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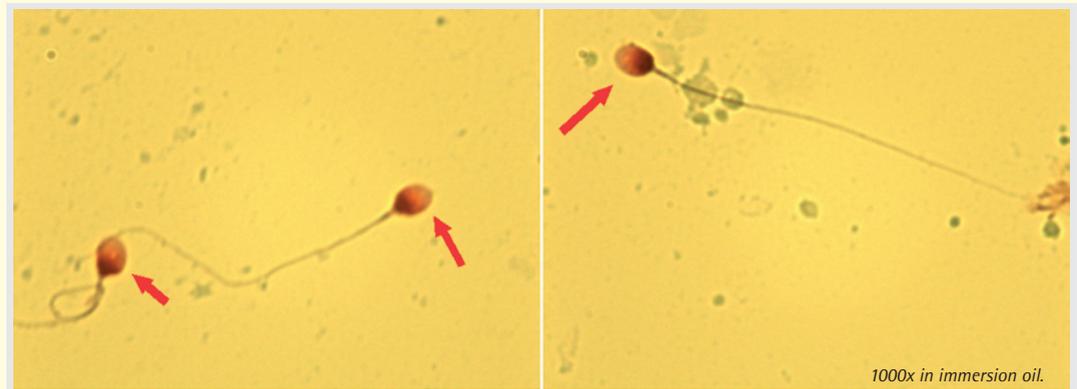


Figure 1 - Spermatozoa found after microcentrifugation technique (2000g for 15 minutes), stained by nuclear fast red and picroindigocarmine (described as NF-PICS or Christmas Tree stain) in an azoospermic man. We observed clear slides obtained with sperm integrity preserved, without cellular debris. (page 134)



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The best treatment for small renal masses has been subject of debates. Despite of the small size, endophytic lesions may represent a challenge, especially in posterior side of the kidneys. In the section Difference of Opinion (pages 3-10), experts of Interventional radiology of Albert Einstein Hospital in São Paulo, claims for cryoablation, while the colleagues from Sonora University, Mexico and Southern California University, are favorable to robotic partial nephrectomy.

At the Review Article, an interesting multidisciplinary study, performed by Tiseo et al., from the Urology and Rheumatology groups from the University of São Paulo, is presented. The authors reviewed extensively the literature since 1970, regarding the influence of the more prevalent rheumatologic diseases and/or treatments on male fertility. These are useful information for the urologist's daily practice, which might include these investigations during the initial anamnesis of infertile men. Among urologists, it is almost consensual to avoid brachytherapy in patients previously submitted to transurethral resection of the prostate (TURP); two Spanish groups from Cantabria reported good oncological and functional outcomes (only 1.7% of urinary incontinence) in 57 patients submitted to dose brachytherapy implantation after TURP, in a medium follow up of 104 months (page 47). Middle East Groups have identified prognostic factors for urinary sepsis after transrectal prostatic biopsy in Lebanon (page 60), and a differential expression of leucocytes and of neutrophil to lymphocyte ratio in Turkish patients with localized testis cancer (page 53).

Moving to minimally invasive surgery, an electronic survey revealed that almost half of Portuguese surgeons are favorable to transvaginal extraction of kidney, after laparoscopic nephrectomies; interestingly, female surgeon gynecologists preferred the vaginal approach (page 78).

A Brazilian group (Marchini et al., page 90), reported the feasibility, the pitfalls and initial results of the laparoscopic single port bilateral nephrectomy in porcine models, performed by post-graduated students.

On page 154, Parente et al. proposed the use of a high pressure balloon to investigate the etiology of ureteropelvic junction obstruction (UPJO) in children: when at fluoroscopy a "waist" in the balloon is verified, an intrinsic UPJO is considered, and is managed by a dismembered pyeloplasty; on the other hand, when there is "no waist" at fluoroscopy, only a vascular hitch is performed regarding the lower pole crossing vessels.

In relation to ischemic priapism (IP), according to Ufuk et al., men with IP present higher mean platelet volumes than healthy controls; probably these elevated platelets volumes contribute in the venno-occlusive mechanisms. In an induced IP rat model, the peritoneal infusion of dipyrindamole was tested as protective drug against the endothelial reperfusion injury processes (page 118).

A new method of semen microcentrifugation, developed by Hallak's group is proposed as an easy, low cost, and reproducible option for the investigation of non-obstructive azospermia. Sperm positivity was identified in 21 of 148 samples (page 132).

Stênio de Cássio Zequi, MD, PhD

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In the current year, the Brazilian Society of Urology celebrates its 90th year of continuous activity and the official scientific journal of the society “International Brazilian Journal of Urology” will present several important modifications throughout this festive year.

The electronic Int Braz J Urol page (www.brazjurol.com.br) was updated and became more interactive and friendly, facilitating the access to all information. From now on, it is possible to directly access all articles published from the first publication of the Brazilian Journal of Urology (the former name of our magazine), providing instant consultation. Visit the new webpage in www.intbrazjurol.com.br

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Sidney Glina, MD, PhD

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Which is the best treatment on a 2 cm complete endophytic tumor on the posterior side of the left kidney?

Opinion: Cryoablation

Rodrigo Gobbo Garcia

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Keywords: Therapeutics: Neoplasms; Cryosurgery; Nephrectomy

The Clinical Problem

With the increased use of advanced imaging techniques, incidental renal mass have become a very frequent finding (1).

Approximately 13 to 27% of abdominal imaging studies incidentally identify a renal lesion, a fact that makes suspected renal cell carcinoma be diagnosed at an early stage (2). Most excised small renal cancers are classified as low grade at the time of diagnosis and synchronous metastases are very infrequent finding associated to such small lesions (3).

Although partial nephrectomy remains the reference standard for treatment of small renal masses, the guidelines of the American Urological Association support consideration of thermal ablative techniques for the treatment of patients with T1a disease (< 4 cm) (4).

Furthermore, the development of ablative techniques has widened the range of treatment options available to these patients and international consensus panels support other indications for ablative therapy for renal tumors (patients with a increased risk of multiple RCC tumors – e.g. von Hippel-Lindau syndrome, clinical conditions not suitable for surgery and solitary or transplanted kidney) (5).

TECHNICAL CONSIDERATIONS

Thermal ablation is performed by inserting needle applicators within the renal tumors to generate lethal temperatures to neoplastic tissues encompassed by ablation zone. Cryoablation and radiofrequency ablation are the most common methods (6).

With refinements in probe size and design, a percutaneous image-guided approach may be preferable to a laparoscopic approach for thermal ablation, since procedure-associated morbidity would be lower (7) (Figures 1-3).

Historically, percutaneous ablation has been reserved for patients with small, exophytic tumors in the posterolateral kidney. However, the increased use of cryoablation

Figure 1 - MR image, T2 sequence. Arrow: A biopsy-proven 2.2 cm clear cell carcinoma on the left kidney.

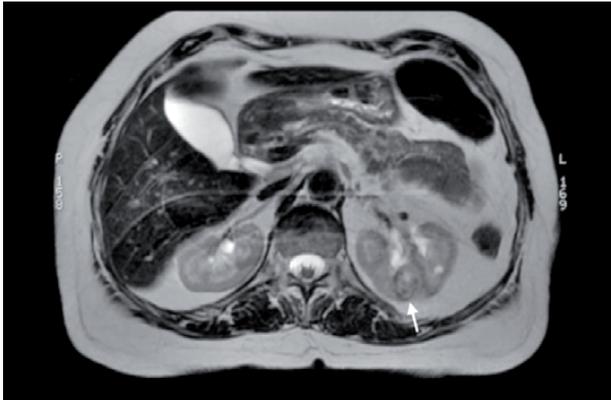


Figure 2 - Interventional CT-scan. Cryoablation with two cryoprobes within target lesion. Dotted circle: edge of ice-ball.

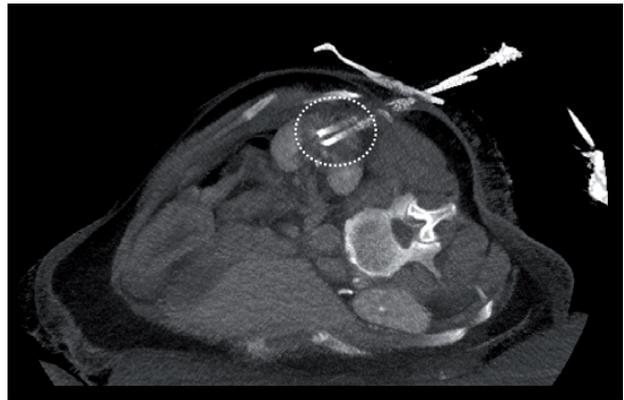
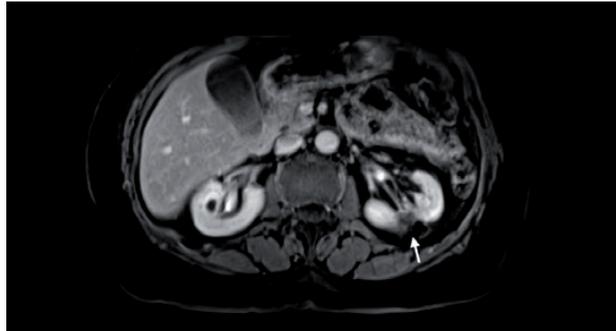


Figure 3 - MR image, T1 post-contrast for surveillance two years post ablation. Arrow: Retracted and non-enhancing ablation zone, depicting successful treatment.



and displacement techniques (8) (e.g. hydrodissection and pneumodissection - infusion of fluid or gas via a small-caliber catheter placed under image guidance) have significantly expanded the number of renal tumors that can be successfully treated percutaneously, including larger tumors, central tumors, and tumors in less accessible locations within the kidney (9).

Cryoablation, rather than radiofrequency ablation, has shown significant promise in treating these larger more complex renal tumors (9).

As the name implies, cryoablation relies on low temperatures to induce cell death. The process of cryoablation obeys the Joule- Thomson effect whereby expanding of some gases (e.g. argon) within a needle-like chamber (the cryoprobe) produces heat sink near the antenna tip that cools the probe to temperatures of -160°C or colder (10). The cell-lethal isotherm is between -20°C and -40°C . Slow freezing produces intra- cellular ice crystals, and fast freezing induces extracellular ice crystals. Both processes induce cell death by different cellular mechanisms. In addition, freeze-thaw cycles can induce cellular dehydration, membrane rupture, vascular thrombosis and tumor cell apoptosis (11).

Proximity of the tumor to the collecting system may represent a relative contraindication to cryoablation due to the risk of urothelial injury and ureteral strictures have been reported, particularly for tumors in the medial lower pole (10).

Placement of a ureteral stent with retrograde warm saline irrigation of the collecting system and a very reliable identification of ureter during the ice ball monitoring may mitigate this risk (12).

Freezing into calyceal structures or intrarenal pelvic collecting system did not cause any apparent strictures or vascular injuries in longer-term follow-up, similar to prior animal data (13).

Relative warming of the ablation zone by large central vessels may limit the ability to achieve cytotoxic temperatures at the central tumor margin, and more aggressive treatment with larger cryoprobes and a greater iceball margin is indicated (14).

Careful preprocedure cross-sectional imaging assessment of a candidate patient's small renal mass is required to minimize complications and maximize therapeutic efficacy. A practical algorithm for procedure planning, ABLATE, has been proposed that takes into account the following tumor characteristics:

A, axial tumor diameter; B, bowel proximity; L, location within the kidney; A, adjacency to the ureter; T, touching renal sinus fat; and E, endophytic or exophytic position (15).

Of all tumor characteristics, the size of the renal mass is the most important factor in achieving local tumor control with ablation (16). This is primarily related to the small size of the ablative zones tissue generated by most ablation devices and some limitations in monitoring its size during the treatment. On that point of view, cryoablation is superior to RFA because the iceball is easily depicted by CT-scan, making more predictable volumes of treatment. The size and shape of the ice ball can be manipulated with multiple cryoprobes synergistically working (10).

Endophytic tumor position (tumor completely surrounded by renal parenchyma) can make ablation procedures more difficult and has been associated with increased local treatment failures. Gupta et al. (17) reported technical failure or recurrence during a mean 18-month follow-up for seven of 46 (15.2%) endophytic tumors versus five of 117 (4.3%) nonendophytic tumors treated by ablation ($p=0.016$). Small endophytic renal tumors that are not confidently visualized with intraprocedural unenhanced CT are particularly challenging to treat.

Ultrasound guidance, ultrasound-CT, or ultrasound-MRI fusion guidance or administration of IV contrast agent (iodinate for CT and microbubbles for ultrasound) may help with localizing endophytic tumors.

Concerning to the location of the renal tumors, an important potential complication to consider before ablation is nerve injury, which can lead to postablative neuralgia and paresthesias. In the context of renal ablation planning, one should consider the position of the intercostal nerves, genitofemoral nerve, and lateral femoral cutaneous nerves. The ablation of posterior masses located close to major psoas muscle draws attention to the danger of damaging the genitofemoral nerve, resulting in chronic pain, tenderness, and diminished sensitivity within the skin area of the ipsilateral groin (18). Displacement techniques (e.g. hydrodissection and torquing handle of the cryoprobe as a lever) can move away the tumor of the psoas, lowering the risks of neural injuries (19).

RESULTS

Local control rates approaching 100% for tumors smaller than 3 cm in select series, with very low tumor recurrence rates (20).

Thompson et al recently compared the oncologic outcomes for cT1a tumors between RFA ($n=180$), PCA ($n=187$), and PN ($n=1057$). On univariable analysis, there was no difference in estimated 3 years disease-free survival between the three treatment modalities (98% for all three arms). Distant metastasis-free survival at 3 years, however, was better for PN (99%) and CA (100%) compared with RFA (93%) (21).

Whitson et al. (22) compared 7704 nephron-sparing surgeries and 1114 renal ablations using data from the Surveillance, Epidemiology, and End Results cancer registry and showed very similar 5 years disease-specific survival rates (98.3% vs 96.6%).

Given that long-term studies have shown durable outcomes for nephron-sparing surgery, the American Urological Association guidelines assert that the most important disadvantage of any ablative technique is a higher local recurrence rate relative to that of partial nephrectomy (4). However, this difference may be overstated in certain patient cohorts and is not entirely evidence based but rather due to a lack of long-term disease-free survival data on ablative management of small RCCs. Results of short- to medium-term studies suggest that appropriate patient selection can yield oncologic outcomes comparable to those of partial nephrectomy with the added benefit of improved preservation of renal function (5). Therefore, the decision to pursue ablative treatments rather than surgery requires careful consideration.

Ideally, the advantages and risks of each approach must be compared and contrasted in consultation with the patient and the local tumor board.

Furthermore, the possibility of repeated ablations in residual lesions or *de novo* tumors, becomes even more attractive the percutaneous approach in comparison to surgery.

In addition, nephron-sparing surgery, including open partial nephrectomy and laparoscopic partial nephrectomy, can be more technically challenging and result in serious complications, such as excessive blood loss and urinary fistula formation (23).

The other obvious advantages of ablation relative to extirpative surgery include reduced morbidity; better preservation of renal parenchymal volume, which correlates with overall renal function; faster recovery time; shorter or no inpatient hospitalization; and possibly being the only treatment option available to patients with serious comorbid conditions who are not considered surgical candidates.

The cost to the health care system may also be reduced with increased use of noninvasive ablative techniques (24).

CONCLUSION

Although nephrectomy (partial or radical) remains the reference standard for renal tumor treatment, physician acceptance and patient interest in percutaneous ablation are growing as intermediate and long-term outcome data become available.

Percutaneous cryoablation of renal tumors under imaging guidance is a curative alternative technique with a low complication rate. The best indications are tumor smaller than 4 cm, even though larger tumors can be effectively treated in selected cases. Percutaneous cryoablation is effective for preserving renal function, even in patients with single kidney. Moreover, in cases of incomplete treatment, cryoablation can be repeated and does not preclude salvage surgery.

So why not cryoablation as a first line therapy for a 2 cm complete endophytic tumor on the posterior side of the left kidney?

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Which is the best treatment on a 2 cm complete endophytic tumor on the posterior side of the left kidney?

Opinion: Robotic partial nephrectomy

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The routine use of abdominal imaging has led to increased detection of small renal masses incidentally, even before they cause symptoms (1). Partial nephrectomy is now the standard therapy for the treatment of small renal masses in stage T1a and even for certain patients with T1b tumors, offering equivalent cancer control to radical nephrectomy with better preservation of renal function and improving survival (2). The recurrence-free survival at 5 years for small renal masses less than 4 cm and 4-7 cm is about 96% and 83% respectively (3). It has been found that radical nephrectomy can lead to an increased risk of chronic kidney disease (4), and is associated with a higher risk of adverse cardiac events, hospitalization and death (5).

The location of the tumor and the endophytic component can be the determining factors on the feasibility and the degree of difficulty in performing a partial nephrectomy, even more important to consider than just the size of the tumor.

A completely intraparenchymal tumor is defined as an injury that is completely surrounded by normal renal parenchyma on all sides (6) or one located at a distance less than 5 mm of the collecting system or hilar vessels without exophytic component (7).

Partial nephrectomy is the treatment of choice for a tumor 2 cm diameter (3, 8) completely intrarenal on the posterior side of the left kidney, considering the context of clinical presentation such as age and comorbidities, even when the contralateral kidney is healthy.

We must consider that partial nephrectomy itself requires many surgical skills, but for a completely intrarenal tumor, partial nephrectomy is very challenging due to difficulties in the exact location of the tumor, complete resection with negative margins but not exceeding the resection of healthy tissue as well as obtaining the perfect hemostasis and renal reconstruction sutured safe, besides the risk of injury to underlying structures of the renal pelvis (9). Intraoperative ultrasonography is absolutely essential especially when tumor is completely intrarenal as it enables optimal placement of the incision (10). Robotic optical system allows three-dimensional vision of deep renal tissue

for better identification of pathological tissue and articulated robotic rotary instruments allows proper closure and suture cavity surrounding healthy tissue. The robotic transabdominal approach offers more working space and greater anatomical references and even when the lesion is located on the posterior side, full kidney can be mobilized to achieve optimal exposure (11) with equivalent results than the open technique (11-13). The robotic system technologies have evolved so fast that the new systems already allow overlapping surgery ultrasonographic images in real time (TilePro™, da Vinci® Si HD, Intuitive Surgical Inc., Sunnyvale, CA) for the best performance in this kind of procedures improving expectations of intraoperative and postoperative results.

The complete removal of the tumor may be performed by a circumferential incision of the affected renal segment, achieving partial nephrectomy that includes all the tumor. In cases where the tumor has a central location and nonpolar, it can be considered a linear vertical incision along the rear face of the kidney, such as that in the anatomic nephrotomy, in order of major greater exposure of the tumor and more healthy tissue preservation, which itself would be a tumorectomy rather than a partial nephrectomy.

Anatomic nephrotomy was described for the first time (14) for staghorn calculi. They used as a reference an imaginary avascular line located between renal portions irrigated by anterior and posterior segmentary branches of the main renal artery, known as Brodel line and thus has been proved that is less likely to injure the intrarenal blood vessels of larger caliber compared to other renal surgery incisions, moreover no other approach offers three-dimensional exposure of deep intrarenal tissue.

This surgical approach has been historically used to treat great complex kidney stones and not for renal tumors. The proven benefits of this approach led us to consider addressing the anatomic nephrotomy for optimal exposure and carry out the removal of a small tumor completely intrarenal (15, 16) as here, seeking maximum preservation of normal renal tissue.

CONCLUSIONS

Robotic partial nephrectomy to a completely intrarenal tumor is feasible, safe and effective in experienced hands. However, it is an advanced technique that should only be performed by surgeons with extensive experience in robotic renal surgery. Intraoperative ultrasound is mandatory to guide tumor resection successfully. Regarding tumorectomy by anatomic incision, may be an option for special cases and in expert hands, prospective studies are needed with larger numbers of patients to evaluate the oncological and functional results and its potential complications of this technique.

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Male fertility potential alteration in rheumatic diseases: a systematic review

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ABSTRACT

Background: Improved targeted therapies for rheumatic diseases were developed recently resulting in a better prognosis for affected patients. Nowadays, patients are living longer and with improved quality of life, including fertility potential. These patients are affected by impaired reproductive function and the causes are often multifactorial related to particularities of each disease. This review highlights how rheumatic diseases and their management affect testicular function and male fertility.

Materials and Methods: A systematic review of literature of all published data after 1970 was conducted. Data was collected about fertility abnormalities in male patients with systemic lupus erythematosus, rheumatoid arthritis, dermatomyositis, ankylosing spondylitis, Behçet disease and gout. Two independent researchers carried out the search in online databases.

Results: A total of 19 articles were included addressing the following diseases: 7 systemic lupus erythematosus, 6 Behçet disease, 4 ankylosing spondylitis, 2 rheumatoid arthritis, 2 dermatomyositis and one gout. Systemic lupus erythematosus clearly affects gonadal function impairing spermatogenesis mainly due to antisperm antibodies and cyclophosphamide therapy. Behçet disease, gout and ankylosing spondylitis patients, including those under anti-TNF therapy in the latter disease, do not seem to have reduced fertility whereas in dermatomyositis, the fertility potential is hampered by disease activity and by alkylating agents. Data regarding rheumatoid arthritis is scarce, gonadal dysfunction observed as consequence of disease activity and antisperm antibodies. **Conclusions:** Reduced fertility potential is not uncommon. Its frequency and severity vary among the different rheumatic diseases. Permanent infertility is rare and often associated with alkylating agent therapy.

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INTRODUCTION

There are 1.3 million adults affected by rheumatoid arthritis (RA) and up to 322.000 by systemic lupus erythematosus (SLE) in United States (1). Improved targeted therapies for rheumatic diseases have been developed recently resulting in better prognosis. In this context health-related quality of life became a major concern, including reproductive issues (2).

Decreased fertility potential is not unusual among patients of both genders with rheumatic diseases, particularly in women (3, 4) with many articles addressing in RA, SLE, ankylosing spondylitis (AS), dermatomyositis (DM), Behçet disease (BD) and gout (5-8). Drug treatment is probably the main factor for gonadal dysfunction (9). Some drugs can cause reversible infertility, such as nonsteroidal antiinflammatory drugs in women

and sulfasalazine/methotrexate in men whereas irreversible infertility is occasionally observed after treatment with alkylating agents (cyclophosphamide-CYC and chlorambucil) in both genders (10). When fertility is an issue, alkylating agents should be used at lowest possible dose and alternative therapies (such as azathioprine or mycophenolate mofetil) must be considered.

The reproduction potential of these male patients is impaired by the disease directly in the testicular tissue or by immunosuppressive therapy (11). The evaluation of male subjects should rely on careful medical history, complete physical examination, semen analysis and sexual hormone profile.

The objective of this systematic review of the literature on rheumatic disease male fertility potential is to provide a better understanding to urologists, andrologists, infertility specialists and rheumatologists of the underlying contributing factors involved, as well as discuss how fertility potential is endangered by diseases management.

SEARCH STRATEGY AND SELECTION CRITERIA

It was conducted a computerized search of English and non-English language articles published after 1970 listed in the electronic databases of SCOPUS, PUBMED/MEDLINE and Cochrane Library. Two independent researchers (MC, BT) conducted the search during May-July 2014. The following terms were used: 'systemic lupus erythematosus', 'ankylosing spondylitis', 'dermatomyositis', 'rheumatoid arthritis', 'Behçet disease', 'gout', 'male infertility', 'pregnancy rate', 'sperm', 'semen', 'spermatozoa', 'sperm quality' and 'rheumatic disease'. The search was performed in English language but articles yielded in other languages were not excluded. The authors graded the abstract of each study identified by the search to determine eligibility. If these criteria remained unclear from the abstract, the full article was retrieved for clarification.

Data extraction was carried out by the investigators using a standardized data collection form with subsequent discussion with all authors. Peer-reviewed observational controlled and non-controlled studies (case-control and co-

hort studies) were selected. All studies were referral centre-, hospital- or population-based studies. The data collected in the selected articles were all related to fertility abnormalities in male patients with SLE, RA, DM, AS, BD and gout. We excluded articles that were case reports and those that did not evaluated male patients.

RESULTS

The article flow through the selection phase is summarized in Figure-1. An initial search of online databases yielded 136 publications from PUBMED/MEDLINE, 112 reviews from Cochrane Library, 136 from Web of Science, and 162 from Scopus. After excluding duplicated publications and applying exclusion criteria, 19 relevant articles were included with the following diseases: 7 SLE, 2 DM, 2 RA, 4 AS, 6 BD and one with gout. There was one article evaluating simultaneously two diseases and another addressing three (Figure-1).

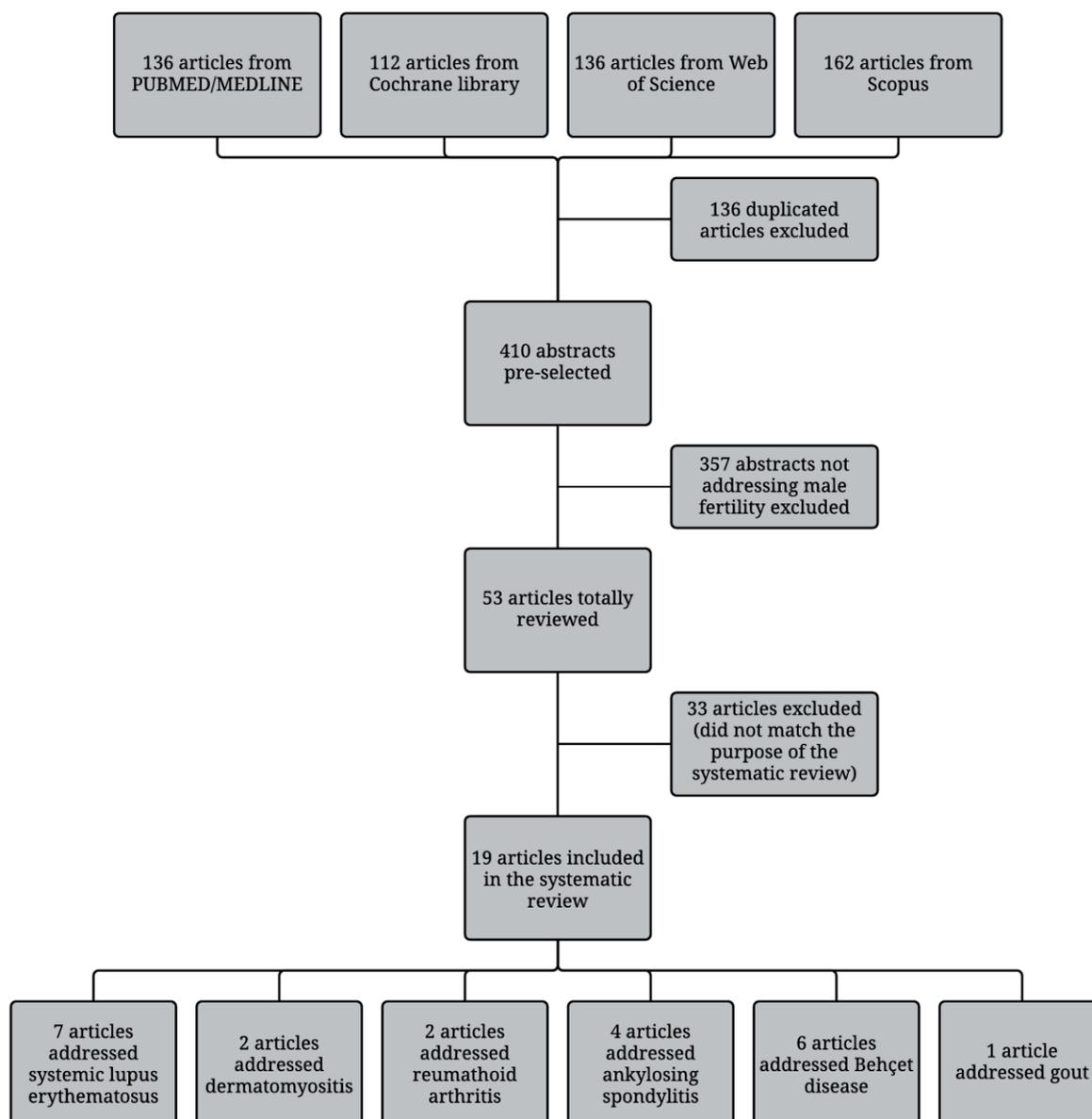
Systemic lupus erythematosus

Publications selected focused on four aspects of male fertility in SLE: gonadal dysfunction, testicular alterations induced by immunosuppressive treatment, presence of anti-sperm antibody and genetic abnormalities (Table-1).

A global gonadal function evaluation was performed by our Group (12) assessing sex hormone profile, semen analysis and antisperm analysis (ASA). Thirty-five patients compared to paired controls had lower testicular volumes, total sperm count and total motile sperm count associated with CYC use.

We investigated Sertoli cell function analyzing inhibin B levels and semen abnormalities in SLE patients. Lower inhibin B level was correlated with diminished sperm count, concentration and total motility count and with elevated FSH and LH levels (13). In addition, it was observed that 20% of SLE patients had erectile dysfunction, 36% of testicles were below the normal volume range and 48% had semen analysis abnormalities associated with CYC therapy (14). The same gonadotoxic effect of CYC was also reported in four patients with juvenile SLE (15).

Serum IgG ASA targeting the sperm head and/or midpiece was reported in 15% and antisperm

Figure 1 - Flow of articles through different phases of the systematic review.

deoxyribonucleic acid antibodies were found in 42% of SLE patients, indicating that autoimmunity is another contributing factor in these patient's (16). This finding was confirmed in 8 patients evaluated by Shiraishi et al. (17).

Recently, Dillon et al. (18) evaluated the karyotype of 316 men with SLE and 1201 healthy controls. Aneuploidies were evidenced in 2.5%

male SLE patients and none in controls. There was three 47, XXY, three patients with mosaic 46, XY/47, XXY, one had 46, XX/47, XXY mosaicism and another one had 46, XX karyotype.

Dermatomyositis

The two publications addressing DM patient's fertility are illustrated in Table-2. Moraes

Table 1 - Systematic lupus erythematosus and male fertility according to gonadal dysfunction, immunosuppressive agents, anti-sperm antibody and sex chromosomes aneuploid.

Author	Year	Study Population	Results	Conclusion
Gonadal dysfunction				
Soares et al. (12)	2007	35 patients with SLE and 35 normal controls	SLE patients had low sperm count, low motile sperm and low normal sperm forms / Sperm abnormalities related to elevated FSH levels	Gonad function is severely affected in male SLE patients due to testicular damage
Suehiro et al. (13)	2008	34 patients with SLE	23% had decreased inhibin B and elevated FSH, 15% had decreased testosterone, 70% sperm analysis alterations, 20% reduced testicular volume	Sertoli cell dysfunction in male SLE affecting inhibin B secretion. It was related to impaired sperm production
Silva et al. (14)	2009	25 patients with SLE and 25 normal controls	20% SLE patients had erectile dysfunction, 36% had elevated FSH and 48% had sperm abnormalities	SLE affects whole male reproductive health, particularly under CYC / No influence of ASA
Testicular damage immunosuppressive agents				
Silva et al. (15)	2002	4 patients with juvenile SLE	1 patient with azoospermia and elevated FSH / 1 patient with severe oligospermia. Both treated with CYC	Alkylating agents may induce testicular damage
Presence of anti-sperm antibody				
D'Cruz et al. (16)	1994	33 patients with SLE, 33 patients with ASA and 20 normal controls	42% patients with SLE had ASA	ASA has high frequency in SLE patients
Shiraishi et al. (17)	2008	32 patients with RA, 14 with BD, 8 with SLE and 80 healthy controls	13% SLE patients had ASA	SLE may induce ASA in patients and affect fertility
Presence of sex chromosomes aneuploid				
Dillon et al. (18)	2012	316 patients with SLE	8 patients with sex chromosomes alterations	Sex chromosomes aneuploid is more common in SLE men and may impact their fertility

Note: SLE = systemic lupus erythematosus; LH = luteinizing hormone; FSH = follicle-stimulating hormone; CYC = cyclophosphamide; RA = rheumatoid arthritis; ASA = antisperm antibody, BD = Behçet disease.

et al. (19) evaluated five patients with juvenile DM and compared with 10 age-matched healthy controls regarding testicular volume, sperm analysis, ASA and sex hormone profile. One patient had used CYC with a cumulative dose of 6.6g and experienced transient azoospermia with normaliza-

tion after 5 years of medication withdrawal. All DM patients had teratospermia, one had ASA and none had abnormal hormone profile.

A later study investigated 10 adult patients and 10 age-matched healthy controls. DM subjects had lower sperm concentration, lower total motile

Table 2 – Dermatomyositis and male fertility.

Author	Year	Study Population	Results	Conclusion
Moraes et al. (19)	2008	5 post-pubertal males with DM and 5 healthy controls	100% teratozoospermia / Azoospermia during CYC treatment	No significant difference between patients and normal controls regarding hormonal levels or sperm analysis
Moraes et al. (20)	2010	10 post-pubertal males with DM and 10 healthy controls	Low concentration, low sperm abnormalities, low motile count, reduced testicular volumes in DM patients	DM may affect testicular function and sex hormones levels / Disease activity and CYC may induce gonadal dysfunction

Note: DM = dermatomyositis; CYC = cyclophosphamide.

sperm count and lower normal sperm morphology percentage. Disease activity seemed to be a relevant factor in four patients and CYC in one of them (20).

Rheumatoid arthritis

Two publications assessed fertility on RA patients (Table-3). Gordon et al. (21) evaluated 31 patients with RA, 33 with AS and 95 healthy controls. Patients with RA had lower serum testosterone levels and higher FSH and LH levels than controls. Ten patients (33%) admitted periods of erectile dysfunction while 15 (50%) also referred decreased libido when suffering from arthritis. Four patients referred difficulty to conceive, among them, two did not seek medical assistance for infertility. Nineteen males had successfully fathered children and the others were still singles.

In 2008, Shiraishi et al. (17) evaluated 32 RA patients and found one with serum ASA. The patient was 74 years old and the disease onset was at the age of 60 years. He had already 2 children before being diagnosed with the disease so the relation between fertility status and the presence of the antibody could not be addressed nor its relation with the disease.

Ankylosing spondylitis

Four publications were selected regarding the AS association with male fertility. The major aspects of each paper are summarized in Table-4. A total of 33 AS patients were evaluated in 1986, reporting four patients with erectile dysfunction and 11 with decreased libido. Only one male with AS had an infertile marriage and did not seek for medical assistance.

Table 3 - Rheumatoid arthritis and male fertility.

Author	Year	Study Population	Results	Conclusion
Gordon et al. (21)	1986	31 patients with RA, 33 with AS patients and 95 normal controls	Low testosterone / Elevated FSH and LH level in patients with RA	Normal pituitary-gonad axis function / Testicular damage by disease activity
Shiraishi et al. (17)	2008	32 men with RA, 14 with BD, 8 with SLE and 80 healthy controls	3% RA patients had ASA	RA may induce ASA in patients and may affect fertility

Note: RA = rheumatoid arthritis; AS = ankylosing spondylitis; BD = Behçet disease; FSH = follicle-stimulating hormone; LH = luteinizing hormone; SLE = systemic lupus erythematosus; ASA = antisperm antibody.

Table 4 – Ankylosing spondylitis and male fertility.

Author	Year	Study Population	Results	Conclusion
Gordon et al. (21)	1986	31 patients with RA, 33 with AS patients and 95 normal controls	Normal testosterone / Normal FSH and LH levels / 13% impotence / 39% decreased libido	AS may affect libido and erectile function / No impact in testicular function
Paschou et al. (7)	2009	4 patients treated with infliximab	All patients had fathered at least one child	AS patients treated with anti-TNF seem not to suffer infertility issues
Nukumizu et al. (23)	2012	20 patients with AS and 24 healthy controls	40% of AS patients had varicocele and was associated to teratospermia	Varicocele is frequent in AS and may affect sperm morphology impairing fertility
Almeida et al. (22)	2013	20 patients with AS and 24 healthy controls	Normal sex hormones levels, including inhibin B / Normal seminal parameters	Sertoli cell function was not affected by AS or by anti-TNF therapy

Note: RA = rheumatoid arthritis; AS = ankylosing spondylitis; TNF = tumor necrosing factor.

ce. Thirteen patients were singles and all other had constituted their families without problems (21). Varicocele was an additional and frequent finding in AS males (40%), of another cohort, and its impact in male fertility remains to be determined, since only mild sperm abnormalities was observed in these patients (22).

Regarding to biological therapy, Paschou et al. (7), in 2009, assessed AS patients who were treated with infliximab and reviewed their medical records. They found that all of them had successfully fathered at least one children even one patient that also received small doses of methotrexate. Reinforcing this finding, our group evaluated 20 AS patients under TNF blockage and compared to 24 normal controls and showed no difference in sex hormone, inhibin B levels and seminal analysis (23).

Behçet disease

There were six papers addressing BD and its relationship with male fertility. They are summarized in Table-5. An early report brought attention to possible side-effects of colchicine used in BD management with oligospermia in 11 of 136 patient's (24). Later, Sarica et al. (25) evaluated

62 men with BD treated only with colchicine and evidenced oligoneospermia in 37% and azoospermia in 3%. However, Fukutani et al. (26) evaluating 27 BD patients did not observe impact seminal parameters or FSH levels in patients treated with colchicine use alone.

Alkylating agents may induce sperm abnormalities in BD patient's, as reported in ten men using chlorambucil that had impairment of semen production: 7 with oligospermia and 3 with azoospermia (27).

A recent study evaluated fertility outcome of a larger retrospective cohort of BD patient's compared to a control Group (28). They observed that 23 out of 130 subjects had infertility and the most common etiology was varicocele. In contrast, none of the 14 men with BD assessed for ASA had antisperm antibodies (17).

Gout

There is only one report addressing gout and fertility in males (Table-5). In a large study with 540 young patients with gout, fertility status was preserved in patients treated with colchicine and none presented fertility issues during 20 years of follow-up (29).

Table 5 - Behçet disease, gout and male fertility.

Author	Year	Study Population	Results	Conclusion
Behçet disease				
Mizushima et al. (24)	1977	157 patients with BD in colchicine use	11 patients had oligospermia	Low side-effects of colchicine use
Fukutani et al. (26)	1981	31 male patients with BD divided in four groups regarding medication use	Only the patients treated with CYC had seminal abnormalities and diminished FSH serum levels	BD did not impair testicular function / Testicular damage related to CYC
Tabbara (27)	1983	10 men with BD treated with chlorambucil	7 patients had oligospermia and the other 3 azoospermia	Chlorambucil should not be used as the first line of therapy in BD
Sarica et al. (25)	1995	62 male patients under colchicine therapy for BD	23 patients (37%) had oligonecrospermia and 2 patients (3%) azoospermia	Urological manifestation of BD and medication adverse reaction should be careful monitored
Shiraishi et al. (17)	2008	32 men with RA, 14 with BD, 8 with SLE and 80 healthy controls	None of BD patients had ASA	BD seems not to be related to ASA
Uzunalan et al. (28)	2013	162 men with BD, 48 with FMF, 79 with AS and 43 healthy controls	23 BD patients had fertility issues, most commonly varicocele / No difference in pregnancies or children conceived	BD does not significantly decrease patient's fertility
Gout				
Yu (29)	1982	518 gout patients treated with colchicine	No fertility issues reported	Neither gout nor colchicine use impacts fertility

Note: BD = Behçet disease; FSH = follicle-stimulating hormone; CYC = cyclophosphamide; AS = ankylosing spondylitis; ASA = antisperm antibody; FMF = familial mediterranean fever

DISCUSSION

SLE is an uncommon disease in men. It affects males in a sex ratio of 1:9 (30), suggesting that sex hormones could modify susceptibility or reduce the expression of SLE (11). Infertility is an important issue for them nowadays due to better prognosis and quality of life. Six publications reported impaired testicular function and decreased semen production in SLE patient's and their possible association with disease and treatment (11-14, 31, 32). The underlying mechanism for disease in-

duced damage to the testis is not completely elucidated although some authors showed that there is immunopathologic lesion of the testis (and ex-current ducts) occurring through T cell-mediated mechanisms triggered by antigens or pathogens that disrupt testicular immune privilege (33, 34). Testicular aggression by alkylating agents has been described since 1972 showing that it impairs the sperm production presenting spermatogenesis abnormalities and Sertoli cell dysfunction (35-37).

Although semen analysis is considered the hallmark of male infertility evaluation, standard

seminal parameters do not detect abnormalities in up to 20% of subfertile males (38). The routine measurements do not reveal seminal defects at molecular levels that might be induced by reactive oxygen species, which are associated with male infertility (39, 40). The 2010 World Health Organization guidelines have reduced the reference limits for seminal parameters and fail to satisfy clinical and statistical standards and pose the risk of misclassifying a subject's true fertility status (41, 42). The introduction of new biomarkers of spermatid function in the clinical practice will allow a more precise evaluation of the real impact of rheumatic disease on male fertility potential. Unfortunately, to date, there is no available information in the literature and these aspects were not covered in the present review.

Autoimmunity also affects fertility by the presence of ASA. Immunologic infertility is characterized by the presence of antibodies against spermatozoa in the serum and/or in the seminal plasma or on the sperm surface (43). The presence of multiple ASA can lead to the immobilization and/or agglutination of spermatozoa, which blocks sperm-egg interaction. They can also prevent implantation or arrest embryo development (44, 45). In SLE patient's ASA have been found in up to 42% of the patient's. However, the real significance of ASA in infertile men is controversial and currently there is no standardized treatment regimens (46). Lastly, aneuploidies are frequent and may also contribute for fertility impairment in SLE patient's; therefore karyotype should be evaluated to complete the fertility analysis of these patient's, especially in those with severely compromised spermatogenesis (47).

The incidence of DM is 1.5 to 0.7 per 100.000 per person-years and there is a trend to affect more women than males in a 1.9 ratio (48, 49). The fertility evaluation of this particular Group has limited publications. The most important contributing factors of infertility in male DM are disease activity and CYC use (6), generally associated with high doses of this alkylating agent (5, 50).

Several large Scandinavian cohorts and a cohort study in the United States demonstrate that women with RA have smaller families and are slower to conceive compared with other wo-

men (51). Even though female infertility has been extensively explored in past years, the evaluation of male fertility was overlooked. The few publications available show gonadal impaired function with elevated LH/FSH, patients with difficult to conceive and also a higher incidence of ASA (17, 21). A more extensive evaluation of this set of patients is necessary to have a clear sense of RA real impact on male fertility.

Most physicians agree that diseases such as RA and AS can cause significant limitations in sexual activity due to diminished desire and impaired mechanical capacity (52, 53). In spite of that, evaluation of sex hormonal levels, seminal analysis and varicocele have shown that AS disease and treatment do not have a major impact in male fertility (7, 21-23).

BD is a multisystemic vasculitis with musculoskeletal, muco-cutaneous, ocular, gastrointestinal and neurological findings. The disease is frequently seen in the Mediterranean basin and the Far East. Young adults in their 20s and 30s are typically affected, during their reproductive years, with no gender predilection. However, males usually have a more severe course of the disease and are prone to present eye and other major organ involvement (28). The available literature about BD relationship with infertility is not robust. The disease itself seems to have no impact on fertility potential, but alkylating agents use is associated with its decrement (26, 27). The aggression to testicular tissue by colchicine reported by some authors in BD (25) was not confirmed in a large gout study (28).

Gout is induced by the deposition of monosodium urate crystals in synovial fluid and other tissues associated with hyperuricemia (54). Patient's with gout are usually older and fertility often is not an issue, as most of them had already constituted their families. Although there is a concern about colchicine impairing reproductive function, decreased fertility has not been found in these patient's (29).

And finally, the modern view of male fertility evaluation gives a new meaning to the term "male infertility management", which goes beyond the simple identification and elimination of the cause. The andrologist's therapeutic strategies have

changed from a recent past of only attempting to achieve a simple increase in the sperm concentration. We are moving forward and now our main target is to improve the “quality” of spermatozoa and/or preserve fertility (55). This approach is thus especially recommended for patients with rheumatic diseases, often not considered potentially fertile.

CONCLUSIONS

Rheumatic diseases comprise a heterogeneous Group of diseases with distinct aspects regarding male infertility. SLE clearly affects gonadal function impairing spermatogenesis due to ASA and CYC therapy. Fertility seems to be not affected in BD and AS patients, including patients under anti-TNF therapy. The fertility potential of DM patients may be affected by the disease activity and by alkylating agents. There are also few data regarding RA, however male gonad may be affected by the disease activity and ASA. Gout patients usually do not have any conception desire at the time of disease manifestation and there are no reports of fertility impairment.

A multidisciplinary Group is essential to assess male reproductive health in rheumatic disease patients with a special focus on improving the fertile potential and sexual dysfunction to minimize the disease and treatment damage.

ABBREVIATIONS

SLE = Systemic lupus erythematosus
 AS = Ankylosing spondylitis
 DM = Dermatomyositis
 RA = Rheumatoid arthritis
 BD = Behçet disease
 T = Testosterone
 FT = Free testosterone
 LH = Luteinizing hormone
 FSH = Follicle-stimulating hormone
 CYC = Cyclophosphamide
 ASA = Antisperm anti-body

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CONFLICT OF INTEREST

None declared.

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Are we following the guidelines on non-muscle invasive bladder cancer?

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ABSTRACT

Objectives: To evaluate the clinical practice of non-muscle invasive bladder cancer (NMIBC) treatment in Brazil in relation to international guidelines: Sociedade Brasileira de Urologia (SBU), European Association of Urology (EAU) and American Urological Association (AUA).

Materials and Methods: Cross-sectional study using questionnaires about urological practice on treatment of NMIBC during the 32nd Brazilian Congress of Urology. A total of 650 question forms were answered.

Results: There were 73% of complete answers (total of 476 question forms). In total, 246 urologists (51.68%) lived in the southeast region and 310 (65.13%) treat 1 to 3 cases of NMIBC per month.

Low risk cancer: Only 35 urologists (7.5%) apply the single intravesical dose of immediate chemotherapy with Mitomicin C recommended by the above guidelines. Adjuvant therapy with BCG 2 to 4 weeks after TUR is used by 167 participants (35.1%) and 271 urologists (56.9%) use only TUR.

High risk tumors: 397 urologists (83.4%) use adjuvant therapy, 375 (78.8%) use BCG 2 to 4 weeks after TUR, of which 306 (64.3%) referred the use for at least one year. Intravesical chemotherapy with Mitomicin C (a controversial recommendation) was used by 22 urologists (4.6%). BCG dose raised a lot of discrepancies. Induction doses of 40, 80 and 120mg were referred by 105 (22%), 193 (40.4%) and 54 (11.3%) respectively. Maintenance doses of 40, 80 and 120mg were referred by 190 (48.7%), 144 (37.0%) and 32 (8.2%) urologists, respectively. Schemes of administration were also varied and the one cited by SWOG protocol was the most used: 142 (29.8%).

Conclusion: SBU, EAU and AUA guidelines are partially respected by Brazilian urologists, particularly in low risk tumors. In high risk tumors, concordance rates are comparable to international data. Further studies are necessary to fully understand the reasons of such disagreement.

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Key words:

Urinary Bladder Neoplasms; BCG Vaccine; Practice Guidelines as Topic

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INTRODUCTION

Bladder tumor is the seventh most common cancer in males and 17th in females. It affects approximately 110.000 men and 70.000 women every year in the World. During 2012, 38.200 and 17.000 deaths were registered due to BC in Europe and USA, respectively (1, 2). According to data from the National Cancer Institute of Brazil, there were 8,940 new cases in 2014 (6.750 men and 2.190 women in the country (3).

Around 75% of bladder tumors are non-muscle invasive (NMSBC-pTa, pTis or PT1), which evolve differently and need adequate classification and treatment (1-3).

NMIBC treatment is standardized by the Brazilian Society of Urology (SBU), European Association of Urology (EAU) and American Urological Association (AUA). There are specific guidelines that show evidence that their use can improve survival rate of treated patients (4-7). However, daily practice may not correspond to what is recommended, mainly in Brazil, where there are no studies about this subject.

The present study was designed to analyze Brazilian urologist's ongoing practice in relation to NMIBC and to compare them to SBU, EUA and AUA guidelines (Table-1) in or-

der to appoint differences between theory and practice in Brazilian hospitals and clinics.

MATERIALS AND METHODS

This cross-sectional study was performed during the 32nd Brazilian Congress of Urology. A total of 650 questionnaires were given to urologists and 476 completed them (73%) regarding their experience and actions on NMIBC of low risk (single tumor, <3cm and low histological grade) and high risk (T1 or high grade or carcinoma in situ) submitted to transurethral resection in the last year (4).

Questionnaires contained two parts. First part had questions about demographic data: state where the physician works, local of practice, number of patients attended and treated each month. Second part comprised questions about treatment of NMIBC according to classification (low and high risk), use of intravesical therapy, drug used (chemotherapy, BCG, other), induction and maintenance doses, follow-up, use of new transurethral resection (re-TUR), etc.

All question forms were included in the study, even those incomplete, comprising 476 urologists.

Results were analyzed in terms of percentage of total answered forms.

Table 1 - Main International Guidelines on NMIBC.

Classification	EAU guideline	AUA guideline
Low risk	TUR -Single dose of intravesical chemotherapy	TUR -Single dose of intravesical chemotherapy (recommended)
Intermediate risk	TUR -Single dose of intravesical chemotherapy followed by: 1-BCG with maintenance for at least 1 year (level A) 2-Intravesical chemotherapy for 6-12 months (level B)	TUR -Induction with BCG or Mitomicin (recommended) -Maintenance with BCG or Mitomicin (option)
High risk	TUR (re-TUR after 4-6 weeks) -Single dose of intravesical chemotherapy followed by: 1-BCG with maintenance for at least 1 year (level A) -Cystectomy may be considered for patients with high risk of progression (level C) or in cases with failure of BCG treatment (level B)	TUR (re-TUR after 4-6 weeks) -Induction and maintenance with BCG (recommended) -Cystectomy (option)

RESULTS

Most participants lived in southeast region of Brazil (246 urologists, 51.68%) and 85.3% were from private practice, but not exclusive. 151 urologists worked in teaching facilities (31.7%) and 325 (68.3%) had no relation to teaching institutions (Table-2). In view of the great number of answers regarding simultaneous practice in public, private and teaching institutions, it was not possible to establish a correlation between adopted treatments and workplace.

Table 2 - Characteristics of Urological Practice.

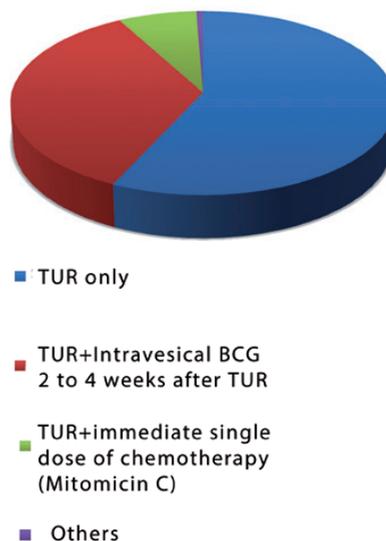
WHERE DO YOU WORK?	TOTAL	TOTAL
Private practice	406	85.3%
Private hospital	279	58.6%
Public hospital	163	34.2%
General or community hospital (noteaching)	162	34.0%
University or school hospital	151	31.7%
Cancer treatment center	57	12.0%
Other	31	6.5%

In relation to experience, 310 (65.13%) urologists treated a media of 1 to 3 cases of NMI-BC per month (12-36 cases per year), reflecting an adequate experience.

