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EDITORIAL

Safety of synthetic mesh in pelvic surgery

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Abstract

Mesh in the form of a midurethral sling is an acceptable and generally safe treatment option for stress urinary incontinence in patients who have failed conservative treatment options such as weight loss and pelvic floor muscle training. In patients with pelvic organ prolapse, when outcomes are measured in terms of improvement in postoperative physical exam (anatomic success), many studies have demonstrated that mesh augmented repairs are superior to prolapse repairs not using mesh (native tissue). However, from a symptomatic standpoint, the outcomes of mesh and native tissue repairs are equivalent. This means that even though the physician may see more prolapse on physical exam after native tissue repair, most patients do not perceive this as a problem because their sensation of a vaginal bulge is gone. The vaginal bulge is one of the most common complaints of a patient prior to pelvic organ prolapse repair. Based on interpretation of the available literature, it does not appear that mesh is superior to native tissue repair for anterior (cystocele) and posterior (rectocele) compartment pelvic organ prolapse repair. However, for apical repairs the native tissue repairs are more technically challenging and it appears that suspension of the apex of the vagina with mesh to the sacrum (sacrocolpopexy) may yield better outcomes. Unfortunately, like all mesh surgeries there is a significant risk of mesh complications with sacrocolpopexy. Surgeons should thoroughly counsel their patients about the permanent nature of synthetic mesh and the potential serious complications related to its use. Mesh augmented pelvic organ prolapse repairs carry unique complications that are not present with native tissue repairs and may not provide better outcomes.

Key words: Complication; Prolapse; Incontinence; Sling; Prosthesis; Graft

Core tip: Mesh does not provide superior results to native tissue repair and has higher rates of dyspareunia and unique potential serious complications. In general, native tissue repairs are more technically challenging than mesh augmented repair and require the surgeon to have a greater understanding of the anatomy of pelvic organ support.

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In the field of pelvic reconstruction, synthetic mesh is commonly used for the treatment of pelvic organ prolapse and stress urinary incontinence (SUI). The use of mesh during pelvic organ prolapse surgery is not more effective for symptomatic relief than native tissue repair and has unique potentially serious complications. Though mesh for stress incontinence also has unique complications, these surgeries appear to be less morbid and equally efficacious to traditional surgeries for stress urinary incontinence.

The most common complications of mesh surgery are mesh exposure and dyspareunia, and the most serious complications are perforation of organs such as the bladder, urethra and bowel. In 2010 the International Continence Society and International Urogynecological Association released a report intended to clarify and standardize the terminology related to complications from insertion of synthetic and biological materials in female

pelvic floor surgery[1]. According to this report, synthetic mesh is termed a prosthesis and a biological implant is termed a graft. Mesh located in the bladder or urethra is termed a perforation and extrusion of mesh through the vagina or skin is termed exposure.

Research has shown that pelvic organ prolapse effects 2.9% of women over the age of 20 and though only 2% of women are symptomatic, women have an approximately 30%-50% lifetime risk of developing pelvic organ prolapse[2,3]. The current prevalence of urinary incontinence in adult women in the United States is much higher than pelvic organ prolapse and is estimated to be between 47% and 51% and increasing[4,5]. Not surprisingly, the rates of surgery for urinary incontinence and pelvic organ prolapse are also increasing[6,7].

Prior to 1998, the most common surgeries for stress urinary incontinence were needle suspensions, autologous pubovaginal slings and collagen injections[8]. In 1998, the Food and Drug Administration (FDA) approved the first midurethral sling for stress urinary incontinence. Then, over the next 10 years the utilization of the midurethral sling increased almost 30 fold and multiple studies have shown its benefit over traditional surgeries for stress urinary incontinence not utilizing mesh[8,9]. However, as the utilization of synthetic mesh increased, problems with mesh exposure and perforation started to become apparent[10].

With interventions such as pelvic floor muscle training, weight loss and pessaries, the initial treatment of symptomatic pelvic organ prolapse should be conservative. When conservative measures fail, the ideal pelvic organ prolapse procedure would restore the body's normal support structure while returning the prolapsed organ to its normal anatomic position with minimal side effects[11]. Prior to 2001, the majority of pelvic surgeons sought to achieve this ideal using native tissue repairs. However, following successful outcomes for mesh for SUI, researchers started looking at mesh to help with pelvic organ prolapse. Starting in 2001, multiple studies were published showing the benefits of mesh augmented repairs for pelvic organ prolapse[12,13]. From 2001 to 2008 mesh augmented pelvic organ prolapse repairs were commonly performed with little discussion regarding the safety of mesh. However, in October 2008, the United States FDA released a public health notification (PHN) alerting the public about potential "rare" complications and problems related to transvaginal mesh for pelvic organ prolapse[14]. In 2011, the FDA modified this alert by removing the term "rare" and stating that surgical mesh does not conclusively improve outcomes over traditional non-mesh or native tissue repair[15]. Paradoxically, after the initial PHN the rate of vaginal mesh implantation increased[16].

The FDA became aware of problems related to synthetic mesh because of information contained in the manufacturer and user facility device experience (MAUDE) database. MAUDE is a database that houses medical device reports (MDRs) of adverse events submitted to the FDA by manufacturers and healthcare professionals. According to MAUDE data, in regards to midurethral slings, from 2008 to 2010 there were 1371 voluntary and involuntary self reported medical device reports of complications[17]. Bladder and urethra perforation were some of the most common reported MDR's. Similarly high, over the same time period, there were 1503 MDRs for synthetic mesh used during pelvic organ prolapse surgery. In July 2011, the FDA released a statement that summarizes their opinion entitled "Urogynecologic Surgical Mesh: Update on the Safety and Effectiveness of Transvaginal Placement for Pelvic Organ Prolapse"[17].

Today, polypropylene is the most commonly used type of synthetic mesh for pelvic surgery. However, surgeons have been using mesh during pelvic surgery for over 50 years. In 1955, Moore and colleagues reported their experience with a screen made from the metallic element tantalum[18]. They found a 100% anatomic cure rate with an unfortunate 40% graft exposure rate. More contemporary studies have shown a 75% to 91% cure rate and a 0% to 5.6% mesh exposure rate[12,19,20].

Polypropylene has become the most commonly implanted material because it is a monofilament with minimal tissue reactivity that can be formatted into mesh with large sized pores. The standard system for classifying mesh was proposed by Amid[21] in 1997 and emphasizes pore size and filament type. Amid classified mesh into four different categories. The ideal mesh type according to Amid is type 1 mesh. Type 1 mesh is made of a monofilament mesh loosely woven with large pores. Mesh is considered to have large pores if the open space between the fibers is greater than 75 μm. This large pore size promotes flexibility, angiogenesis and macrophage penetration[22,23]. Multifilament material can theoretically harbor and promote the growth of bacteria and result in more infection and inflammation. This problem was seen in a 2001 study by Falconer *et al*[24] that showed significantly more histological evidence of inflammation in patients with mersilene suburethral slings compared to patients with polypropylene. In addition, it seems that mesh with smaller pore sizes such as Gore-tex do not become incorporated into tissue and have a high rate of perforation or exposure[25,26].

The management of mesh exposure is within the scope of practice of most pelvic surgeons, however, mesh perforation may require tertiary referral. There are several studies that propose observing any exposure of mesh less than 1 cm because the area may heal spontaneously with mixed results[27-30]. Depending on the preference of the surgeon and the size of the exposure, the next step for intervention may be operative management. Operative management involves excision of the exposed mesh, thorough irrigation with antibiotic solution and closure of vaginal flaps. The addition of topical antibiotics and estrogen may theoretically improve tissue quality prior to surgical intervention. In a series of 48 patients who underwent partial mesh excision, only 6 had persistent exposure[29].

Perforation of mesh slings into the urethra or bladder should be managed with more extensive mesh excision

to the level of the pubic bone or ischiopubic rami. This type of excision leaves behind the arms of the mesh that tunnel into the retropubic space or obturator fossa. It is typically not necessary to enter these spaces because the mesh at this location is no longer under tension and is far from the urethra or bladder. The authors prefer an inverted-U incision because this allows for a vaginal epithelial flap that avoids overlapping suture lines and should decrease the risk of a fistula. In general, reconstruction should involve non-overlapping suture lines and interposition of tissue such as a labial fat pad, greater omentum or autologous fascial sling. In rare cases of mesh complications from slings, when non-operative therapy has failed, such as extreme pain or infection it may be necessary to attempt a complete mesh excision from both sides of the bone. In the case of retropubic slings this involves an abdominal and vaginal incision and in the case of the tans-obturator slings this involves a medial thigh and vaginal incision.

If mesh placed to augment pelvic organ prolapse repair perforates into the bladder or urethra, this is usually best managed with a midline incision and raising flaps of vaginal epithelium. Similar to mesh perforation from slings, prolapse mesh perforation should also be managed with non-overlapping suture lines and interposition of another tissue. Unlike slings, it is often difficult to remove all of the prolapse repair mesh to the level of the pubic bone and ischiopubic rami. The authors attempt to remove the mesh as far away from the bladder or urethra closure as possible and try to avoid tension on any suture lines.

Ranging from 2.7% to 5.7% in the literature, vaginal exposure rates are relatively high with midurethral slings[31,32]. The rate of bladder or urethral perforation with a trocar at the time of surgery is as high as 5.3% and 5.4%[32,33]. Though widely reported, the rate of mesh perforation into the bladder or urethra during midurethral sling surgery is unclear and ranges from 0.6% to 0.75% in the literature[34,35]. Ranging from 3% to 20%, dyspareunia and worsened sexual function are common after midurethral sling surgery[36,37]. The traditional non-mesh repairs for stress urinary incontinence are autologous pubovaginal slings and burch colposuspension. These two procedures are similarly efficacious to midurethral slings, but, have complication rates requiring surgical intervention as high as 13% and 20% in randomized clinical trials[38]. The rates of dyspareunia and sexual dysfunction after a pubovaginal sling and bladder neck suspension in the literature are lower than midurethral slings[39,40]. In another multicenter randomized clinical trial comparing bladder neck suspension to midurethral slings, the former was found to have more postoperative complications and longer recovery with equal efficacy[9].

Mesh exposure rates of synthetic mesh for pelvic organ prolapse range from 0% to 16.9%[12,19,41,42]. Dyspareunia rates after prolapse repair with mesh are as high as 20% with anterior mesh and 63% with posterior mesh[43]. However, a thorough Cochrane review of surgical management of pelvic organ prolapse from 2011 found that mesh repair and native tissue repair had similar rates of dyspareunia[44]. Mesh perforation rates are as high as 0.7%[45]. *De novo* SUI may be more common after mesh POP repair than native tissue repair[46]. Due to concerns about dyspareunia and efficacy, some surgeons advise against the use of synthetic mesh in the posterior compartment and mesh augmentation does not improve outcomes[47]. The lack of benefit from graft use in the posterior compartment might be due to the durable nature of the fascia in the posterior compartment. Abdominal sacral colpopexy with mesh has a lower complication rate than transvaginal apical support surgeries utilizing mesh[48].

When comparing the outcomes of native tissue repair and mesh-augmented repairs using anatomical results only, mesh surgeries have better outcomes[12,49]. However, when focusing on patient reported symptomatic outcomes, the difference between native tissue repair and the use of mesh is minimal[13,28,42]. An analysis of the data from the CARE trial in 2009 found that the absence of vaginal bulge symptoms had the strongest correlation with patient perception of treatment success[50].

It does not appear that transvaginal mesh for pelvic organ prolapse provides more symptomatic benefit than native tissue repair and has common, unique potentially serious complications that are not present with native tissue repair. Unlike mesh for pelvic organ prolapse, mesh with midurethral slings has similar efficacy with less overall morbidity than needle suspensions and pubovaginal slings for SUI. The current perception of many patients is that mesh for vaginal prolapse is a safety concern. Even if future literature demonstrates the safety of transvaginal mesh, some patients may still be reluctant to have foreign material placed in their bodies. Therefore, physicians may need to return to a time when native tissue repairs were more common. Lastly, a reevaluation of how we define a successful outcome may be necessary as many surgeons move away from the use of mesh for pelvic organ prolapse.

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REVIEW

Integrated technologies in the post-genomic era for discovery of bladder cancer urinary markers

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Abstract

The incidence of bladder cancer (BC) continues to rise with high recurrence and mortality rate, especially in the past three decades. The development of accurate and successful BC treatment relies mainly on early diagnosis. BC is a heterogeneous disease reflected by the presence of many potential biomarkers associated with different disease phenotypes. Nowadays, cystoscopy and urinary cytology are considered the gold standard diagnostic tools for BC. There are many limitations to cystoscopy including being invasive, labor-intensive and carcinoma *in situ* of the bladder may easily be missed. Urinary cytology is still a noninvasive technique with high accuracy in high-grade BC with a median sensitivity of 35%. Furthermore, the need for a sensitive, specific, non invasive, easily accessible BC biomarker is a major clinical need. The field of urinary BC biomarkers discovery is still a rapidly evolving discipline in which more recent technologies are evaluated and often optimized if they are not clinically significant to the urologists. Most of the current strategies for BC urinary biomarker detection depend on integration of information gleaned from the fields of genomics, transcriptomics, proteomics, epigenetics, metabolomics and bionanotechnology. Effort is currently being made to identify the most potentially beneficial urinary biomarkers. The purpose of this review is to summarize and explore the efficacy of gathering the information revealed from the cooperation of different omic strategies that paves the way towards various urinary markers discovery for screening, diagnosis and prognosis of human BC.

Key words: Bladder cancer; Urinary biomarkers; Genomics; Proteomics; Bionanotechnology; Metabolomics; Transcriptomics; Epigenetics

Core tip: Capturing information from in silico data, proteomic data, gene expression data and bionanotechnology data outlines a promising approach to discover significant urinary biomarkers whose activity patterns are discriminative of bladder cancer *vs* control.

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INTRODUCTION

It is estimated that urinary bladder cancer (BC) is the sixth most common cancer worldwide, with approximately 382660 new cases of BC each year[1,2].

