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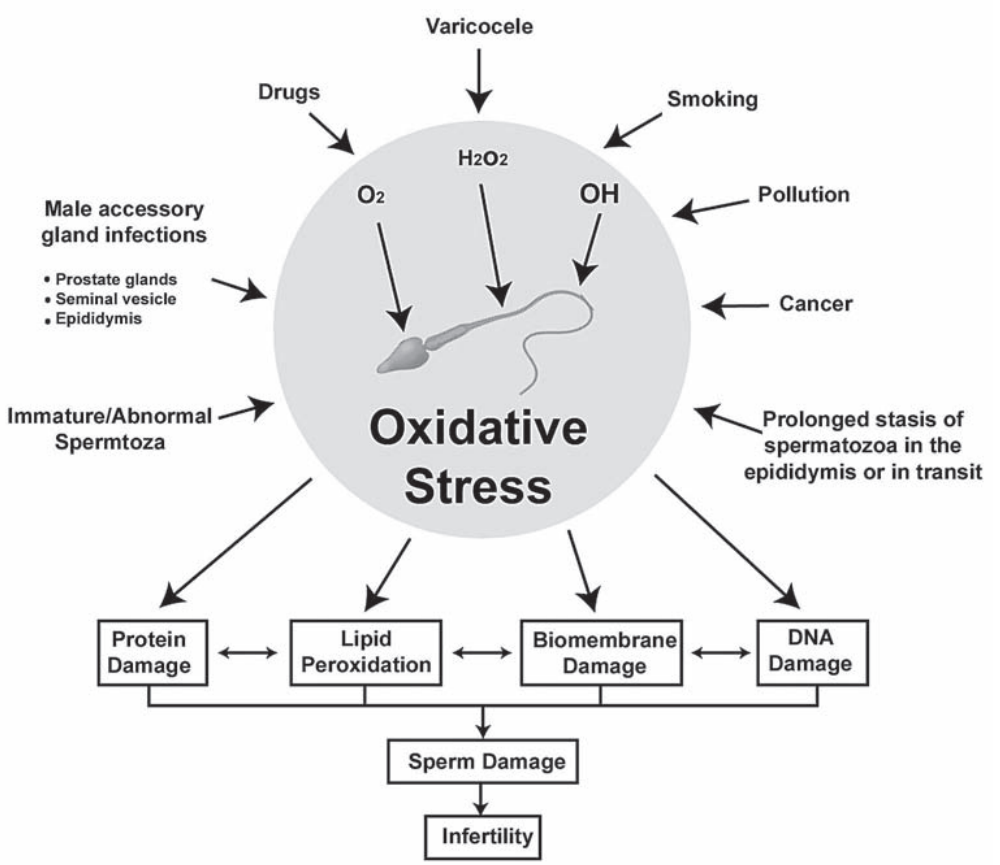


International Braz J Urol

EDITOR'S COMMENT

Oxidative Stress and Sperm Chromatin Damage in Male Infertility

The September - October 2007 issue of the International Braz J Urol presents interesting contributions, and as usual, the editor's comment highlights some papers.

Doctor Cocuzza and collaborators, from the Reproductive Research Center, Cleveland Clinic, Ohio, and Tulane University Health Sciences Center, New Orleans, Louisiana, USA presented on page 603 an important review article on the clinical relevance of oxidative stress and sperm chromatin damage in male infertility. The authors demonstrated that despite the controversial findings in the existing literature, there is now enough evidence to show that sperm DNA damage is detrimental to reproductive outcomes. In addition, spermatozoa of infertile men are suggested to carry more DNA damage than do the spermatozoa from fertile men. Besides impairment of fertility such damage is likely to increase the transmission of genetic diseases during the assisted reproductive procedures. Standardization of protocols to assess reactive oxygen species (ROS) and DNA damage is very important in introducing these tests in such clinical practice. Thus evaluation of seminal ROS levels and extent of sperm DNA damage especially in an infertile male may help develop new therapeutic strategies and improve success of assisted reproductive techniques.

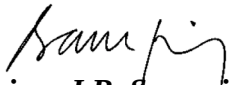
Doctor Mota and co-workers, from the Federal University of Ceara and University of Sao Paulo Ribeirao Preto, Sao Paulo, Brazil, investigate on page 704 the possible protective effect of recombinant human interleukin-11 (rhIL-11) against ifosfamide (IFS)-induced hemorrhagic cystitis (HC). They studied male Swiss mice pretreated with rhIL-11 (25-625 μg, subcutaneously.) 30 min before intraperitoneal injection of IFS (400 mg/kg) or with saline (control group). Twelve hours later, HC was evaluated by bladder wet weight (BWW) to quantify edema, Evans blue extravasation (EBE) to measure vascular permeability, and macroscopic and microscopic analysis. All bladders were assessed by histopathological analysis. rhIL-11 (at 125 and 625 μg) attenuated the IFS- induced increase of BWW (37.48% and 45.44%, respectively, $p < 0.05$) and EBE (62.35% and 56.47%, respectively, $p < 0.05$). The results demonstrate a protective effect of rhIL-11 on experimental IFS- induced HC, which were not previously reported.

Doctor Picolli and colleagues, from the Section of Nephrology, Federal University of Sao Paulo, UNIFESP, Sao Paulo, Brazil investigates on page 622 the association between matrix metalloproteinase-1 (MMP-1) promoter polymorphism and risk of renal cell carcinoma. The authors genotyped 217 individuals, 99 patients with renal cell carcinoma (RCC) and 118 controls without cancer. DNA specimens were extracted from epithelial buccal cells and paraffin-embedded tissue of RCC patients and from epithelial buccal cells and blood cells of healthy controls. The comparison of genotype distribution and frequency of 2G allele in different populations showed a strong variability of 2G allele frequency among the different ethnic groups. This fact may influence on the collaboration of this 2G allele in RCC or others diseases. The data suggested

that the matrix metalloproteinase-1 (MMP-1) promoter polymorphism may not play a significant role in renal cell carcinoma patients in Brazil.

Doctor Nakamura and colleagues, form the Institute Radium of Oncology, Campinas, Sao Paulo, Brazil, identified on page 652 the prognostic factors for late urinary toxicity grade 2-3 after conformal radiation therapy (3DCRT) on patients with prostate cancer. The authors studied 285 patients with localized prostate cancer with a median dose delivered to the prostate of 7920 cGy (7020-8460). On a median follow-up of 53.6 months (3.6-95.3), the 5-year actuarial free from late urinary toxicity grade 2-3 survival was 91.1%. Seven and fifteen patients presented late urinary toxicity grades 2 and 3, respectively. Prior transurethral resection of prostate and radiation dose over 70 Gy on 30% of initial bladder volume were independent prognostic factors for late urinary toxicity grade 2-3. The results suggest that restricting radiation doses to 70 Gy or less on 30% of bladder volume, visualized through CT planning, may reduce late urinary complications. It furthermore suggests that patients with prior transurethral resection of prostate may indicate a group of patients with a greater risk for late urinary toxicity grade 2-3 after 3DCRT. Doctor Michael Pinkawa, from the Department of Radiotherapy, Aachen University, Germany, a world expert in the field, provided interesting editorial comment on this paper.

Doctor Velloso and co-workers, from Federal University of Minas Gerais, Belo Horizonte, Brazil, emphasized on page 639the evaluation of the modified Gleason score agreement in needle biopsies and in surgical specimen, as well as the interobserver variability of this score. Contrary to what was expected, the modified Gleason score was not superior in the agreement between the biopsy score and the specimen, or in interobserver reproducibility, in this study. Doctor Lars Egevad, from Karolinska Hospital, Stockholm, Sweden, Doctor Rodolfo Montironi, from Polytechnic University of the Marche Region, Ancona, Italy, Dr. Liang Cheng, from Indiana University School of Medicine, Indianapolis, USA, and Dr. Jonathan I. Epstein, from The Johns Hopkins Hospital, Baltimore, USA, world renowned experts in prostate pathology, provided excellent and educative editorial comments on this paper.



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

Review Article
International Braz J Urol Vol. 33 (5): 603-621, September - October, 2007

Clinical Relevance of Oxidative Stress and Sperm Chromatin Damage in Male Infertility: An Evidence Based Analysis

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ABSTRACT

Oxidative stress (OS) in the reproductive tract is now a real entity and concern due to the potential harmful effects of high levels of reactive oxygen species (ROS) on sperm number, motility, quality, and function including damage to sperm nuclear DNA. Evaluation of OS related damage to non-functional sperm is highly relevant as intracytoplasmic sperm injection (ICSI) technique, an effective therapy for severe male factor infertility, bypasses the majority of reproductive tract deficiencies. Despite the controversial findings in the existing literature, there is now enough evidence to show that sperm DNA damage is detrimental to reproductive outcomes. In addition, spermatozoa of infertile men are suggested to carry more DNA damage than do the spermatozoa from fertile men. Besides impairment of fertility such damage is likely to increase the transmission of genetic diseases during the assisted reproductive procedures. Standardization of protocols to assess reactive oxygen species and DNA damage is very important in introducing these tests in such clinical practice. Thus evaluation of seminal ROS levels and extent of sperm DNA damage especially in an infertile male may help develop new therapeutic strategies and improve success of assisted reproductive techniques (ART).

***Key words**: free radicals; oxidative stress; sperm; DNA; antioxidants; male infertility*
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INTRODUCTION

A large population of apparently normal males have problem impregnating their partners even when their fertility status by routine semen analysis is considered normal. These cases are classified as idiopathic infertility. Men with idiopathic infertility generally present with significantly higher seminal ROS levels and lower antioxidant potential than healthy fertile controls (1). In addition, high ROS levels have been detected in the semen samples of 25% to 40% of infertile men (2,3).

In the context of human reproduction, a balance called oxidative stress status (OSS) normally exists between ROS production and antioxidant scavenging system in the male reproductive tract (4). Small physiological levels of ROS are essential for the regulation of normal sperm functions such as sperm capacitation, the acrosome reaction, and sperm-oocyte fusion (5,6). However, production of excessive

amounts of ROS in semen especially during leukocytospermia can overwhelm the antioxidant defense mechanisms of spermatozoa and seminal plasma resulting in oxidative stress. Studies suggest that ROS attack the integrity of DNA in the sperm nucleus by causing base modifications, DNA strand breaks, and chromatin cross-linking (7,8). Spermatozoa have limited defense mechanisms against oxidative attack on their DNA mainly due to the complex packaging arrangement of DNA. In vivo, such damage may not be the cause for concern because the collective peroxidative damage to the sperm membrane ensures that spermatozoa susceptible to oxidative stress are unable to participate in the fertilization process. However, these safeguards are circumvented during the course of ICSI and some spermatozoa with significant DNA fragmentation may be used that will produce adverse unfavorable results.

The assessment of sperm DNA damage appears to be a potential tool for evaluating semen samples prior to their use in ART. Testing DNA integrity may help andrologists to select spermatozoa with intact DNA or with the least amount of DNA damage for use in assisted reproduction possibly increasing the success rate. In addition, interest in the physiologic and pathologic effects of ROS on male fertility is growing. Therefore, it is essential for urologists and fertility specialists to understand free radical sources, their generation, sperm damage mechanisms that may affect male reproductive system. In addition, it has been postulated that protective agents against ROS e.g., antioxidants, may be useful for treating male factor infertility. For this reason, deciphering the levels and sources of excessive ROS production in human semen may be useful in developing therapeutic strategies for use in male infertility uses.

This article will discuss in detail about the clinical relevance of oxidative stress in human semen, how excessive ROS damages sperm nuclear DNA as well as how such DNA damage contributes to male infertility and assisted reproductive techniques.

Design: A thorough literature survey was performed using the Medline, EMBASE, BIOSIS and Cochrane databases. We restricted the survey to clinical publications between 1985 and 2006 that were relevant to male infertility with emphasis on oxidative stress and DNA damage.

WHAT ARE REACTIVE OXYGEN SPECIES AND OXIDATIVE STRESS?

Reactive oxygen species (ROS) known as free radicals are oxidizing agents generated as a result of metabolism of oxygen and have at least one unpaired electron that make them very reactive species. Normally, free radicals attack the nearest stable molecule, which becomes a free radical itself, beginning a cascade of chain reaction. These can very rapidly oxidize biomolecules that they encounter in their vicinity thus exerting either a positive or a negative influence on normal cell function (9).

Normal aerobic metabolism is related to optimal levels of ROS because a balance exists between ROS production and antioxidants activity. Oxidative stress (OS) is the term applied when oxidants outnumber the antioxidants due to excessive generation of reactive oxygen species and when antioxidants cannot scavenge these free radicals (10). Such phenomena cause pathological effects, damaging cells, tissues and organs (11).

REACTIVE OXYGEN SPECIES AND SEMINAL OXIDATIVE STRESS

Spermatozoa produce small amounts of ROS that play a significant role in many of the sperm physiological processes such as capacitation, hyperactivation, and sperm-oocyte fusion (12,13). However, ROS must be continuously inactivated to keep only a small amount necessary to maintain normal cell function. Excessive generation of ROS in semen can cause damage to spermatozoa due to its exclusive structural composition. During the maturation process the spermatozoa extrudes cytoplasm, which is the major source of antioxidants. Once this process is slowed down, residual cytoplasm forms a cytoplasmic droplet in the sperm mid region. These spermatozoa carrying cytoplasmic droplets are though to be immature and functionally defective (14). The residual cytoplasm contains high concentration of certain cytoplasmic enzymes (G6PDH, SOD), which are also a source of ROS (15). Lack of cytoplasm results in decreased antioxidant defense. This process is the link between poor sperm quality and elevated ROS.

Human ejaculate consists of different types of cells such as mature and immature spermatozoa, round cells from different stages of the spermatogenic process, leukocytes and epithelial cells. Of these, peroxidase-positive leukocytes and abnormal spermatozoa that produce free radicals continuously (16,17). Spermatozoa are also particularly susceptible to the damage induced by excessive ROS because their plasma membranes contain large quantities of polyunsaturated fatty acids (PUFA), which readily experience lipid peroxidation by ROS, resulting in a loss of membrane integrity (18,19). There are two major systems of ROS production in sperm. One is the nicotinamide adenine dinucleotide-dependent oxidase system at the level of the sperm plasma membrane and the other is NADH-dependent oxido-reductase (diphorase) system at the mitochondrial level (20). There is a strong positive correlation between immature spermatozoa and ROS production, which in turn is negatively correlated with sperm quality (21). Furthermore, it has been noticed that as the concentration of immature spermatozoa in the human ejaculate increases, the concentration of mature spermatozoa with damaged DNA rises (22) (Figure-1).

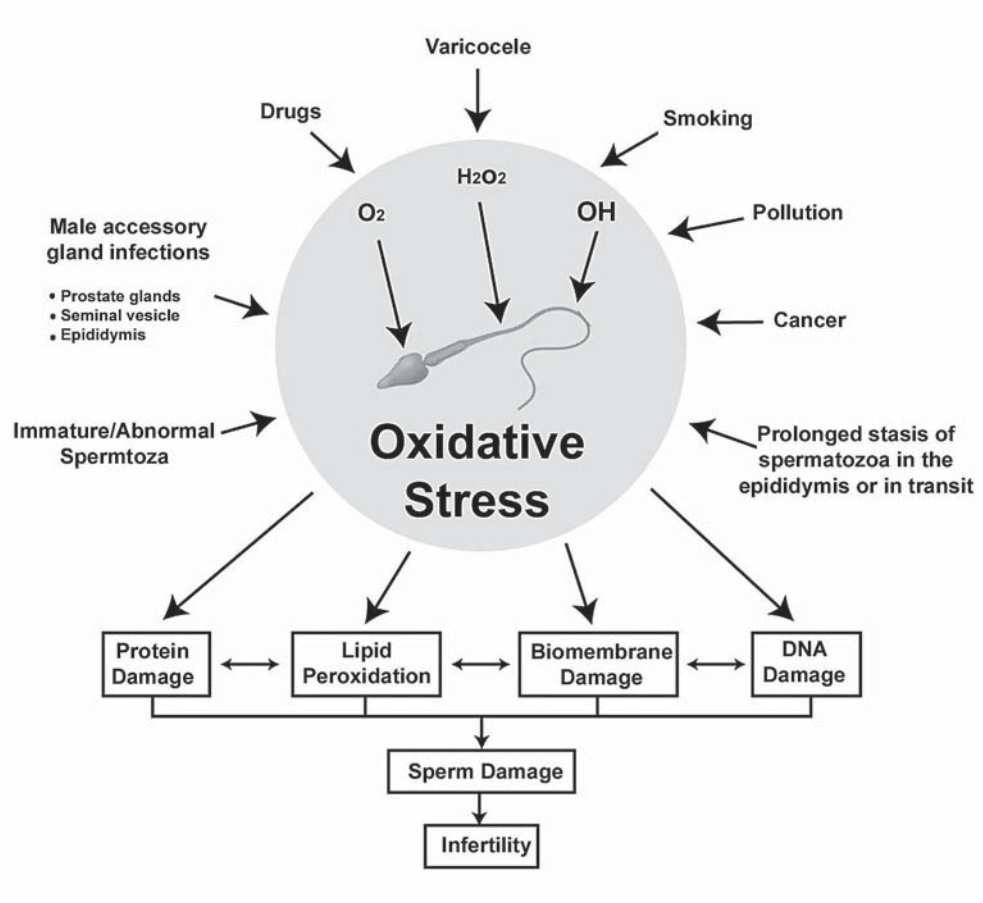

Figure 1 – *Association of increasing reactive oxygen species (ROS) production with infertility.*

OXIDATIVE STRESS AND EFFECT ON SPERM MOTILITY

Seminal ROS levels, when present in excess, possess potentially toxic effects on both sperm quality and function (23,24). Elevated seminal ROS production has been associated with decreased sperm motility, defective acrosome reaction, and loss of fertility (25). Sperm cell dysfunction, a result of ROS damage, is dependent on the nature, amount, and duration of exposure to ROS. The extent of ROS damage is also dependent upon surrounding environmental factors such as oxygen tension and temperature as well as the concentrations of molecular components such as ions, proteins, and ROS scavengers (5).

As reported by Aitken et al., low hydrogen peroxide concentrations do not influence sperm motility, but do suppress human sperm competence during oocyte fusion (26). Possibly ROS levels are not high enough to affect standard seminal parameters but can cause defects in other processes that are required for fertilization, such as sperm-oocyte interaction. These findings suggest an explanation why patients with normal semen parameters can experience idiopathic infertility. Decreased motility is a result of cascade of events including lipid peroxidation (LPO) of sperm plasma membrane that ultimately affect an axonemal protein phosphorylation and sperm immobilization (2).

CLINICAL DIAGNOSIS AND ASSESSMENT OF SEMINAL OXIDATIVE STRESS

Spinal Cord Injury

Recent studies report the detection of increased ROS levels in the semen of 25% to 40% of infertile men (2,3). Padron et al. documented that in men with spinal cord injury, elevated seminal ROS levels are associated with poor sperm motility and morphology. These associations are independent of both ejaculation method and specimen type (3).

Varicocele

The role of ROS in varicocele has been previous reported by our center and others (17,27,28). Excessive nitric oxide release within dilated spermatic veins has been identified in subfertile males with varicocele. This nitric oxide release may cause spermatozoal dysfunction (27,29). Allamaneni et al. report a positive correlation between seminal ROS levels and varicocele grade in which significantly higher levels of seminal ROS are seen in men with varicocele grades 2 and 3 versus men with varicocele grade 1 (30). Varicocele patients also present low seminal plasma TAC levels and increase 8-hydroxy-2'-deoxyguanosine levels, indicating a deficient pro-oxidant defense system and oxidative DNA damage, respectively (17,31). According to a recent meta-analysis, varicocele patients as compared with normal sperm donors have significantly increased oxidative stress parameters such as ROS and lipid peroxidation as well as significantly decreased antioxidant concentrations (32). Antioxidant supplementation may therefore be beneficial to this infertile population with varicocele.

Mostafa et al. first reported that varicocelectomy reduces the seminal plasma ROS levels of infertile men associated with increased seminal plasma concentrations of antioxidants such as superoxide dismutase, catalase, glutathione peroxidase and vitamin E of infertile men (33). Daitch et al. reported that couples who do not achieve pregnancy following varicocelectomy might significantly increase their pregnancy and live birth rates after undergoing intrauterine insemination, despite failing to show improvements in semen parameters (34). It is therefore suggested that pregnancy rate improvement following varicocelectomy may be due to functional factors such as seminal oxidative stress and the spermatozoal DNA integrity not routinely tested during standard semen analysis (34).

Leukocytospermia

ROS in the human ejaculate originate mainly from seminal leukocytes. Leukocytospermia is characterized by abnormally high seminal leukocyte, polymorphonuclear neutrophils, and macrophages (35). Seminal leukocyte ROS production induces spermatozoal damage during ART procedures (1,36). Patients with accessory gland infection demonstrate both leukocytospermia and elevated ROS levels (37). In these patients, sperm function defects are resultant

of abnormal lipid peroxidation, stimulated by the high ROS levels (38).

Genito-Urinary (GU) Tract Infection

During GU infection, the presence of leukocytes in semen has been associated with decreased sperm motility and fertilization capacity (39-41). However, El-Demiry et al. reported no association between standard seminal parameters and leukocyte concentration in human semen (42). This dilemma may be partially due to the different techniques used to determine leukocyte concentration in semen as well as the lack of agreement on the lower leukocyte concentration responsible for sperm damage (43-45). Infections located in the testis and epididymis produce ROS that are particularly harmful to spermatozoa due to its lack of a pro-oxidant defense system. Sperm function may also be indirectly affected by an infection stimulating the presence of ROS in the prostate gland, and seminal vesicles. An association between prostatitis and male infertility has been reported, but the responsible mechanism is still poorly understood (46). Prostatitis is associated with the presence of granulocytes in prostatic fluid. Irrespective of leukocytospermia status, increased seminal oxidative stress is reported in men with chronic prostatitis and prostatodynia (46). Such findings support the controversial prostatitis-infertility relationship debate. Multiple hypotheses discuss male genital tract infections and their relationship with ROS. Specifically, the leukocytes stimulate human spermatozoa to produce ROS. The mechanisms responsible for such stimulation are unknown, but may include the direct contact of sperm and leukocytes or may be regulated by leukocyte release of soluble products (1,47).

Environmental Factors

An association between cigarette smoking and reduced seminal quality has been identified (48). Harmful substances including alkaloids, nitrosamines, nicotine, cotinine and hydroxycotinine are present in cigarettes and produce free radicals (49). In a prospective study, Saleh et al. compared infertile men who smoked cigarettes with nonsmoker infertile men (50). Smoking was associated with a significant increase (approximately 48%) in seminal leukocyte concentrations, a 107% ROS level increase, and a 10 point decrease in ROS-TAC score. The authors concluded that infertile men who smoke cigarettes present higher seminal OS levels than infertile nonsmokers, possibly due to significant increase in leukocyte concentration in their semen. An earlier study also reported an association between cigarette smoking in infertile men and increased leukocyte infiltration in the semen (51). Significantly higher levels of DNA strand breaks in men who smoke have also been identified. DNA strand breaks may be resultant from the presence of carcinogens and mutagens in cigarette smoke (52). In recent decades evidence suggestive of the harmful effects of occupational exposure chemicals known as endocrine disruptors on the reproductive system has gradually accumulated (53). Environmental pollution is a major source of ROS production and has been implicated in the pathogenesis of poor sperm quality (54). In a study conducted by De Rosa et al., tollgate workers with continuous environmental pollutant exposure had inversely correlated blood methaemoglobin and lead levels to sperm parameters in comparison to local male inhabitants not exposed to comparable automobile pollution levels. These findings suggest that nitrogen oxide and lead, both present in the composition of automobile exhaust, adversely affect semen quality (55). In addition, the increase of industrialization has resulted in an elevated deposition of highly toxic heavy metals into the atmosphere. Paternal exposure to heavy metals such as lead, arsenic and mercury is associated with decreased fertility and pregnancy delay according to recent studies (56,57). Oxidative stress is hypothesized to play an important role in the development and progression of adverse health effects due to such environmental exposure (58).

FREE RADICALS AND ASSISTED REPRODUCTIVE TECHNIQUES (ART)

Numerous conditions associated with male infertility, e.g., microdeletions of the Y chromosome, sperm maturational arrest, meiotic defects, aneuploidies, defective centromeres and defects in oocyte activation still lack a specific treatment. However, advances in ART have helped in improving

treatment of male factor infertility (35). Currently, ICSI is the most common ART method, although it is associated with the highest number of miscarriages. One of the explanations can be the poor selection of sperm that are possibly damaged by free radicals during ART procedures.

ROS are produced during ART mainly by oocytes, embryos, cumulus cells and immature spermatozoa (59). Sperm preparation techniques can be used to decrease ROS production to enhance and maintain sperm quality after ejaculation (35). The most common sperm preparation techniques used to preserve and optimize sperm quality after ejaculation is density gradient centrifugation, migration-sedimentation, glass wool filtration, and conventional swim-up (60). The first three preparation techniques are more effective in reducing levels of free radicals than the conventional swim-up technique (60). However, repeated centrifugation causes mechanical injury to spermatozoa and increases ROS production (61). Currently use of antioxidants and other substances to prevent ROS generation during sperm preparation processes are under evaluation.

Aitken et al. reported that men with elevated ROS levels in semen have a sevenfold reduction in conception rates when compared with men having low ROS (47). Also high ROS levels are associated with decreased pregnancy rate following IVF or ICSI and arrested embryo growth. Based on a recent meta-analysis, which included all of the available evidence from the literature, our group found that there is a significant correlation between ROS levels in spermatozoa and the fertilization rate after IVF (estimated overall correlation 0.374, 95% CI 0.520 to 0.205) (62). Thus, measuring ROS levels in semen specimens before IVF may be useful in predicting IVF outcome and in counseling selected patients with male factor or idiopathic infertility.

LABORATORY EVALUATION OF OXIDATIVE STRESS IN INFERTILITY PRACTICE

ROS Measurement

For clinical purposes, it is essential to have a reliable and reproducible method of ROS measurement. Numerous methods are available to measure ROS levels in semen. Direct methods such as electron-spin resonance spectroscopy, also known as electron paramagnetic resonance, have been utilized mainly for research purposes since these are relatively expensive technologies that require fresh samples, and great technical expertise (63,64). This method is used to detect electromagnetic radiation being absorbed in the microwave region by paramagnetic species that are subjected to an external magnetic field. This technique is the only analytical approach that permits the direct detection of free radicals and reports on the magnetic properties of unpaired electrons and their molecular environment (64). However, short life span of ROS makes the application of these techniques difficult.

Indirect techniques, e.g., chemiluminescence method are commonly used for measuring ROS produced by spermatozoa (65,66). This assay quantifies both intracellular and extracellular ROS depending on the probe used. Chemiluminescence determines the amount of ROS, not the level of the sperm-damaging ROS present at any given time. Also, it can differentiate between the production of superoxide and hydrogen peroxide by spermatozoa depending on which probe is used (66). Two probes may be used with the chemiluminescence assay: luminol and lucigenin. A luminol-mediated chemiluminescence signal in spermatozoa occurs when luminol oxidizes at the acrosomal level. Luminol reacts with a variety of ROS and allows both intracellular and extracellular ROS to be measured. Lucigenin, however, yields a chemiluminescence that is more specific for superoxide anions released extracellularly (67,68).

The number of free radicals produced is counted as photons per minute. Presence of leukocytes as a confounding factor and the need of fresh semen samples with high sperm count ($>1 \times 10^6$/mL) are the limitations of this technique (66). Also other multiple factors that affect chemiluminescence include the concentration of reactants, sample volume, reagent injection, temperature control, instrument sensitivity, and background luminescence (69).

A diversity of luminometers is available to measure the light intensity resulting from the

chemiluminescence reaction. Single/double tube luminometers are sensitive and inexpensive but can measure only one or two samples at a given time, which are suitable for small research laboratories. On the other hand multiple tube or plate luminometers are more expensive since they can measure multiple samples at the same time and are suitable for centers that are engaged in regular research work on chemiluminescence (66).

ROS-TAC Score

Since oxidative stress is caused by an imbalance between levels of ROS produced and antioxidant protection at any given time, it is a conceivable that measurement of oxidative stress can be made either by assessment of ROS or total antioxidant capacity (TAC). The TAC is measured by enhanced chemiluminescence assay or colorimetric assay (10,70). Sharma et al. described a ROS-TAC score for assessment of seminal oxidative stress that showed to be superior to ROS or TAC alone in discriminating fertile and infertile population (10). This score minimizes the variability of the individual parameters (ROS or TAC) of oxidative stress. The ROS-TAC score was based on a group of normal healthy fertile men who had very low levels of ROS. Men with male factor or idiopathic infertility had significantly lower seminal ROS-TAC scores compared to normal controls, or the men with initial male factor that eventually were able to initiate pregnancy. The average ROS-TAC score for fertile healthy men was 50 ± 10, which was significantly higher ($p \leq 0.0002$) compared to infertile patient (35.8 ± 15). The probability of successful pregnancy is estimated at < 10% for values of ROS-TAC < 30, but increased as the ROS-TAC score increased.

Leukocyte Evaluation

Since lower leukocyte levels are sometimes associated with significant ROS levels in semen it is important to determine the exact source of ROS in semen because the clinical implications of infiltrating leukocytes are quite different from those of pathological conditions in which spermatozoa themselves are the source of ROS (36,45,71). Methods that are currently used for assessment of seminal OS, such as chemiluminescence assays, do not provide information on the differential contribution of spermatozoa and leukocytes to ROS production in semen. Nitroblue tetrazolium test (NBT) can be used for assessment of seminal oxidative stress, and the differential contribution of cells to ROS generation, and to determine the state of activation of seminal leukocytes. ROS levels measured by chemiluminescence assay are strongly correlated with the results of NBT staining. Also, the NBT reduction test is commonly available, easily performed, inexpensive and has high sensitivity (72).

Oxidative Stress Status (OSS)

Currently there is no consensus regards to the inclusion of ROS measurement as part of the routine clinical evaluation of male infertility mainly because there is a lack of standardization of ROS analytical methods, equipment, and range of normal levels of ROS in semen. Some investigators have defined the basal levels of reactive oxygen species in neat semen specimens of normal healthy donors (45,73). Measurement of ROS levels in neat semen after liquefaction in the presence of seminal antioxidant protection proved to be a better test to evaluate oxidative stress status. The ROS levels for fertile donors with normal genital examination and normal standard semen parameters were 1.5×10^4 cpm/20 million sperm/mL. Using this value as a cutoff, infertile men can be classified as either OS-positive ($> 1.5 \times 10^4$ cpm/20 million sperm/mL) or OS-negative ($\leq 1.5 \times 10^4$ cpm/20 million sperm/mL), irrespective of their clinical diagnosis or results of standard semen analysis (73). Assessing ROS directly in neat semen showed diagnostic and prognostic capabilities identical to those obtained from ROS-TAC score (73).

Earlier studies have shown that sperm washing procedures like multiple centrifugation, resuspension, and vortexing artificially elevate ROS levels (61,74,75). The antioxidant activity of seminal plasma is removed during sperm washing steps, which also results in elevated ROS levels (74). Excessive washing and manipulation including duration of centrifugation was found to be more important than the force of centrifugation for ROS formation by human spermatozoa (76). Therefore procedures that

minimize multiple centrifugation, resuspension, and vortexing should be used for the preparation of spermatozoa for ART (61).

Conflicting studies make it difficult to establish the clinical value of ROS measurement in medical practice since there is no clear evidence whether high ROS levels are a cause or an effect of abnormal semen parameters and sperm damage (77). However, a more recent study reported high levels of ROS as an independent marker of male factor infertility, irrespective of whether these patients have normal or abnormal semen parameters (78). These findings suggest that ROS measurement should be used as a diagnostic tool in infertile men especially in cases of idiopathic infertility and that the reference values of ROS in neat semen can be used to define the pathologic levels of ROS in infertile men and may guide in better therapeutic interventions.

STRATEGIES TO REDUCE SEMINAL OXIDATIVE STRESS

Given the major role of oxidative stress in the pathogenesis of male infertility, treatment strategies with the goal of reducing levels of seminal oxidative stress are necessary for natural as well as assisted reproductive technologies. Spermatozoa produce small amounts of ROS that must be continuously inactivated to keep only the necessary amount to maintain normal physiologic cell function. The pathologic levels of ROS detected in the semen of infertile men are more likely caused by increased ROS production than by reduced antioxidant capacity of the seminal plasma (13). The body has a number of mechanisms to minimize free radical induced damage. Unfortunately, spermatozoa are unable to repair the damage induced by oxidative stress, because they lack the required cytoplasmic enzyme systems to perform the repair (79). Antioxidants are the most important defense mechanisms against OS induced by free radicals. Metal chelators and metal binding proteins that block new ROS formation are classified as preventative antioxidants. Scavenger antioxidants, such as vitamins E and C, beta-carotene and other antioxidant dietary supplements, glutathione and enzymes, act via removing ROS already generated by cellular oxidation.

Many clinical trials have demonstrated the beneficial effect of antioxidants in treating selected cases of male infertility (80-85), whereas others failed to report the same benefits (86-88). Pregnancy, the most relevant outcome parameter of fertility, was reported in only a few of them (80,84,89-91). The majority of the studies analyze multiple antioxidant combinations, different dosages and durations. Also the patient's selection is another important aspect because oxidative stress can not be considered the cause of male infertility in all patients. Recently, Agarwal et al. in an extensive review of literature concluded that many studies suffer from the lack of placebo-controlled, double-blind design, making the effectiveness of antioxidant supplementation in infertile patients still inconclusive (79).

Antioxidants may not be very effective depending on the etiology of infertility (79). Primarily, specific therapeutics directed against the etiological causes of elevated ROS should be attempted. Once the primary cause of infertility have been treated or no specific etiology is identified (idiopathic infertility) patients can be advised to take optimal doses of antioxidants supplementation.

ORIGIN OF DNA DAMAGE IN SPERMATOZOA

Sperm genetic material is structured in a special manner that keeps the nuclear chromatin highly stable and compact. The normal DNA structure is capable of decondensation at appropriate time transferring the packaged genetic information to the egg without defects in the fertilization process. The cause of DNA damage in sperm can be attributed to various pathological conditions including cancer (92), varicocele (93), high prolonged fever (94), advanced age (95) or leukocytospermia (96). Also a variety of environmental conditions can be involved as radiation (97), air pollution, smoking (8), pesticides, chemicals, heat and ART prep protocols (52,97,98). Most of these agents not only disrupt hormone levels but may also

Table 1 – *Etiological factors associated with increased human sperm DNA damage.*

Etiology	Reference	Study Population	DNA Assay	Conclusion
Pollutants	De Rosa et al. 2003 (55)	85 men employed at motorway tollgates 85 controls	Acridine orange	Higher sperm DNA damage in men exposure to pollutants. Nuclear DNA damage was inversely correlated with methaemoglobin levels.
Varicocele	Saleh et al. 2003 (93)	31 infertile men 16 fertile controls	SCSA	Infertile men with varicocele showed significant higher DNA damage that appears to be related to high OS.
Leukocytospermia	Erenpreiss et al. 2002 (96)	187 men	Acridine orange	Normal semen has low DNA integrity and resist to leukocytospermia. Leukocytes increasing primary or provoking potential DNA damage.
	Alvarez et al. 2002 (123)	56 infertile patients 18 healthy fertile men	SCSA	Significant increase in sperm DNA damage in leukocytospermic samples compared to normal controls.
Smoking	Potts et al. 1999 (52)	35 fertile smokers 35 fertile non-smokers	SCSA	Higher sperm DNA damage in smokers compared to non-smokers.
	Singh et al. 2003 (95)	40 infertile men 26 healthy men	Comet	Significant higher DNA damage in men > 36 years old.
Advanced age	Moskovtsev et al. 2006 (124)	1125 infertile men	Acridine orange	DNA damage is significantly higher in men over 40 years old
	Trisini et al. 2004 (125)	252 infertile men	Comet	Significant high DNA damage in men older than 35 years.

Table 1 – *Etiological factors associated with increased human sperm DNA damage. (continued)*

Etiology	Reference	Study Population	DNA Assay	Conclusion
Cancer	Fossa et al. 1997 (126)	39 patients with testicular cancer	SCSA	Sperm DNA damage is higher in men with cancer even before cancer therapy.
		18 healthy controls		Recovery of spermatogenesis is higher when normal SCSA is found before adjuvant treatment.
	Spermon et al. 2006 (127)	22 patients with testicular cancer treated with cisplatin-based chemotherapy	CMA3 TUNEL	High sperm DNA damaged in these patients. Improvement in sperm chromatin packaging after chemotherapy.
	O'Donovan et al. 2005 (92)	8 men with leukemia 12 men with testicular cancer 3 men with lymphoma	Comet	Detrimental effect on chromatin condensation and DNA integrity of cancer and as its treatment.
ROS	Said et al, 2005 (8)	28 infertile men	TUNEL	Increased ROS production showed a positive correlation with sperm DNA damage in a time-dependent manner.
	Henkel et al, 2005 (43)	63 infertile men	TUNEL	DNA fragmentation was strongly positively correlated with intrinsic ROS production, whereas this correlation was weaker for extrinsic ROS production.