In relation to treatment of low risk patients, only 35 urologists (7.5%) followed the guidelines of BSU, EAU and AUA, performing TUR followed by a single immediate dose of intravesical chemotherapy with Mitomicin C (4-7); 271 (56.9%) did not use adjuvant therapy, only TUR and 167 (35%) performed TUR+intravesical BCG 2 to 4 weeks after TUR, totaling 91.9% of divergent treatments in relation to guidelines (Figure-1).

Among patients with high risk tumors, 375 (78.8%) urologists performed TUR followed by adjuvant therapy with BCG 2 to 4 weeks after; however, 360 (75.6%) confirmed the use of the maintenance dose. Still, 22 (4.6%) participants

Figure 1 - Treatment of low risk tumors.



performed immediate intravesical chemotherapy with Mitomicin C following TUR, although being a controversial recommendation of the guidelines (4-7) (Table-3).

Induction was employed by 300 (63.0%) urologists who used doses of 40, 80 and 120mg (105-22.1%, 193-40.5% and 54-11.3% participants, respectively). Induction time was also he-

Table 3 - Treatment of high risk tumors.

Which is your standard treatment for high risk NMIBC tumors?	Total	Total (%)
TUR+intravesical BCG 2 to 4 weeks after TUR	375	78.8%
Re-TUR	322	67.6%
Radical cystectomy	46	9.7%
TUR+immediate single dose of intravesical chemotherapy (Mitomicin C)	22	4.6%
Neoadjuvant chemotherapy followed by surgical resection	17	3.6%
Only TUR	10	2.1%
Others	6	1.3%

terogeneous: 4 weeks–28 (5.9%), 6 weeks–320 (67.2%) and 8 weeks–101 (21.2%).

Maintenance doses were used by 360 urologists (75.6%). The used dose of 40, 80 and 120mg was employed by 190 (39.9%), 144 (37.8%) and 32 (6.7%) urologists respectively.

In relation to interval, the answers were: weekly during 3 weeks on months 3, 6, 12, 18, 24, 30 and 36 (SWOG protocol)–142 (29.8%); monthly during 1 year– 35 (28.4%); monthly during 2 years–25 (5.3%) (Table-4).

Most used strains of BCG included Moreau and Connaught (282–59.3% and 125–26.3%, respectively). Besides, the own patient bought the vaccine without reimbursement, according to 204 (42.9%) participants.

In cases where BCG treatment failed, 152 (31.9%) urologists performed a new intravesical treatment with BCG, 115 (24.2%) used intravesical Mitomicin C and 11 (23.3%) performed radical cystectomy (recommended treatment by BSU, EAU and AUA guidelines in that situation). This

Table 4 - Maintenance regimens of BCG.

When you use maintenance therapy, how many doses are employed, in which interval and for how long?	Total (N)	Total (%)
Weekly for 3 weeks in months 3, 6, 12, 18, 24, 30 e 36	142	29.8%
Monthly for 1 year	135	28.4%
Monthly for 2 years	25	5.3%
Monthly for 6 months	5	1.1%
Weekly for 3 weeks in months 3.6 and 12	1	0.2%
Twice every 3 or 4 months	1	0.2%
Monthly, continuous	1	0.2%
Weekly for 3 weeks in months 6 and 12	1	0.2%
Weekly for 6 months	1	0.2%
Others	75	15.8%
Did not answered	89	18.7%

These data reveal that, when BCG is used, there is a large variation regarding application regimen and at least 306 participants (64.3%) maintain the treatment for at least one year, according to what is recommended by EAU guidelines for the treatment of high risk bladder tumor. In this respect, there was a 64.3% of accordance rate to the referred guideline.

A new TUR (re-TUR) for high risk patients was performed by 322 participants (67.6%) and 41 (29.6%) did not perform it, reflecting the great number of urologists that do not follow the mentioned guidelines. Among those who performed re-TUR, 220 (46.2%), it was carried out 30 to 45 days following initial TUR, while 71 (14.9%) after 46 to 90 days after.

data points out that 67.8% of participants do not follow the recommendations of the main guidelines for BCG-failed patients (4-7).

DISCUSSION

Guidelines are important orientations for medical practice, particularly in diseases where the level of evidence is not high enough to avoid questioning. They gather the highest level of evidences so far available to that particular disease. So, it would be reasonable to expect a great level of accordance between medical practice and guidelines. However, in the present study, we observed great disparity between the proposed guidelines (SBU, EAU and AUA) (4-7) and the current

practice of Brazilian urologists, according to the questionnaires answers.

Treatment of low risk NMIBC showed the lower compliance of guidelines and only 7.5% of participants followed the recommended treatment (TUR followed by intravesical chemotherapy).

A meta-analysis of 1.476 patients showed a reduction of 11.7% of recurrence rates of BC treated with TUR+immediate instillation of Mitomycin C compared to those treated only with TUR (8). In spite of this benefit, in our study Mitomycin C is rarely used by Brazilian urologists as adjuvant therapy of low risk NMIBC.

Another interesting fact relates to the significant number of participants (35%) that used a dose of BCG 2 weeks after TUR for low risk patients. This practice is still controversial in literature, since meta-analysis showed benefits of BCG therapy in recurrence rates only for patients with high risk tumors (9). However, this is not an absolute fact, since there are at least two other meta-analysis that showed superiority of BCG over Mitomycin C in all high risk groups (10, 11). This blurred fact may explain the high use of BCG for low risk patients in this study.

Our study did not analyze patients with intermediate risk. Witjes et al. (12) observed the use of intravesical chemotherapy in only 29.4% of patients with intermediate risk disease but did not analyze low risk patients.

The treatment of high risk bladder tumors showed a good accordance to the guidelines: 83.4% use adjuvant therapy, 4;6% use Mitomycin C, 78.8% use BCG and among these 75.6% use maintenance doses, and also 64.3% use BCG for at least 1 year, in accordance to the guidelines.

The rate of use of BCG for at least one year in North American and European studies (12-16) varies from 39.8% to 78% in patients with high risk tumors.

An North American study (150 with 494 questionnaires available) showed a 89.7% of use of adjuvant intravesical therapy for high risk tumors, and 87.3% used BCG, reflecting a global use of BCG of 78%. Another epidemiological study from Spain (16) analyzed 2.476 cases of BC being 76.7% NMIBC, with a global rate of BCG use of 39.8%. Anyway, Brazilian rates of compliance of

guidelines are comparable to international series.

Isolated TUR was used by 10 participants (2.1%); this number is considerably inferior to that referred by a study performed in European and North American centers, where monotherapy with TUR was used by 9% (12).

Our study detailed the regimen of BCG administration whenever indicated. There was a great variability regarding dose, application interval and total duration of treatment.

According to the guidelines, until now, the evidences show that there is no ideal dose and a reduced one does not lower side effects. However, the distribution of doses probably is based on the concern regarding BCG side effects.

The regimen of BCG use showed a great variation during induction and maintenance. Several regimens were cited by urologists, including never described regimens. However, it is possible to verify that the SWOG protocol was the most used for maintenance (29.8%), what is in accordance to previously described (12, 15).

Guidelines vary in relation to the best regimen to be used; AUA guidelines suggest the use of a standard dose in SWOG regimen (17) for 3 years and EUA guidelines recommend maintenance for at least one year. AUA guidelines are based on a trial with 1.355 patients that used a standard dose and a reduced dose for maintenance during 1 to 3 years with better rates of prevention of recurrence for the 3 year regimen with standard dose, although it was not observed no difference in relation to risk of progression and overall survival (18, 19).

BCG strains are different in relation to phenotype, antigenicity and immunogenic activity, that may influence toxicity, tumor action and clinical efficacy. There are only a few studies that compared effectiveness and collateral effects of different strains, but those used in Brazil are the most common used worldwide (20-22).

Rate of Re-TUR in our study were notably superior in relation to two major international studies (15, 16), where rates varied from 10.4% to 28%. We registered 67.7% of re-TUR.

Following BCG failure, only 23.3% (n=11) indicated cystectomy and not followed international guidelines. Future studies should address criteria for recurrence, failure and intolerance of

BCG. Among these, early failure during intravesical treatment reflects a more aggressive disease, justifying precocious cystectomy, while long term recurrence and intolerance are alternatively treated rather than with cystectomy, what could reflect our results.

Our data have never been published and there are very few studies in Brazilian literature. The lack of compliance and use of different regimens other than the European and North American guidelines reaffirm the complexity and diversity of NMIBC treatment (12, 15).

Limitations of the study include the number of urologists that refrained from answering the questionnaire and the fact that it was realized during the Brazilian Congress of Urology, with a potential bias of selection of professionals who tend to recycle and update their knowledge more frequently. Also, regional comparisons were not made, there was no correlation of data with demographics, evaluation of intermediate tumors was not addressed, pathological data were not reviewed and the factors that influenced treatment were not investigated (lack of access to ideal treatment, money issues, patient choice, urologist's preferences, etc).

CONCLUSIONS

There is not a significant compliance of Brazilian urologists to BSU, EAU and AUA guidelines, particularly in low risk NMIBC. Further studies are necessary to fully understand the reasons for such discrepancy. The results are similar to those of Europe and USA, but with particular aspects.

CONCLUSIONS

SBU = Sociedade Brasileira de Urologia
EAU = European Association of Urology
AUA = American Urological Association

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Brazilian data of renal cell carcinoma in a public university hospital

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ABSTRACT

Purpose: Among renal malignancies, renal cell carcinoma (RCC) accounts for 85% of cases. Stage is a relevant prognostic factor; 5-year survival ranges from 81% to 8% according to the stage of disease. The treatment is based on surgery and molecularly targeted therapy has emerged as a choice for metastatic disease.

Materials and Methods: Retrospective study by reviewing the medical records of patients with RCC treated in the last 10 years at UNIFESP. The primary end point of this trial was to evaluate the overall survival (OS) of the patients. The secondary end point was to evaluate the progression-free survival (PFS) after nephrectomy.

Results: 118 patients with RCC were included. The mean age was 58.3 years, 61.9% men; nephrectomy was performed in 90.7%, clear cell was the histology in 85.6%, 44 patients were classified as stage IV at diagnosis. Among these, 34 had already distant metastasis. 29 patients were treated with sunitinib. The median OS among all patients was 55.8 months. The median PFS after nephrectomy was 79.1 months. Sarcomatoid differentiation HR29.74 (95% CI, 4.31–205.26), clinical stage IV HR1.94 (95% CI, 1.37–2.75) and nephrectomy HR0.32 (95% CI, 0.15–0.67) were OS prognostic factors. Sunitinib had clinical activity.

Conclusions: Patients treated in our hospital achieved median OS compatible with literature. Nevertheless, this study has shown a high number of patients with advanced disease. For patients with advanced disease, treatment with sunitinib achieved median OS of 28.7 months, consistent with the literature.

ARTICLE INFO

Key words:

Renal carcinoma; nephrectomy; sunitinib; survival

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INTRODUCTION

Renal cell carcinomas (RCCs) account for 80–85% of all primary renal malignancies and 2–3% of all cancers in adults (1). Although they are the sixth most common malignance in the USA, there is not any Brazilian epidemiologic data. RCCs are among the most lethal urologic cancers. In the United States, it is estimated that there were 63.920 new cases of kidney and renal pelvis cancer in 2014, and an estimated 13.860 people died of this disease, according to data from the national registry (2).

RCC is more common in male individuals, who outnumber female patients in a ratio of 3:2, and is most frequently diagnosed in the elderly, with a median age of 64 years. There are several established risk factors such as smoking, hypertension, acquired cystic disease of the kidney, and obesity. Most cases are sporadic; however, 2–3% are hereditary. Several genetic syndromes are associated with this disease, of which the best known and most studied is Von-Hippel Lindau disease, which is associated with clear cell carcinoma and other neoplasms (3). RCC is divided into several subtypes, according to histological features,

genetic alterations, and cellular origin. Clear cell carcinomas arise from the proximal tubule and are the most common. Other RCC subtypes include papillary, chromophobe, oncocytic, and collecting-duct carcinomas (4). Translocation carcinoma is a specific subtype of RCC that tends to occur in younger patients and is associated with genomic alterations on chromosome Xp11.2, expression of transcription factor E3, and a poor prognosis (5, 6). Some cases of RCC show sarcomatoid differentiation and are related with poor prognosis.

Recently, an increase in incidence has been observed for all stages of RCC; most frequently, these tumors are detected incidentally in asymptomatic individuals. When in early stages, the gold standard of treatment for these tumors is surgery (radical nephrectomy or other renal-sparing approaches). The clinical presentation of RCC is undetermined and sometimes the symptoms arise late. Because of these facts, almost 20% of cases are diagnosed as advanced disease (2), and systemic therapy is indicated. For many years, the standard of care was cytokines. Interferon [IFN] is marked by low response rates (around 5%) and several adverse effects (7); however, it still is a treatment option, especially when vascular endothelial growth factor (VEGF) inhibitor is unavailable. Interleukin 2 was another cytokine largely studied and although it was the only that achieved cure in some patients, it was related to serious and sometimes life-threatening adverse events (8).

A better understanding of the pathways involved in RCC pathogenesis has enabled the identification of some targets for therapeutic intervention. The most studied target is the VEGF pathway, that led to the development and approval of sunitinib and other VEGF inhibitors (sorafenib, pazopanib, bevacizumab, and axitinib) (9-11). Several studies have reported better response rates, overall survival, and disease-free survival with VEGF inhibitors than with IFN (12).

The main objective of this study was to evaluate the outcomes of various RCC treatments at a Brazilian public hospital.

MATERIALS AND METHODS

Patients

This study included all patients (aged ≥ 18 years) with histologically confirmed RCC, who were treated at Hospital São Paulo, the University Hospital of Federal University of São Paulo, between January 2004 and May 2014. Any disease stage was allowed. The exclusion criteria included patients whose medical records were inadequate and individuals with other synchronous malignancies.

STUDY DESIGN

This retrospective study followed a quantitative approach; medical records were assessed to collect baseline epidemiological and clinical data, in addition to the information pertaining to RCC therapy. The study was approved by the institutional ethics committee and was conducted in accordance with the provisions of resolution 466/12 of the Brazilian National Health Council and Good Clinical Practice guidelines.

TREATMENT PROTOCOLS

The physicians were responsible for decision-making regarding treatment. Molecularly targeted therapy for RCC treatment, specifically sunitinib, has been available in the public health system of the state of São Paulo since 2009. Therefore, aspects related to advanced disease treatment were assessed for these two periods, before and after targeted therapy.

Treatment with IFN involves three subcutaneous infusions per week, with an initial dose of 3 million units (MU) in the first week, 6 MU in the second week, and 9 MU thereafter, if tolerated well. Sunitinib treatment was administered at 50mg/day for 4 weeks, with 2 weeks off treatment. Both protocols could be adjusted in accordance with the adverse effects.

EFFICACY AND SAFETY

The primary endpoint of the study was overall survival (OS), defined as the time from the

diagnosis to death from any cause. The secondary endpoint was progression-free survival (PFS), defined as the time from nephrectomy to the first documentation of objective disease progression or death from any cause. The evaluation for PFS was made according to the investigator's assessment. Imaging studies were performed at intervals set by the physician. Tumor response was assessed by investigators according to the response evaluation criteria in solid tumors (RECIST version 1.1). Therefore, PFS was assessed for a subset of 84 patients who did not have metastasis at diagnosis. The disease was staged at the time of diagnosis according to the guidelines of the American Joint Committee on Cancer version 7.

Statistical analysis

The demographic characteristics were evaluated with descriptive statistics. Time-to-event analyses were performed using the Kaplan–Meier method. A stratified log-rank test and the multivariate Cox regression model were used to evaluate the potential influences of the patient's baseline characteristics, including age, sex, nuclear grade (Fuhrman), and disease stage at diagnosis, on median PFS and median

OS (13). A univariate Cox regression model was used to evaluate the effects of nephrectomy, or different treatments, towing to the limited size of these groups of patients with metastasis (n=34 and n=68, respectively). A p-value of <0.05 was considered statistically significant, and was calculated up to two decimal places. Data on patients who were lost to follow-up were censored at the time of the last evaluation. All authors had access to the primary data and take responsibility for the veracity and completeness of the data reported.

RESULTS

Patients

Between January 2004 and May 2014, medical records of 124 patients with RCC were assessed, but 6 were excluded from the study (5 patients with incomplete data and 1 with synchronous malignancies in the lungs); thus, 118 patients were included in the study. Thirteen patients were lost to follow-up, and their data were censored at the time of the last evaluation.

Table-1 summarizes the baseline characteristics of the patients. The clinical stage at diagno-

Table 1 - Baseline clinical and treatments characteristics.

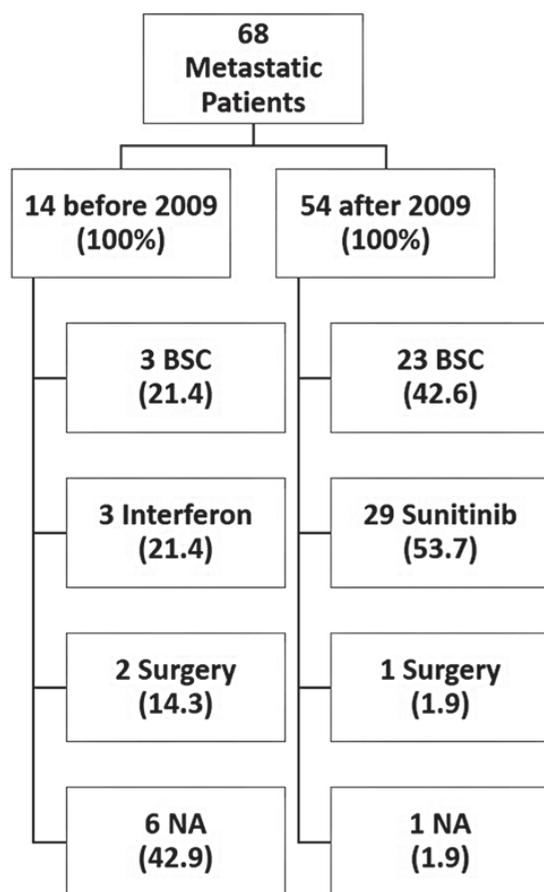
	Mean	Lower	Upper	Median	SD
Age	58.3	22	87	58	13.3
Gender	Female	38.1%			
	Male	61.9%			
Histology Subgroups	Renal Cell	85.6%	Sarcomatoid	2.5%	
	Cromophobe	5.1%	NA	6.8%	
	1	3.4%	3	29.7%	
Furhman Grade	2	46.6%	4	10.2%	
			NA	10.2%	
Clinical Stage	1	29.7%	3	17.8%	
	2	13.6%	4	37.3%	
			NA	1.7%	
Metastasis at Diagnosis	Yes	28.8%			
	No	71.2%			
Metastatic Nephrectomy (n=34)	Yes	73.5%			
	No	26.5%			
Metastatic Treatment (n=68)	Sunitinib	42.6%	Supportive Care	38.2%	
	Interferon	4.4%			
	Surgery	4.4%	Not Avaliable	10.3%	

sis was determined in 116 patients: 35 (30.2%) were stage I, 16 (13.8%) stage II, 21 (18.1%) stage III, and 44 (37.9%) stage IV; 34 (28.8%) had metastasis at diagnosis.

TREATMENT

Nephrectomy was performed in 107 (90.7%) cases. Nephrectomy was performed for 25 (73.5%) of the 34 patients who had metastasis at diagnosis. Figure-1 summarizes the treatment options for metastatic RCC; treatment options have been represented as before and after 2009 in order to account for the availability of sunitinib.

Figure 1 - Treatment for metastatic RCC in Hospital São Paulo.



BSC = Best Supportive Care; **NA** = Not Available.

Note: Sunitinib had been available in public health system since 2009.

PROGRESSION-FREE AND OVERALL SURVIVAL

The median PFS was 79.1 months for all patients who underwent nephrectomy. Nuclear grade IV (Fuhrman) and the clinical stage IV at diagnosis were defined as poor prognostic factors for disease progression, with a hazard ratio (HR) of 2.78 (95% CI, 1.51 to 5.10) and 2.24 (95% CI, 1.49 to 3.37), respectively.

The median OS for all 118 patients was 55.8 months. The presence of sarcomatoid differentiation and clinical stage IV at diagnosis were defined as poor prognostic factors for death (Figures 2 and 3, respectively), with an HR of 29.74 (95% CI, 4.31 to 205.26) and 1.94 (95% CI, 1.37 to 2.75), respectively. Nephrectomy was defined as a positive prognostic factor (Figure-4), with an HR of 0.32 (95% CI, 0.15 to 0.67), for the 34 patients who had metastasis at diagnosis.

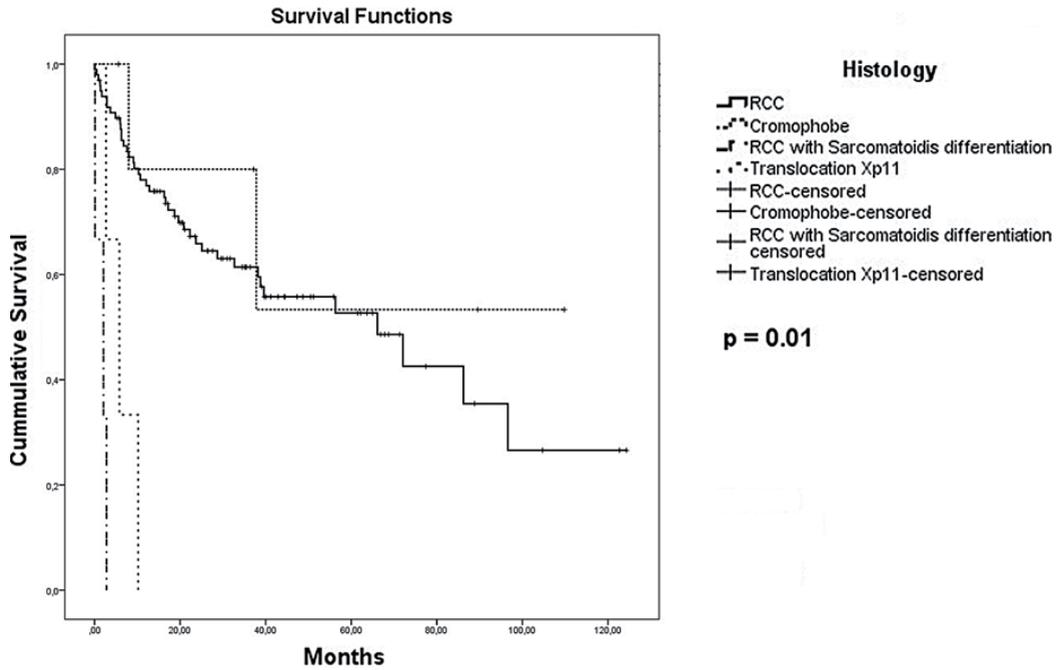
In addition, treatment with sunitinib was defined as a positive prognostic factor (Figure-5) for the 68 patients with metastatic or progressive disease compared to best supportive care, with an HR of 0.22 (95% CI, 0.11 to 0.42). Figure-6 depicts a forest-plot chart that summarizes the analysis of subgroups.

DISCUSSION

Although RCC is not a rare neoplasm, the Brazilian epidemiology remains uncertain. In addition, there is little information about clinical features of this tumor in our country. Despite of these limitations we believe that the clinical outcomes of RCC in our institution are similar to the international literature. This retrospective study showed that patients treated at Hospital São Paulo had a similar prognosis to that reported in the literature (12). It is not possible to understand the entire Brazilian RCC epidemiology and clinical outcomes only based in these results because of the retrospective design of the trial and the short number of included patients.

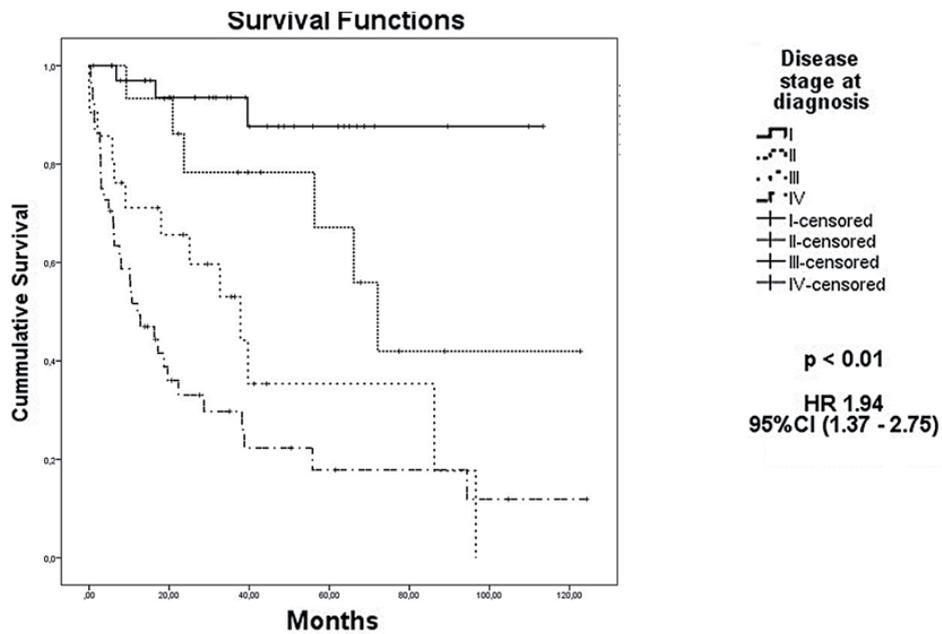
The clinical stage at diagnosis was the most important prognostic factor of the disease, as was already expected. In this study, the median OS was not reached for patients with stage I, and it was 72.1 months for stage II, 37.8 months for

Figure 2 - Kaplan-Meier estimates of Overall Survival per RCC histology



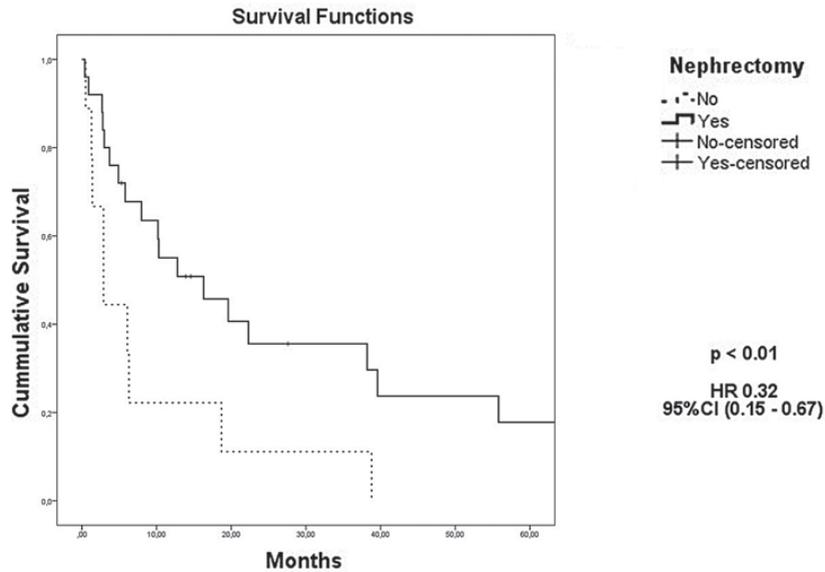
RCC = Renal Cell Carcinoma

Figure 3 - Kaplan-Meier estimates of Overall Survival per clinical stage



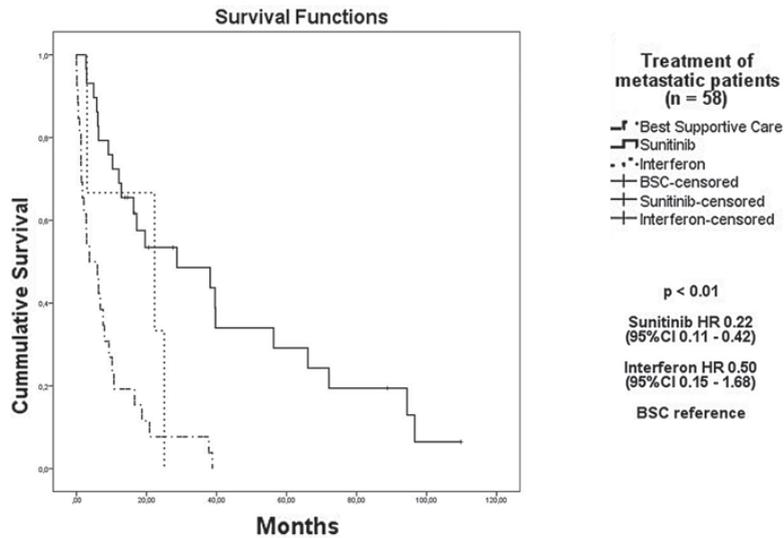
HR = Hazard Ratio; CI95: 95% Confidence Interval

Figure 4 - Kaplan-Meier estimates of Overall survival according to nephrectomy for metastatic patients



HR = Hazard Ratio; CI95: 95% Confidence Interval

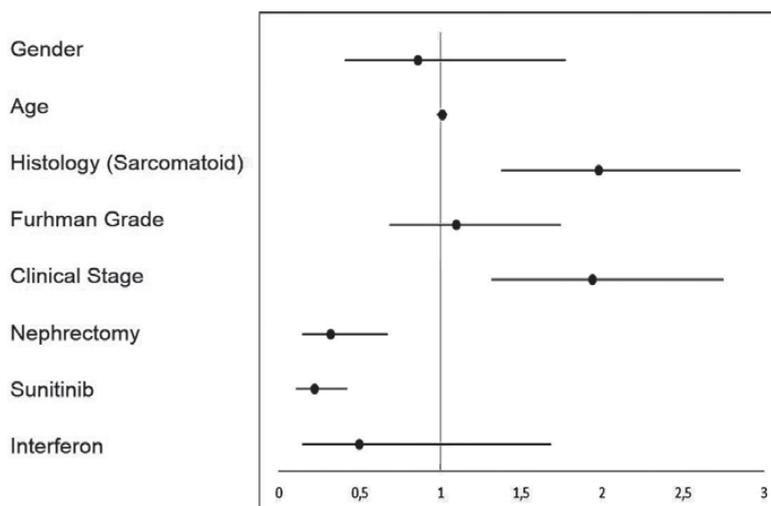
Figure 5 - Kaplan-Meier estimates overall survival according to treatment.



BSC = Best Supportive Care; HR = Hazard Ratio; CI95: 95% Confidence Interval

stage III, and only 12.1 months for stage IV; the five-year survival rate in the aforementioned stages was 85%, 65%, 38%, and 19%, respectively. A majority of patients had advanced disease at diagnosis and it is not possible to correlate this finding with socioeconomic features because in this study some demographic data was not available.

Sarcomatoid differentiation was a poor prognostic factor. Some trials had assessed this issue with similar results. Interestingly, it was also observed in a Mexican trial that has assessed clinical and pathological aspects related to poor prognosis among patients with Stage III or IV RCC (14). In this study, 126 patients were included and

Figure 6 - Forest-plot of subgroup analysis for Overall Survival

8.7% had sarcomatoid differentiation (14). After a multivariate Cox regression analysis, the risk of cancer-specific death was more than 3 times higher among individuals with sarcomatoid differentiation (14). Lymph node invasion was also a poor prognostic factor; however, our trial did not evaluate this aspect.

Nephrectomy is the most important treatment for RCC, even in the advanced stages. Support for this approach comes from an observational study of 314 patients treated with molecularly targeted agents, including 201 patients who underwent cytoreductive nephrectomy (15). Patients who underwent cytoreductive nephrectomy had a significantly longer OS than those who did not have surgery (19.8 versus 9.4 months, $p < 0.01$) (15). This benefit persisted on multivariate analysis after adjusting for other known risk factors (HR 0.7, 95% CI, 0.5 to 1.0) (15). In our cohort, of the 34 patients who had metastasis at diagnosis, 25 were eligible for nephrectomy; of the remaining, only 3 (33.3%) were treated with molecularly targeted therapy alone. The remaining 6 patients (66.6%) were treated with best supportive care (BSC) because of a poor performance status. We found similar results, the median OS among patients whom underwent nephrectomy was 16.3 versus 2.9 months in the control group. However, this large difference might have been overestimated owing to the small number of patients and the compari-

son between patients who underwent surgery versus those who received BSC alone. In this study, there were two cases of complete remission of metastasis after nephrectomy, and the patients have remained free of disease for 8.5 and 10 years. Another patient underwent surgical removal of pulmonary metastasis, and has remained free of disease for 5 years. Based on these results, even the resection of metastases should be encouraged when appropriate.

In the recent years, some trials are providing data on the comparison of different molecularly targeted agents. The efficacy of sunitinib was firstly demonstrated in a phase III study of 750 patients with metastatic RCC who had not received prior systemic therapy. Patients were randomly assigned to sunitinib or IFN α treatments. Sunitinib resulted in a higher overall response rate (47% versus 12%, respectively), a longer PFS (median PFS of 11 versus 5 months, HR 0.54), and a longer OS (median OS of 26.4 versus 21.8 months, HR 0.82, 95% CI, 0.67 to 1.00) (12). In the state of São Paulo, sunitinib was available after 2009 for patients receiving treatment under the Brazilian Health System (Sistema Único de Saúde). Our study assessed 54 patients with metastasis after 2009; 53.7% of patients were treated with sunitinib, whereas 42.6% received BSC. A high proportion of patients underwent best supportive care primarily because of the patient's poor performance status. Sunitinib therapy was effective in improving

OS; the median OS with sunitinib was 28.7 months, versus 3.7 months with BSC. In the Group of patients who received treatment before 2009, of 14 patients with metastasis, 3 were treated with IFN. No statistical significant benefit was obtained with the use of IFN compared to BSC (HR 0.50, 95% CI, 0.15 to 1.68). To date, there are few studies describing the efficacy and safety of sunitinib in a Brazilian population. In 2012, Smaletz et al. have shown a cohort of Latin American patients who has achieved long term clinical benefit (more than 20 months) with the use of sunitinib. Only 29 patients were included and it was hypothesized that young patients with good performance status had the highest benefit (16).

There are some limitations in this study, of which the most important are the small number of patients and the retrospective design. Every evaluation must be done carefully. Furthermore, it is impossible to demonstrate the superiority of one treatment over another because of the retrospective design. Many patient's, especially those with localized disease, were lost to follow-up. Moreover, incomplete and fragmented medical records complicated some analysis. Regardless, this study presents relevant data for subjects treated in a Brazilian public university hospital.

CONFLICT OF INTEREST

None declared.

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Three-dimensional reconstructive kidney volume analyses according to the endophytic degree of tumors during open partial or radical nephrectomy

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ABSTRACT

Objectives: To investigate the renal function outcomes and contralateral kidney volume change measured by using a 3-dimensional reconstructive method after open partial nephrectomy (PN) or open radical nephrectomy (RN) according to the endophytic degree of tumors.

Materials and Methods: We included 214 PN and 220 RN patients. According to the endophytic degree of the tumors, we divided patients into 3 groups. Patients were assessed for renal function and kidney volume change both preoperatively and postoperatively at 6 months. Kidney volume was calculated by using personal computer-based software. Subgroup analyses was performed for tumor >4cm.

Results: Larger and complex tumors were more frequent in the RN group than PN group. Among patients with exophytic and mild endophytic tumors, the mean postoperative renal function was well preserved in PN group and the mean contralateral kidney volume significantly increased in the RN compared to the PN group (PN, 145.55 to 149.98mL; 3.0% versus RN, 143.93 to 169.64mL;17.9% p=0.006). However, in fully endophytic tumors, compensatory hypertrophy of the contralateral kidney was similar between PN and RN (PN, 138.16 to 159.64mL; 15.5 % versus RN, 138.65 to 168.04mL; 21.2% p=0.416) and renal functional outcomes were similar between both groups. These results were also confirmed in tumors >4cm in size.

Conclusions: In fully endophytic tumors, especially large tumors, the postoperative renal function and contralateral kidney volume were similar; therefore, we should consider RN preferentially as surgical option for these tumors.

ARTICLE INFO

Key words:

Kidney; Nephrectomy; Surgical Procedures, Operative; Neoplasms

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INTRODUCTION

Partial nephrectomy (PN) is currently the standard procedure for surgical treatment of small renal cortical tumors, especially clinical T1a tumors (<4cm) (1, 2). For clinical T1b renal tumors, (≥4cm), elective PN is occasionally recommended to be performed in high volume

centers, since present equivalent oncological results to radical nephrectomy (RN) and superior renal functional preservation (3, 4). Even in renal tumors ≥7cm, some reports showed remarkable results with acceptable complication rates and with oncologic outcomes comparable to RN. PN in these large sized tumors could preserve the renal function (5).

However, actual renal functional preservation after PN has not been defined in a standard manner in the literature. Currently, kidney volume is believed to be a measurable parameter to predict renal function. One autopsy study showed kidney volume strongly correlated with the number of functional nephrons (6); kidney volume during donor nephrectomy was correlated with renal function in living donor (7, 8), and Jeon et al. (9) showed that preoperative kidney volume is an independent predictor of renal function in renal cell carcinoma (RCC) patients who underwent PN or RN. However, many cases in real clinic situation had normal contralateral kidney, therefore renal functional recovery might be mainly due to contralateral kidney enlargement. Actually the increasing rate of kidney volume after RN was significantly larger than PN (9). However there were few studies about renal volumetric correlation analysis according to endophytic degree. In the present study, we investigated renal functional outcomes after PN or RN according to endophytic degree using three dimensional reconstructive kidney volumetrics measured by computed tomography (CT) image, and we intended to provide appropriate tumor size criteria of entire endophytic tumor during PN.

MATERIALS AND METHODS

Study population

Our prospectively maintained institutional kidney center database, approved by our Institutional Review Board, was queried to identify all patients from December 2000 to September 2012 undergoing PN or RN with available cross-sectional imaging by CT for assessment. Among them, the patients who had solitary kidney, chronic kidney insufficiency (Modification of Diet in Renal Disease (MDRD) glomerular filtration rate (GFR) $<60\text{mL}/\text{min}/1.73\text{m}^2$) and experience of previous kidney surgery were excluded. Patients who had positive surgical margin after PN were also excluded to reduce bias of tumor effect. And we only enrolled the patients with clear cell type RCC after nephrectomy. Accordingly, 214 patients who underwent PN and 220 patients who underwent RN were included. All patients included in this study

were from South Korean and resided into South Korea. All surgeries were performed by the single surgeon who were specialist in kidney cancer and had a lot experience with PN or RN over hundreds of cases before these series. All PN were conducted under cold ischemia and open method; all RN were also performed via open methods. Surgical technique for open PN introduced previously was applied in all patients (10).

Evaluation and kidney volume measurement

Preoperative CT imaging was reviewed in the axial and coronal planes, and a RENAL nephrometry score (NS) was assigned to all identified lesions, as described by Kutikov and Uzzo (11). The NS was categorized as low (4-6 points), moderate (7-9 points) or high (10-12 points) complexity. Tumor endophytic degree was defined along with E score (1, 2, and 3) of NS system. The E score of NS system assigned a point from 1 to 3 according to endophytic nature of the tumor ($\geq 50\%$ exophytic, $<50\%$ exophytic or endophytic, respectively). Kidney volume analysis was performed via previous reported methods (9). The kidney volume was measured before and after surgery at 6 months using CT (Somatom Plus 4; Siemens Medical Systems, Forchheim, Germany) with the standard clinical abdominopelvic imaging protocol. Venous scans of entire abdomens were performed with a 60-s delay after starting the $2\text{mL}/\text{kg}$ i.v. injection of iodinated contrast agent through an antecubital vein. All axial images were transferred to a workstation running personal computer based software (Rapidia; Infi nitt Co. Ltd, Seoul, Korea), which has been used in previous studies (9, 12). The kidney volume was calculated by summing all the volumes within the normally functioning tissue, excluding tumor tissue or non-enhanced areas in a delayed CT image with a slice thickness of 5mm. Renal volumes were independently measured by three urologists who were blinded to patient characteristics, and final volumes were calculated by averaging the three volumes. The GFR was measured by MDRD equation (13).

Statistical analysis

Demographic and clinical characteristics were compared between PN and RN. Continuous variables

were analyzed by Wilcoxon tests, and categorical variables were examined by chi-square analyses. The renal functional outcomes before and after each nephrectomy according to tumoral endophytic degree were also compared and the ipsilateral and contralateral kidney volume after PN and RN were measured, compared and stratified by degree of endophytic nature. In a sub-analysis, in case of tumor size above 4cm (not clinical T1a), renal functional outcomes and kidney volumetrics were investigated. Intra- and postoperative complications were stratified using the Clavien–Dindo classification system and compared according to surgical methods (14). The prolonged

bleeding and hematuria were defined as persistent symptoms 2 weeks after surgery. Statistical analyses were carried out using SPSS version 15.0 software (Statistical Package for Social Sciences™, Chicago, IL, USA). Two-tailed null hypotheses of no difference were rejected if p-values were less than 0.05.

RESULTS

Demographic and tumor characteristics are summarized in Table-1. The mean age of PN subjects was 53.5 years and of RN subjects was 56.1 years. The mean tumor size was larger in RN group

Table 1 - Descriptive characteristics according to surgical methods.

Variables	Partial nephrectomy	Radical nephrectomy	p-value
N	214	220	
Age (years)±SD	53.52±13.09	56.06±13.83	0.166
Gender (%)			0.893
Male	130 (60.7)	158 (71.8)	
Female	84 (39.3)	62 (28.2)	
Body mass index, kg/m ² ±SD	24.8±5.14	24.2±5.74	0.138
History of hypertension (%)			0.287
Yes	70 (32.7)	82 (37.3)	
No	144 (67.3)	138 (62.7)	
History of diabetes (%)			0.369
Yes	24 (11.2)	30 (13.6)	
No	190 (88.8)	190 (86.4)	
History of smoking (%)			0.530
Yes	37 (17.3)	42 (19.1)	
No	177 (82.7)	178 (80.9)	
ASA score			0.280
1-3	209 (97.7)	213 (96.8)	
>3	5 (2.3)	7 (3.2)	
Tumor size (cm)±SD	4.04±3.56	7.45±3.78	<0.001
R.E.N.A.L. nephrometry score, n (%)			<0.001
Low (4-6)	40 (18.7)	8 (3.6)	
Intermediate (7-9)	150 (70.0)	76 (34.5)	
High (10-12)	24 (11.3)	136 (61.8)	

SD=standard deviation; ASA=American Society of Anesthesiologists

than PN group (7.45 versus 4.04cm, $p < 0.001$); there were more high complexity tumor in RN than PN according to RENAL nephrometry system (61.8% versus 11.3%, $p < 0.001$).

Mean cold ischemic time was 44.5 min under cold ischemia (Table-2). There were no significant differences with respect to age, gender, body mass index and medical history. Mean preoperative renal function was higher in PN group than RN group (GFR 80.56 versus 70.57mL/min/1.73m², $p < 0.001$). Postoperative complications occurred in 20 patients of PN group and in 12 of RN group; severe complication which needed intervention (Clavien III and IV) was registered in 2 cases in PN group and 1 in RN group. There was no postoperative mortality in both groups.

As shown in Table-3, endophytic degree 1 was noted in 104 patients in PN and 70 in RN groups. Among subgroup endophytic degree 1, preoperative renal function was similar between both groups, however postoperative renal function was better preserved in PN group (GFR PN:76.42 versus RN:55.53mL/min/1.73m², $p < 0.001$). Contra-

lateral kidney (non-surgery kidney) volume which was measured 6 months after nephrectomy was significantly enlarged in RN group than in PN group (RN:169.64 versus PN:149.98mL, $p = 0.006$). Mean volume increase rate in contralateral kidney was also higher in RN group than in PN group (17.9% versus 3.0%). Among endophytic degree 2 groups, postoperative renal function also better preserved in PN group than RN group (GFR PN:79.68 versus RN:51.99mL/min/1.73m², $p < 0.001$) and contralateral kidney volume was larger after nephrectomy in RN group (RN:173.92 versus PN:160.18, $p = 0.038$). Mean volume increase rate in contralateral kidney among endophytic degree 2 group was also higher in RN group than in PN group (24.2% versus 11.8%). However, among endophytic degree 3 group, renal function decreased in both groups and contralateral kidney volume was also similar between both groups. Postoperative renal function (GFR PN: 60.92 versus RN:57.02mL/min/1.73m², $p = 0.124$) and contralateral kidney volume (PN: 138.16 to 159.64 versus RN: 138.65 to 168.04mL, $p = 0.416$) were similar in patients with fully endophytic tumors after PN

Table 2 - Perioperative outcomes characteristics according to surgical methods.

Variables	Partial nephrectomy	Radical nephrectomy	p-value
N	214	220	
Mean cold ischemic time (min)±SD	44.52±16.70	0	-
Mean preoperative serum creatinine (mg/dL)±SD	1.04±0.36	1.28±2.44	0.008
Mean preoperative MDRD GFR (mL/min/1.73m ²)±SD	80.56±17.27	70.57±39.48	<0.001
Mean operation time (min)±SD	183.4	175.6	0.881
Mean estimated blood loss (cc)±SD	263.7±144.9	310.3±92.7	0.731
Postoperative complications (%)	20 (9.3)	12 (5.5)	0.984
Clavien classification 1-2	16 (7.5)	10 (4.5)	0.176
Clavien classification 3-4	4 (1.9)	2 (0.9)	0.112
Prolonged ileus	4 (1.9)	7 (3.2)	0.217
Wound problem	5 (2.3)	4 (1.8)	0.495
Urine leakage necessary stent insertion	3 (1.4)	0 (0.0)	-
Prolonged bleeding	5 (2.3)	1 (0.5)	0.083
Prolonged hematuria	3 (1.4)	0 (0.0)	-

MDRD=modification of diet in renal disease; GFR=glomerular filtration rate

Table 3 - Renal functional outcomes and kidney volumetric results according to endophytic degree among patients who underwent nephrectomy.

Variables	Partial nephrectomy	Radical nephrectomy	p-value
Endophytic degree 1, n (%)	104 (59.8)	70 (40.2)	
Preoperative parameters			
Preoperative serum creatinine (mg/dL)±SD	1.10±0.45	1.15±2.29	0.415
Preoperative MDRD-GFR (mL/min/1.73m ²)±SD	73.29±19.45	68.53±40.22	0.303
Contralateral kidney volume (mL)±SD	145.55±33.56	143.93±46.93	0.677
Ipsilateral kidney volume (mL)±SD	154.73±35.58	145.07±70.02	0.458
Postoperative parameters			
Postoperative serum creatinine (mg/dL)±SD	1.07±0.41	1.49±1.76	0.009
Postoperative MDRD-GFR (mL/min/1.73m ²)±SD	76.42±24.20	55.53±24.11	<0.001
Contralateral kidney volume (mL)±SD	149.98±34.14	169.64±51.01	0.006
Increasing rate of contralateral kidney	3.0%	17.9%	<0.001
Ipsilateral kidney volume (mL)±SD	122.24±33.04	0	
Endophytic degree 2, n (%)	86 (43.9)	110 (56.1)	
Preoperative parameters			
Preoperative serum creatinine (mg/dL)±SD	0.98±0.25	1.22±2.30	0.135
Preoperative MDRD-GFR (mL/min/1.73m ²)±SD	78.70±15.04	68.03±39.64	<0.001
Contralateral kidney volume (mL)±SD	143.18±38.30	140.03±58.86	0.782
Ipsilateral kidney volume (mL)±SD	147.84±35.54	133.33±108.30	0.425
Postoperative parameters			
Postoperative serum creatinine (mg/dL)±SD	0.99±0.25	1.79±1.85	0.006
Postoperative MDRD-GFR (mL/min/1.73m ²)±SD	79.68±16.91	51.99±17.13	<0.001
Contralateral kidney volume (mL)±SD	160.18±32.84	173.92±49.05	0.038
Increasing rate of contralateral kidney	11.8%	24.2%	0.021
Ipsilateral kidney volume (mL)±SD	125.54±30.86	0	
Endophytic degree 3, n (%)	24 (37.5)	40 (62.5)	
Preoperative parameters			
Preoperative serum creatinine (mg/dL)±SD	1.27±0.27	1.48±2.25	0.250
Preoperative MDRD-GFR (mL/min/1.73m ²)±SD	68.37±14.35	61.91±34.14	<0.001
Contralateral kidney volume (mL)±SD	138.16±37.01	138.65±36.34	0.370
Ipsilateral kidney volume (mL)±SD	115.60±58.36	116.56±44.53	0.854
Postoperative parameters			
Postoperative serum creatinine (mg/dL)±SD	1.69±0.25	1.75±3.87	0.206
Postoperative MDRD-GFR (mL/min/1.73m ²)±SD	60.92±14.35	57.02±19.36	0.124
Contralateral kidney volume (mL)±SD	159.64±43.55	168.04±69.38	0.416
Increasing rate of contralateral kidney	15.5%	21.2%	0.184
Ipsilateral kidney volume (mL)±SD	113.50±43.41	0	

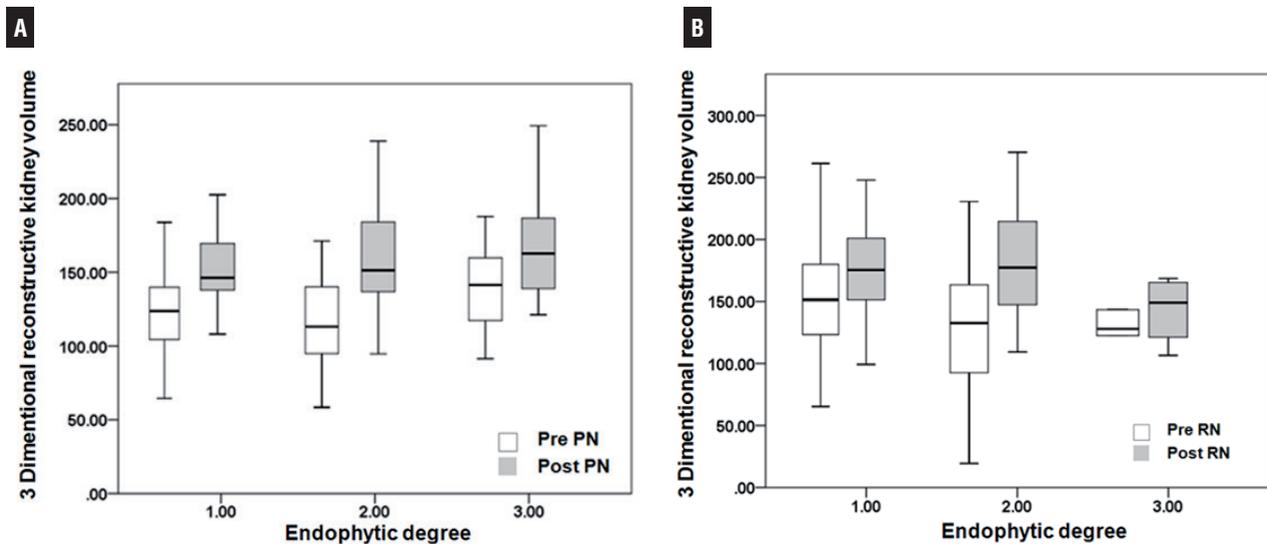
MDRD=modification of diet in renal disease; GFR=glomerular filtration rate

and RN. Figure-1 shows preoperative and postoperative contralateral kidney volume according to endophytic degree. There were significant increase disparity between PN and RN in cases of endophytic degrees 1 and 2, however similar increase was observed in cases of endophytic degree 3 renal tumor.

functional outcomes were similar after PN or RN; contralateral kidney enlargement was also similar between both surgical methods.

