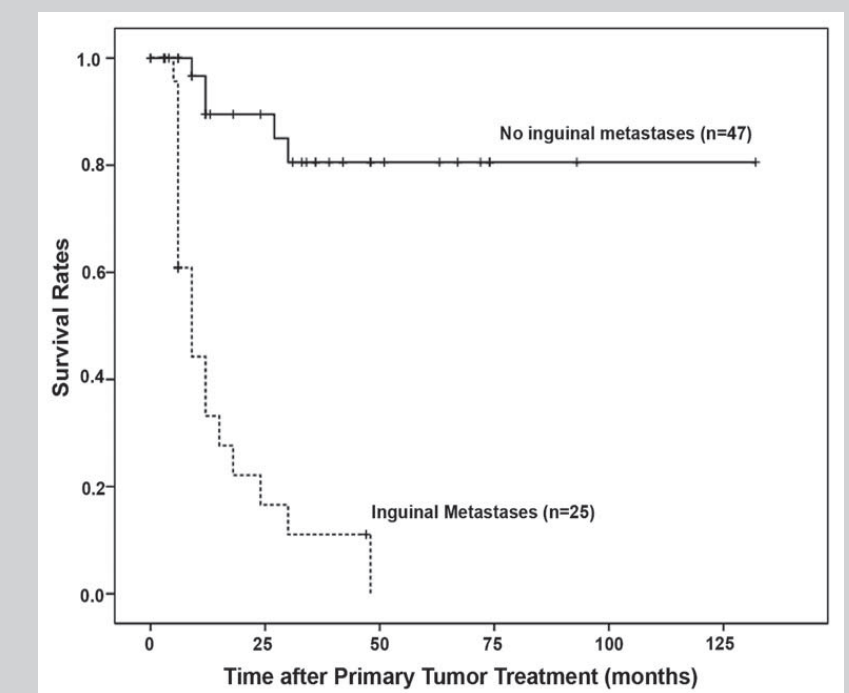
ISSN 1677-5538

# International

# Braz J Urol

Official Journal of the Brazilian Society of Urology
Official Journal of the Confederación Americana de Urologia
Official Journal of the Thai Urological Association
Volume 34, Number 4, July - August, 2008




*Respective 10-year survival rates according to the presence (n = 25) or absence of inguinal metastases (n = 47). Kaplan-Meier method (p < 0.0001, log rank test (Page 467).*

XXXI Brazilian Congress of Urology
November 7 - 11, 2009 - Goiânia - GO - Brazil

# International Braz J Urol

## EDITOR'S COMMENT

### Cryoablation for Clinically Localized Prostate Cancer

The July – August 2008 issue of the International Braz J Urol presents interesting contributions from different countries, and the editor's comment highlights some papers.

Doctor DiBlasio and co-workers, from University of Tennessee, Memphis, USA, evaluated on page 443 the erectile function (EF) and the voiding function following primary targeted cryoablation of the prostate (TCAP) for clinically localized prostate cancer. The authors retrospectively reviewed all patients treated between 2/2000 and 5/2006 with primary TCAP. Variables included age, Gleason sum, pre-TCAP prostate specific antigen (PSA), prostate volume, clinical stage, pre-TCAP hormonal ablation, pre-TCAP EF and American Urologic Association Symptom Score (AUASS). After exclusions, 78 consecutive patients were analyzed with a mean age of 69.2 years and follow-up 39.8 months. Stable voiding function was observed post-TCAP, with an overall incontinence rate of 7.7%. Although erectile dysfunction is common following TCAP, 25.7% of previously potent patients demonstrated erections suitable for intercourse.

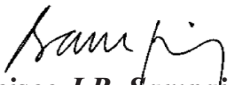
Doctor Kohler and colleagues, from Southern Illinois University, Springfield, IL, USA, evaluated on page 451 the length of the urethra in 109 men with normal genitourinary anatomy undergoing either Foley catheter removal or standard cystoscopy. The authors found the mean urethral length of 22.3 cm with a standard deviation of 2.4 cm. Urethral length varied between 15 cm and 29 cm. No statistically significant correlation was found between urethral length and height, weight, body mass index (BMI), or age. This data adds to basic anatomic information of the male urethra and may be used to optimize genitourinary device design. Dr. K. A. Hutton, from University Hospital of Wales, UK, Dr. Benjamin K. Canales, from University of Florida, Gainesville, FL, USA and Dr. M. M. Koraitim from University of Alexandria, Egypt, provided editorial comments to this paper.

Doctor Zimmermann and collaborators from Universities of Vienna and Innsbruck, and from University of Tuebingen, Tuebingen, Germany, determined on page 457 the expression of the cytokines transforming growth factor-β1 (TGF-β1), interferon-γ (IFN-γ), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) in serum from patients with Peyronie's disease (PD) compared to healthy controls. Ninety-one consecutive PD patients aged 20 - 74 years were included in this study. All patients were diagnosed with symptomatic PD for the first time and had a palpable penile plaque. The authors concluded that the significantly elevated serum level of the profibrotic TGF-β1 cytokine found in this study, underscores the effect of cytokines in the pathophysiology of PD. The significantly decreased TNF-α serum level suggested no acute immunomodulatory process. Therefore, the relevance for therapeutic administration of TNF-α should be further investigated. Quantification of TGF-β1 in serum of PD patients provides a possible diagnostic tool and target for therapy. The data on altered cytokine levels in PD patients also provide a new understanding for etiopathogenesis

of PD, which warrants further investigation. Doctor Joaquim Claro, from University of Sao Paulo, Brazil, provided an editorial comment to this article.

Doctor Scheiner and co-workers, from the National Cancer Institute Rio de Janeiro, Brazil, determined on page 467 the prevalence of human papillomavirus (HPV) DNA in penile cancers in Rio de Janeiro, Brazil. They studied prospectively, 80 consecutive cases of patients with penile cancers who underwent surgical treatment. The parameters observed were the presence or absence of HPV DNA viral type, histological subtypes, clinical stage and overall survival. The authors found HPV DNA in 75% of patients with invasive carcinomas and in 50% of patients with verrucous carcinomas. High risk HPVs were detected in 15 of 54 (27.8%) patients with HPV positive invasive tumors and in 1 of 4 (25%) patients with HPV positive verrucous tumors. HPV 16 was the most frequent type observed. No correlation was observed between HPV status and histological subtype ($p = 0.51$) as well as HPV status and stage stratification ($p = 0.88$). The authors concluded that HPV infection may have contributed to malignant transformation in a large proportion of penile cancer cases but only inguinal metastasis was a prognostic factor for survival in these patients. Dr. P. K. Hegarty from University College Hospital London, UK and Dr. David M. Prowse from the John Vane Science Centre, London, UK, provided editorial comments on this paper.

Doctor Barros and co-workers, from Cleveland Clinic Foundation, Cleveland, Ohio, USA, reported on page 413 their laparoscopic experience with simultaneous laparoscopic radical cystectomy (LRC) and nephroureterectomy. Between August 2000 and June 2007, 8 patients underwent simultaneous laparoscopic radical nephroureterectomy (LNU) (unilateral-6, bilateral-2) and radical cystectomy at their institution. Demographic data, pathologic features, surgical technique and outcomes were retrospectively analyzed. Median estimated blood loss and hospital stay were 755 mL (range 300-2000) and 7.5 days (range 4-90), respectively. There were no intraoperative complications but only 1 major and 2 minor postoperative complications. The overall and cancer specific survival rates were 37.5% and 87.5% respectively at a median follow-up of 9 months (range 1-45). The authors concluded that laparoscopic nephroureterectomy with concomitant cystectomy is technically feasible. Greater number of patients with a longer follow-up is required to confirm our results. Dr. Jose Colombo and Dr. Anuar I. Mitre, from University of Sao Paulo, Brazil, provided an editorial comment to this paper.



***Francisco J.B. Sampaio, M.D.***
Editor-in-Chief

Review Article

International Braz J Urol Vol. 34 (4): 401-412, July - August, 2008

# Difficult Male Urethral Catheterization: A Review of Different Approaches

**Carlos Villanueva, George P. Hemstreet III**

*Section of Urology, University of Nebraska Medical Center, Omaha, Nebraska, USA*

**ABSTRACT**

*Purpose:* To review and compare the different methods for difficult male urethral catheterization described in selected literature.

*Materials and Methods:* A PubMed search was done with the terms "difficult", "failed", or "complications" and "urethral catheterization", "transurethral catheterization", "Foley catheter", "urethral catheter" or "filiforms and followers". All articles addressing the issue of difficult adult male urethral catheterization were included.

*Results:* Six main approaches were identified on the 14 articles included for review: 1) Passage of either a Glidewire, guide wire or filiform under direct vision; 2) Blind passage of a filiform, guide wire, Glidewire or hydrophilic catheter; 3) "The Peel-away® sheath placed on a cystoscope/resectoscope technique"; 4) "The rigid ureteroscope placed inside the 22F Foley technique"; 5) Suprapubic catheterization; and 6) "The instillation of 60 cc of saline through the catheter as it is advanced technique".

*Conclusion:* There is a paucity of prospective data comparing the benefits, risks, success rates and complications of the different approaches for difficult Foley catheter placement. Our suggested approach starts with the initial attempt at urethral catheterization with an 18F coude and a 12F silicone catheter. If these fail, using a flexible cystoscope or the blind Glidewire technique are reasonable alternatives. If dilatation of a stricture is necessary, ureteric dilatators or a urethral balloon dilatator are recommended.

***Key words:*** *urethra; male; catheterization*

***Int Braz J Urol. 2008; 34: 401-12***

## INTRODUCTION

Difficult male urethral catheterization (DUC) is a common problem for the general urologist. Common causes of DUC in normal urethras include a tight external sphincter in an anxious patient, or poor technique. Additional pathologic causes include urethral strictures, phimosis, anasarca, bladder neck contractures, prostate cancer, false passages or benign prostatic hypertrophy (BPH), among others (Figure-1). It is underappreciated that a DUC can result in serious morbidity to the patient. The significance of this problem is exemplified by the complications from difficult catheterizations that include Fournier's gangrene (1), rectal perforation, bleeding requiring transfusion, formation of urethral strictures and sepsis.

This article reviews the alternative methods of approaching the difficult-to-catheterize patient described in the literature.

## MATERIALS AND METHODS

A PubMed search was programmed with the terms "difficult", "failed", or "complications"

and "urethral catheterization", "transurethral catheterization", "Foley catheter", "urethral catheter" or "filiforms and followers" in April of 2008. All articles addressing the issue of difficult adult male urethral catheterization were included. A DUC was defined for the purpose of this article as being unsuccessful at urethral catheterization of the bladder after the initial attempts.

## RESULTS

A summary of the different techniques for urethral catheterization after an unsuccessful attempt is shown in Table-1. Following is a summary of the articles included for review in the chronological order in which they were published. Each paragraph starts with a brief description of the technique. The following information from each article was included when available: risks and benefits of the technique mentioned in the article, complications, success rate, and patient outcomes. All of the statements in each paragraph are derived from the article being discussed.

In 1976, Walden (2) published a technique for DUC in the patient with anasarca using a vaginal speculum. The speculum is passed through the preputial opening down to the glans, and with the use of a long-handled forceps the catheter is advanced into the bladder. He used it in 3 patients, one of whom was a 450-pound (220 kg.) man in which the glans was 11 cm into the swollen prepuce.

Jordan et al. (3) addressed the issue of consultation for DUC in the operating room in 1985. After a through history of previous attempts, past medical history and genitourinary examination, if there are no contraindications to proceed with urethral catheterizations, the first step is the instillation of 20-30 mL of 2% lidocaine jelly or other water-soluble lubricating jelly into the urethra. The authors advocated injecting a large volume of jelly for gentle dilation. A 16-18F coude catheter is preferred over a small catheter, which may not be stiff enough to maneuver the difficult urethra. If this is unsuccessful, proceed with either urethroscopy or urethrography. The information gathered from direct or indirect visualization of the urethra would then lead to either suprapubic catheter placement or passage of filiforms. When to proceed with suprapubic catheterization versus the placement of filiforms was not discussed in their article. The passage of a filiform through the point of obstruction should preferentially be done under direct vision. For the blind passage of filiforms it is imperative not to remove the filiform that meets obstruction but to continue placing filiforms until one passes the point of obstruction. When using filiforms and followers it is recommended to dilate only up to 16-18 F and no larger in order to prevent further damage to the urethra. Finally, a Council tip catheter is advanced over a stylet attached to the filiform. Alternatively, a balloon urethral dilator system that can be passed using a filiform could be used instead. Without supporting data, the authors stated that this balloon system may be better than the use of followers. No specific data regarding success rates or complications was reported in the study.

Krikler (4) described for the first time in 1989 the use of flexible urethroscopy for DUCs. "The cystoscope is negotiated into the bladder, a guidewire is passed through the cystoscope, and the instrument withdrawn leaving the guidewire in place. A ureteric catheter may be passed over the guidewire first and then the tip of a suitable Foley catheter is trimmed to produce an end hole. This allows the catheter to be threaded down the guidewire". He did not make any particular recommendations in the case where a stricture prevented the passage of the flexible cystoscope. This method was recommended for patients in whom suprapubic catheterization is contraindicated or who are known to have false passages or urethral diverticula.

Lowe et al. (5) in 1992 discussed the management of the DUC in patients with multiple false passages, who had just a difficult dilatation with filiforms and followers, in the case of undermining of the bladder neck after trans-urethral resection of the prostate (TURP) or in the early postoperative period after a radical prostatectomy when the catheter came out. The authors described the use of a specially made urethral protective sheath that can be placed on a cystoscope or resectoscope to facilitate guiding the catheter into the bladder. A Peel-Away® Sheath (Cook Urological, Spencer, IN) is placed around the cystoscope or resectoscope at the beginning of the case. Once the

case is completed, the cystoscope/resectoscope is placed in the bladder and then it is removed leaving the sheath in place. A Foley catheter (with or without a catheter guide) is advanced through the sheath into the bladder, the balloon inflated, and the sheath peeled away. In the case of an unexpectedly difficult cystourethroscopy, once in the bladder, a guide wire can be passed, the cystoscope removed and then reinserted over the wire after placing a Peel-Away® sheath over the scope. The main problem encountered with this technique was kinking of the sheath, which can be prevented by not pulling up or downward on the phallus after the cystoscope has been removed. Kinking of the sheath can also be approached by the use of a catheter guide. This technique was used in 20 difficult catheterizations, with 3 failures, 2 due to kinking of the sheath and one because of the development of an erection making the sheath not long enough to reach the bladder. The authors hypothesized that the Teflon sheaths could be less traumatic to the urethra than the resectoscope sheath and that the use of the sheaths over the resectoscope during a TURP may decrease the post-TURP stricture rate although no data was provided to support this statement.

Cancio et al. (6), in 1993, described a series of initial maneuvers when managing the difficult to catheterize patient. "Start with the injection of 10-20 mL of lubricant in the urethra and use a 16-18F catheter first. The use of 1% lidocaine jelly as the lubricant makes the procedure more tolerable for the patient and may prevent sphincter spasm. When a stricture is suspected, either because blockage is encountered soon after entering the meatus or because of a history of instrumentation (e.g., TURP), use a smaller catheter (14-16F). If a 14F catheter will not pass, a smaller catheter will not pass either. For the patient with suspected BPH, use a larger catheter (20-24F). Perineal pressure by an assistant during catheter insertion can help direct the catheter into the prostatic urethra. Coude catheters were recommended for suspected BPH and to prevent injury to the membranous and bulbar urethra.

Beaghler et al. (7) method was described in 1994. This method incorporates the use of a 16F flexible cystoscope, after injecting 2% lidocaine jelly, to pass a 0.038-inch standard guide wire through the obstruction. The urethra is then dilated over the wire with Nottingham dilators 6-12F and 12-18F, and successful catheterization is accomplished by placing a 16F council catheter over the wire. This method was attempted in 54 patients prospectively with a success rate of 96%. These patients were seen if urology consultation was requested because of difficulty in placing a Foley or for complaints of weak urinary stream and urinary retention (26% outpatient clinics, 33% bedside/Intensive care unit, 13% emergency room, 28% operating room). Before attempting the method, catheterization was attempted by standard bedside techniques, including use of various catheters sizes and Coude-tipped catheters. The number of catheters, sizes and types was not specified in the paper, neither was the success rate with these initial maneuvers. The 2 patients in whom this method failed had dense bladder-neck contractures and had a suprapubic catheter placed after a failed attempt at dilating with filiforms and followers. The authors described no complications with little or no discomfort to the patient. The most common causes for difficult Foley catheter placements in this cohort (in order of most to least common) were urethral strictures, bladder-neck contractures, false passages, and locally-advanced prostate cancer.

In 1995, Blitz (8) described a method used in 8 patients that had endoscopic prostate or urethral surgery in which catheters were placed with prior difficulty. With the cystoscope in the bladder, a 0.038 stiff hydrophilic Glidewire is introduced and allowed to coil inside the bladder. Then a "16 G IV catheter with a needle is passed into the distal drainage hole of the urethral catheter and out through the center of the urethral catheter". The needle is then removed and the wire passed through the catheter. After removing the IV catheter, the wire is directed inside the drainage lumen of the urethral catheter. This maneuver avoided the need for a Council tip catheter, and was better than the alternative of cutting the tip of the Foley with scissors affecting the curvature of the tip of the catheter. A variety of urethral catheters can be used with this method. This approach was successful in all 8 patients. Five patients had just undergone a TURP,, 2 patients were status/post direct vision internal urethrotomy, and 1 patient was status/post laser prostatectomy. They stated that their experiences with other wires, including Teflon-coated spiral wound

***Table 1*** *– Techniques for difficult urethral catheterizations.*

| Author/Year | Clinical Scenario | Brief Description of the Method |
|---|---|---|
| Walden 1979 (2) | Difficult urethral catheterization secondary to anasarca | Use of a vaginal speculum to visualize the glans. Use of a long forceps to advance the urethral catheter. |
| Jordan et al. 1985 (3) | Intraoperative urology consultation for difficult urethral catheterization | Initial use of 20-30 mL of lidocaine jelly and 16-18F coude. Urethrography or urethroscopy to determine if a suprapubic catheter should be placed. Filiforms and followers. Council-type catheter with a stylet attached to the filiform. |
| Krikler 1989 (4) | Difficult urethral catheterization | Flexible cystoscope negotiated into the bladder. Guide wire advanced through the cystoscope into the bladder and the cystoscope removed. Ureteral catheter advanced over the wire. Foley catheter with the tip trimmed advanced over the guide wire. |
| Lowe et al. 1992 (5) | Traumatized urethra with false channels, post-transurethral resection of prostate undermined bladder neck, and loss of catheter early after radical retropubic prostatectomy. | Peel-Away® sheath placed on resectoscope or cystoscope. Scope advanced into the bladder. Scope removed leaving sheath in the urethra. Advance Foley through sheath into the bladder. Peel-away the sheath. |
| Cancio et al. 1993 (6) | Initial attempts at urethral catheterization | Use of 10-20 mL of lidocaine jelly. Large-caliber catheter in patient with benign prostatic hyperplasia (20-24F), small-caliber catheter in patients with stricture (14-16F). Use of coude catheters in males. Use of perineal pressure applied by an assistant. |
| Beaghler et al. 1994 (7) | Urology consultations for weak urinary stream or patient with prior attempts at catheter placement in the emergency department, operating room, outpatient clinics and intensive care unit/bedside. | Advancement of a 0.038 guide wire through the cystoscope under direct vision past the area of obstruction. Sequential dilation with Nottingham dilators, first from 6 to 12F, then from 12 to 18F. Placement of 16 F Council-type catheter over the guide wire. |
| Blitz 1995 (8) | Difficult urethral catheterization | Cystoscope inserted into the bladder. Stiff hydrophilic guide wire passed through the cystoscope into the bladder. Urethral catheter with a hole on the tip made with an IV catheter advanced over the guide wire into the bladder. |

***Table 1*** *– Techniques for difficult urethral catheterizations. - continued -*

| | | |
|---|---|---|
| Freid and Smith 1996 (9) | Inability to pass a Foley catheter in cases in which direct visualization urethroscopy is not immediately available | Glidewire is advanced into the bladder. 7 Fr open ended ureteral catheter is advanced over the Glidewire and the Glidewire removed. Placement of a 0.038 inch Teflon coated guide wire through the ureteral catheter. 18F Graham catheter advanced over the ureteral catheter/guide wire unit. Alternatively, dilate to 16-18F followed by the placement of a 16F Council-type catheter. |
| Harkin et al. 1998 (10) | One unsuccessful attempt at urethral catheterization in the absence of major urethral trauma. | Catheter tip syringe with 60 cc of saline is attached to the Foley catheter. The catheter is introduced into the urethra 2-3 cm from the point of obstruction and advanced while simultaneously the syringe of fluid is briskly instilled into the urethra. |
| Rozanski et al. 1998 (11) | Difficult urethral catheterization in the setting of undermining of the bladder trigone after transurethral incision of the prostate or transurethral resection of the prostate. | Short rigid ureteroscope passed through a 22F Foley catheter modified with a catheter punch device. Unit advanced into the bladder under direct vision. Foley secured in place while removing ureteroscope leaving Foley behind. |
| Lachat et al. 2000 (12) | Intraoperative difficulties with transurethral catheterization | 30 cm 0.035 inch J guide wire advance into the bladder. 6f-2L central line or 6F pediatric catheter with the tip cut advanced into the bladder over the wire. |
| Zammit and German 2004 (13) | Patients that failed an initial attempt at urethral catheterization with a 16F Foley catheter. | Hydrophilic guide wire advanced into the bladder. 16F Foley with a hole made in the tip with an IV catheter advanced over the guide wire. Alternatively, placement of a 6F ureteral catheter over the guide wire, followed by advancement of a graduated 6 to 12F semi-rigid ureteral dilator, and then a 12F Foley catheter with a hole made on the tip with an IV Catheter. |
| Athanassopoulos et al. 2005 (14) | Urethral strictures | Straight flexi-tip 0.09 mm hydrophilic guide wire and a 14/16F ureteric access sheath. |
| Mistry et al. 2007 (15) | Patients in acute urinary retention after failed attempt at urethral catheterization with a 12F and a 18F urethral catheters. | Passage of a 12F or 18F hydrophilic catheter. Guide wire passed through the catheter into the bladder and the catheter removed. Advance Council-type balloon retention catheter into the bladder. |
| Chelladurai et al. 2008 (16) | Urinary retention and urethral stricture disease | Negotiate guide wire past stricture under direct vision with a flexible cystoscope. Use serial ureteric dilators over the guide wire. Advance catheter over the guide wire |

guide wires, do not allow such ease of advancement and might cause the catheter to buckle as it is pushed into the bladder. Ex vivo experimentation comparing the Microvasive® Glidewire (Microvasive, Natick, MA) with a Microvasive Lubriglide hydrophilic-coated spiral wound guide wire was nearly equivalent in a subjective evaluation.

Freid and Smith (9) in 1996 described for the first time the use of a 0.038 inch hydrophilic Glidewire (preferentially with an angled or floppy tip) placed blindly into the bladder in a manner similar to a filiform for cases of DUC. The Glidewire is prepared by injecting 5 cc of saline to activate the hydrophilic coating. Lidocaine jelly is then injected in the urethra, followed by the introduction and advancement of the Glidewire with a gentle steady pressure using a gauze pad to grasp it. When resistance is felt, the Glidewire is advanced until either it enters the bladder or the tip appears in the meatus. In the latter situation, the Glidewire is removed and another attempt is made at passing it into the bladder. Entrance into the bladder is inferred by passage of approximately 75 cm of the Glidewire in the urethra without the reappearance of the tip or a coil at the meatus. Then a 7F ureteral catheter (preferentially with a tapered tip) is threaded over the wire and then, following documented urine return corroborating correct placement, the first wire is exchanged for a 0.038 PTFE coated guidewire. An attempt is made to introduce an 18F Graham catheter over the guide wire/ureteral catheter unit, or alternatively the urethra is dilated to 16-18F and a 16F Council catheter is advanced over the wire. The authors recommended this method over standard filiforms and followers when cystoscopy was not immediately available. This method was used most frequently after failed attempts with filiforms and followers. The reported success rate was 95% (19/20). This method failed in a patient who had a pinhole urethral stricture that necessitated cystourethroscopy with direct vision internal urethrotomy. The authors reported no complications. The most common causes of the difficulty in placing the catheter were, in order of most to least common: urethral strictures, bladder neck contracture, BPH and unknown. It is unclear how the causes were determined since it was not stated that the patients underwent cystoscopy or other studies.

Harkin et al. (10), in 1998, introduced an entirely different technique that did not involve wires, cystoscopes, IV catheters, or dilators. Their method consists of connecting a catheter tip syringe loaded with 60 mL of saline to the Foley, inserting the Foley up to where resistance is encountered, and then advancing the Foley while at the same time briskly instilling saline into the distal urethra. It was hypothesized that the flow of fluid distended the urethra facilitating the passage of the catheter, particularly in cases of BPH. The authors recommended using this technique after an unsuccessful attempt at urethral catheterization in the absence of any signs of major urethral trauma. A 100% success rate was reported in over 30 patients. The causes for the difficulty in catheterizing these patients were not mentioned in the study. They recommended aborting the procedure if severe pain or major resistance were encountered.

Rozanski et al. (11) in 1998 described a technique used in 2 patients with significant undermining of the trigone after transurethral incision of the prostate. In these patients a wire was introduced into the bladder (into a barely visible opening at the 12 o'clock position) and multiple attempts to place a Council catheter over the wire failed. Using a 22F Foley with a punch hole at the tip, they inserted a 6 F ureteroscope into the Foley with the tip of the ureteroscope several millimeters beyond the catheter tip, and inserted the ureteroscope and the catheter into the bladder under direct vision. The Foley was grasped securely and the ureteroscope was pulled out. They recommended the use of this technique whenever catheterization is difficult or potentially complicated following transurethral surgery.

Lachat et al. (12) in the year 2000 described a technique for the intraoperative DUC. It consists of advancing a 30 cm 0.035 inch J guide wire through the urethra into the bladder, followed by either a 6F-2L central line or a 6F pediatric catheter with the tip cut off advanced over the wire. This method was used with success in 21 patients undergoing cardiovascular surgery in which difficulties with transurethral catheterization were encountered. In 5 patients, 2 or more attempts were required to advance the wire into the bladder. Fluoroscopic guidance was recommended for placement of the wire when a false passage was suspected.

In 2004, Zammit and German (13) presented a method suggesting the blind passage of a Glidewire (as described by Freid and Smith (9)) as well as the use of an 18 G IV catheter to perforate the tip of the urethral catheter (as described by Blitz (8)). With this method a 0.89 mm Terumo Radiofocus® (Terumo Corp., Tokyo, Japan) Guide Wire M Straight is blindly inserted into the bladder by advancing at least 40 cm of the wire into the urethra (with the urethra being an average of 20 cm, this assures that the wire did not double back on itself). Subsequently an attempt is made to thread a 16F urethral catheter over the wire (tip perforation according to the technique described by Blitz (8)). If this is unsuccessful, the possibility of a urethral stricture is considered and a 6 F ureteral catheter is inserted. If there are no difficulties inserting the ureteral catheter this is removed and a graduated 6F to 12F semi-rigid ureteric dilator (flexible ureteroscopy introducer) is advanced over the wire into the bladder. This allows the insertion of a 12 F urethral catheter over the guide wire using the method described by Blitz. The authors recommended aborting the procedure if there were any difficulties when inserting the 6F ureteral catheter. The success rate was not reported. However, it was mentioned that patients tolerated the procedure well, and that the cases in which this technique were not successful was due to the creation of a false passage at the initial catheterization attempt. Of note, this method was applied after a failed attempt at urethral catheterization with a 16F catheter.

In a letter to Zammit and German (13) Athanassopoulos et al. (14) mentions their unpublished experience using a straight flexi tip hydrophilic Glidewire and a 14/16F ureteric access sheath. They extrapolated the efficacy and atraumatic characteristics of hydrophilic coatings in the ureteric lumen to the urethra. They proposed that "the development of larger diameter hydrophilic sheaths may lead to a totally atraumatic one-step management of urethral strictures".

More recently, Mistry et al. (15) reported in 2007 their experience with the use of hydrophilic-coated urethral catheters in adult males in acute urinary retention with DUC. Criteria for enrollment included a failed attempt at passage of both a 12F and 18F urethral catheters. Preparation of the hydrophilic catheter consisted of immersion for 5 min in sterile water and modification of the tip to convert it into a Council-type catheter. An attempt to pass a 12F or 18F hydrophilic catheter per urethra was made by the urologist. If the hydrophilic catheter successfully passed into the bladder, a guide wire was subsequently passed through the catheter. The hydrophilic catheter was then removed leaving the wire in place, and an attempt to place a standard balloon retention catheter over the guide wire into the bladder was made. If either the hydrophilic catheter did not pass or the balloon retention catheter could not be advanced over the wire, additional intervention was left to the discretion of the physician. Of 44 patients enrolled in the study, 30 (68.2%) underwent successful placement of an indwelling Foley catheter with the use of this method (hydrophilic catheter followed by guide wire followed by balloon retention catheter). The 12F hydrophilic catheter was used to gain access into the bladder in 26 patients and an 18F hydrophilic catheter was used in 4 patients. The patients, in which this method was not successful, underwent flexible cystoscopy, dilatation with filiforms and followers or Amplatz dilators, and/or blind passage of an open ended catheter with subsequent Foley placement. In the 13 patients that underwent cystoscopy the causes for the difficulty in catheterizing were anterior urethral stricture in 7, bladder neck contracture in 3 and false passage in 3. They postulated the success of the 12F hydrophilic catheter may be related to its increased stiffness when compared to the regular 12F latex catheter. In 4 patients, the 12F hydrophilic catheter was inserted into the bladder with subsequent failure to pass the balloon retention catheter over the guide wire. In these patients, benefits were still realized (temporary drainage of painful retention, access to the bladder with a guide wire etc.). The authors concluded that incorporating hydrophilic catheters into the urologic armamentarium for catheterizing the difficult urethra will benefit most patients by avoiding more invasive and costly procedures.

Chelladurai et al. (16) recommend using their technique for patients in urinary retention with urethral stricture disease. Under direct vision with a flexible cystoscope, a guide wire is negotiated past the stricture into the bladder. Well lubricated serial ureteric dilators are introduced over the guide wire into

the bladder. A catheter is then inserted over the guide wire. Delayed definitive treatment of the stricture is undertaken under optimal conditions. They recommend using ureteral over urethral dilators because their narrower caliber, hydrophilic coating and longer length ensure an easier dilatation of the stricture with minimal patient discomfort.

## COMMENTS

The approach to the DUC in the non-trauma setting, where a catheter is needed for urinary retention or to monitor urine output, should start with a through history and physical examination. The history should include, past urologic surgeries (TURP, Radical retropubic prostatectomy), previous difficulties with catheterization, and voiding symptoms, as a clue to the etiology of the problem. Historical information pertaining to previous attempts by nurses or physicians is of utmost importance: the distance at which obstruction was felt (< 16 cm indicating possible urethral stricture, > 16 cm indicating BPH, incorrect technique, or bladder neck contracture), whether the Foley balloon was inflated before urine flow (alerting of possible false passage from urethral trauma) or the types and sizes of catheters used, as well as how many people who previously tried (the higher the number that tried could indicate a more difficult catheterization). A focused genitourinary exam may reveal obvious causes for the difficult catheterization penoscrotal edema, phimosis, meatal stenosis, prostate cancer etc.

If a DUC is anticipated based on the above information, we recommend preparing the field by scrubbing the penoscrotal area with an antiseptic solution and placing a ¾ sheet drape from the scrotum down to the toes. This allows both hands to remain sterile during the procedure to assist in the passage of instruments, and prevents contamination of guide wires, flexible cystoscopes etc. The use of at least 10 cc of a lubricant injected into the urethra should be standard technique. The average volume of the male urethra is 20 mL, suggesting 20 mL may theoretically be better than 10 mL, but this has not been reported in the setting of DUC. Although most urologists use 2% viscous lidocaine, its efficacy as an anesthetic was recently questioned in a meta-analysis in which no difference was found when it was compared to plain gel (17). Despite this, patients are familiar with the drug lidocaine and may derive some placebo effect if they know that the drug is being used to numb their urethras. A tense patient usually means a tight urethral sphincter; therefore, patients should be encouraged to relax their legs by pointing their toes outward, while taking slow deep breaths. A firm grasp of the penis with the non-dominant hand, preferentially with gauze around it, pointing at a 45 degree angle is a key element of urethral catheterization. We recommend detaching the catheter from the Foley bag to allow better sensitivity at detecting when passing through the external sphincter and prostate, and to more readily identify obstruction secondary to a false passage or stricture.

The type and size of catheter to be used initially is a detail that has not yet been reported in the setting of DUCs. In a recent online survey (December 2007) of US urology residents at out institution, revealed an 18F coude catheter was the first catheter used by the majority of residents for the DUC. With appropriate technique, an 18F coude catheter should be advanced with ease into the bladder of the majority of patients in which the difficulty was attributed to an incorrect technique, BPH, or an anxious patient. Only 2 of the articles reviewed mentioned the size / types of catheters employed during the initial attempts at catheterization. In the article by Zammit and German (5), one attempt with a 16F urethral catheter was performed before their method was applied. In the Mistry et al. (15) study, patients must have failed urethral catheterization with an 18F and a 12F urethral catheter as inclusion criteria. As illustrated in Figure-1, there is a high probability that if an 18F coude catheter could not be advanced into the bladder, a narrowing of the urethra (urethral stricture, bladder neck contracture) or a false passage is likely the cause of the problem. Since urethral strictures and bladder neck contractures are 5 to 6 times more common than false passages, we recommend another attempt at urethral catheterization with a smaller catheter. Silicone catheters are stiffer than the regular latex catheters and can theoretically provide an advantage when passing a stricture. We use a 12F silicone catheter after a failed attempt with an 18F coude catheter. The use of more than these 2

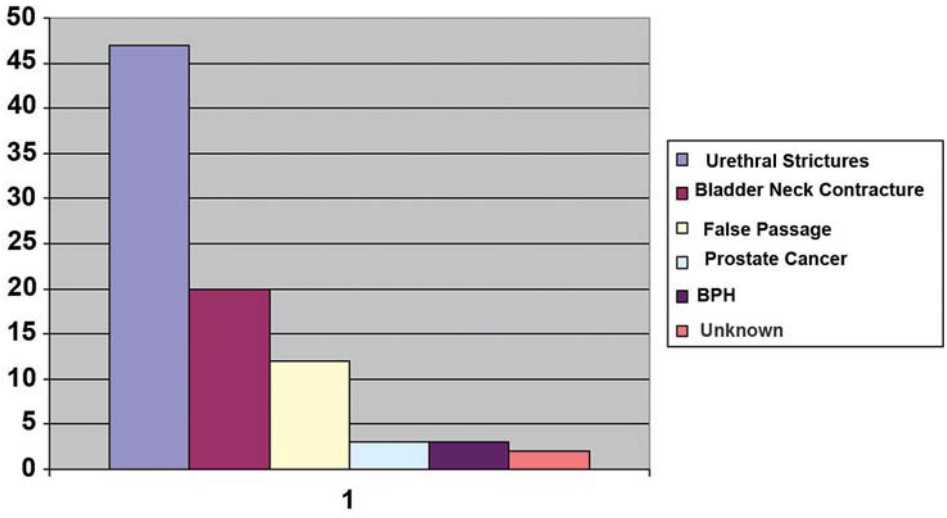



***Figure 1 –*** *Most common causes of difficult urethral catheterization*.*
** Pooled cases from Beaghler et al. (7), Freid and Smith (9) and Mistry et al. (15) series. Included are the 54 patient from Beaghler's series all of which underwent flexible cystoscopy, and the 13 patient in Mistry et al. series that underwent flexible cystoscopy. Twenty patients from Freid and Smith's series were also included, but it was not mentioned in the article how the cause of difficult urethral catheterization was found in these patients.*

catheters, in our opinion, is unlikely to increase the probability of a successful catheterization.

The technique of instilling 60 cc of saline as the catheter is advanced as described by Harkin et al. (10) is a simple one and probably could be attempted before proceeding with any of the other methods if BPH is suspected. This catheter instillation technique utilizes inexpensive and readily available supplies that can be found on any floor of the hospital or the ER. Although Harkin et al. (10) reported a success rate of 100% in over 30 patients; these authors do not delineate the causes for the difficulty in placing the Foley. It is intuitive that this method would not be successful in cases of urethral stricture, false passage, or bladder-neck contracture, the most frequent causes of DUC. Therefore, its use is limited to a small proportion of DUCs. Also, proper technique at urethral catheterization requires one hand to handle the phallus and the other to maneuver the catheter, which means that an assistant is required to push the saline. If the catheter is placed in a false passage and saline is forcefully injected, there is a high probability of making the false passage worse. For these reasons we do not advocate for the use of this technique.

Once a patient failed initial attempts at urethral catheterization, the articles reviewed recommend one of the following general approaches: 1) Passage of either a Glidewire, guide wire or filiform under direct vision (with the use of flexible or rigid cystoscopy) past a visible obstruction (i.e. stricture) or into the bladder, followed by the advancement of a modified urethral catheter over the Glidewire, filiform or guide wire, immediately or after dilatation with followers, ureteral dilators etc; 2) Blind passage of a filiform, guide wire, Glidewire or hydrophilic catheter(which is then exchanged for a guide wire) followed by the advancement of a modified urethral catheter over the Glidewire, filiform or guide wire, immediately or after dilatation with followers, ureteral dilators etc; 3) "The Peel-away® sheath placed on cystoscope/resectoscope technique"; 4) "The rigid ureteroscope placed inside the 22F Foley technique"; 5) Suprapubic catheterization; and 6) "The instillation of 60 cc of saline through the catheter as it is advanced technique". In our recent online survey of US urology residents to ascertain their approaches to the DUC, 60-70% used a flexible cystoscope, 15-20% chose to pass a Glidewire blindly, 7-9% used filiforms and followers blindly and

less than 3% elected a suprapubic catheter after their initial failed attempts at urethral catheterization.

The article by Jordan et al. (3) discusses the blind use of filiforms and followers. They recommend avoiding placing filiforms blindly but also provided recommendations regarding the technique. Freid and Smith (9) described why they stopped using filiforms in favor of their technique. The once popular blind placement of filiforms and followers was chosen only by 7-9% of urology residents in our recent online survey. Other alternative methods and equipment for dilating strictures like Heymans dilators, balloon dilators and ureteral access sheaths, have contributed to the abandonment of the use of filiforms and followers. To out knowledge there is no reported study comparing the use of filiforms and followers with other techniques used for DUC. Cases of perforation of the urethra and entrance into the rectum with followers are not unheard of. We do not consider the blind use of filiforms and followers to be the optimal approach to the DUC because it is difficult to ascertain if the filiform is in the bladder and requires considerable experience to gain this skill.

Several articles discussed above describe the blind placement of a Glidewire. The main advantage of this approach is that it avoids the cost and inconvenience of the use of a flexible cystoscope. Freid and Smith (9) reported a high success rate with no complications using this method. Insertion of a hydrophilic guide wire though the urethra will usually gain access to the bladder without causing any trauma and with minimal discomfort to the patient. If a Glidewire is placed into a false lumen or cannot transverse a stricture it will usually efface the urethral meatus. If most of the Glidewire has been advanced into the bladder without seeing it coming back at the urethral meatus, there is a high probability that access to the bladder was achieved. If there are still doubts about whether the wire is in the bladder or not, a 6F ureteral catheter can be advanced over the wire and urine aspirated or the catheter irrigated with a syringe. Rarely, a bedside plain film of the abdomen is needed to confirm wire placement. There are many types of Glidewires: rigid vs. regular, angled tip vs. straight tip. Freid and Smith's (9) technique used an angled or floppy tip. Zammit and German (13) used a straight tip. Which tip is better to maneuver strictures or a false passage has not been reported. In Mistry et al. (15) article, access to the bladder was gained first with a hydrophilic catheter followed by placement of a guide wire. This technique adds an additional step to the blind placement of a Glidewire. There are probably more chances of being successful at advancing a hydrophilic Glidewire into the bladder than a 12F or 18F hydrophilic catheter. One of the arguments in favor of using the hydrophilic catheter was that they can readily decompress the bladder in distressed patients in urinary retention. The passage of a Glidewire should not take more than a couple of minutes, and once the wire is in, passing a catheter over the wire should be quick in most situations (as it was in 30 of the 34 of their patients in which access to the bladder was achieved with a hydrophilic catheter in their study).

As the availability of flexible cystoscopes and catheterization carts has become commonplace in the hospital ward, the most frequent approach to the DUC among urology residents in the US is the use of a flexible cystoscope. This approach establishes the etiology of the problem in a majority of cases. In the case of false passages, where occasionally a Glidewire cannot be advanced blindly, the cystoscope can usually be maneuvered into the bladder. Guide wires can also be passed through pinpoint urethral strictures or bladder neck contractures under direct vision. The disadvantages of using this approach are that flexible cystoscopes are not always available, they are expensive, and they usually need to be transported in a cart because of the light source. No study has demonstrated the superiority of the use of flexible cystoscopy in the setting of DUC over other more simple techniques like the blind use of a Glidewire. Still, we believe that flexible cystoscopy is needed in a small percentage of DUCs (mainly in cases of false passages and some urethral strictures) if a suprapubic catheter is to be avoided.

The "Peel-away sheath® placed on cystoscope/resectoscope technique" and "The rigid ureteroscope placed inside the 22F Foley technique" are two maneuvers worth remembering that can be used in specific scenarios but probably cannot be used in the majority of consults for DUC. Urethral catheterization will not always be successful despite the use of all the techniques described and suprapubic catheterization will still be required in rare occasions.

In conclusion, there are many approaches to the DUC described in the literature. Prospective randomized trials comparing these approaches are needed to determine the strengths and weaknesses of each technique. The paucity of literature related to one of the most common urologic consults was surprising. Finally our recommended approach to the difficult male urethral catheterization in patients with urinary retention or that need a Foley catheter to monitor urine output is an initial attempt with an 18F coude catheter followed by a 12F silicone catheter. If this approach fails we suggest using the blind Glidewire technique or a flexible cystoscope to pass a Glidewire under direct vision as reasonable options. Once the Glidewire is confirmed to be in the bladder, a 16 F council catheter can usually be advanced into the bladder. In cases of urethral stricture disease, the primary approach would be to pass a 12F silicone catheter (using Blitz (8) technique) over the Glidewire without dilating, with a delayed definitive treatment of the stricture under optimal conditions. In cases of tight strictures in which a 12F did not pass or when treatment of the stricture is desired, serial ureteric dilators or a urethral balloon dilatator passed over the Glidewire are reasonable alternatives.

## CONFLICT OF INTEREST

None declared.

### REFERENCES


1. López Pacios JC, Sánchez Merino JM, Piñeiro Fernández MC, Bouso Montero M, Parra Muntaner L, García Alonso J. Fournier's gangrene secondary to urethral catheterization. Arch Esp Urol. 2005; 58: 167-70.
2. Walden TB: Urethral catheterization in anasarca. Urology. 1979; 13: 82.
3. Jordan GH, Winslow BH, Devine CJ Jr: Intraoperative consultation for the urethra. Urol Clin North Am. 1985; 12: 447-52.
4. Krikler SJ: Flexible urethroscopy: use in difficult male catheterisation. Ann R Coll Surg Engl. 1989; 71: 3.
5. Lowe MA, Defalco AJ: New endourologic technique for catheter placement after TURP, prostatectomy, and difficult urethroscopy. Urology. 1992; 40: 461-3.
6. Cancio LC, Sabanegh ES Jr, Thompson IM: Managing the Foley catheter. Am Fam Physician. 1993; 48: 829-36.
7. Beaghler M, Grasso M 3rd, Loisides P: Inability to pass a urethral catheter: the bedside role of the flexible cystoscope. Urology. 1994; 44: 268-70.
8. Blitz BF: A simple method using hydrophilic guide wires for the difficult urethral catheterization. Urology. 1995; 46: 99-100.
9. Freid RM, Smith AD: The Glidewire technique for overcoming urethral obstruction. J Urol. 1996; 156: 164-5.
10. Harkin DW, Hawe M, Pyper P: A novel technique for difficult male urethral catheterization. Br J Urol. 1998; 82: 752-3.
11. Rozanski TA, Salazar F, Thompson IM: Direct vision bladder catheterization using a short rigid ureteroscope. Urology. 1998; 51: 827-8.
12. Lachat ML, Moehrlen U, Bruetsch HP, Vogt PR: The Seldinger technique for difficult transurethral catheterization: a gentle alternative to suprapubic puncture. Br J Surg. 2000; 87: 1729-30.
13. Zammit PA, German K: The difficult urethral catheterization: use of a hydrophilic guidewire. BJU Int. 2004; 93: 883-4.
14. Athanassopoulos A, Liatsikos EN, Barbalias GA: The difficult urethral catheterization: use of a hydrophilic guidewire. BJU Int. 2005; 95: 192.
15. Mistry S, Goldfarb D, Roth DR: Use of hydrophilic-coated urethral catheters in management of acute urinary retention. Urology. 2007; 70: 25-7.
16. Chelladurai AJ, Srirangam SJ, Blades RA: A novel technique to aid urethral catheterisation in patients presenting with acute urinary retention due to urethral stricture disease. Ann R Coll Surg Engl. 2008; 90: 77-8.
17. Patel AR, Jones JS, Babineau D: Lidocaine 2% gel versus plain lubricating gel for pain reduction during flexible cystoscopy: a meta-analysis of prospective, randomized, controlled trials. J Urol. 2008; 179: 986-90.


*Accepted after revision:*
*June 1, 2008*

**Correspondence address:**
Dr. Carlos Villanueva
Section of Urologic Surgery
University of Nebraska Medical Center
855, N 82ND Plaza Apt 40
Omaha NE 68114, USA
Fax: + 1 402 990-3634
E-mail: cvillanu@unmc.edu

## EDITORIAL COMMENT

In this manuscript, the authors present a review of the literature on the management of difficult male catheterization. Using a limited PubMed search, they retrieved 6 papers on the topic. It is an "information only" type of paper, as it does not provide direct recommendations for clinical practice. However, the various methods proposed are interesting to the reader as well as the creative solutions surgeons have tried in the management of what is a huge problem for both the patient and the care staff.

***Dr. Katherine N. Moore***
*University of Alberta*
*Faculty of Nursing*
*Edmonton, Alberta, Canada*
*E-mail: katherine.moore@ualberta.ca*

## EDITORIAL COMMENT

Difficult male urethral catheterization is still a common problem for the general urologist. It can be a challenge for different reasons, such as urethral stricture, benign prostatic hypertrophy or cancer. It must be reminded that bladder neck contractures and obliterated anastomosis are still present following transurethral resection of the prostate, supra-pubic prostatectomy and radical prostatectomy, in spite of all attempts to avoid them. Unfortunately, the common scenario of "difficult" urethral catheterization after multiple attempts of non-urological stuff is still a part of our daily practice. Sometimes these "emergent calls" come from the emergency or operation room. These circumstances put our young colleagues in a stressful situation making their task more difficult. That is why it is particularly important to construct an algorithm for these situations.

A lot of possible techniques for difficult urethral catheterization have been described (1-4), however there are still no guidelines for practical purposes. The authors of this manuscript not only describe these methods, but also suggest which of them could be use in special situation. They must be encouraged for this attempt to construct a kind of guidelines for difficult urethral catheterization. I hope that this manuscript will be very popular among residents.

### REFERENCES

1. Chelladurai AJ, Srirangam SJ, Blades RA: A novel technique to aid urethral catheterisation in patients presenting with acute urinary retention due to urethral stricture disease. Ann R Coll Surg Engl. 2008; 90: 77-8.
2. Carr LK, Webster GD: Endoscopic management of the obliterated anastomosis following radical prostatectomy. J Urol. 1996; 156: 70-2.
3. Chiang DT, Dewan PA, Ben-Meir D: The difficult urethral catheterization: use of a hydrophilic guidewire. BJU Int. 2004; 94: 447-8.
4. Lachat ML, Moehrlen U, Bruetsch HP, Vogt PR: The Seldinger technique for difficult transurethral catheterization: a gentle alternative to suprapubic puncture. Br J Surg. 2000; 87: 1729-30.

***Dr. Sergey Kravchick***
*Department of Urology*
*Barzilai Medical Center*
*Ashkelon, Israel*
*E-mail: cambell@netvision.net.il*

Clinical Urology
International Braz J Urol Vol. 34 (4): 413-421, July - August, 2008

# Simultaneous Laparoscopic Nephroureterectomy and Cystectomy: A Preliminary Report

**Rodrigo Barros, Rodrigo Frota, Robert J. Stein , Burak Turna, Inderbir S. Gill, Mihir M. Desai**

*Section of Laparoscopic and Robotic Surgery, Glickman Urological Institute, Cleveland Clinic Foundation, Cleveland, Ohio, USA*

**ABSTRACT**

*Purpose:* Patients with muscle-invasive bladder cancer and concomitant upper urinary tract tumors may be candidates for simultaneous cystectomy and nephroureterectomy. Other clinical conditions such as dialysis-dependent end-stage renal disease and non-functioning kidney are also indications for simultaneous removal of the bladder and kidney. In the present study, we report our laparoscopic experience with simultaneous laparoscopic radical cystectomy (LRC) and nephroureterectomy.
*Materials and Methods:* Between August 2000 and June 2007, 8 patients underwent simultaneous laparoscopic radical nephroureterectomy (LNU) (unilateral-6, bilateral-2) and radical cystectomy at our institution. Demographic data, pathologic features, surgical technique and outcomes were retrospectively analyzed.
*Results:* The laparoscopic approach was technically successful in all 8 cases (7 males and 1 female) without the need for open conversion. Median total operative time, including LNU, LRC, pelvic lymphadenectomy and urinary diversion, was 9 hours (range 8-12). Median estimated blood loss and hospital stay were 755 mL (range 300-2000) and 7.5 days (range 4-90), respectively. There were no intraoperative complications but only 1 major and 2 minor postoperative complications. The overall and cancer specific survival rates were 37.5% and 87.5% respectively at a median follow-up of 9 months (range 1-45).
*Conclusions:* Laparoscopic nephroureterectomy with concomitant cystectomy is technically feasible. Greater number of patients with a longer follow-up is required to confirm our results.

***Key words:*** *kidney; ureter; laparoscopy; nephrectomy; cystectomy; TCC*
***Int Braz J Urol. 2008; 34: 413-21***

## INTRODUCTION

Transitional cell carcinoma (TCC) of the bladder is the sixth most common malignancy in the United States, accounting for 10% of cancers in men and 4% in women (1). While open radical cystectomy (ORC) and urinary diversion remain the gold standard for treatment of muscle-invasive TCC of the bladder, laparoscopic radical cystectomy (LRC) has been gaining popularity and presently the worldwide experience includes more than 500 cases (2). Treatment of bladder tumor may be complicated with concurrent upper tract disease. Palou et al. have reported a 1.8% incidence of simultaneous upper tract and bladder TCC, where 46% of the bladder tumors were found to be invasive (3).

Radical nephroureterectomy with bladder cuff excision is considered the standard of care for high-grade, invasive, recurrent, or large volume TCC of the upper urinary tract (UUT). Since the first descrip-

tion of laparoscopic nephroureterectomy (LNU) by Clayman et al. (4), several authors have demonstrated improved recovery with equivalent intermediate-term oncologic outcomes using the laparoscopic approach compared to open radical nephroureterectomy (5-8).

In patients with recurrent high grade or muscle invasive bladder TCC and concomitant UUT tumors, simultaneous cystectomy and nephroureterectomy is the principle oncologic procedure of choice (9-11). Other benign clinical conditions including dialysis-dependent end-stage renal disease (ESRD) or non-functioning kidney are relative indications for simultaneous upper unilateral or bilateral nephroureterectomy and lower tract extirpation (9). The aim of this report is to describe our experience with combined laparoscopic radical cystectomy and nephrouretererectomy.

## MATERIALS AND METHODS

Between August 2000 and June 2007, 8 patients underwent simultaneous laparoscopic radical cystectomy and nephroureterectomy at our institution. All procedures were performed by the same surgical team. Demographic data and pathologic features of the bladder and upper tract tumors were individually recorded. Perioperative outcomes, postoperative pathologic data and oncologic outcomes were retrospectively reviewed and analyzed.

Our surgical technique for the laparoscopic procedure is as follows. Initially, the patient is placed in 60-degree flank position for transperitoneal radical nephroureterectomy. Port placement is depicted in Figure-1 for nephroureterectomy and cystectomy. Notably, the primary port (12 mm) is inserted at the site of the proposed ileal conduit stoma for a right-sided nephroureterectomy or at the edge of the rectus muscle along a line between the umbilicus and anterior-superior iliac spine for a left-sided nephroureterectomy. During right-sided nephroureterectomy, this port will serve as the left hand port during the upper tract procedure and right hand port during the cystectomy. Conversely, for left-sided nephroureterectomy this port serves as the right hand port for the upper tract procedure and left hand port for the pelvic portion. On the right side, ports 2-5 are placed as for our standard nephroureterectomy. Notably, port 4 is used for an Allis clamp locked to the side wall as a self-retaining liver retractor and an instrument placed through port 5 is used for lateral retraction. Left-sided port placement mirrors the right except that a port for liver retraction is not needed. After port placement, transperitoneal LNU is performed in a standard manner and our detailed technique has been published previously (5). For subsequent cystectomy and bilateral lymph node dissection, the patient is re-positioned in a low lithotomy position with a full Trendelenburg tilt. The entire surgical field is re-prepared and re-draped for the lower urinary tract portion of the procedure. Laparoscopic radical cystectomy with bilateral limited or extended lymph node dissection is then performed as previously described (12,13). A 12 mm port site is incised vertically in the midline above the umbilicus to be used as the camera port (Port 6 depicted in Figure-1). Later, this port is extended periumbilically

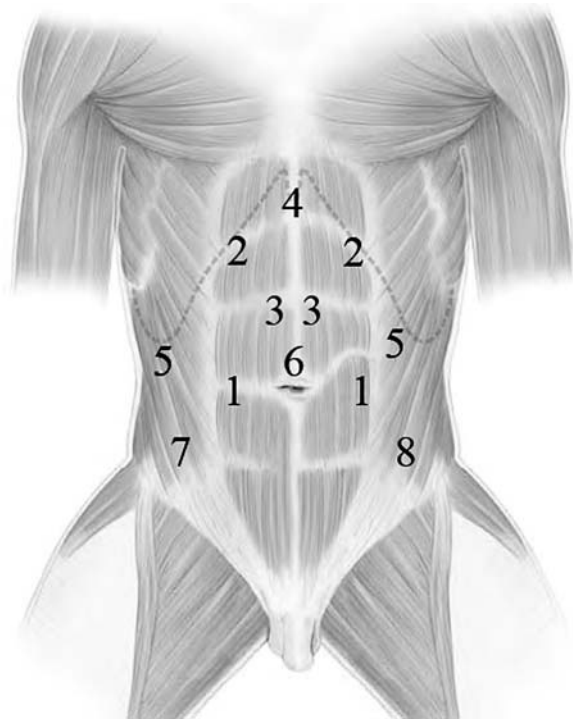



***Figure 1*** *– Illustration representing the port placement on simultaneous laparoscopic radical cystectomy and laparoscopic nephroureterectomy. Ports 1 to 5 are used for nephroureterectomy. Port 4 is used for liver retraction on the right side. Ports 6 to 8 are used for the pelvic component. Port site 1 also serves as the right and left hand ports for the pelvic portion.*

for intact specimen removal and performance of all bowel work including creation of the neobladder or ileal conduit as well as re-establishment of bowel continuity.

## RESULTS

A total of 8 patients (7 males and 1 female) with a median age of 76.5 years (range 65 to 79) underwent LNU and LRC with urinary diversion in the same session. The indication for upper tract surgery was synchronous TCC in 6 patients (unilateral nephroureterectomy) and end-stage renal disease in 2 patients (bilateral nephroureterectomy). Preoperatively, there was a previous history of muscle-invasive or recurrent superficial TCC of the bladder in all patients. Demographic data are detailed in Table-1. Seven (87.5%) patients were classified as ASA score ≥ 3. Of the patients, 7 (87.5%) underwent previous abdominal surgery.

Preoperative tumor characteristics are presented in Table-2. Six patients (75%) had a previous history of intravesical chemo/immunotherapy (mitomycin-2, BCG-4). Five patients (62.5%) presented with carcinoma in situ and 1 patient (12.5 %) had a positive urethral biopsy for tumor. Site of upper tract tumor in 6 patients (right-4, left-2) included ureter in 3, calyx in 1 and multiple locations in 2.

Median total operative time, which included LNU, LRC, pelvic lymph node dissection and urinary diversion, was 9 hours (range 8 to 12). Median estimated blood loss and hospital stay were 755 mL (range 300 to 2000) and 7.5 days (range 4 to 90), respectively. All 8 cases were technically successful without the need to open conversion. There were no intraoperative complications. Table-3 summarizes the intraoperative data.

Postoperatively, 2 (25%) patients had minor complications: prolonged ileus and peritoneal catheter infection in one and wound infection in the other. There was one (12.5%) major complication: sepsis

***Table 1*** *– Demographics data.*

| Patient | Age (years) | Sex | BMI (kg/m²) | Smoking History | ASA Score | Preoperative Serum Creatinine (mg/dL) | Previous Surgery |
|---|---|---|---|---|---|---|---|
| 1 | 76 | M | 28.9 | Yes | III | 1.7 | No |
| 2 | 77 | M | 24 | Yes | III | 7.0 (on dialysis) | Appendectomy, inguinal hernia repair, abdominal aortic aneurysm repair |
| 3 | 65 | M | 26 | Yes | III | 10.2 (on dialysis) | Peritoneal dialysis catheter insertion |
| 4 | 79 | M | 18.9 | No | III | 1.3 | Inguinal hernia repair |
| 5 | 78 | M | 25.1 | Yes | III | 3.8 | Nephrectomy (duplicated system) |
| 6 | 71 | F | 26.5 | Yes | IV | 0.6 | Total abdominal hysterectomy and bilateral salpingo-oophorectomy |
| 7 | 78 | M | 24.9 | No | II | 1.3 | Inguinal hernia repair |
| 8 | 69 | M | 41 | Yes | IV | 2.4 | Cholecystectomy, ureterolithotomy, appendectomy |

** This patient had a long history of superficial bladder cancer and episode of hematuria after 10 years of follow up. ** These patients had TCC of the bladder and end-stage renal disease. They underwent radical cystectomy with concomitant bilateral nephroureterectomy. TURBT = transurethral resection of bladder tumor. CIS = carcinoma in situ. LRC = laparoscopic radical cystectomy, LNU = laparoscopic nephroureterectomy.*

***Table 2*** *– Tumor characteristics.*

| Patient | No Previous TURBT | Previous Intravesical Chemo-therapy | Multifocality | Stage | Grade | CIS | Urethral Involve-ment | Upper Tract Involve-ment | Time from Initial Diagnosis to LRC and LNU (months) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | Yes (BCG) | Multifocal | T1 | G3 | Yes | No | Left renal pelvis and ureter | 120* |
| 2 | 1 | None | Multifocal | T2 | G3 | Yes | Yes | No ** | 3 |
| 3 | 1 | None | Multifocal | T2 | G3 | No | No | No ** | 1 |
| 4 | Multiple | Yes (mito-mycin) | Multifocal | T2 | G3 | No | No | Right ureter | 2 |
| 5 | 1 | Yes (BCG) | Multifocal | Ta | G3 | Yes | No | Left lower calyx | 8 |
| 6 | 1 | Yes (mito-mycin) | Multifocal | Ta | G3 | No | No | Right ureter | 120 |
| 7 | 1 | Yes (BCG) | Multifocal | Tis | G1 | Yes | No | Right ureter | 24 |
| 8 | 1 | Yes (BCG) | Multifocal | T1 | G3 | Yes | No | Right re-nal pelvis and ureter | 2 |

** This patient had a long history of superficial bladder cancer and episode of hematuria after 10 years of follow up. ** These patients had TCC of the bladder and end-stage renal disease. They underwent radical cystectomy with concomitant bilateral nephroureterectomy. TURBT = transurethral resection of bladder tumor. CIS = carcinoma in situ. LRC = laparoscopic radical cystectomy, LNU = laparoscopic nephroureterectomy.*

due to peritonitis from an enterocutaneous fistula and pelvic abscess. Median time for resumption to oral intake was 4 days (range 1 to 19). Table-4 demonstrates the postoperative and pathological data.