DFI = DNA fragmentation index, SCSA = sperm chromatin structure assay; ROS = reactive oxygen species; OS = oxidative stress. TUNEL = terminal deoxynucleotidyl transferase-mediated 2'-deoxyuridine 5'-triphosphate (dUTP)-nick end-labeling.

induce oxidative stress, which could damage sperm DNA (99) (Table-1).

The extent of sperm DNA damage has been closely associated with impaired sperm function as well as male infertility (7). However the precise mechanism(s) responsible for chromatin abnormalities in human spermatozoa is/are most likely to be multi factorial and are not accurately understood at this time (100) (Figure-1). The most important theories proposed as molecular mechanism of sperm DNA damage are: (a) defective chromatin packaging, (b) reactive oxygen species (ROS) (8,101,102), (c) apoptosis mainly during spermatogenesis (7,103), and (d) DNA fragmentation induced by endogenous endonucleases (104).

ROLE OF OXIDATIVE STRESS IN SPERM DNA DAMAGE AS RELATED TO MALE INFERTILITY

Excessive generation of ROS in the reproductive tract not only affect the fluidity of the sperm plasma membrane, but also the integrity of DNA in the sperm nucleus. DNA bases are susceptible to oxidative damage resulting in base modification, strand breaks, and chromatin cross-linking. Oxidative stress-induced DNA damage causes pro-mutagenic change, which in its most severe form affects the quality of the germ line and prevents fertilization. When there is less oxidative damage, fertilization can occur, but the oocyte must repair the DNA strand breaks before the initiation of the first cleavage. Apoptosis and OS are involved in mediating DNA damage in the germ line (105) (Figure-2). The Y chromosome is particularly vulnerable to DNA damage, due to its genetic structure as well as it cannot correct double-stranded DNA deletions.

Fertile healthy men with normal seminal parameters almost consistently have low levels of DNA breakage, whereas infertile men, in particular those with abnormal seminal parameters, have higher fraction of sperm DNA damage (106). Idiopathic infertile men may present normal routine seminal

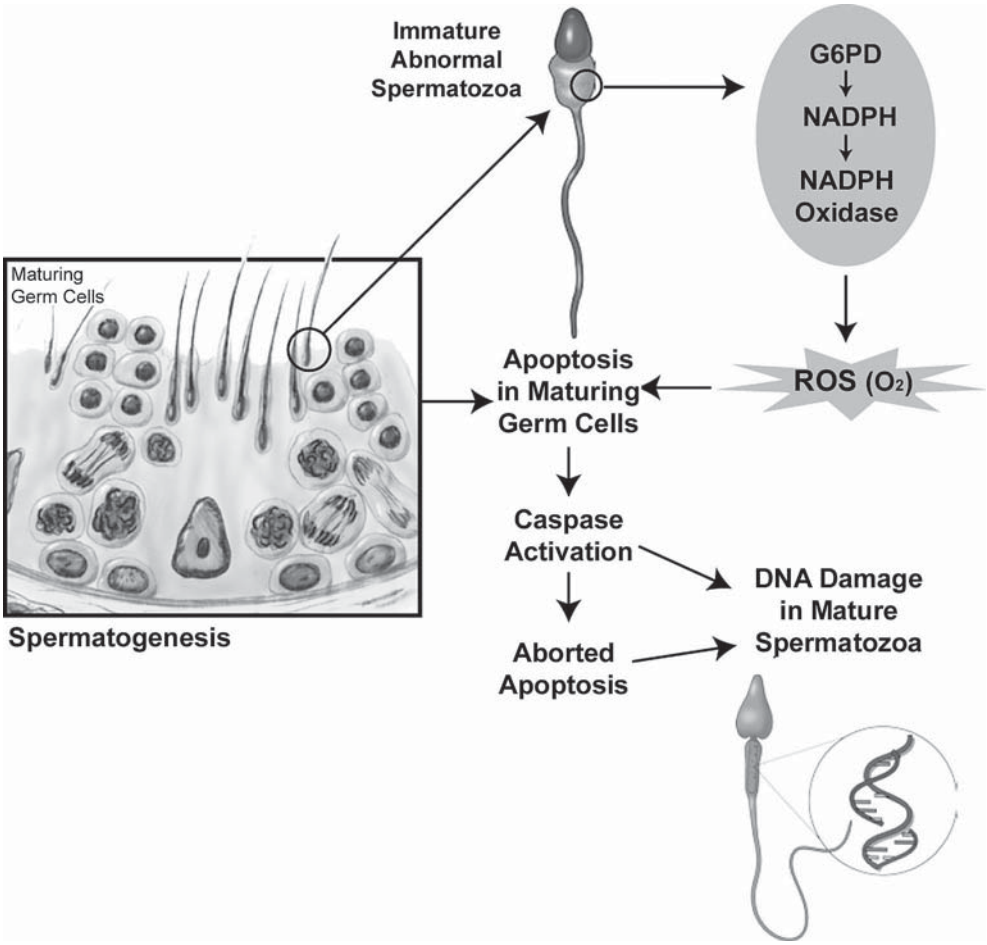

Figure 2 – *Mechanistic pathway showing sperm DNA damage due to oxidative stress.*

parameters (concentration, motility, and morphology) with abnormal DNA integrity (83,106,107). It is of great concern that the most efficient ART techniques used to treat male factor infertility with high degree of sperm DNA damage. During ICSI, it is always desirable to select spermatozoa with normal morphology that reduces the risk of introducing spermatozoa with strand breaks (108). This is sometimes not always true since the traditional sperm parameters such as sperm count, motility and morphology have been proven to be poorly correlated to DNA damage status (109,110). Moreover, this has significant clinical implications because in vitro fertilization using spermatozoa with damaged DNA may lead to paternal transmission of defective genetic material with adverse consequences for embryo development. These findings suggest that an estimate of the percentage of DNA damaged spermatozoa in fertile and infertile men may be important and a future challenge will be to develop methods to identify and select spermatozoa with intact DNA during the IVF/ ICSI procedures.

Recently sperm from infertile men with varicoceles have been associated with significantly high levels of DNA damage (93). The finding of high seminal OS in patients with varicoceles may indicate that OS plays an important role in the pathogenesis of sperm DNA damage in patients with this condition. Although Zini et al. reported that varicocelectomy can improve human sperm DNA integrity in infertile men with clinical varicoceles (28), a limited number of studies has examined potential treatments to reduce sperm DNA damage. Therapeutic conditions have been suggested that avoidance of gonadotoxins (52) (smoking, mediations) and hyperthermia (94) (saunas, hot tubes) may reduce sperm DNA damage. Treatment of GU infection can also be helpful based on the evidence that leukocytospermia induce ROS production and possibly DNA damage (44). Studies suggested that sperm DNA damage can be reduced with oral antioxidants administered during a relatively short time period (111). However, these recommendations have been based on small, uncontrolled studies and to date no treatment for abnormal DNA integrity has been shown to have successful clinical results (107).

ASSESSMENT OF SPERM CHROMATIN INTEGRITY

Several techniques can measure DNA defects in human spermatozoa and the ability of these techniques to accurately estimate sperm DNA damage depends on many technical and biological aspects. However, to establish a threshold level between the fertile population and the lowest sperm DNA integrity required for achieving pregnancy remains extremely challenging. Currently both direct (fragmentation, oxidation) and indirect (sperm chromatin compaction) methods are available to evaluate the integrity of sperm DNA. Direct methods for detecting DNA breaks include (a) the single-gel electrophoresis assay ("Comet assay") and (b) terminal deoxynucleotidyl transferase-mediated 2`-deoxyuridine 5`-triphosphate (dUTP)-nick end-labeling (TUNEL) assay (106,112). Indirect methods mainly sperm chromatin integrity assays (SCSA) for assessing DNA damage uses chromatin and/or DNA intercalating dyes such as acridine orange to differentiate single-stranded and double-stranded DNA (106,109,110).

Less frequent clinical tests for DNA damage include the sperm chromatin dispersion test (SCD) using the Halosperm kit which allow to simultaneously perform DNA fragmentation and chromosomal analyses in the same sperm cell (113), liquid chromatography that detect oxidized DNA nucleotide residues (83) and evaluation of nuclear protein (protamine/histone ratio) levels in sperm samples.

All methods currently lack a threshold, except for the sperm chromatin structure assay (SCSA), which assesses the ability of the DNA to resist denaturation by acid or heat and uses DNA flow cytometry approach. The sperm DNA damage is expressed as the DNA fragmentation index (DFI) (114) that can distinguish fertile and infertile population in clinical practice (115).

DNA DAMAGE AND REPRODUCTIVE OUTCOME

Sperm DNA damage is critical in the context of success of assisted reproductive techniques

(99,116). The main nuisance of ART is that they bypass the natural defense barrier present throughout female reproductive tract responsible for selecting the best spermatozoa for oocyte fertilization. Normally oocytes are capable of repairing partial DNA damage. However, when the damage is severe, embryo death and miscarriages are more likely to happen. Probably that explains why miscarriage rate is higher after ICSI compared to classic IVF (117).

Standard semen parameters do not identify subtle defects in sperm chromatin architecture, which after the advent of ICSI has become more important parameter of sperm functional quality than count, motility or morphology. The emphasis on evaluation of genomic integrity has recently increased due to reports that correlate the degree of DNA damage with various fertility indices including rates of fertilization, embryo cleavage, implantation, pregnancy and live birth (118-120).

Sperm DNA integrity is an essential requirement to achieve pregnancy in natural conception (110) as well as for IVF outcomes where the natural process of fertilization is circumvented (121). A high degree of sperm DNA damage has been found in couples presenting with unexplained recurrent pregnancy loss (117). All male partners of couples who achieved a pregnancy during the first 3 months attempting to conceive had < 30% sperm with fragmented DNA (109), whereas, 10% of the couples who achieved pregnancy in months 4-12 and 20% of couples who never achieved a pregnancy had > 30% sperm with fragmented DNA. Moreover 84% of the men who initiated pregnancy before 3 months had sperm DNA damage levels of < 15%.

Bungum et al. reported that for IUI, there was a significantly higher chance of pregnancy/ delivery in the group with DFI < 27% and HDS (highly DNA stainable) of < 10% than in patients with DFI > 27% and HDS > 10%. Although, no statistical difference between the outcomes of IVF versus ICSI was observed in the group with DFI < 27%, ICSI had significantly better results than those of IVF in patients with DFI > 27%. The authors concluded that combining the two SCSA parameters, DFI and HDS is a useful method for prediction of IUI outcomes.

Henkel et al. reported that even though sperm DNA fragmentation did not correlate with the fertilization and embryo fragmentation rates, patients with a high percentage of TUNEL positive spermatozoa (> 36.5%) showed a significantly lower pregnancy rate compared to those patients with lower than 35.5% TUNEL-positive sperm (118). The decision to incorporate a new test into clinical practice depends on the volume and quality of reports that favor or refute such claims. Although multiple studies have analyzed the relationship between the degree of DNA damage and the fertilization rate, embryo cleavage rate, implantation rate, pregnancy rate, and live birth rate of offspring, existing data on the relationship between abnormal DNA integrity and reproductive outcomes are limited and not analyzed systematically (122). The Practice Committee of the American Society for Reproductive Medicine summarizes the current understanding of the impact of abnormal sperm DNA integrity on reproductive outcomes (107). This Committee concluded that current methods for evaluating sperm DNA integrity alone do not predict pregnancy rates achieved with intercourse, IUI, or IVF and ICSI.

Before sperm DNA damage analysis is introduced routinely in clinical practice, studies with adequate sample size must be conducted evaluating outcomes and role of such tests in the management of male infertility (122).

TAKE HOME MESSAGE

Limited amount of free radicals and oxidative stress have an important role in modulating many physiological functions in reproduction. ROS are being constantly produced in small controlled amounts in the reproductive tract and by a variety of semen components. Many scavenging enzymes and molecules (antioxidants) control the damaging effects of ROS to keep the normal physiological balance. However, when ROS production exceeds the scavenging capacity of the antioxidants a state referred to as oxidative stress is generated that becomes toxic to sperm. High levels of ROS and OS in reproductive tract and semen are associated with sperm dysfunction and damage to sperm nuclear

DNA. Although routine semen analysis remains the backbone of evaluating male infertility, determining the levels and sources of excessive ROS generation in semen may be useful in developing future therapeutic strategies for male infertility.

Current evidence suggests the use of systemic antioxidants for the management of selective cases of male infertility as well as in vitro supplements during various sperm preparation techniques. However, a definitive conclusion cannot be drawn from the available studies, as oxidative stress is not the only cause of male infertility.

Sperm DNA damage is more common in infertile men and has been correlated with poor reproductive outcomes. Although ART is able to compensate for the impairment of sperm chromatin integrity, transmission of abnormal genetic material through ART needs further investigations in order to reduce sperm DNA damage. Current methods for evaluating sperm DNA integrity are not standardized and are not routinely used in clinical laboratories. Also to date no treatment for abnormal DNA integrity has proven to be of clinical value.

A significant percentage of couples, even after extensive infertility evaluation, show no apparent male or female factor and are still unable to conceive. Increased oxidative stress and DNA damage may be responsible for the poor fertility in these patients. Although assisted reproduction provides opportunity to these couples with unexplained infertility, the potential medical risks entailed by multiple-gestation pregnancies and the associated costs are significant. It is important to further decipher the molecular basis of male infertility in order to thoroughly understand the effects of abnormal spermatozoa on fertilization and embryo development. With this understanding, the success of ART and ICSI can be improved significantly.

CONFLICT OF INTEREST

None declared.

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Lack of Association between Matrix Metalloproteinase-1 (MMP-1) Promoter Polymorphism and Risk of Renal Cell Carcinoma

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ABSTRACT

*Objective: I*nvestigate the possible association of insertion/deletion (2G/G) polymorphism at nucleotide -1607 of the MMP-1 promoter with the development and progression of renal cancer.
Materials and Methods: In this study, we genotyped 217 individuals, 99 patients with renal cell carcinoma (RCC) and 118 controls without cancer. DNA specimens were extracted from epithelial buccal cells and paraffin-embedded tissue of RCC patients and from epithelial buccal cells and blood cells of healthy controls.
Results: The difference in frequency of 2G/2G genotype between controls (22.9%) and RCC patients (28.6%) was not statistically significant (p = 0.461). We also did not find correlation between 2G/2G and histological type of RCC. The comparison of genotype distribution and frequency of 2G allele in different populations showed a strong variability of 2G allele frequency among the different ethnic groups. This fact may influence on the collaboration of this 2G allele in RCC or others diseases.
Conclusion: Our data suggest that the matrix metalloproteinase-1 (MMP-1) promoter polymorphism may not play a significant role in renal cell carcinoma patients in Brazil.

***Key words**: MMP-1; polymorphism; renal cell carcinoma*
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INTRODUCTION

The development of cancer is a complex, multistage process during which a normal cell undergoes genetic changes that result in phenotypic alterations and the acquisition of the ability to invade and colonize distant sites (1,2). Although many factors are involved in tumor development, interactions between neoplastic cells and the surrounding microenvironment are crucial to each step of tumorigenesis. The MMP family comprises over 20 enzymes that are associated with degradation of the ECM, including the basement membrane, as their name implies (3). Among the MMPs, matrix metalloproteinase-1 (MMP-1, col-

lagenase-1), is the most highly expressed interstitial collagenase degrading fibrillar collagen, the most abundant protein in the human body (4). MMP-1 expression level is increased in several diseases such as arthritis (5), periodontitis (6) arteriosclerosis (7) and cancer (8,9).

Recently, an insertion/deletion (2G/G) polymorphism was reported at nucleotide -1607 relative to the transcription site of the MMP-1 gene (9). The 2G polymorphism creates a binding site (5'-GGA-'3) for the ETS transcription factor, influencing its transcriptional activity (10,11) Promoter containing 2G allele displays a significantly higher transcriptional activity than 1G promoters (8).

The 2G allele of the MMP-1 promoter polymorphism is relatively common and has a frequency of a little less than 50% of the general population (12). Association studies have been done to determine whether the MMP-1 genotype affects the risk of different types of cancers (5,8,9,11-16).

Renal cell carcinoma accounts for 2 percent of all cancers. Renal cell carcinoma originates in the cortex and accounts for 80 to 85 percent of malignant kidney tumors (17). This carcinoma occurs nearly twice as often in men than in women. Patients are generally over 40 years old at diagnosis, and the disease occurs predominantly in the seventh and eighth decades of life (18). However, small, localized tumors rarely produce symptoms, and for this reason, the diagnosis is often delayed until after the disease is advanced. To improve the prognosis of this disease it is important to clarify the molecular mechanism of invasion and metastasis of renal cell carcinoma.

The aim of this study was to investigate possible correlations between MMP-1 promoter and renal cell carcinoma (RCC) in a Brazilian group.

MATERIALS AND METHODS

Subject selection - This case-control study consisted of 99 patients with renal cell carcinoma (56 men, 43 women mean age 59.97 years) and 118 population-derived, age-matched controls (62 men, 56 women; mean age 60.5 years), all being ethnic Brazilian. This study protocol was approved by the institutional review board. At recruitment, written informed consent was obtained from each subject.

All of the RCC patients were diagnosed histologically and tumors were staged according to the 1997 TNM classification system (19) and graded according to the Fuhrman classification system (20).

Sample acquisition - DNA samples of the patients were obtained from formalin-fixed paraffin-embedded tissue (23 patients), epithelial buccal cells (60 patients) and blood cells (16 patients). Patients were recruited between 2004 and 2006, at the São Paulo Hospital and Public Servant Hospital of São Paulo, Brazil (São Paulo, Brazil), and histopathologically confirmed as a renal cell carcinoma. All DNA samples of the control group were extracted from epithelial buccal cells.

Paraffin-embedded tissue - 10-µm sections were obtained from paraffin-embedded tissue. The sections were deparaffinized by immersing twice in xylene for 2 min, followed by twice 99.5% ethanol for 2 min, and further two times 70% ethanol. Thereafter the samples were digested with 1,000µL 0.1M Tris-HCl (pH 8.0), 0.5 M NaCl, 0.05 M EDTA, 1% sodium dodecyl sulfate (SDS), and 1 unit of proteinase K at 55°C overnight. Then the samples were mixed well, and centrifuged at 10,000 X g for 15 min. The DNA was extracted with phenol/chloroform (1:1). DNA was precipitated by adding cold ethanol, centrifuged and resuspended in 50µL water (21,22).

Epithelial buccal cells - The ephitelial buccal cells were extracted with 100 ng/mL proteinase K (Sigma Chemical Co.) at 37°C for 1 hour. DNA was then purified by sequential phenol/chloroform extraction and salt/ethanol precipitation. DNA was dissolved in 70µL TE buffer (10 mm Tris (pH 7.8), 1mm EDTA), and its concentration was determined by measurements of OD 260 (6).

Statistical analysis - Differences in the genotypes distribution from those expected by the Hardy-Weinberg equilibrium and the significance of differences in the observed frequencies of SNP in both groups were assessed by χ^2 test. We also used the χ^2 test to compare the distribution of genotype and frequency of G2 allele in different populations. T Stu-

Table 1 – *Characteristics of cases and controls.*

	Cases (N = 99)	**Controls** (N = 118)	**p Value**
Male/Female	58/46	62/56	0.726 (a)
Age (mean ± SD)	60.54 ± 13.68	59.90 ± 12.50	0.623 (b)
Smokers (N/%)	30 (28.85%)	29 (24.58%)	0.345 (a)

a = chi square test, b = student t test.

dent test and Fisher test were employed to evaluate the homogeneity of control and case populations. For all tests, the p-values of 0.05 were regarded significant.

RESULTS

We studied a total of 217 individuals: 99 renal cancer patients and 118 non-cancer controls. The baseline characteristics of the patients and controls are summarized in Table-1.

The genotypes of all subjects were clearly determined by PCR-RFLP. Figure-1 shows the RFLP pattern after digestion with BGL II and after digestion with Alw I.

Polymorphism distribution in the control and case population was according to the Hardy-Weinberg principle ($\chi^2 = 0.00$, p = 1.000 and $\chi^2 = 0.04$, p = 0.981 respectively).

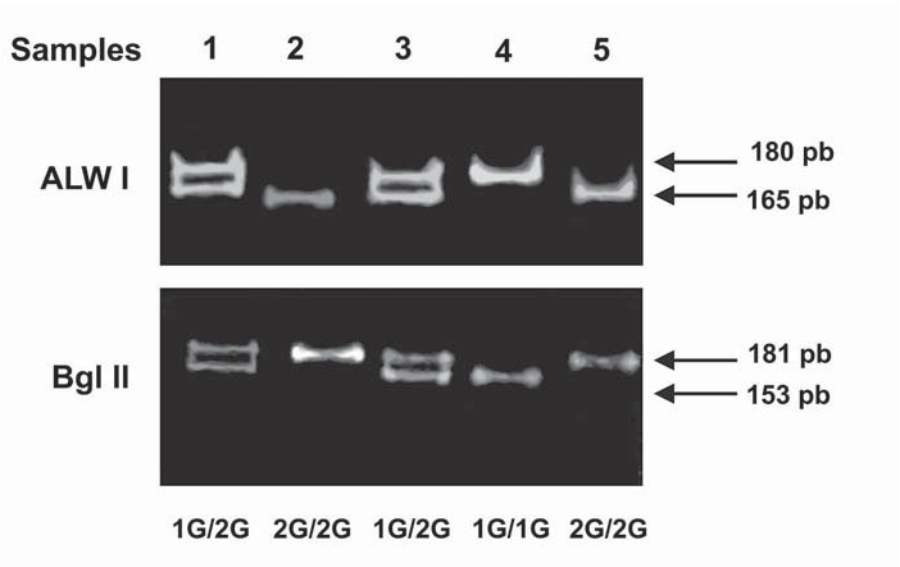

Figure 1 – *RFLP analyses of MMP-1 promoter polymorphism. ALWI digests the 181 bp 2G amplicon (166 bp + 15 bp), leaving the 1G amplicon (180 pb) intact. Bgl II digests the 180 pb 1G (153 bp + 27 bp), leaving the 2G amplicon (181 bp) intact. Samples 1 and 3 are 1G/2G heterozygotes. Samples 2 and 5 are 2G/2G homozygotes. Sample 4 is 1G/1G homozygote.*

Table 2 – *Pathological characteristics of 99 RCC patients.*

		N (%)
Histological type	Clear cells	75 (75.8)
	Chromophobe	15 (15.1)
	Papillary	7 (7.0)
	transitional cell carcinoma	2 (2.0)
TNM stage (a)	T_1	36 (36.4)
	T_2	20 (20.2)
	T_3	27 (27.3)
	T_4	16 (16.2)
Fuhrman grade (b)	G1	9 (9.1)
	G2	55 (55.6)
	G3	17 (17.2)
	G4	3 (3.0)

a = tumor stage according to TNM (20); b = Tumor stage according to the 1997 grading system (19).

We analyzed the correlation between tobacco smoking and RCC and no association was found (p = 0.345) (Table-1).

In the renal cancer group, allele frequency of 2G was 51%, in comparison with 49.0% in control group, did not show any significant statistic difference (p = 0.48). The genotype distribution in RCC patients and controls are shown in Table-2. The frequency of the 2G/2G genotype showed in Table-3 in patients (29.3%) did not significantly differ from the values for normal controls (22.9%) (p = 0.461). Further, we analyzed the a possible correlation between pathological data and genotype frequency of MMP-1 polymorphism in RCC patients and we also found no correlation (Table-4).

In Table-5 we compared the distribution of the MMP-1 genotype in our controls with the data previously reported for other study populations (9,15,16,23-26). Chi-square analysis indicated significant differences in genotype distributions of MMP-1 -1607 promoter between Brazilians and reported data on Japanese (p = 0.003 and p < 0.001), Tai-

Table 3 *– Distribution of MMP-1 polymorphism in case and controls.*

	Cases		Controls		
Genotype	**N**	**%**	**N**	**%**	**p Value ***
2G/2G	29	28.6	27	22.9	
1G/2G	42	48.8	59	50.0	0.461
1G/1G	28	22.6	32	27.1	
Total	99	100	118	100	

** = p Value obtained by chi-square test.*

Table 4 *– Correlation between pathological data and genotype frequency of MMP-1 polymorphism in RCC patients.*

		Genotype			
		2G/2G N (%)	**1G/2G N (%)**	**1G/1G N (%)**	**p Value**
Histological type	Clear cells	22 (73.4%)	29 (70.7%)	24 (85.7%)	0.618 (c)
	Chromophobe	4 (13.4%)	8 (20%)	3 (10.7%)	
	Papillary	2 (6.7%)	4 (10%)	1 (3.6%)	
	Transitional cell carcinoma	2 (6.7%)	0 (0.0%)	0 (0.0%)	
TNM stage (a)	T_1	10 (34.5%)	14 (33.3%)	3 (12.5%)	0.273 (d)
	T_2	5 (17.2%)	8 (19.1%)	16 (66.7%)	
	T_3	8 (27.6%)	16 (38.1%)	4 (16.7%)	
	T_4	6 (20.7%)	4 (9.5%)	6 (21.4%)	
Fuhrman grade (b)	G1	2 (8.3%)	4 (11.1%)	3 (12.5%)	0.803 (c)
	G2	15 (62.5%)	24 (66.7%)	16 (66.7%)	
	G3	5 (20.8%)	8 (22.2%)	4 (16.7%)	
	G4	2 (8.3%)	0 (0.0%)	1 (4.2%)	

a = tumor stage according to TNM (20); b = tumor stage according to the 1997 grading system (19); c = p Value obtained by Fisher exact test; d = p Value obtained by chi-square test.

Table 5 – *Distribution of genotype and frequency of G2 allele in different populations.*

	Genotypes					
Population	**1G/1G (%)**	**1G/2G (%)**	**2G/2G (%)**	**G2 Allele**	**p Value** (*)	**Reference**
Brazilian (n = 118)	32 (27.1)	59 (50.0)	27 (22.9)	0.45		This study
Brazilian (n = 37)	10 (27)	18 (48.7)	9 (24.3)	0.49	0.982	De Souza et al. [6]
Norwegian (n = 364)	87 (25)	197 (52)	80 (23)	0.49	0.949	Wiencke et al. [23]
Caucasian US American (n = 451)	111 (24.61)	196 (43.46)	144 (31.93)	0.53	0.260	Young et al. [24]
Japanese (n = 210)	25 (11.9)	96 (45.7)	89 (42.4)	0.65	< 0.001	Hirata et al. [9]
Japanese (n = 150)	30 (20.0)	56 (37.3)	64 (42.7)	0.62	0.003	Nishioka et al. [25]
Japanese (n = 140)	17 (12.1)	48 (34.3)	75 (53.6)	0.70	< 0.001	Hashimoto et al. [15]
Taiwanese (n = 135)	17 (13)	57 (42)	61 (45)	0.66	< 0.001	Lai et al. [16]
Korean (n = 332)	33 (9.9)	154 (46.4)	145 (43.7)	0.67	< 0.001	W. Ju et al [26]

* = *p Value obtained by chi-square test.*

wanese ($p < 0.001$) and Koreans ($p < 0.001$) (9,15,25). However, there was no difference between Brazilians, Norwegians and Caucasian US Americans (23,24).

COMMENTS

Renal cell carcinoma (RCC) accounts for 3% of adult human cancers and it is becoming more com-

mon (27). Major risk factors include cigarette smoking, obesity and hypertension (28). RCC represents just about 85% of newly diagnosed kidney malignancies, occurring at an estimated rate of 4.4 to 11.1 cases per 100,000 person-years with a steady rise in the rates of RCC of 2.3% to 4.3% annually (29). There is a lack of an effective systemic therapy for RCC, which is necessary for approximately 30% of initially localized disease and 30% of patients presenting RCC with metastases identified at the time of diagnosis, with a 1-year survival rate of 26% (30). Renal tumors are classified as different histopathological subtypes with diverse clinical behavior and genetic mutations that are not completely understood.

A better understanding of the tumor gene activity and its relationships may help on prognosis prediction and on molecular therapies development.

Proteolytic enzymes play a fundamental role in cancer progression providing an access for tumor cells to the vascular and lymphatic systems. Among all proteolytic enzymes, the MMP family has reached an outstanding importance due to their ability to cleave virtually any component of the extracellular matrix (31). Matrix metalloproteinase-1 is a member of this family that is expressed by most normal cell types and there is evidence suggesting that in pathological conditions like cancers the expression is up regulated (5,8,9,11-16).

Rutter et al. (10) reported that a common single nucleotide polymorphism (SNP) in the promoter of MMP-1 is associated with enhanced transcription of this gene. A correlation between the transcription-enhancing insertion of a single G nucleotide in the MMP-1 promoter has been associated with different types of tumors (8,9,11,12). In a recent study Hirata and collaborators reported that distribution of 2G/2G in RCC patients was statistically different from the control group in the Japanese population (9).

The present population-based, control-case in São Paulo, Brazil, did not confirm the relationship between MMP-1 promoter polymorphism and risk of renal cell carcinoma. Using a total of 217 individuals, 99 RCC patients and 118 control individuals we found a lack of association between MMP-1 polymorphism and renal cell carcinoma. The discrepancy among our results and the others (8,9,11,12) may be caused by the relatively small numbers of patients and multi-ethnic composition of the Brazilian group.

Comparing the distribution MMP-1 promoter -1607 genotype in our controls with the data reported previously for other study populations (Table-5), It is clear that the 2G variant genotype is associated to ethnicity. Chi-square analysis showed a significant difference in the genotype distribution between our Brazilian group and data reported for Japanese ($p \leq 0.001$ and $p = 0.003$), Taiwanese ($p \leq 0.001$) and Korean ($p \leq 0.001$) populations (9,25,26). This fact suggests that this polymorphism may be associated with ethnicity and shows the importance of the molecular epidemiological studies in different populations.

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CONFLICT OF INTEREST

None declared.

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Phase II Trial of Neoadjuvant Gemcitabine and Cisplatin in Patients with Resectable Bladder Carcinoma

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ABSTRACT

Objectives: Gemcitabine and cisplatin (GC) is an active combination in the treatment of metastatic bladder cancer. We have prospectively analyzed the efficacy and tolerability of GC as neoadjuvant treatment of invasive bladder cancer.
Materials and Methods: In this single-institution phase II trial, patients with muscle-invasive transitional cell carcinoma received three cycles of gemcitabine 1200 mg/m^2 on days 1 and 8 with cisplatin 75 mg/m^2 on day 1 prior to surgery. Radiologic response was evaluated by computed tomography and magnetic resonance imaging. All patients were referred to surgery after chemotherapy completion.
Results: Between June 2002 and March 2005, 22 patients (19 males) were enrolled. Median age was 63 years. Initial stage was II (T2) in 11 and III (T3-4) in 11 patients. Median follow-up is 26 months (4-43). Partial or complete radiologic response rate was documented in 13 out of 20 assessable patients (70%). One patient was excluded due to sarcomatoid carcinoma at definitive pathologic examination. Cystectomy was performed in 15 patients and pelvic radiotherapy in four patients. Nine out of 21 patients (43%) relapsed and four (19%) died due to disease progression. Complete pathologic response was observed in four patients (26.7% of 15). Median progression-free survival was 27 months (CI 95% not reached) with median overall survival of 36 months (CI 95%: 28.7 - 43.3). Grade III/IV toxicity was infrequent, with no deaths due to chemotherapy.
Conclusions: The combination of GC is effective and well-tolerated when used as neoadjuvant therapy in muscle-invasive bladder cancer. Longer follow-up is necessary to evaluate its impact on the overall survival of these patients.

***Key words**: bladder neoplasms; neoadjuvant therapy; cisplatin; gemcitabine*
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INTRODUCTION

Bladder carcinoma is the second most prevalent genitourinary tract neoplasm in Brazil, with estimated 2000 deaths each year, which represents 1.7% of all cancer deaths (1). Radical cystectomy represents the standard of care in muscle invasive bladder cancer. Nevertheless, only about 50 to 60% of these patients will be cured with surgery alone (2). Therefore, there is a need for additional therapeutic modalities as an adjunct to local treatment in order to improve the outcome of patients with invasive bladder carcinoma.

Systemic chemotherapy is active in advanced bladder cancer, with objective response rates in more than 50% of patients treated with MVAC (combination of methotrexate, vinblastine, adriamycin and cisplatin) (3). It has been administered also in the neoadjuvant and adjuvant settings in patients with bladder cancer at high risk of relapse (4,5). The administration of preoperative chemotherapy has the advantage of immediate treatment of microscopic disease and better tolerability when compared with postsurgical treatment. Another major benefit of the neoadjuvant approach is the ability to assess the response of the primary lesion, which is of prognostic significance. This was illustrated in a report of 125 patients enrolled in multiple trials of cisplatin-based neoadjuvant therapy followed by definitive surgery (6). At a median follow-up of 25 months, 91% of complete responders (defined as ≤ pT1 at cystectomy) were disease-free, in contrast to only 37% of nonresponders (≥ pT2 at cystectomy). In addition, a survival benefit for neoadjuvant cisplatin-based chemotherapy was demonstrated in a 2005 Cochrane database review that included individual patient data from more than 3,000 individuals enrolled in 11 randomized trials comparing neoadjuvant chemotherapy with local therapy alone (7). Neoadjuvant cisplatin-based combination chemotherapy resulted in a significant 14% reduction in the risk of death, which translated into a 5% absolute improvement in five-year overall survival (from 45% to 50%).

Although regimens like MVAC are very active, toxicity has been a major concern and limits its clinical use: grade 3 or 4 mucositis is reported in more than 20% of patients, neutropenic sepsis in more than 10% and with toxic death rate of 3 to 4 % (8,9). Combinations of cisplatin and novel agents have shown promising results in advanced disease. Gemcitabine plus cisplatin (GC) was associated with a similar response rate (49% versus 46%), progression-free survival (7.7 versus 8.3 months), median survival (14 versus 15.2 months) and markedly less toxicity, when compared to MVAC chemotherapy, respectively (10,11). The approval of this combination in the metastatic setting justifies its study as neoadjuvant treatment. The objective of this phase II trial is to evaluate the clinical and pathological response rates of patients with resectable bladder cancer treated with neoadjuvant GC and to assess the toxicity of this regimen in this setting.

MATERIALS AND METHODS

Patients - This is a phase II, nonrandomized, single-institution trial. Patients aged 18 to 70 years, Performance Status (Eastern Cooperative Oncology Group - ECOG) 0 - 2, with histologically confirmed muscle-invasive, resectable (clinical stages T2 - T4a, N0 - 1, M0) transitional cell carcinoma of the bladder and measurable disease on computed tomography (CT) or magnetic resonance imaging (MRI), were included in this trial. Patients should have adequate bone marrow function (hemoglobin > 10g/dL, white blood cell count > 3.0 x 10^9/L, absolute granulocyte count > 1.5 x 10^9/L, platelet count > 150 x 10^9/L), renal function (serum creatinine < 1.3 mg/dL and/or estimated creatinine clearance with Crockoft Formula > 60 mL/min), hepatic function (bilirubin < 1.2 mg/dL, transaminases and alkaline phosphatase < 1.5 x upper normal limit) and absence of concurrent disease precluding surgery. No previous chemotherapy or radiotherapy was allowed. Patients with preexisting peripheral neuropathy, previous history of cancer (except non melanoma skin cancer or in situ cervical cancer) were not eligible for this study.

Treatment schedule - Initial clinical staging included chest X-ray (and CT of the thorax in case of X-ray abnormalities), abdominal and pelvic CT, pelvic MRI, cystoscopy and biopsies of visible tumor. After 3 cycles of chemotherapy the patients were restaged. If operable, radical cystectomy and pelvic lymphadenectomy with or without neobladder reconstruction was offered within 6 weeks after the last course. For those who denied surgery or were not eligible for radical cystectomy and pelvic lymphadenectomy, radiation therapy was offered. Follow-up in the first 3 years included regular clinical assessment (every 2 months) and radiological examination (thorax X-ray and abdominal/pelvic CT every 6 months).

Patients were treated in an outpatient basis. Toxicity was graded according to National Cancer Institute Common Toxicity Criteria version 2.0. Che-

motherapy was given for 3 cycles. Gemcitabine 1200 mg/m^2 was given by intravenous infusion in 250 mL saline solution over 30 minutes on days 1 and 8 of a 21-day cycle. Cisplatin 75 mg/m^2 was given by intravenous infusion in 500 mL of normal saline over 60 minutes on day 1 of the cycle. Intravenous hydration and mannitol were given with each cisplatin infusion. All patients received premedication with intravenous dexamethasone plus ondansetron and postmedication with oral dexamethasone and ondansetron for 3 days as emesis prophylaxis.

