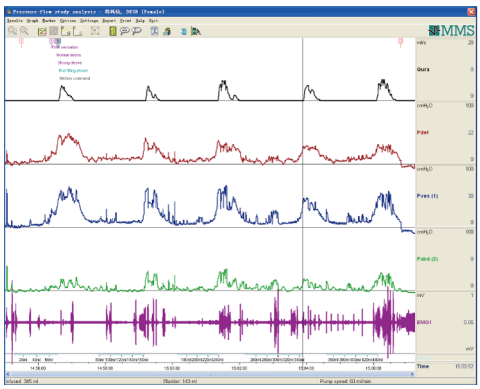

Figure 9 Repeated urodynamic curve of a female patient aged 69 years with urinary urgency and frequency for more than 20 years, proved as idiopathic sphincter overactivity with a functional bladder capacity of 80 mL and cured by baclofen 10 mg, *tid*. The characteristic findings of idiopathic sphincter overactivity were equivocal in the first and fourth urodynamic evaluations and obvious in the remaining ones.

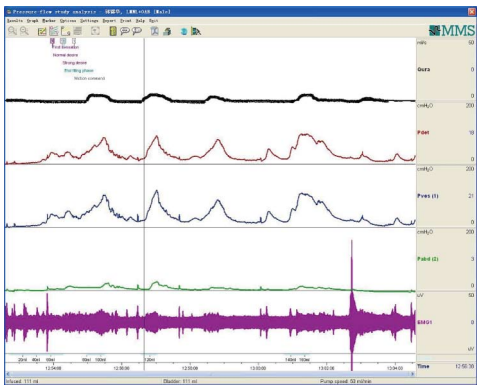

Figure 11 Repeated urodynamic curve of a male patient aged 37 years with urinary retention after lumbar tumor resection for one month, proved as neurogenic detrusor overactivity (*i.e.*, neurogenic detrusor hyperreflexia) and non-sympathetic overactivity (*i.e.*, detrusor-external sphincter dyssynergia).

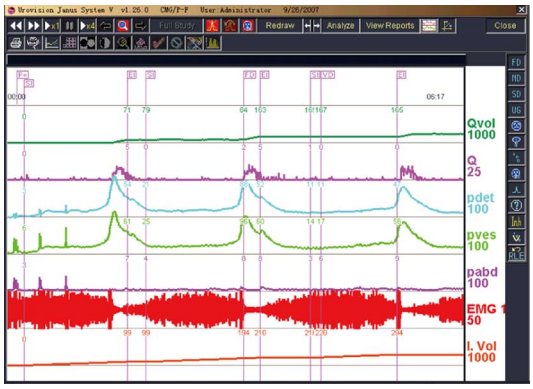

Figure 10 Repeated urodynamic curve of a male patient aged 80 years with urinary urgency and incontinence associated with acute myelitis for more than 11 years, proved as NDO, which had full response to administration of muscarinic blocker.

CMG-PFS-EMG curve: Normal CMG-PFS-EMG curve tells us how the bladder and urethra are performing storage function (bladder distention and urethra contraction) and how they are performing emptying function (at first the bladder contracts and the urethra actively relaxes[28], and then urinary flow produces). Active opening out of the urethra has major effects during emptying stage by stretching backwards the urethra and ceasing extension of urethral elasticity. So the external sphincter would actively relax during micturition: opening of the urethral tube, even to double the original diameter of the urethra[28]. After careful analysis and discussion of the UDS data, ideal options, such as IDO, ISO, DUA, SUI in LUTD, NDO, NSO (neurogenic sphincter overactivity, or detrusor-external sphincter dyssynergia, DESD), DUA in NLUTD, are produced. The process should be repeated more than one time if the curve is doubtful in order to gain a reproducible, stable and typical curve (Figures 9-11).

The main difference between NDO and IDO, or between NSO and ISO, is whether they are related to neurogenic origin or not. Grossly looking the patients may be non-neurogenic, just in the majority of patients with OAB (those with IDO), however, if more meticulous image examination, for example, brain functional magnetic resonance imaging (fMRI), is undertaken, some positive findings could be found in patients with OAB or LUTD responsive to Interstim (sacral neuromodula-

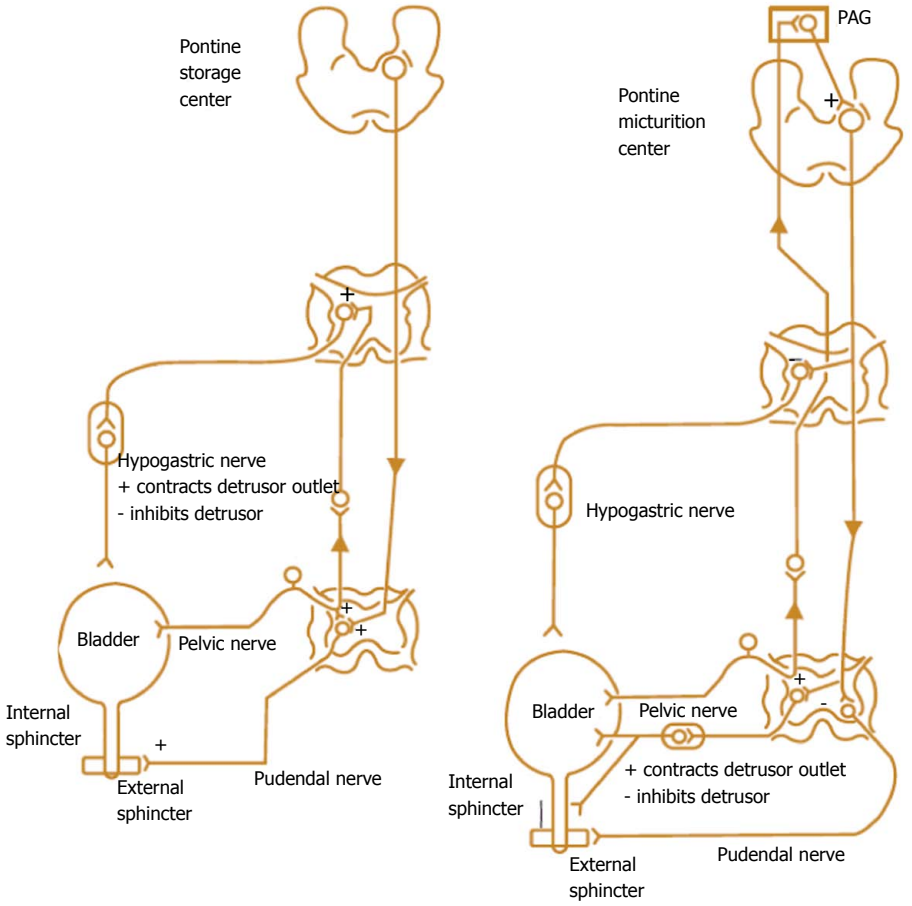

Figure 12 Nerve supply to the bladder and urethra and nerve signal transduction during storage and emptying phase[36]. PAG: Periaqueductal gray.

tion) treatment[29-31]. So many patients may be situated in the equivocal region between neurogenic and non-neurogenic LUTD.

There was some abnormal findings in fMRI in patients with Fowler's syndrome who were responsive to the Interstim procedure[29]. The primary abnormality of the syndrome may be an overactive urethra[29]. This central reflex and sacral guarding reflex have the same nature.

As far as small-vessel diseases of the brain affecting the deep white matter were concerned, they may be associated with some bladder abnormalities. Generally speaking, when we cared for elderly OAB patients, both the brain and the bladder should be looked at[30,31].

IDO and NDO may be treated by monotherapy or combination therapy[32-35]. And now, baclofen, a GABA agonist, may also go into the regimen[17,23]. Combination therapies with α1-blocker plus antimuscarinic, α1-blocker plus 5α-reductase inhibitor, α1-blocker plus PDE inhibitor, and α1-blocker plus 5-ARI have been attempted[32]. The pathophysiologic mechanisms and targets for pharmacotherapy for male LUTS, and nerve supply to the bladder and urethra are displayed below (Figures 12 and 13).

ISO and NSO are displayed by excellent EMG curves. Synonyms of ISO are dysfunctional voiding (DV), non-neurogenic neurogenic bladder, Fowler's syndrome, and Hinman syndrome[38-40]. Chronic idiopathic intestinal pseudo-obstruction (CIPO)[41] may co-exist with ISO.

In patients who complained of symptoms of frequency or urge may actually suffer from ISO, to which baclofen (a GABA-ergic receptor agonist) may be administered as a rational option and obtain good response shown by way of TL value[17,23].

Both storage and emptying symptoms may be caused by ISO. This double link could be explained by guarding reflex[8]. The storage symptom of the patient whose curve was shown in Figure 9 was resolved completely by administration of baclofen. Another female patient complained of urge incontinence for 10 yrs was also cured with baclofen after being confirmed as ISO (Figure 14). A woman aged 26 years complaining of poor-weak flow, voiding difficulty, intermittent or continuous catheterization for 18 years, and even receiving transurethral resection of the bladder neck twice, was also confirmed as ISO (Figure 15). She was eventually cured with baclofen too.

DUA and low compliance curve: DUA, either associated with NLUTS or LUTS, is known as detrusor underactivity, bladder underactivity, underactive bladder, or bladder underactivity/underactive bladder syndrome, either in analogy with the ICS definition of overactive bladder syndrome or not[42,43]. There are many different options as to its terminology, definition, and diagnostic methods. The approach is needed now to gain a consen-

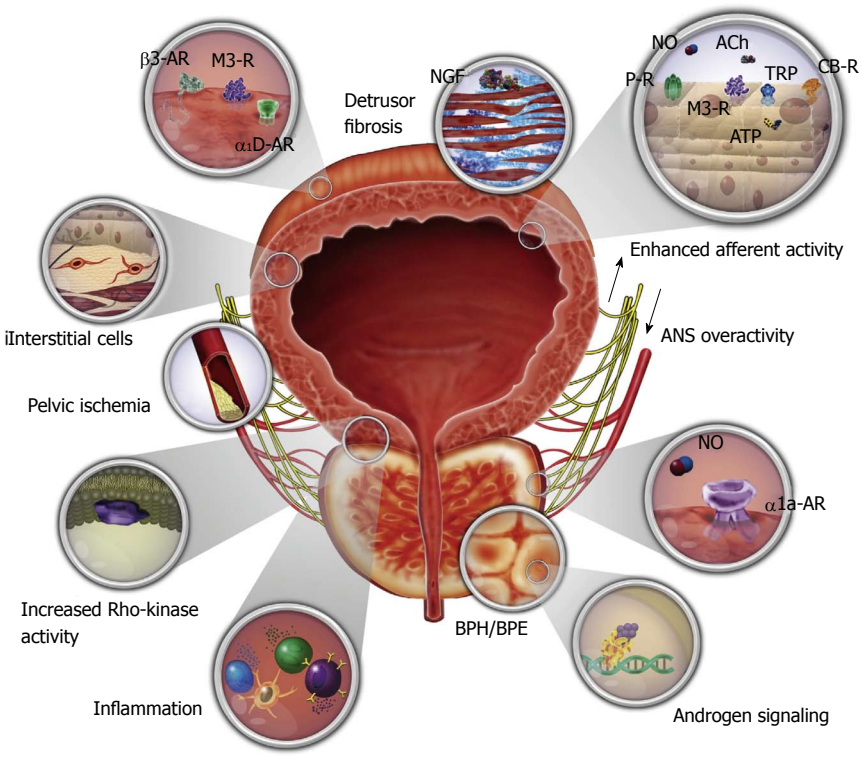

Figure 13 Various autonomic receptors of the bladder and urethra associated with pharmacotherapy for male symptoms of the lower urinary tract[37].

sus on these elements to allow standardisation of the literature and the development of optimal management[43]. No matter what the cause is, the main task of UDS is to show how the detrusor works: either unable to contract or relying on abdominal strain (Figure 16). DUA is associated with dysfunction of the sensory (afferent) nerve, the central nervous system (CNS), the efferent nerve and the target organ, the vesical detrusor itself. Furthermore, impaired voiding function has an age-associated prevalence[42] (Figure 17). As to the treatment of patients with DUA of any cause, the combination therapy (Chinese medicinal herbs, baclofen, and pyridostigmine) and continuous bladder drainage are proposed as feasible options[17]. After UDS, the patients with UDS patterns F and G could not pass urine at all and still needed catheterization, urinary diversion or even artificial urinary sphincter[44], but 56% and 12% of those with patterns F and G had their patterns changed into E or C, respectively, after such conservative treatment. The main function of Chinese medicinal herbs (common clubmoss herb, toothed achyranthes root, semen vaccariae, and so forth) were relaxing the urethral sphincter, decreasing outlet resistance, and promoting diuresis. Baclofen was used to relax the overactive sphincter, and pyridostigmine was used to strengthen the detrusor. And perhaps, the most important thing was absolute resting of the bladder without tube clamping for more than 4-6 wk[17]. Low frequency electrotherapy is also a rational option for selected female DUA patients suffering from neuromuscular deficiency[45].

Normal compliance is more than 10 mL/cmH_2O[46]. Low compliance could co-exist with or without DUA in patients with LUTS or NLUTS. The low bladder compliance patterns in patients with NLUTD had three groups[46]: gradual increase, Group A; terminal increase, Group B; abrupt increase and plateau, Group C. Careful analysis seeking for dominant disorder of the detrusor or sphincter is vital for the patients. Little or no detrusor contraction is needed for complete voiding in some women in whom normal sphincter relaxation is enough to finish the micturition process. These patients are considered to be "normal"[47]. This occult modality of DUA should be considered as asymptomatic DUA (Figure 18 A and B). Furthermore, in male patients with DUA and low compliance potentially related to neurogenic lesions, this pattern of voiding could occur too (Figure 18C).

Low compliance may lead to bilateral hydronephrosis in patients suffering from diabetes insipidus. We found that children with polyuria, nocturnal enuresis and MRI-confirmed pituitary abnormality (hypointensities on T1-weighted MRI) and diabetes insipidus usually had hydroureteronephrosis, enlarged bladder capacity and low bladder compliance at second-half storage phase. Their detrusor and sphincter function had to be evaluated carefully as the first procedure. If the detrusor could contract and sphincter could relax during the voiding phase, the prognosis is good (Figure 19), and vice versa.

Different opinions existed about the newly constructed somatic-autonomic reflex for patients complaining of dysuria and incontinence after spinal cord injury or with tethered spinal cord[48,49]. Whether the operation succeeds or not depends upon the exhibition of detrusor contraction and sphincter dyssynergia or synergia. We have shown the detrusor contraction with or without sphincter overactivity in some patients suffering from SCI who received a successful artificial somatic-autonomic reflex for bladder

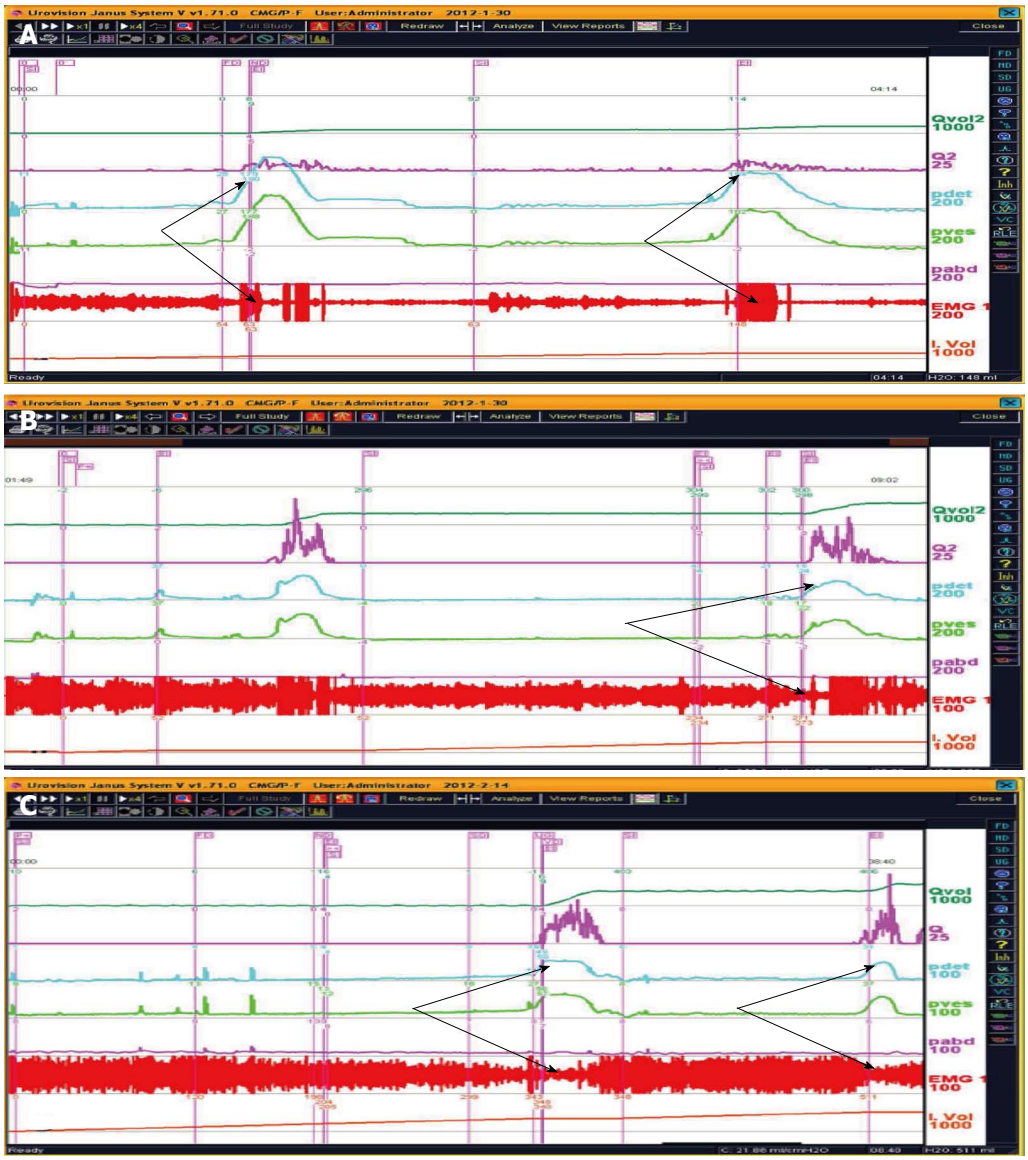

Figure 14 Severe urge incontinence caused by idiopathic sphincter overactivity was cured with baclofen. A: A 39-year-old woman presented with urge incontinence for 10 year. Urodynamics (UDS) showed that she had idiopathic sphincter overactivity (ISO); B: The ISO improved obviously after administration of baclofen for 2 wk; C: Six weeks after the first consultation, her ISO appearance disappeared absolutely.

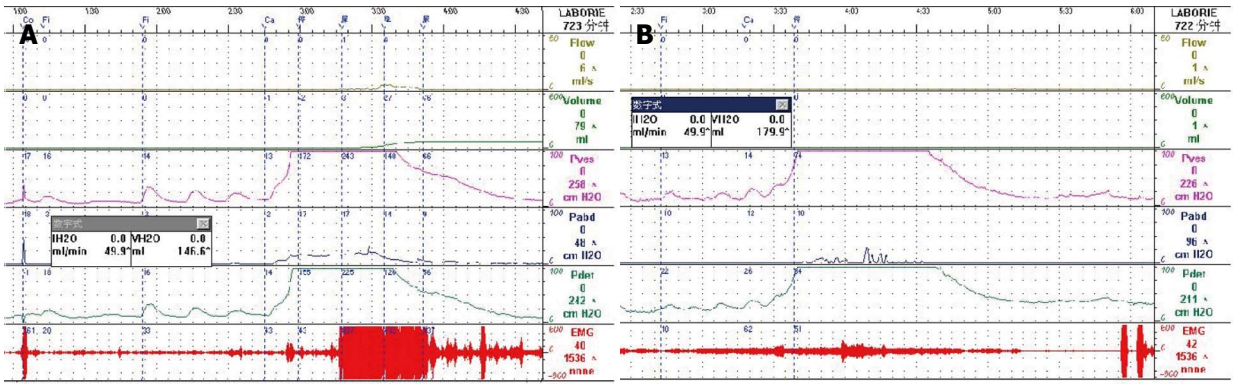

Figure 15 Refractory idiopathic sphincter overactivity-induced bladder outlet obstruction for 18 years cured with baclofen within three weeks. A: Urodynamic (UDS) curve of the woman aged 26 years whose complaints were poor-weak flow and voiding difficulty, undertaken before consultation; B: UDS curve undertaken four years ago.