Median follow-up was 9 months (range 1 to 45). Of the 6 patients undergoing unilateral LNU, 2 (33.4%) required dialysis (one that had a previous contralateral nephrectomy and another due to postoperative renal failure and sepsis). There were no cases of local recurrence and only one (12.5%) patient developed distant metastasis and died 8 months postoperatively. Four other patients have died during follow-up including one during hospital stay, 2 from unknown cause (at 1 and 36 months) and 1 from cardiac disease at 45 months. The patient who died during the hospital stay developed an enterocutaneous fistula due to a small bowel perforation proximal to the ileal anastomosis at postoperative day 7. He underwent fistula resection and drainage of an abscess. However, the urine leak was consistent and he, therefore, underwent a right percutaneous nephrostomy tube placement. However, this patient developed sepsis and renal failure and died at 90 days after surgery. Other patient who died at 1 month

***Table 3*** *– Intraoperative data.*

| | Type of Surgery | Total OR Time* | EBL (units of blood transfusion) | Type of Urinary Diversion | LNU Approach | No. of Pelvic Lymph Nodes Dissected | Type of Incision to Extract Specimen and Perform Bowel Work | Intraoperative Complications |
|---|---|---|---|---|---|---|---|---|
| 1 | Laparoscopic radical cystoprostatectomy + left LNU | 9 | 1500 cc | Ileal conduit ** | Transperitoneal | 0 *** | Pfannenstiel | No |
| 2 | Laparoscopic cystoprostatectomy + bilateral LNU | 9 | 300 cc | None **** | Transperitoneal | 6 | Pfannenstiel | No |
| 3 | Laparoscopic cystoprostatectomy + bilateral LNU | 12 | 1000 cc (2) | None **** | Transperitoneal | 15 | Pfannenstiel | No |
| 4 | Laparascopic cystoprostatectomy + right LNU | 9 | 510 cc | Ileal conduit ** | Transperitoneal | 8 | Small midline incision | No |
| 5 | Laparascopic cystoprostatectomy + left LNU | 8 | 350 cc | None ***** | Transperitoneal | 6 | Small midline incision | No |
| 6 | Laparascopic cystectomy + right LNU | 8 | 350 cc | Ileal conduit | Transperitoneal | 14 | Small midline incision | No |
| 7 | Robotic-assisted laparascopic cystoprostatectomy + right LNU | 9 | 1000 cc | Ileal conduit | Transperitoneal | 2 | Small midline incision | No |
| 8 | Laparascopic cystoprostatectomy + right LNU | 11 | 2000 cc | Ileal conduit | Transperitoneal | 14 | Small midline incision | No |

** Include nephroureterectomy, cystectomy, pelvic lymph node dissection (PLND) and urinary diversion. ** Laparoscopic intracorporeal urinary diversion. *** PLND was not performed because it would not stick to the prognosis and thereby save the patient additional morbidity. **** These patients had transitional cell carcinoma of the bladder and end-stage renal disease. They underwent radical cystectomy with concomitant bilateral nephroureterectomy. ***** Nephroureterectomy in solitary kidney. EBL = estimated blood loss, LNU = laparoscopic nephroureterectomy.*

*Table 4 – Postoperative and pathological data.*

| Patient | LOS (days) | Oral Intake (days) | Postoperative Complications | Blood Transfusion | PSM | PLN | LRC Pathology | LNU Pathology | Site of Upper Tract Tumor |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 90 | 19 | Pyelonephritis, ileus, urine leak, enterocutaneous fistula, pelvic abscess, pneumonia | None | Negative | None | pT1G3 | pT1 | Left renal pelvis and ureter |
| 2 | 5 | 1 | None | None | Negative | 2 out of 6 | pT4 G3 | pT0 | None |
| 3 | 28 | 7 | Prolonged ileus and peritoneal catheter infection | 4 units | Negative | None | pT2 G2 | pT0 | None |
| 4 | 5 | 3 | None | 2 units | Negative | None | pT2 G2 | pT3 | Distal ureter |
| 5 | 4 | 2 | None | None | Negative | None | pT2 G2 | pT2 | Renal pelvis |
| 6 | 6 | 4 | Wound infection | None | Negative | None | pT0 | pTa | Right ureter |
| 7 | 9 | 5 | None | 2 units | Negative | None | pT3 G2 | PTis | Distal ureter |
| 8 | 9 | 4 | None | 2 units | Negative | None | pTis | pTis | Renal pelvis and ureter |

*LOS = length of hospital stay; PSM: positive surgical margin, PLN = positive lymph node.*

was discharged 28 days after surgery and died 2 days later from unexplained causes. The family did not grant permission for a postmortem evaluation. Three patients are alive with an overall survival and cancer specific survival rate of 37.5% and 87.5%, respectively.

## COMMENTS

Synchronous or metachronous presentation of TCC in the upper and lower genitourinary tract has been reported at varying rates throughout the literature. In 1989, Olbring et al. reported 11 cases (1.7%) of subsequent TCC of the renal pelvis or ureter in 657 patients with bladder cancer (14). Of 1,529 patients with primary superficial bladder tumors, Rodriguez et al. reported a 2.6% incidence of upper tract urothelial cancer (15). Herr et al. reviewed a cohort of 86 patients with bladder tumor followed for at least 15 years and found that 21% developed UUT tumors at a median of 7.3 years (16). Accordingly, they have recommended lifelong upper tract surveillance for urothelial cancer in patients with bladder tumor. Miyake et al. reported an incidence of 13.2% of simultaneous bladder and UUT tumors in a total of 106 cases (17). From our report, we noted a 7.9% (6 in 76 for our entire LRC series to date) incidence of concurrent UUT TCC in our LRC series.

Simultaneous nephroureterectomy, radical cystectomy and bilateral pelvic lymph node dissection is a challenging surgical procedure independent of the approach. The patients are often high-risk surgical candidates as demonstrated by the 87.5 % of patients classified as ASA score $\geq 3$ in our series. With regards the technique used, special note must be made for the need to re-position the patient between the nephroureterectomy and cystectomy portions. Moreover, the ureter is never divided during the entire procedure and the urethra at the prostate apex should be sewn shut for intact specimen extraction to prevent any tumor spillage. In our series, 7 (87.5 %) patients underwent previous abdominal surgery suggesting that previous surgery was not a contraindication for the laparoscopic approach.

The various options for the urinary diversion portion of the procedure depend on clinical condition of the patient, the status of the urethra, patient preference and surgeon experience. In this series, all patients primarily underwent extracorporeal ileal conduit urinary diversion because they were all elderly and at high surgical risk with multiple medical and surgical co-morbidities. Our recent report confirms that the open-assisted laparoscopic approach for urinary diversion portion of the procedure is technically more efficient and associated with a quicker recovery profile and decreased complication rates compared with the pure laparoscopic approach during LRC (18).

The extended pelvic lymph node dissection during LRC, adhering to established oncological principles, has been previously shown to be technically feasible (13). The survival appears to be better in patients in whom > 14 lymph nodes were removed (19). In our series, a limited lymphadenectomy was used in patients with a high-risk surgical or in technically difficult cases (i.e. prior surgery) revealing a median yield of 6 lymph nodes. Extended pelvic lymphadenectomy was used later with our evolving experience in patients with better clinical conditions with an increased median yield of 14 lymph nodes. Only one patient had positive nodes. We stress our pelvic lymph node dissection was limited in our early experience.

A simultaneous, bilateral approach is justified in patients with ESRD, because synchronous upper tract TCC has been reported to be more frequent in patients with renal insufficiency (20). In that group, concomitant radical cystectomy with bilateral nephroureterectomy avoids the need for urinary diversion and removes almost all urothelium at risk for tumor recurrence. Care should be taken during renal dissection, mainly in patients with previous surgery, to prevent injury to the adrenal glands for subsequent adrenal insufficiency risk. The specimen can be removed through a Pfannenstiel incision. In female patients, extraction of the specimen en bloc through the vagina is a viable option (21).

For upper tract surgery, the conventional advantages of the laparoscopic approach include earlier resumption of oral intake, reduced narcotic analgesia requirement and decreased length of hospital stay (22).

## CONCLUSIONS

In this study, we have demonstrated the technical feasibility of simultaneous laparoscopic unilateral or bilateral nephroureterectomy and radical cystectomy and urinary diversion in patients with concomitant upper tract TCC or ESRD and bladder cancer. A greater number of patients and increased experience are needed to reduce the total operative duration and complications. Further studies are required to validate our results.

## CONFLICT OF INTEREST

None declared.

### REFERENCES


1. Landis SH, Murray T, Bolden S, Wingo PA: Cancer statistics, 1999. CA Cancer J Clin. 1999; 49: 8-31.
2. Turna B, Aron M, Haber GP, Gill IS, Kaouk JH: Robotic radical cystectomy. Arch Esp Urol. 2007; 60: 439-48.
3. Palou J, Rodríguez-Rubio F, Huguet J, Segarra J, Ribal MJ, Alcaraz A, et al.: Multivariate analysis of clinical parameters of synchronous primary superficial bladder cancer and upper urinary tract tumor. J Urol. 2005; 174: 859-61; discussion 861.
4. Clayman RV, Kavoussi LR, Figenshau RS, Chandhoke PS, Albala DM: Laparoscopic nephroureterectomy: initial clinical case report. J Laparoendosc Surg. 1991; 1: 343-9.
5. Gill IS, Sung GT, Hobart MG, Savage SJ, Meraney AM, Schweizer DK, et al.: Laparoscopic radical nephroureterectomy for upper tract transitional cell carcinoma: the Cleveland Clinic experience. J Urol. 2000; 164: 1513-22.
6. Shalhav AL, Dunn MD, Portis AJ, Elbahnasy AM, McDougall EM, Clayman RV: Laparoscopic nephroureterectomy for upper tract transitional cell cancer: the Washington University experience. J Urol. 2000; 163: 1100-4.
7. Rassweiler JJ, Schulze M, Marrero R, Frede T, Palou Redorta J, Bassi P: Laparoscopic nephroureterectomy for upper urinary tract transitional cell carcinoma: is it better than open surgery? Eur Urol. 2004; 46: 690-7.
8. Manabe D, Saika T, Ebara S, Uehara S, Nagai A, Fujita R, et al.: Comparative study of oncologic outcome of laparoscopic nephroureterectomy and standard nephroureterectomy for upper urinary tract transitional cell carcinoma. Urology. 2007; 69: 457-61.
9. Wu CF, Shee JJ, Ho DR, Chen WC, Chen CS: Different treatment strategies for end stage renal disease in patients with transitional cell carcinoma. J Urol. 2004; 171: 126-9.
10. Takehara K, Nishikido M, Koga S, Miyata Y, Harada T, Tamaru N, et al.: Multifocal transitional cell carcinoma associated with renal cell carcinoma in a patient on long-term haemodialysis. Nephrol Dial Transplant. 2002; 17: 1692-4.
11. Holton MR, Van Zijl PS, Oberle WT, Jacobs SC, Sklar GN: Complete urinary tract extirpation: the University of Maryland experience. Urology. 2006; 68: 65-9.
12. Matin SF, Gill IS: Laparoscopic radical cystectomy with urinary diversion: completely intracorporeal technique. J Endourol. 2002; 16: 335-41; discussion 341.
13. Finelli A, Gill IS, Desai MM, Moinzadeh A, Magi-Galluzzi C, Kaouk JH: Laparoscopic extended pelvic lymphadenectomy for bladder cancer: technique and initial outcomes. J Urol. 2004; 172: 1809-12.
14. Oldbring J, Glifberg I, Mikulowski P, Hellsten S: Carcinoma of the renal pelvis and ureter following bladder carcinoma: frequency, risk factors and clinicopathological findings. J Urol. 1989; 141: 1311-3.
15. Millán-Rodríguez F, Chéchile-Toniolo G, Salvador-Bayarri J, Huguet-Pérez J, Vicente-Rodríguez J: Upper urinary tract tumors after primary superficial bladder tumors: prognostic factors and risk groups. J Urol. 2000; 164: 1183-7.
16. Herr HW, Cookson MS, Soloway SM: Upper tract tumors in patients with primary bladder cancer followed for 15 years. J Urol. 1996; 156: 1286-7.
17. Miyake H, Hara I, Arakawa S, Kamidono S: A clinicopathological study of bladder cancer associated with upper urinary tract. BJU Int. 2000; 85: 37-41.
18. Haber GP, Campbell SC, Colombo JR Jr, Fergany AF, Aron M, Kaouk J, et al.: Perioperative outcomes with laparoscopic radical cystectomy: "pure laparoscopic" and "open-assisted laparoscopic" approaches. Urology. 2007; 70: 910-5.
19. Herr HW: Extent of surgery and pathology evaluation has an impact on bladder cancer outcomes after radical cystectomy. Urology. 2003; 61: 105-8.
20. Chang CH, Yang CM, Yang AH: Renal diagnosis of chronic hemodialysis patients with urinary tract transitional cell carcinoma in Taiwan. Cancer. 2007; 109: 1487-92.

21. Yuan LH, Chung HJ, Chen KK: Laparoscopic radical cystectomy combined with bilateral nephroureterectomy and specimen extraction through the vagina. J Chin Med Assoc. 2007; 70: 260-1.

22. Portis AJ, Elnady M, Clayman RV: Laparoscopic radical/total nephrectomy: a decade of progress. J Endourol. 2001; 15: 345-54; discussion 375-6.

*Accepted after revision:*
*February 12, 2008*

**Correspondence address:**
Dr. Rodrigo Frota
Section of Laparoscopic and Robotic Surgery
The Cleveland Clinic Foundation
9500 Euclid Avenue/A100
Cleveland, OH, 44195, USA
Fax: + 1 216 445-7031
E-mail:rodrigofrotaf@gmail.com

## EDITORIAL COMMENT

Recently, the laparoscopic approach has gained acceptance and more robust data support for radical cystectomy with pelvic lymphadenectomy. The authors should be commended for presenting the feasibility of simultaneous laparoscopic nephroureterectomy and radical cystectomy in a very selective subset of patients from a single, tertiary referral institution with high-volume laparoscopic surgery for urologic malignancy. In this initial series, we noted a high morbidity with two procedure related deaths (< 30 days after discharge) probably due the advanced age and comorbidities of the study subjects, combined with the surgical challenging scenario.

Additionally, this study covers a long timeframe so the major complications observed in cases 1 and 3 might be related to the learning curve of this complex procedure. From the technical standpoint, we should emphasize the high rate of previous pelvic/abdominal surgery, and that not all diversions were performed in an open-assisted manner that is currently the standard-of-care for laparoscopic radical cystectomy, fact that may be contributed for a longer operative time and postoperative complication. Moreover, the extension of the lymphadenectomy was not ideal, what can potentially compromise the specific-survival in these patients. As the authors concluded, large studies are necessary before we can have further conclusions on these preliminary results.

***Dr. José Roberto Colombo and***
***Dr. Anuar Ibrahim Mitre***
*University of Sao Paulo, USP*
*Sao Paulo, Brazil*
*E-mail: anuar@mitre.com.br*

Clinical Urology

International Braz J Urol Vol. 34 (4): 422-432, July - August, 2008

# Urogenital Tuberculosis: Patient Classification in Seven Different Groups According to Clinical and Radiological Presentation

**Andre A. Figueiredo, Antonio M. Lucon, Cristiano M. Gomes, Miguel Srougi**

*Division of Urology, University of Sao Paulo School of Medicine, Sao Paulo, Brazil*

**ABSTRACT**

*Purpose:* To describe and classify 80 cases of urogenital tuberculosis in seven groups of similar clinical and radiological presentation.

*Materials and Methods:* 80 patients (56 males, 70%; median age 34 years; age range 12 to 75) with urogenital tuberculosis were retrospectively reviewed. The patients were divided in seven groups: 1) Bilateral parenchymatous renal lesions; 2) No or minimal changes on radiographic examination; 3) Unilateral renal tuberculosis; 4) Contracted bladder; 5) Contracted bladder with renal failure; 6) Tuberculosis on a transplanted kidney; 7) Isolated genital tuberculosis.

*Results:* 1) Seven (8.8%) patients had multiple bilateral parenchymatous renal lesions with fever and malaise, characteristic of miliary tuberculosis. Three of these patients had AIDS. 2) Six (7.5%) cases had an early diagnosis, with minimal or no radiographic lesions. Two did not have any urologic symptoms. 3) Twelve (15%) patients had unilateral renal tuberculosis with partial (1 case) or total non-function kidney. 4) Thirty-seven (46.3%) patients had contracted bladder associated with unilateral partial (1 case) or total non-function kidney. 5) Ten (12.5%) patients had end stage renal disease due to tuberculosis with contracted bladder. 6) Four (5.0%) patients had tuberculosis on a transplanted kidney, with graft loss in half the cases. 7) Four (5.0%) patients had prostate or epididymis tuberculosis without associated renal lesion.

*Conclusions:* Urogenital tuberculosis is a destructive disease of the urogenital tract with variable clinical and radiographic presentation. A classification according to similar patterns correlating with disease stage is feasible although early diagnosis is the only prevention of the most severe forms.

***Key words:*** *kidney; ureter; bladder; tuberculosis*

***Int Braz J Urol. 2008; 34: 422-32***

## INTRODUCTION

Tuberculosis is a worldwide disease with greater prevalence wherever the population is concentrated in areas with poor sanitation and unfavorable social and economic indicators. Thirty percent of the world's population (1.7 billion people) is estimated to harbor the latent form of Mycobacterium tuberculosis (1-3). In spite of an effective pharmacological treatment and other technological breakthroughs, recent years have witnessed the recrudescence of the infection, due to the appearance of drug-resistant bacilli, population migrations, and the AIDS epidemic (4). Only 22 countries concentrate 80% of the annual cases, with Brazil (80 to 90 thousand new cases a year since 1980) being one of them (5).

From the pulmonary focus, 2 to 20% of the patients go on to develop urogenital tuberculosis. Through hematogenous spread to the kidneys, prostate, and epididymis, through a descending route to the ureters, bladder, and urethra, and through the canalicular route to the genital organs (3,4). Urogenital

tuberculosis affects all age ranges, with predominance of 30 to 50-year-old males (6,7). Because of its insidious evolution and late-onset symptoms, diagnosis and treatment are delayed, with a consequent high rate of urogenital organ destruction and renal failure (8).

As all urogenital organs may be involved, tuberculosis may give rise to all urologic symptoms, adding to the complexity of the clinical and radiographic pictures. We attempted to classify patients with urogenital tuberculosis into identifiable groups with similar clinical and radiographic features.

## MATERIALS AND METHODS

Eighty patients with urogenital tuberculosis, seen during the 1989-2005 period were retrospectively reviewed. These patients were treated in our tertiary teaching hospital that provides medical assistance free of charge to the metropolitan Sao Paulo area. Most of them have poor socio-economic conditions. There were 56 (70 %) males and 24 (30%) females with a median age of 34 years (12 to 75). Figure-1 shows the distributions according to the decades. Urogenital tuberculosis was diagnosed by direct bacilli identification or culture growth in the urine of 36 (45 %) patients; histopathology in 25 (31.3 %) patients; and a combination of strong clinical, laboratory, and radiographic evidence of urogenital tuberculosis with negative bacilli search in the urine of 19 (23.7%) patients.

The clinical features and the organs involved were described. The patients were classified in seven groups according to their patterns of initial clinical and radiographic presentation: 1) Bilateral parenchymatous renal lesions; 2) No or minimal changes on radiographic examination; 3) Unilateral renal tuberculosis; 4) Contracted bladder; 5) Contracted bladder with renal failure; 6) Tuberculosis on a transplanted kidney; 7) Isolated genital tuberculosis.

## RESULTS

Table-1 shows details about the signs and symptoms. Storage symptoms and hematuria were the most frequent symptoms, being present in 72.5% and 56.3% of the patients, respectively. Sixteen patients had some form of immunodeficiency: four because of AIDS, four with a kidney transplant and ongoing immunosuppressive therapy, four with diabetes, and four with alcohol abuse. In 35 (43.8%) patients, there was clinical or radiographic evidence of previous tuberculosis. Table-2 shows the distribution of organ involvement. After tuberculosis diagnosis, all the patients received triple therapy for at least six months.

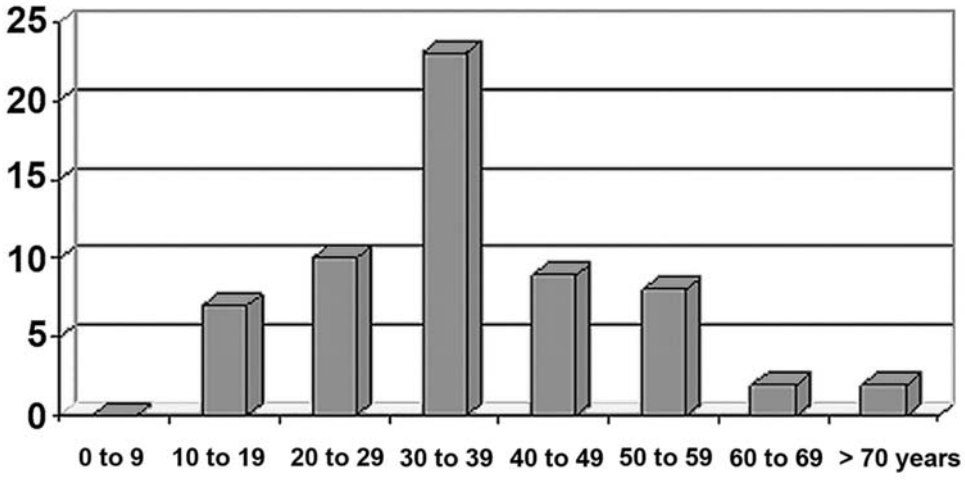



***Figure 1 –*** *Age distribution of the 80 patients with urogenital tuberculosis according to the decades.*

***Table 1*** *– Presenting signs and symptoms in 80 patients with urogenital tuberculosis.*

| Signs and Symptoms | N | % |
|---|---|---|
| Storage symptoms | 58 | 72.5% |
| Hematuria | 45 | 56.3% |
| Urinary tract infection | 14 | 17.5% |
| Lumbar pain | 23 | 28.8% |
| Perineal pain | 2 | 2.5% |
| Scrotal pain | 11 | 13.8% |
| Scrotal mass | 10 | 12.5% |
| Scrotal fistula | 4 | 5.0% |
| LUTS | 7 | 8.8% |
| Urinary retention | 3 | 3.8% |
| Fever, weight loss and asthenia | 36 | 45.0% |
| Urethral fistula | 1 | 1.3% |
| Renal failure | 10 | 12.5% |
| No symptoms from urinary tract | 5 | 6.3% |

*LUTS = lower urinary tract symptoms.*

Initial clinical and radiographic assessment yielded the following patterns of urogenital tuberculosis presentation:

1) Bilateral parenchymatous renal lesions - In seven (8.8%) cases there were multiple bilateral parenchymatous renal lesions (Figure-2). In all cases, there were tuberculosis foci in other organs, fever, and malaise, characteristic of miliary tuberculosis. Three patients had AIDS. Case-fatality was 42.9% (three cases). The latter cases had the urogenital lesions diagnosed at postmortem examination. The other cases were diagnosed on imaging procedures. Three patients had no urologic symptom.

2) No or minimal changes on radiographic examination - In six (7.5%) cases no lesions were found (four patients) or there were minimal lesions (two patients) on radiographic examination with unilateral renal calcification or calyx deformities (Figure-3). Tuberculosis was diagnosed when bacilli were shown in the urine. Four patients had hematuria and the other two had no urologic symptoms but the investigation was undertaken because of previous history of pulmonary tuberculosis. These patients did not require surgery and resolved well with pharmacological treatment alone.

3) Unilateral renal tuberculosis - In 12 (15%) cases there was unilateral renal tuberculosis with obstruction and dilatation of the collecting system due to stenosis (Figure-4). In one case, there was single inferior pole function loss due to infundibular stenosis, which was treated with inferior polar nephrectomy. All the other cases underwent nephrectomy due to a non-functional kidney. In all cases, the contralateral kidney was normal on radiographic examination.

4) Contracted bladder - In 37 (46.3%) patients, there was contracted bladder due to tuberculosis. All the cases had unilateral non-function kidney with dilatation of the collecting system due to stenosis, associated with a contracted bladder, except for one case with polar renal function loss. Voiding cystourethrogram showed no vesicoureteral reflux in 10 cases, bilateral reflux in two cases, and unilateral reflux to the contralateral functional kidney in 25. The latter was the most frequent radiographic finding (Figure-5

***Table 2*** *– Description of the affected organs.*

| Affected Organ | N | % |
|---|---|---|
| Kidney | 72 | 90.0% |
| Bilateral and multiple lesions | 7 | 8.8% |
| Minimal lesions | 2 | 2.5% |
| Unilateral with polar loss of function | 2 | 2.5% |
| Unilateral with loss of function | 47 | 58.8% |
| Renal failure | 10 | 12.5% |
| Transplanted kidney | 4 | 5.0% |
| Ureter | 57 | 71.3% |
| Bladder | 47 | 58.8% |
| Prostate | 6 | 7.5% |
| Prostate abscess | 2 | 2.5% |
| Prostatitis with perineal pain | 2 | 2.5% |
| No symptoms | 2 | 2.5% |
| Epididymis | 10 | 12.5% |
| Unilateral | 8 | 10.0% |
| Bilateral | 2 | 2.5% |
| Cutaneous fistula | 4 | 5.0% |
| Epididymectomy | 5 | 6.3% |
| Seminal vesicles | 2 | 2.5% |

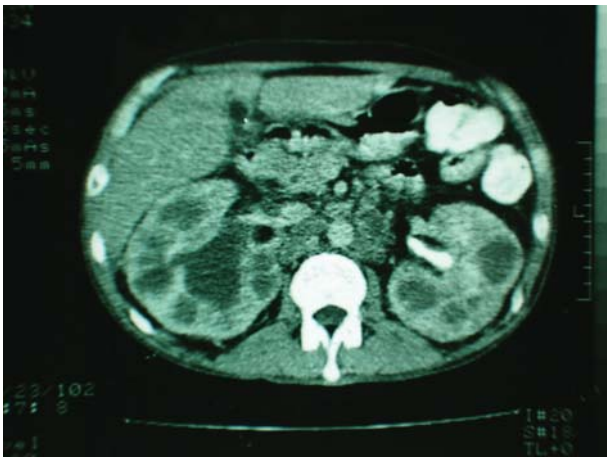


***Figure 2 –*** *Computed tomography showing multiple and bilateral kidney lesions in a patient with miliary tuberculosis.*

and 6). On radiographic examination, the functionally preserved contralateral kidney was normal in 23 cases and with ureterohydronephrosis in 14, one of which showing areas of cortical retraction. In all these cases with ureterohydronephrosis, vesicoureteral reflux to the functionally preserved kidney was observed. The patients underwent bladder augmentation along with total nephrectomy of the non-function kidneys and partial nephrectomy in the case with polar disease. In the cases with high-degree reflux, the ureters were reimplanted. The patients did well but five progressed to chronic renal failure.

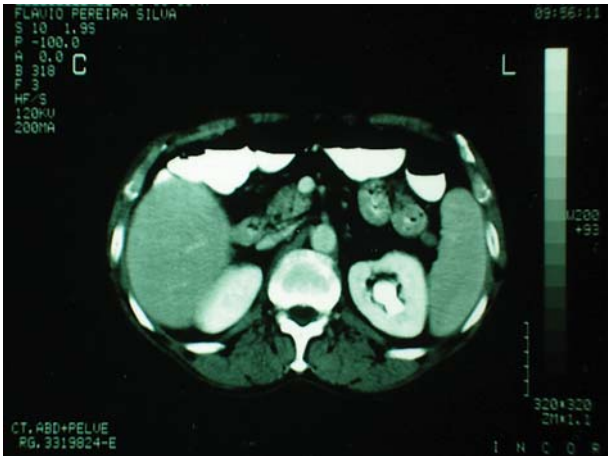



***Figure 3 –*** *Computed tomography showing calyceal dilatation in the left kidney.*

5) Contracted bladder and renal failure - In 10 (12.5%) cases the patients had end-stage renal disease due to tuberculosis. All had contracted bladder and unilateral vesicoureteral reflux. The patients underwent bilateral nephrectomy, bladder augmentation, and renal transplantation.

6) Tuberculosis on a transplanted kidney - In four (5%) cases parenchymatous renal tuberculosis was diagnosed on a transplanted kidney. The patients had creatinine elevation and tuberculosis was diagnosed through biopsy (3 cases) or a finding of bacilli in the urine. There was graft loss in 50% of the cases.

7) Isolated genital tuberculosis - In four (5%) cases there was genital tuberculosis without detectable

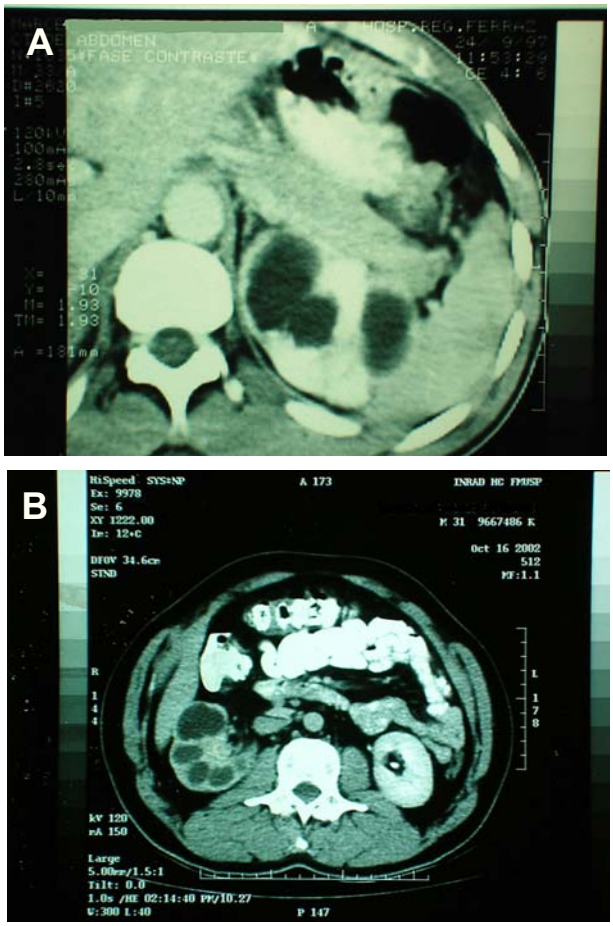



***Figure 4 –*** *A) and B) Computed tomography showing unilateral renal tuberculosis with low function kidney with dilatation of the excretory system in two different patients.*

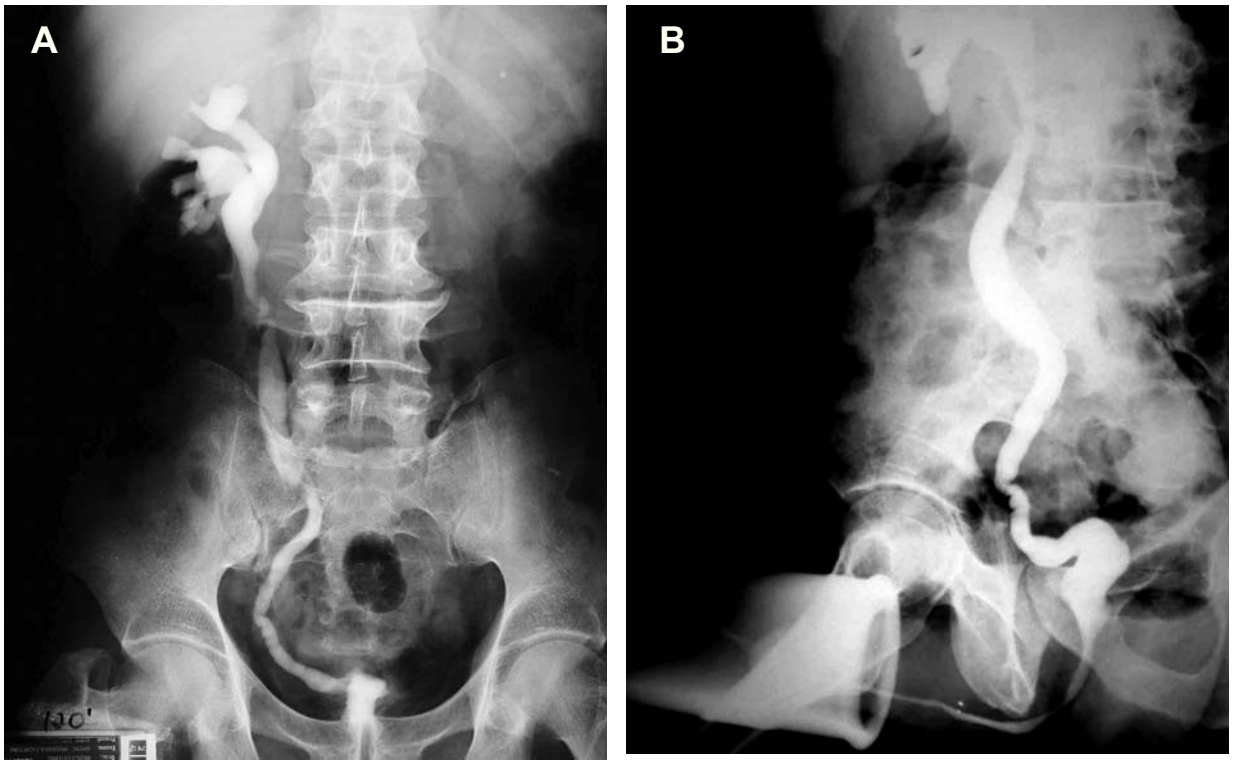



***Figure 5*** *– A) Intravenous urography with left non-function kidney, B) Voiding cystography shows contracted bladder and right vesicoureteral reflux.*

renal lesion. Two patients presented with prostate abscess and two with tuberculosis of the epididymis.

Tuberculosis of the epididymis also occurred in another eight cases with associated renal lesion, with a total of 10 (12.5%) patients with pain and a mass over the epididymis, bilateral in two, and with cutaneous fistulization of an epididymis abscess in four. Because of extensive involvement, orchi-epididymectomy was necessary in 50% of the cases. Besides the two patients with prostate abscess, another four also had prostate tuberculosis. While two had storage symptoms and perineal pain, compatible with chronic prostatitis, two were asymptomatic and were serendipitously diagnosed by histopathology. Tuberculosis of the seminal vesicles occurred in two cases, one of which in the patient with prostate abscess and another as a histopathological finding after radical prostatectomy.

Of the 76 patients without tuberculosis on the transplanted kidney, 15 (19.7%) developed end stage renal disease due to tuberculosis, 10 already at clinical presentation and five on follow-up, despite specific treatment. All 57 patients with tuberculosis-related ureteral stenosis lost the corresponding kidney. Fifty-nine patients required nephrectomy and two needed polar nephrectomy.

## COMMENTS

Of the urogenital tuberculosis cases described in the literature (range 7-39), the male-female distribution was 2-1, with a mean age of 40.7 years (range 5 to 88). In only 36.6% of the cases was there history or radiographic evidence of previous tuberculosis. In cases where the renal lesions are mainly asymptomatic, and only vesical lesions lead to symptoms (7-14), storage symptoms predominate. A total of 48.5% of males had scrotal involvement with an epididymis mass, epididymis hardness, or fistula, on physical examination, findings that point to the importance of these signs. Patients in developed countries have

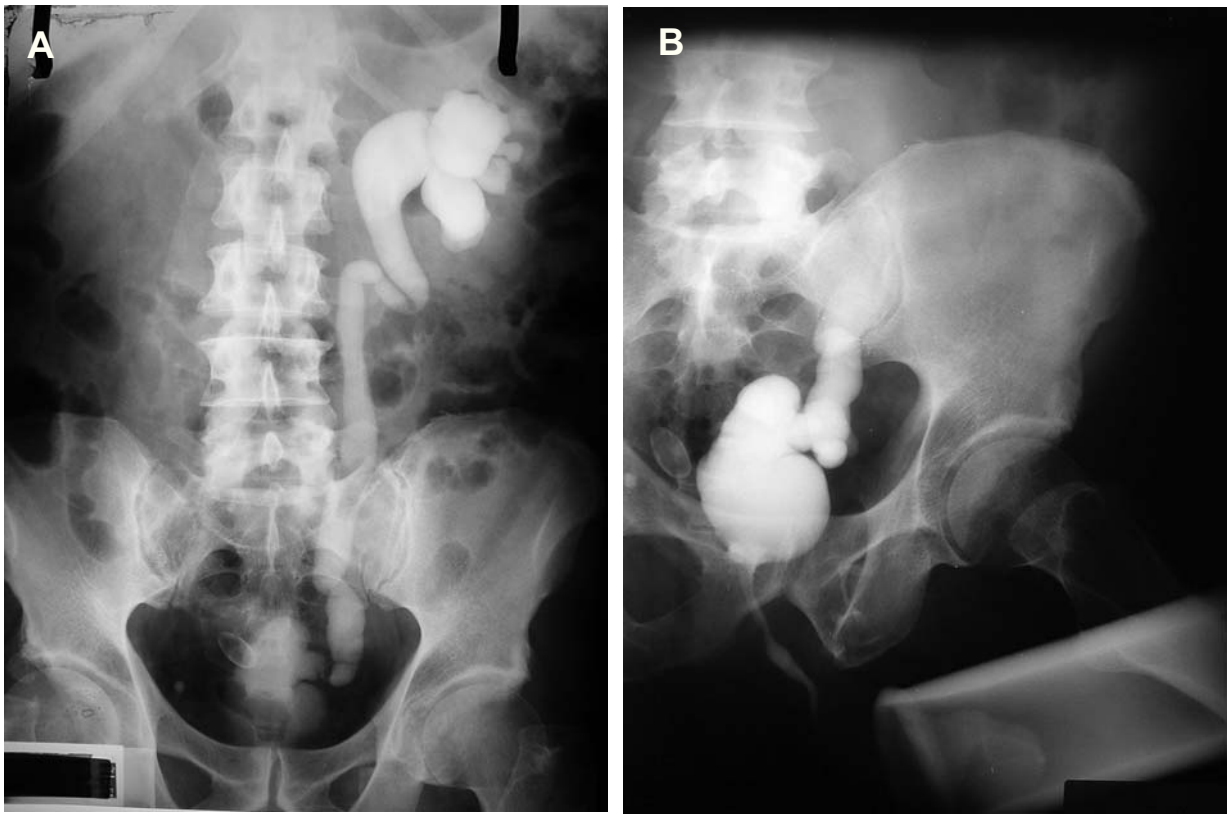



***Figure 6 –*** *A) Intravenous urography with right non-function kidney, B) Voiding cystography shows contracted bladder and left vesicoureteral reflux.*

fewer specific symptoms and smaller percentages of delayed histopathological diagnoses compared with other countries. As a result, the disease tends to be less serious, with fewer instances of renal failure, unilateral non-function kidney, ablative surgeries, and contracted bladder, with more cases presenting without significant lesions of the upper urinary tract on diagnosis. These data point to a correlation of the timing of the diagnosis with the severity of urogenital tuberculosis. Our findings contrast with those from developed countries, with greater rates of histopathological diagnoses, symptomatic patients at presentation, and severe destruction of the urinary tract (57.6% with contracted bladder, 19.7% with end stage renal disease, 12.5% with renal failure at initial presentation, and 71.2% with unilateral renal exclusion in the absence of renal failure or a transplanted kidney). Table-3 shows these features.

Since histopathology provides a delayed urogenital tuberculosis diagnosis, identification of the tuberculosis bacillus in the urine is necessary for early diagnosis. It is achieved through direct smears (Ziehl-Neelsen stain) or through urine culture (Lowestein-Jensen media) (40,41). Direct smears provide a faster result with high specificity (96.7%) but only 42.1 to 52.1% sensitivity (40,41). Culture is the diagnostic gold standard for urogenital tuberculosis, however 3 to 6 early morning mid-stream samples are required, the sensitivity varies widely, from 10% to 90%, and the time to detection of mycobacterium growth may be 4 to 6 weeks (40-42). Faster culture has been achieved with non-radiometric automated or semi-automated liquid culture systems with a 14 to 17 days result (42). Nucleic acid amplification tests, as polymerase chain reaction (PCR), for Mycobacterium tuberculosis identification in the urine may become

***Table 3*** *– Comparison between 3036 cases of urogenital tuberculosis from developed countries (USA, Europe and Japan) with 5925 cases from other countries (Russia, Latin America and Africa) and 80 cases from Brazil.*

| | Developed | Others | p Value | Brazil | Total |
|---|---|---|---|---|---|
| Total | 3036 | 5925 | | 80 | 9041 |
| Men | 62.9% | 65.4% | 0.02 | 70% | 65% |
| Women | 37.1% | 34.6% | 0.02 | 30% | 35% |
| Median age (years) | 42.6 | 39.2 | | 34 | 40.7 |
| Range (years) | 7 to 88 | 5 to 83 | | 12 to 75 | 5 to 88 |
| Previous tuberculosis | 37.9% | 38.4% | 0.66 | 43.8% | 36.6% |
| Signs and symptoms | | | | | |
| Storage symptoms | 44.2% | 55.2% | < 0.01 | 72.5% | 50.7% |
| Dysuria | 33.8% | 46.4% | < 0.01 | 72.5% | 38.2% |
| Lumbar pain | 28.8% | 42.3% | < 0.01 | 28.8% | 34.4% |
| Hematuria | 24.5% | 44.3% | < 0.01 | 56.3% | 35.8% |
| Epididymis lesion * | 20.6% | 47.4% | < 0.01 | 12.5% | 48.5% |
| Fever and malaise | 23.2% | 19.9% | 0.28 | 45.0% | 22.1% |
| No symptoms | 8.4% | 0% | < 0.01 | 6.3% | 6.4% |
| Renal failure | 1.4% | 10.2% | < 0.01 | 19.7% ** | 5.8% |
| Diagnosis | | | | | |
| Urine | 79.0% | 55.4% | < 0.01 | 45.0% | 64.0% |
| Histopathology | 7.8% | 38.3% | < 0.01 | 31.3% | 21.8% |
| Clinico-radiographic | 9.6% | 11.3% | 0.36 | 23.7% | 10.5% |
| Kidney | | | | | |
| Unilateral non-function | 22.8% | 33.1% | < 0.01 | 58.8% | 27.2% |
| Normal | 18.8% | 13.2% | < 0.01 | 10.0% | 15.2% |
| Contracted bladder | 4.0% | 11.6% | < 0.01 | 58.8% | 9.3% |
| Surgeries | 56.6% | 53.6% | < 0.01 | 76.3% | 55.1% |
| Ablative | 35.0% | 26.9% | < 0.01 | 76.3% | 27.6% |
| Nephrectomy | 27.9% | 26.0% | 0.37 | 71.3% | 28.0% |

*p = comparison between developed countries (USA, Europe and Japan) and other countries (Latin America, Africa and Russia) through chi-square test. * in relation to male patients; ** in relation to non-transplanted patients.*

the ideal diagnostic tool, as it gives results in 24 to 48 hours and allows for the diagnosis to be made even when there are few bacilli (40,41). It has been showed 95.6% sensitive and 98.1% specific compared to culture (40) and 94.3% sensitive and 85.7% specific compared to bacteriological, histological, or clinico-radiological diagnoses (41).

Primary pulmonary tuberculosis is usually subclinical and self-limited. From the pulmonary focus there is bacillemia and bacilli implants in other organs, renal parenchymatous and prostate colonization ensuing. After six months, spontaneous cicatrisation of the primary pulmonary tuberculosis lesion occurs and the patients enter a latent phase, with 5% reactivating the disease in the following two years and 5% in their lifetime. In most cases of active pulmonary or extrapulmonary disease, there is foci reactivation due to a breach of immunity brought about by malnutrition, diabetes mellitus, steroid use, immunosuppressor use, and immunodeficiencies (3,43).

The present urogenital tuberculosis patients' classification in seven groups was based on disease

physiopathology and made evident the correlation between symptoms and disease's stage. Renal lesions are initially bilateral, cortical, glomerular, and peri-capillary, typical of the hematogenous spread and concomitant with other hematogenous foci in the prostate and other organs beyond the urogenital system (15,44). These foci usually heal, with the patient entering a latent phase. If any immunodeficiency ensues although, miliary tuberculosis with systemic symptoms develops (3,13),from 25 to 62% of patients with miliary tuberculosis will have a renal lesion with multiple bilateral foci (9,44). In our patients 8.8% had miliary tuberculosis with bilateral parenchymatous renal lesions and systemic symptoms, characterizing a typical clinical presentation of immune deficient patients. Tuberculosis on a transplanted kidney can be also included in this multiple parenchymatous lesion presentation.

The latent period between pulmonary infection with bacillemia and clinically evident urogenital tuberculosis is 22 years on average, ranging from 1 to 46 years, according to the timing of latent foci (renal, prostate, or epididymis) reactivation (12). Isolated prostate or epididymis foci reactivation may occur, characterizing genital tuberculosis without associated renal lesion. In the reactivation of renal foci, infection progresses from a single unilateral focus, with preservation of the contralateral kidney (15). This explains the greater frequency of unilateral renal tuberculosis (12,16). The contiguous involvement of the collecting system leads to bacilluria and descending spread of the infection to the ureter, bladder, and genital organs (44,45). Thus, a typical clinico-radiographic form exists: unilateral renal tuberculosis with preservation of the contralateral kidney and the bladder. This occurred in 15% of our cases, with obstruction and dilatation of the collecting system, with invariable renal functional loss. In fact, obstruction of the collecting system (with distal ureteral stenosis as the most frequent finding) is the main cause of renal functional loss in tuberculosis (11,46,47). The focal origin of tuberculosis reactivation is demonstrated by two of our cases with restriction of the disease to the renal pole. If the diagnosis of the urogenital tuberculosis reactivation is at an early stage, we can detect no or minimal radiographic features such as calyceal dilatation due to initial infundibular stricture as we observed in group 2 patients (41).

Another clinico-radiographic presentation was a contracted bladder without renal failure, occurring in 45% of the patients. There was unilateral non-function kidney in practically all cases, with collecting system obstruction and dilatation, primarily (66.7%) with unilateral reflux to the function kidney, 27.8% without reflux, and only 5.5% with bilateral reflux. All patients with renal failure had bladder contraction and unilateral reflux on investigation. These findings prompted a new hypothesis for the pathophysiology of the urinary tract lesions in urogenital tuberculosis. After reactivation and progression of the unilateral renal focus, the collecting system is implicated, with unilateral descending involvement of the ipsilateral ureter and bladder ensuing (4). Ureteral stenosis with corresponding renal function loss and fibrosis of the bladder wall are the next stages (47). At this stage, the investigations show unilateral non-function kidney with contracted bladder and absence of reflux. Progression of the bladder infection with capacity and compliance reduction leads to distortion of the vesicoureteral junction and vesicoureteral reflux to the functional kidney, the collecting system thus functioning as a buffer for the reduced capacity of the contracted bladder, and with ascending transmission of the high intravesical pressure. Currently, the investigations show unilateral non-function kidney, contracted bladder, and reflux to the function kidney, as seen in most of our patients (Figure-5 and 6). Non-identified and non-treated reflux causes renal lesion due to infection or transmission of intravesical pressure, leading to end stage renal disease. In fact, renal failure patients presented unilateral reflux, underlying the hypothesis that one of the kidneys loses its function because of reflux, and not due to tuberculosis itself. Furthermore, patients with unilateral non-function kidney had ureterohydronephrosis and areas of retraction in the functional kidneys, as well as changes associated with vesicoureteral reflux. The fact that there were no typical tuberculosis lesions in these kidneys, such as ureteral or intra-renal stenosis, underlies the role of reflux nephropathy and not tuberculosis itself, in the pathogenesis of the contralateral involvement.

After the discovery on specific tuberculostatic drugs in the XX century, the profile of urogenital

tuberculosis experienced dramatic changes, with reduced mortality, cure of initial lesions, reduction in the number of ablative surgeries, and increase in the number of reconstructive surgeries. In the last decades, however, no significant change occurred, in spite of technological advances (48). Since the 60`s the importance of an early diagnosis of urogenital tuberculosis has been acknowledged as the main step towards renal preservation (47). A systematic search for urogenital tuberculosis in patients with pulmonary disease yielded 10% of positive cultures (66.7% asymptomatic and 58% with normal urine exam and absence of renal lesions on pyelography) (49). In the other two series, where cultures were routinely grown, a greater frequency of asymptomatic patients without lesions on intravenous pyelography was found (8,50). Although bacilluria is invariably associated with renal lesion, detection of pre-clinical bacilluria allows for an early diagnosis to be made, at a moment when the lesions are amenable to cure and the development of severe and destructive lesions can be avoided (45). Of our patients with minimum or absent lesions, two had no urologic symptoms and the others had isolated hematuria. Thus, a systematic search for urogenital tuberculosis, regardless of symptoms, is warranted for early case detection. We propose that all patients with macroscopic hematuria or persisting microscopic hematuria or leucocituria should be submitted to Mycobacterium tuberculosis urinary culture or PCR analyses. We also propose yearly urinalysis for microscopic hematuria or leucocituria detection in patients with pulmonary tuberculosis history or immunological impairment. However, the better definition of higher risk groups and optimal diagnosis strategy warrants further studies.

## CONFLICT OF INTEREST

None declared.

### REFERENCES


1. Brasil - Ministério da Saúde, Guia de Vigilância Epidemiológica, Brasília. 1998; Cap. 5.34: pp.1-13. Tuberculose.
2. Dias Filho AC, Netto Júnior NR: Aspectos atuais da tuberculose urinária e genital masculina. Jornal Brasileiro de Urologia. 1999; 25: 453-65.
3. Leite OH: Tuberculosis. Problems in General Surgery. 2001; 18: 69-78.
4. Gow JG: Genitourinary tuberculosis. In: Walsh PC, Retik AB, Vaughan ED, Wein AJ. Campbell´s Urology. (7ª ed.), Philadelphia, WB Saunders Company. 1998; Vol. 1: pp 807-36.
5. Hijjar MA, Oliveira MJPR, Teixeira GM: A tuberculose no Brasil e no mundo. Boletim de Pneumologia Sanitária. 2001; 9: 9-16.
6. Schubert GE, Haltaufderheide T, Golz R: Frequency of urogenital tuberculosis in an unselected autopsy series from 1928 to 1949 and 1976 to 1989. Eur Urol. 1992; 21: 216-23.
7. Flechner SM, Gow JG: Role of nephrectomy in the treatment of non-functioning or very poorly functioning unilateral tuberculous kidney. J Urol. 1980; 123: 822-5.
8. Ferrie BG, Rundle JS: Genito-urinary tuberculosis in Glasgow 1970 to 1979: a review of 230 patients. Scott Med J. 1985; 30: 30-4.
9. Simon HB, Weinstein AJ, Pasternak MS, Swartz MN, Kunz LJ: Genitourinary tuberculosis. Clinical features in a general hospital population. Am J Med. 1977; 63: 410-20.
10. Nzerue C, Drayton J, Oster R, Hewan-Lowe K: Genitourinary tuberculosis in patients with HIV infection: clinical features in an inner-city hospital population. Am J Med Sci. 2000; 320: 299-303.
11. Ramanathan R, Kumar A, Kapoor R, Bhandari M: Relief of urinary tract obstruction in tuberculosis to improve renal function. Analysis of predictive factors. Br J Urol. 1998; 81: 199-205.
12. Christensen WI: Genitourinary tuberculosis: review of 102 cases. Medicine (Baltimore). 1974; 53: 377-90.
13. Alvarez S, McCabe WR: Extrapulmonary tuberculosis revisited: a review of experience at Boston City and other hospitals. Medicine (Baltimore). 1984; 63: 25-55.
14. Gokalp A, Gultekin EY, Ozdamar S: Genito-urinary tuberculosis: a review of 83 cases. Br J Clin Pract. 1990; 44: 599-600.
15. Narayana A: Overview of renal tuberculosis. Urology. 1982; 19: 231-7.
16. García-Rodríguez JA, García Sánchez JE, Muñoz Bellido JL, Montes Martínez I, Rodríguez Hernández J, Fernández Gorostarzu J, et al.: Genitourinary tuberculosis in Spain: review of 81 cases. Clin Infect Dis. 1994; 18: 557-61.

17. Mochalova TP, Starikov IY: Reconstructive surgery for treatment of urogenital tuberculosis: 30 years of observation. World J Surg. 1997; 21: 511-5.
18. Kao SC, Fang JT, Tsai CJ, Chen KS, Huang CC: Urinary tract tuberculosis: a 10-year experience. Changgeng Yi Xue Za Zhi. 1996; 19: 1-9.
19. Caro Muñoz M, Corvalán JR, Tenório JP, Vergara JF: Diagnosis of urogenital tuberculosis: a retrospective review (1974 - 1986). Bol Hosp San Juan de Díos. 1988; 35(4): 223-6.
20. Venegas MP, Corti OD, Foneron BA: Genitourinary TBC: experience of Hospital de Valdiva 1979-1984 years. Rev Chil Urol. 1990; 53: 131-4.
21. Núñez Vásquez N, Susaeta S R, Vogel M, Olate C: Genitourinay tuberculosis study: urologic service, Hospital San Juan de Dios 1975-1989. Rev Chil Urol. 1990; 53: 135-7.
22. Allen FJ, de Kock ML: Genito-urinary tuberculosis--experience with 52 urology inpatients. S Afr Med J. 1993; 83: 903-7.
23. Bennani S, Hafiani M, Debbagh A, el Mrini M, Benjelloun S: Urogenital tuberculosis. Diagnostic aspects. J Urol (Paris). 1995; 101: 187-90.
24. Hinostroza Fuschlocher JÁ, Gorena Palominos M, Pastor Arroyo P, Rivera P, Venegas P, Orío M, Peldoza M, et al.: Genitourinary tuberculosis at Araucanía: experience at Hospital Regional de Temuco. Rev Chil Urol. 1998; 63: 113-7.
25. Mnif A, Loussaief H, Ben Hassine L, Chebil M, Ayed M. Aspects of evolving urogenital tuberculosis. 60 cases. Ann Urol (Paris). 1998; 32: 283-9.
26. Lopez de Mesa B, Cruz A, Gomes P: Review of patients with genitourinary tuberculosis in San Juan de Dios Hospital. Urol Colomb. 1998; 7: 43-52.
27. Benchekroun A, Lachkar A, Soumana A, Farih MH, Belahnech Z, Marzouk M, et al.: Urogenital tuberculosis. 80 cases. Ann Urol (Paris). 1998; 32: 89-94.
28. Chtourou M, Kbaier I, Attyaoui F, Nouira Y, Barrak A, Sayari S, et al.: Management of genito-urinary tuberculosis. A report of 225 cases. J Urol. 1999; 161 (Suppl 4): Abstract 34.
29. Buchholz NP, Salahuddin S, Haque R: Genitourinary tuberculosis: a profile of 55 in-patients. J Pak Med Assoc. 2000; 50: 265-9.
30. Hemal AK, Gupta NP, Kumar R: Comparison of retroperitoneoscopic nephrectomy with open surgery for tuberculous nonfunctioning kidneys. J Urol. 2000; 164: 32-5.
31. el Khader K, Lrhorfi MH, el Fassi J, Tazi K, Hachimi M, Lakrissa A. Urogenital tuberculosis. Experience in 10 years. Prog Urol. 2001; 11: 62-7.
32. Gokce G, Kilicarslan H, Ayan S, Tas F, Akar R, Kaya K, et al.: Genitourinary tuberculosis: a review of 174 cases. Scand J Infect Dis. 2002; 34: 338-40.
33. Barrios F, Martinez-Peschard L, Rosas A: Surgical management of tuberculosis. BJU Int. 2002; 90 (Suppl 2): p 26.
34. Ishibashi Y, Takeda T, Nishimura R, Ohshima H: A clinical observation of genitourinary tract tuberculosis during the last decade. Hinyokika Kiyo. 1985; 31: 107-12.
35. Gow JG: Genitourinary tuberculosis: a 7-year review. Br J Urol. 1979; 51: 239-44.
36. Gow JG, Barbosa S: Genitourinary tuberculosis. A study of 1117 cases over a period of 34 years. Br J Urol. 1984; 56: 449-55.
37. Fuse H, Imazu A, Shimazaki J. A clinical observation on urogenital tuberculosis. Hinyokika Kiyo. 1984; 30: 299-304.
38. Fischer M, Flamm J: The value of surgical therapy in the treatment of urogenital tuberculosis. Urologe A. 1990; 29: 261-4.
39. Zwergel U, Wullich B, Rohde V, Zwergel T: Surgical management of urinary tuberculosis: a review of 341 patients. J Urol. 1999; 161 (Suppl 4): Abstract 33.
40. Moussa OM, Eraky I, El-Far MA, Osman HG, Ghoneim MA: Rapid diagnosis of genitourinary tuberculosis by polymerase chain reaction and non-radioactive DNA hybridization. J Urol. 2000; 164: 584-8.
41. Hemal AK, Gupta NP, Rajeev TP, Kumar R, Dar L, Seth P: Polymerase chain reaction in clinically suspected genitourinary tuberculosis: comparison with intravenous urography, bladder biopsy, and urine acid fast bacilli culture. Urology. 2000; 56: 570-4.
42. Dinnes J, Deeks J, Kunst H, Gibson A, Cummins E, Waugh N, et al.: A systematic review of rapid diagnostic tests for detection of tuberculosis infection. Health Technology Assessment. 2007; 11: 1-196.
43. Cos LR, Cockett AT: Genitourinary tuberculosis revisited. Urology. 1982; 20: 111-7.
44. Medlar EM, Spain DM, Holliday RW: Post-mortem compared with clinical diagnosis of genito-urinary tuberculosis in adult males. J Urol. 1949; 61: 1078-88.
45. Medlar EM, Sasano KT: Experimental renal tuberculosis, with special reference to excretory bacilluria. Am Rev Tuberc. 1924; 10: 370-7.
46. Barrie HJ, Kerr WK, Gale GL: The incidence and pathogenesis of tuberculous strictures of the renal pyelus. J Urol. 1967; 98: 584-9.
47. Kerr WK, Gale GL, Peterson KS: Reconstructive surgery for genitourinary tuberculosis. J Urol. 1969; 101: 254-66.