Although the main symptom of BC is hematuria, no symptoms are found in an early stage. About 70% to 80% of patients with newly diagnosed BC present with early stage BC (*i.e.*, stage Ta, Tis, or T1) and low-grade neoplasms that are associated with an excellent prognosis. However, these tumors have a 30% to 70% recur-

rence rate and a strong tendency to progress to invasive cancers in 10% to 30% of patients, with increased risk of metastasis and subsequent mortality. So, early detection of BC is urgently needed to improve prognosis and long-term survival[3].

Nowadays, the standard of care for BC diagnosis and follow-up is through the combination of cystoscopic examination, cytology and histology[4]. However, these methods have a significant financial cost and poor sensitivity for low-grade, well-differentiated lesions. They are also highly subjective investigations and provide little about the molecular characteristics of cancers[5]. Recently, numerous urinary markers have been under study in order to reduce the cost and the frequency of cystoscopies or replace them by non-invasive tests. An ideal test for the detection of bladder tumors should have high sensitivity and specificity; moreover, it is necessary to be objective, accurate, rapid and easy to administer[6].

Urine is an ideal biological fluid representing a gold mine suitable for clinical analysis due to simple, economic and non-invasive collection with large quantities of samples available. Therefore, it has been proposed as a substitute to blood collection as a diagnostic tool or at least as a screening test[7]. Nevertheless, the very low abundance of many candidate targets in urine and the presence of different interfering substances have impeded the development of novel urinary biomarkers that may be clinically useful for BC diagnosis[8].

Integration of different biomolecular signature data set through capturing information from in silico data with multiple omic technologies for genomics, gene expression (transcriptomics) and proteomics is increasingly important to maximize value in biomarker discovery, validation and utilization for early diagnosis or prognosis of cancer[9]. Each one of these technologies provides a snapshot of cell function. However, dynamic understanding of disease processes really needs the integration of all these modalities to the greatest possible extent[10].

LITERATURE SELECTION

The published studies that discussed BC biomarkers were identified by searching PubMed for studies that were published between January 2000 and December 2013. The search terms that were used were "bladder", "carcinoma" or "cancer" and "biomarkers" or "bioinformatics" and "genomic", "proteomic" or "epigenetic", or "nanoparticles" without restrictions. In addition, the reference lists of retrieved papers and recent reviews were also examined.

STUDY SELECTION

Any study that matched the following criteria was included: (1) a case-control study design; (2) an association between BC and biomarkers in humans; and (3) BC confirmed by the accepted diagnostic criteria. To evaluate the eligibility of all the studies retrieved from the databases on the basis of the predetermined selection criteria, two independent investigators were used. Disagreements were resolved by discussion.

BIOINFORMATICS AND BC URINARY BIOMARKERS

BC subtypes and biomarkers have been identified using technologies that combine clustering algorithms and visualization tools into web-based bioinformatic databases and those that analyze high-throughput gene expression data[11]. Common analytical tools include the following: Atlas of Genetics and Cytogenetics in Oncology and Hematology (http://atlasgeneticsoncology.org/) is a database that deals with chromosome abnormalities in cancer and genes involved in cancer. This database is provided by experts in cytogenetics, molecular biology with clinicians in oncology and in hematology, and pathologists[12]; Catalogue of Somatic Mutations in Cancer COSMIC database (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/) stores and supplies information about somatic mutations in cancer. This database collects information about publications, mutations and samples; Human protein atlas database (http://www.proteinatlas.org/) displays protein expression profiles based on immunohistochemistry for a large number of human tissues, cancers and cell lines, subcellular localization in three cell lines and transcript expression levels in three cell lines; OmniBiomarker (http://omnibiomarker.bme.gatech.edu/) is a web-based bioinformatics tool for developing biomarkers in oncology to anticipate the clinical outcome of promising biomolecules as a biomarker; The NCI's Cancer Biomedical Informatics Grid® (caBIG®) initiative is the most widely used tool at every stage of cancer that facilitates biomarker discovery beginning from selection of target groups until clinical validation step. At the same time, caBIG® also provides information related to basic research free of charge; Gene Expression Profile Analysis Suite or GEPAS (http://www.gepas.org) for microarray analysis; Array Express (http://www.ebi.ac.uk/microarray-as/ae/ca) and the Cancer Biomedical Informatics Grid (caBIG) (https://cabig.nci.nih.gov) are used for storage and management of expression data; Biomedical knowledge discovery server, BioGraph (http://www.biograph.be/about/welcome), is a data integration platform for the purpose and discovery of biomedical information[13]. The database offers prioritizations of supposed disease genes, supported by functional hypotheses. BioGraph can retrospectively validate recently discovered disease genes and identify susceptible genes, surpassing recent technologies, without requiring previous domain knowledge. Briefly, such computational methods integrating multi-omics data will be very precious to select molecular targets, biomarker candidates and to translate them into biologically meaningful hypotheses.

GENOMICS IN BC URINARY BIOMARKERS

Genomics is a discipline that applies recombinant DNA,

DNA sequencing methods and bioinformatics to analyze the function and structure of the whole set of DNA within the cell of an organism, allowing increase of the width of the field with the number of newer markers identification[14,15]. Applying technologies such as gene microarray that can analyze huge number of DNA sequences from many patients very quickly, the field of genomics has identified thousands of genetic duplications and aberrations that may take part in bladder carcinogenesis[16]. BC, with tumor cells being bathed in urine, perhaps provides the best potential use of DNA markers. However, such markers will not become clinically significant until easier detection methods are found, marker standardization occurs and more clear and specific applications for primary diagnosis compared to recurrent disease are performed[17]. Some of the common genetic markers including *FGFR-3* mutations, *p53* and retinoblastoma genes have elucidated several molecular pathways in BC development[18,19].

Larré *et al*[20] designed a comparative genome hybridization (CGH) chip, including loci proposed to be associated with BC for the assessment of bladder tissues. The CGH data were used to develop a diagnostic test that could be performed on urothelial cell pellets. This test had an overall diagnostic accuracy of 91% in 44 samples. The detection of specific urothelial gene mutations is also applicable to disease evaluation[20].

Kucukgergin *et al*[21] assessed stromal cell derived factor 1 (*SDF-1*) 3'A, monocyte chemoattractant protein-1 (*MCP-1*) A2518G, and chemokine receptors *CCR2A*, *CCR5* Δ32 and *CXCR4* gene polymorphisms by PCR and PCR-restriction fragment length polymorphism (RFLP) methods in 142 histologically confirmed BC patients and 197 controls in a Turkish population. Their results suggest that the genetic variants of *SDF-1* 3'A, *CCR2A* V64I and *CCR5* Δ32 gene may contribute to muscle invasive BC in a Turkish population[21].

Al-Kashwan *et al*[22] analyzed TP53 alterations by PCR-single strand conformational polymorphism analysis and DNA sequencing in twenty-nine bladder carcinomas. They found infrequent TP53 mutations, especially insertion A and 196 hotspot codons in 37.9% of the cases, while TP53 overexpression occurred in 58.6% among the Iraqi patients who were exposed to war environmental hazards[22]. Eissa *et al*[23] also evaluated diagnostic efficacy of mutant p53 patients by PCR-SSCP followed by DNA sequencing in urine of 100 patients diagnosed with BC, 93 patients with benign urological disorders and 47 healthy volunteers. The sensitivity and specificity were 59% and 91.4% for cytology, and 37% and 100% for mutant p53, with a significant association observed between disease recurrence and mutant p53, stage and lymph node involvement[23].

Wang *et al*[24] adopted co-amplification at lower denaturation temperature-polymerase chain reaction (COLD-PCR) as a straightforward method with no additional reagents requirements or instruments as a highly sensitive, specific and expedient clinical assay for mutation detection in the *H-ras* gene, including exons 1 and 2, in Chinese patients diagnosed with BC, yielding a 36% improvement in mutation detection compared with conventional PCR. They concluded that silent mutations might be important genomic alterations in BC pathogenesis and recurrence[24].

EPIGENETICS IN BC URINARY BIOMARKERS

DNA methylation

Epigenetics is a field that refers to reversible changes in gene expression caused by mechanisms other than any change in genetic sequence[22]. DNA methylation is the most common epigenetic changes addressed in BC biomarkers. DNA methyltransferase catalyzes the transfer of the methyl group to the cytosine ring of the CpG dinucleotides. When these CpGs present in promoter regions of genes at high density, CpG islands and gene silencing may be caused by their methylation[25]. Hypermethylation of tumor suppressor genes is a common event during tumorigenesis[26,27]. A large number of genes and their methylation state were assessed in their relationship to urothelial cancer. DNA methylation analysis is usually carried out by methylation specific PCR, bisulfate sequencing, methylation sensitive restriction enzymes and methylated DNA immunoprecipitation (MeDIP)[21].

An Egyptian study was conducted on 210 BC patients, 61 patients with benign urological conditions and 49 healthy volunteers. Eissa *et al*[28] evaluated promoter methylation of *RARβ(2)* and *APC* in DNA extracted from exfoliated cells by methylation specific PCR. Methylated *RARβ(2)* and *APC* were significantly higher in BC patients (62.8%, 59.5%) than benign (16.4%, 5%) but not detected in healthy volunteers (0%) at ($P < 0.0001$). Both sensitivities and specificities of the methylated genes for BC detection were superior to urine cytology[28].

DNA methylation status of specific gene promoter regions in bladder tumor cells has been proposed as a marker for primary diagnosis and for detection of recurrence. García-Baquero *et al*[29] conducted evaluation of the methylation of 18 tumor suppressor genes using methylation specific multiplex ligation-dependent probe amplification in 2 prospective, training urine sample sets of 120 preparations and validation set of 128 from patients with BC (170) and controls (78). *HLTF*, *DLC1*, *PRDM2*, *BNIP3 ID4*, *H2AFX*, *CACNA1G*, *TGIF* and *CACNA1A* were methylated in BC. The methylation status of 5 genes (*CCND2*, *SCGB3A1*, *BNIP3*, *ID4* and *RUNX3*) was identified as an epigenetic biomarker for BC and achieved very high accuracy when used as a panel in analysis of urine sediments. ROC analysis revealed significant diagnostic accuracy for RUNX3 and CACNA1A in the training set and for RUNX3 and ID4 in the validation set. CACNA1A methylation correlated with recurrence in the training set, while in the validation set, PRDM2 and BNIP3 were significantly associated

with recurrence respectively[29].

Kandimalla *et al*[30] reported a panel of epigenetic target genes. Genome-wide methylation analysis was performed on 44 bladder tumors using human CpG island microarray, then validation was performed using a next generation sequencer in a retrospective group of 77 independent tumors and urine DNA from four healthy males > 50 years of age was used as reference. They found 4 genes, Zic family member 4 (*ZIC4*), T-box 2 (*TBX2*), T-box 3 (*TBX3*) and GATA binding protein 2 (*GATA2*), that were significantly hypermethylated in tumor samples methylation and associated with progression to muscle-invasive disease in pTa tumors. Individually, methylation of *TBX2* alone showed a sensitivity of 100%, a specificity of 80%, a positive predictive value of 78%, and a negative predictive value of 100%. This panel of methylated gene increased the sensitivity to 91.7% and the specificity to 87.6%. They also declared that the multivariate analysis showed that methylation of *TBX3* and *GATA2* are independent predictors of progression when compared to clinicopathological variables. They further identified and validated 110 CpG islands with differential methylation between tumor cells and control urine. This study was limited by the small number of patients analyzed for testing and validation.

Scher group has reported that the methylation of 3 genes (*BCL2*, *CDKN2A* and *NID2*) detected by nested methylation specific polymerase chain is associated with BC. They were able to differentiate BC from other urogenital malignancies and nonmalignant conditions with a sensitivity of 80.9% and a specificity of 86.4%.

The epigenetic markers provide a new paradigm in urinary biomarker development for BC[31]. However, the above mentioned markers have been tested in single institutions and with relatively small case control or pilot studies. At the present time, there is no standard method to assess these markers.

microRNA

Hence, it seems to be a good strategy to find cancer-related genes by categorizing methylated genes and microRNA discovery is another major epigenetic event. MicroRNA as a key post-transcriptional regulator of gene expression is small non-coding RNA of 20-22 nucleotides and involved in crucial biological processes, including development, apoptosis and cell division, through improper pairing with target messenger RNA (mRNA)[32]. Array-based profiling, deep-sequencing technologies and qPCR for miRNA analysis are becoming routine technically. They are suitable for the classification of tumors because of aberrant expression of miRNAs in human cancer[33].

Yamada *et al*[34] found the expression level of miR-96 and miR-183 in urine samples was significantly higher in 100 BC than in healthy controls by qPCR. Their results demonstrated that each microRNA has good sensitivity and specificity (miR-96, 71.0% and 89.2%; and miR-183, 74.0% and 77.3%).

Hanke *et al*[35] monitored a number of 157 microRNA species by quantitative reverse transcriptase-polymerase chain reaction in exfoliated urothelial cells in 36 samples. Subsequently, those microRNAs with a higher abundance in urine samples from BC patients were validated in an independent set of urine samples. The study reported that the ratio of miR-126 to miR-182 achieved 72% sensitivity and 82% specificity in 47 samples.

Differential expression of miRNAs was identified by Wszolek *et al*[36] by microarray analysis between noninvasive and invasive BC cell lines and confirmed using (qRT-PCR) within these cell lines. They reported reduced expression of miR-21, miR-30b, miR-31, miR-141, miR-200 and miR-205 in invasive lesions and overexpressed miR-99a in noninvasive BC lesions. Such a diagnostic test, depending on the three most discriminatory miRNAs in this panel (miR-200c, miR-141 and miR-30b), showed a sensitivity of 100% and a specificity of 96.2%.