Dose adjustment at the start of a treatment cycle was as follows: (1) treatment was delayed by 1 week in patients with an absolute granulocyte count < 1.5 x 10^9/L or platelet count < 100 x 10^9/L (if no recovery occurred after 2 weeks, then the patient was removed from the study); (2) dose of gemcitabine on day 8 was reduced by 25% if absolute granulocyte count between 1.0 to 1.2 x 10^9/L or platelet count between 50 to 75 x 10^9/L and by 50% if absolute granulocyte count between 0.7 to 1.0 x 10^9/L; (3) dose of gemcitabine on day 8 was omitted if absolute granulocyte count < 0.7 x 10^9/L or platelet count < 50 x 10^9/L; (4) treatment was delayed by 1 week if serum creatinine > 1.3 mg/dL (if no recovery occurred after 2 weeks, then the patient was removed from the study); (5) for patients with grade 3 or more nonhematologic toxicity, if no recovery was obtained with 3 weeks delay, the patient was removed from the study (except for nausea, vomiting or alopecia); (6) for patients with recovered grade 3 or more nonhematologic toxicity, both gemcitabine and cisplatin doses were reduced by 25%. Prophylactic use of growth factors was not permitted. Supportive care could include blood transfusions.

Treatment evaluation - Primary endpoint of this trial was to evaluate radiological and pathological response rate with the GC used in the neoadjuvant setting. Secondary endpoints included tolerability of the combination and evaluation of progression-free survival (PFS) and overall survival (OS). Radiological response criteria were as follows: (1) complete response was defined as the disappearance of all known disease at radiological examination (2) partial response was defined as a decrease in more than 30% in the longest diameter of the primary lesion on CT and MRI; (3) progressive disease was defined as increase in more than 20% in longest diameter of the primary lesion; (4) stable disease was defined as no established criteria for disease progression or partial response. Pathological complete response was defined as no residual disease at pathological examination, including patients with residual carcinoma in situ.

Progression-free survival was calculated from the day of treatment initiation until death or progression. Patients who were alive and who had not experienced disease progression were censored for progression-free survival at the date that they were last known to be alive or progression-free. Survival was measured from the date of treatment initiation until death. Patients who had not died were censored for overall survival when they were last known to be alive.

Statistical considerations - The planned sample size of the trial was 35 patients. All analyses were performed using SSPS statistical software. Survival curves for PFS and OS data were based on the Kaplan-Meier method. The study protocol was approved by the Ethics Committee of our institution and each patient signed a written informed consent.

RESULTS

Patients - Between June 2002 and March 2005, 22 patients were entered into the study. Accrual was poor due to overestimation of potentially eligible patients in our institution, as most of them either were older than 70 years at the time of diagnosis or had superficial disease. One of these 22 patients was not considered for response and toxicity evaluation because of protocol violation (sarcomatoid carcinoma at pathological review). Baseline characteristics are shown in Table-1. Median age at diagnosis was 63 years. All patients had Performance Status ECOG 0 or 1. Extravesical disease extension (T3 or T4) was found in 10 patients (48%). Grade 3 tumors were found in all cases. All but 3 patients were current or former smokers (86%).

Chemotherapy - Twenty patients received 3 cycles of neoadjuvant gemcitabine and cisplatin and 1 patient received only one cycle (protocol treatment

Table 1 – *Baseline characteristics.*

	Number	%
Age		
Median	64	
Range	48-68	
Gender		
Male	18	85.7%
Female	3	14.3%
Initial clinical stage		
T2	11	52.4%
T3	2	9.5%
T4	8	38.1%

was interrupted due to renal toxicity - persistent creatinine elevation higher than upper normal limit). Only in 4 cycles (from a total of 61 - 6.5%) it was necessary to delay chemotherapy by one week due to neutropenia ($n = 2$), renal toxicity ($n = 1$) or infection ($n = 1$). Nineteen patients received all full doses of chemotherapy and only 1 patient required 25% reduction in the dose of gemcitabine on day 8 of 2 cycles due to neutropenia. Toxicity was mild and only 38% of the patients presented grade 3 or 4 hematologic toxicity, as shown in Table-2. One patient required red blood cell transfusion and there was 1 episode of uncomplicated febrile neutropenia. Other grade 3 toxicities included nausea (28.6%) and rash (1 patient).

Postchemotherapy evaluation and treatment - As shown in Table-3, radical cystectomy was the definitive treatment of 15 patients. Median time between last chemotherapy cycle and surgery was 47 days (31 - 177). Two patients had delayed surgery not related to neoadjuvant treatment toxicity. Three patients refused surgery and 1 had a myocardial infarction in the preoperative week. No evidence of inoperability was detected in these 4 patients that had radiation therapy to the pelvis as definitive treatment (45Gy in the pelvis with 20 Gy boost to bladder). One patient refused any definitive treatment and another progressed systemically before surgery.

Cystectomy was complicated by wound infection and pyelonepritis in 1 patient, urinary fistula requiring laparotomy in 1 patient and hypertensive crisis followed by cardiac failure and fatal arrhythmia in 1 patient with prior history of cardiac disease.

Radiological response was assessable in 20 patients (1 patient had unmeasurable disease at initial radiological examination). Evaluation with CT was complemented by MRI and is presented in Table-4. Most patients had partial response to treatment (65%).

Table 2 – *Toxicity of gemcitabine plus cisplatin: worst toxicity by patient (n = 21).*

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Hematologic					
Granulocytes	8	3	3	6	1
Platelets	18	2	0	1	0
Hemoglobin	0	18	3	0	0
Alopecia	9	11	1	-	-
Fatigue	8	10	3	0	0
Infection	13	2	5	1	0
Nausea	1	6	8	6	-
Vomiting	7	8	6	0	0
Neuropathy (sensory)	16	2	3	0	0
Fever	19	2	0	0	0
Dermatologic					
Rash	18	2	0	1	0
Injection site reaction	20	1	0	0	-
Weight loss	18	3	1	0	-

Table 3 – *Post-chemotherapy treatment (n = 21).*

	N	%
Surgery	15	71.4%
Bricker	9	
Ileal neobladder	6	
Radiation therapy	4	19.0%
Refused definitive treatment	1	4.75%
Progressed systemically before definitive treatment	1	4.75%

Table 4 – *Radiological response rate (n = 20).*

	N	%
Complete response	1	5.0%
Partial response	13	65.0%
Stable disease	2	10.0%
Progressive disease	4	20.0%

Table 5 – *Post-chemotherapy (surgical) pathological stage.*

	N	%
Stage 0	3	14.3
Stage 0is		
Tis N0	1	4.75
Stage I		
T1N0	1	4.75
Stage II	3	14.3
T2aN0	2	9.50
T2bN0	1	4.75
Stage III	4	18.0
T3aN0	2	9.50
T3bN0	1	4.75
T4aN0	1	4.75
Stage IV	3	14.3
T1 N2	1	4.75
T3bN2	1	4.75
T4aN2	1	4.75
Not available	6	28.6

Post-chemotherapy pathological stage for those submitted to surgery is shown in Table-5. No microscopic evidence of tumor was found in 3 patients and carcinoma in situ in 1 patient (pathological complete response in 4 out of 15 patients - 26.7%). Median number of lymph node dissected was 11 (0 - 25). Three patients had metastasis to pelvic lymph nodes and one patient had positive surgical margins.

Progression-free survival and overall survival - Median follow-up was 26 months (4 - 43). During the follow-up, 9 patients relapsed (42.8%). Median estimated PFS was 27 months (95% CI: not reached), as shown in Figure-1. Six patients had systemic relapse, 2 concomitant locoregional and systemic relapse and 1 exclusive locoregional progression. From these patients, 4 received second-line chemotherapy, 4 are dead and 5 are still on palliative treatment.

During follow-up, 6 patients died: 4 due to bladder cancer relapse, 1 due to postoperative complications and another due to myocardial infarction with no clinical or radiological evidence of disease progression. Median estimated overall survival was 36 months (95% CI: 28.7 - 43.3), as shown in Figure-2.

COMMENTS

The administration of neoadjuvant chemotherapy in patients with operable bladder cancer has been previously studied in randomized trials. The largest trial was performed jointly by the Medical Research Council and the European Organization for Research and Treatment of Cancer (EORTC) (12). Patients with high-grade muscle-invasive bladder transitional cell carcinoma were randomly assigned to three cycles of neoadjuvant CMV (cisplatin, methotrexate, and vinblastine, n = 491) or no chemotherapy (n =

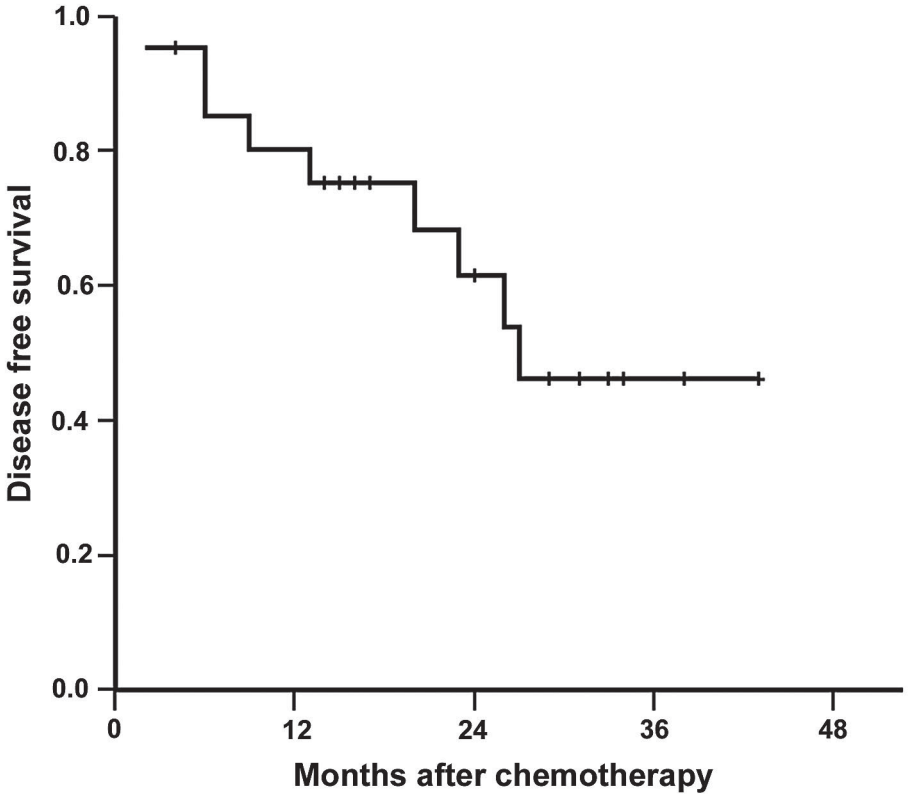

Figure 1 – *Progression-free survival of 21 patients treated with neoadjuvant gemcitabine and cisplatin for invasive bladder cancer.*

485), followed by each institution's choice of local management (radical cystectomy or radiation therapy). The pathological complete response rate in the neoadjuvant group was 33%, and the absolute OS benefit from chemotherapy at three years was 5.5% (55.5% versus 50%), which was not significant. A later report with seven years of follow-up demonstrated a 15% OS benefit with neoadjuvant CMV, but it was still not statistically significant (13).

A sufficiently powered United States Intergroup trial randomly assigned 317 patients with T2-4a N0 bladder cancer to cystectomy with or without three preoperative courses of MVAC (4). In the final report, with a median follow-up of 8.7 years, patients treated with MVAC were significantly more likely to have a pathological complete response (38% versus 15%). The improvements in median OS (77 versus 46 months, p = 0.06), and five year OS (57% versus 43%, p = 0.06) were of borderline statistical significance. These results were obtained after a median follow-up of 8.7 years.

Our trial is the first complete report on the feasibility and efficacy of the combination of gemcitabine and cisplatin as neoadjuvant treatment of invasive bladder carcinoma. The results confirm the excellent tolerability of this regimen and show that it can be safely administered in this setting. Three cycles of this combination resulted in mild toxicity and was completed by 95% of patients. This seems promising compared to neoadjuvant MVAC, which is associated with severe gastrointestinal toxicity in one-third of the patients and grade 3/ 4 hematological adverse effects in at least 60% of the patients (4). Chemotherapy resulted in high percentage of complete/ partial radiological response (70%) and in 4 out of 15 patients submitted to radical cystectomy there was complete pathological response (26.7%). Grossman et al. found 38% of complete pathological response with MVAC chemotherapy (4). Re-

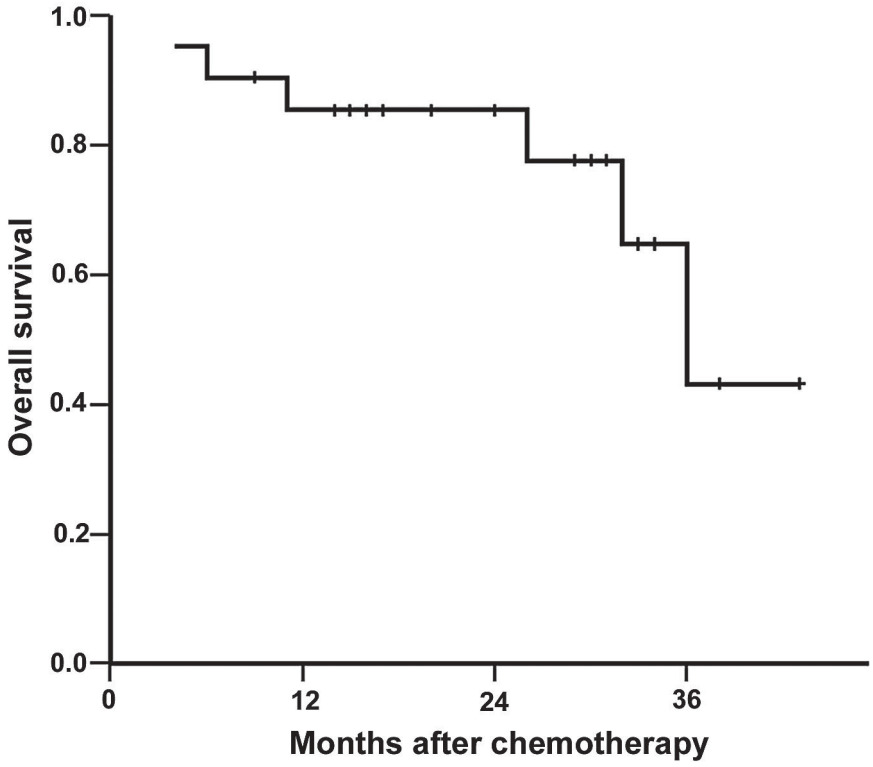

Figure 2 – *Overall survival of 21 patients treated with neoadjuvant gemcitabine and cisplatin for invasive bladder cancer.*

garding survival data, our trial has a limited follow-up of 2.2 years, what justifies the unfavorable median estimated survival of 36 months, as compared to the Intergroup trial (4).

In our trial, nearly 70% of the randomized patients were submitted to radical cystectomy, what is consistent with previous studies evaluating neoadjuvant chemotherapy for invasive bladder cancer (4). Median time from last course of chemotherapy and surgery was 6 weeks, what is in agreement with the protocol schedule. However, extension of lymph node dissection was not uniform and half of the patients had less than 10 nodes removed, what is known to be associated with shorter postcystectomy survival (14). Herr et al. showed that surgical variables associated with longer survival included negative margins and more than 10 lymph nodes removed, using a multivariate model adjusted for neoadjuvant chemotherapy, age, pathologic stage and node status. These results emphasize the importance of surgery in the treatment of bladder cancer.

Other studies have reported initial results with neoadjuvant GC in invasive bladder cancer. Bolotina et al. presented in abstract form the results of another phase II trial of neoadjuvant GC in patients with transitional bladder carcinoma (15). Gemcitabine 1000 mg/m^2 was given on days 1, 8 and 15 and cisplatin 100 mg/m^2 on day 2, for 2 cycles. Complete pathological response was achieved in 6 out of 20 patients (30%) and partial response in 9 patients (45%). No grade 3 or 4 toxicities were observed in the trial.

Additionally, Khaled et al. are conducting a phase III randomized trial of neoadjuvant GC compared to radical cystectomy alone in patients with bladder cancer in Egypt (16). In patients with complete response to neoadjuvant treatment, additional chemotherapy and radiation therapy was recommended in order to preserve the bladder. Radical cystectomy was

recommended to patients with partial response. Most patients had squamous cell carcinoma (59 of 114). Treatment was well tolerated and bladder preservation was feasible in 11 out of 58 patients randomized to neoadjuvant chemotherapy. Although data of this trial cannot be compared with our results once bladder squamous cell carcinomas respond differently to chemotherapy, it shows that effective neoadjuvant treatment might be used for selection of patients for bladder preservation. Sample size and follow-up are also still insufficient for survival evaluation.

Our results suggest that GC might be an attractive alternative to MVAC, as previously reported in the metastatic setting. Nevertheless, it should be noted the small sample size and median follow-up of our trial. Therefore, for definitive conclusions we should wait properly designed randomized trials.

CONFLICT OF INTEREST

Eli Lilly Company funded the trial.

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EDITORIAL COMMENT

Neoadjuvant chemotherapy is an exciting new area of research in the treatment of bladder cancer. It has been previously described that gemcitabine and cisplatin (GC) offers less toxicity than MVAC in both the neoadjuvant and adjuvant setting.

Clearly, there is inadequate statistical power in this study to determine meaningful clinical endpoints. However, the authors do recognize this and address it appropriately in the conclusions section. GC is again well-tolerated in this study; however, this is a known fact.

The authors provided an excellent explanation of lymph nodes obtained and inclusion of the importance of an extended dissection. This is a key point whether one is discussing chemotherapy in the neoadjuvant or adjuvant setting.

I would caution the authors inclusion of the study by Khaled et al (Reference 16) given the high number of patients with primary squamous cell carcinoma (over 50%!). Transitional cell carcinoma responds differently to chemotherapy in general. The statement that this trial shows that neoadjuvant chemotherapy is effective in this population is true. However, it should not be compared with patients who have primary TCC of the bladder.

I am unsure how to word the fact that the scientific community will certainly not base management decisions on such an underpowered study. However, it does promote a basis for continued research and improvements in neoadjuvant chemotherapy in bladder cancer. At the least, it reinforces the need for a large trial evaluating the proposed clinical endpoints.

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Interobserver Agreement of Gleason Score and Modified Gleason Score in Needle Biopsy and in Surgical Specimen of Prostate Cancer

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ABSTRACT

Introduction: Gleason score, which has a high interobserver variability, is used to classify prostate cancer. The most recent consensus valued the tertiary Gleason pattern and recommended its use in the final score of needle biopsies (modified Gleason score). This pattern is considered to be of high prognostic value in surgical specimens. This study emphasized the evaluation of the modified score agreement in needle biopsies and in surgical specimen, as well as the interobserver variability of this score.
Materials and Methods: Three pathologists evaluated the slides of needle biopsies and surgical specimens of 110 patients, reporting primary, secondary and tertiary Gleason patterns and after that, traditional and modified Gleason scores were calculated. Kappa test (K) assessed the interobserver agreement and the agreement between the traditional and modified scores of the biopsy and of the surgical specimen.
Results: Interobserver agreement in the biopsy was K = 0.36 and K = 0.35, and in the surgical specimen it was K = 0.46 and K = 0.36, for the traditional and modified scores, respectively. The tertiary Gleason grade was found in 8%, 0% and 2% of the biopsies and in 8%, 0% and 13% of the surgical specimens, according to observers 1, 2 and 3, respectively. When evaluating the agreement of the traditional and modified Gleason scores in needle biopsy with both scores of the surgical specimen, a similar agreement was found through Kappa.
Conclusion: Contrary to what was expected, the modified Gleason score was not superior in the agreement between the biopsy score and the specimen, or in interobserver reproducibility, in this study.

***Key words**: prostatic neoplasms; biopsy, needle; surgery; pathology*
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INTRODUCTION

Prostate cancer tends to be morphologically heterogeneous (1), showing several patterns of differentiation, classified by Gleason system (2). Prostate needle biopsy provides random samples, which might not represent neoplasia in all its heterogeneity, generally downgrading the tumor (3-5). By clinical accompaniment, a worse prognosis was found in the

patients who had small proportions of Gleason patterns 4 and 5 tumors, which are not mentioned in the Gleason score (6-10). From this observation, the concept of modified Gleason score was created incorporating these small most aggressive patterns in the patient's score and being used in some prognostic nomograms (11,12), Figure-1.

Several studies deal with interobserver agreement of Gleason score, with all sorts of different results (13). Other studies deal with the agreement as regards modified Gleason score in slides (14). Recently, Helpap reported better association between needle biopsy and surgical specimen using the modified Gleason score (11). Gleason histopathological classification shows high level of subjectivity. Despite its undeniable clinical importance, as a diagnostic method, the Gleason score, more precisely the modified score, needs to be evaluated in relation to its reliability. Taking this into account, we tried to evaluate the interobserver agreement and the association between needle biopsy and the surgical specimen adopting Gleason and modified Gleason scores.

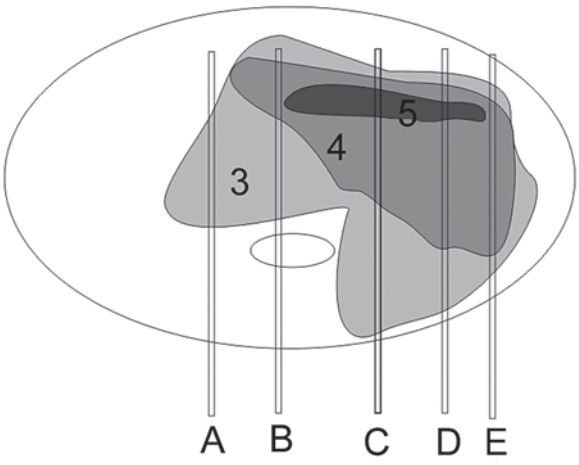

Figure 1 – *Differences between the traditional Gleason score (G) and the modified Gleason score in five hypothetical prostatic needle biopsies with prostate cancer.*
A) G = 3 + 3= 6, modified G = 3 + 3 = 6
B) G = 3 + 4 = 7, modified G = 3 + 4 = 7
C) G = 3 + 4 = 7, modified G= 3 + 5 = 8
D) G = 4 + 3 = 7, modified G = 4 + 5 = 9
E) G = 4 + 3 = 7, modified G = 4 + 3 = 7
The three shades of grey correspond to prostate cancer in Gleason patterns 3, 4 and 5, from lighter to darker respectively.

MATERIALS AND METHODS

A hundred and ten patients suffering from prostate cancer without any previous treatment and who would be referred to a radical prostatectomy agreed to participate in the research. They signed the consent term and sent their needle sextant biopsies, coming from different laboratories, to be reevaluated. Those biopsies had about two cores per sextant, mean total of 12 cores (range 6 to 24 cores). The surgical specimen was processed in the same laboratory, by partial sampling, producing about nine slides per surgical specimen (range 7 to 20), evaluating apex, distal third, mid third, proximal third, bladder neck, right and left seminal vesicles. Thus, the surgical specimen was not processed as a whole. All material was stained with hematoxylin-eosin. All the available slides of the needle biopsy and of the surgical specimens, with or without cancer, were evaluated by the observers.

Three pathologists belonging to different services of Pathological Anatomy examined the slides of the needle and surgical samples of these patients. They did not know the clinical data nor did they know about the pairing between needle biopsy and surgical specimen. They filled in a protocol in which they should classify the primary, secondary and the most aggressive Gleason patterns of each examined area of both specimens. At the end of this task, Gleason score was calculated (the sum of primary and secondary patterns) of each sextant separately (12). The score of the specimen was the highest score found among the evaluated sextants, therefore, the global score was not calculated (4,5,7,15). From the most aggressive Gleason pattern, the tertiary pattern was determined, whenever it was possible. The modified Gleason score was calculated (the sum of primary and tertiary patterns) (12). Similarly, the highest modified score of the examined slides was adopted as the modified Gleason score of the specimen. The primary Gleason pattern was defined as the most frequent Gleason pattern of the sample. The secondary Gleason pattern was the second most frequent pattern, obligatory

higher than 5% of the tumor area (12). When the secondary pattern was less than 5%, the primary pattern was repeated. The tertiary Gleason pattern corresponded to the third Gleason pattern, necessarily more aggressive than the secondary pattern (12). In order to avoid terminology confusion, the Gleason score was called traditional Gleason score, being clearly differentiated from the modified Gleason score.

Data were collected in a data bank and statistically evaluated by Stata program version 9.1 (StatCorp. 4905 Lakeway Dr, College Station, USA). Kappa (K) and weighted Kappa test were used to evaluate the interobserver agreement and the agreement between the Gleason score of the two specimens. The interpretation of the agreement by Kappa value was done by the intervals: K < 0, poor; K = 0-0.2, slight; K = 0.2-0.4, fair; K = 0.4-0.6, moderate; K = 0.6-0.8, substantial; and K = 0.8-1.0, almost perfect (13). In the statistic inferences, in general, the level of significance of 5% was adopted and, consequently, a confidence level of 95% was used.

RESULTS

The samples' mean age was 63.5 +/- 7.7 years old (range 44 to 79 years old). The mean preoperative PSA was 10.2 +/- 8.2 ng/mL (range 1.2 to 53.4 ng/mL). The clinical tumor staging (digital rectal examination) was 46.7% of T1, 47.5% of T2 and 5.8% of T3. In the initial anatomic pathological test, extracapsular tumor extension was found in 17% (pT3a) and in seminal vesicles invasion in 11% (pT3b).

The three pathologists are specialized in the same university even though they nowadays work in different hospitals and laboratories. The experience of working in surgical pathology and the weekly amount of prostate tests, criteria adopted by Taille (13), allow us to classify the observers 1 and 2 as experienced and the observer 3 as less experienced.

Some slides, considered unsatisfactory, were rejected from the research.

In the biopsies, there was a predominance of Gleason pattern 3 in the primary pattern, range from 66% to 86%, and of secondary pattern, range from 63% to 71% among the observers. Similarly, in the surgical specimens, Gleason pattern 3 was more frequent in the primary pattern, being found from 75% to 81%, and as secondary pattern from 60% to 69% of the observations. There was an absolute predominance of Gleason grade 3 in the primary and secondary grade in both specimens. Gleason grade 6 was also predominant in the needle biopsy. In the surgical specimen there was a similar proportion of Gleason score 6 and 7. Table-1 shows the distribution of traditional and modified Gleason scores.

Table 1 *– Frequency of traditional and modified Gleason scores in the specimens according to observers, in percentage.*

Gleason Score	Observer 1				Observer 2				Observer 3			
	Needle Biopsy (N = 98)		Surgical Specimen (N = 109)		Needle Biopsy (N = 71)		Surgical Specimen (N = 85)		Needle Biopsy (N= 100)		Surgical Specimen (N = 110)	
	G	**ModG**	**G**	**ModG**	**G**	**ModG**	**G**	**ModG**	**G**	**ModG**	**G**	**ModG**
4	1%	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	1%	-
6	59%	58%	48%	41%	60%	60%	47%	47%	54%	52%	56%	50%
7	35%	32%	47%	51%	37%	37%	49%	49%	25%	27%	33%	35%
8	4%	6%	2%	3%	3%	3%	4%	4%	2%	2%	7%	9%
9	-	2%	1%	3%	-	-	-	-	1%	1%	2%	5%
10	1%	2%	2%	2%	-	-	-	-	-	-	1%	1%

G = percentage of traditional Gleason score; Mod G = percentage of modified Gleason score.

Interobserver agreement in needle biopsy as regards to primary Gleason grade was reasonable to moderate, according to Kappa. In the surgical specimen, the agreement was moderate to substantial. In the secondary Gleason pattern there was a divergence among the observers, the agreement was generally low, occasionally reasonable. As for the most aggressive Gleason pattern it was from reasonable to moderate (Table-2).

Interobserver agreement of traditional Gleason score in the needle samples was reasonable, with exact agreement among 60% to 68% and agreement +/- 1 Gleason score from 91% to 98%. In the specimens the agreement was from reasonable to moderate, with exact diagnosis from 66% to 71% and accepting difference of one unit from 96% to 99%. Modified Gleason grade presented similar agreement in both specimens, being reasonable to moderate. Exact diagnosis in the biopsy was from 58% to 69% and accepting agreement +/- 1 Gleason score from 86% to 97%. In the specimen the exact diagnosis was from 60% to 64%, accepting divergence of one unit chang-

Table 2 – *Interobserver agreement as regards primary, secondary and the most aggressive Gleason grade in needle biopsies and surgical specimens, considering each patient as an independent event.*

		Observers	Exact Agreement	Expected Agreement	Weighted Kappa	Kappa	Confidence Level	p Value	N
G1	Needle biopsy	1-2-3				0.4020			72
		1-2	85.51%	75.70%	0.4038	0.4036	0.1977 a 0.6095	0.0001	69
		1-3	75.00%	63.80%	0.3079	0.3094	0.1574 a 0.4614	0.0000	96
		2-3	84.29%	64.69%	0.5549	0.5549	0.3368 a 0.8917	0.0000	70
	Surgical specimen	1-2-3				0.5952			85
		1-2	86.90%	64.10%	0.6618	0.6352	0.4391 a 0.8313	0.0000	84
		1-3	85.32%	69.41%	0.5641	0.5201	0.3483 a 0.6919	0.0000	109
		2-3	90.59%	65.02%	0.7509	0.7309	0.5377 a 0.9241	0.0000	85
G2	Needle biopsy	1-2-3				0.1918			72
		1-2	62.32%	58.39%	0.0824	0.0944	-0.1351 a 0.3239	0.2101	69
		1-3	65.63%	55.20%	0.2567	0.2327	0.0399 a 0.4255	0.0090	96
		2-3	64.29%	56.57%	0.1648	0.1776	-0.0454 a 0.4006	0.0593	70
	Surgical specimen	1-2-3				0.2162			85
		1-2	59.52%	53.88%	0.1177	0.1223	-0.0784 a 0.3230	0.1223	84
		1-3	66.06%	51.26%	0.3464	0.3036	0.1363 a 0.4709	0.0002	109
		2-3	63.53%	54.63%	0.2054	0.1962	0.0943 a 0.3959	0.0272	85
Most aggressive G	Needle biopsy	1-2-3				0.4581			72
		1-2	73.91%	48.90%	0.5044	0.4895	0.2788 a 0.7002	0.0000	69
		1-3	72.92%	46.35%	0.4601	0.4951	0.3227 a 0.6675	0.0000	96
		2-3	68.57%	49.47%	0.3892	0.3780	0.1556 a 0.6004	0.0004	70
	Surgical specimen	1-2-3				0.4541			85
		1-2	69.05%	46.46%	0.4610	0.4219	0.2332 a 0.6106	0.0000	84
		1-3	71.56%	42.98%	0.5389	0.5012	0.3468 a 0.6556	0.0000	109
		2-3	68.24%	45.33%	0.4639	0.4190	0.2334 a 0.0646	0.0000	85

G1 = primary Gleason; G2 = secondary Gleason; most aggressive G = the regards most aggressive Gleason grade; weighted Kappa = with linear weight (disagreement by 1 category = 0.67 and disagreement by 2 categories = 0.33). Confidence level of 95% was used.

Table 3 – *Interobserver agreement as regards Gleason score (traditional) and modified Gleason score in needle biopsies and surgical specimens, considering each patient as an independent event.*

		Observers	Exact Agreement	Expected Agreement	Agreement ± 1	Weight Kappa	Kappa	Confidence Level	p Value	N
Gleason score (traditional)	Needle biopsy	1-2-3					0.3641			68
		1-2	68.12%	48.50%	98.55%	0.3996	0.3809	0.1732 a 0.5541	0.0002	69
		1-3	65.63%	42.19%	91.67%	0.4350	0.4054	0.2622 a 0.6676	0.0000	96
		2-3	60.00%	43.63%	92.86%	0.3215	0.2904	0.1119 a 0.4023	0.0007	70
	Surgical specimen	1-2-3					0.4616			84
		1-2	66.67%	45.96%	96.43%	0.4110	0.3832	0.1933 a 0.5765	0.0000	84
		1-3	71.56%	42.62%	99.08%	0.5911	0.5043	0.3566 a 0.6520	0.0000	109
		2-3	70.59%	42.02%	96.47%	0.5235	0.4927	0.3287 a 0.6567	0.0000	85
Modified Gleason score	Needle biopsy	1-2-3					0.3581			68
		1-2	69.57%	47.13%	97.10%	0.4444	0.4243	0.2248 a 0.6238	0.0000	69
		1-3	62.50%	40.61%	86.46%	0.3629	0.3685	0.2280 a 0.5090	0.0000	96
		2-3	58.57%	43.27%	92.86%	0.3080	0.2698	0.0913 a 0.4483	0.0015	70
	Surgical specimen	1-2-3					0.3615			84
		1-2	64.29%	44.52%	94.05%	0.3848	0.3563	0.1760 a 0.5366	0.0001	84
		1-3	63.30%	39.23%	95.41%	0.4901	0.3961	0.2580 a 0.5342	0.0000	109
		2-3	60.00%	40.55%	94.12%	0.3811	0.3271	0.1651 a 0.4891	0.0000	85

Weighted Kappa = with linear weight (disagreement by 1 category = 0.67 and disagreement by 2 categories =0.33). Confidence level of 95% was used.

ing from 94% to 95%. By adopting weighted Kappa, values similar to Kappa (not weighted) were found (Table-3).

Tertiary Gleason pattern was diagnosed in 8%, 0% and 2% of the biopsies and in 8%, 0% and 13% of the surgical specimen according to observers 1, 2 and 3, respectively. Thus, traditional and modified Gleason scores, according to observer 1, were the same in 92% of both specimens. Observer 2 did not consider any pattern as tertiary, having 100% precision between the two Gleason scores. Examiner 3 had 98% of the needle biopsies and 87% of the surgical specimens with the same diagnosis between the two scores.

Traditional and modified Gleason scores were used to evaluate the association among their scores in both specimens by each observer. For observer 1, adopting the traditional score in needle biopsy and in surgical specimen K = 0.24 was found. Adopting the modified score in the biopsy and the traditional one in the specimen, we got K = 0.21. The same happened when using the modified score in the needle biopsy and in the surgical specimen. Examiner 2 did not find any difference in the association of scores between specimens (K = 0.26). When examiner 3 used the traditional score in the needle biopsy and in the surgical specimen, the value for Kappa was 0.18 and when using the modified score in the biopsy and the traditional one in the specimen, Kappa was 0.17. Adopting the traditional Gleason score in both specimens, lower downgrading in needle biopsy was found than by adopting the modified score in both samples (Table-4).

COMMENTS

The sample used reflects a group of patients referred to radical prostatectomy, in other words, young patients, with localized illness and generally low Gleason score. The three observers, also young, had similar academic and professional background and learned the Gleason system during medical residence

Table 4 *– Agreement between traditional and modified Gleason scores in needle biopsies and surgical specimens according to observers. considering each patient as an independent event. Number of needle biopsies whose Gleason score was downgraded or upgraded in relation to the surgical specimen.*

		Exact Agreement	Expected Agreement	Kappa	Confidence Level	p Value	N	Down	Over
Observer 1	Biopsy G x Surgical G	57.73%	44.11%	0.2438	0.0802 a 0.4074	0.0018	97	30 (31%)	11 (11%)
	Biopsy Gmod x Surgical G	54.64%	42.19%	0.2153	0.0609 a 0.3697	0.0031	97	28 (29%)	16 (16%)
	Biopsy Gmod x Surgical Gmod	52.58%	39.45%	0.2168	0.0728 a 0.3608	0.0016	97	33 (34%)	13 (13%)
Observer 2	Biopsy G x Surgical G	60.38%	46.17%	0.2639	0.0319 a 0.4959	0.0129	53	14 (26%)	7 (13%)
	Biopsy Gmod x Surgical G	60.38%	46.17%	0.2639	0.0319 a 0.4959	0.0129	53	14 (26%)	7 (13%)
	Biopsy Gmod x Surgical Gmod	60.38%	46.17%	0.2639	0.0319 a 0.4959	0.0129	53	14 (26%)	7 (13%)
Observer 3	Biopsy G x Surgical G	52.00%	40.79%	0.1893	0.0518 a 0.3268	0.0035	100	20 (20%)	28 (28%)
	Biopsy Gmod x Surgical G	51.00%	40.29%	0.1794	0.0415 a 0.3173	0.0054	100	20 (20%)	29 (29%)
	Biopsy Gmod x Surgical Gmod	48.00%	37.37%	0.1697	0.0361 a 0.3033	0.0064	100	26 (26%)	26 (26%)

Biopsy G = Gleason score in the needle biopsy; Surgical G = Gleason score in the surgical specimen; Biopsy Gmod = modified Gleason score in the needle biopsy; Surgical Gmod = modified Gleason score in the surgical specimen; Down = downgrading of the needle biopsy; Over = overgrading of the biopsy; Confidence level of 95% was used.

in the same institution. Therefore, a good agreement among them would be expected.