48. Ross JC: Renal tuberculosis. Br J Urol. 1953; 25: 277-315.
49. Bentz RR, Dimcheff DG, Nemiroff MJ, Tsang A, Weg JG: The incidence of urine cultures positive for Mycobacterium tuberculosis in a general tuberculosis patient population. Am Rev Respir Dis. 1975; 111: 647-50.
50. Butler MR, O'Flynn JD: Reactivation of genito-urinary tuberculosis: a retrospective review of 838 cases. Eur Urol. 1975; 1: 14-7.

*Accepted after revision:*
*March 17, 2008*

**Correspondence address:**
Dr. André Avarese de Figueiredo
Rua Irineu Marinho 365 / 801, Bloco 3
Juiz de Fora, MG, 36021-580, Brazil
Fax: + 55 11 3287-6922
E-mail: andreavaresef@gmail.com

## EDITORIAL COMMENTS

On May 4, 1953, The New York Times published a report from The World Health Organization that stated 'there has been an "extraordinary" drop in mortality from tuberculosis of the respiratory system since the end of World War II. The development of anti-tuberculosis medication, improved living standards and Public Health measures made tuberculosis an almost forgotten disease particularly in developed countries. Unfortunately, this has not been the case. As noted in the paper tuberculosis continues to be a major world wide public health problem. Poor socio-economic conditions, immune suppression, AIDS are factors that perpetuate tuberculosis. The authors noted that 2 to 20% of patients with pulmonary can manifest urogenital tuberculosis. Their paper 'classified' GU tuberculosis into 7 categories including one category with 'No or minimal' disease.

This paper renews a known caveat for the Urologist. 'Atypical' urologic manifestations such as renal scaring, scrotal masses, idiopathic pyuria or hematuria mandate an assessment for tuberculosis.

***Dr. G. J. Wise***
*Division of Urology*
*Maimonides Medical Center*
*Brooklyn, New York, USA*
*E-mail: gwise@maimonidesmed.org*

Clinical Urology
International Braz J Urol Vol. 34 (4): 433-442, July - August, 2008

# Evaluation of Emergency Extracorporeal Shock Wave Lithotripsy for Obstructing Ureteral Stones

**Ibrahim F. Ghalayini, Mohammed A. Al-Ghazo, Yousef S. Khader**

*Faculty of Medicine, Jordan University of Science & Technology, King Abdullah University Hospital, Irbid, Jordan*

**ABSTRACT**

*Purpose:* To assess the efficacy of extracorporeal shockwave lithotripsy (ESWL) for ureteral calculi during acute renal colic.
*Materials and Methods:* From January 2002 to March 2007, 108 patients were treated by ESWL for obstructing ureteral stones causing acute renal colic. ESWL was performed within 24 hours of the onset of renal colic.
*Results:* The mean age of the patients was 39.5 years (11-72 years). Male/female ratio was 85/23. Mean stone size was 8.45 mm (4-20 mm). They were located in the pelvic (n = 53), iliac (n = 28) or lumbar (n = 27) region. Fragmentation after a single session was complete in 56 patients (52%), incomplete in 28 (26%), and absent in 24 (22%). Patients presenting incomplete fragmentation underwent a second (n = 28) or even a third session (n = 11). Of the 24 patients in whom ESWL had no impact on the stone, 21 underwent ureteroscopy, and in one case open ureterolithotomy for a patient with a hard 17 mm stone, while spontaneous passage occurred in two patients with small stones.
*Conclusion:* Emergency ESWL for obstructing ureteral stones has a satisfactory success rate and very low morbidity. The stone-free rate of retreating ureteral calculi with ESWL decreases significantly after failed initial treatment. Stone size may be the main predictive factor for retreatment. We suggest that no more than 3 treatments should be given for a particular stone due to minimal improvement in the subsequent cumulative treatment success rate.

***Key words:*** *extracorporeal shockwave lithotripsy; ureteral calculi; emergency; treatment outcome*
***Int Braz J Urol. 2008; 34: 433-42***

## INTRODUCTION

Urinary lithiasis can cause a greater or lesser degree of obstruction of the ureter, depending on the size of the calculus, urothelial edema and the degree of impaction, requiring instrumental treatment, sometimes as an urgent procedure. Optimal treatment for ureteral calculi remains controversial. Treatment options vary and include expectant management, passage of ureteral stent, extracorporeal shockwave lithotripsy (ESWL), ureteroscopy with basket extraction or intracorporeal lithotripsy and open ureterolithotomy. Open surgery is rarely used (1). However, a conservative approach is often complicated by recurrent flank pain, multiple visits to the emergency room (ER), absence from work and an increased risk of serious complications, such as obstruction, infection and silent loss of renal function (2). There is a significant risk of long-term renal impairment if patients have unrelieved obstruction for more than 4 weeks regardless of symptoms and stone size (2). ESWL is the treatment of choice for moderately sized, uncomplicated ureteral

stones (3,4). It is a simple, robust and safe procedure and is usually recommended for stones resistant to medical treatment in absence of absolute indication of ureteral drainage (5). Interestingly, the role of ESWL as a first line therapy, applied rapidly after the onset of renal colic, has deserved very limited attention. Few studies have suggested that emergency ESWL is an appealing treatment strategy for symptomatic ureteral stones (6-9).

The success rate of ESWL in the treatment of ureteral stones is about 80% (2). It can be successfully used, without anesthesia, in patients with early recurrence of renal colic (6). Others have used ESWL within 14 days of the onset of acute renal colic but under anesthesia (10) or even during acute renal colic (7) or acute renal failure (11). Moreover, a comparative retrospective analysis has shown that, in emergency situations, ESWL is more effective than nephrostomy or a double J stent and has very low morbidity (12).

We have investigated the efficacy of the lithotripter in the treatment of patients with obstructing ureteral stones during acute renal colic resistant to medical treatment. Also, we compared the success rate of initial shock wave lithotripsy for ureteral calculi with that of subsequent treatments to determine whether more than 1 treatment is justified for any single ureteral stone. Other parameters of treatment outcome were also studied.

## MATERIALS AND METHODS

This study enrolled 108 patients admitted to our department between January 2002 and January 2007 for acute renal colic that proved to be resistant to anti-inflammatory agents or that recurred within 24 hours of such treatment. Admission work-up included: monitoring of vital parameters; temperature; physical examination; blood test for leucocytes, urea, creatinine; urine analysis and culture. All patients should undergo an abdominal X-ray and ultrasound examination. Primary imaging of the patient was performed by helical unenhanced computed tomography of the abdomen, according to current recommendations (13). An intravenous urography (IVU) was only indicated when there was doubt as to the diagnosis. Initial characterization of the stone was based on imaging and included stone size (largest transversal diameter measured by X-ray) and stone location (lumbar, iliac or pelvic ureter).

Patients underwent emergency ESWL using the Dornier lithotripter S (MedTech Europe GmbH, Germany) within 24 h of admission and the calculi were localized with fluoroscopic guidance. All patients were given sedatives and analgesics and the level of shockwave energy was progressively stepped up until satisfactory stone fragmentation within the limits of patient comfort. Patients for whom the therapeutic modality is contraindicated because of pregnancy, urinary tract infection, coagulation disorders or previous ureteral reimplantation, presence of a perirenal urinoma, temperature > 38 C, blood leukocytes > 20,000/dL, solitary kidney, radiolucent stones, or prior history of ureteral stricture or tumor were excluded from the study. Patients with serum creatinine > 1.8 mg/dL, stone located in the renal pelvis or the pyelo-ureteral junction, or if there was any contraindication to ESWL were also excluded.

After defining the indications of treatment, the patients were informed of all the treatment modalities and their probable complications. The need for anesthesia, stent, urethral manipulation, possible complications, need for repeated follow-up especially after ESWL, and the cost factor involved, were explained to the patients.

Baseline medical treatment was started at admission in ER and included IV administration of antispasmodic drug, butylhyoscine 20 mg, and intramuscular non-steroid anti-inflammatory drug (NSAID), diclofenac 75 mg. Thereafter, diclofenac 75 mg was administered routinely every 12 h.

Lumbar ureteral stones were fragmented with the patient in the supine position, while iliac and pelvic stones in the prone position. At the end of the session, patients completed a visual analog pain scale (0-10). Follow-up over three months comprised evaluation of pain, temperature and fragment elimination, and radiological check-ups (abdominal X-ray and/or ultrasound). Patients in whom ESWL fail to completely disintegrate the stone during a first session underwent repeat sessions. Patients in whom ESWL had no impact on the stone during the first session, as evidenced by abdominal X-ray, were subject to repeated treatment, stent insertion or ureteroscopy.

Interventional procedures (double J stent ± ureteroscopy) were performed within 48-72 hours only in cases of worsening symptoms and impossibility to manage patients medically, appearance of fever or modification in laboratory findings.

Results were compared by the Chi-square test. A 0.05 significance level was used. A mean efficiency quotient (EQ) was calculated according to the formula of Denstedt and co-workers (14): Stone free (%) X 100/ (100 + retreatment rate (%) + rate of auxiliary procedures (%)).

## RESULTS

The mean age of the patients was 39.5 years (11-72 years). Male/female ratio was 85/23. Overall, 21 patients were treated as outpatients and 87 were kept in hospital overnight. All the stones were radiopaque. Their mean size was 8.45 mm (4-20 mm). They were located in the pelvic (n = 53), iliac (n = 28) or lumbar (n = 27) region. A total of 108 patients required 163 sessions of lithotripsy with average number of 3710 shock waves at 10-20 kV. The mean number of sessions per patient was 1.5 (1-3). The procedure was completed successfully in 106 patients and aborted in 2 patients due to pain. After ESWL treatment, pain resolved in 58% of patients, persisted in 28%, and required administration of supplementary anti-inflammatory agents or opioids in 14%. Fragmentation after a single session was complete in 56 patients (52%), incomplete in 28 (26%), and absent in 24 (22%). Patient's characteristics at inclusion and in relation to the first session are reported in Table-1.

Patients presenting incomplete fragmentation underwent a second (n = 28) or even third session (n = 11). Two patients with remnants after two or three sessions underwent ureteroscopy. One patient developed acute obstructive pyelonephritis proximal to a pelvic stone fragments which was successfully treated by a

***Table 1 –*** *Patients characteristics and relation to first ESWL session.*

| | Total | Absent Fragmentation | Incomplete Fragmentation | Complete Fragmentation | p Value |
|---|---|---|---|---|---|
| Sex ratio (M/F) | 85/23 | 20/4 | 21/7 | 44/12 | 0.765 |
| Side (L/R) | 56/52 | 13/11 | 14/14 | 29/27 | 0.956 |
| Age (years) | | | | | |
| mean | 39.5 | 36.8 | 38.8 | 41.1 | 0.256 |
| 95% CI lower-upper | 37.4, 41.6 | 32.8, 40.8 | 34.0, 43.6 | 38.2, 43.9 | |
| Stone location, n (%) | | | | | 0.372 |
| Lumbar ureter | 27 | 6 (22.2) | 7 (25.9) | 14 (51.9) | |
| Iliac ureter | 28 | 6 (21.4) | 11 (39.3) | 11 (39.3) | |
| Pelvic ureter | 53 | 12 (22.6) | 10 (18.9) | 31 (58.5) | |
| Total (%) | 108 | 24 (22.2) | 28 (25.9) | 56 (51.9) | |
| Stone size (mm) | | | | | 0.179 |
| Mean size | 8.20 | 8.75 | 8.93 | 7.68 | |
| Median (range) | 8.0 (4, 20) | 8.0 (4, 17) | 9.0 (5, 20) | 7.0 (4, 20) | |
| 4 to < 6 mm | 19 | 4 | 4 | 11 | |
| 6 to < 10 mm | 65 | 15 | 19 | 31 | |
| 10-20 mm | 24 | 6 | 10 | 8 | |
| Hospital stay (day) | | | | | |
| Mean | 2.4 | 4.2 | 2.4 | 1.6 | < 0.0005 |
| Median (range) | 2.0 (0, 7) | 4 (0, 7) | 3.0 (0, 5) | 2.0 (0, 6) | |

double J stent, antibiotics and the fragments passed spontaneously before stent removal. The 24 patients in whom ESWL had no impact on the stone, underwent a second (n = 15) or even third session (n = 6) without success. Of these, ureteroscopy was performed for 21 cases and open ureterolithotomy for one patient with a hard 17 mm stone while spontaneous passage occurred in two patients with small stones.

The stone-free success rate for ESWL (fragmentation + elimination) was 31 % (n = 33) on day 2, 41% (n = 44) on day 15, 68% (n = 73) on day 30, and 77% (n = 83) on day 90. The retreatment rate ranged from 28% to 44% according to the location of the stone, and from 15.8% to 66.7% according to the size of the stone. EQ at 3 months was 49. Results as a function of stone location and size are given in Table-2; both location and size were considered prognostic factors. The mean size of stones that were completely fragmented at a single session (n = 56) was 7.68 mm (4-20 mm), of those requiring a second session (n = 28) was 8.93 mm (5-20 mm), and of those resistant to ESWL (n = 24) was 8.75 mm (4-17 mm). There were no major complications, although eleven patients mentioned macroscopic hematuria afterwards, none requiring specific treatment which is an expected side-effect to treatment.

Group analysis were performed by combining stone location (lumbar vs. iliac or pelvic) and size (largest diameter < 6, 6 to < 10 mm or 10-20 mm). The amplitude of the benefit, however, was more stringent for stones located proximally and with a size >5 mm.

Median and average hospital stay were 2.0 and 2.4 days (95% lower and upper confidence interval: 2.1-2.8 days). This effect largely depended on the rate of fragmentation after the first session as well as the size and location of the stone.

We were able to analyze stones from 32 patients in the study. The majority of the patients had calcium oxalate stones (n = 20) while the remainder had mixed calcium oxalate and phosphate (n = 6), struvite (n = 5), and cystine stones (n = 1).

## COMMENTS

In the last 20 years, the development and constant improvement of minimally invasive techniques such as ureteroscopy with in situ lithotripsy or laser fragmentation and ESWL has prompted urologists toward a more aggressive attitude. Although observation is still recommended for stones measuring less than 4 mm in diameter, most international guidelines today recommend active removal of all stones exceeding 5-7 mm, when proven that they have resisted medical therapy (9). The spontaneous rate of elimination of the stones depends on the stone size and position in the ureter (2). In a recent prospective study using unenhanced helical CT, Coll et al. have demonstrated that the spontaneous passage rate for stones ranged from 87% to 25% according to the size of stones (1 mm in diameter to more than 9 mm) (15). In the same series, spontaneous passage rate was also dependent on stone location (48% for stones in the proximal

***Table 2 –*** *Results of ESWL at 3 months as a function of stone location and size.*

| | N | Mean Size | Success Rate (%) | Retreatment Rate (%) | Hospital Stay **Mean** (SD) |
|---|---|---|---|---|---|
| Size | | | | | |
| Total | 108 | 8.2 | 82 (76.9) | 38 ( 35.2) | 2.4 (1.8) |
| 4 to < 6 mm | 19 | 4.7 | 13 (84.2) | 3 (15.8) | 1.5 (1.8) |
| 6 to < 10 mm | 63 | 7.6 | 65 (77) | 19 (29.2) | 2.2 (1.7) |
| 10-20 mm | 24 | 12.5 | 17 (70.8) | 16 (66.7) | 3.7 (1.5) |
| Location | | | | | |
| Lumbar | 27 | 9.6 | 22 (81.5%) | 12 (44.4) | 3.1 (1.7) |
| Iliac | 28 | 7.7 | 21 (75.0%) | 11 (39.3) | 2.2 (1.7) |
| Pelvic | 53 | 7.8 | 40 (75.5%) | 15 (28.3) | 2.1 (1.8) |

ureter, 60% for mid ureteral stones, 75% for distal stones, and 79% for ureterovesical junction stones). In addition to size and location, there are also other interfering factors such as obesity, level of renal obstruction and type of medical therapy (16). In our study, most of the stones and fragments that passed spontaneously were 7 mm or less and located in the lower ureter. Active removal is also strongly indicated in patient with persistent pain despite adequate medical treatment, acute obstruction with impaired renal function or solitary functional kidney, urinary tract infection, risk or suspicion of urosepsis (2,17). In cases where removal of ureteral stone is warranted, the main debate centers currently around the choice of ESWL or endoscopic management combined with laser or mechanic fragmentation (4,18,19).

Traditionally, the imaging study used for evaluating patients presenting with ureteral colic believed secondary to an acute episode was IVU. Although the examination was often diagnostic, limitations included inability to obtain proper bowel preparation to aid in imaging because of the acute nature of the study, risk of allergy to contrast agents, potential nephrotoxicity, need to assess renal function before contrast injection, inability of conventional radiography to visualize some stones (e.g., uric acid), and the time-consuming nature of the study. Though renal ultrasonography is sometimes useful in detecting the presence of hydronephrosis secondary to an obstructing ureteral stone, the evaluation is very operator dependent. Furthermore, the study is unable to accurately measure the size of the stone and locate ureteral stones in many instances. Computer tomography (CT) scan is able to address many of these issues and, with the introduction of spiral CT, nonenhanced studies are rapidly becoming the standard means of evaluating patients presenting to emergency departments with acute flank pain (13).

In institutions equipped with ESWL the question arises whether applying ESWL shortly after the onset of renal colic could help resolving this issue. Interestingly enough, although ESWL is widely considered as one of the treatments of choice of ureteral stones, its use as an immediate therapeutic tool in an ER setting has not yet deserved much attention. To our knowledge, only reports by Gonzalez Enguita et al. (8), Doublet et al. (6), Tligui et al. (7), and Tombal et al. (9) have addressed its potential interest. Tligui et al. reported in 2003 their experience of 200 patients suffering from acute renal colic and treated with emergency ESWL (EDAP LT-02) within 24 h. Stone-free rate ranged from 79% to 83% according to the location of the stone, and from 75% to 86% according to the size of the stone. Two or three ESWL sessions were required in 79 patients. The 36 patients, in whom ESWL failed, underwent ureteroscopy (n = 23) or lithotripsy with a Dornier® machine (n = 13). Based on this observation, they advocated a more widespread use of the technique based on a high stone free rates after three months and a low morbidity. These are consistent with our findings. The study however was not randomized. We could not do a randomization of our patients in order to collect a representative number of patients to undergo statistical workup.

Tombal et al. in 2005 reported the results of the first randomized trial addressing the role of emergency ESWL in 100 patients requiring hospitalization for the management of renal colic (9). These authors have prospectively compared standard medical treatment with NSAID and antispasmodic to medical treatment plus emergency ESWL, performed without analgesia on a Siemens Lithostar lithotripter (Siemens Medical Systems, AG, Munich, Germany) within 6 h. following admission to the ER. On average, this study showed that ESWL increased the proportion of patients stone-free (SF) after 48 hours (SF-48) by 13% while it increased the median duration of hospitalization by one day. Emergency ESWL increased both SF-48 and proportion of patients discharged from the hospital at 72 hours by respectively 40% and 25% when the stone was located proximally and > 5 mm, and they advocated that it should be strongly recommended in these cases. In contrast, when the stone is located distally from the crossing of the iliac artery, ESWL only slightly increased stone free rate by 5% while decreasing the proportion of patients released from hospitalization at 48 h and 72 h. Their study demonstrated that emergency ESWL is a valuable therapeutic option to improve elimination of ureteral stones and shorten duration of hospital stay, when proven that the stone is located proximally to the iliac vessels.

A better outcome of ESWL has been reported for kidney stones compared to ureter stones, while

others could not demonstrate such differences (20,21). Pace et al. investigated a large number of ESWL cases and demonstrated a superior success rate for upper and mid ureter stones compared to distal calculi (22). The AUA meta-analysis revealed best stone clearance for small stones < 10 mm, with 74% compared to 46% for stones between 11-20 mm (2). For complete stone disintegration, many patients have to undergo 2 or more shockwave sessions (2). There is reported no consensus on the number of shock wave lithotripsy treatments for ureteral calculi that should be administered for a single stone before alternate modalities are used. Pace et al. (22) have reported a low success rate of repeat shock wave lithotripsy for ureteral stones after failed initial treatment. Kim et al. suggested that no more than 3 treatments should be given for a particular stone due to minimal improvement in the subsequent cumulative treatment success rate (23). We compared the success rate of initial shock wave lithotripsy for ureteral calculi with that of subsequent treatments to determine whether more than 1 treatment is justified for any single ureteral stone. In this respect our results are in agreement with the other reported series as none of our patients responded to repeat sessions after failure of the initial treatment. In a series of 1588 patients they had treated 1593 ureteral calculi with the Dornier MFL 5000 lithotripter (Dornier Medical Systems Inc., Kennesaw, GA) over a period from January 1994 to September 1999 (22). The stone free rate after initial treatment was 68% (1086 of 1593 stones), which decreased to 46% for first re-treatment and 31% for second re-treatment. Overall the success rate increased to 77% after 3 treatments compared with 76% after two treatments. Upper and mid ureter stone free rates were significantly higher than those in the lower ureter after initial treatment. Success rate was also greater for smaller stones (10 mm or less versus 11 to 20 mm was 74% versus 43% ($p < 0:001$). In our series, those patients with incomplete fragmentation after the initial treatment were not offered more than 3 sessions of ESWL and all of them were stone free by 3 months. We found that the stone free rate was higher for smaller stones (9 mm or less versus 10 to 20 mm was 78.6% versus 70.8%, $p = 0.428$). Although the difference was not significant, hospital stay was significantly higher for the large stones (mean; 3.7 vs. 2.1, $p < 0.0005$). It was also significantly higher for the lumber ureter ($p = 0.016$) as the stone size increased in the proximal ureter. Upper stone free rate (81.5%) was higher than those in the mid and lower ureter (75% and 75.5%, respectively ($p = 0.804$)) after initial treatment with higher retreatment rate. The rate of retreatment depends on the stone size and position in the ureter. It increased for upper ureteric stones (37%) compared to the mid and lower ureteric stones (28.6% and 24.5%, respectively). This may be explained by the higher mean stone size (9.6 mm) for upper ureter compared to the mid and lower ureter (7.7 and 7.8 mm, respectively). Also, we found difficulty in localization for some cases with mid ureteric stones as overlapped by the iliac bone. Retreatment for a ureteric stone appeared to increase the stone free rate of initial treatment from 58% to 77%. It may be that stone size is the main predictor factor for the retreatment rate.

With the widespread use of ESWL, fewer stones are being analyzed because of difficulties in collecting stone samples. We were able to analyze stones from 32 patients and calcium oxalate stones were the most common type.

More commonly, hospitalization is required to manage intractable pain resistant to oral or intrarectal therapy. While the main goal of therapy should then still be oriented toward fast pain relief and safe stone removal, it is also critical to achieve rapid discharge from the hospital. In our series the majority of the patients had treatments as an inpatient procedure (81%) mainly for 'social' reasons, i.e. ,difficulty in transport, lack of follow-up, health care facility and less commonly for complications. Overall although, there is still considerable scope for improving the process of supplying emergency interventional care and reducing inpatient stay.

Ureteral pre-stenting is only necessary for patients with persistent pain, fever or renal insufficiency due to obstruction. Some authors reported a decreased stone free rate after introduction of an indwelling stent, most probably due to problems in stone detection and interference with the shock waves (4,22). Especially with older lithotripters, focusing on ureter stones was difficult. For this reason pre-stenting was not part of our treatment. If practical, in situ shockwave lithotripsy in acute obstructive ureteric lithiasis seems to be advantageous compared to later

shockwave application in the non-obstructive phase [28]. Arrabal-Martın et al. recently demonstrated, that in situ ESWL for both obstructive and non-obstructive lumbar ureter stones reached 95.5% and 93.15% stone free rate respectively (4).

As kidney stones were thought to show a better response to ESWL, push-back manipulation into the kidney was recommended for proximal ureter stones. We do not recommend this as with improved lithotripsy and stone detection technology, this procedure is now considered being out-dated. Some investigators (21) have reported a better outcome of ESWL after stone manipulation, while others (20) have not found a statistical difference. However it can prove difficult to manipulate an impacted stone, and the possibility of post-treatment obstruction by a large fragment in an edematous ureter remains. This risk can be minimized by stent placement at the time of stone manipulation. Advances in ureteroscopic technology with the introduction of small caliber semi-rigid and flexible ureteroscopes combined with the introduction of the holmium YAG laser have improved stone free rates following ureteroscopy while decreasing the risk of complications (24,25).

Success rates for shock wave lithotripsy may differ according to the lithotripter used. Average stone-free rate for cumulative shock wave lithotripsy series reported in the literature using an HM3 lithotripter is slightly but consistently higher than that achieved with many second and third generation lithotripters and may influence the choice of treatment (26). It is important to stress that the results with shock wave lithotripsy are truly machine specific and cannot be translated to use with other lithotripters (19). The Dornier Lithotripter S that we use proved in different series to be very effective in the treatment of renal and ureteral calculi (18).

In conclusion, rapidly performed ESWL is a valuable therapeutic option to improve elimination of ureteral stones. We agree with the other authors that it could be more widespread in acute renal colic. It presents medical advantages, i.e. no need for prolonged anti-inflammatory treatment, and also possible economic advantages, i.e. no need for anesthesia and routine hospitalization with fewer absences from work. It requires appropriate lithotripter facilities for emergency use and a follow-up period of up to three months. Ultimately, the chosen treatment option (medical treatment, ESWL, or ureteroscopy) is a matter of a joint decision between the physician and the informed patient.

## CONFLICT OF INTEREST

None declared.

### REFERENCES


1. Assimos DG, Boyce WH, Harrison LH, McCullough DL, Kroovand RL, Sweat KR: The role of open stone surgery since extracorporeal shock wave lithotripsy. J Urol. 1989; 142: 263-7.
2. Segura JW, Preminger GM, Assimos DG, Dretler SP, Kahn RI, Lingeman JE, et al.: Ureteral Stones Clinical Guidelines Panel summary report on the management of ureteral calculi. The American Urological Association. J Urol. 1997; 158: 1915-21.
3. Tiselius HG, Ackermann D, Alken P, Buck C, Conort P, Gallucci M, et al.: Guidelines on urolithiasis. Eur Urol. 2001; 40: 362-71.
4. Arrabal-Martín M, Pareja-Vilches M, Gutiérrez-Tejero F, Miján-Ortiz JL, Palao-Yago F, Zuluaga-Gómez A: Therapeutic options in lithiasis of the lumbar ureter. Eur Urol. 2003; 43: 556-63.
5. Tombolini P, Ruoppolo M, Bellorofonte C, Zaatar C, Follini M: Lithotripsy in the treatment of urinary lithiasis. J Nephrol. 2000; 13 (Suppl 3) : S71-82.
6. Doublet JD, Tchala K, Tligui M, Ciofu C, Gattegno B, Thibault P: In situ extracorporeal shock wave lithotripsy for acute renal colic due to obstructing ureteral stones. Scand J Urol Nephrol. 1997; 31: 137-9.
7. Tligui M, El Khadime MR, Tchala K, Haab F, Traxer O, Gattegno B, et al.: Emergency extracorporeal shock wave lithotripsy (ESWL) for obstructing ureteral stones. Eur Urol. 2003; 43: 552-5.
8. González Enguita C, Cabrera Pérez J, Calahorra Fernández FJ, García Cardoso J, Vela Navarrete R. Efficient, immediate or emergency ESWL: an attractive strategic alternative to be considered in the treatment of renal colic! Actas Urol Esp. 2000; 24: 721-7.
9. Tombal B, Mawlawi H, Feyaerts A, Wese FX, Opsomer R, Van Cangh PJ: Prospective randomized evaluation of emergency extracorporeal shock wave lithotripsy (ESWL) on the short-time outcome of symptomatic

ureteral stones. Eur Urol. 2005; 47: 855-9. Erratum in: Eur Urol. 2005; 48: 876.
10. Cass AS: In situ extracorporeal shock wave lithotripsy for obstructing ureteral stones with acute renal colic. J Urol. 1992; 148: 1786-7.
11. Numa H, Yoshida K, Kageyama Y, Hoshino Y. In situ extracorporeal shock wave lithotripsy for ureteral stones causing acute renal failure. Hinyokika Kiyo. 1994; 40: 291-4.
12. Joshi HB, Obadeyi OO, Rao PN: A comparative analysis of nephrostomy, JJ stent and urgent in situ extracorporeal shock wave lithotripsy for obstructing ureteric stones. BJU Int. 1999; 84: 264-9.
13. Wang LJ, Ng CJ, Chen JC, Chiu TF, Wong YC: Diagnosis of acute flank pain caused by ureteral stones: value of combined direct and indirect signs on IVU and unenhanced helical CT. Eur Radiol. 2004; 14: 1634-40.
14. McDougall EM, Denstedt JD, Brown RD, Clayman RV, Preminger GM, McClennan BL: Comparison of extracorporeal shock wave lithotripsy and percutaneous nephrolithotomy for the treatment of renal calculi in lower pole calices. J Endourol. 1989; 3: 265-71.
15. Coll DM, Varanelli MJ, Smith RC: Relationship of spontaneous passage of ureteral calculi to stone size and location as revealed by unenhanced helical CT. AJR Am J Roentgenol. 2002; 178: 101-3.
16. Deliveliotis C, Chrisofos M, Albanis S, Serafetinides E, Varkarakis J, Protogerou V: Management and follow-up of impacted ureteral stones. Urol Int. 2003; 70: 269-72.
17. Anagnostou T, Tolley D: Management of ureteric stones. Eur Urol. 2004; 45: 714-21.
18. Ghalayini IF, Al-Ghazo MA, Khader YS: Extracorporeal shockwave lithotripsy versus ureteroscopy for distal ureteric calculi: efficacy and patient satisfaction. Int Braz J Urol. 2006; 32: 656-64; discussion 664-7.
19. Hochreiter WW, Danuser H, Perrig M, Studer UE: Extracorporeal shock wave lithotripsy for distal ureteral calculi: what a powerful machine can achieve. J Urol. 2003; 169: 878-80.
20. Rassweiler J, Lutz K, Gumpinger R, Eisenberger F: Efficacy of in situ extracorporeal shock wave lithotripsy for upper ureteral calculi. Eur Urol. 1986; 12: 377-86.
21. Graff J, Pastor J, Funke PJ, Mach P, Senge T: Extracorporeal shock wave lithotripsy for ureteral stones: a retrospective analysis of 417 cases. J Urol. 1988; 139: 513-6.
22. Pace KT, Weir MJ, Tariq N, Honey RJ: Low success rate of repeat shock wave lithotripsy for ureteral stones after failed initial treatment. J Urol. 2000; 164: 1905-7.
23. Kim HH, Lee JH, Park MS, Lee SE, Kim SW: In situ extracorporeal shockwave lithotripsy for ureteral calculi: investigation of factors influencing stone fragmentation and appropriate number of sessions for changing treatment modality. J Endourol. 1996; 10: 501-5.
24. Devarajan R, Ashraf M, Beck RO, Lemberger RJ, Taylor MC: Holmium: YAG lasertripsy for ureteric calculi: an experience of 300 procedures. Br J Urol. 1998; 82: 342-7.
25. Scarpa RM, De Lisa A, Porru D, Usai E: Holmium:YAG laser ureterolithotripsy. Eur Urol. 1999; 35: 233-8.
26. Gettman MT, Segura JW: Management of ureteric stones: issues and controversies. BJU Int. 2005; 95 (Suppl 2): 85-93.

*Accepted after revision:*
*May 23, 2008*

**Correspondence address:**
Dr. Ibrahim Fathi Ghalayini
Associate Professor of Urology
P.O. Box 940165
Amman, 11194, Jordan
Fax: + 00962 6 5687422
E-mail: ibrahimg@just.edu.jo

## EDITORIAL COMMENT

We read with interest this original article about the role of shock wave lithotripsy (ESWL) as an emergency treatment of ureteral lithiasis. Unfortunately, while the debate between ESWL and ureteroscopy for such stones is still going on, the importance of ESWL as an emergency approach to face this problem has been merely evaluated (1-3). Wherever lithotripters are available, ESWL may represent the non invasive way to perform an active stone removal, attempting to resolve this common and potentially severe emergency. Its safety leads to an ease of use in most of the cases, excluding those with absolute contraindications (pregnancy, uncorrected bleeding disorders) or complicated features (i.e. ureteral stones associated with urosepsis and /or severe renal function impairment).

One of the crucial points of emergency ESWL trials is the choice of a proper end point: fragmentation and expulsion are achieved gradually after ESWL, and those phases, above all the expulsive one, can last a considerable and variable period of time, depending on stone size, location and ureteral edema. Due to this last consideration, ESWL of ureteral stone is highly recommended within a short period from the onset of acute renal colic. In fact, in uncomplicated cases, like the ones reported in this series, a satisfying stone free rate is rapidly achieved after the treatment. Moreover, one of the main outcomes of this study is that pain was rapidly and definitively controlled after ESWL in 58% of the cases, thus allowing a watchful waiting approach of spontaneous passage of the fragments. Pain relief may enable a faster discharge, and even if only 21 were treated as outpatients, Authors invoked an improve in the delivery process that can be easily achieved. Furthermore, hospitalization, its length and relationship with stone characteristics were properly analyzed in this manuscript.

Since renal colic due to stone disease is a widespread problem, we have previously assessed the role of ESWL as an emergency treatment of ureteral stones associated with mild renal function impairment. Our outcomes focused on the ability of a single session ESWL to decrease rapidly creatinine serum levels, and a normalization of such parameter was evident in 85% of the patients 24 hours after the treatment. A complete stone free condition was then reached gradually (67.5% at 72 hours), and 7 out of 40 patients underwent a successful second session ESWL. Characteristics were properly analyzed in this manuscript.

Since renal colic due to stone disease is a widespread problem, we have previously assessed the role of ESWL as an emergency treatment of ureteral stones associated with mild renal function impairment. Our outcomes focused on the ability of a single session ESWL to decrease rapidly creatinine serum levels, and a normalization of such parameter was evident in 85% of the patients 24 hours after the treatment. A complete stone free condition was then reached gradually (67.5% at 72 hours), and 7 out of 40 patients underwent a successful second session ESWL (4).

Except for this last consideration, our findings are consistent with those reported by the Authors, as ESWL turns out to be effective even as an emergency procedure, potentially reducing the need for an endoscopic management.

Few minor concerns remain, i.e. the tricky focusing of ureteral stones overlapping iliac bone and the role of ureteral stenting, that still represents a matter of debate. Furthermore, we believe that the definite role of repeated sessions have yet to be defined, and greater series in a prospective setting have to assess the value of ESWL multiple treatments.

### REFERENCES

1. Kravchick S, Bunkin I, Stepnov E, Peled R, Agulansky L, Cytron S: Emergency extracorporeal shockwave lithotripsy for acute renal colic caused by upper urinary-tract stones. J Endourol. 2005; 19: 1-4.
2. Doublet JD, Tchala K, Tligui M, Ciofu C, Gattegno B, Thibault P: In situ extracorporeal shock wave lithotripsy for acute renal colic due to obstructing ureteral stones. Scand J Urol Nephrol. 1997; 31: 137-9.
3. Tligui M, El Khadime MR, Tchala K, Haab F, Traxer O, Gattegno B, et al.: Emergency extracorporeal shock wave lithotripsy (ESWL) for obstructing ureteral stones. Eur Urol. 2003; 43: 552-5.

4. Sighinolfi MC, Micali S, DE Stefani S, Grande M, Saredi G, Mofferdin A, et al.: Noninvasive management of obstructing ureteral stones using electromagnetic extracorporeal shock wave lithotripsy. Surg Endosc. 2008 (In press).

***Dr. M. C. Sighinolfi, Dr. S. Micali***
***Dr. S. De Stefani & Dr. G. Bianchi***
*Department of Urology*
*University of Modena and Reggio*
*Modena, Emilia, Italy*
*E-mail: sighinolfic@yahoo.com*

Clinical Urology
International Braz J Urol Vol. 34 (4): 443-450, July - August, 2008

# Contemporary Analysis of Erectile, Voiding, and Oncologic Outcomes Following Primary Targeted Cryoablation of the Prostate for Clinically Localized Prostate Cancer

**Christopher J. DiBlasio, Ithaar H. Derweesh, John B. Malcolm, Michael M. Maddox, Michael A. Aleman, Robert W. Wake**

*Department of Urology, University of Tennessee Health Science Center, Memphis, Tennessee, USA*

**ABSTRACT**

*Purpose:* To evaluate erectile function (EF) and voiding function following primary targeted cryoablation of the prostate (TCAP) for clinically localized prostate cancer (CaP) in a contemporary cohort.
*Materials and Methods:* We retrospectively reviewed all patients treated between 2/2000-5/2006 with primary TCAP. Variables included age, Gleason sum, pre-TCAP prostate specific antigen (PSA), prostate volume, clinical stage, pre-TCAP hormonal ablation, pre-TCAP EF and American Urologic Association Symptom Score (AUASS). EF was recorded as follows: 1 = potent; 2 = sufficient for intercourse; 3 = partial/insufficient; 4 = minimal/insufficient; 5 = none. Voiding function was analyzed by comparing pre/post-TCAP AUASS. Statistical analysis utilized SAS software with $p < 0.05$ considered significant.
*Results:* After exclusions, 78 consecutive patients were analyzed with a mean age of 69.2 years and follow-up 39.8 months. Thirty-five (44.9%) men reported pre-TCAP EF level of 1-2. Post-TCAP, 9 of 35 (25.7%) regained EF of level 1-2 while 1 (2.9%) achieved level 3 EF. Median pre-TCAP AUASS was 8.75 versus 7.50 postoperatively ($p = 0.39$). Six patients (7.7%) experienced post-TCAP urinary incontinence. Lower pre-TCAP PSA ($p = 0.008$) and higher Gleason sum ($p = 0.002$) were associated with higher post-TCAP AUASS while prostate volume demonstrated a trend ($p = 0.07$). Post-TCAP EF and stable AUASS were not associated with increased disease-recurrence ($p = 0.24$ and $p = 0.67$, respectively).
*Conclusions:* Stable voiding function was observed post-TCAP, with an overall incontinence rate of 7.7%. Further, though erectile dysfunction is common following TCAP, 25.7% of previously potent patients demonstrated erections suitable for intercourse. While long-term data is requisite, consideration should be made for prospective evaluation of penile rehabilitation following primary TCAP.

***Key words:*** *prostatic neoplasms; cryoablation; erectile dysfunction; voiding dysfunction*
***Int Braz J Urol. 2008; 34: 443-50***

## INTRODUCTION

With an expected 218,890 new cases and 27,050 deaths estimated in 2007, prostate cancer (CaP) is the most common malignancy in men in the United States (1).With the ongoing stage migration of contemporary CaP, patients diagnosed with apparent organ-confined tumors are faced with a spectrum of treatment modalities including surgical, radiation, medical and surveillance. All curative and palliative treatments for CaP are associated with some degree of morbidity, and the effects on quality of life (QOL) can be quite pronounced (2).

Targeted cryoablation of the prostate (TCAP) has emerged as an accepted therapy for both primary treatment of clinically localized CaP, as well as for

salvage therapy following failed definitive therapy, demonstrating survival and cancer-control at least equivocal to external-beam radiotherapy (3-7). Erectile dysfunction (ED) and voiding dysfunction are common following all potentially curative CaP treatments. ED is reported to be particularly pronounced following TCAP due to the hypothermic impact of the ice ball on the peri-prostatic nerves. However, since no ligation of the neurovascular bundle occurs during TCAP, the potential for axonal regeneration exists (8). As such, several series have reported varying degrees of erectile function (EF) recovery following TCAP (3,9-13). Additionally, "nerve-sparing" TCAP has shown encouraging results with regards to recovery of EF (14,15).

In addition to effects on EF, conflicting reports of the impact TCAP has on voiding function and lower urinary tract symptoms (LUTS) have been documented (3,5,9-12). We investigated our experience with primary TCAP in an attempt to determine EF and voiding function outcomes, as well as predictive factors for improved results, in patients treated for clinically localized CaP.

## MATERIALS AND METHODS

After obtaining institutional review board approval, we retrospectively reviewed all patients with biopsy-proven CaP treated with TCAP between 2/2000-5/2006 at our institution. Patients receiving salvage TCAP and those with incomplete pre- or post-TCAP EF or voiding function data were excluded. All procedures were performed utilizing the Cryocare prostate cryoablation system (Endocare, Inc., Irvine, CA) with real-time transrectal-ultrasound monitoring and a brachytherapy grid for probe/thermosensor placement as has been previously described.(3, 10, 11) All procedures utilized a urethral warmer and employed a six-cryoprobe technique, implementing a double-freeze-thaw cycle.

Clinicopathologic variables included age at TCAP, race, body mass index (BMI), pre-TCAP prostate specific antigen (PSA), Gleason sum (GS), clinical stage, prostate volume, receipt of neoadjuvant androgen deprivation therapy (NADT), and pre-TCAP EF and American Urological Association Symptom Score (AUASS) assessment. NADT was typically employed to downsize glands > 40 $cm^3$. Pre and post-TCAP EF scores and AUASS were recorded at last pre-TCAP follow-up and compared to scores at last post-TCAP follow-up. AUASS were compared as continuous variables, as well as divided categorically according to AUA guidelines: mild (0-7, Category 1), moderate (8-19, Category 2) and severe (20-35, Category 3) (16). Urinary incontinence was defined as any degree of urine leakage, and the number of pads (if any) was recorded. Patients were followed-up with a history/physical examination, PSA, EF score, and AUASS every 3 months for 2 years, every 6 months for the next 2, and annually thereafter. EF was documented using a 5-point scale as follows: 1 = fully potent, 2 = erections sufficient for intercourse, 3 = partial erections insufficient for intercourse, 4 = minimal erections insufficient for intercourse and 5 = no erections. Post-TCAP ED therapy was noted: including phosphodiesterase type-5 inhibitors (PDE5i), prostaglandin E1 analogues (PGE1), vacuum erection device (VED), and inflatable penile prosthesis (IPP). Notably, it was not our routine practice during the study period to encourage penile rehabilitation post-TCAP. Disease-recurrence/progression was defined as: biochemical (BCR) according to the American Society for Therapeutic and Radiology and Oncology (ASTRO) criteria (17), biopsy-proven local recurrence (LCR), distant metastasis, by initiation of salvage ADT (SADT) for rising PSA, or by CaP-related death.

Data analysis utilized Student's-t-test , Chi square and Kruskal-Wallis analysis of variance (where appropriate), as well as univariate/multivariate logistic regression, with all potential explanatory covariates incorporated into models. Independent variables were modeled as continuous and categorical variables as follows: age $\geq$ 70 vs. < 70 years, Gleason grade sum $\geq$ 7 vs. < 7, PSA $\geq$ 10 vs. < 10 ng/mL, prostate volume $\geq$ 30 vs. < 30 $cm^3$ and BMI $\geq$ 30 vs. < 30 $kg/m^2$. All p-values were based on 2-sided tests of significance, with $p < 0.05$ considered statistically significant. The Hosmer-Lemeshow test eliminated models that fit poorly. Statistical analysis utilized SAS computerized software, version 9.1 (SAS Institute Inc., Cary, NC).

## RESULTS

Demographic data and disease characteristics are outlined in Table-1. After exclusions, 78 consecutive patients were analyzed with a mean age of 69.2 years (range: 55.3 - 80.9), pre-TCAP PSA of 9.4 ng/mL (range: 0.8 - 84.0), Gleason grade sum of 6.5 (range: 3 - 9), and prostate volume of 29.7 $cm^3$ (range: 10-50).

### Erectile Function

Overall, 61 (78.2%) patients reported some degree of pre-TCAP EF with or without the use of erectile aids. However, only 35 (of 61, 57.4%) reported pre-TCAP erections sufficient for intercourse (EF levels 1 or 2). Seventeen (21.8%) patients reported pre-TCAP impotence and 2 of these underwent post-TCAP IPP. At a mean follow-up of 39.8 months (range: 0.6-92.4), 10 (16.4%) patients regained EF; 9 (14.8%) achieving level 2 EF with PDE5i only (n = 3), VED only (n = 2), or PDE5i/VED (n = 4) and 1 (1.6%) achieving level 3 EF with PDE5i/VED. Subset analysis of the 35 men who were previously potent (EF level 1-2), however, demonstrated the post-TCAP EF recovery rate to be 25.7%. The mean time to potency restoration was 15.2 months (range: 9.7-29.3). Notably, the 2 patients who underwent IPP were not regarded as potent post-TCAP. No significant clinical predictors of post-TCAP EF were identified on either univariate or multivariate analysis (data not shown). Post-TCAP EF was not associated with an increased risk of disease-recurrence (p = 0.24). EF outcomes are outlined in Table-2.

### Voiding Dysfunction

Median pre-TCAP AUASS was 8.75 (range: 0-31.0) vs. 7.50 (range: 0-33.0) postoperatively and did not change with treatment (p = 0.39). When analyzed categorically, 34 (43.6%) men reported Category 1, 37 (47.4%) reported Category 2, and 7 (9.0%) reported Category 3 LUTS. Post-TCAP, 39 (50.0%), 33 (42.3%), and 6 (7.7%) men reported categories 1, 2, and 3 LUTS, respectively. There was no difference between pre and post-TCAP AUASS when compared categorically (p = 0.74). Six patients (7.7%) experienced urinary incontinence at last follow-up: 1 (1.3%) requiring 0-1 pads/day, 1 (1.3%) requiring 1-2 pads/day, 2 (2.6%) requiring 2-3 pads/day and 1 (1.3%) who developed a bladder neck contracture, which was dilated, resulting in > 3 pads/day incontinence. Overall, 46 patients (59.0%) demonstrated improved or stable AUASS, while 32 (41.0%) reported worsening LUTS. Importantly, the presence of post-TCAP improved/stable AUASS was not associated with an increased risk of cancer recurrence (p = 0.67). Voiding function findings are outlined in Table-3.

On logistic regression (Table-4), lower pre-TCAP PSA (Odds Ratio (OR) 3.06; p = 0.008) and higher Gleason sum (OR 3.80; p = 0.002) were associated with higher post-TCAP AUASS. Larger pre-TCAP prostate volume demonstrated a trend towards worsening LUTS and higher post-TCAP AUASS outcomes (p = 0.07). However, receipt of NADT did not demonstrate a relationship with post-TCAP AUASS (p = 0.67). No patients underwent a pre or post-TCAP transurethral resection/ablation of the prostate for urinary retention or LUTS during the study period.

### Cancer-Control/Disease-Progression

Thirteen (16.7%) patients demonstrated disease-progression: 10 (76.9%) BCR, 2 (15.4%) LCR, and 1 (7.7%) SADT. No CaP-related deaths were noted during the study period. Mean time-to-recurrence was 11.9 months (median 9.9; range: 5.7-23.8). On Kaplan-Meier analysis, BCR-free survival was 97.9% at 1 year, 95.7% at 3 years and 82.9% at 5 years. Progression-free survival was 97.9% at 1 year, 95.7% at 3 years, and 71.1% at 5 years. Four (5.1%) patients died of unrelated causes and were censored at the time of death. Overall survival was 95.9% at 1 year, 94.3% at 3 years, and 94.3% at 5 years.

Multivariate categorical analysis demonstrated African American race (OR 4.46, p = 0.03), GS ≥ 7 (OR 6.4, p = 0.02), pre-TCAP PSA ≥ 10 ng/mL (OR 3.82, p = 0.002), and age ≥ 70 years (OR 2.74, p = 0.01) to predict disease-recurrence, while NADT administration trended towards decreased recurrence (OR 2.23, p = 0.05; Table-5). On continuous variable analysis; however, only age (OR 2.74, p = 0.01) remained a predictor of disease-progression (Table-6).

***Table 1*** *– Demographic and clinicopathologic data on 78 men undergoing primary targeted cryoablation of the prostate (TCAP) for clinically localized prostate cancer.*

| **Variable** | |
|---|---|
| Number of patients | 78 |
| Age at TCAP (years) | |
| Mean | 69.2 |
| Median (range) | 69.6 (55.3 - 80.9) |
| Pretreatment serum PSA level (ng/mL) | |
| Mean | 9.4 |
| Median (range) | 8.9 (0.8 - 84.0) |
| Gleason grade sum (mean (median, range)) | 6.5 (6.0, 3.0 - 9.0) |
| Primary Gleason grade | 3.3 (3.0, 2.0 - 5.0) |
| Secondary Gleason grade | 3.2 (3.0; 1.0 - 5.0) |
| BMI ($kg/m^2$) | |
| Mean | 29.2 |
| Median (range) | 29.1 (14.1 - 46.1) |
| Race (N/%) | |
| African-American | 36 (46.2) |
| Caucasian/Other | 42 (53.8) |
| Prostate Volume ($cm^3$) | |
| Mean | 29.7 |
| Median (range) | 30.0 (10.0 - 50.0) |
| Receipt of NADT (N/%) | 38 (48.7) |
| Number of NADT injections (mean; median (range)) | 1.6 (1.0, 1.0 - 3.0) |
| Clinical Stage (N/%) | |
| T1a/b | 1 (1.3) |
| T1c | 59 (75.6) |
| T2a | 8 (10.3) |
| T2b | 6 (7.7) |
| T2c | 3 (3.8) |
| T3a | 1 (1.3) |
| PSA nadir (ng/mL) | |
| Mean | 0.5 |
| Median (range) | 0.1 (0.0 - 6.0) |
| Time to progression (months) | |
| Mean | 11.9 |
| Median (range) | 9.9 (5.7 - 23.8) |
| Follow Up Time (months) | |
| Mean | 39.8 |
| Median (range) | 37.3 (0.6 - 92.4) |

*NADT = neoadjuvant androgen deprivation therapy.*

***Table 2*** *– Distribution of pre- and post- targeted cryoablation of the prostate (TCAP) erectile function and dysfunction.*

| EF Level | **Pre-TCAP** (N/%) | **Post-TCAP** (N/%) |
|---|---|---|
| 1 | 9 (11.5) | 0 (0) |
| 2 | 26 (33.4) | 9 (11.5) |
| 3 | 17 (21.8) | 1 (1.3) |
| 4 | 9 (11.5) | 0 (0) |
| 5 | 17 (21.8) | 68 (87.2) |

## COMMENTS

TCAP has steadily gained popularity for primary and salvage treatment of clinically localized CaP (3,7). Erectile and voiding dysfunctions are common following all potentially curative CaP therapies. As these morbidities continue to be elucidated, there is increasing interest in improving QOL outcomes for men undergoing CaP-directed treatments, with particular emphasis on ED and voiding outcomes. However, there remains both a paucity of data focusing on these outcomes, as well as considerable variability following primary TCAP (3,5-7,9,11,13). Particular to EF, the thought that TCAP resulted in irreversible ED secondary to hypothermic injury to the cavernous nerves is being questioned. The potential for axonal regeneration after TCAP-related neuropraxia lends credence to reports of EF recovery following TCAP (8).

Bahn et al. reported 7-year outcomes on these endpoints in 590 men undergoing primary TCAP. Of 373 men potent pre-TCAP, only 19 (5.1%) recovered potency at an average of 16.4 months post-TCAP. Regarding voiding function, of 533 previously continent patients, 448 (84.1%) regained continence at an average of 6.1 months (3). In another series, Han et al.

***Table 3*** *– Analysis of pre- and post- targeted cryoablation of the prostate (TCAP) American Urologic Association Symptom Score (AUASS).*

| **Variable** | **Pre-TCAP** | **Post-TCAP** | **p Value** |
|---|---|---|---|
| AUASS | | | |
| Mean | 9.96 | 9.11 | 0.39 |
| Median (range) | 8.75 (0.0 - 31.0) | 7.50 (0.0 - 33.0) | |
| AUASS Category (N/%) | | | |
| Category 1 | 34 (43.6) | 39 (50.0) | 0.75 |
| Category 2 | 37 (47.4) | 33 (42.3) | |
| Category 3 | 7 (9.0) | 6 (7.7) | |

***Table 4*** *– Logistic regression analysis for predicting worsening post- targeted cryoablation of the prostate (TCAP) lower urinary tract symptoms (i.e. increased American Urologic Association Symptom Score).*

| **Variable** | **Odds Ratio** | **p Value** |
|---|---|---|
| Age (years) | 1.68 | 0.112 |
| Pre-TCAP PSA (ng/mL) | 3.05 | 0.008 |
| BMI ($kg/m^2$) | 0.66 | 0.516 |
| Prostate volume ($cm^3$) | 1.93 | 0.070 |
| Gleason sum | 3.80 | 0.002 |

***Table 5 –*** *Logistic regression analysis for predicting disease-recurrence utilizing categorical variable modeling.*

| Variable | Odds Ratio | p Value |
|---|---|---|
| Age ≥ 70 years (vs. < 70) | 2.74 | 0.01 |
| African-American race (vs. other) | 4.46 | 0.03 |
| Pre-TCAP PSA ≥ 10 ng/mL (vs. < 10) | 3.82 | 0.002 |
| BMI ≥ 30 kg/m$^2$ (vs. < 30) | 1.10 | 0.66 |
| Prostate volume ≥ 30 cm$^3$ (vs. < 30) | 0.84 | 0.95 |
| Gleason sum ≥ 7 (vs. < 7) | 6.40 | 0.02 |
| Receipt of NADT (vs. none) | 2.23 | 0.05 |

*TCAP = targeted cryoablation of the prostate; NADT = neoadjuvant androgen deprivation therapy.*

***Table 6 –*** *Logistic regression analysis for predicting disease-recurrence utilizing continuous variable modeling.*

| Variable | Odds Ratio | p Value |
|---|---|---|
| Age (years) | 2.74 | 0.01 |
| Pre-TCAP PSA (ng/mL) | 0.90 | 0.37 |
| BMI (kg/m2) | 0.77 | 0.45 |
| Prostate volume (cm$^3$) | 0.70 | 0.49 |
| Gleason sum | 2.44 | 0.02 |

reported on 106 patients undergoing primary TCAP. They observed impotence rates of 87% for previously potent men, while 3 (3%) required pads for urinary incontinence (5). Similarly, Polascik et al. reported on 50 men undergoing primary TCAP, documenting a response rate of 50% (3 of 6 previously potent men) with the use of PDE5i therapy after TCAP. Further, they found a 3.7% rate of post-TCAP incontinence, requiring 1-2 pads/day (6). In a questionnaire-based study, Anastasiadis et al. reviewed 131 men undergoing primary or salvage TCAP. They found that the most bothersome symptoms following TCAP were sexual, followed by urinary complaints. In particular, ED (90% vs. 86%) and incontinence rates (10% vs. 5.9%) were significantly worse in the salvage versus primary TCAP groups, respectively (9).

Our series demonstrated a recovery rate of EF suitable for intercourse of 25.7% in previously potent men (14.8% overall), with responses to VED, PDE5i, or both in combination (Table-2). It is important to reiterate that it was not our general practice to actively pursue or recommend penile rehabilitation in these patients. Therefore, we infer the potential for further improvement in EF outcomes with implementation of penile rehabilitation protocols post-TCAP. In fact, Ellis et al. reported a recent series of 416 consecutive men undergoing primary TCAP whereby daily VED use was recommended (without constriction ring) beginning 6 weeks post-TCAP for previously potent men along with PDE5i every other day and as needed beginning 6 months post-TCAP (11). They documented progressive EF recovery with this protocol: 29.1% regaining EF at 1 year, 48.5% at 2 years, and 51.3% at 4 years. Similarly, encouraging findings of EF recovery have been documented in other series (13), as well as our own (25.7%), even in the absence of penile rehabilitation. These findings have prompted us to adopt a regimen of aggressive penile rehabilitation following TCAP.

Regarding voiding outcomes, we identified urinary incontinence rates comparable to most series, with 7.7% of men reporting some degree of urine leakage (regardless of pad usage) (3,5,9-12). However, pre-TCAP continence was not recorded consistently in our cohort. Thus, we considered all men to be fully continent prior to TCAP, which may be an overestimation in this regard. Since considerable variations in incontinence definitions exist in the literature, we employed a strict definition of post-TCAP incontinence as any degree of leakage (regardless of pad usage) in order to capture any patient with this complaint.

Our series demonstrated oncologic outcomes similar to most contemporary literature (3,5,6,10,11,13). Specific to our series, it is noteworthy that improved EF or voiding function demonstrated no association with increased risk for cancer recurrence ($p = 0.24$ and $p = 0.67$, respectively). In other words, a suboptimal freeze cannot explain the outcomes seen in our cohort. Another unique finding of our analysis was the increased risk of disease-progression in African-American patients (compared to others) on multivariate analysis (OR 4.46, $p = 0.03$). While previously reported in radiation and prostatectomy series (18,19), to our knowledge, ours is the first series documenting this association in men undergoing primary TCAP.

An additional novel feature of our series is the use of validated objective assessments of LUTS (AUASS) (16). To our knowledge, no prior series has utilized this instrument for LUTS comparisons in men undergoing TCAP. Our series demonstrated stable AUASS, whether analyzed as continuous or categorical variables. Regression analysis demonstrated worsening AUASS to be associated with lower pre-TCAP PSA and higher Gleason sum (Table-4). While the significance of this remains unclear, we hypothesize that higher grade cancers that produce less PSA due to glandular de-differentiating may respond differently to the cryobiology of TCAP, potentially contributing to these findings. Notably, larger prostate volumes demonstrated a trend towards worsening post-TCAP LUTS, though this was not statistically significant ($p = 0.07$). However, NADT administration did not demonstrate a significant relationship with post-TCAP AUASS ($p = 0.67$). We suspect that with larger series and longer follow-up, a relationship between improved LUTS may be realized based on the ability of NADT to reduce the overall prostate volume, potentially offering improved voiding outcomes in this patient population, though we were unable to demonstrate this in our current series.

There are several limitations to this study. Firstly, we report a retrospective review of our findings at a single center and as such, our findings are subject to the inherent biases of this type of analysis. Consequently, patients were not evaluated in a prospective fashion using validated EF instruments such as the Sexual Health Inventory for Men or International Index of Erectile Function questionnaires to document objective pre- and post-TCAP EF. Additionally, our cohort remains relatively small ($n = 78$) with a somewhat short duration of follow-up (39.8 months). Further, selection bias may have occurred as we studied only patients with complete pre- and post-TCAP EF and AUASS data. Thus, these potential biases may limit the ability to demonstrate all potential relationships between variables and the endpoints of the study.

Nonetheless, we feel this data is compelling enough to further investigate the possibility of improving EF outcomes following primary TCAP. Penile rehabilitation has proven useful following radical prostatectomy (20), and results appear encouraging following TCAP, though data is limited (11). For these reasons, we have integrated this strategy into our pre-operative and post-TCAP treatment protocol, utilizing validated questionnaires to objectively determine our outcomes.

With regards to LUTS, TCAP does not seem to improve nor worsen symptoms to any significant degree based on our results. However, a discussion of the potential for urinary incontinence is paramount, as this remains a considerable bother to patients who experience this complaint following TCAP (9).