Tölle *et al*[37] explored the expression of 754 human miRNAs from the Sanger database v14 in the blood and urine samples from 4 controls and from patients suffering from superficial and invasive BC using miRNA microarray. Using the RT-qPCR technique, 6 of the differentially expressed miRNAs were validated in the controls and patients with superficial or invasive tumors. Three blood miRNAs (miR-26b-5p, miR-144-5p, miR-374-5p) were found to be significantly upregulated in invasive bladder tumor patients when compared to the control group. The expression of 2 urinary miRNAs (miR-618, miR-1255b-5p) in patients with invasive tumors was significantly increased in comparison to the control group. The urine miR-1255b-5p had 68% specificity and 85% sensitivity in the diagnosis of invasive bladder tumors.

Pignot *et al*[38] evaluated expression level of miRNAs by quantitative real-time RT-PCR in 11 human normal bladder and 166 bladder tumor samples. The expression level of 804 miRNAs was initially measured and then the differential miRNAs in tumor samples compared to normal bladder tissue were selected for RT-PCR validation in a series of 152 bladder tumors and in six BC cell lines. They reported a panel of 3-miRNA signature (miR-9, miR-182 and miR-200b) was found to be related to bladder tumor aggressiveness and was associated with both recurrence-free and overall survival.

Aberrations in miRNA expression identified between non-muscle invasive BC and muscle-invasive BC provide valuable insight into the molecular mechanisms known to distinguish the unique pathways of bladder carcinogenesis. The limited reproducibility of changes in miRNA expression profiles between studies utilizing in silico miR target-prediction models is due to the heterogeneity of tumor specimens and research methods[39].

TRANSCRIPTOME IN BC URINARY BIOMARKERS

Another component available to be detected in urine is

soluble RNA, including both mRNA and microRNA (miRNA) targets. Quantitative reverse transcription PCR (Q-PCR) and conventional RT-PCR for RNA isolated from exfoliated urothelial cells in urine are the most widely used techniques in novel biomarkers in BC identification and validation[40].

Diverse markers have been discovered but nowadays a very promising mRNA ratio has been assessed[40]. Hanke *et al*[40] isolated RNA from urinary cell pellet and quantified it by reverse transcription quantitative-PCR in 61 patients with BC and 37 healthy donors. The RNA ratio of v-ets erythroblastosis virus E26 oncogene homolog 2 (avian; ETS2) to urokinase plasminogen activator (uPA) enabled the most specific (100%) and sensitive (75.4%) detection of BC from normal urine.

Eissa *et al*[41] evaluated hyaluronidase (HYAL1) and survivin RNA expression by qualitative and semiquantitative reverse transcriptase-polymerase chain reaction in urothelial cells from voided urine in 166 patients with BC, 112 with benign bladder lesions and 100 healthy volunteers. They reported that positivity rates of HYAL1 RNA and survivin RNA on qualitative reverse transcriptase-polymerase chain reaction were significantly different among the study groups. Mean rank using semiquantitative RT-PCR was higher in the malignant compared to the control groups. Using the best cutoffs HYAL1 and survivin RNA sensitivity was 91% and 75%, respectively, with 100% specificity. HYAL1 RNA detected all patients with early stage BC and is more sensitive and specific than urine cytology which is validated in many publications[41-44].

Another study published by this group assessed urinary fibronectin (FN), relative telomerase activity (RTA) and cytokeratin 20 (CK20) mRNA in comparison with voided urine cytology (VUC) in 132 patients with BC, 60 patients with benign bladder diseases, and 48 apparently healthy individuals[45]. Detection of CK20 was carried out by conventional RT-PCR in urothelial cells from voided urine, estimation of fibronectin by ELISA and relative telomerase activity by telomeric repeat amplification protocol (TRAP). The overall sensitivity (89.3%) and specificity (98.4%) were the highest for CK20 mRNA compared to all investigated markers. The efficacy of urinary CK20 mRNA in BC diagnosis was validated in many publications[46-50].

C-X-C chemokine receptor 4 (CXCR4) and CXCR7 were estimated by Yates *et al*[51] in BC cell lines, tissues (normal = 25; BC = 44) and urine specimens (n = 186) by qPCR and/or immunohistochemistry. CXCR7 messenger RNA levels were 5 to 37-fold higher than those for CXCR4. CXCR7 messenger RNA levels and CXCR7 staining scores were significantly higher in BC than in normal tissues. CXCR7 level was elevated in exfoliated urothelial cells from high-grade BC patients (90% sensitivity; 75% specificity) while CXCR4 level was unaltered.

Bongiovanni *et al*[52] performed real-time reverse transcriptase-polymerase chain reaction to evaluate Bradeion/SEPT4 transcript levels in urine samples from 17 healthy controls and 41 patients with BC. Relative quantification analysis of Bradeion transcript showed 92.68% sensitivity and 64.71% specificity. This preliminary study supports the possible usefulness of Bradeion as a urinary marker of BC.

Brems-Eskildsen *et al*[53] measured urinary mRNA levels of PPP1CA, hTERT, MCM5 and SENP1 by q-RT-PCR from 123 prospectively cross-sectional collected urine samples from patients with BC (54 patients with recurrent BC at sampling, 59 patients with previous BC and no tumor at sampling, 10 patients with a primary BC at sampling). The sensitivity and specificity of these mRNA markers were: for hTERT: 86%; SENP1: 71.7%; MCM5: 95.45%; and PPP1CA: 91.3%. Follow-up data resulted in sensitivity and specificity values: for hTERT: 62/84; SENP1: 63%; MCM5: 83.6%; and PPP1CA: 98.5%. The best combination was hTERT and cytology with a sensitivity of 71% and a specificity of 86%, but the combination of hTERT and MCM5 also increased the detection rate.

Rosser *et al*[54] applied cDNA microarray to explore the molecular signatures of BC in urine pellet from 46 individuals. They reported 14 overexpressed and 10 decreased genes in exfoliated tumor cells. Finally, they built a panel of 14 genes as a potential molecular pattern for diagnosing BC with 90% sensitivity and 65% specificity. This study is limited by the small sample size and low specificity but is still significant as it used the exfoliated cells as a source to perform cDNA microarray analysis. This molecular signature motivated another group to validate them in a larger study applying Q-PCR. Holyoake *et al*[55] investigated the expression of 14 different genes by Q-PCR using voided urine from 75 transitional cell carcinoma (TCC) patients and 77 control patients. In their analyses they developed a panel of 4-marker involving CDC-2, HOX-A13, MDK and IGBP-5 mRNAs detected 48%, 90% and 100% of stage Ta, T1, and > T1 TCCs, respectively, at a specificity of 85%.

PROTEOMICS IN BC URINARY BIOMARKERS

Proteomics refers to the large-scale experimental analysis of proteins, mainly their structures and functions using diverse technologies such as 2-dimensional gel electrophoresis (2-DE) and mass spectrometry (MS)[56]. After initial screening, more traditional tests (*e.g.*, ELISA, zymography, western blot) can be carried out to evaluate the clinical efficacy of promising biomarkers[57].

SELDI (surface enhanced laser desorption/ionization) is the best MS-technique used to characterize biomarkers from biological fluids such as urine and blood[58]. Such high throughput technology can analyze only small molecular mass proteins and miss relatively higher molecular mass biomarkers. Several markers have been gleaned from such technology, including TATI (tumor associated trypsin inhibitor), MMPs (matrix metalloproteinase) and CXCL-1[59].

Chen *et al*[60] used 17 biomarkers for BC diagnosis which were already discovered using isobaric tagging absolute and relative quantitation (iTRAQ), then validated by multiple reaction monitoring-based mass spectrometry in urine samples from 57 patients with hernia, 76 BC and 23 urinary tract infection. Prothrombin had the highest sensitivity, 71.1% and 75.0% specificity for discriminating BC from non-cancerous patients. They generated six-peptide panel (apolipoprotein A-II precursor, ceruloplasmin, adiponectin, afamin, complement C4 gamma chain and prothrombin) to differentiate BC subjects from non-cancerous subjects, with a 76.3% positive predictive value and a 77.5% negative predictive value.

Rosse *et al*[61] evaluated the urinary concentration of eight biomarkers (CA9, APOE, MMP-9, PAI-1, VEGF, IL-8, ANG and MMP-10) by ELISA assay in 102 BC subjects and 206 subjects with different urological disorders. They reported that this 7-biomarker model has a sensitivity of 74% and specificity of 90%. This study was limited by being performed on banked urines and the lack of VUC and UroVysion data on controls.

In another study published by Goodison *et al*[62], the urinary concentration of 14 biomarkers (OPN, MMP-9, MMP-10, APOE, CCL18, A1AT, ANG , VEGF, CD44, CA9, PAI-1, IL-8, PTX3 and SDC1) was measured by ELISA in voided urines from 127 patients (64 tumor bearing subjects). They reported a panel of 8-biomarker achieving the most accurate BC diagnosis (sensitivity 92%, specificity 97%) and highly accurate combination of 3 of the 8 biomarkers (IL-8, VEGF and APOE) (sensitivity 90%, specificity 97%) in comparison with the commercial BTA-Trak ELISA test (sensitivity of 79% and a specificity of 83%) and voided urine cytology (33% sensitivity) in the same subjects.

Li *et al*[63] identified 16 urinary proteins including Gc-globulin (GC) from BC patients and normal controls by two-dimensional fluorescent differential gel electrophoresis (2D-DIGE) and matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF/TOF MS). The urinary GC protein from cases and controls were further assessed by western blotting and ELISA showing 82.61% sensitivity and 88.24% specificity. Another Chinese group used 2-dimensional electrophoresis combined with MALDI-TOF/TOF MS and SWISS-PROT database to explore urinary proteins in patients with BC and in normal controls[64]. They identified 14 proteins, including 2 putative proteins [fatty acid-binding protein adipocyte, myoglobin, beta-2-microglobulin isoform 2 of fibrinogen alpha chain, apoA-I, gelsolin, isoform 1 of gelsolin, prostaglandin D(2) synthase 21 kDa (brain), keratin type II cytoskeletal 1, type II cytoskeletal 8, protein AMBP, transthyretin, putative uncharacterized protein ALB, putative uncharacterized protein MASP2 (fragment)]. apoA-I was confirmed by western blot analysis, concluding that proteomic analysis of urine may be a noninvasive and highly efficient strategy for searching for new bladder tumor biomarkers.

Zoidakis *et al*[65] applied immobilized metal affinity chromatography in urine samples from patients with non invasive and invasive BC and the eluted proteins were analyzed by 1D-SDS-PAGE followed by band excision and liquid chromatography tandem MS. They found that MMP9, fibrinogen forms, clusterin, aminopeptidase N, profilin 1 and myeloblastin were differentially expressed in urine from patients with aggressive compared with non aggressive BC and benign controls, then further validated by western blot or ELISA analysis. This study reported that profilin 1 is strongly associated with BC paving the way for its further assessment in BC diagnostics.

Lindén *et al*[66] screened the urine samples from BC patients by mass spectrometry (MS) and western blot (WB)/dot blot (DB). 29 proteins had a significantly higher abundance in BC samples compared with control urine samples. Then four selected proteins were confirmed with western blot: apolipoprotein E, fibrinogen β chain precursor, leucine-rich α-2-glycoprotein 1 and α-1-antitrypsin. Dot blot analysis of a separate urine sample set pointed out fibrinogen β chain and α-1-antitrypsin as the most significant biomarkers with sensitivity and specificity values in the range of 66%-85%. When the Human Protein Atlas (HPA) was explored, it also revealed that BC tumors are the proposed source of these proteins.

Bryan *et al*[67] explored urine samples from 751 patients with BC and 127 controls using MALDI-TOF-MS. They declared that albumin, total protein and hematuria were elevated in T2+ patients. Hematuria was found in 39% of patients with Ta/T1 disease and in 77% of patients with T2+ disease. Taken together, great consideration should be given when applying omic in searching for urinary biomarkers because blood proteins may give false-positive results.

METABOLOMICS IN BC URINARY BIOMARKERS

Metabolomics is defined as "quantitative measurement of the unique chemical fingerprints that elucidate metabolic response of living systems to pathophysiological stimuli or genetic modification"[68]. It provides information that cannot be obtained directly from the gene expression profiles or even the proteomic fingerprint of an individual. Application of urine-based metabolic profiling is achieved using high pressure liquid chromatography (HPLC) and nuclear magnetic resonance (NMR) which may identify specific biomarker patterns that can aid diagnosis of BC[69].

In a study published by the Pasikanti group, gas chromatography mass spectrometry (GC-MS) was used for urinary metabolic profiling of BC patients and non-BC controls and concluded that urinary metabolomics is highly compliant to the noninvasive diagnosis of BC[70].

Huang *et al*[71] enrolled twenty-seven BC patients and 32 healthy volunteers to perform metabolomic profiling to identify a potential unique biomarker pattern in urine

as a noninvasive strategy for BC detection. They utilized a liquid chromatography-mass spectrometry based method. Carnitine C9:1 and component I were identified as a biomarker panel, with 92.6% sensitivity and 96.9% specificity for all patients and 90.5% and 96.9%, respectively, for low-grade BC patients.

Pasikanti *et al*[72] conducted a urinary metabotyping in another study in 38 BC patients and 61 non-BC controls using two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC-TOFMS). Urinary metabotyping characterized 46 metabolites which are human specific to BC with 100% specificity and 71% sensitivity in detecting BC *vs* 100% specificity and 46% sensitivity for cytology. They suggested potential roles of kynurenine in the malignancy and therapy of BC. In addition, altered metabolic pathways extracted from urinary metabotyping shed new insights on the mechanism of BC.

BIONANOTECHNOLOGY IN BC URINARY BIOMARKERS

However, the above mentioned BC diagnostic methods are not very powerful methods in detection of very early stages of cancer[73]. Also, some of them are quite costly and not available for many people. Therefore, the development of novel, specific, reliable and easily accessible technology for detecting BC early is of great importance[74-82].