Rate of PN continues to increase worldwide based on the growing literature supporting its renal function benefits relative to RN. According to 2006 SEER cancer registry, 45% of patients

Figure 1 - Preoperative and postoperative contralateral kidney volume (non-operated kidney) measured by three dimensional reconstructive method according to endophytic degree after PN (A) and after RN (B).



Subgroup analysis of subjects with tumor size above 4cm is shown in Table-4. Among endophytic degree 1 and 2 groups, there was significant better renal functional preservation in PN group than RN group along with significant contralateral kidney enlargement in RN group than PN group. However among endophytic degree 3 group, there was similar postoperative renal function and similar enlargement of contralateral kidney between both groups.

DISCUSSION

In the current study, we observed that PN better preserved renal function than RN in cases of mild and moderate exophytic renal masses despite of significant increase of contralateral kidney enlargement of RN than PN. However in cases of entire endophytic tumor, especially above 4cm, renal

with small renal mass underwent PN, however recent contemporary reports from single major referral centers describe a PN rate of up to 89% for tumors 4cm or less (15, 16). And currently, greater understanding of the biological heterogeneity of small renal masses and increased awareness of the risks of chronic kidney disease have led to greater use of PN for larger and more complex tumors (15). Lane et al. (15) showed 54% of clinical T1b tumors were treated with PN between 2004 and 2012 in a multicenter study. These results might have been originated from renal function preservation concerns: PN could preserve renal parenchymal tissue in some amount. Actually RN had previously been found to be associated with a greater risk of de novo chronic renal failure than PN (17, 18). However in cases with large tumor size and high tumor endophytic degree, ischemic time and perioperative complications should be incre-

Table 4 - Renal functional outcomes and kidney volumetrics results after partial or radical nephrectomy in renal tumor above 4cm.

Endophytic degree	1			2			3		
	PN	RN	P-value	PN	RN	p-value	PN	RN	p-value
Pre-operative parameters									
Serum creatinine (mg/dL)±SD	1.18±0.63	1.09±0.32	0.437	0.98±0.11	1.16±2.31	0.322	1.20±0.01	1.33±0.25	0.528
MDRD-GFR (mL/min/1.73m2)±SD	70.48±18.19	75.27±39.67	0.120	78.75±8.84	66.84±40.37	0.641	66.02±12.41	61.86±46.64	0.405
Contralateral kidney volume (mL)±SD	136.25±36.43	154.81±47.32	0.183	155.97±18.02	147.48±52.41	0.784	168.41±22.40	172.20±27.40	0.617
Ipsilateral kidney volume (mL)±SD	151.13±34.77	144.97±71.07	0.675	163.96±23.34	139.46±109.94	0.626	113.25±14.21	118.73±32.93	0.825
Post-operative parameters									
Serum creatinine (mg/dL)±SD	1.22±0.58	1.80±1.78	0.146	1.06±0.17	1.42±0.47	0.095	1.10±0.17	1.86±0.90	0.339
MDRD-GFR (mL/min/1.73m2)±SD	65.48±24.06	55.47±24.38	0.027	74.19±7.57	53.98±14.68	0.004	59.08±14.83	41.44±15.69	0.228
Contralateral kidney volume (mL)±SD	135.42±32.69	170.53±51.44	0.039	162.70±32.40	178.76±41.96	0.019	176.14±52.14	185.90±31.41	0.191
Ipsilateral kidney volume (mL)±SD	107.32±34.98	0		125.88±46.59			88.30±13.43		

MDRD=modification of diet in renal disease; **GFR**=glomerular filtration rate; **PN**=partial nephrectomy; **RN**=radical nephrectomy
Bold face=significant association p<0.05

ased, therefore NS was introduced (11). Many studies confirmed its usefulness for predicting surgery type and renal functional outcome (19, 20).

Another predictor of renal function after nephrectomy was kidney volume. Kidney volume is an important parameter of renal function in the evaluation and follow-up of patients with end stage renal disease, polycystic kidney and transplanted kidney (21, 22). In these diseases, change of kidney volume became a reliable parameter of disease progression and renal function. By measuring kidney volume with traditional ultrasound, using the dimensions of the 3 orthogonal axes to the ellipsoid formula, there is some error and poor reproducibility (23). However, along with recent improvement of imaging technique, relative accurate kidney volume measurement by CT or mag-

netic resonance imaging was introduced and these methods can be applied after PN. Previous study to investigate kidney volume in 133 patients showed preoperative kidney volume was independent predictor of postoperative GFR in PN or RN patients (9). Simmons et al. (24) also reported that kidney volume measured by cylindrical volume ratio method was well preserved along with NS and indicator of renal function. Gong et al. (25) also showed that kidney volume correlated well with renal function in 539 normal patients.

However, in real clinical situation of PN, we should consider contralateral kidney change. Traditionally RN was thought to be an acceptable surgery due to compensatory recovery of renal function by contralateral kidney. Kidney transplantation could be also acceptable by this reason.

Anderson et al. (26) examined renal function after donor nephrectomy and noted that compensatory hypertrophy was completed 1 week after surgery and that the effective renal plasma flow had increased by 32.5% in the residual kidney. They reported that the effective renal plasma flow recorded 10 years after surgery was still greater than the preoperative level and that the percentage of decrease in the effective renal plasma flow during the 10-year postoperative follow-up did not differ from that of normal controls. Contralateral kidney volume and function after PN were significant higher than after RN. Jeon et al. (9) showed the volume of normal side kidney increased 127.2 to 138.8mL after PN, however normal side kidney after RN increased 142.4 to 166.0mL. One study about effective renal plasma flow using dynamic scintigraphy showed that renal flow increased 3.8% after RN and 0.1% after PN (25). In our study, contralateral kidney volume which was measured 6 months after nephrectomy was significantly more enlarged in RN than PN group in exophytic degrees 1 and 2. Among endophytic degree 3 tumor, which was entirely endophytic mass, similar kidney volume increase after surgery between PN and RN was observed due to poor functional preservation of operative kidney. It might be considered as evidence that remnant operated kidney after PN in entirely endophytic tumor had not good functional contribution to the total kidney function. Especially large masses above 4cm with entirely endophytic feature should consider RN as the method of choice for nephrectomy due to not only less benefit of remnant kidney function but also harmfulness of high complication.

Generally authors also agree with the concept that PN could preserve renal function well, however in cases of entire endophytic tumor we reconsider which choice of surgical methods will be better. Central tumor site is associated with increased complication rates, collecting system entry and ischemia time (27). Tumor endophytic percent is associated with an increased complication rate (28). Another reason is that kidney

volume before nephrectomy in entirely endophytic mass was already increased, meaning that unilateral renal function was already decreased in mass containing kidney. After extraction of central tumor, remnant kidney might not have significant function.

Our study has several limitations. There was sample number disparity according to endophytic degree and surgical methods, despite the consecutive nature of data from our kidney center database. Current many retrospective studies had selectional bias that more complex and large tumors are usually extracted by RN. Also, a small numbers of patients were enrolled in endophytic degree 3 group and these patients had relative low renal function at baseline. Second, preoperative renal scintigraphy was not obtained in the present study and we could not calculate a single GFR for the normal side kidney. Previous study had reported a strong correlation between kidney volume and renal scintigraphy (29). And we could not adjust cold ischemic time in PN group due to its retrospective nature. Furthermore, another study had provided evidence indicating that split renal function could be calculated by measuring the kidney volume (30). Unfortunately, we could not access this method due to its retrospective nature.

CONCLUSIONS

PN preserved renal function better than RN in relative exophytic masses, however in cases of entirely endophytic mass, renal functional outcome after PN was similar to RN especially in large size tumors. Contralateral kidney volume enlargement was significantly increased after RN than PN, except in entirely endophytic masses. Therefore we should consider RN as the preferential surgical option in entirely endophytic masses with large size to reduce PN related complications.

CONFLICT OF INTEREST

None declared.

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Low-dose-rate brachytherapy for patients with transurethral resection before implantation in prostate cancer. Long-term results

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ABSTRACT

Objectives: We analyzed the long-term oncologic outcome for patients with prostate cancer and transurethral resection who were treated using low-dose-rate (LDR) prostate brachytherapy.

Methods and Materials: From January 2001 to December 2005, 57 consecutive patients were treated with clinically localized prostate cancer. No patients received external beam radiation. All of them underwent LDR prostate brachytherapy. Biochemical failure was defined according to the "Phoenix consensus". Patients were stratified as low and intermediate risk based on The Memorial Sloan Kettering group definition.

Results: The median follow-up time for these 57 patients was 104 months. The overall survival according to Kaplan-Meier estimates was 88% ($\pm 6\%$) at 5 years and 77% ($\pm 6\%$) at 12 years. The 5 and 10 years for failure in tumour-free survival (TFS) was 96% and respectively ($\pm 2\%$), whereas for biochemical control was 94% and respectively ($\pm 3\%$) at 5 and 10 years, 98% ($\pm 1\%$) of patients being free of local recurrence. A patient reported incontinence after treatment (1.7%). The chronic genitourinary complains grade I were 7% and grade II, 10%. At six months 94% of patients reported no change in bowel function.

Conclusions: The excellent long-term results and low morbidity presented, as well as the many advantages of prostate brachytherapy over other treatments, demonstrates that brachytherapy is an effective treatment for patients with transurethral resection and clinical organ-confined prostate cancer.

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INTRODUCTION

Brachytherapy has rapidly gained popularity as an accepted, effective and safe therapy for localized prostate cancer. There are robust follow-up data beyond 10 years that show similar biochemical control rates to radical prostatectomy and external beam radiotherapy (1-3).

Many patients with preexisting lower urinary tract symptoms have been considered poor candidates for seed implants; however there have been few rigorous studies of the contraindications for brachytherapy. Several authors (4, 5) reported a higher risk of post-implant urinary incontinence in patients with a prior transurethral prostate resection (TURP).

Transurethral prostate resection, developed in 1930, is a surgical procedure which consists on removal of the prostate parenchyma proximal to the verumontanum and distal to the bladder neck as a treatment for urinary obstruction. It is done without penetrating the prostatic capsule. The incontinence rate from TURP alone is low, ranging from 1% to 5% (6).

There has been little research on the safety and effectiveness of low dose rate brachytherapy performed in patients with prior TURP. The objective of the present study was to report the clinical outcome, side-effects and complications after permanent implantation of 125 I seeds for early prostate cancer in patients with a prior TURP with up to 10 years of follow-up.

MATERIALS AND METHODS

Selection of patients

In all, 57 patients with a TURP prior to brachytherapy were treated between January 2001 and December 2005; the median (range) follow-up was 104 (11–154) months. Patients were staged according to the American Joint Committee on Cancer 6th edition clinical staging guidelines (7) using a directed history, physical examination and TRUS. All patients had their serum PSA level measured and Gleason score histological grading. The tumor characteristics are shown in Table-1.

In all patients TURP was done some months before brachytherapy (mean 70 months, range 4–132 months). All patients underwent small or medium not large TURP. The mean prostate volume, as measured by ultrasound before brachytherapy was 36cc (range 12–66cc). The mean resected volume was small (15g). The resected volume was noted at the time of brachytherapy but did not cause any technical problem to the seed implant to get enough tissue (>1cm) at TURP level.

Definition groups

The Memorial Sloan Kettering group definition (8) was used to classify patients into risk groups; low-risk patients were T1c or T2a, with a PSA level of ≤ 10 ng/mL and Gleason score ≤ 6 ; intermediate risk was T2b, PSA level 11–20ng/mL or Gleason score ≤ 7 ; and high risk was $\geq T2c$, PSA

level > 20 ng/mL or Gleason score > 7 , or two intermediate-risk criteria.

Hormonal Therapy

In our patient population, 40% received hormone therapy before brachytherapy; this treatment was prescribed by the urologist, waiting for the definitive brachytherapy treatment. Hormonal therapy was given for 3 months and then stopped. The mean prostatic volume at implantation was 36 (12–66 cc).

Treatment

All patients received brachytherapy alone with I-125. The prescription dose was 145 Gy to the reference isodose (100%) according to the TG-43 (9). The target volume of the implant was the prostate gland plus a 2–5mm peri-prostatic area.

The technique used in the implantation was based on intra-operative planning with real-time dynamic dose calculation with peripheral loading. The implantation technique has been previously described (10, 11).

To decrease rectal toxicity, transperineal hyaluronic acid injection into the peri-rectal fat was used to consistently displace the rectal wall away from the radiation sources in 6 patients. We considered that the increase in distance (mean 2cm along the length of the prostate) would be enough to provide a significant reduction in radiation dose from LDR brachytherapy (12).

Patients were followed with symptom assessment and PSA determinations every 3 months for the first year, every 6 months for the second year and yearly thereafter.

Toxicity

Morbidity was reported according to the Common Terminology Criteria for Adverse Events (CTCAE 4.0). Toxicity and sexual side effects was scored by the physician.

Statistical considerations

Distant metastases disease was defined by an imaging study or physical examination that demonstrated cancer outside of the prostate and its regional nodes. Failure in tumor-free survival (TFS) analyses was represented as detection of

Table 1 - Patient and tumor characteristics (n=57).

Characteristics	N° Patients (%)
Stage	
≤T2a	50 (88%)
T2b	7 (12%)
Gleason score	
≤6	48 (84%)
=7	8 (14%)
>7	1 (2%)
Pretreatment PSA (ng/mL)	
≤10	41 (72%)
10.1-20	15 (26%)
>20	1 (2%)
Mean: 9/Median 8 (1.4-47)	
Adjuvant hormonal ablation	
Yes	23 (40%)
No	34 (60%)
Age at diagnosis (year)	
≤60	12 (12%)
61-70	25 (44%)
>70	25 (44%)
Risk Level	
Low Risk	48 (84%)
Intermediate Risk	8 (14%)
High Risk by PSA	1 (2%)
Gland Vol. Implant (cc): Mean: 36/Median 35 (12-66)	

local and/or systemic tumor relapse. Biochemical failure was defined according to the “Phoenix definition” (13) consensus panel statement. Estimated likelihood of events was calculated by the Kaplan-Meier method from the time of completion of brachytherapy procedure. The statistical significance of the difference between estimated event-free curves was calculated with the Log Rank test. Multivariate analysis was performed using the Cox proportional hazards model (14).

RESULTS

For the entire cohort of 57 patients, 3 had evidence of biochemical relapse, 2 had a clinical

relapse and 1 died from prostate cancer; 6 patients died of other illnesses.

The overall survival according to Kaplan-Meier estimates was 88% ($\pm 6\%$) at 5 years and 77% ($\pm 6\%$) at 12 years. The 5 and 10 years for failure in tumor-free survival (TFS) were 96% and 96% ($\pm 2\%$) respectively, whereas for biochemical control was 94% and ($\pm 3\%$) at 5 and 10 years respectively, 98% ($\pm 1\%$) of patients being free of local recurrence (Figure-1).

Cox proportional-hazards regression revealed no statistical significant association for clinical T stage, Gleason score, pretreatment PSA, age, brachytherapy dose (D90), hormonal ablative treatment and biochemical failure.

The actuarial biochemical control with Gleason score was 95% and 89% for patients with Gleason score of ≤ 6 and 7, respectively ($P=0.4$). The correlation with pretreatment PSA the biochemical control was 97% and 89% for patients with PSA of ≤ 10 and >10 ng/ml, respectively ($P=0.26$).

T stage was not significant ($P=0.38$) for biochemical control (100% for $\leq T2a$ and 93% for T2b).

Mean patient age was 69 years (range 55-77). The actuarial analysis of biochemical control at ages less than 61, 61 to 70 and greater than 70 years demonstrated no significant difference, as younger and older patients benefited equally ($P=0.26$).

The actuarial biochemical control was the same 93% ($P=0.37$), in patients who received hormones and in those who did not.

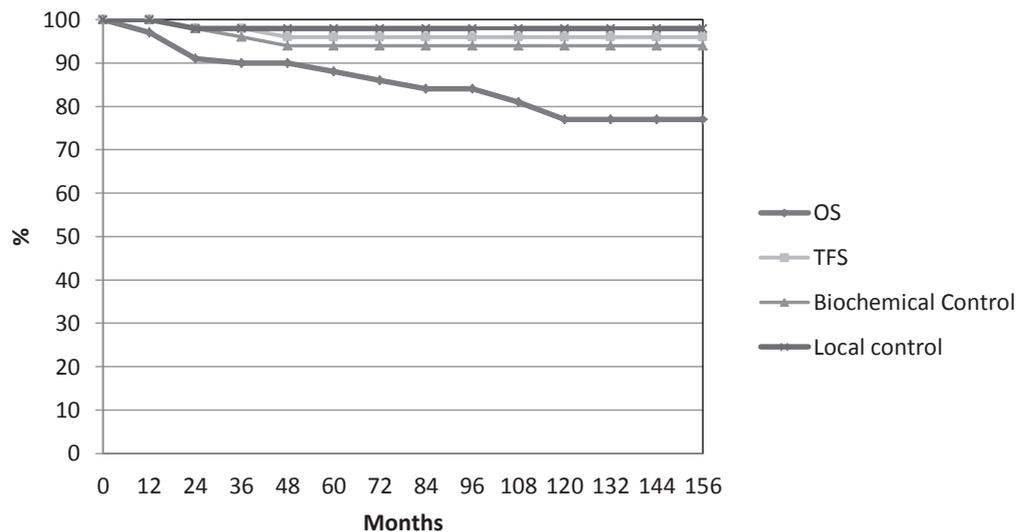
Increasing the dose received by 90% of the prostate volume (D90) from ≤ 160 Gy and >160 Gy was not associated with improved biochemical control ($P=0.37$).

All 57 patients were discharge from the center the same day of the procedure between 6-8 hours of implantation. All patients have been seen in follow-up and the CTCAE toxicity criteria were utilized to score acute and late complications.

Acute and Chronic Urinary Toxicity

Moderate increase in urinary irritation (urethritis) occurred in the third month after treatment; the acute GU grade II toxicity was 9%.

The incontinence rate prior to brachytherapy grade I were 9% (5 patients) and grade II, 3% (2 patients). Only a patient without prior

Figure 1. Actuarial analysis of all 57 patients for overall survival, tumor-free survival and biochemical control and local control.

incontinence reported incontinence after brachytherapy (1.7%). Acute urinary retention was seen in 1 (1.7%) patients, requiring a temporary post-implant bladder catheter. Late urinary retention occurring more than two year after treatment was reported in 1 (1.7%) patients.

The chronic genitourinary complains grade I were 7% and grade II, 10%. A patient had late urethral stricture, requiring urethral dilations.

Lower Gastrointestinal Toxicity

At six months 94% of patients reported no change in bowel function.

The incidence of rectal ulceration and/or recto-urethral fistula (Toxicity grade III-IV) has been observed in 2 patients (3.5%) after rectal biopsy.

Intermittent rectal bleeding was reported in 3 patients (5%). In 6 patients (11%) transperineal hyaluronic acid injection into the peri-rectal fat was used to consistently displace the rectal wall away from the radiation sources; no mucosal damage and no macroscopic rectal bleeding were observed in this group.

No patients with perineal pain were reported.

Sexual function

Of the 17 (30%) patients who were potent preoperatively, 82% were potent postoperative-

ly. Potency was defined as the ability to achieve an erection that was sufficient for intercourse.

DISCUSSION

Our encouraging results are in concordance with the experience of other institutions (15-19). Multivariate and univariate analyses show that the pretreatment PSA level, Gleason score and T stage were not a significant variable for biochemical control. In the present series, hormonal ablative treatment was given for 3-4 months and did not improve biochemical control.

In our series the incontinence grade 1 rate prior to brachytherapy was 9% (5 patients) and grade II, 3% (2 patients) but incontinence chronic toxicity TURP-related after brachytherapy was reported only in a patient (1.7%). Late urethral stricture was reported in 1 (1.7%) patients.

Moran et al. (20) analyzed 171 patients with T1a-T1b prostate cancer who underwent prior TURP. The mean urinary function and bother score for the entire study group was 83.5 ± 19.5 and 82.5 ± 23.7 , respectively. Multivariate analysis revealed higher pretreatment International Prostate Symptom Scores to have significant negative impact on urinary function and bother scores. They concluded that it is feasible LDR brachytherapy in

selected patients with prior TURP, with low impact on urinary function and bother scores.

Wallner et al. (21) in 19 patients reported a 6% incontinence rate in a TURP patient group.

Stone et al. (22) suggest that brachytherapy can be safely performed with a low risk of urinary incontinence if a real-time method combined with peripheral loading is used, but they point out that it could result in a higher risk of urinary incontinence.

Ramirez et al. evaluated urinary incontinence in 16 patients with prior TURP and find lower urinary tract symptoms or urinary incontinence after an average of 30 months (23).

Cesaretti et al. (24) evaluated prostate brachytherapy dosimetry outcomes relative to the transurethral resection of the prostate in 73 patients and they concluded that a visible residual TURP cavity ($\geq 10\%$ of a prostate volume) did not appear to be a statistically significant hindrance to proper dosimetric outcome.

Salembier C et al. (25) evaluated prospectively in a multicenter setting the ability of centers to perform pre-implant permanent prostate brachytherapy planning with dosimetric goals and constraints based on the Groupe de Curiethérapie-European Society for Radiotherapy and Oncology guidelines in patients with prior TURP concluding that it is feasible.

Brachytherapy for patients with a prior TURP and early-stage prostate cancer is effective, with long-term biochemical freedom from recurrence independently of the age of the patients (as younger and older patients benefited equally). The present study showed low toxicity when the dose to any segment of the TURP defects is limited to $\leq 100\%$ of the prescription dose and the actuarial biochemical control was excellent (95% for patients with Gleason score of ≤ 6). The median hospital stay for our patients was 12 h (6-8) h after implantation; there are no other alternative treatments with a shorter hospital stay.

The present complications rates were in accordance with the experience of other institutions using permanent implants of 125 I (19-24) for patients with prior TURP.

In conclusion, with the present long-term data, using intra-operative planning with real-time

dynamic dose calculation with peripheral loading, LDR brachytherapy provides excellent biochemical control rates for patients with localized prostate cancer and prior TURP, and low urinary and gastrointestinal morbidity.

ABBREVIATIONS

AJCC = American Joint Committee on Cancer

D90 = The dose that covers 90% volume of CTV

GU = Genitourinary

PSA = Serum prostate-specific antigen

PTV = Planning target volume

CTAE = Common Toxicity Criteria for Adverse Event.

SPSS = Statistical analysis SPSS

SD = Standard deviations

TFS = Tumour-free survival

TRUS = The trans-rectal ultrasound

TURP = Transurethral prostate resection

LDR = Low dose rate

CONFLICT OF INTEREST

None declared.

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White blood cell counts and neutrophil to lymphocyte ratio in the diagnosis of testicular cancer: a simple secondary serum tumor marker

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ABSTRACT

Purpose: The aim of the study was to investigate white blood cell counts and neutrophil to lymphocyte ratio (NLR) as markers of systemic inflammation in the diagnosis of localized testicular cancer as a malignancy with initially low volume.

Materials and Methods: Thirty-six patients with localized testicular cancer with a mean age of 34.22 ± 14.89 years and 36 healthy controls with a mean age of 26.67 ± 2.89 years were enrolled in the study. White blood cell counts and NLR were calculated from complete blood cell counts.

Results: White blood cell counts and NLR were statistically significantly higher in patients with testicular cancer compared with the control group ($p < 0.0001$ for all).

Conclusions: Both white blood cell counts and NLR can be used as a simple test in the diagnosis of testicular cancer besides the well-known accurate serum tumor markers as AFP (alpha fetoprotein), hCG (human chorionic gonadotropin) and LDH (lactate dehydrogenase).

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INTRODUCTION

All of the cells involved in the immune response are formed via differentiation from pluripotent hematopoietic stem cells of the bone marrow. From common lymphoid progenitor cells among early stage precursor cells, T and B cells of the adaptive immune system and natural killer (NK) cells of natural immunity differentiate. Common myeloid progenitor cell which is another early stage precursor cell firstly differentiates into granulocyte/macrophage cells, then through a series of differentiation phases, they induce formation of dendritic cell, granulocytes (neutrophils, eosinophils, basophils and mast cells), monocyte macro-

phage cells involved in natural immunity. Immune response triggered against various components of microorganisms, macromolecules as protein and polysaccharides or even small chemical components can protect the organism or even lead to deleterious outcomes. As a criteria for the degree of natural and adaptive immune response against antigenic formations as cancer cells, neutrophil to lymphocyte ratio (NLR) can be used.

Testicular cancer (Ca) is a relatively rare disease, accounting for one percent of all neoplasms in men. Despite its low incidence, the investigation of testicular cancer is important because this malignancy occurs in a relatively young male population of 15-35 years. The fact that testicular

cancer is the most curable adult solid tumor, irrespective of the tumor spreading, opens the question of immunological influence on such a favorable outcome. Reports on immunocompetence in testicular cancer patients are scarce.

The role of NLR in the evaluation of progression-free survival and pre-and postoperative treatment of various oncological cases including urogenital and non-urogenital tumors have been investigated in many studies. In our study, preoperative NLR's and neutrophil counts of patients with localized testicular tumors and that of varicocele patients who were included in the study as a control group, were compared. In this pioneer study, we investigate the role of NLR and neutrophil counts in localized testicular Ca in patients with low tumor volume.

MATERIALS AND METHODS

This retrograde study was performed on 72 male (36 testicular cancer, and as a control group 36 varicocele patients) patients. Informed consent forms were taken and institutional review board was approved from hospital ethics committee. Age, number and percentage of neutrophils and lymphocytes, hemoglobin levels and NLR's (neutrophil count divided by the number of lymphocytes; neutrophil-lymphocyte ratio was calculated) in peripheral blood samples obtained during preoperative period were analyzed in detail and subjected to statistical analysis. Patients with an evidence of concomitant infection or inflammation were excluded from the study.

Statistical analysis

For statistical analysis, NCSS (Number Cruncher Statistical System) 2007&PASS (Power Analysis and Sample Size) 2008 Statistical Software (Utah, USA) program was used. Study data were evaluated using descriptive statistical methods (mean, standard deviation, minimum, maximum, median, frequency, and ratio). In the intergroup comparisons of quantitative data, for parameters demonstrating normal distribution Student t test and, for those without normal distribution Mann Whitney U test were used. Statistical significance

was rated at $p < 0.01$ and $p < 0.05$ respectively. The areas under the receiver operating characteristic curves (ROC) were used to assess the discriminative ability of NLR and neutrophil counts in localized testicular Ca.

RESULTS

Thirty-six patients with localized testicular cancer with a mean age of 34.22 ± 14.89 years and 36 healthy controls with a mean age of 26.67 ± 2.89 years were enrolled in the study. The pathological subtypes of the study group consisted of seminomas (n: 6), mixt germ cell carcinoma (n: 18), embryonic carcinoma (n: 7), teratomas (n: 4) and Leydig cell carcinoma (n: 1). Localized testicular Ca is defined as; up to pT2 N0 M0. Ages and blood values and their distribution with respect to groups are shown in Tables 1 and 2, respectively. Statistically and extremely significant intergroup differences were found as for neutrophil counts and percentages ($p = 0.001$ and $p < 0.01$ respectively). Neutrophil counts and percentages noted in patients with testicular tumors were significantly higher when compared with varicocele patients. Neutrophil percentages were statistically and extremely different between both groups ($p = 0.001$ and $p < 0.01$ respectively). Neutrophil percentages of patients with testicular tumors were statistically significantly higher than those of the patients with varicoceles. Lymphocytic measurements did not show a statistically significant difference between groups ($p > 0.05$). A statistically and extremely significant intergroup difference was detected between percentages of lymphocytes ($p = 0.001$ and $p < 0.01$ respectively). Percentages of lymphocytes in patients with testicular tumors were significantly lower. A statistically and extremely significant difference was found between groups as for NLR's of the cases ($p = 0.001$ and $p < 0.01$ respectively). NLR's in cases with testicular tumors were significantly higher than in patients with varicocele (Figure-1). A statistically and extremely significant difference was detected between groups with respect to estimated NLR ($p = 0.001$ and $p < 0.01$ respectively). NLR's in patients with testicular tumors were significantly higher relative to those seen in patients with varicoceles. A sta-

Table 1 - Distribution of age and hematological values.

	Min-Max	Mean±SD	
Age (years)	17.0-48.0	30,44±11,31	
Neutrophil counts (K/μL)	2.6-11.0	4.99±1.81	
Neutrophil ratio (%)	38.9-84.6	60.71±10.55	
Lymphocyte counts (K/μL)	0.9-3.7	2.22±0.68	
Lymphocyte ratio (%)	10.3-47.8	28.59±9.07	
Neutrophil/lymphocyte ratio (%)	0.87-8.50	2.58±1.60	
Neutrophil/lymphocyte ratio (%)	0.86-8.21	2.56±1.57	
Body mass Index (kg/m ²) (n=36)	20.3-35.0	25.71±3.39	
Hemoglobin (gr/dL)	12.1-17.5	14.89±1.09	
B-HCG (mIU/mL) (n=32)	0.5-1987.0	137.17±459.89	
AFP (U/mL) (n=32)	1.2-1656.0	111.70±342.18	
LDH (U/L) (n=32)	106.0-954.0	194.19±146.49	
	n	%	
Group	Testicular tumor	36	50
	Varicocele	36	50

Table 2 - Intergroup comparisons of patients' ages and hematological values.

	Testicular tumor (n=36)	Varicocele (n=36)	a _p
	Mean±SD	Mean±SD	
Neutrophil counts (K/μL)	5.78±1.93	4.21±1.27	0.001**
Neutrophil ratio (%)	64.84±11.04	56.60±8.31	0.001**
Lymphocyte counts (K/μL)	2.11±0.74	2.33±0.59	0.16
Lymphocyte ratio (%)	25.06±8.77	32.11±8.03	0.001**
Hemoglobin (gr/dL)	14.68±1.08	15.10±1.07	0.099
Neutrophil/lymphocyte ratio; (Median)	3.18±1.76 (2.7)	1.99±1.17 (1.8)	^b 0.001**
Neutrophil/lymphocyte ratio (%) (Median)	3.12±1.72 (2.6)	2.01±1.18(1.7)	^b 0.001**

^aStudent-t Test

^bMann-Whitney U Test

**p<0.01

tistically significant difference between groups as for hemoglobin levels was not detected; hemoglobin measurements in patients with testicular tumors were notably lower than those of varicocele patients (p=0.099 and p>0.05 respectively). The area under ROC curve for NLR in localized testicular cancer patients was 0.74, (95% confidence

interval (CI)=0.63-0.86), with a threshold value of 2.06 and sensitivity =69 % and specificity=69%. The area under ROC curve for neutrophil counts in localized testicular cancer patients was 0.76, (95% confidence interval (CI)=0.65-0.87), with a threshold value 4.40(K/μL) and sensitivity=80% and specificity=66% (Figure-2).

Figure 1 - Distribution of neutrophil/lymphocyte ratios between groups.

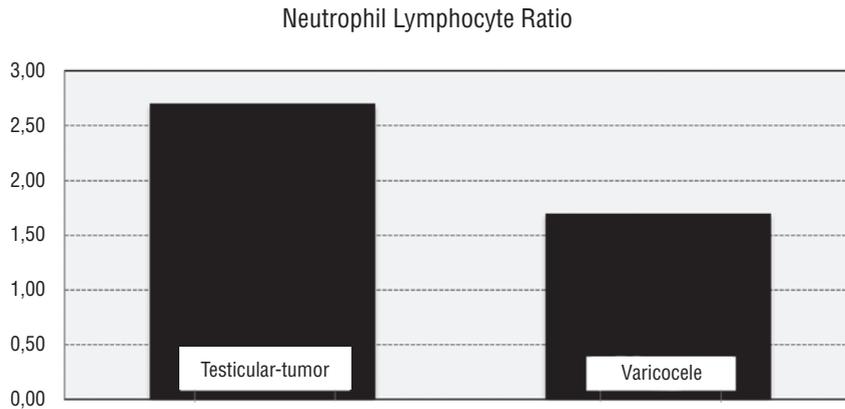
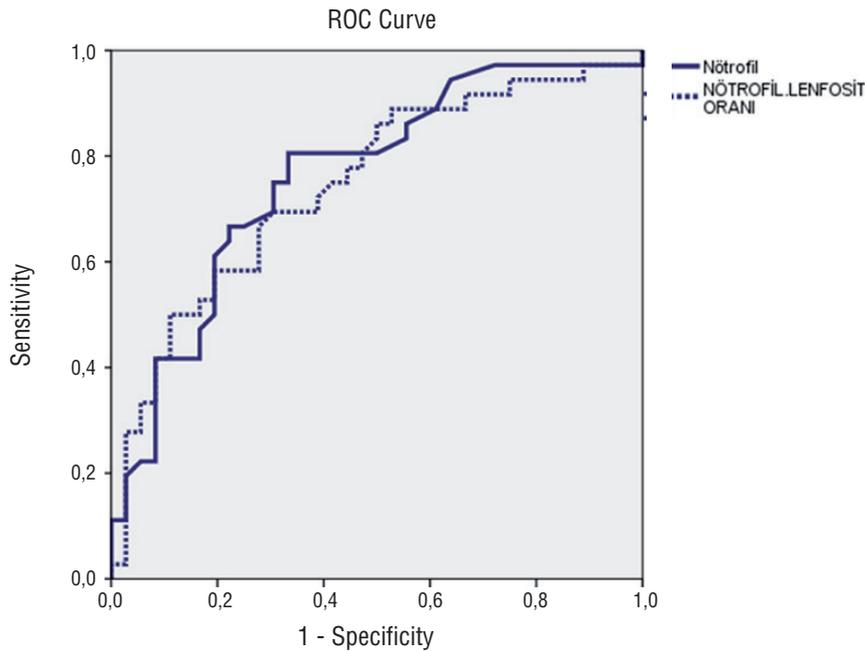


Figure 2 - ROC of NLR and neutrophil counts in localized testicular cancer.



DISCUSSION

Systemic inflammation can be measured by a variety of biochemical and hematological markers. Total leukocyte, neutrophil and lymphocyte count have been used as a marker of inflammation for many years. Neutrophils mediate inflammation by various biochemical mechanisms such as release of arachidonic acid metabolites and platelet aggravating factors (1). Neutrophilia could represent a consequence of ectopic production of

myeloid growth factors as part of a paraneoplastic syndrome (2) or, more likely, a nonspecific response to cancer-related inflammation secondary to tissue destruction and cytokine releases. Experimental data indicate that activated neutrophils may directly and indirectly stimulate tumor growth (3). Lymphopenia is associated with cortisol induced stress response (1). NLR and platelet to lymphocyte ratio (PLR) have also been shown to be reliable markers of systemic inflammation that were provided by many studies (4). Cells with

anti-tumoral activities belong to a wide spectrum including neutrophils, type-2-macrophages, plasmacytoid dendritic cells, suppressive cells derived from myeloid tissue, and mediator T cells (5).

Inflammation plays a critical role in many aspects of cancer, including tumor development, progression, clinical presentations and prognosis (6). The systemic inflammatory and immune responses to tumor cells and tumor cell-secreted peptides vary with the type and extent of malignancy. The tumor/host interaction may have significant influence on patients' outcome. However, this effect is generally not taken into account in current prognostic systems. There is now accumulating evidence that the markers of the systemic inflammatory response, including cytokines, C-reactive protein (CRP), albumin, serum amyloid A and white cell count, can be independent prognostic factors in cancer patients (6). Immune system has a dual function both in the development and progression of cancer. It can destroy tumor cells and on the other hand it can promote growth of active malignant cells, their invasive capacities and metastatic abilities. Excess number of neutrophils in the circulation has been conceived to have an important role in the tumor progression and angiogenesis. Therefore increased neutrophil counts should be related to poor prognosis (7).

Presence of cancer-related systemic inflammatory response has been evaluated in various oncological diseases including renal cell carcinoma, upper urinary tract cancers, bladder cancer, prostate cancer and many studies have demonstrated its effective role in the prediction of surgical margin positivity during postoperative period, and also progression-free survival.

Limited numbers of reports are available on immune resistance in patients with testicular cancer. Considerable evidence supports the view that the biological behavior of tumors and in particular, their capacity to metastasize are in part determined by immunological factors requiring participation of T lymphocytes, B lymphocytes, macrophages and natural killer cells. Immunological reactivity has been analyzed in a wide spectrum of solid tumors and a vast literature indicates a correlation between depressed cell-mediated immunity and the stage of the disease. On the

contrary, there is little evidence about the role of immunological factors in the development and spread of testicular tumors.

In one of the recent studies at this topic, age, female gender, NLR and platelet counts were found to be invasive determinants of urothelial carcinoma. In this study, threshold value for NLR was accepted as 2.5 (8). In our study, NLR in the group with testicular cancer was estimated as 3.18 ± 1.76 (20.7).

In another study, NLR has been indicated as a potentially important criterion in urothelial carcinoma for the detection of extravesical disease (9). Also, NLR has been suggested as one of the factors indicating poor prognosis.

In a study which established cut-off value of NLR as 2.7, the authors indicated that combination of T stage, and NLR could be used for the stratification of recurrence risk in patients with non-metastatic renal cell carcinomas (10). Higher NLR before treatment has been related to poor prognosis for various types of cancer including renal cell carcinoma. In a study on metastatic renal cell carcinoma, it has been reported that decreased NLR might be a criterion for progression-free survival, and treatment response and also an indicator of the balance between host immunity and cancer-related inflammation (11).

Similar studies have been also performed in non-metastatic upper urinary system cancers. In these studies performed using directly neutrophil counts, the value of relevant data for patient counseling and identification of patients with poor prognosis for neoadjuvant chemotherapy has been indicated (12).

Unlike the present one, in previous studies the study groups involved the same oncologic diagnosis within different stages to evaluate the treatment success and as a prognostic factor, however our study design structured in mostly composed of low stage testis cancer patients and had a control group. Thus we claimed that NLR can be added as a helper diagnostic marker of testis cancer. In the unique study with a similar design that analyzed C-reactive protein levels in testicular cancer, the authors demonstrated that C-reactive protein in testicular cancer can be an important marker in the prediction of the development of

secondary non-germ cell cancer which has been accepted as a late-term complication of testicular tumor (13).

An association between high NLR and increased mortality or recurrence has been observed in various solid organ tumors, including lung, pancreatic, hepatocellular and cholangiocarcinoma (14–17).

Testicular cancer is an endocrine malignancy like thyroid cancer. It was reported that higher NLR was associated with increased tumor size and high ATA risk of recurrence in patients with differentiated thyroid cancer (18).

Neutrophils are the first line of natural immune defense against inflammation. Neutrophils may lead to increased endothelial permeability by releasing vasoactive and cytotoxic agents such as reactive oxygen species and digestive proteases during inflammation. Neutrophils contribute to IL-2 induced vascular leak syndrome. It has been also reported that vascular endothelial growth factor (VEGF) has positive correlations with neutrophil and NLR and negative correlation with lymphocyte count (19). Since neutrophils play a dominant role in inflammation, NLR is thought to predict inflammation better than PLR. NLR and PLR are simple and cost effective markers of inflammation when compared with other inflammatory markers such as ILs and TNF- α .

CONCLUSIONS

As we know, localized testicular tumor has a shorter time interval between cancerogenic effect and formation of a macroscopic tumor, relative to other solid tumors and earlier increase in the number of neutrophils. Low cost, easy accessibility and reproducibility of a whole blood count are the other factors that promote its use in clinical practice. This study indicates the potential usefulness of a new predictor of the disease. The limitations of our study were that it was a retrospective one with limited study group and had not a prognostic predictive design. Larger, randomized controlled studies are needed at this field.

CONFLICT OF INTEREST

None declared.

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Incidence of sepsis following transrectal ultrasound guided prostate biopsy at a tertiary-care medical center in Lebanon

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ABSTRACT

Background: Urosepsis is a rare but life-threatening complication following transrectal ultrasound (TRUS) guided needle prostate biopsy. Despite the technological and pharmacological improvements, the problem of bacterial urosepsis after prostate biopsy remains. A strategy for preventing urosepsis following TRUS prostate biopsy in areas with high prevalence of resistant strains or patients presenting risk factors is lacking.

Objectives: The aim of this study was to assess the prevalence of urosepsis, as well its predictors, following TRUS guided needle biopsy of the prostate in a tertiary care medical center in Lebanon.

Materials and Methods: We carried out a retrospective study on all patients who underwent TRUS prostate biopsy at the American University of Beirut Medical Center between January 1, 2011 and June 31, 2013. Patients' hospital charts were reviewed. Data collected included demographic information, pre-procedure disease specific information, as well as post-procedure information. Predictors of urosepsis following TRUS were assessed.

Results: In total, 265 patients were included in this study, where the prevalence of urosepsis following TRUS prostate biopsy was found to be 9.4%. The significant independent predictors of urosepsis were found to be: age with an OR=0.93 (95% CI: 0.88–1.00, p-value=0.03), and hypertension comorbidity with an OR=3.25 (95% CI: 1.19–8.85, p-value=0.02).

Conclusion: We found a high prevalence of urosepsis among patients who have undergone TRUS prostate biopsy, and identified two significant risk factors. The results of this study highlight the importance of implementing strategies for prevention of urosepsis following TRUS prostate biopsy.

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INTRODUCTION

Prostate cancer is the second most commonly diagnosed cancer in men and represents a significant health problem (1). A total of 233,000 new cases of prostate cancer and 29,480 deaths from the disease are anticipated in the United States in 2014 (2). The highest incidence rates of prostate cancer are

reported in Australia/New Zealand, Western and Northern Europe and North America, largely because of the availability of screening programs and the widespread use of prostate-specific antigen (PSA) testing in those regions (3). In Lebanon, the incidence rates for prostate cancer increased during the period 2003–2008 from 29.9 to 39.2 cases per 100,000, and became the most-reported cancer in 2008 (4).

Transrectal ultrasound (TRUS)-guided prostate biopsy remains the gold standard technique to confirm the diagnosis of prostate cancer (5). According to recent estimates, approximately more than 1 million TRUS biopsies are performed per year in Europe and the United States (6). Although TRUS biopsy is generally considered to be a relatively low-risk outpatient procedure, post-biopsy complications and hospital admissions have increased at alarming rates during the last decade due to an increasing rate of infection related complications (7). TRUS biopsy complication rates are reported in up to 50% of cases and range from minor complications, such as hematuria, hematospermia or rectal bleeding, acute urine retention to much more severe complications, such as anemia, fainting, febrile urinary infections, syncope, and even septic shock. The infectious complications, which range from bacteriuria to sepsis, affect 1-4% of the patients who undergo this procedure (8). One study from Ontario, Canada reported that the hospital admission rate for infection-related complications within 30 days of the procedure increased from 1.0% in 1996 to 4.1% in 2005 (9). The reported incidence of urinary tract infections (UTI) after TRUS biopsy typically ranges between 2% and 6% with approximately 30%-50% of these patients having accompanying bacteremia (10). Severe sepsis has been described in 0.1%-3.5% of cases after TRUS biopsy (9). The proposed mechanism of infection is likely the introduction of bacteria into the bladder and bloodstream from the rectum (11). The most common organism responsible for these infectious complications is *E. coli* (7, 12). Moreover, the spread of multiresistant *E. coli* is of particular concern (13). Resistant bacteria are more prevalent in some countries. Antibiotic overuse or misuse has been blamed, but a wider dissemination of resistant organisms resulting from globalization and international travel may also be a factor (13, 14).

Factors that may predict which men are at greatest risk of infectious complications are: underlying medical comorbidities, particularly diabetes mellitus, presence of urethral catheter, and recent hospitalization (15, 16).

Infectious complications can be serious, requiring effective preventative strategies and prompt management. Different methods have

been studied for reducing the rate of infection following TRUS guided biopsy such as the use of prophylactic antibiotic, bowel cleansing enema, and using disposable instruments. Antibiotic prophylaxis is the only measure that has been shown to reduce the rate of infection post TRUS in randomized controlled trial setting, (17, 18). The American Urological Association (AUA) and the European Association of Urology guidelines for antibacterial prophylaxis for TRUS prostate biopsies recommend fluoroquinolones as agents of first choice due to their broad spectrum of activity, excellent penetration into prostatic tissue, and their prolonged post-antibiotic effect (19). The AUA guidelines also recommend aminoglycosides or aztreonam with metronidazole or clindamycin as alternatives to fluoroquinolones (17).

Fluoroquinolone-resistant *E. coli* is emerging globally (20). This poses a clinical challenge to the urologists to tailor the prophylactic regimen according to the resistance pattern in their hospitals. Multiple studies pointed toward the feasibility of using rectal swab culture to guide the prophylactic antibiotic regimen (21). In a survey of 3355 urologists in United States of America, Joel et al. reported 14 different duration of treatment using 10 different classes of antibiotic (22).

The aim of this study was to assess the prevalence of urosepsis following transrectal ultrasound guided needle biopsy of the prostate, as well as its predictors in a tertiary-care medical center in Lebanon.

MATERIALS AND METHODS

Study design and setting

We carried out a retrospective chart review on all patients who underwent TRUS prostate biopsy at the American University of Beirut Medical Center between January 1, 2011 and June 31, 2013.

Inclusion/exclusion criteria

Patients eligible to be included in the study were those undergoing TRUS prostate biopsy, and who had no clinical evidence of prostatitis and had a negative urine culture prior to the biopsy.

Ethical considerations

The institutional review board at the American University of Beirut Medical Center approved the study.

Procedure

During the study period, TRUS prostate biopsy was performed by four urologists in the Radiology Department at the American University of Beirut Medical Center. All patients received prophylactic antibiotics. The adopted regimen by all urologists consisted of a flouroquinolone orally to be started one day prior to the procedure and gentamicin IV or IM injection 30 minutes before the procedure. The procedure was performed while the patient was in the left lateral decubitus position. The anus and perineum were wiped with iodine swabs. A 5 to 9 MHz probe covered by a sterile condom and sterile K-Y Gel was introduced into the rectum and used to measure the prostate size and guide the local anesthesia injection and needle biopsies. An 18 French disposable gun and needle were used, in comparison to previous years, where we used a re-sterilizable automatic gun with a disposable needle. A standard sextant set of biopsies were taken, with the addition of targeted cores as needed to any suspicious lesion.

Outcome

The endpoint in our study was the development of urosepsis after TRUS prostate biopsy. Urosepsis was defined as urinary symptoms, leukocytosis, and/or fever more than 38.0°C orally.

Data collection

Other information collected in this study included demographic information (such as age), lifestyle information (such as smoking), comorbidities (such as cardiac, hypertension, and diabetes), and pre-procedure disease specific information (including prostate size, post-void residual (PVR), PSA value and ratio, presenting urinary symptoms, recent treatment with antibiotic, positive urine analysis, bowel preparation, and previous biopsies). Moreover, post-procedure information were also collected, and included: positive urine analyses, urine culture and the bacteriology, as well as days to develop urosepsis.

Statistical analyses

Data entry and statistical analyses were performed using the Statistical Package for Social Sciences (SPSS), version 21.0. Descriptive analyses were carried out by reporting the number and percent for categorical variables, whereas the mean and standard deviation were calculated for continuous ones. Associations between the different risk factors and the development of urinary tract infection were assessed using the chi-square test for categorical variables or the student's t-test for continuous ones.

To account for the potential confounding effect of the different risk factors on the development of the outcome, multivariate logistic regression analyses were carried out. Included in the model were the multiple variables that could affect urinary infection post biopsy. These variables included: age, smoking status, co-morbidities (such as hypertension and diabetes), prostate size, previous urine analysis, antibiotics use, and mechanical bowel prep.

RESULTS

The demographic characteristics, as well as the pre-and post-procedure information for the total sample (n=265) are presented in Table-1. The average age of the patients was 64.4 years (sd=7.9), where almost half of them were nonsmokers (53.9%). The prevalence of cardiac diseases, hypertension, and diabetes were 13.6%, 38.6%, and 15.2%, respectively. The prostate size prior to the procedure was found to be 49.9 (sd=29.2) and the PSA ratio was 0.2 (sd=0.1). Overall, 58 patients received antibiotics before the prophylactic dose for their lower urinary tract symptoms or as empirical treatment of high PSA (21.9%) and 26.8% had bowel preparation. As for the outcomes, the incidence of urosepsis was 9.4% (25 patients). The urine culture was positive for 18 patients (6.8%), where 13 had E. coli resistance (72.2%). Three patients had E.coli sensitive (16.7%) and 2 patients had Klebsiella pneumoniae (11.1%). The average number of days to develop sepsis was 7.6 days (sd=18.2).

Table 1 - Baseline characteristics of the population.

Variables		n (%)
Total sample		N=265
Demographic	Age	Mean (\pm sd) 64.4 (\pm 7.9)
Lifestyle	Smoking	Non smoker 117 (53.9%)
		Smoker 52 (24.0%)
		Ex-smoker 48 (22.1%)
Comorbidities	Cardiac	No 228 (86.4%)
		Yes 36 (13.6%)
	HTN	No 162 (61.4%)
		Yes 102 (38.6%)
	Diabetes	No 224 (84.8%)
		Yes 40 (15.2%)
Pre-procedure	Prostate size	Mean (\pm sd) 49.9 (\pm 29.2)
	PVR	Mean (\pm sd) 53.5 (\pm 83.5)
	PSA number	Mean (\pm sd) 50.0 (\pm 379.7)
	PSA ratio	Mean (\pm sd) 0.2 (\pm 0.1)
	Symptoms	No 107 (40.4%)
		Yes 158 (59.6%)
	Antibiotics	No 207 (78.1%)
		Yes 58 (21.9%)
	Urine analysis(positive)	No 252 (95.1%)
		Yes 13 (4.9%)
	Enema	No 194 (73.2%)
		Yes 71 (26.8%)
	Biopsy	No 246 (92.8%)
		Yes 19 (7.2%)
Post-procedure	Urine analysis(positive)	No 243 (91.7%)
		Yes 22 (8.3%)
	Sepsis	No 240 (90.6%)
		Yes 25 (9.4%)
	Urine culture	No 247 (93.2%)
		Yes 18 (6.8%)
	Urine culture (bacteriology)	E.choli sensitive 3 (16.7%)
		E.choli resistant 13 (72.2%)
		Klebsiella Pneumoniae 2 (11.1%)
	Hospital admission	No 245 (96.1%)
		Yes 10 (3.9%)
Days to develop sepsis	Mean (\pm sd) 7.6 (\pm 18.2)	

Table-2 summarizes the association between the different patient characteristics and the development of urosepsis. Patients who developed urosepsis were younger (mean=62.8 years, sd=6.3 versus non-septic patients whose age was 64.5, sd=8.0), and more likely to be smokers (33.3% versus non-septic patients, 2.8%), although the association was not significant for both characteristics, p-value=0.31 and 0.51, respectively. Patients who developed urosepsis were more likely to be hypertensive (64.0%) compared to the non-septic ones (36%), with a p-value of 0.006. Simi-

larly, uroseptic patients were more likely to be diabetic (32.0%) as compared to non-septic patients (13.4%), p-value=0.03. As for the pre-procedure information, none of the assessed characteristics was found to be significantly different between the uroseptic and non-septic patients. For instance, uroseptic patients were less likely to have had bowel preparation (16.0%) as compared to the non-septic ones (27.9%), p-value=0.2.