Figure 16 Various detrusor underactivity manifestations coupled with idiopathic sphincter overactivity and abdominal straining. A: A woman complaining of urinary incontinence was confirmed with detrusor underactivity (DUA) and idiopathic sphincter overactivity (ISO); B: A male patient aged 86 years complaining of poor-weak flow after benign prostatic hyperplasia (BPH) operation was confirmed with DUA and abdominal straining with detrusor-sphincter synergia; C: A female patient suffering from bladder overdistention was confirmed with DUA and ISO; D: A female aged 55 years suffering from incontinence was proved with DUA, ISO and nearly normal Qmax.

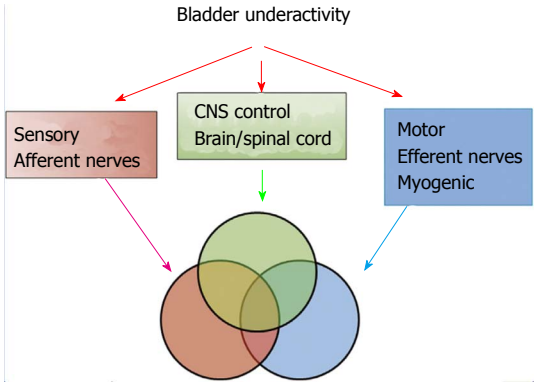

Figure 17 Mechanisms involved in bladder underactivity[42]. CNS: Central nervous system.

control in this institution (Figure 20).

UPP curve: UPP examination is one of the UDS menus and cannot be omitted casually. The UPP curve gives us functional parameter of the urethra and its morphological evaluation, which is very useful for surgical selection of male BPH patients (Figure 21).

LUTS and UDS findings inadequate to explain the disease

UDS findings other than abnormality, in short, subnormal or equivocal UDS findings, may lead to further seeking for potential pathologic factors. At this stage, cystourethroscopic examination, mucus membrane biopsies or imaging study of the LUT, and consult of associated physicians are necessary. Acidophilic cystitis[50] (Figure 22), slight bladder neck contracture, and glandular cystitis may be the possible responsive factors. In female and young male patients, BOO may be associated with inflammation of the bladder neck. In these patients, bladder neck obstruction or contracture, primary or secondary to longstanding ISO, squamous metaplasia or glandular cystitis-like appearance of the bladder neck are always observed. The lining of the bladder neck demonstrates a nontransitional epithelial appearance with epidermoid (squamous metaplasia) or glandular (adenomatous metaplasia) development and later formation of von Brunn's nests in the lamina propria. Squamous metaplasia or glandular cystitis-like lesions in the bladder neck may be responsible for primary bladder neck obstruction in female or young male patients. PFS data gained during emptying phase could reveal the nature of obstruction more precisely (Figure 23).

Extra or intra-LUT symptoms and UDS findings precede pathological findings

LUTS may display ahead of schedule in some disease related or not related with LUT. Multiple system atrophy

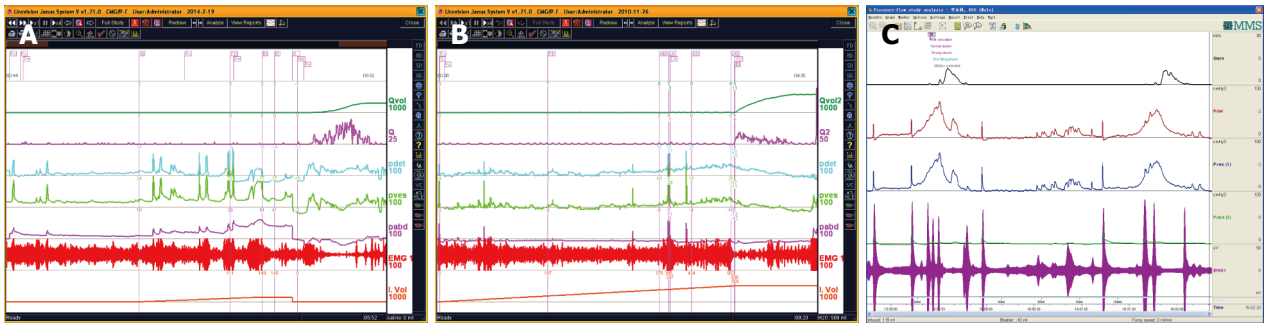

Figure 18 By means of efficient relaxation of the sphincter, satisfactory micturition fulfilled even without detrusor contraction in patients with symptoms of the lower urinary tract or neurogenic symptoms of the lower urinary tract. A: A female patient suffering from stress urinary incontinence had a satisfactory pressure-flow study; B: A woman passed urine fluently without action of the detrusor; C: A male patient with detrusor underactivity and low compliance voided by efficient sphincter relaxation.

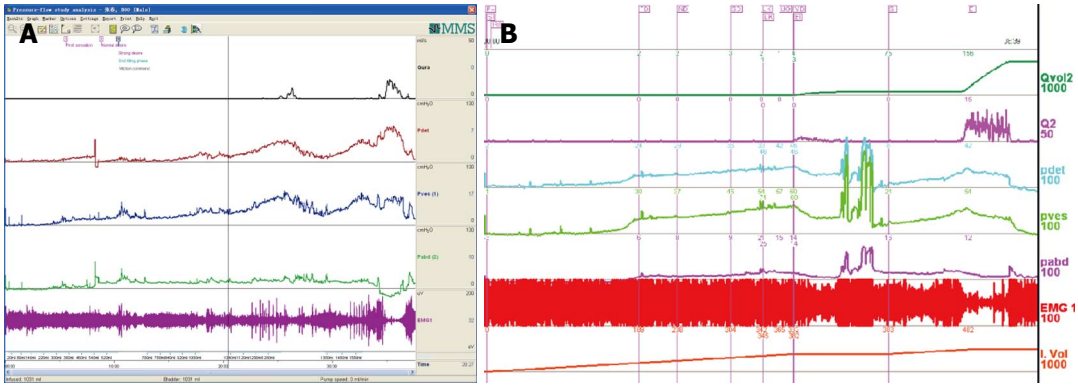

Figure 19 Bilateral hydroureteronephrosis due to bladder low compliance associated with diabetes insipidus and pituitary disorder. A: A male patient aged 42 years complaining of polyuria had low compliance and intact detrusor and sphincter. The residual urine reached 1550 mL after 700 mL saline voided out; B: A male patient aged 23 years had low compliance and no detrusor underactivity or detrusor-external sphincter dyssynergia. His detrusor could contract violently based on raised detrusor pressure and void 900 mL urine.

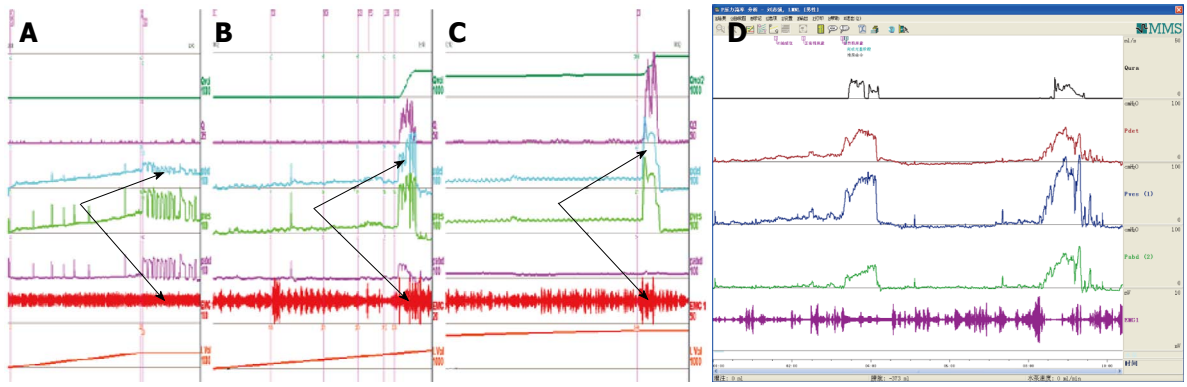

Figure 20 Effective neuroanastomosis resulting in recovery of micturition reflex and the patients getting rid of catheter-dependent state. A female patient aged 46 years had her new reflex succeeded 5 and 9 years after the anastomosis (B and C) as compared with preoperation (A); D: A male patient aged 39 years gained satisfying lower urinary tract function 2 years after the anastomosis, especially with detrusor-sphincter synergia now.

(MSA), multiple sclerosis (MS), spinal cord tumors, idiopathic normal pressure hydrocephalus (iNPH)[51,52] and other occult neurogenic lesions may be responsible for slight degree of DUA or incontinence in some patients (Figure 24). Two patients presented in Figure 23 showed extra or intra-LUT symptoms: incontinence (A) and hydronephrosis (B). The former was proved as MSA two years later after our first consultation, and the potential neurogenic lesion of the latter has not been found so far. Perhaps functional MRI of the nervous system or some forthcoming techniques may aid diagnosis. Whether or not the patients complained of incontinence or hydronephrosis depends on the safe volume of the bladder (bladder volume before *Pdet* reaches 40 cmH_2O) irrespective of the existence of vesico-ureteral reflux. If the functional bladder capacity exceeds it, both condi-

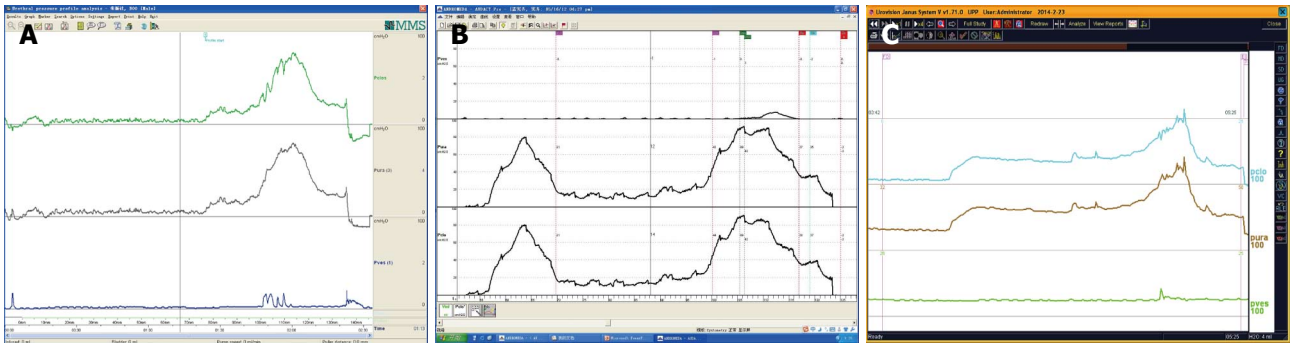

Figure 21 Typical urethral pressure profilometry curves in male patients suffering from benign prostatic hyperplasia. A: Classic urethral pressure profilometry (UPP) curve of male patients with benign prostatic hyperplasia induced bladder outlet obstruction; B: Bimodal UPP curve of patients with enlarged middle lobe; C: Severely big prostate possesses a long functional profile length.

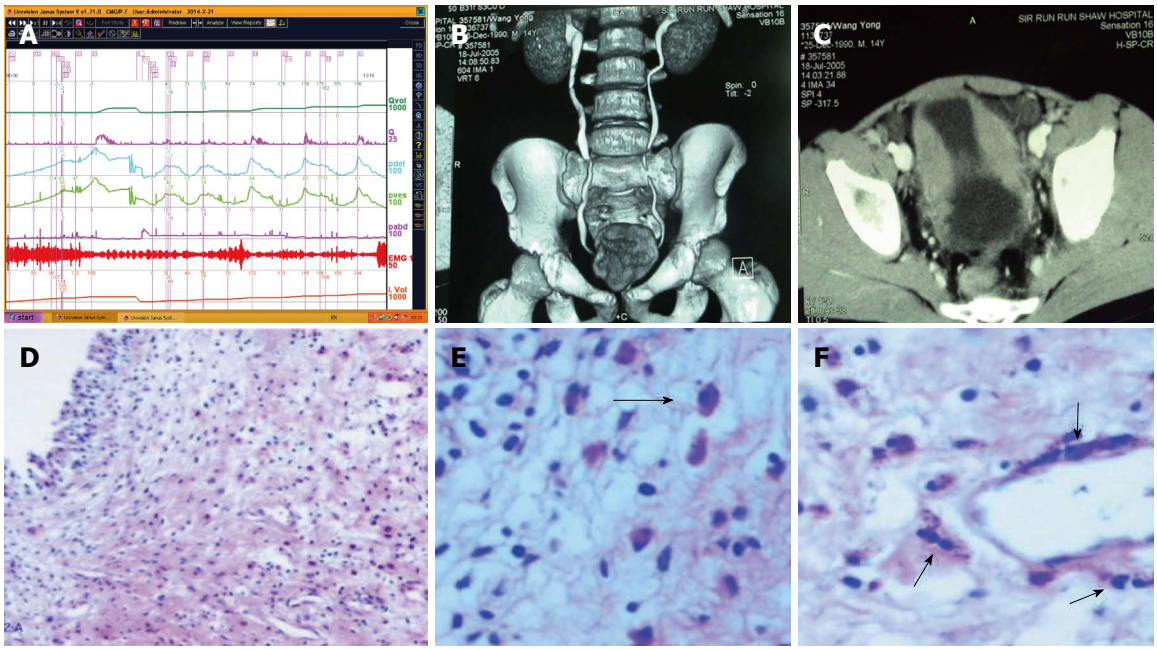

Figure 22 Acidophilic cystitis resulting in poor-weak flow and vomiting in a 15-year-boy was proved as normal detrusor and sphincter function, low compliance, and cured with steroid[50]. A: Although there was neither bladder outlet obstruction (BOO) nor idiopathic sphincter overactivity (ISO), his compliance was low; B: Bladder computed tomography (CT); C: Bladder CT with 3D imaging; D-F: Bladder biopsy specimen: eosinophilic cell suffusion infiltration and proliferation of spindle cells (HE staining).

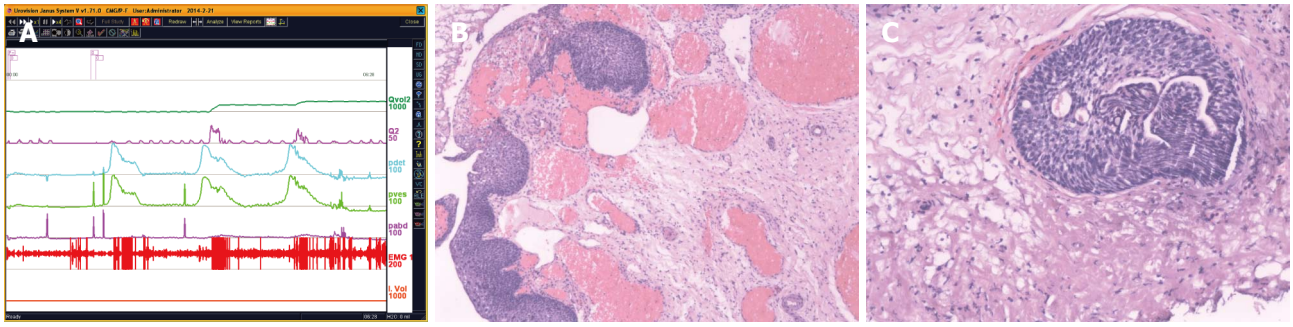

Figure 23 Squamous metaplasia or glandular cystitis of the bladder neck may be caused by longstanding idiopathic sphincter overactivity and produces bladder outlet obstruction-like symptoms. A: A 58-year-old woman presented with urinary obstruction and idiopathic sphincter overactivity and transurethral resection of bladder neck was performed with satisfactory results; B: The bladder transitional epithelium had entered the process of metaplasia, and Brunn nest was forming; C: A Brunn's nest was evident and cavity was in the nest core.

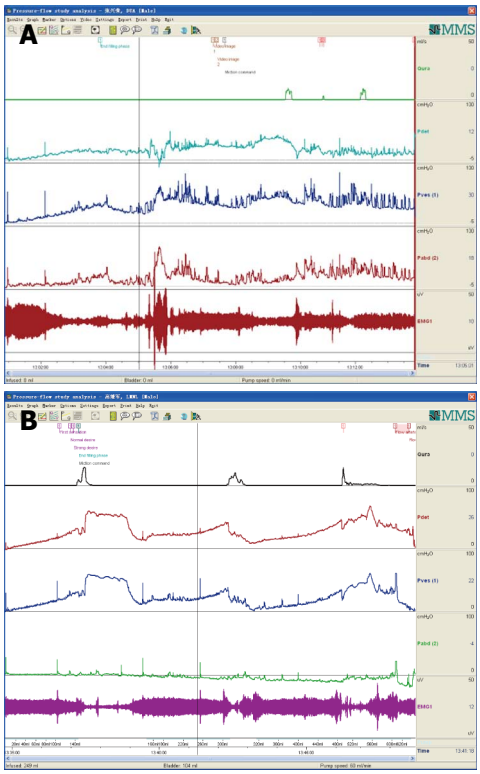

Figure 24 Extra or intra-lower urinary tract symptoms and urodynamic findings in some cases precede pathological abnormality. This phenomenon makes us be convinced of the present medical state. A: A 60-year-old male patient complaining of poor-weak flow and nocturia was proved as having detrusor underactivity (DUA) and low compliance, not responding to TURP, and was proved as having multiple system atrophy 2 years after the first consultation; B: A 52-year-old male patient having received bilateral nephrostomy because of hydronephrosis, was proved as having DUA and low compliance. He could void by efficient sphincter relaxation. However, no abnormal image sign was found at time of consultation.

tions will occur. If the functional capacity is less than the safe volume, distention of the upper urinary tract will not emerge. The latter patient had no imaging confirmed vesico-ureteral reflux indeed. However, bilateral nephrostomies were undertaken because of severe hydronephrosis at our first consultation.

CONCLUSION

Clear definition of UDS entities by means of cutoff values is ideal; however, descriptive recording is often more preferable in practice. TL value is a clear cutoff value for diagnosis of ISO or NSO. By overall view of the whole performance process of micturition (*i.e.*, both the detrusor and sphincter during storage and emptying phases), normal or abnormal UDS findings give us concrete and demonstrable contour of LUTS entities, such as NA, IDO, ISO, DUA, NDO, NSO, BOO and SUI, guide the further option if LUTS and UDS findings are inadequate to explain the disease, and make us be convinced of the present medical state since in some cases extra or intra-LUT symptoms and UDS findings precede pathological abnormality.

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MINIREVIEWS

SCAD syndrome: A vicious cycle of kidney stones, CKD, and AciDosis

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Abstract

Cumulative evidence has shown that kidney stone formers are at high risk for developing end-stage renal disease (ESRD) and cardiovascular disease. The aim of this mini-review is to summarize the present knowledge about the close relationships among kidney stone formation, chronic kidney disease (CKD), and plasma and urine acidosis (SCAD). Part of the cause of the positive relationships between higher risk of developing ESRD and cardiovascular diseases in stone formers may be explained by inflammation and cell death due to the components of kidney stones. In CKD patients, acidic urine and loss of anti-crystallization factors may cause stone formation. Acidosis can promote tissue inflammation and may affect vascular tone. Correction of plasma and urine acidosis may improve renal and cardiovascular outcome of stone formers and CKD patients. More intensive and long-term interventions, which include correction of plasma and urine pH in patients with reduced renal function and correction of urine pH in patients with normal renal function, may be considered in treating patients with SCAD syndrome.

Key words: Endoplasmic reticulum stress response; End-stage renal disease; pH; Endothelial; Epithelial

Core tip: This minireview is written for urology and internal medicine physicians who see kidney stone formers in their daily practice. It is our responsibility to make more serious consideration on the long term outcome of developing end-stage renal disease and cardiovascular diseases in kidney stone formers. The significance of appropriate intervention on acidic condition for these subjects are often neglected. By naming "SCAD syndrome", we can promote more attention on this significant, but sometime forgotten pathological condition.

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INTRODUCTION

Patients with kidney stones usually visit only urologists. However, cumulative evidence indicates that stone formers are at high risk for developing end-stage renal disease (ESRD) and cardiovascular disease. This review will summarize present knowledge about the relationships among stone formation, chronic kidney disease (CKD), and plasma and urine acidosis. Incorporation of these three pathological conditions is needed for the awareness

of urologists and general physicians. To promote such awareness, we would like to give the name SCAD (stones, CKD, and AciDosis) syndrome to this pathological condition.