Nanotechnology has been progressing very rapidly during the last few years and with this, properties of nanoparticles that provide an enriched medium for the selective capture and uptake of urine biomarkers due to their unique optical, chemical and physical magnetic properties[74,83]. Many classes of nanoparticles (such as gold nanoparticles, quantum dots, magnetic nanoparticles) have been proposed to be applicable in diagnosis, monitoring and treatment of disease[75,84-86].

The Wang *et al*[87] has reported that human telomerase activity can be visualized by using primer-modified Au nanoparticles. Our research group developed a gold nanoparticle (AuNP) assay for direct detection of unamplified hepatoma upregulated protein (HURP RNA) in urine samples from 50 bladder carcinoma patients, 25 benign bladder lesions and 25 controls[88]. They purified HURP RNA using magnetic nanoparticles functionalized with HURP RNA-specific oligonucleotides and detected by RT-PCR and gold nanoparticles. The developed HURP RNA AuNP assay has sensitivity and a specificity of 88.5% and 94%, respectively, and a detection limit of 2.4 nmol/L. Nossier *et al*[89] developed a simple colorimetric gold nanoparticle (AuNP) assay for rapid and sensitive detection of urinary HAase activity. The assay depends on charge interaction between polyanionic hyaluronic acid (HA) and cationic AuNPs stabilized with cetyltrimethylammonium bromide (CTAB) led to formation of gold aggregates and a red to blue color shift. HAase digests HA into small fragments preventing the aggregation of cationic AuNPs. The AuNP HAse assay has a sensitivity of 82.5% and a specificity of 96.1% and a short turnaround time of 2 h[89].

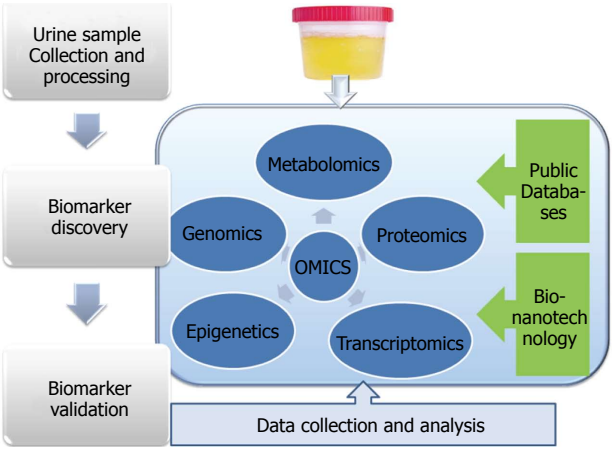

Figure 1 Integration of OMICS strategies for urinary bladder cancer biomarker discovery and validation.

IDENTIFICATION OF WHOLE TUMOR CELLS BY RAMAN SPECTROSCOPY

Raman spectroscopy is a technique based on excitation of vibrational models in the chemical bonds that hold molecules together[90]. Thus, it provides a measure of biologically active molecular groups[91,92]. Many authors applied Raman spectroscopy successfully in discriminating tumor cells from normal cells. Beside accuracy and non invasiveness, Raman spectroscopy is a fast and promising tool for BC screening in high risk populations[93,94]. Shapiro *et al*[95] used a Falcon Raman microscope to diagnose BC from epithelial cells found in urine of 344 patients (116 patients without urothelial cancer, 92 patients with low grade tumors and 132 patients with high grade tumors). They concluded that Raman molecular imaging is a powerful technique for BC diagnosis, with 92% sensitivity and 91% specificity in agreement with many recent studies[96].

Finally, although many policies and guidelines have been developed to evaluate potential BC biomarkers, no proper validation has been achieved until now, except for a few biomarkers[97]. For clinical application, any biomarker should be validated in a large number of samples with different ethnic origin and in different institutes, followed by approval from the FDA[98]. Public and private resources should offer financial support. Collaboration among researchers in universities, clinicians and industrial participants should be encouraged to bring biomarkers from the bench to the clinic[99,100].

CONCLUSION

BC remains an expensive cancer due to life-long surveillance involving upper tract imaging, urinary cytology and cystoscopy. However, as combined cystoscopy with cy-

Table 1 Urinary bladder cancer biomarkers

Biomarker/signature	Technology used	Ref.
MCP-1 A2518G, SDF-1 3'A and chemokine receptors CCR2A V64I, CCR5 Δ32, CCR5 59029 and CXCR4	PCR-restriction fragment length polymorphism	[21]
TP53	PCR-single strand conformational polymorphism analysis, DNA sequencing and immunohistochemical analysis	[22]
H-ras gene mutations	COLD-PCR	[23]
RARβ(2) and *APC* promoter methylation	Methylation specific PCR	[27]
SCGB3A1, *BNIP3*, *ID4* and *RUNX3*	Multiplex ligation-dependent probe amplification	[28]
TBX2, *TBX3*, *GATA2* and *ZIC4*	Genome-wide methylation analysis	[29]
BCL2, *CDKN2A* and *NID2* genes methylation	Nested methylation specific polymerase chain	[30]
miR-96 and miR-183	Q-PCR	[33]
miR-618, miR-1255b-5p	RT-qPCR	[36]
RNA ratio of v-ets erythroblastosis virus E26 oncogene homolog 2 (avian; ETS2) to urokinase plasminogen activator (uPA)	Reverse transcription quantitative-PCR	[41]
HYAL1 and survivin RNA	Qualitative and semiquantitative reverse transcriptase-polymerase chain reaction	[42]
FN, RTA, and CK20	Detection of CK20 by conventional RT-PCR, estimation of fibronectin by ELISA and relative telomerase activity by TRAP	[46]
CXCR4 and CXCR7	qPCR and/or immunohistochemistry	[52]
Bradeion/SEPT4 transcript	Real-time reverse transcriptase-polymerase chain reaction	[53]
hTERT, SENP1, PPP1CA, and MCM5 mRNA	q-RT-PCR	[54]
HOX-A13, IGBP-5, MDK, and CDC-2	cDNA microarray, Q-PCR	[55,56]
Afamin, adiponectin, complement C4 gamma chain, apolipoprotein A-II precursor, ceruloplasmin and prothrombin	iTRAQ	[61]
IL-8, MMP-9, MMP-10, PAI-1, VEGF, ANG, CA9 and APOE	ELISA assay	[62]
IL-8, MMP-9, MMP-10, SDC1, CCL18, PAI-1, CD44, VEGF, ANG, CA9, A1AT, OPN, PTX3 and APOE	ELISA assay	[63]
GC	Two-dimensional fluorescent differential gel electrophoresis and MALDI-TOF/TOF MS	[64]
beta-2-microglobulin, fatty acid-binding protein adipocyte, gelsolin, isoform 1 of gelsolin, myoglobin, isoform 2 of fibrinogen alpha chain, apoA-I, prostaglandin D(2) synthase 21 kDa, protein AMBP, transthyretin, keratin type II cytoskeletal 1, type II cytoskeletal 8, putative uncharacterized protein ALB, putative uncharacterized protein MASP2 (fragment)	2-dimensional electrophoresis combined with MALDI-TOF/TOF MS and SWISS-PROT database	[65]
MMP9, fibrinogen forms, and clusterin, aminopeptidase N, profilin 1 and myeloblastin	1D-SDS-PAGE followed by band excision and liquid chromatography tandem MS	[66]
Fibrinogen α chain precursor, apolipoprotein E, α-1-antitrypsin, and leucine-rich α-2-glycoprotein 1	MS and western blot/dot blot	[67]
Carnitine C9:1 and component I	Liquid chromatography-mass spectrometry based method	[72]
Kynurenine	Two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC-TOFMS)	[73]
HURP RNA	Conventional RT-PCR and AuNP nanoassay	[89]

MCP-1: Monocyte chemoattractant protein-1; SDF-1: Stromal cell derived factor 1; COLD-PCR: Co-amplification at lower denaturation temperature-polymerase chain reaction; ZIC4: Zic family member 4; Q-PCR: Quantitative real time PCR; FN: Fibronectin; TBX2: T-box 2; CXCR4: C-X-C chemokine receptor 4; RTA: Relative telomerase activity; CK20: Cytokeratin 20; GATA2: GATA binding protein 2; HYAL1: Hyaluronidase; TRAP: Telomeric repeat amplification protocol; iTRAQ: Isobaric tagging absolute and relative quantitation; CXCR4: C-X-C chemokine receptor 4; GC: Gc-globulin; MALDI-TOF/TOF MS: Matrix-assisted laser desorption time-of-flight mass spectrometry; MS: Mass spectrometry.

tology is considered the corner stone for BC diagnosis, it is necessary to search for an economical and efficient method to replace these deficient traditional methods. Many of the urinary markers currently available appear to be alternatives to cytology with a lower price and higher sensitivity, especially in detecting low-grade, non-muscle invasive cancers. Modern technologies, including mass spectroscopy, liquid chromatography, next generation sequencing, gene-expression profiling, metabolic profiling, nanoassays and epigenetic markers, are promoting more and more biomarker discoveries each month (Table 1, Figure 1). Finally, these versatile and newer strategies should be integrated to trace which markers may be clinically efficient and refinement of these markers which will help the urologist in critical evaluation of BC. Consequently, further and in-depth studies are required to determine the accuracy and widespread applicability of these modalities in guiding urinary markers discovery in BC.

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MINIREVIEWS

Neural regulation of sexual function in men

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Abstract

Male sexual response is controlled by a series of neurally mediated phenomena regulating libido, motivation, arousal and genital responses such as penile erection and ejaculation. These neural events that occur in a hormonally defined milieu involve different neurophysiological, neurochemical, and neuropsychological parameters controlled by central mechanisms, spinal reflexes and peripheral nervous system. Epidemiologic studies have suggested the high prevalence of male sexual dysfunction worldwide with significant impact on the quality of life of patients suffering from this problem. The incidence of sexual dysfunction is particularly high among men with neurologic disorders. Sexual dysfunction in men, such as loss of sexual desire, erectile dysfunction (ED), changes in arousal, and disturbances in orgasm and ejaculation may involve organic causes, psychological problems, or both. Organic male sexual disorders include a wide variety of neurologic, vasculogenic, neurovascular or hormonal factors that interfere with libido, erection, ejaculation and orgasm. Neurogenic sexual dysfunction may result from a specific neurologic problem or it could be the presenting symptom of a developing neurologic disease. Neurologic ED could result from complications of chronic neurologic disorders, trauma, surgical injury or iatrogenic causes. These etiologic factors and the underlying pathophysiologic conditions could overlap, which should be considered when making a diagnosis and selecting a treatment. A detailed history of physical examination, neurologic disorders, as well as any past history of psychological and psychiatric disturbances, and a thorough neurological examination will provide better understanding of the underlying causes of neurogenic sexual dysfunction. In patients with spinal cord injury, the location of the lesion and the time of onset of injury should be determined. Therapeutic strategies against erectile dysfunction are initiated with the least invasive options using the phosphodiesterase inhibitors. When oral medication options are exhausted, intraurethral and intracavernosal therapies and ultimately vacuum constriction devices and penile implants are considered. Recent basic research has suggested the potential role of stem cell-based therapeutic strategies to protect penile neural integrity and reverse cavernosal neurodegeneration in experimental models. Further insight into the central, spinal and peripheral neural mechanisms of male sexual response may help precise diagnosis and better management of neurogenic sexual dysfunction in men.

Key words: Sexual function; Nerve; Erection; Penis; Neurotransmission

Core tip: Despite considerable advances in our understating of male sexual function over the past two decades, crucial central mechanisms and peripheral pathways of male sexual response are still largely unknown. Neural responses to sexual stimulation precede vascular, smooth muscle, and endothelial cell reactions and play leading role in initiating fundamental pathways of

male sexual arousal, erection, orgasm and ejaculation. These pathways involve a dedicated subset of central mechanisms, spinal reflexes, peripheral nerves, and neurotransmission systems that operate at different levels individually and in conjugation. Further research into the neurophysiology of sexual function may help better management of neurogenic sexual dysfunction in men.

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INTRODUCTION

The nervous system is intricately involved in the regulation of male sexual response. Our knowledge into the central and peripheral neural regulation of male sexual function has gained ground with remarkable scientific advances over the past two decades. However, the precise central neural events and the intercommunication between central, spinal, and peripheral nervous system during male sexual response are still largely unknown. Male sexual arousal involves a dedicated subset of neural mechanisms in central nervous system that depend on fundamental neuronal responses, generalized brain activity, initiation of spinal reflexes, and peripheral neural mechanisms that operate at different levels individually and in conjugation.

Neural aspects of male sexual function are essential to most critical phases of sexual response in men including sexual desire, penile erection, and the development of arousal, orgasm and ejaculation. Penile erection is a complex physiological process involving central and peripheral neural mechanisms, blood vessels, and penile smooth muscle and endothelial cells[1]. Male orgasm is a subjective, perceptual-cognitive event of peak sexual pleasure that coincides with ejaculation. The autonomic nerves mediate one of the most important aspects of the male sexual response as their impulses travel through the cavernous nerves to regulate penile smooth muscle and vascular tone during penile erection and detumescence.

Sexual dysfunction in men involves psychological factors and organic problems. Most cases, however, correlate with organic causes that influence the mechanistic pathways of male sexual response or alter the structure of male sexual organs. Organic sexual dysfunction in men could result from changes in central and peripheral nervous system, hormones, penile vasculature and alterations of erectile tissue endothelium and smooth muscle cells. Loss of sexual drive in men correlates with increase in age[2]. However, the degree of this decline varies, and most men seem to maintain some amount of libido well into their 60s and 70s[2]. Other underlying conditions for loss of sex drive in men include depression, stress, decrease in male sex hormones and changes resulting from medications side effects.

Neurologic disorders compromise penile neural integrity and may lead to neural structural damage, functional deficit, or both[2-5]. Therefore, neurogenic erectile dysfunction (ED) could be an early symptom of progressive neurologic problems. Neurogenic ED may also relate to neural risk factors including alcoholism and other forms of substance abuse, depression, anxiety, stress, surgical treatment of prostate cancer, removal of enlarged prostate, surgical injuries to the pelvic area, and side effects of certain medications[3]. In most cases, however, neurogenic ED relates to impairment of the cavernous nerve pathways by surgical procedures or traumatic injury. An accurate diagnosis and successful treatment of nerve injury associated ED would depend on functional assessment of the prospective nerves and evaluation of the extent of nerve damage using diagnostic methods to accurately confirm cavernous nerve impairment. In this review, we focus attention on the neuroanatomy and neurophysiology of male sexual response.