Finally, Table-3 summarizes the results of the multivariate analyses carried out to identify the predictors of urosepsis among patients

Table 2 - Association between different factors and urosepsis

Variables			All n (%)	No sepsis n (%)	Sepsis n (%)	P value
Total sample			N=265	N=240	N=25	
Demographic	Age	Mean (±sd)	64.4 (±7.9)	64.5 (8.0)	62.8 (6.3)	0.31
Lifestyle	Smoking	Non smoker	117 (53.9%)	106 (54.9%)	11 (45.8%)	0.51
		Smoker	52 (24.0%)	44 (2.8%)	8 (33.3%)	
		Ex-smoker	48 (22.1%)	43 (22.3%)	5 (20.8%)	
Comorbidities	Cardiac	No	228 (86.4%)	207 (86.6%)	21 (84.0%)	0.76
		Yes	36 (13.6%)	32 (13.4%)	4 (16.0%)	
	HTN	No	162 (61.4%)	153 (64.0%)	9 (36.0%)	0.006
		Yes	102 (38.6%)	86 (36.0%)	16 (64.0%)	
	Diabetes	No	224 (84.8%)	207 (86.6%)	17 (68.0%)	0.03
		Yes	40 (15.2%)	32 (13.4%)	8 (32.0%)	
	Prostate size	Mean (±sd)	49.9 (±29.2)	49.3 (±27.9)	54.8 (±39.8)	0.39
	PVR	Mean (±sd)	53.5 (±83.5)	54.8 (±88.4)	45.0 (±36.2)	0.60
	PSA number	Mean (±sd)	50.0 (±379.7)	50.7 (±396.0)	44.4 (±179.2)	0.94
	PSA ratio	Mean (±sd)	0.2 (±0.1)	0.2 (±0.1)	0.2 (±0.1)	0.14
	Symptoms	No	107 (40.4%)	101 (42.1%)	6 (24.0%)	0.08
		Yes	158 (59.6%)	139 (57.9%)	19 (76.0%)	
Pre-procedure	Antibiotics	No	207 (78.1%)	188 (78.3%)	19 (76.0%)	0.79
		Yes	58 (21.9%)	52 (21.7%)	6 (24.0%)	
	Urine analysis	No	252 (95.1%)	228 (95.0%)	24 (96.0%)	1.00
		Yes	13 (4.9%)	12 (5.0%)	1 (4.0%)	
	Enema	No	194 (73.2%)	173 (72.15)	21 (84.0%)	0.20
		Yes	71 (26.8%)	67 (27.9%)	4 (16.0%)	
	Biopsy (previous)	No	246 (92.8%)	222 (92.5%)	24 (96.0%)	1.00
		Yes	19 (7.2%)	18 (7.5%)	1 (4.0%)	

Table 3 - Multivariate analysis for the predictors of urosepsis (Cox regression model).

Predictors		Adjusted OR (95%CI)	P value
Age		0.93 (0.88 – 1.00)	0.03
Smoking	Non smoker	Reference	Reference
	Smoker	1.56 (0.55-4.45)	0.40
	Ex-smoker	0.95 (0.30-3.07)	0.93
Cardiac		0.92 (0.27-3.19)	0.90
HTN		3.25 (1.19-8.85)	0.02
Diabetes		2.18 (0.79-6.01)	0.13
Prostate size		1.00 (0.99-1.02)	0.71
Symptoms		1.83 (0.66-5.04)	0.24
Enema		0.55 (0.17-1.79)	0.32

who underwent TRUS prostate biopsy. Age was found to be a significant predictor, where older patients are less likely to have urosepsis (OR=0.93, 95% CI: 0.88-1.00, p-value=0.03). Hypertension status was also found to be significantly associated with urosepsis (OR=3.25, 95% CI: 1.19-8.85, p-value=0.02). Smokers, diabetics, and patients with symptomatic presentation were at higher chance of developing urosepsis. On the other hand, patients who had bowel preparation or cardiac disease were less likely to develop urosepsis; but none of these associations was statistically significant.

All patients who developed urosepsis were admitted to the hospital for intravenous antibiotic and monitoring. All patient received carbapenem antibiotic for 14 days at least. The average number of days of hospitalization was 5.

DISCUSSION

TRUS prostate biopsy is the standard test to diagnose prostate cancer after a suspicious digital rectal exam and elevated PSA. Occasionally this procedure is associated with significant morbidity. In this retrospective study, charts of patients who underwent TRUS prostate biopsy at the American University of Beirut Medical Center between January 1, 2011 and June 31, 2013 were reviewed to assess the prevalence of urosepsis following transrectal

ultrasound (TRUS) prostate biopsy, as well as its predictors. We found that the prevalence of (TRUS) prostate biopsy urosepsis to be 9.4%. Multivariate analysis identified age and hypertension comorbidity to be significantly associated with an increased risk of developing urosepsis following TRUS prostate biopsy.

The prevalence of urosepsis following TRUS prostate biopsy found in our study (9.4%) was higher than that reported in other studies. The frequency of urosepsis varied among those studies between 0.2% and 3.06%. The lowest rates of urosepsis were reported by Zaytoun et al. in a North American cohort, and Raaijmakers et al. in a European Randomized Study, who reported urosepsis prevalence rates of 0.2%, and 0.5%, respectively (17, 23). However, other series of studies carried out by Carmignani et al., Akduman et al., and Simsir et al. in reported higher rates of urosepsis (2.2%, 3.0%, and 3.06%, respectively) (16, 24, 25). Only one Asian study conducted by Raheem et al. in 2012 reported no septic complications (26). This variation in rates of sepsis among different studies arises from differences in biopsy techniques, prophylactic protocols, consistent reporting, and the definition of urosepsis used. The prophylactic regimes preventing infectious complications may differ with respect to the use of an antibiotic (type of antibiotic used, dose, and method of administration and duration of the therapy), as well as

whether a cleansing rectal enema was used or not (24). Other factors that may increase the antibiotic resistance and thus increasing the rate of sepsis by an antibiotic resistant strain are past history of hospitalization, a past history of exposure to antibiotics, a past history of catheterization, and a past history of urogenital surgery (27, 28). The proponents of the high sepsis rate in our study are cited as following: the high prevalence of *E. coli* resistant to fluoroquinolone in Lebanon which was noted in a recent review of the patterns and trends of bacterial resistance to antimicrobial agents over the last decade in our center, self-medication with antibiotics which is a frequent problem in Beirut area, polypharmacy in patients with co-morbidities which may affect the antibiotic efficacy (29, 30).

As for the risk factors significantly associated with urosepsis following TRUS prostate biopsy, age was found to be a significant risk factor OR=0.93 (95% CI: 0.88–1.00, p-value=0.03), where older patients were less likely to have urosepsis. One possible reason may be that younger patients are more likely to self-report complications compared to older patients. Few studies in the literature reported on the effect of age on sepsis after TRUS prostate biopsy. In a study carried out by Lee et al. between 2003 and 2006 reported no significant difference in the urosepsis rate in relation to age (p-value=0.82) after TRUS prostate biopsy (29). However, several studies reported on higher incidence of general complications after TRUS biopsy in younger patients (30).

Another factor found to be significantly associated with urosepsis following TRUS prostate biopsy in our study was hypertension comorbidity with an OR=3.25 (95% CI: 1.19-8.85, p-value=0.02). The study carried out by Lee et al. did not report any significant association between hypertension and sepsis following TRUS prostate biopsy (p-value=0.18) (29). Further comparison of this association with the literature is hard due to the limited availability of studies assessing this association.

Although many urologists intuitively assume that increased post void residue would predispose to development of post TRUS infec-

tion, clear evidence is lacking as well. Our data did not show any significant difference in the rate of urosepsis among patients with post void residue verses those with no significant residue.

Finally, we should emphasize the limitations of this study, including the small number of patients. In addition, the clinical results were analyzed retrospectively based on charts review.

Notwithstanding those limitations, this study elucidates of the impact of increasing bacteria resistance prevalence on the rate of post TRUS urosepsis.

CONCLUSIONS

Urosepsis after TRUS biopsy represents a great challenge for urologists; sometimes its risks are more important than its benefits. It tips the balance between the risk and the benefit of prostate screening. Implementation of new strategies to prevent urosepsis and early treatment is required; especially in areas where bacterial resistance is endemic. We are considering revisiting our prophylactic regimen in our institution, and developing a follow-up system in which the patients will be contacted every 48 hours by a physician assistant to be screened for early urosepsis symptoms. Larger studies that explore the risks of urosepsis after TRUS biopsy are required. Moreover, identifying biomarkers that are associated with developing urosepsis after TRUS biopsy represents a unique research opportunity.

CONFLICT OF INTEREST

None declared.

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Ischemia modified albumin: does it change during pneumoperitoneum in robotic prostatectomies?

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ABSTRACT

Background: The unique positioning of the patient at steep Trendelenburg with prolonged and increased intra-abdominal pressure (IAP) during robotic radical prostatectomy may increase the risk of splanchnic ischemia. We aimed to investigate the acute effects of IAP and steep Trendelenburg position on the level of ischemia modified albumin (IMA) and to test if serum IMA levels might be used as a surrogate marker for possible covert ischemia during robotic radical prostatectomies.

Patients and Methods: Fifty ASA I-II patients scheduled for elective robotic radical prostatectomy were included in this investigation.

Exclusion criteria: The patients were excluded from the study when an arterial cannulation could not be accomplished, if the case had to be converted to open surgery or if the calculated intraoperative bleeding exceeded 300ml.

All the patients were placed in steep (45 degrees) Trendelenburg position following trocar placement. Throughout the operation the IAP was maintained between 11-14mmHg. Mean arterial blood pressure (MAP), cardiac output (CO) were continuously monitored before the induction and throughout the surgery. Blood gases, electrolytes, urea, creatinine, alanine transferase (ALT), aspartate transferase (AST) were recorded. Additionally, IMA levels were measured before, during and after surgery.

Results: (1) MAP, CO, lactate and hemoglobin (Hb) did not significantly change in any period of surgery ($p>0.05$); (2) sodium ($p<0.01$), potassium ($p<0.05$) and urea ($p<0.05$) levels decreased at postoperative period, and no significant changes at creatinine, AST, ALT levels were observed in these patients; (3) At the end of surgery (180 min) pCO_2 , pO_2 , HCO_3 and BE did not change compared to after induction values ($p>0.05$) but mild acidosis was present in these patients ($p<0.01$ vs. after induction); (4) IMA levels were found to be comparable before induction (0.34 ± 0.04), after induction (0.31 ± 0.06) and at the end of surgery (0.29 ± 0.05) as well.

Conclusion: We did not demonstrate any significant mesenteric-splanchnic ischemia which could be detected by serum IMA levels during robotic radical prostatectomies performed under steep Trendelenburg position and when IAP is maintained in between 11-14 mmHg

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INTRODUCTION

Robot assistance in laparoscopic surgery has undoubtedly contributed to the advancement of minimally invasive oncologic surgery. Robotic surgery provides a number of potential benefits such as improvement in surgical precision, diminished blood loss, reduced postoperative pain, improved cosmetic outcome, shorter convalescence and hence improved patient satisfaction (1, 2). Robot assisted laparoscopic radical prostatectomy (RARP) is the most frequently performed robotic procedure in urology. Despite the established advantages, there are several important issues related to the intra-operative management specific to this procedure. The positioning of patients during RARP impacts the risks related to hemodynamic changes such as increased systemic vascular resistance (SVR), mean arterial pressure (MAP), filling pressures and reduction in cardiac index (CI) (3, 4). High intra-abdominal pressure (IAP), especially if over 15mmHg, increases cerebral blood flow and intracranial pressure, while decreasing portal, hepatic vein flow and the total hepatic microcirculation (5-8). Increased IAP furthermore decreases mesenteric blood flow and impedes gastrointestinal microcirculation (8, 9). The pneumoperitoneum (PP) similarly leads to decreased arterial and venous flow in renal medulla and cortex (10-12).

The fixed positioning of the patients followed by the docking of the robot in steep Trendelenburg position and the relatively long duration of this procedure can thus cause excessive mechanical pressure over the gastrointestinal, respiratory and the cardiovascular systems, increase the risk of hypothermia, intensify the hemodynamic and respiratory adverse effects of the PP and may as well give rise to mesenteric-splanchnic hypoxic ischemia (4).

Serum ischemia modified albumin (IMA) is a new, FDA approved biomarker of ischemia, and increases in patients with acute coronary syndrome (13-16). IMA is produced during an ischemic attack and is present in blood in easily detectable concentrations (13, 17-19). Hypoxia, acidosis and free radical production reduce the ability of human serum albumin to bind metals like cobalt to its N-terminus, which in turn causes IMA pro-

duction. Endothelial or extracellular hypoxia, acidosis, and free oxygen radicals have been shown to cause IMA increase (20, 21), thus IMA can be detected early on the beginning of ischemia. Moreover IMA was found to increase in patients with mesenteric ischemia (22). It has been identified as a helpful marker for determining the alterations in the splanchnic and the visceral blood flow during laparoscopic cholecystectomies (23).

After myocardial ischemia, the serum levels of IMA rise within minutes and continue to increase for 6-12 hours, after which they return to normal (24-28). Increase in IMA concentrations has also been shown to indicate tissue ischemia in other conditions such as peripheral vascular disease, exercise-induced skeletal muscle ischemia, end-stage renal disease patients on haemodialysis, acute stroke, calf-muscle ischemia (29-33).

In the present study, we aimed to investigate the acute effects of IAP and steep Trendelenburg position on the level of ischemia modified albumin (IMA) and to test if serum IMA levels might be used as a surrogate marker for possible covert ischemia during robotic radical prostatectomies.

MATERIALS AND METHODS

Local ethics committee approval for this study (ATADEK No: 2013-456/B.30.2.A CU.00.00.050-06) was provided by ATADEK, Acibadem University, Ethics Committee, Istanbul, Turkey on 01 February 2013.

Fifty male patients, aged 55-75 years, ASA I-II, scheduled for RARP were included in the study and informed consents were taken.

Exclusion Criteria: The patients were excluded from the study when an arterial cannulation could not be accomplished, if the case had to be converted to open surgery or if the calculated intraoperative bleeding exceeded 300ml.

General Procedure

All patients were pre-medicated with midazolam 0.05mg/kg intravenously (i.v.) and standard monitorization, including, electrocardiography (ECG), invasive blood pressure (IBP), pulse oximetry (SpO₂), regional cerebral oxygenation (rSO₂), cardiac output (CO), and end tidal carbon

dioxide (ETCO₂), was applied. Anaesthesia induction was performed by propofol 2.5-3.5mg/kg, remifentanyl 1mic/kg i.v. and muscle relaxation was performed by rocuronium bromide 0.6mg/kg i.v. bolus and infusion 4-15mcg/kg/min. Anaesthesia was maintained by remifentanyl 0.025-0.05mic/kg/min i.v.infusion and by sevoflurane 0.8-1% in O₂:N₂O/40:60 in all patients. PEEP was adjusted between 4.6-4.8mmHg. The patients were placed in steep Trendelenburg position (45 degrees head-down angulation) after trocar placement and until the robot was un-docked. The IAP was maintained between 11-14mmHg throughout the laparoscopic stage of the operation.

MAP and CO were measured constantly as systemic hemodynamic parameters and recorded at particular instants: (1) before induction (BI); (2) after induction (AI); (3) 5thmin; (4) 60thmin; (5) 90thmin; (6) 120thmin; (7) 150thmin; (8) 180thmin of PP. Arterial blood levels of lactate, hemoglobin, pH, pCO₂, pO₂, HCO₃⁻ and base excess were monitored at same intervals. Additionally, serum sodium, potassium, blood urea nitrogen (BUN), creatinine, alanine transferase (ALT) and aspartate transferase (AST) were assessed preoperatively and postoperatively. Blood was sampled for interim measurements of serum ischemia modified albumin levels before induction (BI), after induction (AI) and the end of surgery (ES). The serum samples were stored at -20 degrees until they were sent to laboratory for IMA quantification. All of the serum samples for IMA measurements remained intact.

Determination of Ischemia modified Albumin (IMA)

IMA levels were determined according to the method defined by Bar-Or et al. (24). Briefly, 200uL serum was added to 50uL 0. 1% (w/v) cobalt chloride (Sigma Aldrich, St. Louis, MO; CoCl₂.6H₂O). Af-

ter gentle shaking, 10 minutes were waited to allow cobalt binding to albumin. Then 50uL dithiothreitol (DTT) (Sigma) was added as a colouring agent. As control, 50uL of distilled water was used instead of DTT. After 2 minutes, 1mL of 0.9% NaCl was added to stop the reaction, and the absorbance at 470nm was determined using a spectrophotometer (24). The difference of absorbance units between control and DTT samples were recorded. The results were quantified as absorbance unit (ABSU) and values greater than 0.400 ABSU were accepted as showing lower binding capacity for cobalt, therefore indicative of ischemia, whereas values lower than 0.400 ABSU were interpreted as lack of ischemia (24, 25).

Statistical analysis

Outcomes were reported as the mean±SEM. Statistical analysis was performed using GraphPad Prism version 5.0 for Windows (GraphPad Software, La Jolla, Calif). Results were compared using repeated measures ANOVA-tukey post hoc test used and a p-value of <0.05 was considered statistically significant.

RESULTS

The descriptive characteristics of patients and duration of surgery is summarized in Table-1.

Systemic hemodynamics results

The systemic hemodynamic values are presented in Figure-1. Induction of anaesthesia did not cause a statistically significant effect on MAP (p>0.05). Likewise, CO values were not affected by anaesthesia. At any time of surgery, both MAP and CO values were found to be similar compared to their respective levels after anaesthesia induction (p>0.05).

Table 1 - Characteristics of the patients and duration of pneumoperitoneum(PP).

Age	Height (cm)	Weight (kg)	BSA	PP. duration (min.)
59.7±1.3	171.2±3.0	81.9±1.7	1.95±0.04	186.5±6.54

(Acibadem Kadikoy /Maslak Hospital, 2013)

Blood gases analysis results

Blood gasses and related parameters are presented in Figure-2 and Table-2. The arterial pH was more acidotic at the 150th and 180thmin of PP when compared to 5thmin of PP. pCO₂ values were also found higher concurrently. pO₂ values were higher preoperatively than the pre-induction values during PP. While HCO₃⁻ level was not statistically different, base excess (BE) levels were higher at 60th, 120th and 180thmin. Hemoglobin and lactate values were not statistically altered during the operation.

Routine biochemistry and plasma electrolyte results

Plasma ions, urea, creatinine, ALT, AST levels are presented in Table-3. Postoperative le-

vels of sodium, potassium and urea were lower (p<0.01, p<0.05, p<0.05; respectively), whereas, postoperative levels of creatinine, ALT and AST were not different from the preoperative values (p>0.05).

IMA levels results

IMA assessments at three time intervals are presented in Figure-3. Mean IMA (ABS unit) values were 0.34±0.04, 0.31±0.06 and 0.29±0.05. The variances between intervals were not found to be statistically significant (p>0.05) (Figure-3).

DISCUSSION

RARP is performed while the patient is uniquely placed in a 45 degrees head-down (Tren-

Figure 1 - Systemic hemodynamic parameters.

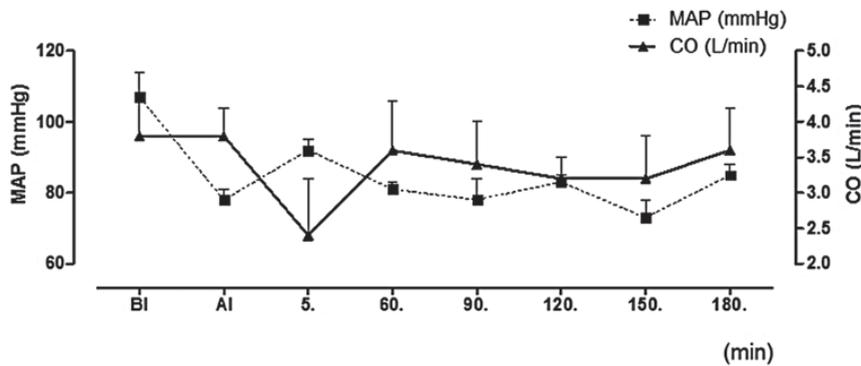


Figure 2 - Lactate and hemoglobin values at all-time points.

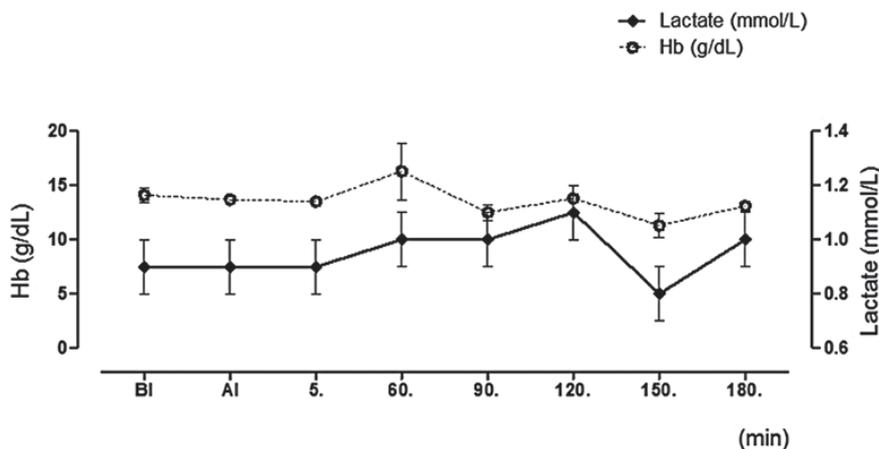


Table 2 - Blood Gases parameters.

	BI	AI	5 th min	60 th min	90 th min	120 th min	150 th min	180 th min
pH	7.41 ± 0.02	7.41 ± 0.01	7.42 ± 0.01	7.42 ± 0.01	7.37 ± 0.01	7.4 ± 0.01	7.33c ± 0.03	7.36 ± 0.01 ^{aabbcccd}
pCO ₂	37.8 ± 2.8	35.6 ± 0.7	33 ± 0.5	32.5b ± 0.6	39.2 ± 2.3	34.3 ± 0.6	43.3 ± 2.3 ^{bcc}	38.5 ± 1.4 ^{bbcccd}
pO ₂	140.2 ± 37.3	211.6 ± 14	157 ± 8.1 ^{aaa}	153 ± 5.9 ^{aaa}	145 ± 16.2	160.8 ± 5.6 ^{aa}	128 ± 8.4	154.7b ± 7.4
HCO ₃ ⁻	25.2 ± 0.7	22.6 ± 0.3	21.6 ± 0.3	24.6 ± 3.8	22.8 ± 0.7	21.2 ± 0.3	22.3 ± 0.9	21.5 ± 0.4
BE (-)	0.8 ± 1.0	1.3 ± 0.3	1.6 ± 0.3	2.4a ± 0.3	1.4 ± 0.7	2.3a ± 0.3	2.3 ± 1.2	2.4a ± 0.4

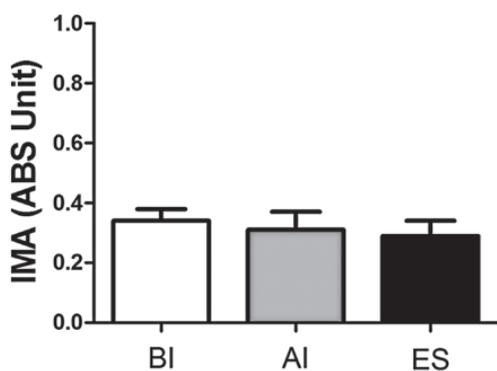
(BI = Before induction, AI = After induction, 5th min: 5th min of PP) (^{aa}p<0.01, ^{aaa}p<0.001 vs AI; ^bp<0.05, ^{bb}p<0.001 vs PP 5th min; ^{cc}p<0.01, ^{ccc}p<0.001 vs PP 60th min; ^dp<0.05 vs PP 120th min) (Acibadem Kadikoy /Maslak Hospital, 2013)

Table 3 - Routine blood chemistry parameters .

	Pre-op	Post-op
Na+ (mmol/L)	140 ± 0	138.2 ± 0.4**
K+ (mmol/L)	4.5 ± 0.1	4.1 ± 0.1*
Urea (mg/dL)	31.7 ± 1.3	24.2 ± 3.7*
Creatinine (mg/dL)	0.9 ± 0	0.8 ± 0.1
AST (U/L)	22.9 ± 1.1	19 ± 1.9
ALT (U/L)	24.7 ± 1.9	21.7 ± 4.1

(*p<0.05, **p<0.01vs Pre-op) (Acibadem Kadikoy /Maslak Hospital, 2013)

Figure 3 - Serum IMA levels at three time points (BI: Before induction, AI: After induction, ES: End of surgery).



delenburg) position and requires CO₂ insufflation to maintain an IAP of 12-15mmHg. We designed this study to investigate a hypothesis that even with a standard IAP, some form of mesenteric-splanchnic injury might be induced due to an extra gravitational pressure or a traction force

caused by the exclusive fixed patient-positioning in addition to the duration of robot-assisted laparoscopic radical prostatectomy. We have come up with a hypothesis of a possible mesenteric-splanchnic injury during RARP subsequent to a simple observation of frequent and prolonged post-operative ileus in patients undergoing this operation. Our purpose was to assess the acute effects of IAP at steep Trendelenburg position during this operation by hemodynamic monitorization, blood gas and electrolyte analyses and utilizing serum IMA as a biomarker for a supposedly overlooked mesenteric-splanchnic ischemia during robotic prostatectomies. However, our research failed to suggest any significant association of this exclusive patient positioning or variances of IAP levels with mesenteric-splanchnic ischemia as assessed by means of serum IMA during robotic radical prostatectomy.

Previous studies evaluating the course of cardiovascular changes during PP pointed out that

the CI gradually increased and systemic vascular resistance decreased 10 minutes after CO₂ insufflation (35-37). Additionally other studies suggested that CO rate decreased by 10-30% in Trendelenburg position (37-39). On the other hand, the mean CO level stayed stable (between 2.4-3.8L/min) in our study. It decreased to 2.4L/min at 5 minutes of PP but this change was statistically insignificant.

Lestar et al. examined the circulatory effects of an extreme Trendelenburg position (45°) on patients during robot-assisted laparoscopic radical prostatectomy and reported an almost 3-fold increase in central venous pressure compared with the initial value. MAP was increased by 35% in this study whereas heart rate (HR), stroke volume (SV), CO, and mixed venous oxygen saturation were unaffected during surgery, as were echocardiographic heart dimensions. In the horizontal position after PP exsufflation, filling pressures and MAP returned to baseline levels (38).

In another study designed to evaluate hemodynamic changes associated with head-down positioning and prolonged PP during RARP, invasive hemodynamic parameters were measured by transpulmonary arterial thermodilution using the PiCCO system with a femoral artery catheter, CI, HR, MAP, systemic vascular resistance index, intrathoracic blood volume, and central venous pressure were recorded with the patient in the supine position, after head-down tilt, intraoperatively after 30 min, 1h, 2hs, 3hs, and 4hs of PP at an insufflation pressure of 12mmHg. Placing the patient in the Trendelenburg position caused a significant increase in CVP, whereas all other hemodynamic parameters remained nearly unaffected. The induction of PP resulted in a significant increase in MAP whereas no other parameter was affected. Even at 4 hours of PP, only mild hemodynamic changes were observed (38). In our study, MAP was lower compared to pre-induction values throughout the operation, except a slight but statistically insignificant increase after PP. In our study, we did not prefer the use of invasive cardiac control.

In the present study, the IAP was 14mmHg at the start of PP and then maintained around 11mmHg for a mean duration of 3 hours. Post-operative levels of AST, ALT were not statistically

different compared to the preoperative values. In an animal study assessing the effect of prolonged PP on liver function and perfusion, it was found that the liver sustained no damage due to prolonged PP during laparoscopic surgery. In that study, the IAP was maintained at 14mmHg and the mean operation time was 6 hours (34).

In our study Group, the blood pH values were significantly lower at 150thmin and 180th min of PP than other intervals. At the same periods, pCO₂ was also significantly higher compared to those measurements at other times. This can be explained by increased CO₂ absorption during that time intervals of PP. In a study assessing the acid-base status and hemodynamic changes during PP, it was reported that there was a significant absorption of CO₂ gas across the peritoneum which caused substantial acidemia and hypercapnia, which is also consistent with our results. In that study by Ho et al., the IAP did not affect metabolic function, acid-base balance or hemodynamics (33). The investigators installed an IAP of 7 and 14mmHg each for 30 minutes to test the IAP ranges for laparoscopic procedures which elicited splanchnic and pulmonary hemodynamic and metabolic changes (33). The effect of low IAP (7mmHg) on splanchnic perfusion was pointed out to be minimal whereas higher IAPs (14mmHg) decreased the portal and hepatic blood flow, lowered the hepatic and intestinal tissue pH. In our study, the IAPs were kept in the range of 11-14mmHg throughout the operations and we found no statistically significant increase in IMA levels, which would have indicated ischemia.

In a study evaluating the effects of PP on mesenteric ischemia-reperfusion injury by measuring intestinal tissue oxygen pressure (Pt_iO₂) and oxidative damage during laparoscopic and open colon surgery, the authors found that during laparoscopic surgery, there was a significant decrease of Pt_iO₂ only when PP was increased to 15mmHg. Although malondialdehyde (MDA) significantly increased in both Groups after mesentery traction and at the end of operation versus baseline levels, there was no difference between techniques (39). When the effects of prolonged PP (4 hours) during RARP were investigated, it was found that MDA concentrations were significantly elevated at

various intervals as compared with the pre-insufflation value and also the intra-mucosal pH value decreased significantly after CO₂ insufflation compared with the pre-insufflation values. It was concluded that prolonged PP in RALP resulted in decreased splanchnic blood flow and PP itself produced oxidative stress (40). In our study, PP lasted 3.5 hours and the difference between pre- and postoperative IMA values was not statistically significant. Therefore, no ischemia was detected by measuring IMA.

Our findings did not reveal any renal injury as there was no significant statistical difference between the preoperative and postoperative BUN and creatinine values, unlike the study of Bishara et al., where renal perfusion and function were decreased by induction of IAP of 14mmHg (41).

The maintenance of the IAP within 11-14mmHg during the RARP operations is necessary in order to avoid the complications of PP. This safety measure certainly protects the patients from the adverse effects of high IAP. The restriction of IAP might be considered a limitation for this clinical study, and the sensitivity of IMA as an ischemic biomarker can further be tested by designing animal studies where IAP may be increased to higher levels. Further studies may as well be performed utilizing novel biomarkers to identify whether there are any mesenteric compartment like syndromes during RARP.

Based upon the findings obtained as a result of current study; although patients were kept in steep (45 degrees) Trendelenburg position during robotic prostatectomies, IAP between 11-14mmHg does not cause any hypoxia/ischemia detected by conventional measurement techniques. Even if there is a hypoxia, which is out of detection limits, this cannot be followed by serum IMA levels.

In conclusion, during robotic radical prostatectomies performed under steep Trendelenburg position and when IAP is maintained in between 11-14mmHg, we did not demonstrate any significant mesenteric-splanchnic ischemia that could be detected by serum IMA levels.

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What about vaginal extraction of the kidney? results of an online survey

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ABSTRACT

Purpose: We aimed to characterize surgeons opinion about the vaginal extraction of the kidney after transperitoneal laparoscopic nephrectomy.

Material and Methods: A 9-item questionnaire was published online (Survey Monkey™) and publicized via email to a multidisciplinary pool of surgeons in Portugal.

Data was collected and statistical analysis was performed using IBM SPSS Statistics, Version 21.0.

Results: Three hundred and fifty nine inquiries were sent, 154 surgeons completed the questionnaires (response rate of 43.0%). Fifty five point eight percent of the participants would choose the transvaginal approach for themselves or for a close relative. The most stated arguments were a better cosmesis (29.0%) expectancy of lower post operative pain (26.0%) and lower rate of incisional hernias (23.0%). Defenders of the transabdominal procedure justified with an expectancy of lower complication rate (39%), namely impairment of sexual function and fertility (22%). The female gender and the familiarity with transvaginal surgery were the stronger predictors of the option for this approach (70.6% vs 48.5%; p=0,016 and 85.3% vs 46.6%; p <0.001 respectively).

Conclusions: Contrasting with similar surveys published on transvaginal NOTES, the vaginal specimen extraction after conventional laparoscopic nephrectomy was fairly accepted by the inquired surgeons.

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INTRODUCTION

Natural orifice transluminal endoscopic surgery (NOTES) is to be implemented for almost a decade. However, because of the lack of appropriate armamentarium and unproven safety, the technique presented in 2006 as “the new paradigm of surgery” (1), has hardly surpassed the initial barriers.

Contrarily, the natural orifices specimen extraction (NOSE) has proven to be feasible and safe (2-6) allowing the retrieval of surgical specimens after standard or mini laparoscopy.

Morcellation is another alternative for specimen retrieval without the need of wound enlargement; however, risks of intra-abdominal lesions, tumor seeding and impaired pathologic examination still elicit concerns in surgical community (7).

In the urological field, the first NOSE procedure was reported by Breda in 1993 (8), who first performed a transvaginal retrieval of a kidney specimen. In 2002 Gill reported a series of 10 laparoscopic radical nephrectomies followed by vaginal extraction (9) and in 2011 Alcaraz tested the safety of the procedure to the limit, reporting a series of 20 living donor laparoscopic nephrectomies with vaginal delivery (6).

However, the technique did not have a great spread, remaining confined to some high specialized centers (10).

We decided to conduct a survey directed to surgeons to better understand their opinion about the vaginal extraction of nephrectomy specimens.

MATERIALS AND METHODS

We designed a survey in Portuguese language, consisting of a 9-item questionnaire (appendix) to evaluate five main items:

Personal and professional data, practice of laparoscopy, practice of transvaginal surgery, personal choice for kidney retrieval and justification of the option.

The inquiry was published online on a proper website (Survey Monkey™, Palo Alto, USA) and publicized via email to a multidisciplinary pool of surgeons, encompassing general surgeons, urologists and gynecologists, from three major surgical societies in Portugal.

No email reminders were sent in order to prevent re-answering.

Data was collected and statistical analysis was performed using IBM SPSS Statistics, Version 21.0 (IBM, New York, USA). Continuous data are expressed as mean and standard deviation. Chi square test was used for comparison of categorical variables with a significance level of 0.05.

RESULTS

Three hundred and fifty nine inquiries were sent and 154 surgeons completed the questionnaires (response rate of 43.0%) (Table-1).

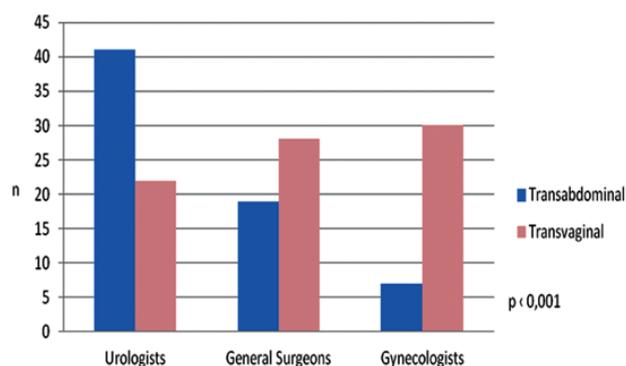
Fifty five point eight percent of the respondents would choose the transvaginal approach for kidney retrieval for themselves or for a close relative.

The most stated arguments were a better cosmesis (29.0%) and expectancy of lower post operative pain (26.0%). Defenders of the transabdominal procedure justified with an expectancy of lower complication rate (39%), namely impairment of sexual function and fertility (22%) (Figures 1 and 2).

Table 1 - Demographic and Professional Data.

Variable	N=154
Age (Mean ±SD)	39.52 (±10.7)
Gender	
Male	66.8%
Differentiation	
Specialist	59,7%
Specialty	
Urologists	44.2%
General Surgeons	31.2%
Gynecologists	24.7%
Practice of Laparoscopy	
No	16.2%
<20 annual procedures	35.7%
≥20 annual procedures	48.1%
Practice of Transvaginal Surgery	22.1%

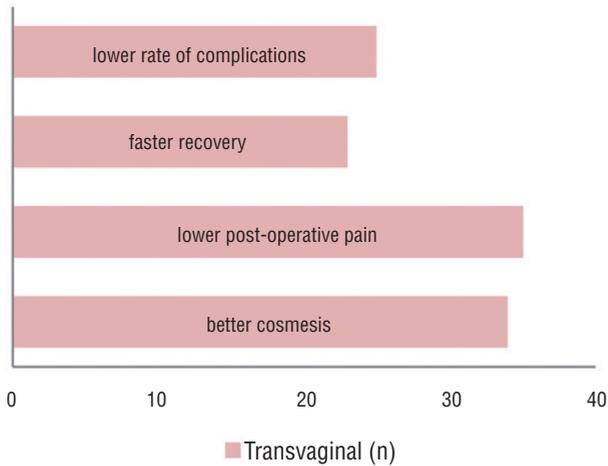
Figure 1 - Surgeons option considering the area of specialization.



Female surgeons showed preference for the transvaginal access (70.6% vs 48.5%; $p=0.016$).

Gynecologists and General surgeons were most likely to choose the transvaginal approach (81.5% and 60.4% respectively), while among the urologists only 38.2% would opt for this access ($p<0.001$) (Figure-3).

Figure 2 - Mentioned arguments favoring the transabdominal approach.



There was no difference in the option between residents and specialists (53.2% versus 57.6% respectively; $p=0.71$).

There was no statistical difference related with the reported number of laparoscopic procedures performed annually and the preference for the transvaginal approach.

The familiarity with transvaginal surgery was the strongest predictor of the option for this approach (85.3% vs. 46.6%; $p<0.001$).

DISCUSSION

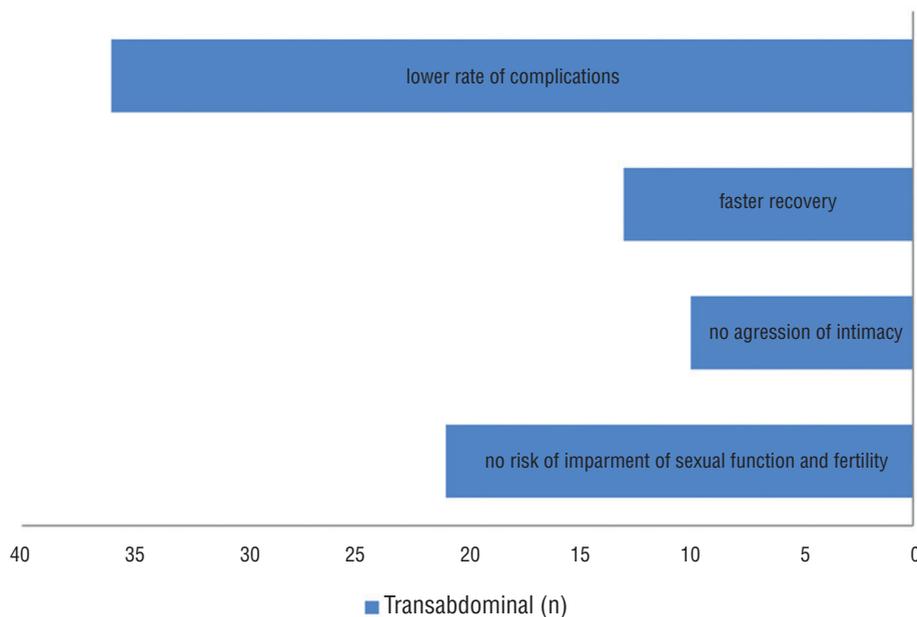
In our survey, the majority of the inquired surgeons privileged the transvaginal extraction of nephrectomy specimens over its transabdominal counterpart.

Gynecologists were the most enthusiasts, which is coherent with the fact that gynecologists perform transvaginal surgery for decades. In fact, 90% of the surgeons that stated practice of transvaginal surgery were gynecologists.

Urologists were the least like to choose the transvaginal approach. A possible explanation may be related with the number of patients amenable for vaginal extraction, which, in the majority of centers is probably too small to sensitize surgeons and to justify specific training. Excluding most of the tumors, because of specimen size, and living donors, which are only performed in specific centers, the majority of urologists can only count on excluded kidneys to get experience on vaginal extraction.

Overall, the expectation of lower post operative pain and better cosmesis were the most mentioned arguments by enthusiasts of the transvaginal retrieval, which is in general agreement with results of similar surveys on NOTES and LESS (11).

Figure 3 - Mentioned arguments favoring the transabdominal approach.



The defenders of the conventional abdominal extraction justified their option with the expectation of lower complication rate and expressed concerns about sexual function and fertility. These concerns are probably restraints to widespread acceptance of transvaginal surgery. Although, studies have shown that per-operative complications are negligible when the transvaginal access is created under direct vision (12) and infection is a rare event ($\leq 1\%$) (12, 13). Sexual function seems not to be affected by the transvaginal access while fertility questions are more controversial. (14-17).

Tanaka M, et al. assessed the long-term complications, including infertility, after transvaginal peritoneal surgery in a group of young patients. They found no evidence that this approach caused infertility or dyspareunia (18).

Female surgeons preferred the transvaginal approach, which is in agreement with similar studies conducted on NOTES, (19-21) that showed that the majority of the inquired women would opt for the transluminal procedures, not only because of the better cosmesis but also expecting a lower post operative pain and lower risk of hernia formation. Probably women see naturally the vagina as a possible route of organ delivery.

Although surgeons with practice in laparoscopy tended to be more prone to choose the vaginal route, it was the experience in vaginal surgery that clearly prompted surgeons to opt for this approach. This suggests that surgeons experienced in vaginal surgery find the vaginal extraction safe and advantageous, and on the other hand, is natural that surgeons with no experience in vaginal surgery tend to "fear" this approach.

Our results also show contrasts with similar studies conducted on NOTES. In the article published by Thele, (22) whereas 69.2% of the inquired surgeons classified transvaginal NOTES as acceptable, only 32.7% considered the procedure appropriate for abdominal surgery and just 28.8% would recommend it. Concerns with infection, visceral lesions and infertility were the most expressed arguments. Volckmann and collaborators surveyed the members of three major surgical societies; 23% respondents demonstrated a great interest on NOTES, however, only 26%

would personally undergo a NOTES procedure. Safety was considered the most important factor in the option. Probably, NOTES and NOSE, do not elicit the same concerns in the surgical community.

Public perception of new surgical procedures may not always consider all their potential risks and benefits; however it is well known the importance of public demanding in the evolution of surgical techniques. Population based surveys found a great acceptance of female public for the transvaginal procedures once safety is assured. In the study of Peterson et al. 73% of the respondents would consider a transvaginal procedure and 68% would opt for the procedure if safety was equivalent to laparoscopy (20). Olakengil et al. surveyed female living donors about transvaginal NOTES nephrectomy; 51% would have opted for this approach if safety was the same (23).

The present study is the first evaluating surgeons opinion on NOSE, however, it has some limitations, namely, the use of a non validated questionnaire and the national character of the survey.

CONCLUSIONS

The transvaginal kidney retrieval was the approach of choice of the majority of the inquired gynecologists and general surgeons but not to the urologists.

The lack of experience in vaginal surgery and the apprehension of long-term effects on sexual function and fertility can be obstacles to the widespread of this technique.

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CONFLICT OF INTEREST

None declared.

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Robotic-assisted radical prostatectomy learning curve for experienced laparoscopic surgeons: does it really exist?

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ABSTRACT

Background: Robotic-assisted radical prostatectomy (RALP) is a minimally invasive procedure that could have a reduced learning curve for unfamiliar laparoscopic surgeon. However, there are no consensuses regarding the impact of previous laparoscopic experience on the learning curve of RALP. We report on a functional and perioperative outcome comparison between our initial 60 cases of RALP and last 60 cases of laparoscopic radical prostatectomy (LRP), performed by three experienced laparoscopic surgeons with a 200+LRP cases experience.

Materials and Methods: Between January 2010 and September 2013, a total of 60 consecutive patients who have undergone RALP were prospectively evaluated and compared to the last 60 cases of LRP. Data included demographic data, operative duration, blood loss, transfusion rate, positive surgical margins, hospital stay, complications and potency and continence rates.

Results: The mean operative time and blood loss were higher in RALP (236 versus 153 minutes, $p < 0.001$ and 245.6 versus 202ml $p < 0.001$). Potency rates at 6 months were higher in RALP (70% versus 50% $p = 0.02$). Positive surgical margins were also higher in RALP (31.6% versus 12.5%, $p = 0.01$). Continence rates at 6 months were similar (93.3% versus 89.3% $p = 0.43$). Patient's age, complication rates and length of hospital stay were similar for both groups.

Conclusions: Experienced laparoscopic surgeons (ELS) present a learning curve for RALP only demonstrated by longer operative time and clinically insignificant blood loss. Our initial results demonstrated similar perioperative and functional outcomes for both approaches. ELS were able to achieve satisfactory oncological and functional results during the learning curve period for RALP.

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INTRODUCTION

Prostate cancer is the most common non-cutaneous men malignancy and the second leading cause of cancer related mortality in Brazil (1).

Minimally invasive approaches for prostate cancer have evolved significantly after 2000.

Laparoscopic radical prostatectomy (LRP) demonstrated improved visualization of the pelvic anatomy, improvements in potency and urinary

rates, lower blood loss, while upholding principles of oncological therapy (2-6). Although, this technique presented a limited expansion due to the steep learning curve, which requires at least 60 cases to obtain proficiency (6).

Recently, robot-assisted radical prostatectomy (RALP) brought several mechanisms which may significantly decrease the learning curve for unfamiliar laparoscopically surgeons (2). The Da Vinci surgical system (Intuitive Surgical, Sunnyvale, California, USA) magnification, robotic-wrist instrumentation and increased degrees of freedom, associated with the 3-dimensional visualization provided surgeons extremely detailed pelvic anatomy which enables the appropriate prostate extirpation (7-9). This minimally invasive technique has received widespread acceptance by physicians and patients and was established as the standard surgical treatment for localized prostate cancer in the US (10-12).

In Brazil, the Da Vinci System was introduced in 2008. However, it was implemented only in 9 hospital centers (Albert Einstein, Sirio Libanes, Oswaldo Cruz, Nove de Julho, INCA, Samaritano, HC Porto Alegre, ICESP and Fundação Pio XII). In addition, this high-cost technology is not provided by health insurances, being mostly performed by private services, which provides low volume of RALP for most urologists.

The aim of this study was to report our initial experience and assess the learning curve of experienced laparoscopic surgeons in robot-assisted radical prostatectomy (RALP). We compared perioperative, functional and oncological outcomes between RALP and LRP.

MATERIALS AND METHODS

The project was approved by the Ethics Committee for Analysis of Research Projects of the involved institutions.

A retrospective review of prospectively collected data was performed from 2008 to 2013, including 120 patients with localized low or intermediate risk of prostate cancer who were indicated for surgical treatment. All selected cases presented previous urinary and potency rates preserved. Patients with previous prostate cancer

treatment, neoadjuvant or adjuvant hormonal treatment were excluded from the study. The robotic procedures were performed at a private hospital while the LRP in public and private hospitals.

Preoperative, perioperative, oncological and functional outcomes of the first 60 cases of robot-assisted radical prostatectomy were compared to the last 60 consecutive cases of laparoscopic radical prostatectomy. All procedures were performed by three experienced surgeons with a 200+experience in LRP, under the same defined protocol.

Data included demographic characteristics, operative parameters (operative time, blood loss, positive surgical margin, complications, conversion and transfusion rates and postoperative (early urinary and potency continence and post-operative stay).

SURGICAL TECHNIQUE

Robotic-assisted laparoscopic radical prostatectomy

The RALP was performed using the S and Si da Vinci Robotic System (Intuitive Surgical, Sunnyvale, CA). First, the patient was positioned supine in low lithotomy in a 15° Trendelenburg position. All cases were performed transperitoneally using the six-port technique as described by Patel et al. (13). Non robotic ports were placed higher or above umbilicus's level in order to provide maximum range of motion to the assistant. Dorsal venous complex was initially isolated and ligated. The seminal vesicles dissection was then performed and prostatic pedicles ligation was carried out. Nerve-sparing surgery was performed when using a clip technique without the use of any kind of thermal energy. Finally, the running vesicourethral anastomosis was performed as described by Van Velthoven et al. with conventional 3-0 barbed sutures.

Laparoscopic radical prostatectomy

Pure laparoscopic cases were performed with five-port extraperitoneal approach described by us previously (14, 15). The patient was placed in supine position with Y-shaped abduction of lower limbs. Optics trocar was inserted in the umbilical

incision, two trocars were inserted in the pararectal external area and two in the iliac fossa. Vascular control of dorsal venous complex was performed using a 2-0 polygalactine suture with CT-1. The bladder neck was incised and the vasa deferentia and seminal vesicles were dissected. Posterior prostate pedicles were clipped and incised. The dorsal vein complex and urethra were incised and the prostate released. Continuous 3-0 monocryl or 3-0 barbed sutures were used to perform the vesicourethral Van Velthoven anastomosis.

Statistical analysis

The statistical analyses were performed using SPSS software (IBM® SPSS® Statistics20; SPSS, Inc., Chicago, IL, USA). The significance level was defined as 0.05 (5%). All confidence intervals used in this study were constructed with a 95% confidence level.

The paired Student t test was used to assess quantitative data and compare means (age, operative time, blood loss, PSA level). The two-samples z test was used to compare intraoperative complications, continence and potency rates, positive surgical margins, transfusion rate, Gleason score,

pathologic stage and nerve sparing between the groups.

RESULTS

Patients who have undergone LRP and RALP were similar in terms of age and ranged from 50 to 70 ($p=0.99$). PSA level, Gleason score and pathologic stage (T2, T3) were also similar between the groups (Table-1). Bilateral nerve sparing was performed in 83.3% in RALP and 73.3% in LRP and both were considered similar ($p=0.18$).

Mean operative time was longer in RALP (236.1 ± 42.95) compared to LRP (153.51 ± 41.8 $p<0.001$). A significant difference was found in the blood loss (245.6 ± 33.71 versus 202 ± 73.3 $p<0.001$). Complications occurred in 10.3% of patients who underwent LRP and 6.6% in RALP. Visceral and rectal injuries, blood transfusion, wound infection, urinary tract infection and retention were included. No conversion to open or laparoscopic surgery was performed (Table-2). The length of hospital stay was similar between the groups ($p=0.92$) and ranged from 1-3 days.

Functional and oncological outcomes are described in Table-3. Continence rates at six mon-

Table 1 - Preoperative patient characteristics. The groups were similar in terms of age, PSA level, Gleason grade, nerve sparing and pathologic stage (T2, T3).

	LRP	RALP	P value
	n=60	n=60	
Age (range, SD) years	60.56±11.6	60.58±7.94	0.99
PSA (range, SD) ng/mL	7.05±3.70	6.17±2.63	0.13
Gleason grade (%)			
≤6	46.25%	46.6%	0.96
7	35.00%	45.0%	0.26
>7	18.75%	8.4%	0.09
Pathologic stage (%)			
pT2	87.20%	81.6%	0.74
pT3	12.80%	18.4%	0.4
Nerve Sparing (%)			
Unilateral	26.7%	16.7%	0.18
Bilateral	73.3%	83.3%	0.18

Table 2 - Perioperative outcomes. Robotic-assisted radical prostatectomy presented longer operative time and higher blood loss when compared to LRP.

	LRP	RALP	P value
	n=60	n=60	
Operative time (minutes)	153.51±41.8	236.1±42.95	<0.001
Blood loss (ml)	202±73.3	245.6±33.71	<0.001
Intraoperative complications (%)	10.30%	6.6%	0.46
Hospital stay (days)	1.38	1.60	0.92
Transfusion rate (%)	0%	0%	1.0

Table 3 - Functional and oncological outcomes. Robotic-assisted radical prostatectomy presented higher percentage of potency continence at six months and positive surgical margins.