## CONCLUSIONS

Primary TCAP resulted in stable postoperative AUASS, while ED remains common. However, 25.7% of previously potent men demonstrated EF suitable for successful intercourse in the absence of penile rehabilitation. Neither the restoration of EF, nor the presence of stable/improved LUTS were associated with disease-recurrence and therefore, not a result of suboptimal cryoablation. While long-term, prospective data employing validated instruments is requisite, implementation of a proactive penile rehabilitation protocol should be considered in order to maximize sexual outcomes following primary TCAP.

## ACKNOWLEDGEMENT

Kimberly D. Lamar, Ph.D. provided statistical analysis of this study.

## CONFLICT OF INTEREST

None declared.

### REFERENCES


1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ: Cancer statistics, 2007. CA Cancer J Clin. 2007; 57: 43-66.
2. Penson DF, Litwin MS: Quality of life after treatment for prostate cancer. Curr Urol Rep. 2003; 4: 185-95.
3. Bahn DK, Lee F, Badalament R, Kumar A, Greski J, Chernick M: Targeted cryoablation of the prostate: 7-year outcomes in the primary treatment of prostate cancer. Urology. 2002; 60: 3-11.
4. Chin JL, Ng CK, Touma NJ, Pus NJ, Hardie R, Abdelhady M, et al.: Randomized trial comparing cryoablation and external beam radiotherapy for T2C-T3B prostate cancer. Prostate Cancer Prostatic Dis. 2008; 11: 40-5.
5. Han KR, Cohen JK, Miller RJ, Pantuck AJ, Freitas DG, Cuevas CA, et al.: Treatment of organ confined prostate cancer with third generation cryosurgery: preliminary multicenter experience. J Urol. 2003; 170: 1126-30.
6. Polascik TJ, Nosnik I, Mayes JM, Mouraviev V: Short-term cancer control after primary cryosurgical ablation for clinically localized prostate cancer using third-generation cryotechnology. Urology. 2007; 70: 117-21.
7. Wake RW, Hollabaugh RS Jr, Bond KH: Cryosurgical ablation of the prostate for localized adenocarcinoma: a preliminary experience. J Urol. 1996; 155: 1663-6.
8. Donnelly BJ, Saliken JC, Ali-Ridha N, Rewcastle JC, White LJ: Histological findings in the prostate two years following cryosurgical ablation. Can J Urol. 2001; 8: 1237-9.
9. Anastasiadis AG, Sachdev R, Salomon L, Ghafar MA, Stisser BC, Shabsigh R, et al.: Comparison of health-related quality of life and prostate-associated symptoms after primary and salvage cryotherapy for prostate cancer. J Cancer Res Clin Oncol. 2003; 129: 676-82.
10. Ellis DS: Cryosurgery as primary treatment for localized prostate cancer: a community hospital experience. Urology. 2002; 60: 34-9.
11. Ellis DS, Manny TB Jr, Rewcastle JC: Cryoablation as primary treatment for localized prostate cancer followed by penile rehabilitation. Urology. 2007; 69: 306-10.
12. Long JP, Bahn D, Lee F, Shinohara K, Chinn DO, Macaluso JN Jr: Five-year retrospective, multi-institutional pooled analysis of cancer-related outcomes after cryosurgical ablation of the prostate. Urology. 2001; 57: 518-23.
13. Robinson JW, Donnelly BJ, Saliken JC, Weber BA, Ernst S, Rewcastle JC: Quality of life and sexuality of men with prostate cancer 3 years after cryosurgery. Urology. 2002; 60: 12-8.
14. Bahn DK, Silverman P, Lee F Sr, Badalament R, Bahn ED, Rewcastle JC: Focal prostate cryoablation: initial results show cancer control and potency preservation. J Endourol. 2006; 20: 688-92.
15. Lambert EH, Bolte K, Masson P, Katz AE: Focal cryosurgery: encouraging health outcomes for unifocal prostate cancer. Urology. 2007; 69: 1117-20.
16. Barry MJ, Fowler FJ Jr, O'Leary MP, Bruskewitz RC, Holtgrewe HL, Mebust WK, et al.: The American Urological Association symptom index for benign prostatic hyperplasia. The Measurement Committee of the American Urological Association. J Urol. 1992; 148: 1549-57; discussion 1564.
17. Horwitz EM, Vicini FA, Ziaja EL, Dmuchowski CF, Stromberg JS, Martinez AA: The correlation between the ASTRO Consensus Panel definition of biochemical failure and clinical outcome for patients with prostate cancer treated with external beam irradiation. American Society of Therapeutic Radiology and Oncology. Int J Radiat Oncol Biol Phys. 1998; 41: 267-72.
18. Powell IJ, Dey J, Dudley A, Pontes JE, Cher ML, Sakr W, et al.: Disease-free survival difference between African Americans and whites after radical prostatectomy for local prostate cancer: a multivariable analysis. Urology. 2002; 59: 907-12.
19. Tewari A, Horninger W, Pelzer AE, Demers R, Crawford ED, Gamito EJ, et al.: Factors contributing to the racial differences in prostate cancer mortality. BJU Int. 2005; 96: 1247-52.
20. Mulhall JP, Morgentaler A: Penile rehabilitation should become the norm for radical prostatectomy patients. J Sex Med. 2007; 4: 538-43.


*Accepted:*
*May 9, 2008*

**Correspondence address:**
Dr. Robert W. Wake
Department of Urology
University of Tennessee Health Science Center
910 Madison Avenue, Room 409
Memphis, TN, 38163, USA
Fax: + 1 901 448-1044
E-mail: rwake@utmem.edu

Clinical Urology
International Braz J Urol Vol. 34 (4): 451-456, July - August, 2008

# The Length of the Male Urethra

**Tobias. S. Kohler, Mitchell Yadven, Ankur Manvar, Nathan Liu, Manoj Monga**

*Division of Urology (TSK), Southern Illinois University, Springfield, IL, Department of Urologic Surgery (MY, NL, MM), University of Minnesota, Minneapolis, MN, Department of Urologic Surgery (AM), Northwestern University, Feinberg School of Medicine, Chicago, Illinois, USA*

**ABSTRACT**

*Purpose:* Catheter-based medical devices are an important component of the urologic armamentarium. To our knowledge, there is no population-based data regarding normal male urethral length. We evaluated the length of the urethra in men with normal genitourinary anatomy undergoing either Foley catheter removal or standard cystoscopy.
*Materials and Methods:* Male urethral length was obtained in 109 men. After study permission was obtained, the subject's penis was placed on a gentle stretch and the catheter was marked at the tip of the penis. The catheter was then removed and the distance from the mark to the beginning of the re-inflated balloon was measured. Alternatively, urethral length was measured at the time of cystoscopy, on removal of the cystoscope. Data on age, weight, and height was obtained in patients when possible.
*Results:* The mean urethral length was 22.3 cm with a standard deviation of 2.4 cm. Urethral length varied between 15 cm and 29 cm. No statistically significant correlation was found between urethral length and height, weight, body mass index (BMI), or age.
*Conclusions:* Literature documenting the length of the normal male adult urethra is scarce. Our data adds to basic anatomic information of the male urethra and may be used to optimize genitourinary device design.

***Key words:*** *genitourinary; catheter; urethra*
***Int Braz J Urol. 2008; 34: 451-6***

## INTRODUCTION

The Egyptians developed the first use of catheters by using an instrument made of reed that was inserted like a plug. The term "katheter" originates from "kathiemai," meaning 'to sound' with a probe. The Greeks further developed this katheter by using a hollow metal tube inserted into the male urethra to empty the bladder.

Modern day catheter use is ubiquitous and catheter-based medical devices are an important component of the urologic armamentarium. Since its development in 1935, use of Frederick Foley's urethral catheter has remained pervasive with little modification to its design. Current urethral catheters typically measure 40-45 cm in length. With recent developments of new catheter-based treatments such as microwave therapy of the prostate or cryotherapy, specialized catheters have been developed. One would expect further novel catheter use in the future, and perhaps a departure from the standard Foley design to optimize bladder drainage, minimize cost and improve patient comfort. In addition, the length of flexible and rigid endoscopes for transurethral applications would benefit from standardization based on the expected length of the male urethra. As such, defining the mean length of the male urethra is critical.

Despite an extensive online literature search and review of anatomic atlases, only one reference to the anatomic length of the male urethra was found. Gray's Anatomy describes a male urethral length of 17.5 to 20 cm (1). However, an exhaustive review of the aforementioned citation's reference list suggests that supportive evidence is lacking. One prior study utilizing retrograde urethrograms noted that the membranous urethral length ranged from 1 to 1.5 cm (2). A second study reported that the average prostatic urethral length was 2.4 cm (3). Lastly, a more detailed anatomical study reports the urethral length for an infant male to be 5.6 cm (4).

## MATERIALS AND METHODS

The study was initiated after IRB consent from all participating institutions was obtained. Patients were recruited from a large Midwest Veteran's Administration Hospital and from a private urologic practice in Florida. Subjects with a history of prostatectomy or urethral surgery were ineligible for the study. To measure urethral length, two methods were employed. The majority of subject data was obtained upon removal of an indwelling Foley catheter. Prior to catheter removal, the subject's penis was placed on a gentle stretch, the balloon of the catheter was gently "cinched" to the bladder neck, and the catheter was marked at the tip of the penis with tape. The catheter was then removed and the distance from the mark to the beginning of the re-inflated balloon was measured in centimeters (n = 79). Alternatively, urethral length was established at the time of flexible cystoscopy upon removal of the cystoscope. The scope was held fixed at the bladder neck with the penis on stretch and the cystoscope was similarly marked with tape at the end of the penis. The cystoscope was then removed and the distance from the mark to the end of the cystoscope was measured in centimeters (n = 30). Stretched penile length has previously been established as surrogate marker for erect penile length (5). No measurements of separate prostatic, membranous, bulbar or pendulous urethral segments were obtained. After measurement was completed, subject data on age, weight, and height was obtained in most patients. BMI was calculated as kg/meter$^2$. All statistics were performed using SPSS software.

## RESULTS

Data was collected on 109 men. The mean urethral length was 22.3 cm with a standard deviation of 2.4 cm. Mean values for age, weight, height, and body mass index (BMI) were 70.7 years, 92 kg, 1.8 meters, and 28.6 kg/m$^2$ (Table-1). Urethral length varied between 15 cm and 29 cm. The distribution of urethral length is shown in Figure-1. No statistically significant correlation was found between urethral length and weight, height, BMI, or age utilizing Pearson's correlation 2-tailed test (Table-2).

## COMMENTS

Literature documenting the length of the normal male adult urethra is scarce. This study determined the average urethral length to be about 22 cm

***Table 1 –*** *Descriptive statistics (n = 109).*

| | Minimum | Maximum | Mean | SD |
|---|---|---|---|---|
| Age | 42.0 | 89.0 | 70.7 | 12.2 |
| Weight (kg) | 61.0 | 141.0 | 92.0 | 17.8 |
| Height (m) | 1.7 | 2.0 | 1.8 | 0.1 |
| BMI (kg/m(m)) | 20.0 | 44.5 | 28.6 | 5.4 |
| Urethra(cm) | 15.0 | 29.0 | 22.3 | 2.4 |

*SD = standard deviation; BMI = body mass index.*

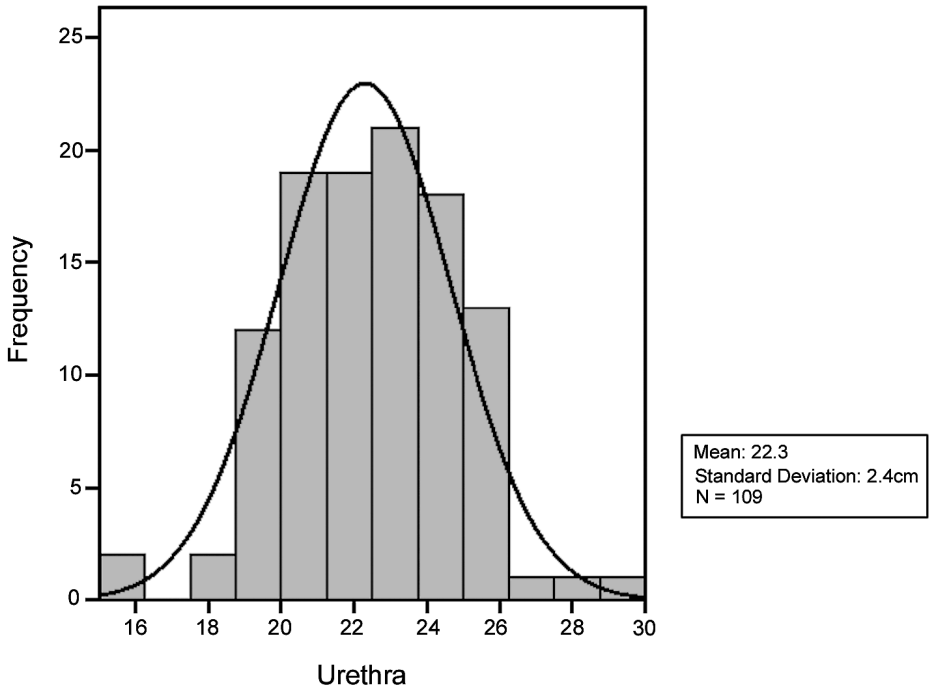



***Figure 1*** *– Urethral length (cm) distribution.*

with a standard deviation of 2.4 cm. Our data adds to basic anatomic information of the male urethra and may be used to optimize genitourinary device design.

***Table 2*** *– Correlations.*

| | **Urethra** |
|---|---|
| Age | |
| Pearson correlation | 0.19 |
| Significance (2-tailed) | p = 0.26 |
| Weight | |
| Pearson correlation | 0.09 |
| Significance (2-tailed) | p = 0.58 |
| Height | |
| Pearson correlation | 0.08 |
| Significance (2-tailed) | p = 0.59 |
| BMI | |
| Pearson correlation | 0.06 |
| Significance (2-tailed) | p = 0.7 |

*BMI = body mass index.*

Current designs have an average length around 40-45 cm, regardless of the lumen size (Table-3). This design is almost double the length of the average male urethral length. Our mean determination or urethral length incorporates differences in penile length and prostatic urethral length, two factors that have a wide variability. Prostatic lengths have been shown to range from (2.5-4.5cm) influenced by both baseline anatomy, and the effect of benign prostatic hyperplasia (6). Our study excluded those with prostatic surgery, but allows for the random variation of prostate size attributable to benign prostatic hyperplasia of our age population. Any variation on prostate length would be accounted for by the standard deviation of our urethral length determination. Similarly, penile lengths are variable in the population. We used stretched penile length in our measurements as it is the only validated surrogate marker for erect length (5). Previous studies have compared both stretched and flaccid penile length to height and found no statistically significant correlation (7). Finding of a positive correlation between urethral length and

***Table 3 –*** *Catheter lengths for 16F adult catheters.*

| **Catheter Manufacturer** | **Length** (inches) | **Length** (cm) |
|---|---|---|
| Bard | 16 | 41 |
| Cook | 13.4 | 34 |
| Boston Scientific | 19.7 | 50 |
| Rochester Medical | 15.9 | 40 |

*Average adult catheter length = 16.2 in or 41 cm.*

height has intrinsic appeal, as one could then predict which catheter length would be appropriate (similar to selecting ureteral stents based on height). However, our findings parallel the penile length data, where no statistically significant correlation between height or BMI and urethral length were found ($p = 0.7$).

By measuring the total male urethral length of our subjects, we are able to obtain a mean length and calculate a standard deviation. Thus, ideal future catheter design seeking to minimize excess catheter material requirements should aim for an intra-urethral length of about 22 cm with variations in length 1-2 standard deviations above and below this length. Based on our results, catheter manufacturers could design catheters that would accommodate the majority of male patient's urethras: 17 to 27 cm. Other authors have determined maximum total penile and urethral extensibility, further justifying the rationale for shorter catheter design (8).

## CONCLUSION

Literature documenting the length of the normal male adult urethra is surprisingly scarce. Our study found the average length of the male urethra was 22.3 cm and there was no statistically significant correlation between urethral length and height. Our data adds to basic anatomic information of the male urethra and may be used to optimize genitourinary device design.

## CONFLICT OF INTEREST

None declared.

## REFERENCES


1. Gray H: Anatomy of the Human Body. Philadelphia, Lea & Fiberger. 1985, 13th ed.
2. McCallum RW: The adult male urethra: normal anatomy, pathology, and method of urethrography. Radiol Clin North Am. 1979; 17: 227-44.
3. Rolnick HC, Arnheim FK: An anatomic study of the external urethral sphincter in relation to prostatic surgery. J Urol. 1949; 61: 591-603.
4. Parker AE: The lymph vessels from the posterior urethra, their regional lymph nodes and relationships to the main posterior abdominal lymph channels. J Urol. 1936; 36:538-57.
5. Wessells H, Lue TF, McAninch JW: Penile length in the flaccid and erect states: guidelines for penile augmentation. J Urol. 1996; 156: 995-7.
6. Elshaikh MA, Angermeier K, Ulchaker JC, Klein EA, Chidel MA, Mahoney S, et al.: Effect of anatomic, procedural, and dosimetric variables on urinary retention after permanent iodine-125 prostate brachytherapy. Urology. 2003; 61: 152-5.
7. Awwad Z, Abu-Hijleh M, Basri S, Shegam N, Murshidi M, Ajlouni K: Penile measurements in normal adult Jordanians and in patients with erectile dysfunction. Int J Impot Res. 2005; 17: 191-5.
8. Da Silva EA, Sampaio FJ: Urethral extensibility applied to reconstructive surgery. J Urol. 2002; 167: 2042-5.


*Accepted after revison:*
*June 10, 2008*

**Correspondence address:**
Dr. Tobias S. Köhler
Southern Illinois University School of Medicine
Division of Urology
PO Box 19638
Springfield, IL, 62794, USA
E-mail: gambitguy@hotmail.com

## EDITORIAL COMMENT

This study assessed urethral length in 109 men aged 42-89 years at the time of Foley catheter removal or whilst undergoing flexible cystoscopy. The authors found a mean urethral length of 22.3 cm (range 15-29, SD 2.4) and suggest the finding might lead to revised catheter designs with intra-urethral lengths ranging from 17 to 27 cm. It is proposed that customized catheter lengths would save on excess catheter materials, reduce costs and improve patient comfort. Although the results are interesting, altering catheter design in relation to the data may not necessarily achieve these aims. Optimal patient comfort, for example, requires sufficient extra-urethral catheter length for ease of appliance attachment and for tension-free catheter immobilization with adhesive tape. In addition, the production of catheters with such a restrictive length would undoubtedly cause problems in the occasional patient with well-above average penile length.

***Dr. K. A. Hutton***
*Department of Pediatric Surgery*
*University Hospital of Wales*
*Heath Park, Cardiff, South Glamorgan*
*Wales, United Kingdom*
*E-mail: kim.hutton@cardiffandvale.wales.nhs.uk*

## EDITORIAL COMMENT

Albert Einstein was quoted to say, "Everything should be as simple as it is, but not simpler", and the study design and methods of this paper could certainly be considered simple. The authors of this study attempt to measure the length of the normal male urethra by either direct cystoscopic measurements (n = 30) or by marking indwelling urethral catheters prior to removal (n = 79). They found that the average stretched male urethral length is 22 cm ± 5 cm. Per their literature review, there has been no previous attempt at a population-based urethral length evaluation. When considering all the times that we as urologists traverse this area blindly (urethral catheter placement, sounds, transurethral microwave thermotherapy, the new prostate SpannerT), I am astounded to find that this is the first study to address this issue. Although no statistically significant predictor of urethral length was identified, I predict that the urethral length conclusion of this study will ("simply" put) be quoted in anatomy textbooks for years to come.

***Dr. Benjamin K. Canales***
*Assistant Professor of Urology*
*University of Florida*
*Gainesville, Florida, USA*
*E-mail: benjycanales@yahoo.com*

## EDITORIAL COMMENT

The authors measured the urethral length in 109 men, 42 to 89 years old, and found that it varied between 15 and 29 cm (mean 22.3 ± 2.4). They noted that the currently used catheters have an average length of 40 to 45 cm, which is almost double the average male urethral length. Thus, they suggest that future catheter design should aim for an intra-urethral length of 22 ± 2 cm to minimize cost. This may be applied also for the design of catheter-based genito-urinary devices.

The authors are to be congratulated for this interesting and unprecedented idea. However, the current study is limited by the relatively small number of patients and by missing the group of patients younger than 42 years. The authors are to be encouraged to corroborate on this issue using a larger number of patients and including all age groups. Thus, they might be able to stratify the length of male urethra - and accordingly the appropriate catheter - into 3 categories: short, medium, and long.

***Dr. M. M. Koraitim***
*Department of Urology*
*University of Alexandria College of Medicine*
*Alexandria, Egypt*
*E-mail: koraitim_mm@yahoo.com*

Clinical Urology

International Braz J Urol Vol. 34 (4): 457-466, July - August, 2008

# Significant Alterations of Serum Cytokine Levels in Patients with Peyronie's Disease

**Reinhold P. Zimmermann, Gerhard Feil, Conny Bock, Lorenz Hoeltl, Arnulf Stenzl**

*Department of Urology (RPZ, LH), Elisabethinen Hospital, University Affiliated Hospital, Universities of Vienna and Innsbruck, Linz, Austria, and Department of Urology (GF, CB, AS), Eberhard-Karls-University of Tuebingen, Tuebingen, Germany*

**ABSTRACT**

*Objective:* To determine the expression of the cytokines transforming growth factor-β1 (TGF-β1), interferon-γ (IFN-γ), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) in serum from patients with Peyronie's disease (PD) compared to healthy controls.

*Materials and Methods:* Ninety-one consecutive PD patients aged 20 - 74 years were included in this study. All patients were diagnosed with symptomatic PD for the first time and had a palpable penile plaque. The patients previously had the disease for 6 - 72 months. None of the patients had a severe infectious disease or known systemic illness. For cytokine analyses, peripheral venous blood samples were obtained before treatment. Fifty healthy male blood donors aged 22 - 64 years served as the control group. TGF-β1, IFN-γ, Il-6, and TNF-α were analyzed quantitatively with commercial immunoassays.

*Results:* Mean cytokine levels in serum from patients were increased for TGF-β1 and IFN-γ compared to healthy controls. The difference for TGF-β1 was considered statistically significant ($p < 0.001$). IL-6 was not detectable in PD patients ($p < 0.01$) and TNF-α was decreased ($p < 0.0001$).

*Conclusion:* The significantly elevated serum level of the profibrotic TGF-β1 cytokine underscores the effect of cytokines in the pathophysiology of PD. The significantly decreased TNF-α serum level suggested no acute immunomodulatory process. Therefore, the relevance for therapeutic administration of TNF-α should be further investigated. Quantification of TGF-β1 in serum of PD patients provides a possible diagnostic tool and target for therapy. The data on altered cytokine levels in PD patients also provide a new understanding for etiopathogenesis of PD, which warrants further investigation.

***Key words:*** *Peyronie's disease; pathophysiology; cytokines; IL-6; TNF-α; TGF-β; IFN-γ*

***Int Braz J Urol. 2008; 34: 457-66***

## INTRODUCTION

Peyronie's disease (PD) has been known for a very long time and even the ancient Egyptians reported PD-like symptoms. In 1743, the French surgeon and court physician François de la Peyronie was the first to scientifically describe penile deviation due to tumors and penile nodes (1). He separated the syndrome from more common sexually transmitted diseases. PD was previously thought to be associated with unnatural or excessive sexual behavior. Degenerative alterations like ageing of the penile fascia layers or penile trauma were considered as possible causes of PD as well as systemic diseases like hyperuricemia, diabetes, rheumatism, and regular alcohol or nicotine consumption. PD is thought to mainly affect 0.15 - 3.0 % of men between the age of 40 - 60 years. The origin of the superficial fibromata with plaque formation between

the corpora cavernosa and the tunica albuginea (TA) still remains unknown (2,3).

The anatomical-pathological correlation of PD might be a traumatically caused inflammation followed by proliferation of fibroblasts and the formation of scar areas such as fibrous plaques and, in some cases, bone formation. The indurations are primarily found on the dorsal surface of the penis and can extend as far as the corpora cavernosa and the deep penile fascia. Smith, in his histological investigations, explained that PD was a form of fibrosis resulting from chronic vasculitis (2). The hypothesis that penile micro-traumata and disorders resulting from the healing of wounds can cause PD has been reported as the most likely explanation (4).

The involvement of the immune system in triggering fibroblast proliferation or an autoimmune reaction to infectious agents, as well as an alteration in the collagen metabolism, have been reported as possibilities due to the fact that erectile tissue in PD patients exhibits a significant increase of collagen fibers (5-7).

Consistently due to the important role of cytokines in mediating inflammation and fibrosis, the identification of appropriate mediators could lead to a better understanding of pathophysiological mechanisms in PD. Accordingly, inflammatory cytokines like IL-6 and TNF-α and the fibrosis-associated cytokines TGF-β and IFN-γ are of particular interest. TGF-β is known to induce fibrosis and IFN-γ is a marker for fibroblast proliferation.

## MATERIALS AND METHODS

Ninety-one consecutive patients initially diagnosed with symptomatic PD were included in this study. Age range of the patients was 20 - 74 (average age 52) years. All patients had a palpable penile plaque, which was verified by means of magnet resonance tomography (MRT) (detection rate 100%). The plaques led to penile deviation during erection in all patients, 70% of these showed a deviation angle between 30 – 60 degrees. The patients previously had the disease for at least 6 months (range 6 - 72 months). None of the patients had a severe infectious disease or known systemic illness. All patients were intended to be treated by extracorporeal shock wave therapy (ESWT) in the context of a prospective randomized placebo controlled study. All additional treatment procedures were stopped at least 12 weeks prior to inclusion in the study. For cytokine analyses, peripheral venous blood samples were obtained before treatment. Fifty healthy male blood donors aged 22 - 64 (average age 41) years with no systemic or local disease served as a control group. Serum samples were immediately stored at -20°C and defrosted for cytokine determination at regular intervals. Cytokine levels were determined quantitatively by commercially available immunoassays (Table-1). Cytokines were measured in duplicates. The assays were performed according to the manufacturer's instructions. Statistical analyses were calculated by t-test using JMP software for personal computers (Version 3.2.6., SAS Institute Inc., Cary, NC)

## RESULTS

Analyses of the fibrosis-associated cytokines TGF-β1 and IFN-γ revealed increased levels in serum from patients compared to healthy male blood donors. TGF-β1 was increased by 14%, whereas IFN-γ was not expressed in the healthy controls (Figures-1 and

***Table 1** – Immunoassays used for cytokine detection.*

| Parameter | Method |
|---|---|
| TGF-β1 | Immunoassay (R&D-Systems Inc., Minneapolis, MN.) |
| INF-γ | Immunoassay (R&D-Systems Inc., Minneapolis, MN.) |
| IL-6 | Immulite-System (DPC, Los Angeles, CA.) |
| TNF-α | Immulite-System (DPC, Los Angeles, CA.) |

2). The difference for TGF-β1 was statistically highly significant ($p < 0.001$). The inflammatory cytokine IL-6 was not detectable in PD patients (Figure-3). Interestingly, TNF-α was 5-fold higher in serum of healthy blood donors (Figure-4). Differences in comparison to the control group were statistically

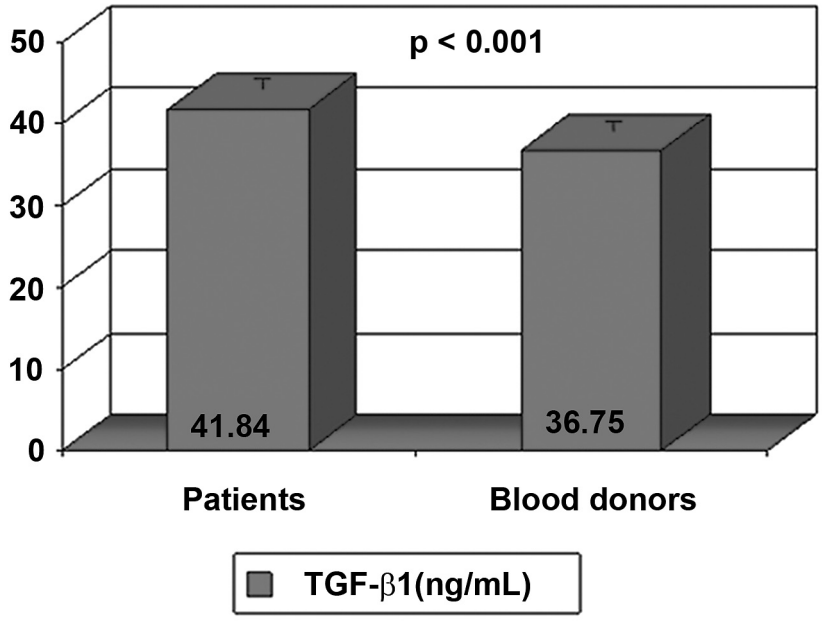



***Figure 1*** *– TGF-β1 serum levels in PD patients and healthy blood donors.*

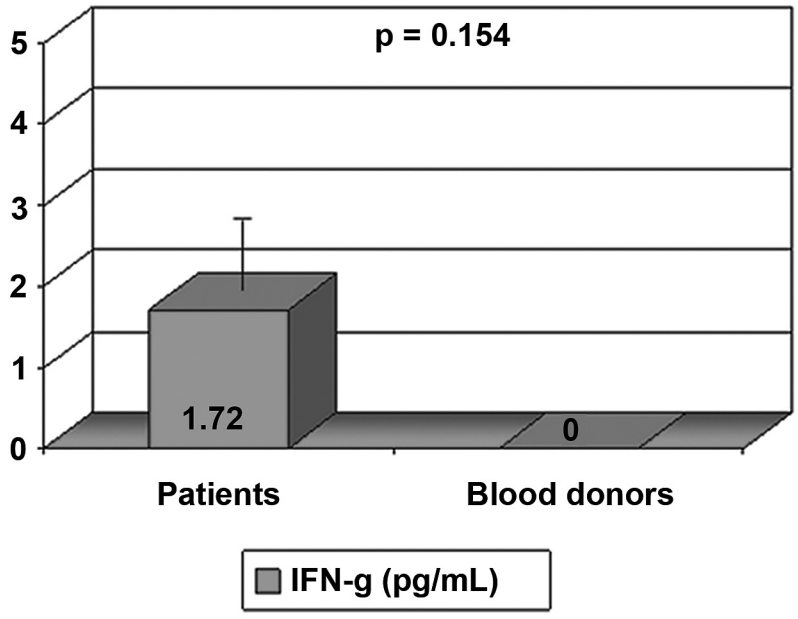



***Figure 2*** *– IFN-γ serum levels in PD patients and healthy blood donors.*

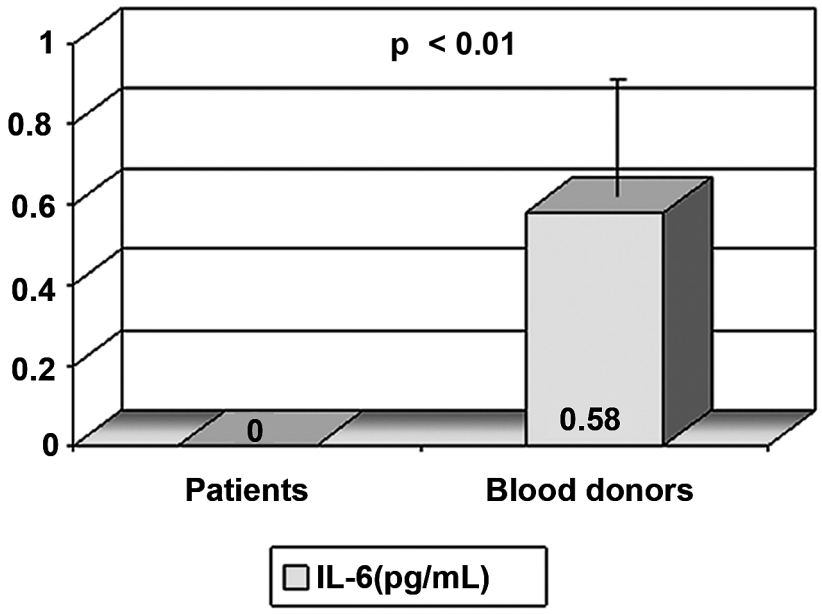



***Figure 3*** *– IL-6 serum levels in PD patients and healthy blood donors.*

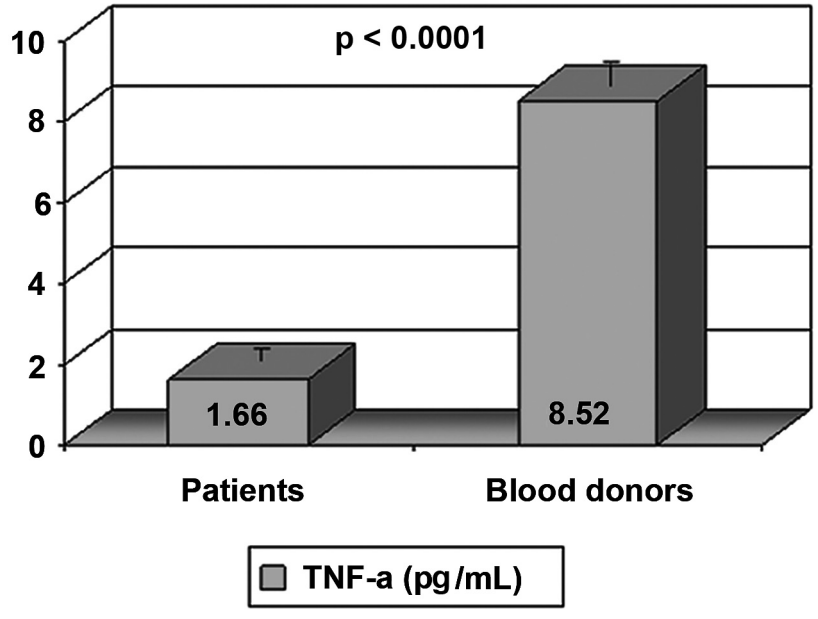



***Figure 4*** *– TNF-α serum levels in PD patients and healthy blood donors.*

significant for IL-6 ($p < 0.01$) and highly significant for TNF-α ($p < 0.0001$). The mean values (± SEM) and ranges of TGF-β1, IFN-γ, IL-6, and TNF-α are shown in Table-2.

***Table 2*** *– Mean values (± SEM, standard error of the mean) and range of cytokine levels in serum of PD patients and male blood donors.*

| Parameter | **Patients** (N = 91) | | **Blood Donors** (N = 50) | | |
|---|---|---|---|---|---|
| | **Mean Value** (± SEM) | **Range** | **Mean Value** (± SEM) | **Range** | **p Value** |
| TGF-β1 (ng/mL) | 41.84 (± 1.15) | 21.70-61.80 | 36.75 (± 0.72) | 27.31-51.39 | < 0.0002 |
| IFN-γ (pg/mL) | 1.72 (± 0.89) | 0.00-63.75 | 0.00 (± 0.00) | | 0.1540 |
| IL-6 (pg/mL) | 0.00 (± 0.00) | | 0.58 (± 0.29) | 0.00-8.90 | 0.0068 |
| TNF-α (pg/mL) | 1.66 (± 0.31) | 0.0-18.20 | 8.52 (± 0.56) | 0.00-20.5 | < 0.0001 |

## COMMENTS

Cytokines promote communication between immunocompetent cells. Thus, if illness activates the immune system, cytokine levels are locally or - despite a short half life - systemically measurable. Previous investigations have opened a possible link between various types of cytokines and PD provoking factors.

All patients with PD over a long period of time, i.e. at least more than 12 months, who were scheduled to be treated within our placebo-controlled prospectively randomized ESWT study, had to meet the strict inclusion criteria. The patients who previously had undergone different medical therapy schedules without exception, had their therapy stopped at least three months before treatment and therefore also prior to cytokine investigation. The majority had been treated by vitamin E or para-aminobenzoic acid. However, at the beginning of our investigation no patients were allowed to undergo any additional therapy either pharmacological or physical. Due to the extremely varying treatment schemes, it made no sense in our opinion to stratify the patients with regards to duration or type of previous treatments. In fact, patient's medical history continued the same course in the vast majority of cases for more than 12 months. This was established by the fact that the "stable disease" was one of the crucial inclusion criteria for our prospectively randomized ESWT study.

Subdividing the patients based on age did not seem reasonable because the particular length of PD medical history was a crucial factor for this study. Therefore, we could not explain why, for example, a patient at the age of 30 with PD for a certain period of time showed different results from a patient of 60 with PD.

However, the majority of our patients were between 40 to 60 years of age.

Cytokines levels after ESWT, to our knowledge, have not been previously investigated. This is regrettable from the current point of view and at the time the ESWT study was completed we did not know the results of the cytokines investigation. Therefore, we did not conclude that it could be of interest to repeat the investigations after the end of ESWT treatment.

The major profibrotic cytokines are IL-4, TGF-β1 and platelet derived growth factor, while IL-6 and TNF-α can act as potent promoters of inflammatory and destructive processes in fibrotic diseases. In contrast, IFN-γ is known as the most potent antifibrotic agent. This was the rational basis for making this choice of cytokines.

Fibrosis is a pathologic process including scar formation and overproduction of extracellular

matrix by connective tissue as a response to tissue damage. It involves inflammation and disruption of normal tissue architecture followed by tissue repair and accumulation of mesenchymal cells. The molecular process is not different from normal formation of connective tissue and extracellular matrix in organs. Pathogenesis of fibrosis has not yet been completely understood. Evidence has been accumulating which suggests that immunologically and cytokine mediated mechanisms are pivotal.

Cytokines may have a causal connection with fibrotic diseases, leading to typical and final alterations of organs and their functionality. They are well known to be involved in many fibrotic diseases (Table-3) of lung, liver, kidney, pancreas, and systemic aggressive fibromatosis as well as colloid and hypertrophic scar formation (8-13). With the exception of a reported series from our group including a smaller patient population (14) to our knowledge there have been no further investigations of serum cytokine levels in PD.

TGF-β can be considered as an immunoregulatory and strong profibrotic factor, which is antagonized in part by IFN-α and -γ. The wound healing process in TA in particular involves TGF-β1, showing a clear correlation to other fibrotic diseases. Pulmonary or liver fibrosis also show elevated local expression of fibrotic factors causing alterations in

***Table 3 –*** *Local and systemic cytokine alterations in various fibrosis-associated diseases.*

| **Disease** | **TGF-beta-1** (profibrotic) | **IFN-gamma** (anti TGF-b1) | **IL-6** (fibrogenic) | **TNF-alpha** (mitogenic) |
|---|---|---|---|---|
| Peyronie's disease | +(S) / +(l) | +(S) | -(S) | -(S) |
| Cystic fibrosis (mild disease) | +(L) | +(L) | | |
| Cystic fibrosis (acute phase) | -(L) | -(L) | | -(L)/(S) |
| Cystic fibrosis (chronic phase) | +(L) | -(L) | +(L) | +(L) |
| Bronchiolitis obliterans | | +(L) | +(L) | |
| Pulmonary fibrosis (chronic) | +(L) | | +(L) (transplant patients) | |
| Chronic obstructive pulmonary disease | +(L) | | +(L) | +(L) |
| Pulmonary fibrosis (idiopathic) | +(L) | | | +(L) |
| Hepatitis viral acute | No change | | +(S) | +(S) |
| Hepatitis viral chronic/cirrhosis | +(S) | -(S) | +(S) | +(S) |
| Hepatitis C (recurrent following lung transplantation) | +(S) | | | |
| Liver fibrosis chronic | | | | +(S) |
| Pancreatitis chronic (onset) | | +(L) | +(L) | +(L) |
| Aggressive fibromatosis | +(L) | | +(L) | +(L) |
| Peritonitis chronic in peritoneal dialysis patients | -(L) | | -(L) | |
| Oral submucosis fibrosis | | -(S) | +(S) | +(S) |
| Hypertrophic scar tissue | +(S) | | | |

*+ = increased level; - = decreased level; L = local alterations (tissue); S = alterations in peripheral blood.*

the composition of connective tissue. For example, patients who had peritonitis during continuous ambulatory peritoneal dialysis showed increased levels of TGF-β1 in the peritoneal dialysate effluent in relation to non-infectious patients, which has been considered as an active release of these proinflammatory cytokines (15).

Idiopathic pulmonary fibrosis, the adult respiratory distress syndrome, and the focal tumor stroma in lung cancer have also revealed a local increase of TGF-β1 (9). Serum concentrations of TNF-α and TGF-β1 have shown to be significantly increased in the majority of patients with chronic viral hepatitis and liver cirrhosis (8). In severely wounded patients elevated serum levels of TGF-β1 have been reported. These authors concluded that this could be due to secretion of wound fibroblasts (16). In the development of renal fibrosis, TGF-β1 is considered to play a key role as regards cytokines and growth factors (10). In contrast, in an animal model the progression of chronic liver fibrosis could have been prevented by inhibition of TGF-β1 (17). In a major series conducted by Hauck and co-workers it was shown that genetic alterations of the TGF-β1 gene could influence the predisposition to PD (18). In the rat penis of a PD animal model (19), as well as in TA of PD patients, local TGF-β1-expression was reported to be 5-fold higher than in healthy subjects. Injections of TGF-β1 or the very similar cytomodulin in TA of rat penis produced chronic cellular infiltration, elastosis, thickening, and clumping of collagen bundles. These PD-like histological alterations resulted in a significant reduction of erectile function (20,21). Incision and suture repair of the rat penis revealed inflammatory reactions similar to those observed in an acute phase of PD, in particular a transient up-regulation of TGF-β1 protein expression. Thus, TGF-β1 has been clearly shown to be an initiation factor of wound healing as well as fibrosis. Possibly, the transformation of the inactive TGF-β1 molecule into an active one is already induced even by minimal tissue lesions with consecutive cellular reparative and fibrotic mechanisms. This fact supports the theory of a traumatic genesis of PD. Based on these findings TGF-β1 may be considered to be one of the central agents in the predisposition and manifestation of PD.

Additionally, other plaque-inducing substances like fibrin (22) might be involved. The significantly higher TGF-β1 levels in serum of PD patients revealed major systemic factors of well known local alterations, particularly as none of the patients showed further signs of fibrotic alterations, but revealed the "typical" pro-fibrotic cytokine constellation in serum. Our results significantly demonstrated a critical role for TGF-β1 in the formation of PD and suggest that anti-TGF-β1 intervention might have a therapeutic effect on fibrotic penile tissue, not only by suppressing fibrosis but also by facilitating regeneration of TA cells.

IL-6 is a multifunctional cytokine and acts as the main synthesis mediator for many acute phase proteins involved in proinflammatoric and cytotoxic conditions. Acute phase proteins may also contribute to the regulation of fibrosis by inhibition of proteases and by binding of cytokines. IL-6 serum levels were increased in the acute inflammation phase of viral hepatitis (23) and in the airways of patients with chronic fibrosis (CF) (24,25). Tissue mRNA expression of IL-6 in patients not only with acute but also with chronic viral hepatitis, was slightly elevated (8,23). In a rat model for chronic pancreatitis IL-6 tissue expression was able to be established (11). IL-6 gene polymorphism led to an increased number of chronic fibrotic lung diseases in transplanted patients (26). In addition, patients with peritoneal dialysis related chronic peritonitis exhibited increased levels of IL-6 in the peritoneal dialysate effluent (15). In contrast, it has been demonstrated in our previously reported investigation that the acute-phase proteins alpha-1-antitrypsin and alpha-2-macroglobulin were not detectable in serum of patients with PD (14). In this study, IL-6 could not be demonstrated in serum of PD patients. Therefore, acute immunological defense mechanisms do not appear to play any particular role in PD. This has been confirmed by the finding that other systemic indicators of acute inflammation like CRP (C-related protein) were only slightly increased in serum of PD patients (14).

TNF-α influences the production of collagenase and fibroblast growth. Local expression of TNF-α occurs in many acute and chronic fibrotic diseases (27-29). TNF-α promotes the course of these diseases and is considered to influence both

the damage and the repair process by regulating additional mediators. Immunological processes or initial perivasculitis might explain the involvement of TNF-α in the etiology of PD. In animals with artificial cochlear inflammation TNF-α induced an amplification of the immune response leading to an increase of inflammation and disease progression (28). In patients with acute exacerbation of cystic fibrosis, the secretion of TNF-α was significantly lower in comparison with healthy controls and returned to normal secretion after treatment (24,25,30). In the airways of CF patients significantly elevated levels of circulating proinflammatory cytokines, in particular TNF-α have been reported (24). Other pulmonary diseases harboring diffuse fibrotic alterations like idiopathic pulmonary fibrosis, adult respiratory distress syndrome, lung cancer and chronic pancreatitis have revealed elevated TNF-α levels (9,11). In serum of patients with severe chronic hepatitis and liver cirrhosis significantly higher TNF-α levels were found compared to healthy controls, whereas patients with mild chronic hepatitis did not exhibit such alterations. In our study, serum TNF-α levels were significantly decreased. This finding does not indicate a systemic involvement of TNF-α in etiopathogenesis of PD. In general, TNF-α seems to be involved in acute and highly active phases of fibrotic diseases. The majority of patients included in our study had a duration of PD longer than 12 months. The decreased TNF-α levels found in our study therefore may possibly be an indicator for the chronic phase of PD in our patients (stable disease). Due to these low TNF-α-levels, it could be concluded that an anti-fibrotic therapy of PD based on TNF-α does not appear to be promising.

IFN-γ can reduce collagen synthesis, proliferation of fibroblasts with subsequent fibrotic activity and increase collagenase production. In the airway epithelium of CF patients increased levels of IFN-γ mRNA should be verified (30). The mRNA of both IFN-γ and IL-6 mRNA were elevated for liver specimens of a transgenic mouse model for chronic hepatitis C-virus liver disease. In spontaneous chronic pancreatitis in a rat model, IFN-γ was related to the progression of chronic pancreatitis (11). These findings are in agreement with the significantly increased IFN-γ serum levels detected in our series of PD patients.

Anti-TGF-β1 agents have been considered to be a therapeutic option in various fibrotic diseases (31,32). The basis of that assumption is a down regulation of the fibrotic protein expression induced by IFN-γ. On the one hand, our results may explain that therapeutically administered local IFN-γ was seen to be ineffective as PD plaques barely responded to local interferon therapy. On the other hand, elevated IFN-γ levels in PD patients might be a type of physiological defense mechanism as in antifibrotic activities.

## CONCLUSION

No straightforward description of the pathophysiological process that leads to the formation of PD is currently known. To our knowledge, the present study is the first reported series with special emphasis on fibrogenic factors in patient's serum.

Cytokines, in particular TGF-β1 and IFN-γ play a local and systemic role in the formation of PD. Local or systemic cytokine patterns might be crucial in different stages of PD. Micro traumata in the tunica albuginea could be the triggering incident.

TGF-β1 may be a target for an antifibrotic PD therapy as well as the first marker for PD therapy response. TGF-β1 serum levels should be integrated in future therapy studies. The role of cytokines in PD, in particular TGF-β1, should be followed-up in clinical as well as in investigative trials.

## ACKNOWLEDGEMENT

Drs. Reinhold P. Zimmermann and Gerhard Feil have both contributed equally to this work.

## CONFLICT OF INTEREST

The authors have nothing to disclose.

### REFERENCES

1. Nöske HD, Hauck EW: Morbus Lapeyronie aus historischer Sicht. Aktuel Urol. 2001; 32 (Suppl 1): 2-6.

2. Smith BH: Peyronie's disease. Am J Clin Pathol. 1966; 45: 670-8.
3. Smith BH: Subclinical Peyronie's disease. Am J Clin Pathol. 1969; 52: 385-90.
4. Devine CJ Jr, Somers KD, Jordan SG, Schlossberg SM: Proposal: trauma as the cause of the Peyronie's lesion. J Urol. 1997; 157: 285-90.
5. Leffell MS: Is there an immunogenetic basis for Peyronie's disease? J Urol. 1997; 157: 295-7.
6. Bichler KH, Lahme S, Mattauch W, Petri E: Collagen metabolism in induratio penis plastica (IPP). Urologe A. 1998; 37: 306-11.
7. Somers KD, Sismour EN, Wright GL Jr, Devine CJ Jr, Gilbert DA, Horton CE: Isolation and characterization of collagen in Peyronie's disease. J Urol. 1989; 141: 629-31.
8. Mammaev SN, Lukina EA, Shul'pekova IuO, Levina AA, Ivashkin VT: Cytokine regulation of liver inflammation and fibrosis during chronic hepatic diseases. Klin Lab Diagn. 2001; 12: 37-40.
9. Martinet Y, Menard O, Vaillant P, Vignaud P, Vignaut JM, Martinet N: Cytokines in human lung fibrosis. Arch Toxicol Suppl. 1996; 18: 127-39.
10. Eddy AA: Molecular basis of renal fibrosis. Pediatr Nephrol. 2000; 15: 290-301.
11. Xie MJ, Motoo Y, Su SB, Sawabu N: Expression of tumor necrosis factor-alpha, interleukin-6, and interferon-gamma in spontaneous chronic pancreatitis in the WBN/Kob rat. Pancreas. 2001; 22: 400-8.
12. Haque MF, Meghji S, Khitab U, Harris M: Oral submucous fibrosis patients have altered levels of cytokine production. J Oral Pathol Med. 2000; 29: 123-8.
13. Mills BG, Frausto A, Brien E: Cytokines associated with the pathophysiology of aggressive fibromatosis. J Orthop Res. 2000; 18: 655-62.
14. Feil G, Bichler KH, Lahme S, Götz T: Investigations on cytokines in serum of patients with Peyronie's disease. Akt Urol. 2001; 32 (Suppl 1): 33-6.
15. Lai KN, Lai KB, Lam CW, Chan TM, Li FK, Leung JC: Changes of cytokine profiles during peritonitis in patients on continuous ambulatory peritoneal dialysis. Am J Kidney Dis. 2000; 35: 644-52.
16. Tredget EE, Wang R, Shen Q, Scott PG, Ghahary A: Transforming growth factor-beta mRNA and protein in hypertrophic scar tissues and fibroblasts: antagonism by IFN-alpha and IFN-gamma in vitro and in vivo. J Interferon Cytokine Res. 2000; 20: 143-51.
17. Nakamura T, Sakata R, Ueno T, Sata M, Ueno H: Inhibition of transforming growth factor beta prevents progression of liver fibrosis and enhances hepatocyte regeneration in dimethylnitrosamine-treated rats. Hepatology. 2000; 32: 247-55.
18. Hauck EW, Hauptmann A, Schmelz HU, Bein G, Weidner W, Hackstein H: Prospective analysis of single nucleotide polymorphisms of the transforming growth factor beta-1 gene in Peyronie's disease. J Urol. 2003; 169: 369-72.
19. Lin CS, Lin G, Wang Z, Maddah SA, Lue TF: Up-regulation of monocyte chemoattractant protein 1 and effects of transforming growth factor-beta 1 in Peyronie's disease.Biochem Biophys Res Commun. 2002; 295: 1014-9.
20. El-Sakka AI, Hassoba HM, Chui RM, Bhatnagar RS, Dahiya R, Lue TF: An animal model of Peyronie's-like condition associated with an increase of transforming growth factor beta mRNA and protein expression. J Urol. 1997; 158: 2284-90.
21. Bivalacqua TJ, Diner EK, Novak TE, Vohra Y, Sikka SC, Champion HC, et al.: A rat model of Peyronie's disease associated with a decrease in erectile activity and an increase in inducible nitric oxide synthase protein expression. J Urol. 2000; 163: 1992-8.
22. Davila HH, Ferrini MG, Rajfer J, Gonzalez-Cadavid NF: Fibrin as an inducer of fibrosis in the tunica albuginea of the rat: a new animal model of Peyronie's disease. BJU Int. 2003; 91: 830-8.
23. Missale G, Ferrari C, Fiaccadori F: Cytokine mediators in acute inflammation and chronic course of viral hepatitis. Ann Ital Med Int. 1995; 10: 14-8.
24. Bonfield TL, Konstan MW, Berger M: Altered respiratory epithelial cell cytokine production in cystic fibrosis. J Allergy Clin Immunol. 1999; 104: 72-8.
25. Osika E, Cavaillon JM, Chadelat K, Boule M, Fitting C, Tournier G, et al.: Distinct sputum cytokine profiles in cystic fibrosis and other chronic inflammatory airway disease. Eur Respir J. 1999; 14: 339-46.
26. Lu KC, Jaramillo A, Lecha RL, Schuessler RB, Aloush A, Trulock EP, et al.: Interleukin-6 and interferon-gamma gene polymorphisms in the development of bronchiolitis obliterans syndrome after lung transplantation. Transplantation. 2002; 74: 1297-302.
27. Elborn JS, Norman D, Delamere FM, Shale DJ: In vitro tumor necrosis factor-alpha secretion by monocytes from patients with cystic fibrosis. Am J Respir Cell Mol Biol. 1992; 6: 207-11.
28. Satoh H, Firestein GS, Billings PB, Harris JP, Keithley EM: Tumor necrosis factor-alpha, an initiator, and etanercept, an inhibitor of cochlear inflammation. Laryngoscope. 2002; 112: 1627-34. Erratum in: Laryngoscope. 2003; 4: 291.

29. Wang X, Chen YX, Xu CF, Zhao GN, Huang YX, Wang QL: Relationship between tumor necrosis factor-alphaand liver fibrosis. World J Gastroenterol. 1998; 4: 18.
30. Wojnarowski C, Frischer T, Hofbauer E, Grabner C, Mosgoeller W, Eichler I, et al.: Cytokine expression in bronchial biopsies of cystic fibrosis patients with and without acute exacerbation. Eur Respir J. 1999; 14: 1136-44.
31. Oldroyd SD, Thomas GL, Gabbiani G, El Nahas AM: Interferon-gamma inhibits experimental renal fibrosis. Kidney Int. 1999; 56: 2116-27.
32. Takahara T, Sugiyama K, Zhang LP, Ando O, Fujii M, Yata Y, et al.: Cotreatment with interferon-alpha and -gamma reduces liver fibrosis in a rat model. Hepatol Res. 2004; 28: 146-154.

*Accepted after revision:*
*May 13, 2008*

**Correspondence address:**
Dr. Gerhard Feil
Department of Urology
Eberhard-Karls-University of Tuebingen
Hoppe-Seyler-Str. 3
72076 Tuebingen, Germany
E-mail: gerhard.feil@med.uni-tuebingen.de

## EDITORIAL COMMENT

Despite the prevalence of Peyronie's disease is increasing fast, until now we do not know the exact pathophysiological mechanism of this disease. The main hypothesis is an inflammatory reaction followed by fibroblasts proliferation and scar tissue formation. However, even this mechanism was not proven yet. So far, how and why fibrosis takes place is not clear. Anyway, cytokines and in particular TGF-beta seem to play a crucial role in Peyronie's disease. If it is so, any information about these very mediators is very welcome. Furthermore, until we do understand why and how fibrosis and Peyronie's disease occur we will not really treat the disease itself.

In our opinion, it is time to search the pathophysiological process that causes Peyronie's disease, in the same that the authors did in this valuable article. In the era that aggressive surgeries to manage Peyronie's disease are being reviewed, probably understanding inflammatory reaction and fibrogenic factors are way to go. In this way, I really believe that cytokines, mainly TGF-beta are the keys to understand what really happens in the tunica albuginea due to Peyronie's disease.

Only after knowing this mechanism, we would treat and cure the disease, instead of manage its complications with poor results.

***Dr. Joaquim A. Claro***
*Division of Urology*
*University of Sao Paulo, USP*
*Sao Paulo, Brazil*
*E-mail: joaquimclaro@hotmail.com*

Clinical Urology
International Braz J Urol Vol. 34 (4): 467-476, July - August, 2008

# Human Papillomavirus and Penile Cancers in Rio de Janeiro, Brazil: HPV Typing and Clinical Features

**Marcos A. Scheiner, Mercia M. Campos, Antonio A. Ornellas, Eduardo W. Chin, Maria H. Ornellas, Maria J. Andrada-Serpa**

*Hematology Service (MAS, MMC), Section of Urology (AAO, EWC) and Bone Marrow Transplant Center (MHO), National Cancer Institute (INCA) and Virology Service (MJAS), Evandro Chagas Institute of Clinical Research, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil*

**ABSTRACT**

*Objective:* To determine the prevalence of human papillomavirus (HPV) DNA in penile cancers in Rio de Janeiro, Brazil.
*Materials and Methods:* We studied, prospectively, 80 consecutive cases of patients with penile cancers who underwent surgical treatment at three different Hospitals in Rio de Janeiro between March 1995 and June 2000. Of these patients, 72 were diagnosed with invasive squamous cell carcinoma and 8 patients with verrucous carcinoma. The following parameters were observed: presence or absence of HPV DNA viral type, histological subtypes, clinical stage and overall survival.
*Results:* HPV DNA was detected in 75% of patients with invasive carcinomas and in 50% of patients with verrucous carcinomas. High risk HPVs were detected in 15 of 54 (27.8%) patients with HPV positive invasive tumors and in 1 of 4 (25%) patients with HPV positive verrucous tumors. HPV 16 was the most frequent type observed. No correlation was observed between HPV status and histological subtype ($p = 0.51$) as well as HPV status and stage stratification ($p = 0.88$). HPV status was also not significantly associated with the presence of regional metastases ($p = 0.89$). The overall survival was related to the presence of lymph node metastases ($p < 0.0001$).
*Conclusions:* HPV infection may have contributed to malignant transformation in a large proportion of our penile cancer cases but only inguinal metastasis was a prognostic factor for survival in these patients with penile carcinoma.

***Key words:*** *human papillomavirus; penile cancer; Brazil*
***Int Braz J Urol. 2008; 34: 467-76***

## INTRODUCTION

Penile cancer prevalence varies according to geographic region and ethnic origin (1). In Brazil, penile cancer represents 2% of all cancers in males and is more frequent in north and northeast regions with an incidence ranging from 1.3 to 2.7 per 100,000, according to the geographic region (2).

The mechanism by which Human Papillomavirus (HPV) leads to malignant transformation is likely mediated through two viral genes, E6 and E7, which are actively transcribed in HPV infected cells. The E6 and E7 proteins bind to and inactivate the host cell's tumor suppressor gene products p53 and pRb, leading to uncontrolled growth. Although HPV genes have been detected in nearly 100% of cervical cancers, the presence of HPV infection in penile cancer is highly variable (3). HPV has been recognized as a possible etiological agent for penile carcinoma but its role in disease development and correlation to prognosis is still unclear.

HPV infections are associated with benign and malignant epithelial lesions and a high-risk HPV group is probably the major cause of anogenital cancers. To date, more than 100 HPV genotypes have been identified. Recently, Munoz et al. (4) pooled data from 11 case-control studies from nine countries and classified the following 15 HPV genotypes into a high-risk group: 16,18,31,33,35,39,45,51,52,56,58 ,59,68,73 and 82. Three other genotypes [26, 53 and 66] will almost certainly also be classified as part of the high-risk group (4).