NEURAL INTEGRITY AND MALE SEXUAL FUNCTION

An impeccable sexual response in men depends on central and peripheral neural integrity for achieving adequate arousal, erection and orgasm. Neural regulation of male sexual function could be disrupted by changes in central control of sexual response, alterations in spinal and peripheral neural pathways, changes in neurotransmission, or loss of neural function due to traumatic injury[5,6]. Neurogenic sexual dysfunction is the inability to initiate and maintain sexual activities due to a neurologic disorder. Underlying causes of neurogenic sexual dysfunction in men includes brain and spinal cord injuries, radical pelvic surgeries, diabetes mellitus, multiple sclerosis, stroke and Parkinson disease[7,8]. The peripheral mechanisms involved in penile erection and ejaculation have been extensively elucidated in the past three decades. However, the contribution of the central mechanisms into sexual response is still less well defined.

Basic research on the central regulation of sexual response using experimental models is currently underway in several institutions. Therapeutic strategies using growth factors and gene therapy have also been used to delay neurodegeneration and stimulate new nerve fiber outgrowth in penile erectile tissue[9,10]. In clinical studies, positron emission tomography scanners and functional magnetic resonance imaging have been used to explore regional brain activities during sexual stimulation, sexual excitement, and penile erection[11-13]. Further insight into the central pathways and peripheral neural mechanisms of male sexual response may lead to more precise diagnosis and treatment of specific neural deficits in neurogenic ED, anorgasmia and ejaculation disorders.

NEUROANATOMY

The neuroanatomy of male sexual response encompasses

a wide variety of anatomical structures in the brain, spinal cord, and peripheral nervous system including autonomic, somatic, sensory and motor neuronal structures[14]. At the spinal cord at the T9 to L4 levels, the intermediolateral column of gray matter gives rise to the sympathetic preganglionic nerve bundles. At the level of S2 to S4, the intermediolateral column gives rise to the parasympathetic nerves[15,16]. Continuation of these nerves assembles the framework of the pelvic and hypogastric plexuses. The penis is innervated by both autonomic and somatic nervous system[15]. At the spinal and peripheral levels, the autonomic (parasympathetic and sympathetic) and somatic (sensory and motor) nerves extend to innervate the penis[17].

Parasympathetic nerves

The neurons in the intermediolateral cell columns of the second, third and fourth sacral spinal cord segments (pelvic nerves) provide parasympathetic nerve fibers to the penis. At the level of the pelvic plexus, the preganglionic nerves are joined by sympathetic nerves originating from the hypogastric plexus. This plexus gives rise to branches that innervate the rectum, bladder, prostate and sphincters. The pelvic plexus give rise to a neural framework called cavernosal nerves that innervate the penile corpora cavernosa including terminal arterioles and erectile tissue[18]. The cavernosal nerves pass the prostate posterolaterally and then extend lateral to the membranous urethra and anterior to the bulbous urethra where they enter the hilum of the penis. The cavernosal nerve may be easily injured during radical pelvic surgery as well as transurethral prostatectomy, external sphincterotomy or any procedure using electrocautery in that region because it is closely applied to the apex of the prostate and membranous urethra.

Studies of penile tissue samples from human and experimental models have suggested that nitrergic nerves contributing to erection originate from the ganglia close to the corpus cavernosum[19,20]. The preganglionic cavernosal nerves are believed to synapse with nitrergic nerves within or near the tunica albuginea[19,20]. Penile erection following stimulation of the pelvic or the cavernosal nerves has been documented in both humans[21] and in animal models[22,23]. However, the precise nature of the cavernous nerve and whether or not it is a purely parasympathetic nerve remains controversial. Retrograde labeling and high resolution autoradiographic studies have suggested that some sympathetic fibers emanating from the lumbosacral sympathetic chain exist in the pelvic nerve of the male rat[24].

Sympathetic nerves

The sympathetic nerves to the male genital organs, which contribute to the regulation of penile detumescence and ejaculation, originate from the preganglionic neurons of the tenth to twelfth thoracic and first and second lumbar segments of the spinal cord. These preganglionic fibers pass *via* rami to the paravertebral sympathetic chain ganglia and descend to make synaptic connections with the postganglionic neurons then travel *via* the pelvic splanchnic nerves to the inferior mesenteric plexus, the hypogastric plexus and the perivesical plexus. Some fibers travel *via* the hypogastric nerve to the pelvic plexus. The hypogastric nerve is a discrete branch from these plexuses that enters the perivesical plexus where it may communicate with parasympathetic nerve fibers. The pelvic plexus is a crucial site in the integration of the autonomic input to the male genitalia.

Studies of experimental models have shown that stimulation of the hypogastric nerve or the sympathetic trunk has no significant effect on intracavernosal pressure in the flaccid state of penis but its stimulation during an erection causes penile detumescence[25]. These observations suggest that some sympathetic fibers may travel *via* the cavernous nerves to the penile corpora cavernosa. In the erect state of the penis, stimulation of the cut distal end of the pudendal nerve results in detumescence[25]. It is thought that some sympathetic fibers, especially the sensory branch, may travel *via* the pudendal nerve. Intracavernosal pressure rise and penile tumescence after stimulation of the sympathetic nerves has been documented in the rat model[26]. The precise mechanism of proerectile activity following sympathetic nerve stimulation remains unclear. One possibility may be the intercommunication between sympathetic fibers and nitregic nerves within the penile erectile tissue to release nitric oxide. Another possibility is sympathetic-mediated pelvic vasoconstriction and shunting of blood flow toward the penile erectile tissue.

Sensory nerves

The sensory nerves of the penis are primarily in the penile skin and glans as free and specialized nerve endings and receptors. The most numerous nerve terminals in the glans penis are free nerve endings (FNEs). Genital end bulbs are denser in the corona and near the frenulum and are present throughout the glans. The ratio of FNE to corpuscular receptors is approximately 10:1[27]. Axon terminals that resemble a tangled web of FNEs are present at the genital end bulbs unique to the glans penis[27]. Sensory nerves relaying pain and pressure sensation are also present in the urethra and corpora cavernosa[27]. Pain mediating signals and temperature sensation travel from free nerve endings *via* small diameter, thinly myelinated or unmyelinated nerve fibers. Large diameter myelinated fibers mediate the sense of vibration, touch and pressure[28]. These nerve fibers merge to assemble the dorsal nerve of the penis[27,28]. The dorsal nerve converges with other perineal nerves to become the internal pudendal nerve, which ascends to the dorsal roots of the second to fourth sacral nerves. The ascending pathways in the spinal cord travel *via* the spinothalamic tract to the thalamus and to the sensory cortex[27,28].

Somatic nerves

The ventral roots of sacral segments two through four along with coalesce form the paired pudendal nerves

provide somatic motor nerves to the penis. These nerves descend together with the internal pudendal vessels as they travel *via* Alcock's canal then provide somatic fibers to the striated muscle of the pelvis. These nerves extend as perineal nerve into the perineum and innervate the bulbocavernous and ischiocavernous muscles. These muscles are believed to provide temporary increases in intracavernosal pressure and contribute to penile rigidity during erection[29]. This is thought to aid in allowing successful vaginal penetration.

Co-existence and co-release of neurotransmitters

Immunohistochemical staining have revealed the co-existence of vesicular acetylcholine transporter, neural nitric oxide synthase (nNOS), vasoactive intestinal polypeptide (VIP), tyrosine hydroxylase, and heme oxygenase in tissue samples from human corpus cavernosum and spongiosum[30]. Immunoreactivity for endothelial nitric oxide synthase (eNOS) and heme oxygenase has been detected in the endothelial lining of corpus cavernosum and penile arteries[30]. Calcitonin gene related peptide has been localized within cavernosal nerves, cavernosal smooth muscle and cavernous arterial wall[31]. Co-release of neuropeptide Y and noradrenaline in autonomic nerves and release of calcitonin gene related peptide in the sensory nerves have been documented in the rat corpus cavernosum[32]. A rich sympathetic adrenergic innervation has been demonstrated in the human penile cavernosal tissue, penile microvasculature and helicine arteries[33,34]. Co-release of norepinephrine and neuropeptide Y from the penile adrenergic nerves has been documented in rats[34]. Downregulation of cavernosal nNOS and eNOS after bilateral cavernosal nerve injury was found simultaneous with upregulation of Rho-associated protein kinase in rat erectile tissue[35]. Inhibition of Rho-kinase was associated with increased nitric oxide (NO) signaling in the rat erectile tissue[35].

NEUROPHYSIOLOGY OF MALE SEXUAL RESPONSE

Male sexual response is a complex multidisciplinary biologic process involving central pathways and peripheral neural mechanisms controlling libido, arousability, penile erection and rigidity, orgasm and ejaculation. Neurologic disorders that can compromise central pathways and peripheral neuronal mechanisms would disrupt physiological sexual response during sexual stimulation. The central, spinal, and peripheral neural mechanisms that regulate male sexual response are summarized below.

Central control of male sexual function

Central regulation of male sexual function is less explored in comparison with the peripheral neural pathways. Multi-regional central neural mechanisms and inter-regional brain communications appear to be involved in male sexual response. It is known that cerebrocortical function is crucial to the initiation of sexual response in men[36,37]. However, the precise areas of the cerebral cortex involved in regulating libido, sexual fantasy and arousal are not well characterized. Studies of patients with traumatic brain injury suggest that the temporal and frontal lobes may play a crucial role in regulating sexual interest and behavior[37]. The septal portion of the hippocampus, the anterior cingulate gyrus, the anterior thalamic nuclei, the mammillothalamic tract and the mammillary bodies control penile erectile activities[36,37]. The medial dorsal nucleus of the thalamus and the medial pre-optic area appear to play crucial roles in the control of penile erection and sexual drive[38,39].

Central neurotransmitters

Central control of male sexual response involves multiple neurotransmitters including serotonin (5-hydroxytryptamine), dopamine, norepinephrine, nitric oxide and many others. Serotonin tends to block the penile erectile pathway at both spinal[40] and supraspinal sites[41]. Gamma amino butyric acid[42], prolactin[43] and endogenous opioid peptides[44] are also known as the central inhibitors of sexual activity in men. Dopamine is thought to regulate erection by acting on oxytocin containing neurons in the paraventricular nucleus of the hypothalamus[45,46].

In experimental animal models, systemic administration of dopamine and dopamine agonists such as apomorphine induce erectile activity *via* central mechanisms[45,46]. Norepinephrine plays various roles in central regulation of male sexual function[47]. Inhibition of central alpha-2 adrenoceptors facilitates sexual function while stimulation of these receptors produces the opposite effect[47]. Increased sexual motivation has been documented after administration of yohimbine, a central alpha-2 receptor blocker[48]. Oxytocin that has been localized in descending pathways from hypothalamus to brain stem is thought to mediate the effects of dopamine on penile erection *via* the oxytocin containing neurons[49,50]. Ascending sensory stimuli from the dorsal penile nerve stimulates oxytocin-containing cells in the supraoptic nucleus[49,50]. Dense nitric oxide synthase is localized in the paraventricular nucleus of the hypothalamus[51]. Administration of nitric oxide synthase blockers to the lateral ventricles or to the hypothalamus prevents erectogenic effects of dopamine agonists and oxytocin in experimental models[52]. The role of adrenocorticotropin and related peptides (melanocortin) in penile erection and ejaculation has been documented in patients with psychogenic erectile dysfunction[53]. A synthetic analogue of alpha-melanocyte stimulating hormone was shown to reverse erectile dysfunction in these patients[53].

Role of spinal reflexes

Spinal reflexes are crucial determinant of both the initiation and the maintenance of male sexual response. The spinal cord, paraspinal sympathetic ganglia, and parasympathetic nerves play a direct role in regulating functional changes of the male genitals. Sympathetic nerve fibers involved in sexual response originate from the interme-

diolateral column of gray matter at the level of T9-L4 in the spinal cord. The intermediolateral column at the levels of S2-S4 gives rise to the parasympathetic nerve fibers that innervate male genitalia. These nerve fibers descend to form the most important plexuses involved in sexual physiology, the pelvic and hypogastric plexus. The cavernosal nerve originates from the pelvic plexus and travels through the pelvic fascia and posterolateral aspect of the prostate. The parasympathetic nerves exit the spinal cord through the ventral roots and constitute the pelvic nerves. Upon sexual stimulation by visual, olfactory, and imaginary stimuli, penile erection takes place as a spinal reflex that is initiated by recruitment of penile stimulation traveling *via* the dorsal penile nerve[36,37]. The reflex that involves both autonomic and somatic efferent is heavily modulated by supraspinal influences. Local segmental reflexes in the lumbosacral cord subserve penile erection[36,37]. These reflexes are generally under the net tonic inhibitory control by higher centers[37].

Peripheral mechanisms

The peripheral neural pathways of sexual response particularly penile erection have received greater research and clinical attention than the central and spinal mechanisms. Basic research on the hemodynamic of penile erection and regulation of penile smooth muscle contractility resulted in the development of oral medications for erectile dysfunction. It was shown that a dedicated subset of neuronal mechanisms involving the adrenergic, cholinergic, and non-adrenergic non-cholinergic neurotransmission regulate cavernosal smooth muscle tone which determines penile tumescence and detumescence[23,54,55].