	LRP	RALP	P value
	n=60	n=60	
Continence rates at six month (%)	89.3%	93.3%	0.43
Potency rates at six month (%)	50%	70%	0.02
Positive Surgical Margins (%)	12.5%	21.6%	0.18
pT2	8%	12.5%	0.7
pT3	33%	50%	0.1

ths was higher in RALP (70% versus 50% $p=0.02$). Potency rates at six months were similar (93.3% versus 89.3% $p=0.43$). Positive surgical margins was higher in RALP when compared to LRP (31.6% versus 12.5% $p=0.01$).

DISCUSSION

Laparoscopic radical prostatectomy was the first successful minimally invasive procedure that provided several benefits concerning potency and urinary continence, blood loss, while upholding principles of oncological therapy (2). However, the two-dimensional image associated with lower range of motion turned LRP into a challenging procedure, which presents a steep learning curve that requires nearly 70 cases to attain proficiency (6, 15).

Robotic assisted radical prostatectomy emerged as an effective alternative to LRP. The Da Vinci 3-dimensional image, magnification, multi-joints devices, increased degrees of freedom sig-

nificantly improved surgical ergonomics and therefore decreased the learning curve of LRP. RALP has received worldwide acceptance by urologists and is on the verge of becoming the preferred surgical treatment of localized prostate cancer (12, 16-18).

However, the high cost of this technology remains as the primary obstacle towards RALP expansion. The Da Vinci system is evaluated at 2 million euros and its maintenance increases financial burden by \$2.698 per patient given an average of 126 cases per year. Previous reports estimated that a total of 75 cases per year with an average operation time of three hours per case are necessary to be cost-effective in the United States (16, 19). In Brazil, this system was introduced in 2008 and was implemented only in 9 hospital centers. INCA's hospital (Instituto Nacional do Câncer) and ICESP (Instituto do Câncer do Estado de São Paulo) were the first public services that provided the Da Vinci System in Brazil. Therefore, based on the medical system without a reference

system of patient's, a low volume of procedures is performed by several urologists who are familiar with this technology. So, this condition may justify the few reports about the current situation of RALP in Brazil.

To our knowledge, this is the first Brazilian series that analyzes the learning curve of experienced laparoscopic surgeons and compare perioperative and functional outcomes between RALP and LRP. In this preliminary report, we found differences and similarities between the groups outcomes.

RALP operative time was longer than LRP, which is in accordance with previous larger series which estimated a range from 140 to 354 min (8, 11, 20-22). Menon et al. reported in early series of RALP a progressive decrease of operative time over time which is not observed in LRP (23). This finding suggests that further experience could lead to similar operation time. Estimated blood loss was higher in RALP and is in accordance with previous reports which reported an average of 234ml with a range of 75-500ml (20-22). Estimated blood loss was higher in RALP (approximately 50ml), however it was clinically insignificant and blood transfusion was not necessary in any case. This difference could be explained by the longer operative time of RALP.

Robotic-assisted radical prostatectomy presents several potential complications. Some authors include catheterization time, symptomatic lymphocele, hematoma, emphysema whereas other uses the Clavien grading system for short-term complications (11, 21, 24). In our initial experience we presented the most common complications and our rate was 10%, in accordance with most reports (22, 24, 25). Both RALP and LRP present similar incidence of conversion to open surgery, which are significantly low (10). In our experience, no procedures needed conversion or transfusions. Length of hospital stay is usually associated with perioperative complications and patient's well-being, and we found no differences between LRP and RALP.

Continence rate at six month was significantly equal between our groups (93.3% versus 89.3%). This finding will be definitive only after a one-year evaluation. Ficarra's et al. meta-analysis

observed that RALP was significantly superior to LRP in terms of 12-month urinary continence recovery. Although he concluded that the prevalence of urinary incontinence after RALP is influenced by several factors including preoperative patient characteristics, surgeon experience, surgical technique and collective methods, which hinder this assessment (7).

However, potency rates were higher in RALP when compared to LRP (70% versus 50%). This finding is in accordance with Ficarra's et al. meta-analysis that demonstrated a significant advantage in favor of RALP in comparison with RRP in terms of 12-month potency rates (26). In addition, this finding suggests that further experience on RALP and longer follow-up could lead to early potency rates, even for experienced laparoscopic surgeons.

Positive surgical margin rates were significantly similar between the groups (21.6% for RALP and 12.5% in LRP). This finding was similar to previous studies which RALP ranged from 12.3% to 17.2% and LRP 11-29%. Most series reported no statistically significant difference between LRP and RALP (16, 20, 23, 27).

Currently, there is no consensus over the superiority of RALP or LRP in the treatment of localized prostate cancer. Several studies compared both techniques and presented different results rather in favor of RALP or LRP (2, 11, 16, 19, 27-30). We believe that the Da Vinci System is a technological evolution which provides more detailed information regarding this complex procedure. On the other hand, considering the low volume of Da Vinci's system installed in Brazil during the 7 last years, most urologists won't have access to robotic surgery in Brazil for a long time, which turns LRP into a feasible alternative. Additionally, LRP may be a shortcut for reducing the learning curve of RALP. We observed that surgeons who are proficient in LRP and have low volume of RALP presents a learning curve that did not jeopardize their oncological and functional outcomes. Similar to USA, where massive RALP expansion turned it to be the established surgical treatment for localized prostate cancer, it will be natural that RALP replace LRP in the future, when technology and trained surgeons could be largely available (10, 23, 27, 28).

In our study we observed that an experienced laparoscopic surgeon was able to attain perioperative and functional outcomes in his/her initial results similar to surgeons who present higher experience in RALP. The previous experience on LRP could decrease the learning curve of RALP, mainly concerning the similarity of surgical steps and pelvic anatomy visualization. Therefore, the learning curve would be mainly related to the management of the robotic system new features such as multi-joints devices and absence of tactile feedback.

We consider the limitations of our initial experience which was performed in a low volume center for both procedures in private hospitals. Our results aid the comparison between LRP and RALP for experienced laparoscopic surgeons, however our results should be considered indicative only. Longer oncologic and functional follow-up are still required.

Experienced laparoscopic surgeons present a learning curve when first performing an RALP, demonstrated only by longer operative time. Even though our perioperative and functional outcomes were similar for both approaches and in accordance with previous reports (11, 21, 31). ELS were able to achieve satisfactory oncological and functional results during the learning curve period for RALP.

CONFLICT OF INTEREST

None declared.

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Specific training for LESS surgery results from a prospective study in the animal model

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ABSTRACT

Objective: to prospectively evaluate the ability of post-graduate students enrolled in a laparoscopy program of the Institute for Teaching and Research to complete single port total nephrectomies.

Materials and Methods: 15 post-graduate students were enrolled in the study, which was performed using the SILSsm port system for single-port procedures. All participants were already proficient in total nephrectomies in animal models and performed a left followed by a right nephrectomy. Analyzed data comprised incision size, complications, and the time taken to complete each part of the procedure. Statistical significance was set at $p < 0.05$.

Results: All students successfully finished the procedure using the single-port system. A total of 30 nephrectomies were analyzed. Mean incision size was 3.61 cm, mean time to trocar insertion was 9.61 min and to dissect the renal hilum was 25.3 min. Mean time to dissect the kidney was 5.18 min and to complete the whole procedure was 39.4 min. Total renal hilum and operative time was 45.8% ($p < 0.001$) and 38% ($p = 0.001$) faster in the second procedure, respectively. Complications included 3 renal vein lesions, 2 kidney lacerations and 1 lesion of a lumbar artery. All were immediately identified and corrected laparoscopically through the single-port system, except for one renal vein lesion, which required the introduction an auxiliary laparoscopic port.

Conclusion: Laparoscopic single-port nephrectomy in the experimental animal model is a feasible but relatively difficult procedure for those with intermediate laparoscopic experience. Intraoperative complications might be successfully treated with the single-port system. Training aids reducing surgical time and improves outcomes.

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INTRODUCTION

Over the last two decades, laparoscopy has revolutionized urological practice. Several series have reported promising results for simple (1) and complex upper tract procedures involving benign (2) and malignant diseases (3).

Laparoendoscopic single-site surgery (LESS) represents the latest innovation in laparoscopic sur-

gery. It aims to minimize postoperative pain and time to complete recovery with improved cosmesis. However, LESS is known to be a challenging procedure since triangulation, a basic principle of laparoscopic surgery, is lost. Therefore, instruments often collide and the procedures are usually associated with poor surgeon and assistant ergonomics (4).

Similarly to a standard laparoscopic surgery, LESS has a learning curve and requires trai-

ning in technical skills and spatial awareness, as these are different from skills required for open surgery or standard laparoscopy. The aim of the present study was to prospectively evaluate the feasibility and morbidity of single-port nephrectomy performed by post-graduate students in live animal models.

MATERIALS AND METHODS

Participants

After having undergone an extended training in urologic experimental laparoscopic surgeries in the animal model at the accredited center of the Institute for Teaching and Research of our Institution, 15 graduated urologists of the post-graduation laparoscopy Urology course were invited to participate in the study. The program comprises a year-long post-graduate course in which students spend three full days per month (one module of a total of ten modules) learning urologic laparoscopic principles and skills. As part of their training, they spend 12 hours per module practicing surgical skills and procedures in live animal models. All invited students were in the two final modules of the annual course and were proficient in laparoscopic total nephrectomies performed in the porcine model. All students accepted to join in the study and were considered suitable.

Single-Port System and Nephrectomy in the Animal Model

The experimental procedures in the wet laboratory consisted in the evaluation of basic nephrectomy tasks (port placement, renal hilum control, and renal dissection) in a porcine model using Single-Incision Laparoscopic Surgery SILS™ (Covidien, Norwalk, CT) port system. The SILS port is an FDA approved, single-incision flexible device, which may be inserted via an open technique through a skin and fascial incision as small as 15mm. It allows access for three 5mm cannulas or one 12mm cannula and two 5mm cannulas. The students had no previous experience with any single-port system and were allowed 15 minutes to familiarize with the instruments immediately before the initiation of the procedure.

Fifteen swine (mini pig BR) weighting 30-35Kg were used in the study. All animals were acquired from the same facility. The protocol was approved by the ethical committee of our institution. In all animals, anesthesia was induced with a combination of intramuscular ketamine (5mg/Kg) and midazolam (0.5mg/Kg) and maintained with continuous intravenous propofol (8mg/Kg) and inhalatory isoflurane (2%) infusions. All procedures were performed with the animal in the flank position. After the first nephrectomy, the incision site was closed and the animal repositioned to the contralateral procedure. A new incision site was used 1cm above or beyond the first one. At the end of the procedures, all pigs were euthanized. All animals were intubated and ventilated and the abdomen was placed on the edge of the bed to prevent instrument collision and mobility limitation.

All students performed exactly the same surgical tasks: a vertical trans-umbilical incision was made and students were oriented to do the smallest incision for trocar placement. The rectal fascia was then identified and opened under direct visualization. Two holding stitches were placed on either side of the fascia to facilitate easier port placement. After abdominal insufflation, a 30° 10mm laparoscope was placed. Renal dissection was performed using a 23cm long grasper, a 23cm-long scissor and the Ethicon Harmonic Scalpel™ (Cincinnati, OH, USA). The peritoneum was incised and the renal hilum was identified. The artery and veins were manually taken separately using 4-0 silk knots. After complete control of the renal hilum, the kidney was completely mobilized using instrument dissection and harmonic energy.

The following parameters were evaluated for each student: incision size (cm), time to insert the SILS Port®, time to mobilize and divide the renal pedicle, time to dissect and mobilize the kidney, and total surgery time. Each student performed a left followed by a right nephrectomy. The same parameters were compared between the first and second procedures to evaluate the learning curve effect. We also evaluated intraoperative complications and the ability to treat them in case they occurred. Each student performed one right and one left total nephrectomy. After the procedures, students were individually questioned

about the two most important technical challenges of single-port.

Statistical analysis

Statistical analysis was performed using SPSS™ version 20 (SPSS, Inc., Chicago, IL, USA). Results were described as mean, standard deviation and range values. Paired T Test was used to compare parameters of first and second procedure for each student. Statistical significance was set at two-tailed $p < 0.05$.

RESULTS

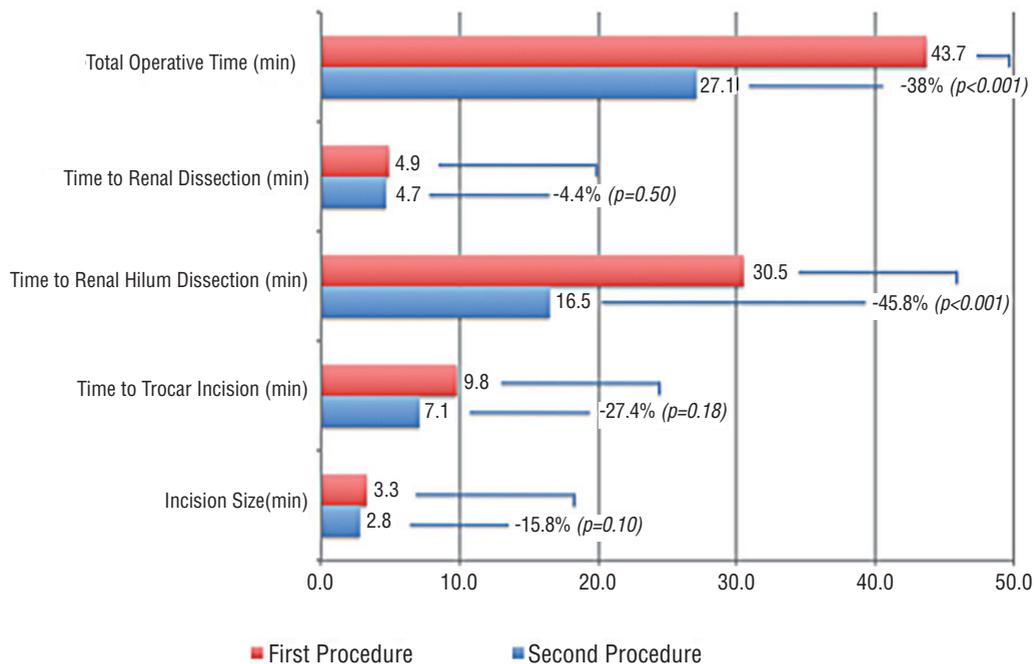
A total of 30 nephrectomies were evaluated. Nephrectomies were successfully completed by all students. Intraoperative data is detailed in Table-1. Mean incision size was 3.61 ± 0.8 (2.5-5) cm. Mean time for trocar insertion was 9.6 ± 3.4 (3-17.4) min. Mean time to dissect and control the renal hilum was 25.3 ± 10.4 (7.9-43) min and to dissect the kidney was 5.2 ± 1.5 (2.1-9.5) min. Finally, mean time to complete the whole procedure was 39.4 ± 12.3 (20.5-59.3) min.

Mean incision size was 0.5cm (15%) shorter in the second procedure, although not statistically significant ($p=0.10$) (Figure-1). Mean time to trocar insertion was 27.4% (2.7 min) faster in the second nephrectomy ($p=0.18$). Although time to dissect renal hilum was faster (45.8%; 13.9 min; $p < 0.001$), time to dissect the kidney was very similar (0.2 min faster in the second surgery; $p=0.5$).

Table 1 - Single-port nephrectomy task and performance evaluation.

Tasks Performance	Mean±SD	range
Incision size (cm)	3.61±0.8	2.5-5.0
Time to trocar insertion (min)	9.61±3.4	3.0-17.4
Time to dissect renal hilum (min)	25.3±10.4	7.9-43.0
Time to kidney dissection (min)	5.18±1.5	2.1-9.5
Total procedure time (min)	39.4±12.3	20.5-59.3
Procedure Feasibility and Morbidity		
Successful procedures	N 30	% 100%
Complications	6	20%
Conversion to standard laparoscopy	1	3.4%

Figure 1 - Individual task comparison between the first and second SILS nephrectomy.



Total operative time was significantly shorter (16.6 min; 38%) in the second nephrectomy ($p=0.001$).

Intraoperative complications were seen in 6 (20%) procedures (Table-1): 3 renal vein lesions, 2 small kidney lacerations, and 1 lesion of a lumbar artery. All complications were immediately identified and lesions were corrected using single-port instruments, except for one renal vein lesion, which required the introduction of an auxiliary 5mm laparoscopic port (3.4% conversion rate). Blood loss was minimal during the procedures and could not be quantified.

Instrument collision and spatial awareness were the most common time consuming problems found by the surgeons. Mobilization of the camera was often related as difficult by the assistants due to the narrow operative field and the small spatial mobilization, occasionally preventing adequate exposure of the operative field.

DISCUSSION

Standard laparoscopic nephrectomy requires fine surgical skills since renal pedicle mobilization may be difficult and vascular complications are usually severe and life threatening. In addition, it requires the introduction of at least 3 ports and each trocar inserted increases the risk of bleeding, internal organ injury, and port-site hernia, also compromising cosmetic results. Single-port access has been developed in order to reduce those complications and with the benefits of less postoperative pain, faster convalescence and better cosmetic results (5). Nevertheless, single-port surgery is known to be a challenging technique, as maneuverability is poor inside and outside the abdomen, there is no triangulation of the conventional laparoscopic instruments, and collision between the instruments and the camera is frequent (6). In order to overcome such limitations, new flexible instruments were developed and intensive training is required to achieve results similar to those of standard laparoscopic technique.

In this study, we evaluated the ability of post-graduate students to complete basic laparoscopic nephrectomy skills using a SILS Port®. All nephrectomies were completed through single-port access and one renal vein lesion required the introduction of an auxiliary 5mm port to control

a small bleeding. Our results show that single port nephrectomy is feasible, even for surgeons with no previous experience with single-port devices. Usually pedicle ligation is achieved with clip ligation of the vessels. However, we decided to use manual knots for such task, in order to better evaluate the procedure in case clip ligation was unavailable or unsuccessful. Despite the known difficulty during pedicle ligation with manual knots, all students successfully completed this task. Although we analyzed the learning curve effect with few cases, we found significant differences from the first to the second procedure. This highlights the importance of training the exact procedure with the correct materials for laparoscopy, leading to faster procedures parallel to better outcomes.

Our results revealed a great variability between students in all steps of the procedure. This was expected and consistent with other early series of laparoscopic and single-port nephrectomies in which the learning curve plays an important role in the duration of the procedure (7). Instrument collision and spatial awareness were the most common time consuming problems found by the surgeons. In addition, according to the assistant report, mobilization of the camera was often difficult since the operative field was narrow and a small spatial mobilization usually prevented ideal visualization of the operative field. Autorino et al. have already compared mini-laparoscopy, laparo-endoscopic single-site surgery and natural orifice transluminal endoscopic surgery for total nephrectomy (8). They found no differences in overall operating time, or time to dissect and manage the renal vascular hilum, however time to gain access was faster with the single-site technique. The subjective perception of the degree of difficulty trended in favour of mini-laparoscopy, but no significant difference was found in regards of surgeon's impression as compared with their expectations.

Single-port access has been introduced as a method that could potentially reduce standard laparoscopic complications, e.g. internal organ and vascular injury, as it does not require needle or blind port placement and there is no need for extra ports (6). Nevertheless, current data shows no benefit of single-port over standard laparoscopy in terms of operative time, blood loss or complication rates (9, 10). This is probably due to a longer learning curve

in acquiring specific skills using single-port access compared to standard laparoscopy. In our study, complication rate was acceptable (20%) and similar to that found during the student's initial laparoscopic experience (data not shown). Both kidney lacerations required no treatment. One small renal vein lesion required an extra 5mm port to facilitate immediate clamping with subsequent suturing of the vessel. Other vascular lesions were small and successfully controlled without suturing.

Suturing was clearly the most demanding task using single-port access and total time noted for all students to complete this task was by far the longest of all steps during the procedure. The introduction of newer flexible and pre-bent graspers will allow better intra-abdominal mobilization of the instruments (11). Stolzenburg and colleagues evaluated pre-bent single-site instruments and verified that time required to perform pedicle dissection was significantly lower in comparison with the results of other studies (9).

Our study has some limitations. An important drawback is the lack of a control group with standard laparoscopy. Nevertheless, the aim of our study was not to compare single-port nephrectomy to standard laparoscopy, but to analyze single-port feasibility in the hands of novice surgeons. In addition, all students were already proficient in porcine laparoscopic nephrectomy and therefore their known expertise would limit such comparison. Also, sample-size is limited, especially because of the course costs and time availability of the post-graduate students. Finally, the present study was based only on a porcine model, in which nephrectomy is known to be less complex than in humans. Thus, the SILS Port® and other single port systems should be further evaluated in clinical setting before solid conclusions are drawn on its efficacy for human surgeries under inexperienced hands. Although feasible in novice hands, only experienced laparoscopic surgeons should perform LESS. In addition, the technique is favored mainly in cases where cosmesis is of paramount importance (12).

CONCLUSIONS

To conclude, laparoscopic single-port nephrectomy using SILS™ in the swine model is a

feasible but relatively difficult procedure for those with intermediate laparoscopic experience. It is potentially associated with significant intraoperative complications, which may be successfully treated with the single-port system. Training aids reducing surgical time ultimately improves outcomes.

CONFLICT OF INTEREST

None declared.

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Comparison of shock wave lithotripsy (SWL) and retrograde intrarenal surgery (RIRS) for treatment of stone disease in horseshoe kidney patients

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ABSTRACT

Objectives: In this study it is aimed to compare the success and complication rates of SWL and RIRS in treatment of HSK stone disease.

Materials and methods: In this retrospective study data of 67 patients treated with either SWL (n=44) or RIRS (n=23) for stone disease in HSK between May 2003 to August 2014 was investigated. age, gender, stone size and multiplicity, stone free status, renal colic episodes and complication rates of the SWL and RIRS groups were compared.

Results: Mean age of the population was 42.5±8.2 (range: 16-78) years and mean stone size was 16.9±4.1 mm. SWL and RIRS groups were similar with regard to demographic characteristics and stone related characteristics. SFR of the SWL and RIRS groups were 47.7%(21/44 patients) and 73.9% (17/23 patients) respectively (p=0.039).

Renal colic episodes were observed in 3 and 16 patients in the RIRS and SWL groups respectively (p=0.024). No statistically significant complications were observed between the SWL (8/44 patients) and RIRS (4/23) groups (p=0.936).

Conclusions: In HSK patients with stone disease, both SWL and RIRS are effective and safe treatment modalities. However RIRS seems to maintain higher SFRs with comparable complication rates.

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Lithotripsy; Surgical Procedures, Operative; Fused Kidney; Therapeutics

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INTRODUCTION

Renal anomalies are associated with increased rates of stone disease and the horseshoe kidney (HSK) is the most common renal fusion anomaly. It is observed in approximately 1 in 400 to 1 in 666 births (1-3). This anomaly leads to anterior displacement of the renal pelvis and associated high insertion of the ureter. This anatomical abnormality causes impaired drainage of the collecting system and urinary stasis and concomitant stone formation (2).

Percutaneous nephrolithotomy (PCNL), shock wave lithotripsy (SWL) and retrograde in-

trarenal surgery (RIRS) are the treatment modalities of choice for HSK stones. PCNL maintains high success rates but it is associated with higher complication rates therefore the latter two alternatives are commonly applied. RIRS is being increasingly used in the treatment of stone disease particularly in HSK patients with holmium laser lithotriptors (4, 5).

Success rates in HSK patients after SWL is highly variable and stone free rates (SFR) of 31-100% were reported in the literature (6-11). SFR of RIRS in the management of HSK patients were reported to be 70% and 88.2% in the two recently published studies (4, 5). However the current lite-

ature lacks studies comparing the success rates and complication rates of SWL and RIRS in treatment of HSK stone disease and in this study it is aimed to compare these two treatment modalities in terms of SFRs and complication rates.

PATIENTS AND METHODS

Data of 67 patients treated with either SWL or RIRS for stone disease in HSK between May 2003 and August 2014 was investigated retrospectively. Stone disease was diagnosed by use of renal ultrasonography (USG), plain abdominal radiography and intravenous urography (IVU) or non-contrast enhanced computerized tomography (NCT). Appropriate antibiotic therapy was prescribed prior to SWL or surgery in case of diagnosed urinary tract infection. Demographic and stone related characteristics collected were: age, gender, stone size, localization and multiplicity and duration of hospitalization for RIRS group.

SWL was performed with ELMED Complit SWL device (Elektronik ve Medikal Sanayi ve Ticaret A.S, Ankara, Turkey). All patients were treated on an outpatient basis without anesthesia but sedation was applied with midazolam 0.1mg/kg intravenously when the patient could not tolerate the procedure. All treatment sessions were limited to 3000 shocks with frequency of 60-120shocks/minute and shock wave intensity was started at 14 kV and gradually increased to 21 kV. None of the patients were stented prior to the procedure.

RIRS procedure was performed with the patient under general anesthesia. The patient was positioned in lithotomy position and in a slight Trendelenburg position to allow the stone fragments to fall into the more upper calices. A 22F cystoscopy was introduced to visualize the ureteral orifice. Ureteral balloon dilation was performed when necessary and a hydrophilic guidewire was introduced. Next a ureteral access sheath, of various sizes (9.5/11.5 F or 12/14F Flexor (Cook Surgical, Indianapolis, IN)) was placed and then the surgeon passed the endoscope into the renal collecting system. Automatic flow irrigation at a pressure of 100cm H₂O associated with the manual pump was used to improve visualization. The laser energy was 0.8-1.2 J and frequency 8-12 Hz.

All the lower pole stones were repositioned to the upper calices. The procedure was ended when there was no visible fragments ≥ 3 mm in direct vision or under fluoroscopy. Double J stent was placed routinely after the procedure. A Foley catheter was inserted and taken out after 12-24 hours to ensure maximal drainage.

Complication rates during the perioperative period for both SWL and RIRS were recorded. All patients were evaluated by plain radiography and either renal USG or NCT at 1-6 weeks after SWL and 2-6 weeks after RIRS to evaluate the SFRs. Stone free status was defined as no residual fragments ≥ 3 mm in size. Renal colic episodes in the postoperative period were recorded and the two treatment modalities were compared for success and complication rates. Macroscopic hematuria was accepted as a complication rather than microscopic hematuria.

Statistical analysis was performed with SPSS ver. 20.0. Chi square test was used to compare categorical variables and Student t-test was applied for continuous variables of the treatment groups. For statistical significance p value of 0.05 was accepted.

RESULTS

A total of 52 stones in 44 patients and 32 stones in 23 patients were treated with SWL and RIRS respectively. Mean age of the population was 42.5 ± 8.2 (range: 16-78) years and mean stone size was 16.9 ± 4.1 mm (range: 6-25mm). SWL and RIRS groups were similar with regard to demographic characteristics and stone related characteristics and the results are summarized in Table-1. Mean duration of hospitalization was 1.8 days (1-3 days) in the RIRS group. Median number of SWL sessions was 3 (range: 1-6).

SFR of the SWL and RIRS groups were 47.7% (21/44 patients) and 73.9% (17/23 patients) respectively (p=0.039). In the SWL group, 10 patients (22.7%) achieved stone free status after a single session of SWL. Renal colic episodes were observed in 3 and 16 patients in the RIRS and SWL groups respectively (p=0.024). The results are summarized in Table-2. Double J stent placement was required in 13 patients (29.5%). SFR of

Table 1 - Demographic characteristics of the groups.

Parameters	SWL group (n=44)	RIRS group (n=23)	P value
Age (mean±SD)	42.8±8.4	44.2±9.9	0.17
Gender			0.62
Male (%)	32 (72.7)	18 (78.3)	
Female (%)	12 (27.3)	5 (21.7)	
Stone size, mm (mean±SD)	16.8±4.4	17.1±5.1	0.27
Multiple stones (%)	8 (18.1)	9 (39.1)	0.06
Stone location			0.917
Lower pole	12 (27.2)	6 (26.1)	
Pelvis and upper pole	32 (72.8)	17 (73.9)	

Table 2 - Treatment results of the groups.

Parameters	SWL group (n=44)	RIRS group (n=23)	P value
SFR (%)*	21 (47.7)	17 (73.9)	0.039
Renal colic episode (%)	16 (36.3)	3 (13)	0.024
Complications	8 (18.1)	4 (17.4)	0.936

*SFR: Stone free rate

the patients in SWL group with or without double J stent placement was found to be similar (6/13 patients vs 15/31 patients, $p=0.892$). Similarly double J stent placed had no effect on prevalence of renal colic episodes (5/13 patients vs 11/31 patients, $p=0.851$).

When the complication rates of the two groups were compared, no statistically significant complications were observed between the SWL (8/44 patients) and RIRS (4/23) groups ($p=0.936$). Hematuria was the most common complication and it was observed in 6 and 3 patients in the SWL and RIRS groups respectively. Fever was observed in 1 patient in each group and perirenal hematoma was observed in 1 patient in the SWL group. Results of the complication rates are summarized in Table-2.

DISCUSSION

HSK is the most common renal fusion anomaly and the abnormal position of the kidney and

unusual course of the upper ureter over the isthmus not only stands as a cause for stone formation but also makes the stone disease treatment more challenging. PCNL has already proved its efficacy on large stones located in HSK but the complicated nature of this method and vulnerability to complications (complication rates 14.3-29.2%) makes SWL and RIRS more feasible options (12-14).

Efficacy of SWL in stone disease of HSK has been studied since 1989 (15) and variable success rates have been reported. SFRs up to 100% were reported in the small sample sized studies (10). Differences in success rates depend on the definition of success, number of SWL sessions and duration of follow-up intervals. Sheir et al. reported 71.4% SFR in their series of 49 patients (9). However, they did not mention data on number of treatment sessions. In another series of 50 patients, 29 patients were available for follow-up and 75.9% SFR was reported (16). An important point in this study is the exclusion of patients with hydronephrosis, delayed drainage in radio-

nuclide scans which takes into mind the selection bias resulting in high success rate with relatively low number of sessions (mean 1.1 sessions). In our cohort, SFR was achieved in 47.7% of the patients after a median of 3 SWL sessions.

Ray et al. reported their series of 61 stones in 41 patients to identify the determinants of SWL success in HSK patients. They argued on incremental benefit of more than 2 sessions of SWL due to the discordance of high stone fragmentation rate (63.6%) and relatively low stone clearance rate (39.1%). However in this study the primary outcome measure was the single session success rate and any patient with more than one SWL sessions was accepted as treatment failure (17). In our study, success rate was not determined based on single SWL session but only 12 patients (21.4%) achieved stone free status after a single session of SWL.

There are a relatively low number of studies in the current literature on success rates of RIRS in HSK patients compared to studies on SWL and PCNL. In the normally located kidneys, RIRS is increasingly used all over the World with potentially higher success rates compared to SWL and lower complication rates compared to PCNL. Therefore not only reporting success and complication rates of RIRS in HSK patients but also comparison of treatment modalities is of importance in this specific patient population. However to our knowledge there is no study published comparing RIRS and SWL.

First series of RIRS in HSK patients was published in 2005 and stone clearance was achieved in 3 of the 4 patients (18). Following that, two larger series were published. In the first study, Molimard et al. reported their experience in 17 patients and SFR was achieved in 15 patients (88.2%) with mean stone size of 16mm. The success rate was comparable to PCNL and better than SWL studies with no major complications. However, the results were achieved by a highly experienced surgeon (4). In the same study 7 (41.2%) patients required more than one session of RIRS. In the second study, 25 renal stones of 20 patients were treated with RIRS and SFR of 70% was reported. The authors mentioned their success rates comparable with PCNL and better than SWL with the advantage of low complication rates (5).

Our study involves the highest number of HSK patients (32 stones in 23 patients) that underwent RIRS. In our study SFR of 73.9% was achieved with acceptable complication rates (4 of the 23 patients) which is comparable to previously published series (4, 5). When the results of SWL and RIRS are compared, significantly higher success rates were achieved with RIRS. Additionally renal colic episodes were observed more frequently in SWL group. One of the main advantages of RIRS compared to SWL is the repositioning of the lower pole stones to upper calices and this may facilitate the stone clearance rates following fragmentation. The main disadvantage of RIRS is the need for general anesthesia. However no major complication related to anesthesia was observed in the current study. Also, no significant difference was observed with regard to complication rates, hematuria being the most common complication in both groups.

The major limitation of the study is the retrospective nature and lack of randomization. Also, the procedures were performed by 4 different surgeons with variable level of experience. Also, neither duration of follow-up nor method of imaging in the preoperative and postoperative period was standardized.

CONCLUSIONS

In HSK patients with stone disease, both SWL and RIRS are effective and safe treatment modalities. However, RIRS seems to maintain higher SFRs with comparable complication rates. To determine the best method for treatment of stone disease in HSK patients, randomized trials comparing SWL, RIRS and PCNL are needed.

CONFLICT OF INTEREST

None declared.

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Effect of alpha1-blockers on stentless ureteroscopic lithotripsy

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ABSTRACT

Objective: To evaluate the clinical efficiency of alpha1-adrenergic antagonists on stentless ureteroscopic lithotripsy treating uncomplicated lower ureteral stones.

Materials and Methods: From January 2007 to January 2013, 84 patients who have uncomplicated lower ureteral stones treated by ureteroscopic intracorporeal lithotripsy with the holmium laser were analyzed. The patients were divided into two groups, group A (44 patients received indwelled double-J stents) and group B (40 patients were treated by alpha1-adrenergic antagonists without stents). All cases of group B were treated with alpha1 blocker for 1 week.

Results: The mean operative time of group A was significantly longer than group B. The incidences of hematuria, flank/abdominal pain, frequency/urgency after surgery were statistically different between both groups. The stone-free rate of each group was 100%.

Conclusions: The effect of alpha1-adrenergic antagonists is more significant than indwelling stent after ureteroscopic lithotripsy in treating uncomplicated lower ureteral stones.

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INTRODUCTION

Indwelling ureteral stent has been a common urological intervention after ureteroscopic stone management in many centers, which was used to reduce upper urinary tract drainage for preventing obstruction, pain, and infection (1-3). However, stent placement is also associated with significant morbidity such as infection, encrustation, hematuria, migration and stent fracture (4, 5). With the application of small-caliber endoscopes during ureteroscopy and better intracorporeal lithotripsy devices, therefore, the necessity for placing a postoperative stent remains question-

able due to the trade-off between lower incidence of postoperative ureteral stricture formation and stent-related complications.

Until now, the use of alpha1-blocker make it possible to treat lower ureteral calculi with ureteroscopic lithotripsy without indwelling ureteral stent. Alpha1-blocker is considered to be a safe and effective treatment for the small lower ureteral calculi. It was reported that alpha1-adrenergic receptors are the most abundant receptors in ureteral smooth muscle cells and it could mimic the stent-related symptoms and the lower urinary tract symptoms due to benign prostatic hyperplasia (6). To avoid the stent-associated complications, the-

refore, an investigation with respect to the possibility of using alpha1-blocker for the purpose of stentless ureteroscopic lithotripsy is necessary.

In view of this, we had performed a prospective study to investigate the effect of alpha1-blockers on stentless ureteroscopic lithotripsy. A series of indicators were measured to certify whether alpha1-blocker Tamsulosin could be used for the purpose of stentless ureteroscopic lithotripsy.

MATERIALS AND METHODS

Patients, Study Population and Ethical Statement

From January 2007 to January 2013, 84 patients who accepted ureteroscopic intracorporeal lithotripsy with the holmium laser were treated in our institution. Exclusion criteria were as follows: (1) acute renal failure, (2) chronic renal failure, (3) active urinary tract infection, (4) pregnant women, (5) history of urinary tract surgery or endoscopic treatment, (6) bladder pathology, (7) benign prostatic hyperplasia.

The patients were divided into two groups: group A included 44 patients who received indwelled double-J stents, and group B had 40 patients without stents. All cases of group B were treated with alpha1-blocker for 1 week. Institutional review board approval was obtained from the dean office and Ethics Committee of the hospital for the study, and informed consent was obtained from patients to allow their information to be used in this study in accordance with the Declaration of Helsinki.

Study procedure

All operations were performed by the same surgeon (JG Zhu). The patients were placed in the dorsal lithotomy position under continuous epidural anesthesia. Then, ureteroscopy was inserted into bladder through urethra with direct vision. With the help of ureteral catheter, ureteroscope went up-straight along the ureter until we found the calculi. The stone was crashed by holmium laser in order to make sure the fragments were less than 0.2cm in diameter. At the end of the procedure, 44 patients of group A were submitted to indwelled double-J stents in the ureter (4.8f, 25cm),

while 40 patients of group B were treated with alpha1-blocker without stents.

Postoperative treatment

Antibiotics were routinely used to prevent from infection for 3 days. All patients were advised to take a minimum of 2L of water daily and evaluated after treatment with KUB in 2 days to identify stone clearance or not and the position of double-J stent. Patients in group B received 0.4mg tamsulosin once daily. On postoperative week 4~5, double-J stent was removed under cystoscopy under local anesthesia in group A.

Indicators

In this study, operation time, hospital stay, and hospital charge were measured to evaluate the clinical outcomes. Furthermore, we evaluate International Prostate Symptom Score/quality of life component of IPSS (IPSS/QoL), Overactive Bladder Questionnaire (OAB-q), and Visual Analogue Pain Scale (VAPS) between the two groups at two weeks after operation. Moreover, we also evaluated complication rate in both groups at the same period (0-2 week post-operation).

Statistical analysis

Data is presented as mean±standard deviation (SD) in the following Table. The Independent-Samples T test was used for the statistical analysis. P values less than 0.05 were considered to be statistically significant (two-tailed). Kruskal-Wallis H test was applied to assess the numeration data. All data were processed by Statistical Product and Service Solutions 13.0 (SPSS 13.0) software.

RESULTS

Demographic Characteristics

A total of 84 patients were enrolled in the study. Forty-four patients were assigned to A group and 40 patients to B group. In all, 84 patients completed the study as specified and 0 patients were withdrawn. By analyzing the clinical characteristics between A and B group, all clinical factors showed no significant differences between these two groups regarding age, gender, body mass index, stone diameters. Our results demon-

trated that the baseline data was comparable in each group (Table-1).

Clinical Results

Placing ureteral stents after ureteroscopy prolonged operative time, the difference of operative time between both groups was statistically significant (p=0.04) (Table-2). The mean hospital stay was 9.64±1.29 days in group A and 6.85±1.34 days in group B (p=0.0000). Stents may add to the cost of patients care due to the price of the stent and the procedure of cystoscopic stent removal (Table-2).

Complication rate

Our results demonstrated that cystoscopic stent removal may raise the infection incidence. Postoperative outcomes are summarized in Table-3. The incidences of hematuria, flank/abdo-

minal pain, frequency/urgency were statistically different between group A and group B.

Comparisons of IPSS/QoL, VAP scale, and OAB-q scores

By Independent-Samples T test, our results demonstrated that no significant differences on IPSS score, QoL score, OAB-q score and VAPS score were detected in both groups at pre-operation. However, we also observed that a lower score of IPSS, QoL, OAB-q e and VAPS score were show in group B than those of in group A at two weeks after operation (further details are given in Table-4).

DISCUSSION

Ureteral stent placement after ureteroscopy is a common procedure. Without the role of stent placement, edema or mucosal inflammation

Table 1 - The demographic characteristics of patients.

Group	A (n=44)	B (n=40)	P values
Age (year)	36.59±11.26	33.05±12.72	0.737
Male/Female	26/18	22/18	0.79
Body Mass Index (Kg/m ²)	23.66±4.06	23.93±3.81	0.771
Stone diameters (mm)	7.77±1.23	8.25±0.91	0.16
Stone-free rate	100%	100%	

Table 2 - Operation time, hospital stay and hospital charges of patients (Mean±SD).

Groups	A (n=44)	B(n=40)	p-value
Operation time (min)	41.50±6.38	26.60±6.60	p=0.04
Hospital stay(day)	9.64±1.29	6.85±1.34	p=0.04
Hospital charges (¥)	8984.91±126.00	6194.90±480.89	p=0.001

Table 3 - Comparison of postoperative complications.

Group	A (n=44)				B (n=40)				p value
	—	Light	Medium	Heavy	—	Light	Medium	Heavy	
Flank/abdominal pain	4	14	16	10	22	14	4	0	p=0.002
Hematuria	6	6	26	6	26	12	2	0	p=0.001
Urinary frequency/urgency	6	10	18	10	26	10	2	2	p=0.002

Table-4 - Comparisons of IPSS/QoL, VAP scale, and OAB-q scores.

Groups	A (n=44)	B(n=40)	p-value
IPSS (storage symptom score)			
Pre-operation	4.31±2.67	4.25±2.54	0.763
2 week Post-operation	7.79±3.42	5.88±2.74	0.025
IPSS (voiding symptom score)			
Pre-operation	4.70±2.61	4.65±2.57	0.814
2 week Post-operation	7.87±3.14	5.36±2.25	0.031
QoI score			
Pre-operation	1.92±1.67	1.83±1.50	0.541
2 week Post-operation	3.98±1.58	2.07±1.03	0.028
OAB-q score			
Pre-operation	7.9±1.4	8.1±1.7	0.221
2 week Post-operation	18.77±2.89	13.63±2.20	0.001
VAPS score			
Pre-operation	2.58±1.49	2.63±1.61	0.149
2 week Post-operation	5.06±1.42	3.02±1.70	0.041

might result in obstructive symptoms and become the potential for ureteral stricture. Zimskind firstly performed temporary ureteral stent placement by cystoscope three decades ago (1). From then on, the stent is considered to decrease the incidence of postoperative ureteral stricture formation (2, 3) after ureteroscopic lithotripsy. However, ureteral stents (4, 5) may also cause significant morbidity such as infection, encrustation, hematuria, migration and stent fracture. It was demonstrated that 76% of patients had urinary symptoms, 70% had severe pain and need to get analgesics, 42% had to reduce their activities and 50% of them felt less healthy in general (7). With the development of small caliber endoscopes of ureteroscopy and intracorporeal lithotripsy devices, ureteric dilation is not necessary for ureteroscopy at present. According to what we mentioned, the need for postoperative stent remains questionable.

Stentless ureteroscopy was first introduced by Hosking in 1999 (8). In the following years, however, such kind of method did not gain much popularity because of the relatively high incidence of obstructive symptoms and potential

ureteral stricture after ureteroscopy. Therefore, the countermeasures for postoperative ureteral stricture formation are essential. As compared with the results obtained in patients with oral tamsulosin (0.4mg once daily), the additional benefits, in the form of hospital stay, lower hospital charges, lighter postoperative complications burden and better rational symptoms, were gained by the concurrent administration of tamsulosin. We also observed that mostly cases with longer operation durations were given stents. However, the reason that induced the longer operation durations was attributed to the procedure of stents implanting. For the patients in A group, the zebra guide wires should be inserted before the stents implanting. Therefore, the procedure of guide wire insertion resulted in longer operation durations compared with B group due to the presentation of tortuous upper ureter in some patients. In addition, a part of patients would re-implant the stents to relieve the stimuli of trigone of bladder. Therefore, the longer operation durations should be observed in patients who were given stents. Due to the same operative technique, supported treatment, clinical characteristics and care of post-operation in A

and B groups, we believe that the excellent results are mainly derived from Tamsulosin. These results were partly consistent with the previous result. Recently, some prospective randomized trials have showed that post-operative pain and irritative voiding symptoms could be reduced with omission of the ureteral stent and there was no difference of stone free status between the stent and the stentless group (9-12).

Tamsulosin is a selective blocker for $\alpha 1A$ and $\alpha 1D$ over $\alpha 1B$ -adrenoceptors, which are prevalent in the distal part of the ureter with a density order like $\alpha 1D > \alpha 1A > \alpha 1B$. It has been demonstrated that tamsulosin treatment may decrease the expulsion time and the frequency of renal colic attacks (13). Moreover, Porpiglia (14) confirmed that both nifedipine and tamsulosin could achieve an excellent expulsion rate with less additional pain medication during the treatment of distal ureteral stones. Therefore, Tamsulosin not only releases the stent-related symptoms but also improves the life quality of patients with ureteral stents (15). Alpha1-blocker can act as a selective antagonist for treating alpha1-adrenoceptor-mediated contraction of prostate, bladder, and proximal urethral smooth muscle (6). Thus, Tamsulosin is helpful for stent-related symptoms which are similar to benign prostatic hyperplasia-related symptoms. Owing to the miniaturization of endoscopes and the utilization of small-caliber holmium YAG laser, the morbidity of endourological procedures has decreased. Considering the effective role of Tamsulosin in dilating the ureteral lumen, it can be widely used in the treatment of uncomplicated lower ureteral stones combined with ureteroscopic lithotripsy.

Because the initial prospective study design was focused on effect of alpha1-blockers on stentless ureteroscopic lithotripsy, the study was presented with a number of challenges including (1 the small number of observations; 2) single center study. Therefore, a larger scale of multi-center clinical trial should be necessary in the future. In addition, some questions would arise concerning the duration for hospital stay is too long for both study groups. In China, however, the mandatory provision determined by the basic medical insurance stipulated that the preoperative examination must be done after hospitalizing, and

all of these preoperative examinations should be completed for two-three days or more. It is the reason why the mean of hospital day is longer than in other countries.

CONCLUSIONS

For patients who have uncomplicated lower ureteral stones treated by ureteroscopic intracorporeal lithotripsy with the holmium laser, the concurrent administration of tamsulosin on post-ureteroscopic intracorporeal lithotripsy should gain better clinical outcomes compared with indwelling ureteral stent. The use of alpha1-blocker make it possible to treat lower ureteral calculi with ureteroscopic lithotripsy without indwelling ureteral stent and avoid to the stent-related complications.

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CONFLICT OF INTEREST

None declared.

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Use of preoperative embolization prior to Transplant nephrectomy

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ABSTRACT

Introduction: After a failed transplant, management of a non-functional graft with pain or recurrent infections can be challenging. Transplant nephrectomy (TN) can be a morbid procedure with the potential for significant blood loss. Embolization of the renal artery alone has been proposed as a method of reducing complications from an in vivo failed kidney transplant. While this does yield less morbidity, it may not address an infected graft or refractory hematuria or rejection. We elected to begin preoperative embolization to assess if this would help decrease the blood loss and transfusion rate associated with TN.

Materials and Methods: We performed a retrospective analysis of all patients who underwent non-emergent TN at our institution. Patients who had functioning grafts that later failed were included in analysis. TN was performed for recurrent infections, pain or hematuria. We evaluated for blood loss (EBL) during TN, transfusion rate and length of hospital stay.

Results: A total of 16 patients were identified. Nine had preoperative embolization or no blood flow to the graft prior to TN. The remaining 7 did not have preoperative embolization. The shortest time from transplant to TN was 8 months and the longest 18 years with an average of 6.3 years. Average EBL for the embolized patients (ETN) was 143.9cc compared to 621.4cc in the non-embolized (NETN) group ($p=0.041$). Average number of units of blood transfused was 0.44 in the ETN with only 3/9 patients requiring transfusion. The NETN patients had average of 1.29 units transfused with 5/7 requiring transfusion. The length of stay was longer for the ETN (5.4 days) compared to 3.9 in the NETN. No intraoperative complications were seen in either group and only one patient had a postoperative ileus in the NETN.

Conclusion: Embolization prior to TN significantly decreases the EBL but does not significantly decrease transfusion rate. However, patients do require a significantly longer hospitalization with embolization due to the time needed for embolization. Larger studies are needed to determine if embolization before transplant nephrectomy reduces the transfusion rates and overall complications.

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INTRODUCTION

Although there have been vast improvements in surgical and immunosuppressive techniques in kidney transplant, transplant failure is

still a significant obstacle. Rates of graft survival beyond 5 years are largely unchanged (1). While rejection is the most common cause of transplant failure, other causes include infection and recurrence of previous kidney disease (2). When

the renal graft fails, several concerns can develop including pain, hematuria, and/or infections. Options for these patients traditionally have only been transplant nephrectomy or supportive care. Recently, embolization alone has shown to be beneficial in chronic pain and hematuria. However, it is not always successful in improving these symptoms. Earlier studies have shown that embolization prior to nephrectomy can eliminate some of the morbidity associated with transplant nephrectomy (1).

Transplant nephrectomy can be a morbid procedure with extensive blood loss and potential for intra and post-operative complications. The overall reported morbidity of transplant nephrectomy ranges from 4.3 to 84.4% and mortality rates have been quoted to be between 1.2 and 38% (1). Vascular complications associated with transplant nephrectomy include hemorrhage, pseudoaneurysm, or death (3).

The presence of a failed allograft *in vivo* can be associated with pain, chronic infection, and even sepsis. A retained failed allograft *in vivo* leads to elevated ESR and CRP. Chronically, this can lead to erythropoietin resistance, decreased albumin, and malnutrition (4). Some studies have shown failed transplants that remain *in vivo* continue to produce anti-HLA immunoglobulin, maintaining the inflammatory response. There have been conflicting studies as to whether transplantectomy affects PRA levels or changes re-transplant graft survival rates (5). The presence of a failed allograft when a patient must return to dialysis is associated with anemia and hypoalbuminemia, which increases the risk of poor outcomes (4).

Recent studies determined that nephrectomy of a failed allograft does not seem to significantly influence the survival of a subsequent graft (6). However, in a recent large study of transplant patients that returned to dialysis after failed kidney transplant, receipt of allograft nephrectomy was associated with a 32% lower adjusted relative risk for all causes of death. (7). Therefore, it seems transplant nephrectomy in a patient who is a surgical candidate may be preferred. However, attempts to reduce the morbidity and mortality of the procedure are still being investigated.

We proposed that performing preoperative embolization prior to transplant nephrectomy

may reduce the morbidity associated with this procedure.

MATERIALS AND METHODS

Data was collected retrospectively on consecutive non-emergent transplant nephrectomies performed at our institution between the years of 2001 and 2013.

Beginning in 2006, evaluation of blood flow to the failed graft was assessed using Doppler ultrasound. This was considered Group-1 regardless of flow status. If flow was identified, these patients went on to have embolization of the graft followed by transplant nephrectomy. If no flow was identified, patients proceeded onto transplant nephrectomy alone. Prior to 2006, no evaluation of blood flow was made and no patients underwent preoperative embolization. This was considered Group-2.

The embolization was performed by interventional radiology. This was done by a standardized technique with access via the right femoral artery. Gelfoam™ slurry was used in most cases to perform the embolization. One case each utilized Embospheres® microspheres or Tornado™ embolization coils. This was done at the preference of the interventional radiologist. Transplant nephrectomy was performed between 1 and 17 days after embolization for all but one patient. Four of the patients in Group-1 remained in the hospital between embolization and nephrectomy. One patient had surgery thirteen months after embolization due to persistent pain at the graft site after initial improvement. Transplant nephrectomy was performed through a modified Gibson incision utilizing the incision performed at the time of transplantation. All surgeries in both groups were performed extraperitoneally, using a subcapsular technique. Clamp hilar control was obtained as fast as safely possible utilizing vascular clamps. The kidney was removed and the vessels were oversewn. After surgery, all patients regardless of preoperative renal blood flow status discontinued immunosuppressive agents.

A retrospective analysis of the data was performed. Endpoints evaluated included estimated blood loss (EBL), blood transfusion rates,

length of hospital stay and peri-and postoperative complications.

RESULTS

Sixteen consecutive non-emergent transplant nephrectomies performed at our institution between the years of 2001 and 2013 were included in the analysis. Nephrectomy for patients in the immediate post-transplant period for acute thrombosis, bleeding, or infectious complications were not analyzed in this setting. Nine patients underwent preoperative embolization or were determined preoperatively to have no blood flow on Doppler ultrasound and were defined as Group-1. Within Group-1, three had no blood flow to the renal artery on preoperative evaluation and six showed flow and subsequently had preoperative renal embolization. Group-2 contained seven patients who did not receive embolization or evaluation of flow prior to nephrectomy. Transplant nephrectomy was performed for recurrent infections in seven, pain in four, hematuria in one, a combination of pain and hematuria in two, persistent anemia in one and disease recurrence in one. Table-1 lists the breakdown of patients in each group. Group-1 had significantly longer time from transplant until nephrectomy ($p=0.0023$). All transplantectomies in both groups were performed at least 6 months after initial graft placement. The age of the two groups ranged from 22-69 and there was no significant difference between them ($p=0.763$).