Higher agreement of primary Gleason pattern was found in the surgical specimen and not in the needle biopsy. By observing smaller areas, it is expected that more attention would be devoted to a specific area and higher agreement would happen. On the other hand, once the specimen is better represented in tissue extension, the suspected areas with borderline pattern were better examined, resulting in higher agreement. This reflects the difficulties in diagnosing secondary pattern, which besides involving the identification of Gleason patterns, demands tumor volume determination. As a rule, secondary Gleason pattern is the one that is more than 5% of the tumor area and with smaller extension than the primary pattern. Determining the tumor extension is not necessary for the diagnosis of the most aggressive Gleason pattern, the recognition of the worst pattern is sufficient. Glaessgen found a weak agreement as regards the diagnosis of the most aggressive patterns and considered that the difficulty in diagnosing them was big-

ger than in determining their volume (14). The experience did not influence the agreement much because it was not higher between the more experienced observers, what contradicts some authors (15,16).

Interobserver agreement of traditional Gleason score was slightly higher in the surgical specimen than in the needle biopsy. By adopting the modified Gleason score, the agreement was similar in needle biopsy and surgical specimen. In general, adopting weighted Kappa, the agreement values were a little higher, but without altering the previous relations. It is interesting to notice that the modified Gleason score did not show any superiority over traditional score, as Glaessgen reported (14). Evaluating the agreement in relation to the patterns, it is higher in the primary pattern and in the most aggressive one (this is intimately related to tertiary pattern) and too low in the secondary pattern. The modified score would be expected to obtain a higher agreement, but this did not happen. This fact might have happened due to the small number of tertiary pattern diagnosed and, as a result, the two scores were similar. However, this number is similar to the one found in Griffiths' study, where the diagnostic proportion of tertiary Gleason pattern was 6% for general pathologists and 9% for uropathologists, showing weak agreement in relation to tertiary pattern (17). This pattern, in general, refers to patterns 4 or 5, which can present borderline structures making the diagnosis more difficult (3). Generally, the studies regarding the use of tertiary pattern use it in the prognostic evaluation, in surgical specimens. (8-10) Mosse, when evaluating the prognosis of patients with tertiary pattern 5, found a worse prognosis in those with Gleason score 6 or 7 in the surgical specimens. (8) It is known that, statistically, those scores are the most frequent ones.

Considering that prostate cancer is heterogeneous and multicentric (1), it is assumed that the biopsy, which samples a small portion of it, might not represent it efficiently (3-5). Traditionally it is believed that Gleason score in needle biopsy tends to downgrade the surgical specimen, because a less differentiated pattern may not have been sampled in the biopsy (4,12). That was observed by observers 1 and 2. Taking the downgrading concept as a starting-point, some authors suggest the use of modified Gleason score, which would better reflect the real tumor characteristics for it values the most aggressive small patterns (6). The International Society of Urological Pathology (ISUP) on Gleason grading recommends the inclusion of tertiary pattern (modified Gleason score) in needle biopsies. (12) In the surgical specimens, however, it is still recommended to mention the tertiary pattern, whenever it is present, without including it in the score (Gleason score). (12) Considering that the needle biopsy downgrades the score, it was expected that the modified Gleason score would have a better agreement with the traditional Gleason score in the surgical specimen. However this fact could not be demonstrated. The modified score in needle biopsy compared to traditional and modified score in the specimen, presented the same Kappa values or even slightly inferior ones when adopting the traditional score in the biopsy. The best representation in the biopsy was not proved when adopting the modified Gleason score. This fact, as previously mentioned, might have happened due to the low diagnosis of tertiary pattern. Helpap, on the contrary, evaluating slides of 368 patients, found improvement of the exact agreement between the two specimens using the modified Gleason score instead of the traditional score, ranging from 58% to 78% (8). However, he did not use the Kappa test to evaluate the real agreement, nor reported the diagnostic proportion of tertiary pattern.

CONCLUSIONS

In this study, the modified Gleason score did not prove to be superior in reproducibility compared to the traditional Gleason score, both in the needle biopsy and in the surgical specimen. Contrary to what was expected, the use of the modified score in the biopsy was not superior to the traditional score, comparing to the Gleason scores of the specimen. Within the aim of the study, the modified Gleason score was not superior to the traditional one. These conclusions might be due to the methodology used, as well as to the observers involved. Isolated morphological analysis is based in criteria of low reproducibility. It is necessary to reevaluate the association between the two Gleason scores, using different samples with a higher amount of tertiary pattern.

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CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

At a consensus conference organized in 2005 by the International Society of Urological Pathology (ISUP), the Gleason grading system underwent its first systematic revision (1). The purpose of the meeting was to standardize both the perception of histological patterns and how the grade information is compiled and reported. One of the decisions of the ISUP working group was that high-grade tumor of any quantity on needle biopsy should be included in the Gleason score. The ISUP recommendations contribute to a general shift upwards of the Gleason scores and it may be necessary to re-iterate some previous studies on grading of prostate cancer. Helpap et al. recently compared conventional and modified Gleason grading in radical prostatectomy specimens and preoperative biopsies and reported on the distribution of modified Gleason score and its correlation with other prognostic factors such as age, stage and serum PSA (2-4). Few studies have been performed on interobserver reproducibility of this new variant of Gleason grading.

In a study by Glaessgen et al., the reproducibility of modified Gleason grading among four genitourinary pathologists was analyzed using a set of 69 consecutive radical prostatectomy specimens (5). Mean weighted kappa for conventional and modified Gleason score were 0.56 (range 0.52-0.66) and 0.58 (range 0.49-0.74), respectively. This study was carried out before the ISUP consensus meeting was held and only addressed the effect of inclusion of tertiary patterns of higher grade in the Gleason score. Hence, recent changes in pattern recognition were not taken into account. Furthermore, the ISUP recommendations to include tertiary higher patterns in the score pertained to needle biopsies, while the study by Glaessgen et al. was done on radical prostatectomy specimens only (5).

Veloso et al., in this paper, present a similar study on the reproducibility of a modified Gleason grading, now done on both needle biopsies and radical prostatectomy specimens. Again, only the effect of inclusion of tertiary higher patterns was studied. In needle biopsies a weighted kappa of 0.36 was reached both with conventional and modified Gleason grading. In radical prostatectomy specimens, the weighted kappa was 0.46 and 0.36, respectively. This interobserver agreement was slightly lower than that of previous studies. For example, in a biopsy study on conventional Gleason score by Glaessgen et al., a weighted kappa of 0.48 to 0.55 (mean 0.51) was reached among 4 genitourinary pathologists using a consecutive series of needle biopsies from 69 men (279 glass slides) (6). Allsbrook et al. circulated 46 needle biopsies containing prostatic carcinoma among 10 genitourinary pathologists (6). The weighted kappa for Gleason score ranged from 0.56 to 0.70. However, the biopsies of this series were selected rather than consecutive which may lead to a better reproducibility.

From studies performed so far, it seems that the interobserver reproducibility of the Gleason grading remains essentially the same with modified Gleason grading and results are probably more influenced by the study design.

Revision of a grading system may be necessary when we gain new knowledge of the biology of cancer. However, it must also be remembered that a revision has consequences in terms of modified prognostic impact of a certain grade and also warrants new studies to verify the value of the novel grading system (7). Whether modified Gleason grading of needle biopsies is superior as predictor of prognosis remains to be seen.

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EDITORIAL COMMENT

This paper by Dr Veloso et al. deal with interobserver agreement of Gleason score and modified Gleason score (1) in needle biopsy and in surgical specimen of prostate cancer. This group of authors found that the modified Gleason score was not superior in the agreement between the biopsy score and the specimen, or in interobserver reproducibility.

The Gleason grading system is a powerful tool to prognosticate and aid in the treatment of men with prostate cancer. The needle biopsy Gleason score correlates with virtually all other pathologic parameters, including tumor volume and margin status in radical prostatectomy specimens, serum PSA levels and many molecular markers. The Gleason score assigned to the tumor at radical prostatectomy is the most powerful predictor of progression following radical prostatectomy. However, there exist significant deficiencies in the practice of this grading system. Not only does there exist problems among practicing pathologists but also a relative lack of interobserver reproducibility among experts.

Correlation

There have been several studies addressing the correlation between Gleason scores in needle biopsies and corresponding radical prostatectomy specimens. Although earlier studies used the thicker (14-gauge) needle biopsies (2,3), more recent series based on thin-core (18-gauge) needles used in conjunction with biopsy guns attached to transrectal ultrasound. Sextant or other modes of systematic sampling are typically performed in the more current series. In a recent compilation of data on 3,789 patients from 18 studies, exact correlation of Gleason scores was found in 43% of cases and correlation plus or minus one Gleason core unit in 77% of cases (4). Under-grading of carcinoma in needle biopsy is the most common problem, occurring in 42% of all reviewed cases. Importantly, over-grading of carcinoma in needle biopsies may also occur, but this was only found in 15% of cases. In general, adverse findings on needle biopsy accurately predict adverse

findings in the radical prostatectomy specimen, whereas favorable findings on the needle biopsy do not necessarily predict favorable findings in the radical prostatectomy specimens in large part due to sampling error.

Sources of Discrepancies

Sampling error

Perhaps the most important factor is sampling error, which relates to the small amount of tissue removed by thin-core needle biopsies. The average 20-mm, 18-gauge core samples approximately 0.04% of the average gland volume (40 cc). The most common type of sampling error occurs when there is a higher grade component present within the radical prostatectomy specimen, which is not sampled on needle biopsy (5). This typically occurs when a needle biopsy tumor is graded as Gleason score 3 + 3 = 6. In the radical prostatectomy, there exists a Gleason pattern 4, which was not sampled on the biopsy, resulting in a prostatectomy Gleason 3 + 4 = 7.

In some instances, under-grading results from an attempt to grade very tiny areas of carcinoma, so-called minimal or limited adenocarcinoma (6). Scores of minimal adenocarcinoma in needle biopsies show a reasonably strong correlation with radical prostatectomy scores, but the Gleason scores do not have the same power to predict extra-prostatic extension and positive margin status as they do in non-minimal carcinomas (6).

Over-grading can result from sampling error in cases where the high-grade pattern is selectively represented in needle biopsy. It may only represent a very minor element in the radical prostatectomy specimen. Even the same cancer focus may have different grades depending on the area sampled.

Borderline cases

The other source of discrepancy between biopsy and radical prostatectomy is borderline cases. In the description of the Gleason grading system, there are some cases that are right at the interface between two different patterns where there will be inter-observer variability and possible even intra-observer variability (7).

Pathology error

Pathology error is most frequently seen when pathologists assigned a Gleason score of ≤ 4 on a needle biopsy, which in fact was Gleason score 5-6. Many pathologists under-grade needle biopsies by confusing quantitative changes with qualitative changes. When there is a limited focus of small glands of cancer on needle biopsy, by definition this is a Gleason pattern 3. Gleason pattern 3 consists of small glands with an infiltrative pattern. Biopsying truly low-grade adenocarcinoma of the prostate could not result in just a few neoplastic glands but rather would be more extensive, as low-grade adenocarcinoma grows as nodules of closely packed glands rather than infiltrating in and amongst normal glands.

Under-grading may result from difficulty in recognizing an infiltrative growth pattern or failing to recognize the presence of small areas of gland fusion (7).

Pathologists' education and experience

The pathologists' experience in grading thin-core needle biopsies can also influence overall correlation with radical prostatectomy results. With experience, pathologists recognize grading pitfalls; in particular, the fact that Gleason scores of 4 and lower are almost non-existent in needle biopsy situation. Furthermore, small areas of fusion in the presence of a predominantly grade 3 background are recognized and will yield a Gleason score of 7, which often correlates well with radical prostatectomy results (8).

Intra-observer and interobserver variability

Reproducibility studies can be categorized as intra-observer and interobserver. For investigations of intra-observer agreement of Gleason grades, exact agreement was reported in 43% to 78% of cases (8,9), and agreement within plus or minus one Gleason score unit was reported in 72% to, 87% of cases. Gleason wrote tat he duplicated exactly his previous histologic scores approximately 50% of times. Highly variable levels of interobserver agreement on Gleason scores have also been reported, with range of 36% to 81% for exact agreement and 69% to 86% observers within plus or minus one Gleason score unit. Improvements in Gleason grading reproducibility can be achieved by

recognizing problematic areas and educating physicians via meetings, courses, website tutorials, and publications that specifically focus on the Gleason grading system (10).

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EDITORIAL COMMENT

In the original Gleason system, the most common and second most common grade patterns are added to arrive at the Gleason score with tertiary patterns not factored in. For example, in a needle biopsy with Gleason score 3 + 4 = 7, a smaller tertiary component of very high grade pattern 5 tumor would not be factored in. In the Consensus Conference on Updating the Gleason grading system, it was recommended that a tertiary component of higher grade tumor on biopsy be included within the Gleason

score by adding the most common and highest grade patterns. In the above example, this would result in a Gleason score of 3 + 5 = 8. This study by Veloso et al. found that the interobserver reproducibility for the modified biopsy Gleason score was not superior to the routine Gleason score and was also not more accurate in predicting radical prostatectomy Gleason score. The major limitation of their study, as they acknowledge, is the limited number of cases with a tertiary pattern on biopsy, ranging from 0%, 2%, to 8% amongst the three observers out of a total of 110 patients. With such small numbers, it would be impossible to show any differences between the routine and modified Gleason score. In a recent paper on 2,370 men with prostate cancer, Patel et al. also found that Gleason score 7 with tertiary pattern 5 was uncommon, occurring in 1.5% of cases (1). However, they documented that Gleason score 7 tumor on biopsy with tertiary pattern 5 has the same prognosis as Gleason score 8 tumor when treated by radiotherapy or radical prostatectomy. These findings are in concert with several studies that have documented the same adverse prognostic significance of tertiary pattern 5 in radical prostatectomy specimens. The growing body of evidence suggests that Gleason score 3 + 4 with tertiary pattern 5, whether on biopsy or radical prostatectomy, should be considered as Gleason score 8.

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Prognostic Factors for Late Urinary Toxicity Grade 2-3 after Conformal Radiation Therapy on Patients with Prostate Cancer

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ABSTRACT

Objective: Identify prognostic factors associated to late urinary toxicity in patients with prostate cancer submitted to radical conformal radiotherapy (3DCRT).
Materials and Methods: From July 1997 to January 2002, 285 patients with localized prostate cancer were consecutively treated with 3DCRT and retrospectively analyzed. Thirty seven (13%) patients were submitted to transurethral prostate resection previously to 3DCRT. The median dose delivered to the prostate was 7920 cGy (7020-8460). Patient and treatment characteristics were analyzed and correlated to late urinary toxicity grade 2-3, especially whether certain radiation doses applied to certain bladder volumes, when visualized through computerized tomography (CT) planning, correlated with the observed actuarial incidences of late urinary complications, using bladder volume as a continuous variable.
Results: On a median follow-up of 53.6 months (3.6-95.3), the 5-year actuarial free from late urinary toxicity grade 2-3 survival was 91.1%. Seven and fifteen patients presented late urinary toxicity grades 2 and 3, respectively. Prior transurethral resection of prostate and radiation dose over 70 Gy on 30% of initial bladder volume were independent prognostic factors for late urinary toxicity grade 2-3.
Conclusions: This study suggests that restricting radiation doses to 70 Gy or less on 30% of bladder volume, visualized through CT planning, may reduce late urinary complications. It furthermore suggests that patients with prior transurethral resection of prostate may indicate a group of patients with a greater risk for late urinary toxicity grade 2-3 after 3DCRT.

***Key words**: prostatic neoplasms; radiotherapy; bladder; toxicity; prognosis*
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INTRODUCTION

Several studies have reported that higher radiation doses improve the control over prostate cancer (1-4). However, by increasing the radiation dose, the risk of developing complications also increases (1,5). Many studies have observed that the incidence of rectal complications is not exclusively associated to the radiation dose, but also to the volume of organ irradiated (2,6,7). Grade 2 or higher late urinary complications occur in 10-13% of patients treated with radiotherapy, but the relation between radiation dose and irradiated bladder volume (dose-volume relation) has not been well documented (2,3,5,8,9). The pur-

pose of this study is to find out the parameters of this dose-volume relation by means of the initial CT planning, as well as factors inherent to the patients that might reduce the rate of late urinary complications.

MATERIALS AND METHODS

Patients - From July 1997 to January 2002, 285 patients with localized prostate cancer were consecutively treated with 3DCRT on a single institution and they were retrospectively analyzed. All patients were staged according to the 2002 American Joint Committee on Cancer. All patients gave written consent prior to treatment. The median age was 70 years (47-86) and 142 (49.8%) patients had associated diseases. Thirty seven (13%) patients were submitted to prior transurethral resection of prostate due to benign prostatic hypertrophy related symptoms. The median prostate weight estimated by transrectal ultrasound previously to 3DCRT was 35 g (11-123). Urinary symptoms before 3DCRT application were not available on medical records.

Treatment characteristics - Neoadjuvant, concomitant and adjuvant androgen suppression were done by discretion of the urologist or the radiation oncologist. On supine position, patients were submitted to urethrography during three-dimensional simulation and 5 mm tomographic slices were obtained. The images were transferred to workstations and the treatment targets, as well as organs at risk, were delineated. The prostate was delineated in all its volume, including the prostate capsule. Regional lymphatic drainage was considered the external and internal iliac vessels drainage, beginning at the caudal portion of the sacroiliac junction, and the obturatory vessels, excluding the perirectal vessels. For movement margins and setup errors, 10 mm were given in all dimensions, except for the seminal vesicles and prostate, which were given only a 3 mm posterior margin. Organs at risk were delineated as follows: a) bladder: delineation of the whole bladder, including its most external layer, b) rectum: delineation of the whole rectum, including contents from the anal-rectum transition to the rectum-sigmoid transition. Energies of 6 or 15 MeV of photons and 5 fields of radiation were used. The radiotherapy planning was divided into phases: pelvis, seminal vesicles and prostate were irradiated in the first phase; in the second phase, the seminal vesicles and prostate were the target volumes; in the last phase, only the prostate was irradiated. Whenever pelvic irradiation was not done, treatment included only the two latter phases: irradiation of the seminal vesicles and prostate, followed by irradiation of the prostate only. After radiotherapy planning was completed, it was then transferred to the linear accelerator and therapy was done with 180 cGy per day, 5 days per week. The patients have been advised to have a full bladder before treatment planning and the daily treatment. 3DCRT on the pelvis and on the seminal vesicles was performed in 50 (17.5%) and 245 (86%) patients, respectively. Neoadjuvant and concomitant androgen suppression therapy were performed in 123 (43.2%) and 146 (51.2%) patients, respectively.

Follow-up - After 3DCRT, patients were followed up between 3 and 6 months with serial PSA and physical examination. Image studies were done when specific complaints occurred.

Urinary Toxicity - Late urinary toxicity was considered after three months of the end of 3DCRT, and was graded according to Common Terminology Criteria for Adverse Events, version 3 (10), Table-1. Information about patient complaint was obtained by physician interview. Only grade 2 or higher toxicities were considered for analysis. Sexual function was not analyzed. The highest grade of late urinary toxicity was considered for statistical analysis when patients presented more than one type of late urinary toxicity.

Statistical analysis - Patient and treatment characteristics were analyzed and correlated to late urinary toxicity, especially whether certain radiation doses applied to certain bladder volumes, visualized through CT planning, correlated with the observed actuarial incidences of late urinary complications, using volume as a continuous variable. Associations between categorical variables for frequency data in contingency tables were performed through the chi-square test. When at least one expected frequency in 2 x 2 tables resulted in less than 5, Fisher's exact test was adopted. The 5% level of significance was considered for all tests. The free from late urinary toxicity

Table 1 – *Common Terminology Criteria for Adverse Events version 3 (10) modified.*

Complication	Grade	Characteristic
Cystitis	1	Asymptomatic
	2	Frequency with dysuria
	3	Intravenous pain medication; bladder irrigation indicated
	4	Major non-elective intervention indicated
	5	Death
Urinary retention	1	Hesitancy or dribbling, no significant residual urine; retention occurring during the immediate postoperative period.
	2	Hesitancy requiring medication; or operative bladder atony requiring indwelling catheter beyond immediate postoperative period but for < 6 weeks
	3	More than daily catheterization indicated; urological intervention indicated (e.g. transurethral resection of prostate, suprapubic tube, urethrotomy)
	4	Life-threatening consequences; organ failure (e.g. bladder rupture); operative intervention requiring organ resection indicated
	5	Death
Bladder hemorrhage	1	Minimal or microscopic bleeding; intervention not indicated
	2	Gross bleeding, medical intervention, or urinary tract irrigation indicated
	3	Transfusional, interventional radiology, endoscopic or operative intervention indicated
	4	Life-threatening consequences; major urgent intervention indicated
	5	Death
Urinary incontinence	1	Occasional (e.g. with coughing, sneezing, etc). pads not indicated.
	2	Spontaneous, pads indicated.
	3	Interfering with daily living; intervention indicated (e.g. clamp, collagen injections)
	4	Operative intervention indicated (e.g. cystectomy or permanent urinary diversion)
	5	—

grade 2-3 survival was defined as the interval between the date of the beginning of 3DCRT and the date of the first reported urinary complaints or the last information for censored observations. The actuarial free from late urinary toxicity grade 2-3 survival was estimated by the Kaplan-Meier method and the log-rank test was applied to compare survival curves with the confidence interval of 95%. All analyses were performed using the statistical software STATA release 7.0 (StataCorp 2001).

RESULTS

On a median follow-up of 53.6 months (3.6-95.3), the 5-year actuarial free from late urinary tox-

Table 2 – *Time and number of patients with urinary complications after 3DCRT.*

Complication	**Grade**	**Number of Patients**	**Median Time of Event Occurrence in Months** (range)
Cystitis	2	2	18.8 (6.7-83.1)
	3	3	
Urinary retention	2	1	30.1 (10.7-56.9)
	3	8	
Bladder hemorrhage	3	8	22.1 (6.1-83.1)
Urinary incontinence	2	4	45.9 (6.1-53.8)

icity grade 2-3 survival was 91.1%. The number of patients with late urinary toxicities according to their grade and the median time of event occurrence are listed on Table-2. The 5-year actuarial free from late urinary toxicity grade 2-3 survival for patients with and without prior transurethral resection of prostate was 74.3% and 93.9%, respectively (p = 0.0002) (Figure-1). For patients who received more than 70 Gy to 30% of bladder volume (54 patients), visualized through CT planning, the 5-year actuarial free from late urinary toxicity grade 2-3 survival was 86.4%, versus 92% for patients who received 70 Gy or less to 30%

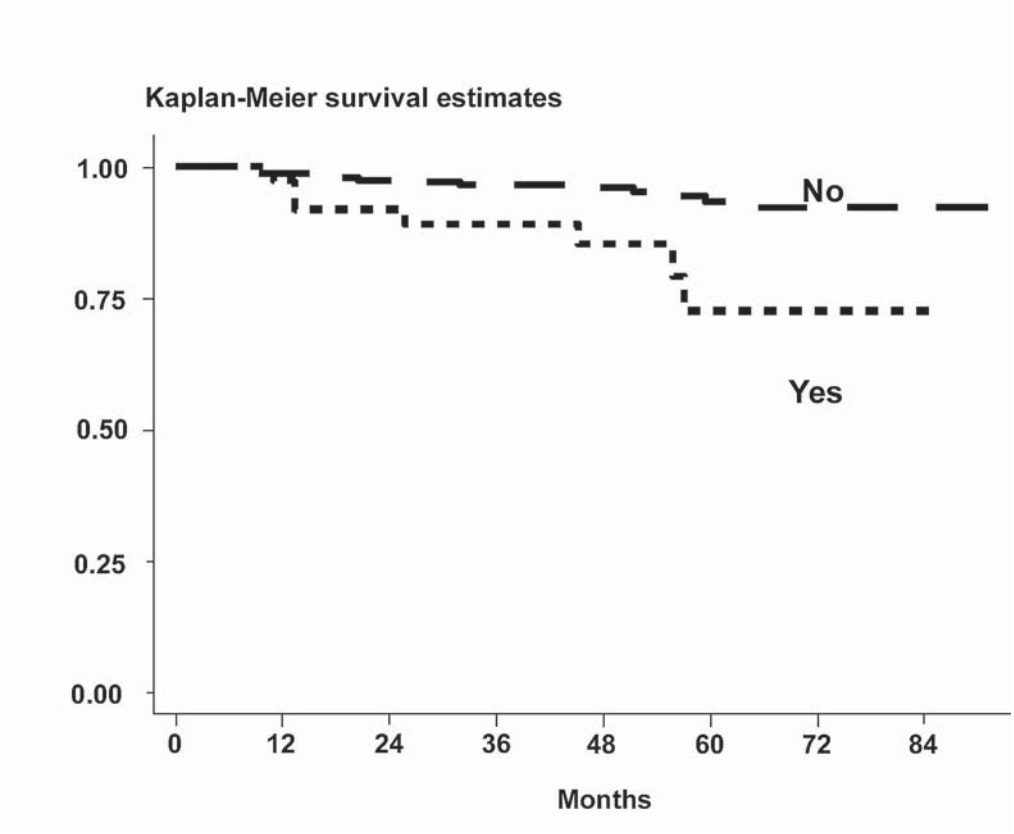

Figure 1 – *Actuarial free from late urinary toxicity grade 2-3 survival according to prior transurethral resection.*

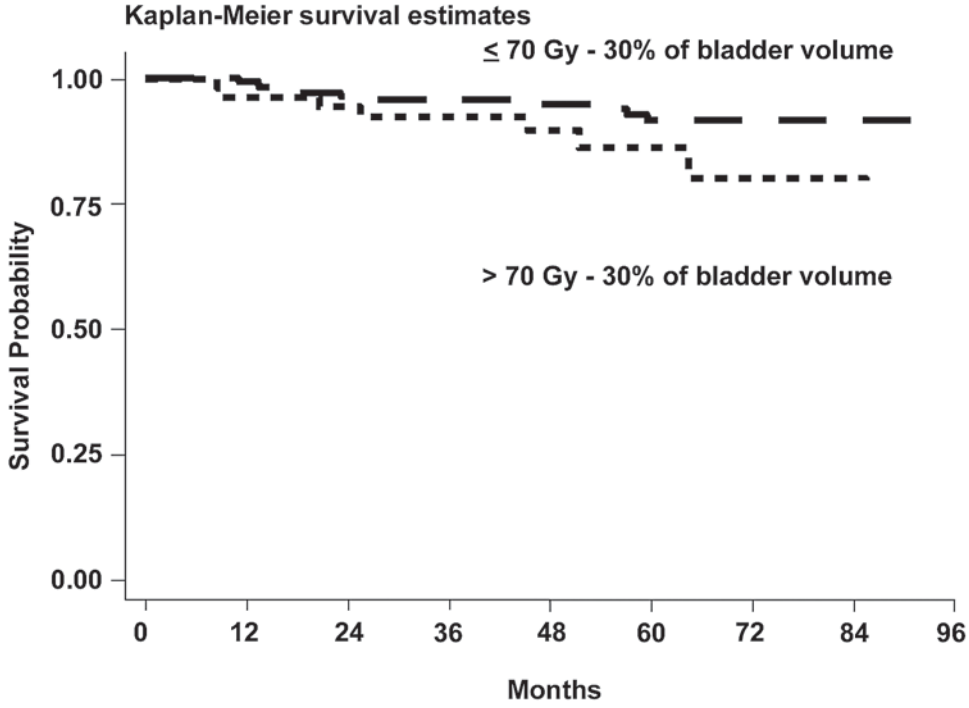

Figure 2 *– Actuarial free from late urinary toxicity grade 2-3 survival according to radiation dose on 30% of initial bladder volume.*

of bladder volume, also visualized through CT planning (p = 0.0264) (Figure-2). Prior transurethral resection of prostate and radiation dose more than 70 Gy to 30% of bladder volume were independent prognostic factors for late urinary toxicity grade 2-3 (Table-3). Analysis of age, ultrasound-estimated prostate weight, associated diseases, Gleason score, initial PSA value, clinical T stage, neoadjuvant, concomitant or adjuvant androgen suppression, irradiation of the pelvis and of the seminal vesicles were not statistically significant for the 5-year actuarial free from late urinary toxicity grade 2-3 survival.

Analyzing each type of late urinary toxicity grade 2-3 (cystitis, bladder hemorrhage, urinary incontinence and urinary retention) with prior transurethral resection and radiation dose more than 70 Gy to

Table 3 – *Independent prognostic factors for late urinary toxicity grade 2-3.*

Variable	**Category**	**Crude Relative Risk** (95% confidence interval)	**Multivariate Relative Risk** (95% confidence interval)
Dose on 30% of bladder volume	> 70 Gy	1.0 (reference)	1.0 (reference)
	≤ 70 Gy	2.7 (1.1 - 6.5)	3.0 (1.2 - 7.7)
Prior transurethral resection	No	1.0 (reference)	1.0 (reference)
	Yes	4.6 (1.9-11.5)	5.7 (2.2-14.5)

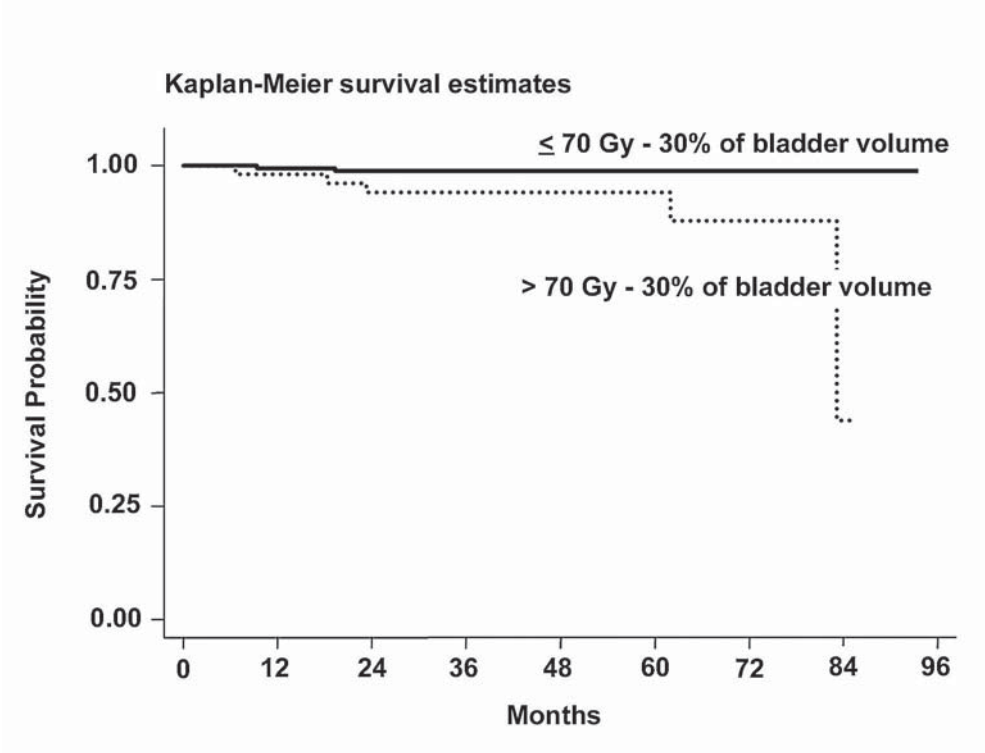

Figure 3 – *Actuarial free from cystitis according to radiation dose on 30% of initial bladder volume.*

30% of bladder volume, only patients with cystitis were associated to radiation dose more than 70 Gy to 30% of bladder volume (p = 0.0008) (Figure-3).

COMMENTS

Various trials did not find any relation between the percentage of bladder volume receiving a certain radiation dose and late urinary toxicity (6,11,12). Kuban et al. (5) postulated that the dose-volume relation is confounded by changes in bladder volume throughout therapy, making it difficult to be evaluated. However, Pinkawa et al. (13) reported that the mean bladder volume can be kept at the same level at the time of the initial treatment planning and during the treatment, if patients are asked to have a full bladder. The M.D. Anderson Cancer Center (6) randomized 189 patients with prostate cancer to receive 70 Gy or 78 Gy. The 5-year Kaplan-Meier risks of Grade 2 or higher late urinary toxicity were 20% and 9% for the 70 Gy and 78 Gy groups, respectively. Late urinary toxicity did not correlate with either the percentage or absolute volume of bladder that received 60 Gy or more, or 70 Gy or more. Koper et al. (11) analyzed 248 patients treated for prostate cancer with radiotherapy in a randomized trial. No association was found between radiation doses applied to certain bladder volumes and late urinary toxicity. However, the total dose of radiation was low (66 Gy on the prostate). Boersma et al. (12) analyzed the radiation dose of certain bladder volumes of 130 patients with prostate cancer treated with 3DCRT in a dose-escalating protocol. The 2-year actuarial incidence of Grade 3 or higher genitourinary complications was 8% and 21% using the RTOG/EORTC and the SOMA/LENT toxicity scales, respectively. They investigated whether the absolute bladder wall volume irradiated by various dose levels of radiation correlated with the actuarial incidence of late bladder complications. Although the crude figures in-

dicated a trend towards higher complication rates with larger irradiated volumes, actuarial analysis did not demonstrate any significant effect. The total radiation dose and the maximum dose applied to the bladder wall did not correlate with the incidence of late bladder complications either. In the first study that found an association between radiation doses applied to certain bladder volumes and low-grade late urinary toxicity, Pinkawa et al. (13) prospectively evaluated the impact of the dose-volume variable in 80 patients with prostate cancer consecutively treated with 3DCRT. The Expanded Prostate Cancer Index Composite (14) was used to grade urinary toxicity. The planned target volume was overlaid by the 90% isodose relative to the ICRU (15) reference point. The total median dose applied to the prostate at the reference point was 70.2 Gy divided into 1.8 Gy daily fractions. It was observed that the initial bladder volume and the percentage of bladder volume receiving 10%-90% of the prescription dose correlated significantly with the urinary function/irritation scales. Bladder volume < 180 mL, planned target volume ≥ 350 mL and area under the dose-volume histogram curve for the bladder ≥ 45% were also prognostic factors for late urinary toxicity. Trying to estimate and to correlate the absolute radiation dose that a percentage of the bladder volume received in this study with low-grade late urinary toxicity, it was found that 25% of bladder volume receiving ≥ 63.2 Gy (90% isodose of 70.2 Gy), 50% of bladder volume receiving ≥ 35.1 Gy (50% isodose of 70.2 Gy) and 65% of bladder volume receiving ≥ 21.1 Gy (30% isodose of 70.2 Gy) resulted in more low-grade late urinary toxicity. The present study used absolute radiation doses applied to a percentage of bladder volume, correlating these variables with late urinary toxicity, because this method is easier to use in clinical practice. It found that more than 70 Gy to 30% of bladder volume, visualized through CT planning, increased the risk of late urinary toxicity grade 2-3 (Figure-2). In medical literature, other factors have been associated to late urinary toxicity after 3DCRT. Peeters et al. (16) analyzed 669 patients with prostate cancer in a randomized trial of dose-escalation therapy. On a median follow-up of 31 months, the 3-year risks of late genitourinary toxicity grade 2 or higher were 28.5% and 30.2% for 68 Gy and 78 Gy, respectively. Androgen suppression therapy, pretreatment genitourinary symptoms and prior transurethral resection of prostate were prognostic factors for late genitourinary grade 2 or higher. Zelefsky et al. (4) analyzed 1100 patients treated with 3DCRT or intensity modulated radiotherapy at Memorial Sloan Kettering Cancer Center and reported a strong relation between radiation dose and late urinary toxicity grade 2. The 5-year actuarial rate of late urinary toxicity grade 2 on patients who received 75.6 Gy or more was 13%, versus 4% on patients who received less than 75.6 Gy ($p < 0.001$). Liu et al. (17) evaluated 1192 patients with prostate cancer treated with radiotherapy and observed that associated genitourinary disease, transurethral resection of prostate previous to radiotherapy and the presence of acute urinary toxicity during treatment were significant prognostic factors for late urinary toxicity grade 3. In the present study, prior transurethral resection of prostate resulted in more late urinary toxicity grade 2-3 (Figure-1). However, data concerning obstructive urinary symptoms before 3DCRT were not available, making it possible that transurethral resection of prostate has selected patients with a higher tendency to urinary toxicity.

CONCLUSIONS

Although bladder volume is not constant during treatment with 3DCRT, restriction of the radiation dose to 70 Gy or less to 30% of bladder volume, visualized through CT planning, seems to be a good measure in order to reduce late urinary toxicity grade 2-3, especially when associated to orientations for the patients to maintain their bladders full during radiotherapy application. Likewise, in spite of the possibility that prior transurethral resection of prostate may have selected patients with a higher propensity for urinary toxicity, the existence of prior transurethral resection of prostate may be an alert to the possibility of late urinary complications.

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

The study focuses on urinary toxicity of 285 patients after conformal radiation therapy for localized prostate cancer after a median follow-up of 54 months. Patient and treatment characteristics were correlated to late urinary toxicity grade 2-3. A dose of > 70Gy to 30% of bladder volume and a prior transurethral prostate resection have been found to predict a greater risk for late urinary toxicity.