Basic research with experimental models have shown that electrical stimulation of the pelvic plexus and the cavernous nerve leads to erection, while stimulation of the hypogastric nerve or the sympathetic trunk induces detumescence[23,54,55]. It was shown that the sacral parasympathetic regulates penile tumescence and that the thoracolumbar sympathetic input mediates detumescence[25-27]. Follow up studies demonstrated that sensory stimuli relating to initiation and maintenance of erection originate primarily from the glans and travel *via* the dorsal nerve of the penis[27,28]. The most crucial step in the peripheral motor control of penile erection depend on smooth muscle tone in the erectile tissue and penile arterioles in the corpora cavernosa[56,57]. Alterations of smooth muscle tone in the tumescence and detumescence states of the penis are regulated by sympathetic and parasympathetic nervous systems and endothelial-mediated mechanisms[54,55]. It was shown that coordinated changes in smooth muscle tone of the penile erectile tissue and arterioles control the amount of blood entering to the cavernosal sinusoids and the amount of blood exiting the corpora[54,55].

Role of the adrenergic nerves

The primary adrenergic transmitter in the penis that controls smooth muscle contraction and induces penile detumescence is norepinephrine[56-59]. The regulation of adrenergic nerve activity and neurotransmission discharge in the penis is complex and appears to involve intercommunication with the cholinergic and the non-adrenergic non-cholinergic systems. As example, norepinephrine release from adrenergic nerves is pre-junctionally regulated by the cholinergic nerves[56]. The alpha and beta adrenergic receptors are localized in both penile blood vessels[57] and cavernous smooth muscle cells[34]. Alpha-1 adrenoceptors are more abundant in the erectile tissue smooth muscle while both alpha-1 and alpha-2 receptors have been localized in the penile arterioles[58,59]. Alpha-2 receptors have been localized both on pre-junctional sites of the adrenergic nerves and on erectile tissue smooth muscle[58].

Alpha-2 adrenoceptors on prejunctional sites mediate the feedback inhibition of norepinephrine discharge from the adrenergic nerves[56]. Upon release from the adrenergic nerves, norepinephrine binds to the pre-junctional alpha-2 adrenoceptor on the adrenergic nerves and inhibits norepinephrine release. This observation suggests that inhibition of alpha-2 receptor with selective antagonists such as yohimbine would inhibit erection by increasing norepinephrine release. It is also suggested that after release from adrenergic nerves, norepinephrine binds to the pre-junctional alpha-2 adrenoceptor on the non-adrenergic, non-cholinergic nerves and inhibits nitric oxide production and bioavailability[56,59,60]. It is thought that inhibition of this reaction by selective alpha-2 receptor antagonists will increase nitric oxide synthesis and promote erection. The smooth muscle alpha-2 adrenoceptors appear to play a role in the mediation of penile smooth muscle cell contraction[60]. Erectile tissue exposure to alpha-2 adrenoceptor agonists results in smooth muscle contraction[58,59]. In contrast, inhibition of smooth muscle alpha-2 adrenoceptors induces penile smooth muscle relaxation and promotes erection[56,59,60].

Role of the cholinergic nerves

Dense cholinergic innervation has been immunostained in penile corpus cavernosum and corpus spongiosum[30]. Immunohistochemical staining has also revealed that penile cholinergic nerves contain NO synthase and VIP. These observations led to the notion that vasodilators such as NO and VIP may be co-released along with acetylcholine from the cholinergic nerves[30,61]. These studies suggested that acetylcholine, whether released from the cholinergic nerves or applied directly to corpus cavernosum, initiates a variety of reactions in the erectile tissue.

Functional assessments of experimental models revealed erectile response to acetylcholine administered systemically or directly into the cavernosal tissue[62-64]. While having no effect on relaxed erectile tissues, acetylcholine produced concentration-dependent relaxation of erectile tissues that has been precontracted with norepinephrine[65,66]. Subsequent mechanistic studies with isolated erectile tissues from human and animals showed that the relaxing effects acetylcholine is partially blocked

by atropine but it could be abolished by removal of the endothelium[65,66]. The relaxing effect of acetylcholine that was markedly attenuated by removal of the endothelium introduced the theory of endothelial derived relaxing factor released from the endothelium under the influence of acetylcholine in the erectile tissue[67]. These findings indicated that acetylcholine may act on adrenergic nerve terminals to suppress the release of norepinephrine[65,66,68]. These observations collectively suggested that acetylcholine may induce cavernosal smooth muscle relaxation by co-release of nitric oxide and perhaps VIP from cholinergic nerve terminals, release of nitric oxide from the vascular endothelium, and suppression of norepinephrine release. The involvement of endothelium was an astonishing finding that led researchers to search for a non-adrenergic non-cholinergic mechanism of penile smooth muscle relaxation.

Role of non-adrenergic non-cholinergic neurotransmission

NO is well established as an important non-adrenergic non-cholinergic (NANC) neurotransmitter in the physiology of penile erection[67-71]. The NO/cyclic guanosine monophosphate signaling pathway has been widely recognized as the primary mediator of cavernosal smooth muscle relaxation and penile erection[67,69]. Mechanistic studies showed relaxation of human and rabbit penile smooth muscle in response to a solution saturated with NO gas[67]. Subsequent studies characterized nitric oxide synthase (NOS) as the enzyme that catalyzes the interaction of L-arginine and molecular oxygen in a process that consumes NADPH to produce NO and L-citrulline[67,69]. NOS exists in constitutive neuronal (nNOS) and endothelial (eNOS) forms, and inducible (iNOS) form. The constitutive forms of the enzyme are coupled to Ca^{2+} and calmodulin and are crucial to penile smooth muscle relaxation and erection.

Basal production of NO is regulated by constitutive NOS that is known to be involved in a variety of physiologic conditions such as cardiac and pulmonary perfusion, heart rate, myocardial contractility, vasodilation and penile erection[70]. iNOS is independent of Ca^{2+} and calmodulin and is believed to be upregulated in cellular stress and pathologic conditions[71]. In experimental models, long-term exposure of penile erectile tissue to ischemia has resulted in progressive downregulation of nNOS and eNOS and a significant increase in iNOS expression[72].

The relaxing role of NO in penile smooth muscle cells involves production and accumulation of the cyclic guanosine-3',5'-monophosphate (cGMP) in erectile tissue. Upon release from cavernous nerves and endothelium, NO diffuses locally into adjacent smooth muscle cells then activates guanylate cyclase to catalyze the formation of cGMP from guanosine-5'-triphosphate[67,69]. The increased levels of cGMP initiate a cascade of intracellular changes leading to activation of protein kinase G, also known as cGMP-dependent protein kinase Ⅰ. These events result in the reduction of cytosolic free calcium by various mechanisms leading to smooth muscle relaxation[67,69].

Relaxation of the trabecular smooth muscle and arterioles results in increased intracavernosal blood flow and activation of corporal veno-occlusive mechanism leading to penile erection. Another cellular mechanism that is thought to maintain penile erection is regulated by phosphatidylinositol 3-kinase (PI3-kinase) pathway that activates the serine/threonine protein kinase Akt, also known as protein kinase B[73]. This induces eNOS phosphorylation, reduces the enzyme's calcium requirement, and enhances NO production[73]. It is believed that after the initiation of erectile process, PI3-kinase/Akt mediated phosphorylation of eNOS result in sustained NO production and penile erection.

Other NANC factors in penile erection

Vasoactive neuropeptides including VIP, substance P, neuropeptide Y, somatostatin, peptide histidine-isoleucine, enkephalins and calcitonin gene-related peptide have been localized along the nerves supplying the penis[74-76]. The precise role of these neuropeptides is not well understood. VIP is believed to be co-released with NO from the cholinergic nerves[74,75]. Vasoconstrictive paracrine factors such as endothelin[77], angiotensin[78], prostaglandin F2-alpha[79], thromboxane[80] and histamine[81] have also been localized in penile erectile tissue but whether they synergize with other neurotransmitters or are modulators of smooth muscle tone is unclear.

Endothelins localized in the penile erectile tissue are potent constrictors of smooth muscle cells[77]. Three isoforms of endothelin called ET-1, ET-2 and ET-3 and two different receptors named ET_A and ET_B have been reported in penile erectile tissue[77]. The ET_A and ET_B receptors are located on vascular smooth muscle and endothelial cells, respectively. ET_A receptor mediates contraction and proliferation while the ET_B receptor contributes to vasodilation[77]. Angiotensin Ⅰ and Ⅱ and two subtypes of angiotensin Ⅱ receptor (AT1 and AT2) have been characterized[82-84]. It was shown that AT1 receptor is expressed in the erectile tissue[83] and that angiotensin Ⅱ causes a dose-dependent contraction of cavernosal smooth muscle[84].

Some of the prostaglandins (PGs) in the penis appear to act as modulators of cavernosal smooth muscle reactivity[85,86]. $PGF_{2\alpha}$, PGI_2 and thromboxane A_2 cause cavernosal smooth muscle contraction while PGE_1 and PGE_2 induce relaxation[87]. In addition to direct vascular smooth muscle relaxation, PGE_1 may also act to inhibit the release of neuronal norepinephrine[88]. A variety of pathologic conditions interfere with the production and action of prostaglandins in erectile tissue. For example, hypoxia was shown to inhibit production of prostanoids in the cavernosal tissue[89,90]. Castration in experimental models was shown to diminish cavernosal smooth muscle relaxation in response to PGE_1, suggesting that androgens may be a prerequisite for their action[91].

Bradykinin relaxes corpus cavernosum tissue and its effects appear to be mediated through cyclic adenosine monophosphate and cGMP[78]. It is thought that bradykinin acts on cavernosal BK2 receptors and stimulates the release of endothelial nitric oxide[92]. Histamine appears to induce endothelium-independent relaxation of erectile tissue and penile microvasculature[81,93]. The relaxatory effects of histamine seem to be mediated by histamine H2 receptors located on vascular smooth muscle. Histamine appears to act on smooth muscle cells without the intervention of nitric oxide or relaxant prostanoids[93].

SUMMARY

Neurophysiology of male sexual response involves multi-regional central neural mechanisms, inter-regional brain communications, and intricate spinal and peripheral neural mechanisms. Our knowledge into the central and peripheral neural regulation of male sexual function continues to gain ground with remarkable scientific advances over the past two decades. Peripheral neural events in male sexual response and the mechanism of penile smooth muscle relaxation have been extensively studied and newer components in these pathways are emerging. A variety of neurologic disorders contribute to the development of male sexual dysfunction and, in some cases, neurologic sexual dysfunction may be a presenting symptom of the impending neurologic disease. Mechanistic knowledge into downstream pathways of NO/cGMP signaling introduced newer concepts in the molecular mechanism of erection and led to the investigation of innovative therapeutic strategies against erectile dysfunction, including the possibility of gene therapy and use of stem cells. However, despite such advances, the precise diagnosis of central problems and peripheral neural factors in neurogenic sexual dysfunction still remain as a major clinical challenge. Nonspecific therapies have been somewhat effective in early-state neurogenic erectile dysfunction but have failed to restore erection in most patients with advanced neurologic problems. Further research into the central, spinal and peripheral neural regulation of sexual function may help the development of more precise diagnostic tools, newer therapeutic strategies, and better management of neurogenic sexual dysfunction in men.

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MINIREVIEWS

Professionalism and patient education in urologic surgery

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Abstract

Medical professionalism provides the guidelines that govern the patient-physician relationship. This implicit contract requires that patients be informed before making decisions regarding their medical care. Educating patients about diagnostic and treatment decisions is critical to an informed decision-making process. Shared decision-making is a recent paradigm shift in patient education that allows patients to make decisions based both on the counsel of their physicians and according to their own preferences and values. This approach moves away from previous models that focused on physicians or third-party payers as the arbiters of diagnostic and treatment choices. Urologic surgeons have been at the forefront of shared decision-making research and continue to promote this concept in the most recent American Urological Association Guideline on Detection of Prostate Cancer. Unfortunately, the fee-for-service financial structure that predominates in the United States' health care system provides a disincentive for shared decision-making. By promoting patient volume rather than time spent with patients, this system rewards physicians who spend less time educating patients about diagnostic and treatment options. Therefore, to promote adherence to the educational responsibility inherent in medical professionalism, we recommend physician payment reform that rewards physicians for time spent with patients rather than the volume of patients seen.

Key words: Urology; Health care reform; Professionalism; Patient education; Decision making; Informed consent

Core tip: Medical professionalism provides the guidelines that govern the patient-physician relationship. This implicit contract requires that patients be educated regarding their diagnostic and treatment decisions. Shared decision-making is a recent paradigm shift in patient education that allows patients to make decisions based both on the counsel of their physicians and according to their own preferences and values. To promote adherence to the educational responsibility inherent in medical professionalism, we recommend physician payment reform that rewards physicians for time spent with patients rather than the volume of patients seen.

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Health care is a dynamic environment. Beyond advances in diagnostic tests and treatments, there is a perpetual shift in the both the landscape of pathology and in the landscape of the health system itself[1-4]. This is particularly true in the United States where the Patient Care and Affordable Care Act will bring significant change to the health care system[5-14]. In the midst of this dynamic space, however, there is a constant and immutable center: medical professionalism. What this term means and its role in the physician-patient relationship will be explored in this piece, as will the interplay between professionalism

and the growing movement behind patient education. Finally, we will explore the potential role for public policy in promoting professionalism and patient education in urologic surgery.

Although medical professionalism is difficult to define, the literature is certainly not bereft of efforts to do so[15-28]. The most notable and durable effort was the publication of Medical Professionalism in the New Millenium: A Physician Charter. A collaborative work by the American Board of Internal Medicine Foundation, the American College of Physicians Foundation, and the European Federation of Internal Medicine, the Charter was published simultaneously in the Annals of Internal Medicine and The Lancet in 2002[29,30]. The Charter defines professionalism as "the basis of medicine's contract with society", asserting that an implicit contract exists between patients and their physicians. Understood in these terms, it is the implied contract of medical professionalism that legitimizes the intimate and often invasive role of physicians in the lives of their patients. In other words, medical professionalism defines the set of standards that physicians must adhere to in exchange for the privilege of diagnosing and treating patients.