THE RELATIONSHIPS BETWEEN STONE FORMATION AND RENAL INJURY

Epidemiology

In patients with rare genetic diseases such as hyperoxaluria, cystinuria, Dent disease, and adenine phosphoribosyltransferase deficiency, it is well established that kidney stone formation causes renal damage[1,2]. In contrast, little attention has been paid to the pathological role of commonly observed kidney stones in the development of renal damage. As was reviewed by Rule *et al*[1] and Gambaro *et al*[2], cumulative evidence has shown a significant association between kidney stone formation and the risk of developing ESRD. Alexander *et al*[3] clearly showed in their prospective cohort study that even a single episode of kidney stones can cause a 2.16-fold higher risk for developing end-stage renal failure in both males and females. Hippisley-Cox and Coupland demonstrated a significant association between kidney stones and the development of end-stage kidney failure only in females in their prospective cohort study[4]. Chen *et al*[5] found an association between sonographically-determined nephrolithiasis and the estimated glomerular filtration rate (eGFR) in their cross-sectional study. Saucier *et al*[6] determined in their case-controlled retrospective cohort study that struvite stone formers and uric acid stone formers are more likely to develop CKD. These associations may not be simply explained by renal damage due to occlusion of the tubules or the urinary tract by stones. Alexander *et al*[7] discovered that a single kidney stone episode can cause a 1.40-fold higher risk of acute myocardial infarction and a 1.26-fold higher risk of stroke.

Mechanisms

Part of the cause of the positive relationship between higher risk of developing ESRD and cardiovascular disease in stone formers may be explained by inflammation and cell death due to the components of the stones. Oxalate has been shown to activate inflammatory cytokine signaling pathways, including the interleukin (IL)-2 and IL-6 signaling pathways, in renal tubular cells[8,9]. Oxalate has also been shown to induce cellular death in vascular endothelial cells, which is enhanced by hypoxia[10,11]. Crystalized uric acid activates toll-like receptors 2 and 4 and promotes inflammation[12]. An increase in intracellular uric acid causes oxidative stress[13]. In addition, during the process of uric acid generation, xanthine oxidase causes oxidative stress[14-16]. Struvite stones are generated at the place of inflammation due to bacterial infection[17]. Hamamoto *et al*[18] demonstrated the similarity in the mechanisms of pathogenesis for stone and atherosclerosis. They have identified the involvement of osteopontin in both pathological condition.

THE RELATIONSHIPS BETWEEN SERUM AND URINE ACIDOSIS AND STONE FORMATION

Epidemiology

Due to the higher prevalence in stone formers of developing CKD, it is difficult to clearly demonstrate the higher incidence of stone formation in CKD subjects. However, several mechanisms have been identified to help speculate that CKD subjects are at high risk for crystal formation.

Mechanisms

According to Coe *et al*[19], there are two major pathways for kidney stone formation. One pathway is based on plaque formation in the basement membrane of the thin limbs of loops of Henle. Stone is formed by the overgrowth of plaque and detaches to the tubular space. Plaque formation correlates with urine volume, pH, and calcium. Another pathway is crystallization in the tubular space. Supersaturated solute, including uric acid or calcium oxalate in the urine, forms crystals. The solubility of these solutes is urine pH dependent. These substances are less soluble in low pH conditions. Overall, urine pH has already been established as the major cause of kidney stone formation. In CKD subjects, reduced eGFR is often associated with decreased excretion of calcium and decreased urine concentration capability[20]. Indeed, Marangella *et al*[21] have demonstrated that subjects with lower GFR may have a lower recurrence rate of calcium stones. Along the same line, metabolic acidosis is often associated with CKD due to the limited capability of acid excretion into urine. But, in contrast to calcium, this does not mean that urine pH is high in CKD subjects, for the following reason. To excrete sufficient acid with limited reduction in urine pH, the kidney uses titration acids, including NH_4^+ and $H_2PO_4^-$. These titration acids can also be reduced in the urine of CKD subjects. Therefore, the pH level easily becomes low in the urine of CKD subjects. Related to this idea, Stettner *et al*[22] recently showed that sulfatide-deficient mice developed metabolic acidosis with lower urine pH in response to acid overload. Low pH causes the crystallization of uric acid and calcium oxalate by limiting their solubility. Systemic acidosis may also promote stone formation by increasing the solute overload into urine. Acidosis promotes calcium release from bone. Starke *et al*[23] showed that, in renal transplant patients, normalization of metabolic acidosis by the administration of potassium citrate has the potential to preserve bone quality, as assessed by bone biopsy. Regarding the molecular mechanism, Geng *et al*[24] showed that serum bicarbonate inhibits osteoclast formation though the activation of soluble adenylyl cyclase. Krieger *et al*[25] showed that metabolic acidosis directly increases fibroblast growth factor 23 (FGF23)

mRNA and protein in mouse bone. CKD and metabolic acidosis may also affect the expression of the Tamm-Horsfall glycoprotein and other factors that inhibit the growth of crystal[26].

THE RELATIONSHIPS BETWEEN SERUM AND URINE ACIDOSIS AND KIDNEY INJURY

Epidemiology

In CKD subjects, high and low serum bicarbonate levels are associated with a risk of mortality and the development of ESRD. Kovesdy *et al*[27] showed in their retrospective cohort study that the group of patients with serum bicarbonate level of 26-29 mmol/L had the lowest mortality rate. The retrospective cohort study of Navaneethan *et al*[28] showed that the group of patients whose serum bicarbonate level was 23-32 mmol/L had the lowest mortality rate. Kanda *et al*[29] showed in their retrospective cohort study that subjects with high serum bicarbonate level (28.8 mmol/L) are less likely to develop ESRD than patients with low serum bicarbonate level (23.4 mmol/L). Recently, several studies have shown the beneficial effect of correcting metabolic acidosis on the decline of GFR in CKD subjects. Susantitaphong *et al*[30] systematically reviewed the effects of sodium bicarbonate in the long term (> 2 mo), and showed an improvement in the eGFR and a lower incidence of initiating dialysis therapy. The beneficial effect of alkali therapy on eGFR in CKD subjects has been shown by the administration of potassium citrate[31].

In non-CKD subjects, acidic urine has been shown to be associated with diabetes and metabolic syndrome (Mets)[32,33]. Maalouf *et al*[32] showed a positive association between acidic urine and a number of the components of Mets in non-CKD subjects. These authors speculated that part of this association may be explained by impaired urine buffering capability due to insulin resistance.

Mechanisms

As was reviewed by Souto *et al*[34], metabolic acidosis can lead to the development of several risk factors for cardiovascular disease, including inflammation, hypertension and disturbed glucose tolerance, due to decreased insulin sensitivity.

Effects on inflamation: Bellocq *et al*[35] and Kellum *et al*[36] showed that acidic condition promotes tumor necrosis factor alpha (TNF-α)-dependent nuclear factor kappa-light-chain-enhancer of activated of B cells (NF-κB) activation in macrophages. More recently, Rajamäki *et al*[37] and Edye *et al*[38] showed pH-dependent secretion of IL-1β and activation of caspase-1 in macrophages. These investigators also have demonstrated the significant role of damage-associated molecular patterns (DAMPs) in this process. Nikoletopoulou *et al*[39] showed that acidosis switches TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis to regulated necrosis in cancer cells. Further investigations are expected to test whether cell death under an acidic condition also shows switching from apoptosis to necrosis. An increase in the proportion of necrosis may promote more inflammation to the injured area. Chen *et al*[40] showed that acidic condition induces the production of cell adhesion molecules, including intercellular adhesion molecule 1 (ICAM-1), E-selectin, and vascular cell adhesion molecule 1 (VCAM-1) in endothelial cells. Acidic condition has been shown to activate complement system. Emeis *et al*[41] and Curley *et al*[42] has shown that acidosis activates C3 and C5. Morita *et al*[43] showed that the administration of sodium bicarbonate in subjects with proteinuria decreases the renal excretion of complement activation products (CAP).

Effects on vessel function: The role of acidosis on vascular tone is controversial. As reviewed by Smith *et al*[44], Smith *et al*[45] and Wray *et al*[46], extracellular and intracellular decreases in pH have been shown to promote vasoconstriction. However, several studies have shown that acidosis enhances nitric oxide (NO) production and promotes vasodilatation[47]. Part of this inconsistency may be explained by oxidative stress. The intracellular acidic condition causes an increase in the fraction of free iron to protein-bound iron in cells, which causes oxidative stress by a Fenton-type biochemical reaction[48]. Oxidative stress itself has been shown to promote vasoconstriction. It reacts with NO and generates the highly toxic peroxynitrite anion ($ONOO^-$). Enhanced NO production also causes high endothelial permeability[49]. Dong *et al*[50] showed that endothelial cells detect the extracellular acidic condition by the proton-sensing G-protein coupled receptor 4 (GPR4), which activates inflammation and the endoplasmic reticulum (ER) stress response.

Effects on tubules: In epithelial cells, adaptation mechanisms to acidic conditions have been well investigated. This is because epithelial cells, including in the intestine and the kidneys, are in the location to be exposed to acidic condition even under physiological conditions. Therefore, these cells are resistant to extracellular acidification. Sodium-hydrogen exchangers (Na^+/H^+ exchangers, NHEs) have an established role in the maintenance of internal pH. Muthusamy *et al*[51] has shown that, in intestinal epithelial cells, acid induces the NHE2 Na^+/H^+ exchanger to regulate internal pH through the induction of early growth response protein 1 (EGR-1). Preisig *et al*[52] and Kwon *et al*[53] showed an increase in NHE3 and the Na/HCO3 cotransporter (NBC1) in the renal proximal tubules. Odunewu and Fliegel showed that acute sustained intracellular acidosis activated NHE1 in human embryonic kidney 293 (HEK293) cells and in Madin-Darby canine kidney (MDCK) cells[54]. Renal epithelial cells have also been shown to activate glutamine transporters, including SN1[55] and mitochondrial

glutamine transporter[56], to increase the production of ammonia, which acts as a titration acid. Ibrahim *et al*[57] proposed that, in proximal tubules, a change in intracellular pH may promote the ER stress response followed by the stabilization of corresponding mRNAs for ammoniagenesis. As for the protective mechanism of renal tubular cells against extracellular acidification, Namba *et al*[58] have demonstrated the significant role of autophagy in the proximal tubules.

CONCLUSION

Kidney stone formation, chronic kidney disease or cardiovascular disease, and metabolic acidosis influence each other and form a vicious cycle. Even a single episode of stone formation in a stone former or an asymptomatic stone former may place those persons at higher risk for the development of CKD and cardiovascular disease. More intensive and long-term interventions, which would include correction of plasma and urine pH in subjects with reduced renal function and correction of urine pH in subjects with normal renal function, may be considered in the strategy for treating subjects with SCAD syndrome.

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Laparoscopic single site surgery: Experience in pediatric urology

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Abstract

Laparoendoscopic single-site surgery (LESS) has been developed to benefit patients by enabling surgeons to perform scarless surgery. In this review we aimed to summarize and critically analyze the available evidence on the current status and future prospects for LESS in pediatric urology, with special emphasis on our experience with LESS in children. The clinical data available clearly demonstrate that LESS can safely and effectively be performed in a variety of pediatric urology settings. As clinical experience increases, expanding indications are expected to be documented and the efficacy of the procedure to improve. So far, the quality of evidence of all available studies remains low; mostly being small case series or case-control studies from selected centers. Thus, the only objective benefit of LESS remains improved cosmetic outcome. Prospective randomized studies are awaited to determine which LESS procedures will be established and which are unlikely to stand the test of time. Technological advances hold promise to minimize the challenging technical nature of scarless surgery. In this respect, robotics may be a driving force in the development of LESS.

Key words: Laparoendoscopic single-site surgery; Pediatric urology; Laparoscopy; Review

Core tip: Laparoendoscopic single-site surgery (LESS) has been developed to benefit patients by enabling surgeons to perform scarless surgery. Clinical data demonstrate that LESS is safe and effective in many pediatric urology settings. As clinical experience increases, expanding indications are expected, along with improved efficacy. Prospective randomized studies are awaited to determine whether LESS procedures will be established as routine and will be able to stand the test of time. Technological advances hold promise to minimize the challenging technical nature of scarless surgery. In this respect, robotics is likely to drive a major paradigm shift in the development of LESS.

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INTRODUCTION

Laparoscopic surgery is beginning to gain acceptance as a standard of care in many intra-abdominal procedures in adult and pediatric urology[1]. Today, laparoscopic procedures are commonly performed and have become widely accepted as alternatives to open surgery, if not the gold standard in some procedures, such as radical or partial nephrectomy[2]. Even the more technically demanding procedures, such as laparoscopic pyeloplasty, laparoscopic-assisted bladder reconstruction, and laparoscopic ureteral reimplantation, have achieved widespread acceptance and are now routinely performed at many centers worldwide. With increasing experience

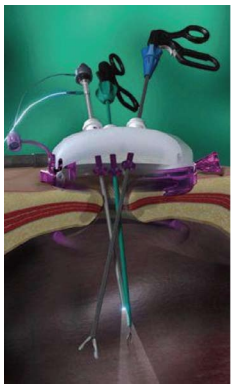

Figure 1 Laparoscopic single site surgery.

in the laparoscopic environment, efforts are now directed at further minimizing morbidity and improving cosmetic outcomes. This has led to the development of techniques, multichannel single-access ports, and novel bent/articulating instruments that allow the laparoscopic procedure to be performed through a single skin incision, often hidden within the umbilicus or utilizing the nature orifices of the human body in order to seal surgical incisions completely. Following this concept natural orifice transluminal endoscopic surgery (NOTES) and laparoendoscopic single-site surgery (LESS) have been developed in an attempt to reduce further the morbidity and scaring associated with surgical intervention[3-5]. Conceptually, these techniques share a common underlying hypothesis that has driven their development-namely, that a reduction in the number of transcutaneous points of access may benefit patients in terms of port-related complications, recovery time, pain, and cosmesis by potentially performing scarless surgery. The first documented one-port single-incision laparoscopy was cholecystectomy in 1997. Ten years later, the first single-port nephrectomy was done. Since then urologists have successfully performed various procedures with LESS, including partial nephrectomy, pyeloplasty, orchiectomy, orchiopexy, varicocelectomy, ureterolithotomy, sacrocolpopexy, renal biopsy, renal cryotherapy, and adrenalectomy[6,7].

In this review, we describe the rationale of the technique, the current clinical applications, the advantages and disadvantages compared to standard laparoscopy, and the results of LESS in pediatric urological surgery, with some attention in robotic surgery.

DEFINITION

LESS

LESS is a minimally invasive surgical procedure in which the surgeon operates almost exclusively through a single entry point, typically the patient's navel. Unlike a traditional multiport laparoscopic approach, LESS leaves only a single small umbilical scar, usually not larger than 2 cm. This particular access can be achieved through a single fascial incision site with a single trocar with multiple ports, or through a single skin incision site with multiple fascial incisions with individual trocars. The most popular technique is the first mentioned above: a single port with multiple channels. Like conventional laparoscopy, there are two principal approaches for renal, adrenal and ureteral surgery: transperitoneal and retroperitoneal[8]. Although the first mentioned above is the best known and usually performed, today sufficient clinical studies have shown the effectiveness and safety of LESS through a retroperitoneal approach, especially in nephrectomy for nonfunctioning kidney and in other extirpative retroperitoneal procedures[9].

With the time and development of the technique, the concept of LESS was diversified and the surgeons proposed different acronyms for LESS and associated procedures. They include: single port access, single incision laparoscopic surgery (SILS), natural orifice transumbilical surgery, transumbilical endoscopic surgery, single-access site laparoscopic surgery, single-site access, one-port umbilical surgery, transumbilical laparoendoscopic single-site surgery, transumbilical laparoscopic assisted surgery, and embryonic natural orifice transluminal endoscopic surgery[10-14]. The common factor is a single small skin incision, usually at the umbilicus (Figure 1).

Robotic LESS

Robotic LESS (R-LESS) uses the Da Vinci Surgery System *via* a one single-port approach to improve ergonomics that limit conventional single-port laparoscopy[15].

HISTORY

Minimally invasive surgery is a changing and evolving field. Since the first documented laparoscopic procedure in humans performed by Hans Christian Jacobaeus in 1910, there has been great progress that has expanded throughout the surgical specialties. In 1918, Goetze developed the first automatic pneumoperitoneum needle. In 1929, Kalk introduced the forward oblique (135 degrees) view lens systems, and in 1938, Veress developed a specially designed spring-loaded needle. Veress did not promote the use of his needle for laparoscopic purposes. He used the Veress Needle for the induction of pneumothorax. To date, the Veress Needle is the most important instrument to create pneumoperitoneum. The real credit for videoscopic surgery goes to Hopkins, who discovered in 1953 the rigid rod lens system that revolutionized the field of laparoscopic surgery. As a result of this development, in 1970, gynecologists started to embrace laparoscopy and thoroughly incorporated the technique into their practice. General surgeons, despite their exposure to laparoscopy remained confined to traditional open surgery until 1977, when the first laparoscopic assisted appendectomy was performed by Dekok. In that setup, the appendix was exteriorized and ligated outside. In the same year, Semm first demonstrated the endoloop suturing technique in laparoscopic surgery. The first documented laparoscopic cholecystectomy was performed

Figure 2 QuadPort + (Olympus) port system.

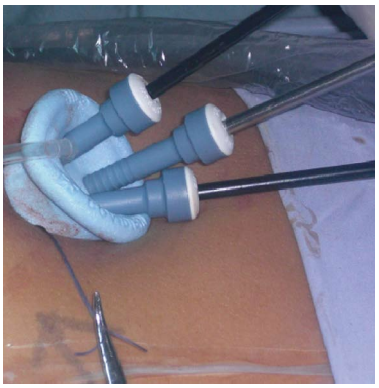

Figure 3 Single incision laparoscopic surgery (Covidien) port system.

by Mühe in Germany in 1985. In 1994, the first robotic arm was designed to hold the telescope, and in 1996, a live telecast of laparoscopic surgery was performed remotely *via* the internet (robotic telesurgery)[16]. As part of the natural development of minimally invasive surgery, in the late 1990s, LESS emerged[3]. The reason for the development of this type of procedure rests on the premise *"LESS pain, LESS scar"*, but the practical advantages in the field are many more than those. As already mentioned, the first urological use of LESS was reported in 2007 with the completion of single-port nephrectomy for a small nonfunctioning kidney, as well as transperitoneal ureterolithotomy[3,6]. To date, there is extensive experience with LESS in adult and pediatric urology in extirpative and nonextirpative procedures such as pyeloplasty, varicocelectomy and orchidopexy[3]. In other specialties such as gynecology, LESS has been used for several years, making single incision laparoscopic tubal ligation one of the most popular procedures in that field[17-19].

EQUIPMENT

Access devices

Multichannel ports can be used during LESS as one access approach. These devices allow for the insertion of instruments and a camera and involve a single fascial incision. TriPort (Advanced Surgical Concepts, Bray, Ireland) is the best-known FDA-approved access system. The size of the TriPort is fully adjustable, and allows a series of instruments to be introduced into any sized abdominal incision, from a 5 mm incision up to a hand-assisted laparoscopic surgery incision. Each device consists of a retractor component and a valve component, where the instruments are inserted. The valve component of TriPort is made of a unique elastomeric material that allows the passage of standard laparoscopic instruments and scopes simultaneously. The TriPort has three inlet valves: one for a 12-mm instrument and two for 5-mm instruments. QuadPort (Olympus, Advanced Surgical Concepts) is also available, and has four ports: two inlets for 12-mm instruments and two for 5-mm instruments. A separate insufflation port is provided through the valve housing in both devices. The high elasticity of the gel valve allows the removal of small specimens, whereas larger specimens are withdrawn into the distal end of the port and removed simultaneously with the device at the end of the procedure (Figure 2).