Group-1 had an average estimated blood loss of 143.89 ccs (range 20-475ccs) and Group-2 lost an average of 621.4ccs (range 50-1500ccs). This difference was significant with a p value of 0.047. However, the number of blood units given was not statistically different with an average of 0.5 units (range 0-2) transfused in Group-1 and 1.29 units (range 0-3) in Group-2 (p -value=0.214). Three of the nine patients in Group-1 required transfusion while five out of the seven in Group-2 required transfusion.

The average intraoperative time in Group-1 was just over 132 minutes. Unfortunately, adequate documentation of intraoperative time was not available for most patients in Group-2. Group-1 had a significantly longer hospitalization at an

average of 5.5 days (range 4-7 days). In comparison, Group-2 stayed on average 3.86 days (Range 2-5 days). There was a significant difference with a p value of 0.013 probably due to the fact that several patients received embolization prior to nephrectomy but during the same hospital stay.

No peri-or postoperative complications were seen in Group-1. Group-2 had one patient that experienced a postoperative ileus which was resolved by postoperative day three and required the patient to stay five total days postoperatively.

DISCUSSION

Approximately 6-16% of transplanted kidneys eventually required explantation (1). This difficult surgery has several known complications, including bleeding, abscesses, wound infection, and vascular injury. This study proves that embolization before nephrectomy reduces blood loss but does not prove to decrease overall transfusion rates.

The estimated blood loss of the embolized patients was significant less than the non-embolized group. However, overall transfusion rates were not significantly different. All patients in the embolized group underwent nephrectomy more recently than the non-embolized group. While the basic surgical technique did not significantly change, improvements in surgical methods, transfusion practices, and particular surgeon's preferences may have changed somewhat between the earlier years when the non-embolized nephrectomies occurred and the later years when embolization began. Most transplant centers report half the use of blood products compared to ten years ago (8). This may have some effect on when blood was given to the surgical patients in this study.

While transfusion rates were not significantly different, estimated blood loss is an important factor in overall surgical morbidity and is still an important consideration in choosing surgical techniques as blood loss is associated with increased morbidity, mortality, and hospital stay (8). Patients undergoing transplant nephrectomy are often anemic or have other comorbidities which may make total blood loss more significant even when it does not affect overall transfusion rate.

Table 1 - Patient Demographics.

Patient	Age at Nephrectomy	Cause of ESRD	Indication for Nx	Time to nephrectomy (years)	Presence of RBF
Group-1					
1	47	HTN	Recurrent UTI	9	Yes
2	58	PCKD	Gross hematuria	18	Yes
3	32	FSGS	Repeat Treatment	12	No
4	20	MPGN	Recurrent UTI	5	No
5	69	HTN	Recurrent UTI	8	No
6	45	HTN	Hematuria, pain	8	Yes
7	40	DM	Pain	8	Yes
8	22	PCKD	Infection	3	Yes
9	51	DM	Pain	8	Yes
Average	42.6			8.78	
Group-2					
1	61	DM	Pain, hematuria	6	Not assessed
2	26	HTN	Pyelonephritis	2	Not assessed
3	67	FSGS	Infection	0.7	Not assessed
4	35	HTN	Pain	3	Not assessed
5	38	PCKD	Pain	1.5	Not assessed
6	38	HTN	Anemia	2	Not assessed
7	44	HTN	Unknown	Unknown	Not assessed
Average	44.1			2.53	
P value	0.76			0.0051	

ESRD = End Stage Renal Disease; **Nx**=Nephrectomy; **DM** = Diabetes Mellitus; **HTN** = Hypertension; **PCKD** = Polycystic Kidney Disease; **FSGS** = Focal Segmental Glomerulosclerosis; **UTI** = Urinary Tract Infection; **MPGN** = Membrano-proliferative glomerulonephritis.

Kidney disease is a major adverse prognostic factor for cardiovascular events (9). Thus, operative blood loss, which creates such complications as hemodilution, hypothermia, clotting factor consumption and acidosis can create situations that are especially precarious in this patient population (8).

Embolization with nephrectomy was found in this study to lead to longer hospital stays. This

can create a new set of concerns such as increased financial burdens and comorbidities such as hospital acquired infections that are associated with longer hospital visits. However, length of stay in the embolized group can partially be accounted for due to the fact that two procedures were performed. Despite longer stays, lack of intra and postoperative bleeding lowers the risk for cardiovascular and he-

Table 2 - Surgical and Post-Operative Characteristics of Group 1 (Embolization) and Group 2 (No Embolization)

Variable	Embolization	No Embolization	P Value
Estimated Blood Loss (cc)	143.89	621.43	0.0417
Units of Blood Transfused	0.44	1.29	0.114
Transfusion Rate (percentage)	0.33	0.714	0.149
Total Hospital Length of Stay (days)	5.44	3.86	0.0112
Intraoperative Complications	0	1	0.3559

modynamic complications (8). Surgical approaches allowing embolization and nephrectomy as one procedure under general anesthesia should be investigated to determine if hospital stays are still longer than nephrectomy alone.

Doppler ultrasound looking for transplant renal blood flow was not routinely performed before nephrectomy until 2006. This practice began as a new surgeon elected to do ultrasounds prior to nephrectomy as standard technique. Therefore, it is reasonable to believe that some of the patients in Group-2 also had no blood flow, placing them in the incorrect study group and possibly altering study results.

Further studies with larger groups are necessary to evaluate whether embolization affects overall complications with transplant nephrectomy. Larger groups have been reviewed in transplant nephrectomy alone without regard to embolization. The paper, "Review of a Transplantation Series" published in *Transplantation Proceedings* in 2015 reviewed 70 transplantectomies and found serious complications (Clavien>III) in 21% of all cases (10). However, this study did not address prior embolization or lack of blood flow to the kidney before surgery.

Similar studies to ours have used comparative numbers to this study. The paper, "Intraoperative Coil Embolization Reduces Transplant Nephrectomy Transfusion Requirement" published in Aug 2007 in *Vascular and Endovascular Surgery* had data on 13 consecutive patients with similar results found in this study (1). Another study published in *Nefrologia* in 2005 addressed embolization alone versus nephrectomy and had a study population of seven (11). Thus, although our study was limited by its small numbers, the rarity

of this surgery makes this one of the larger study populations examined. A multi-center study may be necessary to more accurately look at peri and post-operative complications.

This study was also limited by its retrospective nature. However, prospective studies would again be limited by the infrequent occurrence of this surgery.

CONCLUSIONS

Embolization of the renal transplant prior to nephrectomy is beneficial given that there is less blood loss. However, patients may require a longer hospitalization when embolization is performed as a separate procedure prior to nephrectomy. Larger prospective studies are needed to validate our results as well as to determine if embolization before transplant nephrectomy reduces the transfusion rates and overall complications.

CONFLICT OF INTEREST

None declared.

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A fast, easy circumcision procedure combining a CO₂ laser and cyanoacrylate adhesive: a non-randomized comparative trial

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ABSTRACT

Background: Circumcision is performed as a routine operation in many countries, more commonly for religious and cultural reasons than for indicated conditions, such as phimosis and balanitis. There are many techniques available, and recently electrocautery and both Nd:YAG and CO₂ lasers, instead of blades, have been used for skin and mucosal incisions. However, the infection risk in circumcisions performed using a CO₂ laser was 10% higher. There are also reports of sutureless procedures using cyanoacrylate, but these have higher risks of hematoma and hemorrhage. We combined a CO₂ laser and cyanoacrylate to shorten the operation time and to decrease bleeding complications.

Materials and Methods: Circumcisions were performed under general anesthesia with CO₂ laser and cyanoacrylate combination in 75 6–9-year-old boys between May 2013 and August 2014 only for religious reasons. As a control, we compared them retrospectively with 75 age-matched patients who were circumcised using the conventional guillotine method in our clinic.

Results: No hematomas, bleeding, or wound infections were observed. One wound dehiscence (1.33%) occurred during the early postoperative period and healed without any additional procedures. The median operating time was 7 (range 6–9) minutes. The conventional guillotine group comprised one hematoma (1.3%), two wound dehiscences (2.6%), and two hemorrhages (2.6%), and the median operating time was 22 (range 20–26) minutes. The difference in surgical time was significant ($p < 0.001$), with no significant difference in the rate of complications between the two groups.

Conclusion: The combined CO₂ laser and cyanoacrylate procedure not only decreased the operating time markedly, but also eliminated the disadvantages associated with each individual procedure alone.

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Key words:

Circumcision, Male;
Cyanoacrylates; Lasers

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INTRODUCTION

Circumcision, which is performed more commonly for religious and cultural reasons than for indicated conditions, such as phimosis and balanitis, is a routine operation in many countries

and has a centuries-long history (1). Currently, it is the most common surgical procedure performed in male children (2). Recent studies have shown that it increases penile hygiene and decreases the risks of penile cancer and human immunodeficiency virus (HIV) infection (3-6).

Surgically, circumcision is an easy technique to learn and perform. However, the results must be satisfactory both functionally and esthetically. There are many techniques for performing circumcision. In recent years, circumcision procedures using auxiliary devices have become popular (7, 8). However, the use of these devices increases the recovery time (9).

The guillotine technique is a simple, quick method for circumcision. This technique involves excising the preputial skin circumferentially, preserving the glans penis after separating the adhesions of the coronal sulcus. To preserve the glans penis, the preputial skin is pulled up, and a straight hemostat is applied loosely to the preputium. The excess skin is cut using a scalpel between the glans and hemostat. However, this method has many shortcomings (10).

Electrocautery and Nd:YAG and CO₂ lasers are frequently used for circumcision, instead of blades for the skin and mucosal excisions (11-13). CO₂ lasers have been used since the early 1970s and are widely used for dermatology and plastic surgery procedures (14, 15). Many clinics also routinely use tissue adhesives to treat facial incisions (16). There are some reports on sutureless circumcision using cyanoacrylate (17, 18). However, very recently only one report has used the combination of a CO₂ laser and cyanoacrylate for circumcisions in a small series (30 patients) (19). Therefore, this study examined the combined use of a CO₂ laser and cyanoacrylate for shortening the operating time and reducing complications related to bleeding, in comparison with the conventional guillotine method in a bigger series with 150 patients.

MATERIALS AND METHODS

We performed our technique on 75 boys, aged 6–9 (median 7) years, who underwent circumcision between May 2013 and August 2014. In all cases, the parents had requested circumcision for religious reasons. Routinely, after iodine disinfection under general anesthesia, the adhesions on the coronal sulcus are separated, the preputial skin is pulled up, and a straight hemostat is applied loosely to the preputium. The incision is made distal to the hemostat using a CO₂ laser (UltraPul-

se 5000C, Coherent Medical Group, Santa Clara, USA), applying 350 millijoules energy at 40 pulses/second in continuous mode. No bleeding requiring bipolar use was detected after the excision (Figure-1). Cyanoacrylate (Leukosan Adhesive, BSN Medical, Hamburg, Germany) is applied after approximating the mucosa and remaining skin on the penis (Figure-2). A chlorhexidine wound covering and Coban bandage are used for wound care.

Figure 1 - Image obtained after laser excision.



Figure 2 - Perioperative image obtained after cyanoacrylate application.



The complications and operating time in this group were compared retrospectively with those of 75 age-matched patients from our clinic archive who were circumcised using the conventional guillotine method for religious reasons (supplementary material Video-1).

RESULTS

The patients were followed postoperatively for 12 (range 4–18) months on average. Wound healing took one week. No hematomas, bleeding, or wound infections were observed. Dehiscence occurred in one child (1.3%) during the early postoperative period but healed spontaneously within one week. Six months postoperatively, the cases were similar in appearance to those who underwent the conventional procedure (Figure-3). The parents of the patients were satisfied with the aesthetic results. The median operating time was 7 (range 6–9) minutes. In the conventional guillotine group, one hematoma (1.3%), two wound dehiscences (2.6%), and two hemorrhages (2.6%) were recorded, and the median operating time was 22 (20–26) minutes. The difference in operating times between the groups was significant ($p < 0.001$), while the difference in complications was not ($p > 0.5$) (Table-1).

DISCUSSION

Circumcision is performed routinely in many countries for religious and cultural reasons.

Generally, it is performed in pediatric patients and protects against penile cancer and HIV (3-6).

Commonly used conventional circumcision techniques include the dorsal slit, sleeve, and guillotine techniques (20). Electrocautery or Nd:YAG or CO₂ lasers can be used instead of a scalpel. In addition, non-invasive circumcision using various devices has become popular (7, 8), although the recovery period is increased when these devices are used (9).

Procedures performed using a CO₂ laser can shorten the operating time and decrease bleeding and pain (21). How et al. (13) compared the costs associated with the operating time between CO₂ lasers and the conventional technique. They found that the median operating time was 20 (range 16–21) minutes using the conventional technique and 15 (range 13–17) minutes using a CO₂ laser. When all of the expenses accrued were calculated, the CO₂ laser technique was much more cost effective for circumcision than was the conventional technique, and the morbidity rates were favorable compared with the conventional technique. We could not perform a cost-analysis, because the operating theatre charges in our university hospital are not known due to the health policies in our country. Nevertheless, our median operating time was less than half that of How et al., suggesting that our technique is more cost-effective.

Although the postoperative complication risks of circumcision performed using a scalpel versus a CO₂ laser were similar, the infection risk was 10% higher with the CO₂ laser (10, 22-23).

Figure 3 - Anterior and lateral images obtained during the 6th month.



Table 1 - Comparison of conventional guillotine group and laser+cianoacrylate group.

	Conventional Guillotine Group (75 Patients)	Laser + Cyanoacrylate Group (75 Patients)	p Value
Operation Time (Median)	22 minutes	7 minutes	$p < 0.001$
Hematoma	1(%1.3)	0	$p > 0.5$
Wound Dehiscence	2(%2.6)	1(%1.3)	$p > 0.5$
Hemorrhage	2(%2.6)	0	$p > 0.5$

Recently, the use of tissue adhesives has increased, because they are easy to use, shorten the procedure duration, and achieve similar cosmetic results to those using standard suturing techniques (25-27). Antimicrobial effects, especially on Gram-positive bacteria, are very important for wound care (28). Another important advantage of tissue adhesives is that they decrease the risks of granulation tissue and sinus tract formation (17).

When compared with conventional techniques, circumcision using a CO₂ laser has many advantages, while the increased risk of postoperative infections is the main disadvantage. The antimicrobial characteristics of tissue adhesives overcome this problem associated with laser circumcision. On the other hand, tissue adhesives carry risks of hematoma formation and related suture detachment. However, the high level of hemostasis in circumcisions performed using a CO₂ laser prevents hematoma development and any related detachment.

Complications such as hematoma and hemorrhage, which Kelly et al. (17) reported in 502 subjects undergoing sutureless circumcision, were not encountered using the cyanoacrylate and laser combination in our study. Our complication rates were 1.4% for hematomas and 2.2% for hemorrhage using standard treatment and 0% in the combination group. The dehiscence rate was similar to that in our study (0.8%).

We did not evaluate postoperative pain in this study. Furthermore, a very recently report shown that pain score is not significant different between laser and conventional technique (19).

The parents of the patients in both groups were satisfied with the aesthetic results in our study.

The main reason we developed the combination technique was to prevent complications such as hematoma and hemorrhage by using the laser, while shortening the operation time, decreasing the infection risk, and eliminating the need for postoperative suture removal, which is difficult to perform in children, by using cyanoacrylate.

In the light of our results, we believe that our combination method overcomes the disadvantages observed with each circumcision procedure alone and shortening the operating time.

CONFLICT OF INTEREST

None declared.

Supplementary Material

Video 1 - Conventional Guillotine Technique and Guillotine incision with CO₂ Laser.

Available at: <http://www.intbrazjurol.com.br/videos/ibju-2015-0284video.mp4>

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Does platelet activity play a role in the pathogenesis of idiopathic ischemic priapism?

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ABSTRACT

Purpose: Mean platelet volume (MPV) is used to measure platelet size and is defined as a potential marker of platelet reactivity. Higher MPV levels have been defined as a risk factor for increased incidence of intravascular thrombosis and its associated diseases. We aimed to determine whether a relationship exists between the MPV and veno-occlusive component of idiopathic ischemic priapism (IIP).

Materials and methods: Between 2010 and 2014, 38 subjects were analyzed in two groups. One was composed of 15 patients with diagnosis as IIP in our institute, and the other contained 23 healthy control subjects. Complete blood count reports were retrospectively evaluated in both groups. MPV, platelet count (PLT), platelet distribution width (PDW), white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), reticulocyte distribution width (RDW) were measured in both groups.

Results: The mean ages were similar in IIP patients (45.86 ± 15.82) and control subjects (47.65 ± 10.99). The mean MPV values of IIP patients were significantly higher than control subjects ($p < 0.05$). In contrast, also PLT counts were significantly lower in IIP patients, compared to control subjects ($p < 0.05$). The mean hemoglobin and WBC values were significantly lower in control group ($p < 0.05$). There was no significant difference of RBC, PDW and RDW values in both groups.

Conclusions: We found that the MPV was significantly higher in IIP patients compared to control subjects. The high MPV levels may have contributed to the veno-occlusive etiopathogenesis of IIP disease. We strongly suggest further prospective studies to recommend the use of MPV in routine practice.

ARTICLE INFO

Key words:

Priapism; Mean Platelet Volume; Blood Platelets

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INTRODUCTION

Idiopathic ischemic priapism (IIP) is an emergency condition including a persistent painful rigid erection that continues for longer than 4 hours and unrelieved by ejaculation or orgasm (1). Its incidence is about 1.5 per 100.000 person, and it is a sexual function-threatening andrological emergency (2). The underlying pathophysiology is still not clear. The condition is idiopathic in up to

60% of cases but non-idiopathic pathologies commonly associated with hematologic abnormalities, particularly sickle cell disease, vasoactive intracavernosal injections, psychotropic medications, recreational drugs, and those secondary to malignancy may play a role (3).

Platelet volume indices (PVI) include mean platelet volume (MPV), platelet distribution width (PDW) and total platelet number and variation are indicative of change in platelet function (4).

MPV is used to measure platelet size and its increase is associated to activation of platelets (5). Higher MPV levels are associated with the increase of other markers of platelet activity, including platelet aggregation, thromboxane synthesis, and expression of adhesion molecules (6). Therefore, it is demonstrated that elevated MPV is associated with ischemic pathologies such as unstable angina, myocardial infarction, ischemic stroke and venous thromboembolism (5, 7, 8).

Because IIP is also an ischemic vascular disease, we aimed to determine whether a relationship exists between MPV and veno-occlusive component of IIP in this study.

MATERIALS AND METHODS

Between 2010 and 2014, 15 patients diagnosed with IIP in our institute were included in the study as study group. Control group was composed of 23 healthy subjects of similar ages. All patients and controls were Caucasian.

Patient's who complained of persistent rigid erection that continued longer than 4 hours and not relieving by ejaculation and orgasm, were diagnosed as priapism. After a detailed history and physical examination, cavernosal blood gas examinations and confirmatory penile doppler ultrasound were performed. A complete hematologic evaluation including peripheral blood smear was done. Pelvic magnetic resonance imaging (MRI) was performed in all IIP patients. In all patients, that was the first episode of priapism. However, three patients (20%) complained of erection episodes without sexual stimulation that continued for 1-2 hours and terminated spontaneously in the last two years before the IIP episode.

None of the patients had any of the following diseases: systemic diseases (e.g. coronary artery disease, diabetes mellitus, hypertension), hematological disorders (sickle cell anemia, thrombotic thrombocytopenic purpura (TTP), idiopathic thrombocytopenic purpura (ITP), myeloproliferative disorders, leukemia, Bernard-Soulier syndrome, total number of platelets less than $150 \times 10^3/\mu\text{L}$ or more than $450 \times 10^3/\mu\text{L}$, peripheral vascular diseases, splenectomy, active infectious disease, malignancy, renal or hepatic failure. None of the

subjects were using anti-platelet or anticoagulant drugs, vasoactive intracavernosal injections, psychotropic medications, or recreational drugs.

Complete blood count, including hemoglobin (Hb), mean corpuscular volume (MCV), white blood cell count (WBC), red blood cell count (RBC), platelet number (PLT), mean platelet volume (MPV), reticulocyte distribution width (RDW) and platelet distribution width (PDW) parameters were measured in both groups. Blood samples were drawn from the antecubital vein and they were collected in tubes containing dipotassium ethylenediaminetetraacetic acid. The parameters were measured by an automated blood counter (CELL-DYN® 3700, Abbott Laboratories).

All data were analyzed with Statistical Package for Social Science database program. The Independent Sample t test was used for continuous variables when variables were normally distributed and equal variations were assumed. The Mann-Whitney U test was used for categorical variables or for continuous variables when they were not distributed normally or equal variations were not assumed. Chi-square test was used to find the relationship between two independent categorical variables. A $p < 0.05$ was considered significant in all statistics.

RESULTS

The mean ages were similar in IIP patients (45.86 ± 15.82) and control subjects (47.65 ± 10.99). The duration of rigid erection was 10.26 ± 2.07 in IIP patients. The penile artery flow was found minimal or absent in all IIP patients in the confirmatory penile doppler ultrasound examination. Peripheral blood smear results did not reveal any sign of hematologic disorders. The cavernosal blood gas measurements of IIP patients are shown in Table-1. Patient's had low pH, low $p\text{O}_2$ and high $p\text{CO}_2$ in the cavernosal blood gas measurement. The comparison of complete blood count parameters between control subjects and IIP patients are shown in Table-2. The mean MPV values of IIP patients were significantly higher than control subjects ($p < 0.05$). In contrast, the average number of platelets was significantly lower in IIP patients, compared to control subjects ($p < 0.05$). The mean

Table 1 - The cavernosal blood gas measurements of IIP patients.

		Mean±sd*
Cavernosal blood gas	pH	7.04±0.14
	pO ₂	23.71±4.34
	pCO ₂	77.64±10.2

Sd* = Standard deviation

ted a relationship between MPV and varicocele. They concluded that high MPV values which is a marker of increased platelet activity may play an important role on the varicocele pathophysiology. They reported a correlation between the severity of varicocele and MPV (13). Similarly, Mahdavi-Zafarghandi et al. reported that MPV values with varicocele patients were higher than control group. They found a significant correlation

Table 2 - The comparison of complete blood count parameters between IIP patients and control subjects.

	IIP patients (n=15)	Control subjects (n=23)	p
	Mean±sd*		
Red blood cells (x10⁶/μL)	4.63±0.65	4.99±0.24	0.061
Hemoglobin (gr/dL)	13.90±1.73	12.40±1.06	0.002
White blood cells (x10³/μL)	9.60±3.16	7.48±1.88	0.030
Platelet count (x10³/μL)	221.46±76.79	267.82±51.61	0.032
Mean platelet volume (fL)	8.72±1.82	7.39±0.86	0.017
Platelet distribution width (%)	17.27±1.45	17.42±1.46	0.803
Reticulocyte distribution width (%)	15.06±1.13	15.12±0.12	0.869

Sd* = Standard deviation

Hb values and the number of average WBCs were significantly lower in control group ($p < 0.05$). There was no significant difference of RBC, PDW and RDW values in both groups.

DISCUSSION

Platelets are heterogeneous in size and density regarding their activity. Platelets with larger size are metabolically and enzymatically more active (9, 10) and have greater prothrombotic potential (6). Therefore, platelet volume has been assumed as a marker of platelet reactivity (11). MPV is a parameter of complete blood count that is commonly used to measure the average volume of platelets (12). It is the most validated and prominent platelet volume marker (4) and it can be assumed a potential marker of platelet reactivity (5).

MPV was evaluated for the pathogenesis of several urological diseases. Bozkurt et al. repor-

with varicocele grade and the level of the MPV (12). Ciftci et al. also demonstrated that the MPV values were significantly higher in patients with vasculogenic erectile dysfunction and they suggested that high platelet activity and total platelet count play a role in the pathogenesis of vascular complications such as cavernosal arterial insufficiency in vasculogenic erectile dysfunction (14). On the other hand, several studies found a significant relationship between high MPV levels and ischemic stroke (7, 15, 16). Additionally, Bilgic et al. found that elevated MPV was significantly associated with worse outcomes in acute mesenteric ischemia (17).

In the present study, we found significantly higher MPV levels in IIP patients compared to healthy controls. There are two hypotheses to explain the higher MPV levels in IIP patients. First, the MPV levels may be normal without priapism episodes just as control subjects and it acutely in-

creases at the time of priapism episodes because of various inflammatory conditions or loss of platelets. It is already demonstrated that platelet production is regulated to preserve a constant total body platelet mass (18). The PDW is computed as the coefficient of variation of the average volume of the platelet population. A high PDW indicates that the platelets are more variable in volume than normal (19, 20) and it can be a sign of active platelet release (21). However, we found that the PDW levels were similar between IIP patients and control subjects. Besides that, total platelet count was significantly lower in IIP patients compared to control subjects. Platelets would be down regulated in IIP patients in order to maintain a constant total platelet mass because of higher MPV levels (larger platelets). These findings demonstrated that IIP patients regulated the MPV, PDW and total platelet count values in a chronically time period in order to maintain a constant total platelet mass.

Second, some people may have more active platelets with higher MPV levels and these people may have greater risk for episodes of priapism. Although the etiopathogenesis of IIP remains unclear, the pathophysiology of the condition includes venous outflow occlusion and the resultant cessation of cavernosal arterial inflow leading to hypoxia and micro vascular thrombosis of the corpora cavernosa (22). Upadhyay and colleagues examined the histology of the corpora cavernosa in patients with prolonged priapism. They revealed that an organized thrombus filling the cavernous sinuses caused venous obstruction, stasis and recurrent priapism (23). Probably, metabolically and enzymatically more active platelets with higher MPV levels leading to a tendency of thrombus formation in the cavernous sinuses may have contributed to the pathogenesis of IIP. Also, Braekkan et al. demonstrated that higher MPV levels were significantly associated with higher risk of venous thromboembolism pathogenesis (24). Additionally, higher MPV levels have been defined as a risk factor for increased incidence of intravascular thrombosis and its associated diseases (7, 8, 14-17, 24-27). Consequently, according to these findings, metabolically and enzymatically more active platelets

with higher MPV levels may have contributed to the pathogenesis of IIP. Hence, higher levels of MPV and lower total platelet counts would be predictive for IIP in a risk based approach.

In the present study, WBC and hemoglobin concentrations were found significantly higher in IIP patients compared to control subjects. However, the mean levels of both parameters were ranged in normal spectrum. Acute inflammation would be a possible cause of the slightly increase of leucocytes. In addition, although hemoglobin concentration was higher, red blood cell counts were similar in both groups.

This study has some limitations. The number of IIP patients was relatively small in the present study. While evaluating the prothrombotic activity, other markers of platelet activity including beta-thromboglobulin, platelet factor IV and fibrinolytic status are lacking in the present study. For more insight on the hemodynamics in IIP, studies with large population and assessment of platelet activity with additional markers must be performed.

We found MPV values significantly higher in IIP patients compared to control subjects. The high MPV levels may have contributed to the veno-occlusive pathogenesis of IIP disease. We strongly suggest further prospective studies to recommend the use of MPV in routine practice.

ABBREVIATIONS

IIP = Idiopathic ischemic priapism
 MPV = Mean platelet volume
 MRI = Magnetic resonance imaging
 TTP = Thrombotic thrombocytopenic purpura
 ITP = Idiopathic thrombocytopenic purpura
 Hb = Hemoglobin
 MCV = Mean corpuscular volume
 WBC = White blood cell
 RBC = Red blood cell
 PLT = Platelet number
 PDW = Platelet distribution width
 RDW = Reticulocyte distribution width

CONFLICT OF INTEREST

None declared.

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Severity of erectile dysfunction is highly correlated with the syntax score in patients undergoing coronariography

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ABSTRACT

Objective: To investigate the association between the severity of erectile dysfunction (ED) and coronary artery disease (CAD) in men undergoing coronary angiography for angina or acute myocardial infarct (AMI).

Material and Methods: We studied 132 males who underwent coronary angiography for first time between January and November 2010. ED severity was assessed by the international index of erectile function (IIEF-5) and CAD severity was assessed by the Syntax score. Patients with CAD (cases) and without CAD (controls) had their IIEF-5 compared. In the group with CAD, their IIEF-5 scores were compared to their Syntax score results.

Results: We identified 86 patients with and 46 without CAD. The IIEF-5 score of the group without CAD (22.6 ± 0.8) was significantly higher than the group with CAD (12.5 ± 0.5 ; $p < 0.0001$). In patients without ED, the Syntax score average was 6.3 ± 3.5 , while those with moderate or severe ED had a mean Syntax score of 39.0 ± 11.1 . After adjustment, ED was independently associated to CAD, with an odds ratio of 40.6 (CI 95%, 14.3-115.3, $p < 0.0001$). The accuracy of the logistic model to correctly identify presence or absence of CAD was 87%, with 92% sensitivity and 78% specificity. The average time that ED was present in patients with CAD was 38.8 ± 2.3 months before coronary symptoms, about twice as high as patients without CAD (18.0 ± 5.1 months).

Conclusions: ED severity is strongly and independently correlated with CAD complexity, as assessed by the Syntax score in patients undergoing coronariography for evaluation of new onset coronary symptoms.

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INTRODUCTION

Atherosclerosis is a multifactorial chronic inflammatory disease which occurs as a response to endothelial damage, affecting mostly the intimal layer of multiple diameter arteries. In at least half of the patients, the first presentation of

atherosclerotic disease is an acute coronary event. In 68% of sudden deaths, patients did not have previous symptoms of coronary heart disease (1). There is a huge effort worldwide for early detection of coronary artery disease (CAD), due to its elevated morbidity and mortality rates as well as significant social-economics consequences. It is

estimated that atherothrombotic events will continue to be the leading cause of death as far as 2020 (2).

Although invasive and requiring highly specialized physics, equipment and personnel structure, the gold standard test to determine the presence and severity of CAD is coronariography. The Syntax score developed for the SYNTAX trial (Synergy Between PCI with Taxus and Cardiac Surgery) presents a thorough way to analyze the severity of CAD (3, 4).

Erectile dysfunction (ED) is the inability to achieve and maintain erection for satisfactory sexual intercourse (5) and its global prevalence ranges from 2 to 86% (6). ED has risk factors and physiopathology basis similar to those of CAD, with endothelial dysfunction as a common denominator affecting several vascular beds of multiple diameters (7). ED can be easily assessed by using the abridged International Index of Erectile Function (IIEF-5) questionnaire (8). Besides affecting sexuality, ED has been increasingly recognized for its ability to detect insidious CAD (9, 10).

There is no non-invasive method, clinical sign or laboratory test capable of detecting all individuals who will develop CAD. ED may be an early clinical marker of CAD with association to the degree of ED and the severity of coronary artery disease in a large portion of men. Herein, we evaluated the association between the complexity of CAD measured by the Syntax score and the severity of ED measured by the IIEF-5 in men who underwent coronary angiography.

MATERIAL AND METHODS

Male patients undergoing coronary angiography for the first time between January and November 2010 were included in this case-control study. During this period, 1773 patients underwent coronary angiography. Based on convenience sampling, 132 patients consecutively submitted to first coronary angiography for diagnosis of CAD were selected according to the following criteria: age between 40 and 70 years old, hemodynamic stability, full recovery from the procedure with consciousness and orientation (average recovery time of 3 hours after the procedure), at least 2

risk factors for CAD [hypertension, dyslipidemia, hyperglycemia, smoking, family history of stroke, or acute myocardial infarction (AMI)], absence of previous cardiovascular events (first event).

Exclusion criteria were: ED after radical prostatectomy, ED secondary to a neurological lesion, refusal to participate in the study. Five patients were excluded because they were not fully recovered from the procedure.

Patients were clinically evaluated and filled both a demographic and the IIEF-5 questionnaires. The recorded coronariography films were analyzed with the Syntax Score to evaluate the complexity of the coronary lesions by an experienced cardiologist who was blind to the patient's erectile function status. The SYNTAX score is the sum of the points assigned to each individual lesion with >50% diameter narrowing in vessels >1.5mm diameter in the coronary tree. A computer algorithm is then queried and a summed value is generated.

Patients were divided into two groups according to the exam results: Group-1 (test) had obstruction equal to or greater than 50% of the vessel lumen and Group-2 (control) without CAD detected on coronariography. Patients on Group-2 should have at least one additional exam, such as stress test, stress echocardiography, or myocardial scintigraphy to rule out CAD.

Group-2 patients were further classified according to their indication for the exam in acute (unstable angina, AMI) or chronic (stable angina) cases. The duration of ED was considered in relation to the first coronary symptom.

The IIEF-5 questionnaire was given after the patient fully recovered from the angiographic procedure. The IIEF-5 ranges from 5 to 25 points, classifying ED into one of five possible categories: severe (5-7 points), moderate (8-11), mild to moderate (12-16), mild (17-21), and absence of ED (22-25). The IIEF-5 questions refer to the patient's symptoms in the previous 6 months and not only to the moment when they are answering the questions.

This study was approved by the ethics committee of our Institution under the protocol number 1274.0.000.107-09. Patients were included in the study only after informed consent was obtained.

Statistical analysis

The continuous and categorical variables were described as averages with standard deviation and frequencies with 95% confidence interval, respectively. The Shapiro-Wilk test was used to evaluate the assumption of normality. We used either the Pearson Chi-square or the Fisher's exact test when appropriate to test the hypothesis relative to categorical variables. The Student's t-test for independent samples was used for the comparison between groups with CAD and without CAD. The comparison of the variable "SYNTAX score" between the groups with different degrees of ED was performed with analysis of variance (ANOVA) followed by the Tukey post-hoc. The comparison of the variable "IIEF-5" between the test and control groups was performed with a general linear model with a single factor (group) adjusted for the variables "age" and "use of diuretics". The forward stepwise logistic regression method was used to evaluate ED as a predictor of CAD. The ability of the model to discriminate patients with CAD and without CAD with the receiver operating characteristic (ROC) curve was also analyzed. The ability of the ROC curve to discriminate between cases with CAD and without CAD for the variable "IIEF-5" was also evaluated. The p value of 0.05 was considered significant. The SPSS software version 18.0® was used for statistical analysis.

RESULTS

We evaluated 132 male patients, 86 with CAD and 46 without CAD. Patient's characteristics are shown in Table-1. The mean age was 58.6 ± 8.4 years. Hypertension and diabetes were present in 95.4% and 31% of the patients, respectively. The test and control groups were significantly different regarding mean age, use of diuretics, and IIEF-5 ($p=0.02$, $p<0.0001$, and $p<0.0001$, respectively). The IIEF-5 score of the group without CAD (adjusted mean 22.6 ± 0.8) was significantly higher than the score of the group with CAD (adjusted mean 12.5 ± 0.5) after adjustment for age and use of diuretics, with an average difference of 10.1 ± 0.9 (IC 95%, 8.2–12.0, $p<0.0001$) between the groups (Figure-1). ED was present in 89 patients (67.4%), 46% of which were moderate or severe (Table-2).

In the 86 patients with CAD, the Syntax score averages increased exponentially with increasing severity of ED. In patients with normal erectile function, the Syntax score average was 6.3 ± 3.5 , while those patients with moderate or severe ED had a mean Syntax score of 39.0 ± 11.1 (Figure-2).

After adjustment for age, total cholesterol, LDL cholesterol, and triglycerides, ED was the only variable independently associated to CAD, with odds ratio of 40.6 (CI 95%, 14.3–115.3, $p<0.0001$). The accuracy of the logistic model to correctly identify the presence or absence of CAD was 87.1% after adjustment, with sensibility of 91.9% and specificity of 78.3% for diagnosis of CAD. The area under the ROC curve was 0.851 ± 0.040 ($p<0.001$, CI 95%, 0.773–0.929), demonstrating a good discriminant ability (Table-3).

The IIEF-5 questionnaire had an exceptional discriminant ability for presence or absence of CAD (area under the ROC curve 0.922 ± 0.022 , $p<0.001$), with sensibility of 91.2%, specificity of 78.3%, and an IIEF-5 cutoff value of 21.5 (Figure-3).

The average time that ED was present in patients with CAD was 38.8 ± 2.3 months before the coronariography. The average time of ED in patients with acute disease was 38.4 ± 19.2 months (Table-4).

DISCUSSION

In our study, the IIEF-5 questionnaire had exceptional discriminant ability for the diagnosis of CAD, when compared with the Syntax score. We identified a clear correlation between the severity of ED and the complexity of CAD, which has been previously observed in the literature (11–13). However, the method that we used to evaluate the complexity of coronary disease differed from those used by other authors. Greenstein et al. used the number of vessels with at least one significant lesion. They found that patients with a single vessel affected had erections more frequently, more rigid, and more easily to achieve than patients with 2 or 3 vessels affected (11). Montorsi et al. used the Gensini's score, which estimates the amount of myocardium affected by each coronary

Table 1 - Baseline characteristics.

	Group 1 (n=86) (with CAD)	Group 2 (n=46) (without CAD)	P value
Age	59.8±8.2	56.3±8.8	= 0.02
Systolic BP	156.5±19.0	154.7±17.4	0.6
Diastolic BP	92.7±9.4	92.5±9.0	0.9
BMI	27.2±3.1	27.1±4.2	0.9
AC	103.0±20.1	99.0±10.,8	0.2
Glucose	107.9±39.8	107.9±39.7	0.9
Total cholesterol	214.3±48.6	199.4±72.4	0.2
HDL	39.5±7.4	41.0±9.6	0.2
LDL	139.0±42.8	125.8±64.0	0.2
Triglycerids	178.7±68.3	160.1±69.2	0.2
Total IIEF-5	12.7±5.7	22.03±2.3	< 0.0001
Smoking	48 (55.8%)	21 (45.7%)	0.3
Family history of stroke	28 (32.6%)	12 (26.1%)	0.6
Family history of CVD	30 (34.9%)	15 (32.6%)	0.8
Hypertension	82 (95.3%)	44 (95.7%)	0.9
Diabetes Mellitus	29 (33.7%)	12 (26.1%)	0.4
ETHNICITY			
White	66 (76.74%)	32 (69.57%)	0.4
No-White	14(23.26%)	20 (30.43%)	0.4
MEDICATIONS IN USE			
Diuretics	15 (17.4 %)	25 (54.3%)	< 0.0001
Adrenergic inhibitors	44 (51.2 %)	21 (45.7%)	0.6
Vasodilators	18 (20.9%)	07 (15.2%)	0.5
CCB	09 (10.5%)	08 (17.4%)	0.3
ACE Inhibitors	31 (36.0%)	18 (39.1%)	0.8
ARB	13 (15.1%)	11 (23.9%)	0.2

HDL = high density lipoprotein; **LDL** = low density lipoprotein; **CVD** = cardiovascular disease; **BMI** = body mass index; **AC** = abdominal circumference; **CCB** = calcium channel blockers; **ACE** = angiotensin-converting enzyme; **ARB** = angiotensina II receptor blockers

Figure 1 - IIEF-5 versus Coronary Artery Disease

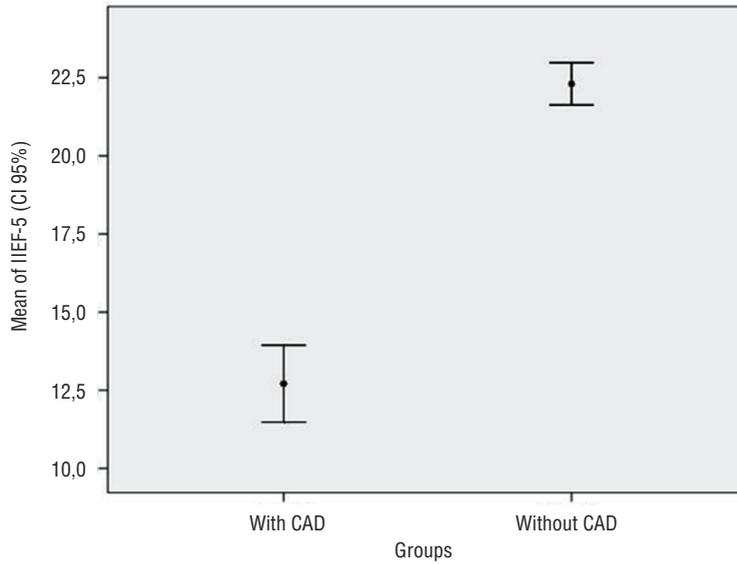


Table 2 – Erectile dysfunction severity (IIEF-5).

Erectile dysfunction severity	n (%)	CI 95%
No ED	43 (32.6 %)	25.0 – 40.2
Mild ED	28 (21.2%)	14.4 – 28.0
Mild to moderate ED	20 (15.2%)	9.1 – 21.2
Moderate ED	19 (14.4%)	8.3 – 20.5
Severe ED	22 (16.7%)	10.6 – 23.5

Figure 2 - Grade of ED according to Syntax Score

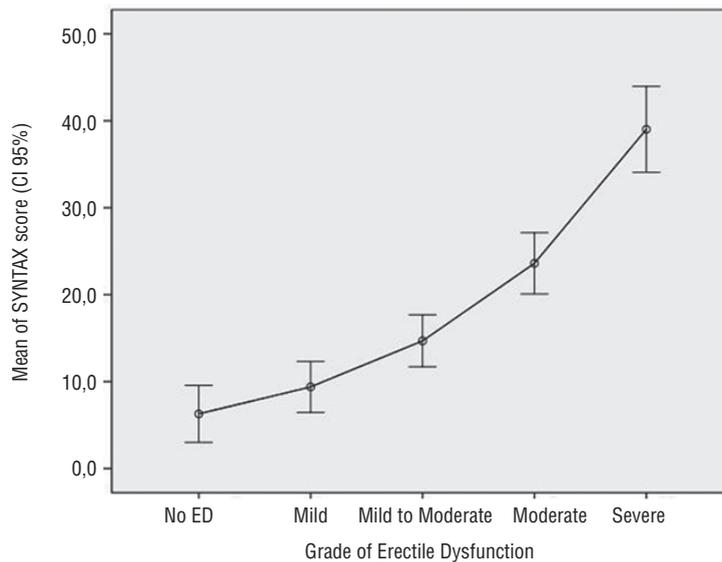
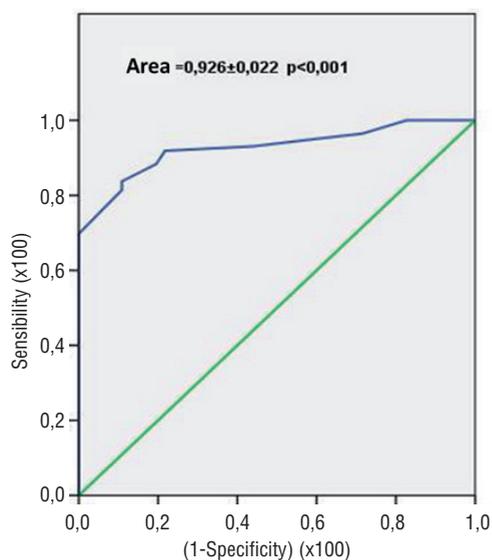


Table 3 - Factors associated with CAD.

Variable	Odds ratio (adjusted)	CI 95%	P
Age	-	-	0.55
Total cholesterol	-	-	0.83
LDL	-	-	0.98
Triglycerids	-	-	0.82
Smoking	-	-	0.78
Erectile Dysfunction	40.6	14.3 -115.3	<0.0001

Figure 3 - ROC curve of IIEF-5 and CAD



lesion. They identified a significant increase of the Gensini's score proportional to the decrease of the IIEF-5 score (14).

To our knowledge, this is the first study to use the Syntax score as the instrument to evaluate the complexity of coronary lesions in patients with ED. The Syntax was developed as a comprehensive angiographic scoring system aiming to assist in patient selection and risk stratification of patients with extensive coronary artery disease undergoing revascularization with either percutaneous coronary intervention (PCI) or coronary-artery bypass grafting (CABG) (15). According to the characteristics of the lesions, the SYNTAX score is able to identify patients who are good candidates for PCI, classifying them into low risk (0-22 points), intermediate risk (23-32 points), and high risk (≥ 33 points) patients. It is also a good predictor of adverse events in patients with multiarterial coronary disease and/or with lesions in the left

Table 4 – Time with Erectile Dysfunction (n=89).

Groups	Time (months)	CI 95%	P
Without DAC	18.0±5.1	7.9–28.1	<0.0001
With DAC	38.8±2.3	34.4–43.3	
Acute cases*	38.4±19.2		0.76
Chronic cases**	39.9±22.9		

* First acute myocardial infarction (AMI) episode, AMI without ST segment elevation or unstable angina; **Stable Angina or thoracic discomfort for > 2 months

coronary artery main trunk who underwent PCI. Hence, this instrument has a good discriminatory power for risk assessment (16).

The highly significant correlation that we found between an increased Syntax score and a decreased IIEF-5 score suggests that the ED severity may be an important factor to be assessed before the indication of myocardial revascularization, either by PCI or CABG, which needs to be confirmed in a study designed for this purpose.

The IIEF-5 is widely used because of its easy application and reproducibility. It has a sensibility of 98% and specificity of 88% (8, 17).

In our study, we did not find any significant difference in the presence of ED between acute and chronic cases. Montorsi et al. found a different prevalence of ED between patients with acute and chronic CAD, with a lower ED rate in acute cases (14).

The fact that we did not reproduce this finding could be possibly explained by the limited number of chronic cases in our study.

In our study, the mean time interval between the onset of ED and coronary symptom was 38.8 months in the group with CAD, more than two times greater than in the control group (18 months). Montorsi et al. found similar numbers in a previous study with 300 men with acute chest pain in whom ED symptoms became clinically evident prior to CAD symptoms in 67% of the patients. They also observed a mean time interval between the onset of ED and CAD of 38.8 months (18).

There is evidence of association between penile vascular alterations and abnormalities in the stress test of asymptomatic individuals (19-21). For this reason, all patients included in the control group of our study were required to have, besides the coronariography, at least one exam (stress test, stress echocardiography, or myocardial scintigraphy) to rule out ischemic heart disease.

ED and CAD share common risk and etiology factors, with atherosclerosis and endothelial dysfunction promoting vascular insufficiency (22). ED is as a highly sensitive, specific, and accurate marker for potential cardiovascular events. A meta-analysis of 7 cohort studies with 45.558 patients showed an adjusted relative risk of 1.47 (95% CI, 1.29-1.66, $P < 0.001$) for

CVD events in patients with ED in comparison with healthy subjects (23).

Age and use of diuretics were the only ED risk factors that differed significantly between the groups. Both are well known risk factors for ED (24, 25). However, the difference of the average IIEF-5 scores between the groups with and without CAD persisted significant even after adjustment for those variables. Riedner et al. observed that men younger than 60 years old with ED are at increased risk for more severe CAD, independently of risk factors for ED and CAD (26).

Limitations of this study include its retrospective nature, which inherently has the potential for selection bias, lack of penile Doppler ultrasound or testosterone levels. Also, the study was conducted in a specific population who carried, at least, two risk factors for atherosclerosis and had an indication for coronary angiography for a clinical suspicion. Hence, its results should be further verified for external validation. Nevertheless, our results associated to the substantial evidence in the literature strongly suggests that the simple assessment of ED severity with a validated tool should not be neglected in the daily practice, as it may aid in the early diagnosis of CAD and prevention of morbidity and mortality associated to coronary disease. The IIEF-5 is an inexpensive and easily applicable tool that may aid in the decision to perform screening exams for CAD in men without coronary symptoms. This holds true especially for patients with other risk factors for CAD. Application of this tool is particularly useful in the daily practice, where methods to detect latent cardiovascular events are extremely important. Health professionals in general should consider ED as a significant medical condition and run a thorough clinical evaluation looking for cardiovascular risk factors. The time when patients first complain of ED may represent a timely window for the early diagnosis and treatment of cardiovascular disease.

CONCLUSIONS

The severity of ED as assessed by the IIEF-5 is strongly and independently correlated with the Syntax score in patients undergoing coronariography for evaluation of new onset coronary

symptoms. This study further confirms the importance of ED and the IIEF-5 questionnaire for the early identification of patients at increased risk for CAD, which could elicit measures to prevent major cardiovascular events in this population. More studies are needed to determine whether application of the IIEF-5 could also be extended to a general population without other CAD risk factors.

CONFLICT OF INTEREST

None declared.

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An easy, reproducible and cost-effective method for andrologists to improve the laboratory diagnosis of non-obstructive azoospermia: a novel microcentrifugation technique

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ABSTRACT

This study describes a new method of microcentrifugation as an improved, viable, cost-effective option to the classical Cytospin apparatus to confirm azoospermia. Azoospermic semen samples were evaluated for cryptozoospermia by a centrifugation method similar to that of World Health Organization guidelines (2010; entire specimen centrifuged at 3000g for 15 min, and aliquots of the pellet examined). Then, if no sperm were detected, the pellet from that procedure was resuspended in culture medium, centrifuged (2000g for 15 min), and the entire pellet spread on a 4 X 6mm area of a slide and stained using the Christmas tree method (Nuclear-Fast solution and picric acid). The entire stained area was examined for the presence or absence of sperm. A total of 148 azoospermic samples (after standard WHO diagnosis) were included in the study and 21 samples (14.2%) were identified as sperm-positive. In all microcentrifugation slides, intact spermatozoa could be easily visualized against a clear background, with no cellular debris. This novel microcentrifugation technique is clearly a simple and effective method, with lower cost, increasing both sensitivity and specificity in confirming the absence or presence of spermatozoa in the ejaculate. It may represent a step forward of prognostic value to be introduced by andrology laboratories in the routine evaluation of patients with azoospermia in the initial semen analysis.

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INTRODUCTION

Non-obstructive azoospermia (NOA) affects approximately 10% of all men presenting with infertility and is responsible for 80% in the subgroup of azoospermic men, whereas obstructive azoospermia contributes to 20% of this subpopulation of infertile men (1). The management of

patients with NOA relies on the correct diagnosis, induction of spermatogenesis to try to produce an ejaculate of viable spermatozoa, and finally techniques for sperm procurement, such as micro-dissection testicular sperm extraction, simple sperm extraction (TESE) or Fine Needle Aspiration (FNA), preferentially followed by either sperm or testicular tissue cryopreservation to subsequent

intracytoplasmic sperm injection (ICSI) use (2-5).

“Should only a few or no spermatozoa be seen at initial evaluation, the sample must be centrifuged and the sediment examined for spermatozoa. The term azoospermia can only be used if no spermatozoa have been found in the sediment” wrote Eliasson 1981 (3). Although apparently simple, the diagnosis of azoospermia has a wide variety of confounding factors including those related to methodology and different evaluation protocols, large errors associated with counting few spermatozoa, the number of microscopy fields to be analyzed, difficulties in examining debris-laden pellets (6). The World Health Organization’s recommended changes include, examining fixed uncentrifuged samples and indicating the sensitivity of the counting methods employed; however, existing centrifugation methods necessary for accumulating sufficient number of cells are also included (6).