The Charter identifies three fundamental principles that define medical professional standards and expounds on these principles with ten specific professional responsibilities. The fundamental principles include the primacy of patient welfare, patient autonomy, and social justice. These principles require physicians to place patient interests above their own, empower patients to make informed decisions, and promote the equitable distribution of health care resources across society. The professional responsibilities most apropos to the current discussion of patient education in urologic surgery include commitments to professional competence, honesty with patients, and maintenance of trust by managing conflicts of interest. Together these responsibilities demand that urologic surgeons commit themselves and their peers to maintaining the knowledge and skills necessary to deliver high quality care and ensure that patients are making medical decisions based on complete information without consideration of physician gain or personal advantage.

The professional obligation to ensure patient autonomy and informed decision-making has led to a new emphasis on patient-centered care[31-42]. Unlike previous eras when decision-making was driven first by physicians and then later by payers, contemporary health reforms now focus on putting patients at the center of care decisions. Nowhere is this more evident than in the sections of the Patient Care and Affordable Care Act that provide grants to promote patient-centered care[43]. Specifically, the Act promotes "shared decision-making" and "patient decision aids" as a means of promoting patient-centered care in those clinical settings where the literature supports multiple diagnostic and/or treatment options. Shared decision-making is defined as a decision-making process that allows patients to consider medical care choices based on clinical evidence and personal preferences, and patient decision aids are the educational tools provided to patients to support this shared decision-making process[44-47]. To illustrate this concept consider a patient diagnosed with clinically localized, intermediate risk prostate cancer. Current evidence supports radiation and surgery as equivalent treatment options for cancer control and survival, although each has a distinct risk profile, while active surveillance is appropriate in certain populations[48]. In the shared decision-making paradigm the patient and his urologic surgeon would discuss the risks and benefits for each option and account for the patient's values and preferences when considering the different approaches. In this example, a patient with bothersome lower urinary tract symptoms might choose surgery over radiation because of a desire to avoid potential radiation injury to the bladder, while a patient with similar disease may choose radiation to avoid the risks of anesthesia. In both instances the urologic surgeon uses shared decision-making to educate patients and ensure that treatment decisions reflect the patients' values and preferences.

Notably, there is a longstanding history between urologic surgery, patient education and shared decision-making. The early research on shared decision-making centered on urologic surgery patients choosing between surgical and non-surgical management of benign prostatic hypertrophy[49,50]. These studies demonstrated that patient preferences had a significant impact on treatment decisions, and that patient preferences flowed from the education that patients were receiving about the treatment options. More recently, the revised 2013 AUA guideline for the early detection of prostate cancer prominently features shared decision-making. For men ages 55 to 69 who are considering prostate cancer screening with a serum prostate specific antigen, the guideline explicitly recommends "shared decision-making" and consideration of each patient's "values and preferences"[51].

To advance physicians' professional obligation to engage patients in shared decision-making will require innovative health care reform. Specifically, physicians should no longer be incentivized to maximize clinical throughput, but should instead be rewarded for spending time with patients to counsel them about their diagnoses and treatment options. One potential mechanism would be to compensate physicians based on the amount of time spent with patients rather than according to fee schedules for particular diagnoses or types of visits. A payment system based on the time spent rather than patients seen would discourage physicians from rushing through clinic visits and elevate the value of the patient-physician relationship. Furthermore, patients could exercise more control over health care spending by comparing the costs and benefits associated with lengthy versus abbreviated clinic visits.

Medical professionalism defines the obligations that urologic surgeons owe to their patients, including ensuring patient autonomy by allowing patients to serve as the primary arbiters of their medical decisions. Towards this end, there has been renewed interest in delivering patient-centered care through patient education. Serving as the nexus between medical professionalism and patient

education, shared decision-making defines the formal process of patients arriving at medical decisions based on the counsel of their urologic surgeon and an evaluation of their own preferences and values. This approach is in sharp contrast to the historically paternalistic medical decision-making process and provides an opportunity to minimize the health care system's disincentives to deliver on medical professionalism's promise of patient autonomy.

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BRIEF ARTICLE

How to improve a urology outpatient service? A survey of patient satisfaction

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Abstract

AIM: To investigate and improve our out-patients department patient satisfaction, provide minimum consultation delay and appropriate consultation duration to meet with targets.

METHODS: We distributed the modified satisfaction with outpatient service (SWOPS) questionnaires developed for use in Irish hospitals by the Health Services Research between August and December 2012. The patient disclosed their age and sex and completed the modified SWOPS questionnaire anonymously. Every patient was eligible to participate in the study who attended any of the Urology Outpatient Clinics. Patients lacking capacity to consent were excluded. Additionally, each patient was only permitted to complete one questionnaire regardless of repeat attendances within the 4 mo study period. The answers to every question were presented as percentages. One-way ANOVA was used to establish whether there was a significant difference in appointment delay and "Overall Satisfaction" on the different clinic days. The unpaired *t*-test was applied to establish whether "Overall Satisfaction" was affected by diagnosis (benign or malignant). Paired *t*-test was used to establish whether "Overall Satisfaction" was affected by appointment delay and appointment length.

RESULTS: Three hundred and forty-eight questionnaires were completed with an overall > 65% participation rate. Eighty-one point six percent were male and 18.4% female with a mean age of 65 ± 21 years. Mean delay time was 32 min, which 30.6% stated should be an improvement priority. The delay times for Wednesday (mean 13 min) were significantly ($P < 0.05$) lower than for other days (mean 36 min). Generally 12-15 min outpatient appointment length is acceptable and adequate for patients as 97.70% suggested, however 31.60% of patients would favour longer duration. Eleven point four nine percent do not want to see different doctors each time, and 31.60% of the patient feel that no change is required. Average satisfaction was 84.65%. There was no significant relationship between satisfaction and clinic day, diagnosis and consultation length, whether the patient was reviewed by a registrar or consultant. Satisfaction was universally high and independent of consultation delay/length and diagnosis. Dissatisfaction in delay times with a significant improvement on Wednesday suggests necessary and achievable improvements. Notably, the Wednesday clinic has less patients per doctor per hour and enforces a 1 patient per 15 min slot with a no over-booking policy.

CONCLUSION: Surveying our patient dissatisfaction would require more frequent audits by clinicians to improve patient satisfaction and to achieve better quality of care.

Key words: Satisfaction with outpatient service ques-

tionnaire; Patient satisfaction; Outpatient department; Survey; Service delivery

Core tip: With our survey we would like to emphasize the need of regular audit activity at the outpatient clinic to improve patient satisfaction and to identify potential pitfalls of the outpatient pathway. Ideally every outpatient clinic or medical practice should conduct a survey yearly for quality improvement purposes to improve patient care and outcomes through systematic review of care against explicit criteria and implement changes if necessary.

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INTRODUCTION

The National Health Service (NHS) in the United Kingdom and the health system in the United States of America seek to develop so called "patient centred care"[1,2], as patient perception of their healthcare experience has gained increasing attention over the past 20 years[3]. Researching patients' healthcare experience is an important method of creating effective action plans for quality improvement in health care organisations[4].

Currently, there is a drive for reducing patient waiting times for the benefit of patients, healthcare providers, managers, and Department of Health. Consequently, Out-patient clinics have also been subject to new targets. The Department of Health's Operating Framework for 2012/13[5] confirmed the NHS Constitution guarantees that patients should expect to receive treatment for non-urgent conditions at an inpatient or outpatient basis within 18 wk. In addition all GP referrals with suspected malignancy should receive specialist review within 2 wk.

As we are searching for ways to make our Outpatient's Department service more responsive to the general public and in order to improve the patient experience at our Urology Out-Patient's Department, we conducted a prospective study of patient satisfaction among patients attending between August 2012 and December 2012. Our modified satisfaction with outpatient service (SWOPS) questionnaires (Figure 1) were used. St Mary's Urology out-patients department targets maximum patient satisfaction as it is one of the key criteria by which the quality of health care service is evaluated[6,7]. This survey aimed to develop a framework for identifying factors affecting patient satisfaction, and to identify potential, and correctable causes in order to improve overall satisfaction and healthcare quality improvement. Therefore we focused on patient dissatisfaction in order to identify reasons which make patients disappointed with the service provided.

MATERIALS AND METHODS

The survey was performed in four Outpatient Urology Clinics held weekly (Monday, Wednesday, Thursday and Friday) at St Mary's Hospital, Imperial Healthcare NHS Trust, London. These are led by the same 2 consultants and 2 registrars. Patients were seen, reviewed and consulted randomly; the next patient was seen by the next available doctor. It is of note, that Wednesday clinic has less patients per doctor per hour and enforces a 1 patient per 15 min slot with a no over-booking policy, which results into a smooth continuously flowing clinic without significant delays.

Data was collected prospectively between August 2012 and December 2012 using our modified SWOPS questionnaires (Figure 1). The SWOPS questionnaire is multi-dimensional outpatient instrument, which was developed for use in Irish hospitals by the Health Services Research Centre at the Department of Psychology, Royal College of Surgeons in Ireland (RCSI). The generic items of the questionnaire make up an overall dimension with an α co-efficient of 0.84. The high reliability co-efficient of each of these dimensions allows users to "select" questions whilst maintaining validity. Therefore the modified SWOPS questionnaire is valid[8], Every patient who attended our four Urology clinics was asked to complete the modified SWOPS questionnaires, the doctor completed sections including consultation length and delay, diagnosis (benign or proven malignant), whether the consultation was Registrar or Consultant led and whether the patient was new or follow-up. The patients disclosed their age and sex and completed 12 of the questions of the modified SWOPS questionnaire anonymously. At the end of the questionnaire 2 open questions were provided for the patients to express their personal view, impression and recommendations. Every patient was eligible to participate in the survey who attended the Urology Outpatient Clinic. Patients with diagnosed mental health disorder and patients lacking capacity to consent to completing the questionnaire were excluded. Additionally, each patient was only permitted to complete one questionnaire regardless of repeat attendances within the 4 mo study period. Doctors were unaware of the collected data and feedback until the end of the survey. The Questionnaire was completed at the end of the consultation by the patient in the waiting area and were collected at the Outpatient Department Reception in a sealed container.

The responses to each question by each patient were recorded onto Microsoft Excel spreadsheet 2010. The number of answers were recorded as percentages. The responses to the "scalable" questions (Questions 1-6 and Questions 8-11) were recorded as a percentage of the maximum score for that question. In order to ensure that these questions were consistently "scalable" for the calculation of "Overall Satisfaction" the following rules were applied: (1) For Question 2, response options 4 and 5 were not taken into account (for the calculation of overall satisfaction); (2) For Question 4, response op-

Urology SWOPS Questionnaire

Please take a few minutes to fill out this survey. The Urology department welcomes your feedback and your answers will be kept confidential.
Thank you for your participation.

Age: _____ years Gender: Male Female

1. Did you have enough time to discuss your health or medical problem with the doctor?
○ Yes, definitely ○ Yes, to some extent ○ No

2. Did the doctor explain the reasons for any treatment or action in a way that you could understand?
○ Yes, definitely ○ Yes, to some extent ○ No ○ I did not need an explanation ○ No treatment or action was needed

3. Did the doctor listen to what you had to say?
○ Yes, definitely ○ Yes, to some extent ○ No

4. If you had an important question to ask the doctor, did you get answers that you could understand?
○ Yes, definitely ○ Yes, to some extent ○ No ○ I did not need to ask ○ I did not have an opportunity to ask

5. Did you have confidence and trust in the doctor examining and treating you?
○ Yes, definitely ○ Yes, to some extent ○ No

6. Did the doctor seem aware of your medical history?
○ He/She knew enough ○ He/She knew something but not enough ○ He/She knew little or nothing ○ Don't know/Can't say

7. Do you have concerns about seeing different doctors each time?
○ I see the same doctor ○ No, I don't mind ○ Yes, I have concerns

8. Were you involved as much as you wanted to be in the decisions made about your care and treatment?
○ Yes, definitely ○ Yes, to some extent ○ No

9. Overall, how would you rate the care that you received in the Outpatients Department?
○ Excellent ○ Very Good ○ Good ○ Fair ○ Poor ○ Very poor

10. Would you recommend this Outpatients Department to your family and friends?
○ Yes, definitely ○ Yes, to some extent ○ No

11. When I left the clinic I knew what was going to happen next and when?
○ Yes, definitely ○ Yes, to some extent ○ No

12. What would you most like to improve in this Outpatients Department?
○ Waiting time in clinic ○ Time spent with doctor Seeing different doctors each time Quality of care ○ No changes needed

13. Are there any areas where you feel we could make improvements?

14. Comments

For office use only
Diagnosis: B / M Type: New / F/u / BBN
Seen by: Cons / Reg
Date of OPD: Scheduled Time:
Start Time: Finish Time:

Thank you for taking the time to fill out our survey. We rely on your feedback to help us improve our services. Your input is greatly appreciated.

Figure 1 Satisfaction with outpatient service (SWOPS) questionnaire.

tions 4 and 5 were not taken into account; (3) For Question 6, response option "Don't Know" was excluded.

The average percentage score, *i.e.*, "Overall Satisfaction" was then calculated.

Statistical analysis

The unpaired *t*-test was applied to establish whether "Overall Satisfaction" was affected by diagnosis (benign or malignant).

The paired *t*-test was used to establish whether "Overall Satisfaction" was affected by appointment delay and appointment length.

Furthermore one-way ANOVA was used to establish whether there was a significant difference in appointment delay and "Overall Satisfaction" on the different clinic days.