The prevalence of HPV infections in penile cancer is similar to those observed in vulvar carcinoma (3). Additionally, specific histological subtypes of penile cancer are consistently associated with HPV infection - basaloid and warty squamous cell carcinoma (3,5).

In Brazil, few studies have reported HPV infection in penile cancer. McCance et al. (6) showed a positivity of 49% using Southern blot for the detection of HPV DNA, while Bezerra et al. (7) reported a prevalence of 30.5% for HPV DNA in paraffin-embedded material using the polymerase chain reaction (PCR).

The aim of the present study was to assess the prevalence of HPV infection in a large series of patients with invasive squamous cell carcinoma and verrucous carcinoma of the penis.

Verrucous carcinoma is a less aggressive variant of squamous cell penile carcinoma, which rarely metastasizes to regional nodes regardless of size, evidence of local invasion or duration of disease (8).

Invasive squamous cell carcinoma of the penis usually metastasizes to the inguinal region though lymphatic channels and approximately 20% of the patients whose nodes appear to be clinically normal have inguinal metastasis at operation.

## MATERIALS AND METHODS

### Histopathological Specimens

Eighty penile cancer specimens were collected from patients from three different Hospitals in Rio de Janeiro: Brazilian National Cancer Institute, Pedro Ernesto University Hospital and Mario Kröeff Cancer Hospital between March 1995 and June 2000. All patients were evaluated prospectively and gave their informed consent to participate in the study. Our Institutional Review Board also approved the study.

### Specimen Processing and DNA Extraction

Two to five cm fragments were collected from the tumor region during surgical procedure and frozen at -80ºC or immediately processed. The specimens were washed in saline and cut into small pieces. Samples were then digested with 50 µL of proteinase K (10 mg/mL) in a volume of 3 mL of cell lyses solution (10 mM Tris-HCl pH 7.6, 10 mM EDTA pH 8.0 and 50 µL of SDS 10%) and incubated at 42ºC for 14 to 16 hours. DNA was recovered after ethanol precipitation, dried at room temperature and dissolved in sterile water. HeLa cell line DNA was used as an HPV positive control (9). All samples were tested for DNA integrity by amplification of a fragment of the β-globin gene using PC04/GH20 as primers.

### PCR Reaction

All samples were first subjected to an amplification using a generic pair of primers (MY09/MY11) for HPV (10) that amplify a fragment of the conserved L1 region. The DNA sample was amplified in 50 µL reactions. DNA from a HeLa cell line infected with HPV-18, was used as a positive control. All positive samples using the generic primers were amplified with a specific pair of primers for HPV-16 and HPV-18. The expected PCR products were fragments of 450-452 bp for generic primers, 268 bp for β globin primers, 120 and 100 bp with HPV 16 and 18 specific primers, respectively. Amplicons were analyzed by electrophoresis in a 1.5% agarose gel stained with 10 µg/mL of ethidium bromide. Samples identified as positive for HPV DNA were genotyped by restriction fragment length polymorphism (RFLP). Specimens were examined without prior knowledge of the histology of the lesions.

DNA samples that were negative for the first round MY9/11 primer sets were re-amplified in a nested PCR using the GP5+/6+ primer pair (11). The

number of cycles was reduced to 30 and 2.5 μL of template was used (12). Strict laboratory conditions were followed in order to avoid contamination.

### Restriction Fragment Length Polymorphism Analysis

The amplicons obtained by the MY9/11 primer sets were submitted to RFLP using the enzymes BamHI, DdeI, HaeIII, RsaI and Sau3AI. The digested and non-digested PCR products were analyzed in 12% polyacrylamide gels. The gels were stained with ethidium bromide and photographed. Fragments were analyzed according to Bernard et al. (13).

### Clinicopathological Data

Specimens from 72 patients histologically diagnosed with invasive squamous cell carcinoma and specimens from 8 patients diagnosed with verrucous carcinoma were analyzed. Patients with verrucous penile carcinoma were studied to assess the prevalence of HPV infection. Patients with invasive squamous cell carcinoma of the penis were studied to assess the prevalence of HPV infection. Stage was based on clinical ground, final stage was determined at the time of presentation according to the 1978 TNM system and were further clinically classified according to the AJCC Cancer Staging Manual [AJCC, 2002] (14). Patients were clinically observed during a median of 15 months. We defined stage stratification into group I (T1N0), II (T1N1,T2N0-1), III (T1N2,T2N2,T3N0-2) and IV (T1-3N3,T4N0-3).

The pathological material collected from patients with invasive squamous cell carcinoma was reviewed and all tumors were classified according to the Broder's grading system.

### Statistical Analysis

Patient follow-up was gathered from medical charts from the Brazilian National Cancer Institute and when necessary through contact with the patient's family. The data obtained were recorded on standard research forms and filed in a database. Analyses were performed using SPSS®. Association with clinical stage, histopathological subtype and HPV status was done using the chi-square test. A "p" value $< 0.05$ was considered statistically significant. Analysis of actuarial survival rates was performed by the Kaplan-Meier method and log rank test. Differences were considered significant when the "p" value was less than 0.05.

## RESULTS

A total of 80 specimens of penile tumors from an equal number of patients were analyzed. Patients were clinically observed during a median of 15 months (1 to 80 months). The mean age of patients was 57.6 years (ranging from 36 to 86 years) and no statistical difference was observed between HPV positive and negative patients.

The glans was the most frequent site involved and tumors were classified as follows: 8 (10%) cases of verrucous carcinoma and 72 (90%) invasive squamous cell carcinoma of penis. Of the 72 patients with invasive carcinoma, 16 (22.2%) patients presented well differentiated, 53 (73.6%) moderately differentiated and 3 (4.2%) poorly differentiated tumors.

The distribution category T in all 72 cases with invasive squamous cell carcinoma of the penis included 8 (11.1%) in clinical stage T1, 23 (31.9%) in clinical stage T2, 24 (33.3%) in clinical stage T3 and 15 (20.8%) in clinical stage T4. The remaining 2 patients (2.8%) in clinical stage TX were included because they had been referred to our institution after surgical treatment of the primary tumor. The N category distribution in the 72 cases included 32 (44.4%) in stage N0, 7 (9.7%) in stage N1, 19 (26.4%) in stage N2 and 13 (18%) in stage N3. The one remaining patient was classified as T2NXM0. The lymph nodes could not be evaluated in one patient (1.4%) classified as stage NX.

Of the 72 cases, 7 (9.7%) were included in group I according to stage stratification, 17 (23.6%) in group II, 22 (30.5%) in group III and 24 (33.3%) in group IV. Two patients did not provide requirements for the inclusion in stage stratification groups. No correlation was observed between HPV status and stage stratification ($p = 0.88$).

Of the 72 patients, 25 (34.7%) presented lymph node metastases. The 5-year disease-free survival rates for patients with negative and positive lymph node involvement were 80% and 0% respectively. Differences between the 2 groups were statistically significant ($p < 0.001$, Figure-1). HPV status was also not significantly associated with the presence of regional metastases ($p = 0.89$).

None of the 8 patients with verrucous carcinoma developed local recurrences or distant metastases and 31/72 patients (43%) with invasive carcinomas died due to progression of the malignancy.

HPV DNA was detected in 44% (35 of 80) of patients using the MY9/11 first round PCR. The overall detection of HPV DNA increased to 72.5% (58 of 80) using the nested GP5+/6+ PCR. A fragment of the β-globin gene was amplified in all specimens as a control of DNA integrity. After RFLP typing of twenty-three HPV+ cases, high risk HPVs were detected in 69% (16 of 23) while low risk HPVs were found in 7 positive cases. The HPV 16 type was observed in 12 of 23 (52%) cases.

Table-1 shows HPV genotyping according to the histopathological subtype. The distribution of positive and negative HPV tumors, according to the histopathological grade of differentiation and clinical stage, is shown in Table-2. No statistical correlation was observed between HPV status and histopathological subtype ($p = 0.51$).

HPV positive patients had better 5-year disease-free survival rates than those with negative HPV results although the differences between the 2 groups were not significant ($p = 0.779$, Figure-2).

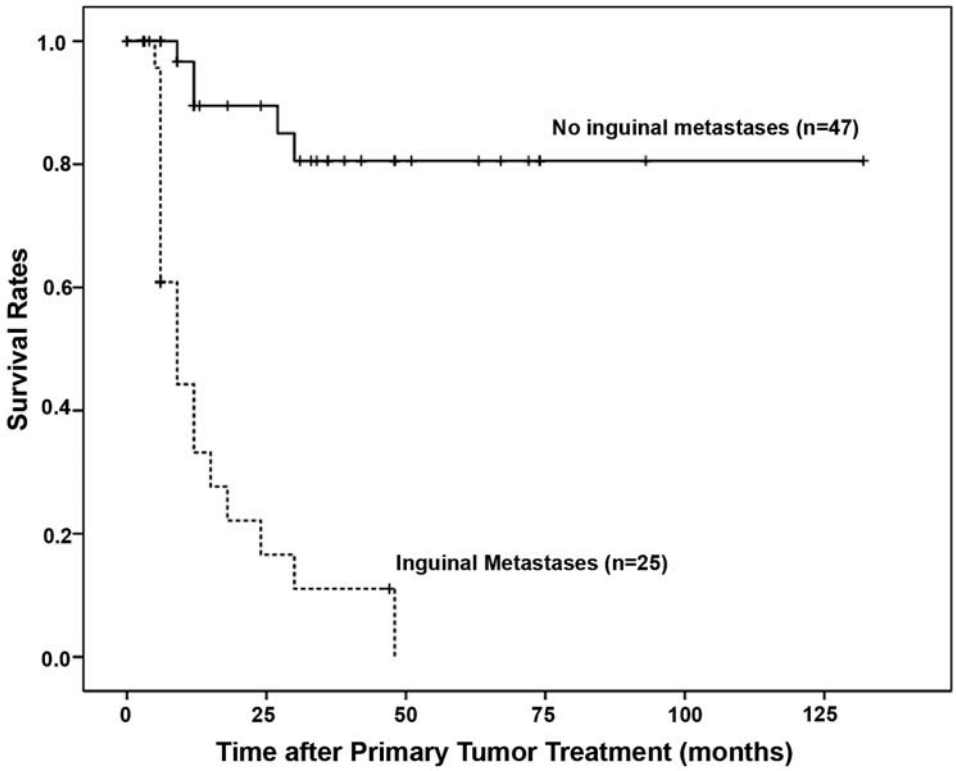



***Figure 1 –*** *Respective 10-year survival rates according to the presence (n = 25) or absence of inguinal metastases (n = 47). Kaplan-Meier method (p < 0.0001, log rank test).*

***Table 1 –*** *HPV genotyping of penile tumors according to the pathological subtype.*

| Histopathological Subtype | N | HPV+ (%) | Genotypes | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 6 | 16* | 18* | 28 | 31* | 33* | 45* | 71 | NT |
| Ver | 8 | 4/80 (5) | 1 | 1* | | | | | | | 2 |
| WD | 16 | 11/80 (13.7) | 2 | 1* | | | | 1* | | 1 | 6 |
| MD | 53 | 40/80 (50) | 1 | 8* | 1* | 1 | 1* | | 1* | | 27 |
| PD | 3 | 3/80 (3.8) | | 2* | | 1 | | | | | 0 |
| Total | 80 | 58/80 (72.5) | 4 | 12* | 1* | 2 | 1* | 1* | 1* | 1 | 35 |

*Ver = verrucous carcinoma; WD = well differentiated squamous cell carcinoma; MD = moderately differentiated squamous cell carcinoma; PD = poorly differentiated squamous cell carcinoma; NT not typed. HPV = human papillomavirus; Asterisks indicate high-risk HPV types.*

## COMMENTS

Age-standardized HPV prevalence varied nearly 20 times between normal populations, from 1.4% (95% CI 0.5-2.2) in Spain to 25.6% (22.4-28.8) in Nigeria (15). The present study evaluated the prevalence of HPV DNA in the largest series of penile tumors, in Rio de Janeiro, Brazil. Squamous cell carcinoma represents 90% of penile cancers. In our series, 70.3% of cases were moderately differentiated SCC. Seventy-two percent (58 of 80) of specimens were HPV DNA positive. This frequency was different from previous reported in Brazil. Bezerra et al. detected HPV DNA in 30.5% (25 out of 82) of penile carcinomas in the State of Sao Paulo (7). Heideman et al. (16) detected HPV 46 of 83 (55%) penile squamous cell carcinomas (SCCs). HPV16 was the predominant type, appearing in 24 (52%) of 46 of penile SCCs. The reported HPV prevalence of penile carcinoma is highly variable, ranging from 15 to 71% (3). Probably the variability in HPV detection in these different studies represents a true difference and or technical discrepancies. High risks HPVs were detected in 27.5% (16 of 58) of our penile carcinomas.

***Table 2 –*** *HPV DNA positive and negative penile cancer according to the histopathological subtypes and clinical stage.*

| Histopathological Subtypes | N of HPV Positive Clinical Stage | | | | N of HPV Negative Clinical Stage | | | |
|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | I | II | III | IV |
| Ver | 0 | 2 | 2 | 0 | 2 | 1 | 1 | 0 |
| WD | 2 | 1 | 3 | 5 | 0 | 0 | 5 | 0 |
| MD | 4 | 10 | 12 | 14 | 1 | 5 | 1 | 4 |
| PD | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| Total | 6 | 14 | 18 | 20 | 3 | 6 | 7 | 4 |

*Ver = verrucous carcinoma; WD = well differentiated squamous cell carcinoma; MD = moderately differentiated squamous cell carcinoma; PD = poorly differentiated squamous cell carcinoma. HPV = human papillomavirus; No correlation was observed between HPV status and histological subtypes (p = 0.51) as well as HPV status and stage stratification (p = 0.88).*

The most frequent viral type detected in penile carcinoma was HPV 16, observed in 20.7% (12 of 58) of cases. This result was similar to that previous reported for anogenital cancers. Low risk HPVs were observed in 7 of 58 (12%) positive carcinomas and HPV 6 was the most frequent type, which was found in 4 cases. In Argentina, Picconi et al. (17) reported a very high prevalence of HPV in penile carcinomas (71%) and HPV 18 was the most frequent type found.

Although, our HPV positive patients had better 5-year disease-free survival rates than those with negative HPV results the differences between the 2 groups were not significant (p = 0.779, Figure-2).

Lont et al. (18) also detected high-risk HPV DNA in 29% of the tumors, with HPV 16 being the predominant type, accounting for 76% of high-risk HPV containing SCCs. Disease-specific 5-year survival in the high-risk HPV-negative group and high-risk HPV-positive group was 78% and 93%, respectively (log-rank test p = 0.03). In multivariate analysis, the HPV status was an independent predictor for disease-specific mortality (p = 0.01). These results indicate that the presence of high-risk HPV (29%) confers a survival advantage in patients with penile carcinoma.

In our study, only one case of HPV 18 was observed. Thirty-five of 58 patients (60.3%) with non-16 and non-18 positive cases could not be typed. No multiple HPV infections were detected. Multiple infections were observed in 11.6% of Chinese women (19) with cervical cancer and in 8.5% of penile tumors (3). Although, multiple HPV types are less frequent, our results may be due to differences in the ability of the set of primers to amplify different amounts and combinations of HPV types within a sample as well as the capacity of RFLP analysis to identify multiple infections (20).

No correlation was observed among histopathological subtypes of invasive squamous cell penile carcinomas and HPV infection ($x^2 = 0.43$ and

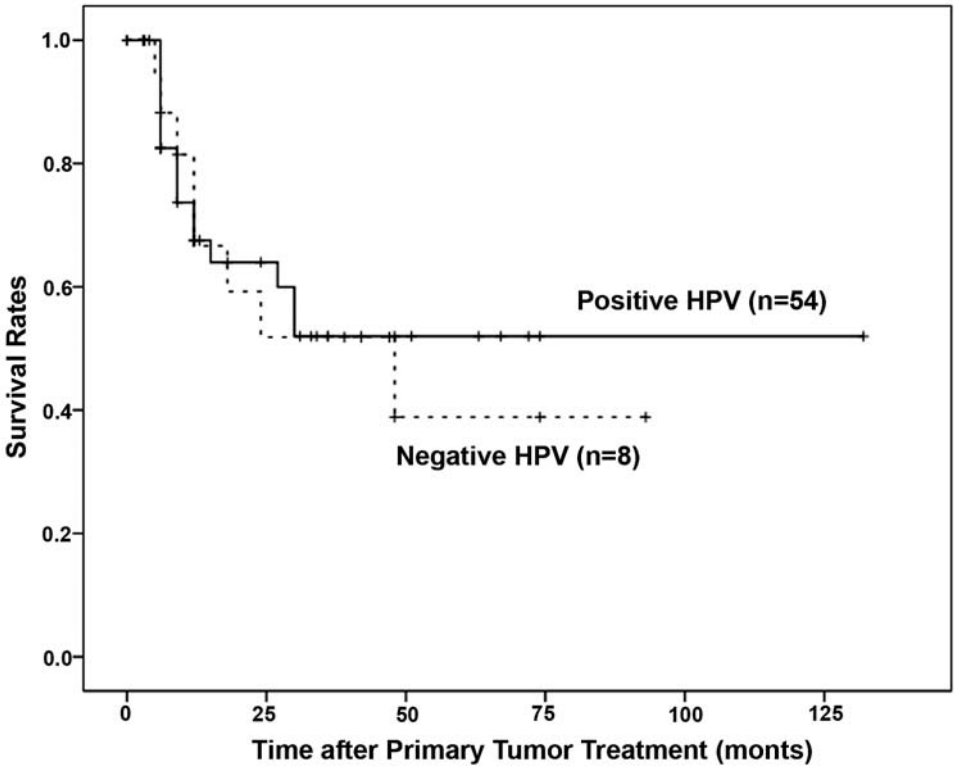



***Figure 2 –*** *Respective 10-year survival rates according to the presence (n = 54) or absence of Human Papillomavirus (n = 18). Kaplan-Meier method (p = 0.779, not significant, log rank test).*

p=0.51), although the majority of HPV DNA positive cases (54) were observed among the moderately and poorly differentiated carcinomas.

Seven of 8 cases of verrucous carcinomas were HPV DNA positive and one presented high risk HPV 16. In a previous report, Rubin et al. (3) related 4 of 12 verrucous carcinomas as being HPV DNA positive including 2 with high-risk HPVs. On the other hand, previous studies showed that verrucous carcinoma is more often HPV negative or low-risk HPV positive (21,22).

The majority of penile cancer cases (40%) occurred in the fifth and sixth decade of life. Lymph node metastasis was associated with poor survival rate and no difference was observed in survival between HPV positive and HPV DNA negative cases.

Although the central role of HPV infection in the etiology of cervical cancer has been recognized, HPV infection alone is insufficient for the malignant transformation in penile cancer. In addition, the presence of HPV DNA could not be considered a prognostic factor. Several epidemiological reports indicate that other factors such as the lack of circumcision, hygiene practices, the presence of other sexually transmitted diseases, the number of sexual partners and cigarette smoking may predispose to penile carcinogenesis, and the exact role of HPV infection in the development of penile cancers remains to be elucidated.

On June 8, 2006, the U.S. Food and Drug Administration approved the use of a new vaccine to prevent infection from four types of HPV. Two of the HPV types targeted by the vaccine (HPV-16 and HPV-18) are responsible for about 70 percent of the cases of cervical cancer worldwide. The other two HPV types (HPV-6 and HPV-11) cause approximately 90 percent of the cases of genital warts. The vaccine is currently recommended for use in young females before they become sexually active, and its possible use in males is under scrutiny. One of several reasons that HPV vaccines have focused on women rather than men is that cervical cancer accounts for 80 percent of HPV-related cancers. Male cancers are obviously in the minority, but 20 percent is still significant, especially considering the prevalence of HPV infection. While women have about 80 percent of the total burden of disease and death, it is certainly not inconsequential for men. Penile cancer affects 100,000 men a year worldwide, and the numbers are increasing. As a global issue, penile cancer is a relevant problem. For heterosexual men, the main benefits of an HPV vaccine will be the prevention of genital warts and, potentially, cervical cancer in women. While studies have not yet been carried out, the hope is that the vaccine may eventually help prevent cancers linked to HPV, including penile cancers. If the vaccine proves successful, the administration of HPV vaccines could eventually become a requirement for boys and girls in middle school or high school as a potential way to reduce HPV infection (23).

Improved sampling techniques of the male genitalia and cohort studies in progress should provide important information on the natural history of HPV infection and disease in men, including risk factors for HPV acquisition and transmission. The impact on HPV infection in males of the vaccination in women will also need to be assessed.

## ACKNOWLEDGEMENTS

This work was supported by the Brazilian Council for Scientific and Technological Development (CNPQ) and Ary Frauzino Foundation, National Institute of Cancer.

## CONFLICT OF INTEREST

None declared

### REFERENCES

1. Dillner J, von Krogh G, Horenblas S, Meijer CJ: Etiology of squamous cell carcinoma of the penis. Scand J Urol Nephrol Suppl. 2000; 205: 189-93.
2. Parkin DM, Whelan SL, Ferlay J, Raymond L, Young J: Cancer Incidence in Five Continents VII. Lyon, IARC Scientific Publications; #143 (www.iarc.fr). 1997.
3. Rubin MA, Kleter B, Zhou M, Ayala G, Cubilla AL, Quint WG, et al.: Detection and typing of human papillomavirus DNA in penile carcinoma: evidence for multiple independent pathways of penile carcinogenesis. Am J Pathol. 2001; 159: 1211-8.
4. Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, et al.: Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med. 2003; 348: 518-27.

5. Bezerra AL, Lopes A, Landman G, Alencar GN, Torloni H, Villa LL: Clinicopathologic features and human papillomavirus dna prevalence of warty and squamous cell carcinoma of the penis. Am J Surg Pathol. 2001; 25: 673-8.
6. McCance DJ, Kalache A, Ashdown K, Andrade L, Menezes F, Smith P, et al.: Human papillomavirus types 16 and 18 in carcinomas of the penis from Brazil. Int J Cancer. 1986; 37: 55-9.
7. Bezerra AL, Lopes A, Santiago GH, Ribeiro KC, Latorre MR, Villa LL: Human papillomavirus as a prognostic factor in carcinoma of the penis: analysis of 82 patients treated with amputation and bilateral lymphadenectomy. Cancer. 2001; 91: 2315-21.
8. Seixas AL, Ornellas AA, Marota A, Wisnescky A, Campos F, de Moraes JR: Verrucous carcinoma of the penis: retrospective analysis of 32 cases. J Urol. 1994; 152: 1476-8; discussion 1478-9.
9. Boshart M, Gissmann L, Ikenberg H, Kleinheinz A, Scheurlen W, zur Hausen H: A new type of papillomavirus DNA, its presence in genital cancer biopsies and in cell lines derived from cervical cancer. EMBO J. 1984; 3: 1151-7.
10. Manos MM, Ting Y, Wright DK, Lewis AJ, Broker TR, Wolinsky SM: The use of polymerase chain reaction amplification for the detection of genital human papillomaviruses. Molecular Diagnostics of Human Cancer. Cancer Cells. 1989; 7: 209-14.
11. Jacobs MV, de Roda Husman AM, van den Brule AJ, Snijders PJ, Meijer CJ, Walboomers JM: Group-specific differentiation between high- and low-risk human papillomavirus genotypes by general primer-mediated PCR and two cocktails of oligonucleotide probes. J Clin Microbiol. 1995; 33: 901-5.
12. Remmerbach TW, Brinckmann UG, Hemprich A, Chekol M, Kühndel K, Liebert UG: PCR detection of human papillomavirus of the mucosa: comparison between MY09/11 and GP5+/6+ primer sets. J Clin Virol. 2004; 30: 302-8.
13. Bernard HU, Chan SY, Manos MM, Ong CK, Villa LL, Delius H, et al.: Identification and assessment of known and novel human papillomaviruses by polymerase chain reaction amplification, restriction fragment length polymorphisms, nucleotide sequence, and phylogenetic algorithms. J Infect Dis. 1994; 170: 1077-85. Erratum in: J Infect Dis 1996; 173: 516.
14. American Joint Committee on Cancer: AJCC Cancer Staging Manual 6th. Penis. New York, Springer. 2002; pp. 303-308.
15. Clifford GM, Gallus S, Herrero R, Muñoz N, Snijders PJ, Vaccarella S, et al.: Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. Lancet. 2005; 366: 991-8.
16. Heideman DA, Waterboer T, Pawlita M, Delis-van Diemen P, Nindl I, Leijte JA, et al.: Human papillomavirus-16 is the predominant type etiologically involved in penile squamous cell carcinoma. J Clin Oncol. 2007; 25: 4550-6.
17. Picconi MA, Eiján AM, Distéfano AL, Pueyo S, Alonio LV, Gorostidi S, et al.: Human papillomavirus (HPV) DNA in penile carcinomas in Argentina: analysis of primary tumors and lymph nodes. J Med Virol. 2000; 61: 65-9.
18. Lont AP, Kroon BK, Horenblas S, Gallee MP, Berkhof J, Meijer CJ, et al.: Presence of high-risk human papillomavirus DNA in penile carcinoma predicts favorable outcome in survival. Int J Cancer. 2006; 119: 1078-81.
19. Chan PK, Li WH, Chan MY, Ma WL, Cheung JL, Cheng AF: High prevalence of human papillomavirus type 58 in Chinese women with cervical cancer and precancerous lesions. J Med Virol. 1999; 59: 232-8.
20. Qu W, Jiang G, Cruz Y, Chang CJ, Ho GY, Klein RS, et al.: PCR detection of human papillomavirus: comparison between MY09/MY11 and GP5+/GP6+ primer systems. J Clin Microbiol. 1997; 35: 1304-10.
21. Gregoire L, Cubilla AL, Reuter VE, Haas GP, Lancaster WD: Preferential association of human papillomavirus with high-grade histologic variants of penile-invasive squamous cell carcinoma. J Natl Cancer Inst. 1995; 87: 1705-9.
22. Noel JC, Vandenbossche M, Peny MO, Sassine A, de Dobbeleer G, Schulman CC, et al.: Verrucous carcinoma of the penis: importance of human papillomavirus typing for diagnosis and therapeutic decision. Eur Urol. 1992; 22: 83-5.
23. Geipert N: Vaccinating men for HPV: new strategy for preventing cervical cancer in women? J Natl Cancer Inst. 2005; 97: 630-1.

*Accepted after revision:*
*May 30, 2008*

**Correspondence address:**
Dr. Antonio Augusto Ornellas
Section of Urology
Instituto Nacional de Câncer
Praça da Cruz Vermelha 23
Rio de Janeiro, RJ, Brazil
E-mail: ornellasa@hotmail.com

## EDITORIAL COMMENT

This study is very welcome as it demonstrates the high rate of human papillomavirus (HPV) associated with penile cancer. The detection rate of 75% approaches the rate of 90% seen in carcinoma in-situ. Other studies show lower HPV rates. It was unclear if this was due to differences in technique, such as immunohistochemistry or due to real variations in biology. Furthermore, the lower rate of HPV (50%) in verrucous type indicates a possible different etiology to this subset of cancer. Studying the subtypes of HPV associated with penile cancer is paramount currently, given the recent development of vaccines against specific subtypes of HPV. It is hard to project the effect mass vaccination of females will have on males, but ought to reduce carriage of HPV among heterosexual males. Vaccinating males may have benefits for females also, as total immunization of the whole female population is unlikely. Reducing the overall population pool of these subtypes is likely to benefit both males and females. The impact on the incidence and type of penile cancer is likely have a lead time of several decades, as only a quarter of men with penile cancer present under the age of 50. Projections must also take into account the increasing life-expectancies in developed countries with more men living into their 70s and 80s, where the disease is more prevalent (see http:// www.oecd.org/statsportal/). Thus in the absence of any vaccination the prevalence is likely to increase, whereas even with successful vaccination changes in demographics may reduce the benefit in the initial years. Finally, the subtypes of HPV being targeted may result in only some cancers being prevented. These factors must be brought together in modeling and scenario planning when formulating strategies for service provision in any healthcare system.

***Dr. P. K. Hegarty***
*Institute of Urology and Pathology*
*University College Hospital London*
*London, UK*
*E-mail: Paulhego@hotmail.com*

## EDITORIAL COMMENT

The prevalence of penile cancer in Brazil is 2% and this is higher than in the USA and Europe, where it accounts for 0.3 to 0.6% of cancers (1,2). Squamous cell carcinoma (SCC) is the most common histological type of penile cancer and represents 95% of cases. However the aetiology of penile cancer is unknown, risk factors include age and lack of circumcision (1,2). Other predisposing factors to the development of penile SCC are the chronic inflammatory disorder penile lichen sclerosus (LS) (also termed balanitis xerotica obliterans) (3-6) or human papillomavirus (HPV) infection (1,2,7,8). A common aetiology for penile and cervical cancer is suggested by the geographical correlation between the incidence of penile and cervical cancers worldwide (9). The persistent infection with sexually transmitted high risk HPV is the main cause of cervical cancer (10,11). The prevalence of HPV penile infections in healthy men is reported to be 39% in Brazil and 3-9% in Western Europe, where there is a lower incidence of penile cancer (12,13). However, HPV detection in penile cancer cases varies from 20-80%, depending on detecti*on* method and geographical location (1,2). This is unlike cervical cancer where HPV infection can be detected in almost all cases (10).

The paper entitled “Human Papillomavirus and Penile Cancers in Rio de Janeiro, Brazil: HPV Typing and Clinical Features” concerns penile cancer HPV infection and survival in 80 consecutive

cases of patients who underwent surgery at Hospitals in Rio de Janeiro between 1995 and 2000. High risk HPV 16 was the predominate HPV type detected and no correlation was observed between HPV status (all types) and penile cancer subtype, stage, regional metastases or survival. HPV 16 has previously been reported to predominate in penile lichen sclerosus and SCC and may be an aetiological agent in the development of a significant proportion of penile cancers (7,14). However the importance of HPV status in penile cancer progression and patient survival is controversial, as high-risk HPV is associated with aggressive variants (8) but recent series examining the relationship of HPV infection with prognosis have revealed either no correlation survival or a favourable survival (15,16). This study from Rio de Janeiro is consistent with only inguinal metastasis being a prognostic factor for penile cancer survival. In summary, high risk HPV infection occurs in penile SCC and it is likely to be an aetiological agent in the development of a significant proportion of penile cancers. These results are important as prophylactic HPV vaccines for prevention of cervical cancer in women could also prevent penile cancers in men. However, several studies, including this one, show that once penile cancer has developed poor prognosis is associated with the occurrence of lymph node metastasis and not HPV status.

**REFERENCES**


1. Micali G, Nasca MR, Innocenzi D, Schwartz RA: Penile cancer. J Am Acad Dermatol. 2006; 54: 369-91.
2. Kayes O, Ahmed HU, Arya M, Minhas S: Molecular and genetic pathways in penile cancer. Lancet Oncol. 2007; 8: 420-9.
3. Nasca MR, Innocenzi D, Micali G: Penile cancer among patients with genital lichen sclerosus. J Am Acad Dermatol. 1999; 41: 911-4.
4. Depasquale I, Park AJ, Bracka A: The treatment of balanitis xerotica obliterans. BJU Int. 2000; 86: 459-65.
5. Powell J, Robson A, Cranston D, Wojnarowska F, Turner R: High incidence of lichen sclerosus in patients with squamous cell carcinoma of the penis. Br J Dermatol. 2001; 145: 85-9.
6. Pietrzak P, Hadway P, Corbishley CM, Watkin NA: Is the association between balanitis xerotica obliterans and penile carcinoma underestimated? BJU Int. 2006; 98: 74-6.
7. Prowse DM, Ktori EN, Chandrasekaran D, Prapa A, Baithun S: Human papillomavirus-associated increase in p16INK4A expression in penile lichen sclerosus and squamous cell carcinoma. Br J Dermatol. 2008; 158: 261-5.
8. Gregoire L, Cubilla AL, Reuter VE, Haas GP, Lancaster WD: Preferential association of human papillomavirus with high-grade histologic variants of penile-invasive squamous cell carcinoma. J Natl Cancer Inst. 1995; 87: 1705-9.
9. Parkin DM, Bray F: Chapter 2: The burden of HPV-related cancers.Vaccine. 2006; (Suppl 3): S11-25.
10. zur Hausen H: Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer. 2002; 2: 342-50.
11. de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H: Classification of papillomaviruses. Virology. 2004;324: 17-27.
12. Franceschi S, Castellsagué X, Dal Maso L, Smith JS, Plummer M, Ngelangel C, et al.: Prevalence and determinants of human papillomavirus genital infection in men. Br J Cancer. 2002; 86: 705-11.
13. Nasca MR, Innocenzi D, Micali G: Association of penile lichen sclerosus and oncogenic human papillomavirus infection. Int J Dermatol. 2006; 45: 681-3.
14. Rubin MA, Kleter B, Zhou M, Ayala G, Cubilla AL, Quint WG, et al.: Detection and typing of human papillomavirus DNA in penile carcinoma: evidence for multiple independent pathways of penile carcinogenesis. Am J Pathol. 2001; 159: 1211-8.
15. Lont AP, Kroon BK, Horenblas S, Gallee MP, Berkhof J, Meijer CJ, et al.: Presence of high-risk human papillomavirus DNA in penile carcinoma predicts favorable outcome in survival. Int J Cancer. 2006; 119: 1078-81.
16. Bezerra AL, Lopes A, Santiago GH, Ribeiro KC, Latorre MR, Villa LL: Human papillomavirus as a prognostic factor in carcinoma of the penis: analysis of 82 patients treated with amputation and bilateral lymphadenectomy. Cancer. 2001; 91: 2315-21.



***Dr. David M. Prowse***

*Centre for Molecular Oncology, Institute of Cancer*
*Bart's and The London Queen Mary's School of*
*Medicine and Dentistry*
*John Vane Science Centre*
*London, United Kingdom*
*E-mail: d.m.prowse@qmul.ac.uk*

Clinical Urology
International Braz J Urol Vol. 34 (4): 477-484, July - August, 2008

# Doppler Sonographic Findings in Testicular Microlithiasis

**Selim Serter, Sebnem Orguc, Bilal Gumus, Veli Ayyildiz, Yuksel Pabuscu**

*Department of Radiology (SS,SO,YP) and Department of Urology (BG), Celal Bayar University, Manisa, Turkey, and Manisa Military Hospital (VA), Manisa, Turkey*

**ABSTRACT**

*Objective:* The aim of this prospective study was to compare the resistive index (RI) values, which is a parameter of testicular parenchymal perfusion, in testicular microlithiasis (TM) cases and normal cases.
*Materials and Methods:* 2179 volunteers, all healthy men (17-42 years of age) from the Annual Army Reserve Officer Training Corps training camp were included in the study. A screening scrotal ultrasound was performed and all men diagnosed with TM underwent a scrotal Doppler ultrasonography scan (US). US examinations were performed for subjects with TM and without TM as a control group and RI was determined.
*Results:* 53 men with TM were identified in the 2179 US. Spectral Doppler examination was applied to 50 randomly selected cases (100 testicles) without TM and 92 testicles with TM, 39 cases (78 testicles) with bilateral and 14 cases with unilateral involvement. However, 48 normal testicles (17 bilateral and 14 unilateral) and 47 testicles with TM (15 bilateral and 17 unilateral, 10 of which were cases with bilateral TM) where flow from the centripetal artery could be obtained and analyzed were included in the statistical analysis for resistive indices. There was no significant difference regarding the RI and spectral examinations between subjects with and without TM. An interesting finding was the twinkling artifact observed in three cases.
*Conclusion:* Microliths did not alter the RI values and thus had no influence on testicular perfusion on Doppler US examination.

***Key words:*** *testis, ultrasonography, lithiasis, Doppler*
***Int Braz J Urol. 2008; 34: 477-84***

## INTRODUCTION

Testicular microlithiasis (TM) is an uncommon condition characterized by intratubular calcifications within a multilayered envelope usually discovered incidentally at ultrasonography (US) (1,2). Although minor microcalcification within a testis is considered normal, the typical US appearance of TM is of multiple nonshadowing echogenic foci measuring 2-3 mm and randomly scattered throughout the testicular parenchyma. The clinical significance of testicular microlithiasis remains unclear. Currently there is no evidence that TM is either a premalignant condition or a causative agent in testicular neoplasia; however, it has been associated with testicular neoplasia in 18-75% of cases (3-7). Some authors concluded that the increase in the relative risk of testicular cancer in the group with TM suggests that the presence of TM in symptomatic men is clinically significant. In contrast, de Castro et al. recently reported only 1 case of germ cell tumor detected in the 5 year follow-up of 63 subjects with TM suggesting US follow-up would do little to improve outcomes associated with testicular cancer and they continued to recommend

testicular self-examination in men at risk (8). It is by no means certain that microlith on its own is predictive of tumor.

Doppler US features of the testes, especially resistive index (RI), which is a reflection of arterial impedance, have been used to evaluate several testicular disorders, such as varicocele, orchitis, scrotal hernias, and others (9-11). Currently, RI has become a diagnostic tool of testicular blood flow in many diseases as established by Middleton et al. (12). Among numerous Doppler US studies of the testes previously published, only two case reports attempted to identify Doppler ultrasonographic parameters for TM, however encountered no specific findings (13,14). This is the first study, to our knowledge, to investigate the Doppler parameters of TM in a large cohort and comparing them with a control group.

The aim of this prospective study was to compare the RI values, which is a parameter of testicular parenchymal perfusion in subjects with or without TM.

Many new studies have addressed the fact that hypoxic stress contributes to many (patho) biological disorders and hypoxic control of cell growth and death may be of general pathophysiological importance (15,16). On these bases, we attempted to evaluate the possible vascular alterations in TM with the hypothesis of compression of microliths leading to increased local pressure in the testicular parenchyma and the possible association of microliths and tumor formation.

## MATERIALS AND METHODS

Between August 2002 and May 2003, 2,179 healthy volunteers from Reserve Officer Training Corps annual training camp were included in the study. A total of 2,179 white male subjects were evaluated by US. The age of the subjects (mean ± SD) was 22.4 ± 3.6 years (range 17-42 years). There was no racial variation in our study cohort. Informed consent was obtained from all participants. The study was approved by the Institutional Review Board and met all guidelines of our institution. A medical history was obtained from all volunteers, who also underwent a genitourinary physical examination. None of the subjects had a urinary disease or any other pathology. Subjects underwent screening scrotal ultrasound with Siemens Sonoline G 50 ultrasound machine (Issaquah, WA). Examinations were performed with the patient in supine position and the scrotum was supported by a towel placed between the thighs and the penis was placed on the abdomen and covered with a towel. The ultrasound gel was warmed with a heated towel before sonography to avoid cremasteric muscle contraction. All examinations were performed using a 10-12 MHz linear array transducer in longitudinal and transversal sections to document the presence or absence of TM. Four different radiologists perform the screening B mod ultrasound. Subsequently the same experienced radiologist (SS) performed the Doppler US examination for cases with TM and the selected control group.

As previously defined, a testicular microlithiasis diagnosis was confirmed when more than 5 high intensity signals 1 to 2 mm in size without acoustic shadowing was detected by US in a testicle (Figure-1). We recorded if the testicles were involved with calcifications unilaterally or bilaterally. The cases with TM were not graded according to the severity of microliths. All men diagnosed with TM underwent testicular Doppler US examination, complete clinical evaluation including a detailed genitourinary history and physical examination and determination of tumor markers (Beta-HCG, AFP and lactate dehydrogenase). Color Doppler US of the scrotum was performed with the same equipment. All Doppler examinations were performed using the same linear array transducer with a range of Doppler frequency 5-7 MHz. Color gain (threshold) was maximized for optimal sensitivity while avoiding excessive color noise. The Doppler scale (range of displayable Doppler frequency shifts) was decreased to its lowest value to maximize sensitivity to slow flow. The Doppler scale was displayed on the right side of all images as a bar containing the red and blue color assignment for different Doppler frequency shifts. Wall filters were adjusted to the lowest possible value. In Doppler examinations resistive indices were obtained from the centripetal artery or its recurrent rami, whichever was visualized optimally. RI was calculated as defined (difference of peak systolic velocity and end diastolic velocity

divided by peak systolic velocity). The RI was measured three times on each testicle.

Data were analyzed using SPSS 11.5 for Windows commercially available computer software. We used independent samples t test and $p < 0.05$ was accepted as statistically significant.

## RESULTS

Fifty-three men with TM were identified in 2179 men, with a prevalence of 2.4% for TM in this asymptomatic population. The mean age ± SD of subjects with TM was 23.9 ± 4.2 years old (range 20 to 31), 39 of the 53 (73%) subjects had bilateral microlithiasis. All cases with microliths displayed diffuse type of TM and no cases of focal TM were detected. All subjects with TM had a normal genitourinary history and physical examination. The tumor markers were within normal limits for all subjects.

Spectral Doppler examination was applied to 50 randomly chosen cases (100 testicles) without TM and 92 testicles with TM, 39 cases (78 testicles) with bilateral and 14 cases with unilateral involvement. However, 48 normal testicles (17 bilateral and 14 unilateral) and 47 testicles with TM (15 bilateral and 17 unilateral, 10 of which were cases with bilateral TM) in which flow from the centripetal artery could be obtained and analyzed were included in the statistical analysis for resistive indices. The percentage of cases where RI could be obtained from the centripetal arteries were approximately the same in both TM and control groups. Resistive indices of subjects with and without TM are shown in Table-1. No finding specific to TM was detected on spectral Doppler ultrasound examination. There was no significant difference regarding the RI's and spectral examinations between subjects with or without TM. Figure-2 demonstrates Doppler spectral analysis of cases with testicular microlithiasis. An additional interesting finding detected during the Color Doppler examination was the twinkling artifact caused by testicular microliths (Figure-3). These artifacts were observed in only three of all cases with TM (5.6%). These artifacts also complicated the examination by mimicking vascular structures while obtaining the Doppler spectrum.

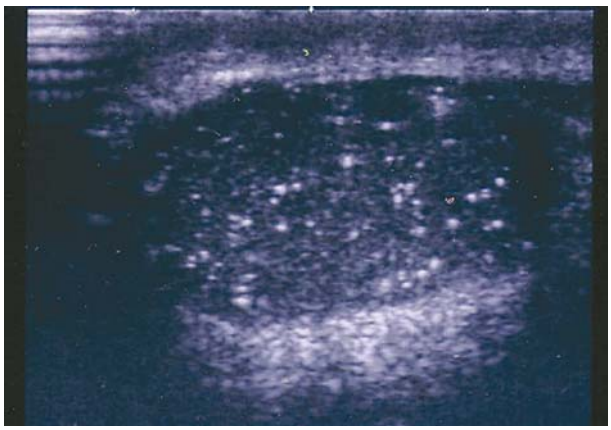


***Figure 1*** *– Longitudinal sonogram of the testicle shows numerous, small, echogenic, foci of calcification without posterior shadowing.*

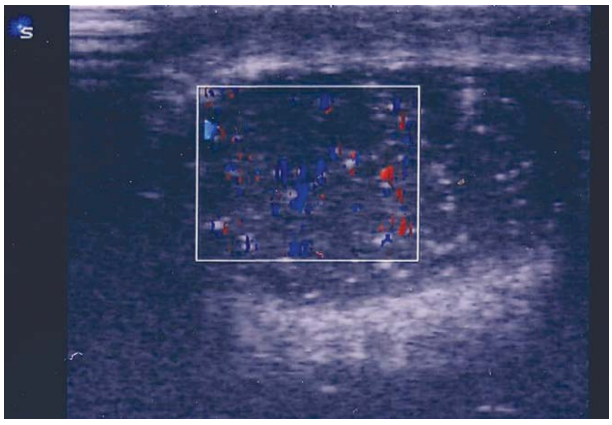


***Figure 2*** *– Spectral Doppler analysis of testicular artery demonstrates resistive index value.*

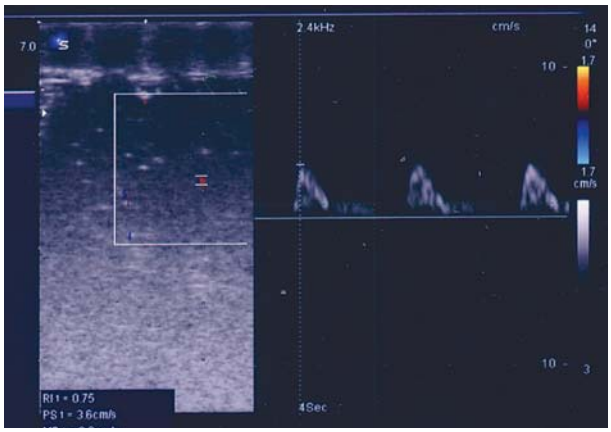



***Figure 3*** *– Color Doppler sonograms shows twinkling artifact appears as rapidly alternating red and blue color Doppler signal behind the microliths.*

***Table 1*** *– Resistive index values of centripetal artery or its recurrent rami transmediastinal testicular artery in subjects with or without testicular microlithiasis.*

| | **Resistive Indices** (mean ± SD) |
|---|---|
| With microlithiasis (n = 48 testes) | 0.72 ± 0.12 |
| Without microlithiasis (n = 47 testes) | 0.71 ± 0.1 |
| p Value | > 0.05 |

## COMMENTS

TM is a rare, asymptomatic disease, suspected to be associated with various benign and malignant urological pathologies and genetic anomalies, usually found incidentally on ultrasound examinations performed for other reasons. TM has a characteristic appearance, classically consisting of multiple, often bilateral microliths scattered throughout the testicular parenchyma. Histologically microliths consist of a central calcified core surrounded by concentric laminations of cellular debris, glycoprotein and collagen (17).

Many new studies have reported that hypoxic stress contributes to many (patho) biological disorders and hypoxic control of cell growth and death may be of general pathophysiological importance (15,16). On these bases, we attempted to evaluate the possible vascular alterations in TM with the hypothesis of compression of microliths leading to increased local pressure in the testicular parenchyma and the possible association of microliths and tumor formation.

This study is the first series that evaluated TM using Doppler ultrasound in a large cohort. There are only two case reports in the literature regarding the Doppler ultrasonographic findings of TM (13,14). Knowledge of the arterial supply of the testis is required for interpretation of color flow Doppler sonography of the testis. The testicular arteries arise from the anterior aspect of the aorta just below the origin of the renal arteries. They course through the inguinal canal with the spermatic cord to the postero-superior aspect of the testis. Upon reaching the testis, the testicular artery divides into branches, which pierce the tunica albuginea and divides over the surface of the testis in a layer known as the tunica vasculosa. Centripetal branches arise from these capsular arteries; these branches course along the septula to converge on the mediastinum. From the mediastinum these branches form recurrent rami that course centrifugally within the testicular parenchyma, where they branch into arterioles and capillaries (18). The velocity waveforms of the normal intratesticular arteries show high levels of antegrade diastolic flow throughout the cardiac cycle, reflecting the low vascular resistance of the testis (12). In this study cohort we evaluated the intratesticular artery (centripetal arteries and its recurrent rami) flow parameters. It is suggested that the resistive indices could be higher in patients with TM due to compression of the intratesticular arteries by the microliths. However, we did not find any differences regarding the Doppler parameters between subjects with or without TM, as reported by Kutlu et al. (14). All Doppler parameters and spectral examination findings were within normal limits in both groups.

An interesting finding was the twinkling artifact seen in three cases. Twinkling artifact was described by Rahmouni et al. (19) as an artifact generated by a strongly reflecting medium. Twinkling artifact appears as a rapidly alternating red and blue color Doppler signal behind certain stationary objects, which gives the appearance of movement. Since its initial description the twinkling artifact has been reported mainly in association with urinary tract calculi (20-22). Recently twinkling artifact has been described behind calcifications in various tissues, such as gallbladder stones, encrustated indwelling ureteral stents, strongly reflecting orbital structures, a calcified liver mass, intestinal pneumatosis and an intracranial microcoil (22-27). This finding has not been previously reported for TM. These artifacts secondary to

hyperechoic microliths have created difficulties in obtaining the Doppler spectral analysis of intratesticular branches.

All subjects with TM were followed-up throughout their military service. At 6 and 12 months of follow-up, the subjects with TM were re-evaluated with a physical examination, testicular tumor markers and scrotal US examination. None of the subjects with TM underwent the biopsy procedure since there were no findings suggesting a testicular tumor such as a hypoechoic area or an irregularity on testicular contour. No testicular tumor was detected during the diagnosis or follow-up.

In conclusion, Doppler ultrasound in TM, previously reported only as case studies in the literature, did not alter the spectral analysis parameters, and thus had no influence on testicular perfusion in Doppler US examination. Additionally we described the twinkling artifact, a misleading finding creating difficulty in spectral analysis secondary to microliths, which has not been reported in TM in the literature.

## CONFLICT OF INTEREST

None declared.

### REFERENCES


1. Janzen DL, Mathieson JR, Marsh JI, Cooperberg PL, del Rio P, Golding RH, et al.: Testicular microlithiasis: sonographic and clinical features. AJR Am J Roentgenol. 1992; 158: 1057-60.
2. Backus ML, Mack LA, Middleton WD, King BF, Winter TC 3rd, True LD: Testicular microlithiasis: imaging appearances and pathologic correlation. Radiology. 1994; 192: 781-5.
3. Jaramillo D, Perez-Atayde A, Teele RL: Sonography of testicular microlithiasis. Urol Radiol. 1989; 11: 55-7.
4. Höbarth K, Susani M, Szabo N, Kratzik C: Incidence of testicular microlithiasis. Urology. 1992; 40: 464-7.
5. Middleton WD, Teefey SA, Santillan CS: Testicular microlithiasis: prospective analysis of prevalence and associated tumor. Radiology. 2002; 224: 425-8.
6. Patel MD, Olcott EW, Kerschmann RL, Callen PW, Gooding GA: Sonographically detected testicular microlithiasis and testicular carcinoma. J Clin Ultrasound. 1993; 21: 447-52.
7. Serter S, Gümüs B, Unlü M, Tunçyürek O, Tarhan S, Ayyildiz V, et al.: Prevalence of testicular microlithiasis in an asymptomatic population. Scand J Urol Nephrol. 2006; 40: 212-4.
8. DeCastro BJ, Peterson AC, Costabile RA: A 5-year followup study of asymptomatic men with testicular microlithiasis. J Urol. 2008; 179: 1420-3; discussion 1423.
9. Tanriverdi O, Miroglu C, Horasanli K, Altay B, Caliskan KC, Gumus E: Testicular blood flow measurements and mean resistive index values after microsurgical and high ligation varicocelectomy. Urology. 2006; 67: 1262-5.
10. Ozturk A, Ozturk E, Zeyrek F, Onur K, Sirmatel O, Kat N: Comparison of brucella and non-specific epididymorchitis: gray scale and color Doppler ultrasonographic features. Eur J Radiol. 2005; 56: 256-62.
11. Turgut AT, Olçücüoglu E, Turan C, Kiliçoglu B, Kosar P, Geyik PO, et al.: Preoperative ultrasonographic evaluation of testicular volume and blood flow in patients with inguinal hernias. J Ultrasound Med. 2007; 26: 1657-66; quiz 1667-9.
12. Middleton WD, Thorne DA, Melson GL: Color Doppler ultrasound of the normal testis. AJR Am J Roentgenol. 1989; 152: 293-7.
13. Yagci C, Ozcan H, Aytaç S, Aydos K, Atasoy C: Testicular microlithiasis associated with seminoma: Gray-scale and color Doppler ultrasound findings. Urol Int. 1996; 57: 255-8.
14. Kutlu R, Sigirci A, Baysal T, Alkan A, Sarac K: Effects of testicular microlithiasis on Doppler parameters: report of three cases. BMC Urol. 2002; 2: 3.
15. Sharifi N, Farrar WL: Perturbations in hypoxia detection: a shared link between hereditary and sporadic tumor formation? Med Hypotheses. 2006; 66: 732-5.
16. Carmeliet P, Dor Y, Herbert JM, Fukumura D, Brusselmans K, Dewerchin M, et al.: Role of HIF-1alpha in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. Nature. 1998; 394: 485-90.
17. Vegni-Talluri M, Bigliardi E, Vanni MG, Tota G: Testicular microliths: their origin and structure. J Urol. 1980; 124: 105-7.
18. Middleton WD, Bell MW: Analysis of intratesticular arterial anatomy with emphasis on transmediastinal arteries. Radiology. 1993; 189: 157-60.
19. Rahmouni A, Bargoin R, Herment A, Bargoin N, Vasile N: Color Doppler twinkling artifact in hyperechoic regions. Radiology. 1996; 199: 269-71.

20. Chelfouh N, Grenier N, Higueret D, Trillaud H, Levantal O, Pariente JL, et al.: Characterization of urinary calculi: in vitro study of "twinkling artifact" revealed by color-flow sonography. AJR Am J Roentgenol. 1998; 171: 1055-60.
21. Lee JY, Kim SH, Cho JY, Han D: Color and power Doppler twinkling artifacts from urinary stones: clinical observations and phantom studies. AJR Am J Roentgenol. 2001; 176: 1441-5.
22. Aytaç SK, Ozcan H: Effect of color Doppler system on the twinkling sign associated with urinary tract calculi. J Clin Ultrasound. 1999; 27: 433-9.
23. Ustymowicz A, Krejza J, Mariak Z: Twinkling artifact in color Doppler imaging of the orbit. J Ultrasound Med. 2002; 21: 559-63.
24. Khan HG, Gailloud P, Martin JB, Khaw N, Spadola L, Rüfenacht DA, et al.: Twinkling artifact on intracerebral color Doppler sonography. AJNR Am J Neuroradiol. 1999; 20: 246-7.
25. Trillaud H, Pariente JL, Rabie A, Grenier N: Detection of encrusted indwelling ureteral stents using a twinkling artifact revealed on color Doppler sonography. AJR Am J Roentgenol. 2001; 176: 1446-8.
26. Ghersin E, Soudack M, Gaitini D: Twinkling artifact in gallbladder adenomyomatosis. J Ultrasound Med. 2003; 22: 229-31.
27. Oktar SO, Yücel C, Erbas G, Ozdemir H: Use of twinkling artifact in sonographic detection of intestinal pneumatosis. Abdom Imaging. 2006; 31: 293-6.

*Accepted after revision:*
*April 20, 2008*

**Correspondence address:**
Dr. Selim Serter
317 sok 20/5
Ucyol, Ýzmir, Turkey
Fax: + 90 236 237-0213
E-mail: serterselim@gmail.com

## EDITORIAL COMMENT

Testicular microlithiasis (TM) corresponds to intratubular calcifications resulting from degenerating cells within the seminiferous tubules. They can be located in the lumen or beneath the epithelium under a thin layer of connective tissue (1). They are well seen with Ultrasound, and occasionally and depending on the number of calcifications, on Computed Tomography.

One of the first reports on testicular microlithiasis was found in the context of pulmonary alveolar microlithiasis in 1970 (2). Microlithiasis has been found in the adult general population with a reported variable incidence of 2.4% (3) and 1.1% in children (4). It has also been observed in association with several other pathologies such as infertility (5), postorchiopexy (6), orchialgia (7), torsion of appendix testis (8), McCune-Albright syndrome (9) and Down syndrome (10). The main concern of testicular microlithiasis is its association with germ cell neoplasia and carcinoma in situ (11).

While microcalcifications do exist in roughly 50% of germ cell tumors, the majority of men with testicular microlithiasis will not develop testicular cancer. Increased emphasis on testicular examination is the recommended follow-up for men identified with this finding (12). Follow-up at this time should be dictated based on risk factors for developing testis cancer rather than on the presence of TM (13).

The recent study on Doppler Sonographic Findings in Testicular Microlithiasis by Serter et al. published in the current issue of the Journal expands the understanding of TM.

**REFERENCES**


1. Smith WS, Brammer HM, Henry M, Frazier H: Testicular microlithiasis: sonographic features with pathologic correlation. AJR Am J Roentgenol. 1991; 157: 1003-4.
2. Coetzee T: Pulmonary alveolar microlithiasis with involvement of the sympathetic nervous system and gonads. Thorax. 1970; 25: 637-42.
3. Serter S, Gümüş B, Unlü M, Tunçyürek O, Tarhan S, Ayyildiz V, et al.: Prevalence of testicular microlithiasis in an asymptomatic population. Scand J Urol Nephrol. 2006; 40: 212-4.
4. Dagash H, Mackinnon EA: Testicular microlithiasis: what does it mean clinically? BJU Int. 2007; 99: 157-60.
5. Schantz A, Milsten R: Testicular microlithiasis with sterility.Fertil Steril. 1976; 27: 801-5.
6. Mullins TL, Sant GR, Ucci AA Jr, Doherty FJ: Testicular microlithiasis occurring in postorchiopexy testis. Urology. 1986; 27: 144-6.
7. MacKinnon J: Testicular microlithiasis: echographic diagnosis of a new cause for orchialgia and infertility. Rev Med Chil. 1990; 118: 287-90.
8. Kwan DJ, Kirsch AJ, Chang DT, Goluboff ET, Berdon WE, Hensle TW: Testicular microlithiasis in a child with torsion of the appendix testis. J Urol. 1995; 153: 183-4.
9. Wasniewska M, De Luca F, Bertelloni S, Matarazzo P, Weber G, Crisafulli G, et al.: Testicular microlithiasis: an unreported feature of McCune-Albright syndrome in males. J Pediatr. 2004; 145: 670-2.
10. Vachon L, Fareau GE, Wilson MG, Chan LS: Testicular microlithiasis in patients with Down syndrome. J Pediatr. 2006; 149: 233-6.
11. Parenti GC, Zago S, Lusa M, Campioni P, Mannella P: Association between testicular microlithiasis and primary malignancy of the testis: our experience and review of the literature. Radiol Med (Torino). 2007; 112: 588-96.
12. Shichijo T, Sakamoto H, Saito K, Ogawa Y, Yoshida H, Kushima M: Relevance of testicular microlithiasis to the testicular carcinoma in situ in the contralateral testicle. Nippon Hinyokika Gakkai Zasshi. 2007; 98: 541-6.
13. Rashid HH, Cos LR, Weinberg E, Messing EM: Testicular microlithiasis: a review and its association with testicular cancer. Urol Oncol. 2004; 22: 285-9.



***Dr. Eugenio O. Gerscovich***
*University of California*
*Davis Medical Center*
*Sacramento, California, USA*
*E-mail: eugenio.gerscovich@ucdmc.ucdavis.edu*


## EDITORIAL COMMENT

In clinical practice, the greatest concern regarding testicular microlithiasis (TM) is whether TM is a premalignant condition. The authors prospectively showed that the prevalence of testicular microlithiasis (TM) was 2.4% of 2179 healthy men, which may be the most important finding in this study because the true prevalence of TM in the general population has not yet been established. This study also showed that TM was not associated with the development of testicular tumors during the 12-month follow-up period. Moreover, the authors compared the resistance index (RI) between men with and without TM in order to evaluate the relationship between TM and testicular perfusion, while also showing that TM did not alter testicular perfusion, which firstly showed based on the findings of a large cohort.