Though daily volume variations will occur, the mean bladder volume can be kept at the same level at the time of the initial treatment planning and during the treatment, if the patients are asked to have a full bladder (1,2). With increasing cystitis rates during radiation therapy (greater bladder volume with a higher dose), the mean bladder volume is likely to decrease during the treatment (3). Written bladder filling instructions for patients might be helpful to improve bladder volume consistency (4).

Radiotherapy with an empty bladder has been recommended in the past by several investigators to reduce prostate mobility during a fractionated treatment (5). A recent organ motion study could demonstrate the same prostate mobility with both a full and empty bladder despite an increased variability of bladder filling with a full bladder (1). The dosimetric advantages of a full bladder compared to an empty bladder are a reduced amount of bladder volume in the high-dose region and additionally a reduced dose to bowel loops, that are shifted superiorly (1).

Several studies have dealt with urinary toxicity after radiation therapy for prostate cancer, but most studies did not find a correlation of the dose-volume-load to the bladder and late urinary toxicity. Prospective health-related quality of life analyses of a more homogeneous group of patients in respect to the total dose, treatment volume and post-treatment period support these results, demonstrating the independence of the initial bladder volume, prostate volume and a neoadjuvant hormonal therapy (6,7). These results are crucial for daily radiotherapy treatment planning.

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EDITORIAL COMMENTS

The purpose of this study was to determine factors that resulted in late urinary toxicity in prostate cancer patients treated with external beam radiation therapy. The authors found that irradiating 30% of the bladder (with a dose of 70 Gy or higher), and previous TURP resulted in an increased risk for at least grade 2, late urinary toxicity. This is an important topic as there is little guidance in the literature regarding specific doses or dosage cut points relating to urinary toxicity.

This is a well-done retrospective study, however it is subject to potential biases inherent to retrospective analysis. It should be pointed out that this a heterogeneous group of prostate cancer patients. Little is known of the patients pre-radiation therapy urinary function.

In the analysis, it was found that irradiating < 30% of the bladder-decreased symptoms by only 5.6% (92% freedom from symptoms vs. 86.4% freedom from symptoms for patients who had < 30% irradiated) though, that was significant. An attempt was made to treat the patients with the bladder full, as a full bladder can minimize the total volume of bladder irradiated, but the actual bladder volumes are not known. Though the patients had been advised to have a full bladder before treatment planning and the daily treatment, it is not known to what extent the patients were able to comply and how this relates to the findings.

The second finding is that patients with previous TURP had a 19.6% higher incidence of urinary toxicity (74.3 vs. 93.9 freedom from symptoms). The authors note that it is possible that patients with previous TURP are a selected group of patients who have a higher propensity for urinary toxicity. I would agree with that conclusion and believe it is related to a select group of patients in whom there has been bladder thickening and irritability due to long standing bladder outlet obstruction that required treatment. It is likely that patients who have had previous TURP already have worse urinary symptoms. It is possible that these patients are more sensitive to irradiation due to physiologic changes (bladder thickening, increased collagen, etc.) associated with bladder outlet obstruction requiring treatment. It is in my opinion there is also a possibility that the effect of bladder irradiation is understated. The investigators used physician interview to determine the side effects and usually patients will underrepresent side effects when talking to the physician as opposed to an anonymous questionnaire.

I believe that this subject is ripe for further study. The authors have opened an important dialogue. I think that in the future it will be important to know specifically how urinary toxicity relates to the bladder's functional status and measurable variables such as bladder capacity, voiding pressures, volume of intravesical prostate, etc.

In summary, I think that this paper addresses very important clinical questions that have the potential to make a difference in every day clinical practice. Clearly more specific information is needed regarding these issues.

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Prognostic Value of Morphologic and Clinical Parameters in pT2 - pT3 Prostate Cancer

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ABSTRACT

Objectives: Verify the efficacy of clinical and morphologic parameters currently applied, including an immunohistochemical panel, in the prognostic of prostate cancer, in specific stages of the disease.
Materials and Methods: In the period from 2002 to 2005, 40 surgical specimens were selected from patients submitted to radical prostatectomy, with their respective diagnostic biopsies. Based on the pathological stage pT2 or pT3, the specimens were separated into two groups, each one with 20 specimens. The results were confronted with pre- and postoperative clinical data. Between the groups studied, the following was also analyzed: the profile of the expression of molecular markers such as PSA, E-caderin, chromogranin-A, synaptofisin, P53 and Ki-67, both in the material coming from the prostatic biopsy and from the surgical specimens of all patients.
Results: Data showed that patients with prostate-confined disease (pT2) presented lower PSA and Gleason score rates, in relation to the group with extra-prostatic disease (pT3). Quantitative measures obtained for the percentage of positive fragments from the biopsy revealed that patients from the pT2 group presented a lower mean percentage when compared to the pT3 group. Positive margins of both groups influenced the need for complementary treatment before biochemical progression. The comparison of the molecular marker expression in both stages was not significantly different.
Conclusion: It is evident the need to improve new methods, predominantly morphologic and molecular, that are able to further exploit the study of the material from the prostatic biopsy. As to the profile of the molecular markers used in both studied groups, there was no significant difference in the sense of outlining an additional prognostic factor in the clinical practice.

Key words*: prostatic neoplasms; biopsy; prostatectomy; immunohistochemistry; pathology; prognosis*
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INTRODUCTION

Prostate cancer is presented as the second malignant disease most commonly diagnosed in men aged more than 50 years and represents a social problem with an important impact to men's health, for 3% of the patients presenting this disease will die, representing in the United States alone approximately 30,200 deaths per year (1).

Prostate cancer incidence presents regional variations and according to the studies of Hsing et al. (2), eastern countries present a lower incidence when

compared to western countries. The improvement of diagnostic methods associated to screening tests have motivated the development of new ways to anticipate the diagnosis, propose specific treatment, minimize unnecessary treatments (3), and to reduce the high percentage of post treatment biochemical recurrence (4). However, it is important to consider that in patients with normal prostatic digital rectal examination the trend to reduce the PSA cut-off level (bellow 4 ng/mL), could lead to the treatment of the so-called insignificant tumors (5).

PSA levels, Gleason score and TNM staging are established and considered essential to the prognostic of prostate cancer, when analyzed separately or jointly (6). Other factors such as the additional clinical-morphologic ones and molecular markers can also contribute substantially (7). Among the factors related to the biopsy, the percentage of positive fragments has presented a positive correlation with the potential risk of biochemical progression after treatment (8).

Today, there is a big concern regarding the research of new prognostic criteria with clinical applicability that precisely define recurrence risk, survival rate and the appropriate medical orientation, for both clinical follow-up and active treatment. In malignant neoplasia, there is a need to go beyond the diagnosis and search information about the most efficient prognostic and therapy for the disease (9). Up to now, clinical factors have offered the basis to build different nomograms that establish the risk and the evolution of the disease. However, before the variability of molecule expression in prostate cancer, the molecular basis and protein expression are not contemplated by these models (10).

The present study tried to assess in locally confined disease (pT2) with extra-prostatic extension (pT3), the efficiency of clinical and morphologic parameters of prognostic presently applied in prostate cancer, correlating them through immuno-histochemistry, with proliferation indexes, cellular adhesion and neuroendocrine differentiation.

MATERIALS AND METHODS

In the period from 2002 to 2005, 40 surgical specimens from patients submitted to radical prostatectomy were selected. When surgical indication was given, all patients presented a clinical stage of localized prostate cancer (T1c/T2c), according to TNM staging system (11). Exclusion criteria involved previous history of hormonal blockage and/or radiotherapy, diagnosis based in material obtained from transurethral resection of the prostate or any clinical evidence of extra-prostatic or metastatic disease.

Selection of surgical specimens was based on the pathological stages pT2 and pT3, which respectively characterize a prostate-confined and extra-prostatic disease. Twenty specimens were selected from each group, being the first, pT2, characterized by specimens from sub-stages pT2a (2 patients) and pT2c (18 patients), while the second was formed by pT3a (16 patients) and pT3b (4 patients).

Mean patient's age with pT2 was 62 years and with pT3 was 65.5 years. After radical prostatectomy, the patients were followed in an outpatient clinic every 4 months, based on total PSA serum concentration and for a mean time of 23 months. Value equal or superior to 0.4 ng/mL was considered as biochemical progression.

Prognostic parameters considered in the preoperative period were age, digital rectal examination, total PSA, free PSA, total of positive fragments on biopsy, positivity percentage per fragment and profile of molecular markers in the prostatic biopsy material. All patients were preoperatively submitted to pelvic computed tomography and bone scintigraphy.

Surgical parameters included positive margins, Gleason score, percentage of positive blocks, pathological stage and molecular markers profile in the surgical specimen.

Indication for ultrasound guided prostatic biopsy, with a removal of an average of 15 fragments, was due to alterations on serum PSA and/or on digital rectal examination.

Biopsy fragments were embedded in paraffin, sectioned and stained with hematoxylin and eosin. Surgical specimens were analyzed by the same pathologist were fixed, stained using India ink and entirely processed by means of a previously established topographic sequence. Characteristics of the surgical specimen and the percentage of positive blocks offered

the basis for staging and indirect calculation of the tumor volume, according to the protocol of the College of American Pathology (12), and of the American Joint Commission on Cancer (2002) (13).

In the immunohistochemical evaluation, the immunoperoxidase technique was used to identify the PSA, Ki-67, p53, chromogranin-A, synaptofisin and E-caderin.

The variables were analyzed by the Kolmogorov-Smirnov test. Statistical tests applied to compare two groups were t-Student for parametric quantitative data and Mann-Whitney for the non-parametric ones. Qualitative data were assessed by the Fisher method and correlation between quantitative data by the Spereman method. The analyses were performed in the SigmaStat program (Jandel Scientific, San Rafael, CA) and the graphics in the Microcal Microcal™ Origin 6.0® (Microcal Software Inc. 1999). Values of $p < 0.05$ were considered statistically significant.

RESULTS

In both the pre and postoperative phases of the disease different clinical and morphological factors in the same stage were studied and correlated as well as between both stages studied (pT2 e pT3). The results obtained in the preoperative phase for stage pT2 (PSA, digital rectal examination, percentage of positive fragments of the biopsy percentage of positivity for the fragment and immunohistochemical study of the biopsy) were studied and correlated to prognostic factors (clinical/morphological) of the postoperative phase, predominantly the data obtained from the analysis of the surgical specimen. The same study

Table 1 – *Clinical and morphologic pre and postoperative data of the patients submitted to radical prostatectomy and classified as pT2.*

Age	**PSA T** (ng/mL)	**% Fragments**	**Gleason** (biopsy)	**TNM Stage**	**% Blocks (+)**	**Gleason** (surgical specimen)	**Positive Margins** (surgical specimen)	**Complementary Treatment**
64	6.4	16.7	3+4	T1c	20.0	3+4	no	no
53	7.4	50.0	4+3	T1c	87.5	3+4	no	no
64	5.3	12.5	3+4	T2a	10.0	3+4	no	no
62	5.9	25.0	3+5	T1c	63.3	3+4	yes	no
62	5.0	50.0	3+6	T2b	34.0	4+3	yes	yes
68	5.8	66.7	3+3	T1c	36.0	3+5	yes	yes
59	5.1	33.3	4+3	T2b	20.0	3+4	no	no
59	8.6	85.7	4+4	T2c	50.0	3+4	yes	yes
65	4.8	33.3	3+3	T2b	16.7	3+4	no	no
59	3.7	16.7	3+4	T1c	14.5	3+4	yes	no
62	4.4	37.5	3+4	T2b	27.8	4+3	no	no
67	8.4	25.0	3+5	T2b	9.7	4+3	no	no
50	4.5	16.7	3+3	T1c	25.8	3+4	no	no
66	5.1	8.7	3+3	T1c	24.4	3+5	no	no
59	2.8	50.0	3+4	T2a	17.4	3+4	no	no
45	6.4	16.7	3+3	T2a	32.9	3+4	yes	no
75	20.0	25.0	3+4	T1c	70.0	3+4	no	no
70	29.0	28.6	3+3	T2b	18.2	3+5	yes	yes
68	3.8	33.3	3+4	T2b	26.3	3+4	no	no
62	7.3	71.4	4+3	T2b	53.3	3+4	no	no

Table 2 – *Clinical and morphologic pre and postoperative data of the patients submitted to radical prostatectomy and classified as pT3.*

Age	PSA T (ng/mL)	% Fragments (+)	Gleason (biopsy)	TNM Stage	% Blocks (+)	Gleason (surgical specimen)	Positive Margins (surgical specimen)	Complementary Treatment
64	18.0	100.0	4+5	T1c	59.2	5+4	yes	yes
69	13.7	33.3	3+5	T1c	34.9	4+3	yes	yes
66	9.4	57.1	3+4	T2b	27.3	3+4	yes	yes
68	27.0	57.1	4+3	T2b	51.8	3+4	no	yes
73	8.0	58.3	4+3	T2b	41.4	4+3	yes	no
70	6.1	28.6	3+5	T1c	33.9	4+3	yes	yes
61	7.8	33.3	4+3	T1c	34.0	4+3	no	no
73	6.6	33.3	4+5	T1c	26.3	4+3	no	no
54	6.1	60.0	3+3	T1c	89.5	3+4	yes	yes
71	23.0	66.7	5+3	T2b	87.2	3+5	yes	yes
58	7.3	16.7	4+4	T1c	57.6	3+4	no	no
73	9.3	66.7	3+4	T2c	79.4	3+4	yes	yes
75	10.3	58.3	4+3	T1c	35.6	3+4	yes	yes
69	19.0	28.6	4+3	T2c	73.9	3+4	yes	yes
56	8.5	83.3	4+3	T2c	76.9	3+4	yes	yes
65	8.8	8.3	3+3	T1c	23.4	3+5	no	no
53	6.9	28.6	4+3	T2b	21.6	4+3	no	yes
70	45.0	50.0	4+3	T2b	44.9	4+3	no	no
63	2.5	66.7	4+4	T2c	47.1	4+3	yes	yes
58	7.5	50.0	3+4	T1c	22.2	3+4	yes	yes

was conducted for stage pT3. We present data from the pre and postoperative phases pT2 (Table-1) and pT3 (Table-2).

Preoperative Total PSA Serum Concentration between the Groups

The first prognostic parameter assessed isolatedly or together with other factors was preoperative total PSA serum concentration. The PSA study showed that patients that had a prostate-confined disease (pT2) presented lower PSA rates (7.5 ng/mL) in relation to the group presenting extra-prostatic disease (pT3) (12.5 ng/mL) (p = 0.002; Mann-Whitney).

Gleason Score

For Gleason score of the biopsy, the results revealed that patients from group pT2 presented inferior mean values of Gleason when compared to patients in pT3 group (p = 0.006; Mann-Whitney).

The comparison between the Gleason score of the biopsy and the surgical specimen, within the same group pT2 or pT3, showed a significant statistical difference only for pT2 (p = 0.008; Mann-Whitney). In pT2 group, 60% of the patients were sub-graduated, i.e., the biopsy Gleason score was inferior to the one of the surgical specimen, while for the pT3 group this percentage was only 10%.

Gleason scores of the surgical specimen between pT2 and pT3 groups were statistically similar

($p > 0.05$). It is worth to mention that in both the predominant Gleason score was 3+4=7 or superior.

Positive Fragments of the Biopsy and Positive Blocks of the Surgical Specimen

The study of the quantitative values obtained for the percentage of positive fragments of the biopsy showed that pT2 group patients presented an inferior mean percentage (35.1%), when compared to pT3 group(49.3%) ($p = 0.049$; Mann-Whitney). A similar result was obtained for the percentage of positive blocks, indicating that the patients from pT2 group presented an inferior tumor volume (32.9%) when compared to pT3 (48.4%) ($p = 0.015$; Mann-Whitney).

Specific analysis for pT2 groups between the percentage of positive fragments of the biopsy and the percentage of positive blocks of the surgical specimen, ($r = 0.465$; $p = 0.0385$; Spearman) or pT3 ($r = 0.576$; $p = 0.007$; Spearman), revealed a weak positive correlation between both, demonstrating that the two variables tend to increase jointly.

Biochemical Recurrence Associated To Prostatic Parameters in Both Pre and Postoperative Phases

Biochemical recurrence varied considerably between pT2 and pT3 stages, being 20% and 70%, respectively. The mean follow-up was of 22.8 months for pT2 stage and 24.4 months for pT3 stage. Among the prognostic parameters assessed the Gleason score (superior to 7), positive surgical margins and tumor volume, were associated to recurrence (Table-3).

The analysis of positive margins and complementary treatment in pT2 or pT3 stages, showed that the total positive margins of the surgical specimen influenced the need for complementary treatment, due to biochemical recurrence. For pT2 stage we have observed that, from the 7 patients with positive margins, 4 of them presented biochemical recurrence ($p = 0.007$; Fischer), while for pT3 this relations was 13 positive margins with 12 recurrences ($p = 0.007$; Fischer). Among the 14 recurrences, in pT3 stage, 12 patients presented positive margins.

For the Gleason score parameter the patients were grouped in two categories ($7 < \text{Gleason} \geq 7$). Among patients with a Gleason score equal or superior to 7, at pT3 stage, 13 presented biochemical recurrence.

In relation to tumor volume, the data show that the patients that presented recurrence presented also a higher tumoral volume (54.3% at pT3 stage).

P53 and Ki-67 Expression

The results for p53 and Ki-67 were expressed in the percentage of labeled cells using the semi-quantitative score method, based on the sum of the immunopositive tumor cells proportion and the intensity of the immunolabelling expression. On Tables-4 and

Table 3 – *Clinical and morphologic variables associated to postoperative recurrence.*

	With Recurrence (number)		**Without Recurrence** (number)	
Variables	pT2	pT3	pT2	pT3
Positive margin	4	12	3	2
Negative margin	0	2	13	5
PSA < 10 ng/mL	3	8	15	5
PSA > 10 ng/mL	1	6	1	1
Gleason < 7	2	1	6	1
Gleason > 7	2	13	10	5
Estimated tumor volume (%)	34.5	54.3	32.5	37.9

Table 4 – *Number of patients that present p53 nuclear expression in the biopsy and in the surgical specimen, in stages (pT2 and pT3). The columns refer to the percentage of cells with expression.*

Stage	0 to 5	6 to 10	11 to 20	> 20
pT2 biopsy	12	3	3	2
pT2 specimen	20	0	0	0
pT3 biopsy	18	1	0	1
pT3 specimen	18	2	0	0

Table 5 – *Number of patients that present Ki-67 nuclear expression in the biopsy and in the surgical specimen, in stages (pT2 and pT3). The columns refer to the percentage of cells with expression.*

Stage	0 to 5	6 to 10	11 to 20	> 20
pT2 biopsy	18	2	0	0
pT2 specimen	20	0	0	0
pT3 biopsy	17	2	1	0
pT3 specimen	18	0	2	0

5, the uniform expression patterns are presented, independently from the stage (pT2 and pT3), both in the biopsy and in the surgical specimen.

Expression of Chromogranin-A and Synaptofisin

The expression of the immunomarkers, chromogranin-A and synaptofisin utilized to differentiate neuroendocrine cells was assessed qualitatively and classified as absent, light, moderate, intense and very intense. The results are presented on Tables-6 and 7, for the different categories. A significant expression of these markers was observed, except in patients with compromised seminal vesicles.

E-caderin Expression

E-caderin was assessed both qualitatively and quantitatively with the respective expression forms (Figure-1). The results obtained showed a variability of the immunomarker. In most of the tissues assessed, including biopsies and surgical specimens, independently from the stage, the e-caderin expression was absent or the cytoplasmic pattern was predominant (Table-8). The usual membrane labeling, identified in normal epithelium, was rarely observed.

PSA Expression

The PSA molecular labeling presented a very intense expression with cytoplasmic granular pattern in all tissues from biopsies and from surgical specimens in pT3 stage.

Molecular Markers and Recurrence

For a global analysis between the recurrence of the disease and the profile of various molecular markers used, it was not possible to establish a correlation between these parameters. The expression of the molecular markers was similar for patients with or without recurrence.

Table 6 – *Number of patients grouped in the different categories, based on the chromogranin-A expression in both stages (pT2 and pT3).*

Stage	Absent	Light	Moderate	Intense	Very Intense
pT2 biopsy	12	4	2	1	0
pT2 specimen	18	0	1	1	0
pT3 biopsy	11	5	4	0	0
pT3 specimen	14	4	2	0	0

Table 7 – *Number of patients grouped in the different categories, based on the synaptofisin expression in both stages (pT2 and pT3).*

Stage	Absent	Light	Moderate	Intense	Very Intense
pT2 biopsy	3	15	1	1	0
pT2 specimen	18	0	2	0	0
pT3 biopsy	14	4	1	1	0
pT3 specimen	15	5	0	0	0

COMMENTS

The present work reinforces the importance of the prognostic parameters already consolidated in nomograms and exhaustively studied in large populations (14). However, the present sample suggests a late diagnosis when we aim at the cure by means of only one form of treatment, confirmed by the high tumor recurrence rate or residual disease in pT3 group.

Mean PSA in pT2 group of 7.5 ng/mL was divergent from the 12.5 ng/mL found for pT3 group. We have observed in both groups a higher levels of PSA, when compared to the cuff of values considered nowadays (16,17).

Total-PSA serum concentration can be altered due to many factors, among which the patient's age and the prostate volume and cannot be considered isolatedly for tumor diagnosis. However, there are evidences that in a population with normal digital rectal examination and PSA between 0 and 4 ng/mL, 15.2% present prostate cancer detected in the biopsy (18).

Gleason score has consolidated its importance as one of the most important prognostic factors in both pre and postoperative phases. A fact that deserves attention in the present sample is the trend to undergraduate the Gleason score in prostatic biopsy material, when compared to the Gleason score of the surgical specimen. An explanation, among other

Table 8 – *Number of patients grouped in the different categories, based on the E-caderin expression in both stages (pT2 and pT3).*

Stage	Absent	Cytoplasmic	Membrane
pT2 biopsy	9	7	4
pT2 specimen	10	7	3
pT3 biopsy	14	5	1
pT3 specimen	16	4	0

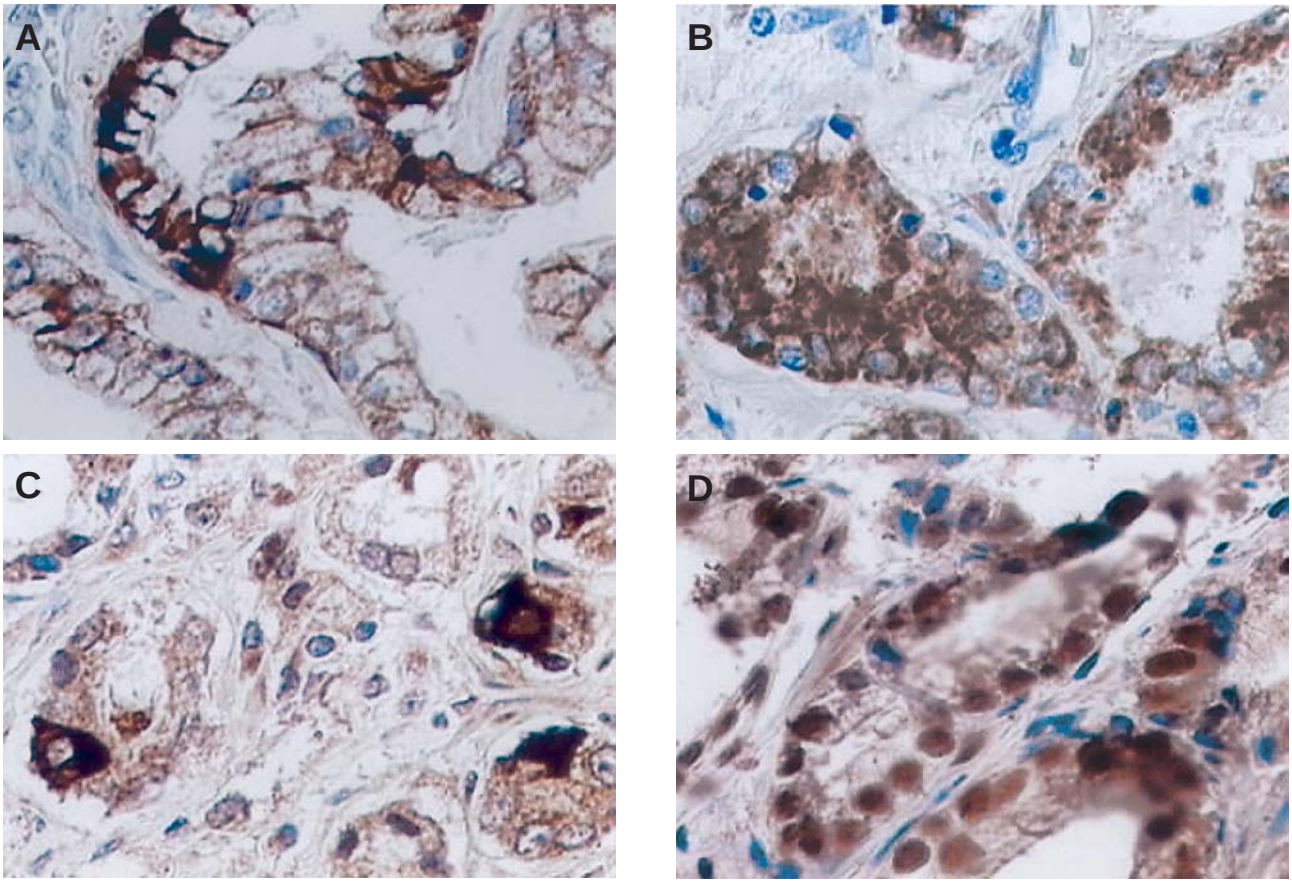

Figure 1 – *Prostatic tumor with expression of different immunomarkers. A) Variation in the E-caderin expression, with focal membrane labeling and cytoplasmic pattern. B) Cytoplasmic expression of E-caderin with loss of membrane expression. C) Neuroendocrine differentiation with positivity for chromogranin-A. D) Strong nuclear expression for p-53.*

variables, is that the limited sampling and the quality of the biopsy material, mainly when in phases where the disease is localized is represented by a small tumor volume. A growing tendency of the Gleason score was verified in pT3 group, confirming the correlation between the increases of the Gleason score with the worst prognostic (19).

Medial results obtained for the percentage of positive fragments reflected a difference in the extension of the tumor between the groups analyzed, being lower in pT2. Gancarczyk et al. showed this parameter to be of great relevance, mainly when associated to pre-treatment PSA and the higher Gleason score of the biopsy, suggesting to be a predictive factor of the pathological staging (20).

The results obtained for immunolabelling related to cellular proliferation, demonstrated a similar standard expression of p53 and Ki-67 in the biopsy and in the surgical specimen when both stages are compared.

The studies of Downing et al. (21) showed a significant association between the nuclear expression of mutant p53 and a higher risk of recurrence, or a lower disease-free survival. The p53 gene mutation blocks apoptosis induction (22). In this work, cellular proliferation markers, in prostatic biopsy, do not influence the prognostic factors related to the recurrence of the disease and compromised margins.

Neuroendocrine cells, usually positive for chromogranin-A are found sparsely in the prostatic

tissue. This quantity can be increased or suffer changes, originating neuro-hormonal stimulations to the tumor microenvironment. Neuroendocrine differentiation in specimens from radical prostatectomies associated to the recurrence of the disease is still an open subject in literature. Some authors found a correlation (23), while others did not obtain the same results (24). Neuroendocrine cells are prominent in only 5 to 10% of the adenocarcinomas, having an important correlation with tumors presenting advanced stages. In gland-confined tumors, its expression does not correlate to the stage of the disease but with the Gleason score (25).

In the present study based on neuroendocrine cell immunomarkers chromogranin-A and synaptofisin, it was observed that in larger volume tumors with compromised seminal vesicle, there was a larger distinct expression or these markers, in two ways. It was observed a neuroendocrine differentiation of tumor cells and also a neuroendocrine cell hyperplasia in the normal tissue.

The PSA expression in pT3 stage was homogeneous and intense with cytoplasmic granular pattern in tissues from biopsies and surgical specimens. It was observed a lower coincident expression with tumors that presented a high Gleason score. Immunohistochemistry applied to PSA presents limitations, because the expression is observed in normal and neoplastic cells, showing that there is no correlation between serum concentration of this antigen and the intensity immunoreactivity of tissue. Immunohistochemistry for this marker can be useful as a method to assess its expression profile all through treatment (26).

The lack of E-caderin expression in the plasma membrane was observed in pT2 and pT3, with a percentage of 30 and 50% respectively. The lack of E-caderin expression relates to the advanced stage of the disease and metastasis. It is important to highlight that in certain metastatic tumors the E-caderin can return to its membrane expression. This finding remains without any clarification (27,28). The lack of E-caderin expression is being related to a high Gleason score, according to the work of Wu et al. (29).

The immunolabelling profile in the present work cannot establish correlations of expressive differences for immunomarkers with neuroendocrine cell expressions and cell proliferation indicators. However, in relation to E-caderin, it was observed lack of expression related to the tumor tissue.

Positive margins with or without post radical prostatectomy extra-prostatic extension has been suffering a wide percentage variation as the medical literature registers (31). Basic influences that origin positive margins reside in the more or less rigorous criteria of selecting the surgical patient and in the surgical strategy utilized.

We have observed in our results a higher incidence of recurrence in the cases with positive surgical margins. All patients of pT2 group with recurrence of the disease presented positive peripheral surgical margins, demonstrating the importance of this parameter mainly in stages that characterize a localized disease. Positive margins in pT2 (35%) and pT3 (65%) groups should warn, based on the consolidated prognostic factors, changes in the surgical strategy (32). Positive surgical margin is represented by the tumor that reaches the previously stained surgical specimen surface, independently from having or not extra-prostatic extension.

When comparing the mean time of biochemical recurrence between both groups it is evident the negative impact originated by the presence of positive margins in pT3 group, predicting the need for complementary treatment in a disease with a worst prognostic. Also, in pT3 group, 20% of the patients presented positive seminal vesicle. Even though there has not been observed any preoperative risk factor suggesting this condition, prognostic factors such as PSA, histological grade and percentage of positive fragments in the biopsy, have been correlated with seminal vesicles involvement (33).

In our sample, among the 20 patients with pT2 disease, none was identified with pT2b, reinforcing the difficulty of classifying such stage based on the surgical specimen (34).

It is worth to highlight that the 7 patients with positive margins and in the pT2 group did not present safe tissue parameters to evaluate the possibility of extra-prostatic extension. Thus, it remains the possibility that these patients were under staged and are in truth pT3.

It is evident the need to improve new methods, predominantly morphologic and molecular, that are able to further exploit the study of the material from the prostatic biopsy. As to the profile of the molecular markers used in both studied groups, there was no significant difference in the sense of outlining an additional prognostic factor in the clinical practice.

CONFLICT OF INTEREST

None declared.

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Erectile Dysfunction in Patients with Chronic Renal Failure

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ABSTRACT

Objective: Determine the prevalence of erectile dysfunction in patients undergoing hemodialysis.
Materials and Methods: This cross-sectional study was carried out to determine the prevalence of erectile dysfunction in a population of 58 patients in hemodialysis program. Erectile dysfunction was assessed by using the International Index of Erectile Function (IIEF). Information on demographic data, renal failure, comorbidities, laboratory tests and search for medical treatment for erectile dysfunction by means of interviews and researches in medical charts was obtained. Student t test was utilized to compare the laboratory results between group of patients with and without erectile dysfunction. The chi-square test was utilized to compare the comorbidities and the characteristics of the population studied between the groups of patients with and without erectile dysfunction. The significance level considered was 5%.
Results: Mean patient age was 50.2 ± 14.6 years and the time of hemodialysis was 30.4 ± 28.4 months. The prevalence of erectile dysfunction was 60.3%. A progressive increase respecting the age was reported. In patients younger than 50 years, this prevalence reached 31.4% and in patients older than 50 years, this prevalence reached 68.6%. With respect to the comorbidities, hypertensive patients prevailed with 94.8% of the total, whilst diabetic patients represented 24.9%. However only the association between diabetes and erectile dysfunction was significant. Patients with erectile dysfunction presented significantly lower values for serum creatinine and Kt/V. There was no variation between the groups with reference to calcium, potassium, phosphorus, hematocrit, hemoglobin, pre- and post-dialysis urea values. There was no correlation between erectile dysfunction and time of dialysis. Amongst patients with erectile dysfunction, 8.6% sought medical care.
Conclusions: The prevalence of erectile dysfunction in patients in hemodialysis program was of 60.3%. Age, diabetes and hemodialysis characteristics are associated to higher incidence of erectile dysfunction.

***Key words**: erectile dysfunction; chronic renal failure; hemodialysis; questionnaires*
Int Braz J Urol. 2007; 33: 673-8

INTRODUCTION

Life expectancy of patients with chronic renal failure (CRF) increased during the last decades with the improvement of renal replacement techniques - dialysis and renal transplantation. However, new complications or aggravation of preexisting diseases do impair the quality of life of these men. Erectile dysfunction (ED) is frequently observed in patients undergoing hemodialysis (HD) program, with prevalence ranging between 41% and 98% (1-12). From 1997, with the development of the International Index of Erectile Function (IIEF) (13), an instrument was created for uniform assessment of erectile dysfunc-

tion, by standardizing the questions and classifying the answers into categories. This study has been developed to assess erectile dysfunction prevalence in patients with Chronic Renal Failure utilizing the IIEF.

MATERIALS AND METHODS

Seventy patients older than 18 years have been selected in two hemodialysis centers and from these 58 patients (83%) agreed to participate in the research and signed the Informed Consent. The patients answered the IIEF questionnaire including the six questions about health-related determinants of the erectile function (questions number 1 to 5 and question number 15). The total score ranges from 1 to 30, by being characterized as severe [1 to 6], moderate [7 to 12], mild to moderate [13 to 18], mild [19 to 24], and no dysfunction [25 to 30].

In the standard questionnaire applied sociodemographic data have been obtained (age, civil status and education level), presence of comorbidities (arterial hypertension and diabetes mellitus - DM) and time of hemodialysis.

Hematocrit, hemoglobin, calcium, phosphorus, potassium, pre- and post-dialysis urea and Kt/V values have been all obtained from the medical charts, as well as the cause of the CRF.

Patients presenting ED were questioned if they have already searched for medical care for this problem.

Descriptive analysis of sociodemographic, clinic and laboratory data of the patients has been performed. The Student t test was utilized to compare laboratory results between patients with and without ED. The chi-square (χ^2) test was utilized to compare the comorbidities and the characteristics of the population studied between the groups of patients with and without ED. The significance level considered was 5%.

RESULTS

The study included fifty-eight (58) patients with age ranging from 21 to 76 years (mean age of 50.2 ± 14.6 years). The proportion between patients younger and older than 50 years was, respectively, 53.5% and 46.5%. Patients undergone hemodialysis for a minimum period of one week and a maximum period of 102 months (mean of 30.4 ± 28.4 months). Patients' data can be found in Table-1.

The prevalence of ED was 60.3% (Figure-1). Progressive increase respecting the age was found. In patients younger than 50 years, the prevalence of ED was 31.4%, reaching 68.6% in those patients older than 50 years ($p < 0.05$).

No statistic association between sociodemographic variables and ED has been found.

Hypertensive patients prevailed in the study (94.8%) with respect to the diabetic patients (24.9%). However only the association between DM and ED was significant (Table-2).

Time of dialysis was not a factor associated to the presence of ED in the population under study.