The Uni-X Single Port Access Laparoscopic System (Pnavel Systems, Cleveland, OH, United States) is a single port with three working channels, which all accommodate specialized 5-mm laparoscopic instruments. The device is placed through an open access technique and requires a 2-cm fascial incision. Once passed into the abdomen, the port is anchored in place using fascial sutures that are placed before attaching the device to the patient. As with TriPort, Uni-X has a separate valve port for insufflation. Once the procedure is complete, the port is untied and the specimen is removed through the initial incision[6]. The GelPOINT system from Applied Medical Technology (Brecksville, OH, United States) accommodates varying abdominal walls and incision sizes, provides continuous access, and ensures improved articulation of 5-12-mm instruments. The Alexis wound protector/retractor offers atraumatic retraction and protection, maintains moisture at the incision site, while providing convenient extracorporeal resection and specimen retrieval. The SILS Port designed by Covidien Tyco Health Care (Mansfield, MA, United States) consists of a blue flexible soft-foam port, with access channels for three cannulae. The 5-mm cannula may be interchanged at any time during the procedure with a 5-12-mm cannula. The SILS Port adapts its configuration to the size of the cannulae while maintaining pneumoperitoneum (Figure 3). We have utilized in all our patients the SILS Port (Covidien, Tyco Health Care). It is our preferential access devise for all LESS procedures. It has a foam port that expands after insertion to prevent air leakage. It is significantly cheaper compared with the others at least on the Israeli market. The access port is easy inserted *via* a 2-cm incision that is performed within the umbilicus. During the procedure the 5-mm trocars can be easily replaced by 10-12-mm trocars during surgery when needed.

Hand instruments

A basic tenet of laparoscopic surgery involves triangu-

Figure 4 Sixty centimeter, 5-mm telescope with right-angle light cord adapter (Karl Storz).

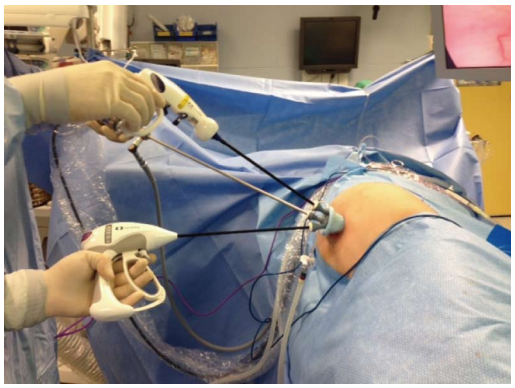

Figure 5 Operating field.

lation of instruments so as to produce adequate intracorporeal working space for anatomical dissection and manipulation of tissues. The parallel and close proximity of the right-hand and left-hand instrument shafts of standard laparoscopic instruments through a solitary port results in crowding of the laparoscope and the instruments, preventing appropriate triangulation. Articulated instruments were designed to overcome these challenges. Some of articulated instruments include the SILS Multiple Instrument Access Port manufactured by Covidien and the Laparo-Angle Articulating Instruments made by Cambridge Endoscopic Devices (Cambridge, MA, United States), articulating laparoscopic graspers (*e.g.*, Real Hand; Novare Surgical Systems, Cupertino, CA, United States and Autonomy Laparo-angle; Cambridge Endo, Framingham, MA, United States), endoshears (Cambridge Endo), and laparoscopic needle drivers (Cambridge Endo). A combination of conventional and flexible (articulating) instruments provides improved intraoperative ergonomics and further facilitates dissection during surgery.

Telescope

There are two types of telescope for LESS: 30° and 0°. Pelvic procedures require the use of a 30° lens directed upwards, whereas upper tract procedures need either a 30° lens facing downward or a 0° lens[20] (Figure 4).

We have utilized a 60-cm, 5-mm, 0° telescope (Karl Storz, Germany) for all LESS intra-abdominal or renal surgery, and a 30° telescope with a right-angle light cord adapter in order to move the camera further from the operating surgeon, minimizing incidental collision of instruments during pelvic surgery. We think that it is crucial to use a long 60-cm telescope with an adaptor that allows receiving a fair laparoscopic picture without interfering with the surgeons within the limited operative field (Figure 5).

The key problem with conventional laparoscopes is that they have a large extracorporeal profile, with a light cable exiting at 90°. This configuration leads to clashing of the instruments and camera during LESS. Thus, the ideal telescope for LESS should remove the light cord and camera head from the operative field. Low-profile camera systems have been introduced for this purpose.

Accessories

Park and colleagues have developed a transabdominal magnetic anchoring and guidance system (MAGS), which can be used to control an intra-abdominal laparoscope and multiple working instruments introduced through a single 1.5-cm port[21]. Once passed into the abdomen, instruments are affixed to the abdominal wall using external magnetic anchors. Currently, the MAGS incorporates an internal camera system, two types of passive tissue retractors, and a robotic arm cauterizer. By fixing internal instruments to external magnetic anchors, this platform allows for unrestricted intra-abdominal movement of surgical instruments, creating the potential benefits of LESS while maintaining an operative perspective similar to that of standard laparoscopy. This system has the added benefit of allowing the surgeon to reposition instruments intraoperatively without additional incisions.

TECHNIQUES

LESS is performed through a single abdominal incision, usually at the umbilicus. We and others have modified routine laparoscopic procedures in order to overcome the limitations of LESS. In general, a single port with multiple channels is used through which the laparoscope and the operative instruments are passed. The procedure usually involves two surgeons. As we have mentioned, pelvic procedures require the use of a 30° lens directed upwards, whereas upper tract procedures need either a 30° lens facing downward or a 0° lens[20].

We have performed LESS nephrectomy by a transperitoneal approach while the patient is in a flank position, utilizing the usual technique. In this setting, the retroperitoneal space is entered through the Told line utilizing Ligasure 5 mm-37 cm (Covidien). An articulating grasper (Covidien) and an articulating dissector (Cambridge Endo) are used in order to develop an operative space. Using both articulating instruments at this stage avoids extracorporeal hand cross and intracorporeal instrument collision. However, we have found particularly useful the use of both articulating instruments only at the initial stage of the surgery. After the initial dissection and the formation of an operative space, the articulating dissector can be easily replaced by a straight instrument such as Ligasure, allowing not only the dissection, but

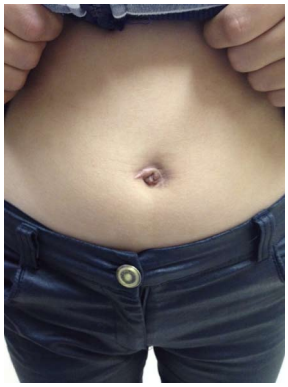

Figure 6 Final incision.

also rapid hemostasis, which shortens the time of the operation. After dissection of the colon away from the kidney, the ureter is identified and transected. Ligation of the hilum vessels is performed utilizing large Auto Suture hemostatic clips (Covidien). The specimen is retrieved into a laparoscopic bag and removed through one of the ports or together with the LESS port. No drain is needed for these cases. In the cases of large hydronephrosis and right-sided kidney, trans-flank holding stitches through the renal pelvis, in the same manner as used in laparoscopic pyeloplasty, can be used in order to facilitate renal dissection.

For single site laparoscopic gonadectomy, the LESS port is inserted in the same manner as for nephrectomy. Vascular control is achieved solely by utilizing the Ligasure system. In the case of varicocelectomy, laparoscopic dissection of the spermatic vein is performed sparing the spermatic artery and dissecting away the lymphatic vessels. Ligation of the spermatic vein is performed utilizing Auto Suture hemostatic clips (Endo Clip; Covidien Tyco Health Care) or using sealing devices such as the Ligasure system only without hemostatic clips.

R-LESS is performed through the same umbilical incision as for conventional LESS. When the SILS port (Covidien Tyco Health Care) is used, a finger is placed to guide introduction of two robotic trocars adjacent to the fascial incision through two separate fascial stab incisions. If using a GelPort (Applied Medical Technology), the access device is placed through the fascial incision and the robot is subsequently docked. The robotic cannulae utilized vary from 8 to 5 mm to accommodate the Endowrist (Intuitive Surgical, Sunnyvale, CA, United States) monopolar shears and the Endowrist Schertel Grasper, depending on the procedure to be performed.

ADVANTAGES AND INDICATIONS

Beyond the obvious better cosmetic results, advantages of LESS include reduced postoperative pain, reduced operative complications related to trocar insertion (*e.g.* wound infections, epigastric vessel injury and organ herniation), and easier specimen removal through a larger incision (specimens may be fragmented in the laparoscopic bag)[22,23]. Those benefits are especially relevant in pediatric and young populations in whom the esthetic outcome is crucial (Figure 6).

LIMITATIONS OF THE TECHNIQUE

Not all patients will be candidates for single-site surgery[24]. As with any other minimally invasive surgical technique (laparoscopy or robotics), patient selection is a composite of clinical judgment, risk, benefits, alternatives and a well-informed patient. Other limitations of this technique are the added cost and the technical challenges of the procedure. The major limitation is the lack of working space. The surgeon and the assistant must maneuver in a small space, resulting in hand and instrument collisions. The laparoscopic surgery concept of triangulation is challenged with the single-port procedure, and the ability to move the scope is significantly limited by other instruments[25].

Specialized equipment for single-port procedures can be used to help overcome these technical challenges, including the use of articulating instruments, a flexible laparoscope or a 30° laparoscope, and instruments of varying lengths. Articulating instruments can help with triangulation because the operator is able to work with two instruments in a similar location inside the abdomen while his or her hands are separated on the outside of the abdomen. Other disadvantages of LESS are related to operative time and learning curve.

CLINICAL STUDIES IN PEDIATRIC UROLOGY

Almost all body cavities can be entered through a small skin incision, therefore, the theoretical applications of LESS seem to be unlimited. However in a practical way, this statement is not entirely correct. Although LESS has successfully been proved for almost all diagnostic, extirpative and reconstructive surgery, there are limitations inherent to patient selection, surgical skills of the team, operative time, setup of the operating room, and availability of devices. In urology, LESS has been principally described for renal, ureteral, and prostatic surgery. In the most specialized centers it is now used for adrenal, bladder and testicular minimally invasive surgery as well. Despite the slower introduction of LESS in the pediatric population, today various LESS procedures have been described in pediatric urology with apparently good results.

Nephrectomy for nonfunctioning kidney is a good example. In 2010 Koh *et al*[26] reported outcome in 11 LESS nephrectomies in pediatric patients (age range: 0.1-16.2 years, with a mean age of 5.7 years) using an umbilical incision. None of the patients required conversion to conventional laparoscopy or open surgery. However, an accessory port was used in five of 11 cases. Of the 11 patients, two were infants, aged 39 d and 3.5 mo. The mean operative time was 139 min (range:

85-205 min), and the mean hospital stay was 1.5 d (range: 1.0-2.1 d)[26]. Ham *et al*[27] reported their results in four LESS nephrectomies and two nephroureterectomies through a homemade transumbilical port in children, without intraoperative or postoperative complications. The median operation time was 112 min (range: 90-148 min), and the median blood loss was 30 mL (range: 0-50 mL). All patients were discharged on postoperative day 2. As the surgeon had gained experience, the length of the umbilical incision was decreased from 2.0 to 1.0 cm[27]. In another recent study, Ganpule *et al*[28] reported on 10 patients who underwent different LESS procedures through the umbilicus. Seven patients underwent nephrectomy and three pyeloplasty. Mean age of the nephrectomized patients was 3.14 ± 1.7 years; the mean operating room time was 97.5 ± 12.54 min. All procedures were technically successful[28].

Another usual application of LESS in pediatric urology is varicocelectomy. Kaouk *et al*[29] reported three consecutive adolescent patients who underwent transumbilical varicocelectomy without placement of any additional ports or conversion to open surgery. The mean operative duration was < 1 h and all patients were discharged on the same day as their surgery and none required rehospitalization. There was no varicocele recurrence, or intraoperative or postoperative complications including wound infection, hydrocele, or incision site herniation[29].

LESS pyeloplasty is another popular but technically demanding procedure. Desai *et al*[30] performed 17 pyeloplasties; two with robotic assistance. The mean operative time and blood loss were 236 min and 79 mL, respectively. There were no complications, but all cases required an additional 2-mm port to aid suturing. One case was converted to conventional laparoscopy. All patients were symptom-free post-procedure and postoperative imaging showed unobstructed drainage in 15 of the 16 patients for whom data were available[30]. White *et al*[7] performed eight pyeloplasties; one with the aid of the Da Vinci robotic platform. The mean operative time and blood loss were 233 min and 62.5 mL, respectively. Renographic follow-up was documented as within normal limits and there were no complications apart from a wound site hernia[7]. One of the most recent studies was done at the Bayi Children's Hospital and included 24 pediatric patients with ureteropelvic junction obstruction who underwent transumbilical LESS pyeloplasty. All operations were successful. None was converted to open surgery and no additional sheath tube or incision besides umbilicus was needed. No intraoperative complications occurred. The mean operative time was 145 min, and the average blood loss -10 mL. Two patients had postoperative urinary fistula, which naturally disappeared at 4 and 7 d postoperatively, respectively. In follow-up, 23 of 24 patients demonstrated a significant decrease in renal pelvis diameter[31].

Orchidopexy has been performed with LESS as well. Noh *et al*[32] published the results of LESS orchidopexy in 17 patients with a median age of 11 mo (range: 3-43 mo). The study included two bilateral procedures and five primary Fowler-Stephens (FS) procedures. One patient underwent a staged FS orchidopexy, with the LESS technique utilized during the second stage. Median laparoscopic dissection time for each testis was 35 min (range: 22-40 min). There was no blood loss or intraoperative complications. In follow-up, all testes were noted to be in the scrotum without testicular atrophy[32].

Other LESS procedures have also been performed in pediatric urology, such as ureteral reimplantation and bladder augmentation. The data for this type of surgery is limited to case reports and small series[33,34] (Table 1).

OUR EXPERIENCE

Since 2011 a total of 18 patients underwent 23 procedures at our department: eight patients underwent nephrectomy due to nonfunctioning kidneys; four had removal of bilateral intra-abdominal gonads; four had high ligation of spermatic vein (HLSV); one underwent hysterectomy; and the remaining one had bilateral HLSV. A 1-year-old child who required hysterectomy was diagnosed with 46 XY ovotestis disorder of sexual differentiation and was raised as a boy. He required the removal of ovary and hypoplastic uterus. In all the patients a multichannel single laparoscopic port (Covidien) inserted through a 2-cm skin incision was used in order to obtain access into the abdominal cavity. All the patients underwent LESS without complications within a reasonable operating time. No one required conversion to open or conventional laparoscopic surgery. In two patients with large hydronephrosis we utilized a transcutaneous holding stitch, which was introduced through the renal pelvis and allowed additional manipulation of the severely hydronephrotic kidney, facilitating dissection and avoiding a need for additional trocar insertion. All but one patient were discharged on the day of surgery or on the day after[35] (Table 1).

LIMITATIONS OF CLINICAL STUDIES

Thus far, LESS is no longer an experimental technique; however, there are only a few retrospective studies with a significant number of cases that can prove the efficacy and safety of this technique for different indications. The advantages of LESS still exist at a theoretical level, because no clear benefit on postoperative course and patient convalescence has been definitively proven. The only potential benefit of LESS remains the claimed cosmetic outcome. Another obvious limitation is the lack of comparative studies between LESS and standard laparoscopy in terms of clinical outcome. Only a few retrospective case-control studies have compared LESS with standard laparoscopic techniques. In one such study, LESS nephrectomy (11 procedures) demonstrated no difference in median operating room time (122 min *vs* 125 min), change in hemoglobin levels, analgesic use, length of hospital stay, or complication rate compared to

Table 1 Clinical studies in pediatric urology

Ref.	Type of study	Procedure	Nopts	Operating time	Need for conversion	Complications/events	Blood loss
Koh *et al*[26]	Retrospective	Nephrectomy	11	139 min	No	Acc. port in 5	20 mL
Kocherov *et al*[35]	Case Control	Nephrectomy	8	65 min	No	None	None
Ganpule *et al*[28]	Retrospective	Nephrectomy	7	97.5 min	No	None	None
Ham *et al*[27]	Prospective	Nephrectomy	4	112 min	No	None	0-50 mL
Ham *et al*[27]	Prospective	Nephro-UBil	2	112 min	No	None	0-50 mL
Kocherov *et al*[35]	Case Control	Gonadectomy	4	37.5 min	No	None	None
Kocherov *et al*[35]	Case Control	Varicocelectomy	6	26 min	No	None	None
Kaouk *et al*[29]	Retrospective	Varicocelectomy	3	< 1 h	No	None	None
Noh *et al*[32]	Retrospective	Orchidopexy	17	35 min	No	None	None
Desai *et al*[30]	Retrospective	Pyeloplasty	17	236 min	No	Acc. port in all	79 mL
White *et al*[7]	Prospective	Pyeloplasty	8	233 min	No	None	136 mL
Zhou *et al*[31]	Prospective	Pyeloplasty	24	145 min	No	Two urinary fistulas	10 mL

standard laparoscopic nephrectomy (22 procedures)[22]. A limitation of this study, in addition to it being retrospective, was that patients had their nephrectomy specimens removed through an extension of the umbilical incision up to 4-6 cm, thus obscuring the possible benefits of LESS, such as shorter convalescence and reduced postoperative pain, compared with standard laparoscopy. These results might not indicate any advantages of LESS over standard laparoscopy[36]. We have also identified a similar historic group of patients from our database who underwent conventional laparoscopy and have compared their outcome to those patients undergoing the LESS technique[35]. This group included two patients with androgen insensitivity that underwent gonadectomy; four with nonfunctioning kidneys who underwent nephrectomy; and four who underwent HLSV. All patients in this group had similar parameters in terms of age and indications for surgery as the LESS group. Outcome data regarding operative time, narcotic requirements, length of hospitalization, and complication rate were obtained following chart review. In spite of the fact that in those patients who underwent LESS the operating time seemed to be longer, there was no difference in the length of surgery and intraoperative narcotic requirements between conventional laparoscopy and LESS. None of the patients in the LESS group required narcotic administration compared with three patients from the conventional laparoscopy group (one gonadectomy and two nephrectomies) who required postoperative narcotic treatment. LESS patients had shorter hospitalization compared with the conventional laparoscopy group, but only in the nephrectomy group.

CONCLUSION

LESS has proved to be immediately applicable in the clinical field of pediatric urology, being safe and feasible in the hands of experienced laparoscopic surgeons in well-selected patients. We believe that one of the future challenges for LESS in the pediatric population may be the treatment of nephrolithiasis. Despite promising early outcomes, the benefits of LESS are not obvious at present, with the only claimed advantage being cosmetic. Prospective randomized studies are required to define the benefits of this technique for patients as well as to elucidate the cost-effectiveness of the approach.

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RETROSPECTIVE STUDY

Cardiopulmonary bypass with brain perfusion for renal cell carcinoma with caval thrombosis

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Abstract

AIM: To compare a modified technique preserving brain circulation during cardiopulmonary by-pass (CPB) for radical nephrectomy and caval thrombectomy, to the standard technique.

METHODS: Retrospective evaluation of an institutional database that collects the data of patients submitted to nephrectomy and removal of caval thrombosis with CPB since 1998. In period between 1998 and 2007, CPB followed a standard technique (group sCPB); then, since 2008, a variation in the perfusional technique was introduced, allowing the anterograde perfusion of brain circulation during circulatory arrest (group CPB + BP) with the aim to reduce the risk of ischemic damage to the brain and also the need of deeper hypothermia. Patients (age, gender, comorbidity) and tumor characteristics (side, histology, staging, level of thrombosis), as well as parameters of CPB (times of CPB, aortic clamping and circulatory arrest, minimum temperature reached during hypothermia), intra- and perioperative morbidity (complications in general, bleeding, renal and hepatic failure) and mortality were analyzed and compared between 2 groups (sCPB *vs* CPB + BP)

RESULTS: The data of 24 patients, respectively 9 in sCPB group and 15 in CPB + BP group, have been reviewed. No differences in the characteristics of patients and tumors were observed. Only 1 (11.1%) and 4 (26.0%) of sCPB and CPB + BP patients, respectively, didn't experience any event of complication. In sCPB group were observed 15 events of complication (5 of which Clavien ≥ 3, 33% of the events), for a mean of 1.66 events/patient; 29 events (10 Clavien ≥ 3, 30.3%), in the CPB + BP group, for a mean of 2.1 events/patient. 1 (11.1%) and 2 (14.2%) deaths occurred, respectively. For patients submitted to CPB + BP, the minimum temperature reached was significantly higher (29.9 ℃ *vs* 26.4 ℃, $P = 0.001$), the time of circulatory arrest was longer (17.4 min *vs* 13.7 min, NS), but the overall time of CPB shorter (76.1 min *vs* 92.5 min, NS), albeit these latter differences were not statistically significant. No differences in terms of bleeding, impairment of renal function (post-operative Cr > 2.0 mg/dL respectively in 44.4% *vs* 35.7% of cases, in the two groups, NS) or hepatic insufficiency (post-operative GOT or GPT > 50 U/L respectively in 44.4% and 66.7% of patients, NS) were noted. Average follow-up was 51 mo in patients undergoing a sCPB and 12 mo in the CPB + BP group of patients; at the last follow-up, 7 patients had died of progression of the condition (4 in the first group and 3 in the second group, respectively), 7 were alive in progression and 10 had no evidence of the disease.