However, this study had some limitations as follows: 1) Various ultrasonographic diagnostic

criteria were used in different studies including this study, which may thus have influenced the prevalence of TM. 2) While the etiology of TM has not yet been verified, TM may originate from the degeneration of seminiferous tubules. Therefore, TM itself may be associated with an alteration of testicular perfusions. Moreover, the number and distribution of microliths may be related to testicular perfusion. 3) The number of men with TM is small. In addition, a failure to measure the RI was observed in about one-half of all males with and without TM. These factors may thus have influenced the results and they may also be related to the impairment of testicular perfusion. Therefore, further study is necessary to verify the relationship between testicular perfusion and TM. 4) Few studies have so far shown what the measurement of RI means in healthy men. Unfortunately, comparisons of the RI findings between men with and without TM in order to elucidate the relationship between TM and testicular perfusion may not be informative for readers. I think that a study, which evaluates the testicular function including the semen profiles, may be more useful for elucidating the relationship between TM and testicular perfusion.

Finally, TM itself and testicular tumors have not yet been verified as a premalignant condition, while TM has been reported to be associated with testicular tumors and carcinoma in situ. Therefore, the necessity of regular follow-up in normal men with TM has not yet been conclusively proven.

***Dr. Hideo Sakamoto***
*Department of Urology,*
*Showa University School of Medicine*
*Tokyo, Japan*
*E-mail: hs-showa-u@med.showa-u.ac.jp*

Clinical Urology

International Braz J Urol Vol. 34 (4): 485-491, July - August, 2008

# Activity of Antioxidant Enzymes in Seminal Plasma and their Relationship with Lipid Peroxidation of Spermatozoa

**Heidar Tavilani, Mohamad T. Goodarzi, Asad Vaisi-raygani, Saeedeh Salimi, Taghi Hassanzadeh**

*Department of Biochemistry (HT, MTG, THG), Medical School, Hamadan University of Medical Sciences, Hamadan, Iran, Department of Clinical Biochemistry (AVR), Faculty of Medicine, Kermanshah University of Medical Science, Kermanshah, Iran and Department of Biochemistry (SS), School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran*

**ABSTRACT**

*Purpose:* To determine the activity of seminal plasma catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) and their relationship with malondialdehyde (MDA), as a marker of lipid peroxidation, content of spermatozoa and seminal plasma in normozoospermic and asthenozoospermic males.
*Materials and Methods:* Semen samples were obtained from 15 normozoospermic and 30 asthenozoospermic men.
*Results:* We observed inverse correlations between activities of CAT (k/mL) and SOD (U/mL) in seminal plasma with MDA content of spermatozoa from normozoospermic samples ($r = -0.43$, $p < 0.05$ and $r = -0.5$, $p < 0.05$, respectively). Significant correlations were observed between total activity CAT (k/total seminal plasma) with total SOD (U/total seminal plasma) and GPX activity (mU/total seminal plasma) in seminal plasma from normozoospermic samples ($r = 0.67$, $p = 0.008$ and $r = 0.455$, $p = 0.047$, respectively). Furthermore, we found positive correlations between total activities of CAT, SOD and GPX with total content of MDA in seminal plasma (nmoL/total seminal plasma) from normozoospermic samples ($r = 0.67$, $p = 0.003$; $r = 0.73$, $p = 0.003$; $r = 0.74$, $p = 0.004$, respectively). In asthenozoospermic samples, there were no significant correlations observed between activities of CAT (k/mL), SOD (U/mL) and GPX (mU/mL) of seminal plasma with MDA content of spermatozoa. However, we found significant correlations between total activities of CAT (k/total seminal plasma) and SOD (U/total seminal plasma) with total content of MDA in seminal plasma ($r = 0.4$, $p = 0.018$ and $r = 0.34$, $p = 0.03$, respectively).
*Conclusion:* These findings indicate a protective role for antioxidant enzymes of seminal plasma against lipid peroxidation of spermatozoa in normozoospermic samples.

***Key words:*** *catalase; superoxide dismutase; glutathione peroxidase; semen; malondialdehyde*
***Int Braz J Urol. 2008; 34: 485-91***

## INTRODUCTION

Aerobic metabolism of human sperm produces various reactive oxygen species (ROS), which are potentially harmful to the sperm plasma membrane with its high content of polyunsaturated fatty acids (1-3). There is growing evidence that lipid peroxidation damage to the plasma membrane of spermatozoa plays an important role in the mechanism of male infertility (4-6). The toxic lipid peroxides are known to cause various impairments of the sperm cell, such as membrane damage and decrease in motility (7,8). Control of lipid peroxidation in the male reproductive tract is exerted by antioxidant molecules and protec-

tive enzymes within the spermatozoa and seminal plasma (9). Seminal plasma contains enzymatic ROS scavengers such as superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT). These enzymes act as an antioxidant and inhibitor of lipid peroxidation. Thus, peroxidative damage in spermatozoa not only depends on ROS production, but also on sperm and seminal plasma antioxidant defenses (10).

The question of whether seminal plasma SOD, GPX and CAT can act coordinately to protect human spermatozoa from lipid peroxidation has not to date been systematically addressed, although the presence of SOD, GPX and CAT activity in seminal plasma from fertile and infertile men has been reported (11-15). Since lipid peroxidation leads to loss of motility in human spermatozoa, the possibility exists that asthenozoospermic sperm suffers from the lack of protection against lipid peroxidation due to lack of adequate or non-coordination between SOD, GPX and CAT activity in seminal plasma. Whether the protective role of seminal antioxidant enzymes, against peroxidation, can affect products of spermatozoa or seminal plasma by lipid peroxidation remains unknown? The main objective of this study was to determine the activity of seminal plasma GPX, SOD and CAT and their relationship to MDA, as a marker of lipid peroxidation, content of spermatozoa and seminal plasma in normozoospermic and asthenozoospermic males.

## MATERIALS AND METHODS

### Semen Samples

Semen specimens were obtained in 30 asthenozoospermic patients who attended the Omid Fertility Clinic for infertility evaluation. In addition, 15 healthy men with normal semen parameters according to World Health Organization (WHO) criteria were enrolled as controls (16). The two groups were similar as regards mean age (20-40 years of age). Patients had no systemic diseases were non smokers and had no alcohol dependence, and none were taking an oral antioxidant supplement for three months prior tot the study. Patients fulfilling the inclusion criteria were asked to participate in this research project, which was duly explained to them. Written informed consent was obtained from all enrollees, according to the criteria of the Ethical Committee of Tehran University of Medical Sciences. All semen samples were collected by masturbation following 3 days of abstinence. After liquefaction, semen volume, sperm concentration (hemocytometer), total sperm count, morphology (Pap smear), motility grades: a (rapid progressive), b (slow progressive), c (non-progressive), d (immotile) were determined using WHO standard procedures (16). All major determinations were carried out in duplicate. Semen samples with more than $1\times10^6$/mL neutrophils using peroxidase staining (16) or other round cells were excluded. Asthenozoospermia was indicated by a sperm concentration of $\geq 20\times10^6$/mL and motility (grade a+b) of < 50%, irrespective of the morphology results. Normozoospermia was indicated by a sperm concentration of $\geq 20\times10^6$/mL and a motility (grade a+b) $\geq$ 50% and a normal morphology of $\geq$ 14%. Following semen analysis, a volume of semen containing at least 50 million sperm (or more) was transferred into a conical centrifuge tube and was centrifuged at 1000 × g for 10 min at room temperature. Immediately after the centrifugation, the supernatant was collected and stored at -80º C and the pellet from each sample was resuspended in 0.2 mL phosphate buffer saline (17).

### Measurement of Antioxidant Enzymes Activity

Seminal plasma SOD activity was measured using a Ransod kit (Randox Laboratories, Crumlin, U.K.) with xanthine and xanthine oxidase to generate superoxide radicals which react with 2- (4 - iodophenyl) - 3 - (4- nitrophenol)-5-phenyltetrazolium chloride (I.N.T) to form a red formazan dye. Seminal plasma was diluted 31-fold with l0 mM phosphate buffer, pH 7. One unit of SOD was the amount that caused a 50% inhibition in the rate of I.N.T. reduction. The SOD activity was expressed as specific activity (U/mL seminal plasma) and total activity (U/total seminal plasma).

Seminal plasma GPX was measured by a Ransel kit (Randox Laboratories Ltd., London, U.K.). GPX catalyses the oxidation of glutathione by cumene

hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione was immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm was measured. The GPX activity was expressed as specific activity (mU/mL seminal plasma) and total activity (mU/total seminal plasma).

Catalase activity was measured according to Abei (18) by monitoring the initial rate of disappearance of hydrogen peroxide (initial concentration 10 mM) at 240 nm. The catalase activity was expressed as specific activity (k/mL seminal plasma) and total activity (k/total seminal plasma).

## Measurement of Malondialdehyde Levels

Lipid peroxidation in spermatozoa and seminal plasma was measured by reaction of thiobarbiuric acid (TBA) with malondialdehyde (MDA) according to Yagi (19). Content of MDA was measured spectrofluorometrically using a Jasco (FP-6200) spectrofluorometer (excitation 515 nm, emission 553 nm). The MDA fluorescence intensity of spermatozoa and seminal plasma was determined using various concentrations of tetraethoxypropane as standards. The results were expressed as nmoL MDA/$10\times10^6$ cells, nmoL MDA/mL seminal plasma and nmoL MDA/total seminal plasma.

## Statistical Analysis

Due to the fact that sperm concentration, motility, morphology, MDA and various other determined semen parameters were not normally distributed, the Mann-Whitney U test was applied to compare the asthenozoospermic and normozoospermic groups. To assess seminal plasma CAT, SOD, GPX activities and sperm count, one tailed two-independent sample t-test was used. Correlation between variables was assessed using non-parametric Spearman's coefficient (r). Data were expressed M ± Standard Error.

## RESULTS

The semen profiles of normozoospermic and asthenozoospermic samples are shown in Table-1. Percent of motility grade a+b and spermatozoa with normal morphology was higher in normozoospermic compared to asthenozoospermic samples ($p < 0.001$). Results of seminal plasma CAT, SOD and GPX activities in normozoospermic and asthenozoospermic groups are shown in Table-2. Mean seminal plasma specific and total activity of SOD, GPX and CAT were not significantly different in two groups. MDA content in the spermatozoa of asthenozoospermic was significantly higher than in normozoospermic samples ($0.14 \pm 0.004$ and $0.09 \pm 0.004$ nmoL/$10^7$ spermatozoa, respectively). The mean ± SE value of MDA in the seminal plasma of asthenozoospermic and normozoospermic were not significantly different (Table-2).

Correlations between CAT, SOD and GPX activities with MDA content of spermatozoa and seminal plasma from normozoospermic samples are shown in Table-3. There were negative and significant correlations between activities of CAT and SOD in seminal plasma with MDA content of spermatozoa from normozoospermic samples. In addition, we observed high positive correlations between total activities of CAT, SOD and GPX with total content of MDA from seminal plasma.

In asthenozoospermic samples, there were no significant correlations between specific activities of CAT, SOD and GPX of seminal plasma with MDA content of spermatozoa (Table-4). However, we found positive and significant correlations between total activities of CAT and SOD with total content of MDA in seminal plasma (Table-4).

Significant correlations were found between total activity CAT with total activity SOD and total activity GPX in seminal plasma from normozoospermic samples ($r = 0.67$, $p = 0.008$ and $r = 0.455$, $p < 0.05$, respectively). In addition, there was a significant correlation between specific activity CAT and specific activity SOD in normozoospermic samples ($r = 0.58$, $p = 0.022$). Moreover, we observed a significant correlation between total CAT and SOD activity in seminal plasma of asthenozoospermic samples ($r = 0.33$, $p < 0.05$).

## COMMENTS

In the present study, we were able to determine the SOD, GPX and CAT activity in the seminal

***Table 1 –*** *Basic semen parameters (mean ± SE) from normozoospermic and asthenozoospermic subjects.*

| **Semen Parameters** | **Normozoospermic** (n = 15) | **Asthenozoospermic** (n = 30) |
|---|---|---|
| Volume (mL) | 3.68 ± 0.34 | 3.45 ± 0.22 |
| Sperm concentration ($10^6$/mL) | 112 ± 19 | 83.9 ± 5* |
| Total sperm count ($10^6$ sperm /ejaculate) | 383 ± 63 | 275 ± 26 |
| Normal sperm form (%) | 22 ± 1.7 | 8.1 ± 0.6** |
| White Blood Cell ($10^6$/mL) | 0.68 ± 0.09 | 0.71 ± 0.08 |
| Motility grade a+b (1) (%) | 58.7 ± 2.9 | 27.5 ± 2** |

*(1) = Grade of sperm movement according to World Health Organization criteria (16). a = rapid progressive; b = slow progressive, * p < 0.05; ** p < 0.001.*

plasma and the MDA content of the spermatozoa and seminal plasma in normozoospermic and asthenozoospermic samples. Jones et al. showed that the mechanism by which oxidative stress induced motility loss in mammalian spermatozoa involved the induction of peroxidative damage to the sperm plasma membrane (1). Human spermatozoa are particularly vulnerable to lipid peroxidation because their plasma membranes are enriched with polyunsaturated fatty acids, particularly docosahexaenoic acid with six double bonds (6,20). These polyunsaturated fatty acids are essential to produce plasma membrane fluidity that is required to participate in the membrane fusion events associated with fertilization (1).

***Table 2 –*** *Specific and total activity of catalase (CAT), glutathione peroxidase (GPX), superoxide dismutase (SOD) of seminal plasma and malondialdehyde (MDA) content of spermatozoa and seminal plasma from normozoospermic and asthenozoospermic samples.*

| **Parameters** | **Normozoospermic** (n = 15) | **Asthenozoospermic** (n = 30) |
|---|---|---|
| Specific activity of CAT (k/mL) | $0.3 \pm 0.1 \times 10^{-3}$ | $0.18 \pm 0.04 \times 10^{-3}$ |
| Total activity of CAT (k/total seminal plasma) | $1.14 \pm 0.39 \times 10^{-3}$ | $0.6 \pm 0.11 \times 10^{-3}$ |
| Specific activity of SOD (U/mL) | 6.88 ± 0.66 | 7.79 ± 0.64 |
| Total activity of SOD (U/ total seminal plasma) | 24.6 ± 3.6 | 25 ± 1.8 |
| Specific activity of GPX (mU/mL seminal plasma) | 352 ± 58 | 366 ± 33 |
| Total activity of GPX (mU/ total seminal plasma) | 1318 ± 251 | 1243 ± 111 |
| Sperm MDA content (nmoL/$10^7$ spermatozoa | 0.09 ± 0.004 | 0.14 ± 0.004* |
| Seminal plasma MDA content (nmoL/ mL seminal plasma) | 1.2 ± 0.077 | 1.35 ± 0.076 |
| Total seminal plasma MDA content (nmoL/ total seminal plasma) | 4.1 ± 0.36 | 4.6 ± 0.31 |

**p < 0.05*

***Table 3 –*** *Correlations between catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities with malondialdehyde (MDA) content of spermatozoa and seminal plasma from normozoospermic (n = 15) samples.*

| **Enzyme Activity** | **MDA of Spermatozoa** (nmoL/$10^7$ spermatozoa) | | **MDA in Total Seminal Plasma** (nmoL/total seminal plasma) | |
|---|---|---|---|---|
| | **r** | **p Value** | **r** | **p Value** |
| CAT (k/mL) | -0.43 | 0.048 | 0.36 | 0.1 |
| SOD (U/mL) | -0.5 | 0.046 | 0.27 | 0.19 |
| GPX (mU/mL) | -0.161 | 0.3 | 0.3 | 0.17 |
| CAT (k/total seminal plasma) | -0.38 | 0.10 | 0.67 | 0.003 |
| SOD (U/total seminal plasma) | -0.19 | 0.2 | 0.73 | 0.003 |
| GPX (mU/total seminal plasma) | -0.02 | 0.4 | 0.74 | 0.004 |

***Table 4 –*** *Correlations between catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities with malondialdehyde (MDA) content of spermatozoa and seminal plasma from asthenozoospermic (n = 30) samples.*

| **Enzyme Activity** | **MDA of Spermatozoa** (nmoL/$10^7$ spermatozoa) | | **MDA in Total Seminal Plasma** (nmoL/total seminal plasma) | |
|---|---|---|---|---|
| | **r** | **p Value** | **r** | **p Value** |
| CAT (k/mL) | -0.23 | 0.12 | 0.07 | 0.3 |
| SOD (U/mL) | -0.2 | 0.14 | -0.28 | 0.06 |
| GPX (mU/mL) | 0.041 | 0.4 | -0.18 | 0.18 |
| CAT (k/total seminal plasma) | -0.004 | 0.49 | 0.4 | 0.018 |
| SOD (U/total seminal plasma) | 0.04 | 0.4 | 0.34 | 0.03 |
| GPX (mU/total seminal plasma) | 0.27 | 0.09 | 0.14 | 0.24 |

Our study showed that asthenozoospermic men compared with normozoospermic do not have deficient seminal plasma SOD, GPX and CAT activities. In contrast, there were no significant differences between specific and total activity of SOD, GPX and CAT in seminal plasma of two groups. Different studies have investigated antioxidant enzymes of seminal plasma in asthenozoospermic samples or other altered semen parameters but their results remain controversial (11-15). Results based on this study showed a negative correlation between specific activity of CAT and SOD with MDA content of spermatozoa from normozoospermic samples. This observation suggests that CAT and SOD of seminal plasma may play a role in the protection against lipid peroxidation in the normozoospermic samples. In our study, we observed higher content of lipid peroxidation product malondialdehyde (MDA) in spermatozoa of asthenozoospermic compared with normozoospermic samples ($p < 0.05$). Although, the difference between MDA of seminal plasma was not significant between two groups. In addition, we did not find any significant correlation between spermatozoa MDA and activity of antioxidant enzymes of seminal plasma. Moreover,

the activity of seminal antioxidant enzymes could not have protected spermatozoa from asthenozoospermic samples against lipid peroxidation. Our results are in agreement with Jones et al. who reported that the addition of SOD, GPX and CAT to the medium of spermatozoa (which contain generating system of oxygen free radicals; sodium ascorbate and $FeSO_4$) did not inhibit MDA formation (21). There is evidence for transferring of various proteins to the spermatozoa, and the role of post testicular maturation of the sperm cells have been well documented (22). We suggest that in the normozoospermic samples, the membrane structure of spermatozoa is influenced to allow adsorption of seminal plasma CAT and SOD onto the membrane, thereby providing the protective action of CAT and SOD against lipid peroxidation. However, in asthenozoospermic samples, seminal antioxidant enzymes cannot be adsorbed to the plasma membrane of spermatozoa. Sperm membrane has been reported to be adversely affected by peroxidation of polyunsaturated fatty acids and accumulation of organic hydroperoides (21). Since, we found higher content of lipid peroxidation product (MDA) in asthenozoospermic samples, we suggest that membrane of spermatozoa was affected by lipid peroxidation and thereby could not have adsorbed antioxidant enzymes of seminal plasma. While there may be many reasons for increased lipid peroxidation product in spermatozoa from asthenozoospermic males, one reason may be partly due to non adsorption of seminal antioxidant enzymes to spermatozoa membrane and subsequent reduction in lipid protection.

In this study, we found a positive correlation between total activity of CAT, SOD and GPX with total content of MDA in seminal plasma (nmoL/total seminal plasma) from normozoospermic samples. In addition, our data showed the significant correlation between total activity of CAT with total activity of SOD and GPX in normozoospermic samples. These findings may indicate a cooperation and coordination between function of antioxidant enzymes in normozoospermic samples. We suggest that the activity of seminal antioxidant enzymes may be regulated by MDA content of seminal plasma. Thus, further studies are needed to clarify the role of MDA on activity of antioxidant enzymes of seminal plasma from normozoospermic and asthenozoospermic samples.

In conclusion, these findings indicate a protective role for antioxidant enzyme of seminal plasma against lipid peroxidation of spermatozoa in normozoospermic samples. We suspect that under pathological conditions (e.g. asthenozoospermia) the activity of seminal antioxidant enzymes can not protect spermatozoa and may cause an increase of lipid peroxidation from spermatozoa.

## ACKNOWLEDGEMENTS

The authors are grateful to Professor Bayard T. Storey, University of Pennsylvania, for his review of this manuscript. Research was supported by Hamadan University of Medical Sciences.

## CONFLICT OF INTEREST

None declared.

### REFERENCES

1. Jones R, Mann T, Sherins R: Peroxidative breakdown of phospholipids in human spermatozoa, spermicidal properties of fatty acid peroxides, and protective action of seminal plasma. Fertil Steril. 1979; 31: 531-7.
2. Cocuzza M, Sikka SC, Athayde KS, Agarwal A: Clinical relevance of oxidative stress and sperm chromatin damage in male infertility: an evidence based analysis. Int Braz J Urol. 2007; 33: 603-21.
3. Lenzi A, Gandini L, Picardo M, Tramer F, Sandri G, Panfili E: Lipoperoxidation damage of spermatozoa polyunsaturated fatty acids (PUFA): scavenger mechanisms and possible scavenger therapies. Front Biosci. 2000; 5: E1-E15.
4. Aitken RJ, Buckingham D, West K, Wu FC, Zikopoulos K, Richardson DW: Differential contribution of leucocytes and spermatozoa to the generation of reactive oxygen species in the ejaculates of oligozoospermic patients and fertile donors. J Reprod Fertil. 1992; 94: 451-62.
5. Zalata A, Hafez T, Comhaire F: Evaluation of the role of reactive oxygen species in male infertility. Hum Reprod. 1995; 10: 1444-51.

6. Khosrowbeygi A, Zarghami N: Fatty acid composition of human spermatozoa and seminal plasma levels of oxidative stress biomarkers in subfertile males. Prostaglandins Leukot Essent Fatty Acids. 2007; 77: 117-21.
7. Alvarez JG, Touchstone JC, Blasco L, Storey BT: Spontaneous lipid peroxidation and production of hydrogen peroxide and superoxide in human spermatozoa. Superoxide dismutase as major enzyme protectant against oxygen toxicity. J Androl. 1987; 8: 338-48.
8. Aitken RJ, Harkiss D, Buckingham D: Relationship between iron-catalysed lipid peroxidation potential and human sperm function. J Reprod Fertil. 1993; 98: 257-65.
9. Storey BT: Biochemistry of the induction and prevention of lipoperoxidative damage in human spermatozoa. Mol Hum Reprod. 1997; 3: 203-13.
10. Garrido N, Meseguer M, Simon C, Pellicer A, Remohi J: Pro-oxidative and anti-oxidative imbalance in human semen and its relation with male fertility. Asian J Androl. 2004; 6: 59-65.
11. Zini A, Garrels K, Phang D: Antioxidant activity in the semen of fertile and infertile men. Urology. 2000; 55: 922-6.
12. Sanocka D, Miesel R, Jedrzejczak P, Kurpisz MK: Oxidative stress and male infertility. J Androl. 1996; 17: 449-54.
13. Hsieh YY, Sun YL, Chang CC, Lee YS, Tsai HD, Lin CS: Superoxide dismutase activities of spermatozoa and seminal plasma are not correlated with male infertility. J Clin Lab Anal. 2002; 16: 127-31.
14. Alkan I, Simsek F, Haklar G, Kervancioglu E, Ozveri H, Yalçin S, et al.: Reactive oxygen species production by the spermatozoa of patients with idiopathic infertility: relationship to seminal plasma antioxidants. J Urol. 1997; 157: 140-3.
15. Khosrowbeygi A, Zarghami N: Levels of oxidative stress biomarkers in seminal plasma and their relationship with seminal parameters. BMC Clin Pathol. 2007; 7: 6.
16. World Health Organization WHO: Laboratory Manual for the Examination of Human Semen and Semen - Cervical Mucus Interaction, 4 th (ed.), Cambridge University Press. 1999; p. 4-23.
17. Engel S, Schreiner T, Petzoldt R: Lipid peroxidation in human spermatozoa and maintenance of progressive sperm motility. Andrologia. 1999; 31: 17-22.
18. Aebi H: Catalase in vitro. Methods Enzymol. 1984; 105: 121-6.
19. Yagi K: Assay for blood plasma or serum. Methods Enzymol. 1984; 105: 328-31.
20. Tavilani H, Doosti M, Nourmohammadi I, Mahjub H, Vaisiraygani A, Salimi S, et al.: Lipid composition of spermatozoa in normozoospermic and asthenozoospermic males. Prostaglandins Leukot Essent Fatty Acids. 2007; 77: 45-50.
21. Christova Y, James PS, Jones R: Lipid diffusion in sperm plasma membranes exposed to peroxidative injury from oxygen free radicals. Mol Reprod Dev. 2004; 68: 365-72.
22. Saez F, Frenette G, Sullivan R: Epididymosomes and prostasomes: their roles in posttesticular maturation of the sperm cells. J Androl. 2003; 24: 149-54.

*Accepted after revision:*
*April 4, 2008*

**Correspondence address:**
Dr. Heidar Tavilani
Department of Biochemistry
Medical School, Hamadan Univ of Medical Sciences
Hamadan, Iran
Fax: + 98 811 827-6299
E-mail: tavilani@gmail.com

Investigative Urology

International Braz J Urol Vol. 34 (4): 492-502, July - August, 2008

# In Vitro Evidence for a New Therapeutic Approach in Renal Cell Carcinoma

**Carmine Pittoggi, Gianni Martis, Giorgia Mastrangeli, Bruno Mastrangeli, Corrado Spadafora**

*SBGSA (CP, CS), Italian National Institute of Health, Rome, Italy, Department of Urology (GM, BM), San Camillo De Lellis Hospital, Rieti, Italy and MIPI (GM), Italian National Institute of Health, Rome, Italy*

**ABSTRACT**

*Purpose:* Renal cell carcinoma (RCC) is the most lethal among the common urologic malignancies, comprising 3% of all human neoplasias; approximately 40% of patients eventually die of cancer progression. One third of patients who present with metastatic disease and up to 40% treated for localized disease generally experience recurrence. RCCs are characterized by high resistance to chemo-, radio- and immunotherapy. We recently discovered an endogenous enzymatic activity, which is particularly expressed in tumorigenic cell, endogenous non-telomerase reverse transcriptase (RT) of retrotrasposon / retroviral origin, as a specific target to induce proliferation arrest in a number of human carcinogenesis in vitro culture cell lines.

*Methods:* To address this possibility, we have employed RCC primary cell culture testing pharmacological inhibition, in vitro, by two characterized non nucleosidic RT inhibitors, nevirapine and efavirenz; next, we assessed morphological effects and analyzed putative modulation on gene expression profile.

*Results:* Both treatments reduced cell proliferation rate and induced morphological differentiation and gene expression reprogramming in different RCC analyzed tumor biomarkers.

*Conclusion:* In this study we describe a new potential therapeutic approach to obtain considerable future benefits in renal carcinoma cure and attempt to establish a new possible pharmacological therapy based on oral drugs administration in renal RCC treatment.

***Key words:*** *renal cell carcinoma; reverse transcriptase; gene expression; therapy*

***Int Braz J Urol. 2008; 34: 492-502***

## INTRODUCTION

Renal cell carcinomas (RCCs) are the most frequent tumors of the kidney. They comprise 3% of all human neoplastic diseases and are increasing in incidence. The etiology of RCC remains unknown and although the majority of renal tumors develop as sporadic forms, rare familiar forms and distinct genetic abnormalities have been observed. In particular, it seems that clear cell carcinoma originates from the proximal tubular epithelium in the renal cortex. Molecular analysis of this solid tumor is furthermore complicated by the mixture of tumor cells and normal cells, composed of leukocytes and connective tissue cells. To overcome this problem the tumor cells may be adapted to grow in vitro, as primary cell cultures, in order to obtain a more homogenous cellular material to study the biochemical and molecular changes associated with neoplastic status.

RCCs can be classified into clear cell, papillary and chromophobe carcinomas based on their histological appearance.

In these tumors, a crucial role for prevention should include accurate prognosis and systematic investigation of gene expression and/or proteomic profile to identify protein changes caused by disease processes (1). The most frequent type of renal cancer is the clear cell variant, accounting for 80-85% of adult renal neoplasms.

RCCs are characterized by high resistance to chemo-, radio- and immunotherapy. Recently, new approaches, based on anti-angiogenic drugs, have emerged as potential therapies in the treatment of metastatic renal cell carcinoma (mRCC). Tyrosine kinase receptors (RTKs) are transmembrane proteins involved in signal transduction. Overexpression and/or the structural alteration of different RTKs classes are generally associated with cancer and their abnormal activation often generates cancer growth, angiogenesis and metastatization. Sunitinib malate is a molecule able to link intracellular tyrosine kinase domain of RTKs, which have an anticancer and antiangiogenetic activity. Sunitinib targets selectively vascular endothelial growth factor, KIT, Flt3 and platelet-derived growth factor receptors and the receptor encoded by the ret proto-oncogene. This drug is currently used in the treatment of gastrointestinal stromal tumors resistant to imatinib and mRCC (2). Sorafenib (Nexavar, BAY43-9006) is a new small-molecule, oral, multi-kinase inhibitor for the treatment of patients with advanced RCC. The response rate to sorafenib is partial (10%). In sorafenib treated patients there was a significant prolongation of progression-free survival. Overall survival results are still preliminary. The principal toxicities in the sorafenib patients included reversible skin rashes in 40% and hand-foot skin reaction in 30% (3). Another compound recently used in mRCC cancer therapy is temsirolimus, a specific inhibitor for the mammalian target of rapamycin kinase. Interferon alpha is widely used for mRCC but has limited efficacy and tolerability. As compared with interferon alpha, temsirolimus has been shown to improve overall survival among patients with metastatic renal-cell carcinoma and a poor prognosis (4).

This prompted us to evaluate the possibility of applying our findings for a therapeutic application in combination with kinase inhibitors recently used in mRCC, as a new class of biological agents , which have begun to break the resistance barrier (5).

Cancer progression and development have been accompanied by profound changes at cellular and molecular level, involving RNA, DNA and protein function and structure.

Recently, we have defined an endogenous activity, reverse transcriptase (RT), which is particularly expressed in tumor tissues as compared to normal tissues. In vitro cell culture treatment with specific inhibitors of the endogenous RT activity (6) has led to cell cycle arrest and induced morphological differentiation and gene expression reprogramming.

In this study, we used a human renal carcinoma derived primary cell culture to assess the cell cycle progression in vitro demonstrating that RT non nucleosidic inhibitors (nevirapine and efavirenz) induce cell cycle arrest in RCCs, morphological changes and gene markers modulation.

Our results demonstrate the treatment efficacy of these new drugs as potential therapy for in vitro RCC culture.

## MATERIALS AND METHODS

### Tissue Samples

Samples from 3 patients with clear RCC, containing control non tumoral ("normal") and tumor tissue were obtained from fresh nephrectomy specimens. After surgical resection the samples were immediately snap frozen in liquid nitrogen and stored at -80°C at the Department of Urology of the San Camillo De Lellis Hospital in Rieti, Italy. Paraffin sections from each specimen were reviewed by a pathologist and classified histologically according to UICC-TNM.

### Primary Tissue RCC Cultures and Derived Cell Line

Autologous tumor and cortex renal tissue specimens were collected, after surgery, in cold DMEM medium containing 1% penicillin/streptomycin, 1% amphotericin, 0.5% glutamine, 20% fetal

calf serum (FCS) and kept at 4°C until processing (within 18 h). Tissues , normal and neoplastic, were vigorously washed 4-5 times vortexing in phosphate-buffered saline (PBS) pH 7.2 at 37°C and minced in 1-$mm^3$ fragments, in a Petri dish containing PBS, and vigorously re-washed vortexing with PBS at 37°C. The small fragments were left for 1 h in a dish in the presence of medium at 37°C and then 10 pieces were definitively plated in a new 10 cm Petri dish and covered by DMEM. Four Petri dishes were routinely prepared for each autologous tissue and incubated at 37°C in 5% $CO_2$. The first medium change was performed after 5 days, when the tissue fragments were removed. Cultures were fed twice weekly and passed in new dishes when 90% confluent after trypsinization and 1:2 split. Aliquots of cells were cryopreserved in 90% FCS/10% dimethyl sulfoxide and stored in liquid nitrogen after 1-2 passages. All experiments were conducted on the third passage. Primary RCC cell line obtained was well identified as T1 N0 M0 stage, II° grade, according to standard nomenclature defined by Fuhrman.

## Cell Cultures

Human RCC primary culture cells were seeded in six –well plates at a density of $10^4$ to 5 x $10^4$ cells/well and cultured in RPMI 1640 medium with 10% fetal bovine serum. Nevirapine and efevirenz were purified from commercially available Viramune® (Boehringer-Ingelheim) and Sustiva® (Bristol-Myers Squibb) as described (7). The drugs were made 350 and 15 μM (final concentration) in dimethyl sulfoxide (DMSO, Sigma Aldrich), respectively, and added to cells 5 h after seeding, the same DMSO volume (0.2% final concentration) was added to controls. Every 48 h fresh RT inhibitors-containing medium was changed. Cells were harvested every 96 h, counted in a Burker's chamber and replated at the same density.

## RNA Extraction and Semi-quantitative RT-PCR

Total RNA was extracted from RCC primary cell culture (10 x $10^6$ cell) treated with nevirapine and efavirenz and untreated (CTR); the RNAeasy mini kit (Qiagen, Germany) was used. Trace amounts of contaminant chromosomal DNA was eliminated by incubation with Rnase-free Dnase I (Invitrogen, Carlsbad, CA ), 1 U/μg of total RNA, for 15 min at room temperature; 200 ng of each RNA sample was used in a oligo(dT) cDNA synthesis, performed using the Thermoscript RT-PCR system (Invitrogen, USA). 2 μL for each cDNA sample were PCR amplified using the Platinum Taq DNA Polymerase I (Invitrogen, USA), in a 50 μL reaction mixture containing 30 pmoL of specific oligonucleotides (MWGBiotech, Ebersberg, Germany) in an initial 2-min step at 94°C, followed by cycles of 30 s at 94°C, 30 s at 58-62°C, 1 min at 72°C. Each oligo pair was used in sequential amplification series with increasing numbers (30-40) of cycles. PCR products were fractioned through 1.4% agarose gels and visualized with UV transilluminator light.

Set of primers used for standard PCR are designed forward (fwd) and reverse (rev). Oligonucleotide sequences and expected product sizes are listed below:

NNMT (NM 006169); PCR product size:188 bp
NNMT fwd 5'-tcaagcaggtgctgaagtgt-3'
NNMT rev 5'-atccatgatcaccaggaagc-3'
NNMT int 5'-agcacactgtgtctggatgc-3'

AFP (NM 001134); PCR product size:113 bp
AFP fwd 5'-agcttggtggtggatgaaac-3'
AFP rev 5'-tcttgcttcatcgtttgcag-3'
AFP int 5'-tccctcctgcattctctgat-3'

CD70 (EF 064709); PCR product size:110 bp
CD70 fwd 5'-aatcacacaggacctcagcaggacc-3'
CD70 rev 5'-agcagatggccagcgtcacc-3'
CD70 int 5'-agccgtagtaatggaatccca-3'

FRA2 (X 16706); PCR product size:125 bp
FRA2 fwd 5'-ccctgcacacccccatcgtg-3'
FRA2 rev 5'-tgattggtccccgctgctactgctt-3'
FRA2 int 5'-tccttagataatgcatccagtaa-3'

p 27 KIP1 (NM 004064); PCR product size: 184 bp
p 27 fwd 5'-gccctccccagtctctctta-3'
p 27 rev 5'-acagcccgaagtgaaaagaa-3'
p 27 int 5'-caggtagtttggggcaaaaa-3'

GAPDH (NM 002046); PCR product size: 650 bp
Gapdh fwd 5'-ATTCAACGGCACAGTCAAGG-3'
Gapdh rev 5'-AAGGTGGAAGAGTGGGAGTT-3'
Gapdh int 5'-GGGAAGCCCATCACCATCT-3'.

Internal oligonucleotides were used as probe to hybridize PCR DNA transferred to Hybond N+ nylon filters. After washing, filters were exposed to FUJI XR-E 30 films for the requested time.

## Tumor Markers Gene Expression Profile

The induction of morphological differentiation suggests that critical regulatory genes are modulated in response to the RT inhibitory treatment. This was investigated in semi-quantitative RT-PCR analysis of cultures treated with DMSO alone, or nevirapine or efavirenz for three cycles. In RCC derived primary cell culture, we focused on a set of five genes: the CD70 gene, a new diagnostic biomarker, known as a member of the tumor necrosis factor (ligand) superfamily (8); the FRA 2 gene, Fos Related Antigen 2, associated also with apoptosis and regulator of cell proliferation, differentiation and transformation (9); the NNMT gene; the AFP gene; p27KIP1 gene.

RT-PCR results, in Figure-1, indicate that the CD70 gene and the FRA 2 gene were markedly down - regulated, in contrast with not treated tumor cells where expression levels for the same genes were considerably higher; whereas, the AFP gene, which encodes for a major plasma protein, which expression in adults is often associated with hepatoma and teratoma (10), and is thought to be the fetal counterpart of serum albumin, in RT-inhibited RCC, results in a down regulation of its mRNA expression level. In contrast, the NNMT gene, encoding for a cytokine that belongs to the tumor necrosis factor ligand family, involved in T cell antigen-presenting cell interactions, and along with CD70 shown to provide CD28-independent costimulatory signals (11), results in mRNA expression levels similar to DMSO control expression. We extended mRNA expression to another possible tumor marker: p27 KIP1. This gene encodes a cyclin-dependent kinase inhibitor, which shares a limited similarity with CDK inhibitor CDKN1A/p21. The encoded protein binds to and prevents the activation of cyclin E-CDK2 or cyclin D-CDK4 complexes and thus control the cell cycle progression at G1. The degradation of this protein is required for the cellular transition from quiescence to the proliferation state. Our results show a significant p27 over-expression in nevirapine and efavirenz treated cells, confirming that the p27 expression increase was correlated to a decreasing tumor stage (12).

Thus, RT inhibitory drugs modulate the expression of critical genes implicated in the development of transformed cells, concomitantly with the induction of differentiation-like state relative to quiescence; also, this reprogramming is reversible and is abolished when RT-inhibition is released (data not shown).

## Preparation of Cell-Free RCC Lysates and RT Activity Assay

Cell free RCC lysates were prepared by lysing control, nevirapine and efavirenz treated

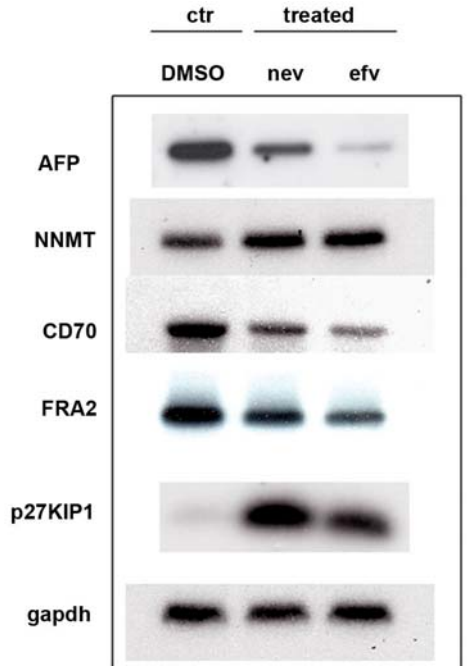



***Figure 1*** *– RT inhibitors modulate gene expression in primary RCC cell culture. RNA extracted from cells treated with DMSO (ctr), nevirapine (nev) or efavirenz (efv), was amplified by RT-PCR, blotted and hybridized with internal oligonucleotides.*

RCC cells grown till the third cycle (288 h) in vitro, using ice-cold lysis buffer (10mM Tris-Hcl, pH=7.5; 1 mM $MgCl_2$; 1mM EGTA; 0.1 mM PMSF; 5 mM β-mercaptoethanol; 0.5% CHAPS; 10% glycerol).

Cell lysates ($5x10^6$ cells /100 µL of lysis buffer) were subjected to three freeze-and-thaw (liquid nitrogen/37°C) cycles, incubated for 30 min on ice and centrifuged for 30 min at 14,000 rpm at 4°C. The supernatant containing the RT activity was aliquoted, quickly frozen in dry ice and stored at -70°C.

The protein concentration was determined by the standard Coomassie (Pierce Chemical Co., Chester, England). RT was tested in a PCR-based (PBRT) assay as previously described (3) with minor modifications. Briefly, PBRT reactions contained cells lysate aliquots corresponding to 6 ug of protein, 10 ng of bacteriophage MS2 RNA (Roche Diagnostics, Hertfordshire, England), 50 mM Tris acetate (pH 8.4), 75 mM K acetate, 40 mM Mg acetate, 5 mM DTT, 1mM of four nucleotide triphosphate mix, 2 U of Rnase OUT and 30 pmoL of MS2 reverse primer (R) (see below for the sequence) in a final volume of 20 uL. Reaction mixture were incubated at 55°C for 1 hour followed by 5 min at 85°C. One microliter of E. Coli RNaseH (2U/uL) was added to each sample and further incubated at 37°C for 20 min. Control reactions were set up by omitting cell lysate (negative controls), or adding 1 uL of ThermoScript RT (Invitrogen, Karlsruhe, Germany) 15 U/uL (positive control). Two microliters from each reaction were mixed with 30 pmoL each of forward (F) 5'-TCCTGCTCAACTTCCTGTCGAG-3' and reverse (R) 5'-CATAGGTCAAACCTCCTAGGAATG-3' MS2 primers and PCR-amplified using ThermoScript RT PCR kit (Invitrogen). PCR conditions were as follows: 95°C for 2 min; followed by 30 cycles of 94°C for 30 sec, 58°C for 45 sec and 72°C for 1 min. The amplification product is a 112-bp DNA fragment spanning positions 21-132 at the 5' end of the MS2 RNA (GeneBank J02467). PCR products were fractioned through 1.5% agarose gel electrophoresis; Southern blotted filters were hybridized with end-labeled internal oligonucleotide, 5'-TTAATGTCTTAGCGAGACGC-3'.

## RESULTS

### In Vitro Treatment with RT Inhibitors Induces RCCs Proliferation Arrest

The in vitro treatment with RT non-nucleosidic inhibitors induced cell growth arrest in several human cell line cultures (6) and caused, in vivo, a growth arrest in nude mice inoculated human tumor (13). In a previous work, we reported that the RT inhibitor nevirapine, largely used in anti-HIV therapy, blocks the enzymatic activity of endogenous RTs in non infected proliferating cells, as revealed using a highly sensitive RT-PCR based in vitro assay (7), and, concomitantly, reduces the growth of human primary RCC cell culture (T1 N0 M0 stage, II° grade) to prolonged exposure to RT inhibitors. Two well-defined RT inhibitors, nevirapine and efavirenz, were used. Primary culture cells were passed, counted and replated every 96 h with continuous drug re-addition (or DMSO alone in control cultures) for at least three 96 h-cycles. As shown in Figure-2, both inhibitors effectively reduced cell growth in primary cell culture, in nevirapine and efavirenz treated cells, comparing with control, with a stable effect during prolonged exposure. In Figure-2, we also show the relative rate of proliferation in nevirapine and efavirenz RCC primary culture relative to control culture (tumor derived primary RCC culture, not treated). During the first cycle, the inhibition relative to control remained at approximately 68% for nevirapine sample and 72% for efavirenz one (Figure-2). After the second and the third cycle, treated cells reduced the rate of their proliferation respectively to 31% (nevirapine treated) and 33% (efavirenz treated); it is clear that RT inhibitors were responsible for a 60% growth inhibition compared to control. We also defined the relative counts regarding cell death obtaining values similar to control (Figure-2).

### RT Inhibitors Induce Differentiation in RCC Cells

It was relevant to determine whether RT inhibitors induced a differentiation-like state concomitant with reduced cell growth. We first examined RCC cells, which acquire a typical dendritic-like phenotype in response to certain inducers of differentiation (14).

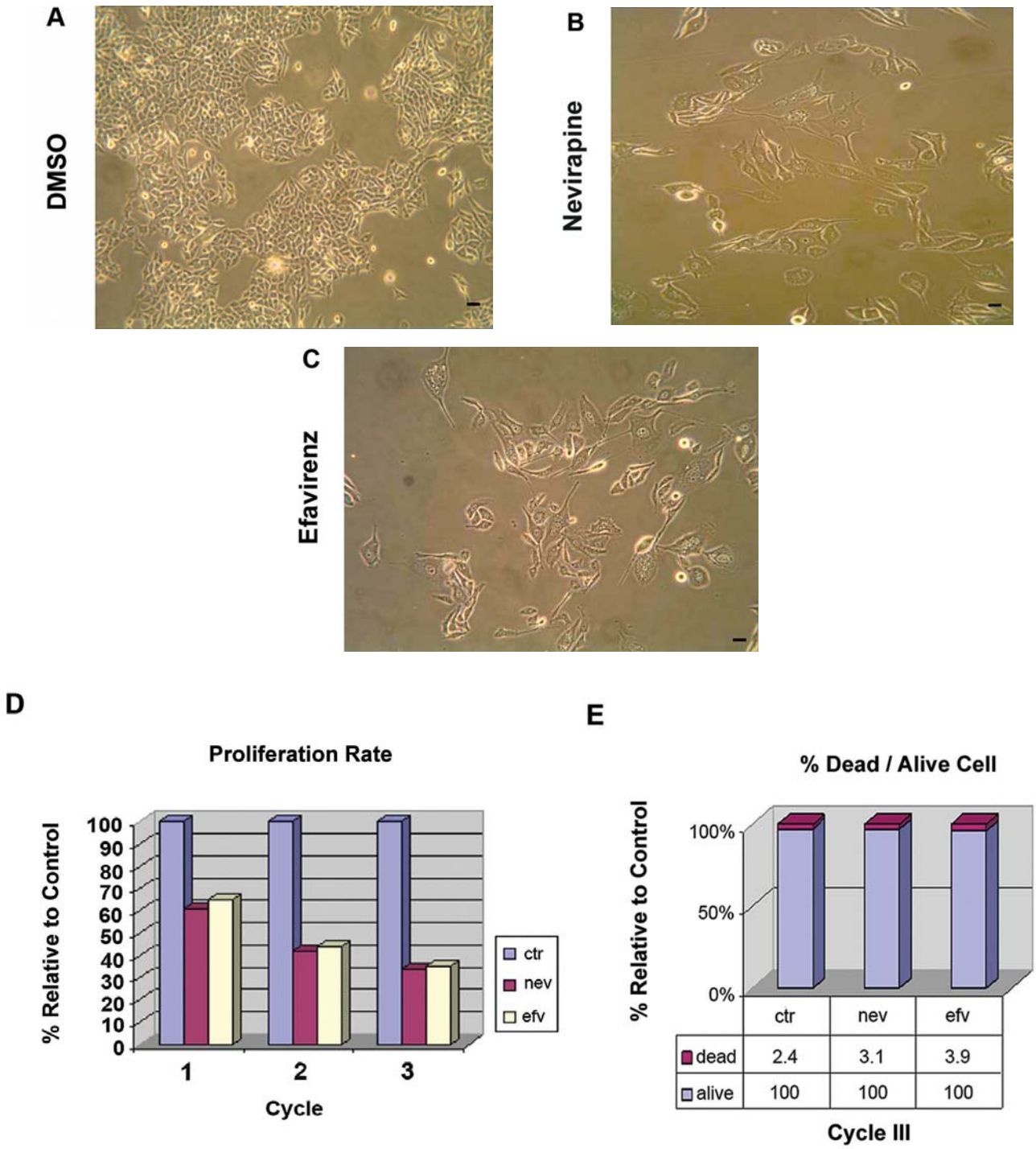



***Figure 2 –*** *Renal carcinoma cells in vitro treatment with RT inhibitors. Treated RCC primary culture compared to ctr DMSO cultured cells (A), observed under phase-contrast microscope. Morphological differentiation in nevirapine (B) and efavirenz (C), bar, 10 µm. D) Inhibition of proliferation by anti - RT drugs: cell growth in cultures treated with DMSO (control, blue), nevirapine (nev, pink) and efavirenz (efv, white). Cells were counted and re-plated every 96 h for three cycles (1-3). Counted cells are expressed as the % of controls, taken as 100. Values represent pooled data from two experiments. E) Relative percentage of dead treated and un-treated cells relative to total alive cell; counted cells are expressed as the % total alive cell, taken as 100. Data represent medium values from three cycles.*

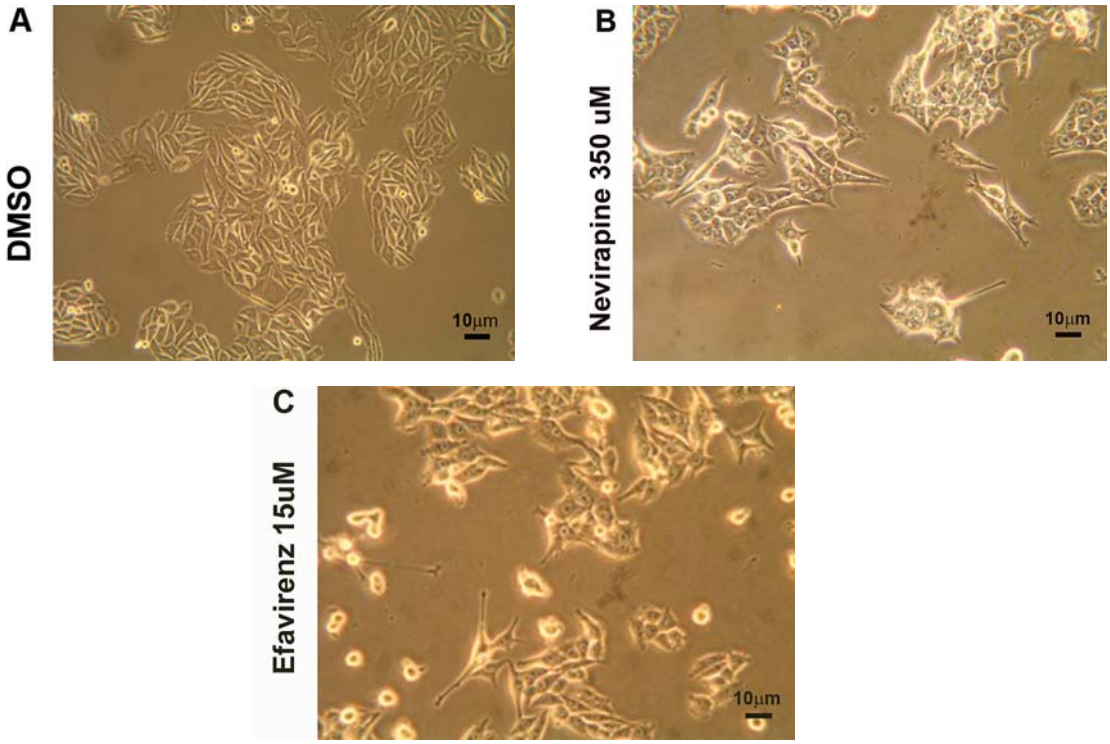



***Figure 3*** *– Morphological differentiation of RCC in vitro primary culture after drug treatment: A) DMSO - RCC primary cell culture, control; B) In primary culture, nevirapine incubation determined dendritic like cell shape; C) As in nevirapine, also in efavirenz treated cells, there is a morphological differentiation: in this case, the dendritic extensions are less evident, but they are also present, indicating a general redistribution of cytoplasmic shape. Bars, 10 μm.*

As shown in Figure-3, morphological change, revealed by cell shape and dendritic-like extensions, became evident within 4-5 days (96 h) of exposure to nevirapine or efavirenz, compared to DMSO-treated controls.

## RT Enzymatic Activity Reduction in RCC Primary Cell Culture

In a previous study we detected a functional RT activity in murine F9 cell line and in several human cell line (6). To assess whether an endogenous RT is also functional in RCC cell culture, we tested the ability of cell-free lysates prepared from in vitro RCC cell culture (tissue derived primary culture), inhibitors treated and not treated (DMSO), to retrotranscribe purified MS2 phage RNA. Lysate aliquots from RCC cell line (DMSO), nevirapine treated RCC cell line and efavirenz treated RCC cell line were incubated with purified MS2 phage RNA. Incubation mixtures where then subjected to direct PCR amplification using MS2-specific oligonucleotide pairs to establish whether MS2 cDNA molecules had been newly synthesized. As shown in Figure-4, a MS2-specific cDNA product of the expected size (112 bp) was retrotranscribed from the RNA template by RCC cell line lysates (lane 1 and 2). There was no difference between the RT activity in free RCC line cell culture and RCC cell culture in presence of 0.1% DMSO.

The 112 bp-long cDNA product was only obtained when cell lysates and phage RNA were incubated together, but not when RNA (lane 7) or lysate (lane 8) were omitted from incubation mixtures.

Furthermore, we sought to establish whether RT activity was sensitive to inhibition by nevirapine and efavirenz. The mechanism of nevirapine action is well characterized and the binding site of the molecule maps a hydrophobic pocket of the RT p66 subunit,

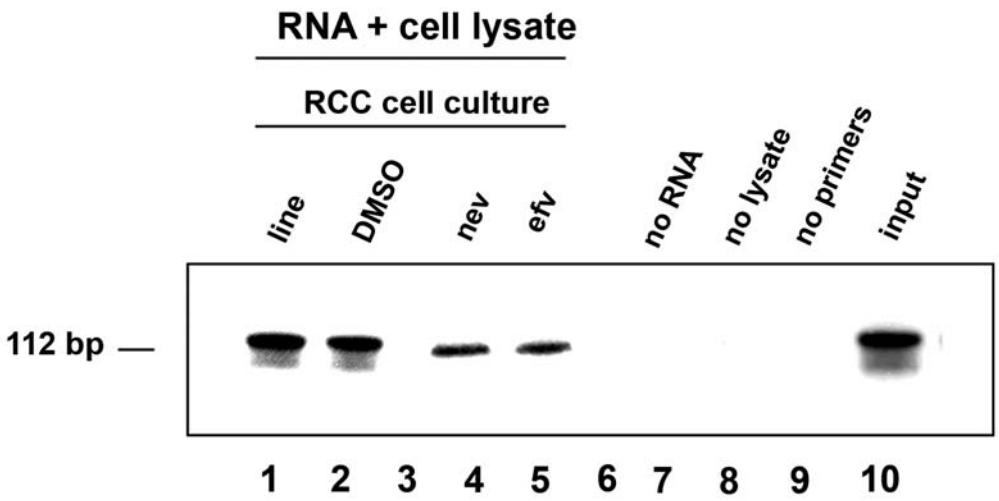



***Figure 4*** *– Functional assay of RT activity from in vitro RCC cell culture lysates and inhibition by nevirapine and efavirenz. Endogenous RT activity was tested in vitro using MS2 phage RNA as the template and lysates from RCC primary culture cell line (lane 1) and DMSO in vitro RCC cell culture (lane 2, 4 and 5). Endogenous RT activity was tested in the absence (lane 1 and 2) or presence of RT inhibitors (nevirapine, lane 4; efavirenz, lane 5). Negative controls were selectively depleted of MS2 RNA (lane 7), cell lysate (lane 8) or PCR primers (lane 9). A positive control for the reaction utilized commercial RT instead of cell-lysate (lane 10).*

near - though not overlapping with - the polymerase active site.

Preincubation of cell lysates with nevirapine and efavirenz inhibited retrotrascription of the RNA template significantly, though not totally, as shown in Figure-4, lanes 4 and 5, respectively. These results indicate that an endogenous RT activity is biologically functional in RCC in vitro cell culture and is sensitive to inhibition by nevirapine and efavirenz.

## COMMENTS

This work highlights two unexpected aspects of the human genome that have implications for cancer: first, LINE L-1 elements, derived from active retroposon sequences, described by Brouha and coll. (15), have been identified as active components of a mechanism involved in the control of cell differentiation and proliferation; second, pharmacological inhibition of the endogenous RT activity which they encode, can restore control of these traits in transformed cells.

The RT inhibitors nevirapine and efavirenz, used in our study, share a common biochemical mechanism of action by binding the hydrophobic pocket in the p66 of retroviral reverse transcriptase (HIV) of RT enzymes. Although originally designed to target the HIV encoded RT, nevirapine was also able to inhibit the endogenous retro-transcriptase activity present in non infected cells (6) as shown in a highly sensitive in vitro assay (7). We also demonstrated that both drugs reduce proliferation of RCC primary cultures derived transformed cells, largely independent from cell death. Concomitant with this, RT inhibitors treatment induced a morphological transformation of the cells shape. The induction of morphological changes is rapid, different from phenotypic changes elicited by inhibitors of the telomerase-associated RT (TERT), which require an extensive treatment period which can be as long as 120 days (16).

Cytokine therapies are used in treatment of metastatic renal cell carcinoma.. However, these new biological agents only provide clinical benefit to a small subset of patients and are associated with significant toxicity (2-4). A better understanding of the

molecular biology of RCC has identified the vascular endothelial growth factor and platelet-derived growth factor signaling pathways as rational targets for anticancer therapy. The multitargeted receptor tyrosine kinase inhibitors sunitinib and sorafenib have both demonstrated improved efficacy as second-line therapy in patients with RCC. Based on these partial results, the discovery of new antitumoral targets checked by antiretroviral agents could suggest the possibility of a synergic therapy against neoplastic proliferation. Therefore, nevirapine and efavirenz could produce a cytostatic effect permitting multikinases inhibitors to enhance their tumor regression activity.

Together with growth reduction and induction of partial differentiation, RT inhibition was responsible for the reprogramming of gene expression: this implicates endogenous RT in modulation of expression for genes that promote the transition from highly proliferating, transformed phenotypes to low proliferating, differentiated phenotypes, suggesting that genome function could be the ultimate target of pharmaceutical inhibition of RT activity.

In the present study, we analyzed the expression of four genes, indicated as molecular biomarkers in RCC cells: AFP, NNMT, CD70, FRA 2 and p21KIP1 genes.

CD70 gene is strongly down-regulated in treated RCC derived primary culture, as regards control of DMSO tumor cell culture, and this pattern is very similar to differentiated renal cells; although the role of this gene in cancer development and progression remains unclear, the encoded protein (type II trans-membrane glycoprotein) seems to mediate the interaction between T and B-lymphocytes and the natural killer cell-activation; it is also implicated in processes like cell proliferation, cell to cell signaling and induction of apoptosis by binding to its receptor CD27 (17). In several studies, high expression of CD70 has been found in malignant lymphomas and nasopharyngeal tumors (18) and in all examined clear cell tumors, whereas no expression occurred in the related normal epithelial cells.

Given that RCC can be considered as an immunogenic tumor, it is tempting to speculate that a CD70 over-expression can be associated to a possible immuno-escape for clear cell RCC: a strong reduction in expression level after RT inhibitors could induce a reprogramming status where cell progression is arrested and the cell partially differentiated.

The Fos protein FRA2 forms transcription factor complexes and has been described as a regulator of cell proliferation, differentiation and transformation and in some cases the expression of FOS gene has also been associated with apoptotic cell death (19). The mRNA expression pattern of FRA2 is differently regulated in treated and untreated cell culture: in the first case, the gene was up-regulated, while in the control sample the expression level was much lower.