Table 1 – *Characteristics of the population studied.*

	Number of Patients	%
Age		
21 to 30	5	9
31 to 40	12	21
41 to 50	14	24
51 to 60	9	16
61 to 70	14	24
71 to 80	4	7
Ethnic group		
White	49	84.5
Non-white	9	15.5
Education		
None	5	8.6
Primary school	42	72.4
Secondary school	11	19
Civil status		
Single	6	10.3
Married	51	87.9
Widower	1	1.7
Diabetes mellitus		
Yes	15	24.9
No	43	74.1
Arterial hypertension		
Yes	55	94.8
No	3	5.2

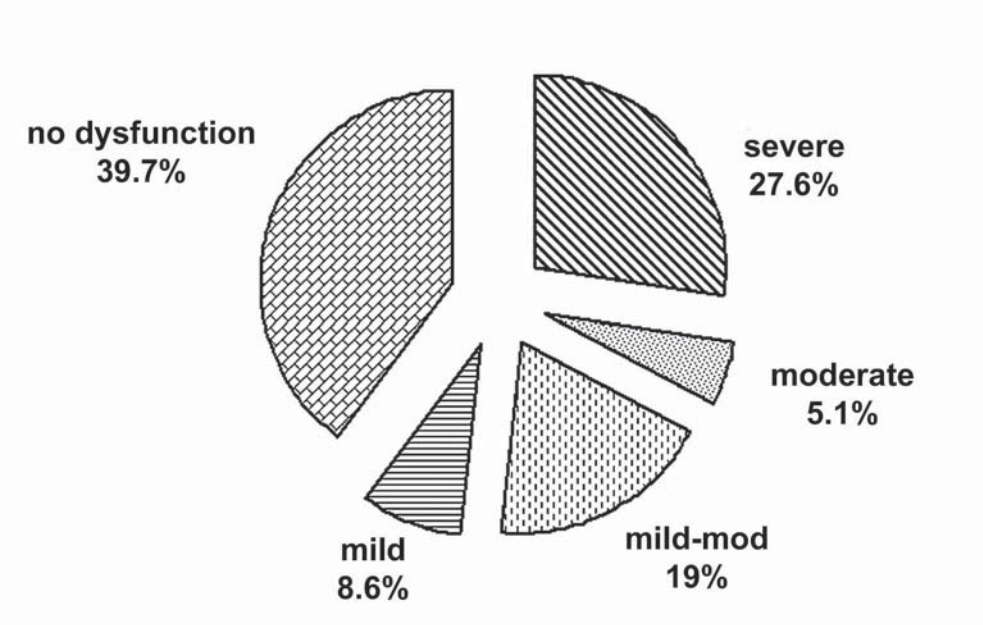

Figure 1 – *Erectile dysfunction in patients with chronic renal failure.*

Table 2 – *Results found in patients with and without erectile dysfunction (ED).*

	Without ED (N = 23)	**With ED** (N = 35)
Age		
Up to 50 years of age	20	11
Older than 51 years of age	3	24*
Diabetes mellitus		
No	22	21
Yes	1	14*
Arterial hypertension		
No	0	3
Yes	23	32
Serum creatinine (mg/dL)⁺	15.9 ± 4.5	11.6 ± 4.3*
Serum calcium (mg/dL)⁺	12.4 ± 17.8	8.7 ± 0.8
KT/V°	1.2 ± 0.4	0.9 ± 0.2*
Serum potassium (mEq)⁺	4.8 ± 0.7	4.7 ± 0.7
Hematocrit (%)⁺	30.9 ± 7.3	28.5 ± 7.5
Hemoglobin (g/dL)⁺	9.8 ± 2.3	8.9 ± 2.4
Phosphorus (mg/dL)⁺	8.9 ± 9.7	6.3 ± 1.7
Pre urea (mg/dL)⁺	155.1 ± 31.1	151.9 ± 38.8
Post urea (mg/dL)°	62.0 ± 24.5	72.3 ± 28.1

⁺ *= data expressed as mean ± standard deviation, * = p < 0.05*

Amongst the laboratory tests, only creatinine and the Kt/V presented static statistic relation with the ED (Table-2).

Only three patients (8.6%) searched medical treatment for ED.

COMMENTS

In our sample, the prevalence of ED was 60.3%. Taking into consideration the only severe category, the prevalence was 27.6%. Initial studies reported that ED in patients undergoing hemodialysis ranged between 41% and 93% (1-3). Since the methodology of such studies was not uniform, the comparison of the results found was not adequate. In studies in which the International Index of Erectile Function (IIEF) was utilized, the prevalence of ED ranged between 57.9% and 86.4% (4-12), evidencing that such dysfunction is frequent in patients with CRF (Table-3). Severe ED affects from 28% to 45% of these patients.

It is found in literature that ED is age-related (6-8,12,14). By stratifying the age of our patients below and above 50 yeas of age, we found, respectively, 31.4% and 68.6%, of ED prevalence.

The presence of hypertension found in our patients was 94.8%. However, this association was not significant. Several studies with chronic renal patients found in literature do confirm this finding (1-8). Feldman et al. (15) pointed out the occurrence of severe ED in 15% of hypertensive patients treated against 9.6% in the general population. The study does not indicate, however, if this association was originated from the hypertension or from the use of antihypertensive medication by considering that there is also an association between ED and the use of hypotensives. It can be argued that the high degree of vascular impairment (atherosclerosis) present in men with CRF undergoing hemodialysis is at least partially responsible for the erectile mechanism aggravation (15).

With reference to the DM, there was a significant statistic association to the ED. This study indicated that only one amongst the fifteen patients did not have ED. In the patients evaluated by Cerqueira et al. (5), 99% of the diabetic patients presented ED. This association has been also found in other studies (4,8,10,15).

No statistic association of ED has been found with reference to civil status, education or ethnic groups. In the MMAS longitudinal study, Johannes et al. (14) reported that the risk of developing age-related ED was higher in men with lower education degree. In our group, Moreira et al. (16) found that the education was inversely correlated to ED. Study carried out in four North-American cities, including 1,680 men older than 40 years of age, demonstrated that ED was not related to ethnic groups (17).

Time of dialysis was not also a factor associated to the presence of ED in the population under study, similar result was found by other authors (6-8).

Kt/V, measure of the quantity of plasma cleared of urea (K x t) divided by the volume of urea distribution (V) is an index utilized to assess dialysis adequacy. Patients with erectile dysfunction presented statistically significant lower values for Kt/V (0.9) when compared to patients without erectile dysfunction (1.2). Some authors (18,19) recommend one Kt/V of 1.3 for providing adequate hemodialysis. Therefore, the indexes found in our study can indicate that a hemodialysis within acceptable standards may contribute to prevent ED. This relation has not been found in other studies (1,4,7).

Miyata et al. (10) found in literature that higher values of hemoglobin were associated to severe ED. In our study, hematocrit and hemoglobin were both found to be higher in patients without ED, when compared with those with ED, but they show no statistic difference.

Table 3 – *International Index of Erectile Function (IIEF) in man with chronic renal failure.*

Author	Year	N	% DE
Turk, S et al.	2001	35	71
Rosas et al.	2001	302	82
Cerqueira et al.	2002	119	57.9
Arslan et al.	2002	187	80.7
Naya et al.	2002	174	86.2
Fernandes Neto et al.	2002	118	86.4
Ali ME et al.	2005	75	82.5

Amongst patients with ED, only three of them (8.6%) sought medical guidance. In the studies with chronic renal patients, the search for medical treatment ranged between 1% and 9.6% (7,8). It is found in literature that less than 10% of men seek medical care for ED (20). Moreira et al. (21) found that the number of individuals with ED that sought medical help was of 42%. The lack of approach regarding sexuality is caused, mostly, by health professionals that are not used to question the topic. The majority of the men doctors (62.5%) and women doctors (71.5%) reported that they do not routinely investigate the sexual function of the male patients. In a routine consultation, only 11.1% and 8.7% of the men doctors and women doctors, respectively, reported they were used to always investigate the sexual function of male patients. Amongst the patients, 78% of them did not refer about having problems to discuss sexual issues with their physicians (21).

CONCLUSIONS

The prevalence of ED in patients in hemodialysis program was 60.3%. However, only 8.6% of these patients search for medical help. Age, diabetes and hemodialysis characteristics are associated with higher incidence of ED. Physicians and other health professionals shall pay attention to the erection problems in this group of patients in order to provide directions for an adequate medical treatment.

CONFLICT OF INTEREST

None declared.

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Case Report
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Acute Renal Insufficiency after Radiofrequency of Renal Tumor

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ABSTRACT

Recent advances in techniques of imaging and ablation have led to the application of several minimally invasive modalities, such as radiofrequency ablation (RFA) with a success rate varying from 79 to 96% and a serious complication rate of 1 to 4% in the treatment of small renal tumors.
The authors report on the case of a 67-year-old patient with a radiofrequency ablation complication, stenosis of the ureteropelvic junction in one kidney, and analyze the results of this modality for the treatment of renal tumors.

***Key words**: kidney neoplasms; catheter ablation; renal insufficiency*
Int Braz J Urol. 2007; 33: 679-82

INTRODUCTION

Traditionally, the treatment of renal tumors included radical or partial nephrectomy. Minimally invasive treatment modalities such as cryotherapy and radiofrequency ablation (RFA) by percutaneous approach have been used in the treatment of carcinoma of the renal cells, offering some advantages, such as shorter convalescence, lesser pain, lower costs and better esthetic effect, if compared to conventional surgery (2).

The authors report on the case of a 67-year-old patient with a radiofrequency ablation complication, stenosis of the ureteropelvic junction (UPJ), in one kidney, and analyze the results of this modality for the treatment of renal tumors.

CASE REPORT

A 67-year-old man had undergone a radical left nephrectomy 5 years before due to a grade-1, 8 cm, carcinoma of the renal cells. During a routine consultation, using computed tomography (CT) a solid lesion was located in the lower medial region, in the remaining kidney (Figure-1). Biochemical assessment presented urea at 38 mg/dL and creatinine at 0.9 mg/dL. The patient was submitted to percutaneous RFA in February 2003, after which acute renal insufficiency developed (creatinine at 7.0 mg/dL), requiring urgent nephrostomy after fifteen days. Imaging confirmed the presence of stenosis of the UPJ (Figure-2), the patient having been submitted to endopielotomy with an unsuccessful attempt to place a double iota catheter.

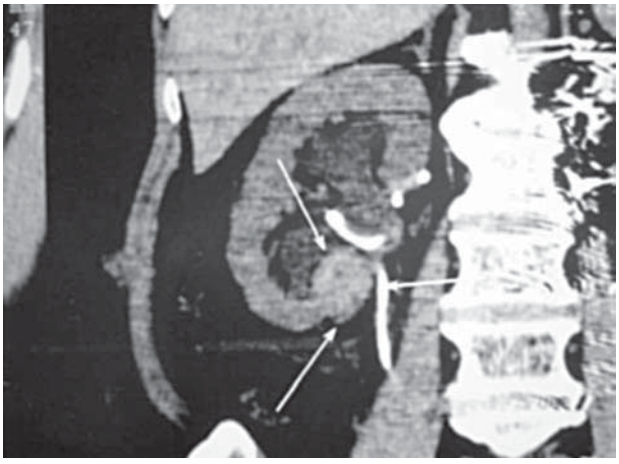

Figure 1 – *Computed tomography demonstrating renal nodule of 3.5 cm in remaining kidney.*

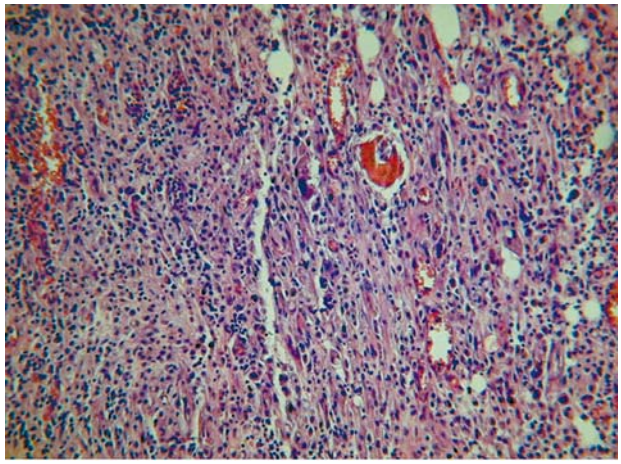

Figure 3 – *Necrotic area, fibrosis and granulomatose reaction of the foreign-body type. Absence of viable residual neoplasia (HE X400).*

The patient sought out our institution to verify the therapeutic possibilities. Open pyeloplasty was recommended, followed by the enucleation of the renal nodule. The pathological examination revealed a necrotic area with the formation of abscesses, fibrosis and a granulomatose reaction of the foreign-body type, with no evidence of a viable tumor (Figure-3). The patient progressed well (Figure-4), currently having a creatinine level of 1.9 mg/dL.

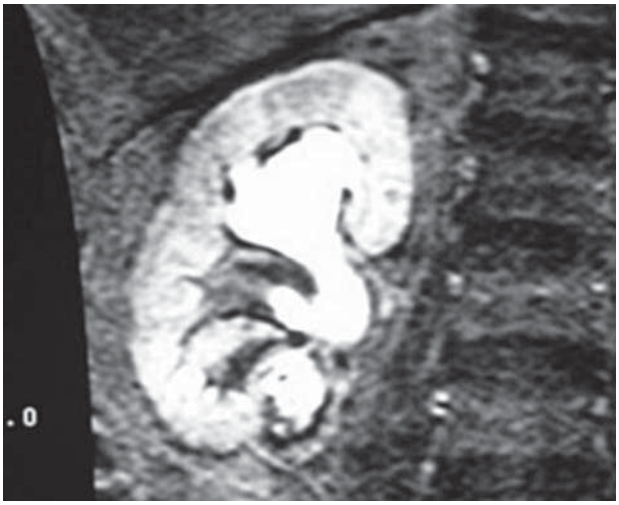

Figure 2 – *Stenosis of ureteropelvic junction by magnetic resonance.*

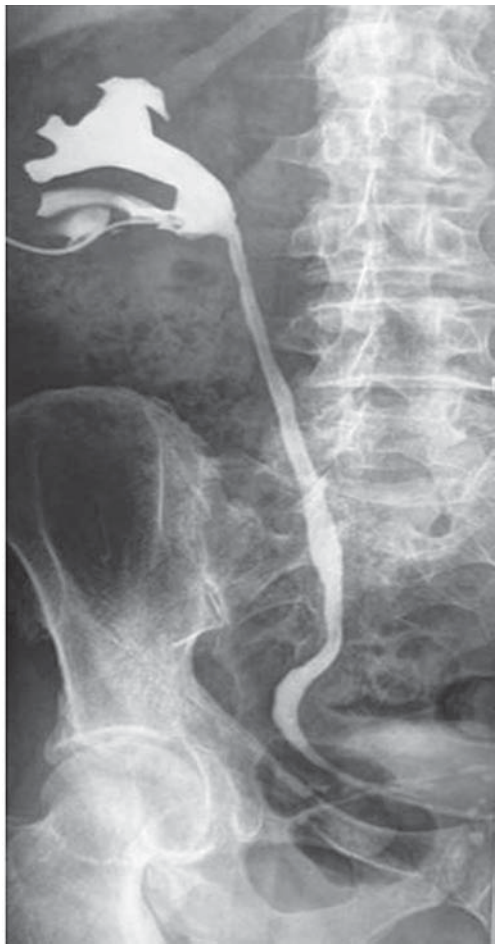

Figure 4 – *Antegrade pyelogram demonstrating evident ureteropelvic junction.*

COMMENTS

RFA has been used recently as a new treatment option for small renal tumors with a success rate of 79 to 96% of the cases (2,3), the incidence of serious complications, such as intestinal lesion, cutaneous fistula, urethral stenosis and pneumothorax, occurs in 1 to 4% (1,2).

Radiofrequency ablation (RFA) is to be recommended for the treatment of renal tumors of less than 3 cm, which have given signs of growth during the period of one year. Surgical approach may vary either by means of percutaneous puncture (3) or by laparoscopy (2). The principle of RFA involves heating to high temperatures (< 70 degrees C) thus provoking necrosis of coagulation and cell death (2,3). The criteria of inclusion for RFA are solid lesions < 3 cm, which have been growing over the previous year, creatinine below 2.0 mg/dL and 24-hour creatinine clearance greater than 60 mL/min (2,3). The position of the tumor (posterior, lateral or medial) has not been considered among the exclusion criteria, although the proximity of the colon, duodenum or of important vessels is a limiting factor for this technique (1-3). The most frequent complications arising from RFA are hematuria (4 to 8%), proteinuria (16%), low back pain (16%) and perirenal hematoma (4%), and these are treated conservatively (2,3).

The criterion of cure is confirmed by the absence of the visualization of contrast (< 10UH) on tomography, with a success rate of 79 to 96% (2,3).

Stenosis of the UPJ may occur in 4% of the cases, being presented after two months (2). According to some authors, the position of the tumor does not constitute a criterion of exclusion, but rather a limitation of the applicability of the technique (2,3). According to Hwang et al., open pyeloplasty is the best way to deal with this complication (2), particularly in the reported case, as we are dealing with a sole kidney in a rather delicate situation. An alternative technique in the case of an extensive lesion would be the interposition of the loop ileal.

The RFA of small renal tumors, whether by percutaneous approach or laparoscopy, still requires further study for the assessment of the method's efficiency and safety. If the long-term results are favorable, then RFA could be an attractive treatment option for solid renal lesions.

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

The management of small renal tumors is changing over the years to a nephron-sparing surgery. Of the various ablation techniques, radiofrequency ablation and cryotherapy are being increasingly applied clinically (1). They can be performed both laparoscopically or percutaneously using a combination of probes and imaging techniques for focusing and monitoring the therapy. Noninvasive tumor ablation by high-intensity focused ultrasound, and other techniques, are still on experimental stage.

Although the initial outcomes of cryoablation and radiofrequency ablation are encouraging, long-term data are necessary to confirm their efficacy. Early reports of the technique's effectiveness are promising (2). Dr Inderbir Gill from the Cleveland Clinic published 51 patients undergoing cryotherapy for a unilateral, sporadic renal tumor with a 3-year cancer specific survival of 98%. There was no open conversion, kidney loss, urinary fistula, dialysis requirement, or perirenal or port site recurrence in any patient.

These ablative techniques should be reserved for carefully selected patients, the data should be prospectively studied and the results should be compared to the standard treatment, open or laparoscopic partial nephrectomy.

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Intracaval and Intracardiac Extension of Wilms' Tumor. The Influence of Preoperative Chemotherapy on Surgical Morbidity

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ABSTRACT

Objectives: The aim of this retrospective study is to compare surgical complications and long-term survival in children with Wilms' tumor (WT) and tumor thrombus receiving or not preoperative chemotherapy.
Materials and Methods: Review of the charts of 155 children with WT treated between 1983 and 2005, and analysis of 16/155 (10.3%) children with WT who presented cavoatrial tumor extension, being 8/16 IVC and 8/16 atrial thrombus.
Results: Median age was 54 months. 2/16 had cardiac failure as the first symptom. 11/16(7 IVC and 4 atrial extension) (67%) were submitted to preoperative chemotherapy with vincristine plus actinomycin D, and 5/16(1 IVC and 4 atrial) (33%) underwent initial nephrectomy and thrombus resection. So, 11 patients were submitted to preoperative VCR/ACTD and 2/11 (18.1%) had complete regression of the thrombus, 6/11(54.5%) partial regression and 3/11 (27%) had no response. Among the partial responders, nephrectomy with thrombus removal was performed in all, including one patient with previous intracardiac involvement, without extracorporeal circulation procedures. In two of the three non-responders, cardiopulmonary bypass was necessary for thrombus removal. There were no surgical related deaths. Long-term survival is 91% in the group submitted to preoperative chemotherapy and 100% in the group who had surgery as first approach.
Conclusion: Preoperative chemotherapy was able to reduce thrombus extension in 8/11 (73%) treated patients and cardiopulmonary bypass was avoided in 2 patients with atrial thrombus. Surgical resection of tumor and thrombus was successful in all cases, receiving or not preoperative chemotherapy and overall survival was similar in both groups.

***Key words**: Wilms tumors; thrombus; vena cava; cardiac; chemotherapy; surgery*
Int Braz J Urol. 2007; 33: 683-9

INTRODUCTION

Inferior vena cava (IVC) involvement by Wilms' tumor occurs in 4-10% of patients and right atrium thrombus extension in less than 1% (1). This complication does not influence on the prognosis of the malignancy, but it makes surgical procedures more challenging, mostly when there is intracardiac involvement. Surgery used to be the first recommended approach, but some authors report the effectiveness of preoperative chemotherapy in reducing or eradicating the thrombus, and also reducing the tumor dimensions and making surgery easier to perform (2).

The aim of this retrospective study is to compare surgical morbidity and outcome of patients

with Wilms' tumor and cavoatrial thrombus who received or not preoperative chemotherapy.

MATERIALS AND METHODS

A retrospective review of the charts of 155 children with Wilms' tumor admitted to the University of Sao Paulo from June/1983 through April/2005 was performed in order to select those with intracaval (IVC) or intracardiac thrombus. Among them, 16/155 (10.3%) children presented intravascular thrombus, being the last one diagnosed in April/2001. The patients were treated with an institutional protocol from 1983-2001. These 16 patients were analyzed for the use or not of preoperative chemotherapy, effect of preoperative chemotherapy regarding thrombus extension, surgical morbidity, intraoperatory time, use of cardiopulmonary bypass, postoperative complications, number of hospitalization days, transfusion amount and influence on disease outcome. Fisher's test, Mann-Whitney and Kaplan-Meyer curve were employed for statistical analysis.

RESULTS

Sixteen patients were selected. Eight had IVC (5.15%) and eight (5.15%) had atrial tumor involvement. There were nine females and seven males. Median age was 54 months, ranging from one year through 8 years. In 13 patients, the tumor arose from the right kidney and in 3 from the left. Hematuria was presented by 4/16 patients, hypertension by 2/16 and 2/16 had cardiac failure as first symptom. All thrombus were preoperatively detected by ultrasonography in 13/16 patients, Doppler echography in 5/16, abdominal CT in 3/16 (Figure-1) and IVU in 3/16.

Median tumor dimension at diagnosis was 120 X 80 mm. Stage II disease was observed in six patients, stage III in seven and stage IV in three. Histology was favorable in 13 patients and unfavorable in three.

Among the 8 patients with tumor extension into the IVC, in 5/8 the thrombus was infrahepatic and in 3/8 there was a suprahepatic involvement.

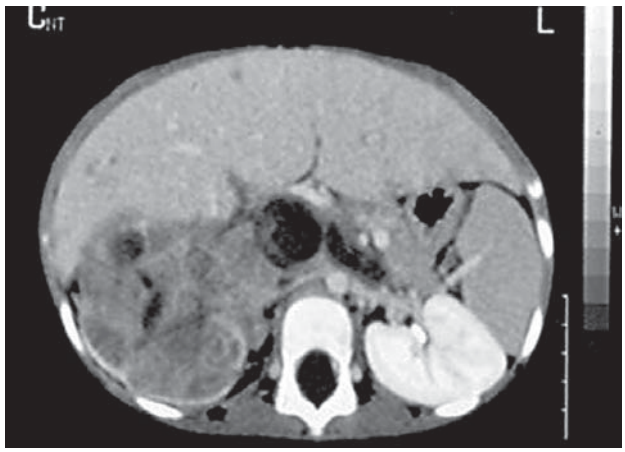

Figure 1 – *CT scan revealing thrombus in the vena cava in a patient with Wilms' tumor.*

Preoperative chemotherapy with vincristine 1.5 mg/ m^2/ day and 21 plus actinomycinD 1.5 mg/m^2/ day for four to six weeks was administered to 7/8 of these patients with IVC involvement and in 1/8 IVC thrombus, a primary surgical resection was carried out.

In the group of 8 patients with intracardiac thrombus extension, 4/8 were submitted to the same preoperative chemotherapy schedule and 4/8 went to surgery after diagnosis because of poor clinical situation (2 cardiac failure) or surgeon's preference (2 cases of the early 80's).

A total of 11/16 patients were submitted to preoperative VCR/ACTD for four to six weeks and 2/11 (18.1%) had complete regression of the thrombus, 6/11(54.5%) partial regression and 3/11 (27.2%) had no response (Figure-2). There was no correlation between the duration of preoperative chemotherapy and response. Among the partial responders, nephrectomy with thrombus removal was performed without cardiopulmonary bypass in all, including one patient with previous intracardiac involvement, obviating the use of cardiopulmonary bypass. In two of the three non-responders cardiopulmonary bypass was necessary for thrombus removal (Table-1). Hypothermia was used in four patients with atrial thrombus. Radiotherapy was not used in any patient previously to surgery.

Transfusion mean amount was 803 mL (± 678 mL) in the preoperative chemotherapy group and 1536

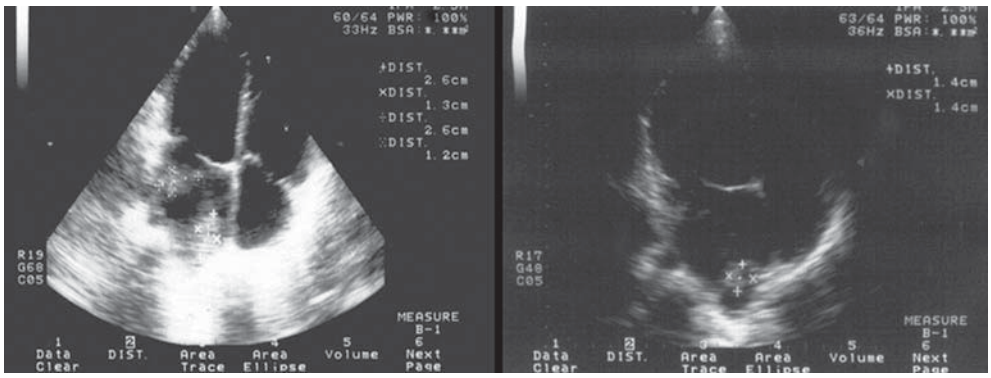

Figure 2 – *Echocardiographic evaluation of a cardiac thrombus before and after preoperative chemotherapy: absence of response.*

mL (± 1001 mL) in the other group, statistically not different values (p = 0.2159). Cardiopulmonary bypass was more frequently used in the non-preoperative chemotherapy group (p = 0.036). Comparing patients not submitted to CBP with patients submitted to CBP, we have observed that the first group had lesser transfusion amount (p < 0.001), shorter operative time (p = 0.001) and shorter hospital stay (p = 0.001).

Mean operative time was 227.7 minutes (±89.2 min.) in the preoperative chemotherapy group and 369 minutes (±110 min.) in the other one, revealing a quite longer time for the non- preoperative chemotherapy group (p = 0.0263). Mean hospital stay was 5.72 days (± 7.1 days) in the preoperative chemotherapy group and 9 days (± 4.9 days) in the other one, a significant difference (p = 0.0342).

Postoperative infectious complications were observed in two patients, one with and one without preoperative chemotherapy, resulting in a longer hospitalization period. No other complications were presented.

The histopathological analysis of the removed thrombus revealed viable tumor in 6/11 (54.5%) patients submitted to preoperative chemotherapy, all of them being favorable histology tumors.

Long-term survival was 91% in the previously treated group (one child with a chemotherapy resistant anaplastic tumor had a local relapse and died), and 100% in the group not submitted to preoperative chemotherapy. There was no difference between both groups regarding survival rate (p = 0.50), Figure-3. The median follow-up is 177.3 months for the whole group.

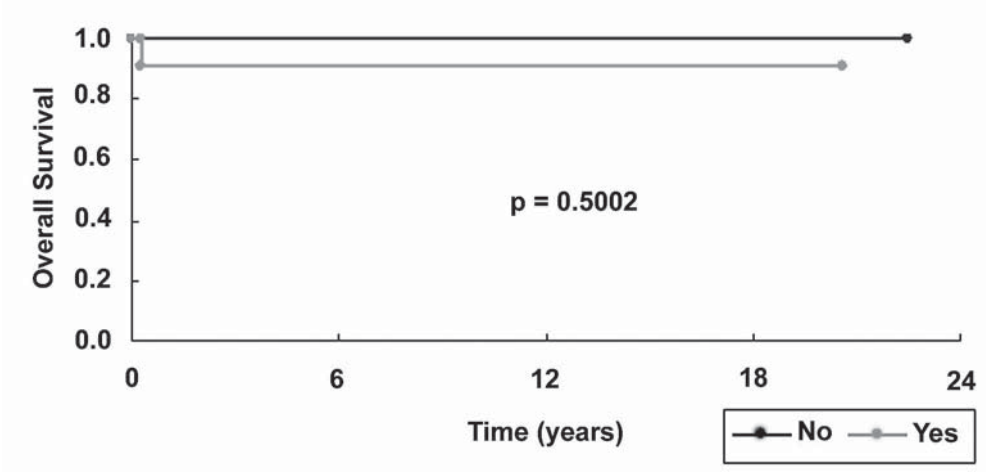

Figure 3 – *Overall survival of patients with Wilms' tumor and intravascular thrombus, receiving or not preoperative chemotherapy.*

Table 1 – *Study population: characteristics of the thrombus at diagnosis and at surgery, use or not of preoperative chemotherapy, cardiopulmonary bypass, hypothermia, transfusion amount, surgical time, hospital stay and stage.*

Case	Thrombus Extension at Diagnosis	Preoperative VCR/ACTD	Response	Thrombus Extension at Surgery	CBP	Hypothermia	Transfusion (mL)	Surgical Time	Hospital Stay (d)	Stage
1	vena cava infrahepatic	yes	complete	-	no	no	-	4h	3	III
2	vena cava infrahepatic	yes	partial	vena cava infrahepatic	no	no	250	3h	5	II
3	vena cava infrahepatic	yes	partial	vena cava infrahepatic	no	no	-	3h30min	5	III
4	vena cava suprahepatic	yes	partial	renal vein	no	no	-	2h30min	4	II
5	vena cava infrahepatic	yes	partial	renal vein	no	no	-	3h	3	II
6	vena cava suprahepatic	yes	partial	vena cava infrahepatic	no	no	-	2h45min	2	II
7	vena cava infrahepatic	yes	none	vena cava infrahepatic	no	no	-	4h30min	3	IV
8	vena cava suprahepatic	no	-	vena cava suprahepatic	no	no	-	4h	4	III
9	right atrium	yes	partial	vena cava infrahepatic	no	no	-	3h	2	III
10	right atrium	yes	none	right atrium	yes	yes	600	6h	27	II
11	right atrium	yes	none	right atrium	yes	yes	1560	7h	6	II
12	right atrium	yes	complete	-	no	no	-	2h30min	3	IV
13	right atrium	no	-	right atrium	yes	yes	1750	5h	7	IV
14	right atrium	no	-	right atrium	yes	no	2900	8h45min	17	III
15	right atrium	no	-	right atrium	yes	yes	700	6h	7	III
16	right atrium	no	-	right atrium	yes	no	900	7h	10	III

COMMENTS

The incidence of intravascular thrombus extension in our study population was 10.3%, similar to other authors' findings, but intracardiac involvement was 5.5%, a little higher than the reported experience (3,4). The thrombus occurrence represents a remarkable difficult factor for surgical procedures, increasing morbidity. In the NWTSG-4 intravascular tumor extension presented an increased risk for complications (odds ratio 3.8, 95% confidence interval) (5). It should be an elective procedure, performed by a multidisciplinary team. Tumor thrombus extending into the suprahepatic IVC (type III) and right atrium (type IV) requires cardiopulmonary bypass, with or without circulatory arrest, for removal (6). Cardiopulmonary bypass includes the use of median sternotomy, atriotomy and systemic anticoagulation (7). It elongates intraoperatory time, exposing the patient to hypothermia, blood transfusion, cardiac arrest, pericardic-patch and to the complications related to these methods (8). Preoperative chemotherapy is recommended by many authors because it is able to promote significant tumor and thrombus shrinkage, and may facilitate the surgical approach and tumor resection, avoiding tumor rupture and neoplastic cells spillage (9), but in some cases, mostly in patients with cardiac thrombus, the risks of immediate cardiorespiratory dysfunction due to thromboembolism makes surgery the first recommended approach (10).

In our retrospective analysis, preoperative chemotherapy with VCR / ACTD has induced thrombus shrinkage in 8/11(72.7%) treated patients, but in 3/11(27.3%) it was ineffective regarding thrombus extension. Shamberger et al. reported a 79.5% incidence of tumor regression in similar cases (11). Tumor or thrombus progression or toxicity during preoperative chemotherapy is a concern for some authors, but it has not occurred in our patients (12,13).

The 7/8 patients with intracaval thrombus that were submitted to preoperative chemotherapy presented 1/7(14.3%) complete regression of the thrombus, 1/7(14.3%) failure and 5/7(71.4%) partial response. As CBP is the reason for a longer operative time ($p < 0.001$), higher transfusion amount ($p < 0.001$) and longer hospital stay ($p < 0.001$), avoiding CBP is the objective of the use of preoperative chemotherapy. Cardiopulmonary bypass was not necessary for thrombus removal in all cases of IVC thrombus and in 2/4 patients with intracardiac thrombus, who were supposed to be submitted to invasive surgical procedures with CBP, and after receiving preoperative chemotherapy presented a partial or total thrombus decrease. The remaining 2/4 presented no change in thrombus extension and surgery with cardiopulmonary bypass was performed with success. The other 4/8 children with intracardiac thrombus were treated exclusively with surgery, two due to critical clinical situation at diagnosis (cardiac failure) demanding prompt intervention and the two others due to surgeons' own decision. These cases were treated at the early 80's and a more aggressive initial approach was recommended at our hospital. All were successful procedures, with no intra or postoperative deaths.

The mean transfusion amount was similar in both groups ($p = 0.2159$), but the group submitted to preoperative chemotherapy had advantages such as shorter operative time ($p = 0.0263$) and shorter hospital stay ($p = 0.0342$).

Complications like infection, tumor progression, tumor rupture, thromboembolism, hemorrhage and death are a major concern in this situation. The NWTS-4 reports a complication incidence rate of 26% for children with initial surgical resection and 13.2% for those with preoperative chemotherapy ($p = 0.053$) (11). The complications incidence rate in NWTS-3 is 43% (14, 15). The SIOP/GPOH group reports 18.18% of complications in 33 children with Wilms' tumor and thrombus extension, 29/33 submitted to preoperative chemotherapy (16). The UKW3 trial reports 13.6% of hemorrhagic complications, including 3 deaths (17). In our study, 7/16 (44%) patients required blood transfusions due to the surgical procedures, and 2/16(12.5%) patients had infectious complications after surgery, one patient with and one without preoperative treatment. No intraoperative or postoperative deaths were observed.

This is a twenty-three-year experience and along this long period of time some changes in medical approach of this situation have occurred in our institution, including the patterns for indication of initial

surgery, the indication and the drugs used for preoperative chemotherapy and the postoperative management. Some authors recommend preoperative chemotherapy for those patients whose tumors are at or above the supra-hepatic vena cava (COG/NWTS), but many years ago, a surgical first approach was considered by some surgeons in our institution.

The five-year overall survival was 100% in the not treated group and 91% in the other, with one death due to local relapse in a patient with anaplastic tumor submitted to preoperative chemotherapy with partial response.

CONCLUSION

In conclusion, preoperative chemotherapy was able to reduce thrombus extension in 8/11 patients and cardiopulmonary bypass was avoidable in at least two patients. Although surgical procedures were successful in all cases, receiving or not preoperative chemotherapy, the first group had some significant advantages such as shorter operative time and shorter hospital stay, suggesting the benefits of the preoperative use of VCR/ACTD in patients with Wilms' tumor and intravascular thrombus extension.

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

Vascular extension in patients with Wilms' tumor occurs in 5 - 10%. This situation consists in a surgical challenge. Preoperative chemotherapy seems to benefit those patients, although there are few references about tumor vascular extension in literature (1,2). Both NWTS and SIOP protocols recommend that preoperative chemotherapy should be done. The presented paper shows that preoperative chemotherapy does not change the survival probability and have some advantages when compared to the non preoperative chemotherapy group, such as shorter operative times, blood loss and days of hospitalization. It also prevented some patients from cardiopulmonary bypass. However, the authors did not make any relation between blood loss and the type of procedure performed. They reported that 11 patients received preoperative chemotherapy. Four of those had extension until the right atrium, but only two needed cardiac bypass. The group of patients without preoperative chemotherapy (n = 5), four had extension until the right atrium and all of them were operated with cardiac bypass, which lead to a greater blood loss (bias). Therefore, this paper shows one institution's experience in a rare situation and confirms the results of literature.

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Pubovaginal Sling with a Low-Cost Polypropylene Mesh

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ABSTRACT

Objective: The aim of this study was to present the results of pubovaginal sling with a low-cost polypropylene mesh in the treatment of stress urinary incontinence.
Materials and Methods: 118 women diagnosed with stress urinary incontinence (SUI) due to urethral hypermobility or intrinsic sphincteric deficiency, treated with pubovaginal sling (PVS) with a low-cost polypropylene mesh confectioned by the surgeon, were analyzed. All patients had a basic evaluation that included a medical history, physical examination, stress tests and urodynamic investigation.
Results: The average follow up was of 42 months. Urethral hypermobility was observed in 67% of the cases. The process was carried out on an outpatient basis on 67 patients. Intra-operative complications included 4 vesical injuries, treated with catheterization for 3 days. There were 13 early complications, of which 8 were urinary retentions treated with vesical drainage for 1 to 3 weeks and 3 vaginal extrusions of the mesh treated with covering of the sections with mucous membrane. There was a need for 6 urethrolysis in patients who presented irritative and postoperative obstructive symptoms; 81.3% of the patients were considered cured, while 9.3% had significant improvement. Three initially unsuccessful cases required sling reconfiguration. All cases were eventually cured.
Conclusion: The construction of a pubovaginal sling using a low-cost polypropylene mesh is a safe and effective technique for the relief of SUI. It should be considered an alternative, especially for patients in public health systems with low financial resources.

***Key words**: urinary incontinence, stress; prostheses and implants; suburethral slings; polypropylene*
Int Braz J Urol. 2007; 33: 690-94

INTRODUCTION

Suburethral slings have become the preferred technique for the treatment of stress urinary incontinence (SUI) (1). The Integral Theory and concept of a medium tension free urethral sling, surgical refinements and new materials allowed these procedures to be carried out in a non invasive manner, with low morbidity rates and satisfactory results (2-4).