RESULTS

The survey had an overall > 65% participation rate. A total of 348 patients completed and returned the questionnaire. The demographic characteristics of the survey consisted of 284 males (81.6%) and 64 females (18.4%) with an average age of 61 years and a mean age 65 ± 21 years. 29.88% of patients (n = 104) were new referrals to the clinic with no previous experience with the Department and 244 (70.12%) were at least seen once previously so called follow up patient. All clinics were a mixture of patients diagnosed with benign and malignant urological diseases. Overall 214 (61.49%) patients attended clinic for benign urological problems and 134 (38.51%) for a histologically proven malignancy either as a new diagnosis or follow up. One hundred and fifty-six (44.82%) patients were seen by the Consultant and 192 (55.17%) patients by the registrars. Groups seen by Consultant and registrar were identical demographically (age, sex), in terms of medical condition (new or follow up) and histological diagnosis (benign or malignant) as a result of random outpatient consultation. Overall statis-

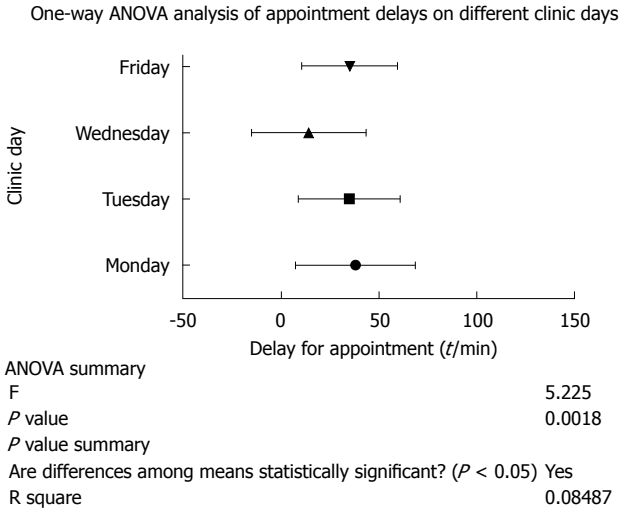

Figure 2 One-way ANOVA analysis of appointment delays for appointment on different clinic days.

Table 1 Analysis of consultant *vs* registrar led consultation duration time, delay of consultation, and patient satisfaction

	Consultant led clinic		Registrar led clinic	
	Mean	SD	Mean	SD
Consultation time (min)	13.61	10.58	13.81	10.19
Delay of consultation (min)	33.71	18.84	36.48	20.22
Patient satisfaction	0.90	0.14	0.89	0.12

tical analysis did not reveal any significant difference between consultant and registrar led consultation in duration time, delay of consultation, and patient satisfaction (Table 1).

The number of patients who completed the questionnaire for every clinic were respectively for the Monday clinic 98 (28.16%), Wednesday clinic 52 (14.94%), Thursday clinic 74 (21.26%), Friday clinic 124 (35.63%).

Analysis of the Appointment Length and Appointment Delay Times (Table 2), clearly showed similarities in appointment or consultation length by average of 13 min per consultation. On Question 12, only 16.66% (n = 58) of the patients found this consultation length inadequate. Further analysis of Appointment Delay time to patient schedule showed that notably Wednesday appointment delay length was only 14 min far less compared with other clinics, where patients have nearly 35-min delay to see the doctor. One-way ANOVA Analysis of Appointment Delays on Different Clinic Days (Figure 2) is statistically significantly different with a P value of less than 0.0018. On Question 12, 31.60% (n = 110) of patients stated the waiting time in clinic needs to improve.

Analysis of the individual Clinic days (Table 3), showed no statistically significant difference between the clinic days or whether the visit was a result of a benign or malignant diagnosis. However, the Wednesday clinic had the highest overall satisfaction rate with 87.7% of the attending patients with the lowest standard deviation compared with any other clinic days.

The individual answers to the questions of the questionnaire are presented in percentages in Table 4.

DISCUSSION

A prospectively administered exit survey questionnaire is a reasonably effective way of eliciting the view of members of the public but it is important to be aware of the limitation of this method. Firstly response rate can be often quite low and depends on the quality of the questionnaire, the clinical set up, the selling point of the questionnaire and also the willingness of those approached in this way to respond truly. Patients who are extremely satisfied or dissatisfied will more commonly be willing to express their feelings, and fill the questionnaire which can result in a significant bias for the study. Our survey participation rate after excluding the ineligible patients was 65%.

For better understanding of the large amount of data collected, we divided the questionnaires into 3 major areas. First was the sufficient appointment length surveyed by the first question. Generally a 12-15 min outpatient appointment length is acceptable and adequate for patients as 97.70% were not dissatisfied, however, when patients answered the last question 16.66% of them would like to spend more time with their doctor. We concluded that an average of 20 min consultation time (3 patients/h) would be satisfactory for both doctors and patients. This would result at an average of 12 patients over a half day clinical session. The second part is focus on doctor-patient interaction and communication (Q 2, 3, 4, 5, 6, 7, 8, 11). Good communication between patients and healthcare professionals has long been seen as the bedrock of quality from the patient's perspective[9]. Generally doctors and healthcare professionals are appreciated more when they are genuinely interested in what patients have to say or ask, when they provide clear explanation and examples about the possible treatment options, as well as offer sufficient time for patient interaction. Direct patient involvement by having the opportunity to choose treatment options or decision how to manage their condition is becoming more common[10]. A shared decision approach may be the preferred way, however the extent of patient involvement in their decision making process is dependent upon the background knowledge and education. Our survey results showed that generally patients were happy, however, in some cases the lack of notes resulted in lack of confidence and dissatisfaction for the doctor and the Department. Therefore this group of questions focused on the received care, expectations and area of improvement (Q9, 10, 12).

Furthermore we concluded the following from the patient answers (Table 3): Question two highlights the importance of good patient/doctor interaction and involvement in the decision making process to improve patient understanding of their disease and medical management plan, resulting in improvement of patient

Table 2 Mean ± SD appointment delay time and appointment length times on various days of the week in our Urology Outpatient Clinics

Clinic day	Appointment delayed length mean (min)	Appointment delayed ± SD (min)	Appointment length mean (min)	Appointment length ± SD
Monday	38	30	13	7
Wednesday	14	28	12	9
Thursday	35	25	14	12
Friday	35	23	14	11

Table 3 Patient satisfaction outcomes and their standard deviation by clinic days and according to patient histological diagnosis

Factor	Satisfaction (%)	SD
Monday clinic	83.4	16
Wednesday clinic	87.7	15.7
Thursday clinic	83.2	17.7
Friday clinic	84.3	18.9
Benign diagnosis	83	18.6
Malignant diagnosis	86	15.3

compliance to treatment. Question three indicates the importance of doctor/patient interaction, especially underlining the importance of active listening to the patient. Question four demonstrated that answering patient questions about their problem is extremely important, however, that should be explained in a way that the patient understands. In question five 1.72% of the patients have no confidence in their doctor, a fact probably difficult to correct, however 74.71% answered "Yes, definitely" and 23.56% answered "Yes, to some extent" being confident in their doctor. Patient perception of the delivered quality of care is commonly measured by the doctors' knowledge of patient's disease and past medical history. Question six supported that most patients were generally satisfied (92.52%) with our service, however, improvement was required to satisfy the remaining 7.5%. Question seven surprisingly revealed, that patients not necessarily wanted to see the same doctor, providing that their medical records were updated and the presently treating medical doctor were made aware of their condition. Question eight suggested that 97.70% of patients feel that the consultation was adequately managed, and they were involved in the decision making process fully in 58.62%. Question nine revealed that an overall 86.20% had excellent or good opinion about the care that they received, 8.62% found it fair, however 6.32% of the patients were dissatisfied (18 patients in total). Both the 14 questionnaires which found our service poor and the remaining 4 that found it very poor were analysed independently. Overall from the group of these 18 patients, 17 would probably have been satisfied with the department, however their average delay to be seen was more than 65 min, which resulted to major disappointment. The remaining patient was dissatisfied as he was delayed by 45 min, his notes were missing and the doctor was unaware of his history. Question ten confirmed however, when it is about recommending the department to family members only 3.44% of the patients would not make such a recommendation, which might suggest individual or personal issues, and was independent from the quality of care which they received. Question eleven underlines the importance of sharing future management plan with patients to improve patient compliance. At last but not least question twelve highlights the expectation of the patient and suggestion for further improvement. Thirty-one point six percent of patients seemed happy with the present status quo, however that does not necessarily mean a happy customer. Moreover there is plenty of room for improvement as 76.43% would favour changes to improve service delivery. By far more than 31.6% of patients would like to reduce the waiting time in the clinic (time spent in the department waiting for consultation), which turned out one of the independent factors influencing patient satisfaction.

Some outpatient urology clinics are performing minimal invasive procedures as well during their outpatient consultation, such as flexible cystoscopies, prostatic biopsies or even ureteric stenting[11]. In these circumstances a sufficient pain management is mandatory and could result in further improvement in patient satisfaction[12] and outpatient service.

Alternatively for the measurement of patient satisfaction the Patient Satisfaction Questionnaire Short Form (PSQ-18) can also be used, which is an adaptable, reliable, and validated tool that may be applied to various settings, as well as comparing interventions[13,14].

It seems that in the era of financial constraints, hospital managers focus more on the number of patients seen in clinic in order to reduce waiting times, satisfy targets and earn more financially for their hospitals. However, quantity is not only what matters, quality in the delivery of care has to be a priority for every physician. Therefore it is very important to evaluate patient satisfaction and implement new strategies to provide quality care to our patients.

COMMENTS

Background

Patient satisfaction gained increasing attention over the past 20 years as one of the primary factors to measure quality of care. The findings of this study are important because, to our knowledge, such specific outpatient survey and feedback is rarely undertaken in the hospital setting. Satisfaction surveys are mainly used by hospital managers who evaluate their staff in their working environment.

Research frontiers

In an era when reductions in patient waiting times (and to meet the increasing

Table 4 Individual question results of the satisfaction with outpatient service questionnaire *n* (%)

Question	Total responses	Response	Responses
Question 1 Did you have enough time to discuss your health or medical problem with the doctor?	348	Yes, definitely	242 (69.54)
		Yes, to some extent	98 (28.16)
		No	8 (2.29)
Question 2 Did the doctor explain the reasons for any treatment or action in a way that you could understand?	348	Yes, definitely	252 (72.41)
		Yes, to some extent	72 (20.68)
		No	8 (2.29)
		I did not need an explanation	6 (1.72)
		No treatment or action was needed	10 (2.87)
Question 3 Did the doctor listen to what you had to say?	348	Yes, definitely	268 (77.01)
		Yes, to some extent	80 (22.98)
		No	0 (0)
Question 4 If you had an important question to ask the doctor, did you get the answers that you could understand?	348	Yes, definitely	202 (58.04)
		Yes, to some extent	94 (27.01)
		No	14 (4.02)
		I did not need to ask	30 (8.62)
		I did not have an opportunity to ask	8 (2.29)
Question 5 Did you have the confidence and trust in the doctor examining treating you?	348	Yes, definitely	260 (74.71)
		Yes, to some extent	82 (23.56)
		No	6 (1.72)
Question 6 Did the doctor seem aware of yor medical history?	348	He/she new enough	234 (67.24)
		He/she knew something but not enough	88 (25.28)
		He/she knew little or nothing	16 (4.59)
		Don't know/Can't say	10 (2.87)
Question 7 Do you have concerns about seeing different doctors each time?	348	I see the same doctor	74 (21.26)
		No, I don't mind	214 (61.49)
		Yes, I have concerns	60 (17.24)
Question 8 Were you involved as much as you wanted to be in the decisions made about your care and treatment?	348	Yes, definitely	204 (58.62)
		Yes, to some extent	136 (39.08)
		No	8 (2.29)
Question 9 Overall, how would you rate the care that you received in the Outpatients Department?	348	Excellent	120 (34.48)
		Very good	100 (28.73)
		Good	80 (22.98)
		Fair	30 (8.62)
		Poor	14 (4.02)
		Very poor	4 (1.11)
Question 10 Would you recommend this Outpatients Department to your family and friends?	348	Yes, definitely	212 (60.91)
		Yes, to some extent	124 (35.63)
		No	12 (3.44)
Question 11 When I left the clinic I knew what was going to happen next and when?	348	Yes, definitely	246 (70.68)
		Yes, to some extent	94 (27.01)
		No	8 (2.29)
Question 12 What would you most like to improve in this Outpatients Department?	348	Waiting time in clinic	110 (30.60)
		Time spent with doctor	58 (16.66)
		Seeing different doctors each time	40 (11.49)
		Quality of care	30 (8.62)
		No changes needed	110 (31.60)

demand) is the main priority of all parties (patient, healthcare provider, managers, and ministry), organizing and running an effective outpatient clinic could face a major challenge. On this ground more and more clinicians are trying to come up with a solution to improve patient satisfaction by not compromising managerial and financial targets at the same time.

Innovations and breakthroughs

Satisfaction with outpatient service (SWOPS) questionnaire is multi-dimensional outpatient instrument, was developed by the Health Services Research Centre at the Department of Psychology, Royal College of Surgeons in Ireland for use in Irish hospitals. The generic items of the questionnaire make up an overall dimension with an α co-efficient of 0.84. The high reliability co-efficient of each of these dimensions allows users to "select" questions whilst maintaining validity, providing a valid questionnaire for researchers and clinicians to audit their patient satisfaction and outcomes.

Applications

SWOPS questionnaire can be used in any clinical setting from outpatient to inpatient and ward setting. Also it is a useful tool in the primary care to monitor quality of care.

Terminology

Patient satisfaction is the perception of the patient of one or more aspects of the received care, thus a tool for measuring quality of care.

Peer review

This is an interesting topic as outpatient department service is very important in the diagnostic, therapeutic and follow-up process of many urological pathologies.

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The topic covers general clinical diagnosis, differential diagnosis, pathological diagnosis, laboratory diagnosis, imaging diagnosis, endoscopic diagnosis, biotechnological diagnosis, functional diagnosis, and physical diagnosis; (4) Therapeutics Advances: The editorial board members are invited to write high-quality therapeutic advances in their field to help improve the therapeutic skills of readers. The topic covers medication therapy, psychotherapy, physical therapy, replacement therapy, interventional therapy, minimally invasive therapy, endoscopic therapy, transplantation therapy, and surgical therapy; (5) Field of Vision: The editorial board members are invited to write commentaries on classic articles, hot topic articles, or latest articles to keep readers at the forefront of research and increase their levels of clinical research. 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6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

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9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

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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

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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