AFP gene encodes alpha-fetoprotein, a major plasma produced by the yolk sac and liver during fetal life. Alpha-fetoprotein expression in adults is often associated with hepatoma or teratoma. AFP mRNA is a more reliable marker of metastasis compared to serum AFP (20).

p 27 KIP1 gene encodes a cyclin-dependent kinase inhibitor, which shares a limited similarity with CDK inhibitor CDKN1A/p21. The encodec protein binds to and prevents the activation of cyclin E-CDK2 or cyclin D-CDK4 complexes, and thus controls the cell cycle progression at G1. The degradation of this protein, which is triggered by its CDK dependent phosphorylation and subsequent ubiquitination by SCF complexes, is required for the cellular transition from quiescence to the proliferative state.

Decreased p27 expression has been shown to be associated with aggressive tumor behavior and decreased patient survival in numerous human malignancies (12). Expression level of p27 mRNA in treated renal carcinoma cells is strongly up-regulated demonstrating that cells could reverse to a quiescent condition, prior differentiation.

Changes in gene expression are not inherited through cell division, but are reversible when RT inhibition is released (data not shown). The reversibility of examined features after release of the inhibition suggest that LINE-1 encoded RT is part of an epigenetic mechanism that can modulate gene expression and contributes to the molecular mechanisms underlying cell proliferation and differentiation.

## CONCLUSIONS

The prospect of using RT inhibitors in RCC cancer therapy could have obvious advantages given

their resistance to many therapeutical approaches such as chemo-, radio- and immunotherapy.

The finding of a stable inhibition of endogenous reverse transcriptase activity in tumor or proliferative RCC cells opens the possibility for the involvement of retro-elements and retrotrasposon-sequences in the control of the proliferative process. In this study, we attempted to establish a new therapeutic approach to arrest in vitro cell growth in a RCC-derived primary cell culture as a possible useful application in cancer treatment.

## ACKNOWLEDGEMENT

This work was supported by National Italian Institute of Health.

## CONFLICT OF INTEREST

None declared.

### REFERENCES

1. Thoenes W, Störkel S, Rumpelt HJ, Jacobi GH: Renal cell carcinoma- a classification based on cytomorphological criteria. Zentralbl Allg Pathol. 1986; 132: 503-13.
2. Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Rixe O, et al.: Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. N Engl J Med. 2007; 356: 115-24.
3. Escudier B, Eisen T, Stadler WM, Szczylik C, Oudard S, Siebels M, et al.: Sorafenib in advanced clear-cell renal-cell carcinoma. N Engl J Med. 2007; 356: 125-34. Erratum in: N Engl J Med. 2007; 357: 203.
4. Hudes G, Carducci M, Tomczak P, Dutcher J, Figlin R, Kapoor A, et al.: Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. N Engl J Med. 2007; 356: 2271-81.
5. Costa LJ, Drabkin HA: Renal cell carcinoma: new developments in molecular biology and potential for targeted therapies. Oncologist. 2007; 12: 1404-15.
6. Mangiacasale R, Pittoggi C, Sciamanna I, Careddu A, Mattei E, Lorenzini R, et al.: Exposure of normal and transformed cells to nevirapine, a reverse transcriptase inhibitor, reduces cell growth and promotes differentiation. Oncogene. 2003; 22: 2750-61.
7. Pyra H, Böni J, Schüpbach J: Ultrasensitive retrovirus detection by a reverse transcriptase assay based on product enhancement. Proc Natl Acad Sci U S A. 1994; 91: 1544-8.
8. Diegmann J, Junker K, Gerstmayer B, Bosio A, Hindermann W, Rosenhahn J, et al.: Identification of CD70 as a diagnostic biomarker for clear cell renal cell carcinoma by gene expression profiling, real-time RT-PCR and immunohistochemistry. Eur J Cancer. 2005; 41: 1794-801.
9. Shirsat NV, Shaikh SA: Overexpression of the immediate early gene fra-1 inhibits proliferation, induces apoptosis, and reduces tumourigenicity of c6 glioma cells. Exp Cell Res. 2003; 291: 91-100.
10. Giannelli G, Marinosci F, Trerotoli P, Volpe A, Quaranta M, Dentico P, et al.: SCCA antigen combined with alpha-fetoprotein as serologic markers of HCC. Int J Cancer. 2005; 117: 506-9.
11. Imura A, Hori T, Imada K, Kawamata S, Tanaka Y, Imamura S, et al.: OX40 expressed on fresh leukemic cells from adult T-cell leukemia patients mediates cell adhesion to vascular endothelial cells: implication for the possible involvement of OX40 in leukemic cell infiltration. Blood. 1997; 89: 2951-8.
12. Anastasiadis AG, Calvo-Sanchez D, Franke KH, Ebert T, Heydthausen M, Schulz WA, et al.: p27KIP1-expression in human renal cell cancers: implications for clinical outcome. Anticancer Res. 2003; 23: 217-21.
13. Sciamanna I, Landriscina M, Pittoggi C, Quirino M, Mearelli C, Beraldi R, et al.: Inhibition of endogenous reverse transcriptase antagonizes human tumor growth. Oncogene. 2005; 24: 3923-31.
14. Sauane M, Gopalkrishnan RV, Sarkar D, Su ZZ, Lebedeva IV, Dent P, et al.: MDA-7/IL-24: novel cancer growth suppressing and apoptosis inducing cytokine. Cytokine Growth Factor Rev. 2003; 14: 35-51.
15. Brouha B, Schustak J, Badge RM, Lutz-Prigge S, Farley AH, Moran JV, et al.: Hot L1s account for the bulk of retrotransposition in the human population. Proc Natl Acad Sci U S A. 2003; 100: 5280-5.
16. Damm K, Hemmann U, Garin-Chesa P, Hauel N, Kauffmann I, Priepke H, et al.: A highly selective telomerase inhibitor limiting human cancer cell proliferation. EMBO J. 2001; 20: 6958-68.
17. Wischhusen J, Jung G, Radovanovic I, Beier C, Steinbach JP, Rimner A, et al.: Identification of CD70-mediated apoptosis of immune effector cells as a novel im-

mune escape pathway of human glioblastoma. Cancer Res. 2002; 62: 2592-9.
18. Douin-Echinard V, Bornes S, Rochaix P, Tilkin AF, Peron JM, Bonnet J, et al.: The expression of CD70 and CD80 by gene-modified tumor cells induces an antitumor response depending on the MHC status. Cancer Gene Ther. 2000; 7: 1543-56.
19. Tilghman SM: The structure and regulation of the alpha-fetoprotein and albumin genes. Oxf Surv Eukaryot Genes. 1985; 2: 160-206.
20. Yao F, Guo JM, Xu CF, Lou YL, Xiao BX, Zhou WH, et al.: Detecting AFP mRNA in peripheral blood of the patients with hepatocellular carcinoma, liver cirrhosis and hepatitis. Clin Chim Acta. 2005; 361: 119-27.

*Accepted after revision:*
*May 23, 2008*

**Correspondence address:**
Dr. Carmine Pittoggi
S.B.G.S.A, Italian National Institute of Health
Viale Regina Elena, 299
Rome, 00161, Italy
E-mail: carminepittoggi@hotmail.com

Investigative Urology

International Braz J Urol Vol. 34 (4): 503-511, July - August, 2008

# Urinary Glycosaminoglycans Excretion and the Effect of Dimethyl Sulfoxide in an Experimental Model of Non-Bacterial Cystitis

**Roberto Soler, Homero Bruschini, Jose C. Truzzi, Joao R. Martins, Niels O. Camara, Maria T. Alves, Katia R. Leite, Helena B. Nader, Miguel Srougi, Valdemar Ortiz**

*Division of Urology (RS, JCT, VO), Division of Molecular Biology (JRM, HBN), Division of Nephrology (NOC) and Division of Pathology (MTA), Federal University of Sao Paulo, UNIFESP, and Division of Urology (HB, KRL, MS), University of Sao Paulo, USP, Sao Paulo, SP, Brazil*

**ABSTRACT**

*Purpose:* We reproduced a non-bacterial experimental model to assess bladder inflammation and urinary glycosaminoglycans (GAG) excretion and examined the effect of dimethyl sulfoxide (DMSO).

*Materials and Methods:* Female rats were instilled with either protamine sulfate (PS groups) or sterile saline (control groups). At different days after the procedure, 24 h urine and bladder samples were obtained. Urinary levels of hyaluronic acid (HA) and sulfated glycosaminoglycans (S-GAG) were determined. Also to evaluate the effect of DMSO animals were instilled with either 50% DMSO or saline 6 hours after PS instillation. To evaluate the effect of DMSO in healthy bladders, rats were instilled with 50% DMSO and controls with saline.

*Results:* In the PS groups, bladder inflammation was observed, with polymorphonuclear cells during the first days and lymphomononuclear in the last days. HA and S-GAG had 2 peaks of urinary excretion, at the 1st and 7th day after PS injection. DMSO significantly reduced bladder inflammation. In contrast, in healthy bladders, DMSO produced mild inflammation and an increase in urinary HA levels after 1 and 7 days and an increase of S-GAG level in 7 days. Animals instilled with PS and treated with DMSO had significantly reduced levels of urinary HA only at the 1st day. Urinary S-GAG/Cr levels were similar in all groups.

*Conclusions:* Increased urinary levels of GAG were associated with bladder inflammation in a PS-induced cystitis model. DMSO significantly reduced the inflammatory process after urothelial injury. Conversely, this drug provoked mild inflammation in normal mucosa. DMSO treatment was shown to influence urinary HA excretion.

***Key words:*** *cystitis; dimethyl sulfoxide; glycosaminoglycans; rats; protamines*

***Int Braz J Urol. 2008; 34: 503-11***

## INTRODUCTION

Interstitial cystitis (IC) is a heterogeneous syndrome, diagnosed mainly in women, characterized by painful bladder symptoms, nocturia, urinary frequency and urgency. These symptoms usually appear acutely and follow a waxing and waning course (1).

Dimethyl sulfoxide (DMSO) has been used as an intravesical therapy for IC due to its anti-inflammatory and analgesic properties. Although its mechanisms of action have not yet been fully elucidated, it is a well established treatment for IC, with proven feasibility (2).

Impaired barrier function of bladder epithelium and subsequent infiltration of urine contents are

expected initial events in the pathophysiology of IC. Glycosaminoglycans (GAG) are complex long-chain polysaccharides components of the extra cellular matrix and cell surface, which play multiple physiological functions (3). The GAG layer is thought to create a hydrophilic coating in the underlying cells by binding water, via their sulfated groups. Alterations of the urothelial GAG layer, which may lead to higher bladder permeability, might also be present in IC (4).

Considering the lack of a defined cause and also generally effective treatments, experimental models of IC are a challenging field. Protamine sulfate (PS) has been used in experimental models of IC, which at a low concentration (10 mg/mL) represents a noncytotoxic affront to urothelial barrier instead of a bladder irritant (5,6) PS is a polycationic quaternary amine which changes the permeability of the urothelium and other types of epithelia (7). In this study, we examined the urinary GAG excretion and its correlation with inflammation and the effect of treatment with DMSO in an experimental model of IC, induced by PS.

## MATERIALS AND METHODS

### Animals and Induction of Bladder Inflammation

Adult female Wistar rats (180 to 200 g) were housed in light and temperature controlled rooms on a 12/12 hours light-dark cycle, with free access to water and food, prior to their use in experimental studies. All animal studies were carried out with the approval of the University Ethics Committee.

Rats were anesthetized with an intraperitoneal injection of xylazine (4 mg/kg) and ketamine (90 mg/kg). External genitalia were cleansed with povidone-iodine and a small quantity of 2% lidocaine lubricant was applied to the external urethra. A 24 gauge ¾-inch catheter was inserted into the bladder and the urine was drained. Bladder injury was induced with grade X PS (Sigma, St. Louis, MO), 10 mg in 200 µL sterile 0.9% saline applied intravesically. After 30 minutes, the bladder was drained and washed with 200 µL 0.9% saline. The catheter was removed and the rats were allowed to recover. Control rats were initially injected with 200 µL saline and the same procedure was followed.

### Experimental Groups

PS groups (n = 7 per day) and control groups (n = 5 per day) were analyzed at different days following the procedure: 1st to 7th , 10th and 14th days (total n = 108). The rats were housed 24 hours prior to sacrifice in a metabolic cage in order to collect urine. The bladder was removed and fixed in normal 10% buffered formalin and urine was immediately centrifuged to remove exfoliated cells and urinary debris and stored at -20°C for further analyses. Five non-manipulated animals (day 0) also had their urine collected. Prior to assay, the creatinine (Cr) content was measured by a kit purchased from Sigma Chemical Co. (St. Louis, MO.).

To evaluate the effect of DMSO on this experimental model, 6 hours after PS instillation, the animals were intravesically instilled with 200 µL of either 50% DMSO (n = 5 per day) or saline (n = 5 per day) for 30 minutes. To assess the effect of DMSO in healthy bladders, rats (n = 5 per day) were instilled with 200 µL 50% DMSO and controls (n = 5 per day) with 200 µL saline for 30 minutes. One and 7 days afterwards, 24 hrs urine was collected and bladders were removed for histopathological analysis.

### Histopathology

Approximately 5 µm thick paraffin sections were stained with hematoxylin and eosin for general morphology. The samples were blinded reviewed by two pathologists. Edema and vascular congestion were graded 0 (absent), 1 (mild), 2 (moderate) and 3 (severe). Each inflammatory cell type was counted (polymorphonuclear - PMN, mast cell and lymphomononuclear - LMN) in 5 cross sections at X400 magnification, at the most infiltrated area.

### Measurement of Urinary Hyaluronic Acid

Urinary hyaluronic acid (HA) levels were measured by a noncompetitive and nonisotopic fluoroassay. Plates were coated with hyaluronan binding

proteins (HABP) and successively incubated with samples containing standard solutions of HA or urine samples from the different groups, biotin-conjugated HABP and europium-labeled streptavidin (Amersham Life Science, Buckinghamshire, England). After release of europium from streptavidin with enhancement solution (Perkin-Elmer Life Sciences-WaIlac Oy, Turku, Finland) the final fluorescence was measured in a fluorometer (8). HA concentration was normalized to Cr and expressed in ng/mg Cr.

## Measurement of Urinary Sulfated GAG

Two milliliters of urine from the different groups of animals were applied in a Sephadex G25 column, equilibrated with distilled water, which separates GAG from salt, pigments, smaller compounds and other impurities. The inclusion volume was discarded and the following 4 mL flow-through were collected, vacuum dried and then, dissolved in 10 µL of distilled water and kept frozen at -20°C for analysis. Afterwards, 5 µL of the stored samples and 5 µL of an aqueous mixture of 1 mg/mL of standard GAG (Chondroitin 4- and 6-sulfate (CS), Dermatan sulfate (DS) and Heparan sulfate (HS)) were applied in 0.2-cm thick agarose gel slabs (0.55% agarose in 50 nM 1.3-diaminopropane/acetate buffer, pH 9.0) to proceed with the electrophoresis (9). The gel slabs were then fixed with 0.1% cetyltrimethyl-ammonium bromide, dried, stained with toluidine blue and quantified by densitometry at 595 nm. GAG concentration was normalized to Cr and expressed in µg/mg Cr.

## Statistical Analysis

Comparison between treated groups and their respective control groups was carried out by performing Student's-t-test for parametric data and Mann-Whitney U test for non-parametric data. Comparison between all the groups was carried out by ANOVA, when normal distribution or Kruskal-Wallis test, without normal distribution, followed by Dunn's or Tukey multiple comparison tests. We used the Sigma Stat software for Windows, 2.0, 1999 (SSPS Inc., Chicago, IL).

# RESULTS

## Histopathology

### *PS and Control Groups*

Edema was more pronounced on the first three days after PS injection. Vascular congestion grade was in overall higher in PS groups and was considered statistically different on the first three days and between the 6th and 10th days (Figure-1A).

The bladder sections showed focal inflammatory changes. Control animals had low number of inflammatory cells during all analyzed days. PMN ranged from 0 to 10, mast cells from 0 to 6 and LMN from 0 to 5/cross section (data not shown). In the PS groups, there was a predominance of PMN in the first four days (Figure-1B). The occurrence of mast cells during all days was not influenced by either PS or saline injection ($p = 0.074$) (Figure-1C). In contrast, LMN infiltrate was more prominent at the 6th to 14th day (Figure-1D).

### *DMSO Groups*

Rats treated with DMSO had a significantly reduced grade of edema, vascular congestion and PMN count at the 1st day after PS instillation. At the 7th day, edema, vascular congestion and inflammatory infiltrates (LMN) were also significantly reduced in the DMSO treated-group. Examples of these inflammatory alterations are shown in Figure-2. Conversely, when DMSO was injected in healthy bladders, there was a more pronounced PMN infiltrate at the 1st and 7th days and edema at the 1st day. Mast cell count was increased only on the 7th day in the DMSO group (Table-1).

## GAG Measurement

### *PS and Control Groups*

Urinary HA/Cr levels were higher in all days following intravesical PS injection. There were two peaks of urinary HA excretion, at the 1st day and 7th days. Compared with the levels detected at day 0 animals, HA/Cr on the 1st day was increased 2.4-fold and on the 7th day, 2.1-fold ($p < 0.05$) (Figure-3A).

Urinary S-GAG/Cr followed an almost similar pattern of excretion following PS injection,

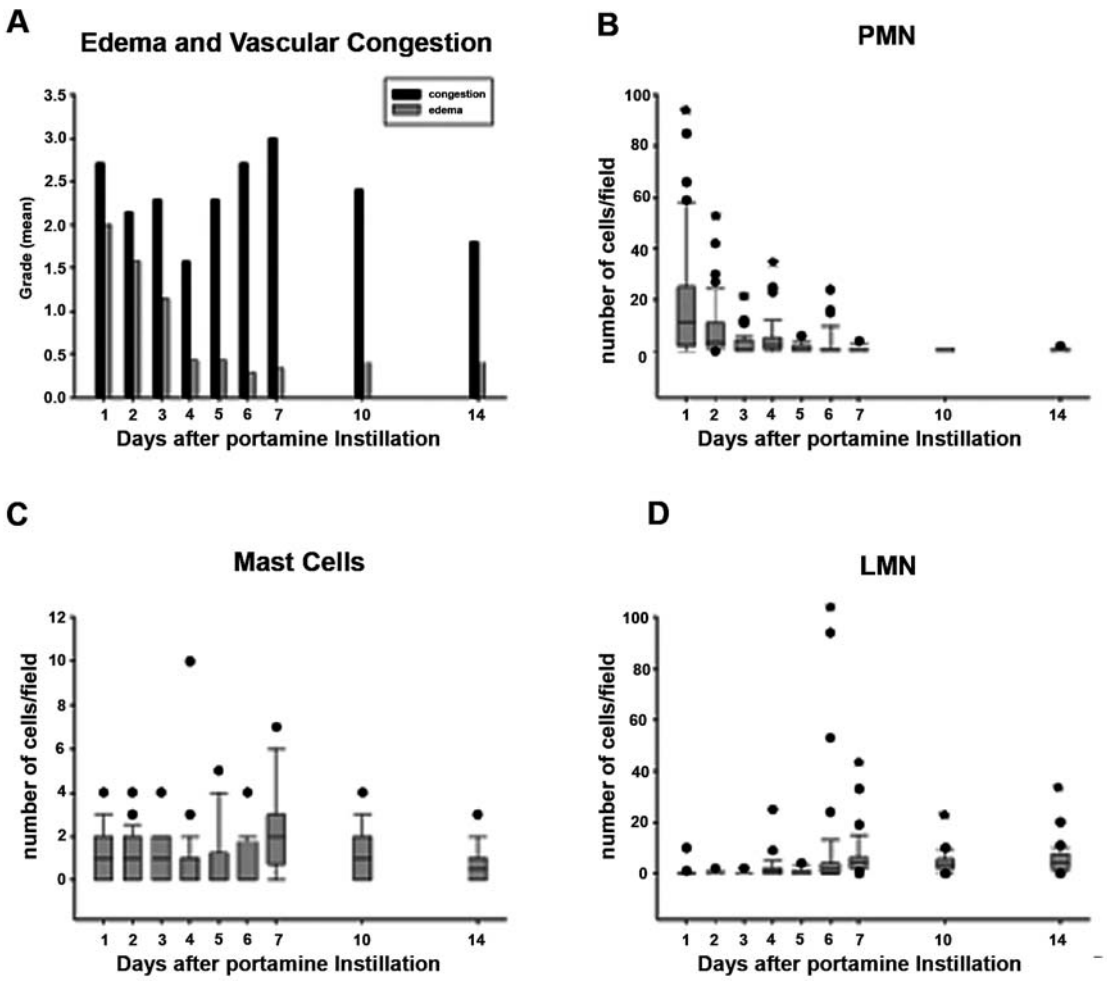



***Figure 1 –*** *Histopathological analysis. Time course of effects of intravesical PS instillation on edema and vascular congestion grade (A), on PMN count/field (B), on mast cell count/field (C) and on LMN count/field (D). Values are means in the bars and median with interval (box plots) * $p < 0.05$, points on the PS time course vs. those on the control time course.*

increasing at the first day with a more remarkable peak at the 7th day. Urinary S-GAG/Cr levels at 7th day were increased 2.1-fold compared to levels on day 0 ($p < 0.05$) (Figure-3B).

***DMSO Groups***

The instillation of DMSO in healthy bladders provoked an increase in urinary HA/Cr levels after 1 and 7 days and an increase of S-GAG/Cr level after 7 days. Animals instilled with PS and treated with DMSO had significantly reduced level of urinary HA only at the 1st day. Urinary S-GAG/Cr levels were similar in all groups (Table-2). There were no differences in urinary creatinine levels between the groups.

## COMMENTS

The polycationic quaternary amine, PS, has a well documented effect of increasing urothelial permeability. PS increased water and urea permeability, reduced the transepithelial resistance, provoked patchy umbrella cell lysis and damage to the tight

***Table 1** – Pathological features in DMSO groups.*

| Group | **Edema** (grade) | | **Vascular Congestion** (grade) | | **PMN Count** (median) | | **Mast Cell Count** (median) | | **LMN Count** (median) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **1 day** (n = 5) | **7 days** (n = 5) | **1 day** (n = 5) | **7 days** (n = 5) | **1 day** (n = 5) | **7 days** (n = 5) | **1 day** (n = 5) | **7 days** (n = 5) | **1 day** (n = 5) | **7 days** (n = 5) |
| Saline | 0.6 ± 0.5 | 0.4 ± 0.5 | 1.2 ± 0.4 | 1.0 ± 1.2 | 0 | 0 | 1 | 0 | 0 | 0 |
| DMSO | 1.8 ± 0.8* | 1.0 ± 1.0 | 2.0 ± 0.7 | 1.8 ± 0.8 | 3* | 1* | 1 | 1* | 0 | 0 |
| PS + Saline | 3.0 ± 0‡ | 1.4 ± 0.5‡ | 2.6 ± 0.5‡ | 2.2 ± 0.4‡ | 20‡ | 0 | 0 | 0 | 3‡ | 0 |
| PS + DMSO | 1.6 ± 0.5 | 0.4 ± 0.5 | 1.4 ± 0.5 | 1.2 ± 0.4 | 3 | 0 | 0 | 0 | 0 | 0 |

** p < 0.05 - saline group X DMSO group at day 1 or 7 (Mann-Whitney U test); ‡ p < 0.05 - PS+saline group X PS+DMSO group at day 1 or 7 (Mann-Whitney U test), DMSO = dimethyl sulfoxide.*

junctions, with reduced expression of uroplakins and ZO-1, in an animal model (10). These findings were also described in biopsies and cell culture obtained from patients with IC (11).

Due to these actions, intravesical instillation of PS could provide an effective and reliable model of urothelial damage. We therefore chose it to study the consequences of the increase of permeability on the urothelium, focusing on bladder inflammation and on urinary GAG behavior.

The histological sections demonstrated a clear difference between PS and control groups. The inflammation was focal in the sections, in accordance with other animal studies and with findings in biopsies from IC patients (10,12). Features associated with IC such as edema and congestion were detectable and significant within the PS groups. There was an apparent temporal evolution of the inflammation, from PMN infiltrate in the first four days to a LMN infiltrate after the 6$^{th}$ day, which lasted until the 14$^{th}$ day. These results demonstrate that even after the removal of PS from the bladder by washing it with saline, there is a persistence of local inflammatory process, possibly due to the increase of permeability and leakage of urinary components.

DMSO, a U.S. Federal Drug and Food Administration-approved intravesical therapy for IC, has been used to provide symptomatic relief of chronic pain in these patients. DMSO has anti-inflammatory and reactive oxygen scavenger actions, crosses membranes easily, impairs the nerve conduction of C-fibers, prevents depolymerization of HA, inhibits angiogenesis of endothelial cells and has local anesthetic properties (2,13,14). However, its mechanism of action and effects on bladder tissue function are not completely understood.

As we found two major peaks of inflammation, we examined the effect of DMSO on these inflammatory changes and also on healthy bladders. DMSO significantly reduced the inflammatory process at both 1$^{st}$ and 7$^{th}$ days after the urothelial injury, which proves its anti-inflammatory action in the bladder. Conversely, this drug provoked mild inflammation in normal mucosa. This finding might support the initial complaint from some patients of exacerbated urethral burning and pelvic pain after DMSO instillation (2).

The GAG layer on the epithelial bladder surface, with its hydrophilic characteristics , is thought to shield the urothelium from microcrystals, proteins, pathogens and noxious substances (15). GAG are composed by repeating disaccharide units, consisting of alternating hexosamine and uronic acid. Hyaluronic acid (HA) is a nonsulfated GAG, most abundantly found in loose connective tissue. In the bladder, HA is more abundant in the underlying connective tissue

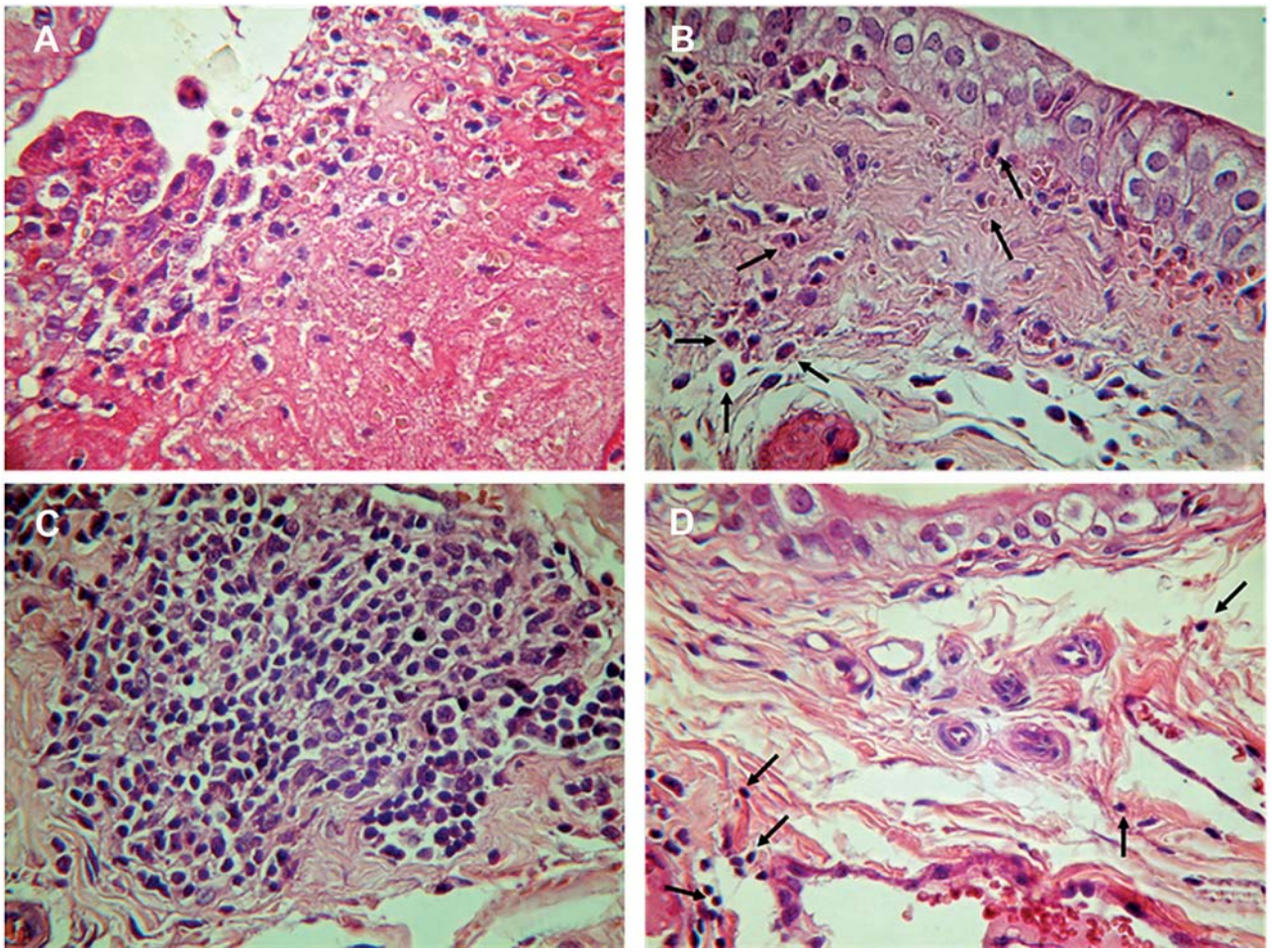



***Figure 2 –*** *Light microscopy of dimethyl sulfoxide (DMSO) groups. A) PS + saline (1 day) - exulceration of the urothelium with intense neutrophilic infiltrate. Note the complete loss of the urothelial architecture. B) PS + DMSO (1 day) - scarce neutrophils in the lamina propria (arrows). Note the intact urothelium. C) PS + saline (7 days) - exuberant aglomeration of lymphocytes in the lamina propria with some edema in the underlying layers. D) PS + DMSO (7 days), rare lymphocytes in the lamina propria (arrows). Note the intact urothelium. (HE, X400)*

of the mucosa and between the smooth muscle layers (16). Sulfated GAG (S-GAG) occur covalently linked to protein cores, forming proteoglycans. The cell surface proteoglycans are composed by proteins and a dense layer of intercalated GAG, forming the so-called GAG layer.

Based on the theory proposed by Parsons et al. of an urothelial deficiency of GAG as an etiological factor for IC (4), urinary concentration of these compounds have been measured in order to establish a disease marker. Despite the hypothesis of a deficient GAG layer on the cell surface, the amount of GAG in the urine does not necessarily reflect this condition. Different studies have described either decreased or elevated total GAG and HA levels in patients with IC (17). Recently, increased urinary S-GAG and HA levels were associated with severe IC, based on a symptom questionnaire (18,19).

We assessed urinary GAG to verify their excretion in this model of urothelial injury. Urinary HA and S-GAG had a similar pattern of excretion, since two urinary peaks were detected, at the 1st and

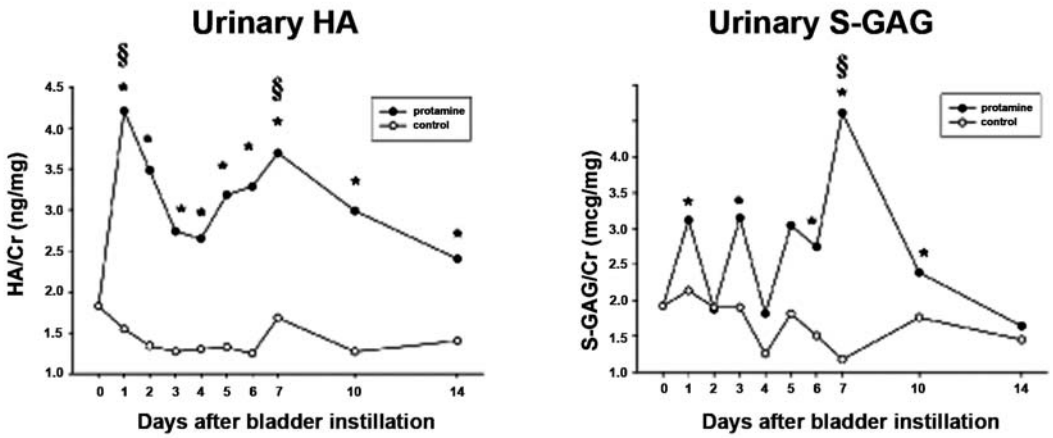



***Figure 3 –*** *Urinary GAG measurement. Time course of effects of intravesical PS or saline instillation on urinary HA (A) and urinary S-GAG (B). The concentrations were normalized by urinary creatinine. Values are means ± SD. * P < 0.05, points on the PS time course vs. those on the control time course. § P < 0.05, comparison between PS groups (HA: day 1 vs. day 0; day 7 vs. day 0) (S-GAG: day 7 vs. days 0, 2, 4 and 14).*

at 7th day. These increased levels were concomitant with more pronounced PMN infiltrate and edema, and LMN infiltrate and vascular congestion, respectively. These data might suggest that increased urinary levels may be associated with bladder inflammation.

Urinary HA levels seemed to be associated with the occurrence of PMN infiltrate. DMSO treatment only influenced the urinary HA excretion at the 1st day, concomitantly with reduced PMN infiltrate. Additionally, higher levels of this compound were associated with this type of inflammatory infiltrate when DMSO was instilled in healthy bladders. DMSO treatment did not influence urinary S-GAG excretion.

HA also has an influence on inflammation process, such as migration, differentiation, cellular proliferation, angiogenesis and induction of proinflammatory cytokines and chemokines. During inflammation or after injury, HA may either be degraded

***Table 2 –*** *Urinary GAG levels in DMSO groups.*

| **Group** | **HA/Cr** (ng/mg) | | **S-GAG** (μg/mg) | |
|---|---|---|---|---|
| | **1 day** (n = 5) | **7 days** (n = 5) | **1 day** (n = 5) | **7 days** (n = 5) |
| Saline | 1.6 ± 0.3 | 1.5 ± 0.4 | 2.0 ± 0.4 | 1.3 ± 0.3 |
| DMSO | 4.5 ± 2.2* | 6.0 ± 2.0* | 1.9 ± 0.7 | 2.6 ± 0.8* |
| PS + Saline | 7.6 ± 3.0‡ | 8.6 ± 2.3 | 4.3 ± 1.2 | 3.8 ± 0.9 |
| PS + DMSO | 3.4 ± 0.7 | 5.4 ± 0.7 | 3.3 ± 1.3 | 3.8 ± 1.9 |

** p < 0.05 - saline group X DMSO group at day 1 or 7 (Mann-Whitney U test); ‡ p < 0.05 - PS+saline group X PS+DMSO group at day 1 or 7 (Mann-Whitney U test); DMSO = dimethyl sulfoxide.*

into smaller weight molecules, by hyaluronidases, or have its synthesis enhanced, by hyaluronan synthases. Degradation could also occur mediated by free radicals (20). As DMSO has an anti-inflammatory effect, depolymerization of HA and is know to be a scavenger of the intracellular hydroxyl radical, the concomitant reduced inflammation and urinary HA levels in the DMSO-treated group might represent an impairment of either degradation or synthesis of this compound.

Sulfated GAG also play role in inflammation and wound healing process. After injury they become soluble as they are released from their protein core. Likewise, their synthesis is also enhanced during inflammation. Both synthesized and released S-GAG participate in different phases of the inflammation and tissue repair (20). The treatment with DMSO did not significantly alter the urinary S-GAG excretion in this experimental model. At the first day after the injury, the higher urinary S-GAG concentration may have corresponded to a desquamation of the superficial urothelial layers that occurred due to the instillation of PS after the treatment with DMSO. The second peak of urinary excretion might represent the urothelial recovery process, in which these compounds may play a part.

Although this is an acute model of urothelial injury and so, not necessarily representative of the IC patient, it supplies valuable information concerning urinary GAG excretion and its relationship with bladder inflammation, validated by the changes occurred after DMSO treatment. In an acute phase there is an increased GAG excretion, which may be observed in phases of worsening symptoms. However, in a chronic phase, GAG production and excretion may reach equilibrium and urinary levels might not reflect any changes.

## CONCLUSIONS

Intravesical instillation of PS promoted focal inflammatory changes, with two distinct types of infiltrate, PMN initially and LMN afterwards. Elevated levels of urinary GAG were associated with bladder inflammation. Two peaks of urinary excretion were concomitant with PMN and LMN infiltrate. The treatment with DMSO reduced these inflammatory changes. This local anti-inflammatory action may be a mechanism by which it exerts a beneficial effect on IC. On the other hand, it caused inflammation in normal mucosa, which could explain the initial flare-up of symptoms that some patients relate. Urinary HA levels seemed to be associated with the occurrence of PMN infiltrate, since lower urinary HA levels and reduced PMN infiltrate were concomitant findings after DMSO instillation. DMSO treatment did not influence urinary S-GAG excretion.

## CONFLICT OF INTEREST

None declared.

## REFERENCES


1. Driscoll A, Teichman JM: How do patients with interstitial cystitis present? J Urol. 2001; 166: 2118-20.
2. Rössberger J, Fall M, Peeker R: Critical appraisal of dimethyl sulfoxide treatment for interstitial cystitis: discomfort, side-effects and treatment outcome. Scand J Urol Nephrol. 2005; 39: 73-7.
3. Dietrich CP, Sampaio LO, Toledo OM: Characteristic distribution of sulfated mucopolysaccharides in different tissues and in their respective mitochondria. Biochem Biophys Res Commun. 1976; 71: 1-10.
4. Parsons CL, Lilly JD, Stein P: Epithelial dysfunction in nonbacterial cystitis (interstitial cystitis).J Urol. 1991; 145: 732-5.
5. Fraser MO, Chuang YC, Lavelle JP, Yoshimura N, de Groat WC, Chancellor MB: A reliable, nondestructive animal model for interstitial cystitis: intravesical low-dose protamine sulfate combined with physiological concentrations of potassium chloride. Urology. 2001; 57: 112.
6. Westropp JL, Buffington CA: In vivo models of interstitial cystitis. J Urol. 2002; 167: 694-702.
7. Peterson MW, Gruenhaupt D: Protamine interaction with the epithelial cell surface. J Appl Physiol. 1992; 72: 236-41.
8. Martins JR, Passerotti CC, Maciel RM, Sampaio LO, Dietrich CP, Nader HB: Practical determination of hyaluronan by a new noncompetitive fluorescence-based assay on serum of normal and cirrhotic patients. Anal Biochem. 2003; 319: 65-72.
9. Dietrich CP, Dietrich SM: Electrophoretic behaviour of acidic mucopolysaccharides in diamine buffers. Anal Biochem. 1976; 70: 645-7.

10. Lavelle J, Meyers S, Ramage R, Bastacky S, Doty D, Apodaca G, et al.: Bladder permeability barrier: recovery from selective injury of surface epithelial cells. Am J Physiol Renal Physiol. 2002; 283: F242-53.
11. Slobodov G, Feloney M, Gran C, Kyker KD, Hurst RE, Culkin DJ: Abnormal expression of molecular markers for bladder impermeability and differentiation in the urothelium of patients with interstitial cystitis. J Urol. 2004; 171: 1554-8.
12. Rosamilia A, Igawa Y, Higashi S: Pathology of interstitial cystitis. Int J Urol. 2003; 10 (Suppl): S11-5.
13. Parcell S: Sulfur in human nutrition and applications in medicine. Altern Med Rev. 2002; 7: 22-44.
14. Santos NC, Figueira-Coelho J, Martins-Silva J, Saldanha C: Multidisciplinary utilization of dimethyl sulfoxide: pharmacological, cellular, and molecular aspects. Biochem Pharmacol. 2003; 65: 1035-41.
15. Parsons CL, Boychuk D, Jones S, Hurst R, Callahan H: Bladder surface glycosaminoglycans: an epithelial permeability barrier. J Urol. 1990; 143: 139-42.
16. Laurent C, Hellström S, Engström-Laurent A, Wells AF, Bergh A: Localization and quantity of hyaluronan in urogenital organs of male and female rats. Cell Tissue Res. 1995; 279: 241-8.
17. Akçay T, Konukoglu D: Glycosaminoglycans excretion in interstitial cystitis. Int Urol Nephrol. 1999; 31: 431-5.
18. Lokeshwar VB, Selzer MG, Cerwinka WH, Gomez MF, Kester RR, Bejany DE, et al.: Urinary uronate and sulfated glycosaminoglycan levels: markers for interstitial cystitis severity. J Urol. 2005; 174: 344-9.
19. Lokeshwar VB, Selzer MG, Unwala DJ, Estrella V, Gomez MF, Golshani R, et al.: Uronate peaks and urinary hyaluronic acid levels correlate with interstitial cystitis severity. J Urol. 2006; 176: 1001-7.
20. Taylor KR, Gallo RL: Glycosaminoglycans and their proteoglycans: host-associated molecular patterns for initiation and modulation of inflammation. FASEB J. 2006; 20: 9-22.

*Accepted after revision:*
*March 21, 2008*

**Correspondence address:**
Dr. Homero Bruschini
R. Barata Ribeiro, 414 / 35
São Paulo, SP, 01302-000, Brazil
Fax: + 55 11 3218-8283
E-mail: bruschini@uol.com.br

## EDITORIAL COMMENT

A number of models of urothelial damage have been used to investigate the effects of loss of barrier function. None has been particularly well characterized. This study characterizes the response over several days of the protamine sulfate model and shows that the anti-inflammatory DMSO is capable of markedly reducing the inflammatory response in animals subjected to the urothelial damage protocol. Interestingly, DMSO induces a mild inflammatory response in normal bladder, which may, in part, explain its action in interstitial cystitis in both helping and eventually harming patients.

***Dr. Robert E. Hurst***
*Oklahoma University Cancer Institute and*
*Oklahoma University Health Sciences Center*
*Oklahoma City, Oklahoma, USA*
*E-mail: robert-hurst@ouhsc.edu*

Letter to the Editor
International Braz J Urol Vol. 34 (4): 512-515, July - August, 2008

# Re: Comparison of Radical Prostatectomy Techniques: Open, Laparoscopic and Robotic Assisted

**Rodrigo Frota, Burak Turna, Rodrigo Barros, Inderbir S. Gill**

*Section of Laparoscopic and Robotic Surgery, Glickman Urological Institute, Cleveland Clinic Foundation, Cleveland, Ohio, USA*

Int Braz J Urol. 2008; 34: 259-69

To the Editor,

This timely paper reviews the current status and respective roles of laparoscopic, robotically-assisted and open radical prostatectomy in the management of localized prostate cancer. While open radical prostatectomy remains the gold standard of treatment, a minimally-invasive approach has been available since 1997 in the form of laparoscopic radical prostatectomy. Minimally-invasive approaches to radical prostatectomy hope to duplicate the benefits of this approach seen with other procedures, including decreased patient blood loss and post-operative recovery time. The increased visualization, through digitally enhanced images that both magnify and illuminate the operative field, contributes greatly to the performance of this technically challenging procedure. However, laparoscopic surgery requires the acquisition of new anatomical perspectives, hand-eye coordination and the capacity to operate with limited tactile feedback and lack of 3-dimensional vision, all of which contributes to its undeniably steep learning curve. More recently, robotic systems have been used as an additional tool for the laparoscopic approach, with the hypothesis that they might improve the precision and accuracy of the anatomical dissection for the reasons outlined in the introduction of the current paper.

The authors concisely summarize the available contemporary literature, paying most attention to larger series from centers with established reputations in this field and with longer term follow-up. Criteria for comparison include operative, oncological and functional outcomes, as well as a pertinent discussion of financial considerations. Advantages of the minimally-invasive approaches are seen in generally lower operative blood loss, marginally decreased complication rates and shorter duration of catheterization. Analgesia requirements appear to be comparable and length of hospital stay often depends on more than simply the operative technique involved. Data concerning functional outcomes appears to be similar across the different techniques, but the authors rightly point out the difficulties comparing like with like in these studies, in terms of definitions of continence and potency and the use of validated questionnaires. The long term oncological efficacy of RRP is well studied but as yet limited long-term follow up is available for the minimally-invasive approaches. PSA progression-free survival appears comparable in the short to medium term, and what comparative studies exist show no significant differences in positive margin rates.

Our own unit recently published a direct comparison of robotic-assisted versus pure laparoscopic radical prostatectomy (1). No significant differences were observed between the pure laparoscopic and the robotic-assisted procedure with regard to operative time, operative blood loss, length of hospital stay or bladder catheterization. A higher transfusion rate was seen in the robotic-assisted group (9.8%) compared to the pure laparoscopic group, though this finding has not been borne out in other similar studies (2,3). No significant differences were seen in the rate of major

complications between the 2 groups. The rate of margin positivity did not significantly differ between pure laparosocopy (15.8%) and the robotic-assisted procedure (19.5%). Our conclusion was that pure laparoscopic extra-peritoneal radical prostatectomy is equivalent to the robotic-assisted procedure in a centre experienced in laparoscopic techniques.

The current review is a welcome addition to the comparative literature regarding the status of minimally-invasive techniques against the well-established gold standard of open surgery. Tooher et al., in their comprehensive review of this topic, concluded that any conclusions that can be drawn from these comparisons are limited by the nature of the available data (4). Well performed, randomized, controlled trials are urgently required to provide stronger evidence when comparing these techniques. Sufficient follow-up and the use of internationally validated measures of functional outcomes are essential.

**REFERENCES**

1. Rozet F, Jaffe J, Braud G, Harmon J, Cathelineau X, Barret E, et al.: A direct comparison of robotic assisted versus pure laparoscopic radical prostatectomy: a single institution experience. J Urol. 2007; 178: 478-82.
2. Menon M, Shrivastava A, Tewari A: Laparoscopic radical prostatectomy: conventional and robotic. Urology. 2005; 66 (5 Suppl): 101-4.
3. Joseph JV, Vicente I, Madeb R, Erturk E, Patel HR: Robot-assisted vs pure laparoscopic radical prostatectomy: are there any differences? BJU Int. 2005; 96: 39-42.
4. Tooher R, Swindle P, Woo H, Miller J, Maddern G: Laparoscopic radical prostatectomy for localized prostate cancer: a systematic review of comparative studies. J Urol. 2006; 175: 2011-7.

***Dr. François Rozet &***
***Dr. Gordon P. Smith***
*Department of Urology, Institut Montsouris*
*Université Paris Descartes*
*42, Bd Jourdan, 75014*
*Paris, France*
*E-mail: francois.rozet@imm.fr*

## Systemic Treatment for Invasive Bladder Cancer: Neoadjuvant Chemotherapy and Laparoscopic Radical Cystectomy

To the Editor,

The standard treatment for invasive transitional cell carcinoma (TCC) is radical cystectomy (RC) with lymphadenectomy; however, defining adequate therapy in every patient with invasive TCC remains difficult, because multiple biologic behavior patterns can be found in this disease (1).

Laparoscopy has come forward in oncologic urologic surgery to reproduce traditional operations in the endoscopic environment in order to minimize morbidity without compromising cancer outcomes. Laparoscopic radical cystectomy (LRC) was conceived as a procedure that could actually diminishes the associated morbidity of RC, while maintaining the oncological objectives.

Quality indicators in RC are well established nowadays: Mortality should not be higher than 2%

to 4%. Positive surgical margin rates should be lower than 10% overall and 15% in pT3 or pT4 and the median number of pelvic nodes retrieved in the lymphadenectomy should be 10-14 (2). Simultaneously, orthotopic neobladder has become a surgical standard that improved the quality of life of these patients (3).

The surgical technique for radical cystectomy has specific technical objectives that should be met in every case (2):

1. Complete bladder cancer resection even in locally advanced tumors.
2. Minimal blood loss with early vascular control of superior and inferior vesical arteries.
3. Complete pelvic lymph node dissection.
4. Avoidance of tumor cell spillage.

Nowadays, the best outcomes in bladder cancer therapeutics are probably obtained when there is radical cystectomy in a systemic treatment setting. Neoadjuvant treatment has shown interesting advantages in patients with bladder cancer because it offers 5% of survival and 14% decreased risk of associated disease mortality (1). One might argue that two third of the patients would be treated without any response and survival advantage may be outweighed by potential treatment morbidity, with an important number of patients receiving chemotherapy to reach the 5% benefit, however, selection of the population incorporated in the protocols should address this issue.

Adequate surgical endoscopic skill developed in the last two decades and advances accomplished in the management of pulmonary, cardiovascular and hemodynamic effects of pneumoperitoneum allows offering laparoscopy as a safe alternative for these patients and recent data (4). Furthermore, as LRC has been reported with perioperative and functional outcomes comparable with open surgery and adequate mid-term cancer control (5), combining neoadjuvant therapy and LRC, would add the benefits of each one, and perhaps offer a more effective treatment for patients with invasive bladder cancer: The objective would be oncological efficacy with less morbidity. Clinical protocols addressing results of this mentioned way of treatment would be responsible for final answers in this matter and this constitutes our proposal for laparoscopy teams and medical oncologist, to unite for a common objective.

At the beginning of our experience with LRC the main consideration for surgery in bladder carcinoma was the precarious health of this patient's population. Things have not changed much; Haber and Gill (6) have reported important percentages of smokers (65%), hypertension (59%) and cardiac disease (17%) in there series of long term follow-up for LRC. Today, we know that physiological changes incurred as a result of pneumoperitoneum have minimal adverse effects in the majority of patients undergoing laparoscopic surgery; therefore, in the setting of systemic treatment, LRC might represents the low morbidity surgical option for the patient who had neoadjuvant therapy. Minimizing operative trauma becomes even more important for these patients. To open the path, there is need for clinical protocols incorporating these therapeutical options in order to address initially the morbidity and mortality while keeping in mind the oncological safety.

## Take Home Message

The combination of two effective treatments -medical and surgical- would probably offer a great advantage to patients with invasive bladder cancer. Laparoscopic cystectomy might represent a low morbidity surgical option to patients who have previously received chemotherapy for invasive bladder carcinoma.

## Acknowledgement

The Institut Mutualiste Montsouris has started a protocol on neoadjuvant chemotherapy and laparoscopic cystectomy, funded in part by a Clinical Research Grant from Oficina de Investigacion, Confederacion Americana de Urologia, CAU.

### REFERENCES

1. Herr HW, Dotan Z, Donat SM, Bajorin DF: Defining optimal therapy for muscle invasive bladder cancer. J Urol. 2007; 177: 437-43.

2. Herr HW, Smith JA, Montie JE: Standardization of radical cystectomy: time to count and be counted. BJU Int. 2004; 94: 481-2.
3. Hautmann RE, Volkmer BG, Schumacher MC, Gschwend JE, Studer UE: Long-term results of standard procedures in urology: the ileal neobladder. World J Urol. 2006; 24: 305-14.
4. O'Malley C, Cunningham AJ: Physiologic changes during laparoscopy. Anesthesiol Clin North America. 2001; 19: 1-19.
5. Haber GP, Crouzet S, Gill IS: Laparoscopic and robotic assisted radical cystectomy for bladder cancer: a critical analysis. Eur Urol. 2008; 54: 54-64.
6. Haber GP, Gill IS: Laparoscopic radical cystectomy for cancer: oncological outcomes at up to 5 years. BJU Int. 2007; 100: 137-42.

***Dr. Eric Barret, Dr. Rafael Sanchez-Salas & Dr. Guy Vallancien***
*Department of Urology, Institut Montsouris*
*Université Paris Descartes*
*42, Bd Jourdan, 75014*
*Paris, France*
*E-mail: eric.barret@imm.fr*

International Braz J Urol
Vol. 34 (4):516-538, July - August, 2008

# UROLOGICAL SURVEY

**Francisco J.B. Sampaio**
Urogenital Research Unit
State University of Rio de Janeiro

**Athanase Billis**
State University of Campinas
Campinas, SP, Brazil

**Andreas Böhle**
Helios Agnes Karll Hospital
Bad Schwartau, Germany

**Steven B. Brandes**
Washington University in St. Louis
St. Louis, Missouri, USA

**Fernando J. Kim**
Univ Colorado Health Sci Ctr
Denver, Colorado, USA

**Manoj Monga**
University of Minnesota
Edina, MN, USA

**Steven P. Petrou**
Mayo Medical School
Jacksonville, Florida, USA

**Adilson Prando**
Vera Cruz Hospital
Campinas, SP, Brazil

**Brent W. Snow**
University of Utah
Salt Lake City, Utah, USA

**Arnulf Stenzl**
University of Tuenbingen
Tuebingen, Germany

## STONE DISEASE

### Climate-related increase in the prevalence of urolithiasis in the United States

Brikowski TH, Lotan Y, Pearle MS
*Geosciences Department, University of Texas at Dallas, Richardson, TX 75080-3021, USA*
Proc Natl Acad Sci USA. 2008; 105: 9841-6

An unanticipated result of global warming is the likely northward expansion of the present-day southeastern U.S. kidney stone "belt." The fraction of the U.S. population living in high-risk zones for nephrolithiasis will grow from 40% in 2000 to 56% by 2050, and to 70% by 2095. Predictions based on a climate model of intermediate severity warming (SRESa1b) indicate a climate-related increase of 1.6-2.2 million lifetime cases of nephrolithiasis by 2050, representing up to a 30% increase in some climate divisions. Nationwide, the cost increase associated with this rise in nephrolithiasis would be $0.9-1.3 billion annually (year-2000 dollars), representing a 25% increase over current expenditures. The impact of these changes will be geographically concentrated, depending on the precise relationship between temperature and stone risk. Stone risk may abruptly increase at a threshold temperature (nonlinear model) or increase steadily with temperature change (linear model) or some combination thereof. The linear model predicts increases by 2050 that are concentrated in California, Texas, Florida, and the Eastern Seaboard; the nonlinear model predicts concentration in a geographic band stretching from Kansas to Kentucky and Northern California, immediately south of the threshold isotherm.

**Editorial Comment**

This novel study raises important concerns and provokes many unique avenues for future investigation. It is ironic that as the polar ice melts, and water levels rise, we may need this water to prevent kidney stone disease!

In developed countries, we live in climate-control; ambient temperature set at 65 or 70 degrees F, irrespective of time of season. The health risk posed by rises in mean annual temperature (MAT) and heat index will be felt heaviest by those with occupations that demand a significant time outdoors (agriculture, construction etc.) The risks of global warming on stone formation will be more acutely felt by those living in areas not fortunate to have air-conditioning.

The authors note that heat stress and heat index may have a closer link to the distribution of the stone belt than MAT. As scientists debate the "positive water vapor feedback" that links humidity with global warming, it will be important to consider this for stone risk projections. In addition, the interplay between vitamin-D metabolism, stone risk and atmospheric changes deserves further study.

***Dr. Manoj Monga***
*Professor, Department of Urology*
*University of Minnesota*
*Edina, Minnesota, USA*
*E-mail: endourol@yahoo.com*

### Accuracy of urinary dipstick testing for pH manipulation therapy

Desai RA, Assimos DG
*Department of Urology, Wake Forest University, Winston Salem, North Carolina, USA*
J Endourol. 2008; 22: 1367-70

Purpose: To determine the accuracy of urinary dipstick testing for pH manipulation therapy.
Materials and Methods: Three commercial brands of dipstick paper were used to measure the pH of 100 fresh urine specimens from patients with urologic diseases. These were all read by an experienced medical technician. The pH of these specimens was also measured with an electrochemical pH meter ("gold standard") performed by another experienced technician. Both were blinded to each other's results. The influence of urinary microscopic findings was also assessed. Student t test and analysis of variance were used to analyze the data.
Results: The accuracies of the dipsticks for determining pH were as follows: 54.8% to 92.8% for less than 6, 45% to 97.5% for 6 to 7, 72.2% to 83.3% for greater than 7. One of the dipsticks assessed had the lowest accuracy for all three ranges. There was a statistically significant difference between the performances of the other two as compared with the least accurate one. There were no statistically significant differences between the two more accurate dipsticks. Urinary microscopic findings and other dipstick results did not influence results.
Conclusion: The targeted pH range for urinary pH manipulation therapy is 6 to 7. These results indicate that dipstick testing may be applicable to monitor patients on pH manipulation therapy and modify treatment when necessary. The accuracy of the device used for this purpose, however, must be determined before use.

**Editorial Comment**

The authors conducted a well-designed and elegant evaluation of an important question that impacts clinical practice. This study evaluated trained medical technicians - it would be critical to evaluate the ability of the patient to correctly read the urine pH using a dipstick, as this strategy is best suited for home-monitoring. Monitoring pH levels over 7 is of particular importance to avoid increasing the risk of calcium phosphate crystallization, and as such, the litmus paper proved superior in this regard. Similarly, the litmus paper was most accurate at providing "positive feedback" in the face of a therapeutic pH of 6-7. The authors plan to evaluate a handheld pH meter accurate to within 0.1 pH units for home therapy that costs less than $100. The authors recommend checking the urinary pH three times a day during initial titration of therapy.

***Dr. Manoj Monga***
*Professor, Department of Urology*
*University of Minnesota*
*Edina, Minnesota, USA*
*E-mail: endourol@yahoo.com*

## ENDOUROLOGY & LAPAROSCOPY

### Risk score and metastasectomy independently impact prognosis of patients with recurrent renal cell carcinoma

Eggener SE, Yossepowitch O, Kundu S, Motzer RJ, Russo P.
*Department of Urology and Division of Solid Tumor Oncology, Memorial Sloan-Kettering Cancer Center, New York, New York, USA*
J Urol. 2008; 180: 873-8; discussion 878

Purpose: We evaluated the prognostic roles of metastasectomy and an established risk stratification system in patients with disease recurrence following nephrectomy for nonmetastatic renal cell carcinoma.
Materials and Methods: A retrospective analysis was performed in 129 patients with localized renal cell carcinoma treated with partial or radical nephrectomy and subsequently diagnosed with disease recurrence. At

recurrence a previously validated risk score based on Karnofsky performance status, interval from nephrectomy, and serum hemoglobin, calcium and lactate dehydrogenase was used to categorize patients as being at favorable, intermediate or poor risk. Survival from time of recurrence was assessed based on risk categorization and metastasectomy.
Results: Median time from nephrectomy to recurrence was 16 months. The risk score was strongly associated with median survival and the 2-year survival rate, including 73 months and 81% for favorable risk, 28 months and 54% for intermediate risk, and 6 months and 11% for poor risk, respectively (log rank $< 0.001$). Metastasectomy performed in 44 patients (34%) was found to be of clinical benefit across the various risk categories (interaction analysis $p = 0.8$). On multivariate analysis a better risk category and metastasectomy were each independently associated with more favorable survival (each $p < 0.001$). When combined, they provided 6 risk categories with an estimated 2-year survival of 0% to 93%.
Conclusions: The clinical course in patients with recurrent renal cell carcinoma following nephrectomy can be variable. It is independently impacted by an objectively determined risk score and whether the patient undergoes metastasectomy.

**Editorial Comment**

This retrospective study demonstrated prognostic roles of metastasectomy and an established risk stratification system in patients with disease recurrence following nephrectomy for nonmetastatic renal cell carcinoma.

Although the metastectomy may improve survival in the favorable group, the limitations of this retrospective study still do not answer all the questions for the less favorable group of patients.

With the advent of new targeted therapy drugs and better stratification of these patients it is possible that we will improve the lives of these patients.