Various materials were used in the slings, from autogenous tissues to synthetic materials, and the choices were based upon well-defined criteria, in which the cost was one of the most important factors for the selection of materials, mainly in countries with limited public health resources (5).

Studies confirm that the choice of a tension free polypropylene mesh allow high success rates and the TVT® simplified the SUI therapy, becoming one of most common options for the treatment of this disease (3,6). Thus, the industry has offered different kits to make the slings, but most of the time the costs are prohibitive for public health systems with few financial resources.

The objective of this study is to analyze the results of pubovaginal sling (PVS) using a low-cost polypropylene mesh for the treatment of stress urinary incontinence (SUI).

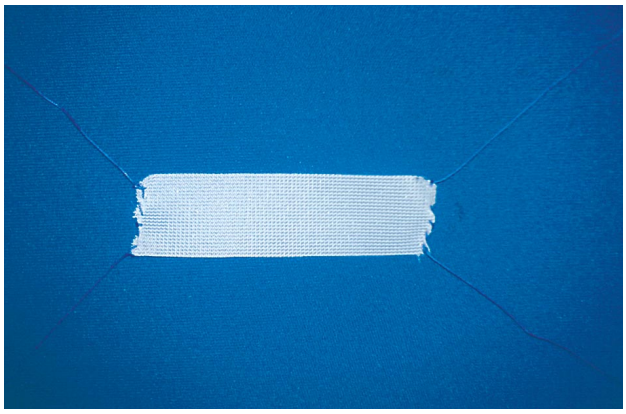

Figure 1 *– Polypropylene mesh with poygalactyne-0 thread fixed to the extremities, confectioned by the surgeon.*

MATERIALS AND METHODS

A retrospective study of 118 women with SUI treated with PS using a low cost polypropylene mesh was conducted from April 2002 to April 2006. The preoperative evaluation consisted of the medical history, urogynecological examination, stress tests and a urodynamic assessment cystometry, measure of the leak point pressure and flux-pressure study. The etiology of SUI was considered urethral hypermobility when the leak point pressure under stress (VLPP) was greater than 60 cmH_2O, and intrinsic sphincteric deficiency (ISD) when the pressure was below that value.

The follow up was carried out with consultations in the first month after the treatment and every 4 months, in which stress tests and clinical histories were obtained. Urodynamic studies were indicated in patients with persistence of SUI, irritative and/or moderate obstructive symptoms, or urinary retention 30 days after the surgical procedure.

Patients who related satisfaction and did not express urinary loss after stress tests were considered cured. Patients in use of pads for precaution, due to minimal urinary loss, but satisfied with the results were considered patients with significant recovery. The other cases were classified as failures.

Surgical Technique

All the procedures were carried out under spinal anesthesia, except 1 patient who underwent concomitant laparoscopic surgery. Patients received first generation cephalosporin as a prophylactic antibiotic. The surgical technique adopted was similar to the technique described by Almeida and Raz (6), using a polypropylene mesh of 10 x 1.5 cm, with polygalactyne-0 threads fixed at each extremity of the mesh, confectioned by the surgeon (Figure-1).

A longitudinal incision of 2 cm was made on the anterior vaginal wall, 1 cm from the urethral meatus, and dissection of the periurethral spaces was done. The endopelvic fascia was opened on each side, with access to the retropubic space. A hypogastric incision of 2 cm was made on the upper border of the pubic bone, allowing the passage of the long tongs to the retropubic space, bringing them out through the vaginal incision at each side of the urethra. The threads at the extremities of the mesh were held by the tongs and pulled till the abdominal incision, where they were tied after confirmation of the absence of vesical injuries by cystoscopy, in such a manner that the mesh stays below the urethra, free of tension.

RESULTS

The average age was 52 years (29-77 years). From the 118 patients, 2 had been treated previously with Burch colposuspension and 23 received surgical indications after failure of perineal physiotherapy. Eighty patients were diagnosed with urethral hypermobility (67%), from which 4 presented detrusor hyperactivity, and 38 were diagnosed with ISD

(33%). The stress test was positive in all cases. Fifty six women (48%) used pads daily, with an average of 1.7 pad/day. The medium VLPP was 84 cmH_2O. Fifty-two patients (44%) presented irritative symptoms before the surgery.

Procedures associated to PVS were carried out in 17 cases, described in Table-1. The average operative time was 49 minutes, including other concomitant procedures. The surgery was carried out on an outpatient basis on 67 patients (56%), with an average operative time of 28 minutes. Four vesical injuries (3.3%) were observed during the procedures and were treated with a vesical tube for 3 days. No cases of substantial hemorrhage were observed.

Post-operative complications are described in Table-2. All cases of acute urinary retention were resolved with vesical tubes for 1-3 weeks. The 3 cases of vaginal mesh extrusion (2.5%) were treated with coverage by the vaginal mucosa, with success in 2 patients. The third patient presented recurrent erosion, requiring removal of the mesh segment, but persisting continent.

Average post-operative follow up was 42 months (14-61 months). After the procedure, 5 patients presented irritative urinary symptoms "de novo" (4.2%), without detrusor hyperactivity on postoperative urodynamics. All patients with preoperative detrusor hyperactivity continued with irritative symptoms, however without SUI. From the 52 patients with preoperative irritative symptoms, 40 (76.9%) reported improvement of symptoms. There was necessity for urethrolysis in 6 patients (5%) who continued with significant irritative symptoms and a suspicious infravesical obstruction on urodynamics. Three patients from this group continued continent, while the others underwent a new PVS using the initial technique, and presented incontinence resolution.

Ninety six patients (81.3%) were considered cured, and 11 patients (9.3%) presented significant improvement of incontinence. From the 11 women who did not benefit from the treatment, 3 underwent a new PVS, achieving continence. The others are being followed up by the perineal physiotherapy staff.

Table 1 – *Procedures associated with the pubovaginal sling.*

Procedures	Number of Cases
Posterior perineoplasty	10
Vaginal hysterectomy + perineoplasty	2
Vaginal hysterectomy	1
Excision of Gardner cyst	1
Abdominal hysterectomy	1
Paraurethral cyst correction	1
Laparoscopic treatment of renal cyst	1

COMMENTS

The Integral Theory postulates that female urinary continence occurs due to the closing of the medium portion of the urethra, which depends on the integrity of pubourethral ligaments and the suburethral support given by the anterior wall of the vagina. Similar to TVT®, the technique discussed here is based on this theory, positioning the sling in the medial third of the urethra, free of tension. The principal difference

Table 2 – *Postoperative complications and treatments.*

Complication	Number of Cases	Treatment
Pubic hematoma	1	Conservative
Acute vaginitis	1	Antibiotics
Vaginal erosion of mesh	3	Covering of the mesh with vaginal mucosa Mesh removal (1 case)
Acute urinary retention	8 cases	Bladder drainage (1 to 3 weeks)

lies in the cost of the material used, since in this study the same propylene mesh was used, however it was prepared by the surgeon, instead of the specific commercialized material for this procedure. The propylene mesh and polygalactyne thread approximately costs U$ 15.00.

The sling made of autogenous tissue, like the abdominal rectus fascia or fascia lata, has shown good long-term results. However, the high morbidity rates associated to the procedure pose a disadvantage (7-9). The use of cadaverous fascia would eliminate some inconveniences and, in short term, produce similar results to that observed with autogenous slings (10); however, further studies have not confirmed these initial good results (11). This scenario led to the development of synthetic materials for slings, especially polypropylene, which is very durable, has low indices of rejection and is easily available.

The main intraoperative complications of PVS were vesical perforation and hemorrhage. Bousted (4), in a metanalysis, observed 6.9% of vesical perforations in 160.000 patients treated with TVT®. Tamoussino et al. (12) verified vesical injuries in 2.7% of 2.795 patients from the Austrian series. According to data from Meshia et al. (13), bleeding was reported in 15 % of the cases. In this series, low incidence of intraoperative complications was observed, including 4 vesical injuries (3.3%), and there were no significant cases of hemorrhage.

Postoperative complications included 8 cases of urinary retention (6.7%), and vaginal erosion of the mesh in 3 patients (2.5%); there were also isolated cases of pubic hematoma and acute vaginitis. A variable incidence of urinary retention after the TVT® was reported (2.3%-43%), in which an increase is observed when associated to other pelvic procedures (14,15). The vaginal erosion of the mesh was observed in approximately 1% of the cases. Patients who presented this complication were treated with simple procedures, with good results.

Resolution of preoperative irritable symptoms was observed in 76.9% of the women, and 4.2% presented “de novo” urinary urgency, without detrusor hyperactivity. The incidence of this symptom is not consistent in literature, being reported in 1% to 35% of patients (16,17). In this study, obstructive and/or irritative symptoms persisted in 6 patients (5%), and all underwent urethrolysis, with improvement in all cases.

Bousted analyzed the results from 16 studies about TVT®, with a minimum follow up of 12 months, observing objective cure rates of 80% to 96%, and 5% to 17% of significant improvement (4). In the present study, the cure rate (81.3%) and significant improvement (9.3%) are similar to those obtained by TVT®, even in patients diagnosed with ISD, whose results tend to be worse. Rezapour et al. (18) obtained cure in 74% and an important improvement in 12% of the patients with ISD that underwent sling with TVT®.

CONCLUSION

Complications and cure rates of the pubovaginal sling (PVS) using a low cost polypropylene mesh can be compared to those of TVT® for the treatment of SUI, with an advantage of lower costs. This procedure should be considered as an alternative to PVS with commercial kits, mainly for patients of public health systems with few financial resources.

CONFLICT OF INTEREST

None declared.

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Clinical and Urodynamic Evaluation in Women with Stress Urinary Incontinence Treated by Periurethral Collagen Injection

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ABSTRACT

Objective: To evaluate the success of treatment with periurethral collagen injections in patients suffering from stress urinary incontinence (SUI) with bladder neck hypermobility and intrinsic sphincter deficiency.
Materials and Methods: Forty women suffering from (SUI) were selected and divided into GI (consisting of 13 women with SUI and bladder neck hypermobility) and GII (consisting of 27 women with SUI and intrinsic sphincter deficiency). Periurethral collagen was injected followed by a subjective evaluation (the need for urinary protectors) and an objective evaluation through urodynamic study before and after the treatment.
Results: It was noticed that after 9 months there was a decrease in the need of urinary protectors in the two groups. It was observed through the urodynamic study that either cure or improvement was achieved in 46% in GI and 40.7% in GII. There was a significant increase in the leak pressure in GII. Moreover, there was a decrease in the volume of urine leak in the two groups, being the results in GII statistically significant.
Conclusions: It was concluded that the periurethral collagen injection is useful for the treatment of the SUI. The results in hypermobility are similar to those in intrinsic sphincter deficiency. In fact, it is a very simple out patient's procedure, with little side effects.

***Key words**: urinary incontinence, stress; urinary sphincter; injections; collagen*
Int Braz J Urol. 2007; 33: 695-703

INTRODUCTION

In its latest publication, the ICS (International Continence Society) defines the urinary incontinence as the complaint of any involuntary leakage of urine. The stress urinary incontinence (SUI) is the complaint of involuntary leakage on effort or exertion, or on sneezing or coughing. It is the most common type of urinary incontinence (1).

The stress urinary incontinence affects 10% to 30% of women above 50 years of age. Patients with intrinsic sphincter deficiency (ISD) present high grade stress urinary incontinence and have low abdominal leak point pressures on urodynamic studies.

On the other hand, those with bladder neck hypermobility present low grade stress urinary incontinence (2).

Periurethral injection is being used for almost one century for the treatment of stress urinary incontinence. Several substances have been employed, and among them Teflon, autologous fat, silicone microimplants, Durasphere, Zuidex and bovine collagen (2-4). The injection aims at increasing urethral strength, avoiding thus urinary leak.

Periurethral collagen injection (PCI) has been used in the treatment of SUI due to intrinsic sphincter deficiency since 1993 when it was first approved for this application by the U.S. Food and Drug Administration (5).

It has been reported that stress urinary incontinence associated to intrinsic sphincter deficiency can be treated with reasonable success by means of periurethral collagen injections (6).

However, periurethral collagen injections have also been efficacious in patients with bladder neck hypermobility. The use of collagen in bladder neck hypermobility was evaluated in a nonrandomized prospective study that concluded that this therapy is appropriate in those patients who wish to avoid surgical risks and to whom surgery is ill advised (7).

Because collagen is less invasive than surgery (i.e., retropubic bladder neck suspension or slings), it could represent an interesting alternative for the treatment of SUI. The side effects of collagen injection are generally transient (e.g., urgency, frequency syndrome, retention) (7,8).

Long-term outcomes for the most commercially available bulking agents including collagen demonstrate a cure rate of 25% to 45% and an improvement rate of 25% to 70%. However, due to the decreased effectiveness of collagen with time, repeating injections may be necessary (9).

Most of the studies demonstrate that patient selection is important in the outcomes with PCI. The ideal patient should have diminished urethral function with minimal proximal urethral hypermobility (10).

Thus, in the world literature there is no consensus of opinion that patients with hypermobility will not benefit from PCI.

We evaluated women with SUI with hypermobility and intrinsic sphincter deficiency treated with PCI through clinical criteria (number of urinary protectors) and urodynamic parameters.

MATERIALS AND METHODS

Between January 2004 and January 2005 40 women with stress urinary incontinence were studied: 13 with bladder neck hipermobility (GI) and 27 with intrinsic sphincteric deficiency (GII).

All patients underwent a meticulous baseline evaluation, including a complete history, physical examination and urine culture.

They also underwent a urodynamic evaluation that confirmed the diagnosis of stress urinary incontinence in both groups. The urodynamic evaluation was repeated four months after the PCI.

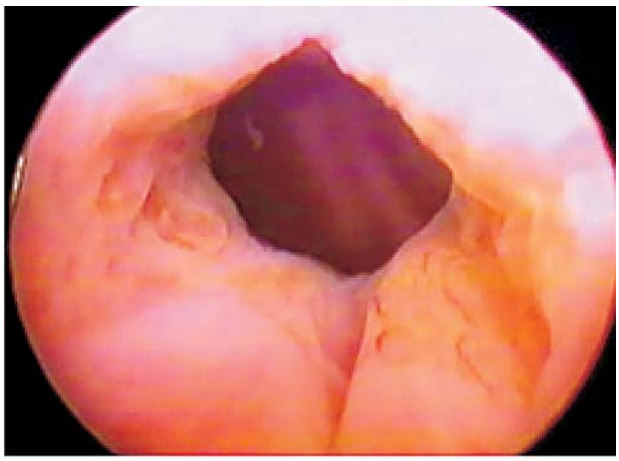
In GII, the ISD was defined as an VLPP of less than 60 cm H_2O. Bladder neck displacement greater than 10 mm measured by transperineal ultrasound was used to define bladder neck hipermobility in GI.

The age ranged from 36 to 81 (mean of 60.4). Age was homogeneous between GI and GII. The groups were also homogeneous as to the number of previous surgeries for stress urinary incontinence.

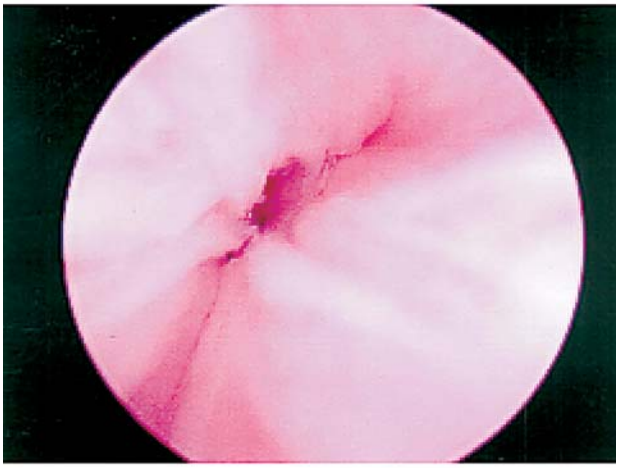
Women who presented contraindications to collagen injections (allergic reaction) were excluded. Subjects with neurogenic bladder, interstitial cystitis and pelvic prolapse higher than stage II were also excluded.

It was agreed that intraurethral collagen submucosal injection under local anesthesia (3 to 4 mL xylocaine 1%) as an outpatient procedure would be used for all patients. Collagen was injected at the 3-o'clock, and 9-o'clock positions until coaptation of the urethral mucosa was obtained (Figure-1). Sequential injections were given 1 month apart until continence was achieved or until it was predicted that further injections would not provide success. Follow-up visits were conducted at 1, 3, 4, 6 and 9 months after collagen injection.

The success of the intervention was evaluated by means subjective and objective criteria (num-



BEFORE



AFTER

Figure 1 – Bladder neck before and after the periurethral injection of collagen.

ber of urinary protectors and urodynamic parameters respectively).

Cure was defined as the absence of urine leak during cistometry performed four months after collagen periurethral injections, improvement when there was urine leak with a volume 50% bigger than those before treatment and we consider failure when the urine leak occurred with a volume similar to that before PCI.

The study was approved by the Medical Ethics Committee of the Federal University of Sao Paulo, Escola Paulista de Medicina. All patients gave informed consent to participate in the study.

Statistical analysis was performed with the software Analyze-it® for Microsoft® Excel. Statistical significance of differences among the number of urinary protectors, Valsalva leak point pressure, maximum urethral closure pressure and volume of urine leak before and after PCI were assessed using non-parametric tests (Kruskal-Wallis or Mann-Whitney tests, as appropriate). The occurrence of the cure, improvement or failure was assessed using chi-square test. $P < 0.05$ was considered statistically significant.

RESULTS

The need to use urinary protectors before treatment in GI was smaller than GII. However, after the treatment it was similar in the two groups (Figure-2).

It was noticed that after 9 months there was a significant decrease in the need to use urinary protectors in the two groups. (Figure-3).

It was observed through the urodynamic study that either cure or improvement was achieved in 46% in GI and 40.7% in GII ($p > 0.05$) (Table-1).

There was an increase in the leak pressure in both groups, but it was significant only in GII (Graphic-2). We also compared the maximum urethral closure pressure (MUCP) in both groups before and after the treatment with periurethral collagen injections and we could not find significant differences (Figure-4).

Besides, there was a decrease in the volume of leakage of urine during urodynamic evaluation in the two groups, being the results in group II statistically significant (Figure-5).

In our series additional injections were given in seven cases of GI and fifteen of GII ($p > 0.05$). As for the volume of injection, there were no differences between GI and GII.

As far as side effects are concerned, there was no case of urinary retention. There was a case of urinary infection in GI and another in GII, both were successfully treated.

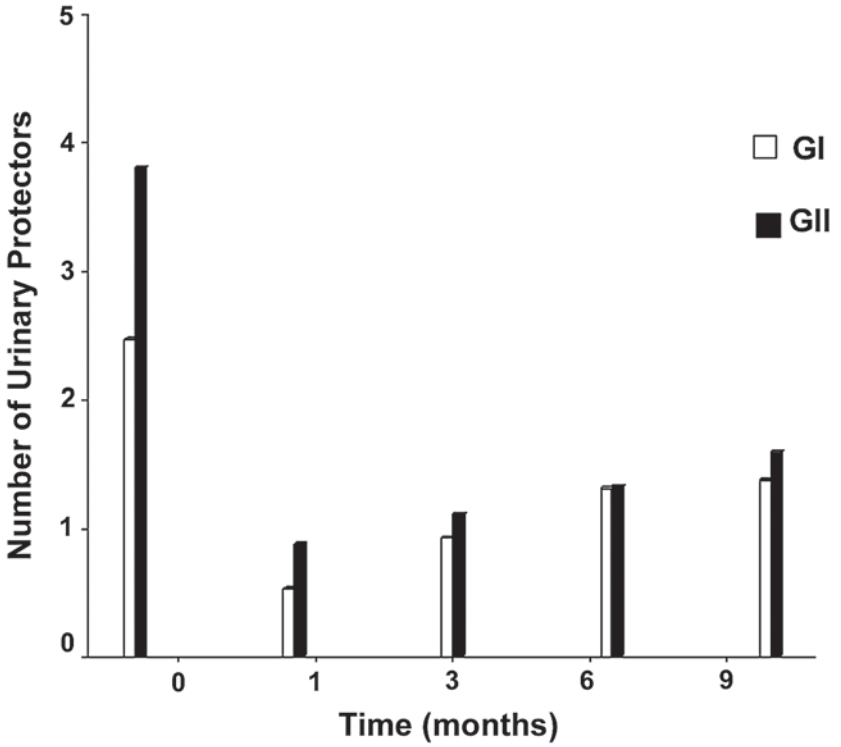


Figure 2 – *Number of urinary protectors before and after periurethral injections. Before (GI X GII): Kruskal-Wallis test (p < 0,05) ∴ GII > GI. After - 1, 3, 6 and 9 months: (GI X GII): Kruskal-Wallis test (p > 0.05); 1, 3, 6 and 9 months: (GI X GI): Kruskal-Wallis test (p < 0.05); 1, 3, 6 and 9 months: (GII X GII): Kruskal-Wallis test (p < 0.05).*

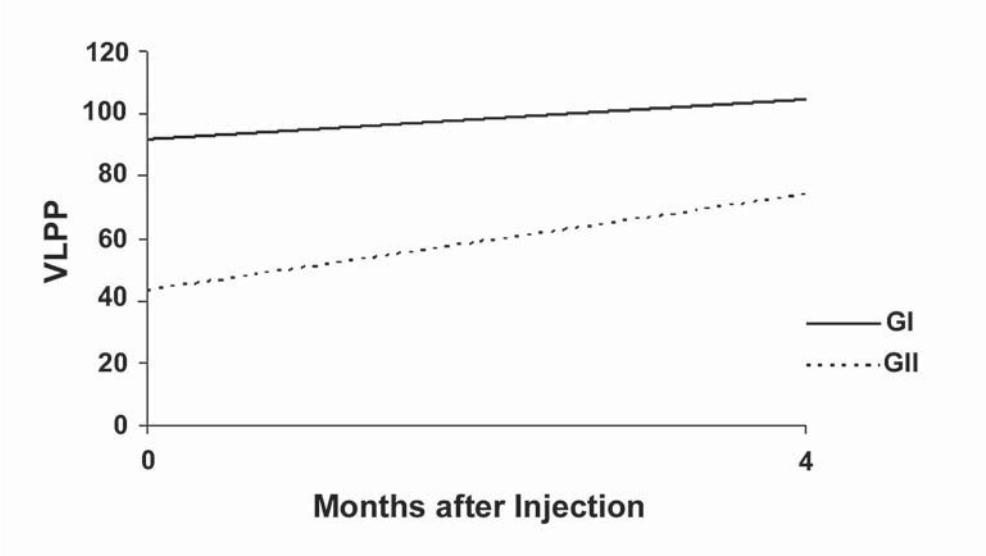


Figure 3 – *Behavior of Valsalva leak point pressure before and after treatment with periurethral injection.GI: Kruskal-Wallis test: p > 0.05, GII: Kruskal-Wallis test: p < 0.05.*

Table 1 – *Cystometric evaluation in the different groups.*

Groups	Outcome			
	Cure	**Improvement**	**Failure**	**Total**
GI	3 (23%)	3 (23%)	7 (54%)	13
GII	3 (11.1%)	8 (29.6%)	16 (59.3%)	27
Total	6	11	23	40

χ^2 statistic = 1.0; p = 0.6007.

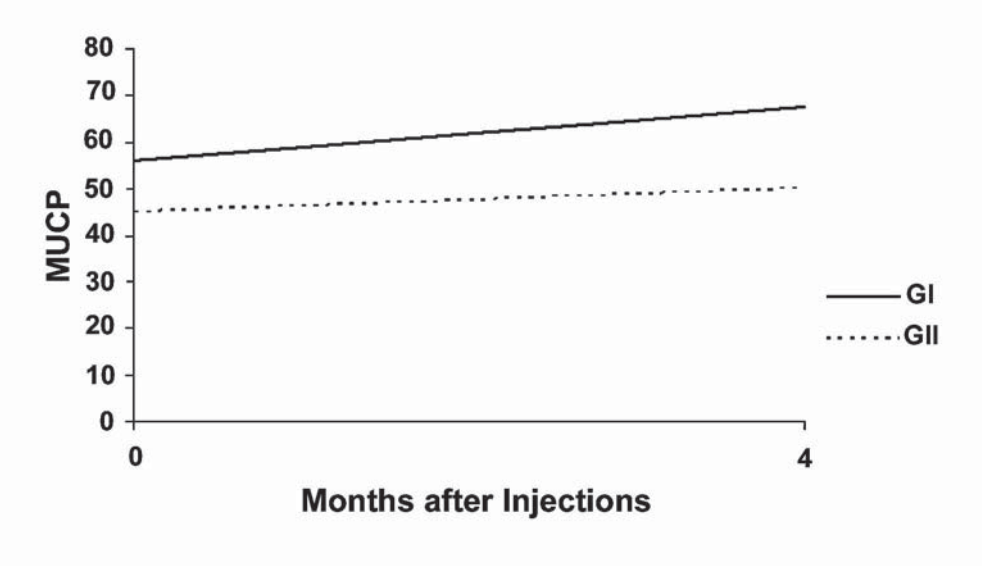


Figure 4 – *Behavior of maximum urethral closure pressure (MUCP) before and after treatment with periurethral injection.GI: Kruskal-Wallis test: p > 0.05, GII: Kruskal-Wallis test: p > 0.05*

COMMENTS

Collagen is a popular, safe and effective periurethral bulking agent for the treatment of stress urinary incontinence primarily due to intrinsic sphincter deficiency.

The procedure may be done under local anesthesia, the period of convalescence is short, and complications are minimal (11).

The reported success rate of collagen injection varies considerably according to patient selection and follow-up duration and also according to the in-

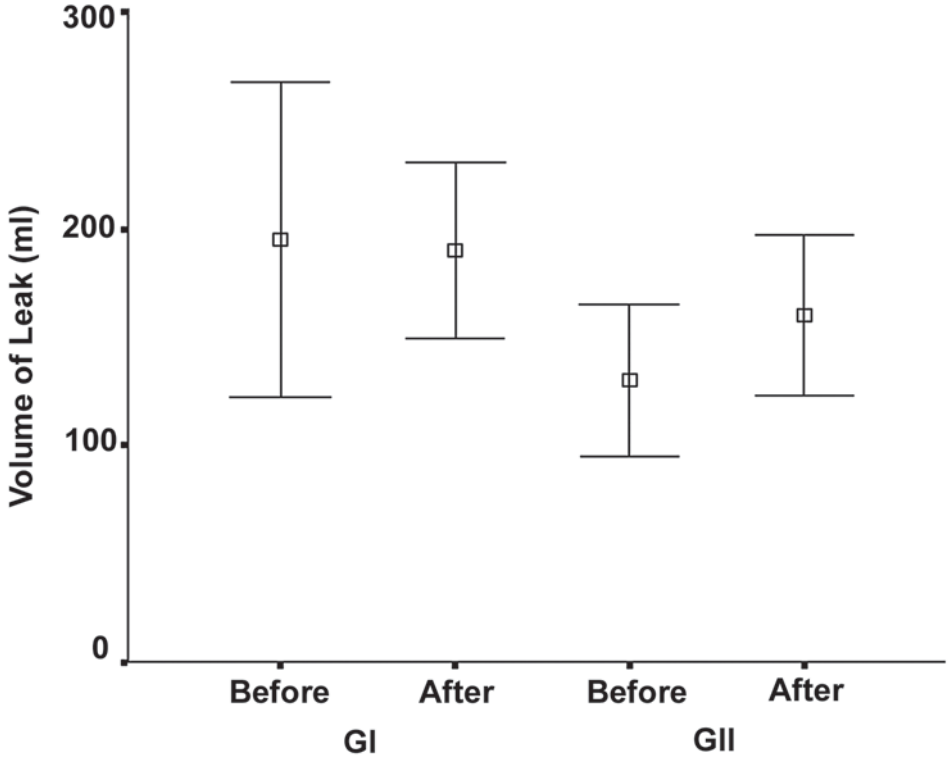


Figure 5 – *Volume of leakage before and after treatment with periurethral injections. GI (Before X After): Anova – p > 0.05, GII (Before X After): Anova – p < 0.05.*

Descriptive Statistics:

		N	Minimum	Maximum	Mean	SD
GI	Before	13	100,00	400,00	188,4615	91,6375
	After	10	100,00	300,00	190,0000	56,7646
GII	Before	27	100,00	200,00	129,6296	46,5322
	After	24	100,00	200,00	162,5000	49,4535

vestigator definition of cure, improvement and failure.

There has been a previous collagen outcome assessed by direct patient questioning on symptom severity and pad requirements (12,13). The outcome reported cure in 23% to 74% of cases, improvement in 20% to 52% and failure in 6% to 33%.

Our results are similar from those concerning the cure and improvement rates in the world literature. However, we believe that most cases reported as cured in previous studies would be reclassified as improved by our strict criteria. Moreover, cure should imply the reestablishment of normal voiding patterns but in most studies cure denotes that the patient no longer had stress urinary incontinence. Thus, in most cases our new onset urge incontinence, urinary urgency or difficult voiding may have been present.

As opposed to these favorable long-term results, collagen injection is not considered to be a durable procedure and most patients need additional treatment sessions to achieve and maintain improvement or cure. In our series in seven cases additional injections were given in GI and fifteen in GII ($p > 0.05$). In

addition, there have not been differences of injection volume between the two groups.

Our study observed a graduated increase of the need of urinary protectors through consecutive months after PCI (Graphic-1). This can be an evidence of the low durability of this procedure.

We also observed urodynamic parameters that have denoted that PCI can be useful in SUI with intrinsic sphincter deficiency. In this group there was a significant increase of the VLPP (Graphic-2). This finding was also reported by other authors (14,15). Overall the literature is inconclusive on the association of improved incontinence grade and increased leak point pressure after treatment as well as the predictive nature of baseline leak point pressure (7).

Among some of the issues addressed in this paper there are the value of collagen injections in patients with hypermobility and ISD. Regarding hypermobility, ISD became the sole indication for the use of collagen in patients with stress urinary incontinence as a result of the US multicentric trial (16). Since then a number of reports have demonstrated the use of collagen in patients with hypermobility. Herschorn & Radomski (17) found no difference in outcomes with stress urinary incontinence with hipermobility and ISD. The series of Moore et al. (18) included patients with both types of SUI.

In the editorial by McGuire & Appell (6), the results at more than 1 year in women with ISD were statistically similar to those in women with hypermobility, although Appell (19) subsequently reported that the all patients with hypermobility required bladder neck surgery with 2 years.

In our study, we found similar decrease in the need to use urinary protectors in both groups. Moreover, cystometric evaluation allowed us to infer that outcome results for hypermobility or ISD are similar.

Therefore, in the light of these several recent studies, including our own, we concur that urethral hypermobility is not a contraindication to injection therapy.

CONCLUSIONS

In our series either cure or improvement was achieved in 46% of the patients in GI and 40.7% in GII. Therefore, periurethral collagen injection may provide a minimally invasive means to treat both types of stress urinary incontinence. We concur that bladder neck hypermobility is not a contraindication to injection therapy.

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

The authors present their results of periurethral collagen injections in 27 women with stress urinary incontinence.

Most studies to date have some urodynamic outcomes included. Most are also of longer duration. It is not clear from the introduction just what question the authors want to address.

It is also not mentioned whether patients were given additional injections. It is well known that injectables, especially collagen, may require a few initial sessions for success. If this was not done, it may have compromised the continence outcome.

Regarding the treatment outcomes, the demonstrated leak on cystometry is understood, but there are many patients who do not leak with catheters in place. Do they possibly mean a cough-stress test as an outcome measure? Furthermore, what do they mean by volume of leakage? Is it volume in the blad-

der at which the leakage occurred? If so, then they should provide evidence that this test has been validated and standardized as an outcome measure.

It appears that the results of treatment in both groups are similar, despite the pre-treatment testing, and despite some discrepancies in urodynamic results.

In the Comments, the authors point to discrepancies between their results and previously published outcomes. From the data they present, the success rates appear relatively similar. Longer term results are mostly less favorable than short-term results. Furthermore, there are clinical outcome measures that are valid in SUI studies. This study has no clinical outcome measures, e.g. validated questionnaires, against which to compare the urodynamic results.

The authors indicate the lack of durability as evidenced by the increasing need for pads. They then state that collagen can be useful in SUI with ISD. However, they actually showed that the results in patients with normal sphincters (GII?) were the same.

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EDITORIAL COMMENT

This looks at 40 women suffering from stress urinary incontinence over a one-year period. It is not clear on what basis patients were differentiated into the group with intrinsic sphincter deficiency and those with normal sphincters – it is a very vague area and it is difficult to separate out the groups. Clearly though it is a consecutive series followed up prospectively – the authors do not mention Zuidex, which is a new agent, which has been around for some time now, and this is an oversight in their literature survey.

Under results, it is interesting to see that there is limited cure shown urodynamically.

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Investigative Urology
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Interleukin-11 Attenuates Ifosfamide-Induced Hemorrhagic Cystitis

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ABSTRACT

Objective: To investigate the possible protective effect of recombinant human interleukin-11 (rhIL-11) against ifosfamide (IFS)-induced hemorrhagic cystitis (HC).
Materials and Methods: Male Swiss mice (20-30g) were pretreated with rhIL-11 (25-625 μg, subcutaneously.) 30 min before intraperitoneal injection of IFS (400 mg/kg) or with saline (control group). Twelve hours later, HC was evaluated by bladder wet weight (BWW) to quantify edema, Evans blue extravasation (EBE) to measure vascular permeability, and macroscopic and microscopic analysis. All bladders were assessed by histopathological analysis.
Results: rhIL-11 (at 125 and 625 μg) attenuated the IFS- induced increase of BWW (37.48% and 45.44%, respectively, $p < 0.05$) and EBE (62.35% and 56.47%, respectively, $p < 0.05$). IFS- induced macroscopic edema and hemorrhage and microscopic alterations, were also prevented by rhIL-11 at 625 μg. ($p < 0.05$).
Conclusion: Our results demonstrate a protective effect of rhIL-11 on experimental IFS- induced HC, not previously reported.

***Key words**: bladder; ifosfamide; cystitis; interleukin-11; rats*
Int Braz J Urol. 2007; 33: 704-10

INTRODUCTION

Interleukin-11 (IL-11, thrombopoietin) is a pleiotropic 178-amino acid polypeptide with a molecular weight of 18 kDa which is expressed by a wide range of mesenchymal tissues (1,2) used in clinical practice as a megakaryocytopoiesis stimulator in patients with thrombocytopenia (3). It is well known that IL-11 protects the oral gastrointestinal mucosa against radiation (4) and 5-fluorouracil (5) damage by attenuating TNF-α and IL-1β expression in hamsters. In attempt to elucidate this anti-inflammatory effect, we investigated if IL-11 could protect against ifosfamide (IFS)-induced hemorrhagic cystitis (HC).

IFS is an alkylating agent from the oxazaphosphorine group with a broad spectrum of antineoplasic activity. In the absence of adequate uroprotection, patients treated with IFS for malignant tumors or immunosuppression develop a dose-limiting HC with an average incidence of 40%. Such toxicity

is attributed to the intravesical release of acrolein (ACR), an IFS highly urotoxic metabolite. It has been proposed that urothelial damage occurs by direct contact with ACR, causing edema, ulceration, neovascularization, hemorrhage and necrosis (6). Despite the preventive use of mesna, the occurrence of hematuria due to HC has been observed in 33% of patients (7). These facts increase the importance of studies to investigate novel therapies and elucidate the mechanisms involved in bladder lesion resulting from alkylating agent therapy.

Recombinant human IL-11 (rhIL-11, oprevelkin) has a number of biological activities which could impact HC including inhibition of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin-1β (IL-1β), nitric oxide (NO) synthesis and apoptosis (8,9), and stimulation of cell proliferation and differentiation and protection of connective tissue (8). For that reason, the present study investigated if rhIL-11 has a protective effect against IFS- induced HC in mice.

MATERIALS AND METHODS

Animals - Male Swiss mice (20-30 g), provided from the Central Bioterium of Pici, were kept in a temperature-controlled room with food ad libitum and water restriction 12 hours before they were sacrificed. All animal treatments and surgical procedures were performed in accordance with the Guide for Care and Use of Laboratory Animals, National Institutes of Health (Bethesda, MD, USA).

Drugs - Ifosfamide (IFS, Holoxane®: ASTA - AG, Frankfurt Germany; 1g), recombinant human interleukin-11 (rhIL-11, Neumega®, Oprevelkin: Wyeth, 5 mg) and Evans blue (Sigma Chemical Co.) were dissolved in sterile saline. All other reagents were obtained from Sigma Chemicals Co (St. Louis, MO).

Effect of IL-11 on ifosfamide-induced hemorrhagic cystitis - Groups of 6 mice were pretreated with subcutaneous (s.c.) administration of sterile saline or rhIL-11 at 25, 125 or 625 μg 30 minutes before the induction of HC with intraperitoneal injection of IFS at 400 mg/kg. The control group received only sterile saline. Twelve hours after IFS treatment, animals were euthanized, and the bladders were removed by careful dissection and emptied of urine. Bladder wet weight (BWW) was measured as a parameter of vesical edema and expressed as g/20 g body weight (mean ± SEM).