CONCLUSION: The perfusional technique that maintains brain perfusion during circulatory arrest limits hypothermia and lowers time of CPB, without rising the risk of renal and hepatic injury.

Key words: Renal carcinoma; Vena cava thrombosis; Cardiopulmonary bypass; Cerebral perfusion; Circulatory arrest

Core tip: Surgery for renal cell carcinoma with caval thrombosis extended to the diaphragm or right atrium is burdened by a high risk of complications. The adoption of a modified technique of cardiopulmonary bypass that maintans the perfusion of brain circulation, doesn't add morbidity to the procedure and can be in principle of benefit, since it shortens the duration of surgery and requires a less deep hypothermia.

Antonelli A, Bisleri G, Mittino I, Moggi A, Muneretto C, Cosciani Cunico S, Simeone C. Cardiopulmonary bypass with brain perfusion for renal cell carcinoma with caval thrombosis. *World J Clin Urol* 2014; 3(2): 127-133 Available from: URL: http://www.wjgnet.com/2219-2816/full/v3/i2/127.htm DOI: http://dx.doi.org/10.5410/wjcu.v3.i2.127

INTRODUCTION

Renal carcinoma (RCC) is extremely angiotropic and may extend into the venous circulation, from the renal vein to the right sections of the heart[1]. Lacking an effective medical treatment, surgery is still the only available treatment, leading to 5-year cancer-specific survival rates of up to 70%[2,3].

When the thrombosis reaches the diaphragm, the surgical treatment requires a combination of abdominal and thoracic approaches in order to achieve an extensive removal of the thrombus within the inferior vena cava and the right atrium by means of cardiopulmonary bypass (CPB) and circulatory arrest. Even if other strategies have been also suggested to avoid circulatory arrest or even CPB (*i.e.*, CPB under mild hypothermia without cardiac arrest, veno-venous by-pass by means of cavo-atrial shunt and "milking" manoeuvre below the major hepatic veins without CPB[4]), CBP with circulatory arrest is probably the most widely accepted surgical strategy for these patients.

This procedure itself is burdened by a high risk of bleeding, which is in part due to the need of blood heparinization during the exanguination of the patient, but also to the coagulation disorders caused by deep hypothermia, used in order to minimise the potential parenchymal damage (in particular to the brain) caused by the circulatory arrest.

In an attempt to further improve patients' clinical outcomes, at our institution since 2007 a novel CPB technique has been adopted which allows for antegrade cerebral perfusion during circulatory arrest in mild hypothermia. The purpose of this study was therefore to review the results achieved by means of this novel technique (cardiopulmonary bypass with cerebral perfusion-CPB + BP, Figure 1) and compare it with the conventional technique (standard cardiopulmonary bypass without cerebral perfusion-sCPB), with respect to intra- and post-operative outcomes.

MATERIALS AND METHODS

This study included all patients undergoing a procedure including nephrectomy and removal of thrombosis with CPB at our Institution since 1998, because since that year a specific database to store all the surgical data of these patients was generated.

The indication for the removal of thrombosis with the aid of CPB was done for all those cases in which the preoperative assessments revealed that the upper level of thrombosis reached the right atrium or above. All the patients were studied by electronic computer X-ray tomography technique (CT), magnetic resnane iamge (MRI), trans-esophageal echocardiography and coronarography, in order to evaluate the extent and features of the thrombosis as well to rule out any potential concomitant heart disease.

The surgical approach implies a bilateral subcostal incision and a median sternotomy, separated by a short skin bridge, with the aim to improve the healing of the sternal incision by keeping it separate from the abdominal one. Once the abdominal vena cava and aorta have been isolated and the renal artery ligated, the kidney is mobilised while preserving only the renal vein; once the CPB has been instituted, before the cavotomy, the inferior vena cava caudal to the thrombosis and the contralateral renal vein are clamped with tourniquets. Then, once mild hypothermia is achieved, cardioplegia solution is delivered and the general circulation is arrested; finally, the right atrium and the lower abdominal vena cava are opened in order to remove the thrombosis and to complete the nephrectomy.

From 1998 to 2007, CPB has been carried out using a standard central cannulation technique, achieving moderate hypothermia (25 ℃) during circulatory arrest (sCPB group).

Instead, since 2007 to date, a variation of such technique has been adopted (CPB + BP group), to maintain cerebral perfusion so that mild hypothermia was sufficient during the circulatory arrest. As we previously described[5], once the pericardium is opened, the aortic arch is prepared as to allow exposure of the supra-aortic vessels; the ascending aorta is cannulated, as well as the right atrium and the superior vena cava. Once moderate-mild hypothermia has been reached (30 ℃), the aorta is clamped and a cardioplegic solution is administered; then, an additional clamp is positioned on the aortic arch between the left common carotid artery and the left subclavian artery. Following opening of the right atrium, venous drainage is obtained exclusively *via* the cannula previously inserted into the superior vena cava: therefore, cerebral perfusion is maintained through the aortic arch while the patient is on systemic circulatory arrest, allowing for a bloodless field both on the cardiac and abdominal sides.

In the present study, the level reached by the thrombosis was classified according to the Mayo Clinic system[6]; co-

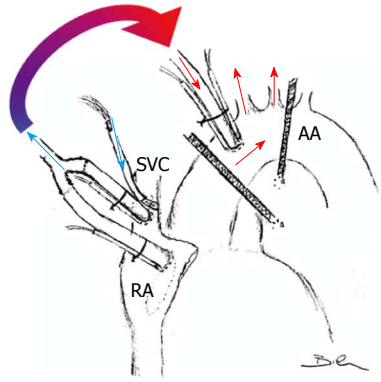

Figure 1 Sketch of the technique of incannulation adopted for CPB+BP: when right atrium and superior vena cava are cannulated, venous flow (blue arrows) after oxygenation is pumped into the aortic arch clamped (aclamps) between the brachio-cefalic trunk and the left carotid artery. RA: Right atrium; SVC: Superior vena cava; AA: Aortic arch.

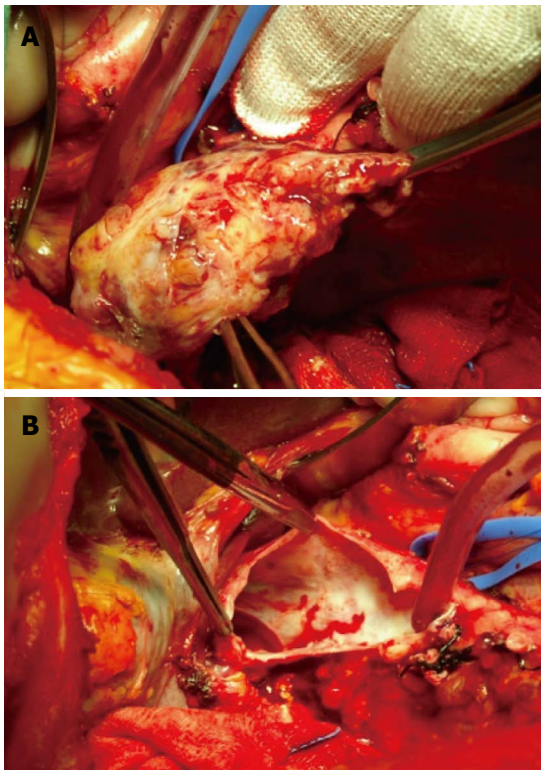

Figure 2 Extraction of the thrombus through a cavotomy (A) and examination of the caval lumen in a completely bloodless field (B).

morbidities were scored according to the Charlson-Romano score[7]; post-operative complications were recorded up to 30 d after surgery according to the Clavien-Dindo system[8].

Statistical analysis

Discrete variables were analysed by Fisher exact test or χ^2 test, while Mann-Whitney test was utilised for continuous variables (SPSS v.13, SPSS Inc, Chicago, IL, United States).

RESULTS

During the period of the study (1998-2013), 1477 patients underwent surgery for renal cancer at our Institution. Overall, 23 patients with venous thrombosis underwent surgery with the aid of CPB, 9 with sCPB, 15 with a CPB + BP technique.

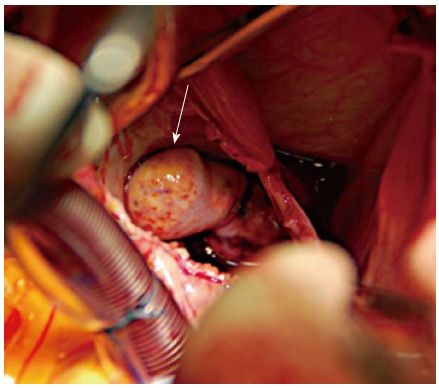

Figure 3 The head of the thrombus (arrow) seen from an upper point of view through the atriotomy.

The two groups had comparable characteristics, as listed in Table 1. Even in all the patients at the preoperative evaluation the head of the thrombosis was deemed as inside of the right atrium, in 12 patients at surgical exploration, it was close but outside the atrium, so that they have been down-classified as level 3, again in a similar rate in the two groups.

Only 1 (11.1%) and 4 (26.0%) patients in the sCPB and in the CPB+BP group, respectively, did not experienced any postoperative complications; 15 complications (5 of which were grade $\geqslant$ 3, equal to 33% of the events) happened in the sCPB group, which means on average 1.66 events per patient; 29 complications (10 of which were grade $\geqslant$ 3, equal to 30.3% of the events) in the CPB + BP group, which means on average 2.1 events per patient (Table 2).

Table 3 summarizes the comparison of CPB parameters and indicators of morbidity. For the patients of the CPB+BP group was noted a significantly less deep hypothermia and, even without statistical significance, a prolonged time of circulatory arrest (CPB + BP 17.4 min *vs* sCPB 13.7 min), but a shorter overall CPB time (CPB + BP 76.1 min *vs* sCPB 92.5 min) due to the faster cooling and rewarming of the patient. Finally, no differences in terms of indicators of hepatic, renal, brain and coagulative impairment were noted, with a shorter overall length of stay for the CPB + BP group.

Average follow-up was 51 mo in patients undergoing a sCPB and 12 mo in the CPB + BP group of patients; at the last follow-up, 7 patients had died of progression of the condition (4 in the first group and 3 in the second group, respectively), 7 were alive in progression and 10 had no evidence of the disease.

DISCUSSION

Renal cancer is extremely angiotropic, which may result in a macroscopic invasion of the large venous vessels in up to 10% of cases[9]. While a regression with targeted

Table 1 Details of the two groups

Patients and tumors characteristics	sCPB	CPB + BP	*P*
Age (yr)	64.0 ± 9.2	66.2 ± 10.2	NS
Gender			
Males	7/9 (77.8%)	9/15 (60%)	NS
Females	2/9 (22.2%)	6/15 (40%)	
Pre-operative ejection fraction	59.7% ± 0.8%	57.6% ± 4.6%	NS
Comorbidity			
Charlson score 0	2/9 (22.2%)	6/15 (40%)	NS
Charlson score ⩾ 1	7/9 (77.8%)	9/15 (60%)	
Tumor side			
Right	7/9 (77.8%)	10/15 (66.7%)	NS
Left	2/9 (22.2%)	5/15 (33.3%)	
Tumor diameter (cm)	9.6 ± 2.5	9.6 ± 2.9	NS
Level of thrombosis			
3	5/9 (56.6%)	8/15 (53.3%)	NS
4	4/9 (44.4%)	7/15 (46.6%)	
Histology			
Clear Cell renal carcinoma	7/9 (77.8%)	13/15 (86.6%)	NS
Other	2/9 (22.2%)	2/15 (13.3%)	
Infiltration of perirenal tissues	7 (77.8%)	10 (66.7%)	NS
Infiltration of venous wall	3 (33.3%)	4 (26.6%)	NS
N+	1 (11.1%)	2 (13.3%)	NS
M+	2 (22.2%)	5 (33.3%)	NS

NS: Not significant; CPB: Cardiopulmonary by-pass.

therapies has been occasionally reported[10,11], only radical surgery can effectively treat patients albeit with variable 5-year survival rates, ranging between 34% and 72%[2,3]: such outcomes may be influenced mostly by unfavourable prognostic factors which are frequently associated with the occurrence of neoplastic thrombosis rather than the cranial extension of the thrombosis itself[12].

Surgery of caval thrombosis is technically complex and has high morbidity and mortality rates, up to 22% in a recent series[11].

In patients in which the thrombosis has extended over the diaphragm, the use of a CPB with circulatory arrest has been widely utilised in order to allow for a better control of the cranial end of the thrombosis through an atriotomy as well as to achieve a bloodless surgical field once the inferior vena cava has been opened (Figures 2, 3). To reduce the metabolic requirements of the tissues and thus increase the resistance to ischemic damage during the circulatory arrest, deep hypothermia (< 20 ℃) has been routinely used[13]. In particular, the brain yields an higher risk of ischemic damage and its metabolism during circulatory arrest must be therefore significantly reduced by means of hypothermia, with an ischemic tolerance lasting up to 5 min at 37 ℃, 15 min at 25 ℃, 24 min at 20 ℃, and 45 min at 10 ℃[14-17].

There is a high risk of bleeding during CPB, which is related to the heparin infused when blood is diverted into the reservoir and the patient is exanguinated, and then antagonised by protamin when the physiologic circulation is restored, but also to hypothermia itself that could impair the platelet function and the production of coagulation factors.

The evidence that hypothermia may exert a negative impact is debated and recent publications, on the contrary, advocate its adoption on the basis of a better clinical outcome[18,19]. At the same time a diffuse concern exists, since several authors suggested alternative techniques to avoid the circulatory arrest[20,21], rather accepting a higher risk of a bloody surgical field, embolisms and tears to the caval wall due to the lack of a direct control over the cranial end of the thrombosis[22].

CPB with antegrade cerebral perfusion has been widely utilised for the surgery of the aortic arch[23], and it has also been reported in the treatment of thrombosis from renal carcinoma by our and other groups[5,24]. By the antegrade perfusion of the brain, hypothermia can be limited, thus reducing the theoretical risk of hypothermia-induced coagulation failure as well as the sure waste of time required to cool down and warm up the patient, but still providing the same bloodless operative field offered by a conventional deep hypothermic circulatory arrest.

The present study reviews the experience of a single academic institution with 23 consecutive patients treated in the last 15 years, using a CPB for caval thrombosis over the level of diaphragm. The number of patients, limited in absolute terms, is comparable to the main reports that have been published so far; in addition, for the present study the data collection has been planned for the specific purpose of estimating the morbidity of the procedure.

It has been confirmed that extensive caval thrombosis is associated with other unfavourable pathologic prognostic factors (invasion of peri-renal tissues, lymphnodes and distant metastasis), which may account for the poor prognosis[1,5,11]: in this study, the 5-year cancer-specific survival rate was 35%, but reached 62% in patients without lymphnodes or distant metastasis, thus confirming that in such cases surgery may promote a prolonged survival.

In the 9 cases treated in the first period of this study (sCPB), patients were cooled down to an average temperature of approximately 26 ℃ during the circulatory arrest, while in the 15 cases treated in the second period (CBP + BP), changing the technique of perfusion resulted in a remarkably lesser hypothermia, with an average minimum temperature of approximately 30 ℃. Such level of hypothermia was maintained for a more prolonged time of circulatory arrest (CPB + BP 17.4 min *vs* sCPB 13.7 min), but within a shorter overall CPB time (CPB + BP 76.1 min *vs* sCPB 92.5 min) due to the less time needed to cool and rewarm the patient.

No differences were found between the two groups in terms of pre-operative co-morbidity or characteristics of tumors. Post-operative complications, systematically recorded, were very frequent, with at least one event in 83% of cases and a 30-d perioperative mortality rate of 12.5%, as shown in Table 3. The number of events of complications and the rate of major complications were similar between the 2 groups, as the mortality rate. However the length of stay was slightly shorter in CPB + BP group, suggesting a less detrimental effect in these patients from the events of complication. More interest-

Table 2 Complications observed in the 2 groups, according to the Clavien-Dindo's scale

Clavien grade	sCPB	CPB + BP
1	4 acute renal failures	5 acute renal failures
2	3 transfusions	7 transfusions
	3 atrial fibrillations	7 atrial fibrillations
3a	1 sternal wound dehiscence	
3b	1 bleeding requiring re-laparotomy	1 bleeding requiring re-laparotomy
		1 ligature of the contralateral ureter, requiring ureteral stenting
4a	2 =respiratory failures	1 brain stroke
		1 pulmonary embolism
		3 respiratory failures
4b	-	1 multi-organ failure
5	1 (1st post-operative day)	2 (3rd and 25th post-operative day)

CPB: Cardiopulmonary by-pass. NS: not significant.

Table 3 Comparison in terms of intra- and peri-operative characteristics between the 2 groups

Intra- and post-operative variables	sCPB	CPB + BP	*P*
CPB time (min)	92.5 ± 35.7	76.1 ± 18.5	NS
Aortic Clamp time (min)	30.8 ± 15.8	26.5 ± 19.6	NS
Circulatory arrest time (min)	13.7 ± 8.9	17.4 ± 5.3	NS
Minimum temperature (℃)	26.4 ± 2.5	29.9 ± 1.9	0.001
Intraoperative blood units transfused (no.)	12 (4-12)	13.2 (6-20)	NS
ICU stay (in days)	2 (2-2)	2.7 (1-3)	NS
Hospital stay (in days)	27 (22-57)	21 (10-25)	NS
Post-operative Cr > 2 mg/dL	4/9 (44.4%)	5/15 (33.3%)	NS
Post-operative GOT or GPT > 50 U/L	4/9 (44.4%)	8/15 (53.3%)	NS
Post-operative bleeding	5/9 (55.6%)	8/15 (53.3%)	NS
Peri-operative mortality	1/11 (11.1%)	2/15 (13.3%)	NS

CPB: Cardiopulmonary by-pass. NS: not significant; GOT: Glutamic-oxalacetic transaminase; GPT: Glutamic-pyruvic transaminase.

ingly, the number of renal and liver failure events turned out to be comparable, confirming that these parenchyma may tolerate temperatures above 20 ℃-25 ℃, at least for the time of circulatory arrest that such kind of surgery requires. At the same time, from our results nor advantages neither disadvantages in terms of bleeding or neurologic sequelae cannot be proved, as the only neurologic event happened just in the CPB + BP group.

So, the present study shows that the CPB + BP technique is not inferior to a standard CPB in terms of radicality of surgery and overall complications, while gives an advangtage in terms of duration of surgery. Surely, these conclusions should be taken with caution, because the study suffers from some limitations. First, the retrospective design, in spite of the perspective manner in which data has been collected: even if it is reasonable that the maintenance of cerebral circulation could add an advantage on neurological functions, the study fails to offer sure evidences of this, lacking of an established indicator that could measure cerebral metabolism prior, during and after the intervention. Second, the limited number of patients, enrolled in a long timeframe, that lowered the validity of statistical comparisons between groups; however, RCC with caval thrombosis treated with CPB is a rare condition, so that a perspective study probably will never be designed, even with a multicentric data collection, since different institutions usually adopt a personal approach.

The variation of the technique of perfusion proposed limits the need for hypothermia to moderate levels (30 ℃), with the theoretical benefits on coagulation function and a sure advantages in terms of duration of surgery, without affecting the radicality of surgery or provoking a higher number of sequelae than a CPB with deeper hypothermia.

COMMENTS

Background

Surgery is the only option to cure some of the patients affected by a renal cancer that involves the inferior vena cava, However, this surgery suffers from high risks of intra and postoperative morbidity, which mainly depend on the cranial extension of the thrombus. Indeed, morbidity is higher for thrombosis extended up to the retroepatic vena cava or the right atrium, since in such conditions cardiopulmonary by-pass (CPB).

Research frontiers

The exsanguination by CPB is required to extract of caval thrombosis extended up to the diaphragm or above in a bloodless field after the vena cava and/or the atrium are opened. To permit CPB, deep hypothermia is required, so that the organs - and in particular the brain - will suffer in a less extent from ischemia. Hypothermia can provoke an impairment of coagulation and prolongs the duration of the operation, worsening the morbidity of the procedure. Many authors investigated means alternative to CPB to reduce the morbidity of surgery in renal cancer with extended thrombosis. These procedures avoid at all the CPB,

adopting an exclusive abdominal approach, by the complete mobilization of the liver and the exposition of the retrohepatic vena cava, or by a veno-venous bypass that diverts the venous flow from the vena cava but maintaining the cardiac activity and the arterial perfusion. There are, however, risks of incomplete radicality, if the thrombosis infiltrates a portion of the caval wall out which is not visible from the abdominal access, of embolism due to the detachment of part of the thrombus without the upper control through atriotomy, and of bleeding, related to the backflow from the lumbar veins.