Whereas that presence of any spermatozoa in a complementary test of azoospermic patient may determine the clinical approach to be adopted, the centrifugation method of cell suspension on slides (Shandon CytoSpin III Cyto-centrifuge, Thermo Scientific, Waltham, MA, USA) is widely used to concentrate the ejaculate in a single droplet to enable checking for the presence of spermatozoa in an optical microscope (7, 8). The Cytospin apparatus is a bench-top centrifuge with a specially-designed rotor, and sample chambers in which a special micro-slide is vertically placed after being filled with 0.1mL of well-mixed whole semen and an equal volume of sterile saline added in situ. This method of diluting the specimen is preferable to using a premixed dilution and minimizes cell-sampling errors, and should be used on specimens in which “no spermatozoa” were detected in a wet preparation. After centrifugation the cells are deposited in a uniform monolayer in a compact area (32mm²) and even with the use of stains such as nuclear fast red and picroindigocarmine (described as NF-PICS or Christmas Tree stain; Sigma-Aldrich, St. Louis MO, USA) (9, 10), is not always easy to identify isolated sperm heads owing to the large amount of cellular debris. Therefore, our group has developed a simple and

cost-effective technique as an alternative to the Cytospin method: the microcentrifugation technique to confirm azoospermia (Labnet, Woodbridge NJ, USA). The aim of this study is to demonstrate an alternative method to confirm and improve the laboratory diagnosis of non-obstructive azoospermia.

PATIENTS AND METHODS

Study population

A retrospective study was performed involving 148 slides from semen of non-obstructive azoospermic patients in the reproductive age (mean 40.66; standard deviation 9.40) presenting to male infertility evaluation at Androscience, High Complexity Clinical and Research Andrology Laboratories, São Paulo, Brazil, between November 2008 and July 2013. Institutional Review Board approval was obtained from the University of São Paulo Research Ethics Committee and before that all samples were collected after informed consent signature.

Seminal Analysis

Semen sample was obtained by masturbation after 48 to 72 hours of sexual abstinence. All semen analysis was performed manually by the same investigator (RACM).

After liquefaction, macroscopic and microscopic parameters were analyzed according to World Health Organization (WHO) guidelines of 1999 and 2010 (6, 11). Semen was evaluated for cryptozoospermia by a centrifugation method similar to that in WHO (2010; entire specimen centrifuged at 3000g for 15 min, and aliquots of the pellet examined), following by triplicate sediment evaluation (100µL) in all field of Neubauer chamber (6). If any spermatozoa were present in the pellet obtained after this first centrifugation the sample was classified as cryptozoospermic and excluded. In the absence of spermatozoa after the first centrifugation, a standard WHO diagnosis of azoospermia was given and the samples were further alternately processed by the microcentrifugation technique, proposed by this study.

The samples had all of the sediment left after the first centrifugation resuspended in a

small amount (100µL) of Human Tubal Fluid (Modified HTF Medium, Irvine Scientific, Santa Ana, CA, USA) and centrifuged in a mini-centrifuge (Labnet, Woodbridge NJ, USA) at 2000g for 15 minutes. After removal of the supernatant, the pellet was deposited on and gently spread over a glass slide with a pipette in order to cover an area of 2 to 4cm².

The slides obtained were then fixed in absolute ethanol for 15 minutes, and air dried. Nuclear-Fast Red solution [73.1mM Aluminum Sulfate (cat#202614, Sigma Aldrich, St. Louis, MO, USA) and 1.49mM Fast Nuclear Red (cat#N8002, Sigma Aldrich, St. Louis, MO, USA) in 50mL distilled water], was placed over the slide for 15 minutes, after which the slides were carefully rinsed with distilled water, covered with picroindigocarmine stain [7.15mM Indigo Carmine (cat#18130, Sigma Aldrich, St. Louis, MO, USA) and 50mL of Picric Acid (cat#92540, Sigma Aldrich, St. Louis, MO, USA)] for 15 seconds, immediately rinsed with absolute ethanol, left to air dry, covered with coverslips and mounted with Entellan (Merck Millipore, Darmstadt, Germany) (8).

The slides were examined at 1000×magnification in optical microscope (Nikon Eclipse E200, Japan) for the following parameters: sperm presence or absence, sperm integrity, and for the presence of cellular debris.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 19 for Windows. We calculated the mean of age (years) and frequency of events in the study (%).

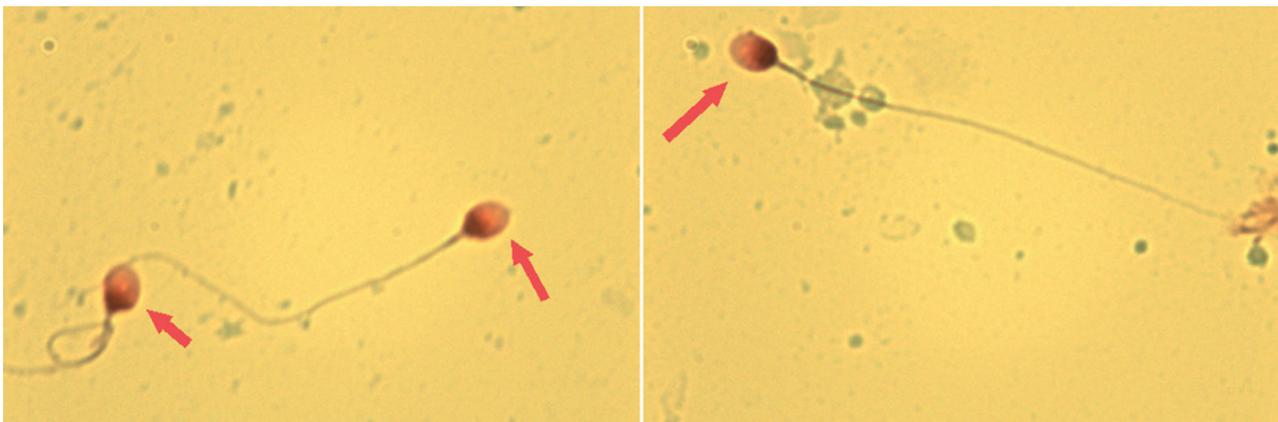
RESULTS

A total of 148 azoospermic samples (after standard WHO diagnosis) were included in the study. Twenty one samples (14.2%) were identified as sperm-positive. In addition, intact spermatozoa could be visualized against a clear background, with minimal cellular debris in all slides (Figure-1).

DISCUSSION

Although intracytoplasmic sperm injection (ICSI) is a remarkably effective technique in allowing fatherhood in men previously considered sterile, sperm retrieval from the epididymis or testis should be the last option to be given to men. Gnoth et al. showed that the source of spermatozoa has not relevant impact on the results of ICSI cycles as long as fresh motile, morphologically normal spermatozoa are used, proposing, in case of cryptozoospermia, to preferentially use ejaculated spermatozoa to prevent those men from an unnecessary testicular biopsy avoiding risks and costs implied (12). In addition, the

Figure 1 - Spermatozoa found after microcentrifugation technique (2000g for 15 minutes), stained by nuclear fast red and picroindigocarmine (described as NF-PICS or Christmas Tree stain) in an azoospermic man. We observed clear slides obtained with sperm integrity preserved, without cellular debris.



1000x in immersion oil.

use of a single sperm found in ejaculate in ICSI resulting in successful pregnancy was demonstrated by Desai et al. (13). Thus, azoospermia requires a diligent search for reversible factors and treatment to restore natural fertility and a pragmatic 'default to ICSI' can be avoided (14).

Semen analysis is the single most important diagnostic tool method for the assessment of male infertility and the gold standard according to the World Health Organization. The WHO guidelines offer detailed advice on every aspect of semen analysis and consequently has become the gold standard for the field (6). According to these guidelines, a patient is diagnosed as azoospermic when few or no sperm cells are found in wet preparations, the sample is centrifuged and there is also absence of sperm in the sediment (3). However, the presence or not of spermatozoa in the sediment depends on the centrifugation time and speed and on how much of the sediment is examined and how thoroughly (6). No technique more stringent is proposed by the WHO guidelines to characterize the sample further after the routine seminal analysis does not identify any spermatozoa.

Shandon Cytospin emerged in the 1970s as an apparatus suited for cell concentration and has since been used for sediment analysis of many fluids. In the late 1980s it was first used to analyze the presence of germ cells exfoliate in semen samples and identification of carcinoma in situ of the testis (15). Afterwards Cytospin was introduced in Andrology laboratories as an additional test to confirm azoospermia. Despite being a simple and rapid method, processing the sample often causes damage to the structure of the sperm cells, such as the separation of the heads from mid piece and tail. Furthermore, the presence of cellular debris makes a detailed analysis difficult and time-consuming. In the view of these disadvantages, the microcentrifugation technique developed by our group proved to be as effective and possibly increased the sensitivity and specificity in confirming azoospermia, beyond the cost 5 times lower, since it uses the whole sediment obtained after the first centrifugation, minimizing cell losses but maximizing debris washout.

We purposed to demonstrate a method detection of single sperm cells in semen sediments as Cytospin, but with visualization and identification of isolated sperm cells much easier. From the technical

standpoint, the differences between the two methods presented here are: a second wash to remove cellular debris, the rotational speed applied to the sample, and the way the final sample is "deposited" on the slide. More important than the time and rotation speed, the new technique proposed uses another wash with culture medium which can contribute to both the concentration of the sediment and the removal of cellular debris. The centrifugation and pellet resuspension technique is widely used in laboratory routine to eliminate cell debris and was applied in our method. Additionally, the sample deposition on slide is carried out differently: while Cytospin method concentrated all sample in a small area of slide, the microcentrifugation allows the sample to spread over a larger area of slide surface with a pipette.

There is no consensus on which rotation is best for the processing of azoospermic semen samples. Whereas the Cytospin centrifuge uses 700g for five minutes, the microcentrifugation proposed by us used 2000g for 15 minutes. In the literature, the speed centrifugation of semen samples remains controversial. Corea and colleagues (16) showed that centrifugation should apply at least 1000g for 5 minutes in order to find sperm cells in suspected cases of azoospermia but the WHO guidelines (6) suggests centrifugation at 3000g for 15 minutes for samples in which no sperm cell is found. Other studies showed that centrifugation at 1000g for 15 minutes is effective (17, 18). Our hypothesis of 2000g for 15 minutes was based on laboratory practice and literature, considering that (I) little centrifugation time was not enough for the pellet formation and (II) 3000g damaged the sperm integrity.

The NF-PICS or Christmas Tree stain is one of the most widely used for histological tests for the identification of sperm in sexual assault cases (10). With this stain it is possible to color both the post-acrosomal region of the sperm head, that stains pink, and the acrosome itself, that stains light pink, while cell debris are colored green, allowing easy differentiation of spermatozoa from cellular debris and thus facilitating the reading of the slide. Other cellular dyes, such as eosin-nigrosin, Hoechst 33342 fluorescent stain and Diff-Quick can be suggested to replace the NF-PICS (6, 19). Therefore, as azoospermia is a complex diagnosis and subjected to doubts, it is important to preserve the slide with mounted coverslip

after staining not only for subsequent reading but also to make it available for a second opinion review by other professionals.

In conclusion, the microcentrifugation method developed by our group showed to be a simple, effective, and low cost technique able to increase the sensitivity of confirming azoospermia, an important step of prognostic value to be used hand in hand with clinical and surgical approaches aimed to reverse azoospermic state.

ABBREVIATIONS

NOA = Non-obstructive azoospermia
 TESE = Testicular sperm extraction
 FNA = Fine Needle Aspiration
 ICSI = Intracytoplasmic sperm injection
 WHO = World Health Organization
 NF-PICS = Nuclear fast red and picroindigocarmine

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CONFLICT OF INTEREST

None declared

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EDITORIAL COMMENT

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In this issue of *Int Braz J Urol*, a paper by Monteiro and colleagues reports on a novel semen analysis method to increase precision in the diagnosis of azoospermia (1). This paper is important given the recent changes in the World Health Organization (WHO) laboratory methods for evaluation of human semen (2) and the current discussion on how to count sperm properly (3). This editorial commentary is aimed to highlight Monteiro and colleagues findings and to expand the discussion on the clinical importance of laboratory andrology.

Azoospermia is a laboratory diagnosis of ejaculates that lack spermatozoa (4). Despite being a descriptive term that does not imply any specific underlying cause, the absence of spermatozoa in a semen analysis has many ramifications for both clinical practice and research. The reliable diagnosis of azoospermia is important not only for male infertility and to ensure the success of vasectomy but also for assessing efficacy of male contraceptive trials. Moreover, the confirmation for the presence of semen in forensic studies is based on the direct identification of spermatozoa.

Seminal fluid is a complex mixture of secretions from the seminal vesicle, the prostate gland and the combined contribution of the epididymis, testicles and bulbourethral glands (5). An average male ejaculates around 3.2 mL of semen. Each milliliter contains approximately 64 million spermatozoa (6). This number can vary with the age of the male, and may be affected by other factors, including medical conditions, genetic background, ejaculatory abstinence and life-style habits such as diet, tobacco and illicit drug use (7). On the contrary, azoospermia affects approximately 1% of males in the general population and 10-15% of those facing infertility (8).

In the context of infertility, men with azoospermia do not have an unattainable potential to initiate a pregnancy. From the laboratory standpoint, the assessment of an initially azoospermic ejaculate should be followed by the examination of the pelleted semen to exclude cryp-

tozoospermia, which is defined by the presence of very small number of live sperm (8). This is important because the finding of live sperm may allow intracytoplasmic sperm injection to take place without the need of surgical sperm retrieval. Since the diagnosis of azoospermia or cryptoospermia is based upon semen analysis, proper laboratory techniques are crucial to reduce analytical errors and enhance precision (9).

Accuracy, the degree to which the measurement reflects the true value, and precision, the reproducibility of the results, are important for clinicians who rely upon the values provided by the laboratory to direct the further work-up and to counsel men seeking fertility (9). Interestingly, data from surveys of laboratory practice in the United States and the United Kingdom revealed that semen analysis techniques are poorly standardized. Interestingly, less than half of the laboratories providing semen analysis services have implemented quality control practices and only about 5% fully complied with the WHO manual for the laboratory assessment of human semen (10, 11).

Owed to its complex nature, we have advocated that semen analysis should be ideally carried out in Andrology Laboratories equipped with experienced technicians and validated systems, and enrolled in internal and external quality control programs (3, 8, 9, 12). In this current issue of *Int Braz J Urol*, Monteiro and colleagues shed light on this very same issue (1). The authors studied semen specimens from men with nonobstructive azoospermia, all of which were negative for the presence of sperm after processing according to the new WHO guidelines. Of note, the WHO method recommends centrifugation of a 1 mL aliquot of semen for 15 minutes using high *g* force and examination of two aliquots of the pelleted semen (2). The use of high centrifugation force is warranted because the accuracy of any centrifugation protocol using low *g* forces in pelleting all spermatozoa in a given ejaculate is uncertain (13). However, the aforementioned WHO method is flawed by not

recommending examination of the whole specimen. Furthermore, the pelleted semen gives rise to large amount of debris that might make it difficult to identify isolated spermatozoa (13). Monteiro et al. proposed an additional centrifugation step using high g force, followed by examination of the whole pellet deposited in a large area of a microscope glass slide (1). For this, the slides were fixed and stained with nuclear fast red and picroindigocarmine. Interestingly, clear slides were obtained with minimum debris, and isolated spermatozoa were identified in about 14% of the specimens evaluated. These patients were reclassified as having cryptozoospermia, with obvious diagnostic and management implications.

Although a comparative analysis with other centrifugation and staining techniques has

not been included, Monteiro and colleagues' method was clearly superior to the WHO method for evaluating azoospermic specimens. The finding of any sperm in a semen analysis meticulously performed, such as the one object of this commentary, has important implications for men with nonobstructive azoospermia. It is not only a proof that residual sperm production exists, thus allowing the affected men to achieve biological offspring with the aid of assisted reproductive technology, but also imply that sperm retrieval is likely to be successful whenever required.

Collectively, the study of Monteiro and colleagues highlights the importance of semen analysis in the context of azoospermia and the role of Andrology Laboratories to improve precision and reproducibility of reported results.

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Short hairpin RNA targeting insulin-like growth factor binding protein-3 restores the bioavailability of insulin-like growth factor-1 in diabetic rats

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ABSTRACT

Purpose: To investigate whether intracavernosal injection of short hairpin RNA for IGFBP-3 could improve erectile function in streptozotocin-induced diabetic rats.

Materials and methods: After 12 weeks of IGFBP-3 short hairpin RNA injection treatment, intracavernous pressure responses to electrical stimulation of cavernous nerves were evaluated. The expression of IGFBP-3 and IGF-1 at mRNA and protein levels were detected by quantitative real-time PCR analysis and Western blot, respectively. The concentration of cavernous cyclic guanosine monophosphate was detected by enzyme-linked immunosorbent assay.

Results: At 12 weeks after intracavernous administration of IGFBP-3 shRNA, the cavernosal pressure was significantly increased in response to the cavernous nerves stimulation compared to the diabetic group ($P < 0.05$). Cavernous IGFBP-3 expression at both mRNA and protein levels was significantly inhibited. At the same time, cavernous IGF-1 expression was significantly increased in the IGFBP-3 shRNA treatment group compared to the diabetic group ($P < 0.01$). Cavernous cyclic guanosine monophosphate concentration was significantly increased in the IGFBP-3 shRNA treatment group compared to the diabetic group ($P < 0.01$).

Conclusions: Gene transfer of IGFBP-3 shRNA could improve erectile function via the restoration of cavernous IGF-1 bioavailability and an increase of cavernous cGMP concentration in the pathogenesis of erectile dysfunction in streptozotocin-induced diabetic rats.

ARTICLE INFO

Key words:

Insulin-like growth factor binding protein-3; insulin-like growth factor-1; erectile dysfunction

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INTRODUCTION

Erectile dysfunction (ED) has been increasingly recognized as a public health problem, estimated to affect approximately 150 million men worldwide (1). Current research on erectile physiology has focused on the pathogenesis of ED and provided convincing evidence that diabetes is one of the most prevalent causes of ED (2).

Insulin-like growth factor binding protein-3 (IGFBP-3) is a member of the growth factor family (3). As an important growth factor, IGFBP-3 has been reported that it is highly correlated with erectile function in diabetic rat model and the increased expression of IGFBP-3 could elicit ED in patients with diabetes mellitus (DM) (2). In some previous studies, it has been also reported that the increased expression of IGFBP-3 is associated with

ED in hypertensive and streptozotocin (STZ)-induced diabetic rats (4, 5). IGFBP-3 may limit the bioavailability of insulin-like growth factor-1 (IGF-1), while IGF-1 is associated with ED in rat model (6, 7).

Since short hairpin RNA (shRNA) emerges as a technology to silence gene expression by inhibiting mRNA translation and/or inducing its degradation (8), therefore, in this study we employed shRNA technology to investigate whether intracavernosal injection of shRNA for IGFBP-3 can ameliorate diabetes-related ED.

MATERIAL AND METHODS

IGFBP-3 shRNA Constructs

To design IGFBP-3 shRNA construct, the rat IGFBP-3 gene sequence (GI:M31837) was analyzed for a potential siRNA target using the web-based siRNA target finder and design tool provided on the Ambion website (Ambion, Inc., Austin, TX). Double-stranded siRNAs (nucleotide position: 611) were transcribed "in vitro" using the Silencer siRNA construction kit (Ambion) following the manufacturer's instructions. The inhibitory siRNA (5'-GCGCTACA-AAGTTGACTATGA-3', nucleotide position 611) was then cloned into the pGPU6/GFP/Neo plasmid vector (2nd version, Ambion) as a short hairpin DNA sequence (5' sense strand:5'-CACCGCGCTACAAAG TTGACTATGATTCAAGAGATCATAGTCAACTTTGTA GCGCTTTTTT-3') according to the manufacturer's instructions. The pGPU6/GFP/Neo-IGFBP-3 shRNA plasmid was purified using Endo-free Maxi kit (Qiagen, Valencia, CA). Plasmids were quantitated by spectrophotometry and prepared in 0.9% saline solution at a concentration of 1.0 μ g/ μ L.

Experimental Animals

Twenty seven adult male Wistar rats (Grade SPF, 3-month-old, weight 310-330g, certificate No. scxk (E) 2008-0005) were obtained from Hubei Research Center of Laboratory Animal (Wuhan, China). The rats were randomly divided into three groups with nine rats in each group. Group 1 included 9 normal control rats that received i.p. injection of citrate buffer (100mmol/L citric acid and 200mmol/L disodium phosphate, pH 7.0). The other 18 rats received i.p. injection of STZ at a dose of 65mg/kg. Rats were considered diabetic

if blood glucose level was greater than 200mg/dL (Table-1). Animals received 60 μ L citrate buffer (group 1 and 2) and 60 μ L IGFBP-3 shRNA (10 μ L/kg) (group 3) into the corpus cavernosum at 12 weeks after STZ induction, respectively. Half of the dose was administered in each crus. During intracavernosal injection, a constriction band was applied at the base of the penis, and the needle was left in place for 5 min to allow the medication (lipofectamine-plasmid complex) to diffuse throughout the cavernous space (7). The animal experiments were approved by Wuhan University Animal Care and Use Committee.

Measurement of Erectile Responses

Erectile function was assessed by measuring intracavernous pressure (ICP) following electrostimulation of the cavernous nerves at 12 weeks after IGFBP-3 shRNA administration, as previously described (7). Mean arterial pressure (MAP) and ICP lines were connected to a pressure transducer, which was then connected via a transducer amplifier to a data acquisition board (RM6240, Chengdu Instrument, Chengdu, China). Electrical stimulation of the cavernous nerves (1ms pulse, 60s, 15Hz, 2.5V) was performed. The ratio of maximal ICP-to-MAP (ICP/MAP) and total ICP (the area under curve) were recorded for each rat. After measurement of the erectile responses, all rats were killed with an i.p. overdose of pentobarbital (80mg/kg) and the penile shaft was removed for other analysis.

Quantitative Real-Time PCR Analysis

Total RNA was extracted from rat penile samples using TRIzol™ reagent (Invitrogen, Merelbeke, Belgium) according to the manufacturer's protocol and re-suspended in RNase-free water. Total RNA was stored at -80°C until analysis of IGFBP-3 and IGF-1 mRNA levels by MyiQ single color real-time PCR detection system (Bio-Rad laboratories, Hercules, CA) with following primers: IGFBP-3, forward 5'-AGCCGTCTCCTGGAAACACC-3' and reverse 5'-CCCGCTTCTGCCTTTGG-3'; IGF-1, forward 5'-GACATGCCCAAGACCCAGAAGGA-3' and reverse 5'-CGGTGGCATGTCACTCTTCACTC-3'.

Quantitative mRNA measurements were performed in triplicate and normalized to an in-

ternal control of GAPDH. After the PCR program, data were analyzed with the ABI 7300 SDS software (Applied Biosystems, Foster City, CA, USA).

Western Blot Analysis

As previously described (7), the rat penile samples were lysed in RIPA buffer (1xTBS, 0.1% SDS, 0.004% sodium azide, 1% Nonidet P-40, 10 μ L/ml PMSF, 10 μ L/mL protease inhibitor cocktail, 0.5% sodium deoxycholate, 10 μ L/mL sodium orthovanadate). Protein concentration was measured using the Coomassie Plus Protein Assay Reagent™ (Pierce Biotechnology, Rockford, IL). Equal quantities (30 μ g) of lysates were separated on 10% sodium dodecyl sulfate gradient polyacrylamide gels and electroblotted onto PVDF membranes (Bio-Rad, Hercules, CA, USA). Then the membranes were blocked and incubated with rabbit anti-IGFBP-3 antibody (Sc-9028) or anti-IGF-1 (Sc-9013) antibody at 1:1000 dilutions, respectively (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Chemiluminescence was detected using ECL Western blotting detection reagents (Amersham, Buckinghamshire, UK). The IGFBP-3 protein expression was visualized by densitometry using the Mivnt Image analysis system (Shanghai Institute of Optical Instruments, Shanghai, China).

Enzyme-linked Immunosorbent Assay

Cyclic guanosine monophosphate (cGMP) concentration in the lysate of rat penile tissue was determined using an enzyme-linked immunosorbent assay (ELISA) cGMP detection kit (R&D Systems, Minneapolis, MN, USA) following the manufacturer's instruction.

Statistical analysis

Data were expressed as mean \pm SD. Differences were considered significant at the level of $p < 0.05$ using two-tailed unpaired t test.

RESULTS

IGFBP-3 shRNA Improves Erectile Function in Diabetic Rats

We measured ICP/MAP ratio and total ICP (the area under curve) during electrostimulation of the cavernous nerves in three groups at

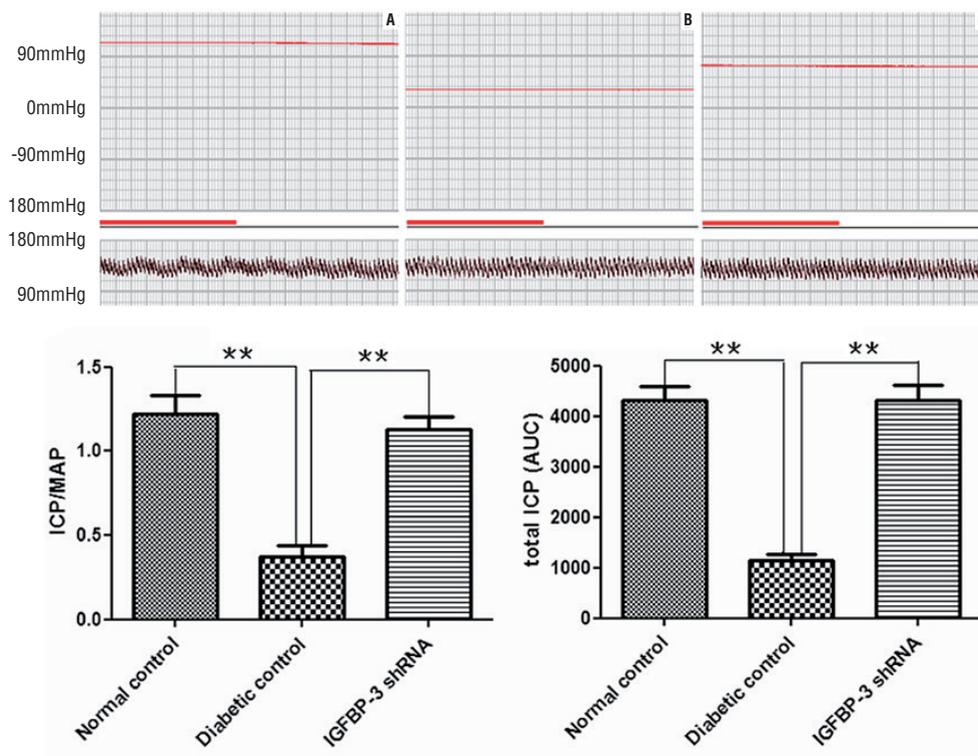
12 weeks after intracavernous administration of IGFBP-3 shRNA. The representative ICP tracing in response to electrostimulation of the cavernous nerves is shown in Figure-1 (upper panel). Electrostimulation in IGFBP-3 shRNA treatment group elicited significantly increased ICP/MAP ratio (Figure-1, lower: left) and total ICP (Figure-1, lower: right) compared to those in diabetic control group ($P < 0.01$, respectively). These data suggest that IGFBP-3 shRNA treatment improved erectile function in diabetic rats.

IGFBP-3 shRNA Inhibits IGFBP-3 Expression and Improves IGF-1 bioavailability in Rat Cavernous Tissue

To confirm that inhibition of IGFBP-3 expression contributes to IGFBP-3 shRNA mediated improvement in erectile function in diabetic rats, we examined IGFBP-3 expression level in rat cavernous tissues at 12 weeks after intracavernous administration of IGFBP-3 shRNA. Real-time qPCR analysis using GAPDH as a housekeeping gene showed that cavernous IGFBP-3 mRNA level in IGFBP-3 shRNA treatment group was significantly lower than in diabetic control group ($P < 0.01$, Figure-2). In addition, as shown in Figure-2, it was showed that cavernous IGF-1 mRNA level in IGFBP-3 shRNA treatment group was significantly higher than in diabetic control group ($P < 0.01$, Figure-2). Accordingly, Western blot analysis showed that cavernous IGFBP-3 protein level in the IGFBP-3 shRNA treatment group was significantly lower than in the diabetic control group ($P < 0.01$, Figure 3 B and D), while it was showed that cavernous IGF-1 protein level in IGFBP-3 shRNA treatment group was significantly higher than in diabetic control group ($P < 0.01$, Figures 3 A and C). These results were consistent with the results of real-time qPCR analysis and proved that IGFBP-3 shRNA inhibits IGFBP-3 expression and improves IGF-1 bioavailability in rat cavernous tissue.

IGFBP-3 shRNA Increases cGMP Concentration in Rat Cavernous Tissue

At 12 weeks after intracavernous administration of IGFBP-3 shRNA cavernous cGMP concentration was significantly increased in IGFBP-3 shRNA treatment group compared to that in diabetic control group ($P < 0.01$, Figure-4).

Figure 1 - IGFBP-3 shRNA improves erectile function in diabetic rats.

Representative ICP responses to cavernous nerves electrical stimulation at 12 weeks after intracavernous administration of IGFBP-3 shRNA in normal control (A), diabetic control (B) and IGFBP-3 shRNA treatment (C) (n=9). Bold line indicated 1 min of electrical stimulation to cavernous nerve. The data of ICP/MAP (lower, left) and total ICP (the area under curve) (lower, right) were collected from nine rats in each group. * P<0.05, compared to diabetic control, **: P<0.01, compared to diabetic control. MAP = mean arterial pressure; ICP = intracavernous pressure.

DISCUSSION

In the current study we demonstrated that intracavernosal injection of IGFBP-3 shRNA could improve erectile responses in STZ-induced diabetic rats. We also presented evidence that improved erectile responses derived from IGFBP-3 shRNA might be caused by the restoration of IGF-1 activities and an increase of cGMP concentration in rat penile tissue.

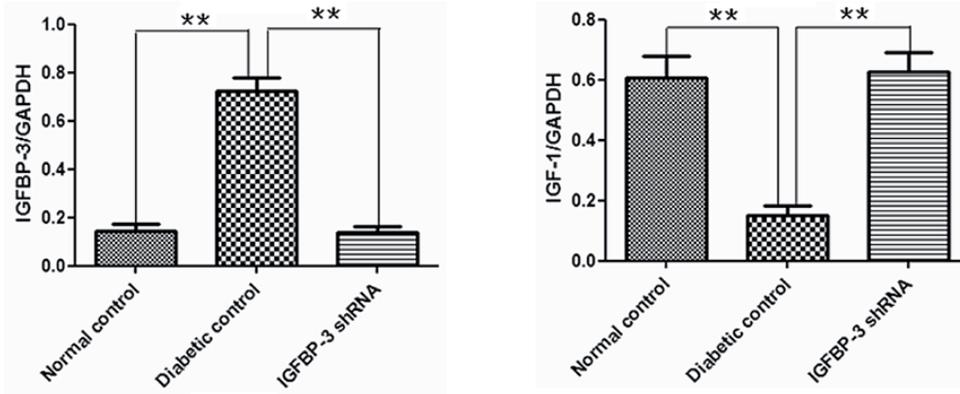
A previous finding shows that IGFBP-3 can prevent its interaction with membrane receptors as a potent inhibitor of cell growth by virtue of its ability to bind insulin-like growth factor (IGF) with high affinity, including IGF-1 (2), while IGF-1 plays a key role in the regeneration of nitric oxide synthase (NOS)-containing nerve fibers in the dorsal and intracavernosal nerves (7). The

nitric oxide (NO) derived from these nerves can cause vasodilatation, increase blood flow, smooth muscle relaxation, and penile erection (9, 10). Previously, it has been reported that there is a down-regulation of IGF-1 protein expression in penile cavernosum of diabetic rats with ED (11). Here, we confirmed that cavernous IGFBP-3 mRNA and protein levels were significantly lower in the IGFBP-3 shRNA treatment group than those in the diabetic control group (p<0.01). The improved erectile responses might be caused by an increase of IGF-1 bioavailability after intracavernosal injection of IGFBP-3 shRNA. However, this is in contrast with published results in which no variations of IGF-1 mRNA is reported in rats after STZ diabetes induction (2).

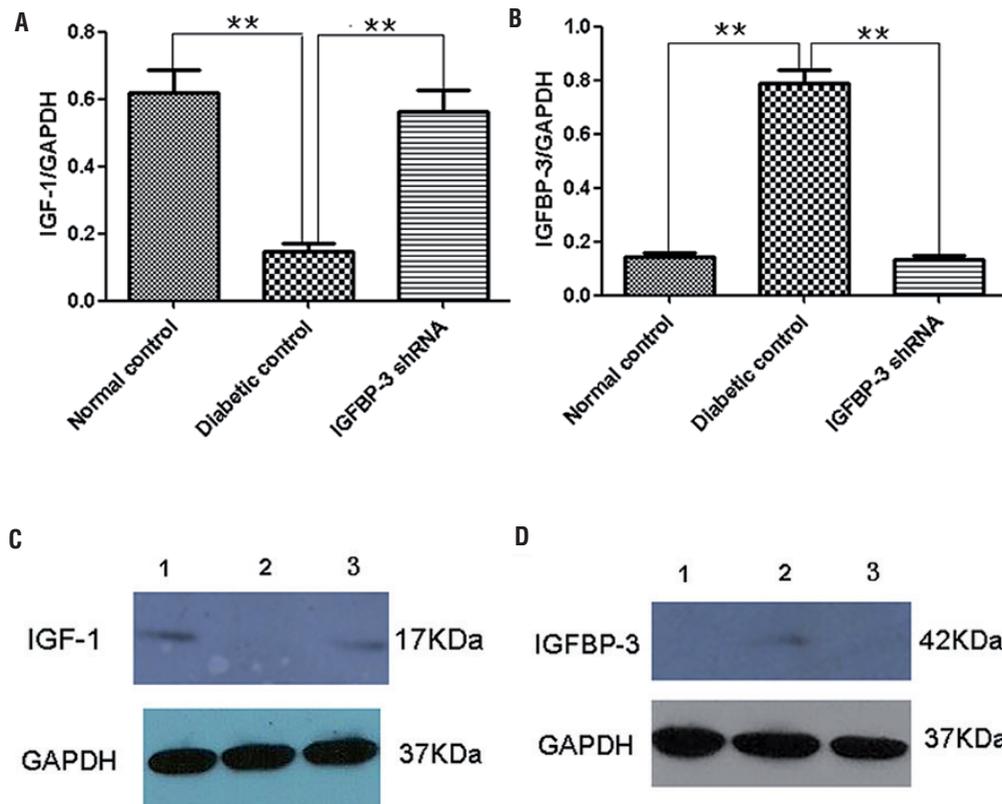
On the other hand, evidence reported so far suggests that ED in diabetic animals and patients

Figures 2 and 3 - IGFBP-3 shRNA Inhibits IGFBP-3 Expression and Improves IGF-1 bioavailability in Rat Cavernous Tissue.

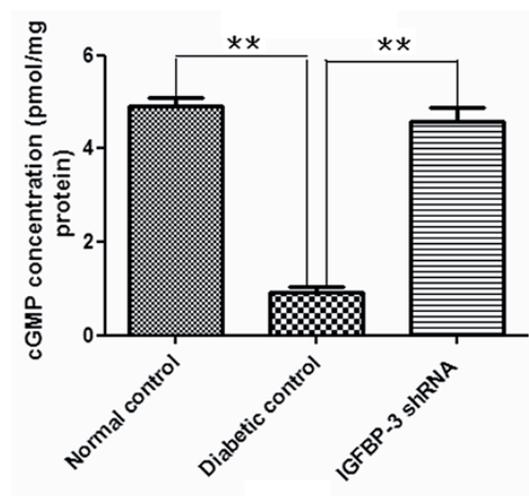
2



3



Quantitative real time-PCR and Western blot analyses of IGFBP-3 and IGF-1 mRNA and protein levels in rat cavernous tissue at 12 weeks after intracavernous administration of IGFBP-3 shRNA. The data were collected from nine rats in each group. ** P<0.01, compared to diabetic control.

Figure 4 - cGMP Concentrations in Rat Cavernous Tissue.

cGMP concentrations in rat penile tissue at 12 weeks after intracavernous administration of IGFBP-3 shRNA were determined by ELISA. The data were collected from nine rats in each group.

** $p < 0.01$, compared to diabetic control.

is mainly caused by the impairment of NO-cGMP signaling activities (10). The major subcellular mechanism by which erection occurs involves NO-induced activation of soluble guanylyl cyclase (sGC) and increased cGMP concentration (12, 13). The subsequent activation of cGMP-dependent protein kinase 1 (PKG1) causes smooth muscle relaxation through the inhibition of calcium flux (14). A decrease of cavernous cGMP concentration is associated with an impairment of cavernosal smooth muscle relaxation with a resultant decrease in erectile function (10, 15). As expected, agents that may increase cGMP concentration should enhance the relaxation of cavernosal smooth muscle and thereby can be applied for treatment of ED. In this study we measured cGMP concentration in rat penile tissue and found that cGMP concentration was significantly increased in the IGFBP-3 shRNA treatment group than that in the diabetic control group, indicating that the NO-cGMP signaling is restored by gene transfer of IGFBP-3 shRNA in diabetic rats. Taken together, these data strongly suggest that IGFBP-3 shRNA could antagonize downregulation of the NO-cGMP signaling and ameliorate diabetes-related ED. These results were also in agreement with physiological studies showing that gene transfer of shRNA-IGFBP-3 could

improve erectile function in STZ-induced DM rats by an increase in the cyclic guanosine monophosphate concentration in cavernous tissue (5). However, long-term efficacy and safety studies of the current procedure are needed in future.

CONCLUSIONS

Here we provide two lines of evidence that gene transfer of IGFBP-3 shRNA could improve erectile function via the restoration of cavernous IGF-1 bioavailability and an increase of cavernous cGMP concentration in the pathogenesis of erectile dysfunction in STZ-induced diabetic rats. However, several concerns should be addressed in future investigations to pave the way for its translation into clinical practice. For example, we need to evaluate the long-term efficacy and safety of this procedure.

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CONFLICT OF INTEREST

None declared.

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The possible protective effects of dipyridamole on ischemic reperfusion injury of priapism

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ABSTRACT

Purpose: To investigate the protective effects against ischemia reperfusion injury of dipyridamole in a model of induced priapism in rats.

Materials and Methods: Twenty-four male Sprague-Dawley rats were divided into four groups, control, P/R, P/R+DMSO and P/R+D. 3ml blood specimens were collected from vena cava inferior in order to determine serum MDA, IMA, TAS, TOS and OSI values, and penile tissue was taken for histopathological examination in control group. Priapism was induced in P/R group. After 1h, priapism was concluded and 30 min reperfusion was performed. In P/R+DMSO group 1ml/kg DMSO was administered intraperitoneally 30 min before reperfusion, while in P/R+D group 10mg/kg dipyridamole was administered intraperitoneally 30 min before reperfusion. Blood and penis specimens were collected after the end of 30 min reperfusion period. Sinusoidal area (μm^2), tears in tunica albuginea and injury parameters in sinusoidal endothelium of penis were investigated.

Results: Histopathological examination revealed no significant changes in term of sinusoidal area. A decrease in tears was observed in P/R+D group compared to P/R group ($p<0.05$). Endothelial injury decreased in P/R+D group compared to P/R group ($p>0.05$). There were no significant differences in MDA and IMA values between groups. A significant increase in TOS and OSI values was observed in P/R+D group compared to P/R group. A significant decrease in TAS levels was observed in P/R+D group compared to the P/R group.

Conclusions: The administration of dipyridamole before reperfusion in ischemic priapism model has a potential protective effect against histopathological injury of the penis.

ARTICLE INFO

Key words:

Priapism; Reperfusion Injury; Ischemia; Dipyridamole

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INTRODUCTION

Priapism is defined as complete or partial penile tumescence exceeding 4 h in length (1, 2). There are three types of priapism, ischemic (low flow, veno-occlusive), non-ischemic (high flow,

arterial) and stuttering (repeating). Ischemic priapism is the most common form and represents a urological emergency (3). In low-flow priapism, a reduced venous flow in cavernous tissues, hypoxia related to venous stasis and histopathological changes associated with acidosis are observed. Ir-

reversible corporal tissue necrosis and fibrotic tissue formation may occur if ischemia exceeds 4-6 h (4, 5).

Ischemia-reperfusion (I/R) injury occurs with termination of priapism (6). Although reperfusion is a necessary mechanism for the protection of ischemic tissue, reperfusion itself initiates a pathophysiological process that leads to injury. With I/R injury neutrophils, inflammatory cytokines and adhesion molecules with increased thrombogenicity are activated, and free oxygen radicals develop with the release of massive intracellular Ca (7, 8). Oxidative stress damages the cell membrane and has damaging effects on tissue by leading to permanent cellular injury.

Dipyridamole is a platelet inhibitor with antithrombotic effects. It is a cyclic guanosine monophosphate (cGMP)-dependent phosphodiesterase and adenosine carrier inhibitor with antioxidant properties that prevents the formation of reactive oxygen radicals in endothelial cells and thrombocytes (9, 10). In addition to antiaggregant effects, it also possesses anti-inflammatory and neuroprotective effects. Experimental studies have shown that dipyridamole reduces I/R injury in various organs and tissues, such as the testis, liver and myocardium (11-13).

The purpose of this study was to determine the protective effects of dipyridamole against I/R injury in an induced experimental model of acute ischemic priapism in rats.

MATERIALS AND METHODS

All animal experiments were performed following Karadeniz Technical University Animal Care and Ethics Committee approval, in compliance with the principles of laboratory animal care (National Institutes of Health publication no. 85-23, revised 1985). Twenty-four adult (approximately 4 months) male Sprague Dawley rats weighing 400-450 gr were used in the study. Rats were placed in cages 1 week before the study for adaptation and given standard rat chow and tap water. Rats were numbered and randomized by lots. Following randomization, rats were divided into four groups of six rats each (n=6). Throughout the experiment all animals were kept under standard

room temperature ($23\pm 2^{\circ}\text{C}$), lighting (12 h light/12 h dark) and humidity conditions ($50\%\pm 10$).

Experimental design and groups

Anesthesia in all surgical procedures was established with 80 mg/kg ketamine and 10 mg/kg xylazine. The priapism model was induced using the vacuum and constrictor band previously described in the literature (14). A 50 cc irrigation injector was attached to the base of the flaccid rat penis and used like a vacuum device. A constriction band, which was cut from a 16 Fr Foley catheter in 2 mm slices, was attached to the tip of the vacuum erection device before vacuum was applied to the penis. Erection was induced by placing the tip of the syringe to the base of the rat penis and withdrawing it to create a 20 cc negative pressure. Once sufficient erection had been induced, the constriction band was removed from the syringe and attached to the base of the penis (Figure-1). The powdered dipyridamole (Koçak Farma, Turkey) used in the experiment was dissolved in DMSO (dimethyl sulfoxide) at 10mg/ml. This was injected intraperitoneally at a dose of 10 mg/kg into rats weighing 400-450 gr. Drug dose

Figure 1 - Priapism model induced in the rat penis using a constrictor band.



was determined based on the report demonstrated that dipyridamole protects rat testis against testicular I/R injury (11).

Control Group: Three-milliliter blood specimens were collected from the vena cava inferior (VCI) in order to determine basal serum malondialdehyde (MDA), serum ischemia modified albumin (IMA), serum total antioxidant status (TAS), serum total oxidant status (TOS) and serum oxidative stress index (OSI) values. Penectomy was performed and tissues were placed in a 10% formaldehyde solution for basal histopathological examination of penile tissues.

Priapism/Reperfusion (P/R) Group: Thirty minute reperfusion was established by removing the constrictor band on the penis following 1 h priapism.

Priapism/Reperfusion+DMSO (P/R+DMSO) Group: The same surgical procedure was performed as in the P/R group. This group received the same volume of DMSO solution as the P/R+D group without dipyridamole intraperitoneally, 1/2 h before reperfusion (Figure-2).

Priapism/Reperfusion+Dipyridamole (P/R+D) Group: The same surgical procedure was performed as in the P/R group. Half an hour before reperfusion, 10 mg/kg dipyridamole were administered intraperitoneally.

Following 30 min reperfusion, 3ml blood specimens were collected from all animals in order to determine serum MDA, IMA, TAS, TOS and OSI values. Penectomy was performed on all animals at the end of the experiment and penises were placed in 10% formaldehyde solution for histopathological examination of penile tissues.

Histopathological analysis

Penis tissue was extracted from rats from all groups at the end of the study. Penis tissues, taken from approximately the same areas, were kept for 72 h in 10% formaldehyde solution for histopathological examination. Tissue samples were passed through 70%, 90%, 96% and 100% alcohol series for dehydration. They were then passed through xylene solution. Tissues were fixed in paraffin blocks and sections 5 µm in thickness were taken using a fully automated micro-

Figure 2 - Dissected rat penis.



tome. Following deparaffinization, sections were stained with Masson's trichrome for detailed histological examination and for better investigation of the cavernous structure (15).

Preparates were evaluated by an experienced and blinded histologist using a light microscope (Olympus BX-51, Olympus Optical Co., Tokyo, Japan). General histological architecture was evaluated. Pathological findings (tunica albuginea tear, endothelial injury) were evaluated semi-quantitatively on a scale of 0 to 3 (0: None, 1: Mild, 2: Moderate, 3: Severe). Groups were also examined using a light microscope at a magnification of 200X on Analysis 5 Research (Olympus Soft Imaging Solutions, Münster, Germany) software for measurement of cavernous tissue sinusoidal area. Means were calculated for sinusoidal area measurements in five different areas for each group.

Masson's Trichrome Staining: Following deparaffinization, samples were kept in bovine solution in an oven at 56°C for 1 h. Samples were then kept until they turned yellow. After being kept in water for 2 min they were again washed in water. They were then kept for 5 min in combined trichrome dye. They were then again washed in water, and finally dried after being passed through alcohol.

BIOCHEMICAL ANALYSIS

Serum MDA Activity Assay

The method described by Yagi was used to determine lipid peroxidation in rat serum samples in the form of MDA concentration (16). Tetramethoxypropane was employed as a standard, and MDA levels were expressed as nmol/mL.

Measurement of Ischemia-Modified Albumin (IMA)

Assessment of reduced cobalt to albumin binding capacity (IMA level) was performed based on the colorimetric method described by Bar-Or et al. (17). Briefly, 200 μ L of rat serum was added to 50 μ L of 0.1% cobalt chloride (Sigma, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$) in H_2O . The solution was gently shaken and then left for 10 minutes to ensure sufficient cobalt albumin binding. Fifty microliters of dithiothreitol (DTT) (Sigma, USA 1.5 mg/ml H_2O) was added as a colorizing agent. After 2 min the reaction was quenched with the addition of 1.0 mL of 0.9% NaCl. Colorimetric control specimens were prepared for preoperative and postoperative serum samples with 50 μ L of distilled water replacing 50 μ L of 1.5 mg/mL DTT. Specimen absorbencies were analyzed at 470 nm on a spectrophotometer (Shimadzu UV1601, Ausburn, Australia). The color of the specimens with DTT was compared against the colorimetric control tubes. The results were expressed as absorbance units (ABSUs).

Measurement of Total Oxidant Status (TOS)

TOS levels were determined using a method previously described by Erel (18). Serum TOS levels were calculated in $\mu\text{mol H}_2\text{O}_2$ equivalent/L.

Measurement of total antioxidant status (TAS)

Total antioxidant status levels were calculated based on the method described by Erel (19) and expressed as mmol Trolox equivalent/L.

Calculation of oxidative stress index (OSI)

OSI was calculated as the TOS: TAS ratio. TAS units in the form of mmol Trolox equivalent/L were converted to $\mu\text{mol Trolox equivalent/L}$, with OSI being determined using the formula $\text{OSI} = [(\text{TOS}, \mu\text{mol H}_2\text{O}_2 \text{ equivalent/L}) / (\text{TAS}, \mu\text{mol Trolox equivalent/L}) \times 100]$ (20).

Statistical analysis

Kruskal-Wallis analysis of variance (Bonferroni-corrected Mann Whitney U test) was used to compare the groups. $P < 0.05$ was regarded as statistically significant.

RESULTS

Histopathological Findings

Common parameters in all tissues, sinusoidal area (μm^2), tears in the tunica albuginea and injury in the sinusoidal endothelium were investigated. Assessment was performed semi-quantitatively, with findings being scored between 0 and 3 (0: None, 1: Mild, 2: Moderate, 3: Severe). Distribution of histopathological parameters in all groups is shown in Table-1. No significant difference was determined between the groups in terms of sinusoidal area (μm^2) ($p > 0.05$). Significantly more tears in the tunica albuginea were determined in the P/R group compared to the control group ($p < 0.05$). Comparing the P/R+D and P/R groups, dipyridamole resulted in significantly fewer tears in the

Table 1 - Groups' histological data.

Groups	Control	P/R	P/R+DMSO	P/R+D	p
Sinusoidal area (μm^2)	3550 \pm 1096	4959 \pm 1187	4349 \pm 600.6	3550 \pm 393.9	0.0312
Tunica albuginea tear	0.3333 \pm 0.5164	2.5 \pm 0.5477	0.8333 \pm 0.4082	1.167 \pm 0.4082	0.0005
Endothelial injury	0.1667 \pm 0.4082	2.333 \pm 0.5164	0.5 \pm 0.5477	1.5 \pm 0.5477	0.0005

tunica albuginea ($p < 0.05$). In terms of endothelial injury, significantly more damage was determined in the P/R group compared to the control group ($p < 0.05$). Comparing the P/R+D and P/R groups, dipyridamole caused a decrease in endothelial injury, but the difference was not statistically significant ($p > 0.05$).

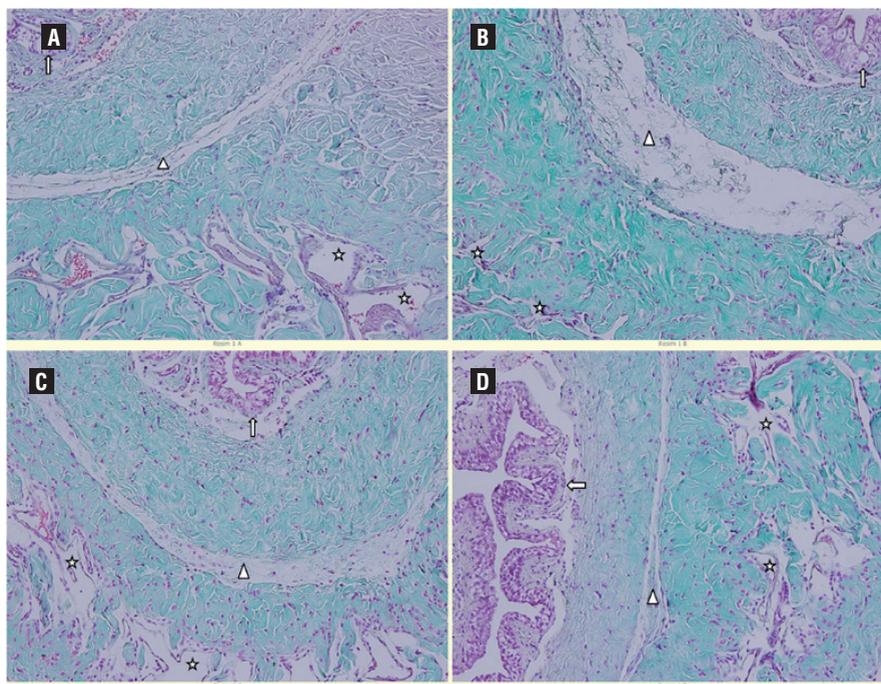
A normal corpus cavernosum and corpus spongiosum appearance was observed in the control group. Normal morphology was also observed in the surrounding tunica albuginea and the external surrounding superficial fascia. Histological data for vascular spaces and the surrounding smooth muscle layer were normal (Figure-3A). In the P/R group, tears in the tunica albuginea surrounding the corpus spongiosum and connective tissue irregularities were present. The urethral epithelium had a normal appearance. Damage was observed in the endothelial cells lining vascular spaces in the corpus cavernosum. Sinusoidal areas had decreased in size (Figure-3B). In the P/R+DMSO group, a normal appearance was observed in the urethral structure and the tunica albu-

ginea around the corpus spongiosum and corpus cavernosum. The vascular endothelium around the corpus cavernosum had a normal morphology (Figure-3C). In the P/R+D group, the tunica albuginea surrounding the corpus spongiosum and urethral epithelium had a normal appearance. Although occasional damage was observed in the endothelial cells around the vascular spaces in the corpus spongiosum, a close to normal appearance was observed in most of the area (Figure-3D).