***Dr. Fernando J. Kim***
*Chief of Urology, Denver Health Med Ctr*
*Assistant Professor, Univ Colorado Health Sci Ctr*
*Denver, Colorado, USA*
*E-mail: fernando.kim@uchsc.edu*

## Conversion during laparoscopic surgery: frequency, indications and risk factors

Richstone L, Seideman C, Baldinger L, Permpongkosol S, Jarrett TW, Su LM, Pavlovich C, Kavoussi LR
*North Shore-Long Island Jewish Health System, New Hyde Park, New York, USA*
J Urol. 2008; 180: 855-9

Purpose: There are limited data on the indications for open conversion during laparoscopic surgery. The frequency of conversion for various procedures is poorly quantified and the degree to which this changes with time is not well understood. Risk factors for conversion are not defined. We addressed these issues in a large series of laparoscopic operations.
Materials and Methods: We reviewed our database of 2,128 laparoscopic operations performed between 1993 and 2005, including radical nephrectomy in 549 patients, simple nephrectomy in 186, partial nephrectomy in 347, donor nephrectomy in 553, pyeloplasty in 301, nephroureterectomy in 106 and retroperitoneal lymph node dissection in 86. Open conversions were identified and the frequency of conversion for the total cohort and specific procedures was determined. Trends in conversion with time were assessed and indications analyzed.

Clinicopathological features between patients requiring conversion and those who did not were compared.
Results: We identified 68 patients (3.3%) who underwent conversion to open surgery (group 1) and 2,011 (96.7%) who did not (group 2). The frequency of conversion was greatest during nephroureterectomy (8.49%), followed by simple nephrectomy (5.91%), retroperitoneal lymph node dissection (4.65%), partial nephrectomy (4.32%), radical nephrectomy (2.91%), donor nephrectomy (2.53%) and pyeloplasty (0.33%). The absolute number of conversions and conversions/cases performed per year decreased significantly with time, reaching a nadir of less than 1% per year. Conversion was inversely related to case volume and cumulative experience. Indications included vascular injury in 38.5% of cases, concern with margins in 13.5%, bowel injury in 13.5%, failure to progress in 11.5%, adhesions in 9.6%, diaphragmatic injury in 1.9% and other in 11.5%. The distribution of indications remained similar with time. There were no differences in patient age, gender, surgical history, American Society of Anesthesiologists score or tumor stage between groups 1 and 2. In groups 1 and 2 mean operative time was 304 vs. 219 minutes and estimated blood loss was 904 vs. 255 cc (each $p < 0.0001$).
Conclusions: The rate of conversion during laparoscopic surgery is not uniform across procedures and it is important for patient counseling. The most common indication for conversion is vascular injury. Importantly the frequency of conversion is dynamic and likely related to case volume and cumulative experience.

**Editorial Comment**

Conversion of laparoscopic to open surgery is not a complication in my view.

The escalation of surgical technique during a difficult case may provide the safe outcome desired for the patient. This large series of laparoscopic cases demonstrate that the vascular injuries are responsible for the majority of the conversions. The longer the clinical experience the rate of conversion tends to decrease even in complex cases. The authors ought to be congratulated to demonstrate that conversion is beneficial for the well being of the patient encouraging novice surgeons to perform it when suited.

***Dr. Fernando J. Kim***
*Chief of Urology, Denver Health Med Ctr*
*Assistant Professor, Univ Colorado Health Sci Ctr*
*Denver, Colorado, USA*
*E-mail: fernando.kim@uchsc.edu*

## IMAGING

### Development of renal scars on CT after abdominal trauma: does grade of injury matter?

Dunfee BL, Lucey BC, Soto JA
*Department of Radiology, Division of Body Imaging, Boston University Medical Center, Boston, MA, USA*
AJR Am J Roentgenol. 2008; 190: 1174-9

Objective: The objective of our study was to determine whether there is an association between the grade of a traumatic renal injury and the subsequent development of renal parenchymal scars on CT.
Materials and Methods: We performed a retrospective study encompassing all acute trauma patients admitted to our institution over a 42-month period found to have renal parenchyma injuries on initial MDCT and also to have undergone a follow-up CT performed at least 1 month after trauma. We identified 54 patients who sustained blunt (n = 44) or penetrating (n = 10) abdominal trauma. The renal injuries were graded by two

radiologists according to the Organ Injury Scaling Committee of the American Association for the Surgery of Trauma (AAST), grades I through V. Follow-up CT was reviewed for the presence of parenchymal distortion, scarring, or perfusion defects.
Results: Of the 54 patients, 12 had grade I injury, eight had grade II injury, 22 had grade III injury, 10 had grade IV injury, and two had grade V injury. Grades I and II traumatic renal injuries were undetectable on follow-up CT. Grade III injuries resulted in the development of renal scars in 14 of 22 (64%) patients. Scarring resulted in all patients with grades IV and V injuries.
Conclusion: Grades I and II renal injuries heal completely, whereas higher grades of renal trauma result in permanent parenchymal scarring. Hence, incidentally discovered renal scars in patients with a history of minor renal trauma should be attributed tentatively to other causes that may or may not require additional investigation.

**Editorial Comment**

Since the preservation of long-term renal function is often better when renal injuries are treated nonoperatively, in stable patients, conservative management may be preferable even in high-grade injuries. Surgery or interventional radiographic procedures will be used mainly in patients presenting extensive devitalized renal tissue, active hemorrhage, or a large injury to the collecting system with progressive renal compression on follow-up or with ureteral disruption, Overall, with modern management techniques, renal salvage rates approach 85-90%. This report focuses on the follow-up of traumatic blunt or penetrating renal parenchymal damage. The authors used initial and a follow-up CT, which was performed at least 1 month after trauma. The authors concluded that Grades I and II renal injuries heal completely but most of Grade III an all Grades IV and V were associated with variable degree of parenchymal distortion, scarring or perfusion defects. The healing and scar formation were directly correlated with the severity of injury. This is an important observation since areas of parenchymal renal scarring is not an infrequent finding on abdominal CT performed for many other clinical reasons. Radiologist should consider sequelae of high grade renal lesion among the causes of renal scarring such as pyelonephritis, renal emboli and systemic vasculites. We have also to remember that other late complications after renal trauma are hydronephrosis and calculus formation (both secondary to scarring in the region of renal pelvis), arteriovenous fistula (usually after stab wound) and delayed hypertension.

***Dr. Adilson Prando***
*Chief, Department of Radiology and*
*Diagnostic Imaging, Vera Cruz Hospital*
*Campinas, São Paulo, Brazil*
*E-mail: adilson.prando@gmail.com*

## Prostate cancer: is inapparent tumor at endorectal MR and MR spectroscopic imaging a favorable prognostic finding in patients who select active surveillance?

Cabrera AR, Coakley FV, Westphalen AC, Lu Y, Zhao S, Shinohara K, Carroll PR, Kurhanewicz J
*Department of Radiology, University of California, San Francisco, CA, USA*
Radiology. 2008; 247: 444-50

Purpose: To retrospectively determine whether inapparent tumor at endorectal magnetic resonance (MR) imaging and MR spectroscopic imaging is a favorable prognostic finding in prostate cancer patients who select active surveillance for management.
Materials and Methods: Committee on Human Research approval was obtained and compliance with HIPAA regulations was observed, with waiver of requirement for written consent. Ninety-two men (mean age, 64 years; range, 43-85 years) were retrospectively identified who had biopsy-proved prostate cancer, who had undergone

baseline endorectal MR imaging and MR spectroscopic imaging, and who had selected active surveillance for management. Their mean baseline serum prostate-specific antigen (PSA) level was 5.5 ng/mL, and the median Gleason score was 6. Two readers with 10 and 3 years of experience independently reviewed all MR images and determined whether tumor was apparent on the basis of evaluation of established morphologic and metabolic findings. Another investigator compiled data about baseline clinical stage, biopsy findings, and serum PSA measurements. Multiple logistic regression analysis was used to investigate the relationship between the clinical parameters and tumor apparency at MR imaging and the biochemical outcome.
Results: At baseline MR imaging, readers 1 and 2 considered 54 and 26 patients, respectively, to have inapparent tumor (fair interobserver agreement; kappa = 0.30). During a mean follow-up of 4.8 years, 52 patients had a stable PSA level and 40 had an increasing PSA level. In multivariate analysis, no significant association was found between the baseline clinical stage, Gleason score, serum PSA level, or the presence of apparent tumor at endorectal MR imaging and MR spectroscopic imaging for either reader and the biochemical outcome ($P > .05$ for all).
Conclusion: Endorectal MR imaging and MR spectroscopic imaging findings of tumor apparency or inapparency in prostate cancer patients who select active surveillance for management do not appear to be of prognostic value. (c) RSNA, 2008.

**Editorial Comment**

Endorectal MR imaging (MRI) and magnetic resonance spectroscopic imaging (MRSI) is emerging as a useful technique for detection and local evaluation of prostate cancer extent and aggressiveness. Combined MRI/MRSI has shown excellent sensitivity and specificity for detecting cancer in the peripheral zone. These techniques are also capable of detecting tumor in the transition zone and may reduce the rate of false-negative biopsies and hence decrease the need for more extensive biopsy protocols and multiple repeat biopsy procedures. The authors of this retrospective study show that tumor apparency or inapparency on MRI/MRSI has no predictive value in the active-surveillance population. In other words, in patients with-low risk prostate cancer, tumor apparency or inapparency on baseline imaging studies are not helpful in predicting disease progression. Patients with negative MRI+MRSI examinations were just as likely to develop an increasing PSA level (progression of disease) as those with radiologically apparent tumors. We agree with the authors' statement that the results of this study do not undermine the role of MRI/MRSI in the evaluation of prostate cancer. In a previous study using extended prostate biopsy (12 cores) as a reference, MRI/MRSI showed a negative predictive value of 100% for the detection of prostate cancer (1). In our small sample, all patients with tumor inapparency on MRI/MRSI had negative extended biopsy. Since published data from the Prostate Cancer Prevention Trial demonstrated that there is no PSA level below which the risk of having prostate cancer is zero, probably the same is happening with currently available armamentarium used to predict its progression. As shown in this study PSA levels and Gleason scores, similar to MRI/MRSI, are of limited value in predicting disease progression. For this purpose, probably we will need a new and more specific biologic marker.

**Reference**

1. Prando A, Kurhanewicz J, Borges AP, Oliveira EM Jr, Figueiredo E: Prostatic biopsy directed with endorectal MR spectroscopic imaging findings in patients with elevated prostate specific antigen levels and prior negative biopsy findings: early experience. Radiology. 2005; 236: 903-10.

***Dr. Adilson Prando***
*Chief, Department of Radiology and*
*Diagnostic Imaging, Vera Cruz Hospital*
*Campinas, São Paulo, Brazil*
*E-mail: adilson.prando@gmail.com*

## UROGENITAL TRAUMA

### Penetrating external genital trauma: a 30-year single institution experience

Phonsombat S, Master VA, McAninch JW
*Department of Urology, San Francisco General Hospital and University of California, San Francisco, San Francisco, California, USA*
J Urol. 2008; 180: 192-5; discussion 195-6

Purpose: We examine the characteristics, outcomes and incidence of penetrating external genital trauma at our level I trauma center.
Materials and Methods: Patient records entered into our urological trauma registry were reviewed from 1977 to August 2006.
Results: A total of 110 patients sustained penetrating external genital trauma. Injuries were divided into gunshot wounds (49%), stab wounds/lacerations (44%) and bites (7%). Half of the stab wounds/lacerations were self-emasculation injuries. Operative exploration was performed in 78%, 63% and 75% of gunshot wounds, stab wounds/lacerations and bite injuries, respectively. Of 6 patients with complete penile amputations 5 underwent replantation with an 80% success rate. Testicular injury occurred in 39% and 27% of patients with gunshot wounds and stab wounds/lacerations, respectively. Of the 24 testicles injured via gunshot wounds 18 were reconstructed (75%). Testicular salvage rates were 24% (4 of 17) for self-emasculation stab wounds and 20% (1 of 5) for all other stab wounds/lacerations injuries. Of patients with penetrating external genital trauma 11% also had associated urethral injuries. The incidence of penetrating external genital trauma has remained stable during the last 30 years ($r(2) = 0.98$). Of patients treated with operative exploration 8% and of those treated nonoperatively 4% reported complications.
Conclusions: Conservative débridement of penetrating injuries to the external genitalia should be stressed to maximize tissue preservation. Testicular salvage rates are significantly higher in gunshot wound injuries (75%) compared to stab wounds/lacerations injuries (23%) ($p < 0.001$). A select group of patients with penile and scrotal injuries (ie those with injuries superficial to Buck's or dartos fascia) may undergo nonsurgical treatment of the penetrating external genital injury with minimal morbidity.

### Evaluation and management of gunshot wounds of the penis: 20-year experience at an urban trauma center

Kunkle DA, Lebed BD, Mydlo JH, Pontari MA
*Department of Urology, Temple University Hospital, Philadelphia, Pennsylvania, USA*
J Trauma. 2008; 64: 1038-42

Background: Although gunshot injuries to the penis occur relatively infrequently in patients with penetrating trauma, they often present dilemmas of subsequent evaluation and management. We review our extensive experience with gunshot wounds to the penis at a high volume urban trauma center.
Methods: The urologic trauma database was retrospectively reviewed to extract and compile information from the records of 63 patients treated for gunshot wounds to the penis. Data were accumulated for a 20-year period from 1985 to 2004 with regard to findings on physical examination, diagnostic evaluation, associated injuries, management, and outcome. We detail our technique of penile exploration and artificial erection in the management of these injuries.

Results: Penile gunshot wounds were associated with additional injuries in 53 of 63 (84%) patients. A total of 48 (76%) patients were taken to the operating room and 44 (70%) penile explorations were performed. Evaluation included retrograde urethrogram in 50 of 63 (79%) patients and was diagnostic for urethral injury in 11 of 12 (92%) cases. Primary urethral repair was performed in 8 of 12 (67%) patients with urethral injury versus 4 of 12 (33%) who underwent urinary diversion by means of suprapubic cystotomy.
Conclusions: Evaluation and management of gunshot wounds to the penis may potentially be complex. Retrograde urethrogram should be performed in all cases except the most insignificant and superficial wounds. We describe our technique of penile exploration and artificial erection, noting excellent results in patients for whom follow-up is available. Additional studies are needed to prospectively evaluate techniques for management of gunshot urethral injuries.

**Editorial Comment**

The above two articles are from major trauma centers in the US, from San Francisco and Philadelphia. The San Francisco paper is unique in that the 30 year experience is the cumulative experience of one surgeon (an authority in the field) over the course of his career. This continuity and consistency of care, strengthens the conclusions of this paper.

Overall, both papers illustrate that penetrating genital injuries occur uncommonly – even in major trauma centers, only 3 or so cases per year. Such rare events, further values the conclusions and cumulative experience of papers over such long study period. Aside from evaluating the injury to the genitals, all patients need to be evaluated according to AAST trauma protocols, including routine radiographs of the chest and abdomen, with entrance and exit wounds marked with radio-opaque markers. General surgical principles for managing penetrating injuries apply well to external genitalia trauma, except for wounds of the corpora cavernosum and spongiosum, which should be treated like vasculature, with limited debridement and good hemostatic closure, except repaired with absorbable suture material. General management consists of meticulous hemostasis, vigorous saline lavage, removal of foreign bodies, hematoma evacuation, conservative debridement of devitalized tissue, repair of associated injuries, and primary wound closure. Infection is rare in properly debrided wounds.

Penetrating injuries to the penis (deep to Buck's fascia) demand evaluation for associated urethral injury by either retrograde urethrography or cystoscopy. Surgical exploration should be performed in all cases except with the most insignificant and superficial wound. Blood at the meatus or gross hematuria highly suggests a urethral injury and warrant evaluation. Corporal injuries should be repaired primarily with absorbable sutures. Low velocity penetrating urethral injuries should be repaired primarily – typically by an anastomotic urethroplasty. Primary realignment for such urethral injuries often results in high urethral strictures rates. Staged urethral injury repair is often reserved for extensive injuries – as is often seen in high velocity gunshot wound tissue injuries Patients with injuries to the scrotum deep to Dartos fascia or with scrotal swelling also warrant exploration. Penetrating wounds to the scrotum damage a testis or cord roughly half the time. Once the testis is struck, the chance to salvage the testis after a low velocity GSW is 25 -50%. This contrasts sharply for high velocity injuries of the battlefield, where salvage is rare. Scrotal stab wounds seem to more commonly involve the vascular cord, and thus explaining the reported poor salvage rate.

***Dr. Steven B. Brandes***
*Associate Professor, Division of Urologic Surgery*
*Washington University in St. Louis*
*St. Louis, Missouri, USA*
*E-mail: brandess@wudosis.wustl.edu*

## PATHOLOGY

### Diffuse adenosis of the peripheral zone in prostate needle biopsy and prostatectomy specimens

Lotan TL, Epstein JI
*Departments of Pathology and Urology Oncology, The Johns Hopkins Medical Institutions, Baltimore, MD, USA*
Am J Surg Pathol. 2008; 30: Epub ahead of print

We have observed a group of typically younger patients with multiple foci of small, nonlobular, crowded, but relatively bland acini on needle biopsy and in prostatectomy specimens. It is unclear whether this architectural pattern, which we have termed diffuse adenosis of the peripheral zone (DAPZ), is simply a crowded glandular variant of normal prostate morphology or whether it represents a risk factor for the development of prostatic carcinoma. We studied 60 cases of DAPZ on needle biopsy in our consult practice from 2001 to 2007. Cases, on average, showed 72% of cores involved by DAPZ. Average patient age was 49 years (range: 34 to 73) and the average prostate specific antigen (PSA) level at the time of biopsy was 5.2 ng/mL (n = 42). Forty-three (72%) men had available clinical follow-up with 35 (81%) patients undergoing rebiopsy and 8 (19%) followed with serial PSA measurements. Patients who were rebiopsied after DAPZ diagnosis had higher PSA levels than those who were followed by PSA levels alone (6.2 vs. 3.1 ng/mL, P = 0.04). Of the rebiopsied cases, 20 (57%) were subsequently diagnosed with carcinoma, with an average of 15 months elapsed between initial biopsy and carcinoma diagnosis. Although the majority of tissue sampled in a typical DAPZ case had no cytologic atypia, in 65% of cases there were admixed rare foci of atypical glands with prominent nucleoli comprising < 1% of submitted tissue. Patients with a subsequent diagnosis of carcinoma were more likely to have had DAPZ with focal atypia, although this did not reach statistical significance (70% vs. 36%, P = 0.08). We histologically confirmed the carcinoma diagnosis in 18/20 cases. In 12/14 radical prostatectomies, we were able to review the slides. Eleven had Gleason score 3+3=6 adenocarcinoma in addition to background DAPZ; 9 showed peripheral zone organ-confined cancer, and 2 had focal extraprostatic extension. In one case of DAPZ misdiagnosed as cancer on biopsy, no carcinoma was found at prostatectomy. DAPZ is a newly described and diagnostically challenging mimicker of prostate cancer seen in prostate needle biopsies from typically younger patients. Our findings suggest that DAPZ should be considered a risk factor for prostate cancer and that patients with this finding should be followed closely and rebiopsied.

**Editorial Comment**

Adenosis is a focal lesion that may be confused with carcinoma in transurethral resection specimens (1) or in needle biopsy specimens (2). Another commonly used term for adenosis is atypical adenomatous hyperplasia (3). Epstein prefers the term adenosis, as prefacing adenomatous hyperplasia with the term atypical has adverse consequences in terms of practical patient management considering that there are little data in support of a relation between adenosis and carcinoma. By designating these lesions as atypical, many patients will be subjected to unnecessary repeat biopsies.

In general this lesion is not reported by the pathologist being only a problem in the differential diagnosis with adenocarcinoma. Immunohistochemistry is useful for the correct diagnosis. Lotan and Epstein report a variant of adenosis that is diffuse and seen in younger patients in prostate needle biopsies. Forty-three (72%) men had available clinical follow-up with 35 (81%) patients undergoing rebiopsy. Of the rebiopsied patients, 20 (57%) were subsequently diagnosed with carcinoma, with an average of 15 months elapsed between initial biopsy and carcinoma diagnosis.

The authors consider this newly described variant of adenosis diagnostically challenging mimicker of prostate cancer seen in prostate needle biopsies from typically younger patients (average patient age 49 years). The findings suggest that diffuse adenosis of the peripheral zone should be considered a risk factor for pros-

tate cancer and that patients with this finding should be followed closely and rebiopsied. Therefore this lesion should be reported by the pathologists.

**References**

1. Gaudin PB, Epstein JI: Adenosis of the prostate. Histologic features in transurethral resection specimens. Am J Surg Pathol. 1994; 18: 863-70.
2. Gaudin PB, Epstein JI: Adenosis of the prostate. Histologic features in needle biopsy specimens. Am J Surg Pathol. 1995; 19: 737-47.
3. Bostwick DG, Srigley J, Grignon D, Maksem J, Humphrey P, van der Kwast TH, et al.: Atypical adenomatous hyperplasia of the prostate: morphologic criteria for its distinction from well-differentiated carcinoma. Hum Pathol. 1993; 24: 819-32.

***Dr. Athanase Billis***
*Full-Professor of Pathology*
*State University of Campinas, Unicamp*
*Campinas, São Paulo, Brazil*
*E-mail: athanase@fcm.unicamp.br*

## Positive surgical margins in areas of capsular incision in otherwise organ-confined disease at radical prostatectomy: histologic features and pitfalls

Chuang AY, Epstein JI
*Department of Pathology, Koo Foundation Sun Yat-Sen Cancer Center, Taipei, Taiwan*
Am J Surg Pathol. 2008; 32: 1201-6

Capsular incision (CI) refers to the urologist transecting either benign or malignant prostatic tissue, where the edge of the prostate in this region is left within the patient. Histologic assessment of CI is difficult and its diagnosis varies among pathologists. Between 1993 and 2004, we reviewed 186 radical prostatectomies that were signed out as either: (1) CI into tumor in otherwise organ-confined disease [elsewhere no extra-prostatic extension (EPE), seminal vesicle invasion, or lymph node spread] (n = 143); (2) positive surgical margin in an area difficult to distinguish EPE from CI into tumor in otherwise organ-confined disease (n = 36); or (3) equivocal positive surgical margin in an area difficult to distinguish organ-confined disease with tumor close to resection margins (OC M-) from CI into tumor in otherwise organ-confined disease (n = 7). On review, CI with a positive margin was confirmed in 83.2% of cases. Of cases signed out with margins positive where it was difficult to distinguish CI from EPE, CI was confirmed in 52.8% of cases. Cases with equivocal positive margins with either CI or OC M- were considered CI with positive margins in 57.1% of cases on review. Cases in all 3 groups not considered positive margins with CI were on review equally divided between diagnoses of organ-confined margin negative and EPE with positive margins. The locations of the 39 cases originally misdiagnosed as definitive or questionable CI with positive margins were posterolateral (N = 19, 48.7%), distal (N = 12, 30.8%), posterior (N = 6, 15.4%), and anterolateral (N = 2, 5.1%). Familiarity with different patterns of EPE in different anatomic locations and applying strict criteria for diagnosing CI into tumor can minimize overcalling CI and can provide accurate feedback to urologists to prevent iatrogenic positive margins.

**Editorial Comment**

Positive surgical margin (vesical, urethral or circumferential) in radical prostatectomy specimens is a well established adverse finding for biochemical (PSA) progression following surgery. The frequency of this

progression varies from 36% to 72% in the literature (1). In our Institution, the progression in 300 patients was 37% after 5 years of follow-up.

It is important for the urologist the definition and the description of the several kinds of positive surgical margins (2):

a) Positive surgical margins are defined as cancer cells touching the inked surface of the prostate;

b) Iatrogenic surgical margin occurs whenever there is a transection of the intraprostatic tumor. If this occurs, one cannot determine whether there is extraprostatic extension in the region of incision into the prostate as the edge of the prostate has been left in the patient. Unless there is extraprostatic extension in other areas of the surgical specimen, the pathologic stage is called pT2+;

c) Non-iatrogenic surgical positive margin occurs whenever there is an inability to widely excise tumor showing extraprostatic extension.

It is worth mentioning the possibility of positive surgical margins in normal prostatic glands. This is not routinely reported by the pathologist; however, it is very important to report in cases of limited carcinoma in the surgical specimen. In these cases, biochemical (PSA) progression following surgery may be due to normal glands left in the patient. In our Institution, no patient with limited carcinoma in the specimen had biochemical progression, except 3 patients. Reviewing the prostatectomy slides, we found that all 3 patients had frequent and extensive positive surgical margins in normal glands.

**References**

1. Epstein JI, Amin M, Boccon-Gibod L, Egevad L, Humphrey PA, Mikuz G, et al.: Prognostic factors and reporting of prostate carcinoma in radical prostatectomy and pelvic lymphadenectomy specimens. Scand J Urol Nephrol Suppl. 2005; 216: 34-63.
2. Billis A, Watanabe IC, Costa MV, Telles GH, Magna LA: Iatrogenic and non-iatrogenic positive margins: incidence, site, factors involved, and time to PSA progression following radical prostatectomy. Int Urol Nephrol. 2008; 40: 105-11.

***Dr. Athanase Billis***
*Full-Professor of Pathology*
*State University of Campinas, Unicamp*
*Campinas, São Paulo, Brazil*
*E-mail: athanase@fcm.unicamp.br*

## INVESTIGATIVE UROLOGY

### Protein oxidation as a novel biomarker of bladder decompensation

Kalorin CM, Mannikarottu A, Neumann P, Leggett R, Weisbrot J, Johnson A, Kogan BA, Levin RM
*Albany Medical College, Albany, NY, USA*
BJU Int. 2008; 102: 495-9

Objective: To measure the degree to which partial bladder outlet obstruction (PBOO) results in oxidative bladder damage, which subcellular components of the bladder are affected and whether these changes correlate with bladder function.

Materials and Methods: In all, 32 rabbits were divided into four groups. Each group underwent PBOO for 1, 2, 4, and 8 weeks, respectively. Bladder tissue from each group was homogenized and separated into subcellular

fractions via differential centrifugation. The carbonyl content within the subcellular fractions, including the nuclear, mitochondrial, and microsomal pellets, was then quantified by dot blot analysis.
Results: Total bladder oxidation increased with duration of obstruction across all subcellular fractions. The largest increase in total oxidation occurred between 4 and 8 weeks. Protein oxidation density in the nuclear and microsomal fractions both showed increases at 2 weeks obstruction, decreases at 4 weeks, and then large increases at 8 weeks. The increase in protein oxidation density between 4 and 8 weeks obstruction was most pronounced in the microsomal fraction.
Conclusions: Overall bladder protein oxidation increased with the duration of obstruction and increased at a greater rate during the transition to decompensation. Furthermore, the subcellular fraction that exhibited the most oxidation was the microsomal pellet. The amount of protein oxidation correlated with the functional changes in the bladder.

**Editorial Comment**

In this interesting and welcome experimental study, the authors created surgically partial bladder outlet obstruction (PBOO) in 32 rabbits. They were interested to analyze whether oxidative stress measured after PBOO would correlate with the function of the bladder and whether markers of oxidative stress might serve as a biomarker of the progression to bladder decompensation.

The authors presented clear evidence that protein oxidation occurs to a significant degree in the PBOO rabbit bladder. They concluded that overall bladder protein oxidation increases with the duration of obstruction and increases at a greater rate during the transition to full decompensation. They speculated that in the clinical setting, the urologist could obtain tissue from the bladder of a patient with BPH and analyze it specifically for microsomal protein oxidation and determine the degree to which the patient is moving towards decompensation. Of course, theoretically, it could be done, but probably it will be hard to put in clinical use.

The authors are to be congratulated for this elegant study that opens new avenue for the understanding and management of benign prostatic hyperplasia consequences.

***Dr. Francisco J. B. Sampaio***
*Full-Professor and Chair, Urogenital Research Unit*
*State University of Rio de Janeiro*
*Rio de Janeiro, RJ, Brazil*
*E-mail: sampaio@urogenitalresearch.org*

## The potential of hormones and selective oestrogen receptor modulators in preventing voiding dysfunction in rats

Tantiwongse K, Fandel TM, Wang G, Breyer BN, Walsh TJ, Bella AJ, Lue TF
*Knuppe Molecular Urology Laboratory, Department of Urology, University of California-San Francisco, San Francisco, CA, USA*
BJU Int. 2008; 102: 242-6

Objective: To investigate whether oestrogen, selective oestrogen receptor modulators (SERMs), and growth hormone (GH) can prevent the development of voiding dysfunction in a postpartum postmenopausal rat model of voiding dysfunction.
Materials and Methods: Immediately after spontaneous delivery, nine primiparous Sprague-Dawley rats served as uninjured controls (sham group) and 54 underwent intravaginal balloon dilation. On day 7, the 54 subject rats underwent bilateral ovariectomy. A week later, six treatment groups of nine rats were randomized to receive: normal saline (injured control group), 17beta-oestradiol (E(2)), raloxifene, levormeloxifene, GH, or

GH + E(2). The treatment groups received daily subcutaneous injections for 3 weeks. The effects of hormone treatment were examined by conscious cystometry at the end of the study. Voiding dysfunction was defined to include overactive bladder and sphincter deficiency.
Results: The sham rats had a mean (sd) voiding frequency of 3 (0.87) times in 10 min and a bladder capacity of 0.43 (0.13) mL with smooth cystometry curves. The number of rats in each treatment group (each group contained nine rats) that had voiding dysfunction was as follows: E(2), three; raloxifene, six; levormeloxifene, four; and controls, four ($P > 0.05$ among the groups). Only one rat in the GH-treated group and no rats in the GH + E(2)-treated group had voiding dysfunction, which was significantly less in the GH + E(2)-treated group than in the controls ($P = 0.041$).
Conclusion: This functional data suggest that the development of voiding dysfunction can be prevented by short-term administration of GH and GH + E(2) in our rat model. SERMs and E(2) alone seem to have no therapeutic effect.

**Editorial Comment**

This is a wished study by Dr. Lue and collaborators that have been working on this topic for the last years. They analyzed if short-term therapy with ultra-low dose of estrogen, selective estrogen receptor modulators (SERMs), and growth hormone (GH) can prevent the development of voiding dysfunction in a postpartum, postmenopausal voiding dysfunction rat model. By using conscious cistometry, developed in its own laboratory, the authors found that short-term therapy with E2, SERMs and GH suggest that, in the dosage and duration used, GH and GH + E2 seem to prevent the development of voiding dysfunction while E2 alone and SERMs do not have significant effects. With this paper, we are able to better understand the effect of these hormones on voiding, with the consequent clinical implications for treating and preventing post-partum and postmenopausal voiding dysfunction.

***Dr. Francisco J. B. Sampaio***
*Full-Professor and Chair, Urogenital Research Unit*
*State University of Rio de Janeiro*
*Rio de Janeiro, RJ, Brazil*
*E-mail: sampaio@urogenitalresearch.org*

## RECONSTRUCTIVE UROLOGY

### A collagen matrix derived from bladder can be used to engineer smooth muscle tissue

Kim BS, Atala A, Yoo JJ
*Department of Bioengineering, Hanyang University, Seoul, South Korea*
World J Urol. 2008 Jul 2 [Epub ahead of print]

We have previously demonstrated that a collagen matrix derived from lamina propria, commonly known as bladder submucosa (BSM matrix), is a suitable biomaterial for several urologic applications, including reconstruction of the bladder and urethra in experimental models and clinical trials. In the present study, we evaluated the physical properties of BSM as well as its biocompatibility, cellular interactions, and ability to support the formation of functional tissue in order to determine whether this biomaterial could serve as a matrix for urinary smooth muscle tissue engineering. BSM matrix resembles the extracellular matrix of bladder submucosa in its native structure, composition, and mechanical properties. BSM matrix supported normal

mitochondrial metabolic and proliferative functions of human urinary smooth muscle cells and did not induce cytotoxic effects in vitro. When implanted in vivo, BSM matrix promoted the regeneration of urinary smooth muscle tissues with contractility, which is a smooth muscle-specific tissue function. These results suggest that BSM matrix would be a useful biomaterial for urinary smooth muscle reconstruction.

**Editorial Comment**

Using scaffolds to regenerate tissue especially in the urological field has been the aim for the last decade. Which scaffold might be the best still seems to be not clear. The paper of Kim et al. investigated the native structure of Bladder Submucosa Matrix (BSM), seeded with smooth muscle cells as a composition and its mechanical properties. Compared to previous publications the extended investigation was performed in a tissue-engineered seeded fashion, but as Piechota et al. (1) previously demonstrated (and further investigated by Dahms et al. (2), the acellular Bladder Matrix Graft (BMG) fully regenerated and functioned as native bladder tissue.

The use of organ-specific scaffolds was extended to other urological organs such as urethra and ureter (3). However, the use of SIS® by Cook in the context of pre-seeding scaffold did not always demonstrate the expected success (4). Through the investigation of BSM, Kim et al. compared unseeded scaffolds; they found that BSM demonstrated a faster functional regeneration, thus underlining, depending on its thickness, that an organ-specific scaffold might be more favorable (5).

**References**

1. Piechota HJ, Gleason CA, Dahms SE, Dahiya R, Nunes LS, Lue TF, et al.: Bladder acellular matrix graft: in vivo functional properties of the regenerated rat bladder. Urol Res. 1999; 27: 206-13.
2. Dahms SE, Piechota HJ, Dahiya R, Gleason CA, Hohenfellner M, Tanagho EA: Bladder acellular matrix graft in rats: its neurophysiologic properties and mRNA expression of growth factors TGF-alpha and TGF-beta. Neurourol Urodyn. 1998; 17: 37-54.
3. Sievert KD, Tanagho EA: Organ-specific acellular matrix for reconstruction of the urinary tract. World J Urol. 2000; 18: 19-25.
4. Feil G, Christ-Adler M, Maurer S, Corvin S, Rennekampff HO, Krug J, et al.: Investigations of urothelial cells seeded on commercially available small intestine submucosa. Eur Urol. 2006; 50: 1330-7.
5. Sievert KD, Wefer J, Bakircioglu ME, Nunes L, Dahiya R, Tanagho EA: Heterologous acellular matrix graft for reconstruction of the rabbit urethra: histological and functional evaluation. J Urol. 2001; 165: 2096-102.

***Dr. Karl-Dietrich Sievert &***
***Dr. Arnulf Stenzl***
*Department of Urology*
*Eberhard-Karls-University Tuebingen*
*Tuebingen, Germany*
*E-mail: arnulf.stenzl@med.uni-tuebingen.de*

## Laparoscopic ureteroneocystostomy and psoas hitch for post-hysterectomy ureterovaginal fistula

Modi P, Gupta R, Rizvi SJ
*Department of Urology and Transplantation Surgery, Institute of Kidney Diseases and Research Centre, Institute of Transplantation Sciences, Civil Hospital Campus, Asarwa, Ahmedabad, Gujarat, India*
J Urol. 2008; 180: 615-7

Purpose: We assessed the results of laparoscopic ureteroneocystostomy with a psoas hitch for iatrogenic lower ureteral injuries leading to a ureterovaginal fistula.
Materials and Methods: Between July 2003 and November 2007, 18 patients with iatrogenic lower ureteral injuries during hysterectomy leading to ureterovaginal fistula underwent laparoscopic ureteroneocystostomy with a psoas hitch. Of the patients 17 underwent abdominal or vaginal hysterectomy, while in 1 with a ruptured gravid uterus emergency hysterectomy was done for uncontrolled bleeding. Mean patient age was 35.5 years (range 23 to 45) and mean time to surgery since the injury was 2.2 months (range 1.5 to 3.5). Transperitoneal 3 or 4 port laparoscopic ureteroneocystostomy with a psoas hitch was performed.
Results: Of the procedures 17 were completed successfully. Intraoperative cardiac arrhythmia occurred in 1 patient due to pneumoperitoneum and hypercarbia, requiring open conversion. Mean operative time was 2.5 hours (range 1.9 to 2.8) hours, mean blood loss was 90 ml (range 45 to 150) and total hospital stay was 5.3 days (range 2.9 to 8). The nephrostomy tube was blocked on the table in all patients and it was removed on day 7. At an average followup of 26.4 months (range 3 to 52) postoperative excretory urography did not reveal obstruction in any patient. One patient had vesicoureteral reflux on voiding cystogram.
Conclusions: Laparoscopic ureteroneocystostomy with a psoas hitch for ureterovaginal fistula secondary to hysterectomy is safe and effective, and associated with a low incidence of postoperative reflux and obstruction.

**Editorial Comment**

Using a minimally invasive approach, a laparoscopic ureter reimplantation in an anti-refluxive fashion, is a logical approach if a fistula occurs after a transvaginal hysterectomy. Mondi et al. presented 18 cases using a laparoscopic ureteroneocystostomy in a psoas hitch technique with a no-refluxing Lich-Gregoir only technique, which seems to be very convincing and is supported with the recent publication of Patill et al. (1,2).

With the increased integration of laparoscopic surgery in our department, similar cases have been treated. From our recent experiences, we propose a modified approach: because of the fistula tissue we try to avoid any foreign material and comparatively use a clip at the distal ureter thermofusion to seal the ureter (3). Further, most commonly the fistula is not associated with an obstruction or even stricture of the ureter. A double-J-stent usually secures drainage of the kidney without the requirement of a nephrostomy tube. Only in those cases with a stricture a nephrostomy tube is required, which will be replaced intraoperatively while performing the ureteroneocystostomy into the bladder dome using a double-J-stent. After four weeks in particular, in women the double-J-stent can be removed without the need of anesthesia. In the context of mini-percutaneous nephrolithomy, we evaluated the patient's preference and concluded that the double-J-stents causes less pain, its removal is less traumatic to the patient than a nephrostomy tube and also needs to stay in place even if it is only for a week (4).

Overall we believe the laparoscopic approach to treat distal uretral fistulas or strictures are feasible. As the authors mentioned, the patient recovers faster. However, the laparoscopic approach with the implantation of the ureter into the ventral bladder wall - with a bigger distance to the former fistula location -, compared to the open procedure where the ureter is placed dorsally, needs to be evaluated over time and compared against the open procedure.

**References**

1. Modi P, Gupta R, Rizvi SJ: Laparoscopic ureteroneocystostomy and psoas hitch for post-hysterectomy ureterovaginal fistula. J Urol. 2008; 180: 615-7.
2. Patil NN, Mottrie A, Sundaram B, Patel VR: Robotic-assisted laparoscopic ureteral reimplantation with psoas hitch: a multi-institutional, multinational evaluation. Urology. 2008; 72: 47-50; discussion 50.
3. Nagele U, Merseburger AS, Horstmann M, Amend A, Schilling D, Kuczyk M, et al.: Thermofused bladder cuff resection in nephroureterectomy - a new approach, J Urol. 2008; 179: 346 (Abst# V1058).

4. Nagele U, Schilling D, Anastasiadis AG, Corvin S, Seibold J, Kuczyk M, et al.: Closing the tract of mini-percutaneous nephrolithotomy with gelatine matrix hemostatic sealant can replace nephrostomy tube placement. Urology. 2006; 68: 489-93; discussion 493-4.

***Dr. Karl-Dietrich Sievert,***
***Dr. Udo Nagele & Dr. Arnulf Stenzl***
*Department of Urology*
*Eberhard-Karls-University Tuebingen*
*Tuebingen, Germany*
*E-mail: arnulf.stenzl@med.uni-tuebingen.de*

## UROLOGICAL ONCOLOGY

### Renal cell carcinoma in adults 40 years old or less: young age is an independent prognostic factor for cancer-specific survival

Taccoen X, Valeri A, Descotes JL, Morin V, Stindel E, Doucet L, Joulin V, Bocqueraz F, Coulange C, Rambeaud JJ, Fournier G, Mejean A; Oncology Committee of the Association Française d'Urologie
*Service d'Urologie, Centre Hospitalier et Universitaire, 29609 Brest Cedex, France*
Eur Urol. 2007; 51: 980-7

Objectives: Renal cell carcinoma (RCC) is uncommon in young adults. Based on the few studies published to date, it is difficult to determine whether this tumour has a particular progression pattern. This retrospective, multicentre study analysed RCC in young patients, defined as </= 40 yr old, compared to RCC in older patients.
Methods: Between 1988 and 2000, 1233 patients, 93 under 40 yr old and 1140 older (mean ages, 34.2 and 61.9 years, respectively) underwent surgery for RCC in four teaching hospitals. Clinical and biologic parameters at diagnosis were compared and subjected to univariate and multivariate analyses to study survival. Mean follow-up was 4.5 yr for young and 4.1 yr for older patients.
Results: When comparing younger to older patients, respectively, they had a lower male-to-female ratio (1.2 vs. 2.5), lower stage (84.9% vs. 67.4% pT1-pT2N0M0; $p = 0.001$), and fewer clear-cell carcinomas (73.1% vs. 82%), but more papillary carcinomas (20.4% vs. 11.4%; $p = 0.01$) and better 5-yr cancer-specific survival rates (90.8% vs. 78.3%; $p = 0.005$). Independent prognostic factors for survival, in the order of decreasing impact, were tumor stage ($p < 0.0001$), Fuhrman nuclear grade ($p < 0.0001$), and age </= 40 yr at diagnosis (risk ratio 0.4, $p < 0.047$). Young patients tended to have a better 5-yr progression-free survival (80.5% vs. 70.7%; $p = 0.05$). Conclusions: RCC in young adults was more often localised at diagnosis and had a better prognosis than the disease in older subjects. Age under 40 yr old was an independent prognostic factor for survival.

**Editorial Comment**

This report focuses on a large database of roughly 1300 patients with renal cell carcinoma from several hospitals in France. 10% of these patients were less than 40 years old and were analyzed in comparison to the older ones. Interestingly, young patients had a better 5 year progression-free prognosis.

One of the factors that differed between these groups was that younger patients had more symptomatic tumors (60,2% vs. 50,4%), which , however, was not due to a different tumor size (5.8 cm vs. 6 cm). Aggressive growth showed differences, as favourable pT1 and pT2 tumors were more often among younger patients

(84.9% vs. 67.4%). The differences between the age groups is interesting and, to my opinion, might be due to a shift in immunologic control with age. This should be focused in further scientific approaches on renal cell cancer.

***Dr. Andreas Bohle***
*Professor of Urology*
*HELIOS Agnes Karll Hospital*
*Bad Schwartau, Germany*
*E-mail: boehle@urologie-bad-schwartau.de*

**Should we replace the Gleason score with the amount of high-grade prostate cancer?**

Vis AN, Roemeling S, Kranse R, Schröder FH, van der Kwast TH
*Department of Urology, Erasmus Medical Center, Rotterdam, The Netherlands*
Eur Urol. 2007; 51: 931-9

Objectives: The stage and grade shift of currently diagnosed prostate cancer has led to a diminished prognostic power of the Gleason score system. We investigated the predictive value of the amount of high-grade cancer (Gleason growth patterns 4/5) in the biopsy for prostate-specific antigen (PSA) and clinical relapse after radical prostatectomy.
Methods: PSA-tested participants (N = 281) of the European Randomized Study of Screening for Prostate Cancer (ERSPC) who underwent radical prostatectomy were analyzed. Besides clinical features, and serum-PSA, histopathologic features as determined in the diagnostic biopsy and matching radical prostatectomy specimen were related to patient outcome.
Results: At a median follow-up of 7 yr, 39 (13.9%), 24 (8.5%), and 12 (4.3%) patients had PSA >/= 0.1 ng/ml, PSA >/= 1.0 ng/ml, and clinical relapse after radical prostatectomy, respectively. Using Cox proportional hazards, PSA level (p = 0.002), length of tumour (p = 0.040), and length of high-grade cancer (p = 0.006) in the biopsy, but not Gleason score, were independent prognostic factors for biochemical relapse (PSA >/= 0.1 ng/ml) when assessed as continuous variables. In radical prostatectomies, the proportion of high-grade cancer (p < 0.001) was most predictive of relapse (PSA >/= 0.1 ng/ml). For PSA >/= 1.0 ng/ml and clinical relapse, the amount of high-grade cancer, both in the biopsy specimen (p = 0.016 and p = 0.004, respectively) and radical prostatectomy specimen (p = 0.002 and p = 0.005, respectively), but not Gleason score, was an independent predictor.
Conclusions: In biopsy and radical prostatectomy specimens of surgically treated prostate cancer, the amount of high-grade cancer is superior to the Gleason grading system in predicting patient outcome. We propose that, in addition to the Gleason score, the amount of Gleason growth patterns 4/5 in the biopsy (whether absolute length or proportion) should be mentioned in the pathology report.

**Editorial Comment**

Gleason sum score is widely used for tayloring treatment to patients with prostate carcinoma. In this report, the authors compare the usual Gleason sum score to the amount of Gleason 4/5 (aggressive growth pattern) in the biopsy in predicting outcome after radical prostatcetomy. They found that the proportion of aggressive tumor correlates very well with PSA relapse after radical prostatectomy and suggest to indicate this proportion in the pathological report.

Indeed, from these data and other reports this approach can only be emphasized and every pathologist should be asked for this additional service. The only caveat may be the difficulty to define the proportion of

aggressive tumor growth (Gleason 4/5) in biopsies with small amount of tumors. Still, this approach may be very helpful in clinical practice.

***Dr. Andreas Bohle***
*Professor of Urology*
*HELIOS Agnes Karll Hospital*
*Bad Schwartau, Germany*
*E-mail: boehle@urologie-bad-schwartau.de*

## NEUROUROLOGY & FEMALE UROLOGY

### Development of de novo urge incontinence in women post sling: The role of preoperative urodynamics in assessing the risk

Alperin M, Abrahams-Gessel S, Wakamatsu MM
*Division of Urogynecology, Department of Obstetrics and Gynecology and Reproductive Sciences, Magee-Womens Hospital, University of Pittsburgh, Pennsylvania*
Neurourol Urodyn. 2008; 27: 407-11

Aims: The study was undertaken to investigate if there are specific identifiable risk factors on the preoperative history or urodynamics testing associated with an increased risk for the development of symptoms of de novo urge urinary incontinence after a minimally invasive sling procedure.
Methods: Two hundred eighty-one women who had undergone minimally invasive sling surgery for stress urinary incontinence between January 2000 and December 2003 were identified. The records of 92 patients were included in this review.
Results: Twenty-five patients (27%) reported urge urinary incontinence on postoperative questioning. Clinical and urodynamic parameters were correlated with the development of de novo urge urinary incontinence. Preoperative history parameters were not predictive of the increased risk of de novo urge urinary incontinence, with the exception of increased preoperative daytime frequency (OR 3.3 (1.2, 9.1)). Of 16 women whose detrusor pressure during the filling phase of cystometry exceeded 15 cm H2O, de novo urge urinary incontinence developed in 9 (56%) vs. 16 (21%) of 76 women, whose detrusor pressure was </= 15 cm H2O (OR 4.6 (1.4, 15.0)).
Conclusions: Directed patient history is only minimally helpful in the identification of women at increased risk for the development of de novo urge urinary incontinence, with the exception of the complaint of increased daytime frequency. Women with elevated detrusor pressure during the filling phase of cystometry were more likely to develop urge urinary incontinence postoperatively. Therefore, we suggest that preoperative urodynamic evaluation, and specifically detrusor pressure > 15 cm H2O may help identify patients at increased risk of developing de novo urge urinary incontinence following the minimally invasive sling procedure. Neurourol. Urodynam. 27:407-411, 2008. (c) 2007 Wiley-Liss, Inc.

**Editorial Comment**

The authors reviewed a population of women who had undergone a midurethral sling. Out of this population, 92 women were identified as having had no complaints and/or urodynamic evidence of urge urinary incontinence or detrusor overactivity before their operation. Of those 92 women, 25 (27%) developed de novo postoperative urge urinary incontinence after their surgery. The authors found that of all the preoperative variables examined, only a history of daytime urinary frequency or a bladder filling pressure of > 15 cm of

water predicted an increased risk for the development of de novo urge urinary incontinence. All the patients underwent a midurethral retropubic operation with none receiving a transobturator sling.

This manuscript points out the definite morbidity of new onset urinary urge incontinence after an anti-incontinence operation for stress urinary incontinence. A 27% incidence rate seems high but is very realistic. Great interest would be if the authors would expand their study in the future to look at patients who underwent a transobturator technique to see if the rates of new onset urinary urge incontinence would be the same given the potential for less obstruction with this newer technique. In addition, in view of the large number of patients available for review, it would be very beneficial for the data base to be re-mined to note if the remaining 189 patients who were excluded for history of reported urinary urge incontinence preoperatively or evidence of detrusor overactivity on preoperative evaluation had resolution of their complaint(s) on a historical basis. This is a topic that has been examined for greater than two decades. Readers should revisit the article written by Dr. E. McGuire, almost exactly 20 years ago in the same journal on this very topic (1).

**Reference**

1. McGuire E: Bladder instability and stress incontinence. Neurourol Urodyn. 1988; 7: 563-7.

***Steven P. Petrou, M.D***
*Associate Professor of Urology*
*Chief of Surgery, St. Luke's Hospital*
*Associate Dean, Mayo School of Graduate Medical Education*
*Jacksonville, Florida, USA*
*E-mail: petrou.steven@mayo.edu*

## Urodynamic characteristics of mixed urinary incontinence and idiopathic urge urinary incontinence

Chou EC, Blaivas JG, Chou LW, Flisser AJ, Panagopoulos G
*Department of Urology, School of Medicine, China Medical University, Taichung, Taiwan*
Neurourol Urodyn. 2008; 27: 376-8

Purpose: To evaluate and compare the clinical and urodynamic findings in patients with either mixed urinary incontinence (MUI) or simple urge urinary incontinence (UUI).
Materials and Methods: A series of 100 consecutive female patients with MUI and UUI were identified from a database. Patients with neurogenic bladder, fistula, urethral diverticulum, prior urologic surgery or known urinary tract obstruction were excluded. All patients were classified according to the urodynamic classification of overactive bladder of Flisser et al. and all patients underwent history, physical examination, validated incontinence questionnaire, 24-hour voiding diary, 24-hour pad test, video urodynamic study (VUDS), and cystoscopy.
Results: A significantly higher proportion of patients with UUI exhibited detrusor overactivity at VUDS, (67% of the patients with UUI vs. 24% of the MUI, $P < 0.05$). Patients with UUI had fewer episodes of incontinence (6.7 vs. 4.2, $P < 0.05$) with slightly less objective urine loss (24-hour pad test 94 gm vs. 128 g of loss, $P < 0.05$) and voided at higher pressures (p(det) at Q(max) 21.4 vs. 15.6 cm H2O, $P < 0.05$). Patients in both groups had functional and urodynamic bladder capacities that were not statistically different.
Conclusions: Women with UUI were more likely to exhibit detrusor overactivity but experienced fewer episodes of incontinence and less urinary loss when compared with women who had MUI. The "urge incontinence" component of MUI appears to be different than that of UUI, and suggests that urge incontinence may

be overdiagnosed in patients with SUI who misinterpret their fear of leaking (because of SUI) for urge incontinence. Neurourol. Urodynam. 27:376-378, 2008. (c) 2008 Wiley-Liss, Inc.

**Editorial Comment**

A straightforward report from leaders in the field comparing the urodynamic characteristics and variables of patients suffering from stress urinary incontinence combined with urinary urge incontinence versus those plagued with urinary urge incontinence alone. The authors started with 100 patients in the study population then parsed the group down to a total of 72 patients: 45 patients with mixed urinary incontinence versus 27 patients with urinary urge incontinence alone (patients were excluded from the original 100 if they had a neurogenic bladder, urinary fistula, urethral diverticulum, prior urologic surgery, or known infravesical outlet obstruction). The patient's overactive bladder was classified by the criteria of Flisser et al. (1). Significant differences were noted upon analysis with regards to the presence of absence of detrusor overactivity, episodes of urinary incontinence for 24 hour period, voiding pressure, functional bladder capacity, as well as severity of urinary incontinence on a 24 hour pad test.

A well written paper with an excellent discussion on urinary urge incontinence in patients with and without stress urinary incontinence. The presentation does raise an excellent point with regards to the presence of urinary urge incontinence in patients classified with mixed urinary incontinence: are these patients really suffering from urge episode or do they just void often to minimize bladder volume and potential leakage episodes? This paper is an appropriate companion to the other reviewed article in this month's journal to engender thought on urinary urge incontinence and its role in anti-incontinence surgery success rates.

**Reference**

1. Flisser AJ, Walmsley K, Blaivas JG: Urodynamic classification of patients with symptoms of overactive bladder. J Urol. 2003; 169: 529-33; discussion 533-4.

***Steven P. Petrou, M.D***
*Associate Professor of Urology*
*Chief of Surgery, St. Luke's Hospital*
*Associate Dean, Mayo School of Graduate Medical Education*
*Jacksonville, Florida, USA*
*E-mail: petrou.steven@mayo.edu*

## PEDIATRIC UROLOGY

### A long-term prospective analysis of pediatric unilateral inguinal hernias: should laparoscopy or anything else influence the management of the contralateral side?

Maddox MM, Smith DP
*East Tennessee Children's Hospital, Knoxville, TN, USA*
J Pediatr Urol. 2008; 4: 141-5

Purpose: To prospectively determine if children who present with a unilateral inguinal hernia can be identified as at risk for developing a metachronous inguinal hernia (MIH) based on risk factors and laparoscopic findings of the contralateral internal ring. Materials and Methods: Between April 2000 and October 2004, 299 patients with a unilateral inguinal hernia were followed prospectively. Laparoscopy was attempted in each child. Bilateral repair was only performed in those with contralateral swelling or crepitus during laparoscopic evalua-

tion. All other children were followed regardless of laparoscopic findings. Risk factors to include premature delivery, family history and increased abdominal pressure were recorded. Clinical follow up and annual phone interviews were performed to determine the development of a MIH.
Results: Thirteen patients underwent initial bilateral inguinal hernia repair. Of the remaining 286 patients (272 boys, 14 girls; ages 54 +/- 50.8 months), laparoscopy revealed 127 closed, 48 cleft and 67 open (contralateral patent processus vaginalis) contralateral internal rings, and in 44 laparoscopy was not possible due to a small hernia. Of 222 patients followed for 53.2 months (30.1-82.5 months), 15 (6.8%) developed a MIH. When comparing age, gender, laterality, laparoscopic findings, family history, premature birth and intra-abdominal pressure, only family history exhibited a significant risk for MIH (33% vs. 7.7%). However, 16/21 children with a family history never developed a MIH, and 47/53 children with a contralateral patent processus vaginalis have yet to develop one.
Conclusions: Risk factors and laparoscopic findings failed to predict the few children who would develop a MIH. The contralateral side should not be routinely explored by any methodology.

**Editorial Comment**

This manuscript studied the questions of whether laparoscopy or any other diagnostic treatment modality should be used to evaluate the contralateral inguinal canal for hernia development. These authors studied 299 patients prospectively over about 4 years and inguinal herniorrhaphies on the contralateral side were only performed if the child demonstrated an inguinal swelling or during laparoscopy palpable crepitans. The laparoscopic exam of the contralateral internal ring was divided into three categories: closed, cleft or open.

Thirteen of their initial patients underwent surgery at the same time on the contralateral groin because of inguinal swelling or crepitance at the time of laparoscopy. 23% of the patients had a contralateral patent processus vaginalis. 44% were closed and 17% had a cleft and 15% did not undergo laparoscopic evaluation because of technical issues. After 19 months, 9 patients (3.6%) had developed a contralateral inguinal hernia, and after a minimum of 30 months, 6 more children had developed an inguinal hernia on the opposite side for a 6.8% rate. There were no predictive factors in the history or physical exam that were helpful, except a positive family history.

In this study a contralateral patent processus vaginalis only predicted 11% of patients that went on to develop an inguinal hernia. The manuscript did not show any age factors as predictive indicators and this group of patients did not show a laterality difference. The authors conclude that the contralateral side should not routinely be explored by any method.

For years, what to do with the opposite inguinal canal when a clinical hernia is present has been studied and debated. This manuscript and references cited within it seem to suggest that there is no reason to explore an asymptomatic inguinal canal, nor is there a reason to look at it laparoscopically. With only a 7% metachronous hernia rate, many unnecessary procedures can be avoided.

***Dr. Brent W. Snow***
*Division of Urology*
*University of Utah Health Sci Ctr*
*Salt Lake City, Utah, USA*
*E-mail: brent.snow@hsc.utah.edu*

## Ileal enterocystoplasty and B12 deficiency in pediatric patients

Rosenbaum DH, Cain MP, Kaefer M, Meldrum KK, King SJ, Misseri R, Rink RC
*Division of Pediatric Urology, Riley Hospital for Children, Indianapolis, Indiana, USA*
J Urol. 2008; 179: 1544-7; discussion 1547-8

Purpose: Vitamin B12 deficiency is a feared complication of enterocystoplasty but it has never been demonstrated in pediatric patients who have undergone ileal enterocystoplasty. We reviewed our series of more than 500 bladder augmentations in an attempt to define the timing and risk of vitamin B12 deficiency in pediatric patients after bladder augmentation.
Materials and Methods: From October 2004 to present we obtained serum B12 values in patients who had undergone bladder augmentation at our institution. We looked at patients who had undergone ileal enterocystoplasty and who were 18 years or younger at the time of augmentation. Any B12 value that was obtained while on any form of B12 supplementation was excluded. These criteria resulted in 79 patients with 105 B12 values. B12 values of 200 pg/mL or less were considered "low", and values between 201 and 300 pg/mL were considered "low-normal".
Results: There was a statistically significant correlation between follow-up time and serum B12 ($p = 0.0001$). The probability of low B12 increased as follow-up time increased ($p = 0.007$), as did the probability of low-normal B12 ($p = 0.005$). Starting at 7 years postoperatively 6 of 29 patients (21%) had low B12 values, while 12 of 29 (41%) had low-normal values.
Conclusions: Pediatric patients who have undergone ileal enterocystoplasty are at risk for development of vitamin B12 deficiency. These patients are at the highest risk beginning at 7 years postoperatively, and the risk increases with time. We recommend an annual serum B12 value in children beginning at 5 years following bladder augmentation.

**Editorial Comment**

This research project involved the measurement of B12 levels starting in October 2004 on all bladder augmentation patients that had terminal ileum utilized for the bladder augmentation. Eighty-six patients with B12 levels were available for evaluation and 10 of those patients were being treated for B12 deficiency and were excluded. Seventy-nine percent were studied with B12 levels. Seven of 79 patients (9%) had low B12 levels and 29% had low normal levels. The patients with the longest follow up had the lower B12 levels in general. Sixty-two percent of patients who had been followed for longer than 7 years (29 patients), had lower or normal B12 values. The authors suggest that B12 levels be obtained in patients who have had an ileocystoplasty beginning at 5 years postoperatively.

It is not surprising that if terminal ileum has been "resected" and used as a bladder augmentation that B12 metabolism may be affected. There were only 7 patients who were truly below the lowest limits of normal B12 values in their institution and the authors include a number of patients that have values in the normal range and consider them low normal. There was no megaloblastic anemia in their study and no neurologic deficits, although there study raises the concern that long-term follow up will be necessary and treatment before the megaloblastic anemia or neurologic symptoms occur, would obviously be in the patient's best interest.

***Dr. Brent W. Snow***
*Division of Urology*
*University of Utah Health Sci Ctr*
*Salt Lake City, Utah, USA*
*E-mail: brent.snow@hsc.utah.edu*