Innovations and breakthroughs

The CPB with brain perfusion (CPB + BP) is a technique developed by cardiac surgeons with the intent to preserve cerebral perfusion during aortic arch replacement, showing a lower morbidity in respect to a standard CPB, in principle related to the less deep hypothermia required and to the maintenance of cerebral perfusion. At authors' institution this technique has been applied to the surgery of renal cancer with extended venous thrombosis. At now only a very few cases have been reported, while this study reported a larger number of patients, comparing the results with an homogeneous historical group in which a standard CPB was adopted

Applications

The tecnhique can be suggested to the institutions actually treating these patients by a standard CPB, since the authors showed a non-inferiority of this modified technique in terms of renal and hepatic impairment, as of morbidity in general, with a less deep hypothermia and shorter operative times. The study can be the basis for future studies to investigate the impact on cerebral metabolism of this technique.

Terminology

Cardiopulmonary by-pass: a procedure to convey blood from normal circulation to a machine that oxygenates blood.

Peer review

The article is a well-written manuscript comparing two techniques of cardiopulmonary bypass for excision of renal tumours with vena caval extension.

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RETROSPECTIVE STUDY

Penile prosthesis: Patient satisfaction, use and preference for malleable *vs* inflatable

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Abstract

AIM: To evaluate and compare long-term patient satisfaction and use after either malleable penile prosthesis (MPP) or inflatable penile prosthesis (IPP) implantation.

METHODS: we present a retrospective unicenter study of 108 patients implanted with either 2 or 3-piece American Medical System™ (AMS™) or Coloplast™ inflatable penile prosthesis (AMS 700CX™, AMS 700CXR ™, AMS Ambicor™ or Coloplast TITAN™) or malleable (AMS Spectra™ or Coloplast Genesis™) in our Centre between 1993 and 2011. We collected data from the medical record including follow-up, age and type of prosthesis. We used a four-question telephone survey designed ad hoc by urologist in our Department, with three multiple choice questions and a grading answer. After verbal consent was obtained, proposed questions concerned global satisfaction regarding to the procedure, quality of sexual intercourses graded from 0 to 10, frequency of sexual intercourse and about undergoing the same procedure again. SPSS™ version 20.0 was used for the descriptive analysis of the data.

RESULTS: Sixty seven (64%) patients underwent a MPP and 41 (36%) an IPP. The mean age was 52.6 ± 3.6 years in the MPP group and 57.2 ± 2.8 years in the IPP group (P = 0.02). Total respond rate was 55.5% (60/108). Twenty six out of 33 MPP patients (78.9%) and 19 of the 27 IPP subjects (70.3%) were satisfied or very satisfied with the procedure. The quality of sexual intercourse was rated 7.13 ± 0.39 points in the MPP group and 6.16 ± 0.47 points in the IPP group. Frequency of sexual intercourse was 1 or more times per week in 15 (46.9%) patients with MPP and in 12 (46.1%) of the IPP patients. Twenty-eight (84.9%) patients who received a MPP would undergo the procedure for the same device again as well as 24 (88.9%) of the IPP group. There were no statistical differences between groups regarding the four items in the survey.

CONCLUSION: Patients show high satisfaction rate and no statistical differences exist regarding to global satisfaction, use of the device and quality of sexual intercourse depending on the type of penile prosthesis.

Key words: Penile prosthesis; Patient satisfaction; Erectile dysfunction; Medical survey

Core tip: retrospective study of 108 patients implanted with either malleable penile prosthesis or inflatable penile prosthesis designed to investigate results in

terms of use of the device and satisfaction. Patient satisfaction after prosthetic surgery is multifactorial and it should be considered when exposing the pros and cons of prosthesis to patient before surgery. We collected data with a four-question telephone survey. After analyzing our results, we concluded that patients show high satisfaction rate and no statistical differences exist regarding to global satisfaction, use of the device and quality of sexual intercourse depending on the type of penile prosthesis.

Rogel Bertó R, López-Acón JD, Luján Marco S, Ordaz Jurado DG, Delgado Oliva F, Conca Baenas MA, Boronat Tormo F. Penile prosthesis: Patient satisfaction, use and preference for malleable *vs* inflatable. *World J Clin Urol* 2014; 3(2): 134-138 Available from: URL: http://www.wjgnet.com/2219-2816/full/v3/i2/134.htm DOI: http://dx.doi.org/10.5410/wjcu.v3.i2.134

INTRODUCTION

Surgical treatment for erectile dysfunction (ED) by implantation of penile prosthesis is considered a safe and efficient option to treat those cases non responding to pharmacological agents[1]. Both, prosthesis materials and prosthesis design, have evolved in time, in the same way that surgical techniques have, in order to achieve a better durability and quality of the device.

Patient satisfaction after the penile prosthesis surgery is considered multifactorial and depends on issues like presurgery expectations and the success of the implant as ED treatment option[2]. If compared with the other options to treat ED like phosphodiesterase five (PDE-5) inhibitors, intraurethral alprostadil, intracavernous injection of alprostadil or mechanical devices, penile prosthesis is the one showing better satisfaction rates[3].

The objective of our study was to evaluate and compare long-term patient satisfaction and use after either malleable or inflatable penile prosthesis implantation. Patients were offered both types of prosthesis if no contraindication. Most of the papers published so far concerning patient satisfaction with the device have used non-validated questionnaires designed by each Hospital or Investigation Group[1]. Two validated questionnaries exist concerning sexual intercourse satisfaction: the satisfaction domain of the Internation Index of Erectile Function (IIEF) and the Erectile Dysfunction Inventory of Treatment Satisfaction (EDITS)[4-7].

MATERIALS AND METHODS

We present a retrospective study on 108 patients implanted with either 2 or 3-piece American Medical System™ (AMS™) or Coloplast™ inflatable penile prosthesis (AMS 700CX™, AMS 700CXR™, AMS Ambicor™ or Coloplast TITAN™) or malleable (AMS Spectra™ or Coloplast Genesis™) in our Hospital between 1993 and 2011. We collected data from medical record like follow-up, age and type of prosthesis. We made contact with each patient for a telephone survey. Prior to the survey each patient was informed of the content and objective of the survey and consent was obtained. Survey was designed by the authors based in the penile prosthesis satisfaction papers published to date. We configured a first version which was examined by several other urologists in the Department to evaluate clarity and precision of the questions. After this first version, we configured the final version. We obtained a four-question telephone survey with three multiple choice questions and a grading answer question. First of them made reference to global satisfaction concerning the procedure with four possible answers being: (1) Not satisfied; (2) Partially satisfied; (3) Satisfied; and (4) Very satisfied. Second question asked about the quality of sexual intercourses graded from 0 to 10, being zero "very bad quality" and 10 "very good quality". We asked in question number three about the frequency of sexual intercourse being answer: (1) More than once per week; (2) Once per week; (3) Once per for night; (4) Once per month; and (5) Less than once per month; and last, we requested about the fact of undergoing the same procedure again, and the two possible answer were (1) Yes or (2) No.

Statistical analysis

Data were analysed using the SPSS™ 20.0 (IBM corp™). Statistical analysis was performed using Fisher test to detect differences between differents groups. A value of $P < 0.05$ was considered to be statistically significant.

Table 1 Etiology, infection rate and reoperation *n* (%)

		MPP	IPP	*P*
Main etiology	Diabetes mellitus	21 (31.3)	17 (41.5)	0.505
	Vasculogenic	14 (20.9)	8 (19.5)	
	LaPeyronie disease	9 (13.4)	5 (12.2)	
	Radical Prostatectomy	2 (3)	3 (7.3)	
	Neurogenic	8 (11.9)	4 (9.7)	
	Unknown	13 (19.4)	4 (9.7)	
Implant Infection		7 (10.5)	5 (12.2)	0.202
Reoperation		10 (14.9)	7 (17.5)	0.787

MPP: Malleable penile prosthesis; IPP: Inflatable penile prosthesis.

RESULTS

A total of 67 (64%) patients underwent a MPP and 41 (36%) an IPP. The mean age was 52.6 ± 3.6 years in the MPP group and 57.2 ± 2.8 years in the IPP group ($P = 0.02$). Total respond rate was 55.5% (60/108); 27 (25%) had deceased and the remaining 21 (19.4%) did not respond. Among those who attended to the survey, 33 patients (55%) had MPP and 27 (45%) had IPP. There were no statistical differences between the groups regarding to etiology of erectile dysfunction (ED) ($P = 0.505$), incidence of implant infection ($P = 0.202$) or reoperation rate ($P = 0.787$) (Table 1).

The median time from surgery to the survey was 161 (6-199) mo for the MPP group and 37 (3-161) for the IPP group. As shown in Table 2, 26 of the 33 MPP

Table 2 Results of the survey *n* (%)

Question	MPP		IPP		*P*
Satisfaction	No satisfied	2 (6.1)	No satisfied	6 (22.2)	0.157
	Partially satisf.	5 (15.2)	Partially satisf.	2 (7.4)	
	Satisfied	15 (45.5)	Satisfied	13 (48.1)	
	Very satisfied	11 (33.3)	Very satisfied	6 (22.2)	
Quality	7.13 ± 0.39		6.16 ± 0.47		0.314
Frequency	> 1/wk	8 (25)	> 1/wk	7 (26.9)	0.413
	1/wk	7 (21.9)	1/wk	5 (19.2)	
	1/15 d	5 (15.6)	1/15 d	5 (19.2)	
	1/mo	3 (9.4)	1/mo	3 (11.2)	
	> 1/mo	9 (13.4)	> 1/mo	6 (23.1)	
Undergo again	YES	28 (84.9)	YES	24 (88.9)	0.774
	NO	5 (15.1)	NO	3 (11.1)	

MPP: Malleable penile prosthesis; IPP: Inflatable penile prosthesis.

patients (78.9%) and 19 of the 27 IPP subjects (70.3%) were satisfied or very satisfied with the procedure. The quality of sexual intercourse was rated 7.13 ± 0.39 points in the MPP group and 6.16 ± 0.47 points in the IPP group. Frequency of sexual intercourse was 1 or more times per week in 15 (46.9%) patients with MPP and in 12 (46.1%) of the IPP patients. Twenty eight (84.9%) patients who received a MPP would undergo the same device procedure again as well as 24 (88.9%) of the IPP group. There were no statistical differences between groups regarding the four items investigated in the survey.

DISCUSSION

Penile prosthesis as an ED treatment option is considered to have a high satisfaction rate among the patients implanted[3]. On the other hand, there is a small number of unsatisfied patients with surgery, esthetic and/or functional results. When perceived, it is about rigidity, length, infection of the device, spontaneous deflation or mechanical failure the main reasons for those cases of patient dissatisfaction. Patient has to know before surgey which are the real expectations, the way prosthesis will modify or not penile length and girth, penile sensitivity, glans status, if circumcision is going to be performed as well as infection, mechanical failure and prosthesis removal rates[1,8].

In our unicenter retrospective study we have obtained a high rate global satisfaction in both groups showing no statistically significant differences between them. Another result to pay attention is that most of patients are located in highest or lowest frequency groups of use, at the expense of the middle positions. More than 1 per week is 25% in MPP group and 26.9% in IPP; and the opposite position, less than 1 per month is 13.4% in MPP group and 23.1% in IPP. Concerning to satisfaction, most of the patients in both groups would undergo the procedure again.

Focusing in global satisfaction with the inflatable prosthesis, our percentages of 70.3% of satisfied or very satisfied, are less evident than the ones in other references. In a study that involved 145 patients implanted with IPP AMS 700 Ultrex™, after the satisfaction questionnaire, 85% were satisfied against 76% of partner satisfaction[9]. Another study of 207 patients implanted with IPP AMS 700CX™, performing a telephone survey, showed 79% use the device at least twice monthly and 88.2% would recommend an implant to a relative or friend[10]. Two other studies, one conducted with 200 consecutive patient who underwent IPP AMS Ultrex™ and CX™ showed an overall satisfaction of 92%[11]. The other one, 80 cases implanted with IPP AMS 700CX™ responded to a nine-point telephone survey and 97% of patients use the device frequently; 69% affirmed they never had problems with its use; and 97% reported they would suggest this treatment to other people[12].

In relation to IPP Mentor alpha-1™, one study showed that 89% of men had fulfilled expectations with the prosthesis. Regarding intercourse ability, confidence and device rigidity and function satisfaction rate was 80% or greater[13].

One study was conducted to rate patient satisfaction with 3 types of penile prosthesis. A random sample of 330 patients (of 1298 patients implanted) with either AMS700™, Mentor Alpha 1™ or Mentor Alpha NB™ responded to a computer assisted telephone survey. The overall satisfaction rate was 69%, and there was no significant difference by implant type[14].

Another multicentre study comprising several types of prosthesis (IPP AMS700 CX™, AMS Ambicor™ and AMS 600-650™) in terms of satisfaction, use the EDITS validated questionnaire. Patient satisfaction rates were 97%, 81% and 75% respectively[15].

Two different papers included two-piece IPP patient satisfaction. The first one evaluated 146 patients implanted with IPP AMS Ambicor™ and found that 91% said that it was easy to use. Overall patient satisfaction was 85%, and 86% would recommend the prosthesis to friends or undergo the procedure again if necessary [4]. The second paper regarding IPP AMS Ambicor™ satisfaction involved 131 patients and they collected date from their own mailed questionnaire and from a modified EDITS mailed questionnaire. Overall patient satisfaction was 96.4% and 92.9% would recommend it to others. Of the 85 men who completed the modified EDITS survey,

90.6% were satisfied and 82.6% were very satisfied with the prosthesis[16].

A review article published recently 2012 concluded that nine studies, which met their criteria for review over the past 20 years, showed high satisfaction rates with the 3-piece IPP[1].

The limitations of this study are our low number of patients collected, which downs the statistical potency. In the same way, results might have been affected by the fact of being unicenter and retrospective. We consider that prostheses conditions and characteristics through time and the different surgeons performing the implantation could have modified the patients satisfaction as well, and it has not been taken into account to perform the analysis. Another important issue is that we have not used a standarized and validated questionnaire which makes our results difficult to correlate or compare with the ones published by other authors using them.

On the other hand, we present a long term follow up study with low representation in the literature by the fact that we compare malleable and inflatable two or three component in terms of satisfaction. This results offer a new extra tool for the urologist. Because of the absence of differences between malleable or inflatable penile prosthesis in terms of satisfaction, frequency or quality of sexual intercourse, these results could be used as an extra parameter to consider and should be added to the ones used normally to assist ourselves and the patient to choose the more suitable type of prothesis.

Using a non validated questionnaire in our retrospective, unicenter study, our results show high satisfaction rate in patients implanted with either IPP or MPP, similar to literature, and indicate that there are no statistical differences with regard to patient global satisfaction, frequency use of the device and quality of sexual intercourse depending on the type of penile prosthesis. More prospective studies using validated questionnaires are needed in order to obtain more powerful results and conclusions regarding satisfaction in patients implanted with penile prosthesis. In terms of sexual satisfaction, those studies should consider partner satisfaction as well.

COMMENTS

Background

Surgical treatment for erectile dysfunction (ED) by implantation of penile prosthesis is considered a safe option. High satisfaction rates have been reported in the literature.

Applications

Satisfaction rates should be used as an extra item to consider and should be added to the ones used normally (infection and mechanical failure rates, possibility of prosthesis removal, patient preference, anatomical conditions and comorbidities) to assist ourselves and the patient to choose the more suitable type of prothesis.

Peer review

This is a interesting article using a retrospective design, but less innovation. The results of the data are acceptable. Overall the manuscript is well written especially for the authors with English as non-native language.

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OBSERVATIONAL STUDY

Bone markers predict survival in castration-resistant prostate cancer patients treated with docetaxel

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Abstract

AIM: To investigate the relationship between clinicopathological features and bone turnover markers in castration-resistant prostate cancer (CRPC) patients treated with docetaxel.

METHODS: Thirty-three patients were enrolled in this study. Serum levels of carboxyterminal cross-linked telopeptide of type 1 collagen generated by metalloproteinases (1CTP) and alkaline phosphatase (ALP) were measured at the start of docetaxel chemotherapy. We examined the relationship between clinicopathological features and serum levels of 1CTP and ALP levels in CRPC patients treated with docetaxel.

RESULTS: For the total patient group, the mean ± standard deviation (SD) values for docetaxel chemotherapy dose, dose intensity, dosage interval, and number of cycles were 59.3 ± 10.6 mg/m^2, 13.9 ± 5.2 mg/m^2 per week, 4.7 ± 1.2 wk, and 11.2 ± 7.4, respectively. Fourteen patients died from prostate cancer. Patients were divided into two groups according to mean + SD of serum 1CTP (8.2 ng/mL) and ALP (538.2 IU/L) levels at the start of docetaxel chemotherapy. Patients with lower levels of serum 1CTP and ALP had significantly better survivals than those with higher serum levels ($P < 0.05$).

CONCLUSION: Serum levels of 1CTP and ALP are predictors of survival in patients with CRPC who are treated with docetaxel.

Key words: Prostate cancer; Docetaxel chemotherapy; Carboxy-terminal pyridinoline cross-linked telopeptide parts of type-1 collagen; Alkaline phosphatase; Prognostic factor

Core tip: This study examined the relationship between clinicopathological features and serum levels of carboxy-terminal pyridinoline cross-linked telopeptide parts of type-1 collagen (1CTP) and alkaline phosphatase (ALP) in castration-resistant prostate cancer patients treated with docetaxel. Patients were divided into two groups according to mean + SD of serum 1CTP (8.2 ng/mL) and ALP (538.2 IU/L) levels at the start of docetaxel chemotherapy. Patients with lower levels of serum 1CTP and ALP had significantly better survivals than those with higher serum levels. Serum levels of 1CTP and ALP are predictors of survival in patients with CRPC who are treated with docetaxel.

Endo T, Kamiya N, Suzuki H, Oka R, Lee FC, Utsumi T, Yano M, Kamijima S, Kawamura K, Imamoto T, Ichikawa T. Bone markers predict survival in castration-resistant prostate cancer patients treated with docetaxel. *World J Clin*

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INTRODUCTION

Prostate cancer (PCa) is the most common cancer among men in Western countries[1] and is the second leading cause of death in men[2]. In Japan, the incidence of PCa is rapidly increasing[3]. The clinical course of this cancer varies markedly due to its biological heterogeneity[4]. PCa is androgen-dependent, so androgen deprivation therapy (ADT) is generally used for patients with locally advanced and metastatic PCa[5,6]. ADT is effective for several years, but the disease may ultimately evolve into castration-resistant prostate cancer (CRPC)[7,8].

Docetaxel chemotherapy is the first chemotherapy regimen to demonstrate a survival benefit in CRPC patients[9,10], but not all patients gain benefit from this therapy. Thus, identification of factors that can predict favorable responses to docetaxel would be of benefit for rational selection of therapy for patients with CRPC.

PCa commonly metastasizes to bone, and these types of metastases are associated with various complications and significant morbidity, including severe bone pain, prolonged hospital stay, reduced mobility, hypercalcemia, and pathologic fractures. Furthermore, skeletal-related events correlate with reduced overall and median survival and quality of life of patients with PCa. Biochemical markers of bone metabolism may be useful, non-invasive, and sensitive surrogates of skeletal health. Carboxyterminal cross-linked telopeptide of type 1 collagen generated by metalloproteinases (1CTP) is a marker of bone resorption and is a metabolic product of mature type 1 collagen resorption[11]. Several studies have reported that serum 1CTP levels were significantly higher in PCa patients with bone metastasis than in PCa patients without bone metastasis[12]. Alkaline phosphatase (ALP) is a marker of bone formation and is widely used for assessing bone metastases[13]. We previously reported that serum 1CTP and ALP levels were reliable for the detection of bone metastatic spread and for the prediction of survival in PCa patients with bone metastasis[14].

The goal of the present study was to focus on bone resorption and formation markers by examining the relationship between clinicopathological features and serum levels of 1CTP and ALP in patients with CRPC treated with docetaxel.

MATERIALS AND METHODS

A total of 33 patients treated at our medical institutions between 2005 and 2009 were investigated in this study. All patients had histologically confirmed PCa. They all eventually became resistant to ADT, progressed to metastatic CRPC, and received docetaxel chemotherapy. The dose of docetaxel was 88.1 ± 24.7 (range, 40-130) mg/body, and the median number of docetaxel chemotherapy cycles was 11 ± 8.5 (range, 1-33). Other patient characteristics are described in Table 1.