Biochemical Findings

Distribution of biochemical parameters in all groups is shown in Table-2. Although an increase was observed in MDA values in the P/R group, no statistically significant difference in MDA was observed among the groups ($p > 0.05$). Although an increase in serum IMA levels was observed in rats in the P/R group, no significant improvement in IMA levels was observed in the P/R+D group ($p > 0.05$). A significant increase in TOS values was observed in the P/R+D group compared to the P/R group ($p < 0.05$). A significant increase in terms

Figure 3 - Histopathological examination of the penis from each experimental group (Masson's trichrome staining x200). A: Group 1, B: Group 2, C: Group 3, D: Group 4.



A) Urethral epithelium (↑), tunica albuginea (Δ), sinusoidal area (star); **B)** Urethral epithelium (↑), tunica albuginea (Δ), sinusoidal area (star); **C)** Urethral epithelium (↑), tunica albuginea (Δ), sinusoidal area (star); **D)** Urethral epithelium (↑), tunica albuginea (Δ), sinusoidal area (star).

Table 2 - Groups' biochemical data.

Groups	Control	P/R	P/R+DMSO	P/R+D	p
TOS ($\mu\text{molH}_2\text{O}_2\text{Eq/L}$)	11.06 \pm 2.224	10.08 \pm 3.029	22.06 \pm 7.188	19.44 \pm 4.715	0.0008
TAS (mmol/L)	2.404 \pm 0.1754	2.808 \pm 0.140	2.748 \pm 0.161	2.495 \pm 0.209	0.0075
OSI	0.4625 \pm 0.102	0.358 \pm 0.104	0.795 \pm 0.226	0.7918 \pm 0.233	0.0012
Serum MDA (nmol/L)	0.3087 \pm 0.044	0.3865 \pm 0.088	0.3515 \pm 0.089	0.3803 \pm 0.061	0.2245
IMA (ABSU/gr)	0.7398 \pm 0.051	0.7578 \pm 0.011	0.8015 \pm 0.036	0.7315 \pm 0.044	0.0452

of TAS was observed in the P/R group compared to the control group ($p < 0.05$), while a decrease was observed in the P/R+D group compared to the P/R group ($p > 0.05$). OSI values were similar in the control and P/R groups ($p > 0.05$), while an increase was observed in the P/R+D group compared to the P/R group ($p < 0.05$).

DISCUSSION

Priapism may be defined as a prolonged erection due to disturbance of the mechanisms that control penile tumescence, rigidity and flaccidity (21). Ischemic priapism refers to a painful and continuing rigid erection characterized by little or no cavernous blood flow. Hypoxia, hypercarbia and acidosis develops in cavernous blood gas (22).

Ultrastructural changes in smooth muscle occur after 12 h in ischemic priapism, focal necrosis after 24 hs and finally necrosis and transformation of fibroblast-like cells after 48 h. If priapism is untreated or treated late (> 24 hs) necrosis in cavernous smooth muscle, irreversible corporal fibrosis and permanent erectile dysfunction occur (4). The most significant determining factor in the prevention of tissue damage after ischemic priapism is duration of ischemia, which is directly correlated with reperfusion injury. Several studies have investigated I/R injury in different tissues (6, 11, 23). The purpose of this study was to investigate early histological and biochemical changes and the probable protective effects against I/R injury of dipyridamole before the emergence of irreversible damage following 1 h acute ischemic priapism in a model of ischemic priapism experimentally induced in rats.

Dipyridamole is a platelet inhibitor that increases cAMP levels by inhibiting the enzyme phosphodiesterase in platelets. It blocks reabsorption of adenosine by cells and increases interstitial adenosine concentrations (24, 25). It causes vasodilation by increasing adenosine formation, and probably improves tissue perfusion through a combination of its antiplatelet and vasodilator effects (10). Various studies have investigated the effects of dipyridamole on I/R injury. In a study of the antioxidant properties of dipyridamole, Vargas et al. reported that dipyridamole scavenges reactive oxygen radicals (ROS) released by human polymorphonuclear leukocytes (26). Iuliano et al. reported that dipyridamole possesses antioxidant properties (27). An experimental study determined that dipyridamole protects liver cells against warm I/R injury (28). Karagüzel et al. reported that the use of dipyridamole before testicular reperfusion in testicular torsion protects the testis against the long-term effects of I/R injury (11). The intraperitoneal 10 mg/kg dose of dipyridamole used in that study of testicular torsion was also employed in our priapism model which is another model of I/R injury. It may be possible for further studies to use different doses of dipyridamole to show increased effectiveness.

Very few experimental drug studies have investigated protective efficacy against I/R injury occurring in priapism (14, 29). Ours is the first study in the literature to investigate the effect of dipyridamole in an experimental priapism model. Although dipyridamole is a molecule that is safely used in various indications because of its anti-

-aggregating properties in humans, there are no studies concerning its use in humans with clinical priapism. There is a strong probability that as a result of this and future studies, dipyridamole will be used to prevent potential I/R injury in priapism, a new indication in humans, through investigation of the most appropriate and effective dosage.

Although histopathological evaluation of tunica albuginea and endothelial injury parameters in this study revealed ischemic changes, no change in favor of ischemia was determined in the levels of serum IMA and MDA and other biochemical parameters. Although a decrease was determined in TAS and IMA values in the group receiving dipyridamole compared to the P/R group, this was not statistically significant. Biochemical data not exhibiting significant differences in ischemic priapism, a compartment syndrome analogue, may be attributed to the 30 min reperfusion time we used being too short for ischemic tissue products to enter the circulation with reperfusion and establish a systemic effect. A longer reperfusion process is needed for penile ischemic products to exhibit systemic effects and for significant changes to be identified in ischemic parameters in serum. This study represents the first investigation in a priapism model of IMA, a biochemical parameter that has been investigated and found to be of value in such different models of I/R injury as testicular torsion (30, 31).

The effects of I/R injury occurring in priapism and the probable protective effects of dipyridamole were investigated through histological examination of the sinusoidal area, tears in the tunica albuginea and damage parameters in the sinusoidal endothelium. Examination of I/R injury in the P/R group revealed tears in the tunica albuginea surrounding the penis corpus spongiosum and irregularities in connective tissue, injury to the endothelial cells around the vascular spaces in the penis corpus cavernosum and contractions in sinusoidal areas. Examination of the penises of rats given dipyridamole revealed improvement in tunica albuginea and endothelial injury and showed that dipyridamole, which has antioxidant and anti-inflammatory properties, exhibits histopathological protective effects in I/R injury caused by priapism.

A certain amount of I/R injury will develop in penis corpus cavernosum tissue in patients with

priapism due to various etiological causes, even if treated in the early period. Further studies are needed on the subject of the clinical outcomes that I/R may cause in the long-term. This study represents one of the rare examples of medical therapies that can be used in order to reduce I/R injury that may occur in patients treated in the early period.

The main limitation of this study is that the length of ischemia in the model of priapism used was limited to 1 hour. More significant results in terms of biochemical data may be obtained with a longer duration of ischemia. Another limitation is the difficulty in applying the animal model used in this study to humans and in determining appropriate dose selection.

CONCLUSIONS

In conclusion, this experimental study shows that the application of dipyridamole before reperfusion in a model of ischemic priapism has potential protective effects against histopathological injury that may develop in the penis. Further and more comprehensive studies are now needed in order to prevent I/R injury in ischemic priapism.

CONFLICT OF INTEREST

None declared.

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High-pressure balloon assessment of pelviureteric junction prior to laparoscopic “vascular hitch”

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ABSTRACT

Aim: To assess if calibration of the ureteropelvic junction (UPJ) using a high-pressure balloon inflated at the UPJ level in patients with suspected crossing vessels (CV) could differentiate between intrinsic and extrinsic stenosis prior to laparoscopic vascular hitch (VH).

Materials and Methods: We reviewed patients with UPJO diagnosed at childhood or adolescence without previous evidence of antenatal or infant hydronephrosis (10 patients). By cystoscopy, a high-pressure balloon is sited at the UPJ and the balloon inflated to 8-12 atm under radiological screening. We considered intrinsic PUJO to be present where a ‘waist’ was observed at the PUJ on inflation of the balloon and a laparoscopic dismembered pyeloplasty is performed. When no ‘waist’ is observed we considered this to represent extrinsic stenosis and a laparoscopic VH was performed. Patients with absence of intrinsic PUJ stenosis documented with this method are included for the study.

Results: Six patients presented pure extrinsic stenosis. The mean age at presentation was 10.8 years. Mean duration of surgery was 99 min and mean hospital stay was 24 hours in all cases. We found no intraoperative or postoperative complications. All children remain symptoms free at a mean follow up of 14 months. Ultrasound and renogram improved in all cases.

Conclusion: When no ‘waist’ is observed we considered this to represent extrinsic stenosis and a laparoscopic VH was performed. In these patients, laparoscopic transposition of lower pole crossing vessels (‘vascular hitch’) may be a safe and reliable surgical technique.

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Key words:

Multicystic renal dysplasia, bilateral [Supplementary Concept]; Laparoscopy; Child

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INTRODUCTION

The incidence of crossing vessels (CV) in the etiology of Ureteropelvic Junction Obstruction (UPJO) in children ranges from 11% to 15% (1), but has been reported as frequently as 58% in a series of older children with symptomatic UPJO and normal prenatal ultrasonography (2). This is especially common in children who report a history of intermittent abdominal pain coincident with abundant fluid ingestion and preserved renal function.

So far, the gold-standard treatment for these patients is pyeloplasty, with laparoscopic pyeloplasty (LP) being recommended in older children. Recently however, cranial relocation of the lower pole crossing vessels or “vascular-hitch” (VH) (3-6) as described by Hellström (3) has gained popularity. The main limitation of vascular hitch is the difficulty in distinguishing between intrinsic and extrinsic stenosis in order to select the least invasive surgical option.

Although laparoscopic pyeloplasty is a common, low morbidity technique, VH provides

its own benefit, namely a reduction in operative time and preservation of the intact urinary tract that removes the risk of urinary leakage and anastomotic stricture formation. Furthermore, drains or stents are not required, reducing the length of stay and avoiding the need for a later cystoscopic stent removal.

We propose that calibration of the ureteropelvic junction (UPJ) be performed using a high-pressure balloon inflated at the UPJ level in patients with suspected CV to differentiate between intrinsic and extrinsic stenosis. Those patients with demonstrated extrinsic outflow obstruction may benefit from VH and the surgeon will be able to be more confident in the ability to select only the required intervention at the time of surgery.

MATERIALS AND METHODS

We reviewed patients with UPJO diagnosed at childhood or adolescence without previous evidence of antenatal or infant hydronephrosis. Those with a diagnosis of crossing vessels made by magnetic resonance urography (MRU) were included. Diagnosis of UPJO was made by ultrasound and diuretic renogram. When the imaging and clinical assessment were consistent with the existence of crossing vessels, MRU was performed as per departmental protocol, namely children over 4 years with no antenatal or perinatal history of hydronephrosis, with episodes of lumbar pain, evidence of crossing vessels on ultrasound or fluctuating hydronephrosis.

Surgical intervention was considered in the presence of loin or lumbar pain with intermittent obstructive hydronephrosis and/or UTIs, with hydronephrosis grade III or IV on ultrasound and the presence of an obstructive renogram.

When the MRU demonstrated CV a decision was made to treat with laparoscopic dismembered pyeloplasty or laparoscopic vascular hitch based on the PUJ calibration (intrinsic stenosis versus extrinsic).

Patients presenting with severe loss of renal function or other associated anomalies were excluded.

Calibration

Under cystoscopic guidance a retrograde pyelography is performed. A 0.014” ureteral guidewire is then introduced into the renal pelvis. A high-pressure balloon (Rx Muso® Terumo Corp., Somerset, NJ, USA) is sited at the UPJ and the balloon inflated to 8-12atm under radiological screening.

We considered intrinsic PUJO to be present when a ‘waist’ was observed at the PUJ on inflation of the balloon (Figure-1). A laparoscopic dismembered pyeloplasty was then performed as a continuation of this procedure under the same general anaesthetic.

When no ‘waist’ is observed we considered this to represent extrinsic stenosis (Figure-2) and a laparoscopic VH was performed.

“Vascular Hitch”

This technique consists of a laparoscopic transperitoneal exposure of the lower pole vessels with the patient in a lateral position. Three 5mm

Figure 1 - We considered intrinsic PUJO to be present when a ‘waist’ was observed at the PUJ on inflation of the balloon.

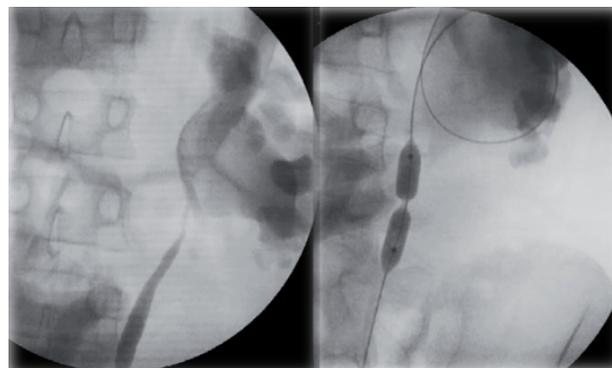
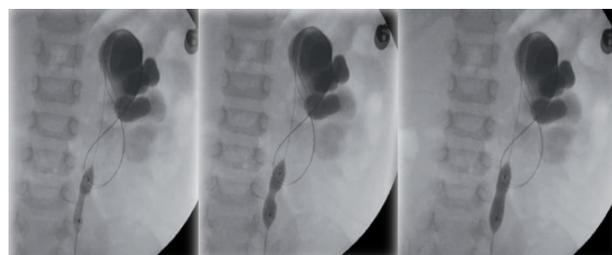


Figure 2 - When no ‘waist’ is observed we considered this to represent extrinsic stenosis.



ports were used in all cases. The lower pole vessels were dissected free from the PUJ and full mobility of the pelvis and PUJ was confirmed by the 'shoe shine' manoeuvre. The PUJ was carefully inspected for intrinsic stenosis, and inspection for evidence of peristalsis across the junction was performed. To remove doubt, the pelvis was also distended with saline from a fine-bore needle to assess drainage across the PUJ. The lower pole vessels were then fixed in a cephalic position away from the PUJ by suturing the pelvis on either side of the vessels with two to three absorbable sutures without tension (Figure-3).

RESULTS

Six children (4m, 2f) were included in the study. Median age at presentation was 10.8 years (6-15 years) and the median duration of symptoms was 22 months (6 months-4 years). All children had intermittent loin pain, one presented with a

UTI and one had haematuria. Grade IV hydronephrosis was demonstrated on USS in 4 cases (with mild parenchymal thinning) and grade III in 2. The anteroposterior diameter of the pelvis was 41mm (60-22mm). In all cases an obstructive renogram was found, 3 retaining preserved function and 2 with mild loss of function (33% and 37%).

The right kidney was affected in 3 patients and left kidney in 3. The median duration of surgery was 99 min (85-110 min) and mean hospital stay was 24h in all cases. In no case was a drain or double J stent used. No intraoperative or postoperative complications were encountered.

All children remained symptom free at a median follow-up of 14 months (6-24). Routine ultrasound showed a decreased degree of hydronephrosis and anteroposterior diameter of the pelvis in all cases with a median value of 14mm (7-20mm). MAG3-lasix renograms demonstrated improved drainage in all children and no change in renal function (Table-1).

Figure 3 - Cranial relocation of lower pole crossing vessels or "vascular-hitch". A-B: The lower pole vessels were dissected free from the PUJ; C-D: full mobility of the pelvis and PUJ was confirmed by the 'shoe shine' manoeuvre; E: The lower pole vessels were then fixed in a cephalic position away from the PUJ by suturing the pelvis on either side of the vessels with two to three absorbable sutures without tension. 1: Crossing vessels; 2: Renal pelvis; 3: Ureter.

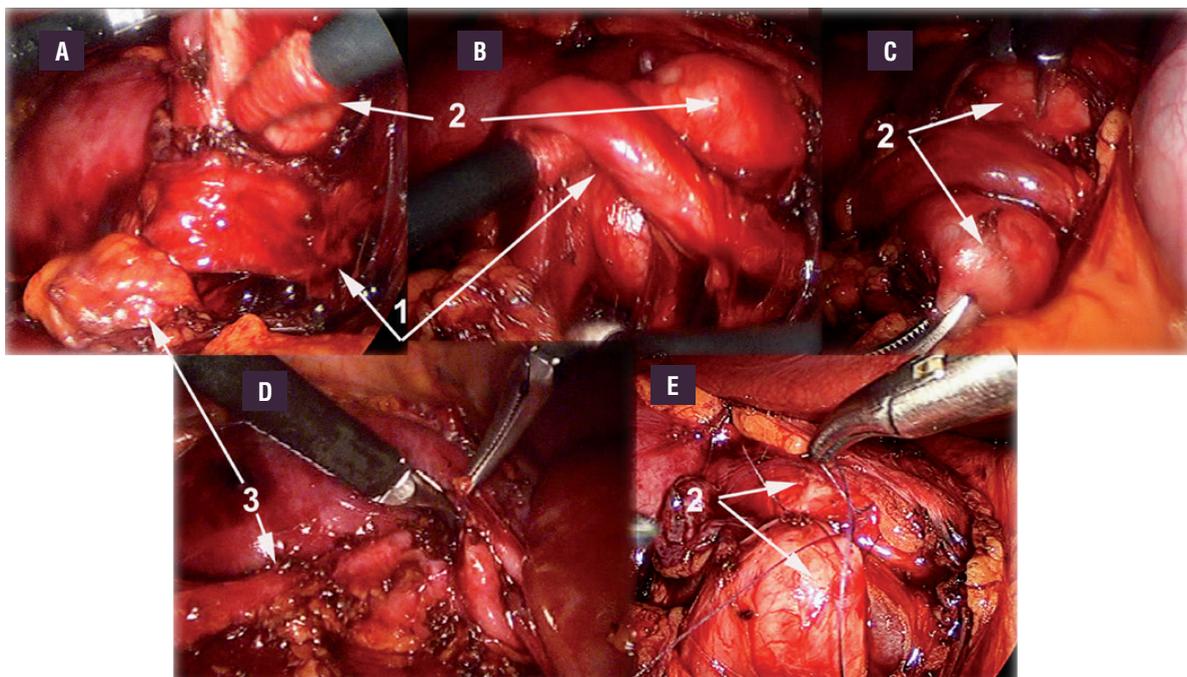


Table 1 - Patient data.

Age	Preoperative hydronephrosis grade	Preoperative APD renal pelvis	Preoperative parenchymal thinning	Preoperative differential renal function	Preoperative symptoms	Postoperative hydronephrosis grade	Postoperative APD renal pelvis	Postoperative differential renal function	Postoperative curve renogram	Duration of follow-up (months)
13	IV	60	+	45	Pain	II	20	47	Normal	20
11	IV	40	+	43	Pain	I	7	48	Normal	24
15	IV	47	++	36	Pain	II	13	40	Normal	11
9	III	22	-	45	Pain + UTI	II	11	47	Normal	9
6	IV	34	+	31	Pain + haematuria	III	20	35	Semiobstructive	6
12	IV	55	++	30	Pain	II	23	31	Normal	12

APD = Anteroposterior diameter

UTI = Urinary tract infection

DISCUSSION

Although prenatal hydronephrosis and hydronephrosis diagnosed in childhood or adolescence may appear to be the same condition, doubts remain about whether it is actually the same entity. Thus, the need to differentiate the aberrant polar vessels associated with intrinsic UPJ stenosis or diagnosing a polar vessel as a single extrinsic cause of obstruction becomes relevant in the older age group.

PUJ obstruction diagnosed antenatally or in the first few years of life usually have an intrinsic stenosis as the cause of their obstruction. In these patients, the existence of a polar vessel is treated as an incidental finding (7, 8). Those that present in late childhood or adulthood generally report intermittent symptoms and a significant proportion are associated with accessory lower pole vessels (9, 10). The debate rests in whether these vessels are merely an anatomical variation with no pathological significance or whether they play a role in the pathogenesis of the outflow impairment or obstruction at the PUJ level.

Histological studies examining intrinsic obstruction have postulated that the obstruction is caused by aperistaltic segments with abnormal amounts of muscle and collagen deposition (11).

Extrinsic obstruction is thought to originate from an overlying renal vessel. Whether the vessel alone causes obstruction or whether the-

re is also a component of underlying UPJ fibrosis remains unclear and controversial (12). Some studies have confirmed that muscle density significantly increases in those with CV obstruction compared to intrinsic obstruction (13, 14). This may mean that extrinsic stenosis produces different histological changes in the UPJ when compared to intrinsic stenosis. However, it is not clear if these changes later form obstructions.

Numerous imaging modalities have been used to identify crossing renal vessels, such as angiography, endoluminal ultrasound, Doppler ultrasound, spiral CT, and MRI (1). However, none can prove whether crossing renal vessels are obstructive or are merely incidental findings (15). Many paediatric urologists believe that during a dismembered pyeloplasty in older symptomatic children with lower pole vessels, there is a lack of a macroscopically obvious intrinsic obstruction at the PUJ (3).

Some research groups perform a VH if the patient meets certain criteria: a normal calibre ureter, a normal appearance UPJ without stenosis or narrowing and good peristalsis across the UPJ (4). Other groups perform a water overload test intraoperatively after freeing the lower pole vessels. This consists of inducing hydronephrosis using intravenous hyperhydration and diuretic medications injected during the first stages of the procedure (5).

We hypothesise that the calibration of the PUJ with a balloon under radiological screening

while assessing the presence of intrinsic stenosis is a reliable and safe way to decide which patients may benefit from the VH.

Although it may be felt that calibration may prolong the procedure, this was not demonstrated in our series. Our operative time is comparable to other series recently published (3-5). There is no increase in complication rates or postoperative hospital stay associated with this modification. While these steps may increase the cost of the procedure the costs of operation for stent removal are avoided. Therefore, we think that assessment of the PUJ with a dilating balloon intraoperatively is a useful tool to avoid inappropriate surgical technique being employed.

From our previous experience dilating the UPJ endoscopically in infants with PUJO we believe that intrinsic stenosis always demonstrates an indentation or 'waist' visible in the high-pressure balloon under radiological screening. This narrowing requires a pressure of over 8-10atm (16) in the balloon to overcome it. In patients with extrinsic stenosis this balloon inflation did not show any indentation or waist.

Previous descriptions of "vascular hitch" have used exclusively laparoscopic observation of the ureteropelvic junction to decide there is no intrinsic cause of stenosis and therefore perform a VH. In our series, in addition to internal or endourologic observation we also examine the ureteropelvic junction radiologically (external observation), using additional information to decide on when purely extrinsic stenosis is present (4, 5). We believe that this assessment of the PUJ increases the safety and accuracy of the surgical decision making process.

Despite the small number of patients in this study, the postoperative results are satisfactory after a follow-up period longer than 6 months in all cases. A large number of patients is now required to provide further reliability to the calibration process for the diagnosis of intrinsic stenosis.

We feel that the additional steps described here do not add any increased morbidity to this operation, instead we believe they benefit the patient by ensuring that only the procedures required are performed. Retrograde instrumentation of urinary tract prior to laparoscopic pyeloplasty

is still used by some groups to insert a double J (17), this has been reported as adding technical difficulty to pyeloplasty suture. Our approach is safe, as it has not increased the difficulty of the procedure and no intraoperative or postoperative complications were registered. Our success in calibrating the PUJ relies on consistent technique and adequate instrumentation.

The limitations of this study are that it is a retrospective study without a control group. The absence of the control group (laparoscopic "vascular hitch" in the presence of an extrinsic "waist" during calibration) is due to our feeling that to offer VH without assessing for intrinsic obstruction leaves open the potential for repeat surgery to be required at a later date that could have been avoided had this balloon assessment being completed. The small number of patients make this study statistically underpowered and represents a challenge to validate the technique.

CONCLUSION

Assessment of PUJ with high-pressure balloon calibration in patients with PUJO and crossing vessels may allow us to better differentiate between those cases with extrinsic stenosis of the PUJ from those with associated intrinsic obstruction. Consequently, these patients would receive a less invasive surgery preserving the integrity of the urinary tract and avoiding the need for stenting, reducing their associated morbidity.

In these patients, laparoscopic transposition of lower pole crossing vessels ('vascular hitch') may be a safe and reliable surgical technique.

CONFLICT OF INTEREST

None declared.

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Management of full-length complete ureteral avulsion

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ABSTRACT

Introduction: Complete ureteral avulsion is one of the most serious complications of ureteroscopy. The aim of this report was to look for a good solution to full-length complete ureteral avulsion.

Case presentation: A 40-year-old man underwent ureteroscopic management. Full-length complete avulsion of ureter occurred during ureteroscopy. Pyeloureterostomy plus greater omentum investment outside the avulsed ureter and ureterovesical anastomosis were performed 6 hours after ureteral avulsion. The patient was followed-up during 34 months. Double-J tube was removed at 3 months after operation. Twenty three months after the first operation, the patient developed hydronephrosis because of a new ureter upside stone, then rigid ureteroscopy and holmium laser lithotripsy were used successfully.

Conclusion: Pyeloureterostomy plus greater omentum investment outside the avulsed ureter and ureterovesical anastomosis may be a good choice for full-length complete ureteral avulsion.

ARTICLE INFO

Key words:

Ureteral avulsion, Greater omentum, Pyeloureterostomy, Ureterovesical anastomosis

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INTRODUCTION

Urolithiasis is one of the most common diseases of urinary system. With the wide application of ureteroscopes, percutaneous nephroscopes, and endoscopic stone extractors, the incidence of iatrogenic ureteral avulsion tends to grow year by year (1). Ureteral avulsion refers to discontinuation of the full thickness of the ureter. Inappropriate management of this serious condition may lead to nephrectomy (2). How to manage ureteral avulsion has become a challenge to urologists. Here, we presented the management of full-length complete ureteral avulsion.

Case presentation

A 40-year-old male presented to us with right flank pain experienced for two weeks. Pain was colicky in nature, radiating to genitalia, asso-

ciated with vomiting. Bowel habits were normal. There was no history suggestive of any other system involvement. Examination was unremarkable. Computed Tomography (CT) of urinary system revealed right hydronephrosis and a calculus measured 0.9x0.8x0.6cm located in the right upper ureter, and the distance between the stone and renal pelvis was 7.44cm (Figure-1a).

Ureteroscopic removal was planned. Forceful placement of rigid ureteroscope resulted in instrument drag, which hampered its maneuverability. An attempt at extraction produced full-length complete avulsion of ureter. The avulsed ureter was pulled out of body (Figure-1b), and the native ureter was preserved in physiological saline. The reconstruction treatment selection was a decision made for the patient after extensive discussion with urologists of the Affiliated Hospital of Guizhou Medical University. After discussing the

complication with the patient, his spouse and his family members, we underwent ureteral reconstruction by standard open surgical techniques. About 6 hours after ureteral avulsion, pyeloureterostomy plus greater omentum investment outside the avulsed ureter and ureterovesical anastomosis were performed for the patient. A single double-J stent tube (6F, Budd Company) was placed inside the ureter (Figures 1c-f).

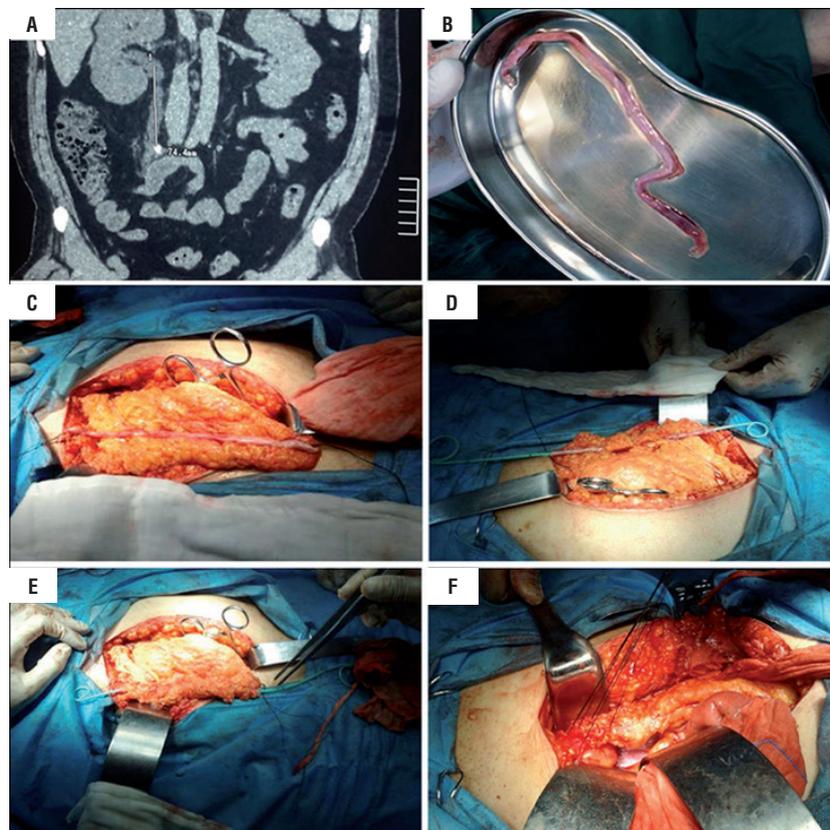
The patient was followed-up for 34 months. Plain abdominal radiography (KUB) and CT indicated that there was no hydronephrosis and the position of double-J tube was normal (Figures 2a and b). At 3 months, CT indicated that there was a stone like-material attached to the double-J tube (Figure-2c). After extensive discussion with urologists and with the patient, his spouse and

his family members, we decided to pull out the double-J tube finally. At 5 months, CT indicated that there were no hydronephrosis and other abnormalities (Figure-2d). At 23 months after first operation, CT revealed right hydronephrosis and a new upper ureteral stone (Figure-2e). Rigid ureteroscopy and holmium laser lithotripsy were used, and a single double-J stent tube was placed inside the ureter after management, which was removed one month later. At 34 months, CT of urinary system revealed no hydronephrosis, renal atrophy or other complication (Figure-2f).

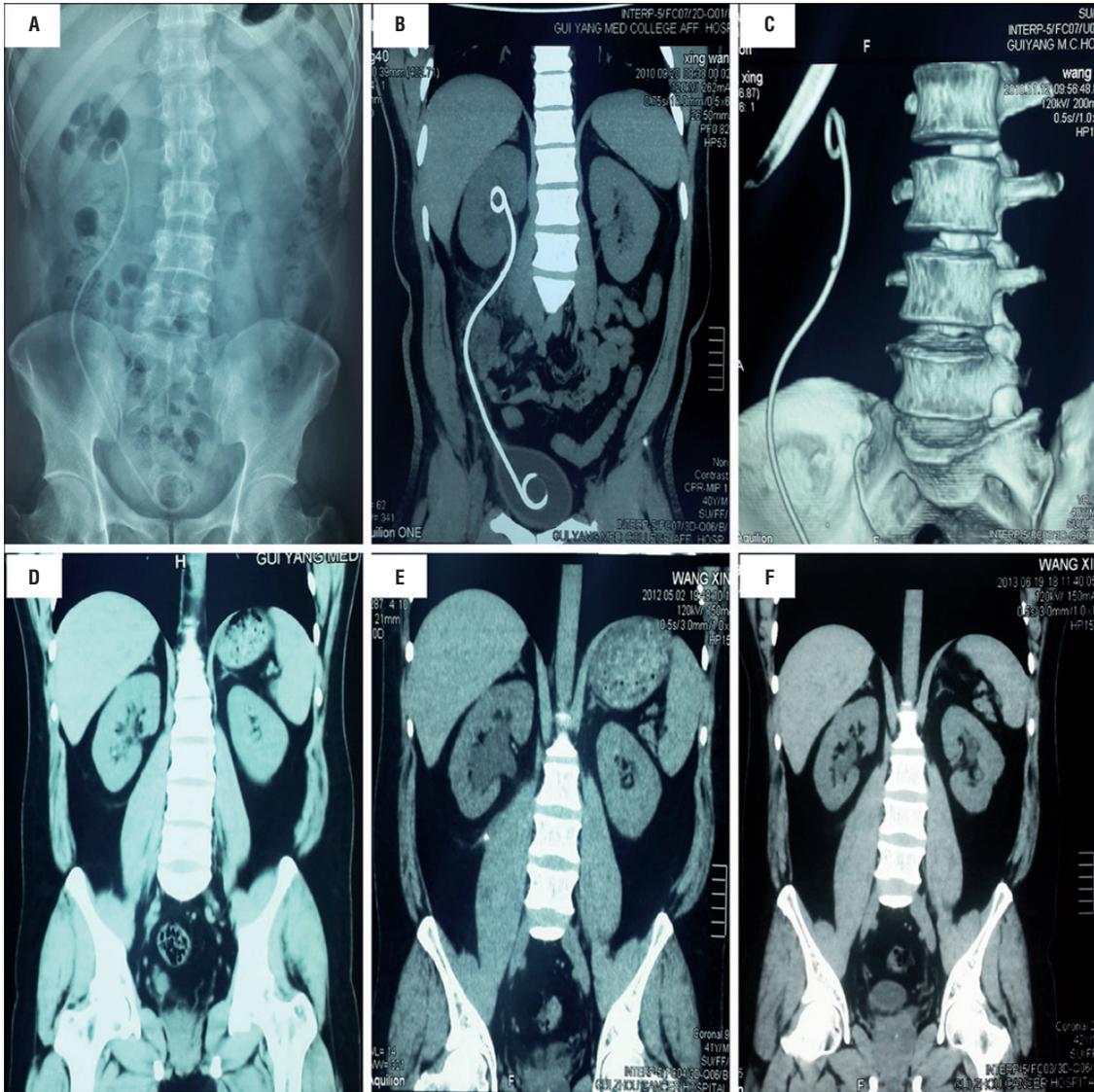
DISCUSSION

Urolithiasis is a very common and major disease in urology department, the lifetime risk of

Figure 1 - A) Right hydronephrosis secondary to a stone located in the ureter; B) The avulsed ureter; C) Free vascularized greater omentum in order to adapt to the avulsed ureter length; D) A single double-J stent tube was placed inside the ureter, package of ureter with greater omentum from the inside to the outside; E) The greater omentum was sutured around the ureter closely; F) pyeloureterostomy and ureterovesical anastomosis, respectively.



Figures 2 - A and B) KUB and CT at one month; C) CT indicated that there was a stone like-material attached to the double-J tube at 3 months; D) CT indicated that there were no right hydronephrosis or other abnormalities at 5 months; E) CT revealed hydronephrosis secondary to a stone located in the upper ureter at 23 months; F) CT of urinary system revealed no hydronephrosis, renal atrophy or other complications at 34 months.



urolithiasis in the general population is 13% (3, 4). Ureterscopy is considered a reasonable therapeutic option for patients with ureteral stones (5). However, ureteroscopic examination or treatment procedures may lead to various complications, such as stone residuals, mucosa injury, perforation, bleeding, and edema (6). Ureteral avulsion is a rare but extremely serious complication, in-

cidence of which has been reported at 0-3.75% (7), which is very difficult to manage. Many treatments may be considered: autotransplantation of kidney, ureterovesical anastomosis; replacement of the ureter with the ileum, ureterocalicostomy; and ureteral-ureteral end-end anastomosis, extended spiral bladder flap treatment of upper ureteral loss, pyeloureterostomy plus greater omentum in-

vestment outside the avulsed ureter and ureterovesical anastomosis and so on (8-11). The pros and cons of all treatment options in the management of ureteral avulsion are listed in Table-1. The actual surgical procedure depends on the site and severity of injury.

The treatment of ureteral avulsion is challenging and remains controversial. Accord-

good solution to full-length complete ureteral avulsion.

CONSENT

Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of

Table 1 - The pros and cons of all treatment options in the management of ureteral avulsion.

Methods of reconstruction	Pros	Cons
Autotransplantation of kidney (12)	Priority selection for isolated kidney, renal insufficiency and complete ureteral avulsion	The operation was difficulty, and patients and their families is difficult to accept
Ureterovesical anastomosis, ureterocalicostomy and Ureteral-ureteral end-end anastomosis (9, 13)	The operation was simple, less trauma	Anastomosis stenosis or leakage, and not suitable for complete ureteral avulsion
Replacement of the ureter with the ileum (14, 15)	High success rate	Obstruction, delayed formation of mucus, stones, recurrent infection, ischemic necrosis of intestine, electrolyte disorder and preoperative bowel preparation
Extended spiral bladder flap treatment of upper ureteral loss (11)	The recovery of renal function was good, less complications	Repair ureter injury length is limited
Pyeloureterostomy plus greater omentum investment outside the avulsed ureter and ureterovesical anastomosis (8)	The renal function recovered well, especially suitable for full-length ureteral avulsion	The operation was difficulty, fibrosis tissue was forming outside ureter and anastomotic atresia of ureter-bladder
Nephrectomy (7)	The operation was easily	Patients and their families are difficult to accept

ding to this case report with full-length complete ureteral avulsion, it is suitable for pyeloureterostomy plus greater omentum investment outside the avulsed ureter and ureterovesical anastomosis. Previous studies showed that the blood supplies of greater omentum could nourish the avulsed ureter (8, 9).

We believe that pyeloureterostomy plus greater omentum investment outside the avulsed ureter and ureterovesical anastomosis may be a

the written consent is available for review by the Editor of this journal.

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CONFLICT OF INTEREST

None declared.

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Renal pseudoaneurysm after core-needle biopsy of renal allograft successfully managed with superselective embolization

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INTRODUCTION

Renal biopsy of the allograft is important to evaluate renal dysfunction (1). Rare complications like pseudoaneurysm (PA) can develop and could lead to life-threatening bleeding (2, 3). It can be safely and effectively managed by endovascular embolization yielding good renal function in the long term follow-up (4, 5). We describe a PA of a kidney transplant (KTX) associated with arteriovenous fistula (AVF) at the site of a core needle percutaneous biopsy (CNPB).

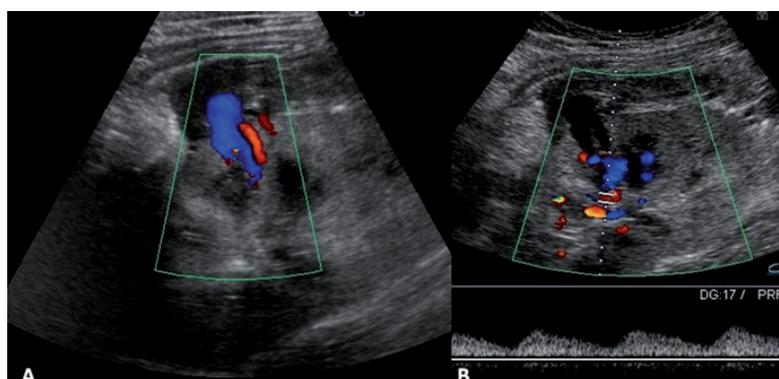
CASE DESCRIPTION

A 39-year old woman with nephrosclerosis and in hemodialysis for the last 3 years re-

ceived a KTX from a deceased 20-year old male donor that had a cranio-cerebral trauma. The vascular anastomoses were performed at the right iliac vessels in an end-to-side fashion after 23 hours of cold ischemia.

A CNPB, guided by ultrasonography, was indicated due to delayed graft function at postoperative day 10 which revealed acute tubular necrosis. Shortly after the CNPB she experienced tachycardia, hypotension and decreased blood levels requiring 2 units of blood transfusion and remained stable and developed mild hematuria. An allograft ultrasonography performed five days later revealed an AVF and a PA at the middle pole of the allograft and a peri-renal hematoma around the upper pole with 200cc (Figure-1). A superselective catheter-

Figure 1 - A-Doppler ultrasound with reverse diastole in interlobular artery and lesion suggestive of pseudoaneurysm at the middle pole of the kidney. Cystic formation which implies the renal parenchyma toward the collection, that measured about 1.2cm. This structure has bidirectional blood flow, suggestive of a pseudoaneurysm. B-Pulsatile flow in the vein suggestive of AVF.



terization was then performed, six days after the CNPB with embolization of the PA with two coils. AVF was not observed (Figures 2 and 3). A control by ultrasound 5 days after the pro-

cedure assured the closure of the pseudoaneurysm (Figure-4). The patient did well and gradually recovered renal function (creatinine of 1.09mg/dL after two months).

Figure 2 - Renal transplanted arteriography: (A)-Sacular formation in the arterial phase of the study, suggestive of pseudoaneurysm (Arrow), (B)-a microcatheter (inferior arrow) has been advanced superselectively in the lesion arterial branch with Vortex coil (superior arrow).

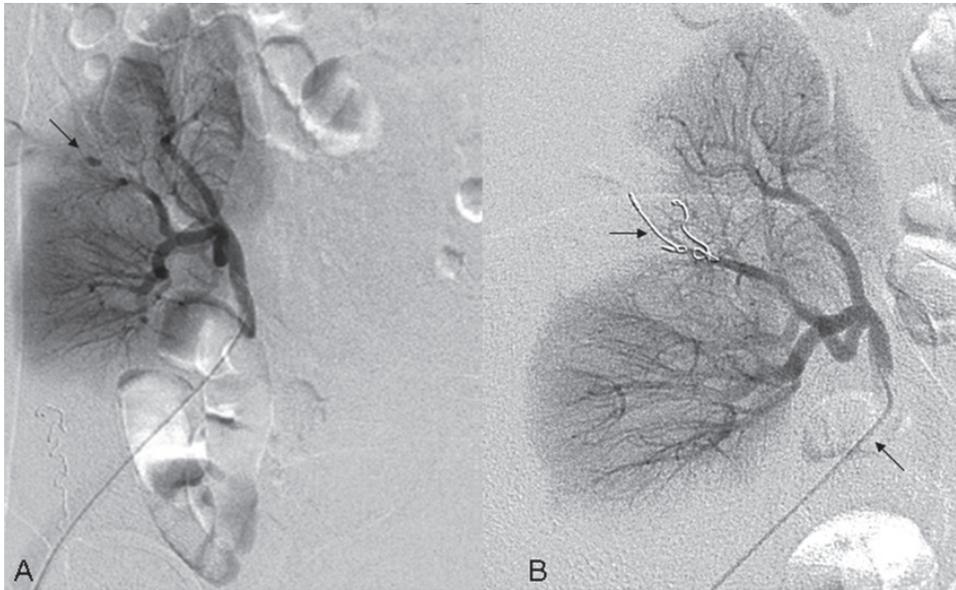
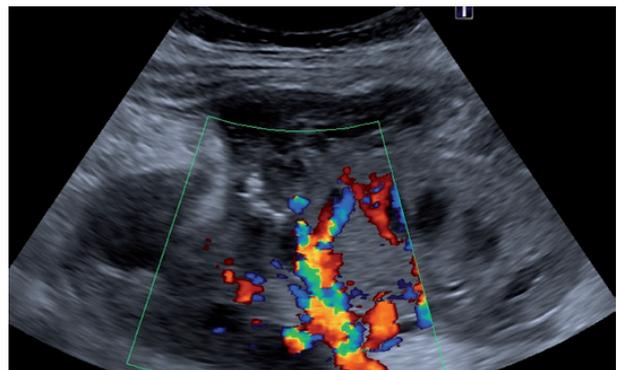


Figure 3 - Superselective catheterization of the interlobular artery.



Figure 4 - Doppler control demonstrating the closure of the PA.



CONFLICT OF INTEREST

None declared.

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Robotic repair of vesicovaginal fistula – initial experience

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ABSTRACT

Objective: The most common acquired fistula of the urinary tract is Vesicovaginal fistulae (VVF) (1) posing social stigmata for the patient as well as a surgical challenge for the urologist. Here we present our initial experience with Robotic assisted laparoscopic repair of VVF, its safety and efficacy.

Materials and Methods: Seven out of eight fistulas were post hysterectomy; five had undergone abdominal while two had laparoscopic hysterectomy while one was due to prolonged labour. Two had associated ureteric injury. All underwent robotic assisted laparoscopic trans abdominal extravesical approach. Three 8 mm ports for robotic arms, one 12 mm port for camera and another 12 mm for assistant were used in a fan shaped manner. All had preoperative ureteric catheter placed. Bladder was closed in two layers and vagina in one layer. Omental flap placed in all cases except two where it was not possible. Drain and per urethral catheter placed in all cases. Double J stents were placed in two cases requiring ureteric implantation additionally.

Results: The mean age of presentation was 39.25 years (26-47 range) with mean BMI being 26.25 kg/m² (21-32 range). Mean duration between insult and repair was 9.37 months (3-24 months). Only in single case there was history of previous repair attempt. On cystoscopy four had supratrigonal VVF and four were trigonal with mean size of 13.37 mm (7-20 mm). Mean operative time was 117.5 minutes (90-150). There were no intraoperative/postoperative complications or need for open conversion. Mean haemoglobin drop was 1.4 gm/dL (0.3-2 gm). Drain was removed once 24-48 hours output is negligible. One patient had post-operative urinary leak at 2 weeks which ceased with continuation of catheterisation for another 2 weeks. Catheter was removed after voiding cystourethrogram showed no leak at 2-3 weeks postoperatively. Mean duration of drain was 3.75 days (3-5) and per urethral catheterisation (which was removed after voiding cystourethrography) was 15.75 days (9-28). Mean hospital stay was 6.62 days (4-14). Post-operative bladder capacity was 324.28 cc (280-350) on voiding diary. Follow up ranged from 3-9 months. At 3 months of follow-up, these patients continued to void normally and there was no evidence of recurrence of VVF.

Conclusion: Robotic repair of VVF is safe and feasible and has additional advantages in the form of precise suturing under 3D vision and certainly a more striking and effective option especially in complex VVF repair associated with ureteric injuries (2).

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EDITORIAL COMMENT

These authors have reported one of the largest series of robotic assisted laparoscopic repair of vesico-vaginal fistula (VVF). They have demonstrated that robotic repair of VVF is associated with minimal morbidity with excellent 3 month follow up outcomes. As urologists have advanced their robotic expertise, they are now able to tackle the more difficult cases of pelvic

reconstruction. VVF can represent a challenge due to both etiology and variability in anatomy. The authors are correct in their commentary that the robotic platform with its 3D vision system and precise instrumentation in the pelvis allow it to be an effective option when considering VVF repair. While urologists can add this to their robotic armamentarium, I would caution that such cases should be only attempted by seasoned robotic surgeons.

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Hemostatic completion of percutaneous nephrolithotomy using electrocauterization and a clear amplatz renal sheath

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ABSTRACT

Background and Purpose: A tubeless PCNL can reduce postoperative pain, the need for analgesics, hospital stay, and postoperative urinary leakage. However, perioperative or delayed bleeding remains the primary postoperative concern. We demonstrate a simple and cost-effective method to develop a clear nephrostomy tract after completion of a tubeless PCNL.

Materials and Methods: Four consecutive patients with renal calculi >3cm underwent a tubeless PCNL. We used a 24 Fr nephroscope and a 24 Fr transurethral resectoscope. Intraoperative urologist-directed percutaneous renal access was performed under fluoroscopy. After calculi removal, active bleeders were identified via a clear Amplatz renal sheath. The sheath provided excellent visualization of the nephrostomy tract for the detection of bleeders and surrounding structures. Bleeders were electrocauterized using a roller barrel electrode. During extraction of the renal sheath, the surgeon can confirm hemostasis in the tract and apply intermittent suction.

Results: Bleeding primarily originated from the torn calyceal mucosa and the parenchyma. Tract electrocauterization was successful. All patients had mild hematuria, which resolved within two days. The average hemoglobin decrease was 1.65g/dL (0.8-2.1) and no patients required a transfusion. No perioperative complications occurred. On postoperative day 2, the patients could ambulate without a Foley catheter. During three months of follow-up, delayed bleeding or percutaneous urine leakage did not occur.

Conclusions: Electrocauterization with a roller barrel electrode and a clear Amplatz renal sheath is an effective method to obtain hemostasis after completion of a PCNL. Our technique is cost-effective and readily adapted without the need for additional instruments.

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EDITORIAL COMMENT

In the present video submission by Yu et al., the authors nicely depict a novel way bleeding encountered at the time of a percutaneous nephrolithotomy can be controlled using electrocautery and a clear Amplatz sheath. This is clearly one of the most challenging clinical scenarios encoun-

tered at time of such procedures can be addressed minimizing perioperative morbidity and the necessity for possible additional ancillary procedures. Developing innovative solutions to manage such surgical complications are strongly encouraged and I congratulate the authors in doing so particularly as this requires readily available surgical equipment at our disposition.

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RE: Ultrasonic Measurement of Testicular Tumors and the Correlation with Pathologic Specimen Sizes

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To the editor,

We read with great interest the article “The value of testicular ultrasound in the prediction of the type and size of testicular tumors” by Shtricker et al. (1). They aimed to assess the correlation between ultrasound (US) findings and testicular tumor type and size. The authors concluded that the testis US findings underestimated the size in 25% of the malignant testicular lesions and 16% of the cases were proven to be benign. Thus they recommended putting into practice frozen sections for borderline cases. This study gives substantial information on this clinically relevant condition. The awareness of this diagnostic finding and its clinical results may increase the accuracy of preoperative management of the patients with testicular lesions. Thanks to the authors for this contribution.

Several medical subspecialties manage their treatments with respect to anatomic measurements. The reproducibility and accuracy of the measurements are especially crucial in radiology as important clinical decisions are often based on the assumption that radiologic measurements are accurate and any measurement differences on follow-up imagings represent a real change in size. Favorably, measurements of the tumors on images should be accurate, reproducible, and practiced in a standardized method with low rates of intra-and interobserver variability. Even so, there a lot of factors, which may affect the consistency of the measurement, including patient-dependent factors, technical factors and radiologist-dependent factors (2,3).

World Health Organization criteria (WHO) (4) and the Response Evaluation Criteria in Solid Tumors (RECIST) (5) are two widely accepted guidelines of measurement methods to obtain standardized results (6). WHO criteria recommend the measurement method on the basis of an approximation of cross-sectional area (bidimensional measurement), whereas RECIST suggests to measure only the tumor’s greatest diameter (unidimensional measurement) on a transverse plane (7).

Shtricker et al. have designed this study as a multicenter study (1). This design may increase the variabilities in the tumor measurement. However, the authors did not mention any measurement method for standardization in the study. A lot of published studies based on the variability and reproducibility of tumor measurements define their measurement methods and they generally use WHO or RECIST criteria. Therefore a measurement variability might occur due to lack of measurement standardization.

In conclusion, we strongly believe that those findings obtained from the current study will lead to further studies examining the correlation between testis US findings and histopathology. One should keep in mind that measurement standardization and comparison with pathologic specimens in optimized conditions are essential for valuable results.

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- Sabiston DC: Textbook of Surgery. Philadelphia, WB Saunders. 1986; vol. 1, p. 25.

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