Blood samples were taken with informed consent. Serum samples, such as prostate specific antigen (PSA), C-reactive protein (CRP), 1CTP, and ALP, were measured at the start of docetaxel chemotherapy. All serum samples were immediately frozen and stored at -20 ℃ until analysis. Serum PSA (ARCHITECT, Abbott Laboratories, Abbott Park, IL, United States) and serum CRP (Alpha Diagnostic Intl. Inc., San Antonio, TX, United States) were measured. Serum 1CTP levels were measured by radioimmunoassay (Immundiagnostik, Bensheim, Germany) to avoid the instability of radioiodinated reagents. Serum ALP levels were measured with IATROLQ ALP (Mitsubishi Chemical Medience Corporation, Tokyo, Japan). The rate of decrease in PSA was stratified according to > decline in 30%PSA and < decline in PSA. AEs were classified using common terminology criteria for adverse events (CTCAE) v4.0.

Statistical analysis

Statistical significance was examined using the Mann-Whitney *U*-test and Student's *t*-test, and cause-specific survival curves were created using the Kaplan-Meier method with the log-rank test. For all analyses, differences were considered statistically significant at $P < 0.05$. All statistical analyses were performed using SPSS version 11.0 (SPSS Inc., Chicago, IL, United States).

RESULTS

For the total patient group, the mean ± SD values for docetaxel chemotherapy dose, dose intensity, dosage interval, and number of cycles were 59.3 ± 10.6 mg/m^2 (range 20-75 mg/m^2; median 60 mg/m^2), 13.9 ± 5.2 mg/m^2 per week (range 3.3-25 mg/m^2 per week; median 13.8 mg/m^2 per week), 4.7 ± 1.2 wk (range 3-8 wk; median 4 wk), and 11.2 ± 7.4 (range 4-37; median 9), respectively. Mean follow-up time after the start of docetaxel chemotherapy was 16.2 ± 12.0 mo (range, 0.2-38.6 mo). During the follow-up period, 14 patients (42%) died from PCa. The median survival period of all patients was 16.2 ± 12.0 mo (range, 0.2-38.6 mo).

Fourteen patients had a PSA decrease > 30%, and 19 patients had a PSA decrease < 30%; thus, the PSA response rate was calculated to be 42.4%. The age at the start of chemotherapy, PSA level at the start of docetaxel chemotherapy, with or without PSA flare , Gleason sum and Extent of disease (EOD) score were not statistically related to survival (data was not shown). However, the number of chemotherapy cycles was significantly related to survival (data was not shown).

A number of adverse events (AEs) were reported in this study. Nausea was reported in seven (21%) cases, including two (6%) cases with grade 2 nausea, and three (9%) cases with grade 3 nausea. Grade 2 constipation was reported in three (9%) cases. Grade 3 lymphedema

Table 1 Clinicopathologic characteristics of castration-resistant prostate cancer patients *n* (%)

Variable	Value
Patients, *n*	33
Median (range)	
Age at start of chemotherapy, years	71.5 ± 7.4 (55-83)
PSA levels at start of chemotherapy, ng/mL	93.3 ± 131.3 (6.3-744.5)
PSA levels (initial diagnosis), ng/mL	877.8 ± 2259.1 (21.0-12490)
Dose of docetaxel, mg/body	88.1 ± 24.7 (40-130)
Dose of docetaxel, mg/m^2	53.3 ± 13.1 (30-70)
Number of cycles, *n*	11 ± 8.5 (1-33)
Survival period after chemotherapy, mo	16.2 ± 12.0 (0.2-38.6)
Clinical T stage	
3	28 (84.8)
4	5 (15.2)
Lymph node status	
0	15 (45.5)
1	18 (54.5)
Gleason sum (initial diagnosis)	
< 6	3 (10.0)
7	7 (23.3)
8	5 (16.7)
9	11 (36.7)
10	4 (13.3)
EOD score (initial diagnosis)	
0	12 (36.4)
1	3 (9.1)
2	10 (30.3)
3	8 (24.2)

CRPC: Castration-resistant prostate cancer; PSA: Prostate specific antigen; EOD: Extent of disease.

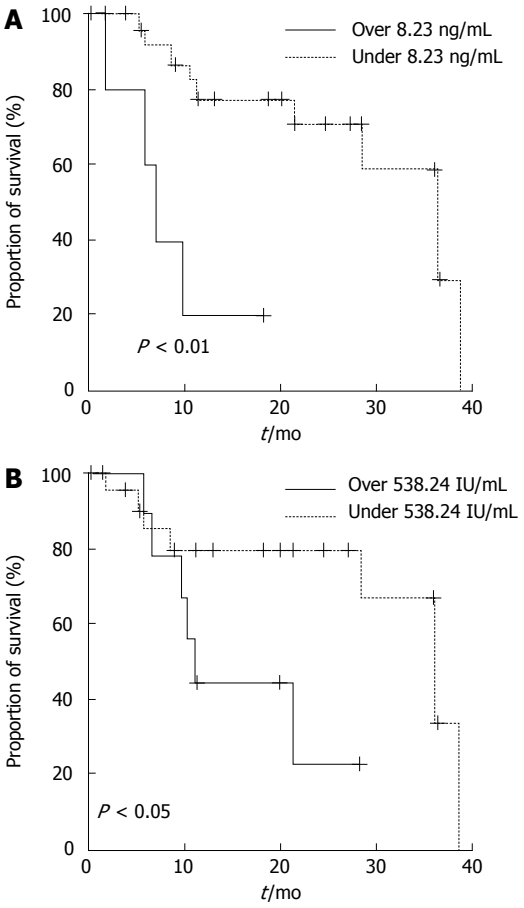

Figure 1 Cause-specific survival curves of castration-resistant prostate cance patients according to serum carboxy-terminal pyridinoline cross-linked telopeptide parts of type-1 collagen levels (A) and alkaline phosphatase levels (B).

of the limb was seen in two (6%) cases; this complication interfered with activities of daily living and resulted in a decreased quality of life. Grade 3 leukopenia was found in five (15%) cases.

During docetaxel chemotherapy up to five cycles, PSA flare was found in five (15%) cases. However, statistical analysis using the Kaplan-Meier method showed that PSA flare was not related to survival.

CRPC patients were stratified according to higher versus lower serum 1CTP levels using the mean + SD (8.23 ng/mL). Six patients had higher levels, and 27 patients had lower levels. Using the Kaplan-Meier method to construct cause-specific survival curves, we established that patients with higher serum 1CTP levels showed statistically significantly worse survival when compared with those with lower levels of 1CTP ($P < 0.01$) (Figure 1A).

CRPC patients were also stratified according to higher versus lower serum ALP levels using the mean + SD (538.24 IU/L). Nine patients had higher ALP levels, and 24 patients had lower ALP levels. Patients with higher serum ALP levels showed statistically significantly worse survival than those with lower ALP levels ($P < 0.05$) (Figure 1B).

Finally, CRPC patients were stratified according to higher (≥ 1 mg/dL) versus lower (< 1 mg/dL) serum CRP levels. Twenty-four patients had higher levels of CRP, and four patients had lower levels of CRP. Statistical analysis using the Kaplan-Meier method to construct cause-specific survival curves showed that patients with higher serum CRP levels had significantly worse survival than those with low CRP levels ($P < 0.01$) (data not shown).

There was no significant difference in the dose of docetaxel when comparing patients with higher and lower levels of 1CTP, ALP, or CRP, respectively.

DISCUSSION

Two large randomized phase Ⅲ studies (SWOG 99-16 and TAX 327) showed that docetaxel, when combined with either estramustine phosphate (EMP) or prednisone, significantly prolongs overall survival in men with metastatic CRPC when compared with the former standard treatment[9,10,15]. The SWOG 99-16 study using EMP in combination with docetaxel confirmed the survival advantage of docetaxel-based chemotherapy[15]. The TAX-327 study randomized 1006 patients to receive either docetaxel or mitoxantrone, each given with low-dose prednisone, and showed an extension of overall survival, improvement in quality of life, pain control, PSA decline, and objective tumor response[9]. Data from these two landmark trials and from other studies demonstrating promising activity for docetaxel against CRPC have resulted in the use

of docetaxel-based chemotherapy as a standard treatment for metastatic CRPC[16,17]. Indeed, docetaxel-based chemotherapy against metastatic CRPC is recommended by the National Comprehensive Cancer Network (NCCN) and the European Association of Urology (EAU) guidelines and is widely used in Japan.

Since the data described above were published, further data have become available from TAX-327, and prognostic factors and nomograms for docetaxel therapy have been proposed[18-21]. In 2010, Armstrong *et al*[20] reported that four independent risk factors predicted a ⩾ 30% increase in PSA within 3 mo of starting chemotherapy, pain, as well visceral metastases, anemia, and bone scan progression following treatment with docetaxel. Furthermore, a nomogram integrating several pretreatment factors (*e.g.*, pain, performance status, ALP, number of sites of metastatic disease, liver metastases, hemoglobin, PSA, and time since diagnosis) was validated for the prediction of post-progression survival. Armstrong *et al*[21] described evidence for the benefit of continuation of chemotherapy beyond progression only for men who had isolated worsening of pain. We similarly demonstrated that serum ALP level was a prognostic factor and that serum 1CTP level was also a useful prognostic factor for cause-specific survival in patients with CRPC who are treated with docetaxel chemotherapy.

Bone metastases are present in almost all CRPC patients receiving docetaxel therapy. In our previous study, we reported that serum ALP levels were useful for the detection of bone metastatic spread and for predicting survival probability in PCa patients with bone metastasis[14]. Sonpavde *et al*[22] investigated patients with bone metastasis and high baseline ALP who were treated with docetaxel and reported that normalization of ALP by day 90 was predictive of better survival independent of whether or not a 30% decline in PSA was achieved. An increase in ALP by day 90 was also predictive of poor survival independent of whether or not a 50% increase in PSA occurred.

Serum ALP level is a useful biomarker in patients with prostate cancer that is characterized by osteosclerotic bone metastasis. Based on data from the present study, serum 1CTP (a bone formation marker) may also be a reliable biomarker in CRPC patients. Indeed, serum 1CTP level was an independent predictor of bone metastasis according to univariate and multivariate analysis in our previous paper. Furthermore, as the EOD score increased, serum levels of 1CTP also significantly increased. Serum 1CTP level was also a significant independent predictor of cause-specific survival according to univariate and multivariate analysis[13]. Patients with higher levels of 1CTP showed worse prognosis, and our analysis suggests that therapies other than docetaxel should be considered for patients with higher levels of 1CTP.

A previous study reported that CRP is an independent prognostic factor for overall survival in patients with CRPC treated with docetaxel[23]. Similarly, we found that patients with lower CRP levels (< 1 mg/dL) had better outcomes than those with higher CRP levels (⩾ 1 mg/dL) ($P < 0.01$). These data suggest that CRP level can predict outcomes in patients with CRPC treated with docetaxel. Narita *et al*[24] used multivariate analysis to demonstrate that serum lactate dehydrogenase (LDH) was an independent prognostic factor for overall survival. Another analysis suggested that levels of serum markers of angiogenesis [*e.g.*, endothelin-1 (ET-1) and tissue factor (TF)] and/or markers of vascular damage [*e.g.*, circulating endothelial cells] could predict overall survival in CRPC patients treated with docetaxel[25].

Measurement of serum bone turnover markers, such as 1CTP or ALP, is useful when PCa patients are diagnosed with CRPC. If high serum bone turnover marker levels are observed, the patient will need to be treated by immediate and adequate intervention (*i.e.*, docetaxel chemotherapy or bone targeted therapy). This study has several limitations, further investigation is necessary to confirm our results.

In conclusion, the present study demonstrates that serum levels of 1CTP and ALP are predictors of survival in patients with CRPC who are treated with docetaxel. The novel agents (*i.e.*, abiraterone acetate, enzalutamide, radium-223, and cabozantinib) offer new options for the treatment of patients with CRPC, including those with disease that is resistant to docetaxel chemotherapy in Western countries.

COMMENTS

Background

Docetaxel chemotherapy has been widely administered to men with metastatic castration resistant prostate cancer (CRPC). To investigate the relationship between clinicopathological features and serum levels of bone turnover markaers in CRPC patients treated with docetaxel.

Innovations and breakthroughs

Serum bone turnover markers can be determined frequently and easily, with negligible disturbance to the patient. This present study suggested that both bone formation markers [alkaline phosphatase (ALP)] and bone resorption markers [type 1 collagen generated by metalloproteinases (1CTP)] might be useful predictors of survival in patients with CRPC who are treated with docetaxel.

Applications

The authors might help the treatment strategy for CRPC in using a novel agents (*i.e.*, abiraterone acetate, enzalutamide, radium-223, and cabozantinib), and avoid the need for frequent electronic computer X-ray tomography technique or bone scintigraphy by using bone formation markers (ALP) and bone resorption markers (1CTP).

Peer review

The article supplements the known literature on risk factors for progression on chemotherapy in patients with hormone refractory prostate cancer.

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PROSPECTIVE STUDY

Alternative mechanisms for prostate-specific antigen elevation: A prospective analysis of 222 transurethral resections of prostate patients

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Abstract

AIM: To investigate the relationship between prostate-specific antigen (PSA) levels and (1) bladder outlet obstruction (BOO) and (2) the severity of prostate inflammation.

METHODS: Two hundred and twenty-two consecutive patients undergoing transurethral resection of the prostate (TURP) were prospectively included. Patients with proven urinary tract infection and/or known prostate cancer were excluded. PSA levels, International Prostate Symptoms Score (IPSS), prostate weight, post residual volume and pressure flow parameters were determined. A histopathological assessment of the presence and severity of inflammation was also performed.

RESULTS: Patients had a mean age of 69.1 ± 8.6 years (45-90 years), with mean preoperative PSA levels of 4.7 ± 5.4 ng/mL (0.2-32.5 ng/mL) and IPSS of 15.7 ± 6.9 (0-32). Mean $PdetQ_{max}$ was 96.3 ± 34.4 cmH_2O (10-220 cmH_2O). The mean resected prostate weight was 39.4 ± 27.3 g (3-189 g). Correlations were observed between PSA (logarithmic) and resected prostate weight (r = 0.54; P < 0.001), PSA (logarithmic) and $PdetQ_{max}$ (r = 0.17; P = 0.032), and resected prostate weight and $PdetQ_{max}$ (r = 0.39; P < 0.001). Furthermore, low correlations were observed between PSA (logarithmic) and active (r = 0.21; P < 0.0001) and chronic (r = 0.19; P = 0.005) inflammation.

CONCLUSION: In this study we showed a correlation between BOO ($PdetQ_{max}$) and PSA (logarithmic). Furthermore, we demonstrated a weak correlation between PSA (logarithmic) and active as well as chronic prostatic inflammation.

Key words: Transurethral resection of the prostate; Prostate-specific antigen; Bladder outlet obstruction; Lower urinary tract symptoms; Prostate inflammation

Core tip: The goal was to investigate the relationship between prostate-specific antigen (PSA) levels and (1)

bladder outlet obstruction (BOO) and (2) the severity of prostate inflammation. We performed a prospective study on 222 consecutive patients undergoing transurethral resection of the prostate. Patients with proven urinary tract infection and/or known prostate cancer were excluded. PSA levels, International Prostate Symptoms Score, prostate weight, post residual volume and pressure flow parameters were determined. A histopathological assessment of the presence and severity of inflammation was also performed. In this study we showed a correlation between BOO ($PdetQ_{max}$) and PSA (logarithmic). Furthermore, we demonstrated a weak correlation between PSA (logarithmic) and active as well as chronic prostatic inflammation.

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INTRODUCTION

Since its introduction, prostate-specific antigen (PSA) has played a key role in prostatic evaluation[1]. Elevated or rising PSA levels might indicate prostate cancer, a highly prevalent cancer in men with an incidence rate surpassing 20%[2]. The downside of PSA testing is that it is not cancer specific but merely organ specific.

Consequently, elevated PSA levels are a challenging problem for urologists in assessing or excluding potential life-threatening prostate cancer. However, to confirm the diagnosis of prostate cancer, an additional histological evaluation from prostate biopsies is still required. These biopsies can be falsely negative and cannot be repeated infinitely[3]. Furthermore, taking a prostate biopsy is a moderately invasive examination that has, although infrequently, potential life threatening complications[4].

Therefore, a better understanding of other possible mechanisms causing PSA elevation is of utmost importance as this may help to avoid unnecessary biopsies and prevent patient anxiety. Furthermore, patients do not have to be bothered with other, sometimes expensive diagnostic tests, including magnetic resonance imaging[5], advanced transrectal ultrasound imaging[6] or molecular diagnostics[7].

Benign prostate hyperplasia (BPH) is a very common condition in ageing males. As prostatic enlargement can be asymptomatic, the exact incidence of BPH is unknown, ranging between 28% and 60% of the population[8,9]. Additionally, BPH seems to be the second most common reason for surgery in men over 60[10]. Currently, the exact pathogenesis of BPH is not fully understood. Amongst other factors, including hormonal influence, prostatic inflammation could stimulate prostatic growth. Moreover, data on the association between inflammation, prostatic volume, PSA levels and acute urinary retention risk have been published[11].

Therefore, the aim of this study was to investigate the relationship between PSA and the degree of prostate inflammation and to investigate whether PSA levels can be used as a biomarker for bladder outlet obstruction (BOO).

MATERIALS AND METHODS

The study was approved by the hospital's Ethics Committee (07.58/uro07.02) and was conducted according to the established GCP criteria. In this prospective study, 222 consecutive patients undergoing transurethral resection of the prostate (TURP) between May 2008 and June 2010 were included. A single high volume surgeon in a non-academic referral center operated on all patients. As indicated in the EAU guidelines[12], surgery was performed only on patients with a clear indication for TURP, including patients who did not improve after medical therapy, patients with (recurrent) acute urinary retention, high post void residual volume, obstruction characterised by pressure flow analysis or post renal kidney insufficiency. However, patients who were treated with 5ARIs were excluded because of the possible impact on PSA values. Patients with a proven urinary tract infection were excluded, except for patients with catheters, which are colonised by definition. Additionally, patients with known prostate cancer were excluded in order to prevent influence on PSA by cancer cells.

Before surgery, PSA levels were determined for all patients by GPs. The International Prostate Symptom Score (IPSS) was determined. Full urodynamic studies were performed in 154 patients using Laborie Medical Technologies INC/UDS-64-IIs and were evaluated by PIs. Urodynamic studies were not performed in cases of acute urinary retention or high post-residual volume. All patients were treated with low-dose quinolone prophylaxis for 48 hours, starting the day before urodynamic testing. Filling was done standing with a filling speed of 35 mL/minute, using a 6F-filling catheter (double lumen). When indicated, pressure flow analysis was performed according to the International Continence Society criteria[13]. Endoscopic procedures were performed under loco-regional anesthesia using an Olympus resectoscope 26 (6%) or 28 Charrière (94%), depending on the estimated prostate volume. The resected prostate specimens were weighed and the fragments were embedded until four cassettes, each containing 2 g of tissue, were filled. Each additional 10 g of prostate tissue was used to fill an extra cassette[14]. The tissue was fixed in formalin and embedded in paraffin. One slide was made from every paraffin block and examined after staining by hematoxylin and eosin. The inflammatory infiltrate was first divided into an active (mixed infiltrate of lymphocytes, plasma cells and polynuclear cells) and chronic (mononuclear infiltrate of lymphocytes and plasma cells) component, after which the density of both components

Reference tissue (A0 and C0)

A1	A2	A3
C1	C2	C3

Figure 1 Scores for active and chronic inflammation. A: Active inflammation; C: Chronic inflammation. Semi-quantitative scoring scale from 0 (no infiltrate) to 3 (severe infiltrate).

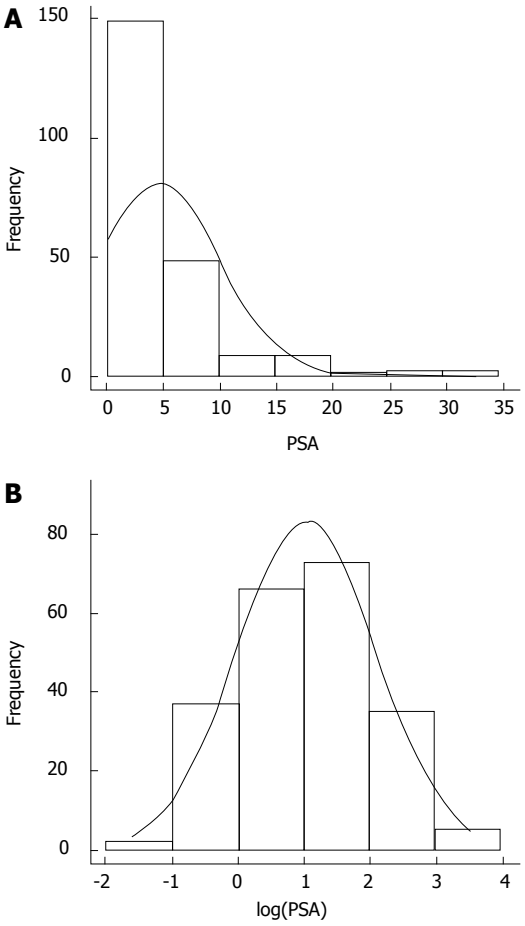

Figure 2 Histogram for prostate-specific antigen (A) and log prostate-specific antigen (B) with a normal density curve superimposed. PSA: Prostate-specific antigen.

was scored 0-3 according to a semi-quantitative scoring system: 0 being no infiltrate and 3 severe infiltrate (Figure 1). Two senior pathologists analyzed all tissue sections of each patient independently and were blinded for clinical data. They scored the mean value of the infiltrate, considering all tissue sections.

Statistical analysis

In an initial step, data were analyzed with descriptive statistics and Pearson correlation was used to investigate simple correlations between different variables. Appropriate linearity tests and lack of fit tests were performed to ensure that these relationships were indeed linear and adequate. Secondly, in a more in depth analysis the relationship between PSA levels and the different potential covariates was analyzed with a multiple regression model. Since the histogram of PSA is severely skewed due to the prevalence of lower PSA values in a normal population (Figure 2), PSA values were transformed with a natural log transformation, resulting in a more realistic normality assumption of the log PSA than the original PSA scale. Selection of the variables included in the model for log PSA was based on the AIC criterion and the adjusted r-square. With respect to the variable active inflammation, it might not seem realistic from a clinical point of view to assume that the difference between any consecutive levels of the active inflammation was the same or the increase was linear in nature. For this reason, the choice was made to treat inflammation as a categorical rather than a continuous variable. Furthermore this choice resulted in a better fit of the model.

Table 1 Characteristics of the 222 consecutive patients undergoing transurethral resection of the prostate between May 2008 and June 2010

	n	Mean	SD	Min	Max
PSA (ng/mL)	218	4.7	5.4	0.2	32.5
Age (yr)	222	69.1	8.6	45	90.0
UF (mL/s)	204	10.7	6.5	1	47.2
IPSS	222	15.7	6.9	0	32.0
PRV (mL)	208	83.8	130.3	0	890
P/F (cm H_2O)	154	96.3	34.4	10	220
Operation time (min)	222	31.1	11.9	10	110
TURP weight (g)	222	39.4	27.3	3	189

SD: Standard deviation; PSA: Prostate specific antigen; UF: Uroflow; IPSS: International prostate symptoms score; PRV: Post residual volume; P/F: Pressure flowmetry; TURP: Transurethral resection of the prostate; Min: Minimum; Max: Maximum.

RESULTS

Patient characteristics

The patients were between 45 years and 90 years old (mean age: 69.1 ± 8.6 years) (Table 1) and had an indication for TURP. Mean preoperative PSA value was 4.7 ± 5.4 ng/mL (0.2 to 32.5 ng/mL). Mean IPSS was 15.7 ± 6.9 (0 to 32). Mean peak flow was 10.7 ± 6.5 mL/s (1 to 47 mL/s). Post residual volume ranged from 0 to 890 mL with a mean value of 83.8 ± 130.3 mL. When indicated (n = 154), pressure flow analysis was executed and showed a mean value of $PdetQ_{max}$ of 96.3 ± 34.4 cmH_2O (10 to 220 cmH_2O). The mean resected prostate weight was 39.4 ± 27.3 g (3 to 189 g). Mean operating time was 31.1 ± 11.9 min (10 to 110 min).

Correlations with PSA

We investigated the relationship between log PSA and several parameters, including IPSS, maximum uroflow, post voided residual volume, pressure flowmetry parameter $PdetQ_{max}$ and the weight of the resected prostate tissue (Table 2). A significant correlation between log PSA and the resected prostate weight was found (r = 0.54; $P < 0.001$). Furthermore, log PSA was correlated with $PdetQ_{max}$ (r = 0.17; P = 0.032). A negative correlation was encountered between log PSA and IPSS (r =

Table 2 Correlation between log (prostate specific antigen) and the corresponding predictor variables

Variable	Linearity (*P* value)	Significance (*P* value)	Correlation (*r*)
IPSS	0.488	0.04	-0.14
Age (decades)	0.563	< 0.0001	0.29
UF (mL/s)	0.929	0.986	0.00
PRV (mL)	0.766	0.276	0.08
P/F (cm H_2O)	0.443	0.032	0.17
TURP weight (g)	0.504	< 0.0001	0.54

IPSS: International prostate symptoms score; UF: Uroflow; PRV: Post residual volume; P/F: Pressure flowmetry; TURP: Transurethral resection of the prostate.

Table 3 Multiple regression model results

Effect	Estimate(st.err)	*P* value
Age (decades)	0.18 (0.069)	0.010
IPSS	-0.02 (0.008)	0.008
Active_0	-1.58 (0.47)	0.001
Active_1	-0.96 (0.53)	0.073
Active_2	-0.78 (0.54)	0.146
Active_3	-0.80 (0.55)	0.150
TURP weight	0.034 (0.006)	< 0.0001
R^2 adj	0.377	

IPSS: International prostate symptoms score; Active 0: No active inflammation; Active 3: Most severe inflammation; TURP: Transurethral resection of the prostate.

-0.14; $P = 0.04$). No correlation was observed between log PSA and peak flow on uroflowmetry ($r = 0.00$; $P = 0.99$). The correlation between log PSA and the post residual volume ($r = 0.08$; $P = 0.28$) was not significant. Additionally, we did not find any correlation between IPSS and $PdetQ_{max}$ ($r = 0.051$; $P = 0.53$). Last but not least, a significant correlation between the weight of the resected tissue and $PdetQ_{max}$ ($r = 0.39$; $P < 0.001$) was observed. These relationships were confirmed in a multiple regression model (Table 3).

Correlations with inflammation (active and chronic)

We also investigated the relationship between inflammation (active and chronic) and log PSA, IPSS, age and the resected prostate tissue weight (Tables 4 and 5). A significant correlation between log PSA and active ($r = 0.21$; $P < 0.0001$) and chronic ($r = 0.19$; $P = 0.005$) inflammation was observed. Age was related to active (r = 0.24; $P < 0.0001$) but not to chronic ($r = 0.09$; $P = 0.08$) inflammation. Similarly, the weight of the resected prostate tissue was related to active inflammation ($r = 0.13$; $P = 0.011$) but not to chronic inflammation ($r = 0.1$; $P = 0.34$). However, IPSS was not correlated with active inflammation ($r = 0.03$; $P = 0.6$) or chronic inflammation ($r = - 0.03$; $P = 0.91$). Additionally, categorization of IPSS into 3 categories did not result in any association with active and chronic inflammations (Table 6).

We also evaluated the influence of pre-operatively placed suprapubic catheters, trans-urethral catheters or previous prostate puncture biopsies and TURP on the relationship with active and chronic inflammation (Table 7). Only a weak negative correlation was found ($r = -0.16$; $P = 0.003$) between active inflammation and the presence of a suprapubic catheter. No significant correlation was observed with the other variables (Table 7). However, for some categories of degree of inflammation no data were obtained, complicating comparisons.

DISCUSSION

PSA measurement is one of the cornerstones in prostate evaluation. Besides BPH and acute prostatitis, prostate cancer can be one of the reasons for PSA elevation, sensitizing and alarming many patients and care givers. Dealing with a patient with elevated/rising PSA levels is always a challenge, especially in ruling out potential life-threatening prostate cancer.

Relationship between PSA and BPH and/or BOO

Multiple papers have been published indicating the relationship between PSA levels and BOO. In patients with clinical BPH, PSA levels are shown to be positively correlated with the five year cumulative risk of invasive BPH treatment[15]. Additionally, in men with BPH, prostate volume and PSA have been evaluated as predictors of acute urinary retention[16,17]. Consequently, elevated PSA levels are predictive for the need of BPH-related surgery. PSA has also been shown to be a strong predictor of future prostate growth[18], which is related to a higher risk of acute urinary retention and, subsequently, to the need for BPH-related surgery. A broader approach for the use of PSA seems justified. In a multicenter study in men suffering from LUTS, a correlation between PSA and the category of BOO was observed[19], Above a PSA-level of 4 ng/mL, mild or definite BOO was observed (in 89% of the cases) and below 2 ng/mL, the chances of a patient not suffering from BOO were one in three[19]. In previous papers, we have shown that BOO can be expected in a very particular group of patients with elevated and/or rising PSA, (multiple) negative multisided prostate biopsies and minor LUTS (mild + moderate IPSS)[20,21]. In a retrospective analysis of 82 consecutive patients, 95.9% were clearly obstructed ($PdetQ_{max} \geqslant 40$ cm H_2O)[18]. Similar results were obtained in a prospective analysis ($n = 33$), with a mean $PdetQ_{max}$ of 80.3 cm H_2O[21]. A positive correlation between PSA velocity and $PdetQ_{max}$ was found ($r = 0.5014$; $P = 0.006$)[22]. As already mentioned, rising PSA levels can also be observed in patients with prostate cancer. Notwithstanding the presence of a tumor in these patients, the elevated PSA levels can also be influenced by BOO, implying that PSA should be included in the pre-treatment workup and the post-therapy evaluation of these patients. Therefore, PSA should be considered an additional indicator in the BOO decision tree.

We found some interesting correlations in this pro-

Table 4 Correlation between active inflammation and prostate related continuous variables

	Active inflammation				*P* value	Correlation, *r*
	0	1	2	3		
Log PSA, *n*	25	91	88	14		
Mean (SD)	1.4 (1.6)	4.4 (4.3)	5.9 (6.4)	4.6 (6.6)	<0.0001	0.21
IPSS, *n*	25	94	88	15		
Mean (SD)	14.4 (6.3)	16.2 (7.0)	15.3 (7.0)	16.6 (7.1)	0.595	0.03
Age, *n*	25	94	88	15		
Mean (SD)	59.7 (5.4)	70.1 (8.1)	70.8 (8.2)	68.4 (9.5)	<0.0001	0.24
PRV, *n*	25	90	81	15		
Mean (SD)	75.0 (103.9)	83.8 (110.6)	68.9 (110.0)	202.8 (303.2)	0.001	0.09
TURP weight, *n*	25	94	88	15		
Mean (SD)	24.7 (10.7)	39.5 (27.4)	44.2 (30.4)	32.6 (14.0)	0.011	0.13

SD: Standard deviation; PSA: Prostate specific antigen; IPSS: International prostate symptoms score; PRV: Post residual volume; TURP: Transurethral resection of the prostate.

Table 5 Correlation between chronic inflammation and prostate related continuous variables

	Chronic inflammation				*P* value	Correlation, *r*
	0	1	2	3		
Log PSA, *n*	4	109	91	14		
Mean (SD)	1.0 (0.7)	4.0 (5.0)	5.7 (5.9)	4.2 (4.4)	0.005	0.19
IPSS, *n*	4	110	93	15		
Mean (SD)	14.8 (7.9)	16.0 (6.5)	15.3 (7.2)	15.7 (8.3)	0.91	-0.03
Age, *n*	4	110	93	15		
Mean (SD)	61.5 (5.2)	68.4 (8.75)	70.5 (8.31)	67.6 (9.3)	0.08	0.09
PRV, *n*	4	105	86	13		
Mean (SD)	68.5 (137.0)	80.3 (129.5)	83.8 (121.8)	116.9 (191.4)	0.88	0.05
TURP weight, *n*	4	110	93	15		
Mean (SD)	25.0 (5.2)	36.8 (25.4)	42.5 (28.9)	40.1 (31.6)	0.34	0.1

SD: Standard deviation; PSA: Prostate specific antigen; IPSS: International prostate symptoms score; PRV: Post residual volume; TURP: Transurethral resection of the prostate.

Table 6 Relationship between International Prostate Symptoms Score (categorized) and inflammation

		IPSS Categorized			*P* value
		Mild	Moderate	Severe	
Active inflammation	0	4	16	5	0.68
	1	11	47	36	
	2	15	43	30	
	3	2	7	6	
Chronic inflammation	0	1	1	2	0.13
	1	10	65	35	
	2	18	41	34	
	3	3	6	6	

IOSS: International Prostate Symptoms Score. 0: No inflammation; 3: Most severe inflammation.

spective analysis of 222 consecutive patients undergoing TURP for bothersome LUTS. A significant correlation was shown between log PSA and $PdetQ_{max}$. In line with the literature[22], log PSA was also correlated with prostate volume (the resected prostate weight) (r = 0.54; P < 0.001). Interestingly, a negative correlation between IPSS and log PSA was observed (r = -0.14; P = 0.04), confirming the findings of our previous studies. This negative correlation could partly be explained by the correlation between high PSA levels and high detrusor pressures due to an increased compensation level of the detrusor for the urethral resistance, resulting in high flow rates and less symptoms.

On the other hand, no statistically significant correlation was observed between log PSA and post voiding residual volume, and between IPSS and the weight of the resected tissue. This might mean that the response of the detrusor to obstruction is more related to the symptoms than the degree of obstruction as such. Last but not least, a highly significant correlation between the weight of the resected tissue and $PdetQ_{max}$ (r = 0.39; P < 0.001) was observed, implying that bigger prostates are more frequently associated with obstruction and therefore are more prone to BPH-related surgery.

Relationship between PSA and prostate inflammation

The role of chronic prostate inflammation in PSA elevation in asymptomatic men is still unclear. Many papers have covered this subject with various and sometimes contradictory outcomes and conclusions. In a prospective analysis of a small group of asymptomatic patients (n = 51) who underwent prostate puncture biopsies, a statistically significant correlation was found between the inflammatory process and the PSA values (P = 0.02)[23].

Table 7 Correlation between inflammation (active and chronic) and risk factors for inflammation

		Active inflammation				*P* value	Correlation, r_c	Chronic inflammation				*P* value
		0	1	2	3			0	1	2	3	
SPC	Yes	1	6	6	5	0.003	-0.16	0	8	7	3	0.34
	No	124	88	82	10			4	102	86	12	
TUC	Yes	0	1	3	0	0.710	[1]	0	2	2	0	1
	No	25	93	85	15			4	108	91	15	
PPB	Yes	2	29	27	4	0.130	[1]	1	25	34	2	0.08
	No	23	65	61	11			3	85	59	13	
Re-TURP	Yes	2	13	11	2	0.930	[1]	0	14	12	2	1
	No	23	81	77	13			4	96	81	13	

[1]No significant correlation. SPC: Presence of a suprapubic catheter; TUC: Presence of a transurethral catheter; PPB: Prostate biopsy punctures; Re-TURP: Previous transurethral resection of the prostate.

Similarly, in a retrospective analysis of 238 men, a correlation between PSA and inflammation was observed[24]. In an analysis of the prostate puncture biopsy of 80 asymptomatic patients, the extent of inflammation was positively correlated with total PSA levels ($P < 0.001$)[25]. In all these studies, histological examination was performed on prostate puncture biopsies in asymptomatic men, limiting the results obtained.

In contrast to the previously mentioned reports, multiple papers claimed the opposite and concluded that there is no correlation between PSA and inflammation. In a retrospective analysis of cancer negative prostate biopsies ($n = 233$), it was concluded that the degree of chronic inflammation did not correlate with PSA levels[26].

Chronic prostatitis was encountered in 68.3% of 284 patients with negative prostate puncture biopsies, while active prostatitis was observed in 8.4% of patients[27]. However, inflammation did not correlate with total PSA levels. In a prostate puncture biopsy driven evaluation ($n = 49$), the presence of inflammation did not correlate statistically with the PSA levels[28]. Finally, Nickel *et al*[29] studied a cohort of 80 patients without a history of prostatitis who underwent TURP. After histological examination on the resected tissue, prostatic inflammation was found to be extremely common. On the other hand, no correlation was observed between inflammation and PSA levels.

We performed a prospective analysis in 222 patients undergoing TURP according to the EAU guidelines[30] but without proven infection, known prostate cancer and a history of chronic prostatitis. We made a histological distinction between active and chronic inflammation, each with 4 subcategories. Additionally, since we used TURP tissue and not prostate puncture biopsy derived tissue, we were able to evaluate a substantial prostatic tissue volume. Our results showed that most patients have some degree of inflammation. Especially in the active inflammation group, a correlation between log PSA and inflammatory parameters was observed ($r = 0.21$; $P < 0.0001$). However, we did not encounter a statistically relevant correlation between symptoms (IPSS) and inflammation. The correlation between age and active inflammation ($r = 0.24$; $P < 0.0001$) is interesting to note, which was not the case for the chronic inflammation group ($r = 0.09$; $P = 0.08$). A possible explanation for this finding could be the accumulation of debris in the prostate during the lifetime, resulting in more frequent active inflammations. This finding may also be the explanation for the correlation between prostate weight (TURP) and active ($r = 0.13$; $P = 0.011$) and chronic ($r = 0.1$; $P = 0.34$) inflammation.

In conclusion, elevated and/or rising PSA levels with regard to underlying prostate cancer and prostatic inflammations are well known. In this paper, we have shown that PSA could also be indicative of BOO, not only with respect to observational data but also with regard to statistically relevant correlations between PSA and $PdetQ_{max}$. Therefore, PSA levels should be taken into account and could even be used as a biomarker when a treatment strategy is determined or executed for patients suffering from clinical BPH. Additionally, the results obtained in this study indicate that inflammation can be correlated with PSA levels. This implies that elevated PSA levels should also be considered as predictive for prostate inflammation as well as for prostate cancer.

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COMMENTS

Background

Since its introduction, prostate-specific antigen (PSA) has played a key role in prostatic evaluation. However, it is not a cancer specific parameter but merely organ specific. Consequently, elevated PSA levels are a challenging problem for urologists in assessing or excluding potential life-threatening prostate cancer. A better understanding of other possible mechanisms causing PSA elevation is required. Therefore, the aim of this study was to investigate the relationship between PSA and the degree of prostate inflammation and to investigate whether PSA levels can be used as a biomarker for bladder outlet obstruction (BOO).

Research frontiers

The cause of elevated PSA serum levels is not always prostate cancer. In the area of interpreting PSA elevation, the research hotspot is to have an insight in to other mechanisms that can be responsible for a PSA elevation, apart from prostate cancer.

Innovations and breakthroughs

In this study the authors showed a correlation between BOO ($PdetQ_{max}$) and PSA (logarithmic). Furthermore, the authors demonstrated a weak correlation between PSA (logarithmic) and active as well as chronic prostatic inflammation.

Applications

PSA could also be indicative of BOO, not only with respect to observational data but also with regard to statistically relevant correlations between PSA and $PdetQ_{max}$. Therefore, PSA levels should also be taken into account and could even be used as a biomarker when a treatment strategy is determined or executed for patients suffering from clinical benign prostate hyperplasia (BPH). Additionally, the results obtained in this study indicate that inflammation can be correlated with PSA levels. This implies that elevated PSA levels should also be considered as predictive for prostate inflammation as well as for prostate cancer.

Terminology

BOO: a blockage at the base of the bladder that reduces or prevents the flow of urine into the urethra, the tube that carries urine out of the body. This condition is most common in aging men. It is often caused by BPH. As a man ages, his chance of developing these diseases increases dramatically; Prostate inflammation: inflammation of the prostate gland. There are four types of prostatitis: acute bacterial prostatitis, chronic bacterial prostatitis, chronic prostatitis without infection, asymptomatic inflammatory prostatitis; PSA (prostate-specific antigen): a protein manufactured exclusively by the prostate gland. PSA is produced for the ejaculate where it liquefies the semen and allows sperm cells to swim freely. Elevated levels of PSA in blood serum are associated with benign prostatic hyperplasia and prostate cancer.

Peer review

This prospective analysis investigates the association of high PSA levels and bladder outflow obstruction and prostatic inflammation. Its results, as described in the discussion, add to the literature and the controversy that exists in these issues among other studies.

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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

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