Macroscopic evaluation - Bladders were grossly examined for edema and hemorrhage. According to Gray´s scoring criteria (12), edema was considered severe (3+) when fluid was seen externally and internally in the bladder walls, moderate (2+) when confined to the internal mucosa, mild (1+) between normal and moderate and normal (0) when no edema was observed. Hemorrhage was scored as follows: 3+, intravesical clots; 2+, mucosal hematomas; 1+, telangiectasia or dilatation of bladder vessels; and 0, normal.

Microscopic evaluation - Bladders were fixed in formalin at 10%, embedded in paraffin and processed for hematoxylin and eosin (HE, Reagen) staining. Histopathological analysis was performed by a person (GACB) who was unaware of the treatments and group divisions, and scored as follows: (0) normal epithelium and absence of inflammatory cell infiltration and ulceration, (1) mild changes involving reduction of urothelial cells, flattening with submucosal edema, mild hemorrhage and few ulcerations, and (2) severe changes including mucosal erosion, inflammatory cell infiltration, fibrin deposition, hemorrhage and multiple ulcerations (10).

Evans blue extravasation assay - Vesical vascular permeability was evaluated by the Evans blue extravasation technique. Following the same previous group division (n = 6) and protocol, 2.5% Evans blue (25 mg/kg) was injected intravenously via the retro orbital plexus 30 minutes before the animals were sacrificed. Bladders were then excised, dissected and placed into glass tubes containing a formamide solution (1 mL/bladder) at 56°C overnight to extract the stain. The total extracted dye was determined by measuring the absorbance change at 630 nm (ELISA). At the same time, an absorbance-concentration curve was determined. The results were then reported as in micrograms of Evans blue per bladder (mean ± SEM).

Statistical analysis - Data were reported as the mean ± SEM (bladder wet weight, Evans blue extravasation and NOS activity) or the median values

followed by range values (macroscopic and histopathological scores) of groups of six mice. Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Newman-Keuls test, when appropriate. Macroscopic and microscopic scores were evaluated by Kruskal Wallis non-parametric test followed by Dunn's multiple comparison. Statistical significance was set at $p < 0.05$.

RESULTS

Protective effect of IL-11 against ifosfamide-induced increase in bladder wet weight - IFS at 400 mg/kg i.p. induced a marked increase (51.40 ± 7.10 g/20g, 211.51% increase, $p < 0.05$) in bladder wet weight 12 hours after its administration when compared to the control group which received only sterile saline (16.50 ± 0.40 g/20g). In a dose-dependent manner, rhIL-11 provided partial prevention (Figure-1) of the bladder wet weight increase induced by IFS, reaching a maximal effect at 125 μg (38.32 ± 2.89 g/20g, 37.48% reduction, $p < 0.05$) and 625 μg (35.54 ± 2.67 g/20g, 45.44% reduction, $p < 0.05$). The effect of rhIL-11 at 25 μg did not reach statistical significance (42.28 ± 0.93 g/20g, 26.13% reduction, $p > 0.05$).

Protective effect of IL-11 against ifosfamide-induced macroscopic and microscopic changes - As demonstrated in Table-1, bladders from animals treated with IFS only developed significant edema (median = 2, $p < 0.05$) and hemorrhage (median = 2, $p < 0.05$), when compared to the control group (median = 0 for both parameters). rhIL-11 at 625 μg significantly protected ifosfamide- induced gross edema and hemorrhage (median = 1, $p < 0.05$, for both parameters). Table-1 also shows that IFS induced microscopic alterations, when compared to the control bladders, such

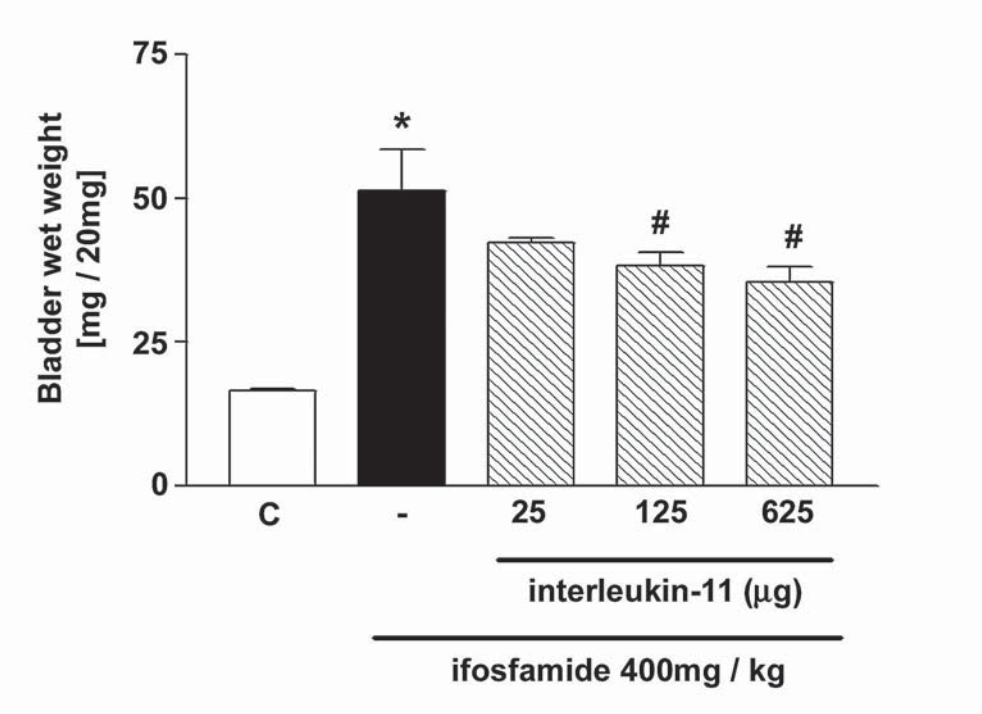

Figure 1 – *Protective effect of rhIL-11 against ifosfamide-induced bladder wet weight increase. Ifosfamide at 400 mg/kg induced an increase in BWW when compared to the control group (C), and rhIL-11 at 125 μg and 625 μg protected against this side effect. * $p < 0.05$ compared with control group. # $p < 0.05$ compared to ifosfamide only group. ANOVA followed by Newman-Keuls.*

Table 1 – *Macroscopic and microscopic analysis of rhIL-11 effect on ifosfamide-induced hemorrhagic cystitis.*

Experimental Groups	Macroscopic Analysis (edema)	Macroscopic Analysis (hemorrhage)	Microscopic Analysis
C	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)
IFS	2 (1 - 3)[a]	2 (1 - 3)[a]	2 (2 - 2)[a]
IL-11 25	2 (1 - 3)	2 (1 - 3)	1 (1 - 2)
IL-11 125	2 (1 - 2)	2-3 (1 - 3)	2 (1 - 2)
IL-11 625	1 (0 - 1)[b]	1 (0 - 2)[b]	1 (1 - 1)[b]

Ifosfamide (400 mg/kg, i.p.) induced macroscopic (edema and hemorrhage) and microscopic alterations (fibrin deposition, edema, urothelial desquamation, hemorrhage and leukocyte infiltration). Pretreatment with rhIL-11 at 625 µg protected against those side effects at 625 µg. C, control group treated with saline alone; IFS, ifosfamide only treated group; rhIL-11 25, 125 and 625, pretreated groups that received 25, 125 and 625 µg of rhIL-11, respectively. The results are reported as medians and range (n = 6). [a] $p < 0.05$ compared to the control group. [b] $p < 0.05$ compared to the IFS group. Kruskal-Wallis and Dunn´s test.

as edema, hemorrhage, fibrin deposition, neutrophil infiltration and vascular congestion (median = 2, $p < 0.05$). rhIL-11 at 625 µg partially prevented these side effects (median = 1, $p < 0.05$) (Table-1, Figure-2). In macroscopic and microscopic evaluation, rhIL-11 at 25 and 125 µg did not reach statistical differ-

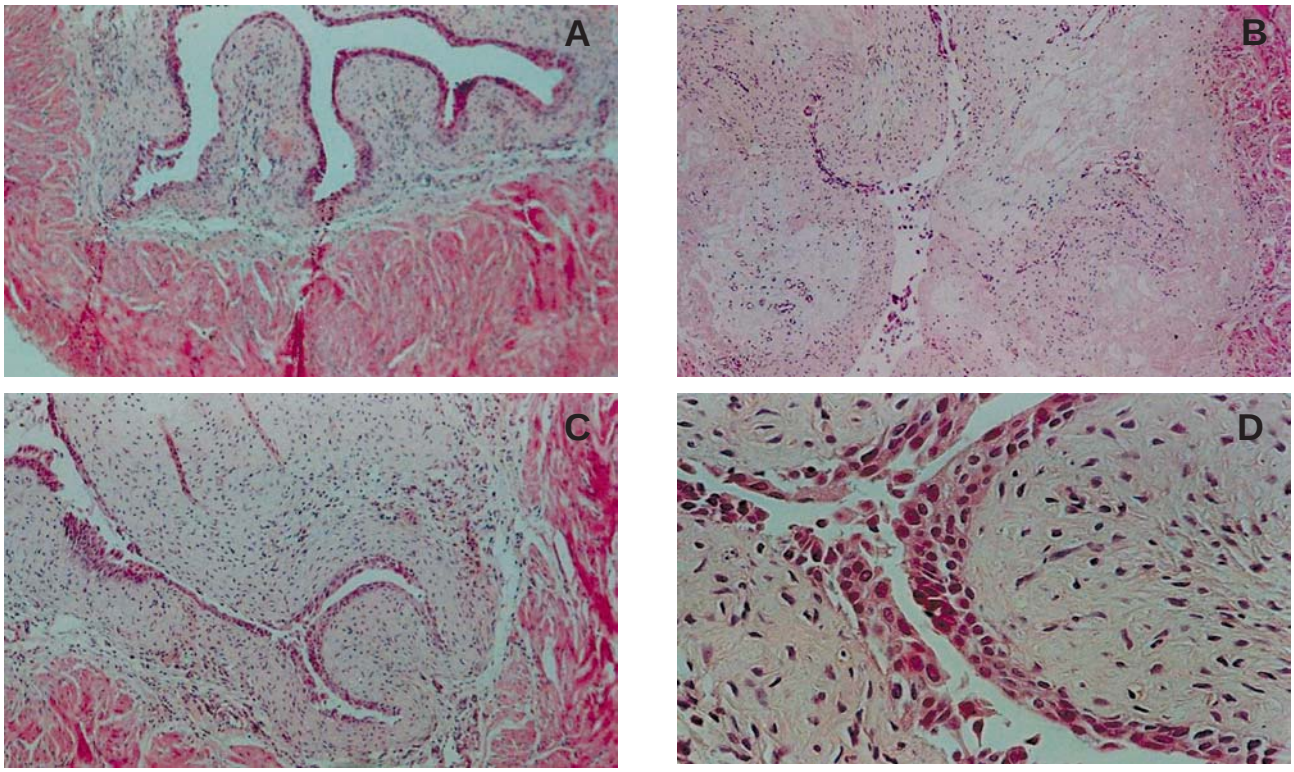

Figure 2 – *Histological analysis of cross sections of representative bladder walls. A) Control group which received only saline. B) Ifosfamide at 400 mg/kg induced urothelium desquamation, edema, pronounced hemorrhage and fibrin deposition and leukocyte infiltration when compared to control, shown in A. C) and D) Pretreatment with rhIL-11 at 625 µg was capable of reducing these side effects. (HE, A, B and C X100 and D X400).*

ence compared of the group that received only IFS ($p > 0.05$).

Protective effect of IL-11 against ifosfamide-induced increase of Evans blue extravasation in bladder - IFS at 400 mg/kg induced significant Evans blue extravasation in the bladders (6.08 ± 1.27 μg/bladder, 4576.92% increase, $p < 0.05$) when compared to control group (0.13 ± 0.12 μg/bladder). rhIL-11 in a dose-dependent fashion prevented the IFS- induced Evans blue extravasation (Figure-3), maximally at 125 μg (2.37 ± 0.59 μg/bladder, 62.35% reduction, $p < 0.05$) and 625 μg (2.72 ± 0.41 μg/bladder, 56.47% reduction, $p < 0.05$). rhIL-11 at 25 μg did not reach statistical significance (3.79 ± 0.52 μg/bladder, $p > 0.05$).

COMMENTS

The present study demonstrates the protective effect of rhIL-11 on IFS-induced hemorrhagic cystitis (HC). Our results show evidence that pretreatment with rhIL-11 in a dose-dependent manner prevents the increase of bladder wet weight, (parameter used to measure edema) and Evans blue extravasation in bladder (parameter used to measure vascular permeability), induced by IFS at 400 mg/kg. The results obtained were confirmed by macroscopic and microscopical analysis, in which only 625 μg, but not 125 μg dose, was effective in protecting against IFS-induced macroscopic edema and hemorrhage and microscopic alterations. Consequently, our preclinical

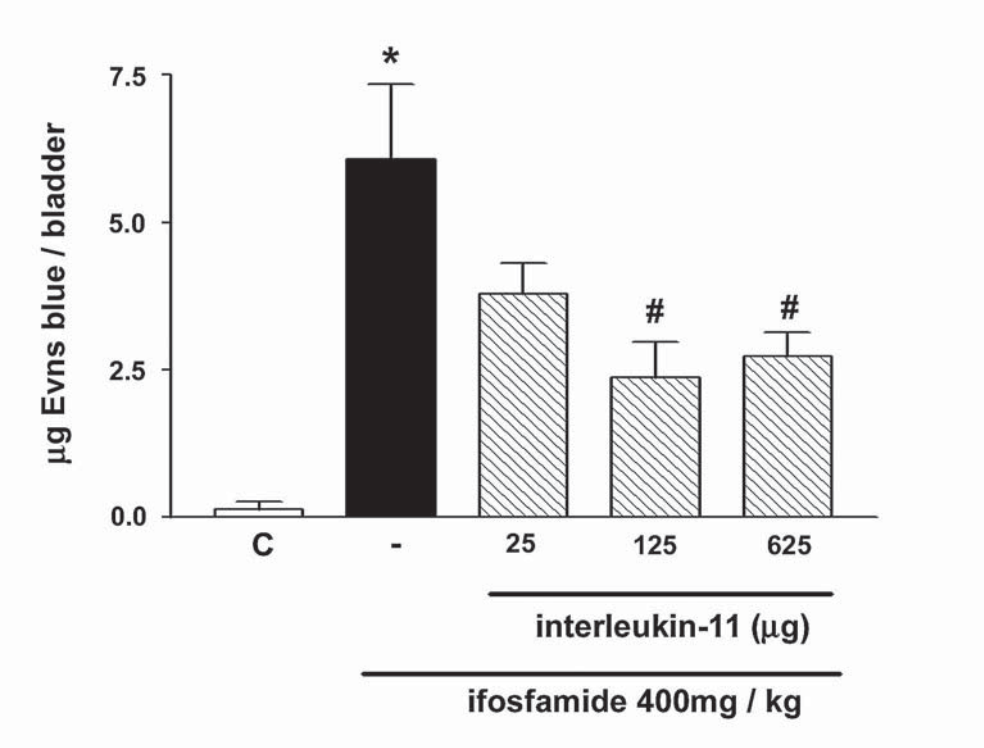

Figure 3 *– Protective effect of rhIL-11 against ifosfamide-induced Evans blue extravasation in bladder. Ifosfamide at 400 mg/kg induced an increase in Evans blue extravasation when compared to the control group (C), thus demonstrating an increase in vascular permeability. rhIL-11 at 125 μg and 625 μg reduced ifosfamide-induced Evans blue extravasation. * $p < 0.05$ compared with control group. # $p < 0.05$ compared to ifosfamide only group. ANOVA followed by Newman-Keuls.*

study suggests a previously unreported effect of rhIL-11 for the treatment of experimental ifosfamide-induced hemorrhagic cystitis.

Recently, some important mechanisms of experimental ifosfamide-induced hemorrhagic cystitis have been elucidated. Pro-inflammatory cytokines such as platelet-activating factor (PAF), TNF-α and IL-1β have been implicated in its pathogenesis. Previously, we demonstrated the participation of interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) pathway in the induction of nitric oxide (NO) production as an important pathway in the pathogenesis of lesion in hemorrhagic cystitis induced by alkylating agents. NO was demonstrated to be the final mediator of urothelial damage and hemorrhage in that type of HC (11). TNF-α and IL-1β have been shown to be important mediators of NO synthesis, since treatment with anti-TNF-α and anti-IL-1β decreased cyclophosphamide vesical damage as well the rise of inducible NO synthase expression and activity (12,13).

A previous study from our laboratory proved that dexamethasone alone was ineffective in preventing experimental IFS-induced hemorrhagic cystitis, but when combined with mesna, this corticosteroid potentiated its protective effect (14). More recently, we showed that amifostine and glutathione prevent ifosfamide and acrolein-induced hemorrhagic cystitis in mice (15). Therefore, it is becoming important to establish novel therapies for hemorrhagic cystitis, for the reason that, as previously demonstrated, 33% of patients using alkylating agents for the treatment of cancer develop hematuria (hemorrhagic cystitis sign) despite the preventive use of mesna (7). An experimental study in rats reinforced that the notion that there a high percentage of non protection despite preventive mesna usage (14).

Interleukin-11 (IL-11) has been extensively investigated as a protective agent for inflammatory events, such as inflammatory bowel disease, psoriasis, autoimmune joint disease, and many other inflammatory diseases (16). rhIL-11 has been shown to down regulate macrophage production in vitro of IL-1β, TNF-α, IL-12 and nitric oxide, without inducing anti-inflammatory cytokines such as IL-10, TGF-b, and IL-6 (8). It was also observed that rhIL-11 attenuates Th1 cytokines production in human psoriasis lesions, noting that its effect is not restricted to macrophages (17). More recent works demonstrate the rhIL-11 property of inhibiting NF-κB and AP-1 activation in islets to prevent streptozotocin-induced diabetes (18). Other studies have successfully used IL-11 for the prevention of intestinal ischemia-reperfusion lesion (19) and intestinal mucosa damage in response to chemotherapy and radiation due to diminished cell apoptosis and consequent death (9). On the other hand, other authors demonstrated that IL-11 does not prevent methotrexate-induced intestinal cell apoptosis but reduces the damage by compensatory crypt cell proliferation (20). Our data introduce a novel anti-inflammatory effect of IL-11 and are in accordance with previous reports in the literature.

In conclusion, the present study demonstrated that the pleiotropic cytokine rhIL-11 partially prevents IFS-induced experimental HC, an inflammatory event that depends on TNF-α, IL-1β and nitric oxide release. Possibly, rhIL-11 acts on an inhibitory pathway of these inflammatory mediators. Taking into account that rhIL-11 is already being used in clinical practice, it is possible to propose a clinical trial to investigate its effect on human HC.

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CONFLICT OF INTEREST

None declared.

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Letter to the Editor

International Braz J Urol 33 (5): 711-712, September -October, 2007
Official Journal of the Brazilian Society of Urology

Re: The Tunica Vaginalis Dorsal Graft Urethroplasty: Initial Experience

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Int Braz J Urol, 33: 523-531, 2007

To the Editor:

After, in 1996, long term poor results of urethrotomy have been shown, urethral reconstruction procedures have significantly expanded. However, it is of interest that in US (1,2), 93% of urologists perform still urethrotomy for the treatment of urethral strictures, while only a little minority (4.2%) perform buccal mucosa urethroplasty.

The use of buccal mucosa is today the best available option for urethral reconstruction, and the high success rate of the procedure has probably slowed down the search for new urethral substitution materials. However, the search for the "graal" of the ideal urethral substitute is still active, including small intestinal submucosa (3), tongue mucosa (4), acellular matrix (5), which have been all proposed in recent years for urethroplasty. In the next future, tissue engineering might offer the definite answer (6).

The authors report they experience with tunica vaginalis urethroplasty in 11 male patients; in nearly half the patients, the urethral stricture was recurrent after urethrotomy.

The use of tunica vaginalis is not completely new. A pedicled tubularized flap of tunica vaginalis was used for urethral reconstruction in 1992 in 3 patients (7). Nevertheless, the tunica vaginalis has been used seldom, and always as a flap. This is the first report on the use of a free graft of tunica vaginalis for urethral reconstruction.

Though the seek for new urethroplasty options should be encouraged, we must emphasize that we have now long term (7-10 years) studies (8) on the results of buccal mucosa urethroplasty available.

As the authors rightly state, this study should be considered investigational, due to very short follow-up and the small number of cases.

To date, buccal mucosa urethroplasty should be the best graft procedure to offer to patients with bulbar (longer than 2 cm) or penile stricture.

The authors used the dorsal approach popularized by Barbagli. Noteworthy, recently Barbagli himself (9) has questioned the real advantage of this approach compared to lateral and ventral approach.

We congratulate with the Brazilian Urology, which is very active in urethral reconstruction.

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UROLOGICAL SURVEY

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STONE DISEASE

Third prize: contemporary percutaneous nephrolithotripsy: 1585 procedures in 1338 consecutive patients

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J Endourol. 2007; 21: 824-9

Background and Purpose: The approach to urinary-stone disease has changed dramatically over the last three decades with a transition from open surgery to minimally invasive procedures. Percutaneous nephrolithotripsy (PCNL) is a cornerstone of the treatment of kidney and selected upper-ureteral stones and continues to evolve with advances in techniques and instrumentation. The purpose of this study was to assess outcomes and trends prospectively in a large contemporary group of patients undergoing PCNL.
Patients and Methods: Between July 1990 and December 2005, all 1338 patients at a single center scheduled for PCNL (N = 1585 procedures) were enrolled. Their mean age was 53 years (range 4-89 years). Data including comorbidities, stone burden, stone location, surgical time, hospital length of stay, rate of secondary procedures, and adverse events were collected prospectively. The primary outcome measures were stone-free rate and complications.
Results: There was a substantial incidence of comorbid medical conditions (48.8%) and anatomic renal abnormalities (25.3%), demonstrating the diverse and challenging patient population in this contemporary series. The overall stone-free rate at 3 to 6 months of follow-up was 94.8%.
Conclusions: Percutaneous nephrolithotripsy is a highly effective procedure and may be performed in a diverse group of patients with comorbid conditions and renal abnormalities. Improved intracorporeal lithotripters, balloon dilation of the tract, use of flexible instruments, and liberal use of secondary nephroscopy result in excellent stone-free rates with low morbidity.

Editorial Comment

This large contemporary series provides important information that is helpful with regards to counseling patients on the risk:benefit ratio of percutaneous nephrolithotomy (PCNL). It is important to emphasize that these results are from a very experienced tertiary center with a large volume of procedures (over 100 PCNL's per year), and one might expect that success rates may be somewhat lower and complication rates somewhat higher at sites with lower surgical volumes.

The broad applicability of the PCNL is supported by the wide age range treated (4-89), the substantial comorbidity (in 49% of patients) and the significant proportion of patients with renal abnormalities (25%). In spite of this challenging patient population, the success rate of 90% at time of discharge is commendable. We should note that no computerized tomography was utilized for postoperative follow-up – recent studies would suggest that more sensitive CT scans would detect residual stones in approximately 20% of those deemed stone-free by traditional imaging.

Importantly, the high success rate in this series was obtained without the need for routine upper pole access as has been recommended by other investigators. It may have been useful to stratify efficacy and safety based on the presence of renal anomalies and patient comorbidites or based on the need for multiple or supracostal access.

The authors report that they converted from a serial Amplatz dilator system to a balloon dilation system in 1995, following reports by other investigators of decrease in bleeding with this approach. It would have been interesting to know if their 7% bleeding complication rate (minor and major) decreased after the switch to

balloon dilation. The low rates of pulmonary complications and major bleeding set new standards for preoperative counseling of patients.

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Determination of ideal stent length for endourologic surgery

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J Endourol. 2007; 21: 906-10

Purpose: To assess whether direct measurement of ureteral length correlates with patient height or the ureteral length measured on intravenous urography in order to determine the appropriate ureteral stent length to be used for ureteroscopic surgery.
Patients and Methods: Sixty-five patients (70 ureters) who underwent ureteroscopic procedures were evaluated. The ureteral length between the ureteropelvic and ureterovesical junctions was determined either by preoperative intravenous urography (straight ureteral length; SUL) or intraoperatively with the aid of a guidewire (practical ureteral length; PUL). We regarded the PUL as a clinically useful measurement. The height, SUL, and PUL for each patient was determined. For a postoperative comparison of proper stent position, we selected another 36 patients in whom the length of the stent was based on patient height.
Results: The SUL values correlated significantly with the PUL ($R2 = 0.482$ on the right v 0.564 on the left side) and might be used as a predictor of stent length. However, patient height did not correlate with the PUL. Postoperative stent position tended to be better in the patients who had direct ureteral measurements than in those with stents chosen on the basis of patient height.
Conclusion: Determination of stent length according to patient height does not correlate well with the length needed for endoscopic procedures. Direct measurement of the ureteral length is easy and minimizes stent-associated complications and stent migration.

Editorial Comment

The routine use of noncontrast cross-sectional imaging for the diagnosis and preoperative planning for urolithiasis makes the determination of ureteral length on IVP to a certain degree obsolete. As such, alternative methods to determine ureteral length at the time of surgery are attractive. The concept of direct ureteral length measuring at the end of the procedure is attractive. This can be accomplished as described in this study, by passing a guidewire. Alternatively, one can measure the distance on the ureteroscope as it is withdrawn from the UPJ to the UVJ. Lastly, one could use an open-ended ureteral catheter with inked-measurements. The authors note that ureteral dilation at the end of a ureteroscopic stone extraction could lead to overestimation by the PUL method.

The stent sizes utilized based on patient height were longer than we would traditionally utilize. For example, we commonly utilize 22 cm stents for patients shorter than 5’4”, and though the shortest patient in this study was 4’8”, the shortest ureteral stent placed was 24 cm.

The authors’ hypothesis that a poorly placed stent that crosses the midline is somewhat speculative without the evaluation of urinary symptoms and flank pain in this study. One could make a counter-argument that

a coil sitting flush on the sensitive trigone could cause more discomfort than one that has extra length in the bladder. As such, the impact of stent positioning on patient outcomes remains an area ripe for investigation.

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ENDOUROLOGY & LAPAROSCOPY

Open versus laparoscopic live donor nephrectomy: a focus on the safety of donors and the need for a donor registry

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J Urol. 2007; 178: 1860-6

Purpose: A review of the existing literature showed that the subject of live donor nephrectomy is a seat of underreporting and underestimation of complications. We provide a systematic comparison between laparoscopic and open live donor nephrectomy with special emphasis on the safety of donors and grafts.
Materials and Methods: The PubMed(R) literature database was searched from inception to October 2006. A comparison was made between laparoscopic and open live donor nephrectomy regarding donor safety and graft efficacy.
Results: The review included 69 studies. There were 7 randomized controlled trials, 5 prospective nonrandomized studies, 22 retrospective controlled studies, 26 large (greater than 100 donors), retrospective, noncontrolled studies, 8 case reports and 1 experimental study. Most investigators concluded that, compared to open live donor nephrectomy, laparoscopic live donor nephrectomy provides equal graft function, an equal rejection rate, equal urological complications, and equal patient and graft survival. Analgesic requirements, pain data, hospital stay and time to return to work are significantly in favor of the laparoscopic procedure. On the other hand, laparoscopic live donor nephrectomy has the disadvantages of increased operative time, increased warm ischemia time and increased major complications requiring reoperation. In terms of donor safety at least 8 perioperative deaths were recorded after laparoscopic live donor nephrectomy. These perioperative deaths were not documented in recent review articles. Ten perioperative deaths were reported with open live donor nephrectomy by 1991. No perioperative mortalities have been recorded following open live donor nephrectomy since 1991. Regarding graft safety, at least 15 graft losses directly related to the surgical technique of laparoscopic live donor nephrectomy were found but none was emphasized in recent review articles. The incidence of graft loss due to technical reasons in the early reports of open live donor nephrectomy was not properly documented in the literature.
Conclusions: We are in need of a live organ donor registry to determine the combined experience of complications and long-term outcomes, rather than short-term reports from single institutions. Like all other new techniques, laparoscopic live donor nephrectomy should be developed and improved at a few centers of excellence to avoid the loss of a donor or a graft.

Editorial Comment
The author performed a very comprehensive review of the literature (live donor laparoscopic nephrectomy) revealing only 7 randomized trials that concluded that when compared to open live donor nephrectomy, laparoscopic

live donor nephrectomy provides equal graft function at 1 year, an equal rejection rate, equal urological complications, and equal patient and graft survival. Analgesic requirements, pain data, hospital stay and time to return to work are significantly in favor of the laparoscopic procedure.
On the other hand, laparoscopic live donor nephrectomy has the disadvantages of increased operative time, increased warm ischemia time and increased major complications requiring reoperation. These complications may decrease with more operative experience. The learning curve for laparoscopic procedures has been extensively discussed in the literature. It is pivotal that better simulation, education models can be created to decrease the challenging issues of learning this new operative technique.

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Prospective radiographic followup after en bloc ligation of the renal hilum

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J Urol. 2007; 178: 1888-91

Purpose: We determined the risk of arteriovenous fistula after en bloc ligation of the renal hilum.
Materials and Methods: A prospective evaluation of all patients who underwent en bloc ligation of the renal hilum during nephrectomy for malignant disease was performed. Pertinent operative data were recorded and patients were followed for clinical evidence of arteriovenous fistula formation, including hypertension, abdominal bruit and new onset congestive heart failure. Patients with at least 12 months of followup underwent computerized tomographic arteriography to assess arteriovenous fistula formation.
Results: A total of 94 patients underwent en bloc renal hilar ligation during open (43) and laparoscopic (51) nephrectomy using a 45 mm titanium endovascular stapler. Of this cohort 11 patients were lost to followup and 3 died of disease. The remaining 80 patients were followed an average of 35.2 months with no clinical evidence of arteriovenous fistula formation. Specifically there was no statistically significant difference in preoperative and postoperative blood pressure ($p = 0.18$ and 0.62, respectively), no evidence of abdominal bruit on examination and no new onset congestive heart failure. A total of 32 had increased serum creatinine and, therefore, they were excluded from followup computerized tomographic arteriography. Eight patients had a followup of less than 1 year and they were not yet eligible for evaluation. In the 40 patients who underwent computerized tomographic arteriography no fistulas were noted.
Conclusions: Based on clinical followup and prospective radiographic evaluation there appears to be a low risk of arteriovenous fistula formation after en bloc ligation of the renal hilum using a titanium endovascular stapler.

Editorial Comment

The authors should be congratulated to perform this prospective study. The first case of fistula formation after en bloc ligation of the renal pedicle was reported by Hollingsworth (1934) in a patient with tuberculosis renal disease. Few other cases of fistula formation after en bloc ligation of the renal pedicle were reported. Approximately 60 case reports of fistula formation after mass ligation of the renal pedicle were published of which most developed in the setting of infection or inflammation.

The authors performed the “en bloc” endovascular renal hilar ligation using endovascular staplers during open and hand assisted laparoscopic nephrectomies. One should be careful and aware of possible misfiring and

different types of laparoscopic endovascular staplers so possible complications can be minimized or completely avoided.

In summary, “en bloc” renal hilar ligation using endovascular staplers could be considered in cases of renal cell carcinoma when the absence of infection and/or severe inflammation may contribute for possible arterio-venous fistula formation.

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IMAGING

Combined T2-weighted and diffusion-weighted MRI for localization of prostate cancer

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Objective: The objective of our study was to compare T2-weighted MRI alone and T2 combined with diffusion-weighted imaging (DWI) for the localization of prostate cancer.
Subjects and Methods: T2-weighted imaging and DWI (b value = 600 s/mm2) were performed in 49 patients before radical prostatectomy using an endorectal coil at 1.5 T in this prospective trial. The peripheral zone of the prostate was divided into sextants and the transition zone into left and right halves. T2 images alone and then T2 images combined with apparent diffusion coefficient (ADC) maps (T2 + DWI) were scored for the likelihood of tumor and were compared with whole-mount histology results. Fixed window and level settings were used to display the ADC maps. Only tumors with an area of more than 0.13 cm2 (> 4 mm diameter) and a Gleason score of > or = 6 were considered significant. The area under the receiver operating characteristic curve (A(z)) was used to assess accuracy.
Results: In the peripheral zone, the A(z) value was significantly higher (p = 0.004) for T2 plus DWI (A(z) = 0.89) than for T2 imaging alone (A(z) = 0.81). Performance was poorer in the transition zone for both T2 plus DWI (A(z) = 0.78) and T2 (A(z) = 0.79). For the whole prostate, sensitivity was significantly higher (p < 0.001) with T2 plus DWI (81% [120/149]) than with T2 imaging alone (54% [81/149]), with T2 plus DWI showing only a slight loss in specificity compared with T2 imaging alone (84% [204/243] vs 91% [222/243], respectively).
Conclusion: Combined T2 and DWI MRI is better than T2 imaging alone in the detection of significant cancer (Gleason score > or = 6 and diameter > 4 mm) within the peripheral zone of the prostate.

Editorial Comment

Localization of prostate cancer is important for adequate tumor staging, adequate targeting for transrectal ultrasound biopsy and for adequate conservative therapies such as intensity-modulated radiation therapy, interstitial brachytherapy and cryosurgery. Endorectal magnetic resonance techniques that can be used for identification of prostate cancer are conventional T2-weighted image, 3D-spectroscopy, diffusion-weighted image (DWI) and dynamic contrast enhanced technique (DCE). Since the appearance of cancer on T2-weighted image is not specific, several studies have demonstrated that the combination of endorectal MR imaging and

magnetic resonance spectroscopic imaging, can lead to high sensitivity and specificity for peripheral zone tumor localization. DWI is a technique of imaging prostate cancer based on the fact that cancer tissue presents with restriction of the movements of the molecules of water compared with the movement of the molecules of water within normal prostatic tissue. In other words, cancer appears with low apparent diffusion-coefficient values (ADC).Though the authors state that sensitivity of combined T2 and DWI MRI is significantly higher than with T2 imaging alone, we should be alert because both techniques can present false positive (due to prostatitis, focal prostatic atrophy, etc) or false negative results. In our institution, we have been using routinely, in the last 3 years, the combination of these four different techniques: T2-weighted image, 3D-spectroscopic imaging, DWI and dynamic contrast enhanced imaging. Preliminary analysis of our materials has been shown that combining these four techniques provides better sensitivity and specificity for cancer detection and localization.

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Split-bolus MDCT urography with synchronous nephrographic and excretory phase enhancement.
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Objective: Our purpose was to evaluate the utility of CT urography performed using a split contrast bolus that yields synchronous nephrographic and excretory phase enhancement.
Materials and Methods: Five hundred consecutive patients referred for evaluation of possible urinary tract abnormalities (327 for painless hematuria) underwent CT urography with unenhanced scanning of the abdomen and pelvis and scanning during concurrent nephrographic and excretory phase enhancement produced by administration of a split contrast bolus. The enhanced abdomen scan was obtained with abdominal compression; the enhanced pelvis scan was obtained after release of compression. Findings from axial sections and coronal maximum intensity projections were correlated with clinical follow-up and, as available, with laboratory and other imaging studies including cystoscopy, ureteroscopy, urine cytology, surgery, and pathology. Follow-up management for each patient was determined by the clinical judgment of the referring physician.
Results: CT urography identified 100% of pathologically confirmed renal cell carcinomas (n = 10) and uroepithelial malignancies involving the renal collecting system or ureter (n = 8). An additional nine renal masses were identified for which no pathologic proof has yet been obtained, including eight subcentimeter solid renal masses and one multiloculated lesion. Fourteen of 19 confirmed cases of uroepithelial neoplasm involving the bladder were identified. CT urography yielded one false-positive for bladder tumor, two false-positives for ureteral tumor, and one patient with a bladder mass who refused further evaluation. CT urography yielded sensitivity and specificity of 100% and 99% and 74% and 99% and positive predictive value and negative predictive value of 80% and 100% and 93% and 99% for the renal collecting system and ureter and bladder, respectively. CT urography was ineffective in identifying 11 cases of noninfectious cystitis. CT urography also depicted numerous other congenital and acquired abnormalities of the urinary tract.
Conclusion: Split-bolus MDCT urography detected all proven cases of tumors of the upper urinary tract, yielding high sensitivity and specificity. The split-bolus technique has the potential to reduce both radiation dose and the number of images generated by MDCT urography.

Editorial Comment

Multidetector CT-urography (MDCT-urography) has been shown to be an effective single comprehensive examination in the evaluation of patients with hematuria or with risk for the development of urothelial malignancies. Since protocols for MDCT urography varies from each institution, most MDCT urography images are obtained in the unenhanced phase (detection of calculi), nephrographic-phase (detection of renal masses) and excretory-phase (detection of urothelial lesions). The authors present their results with a new protocol called split-bolus MDCT urography where the unenhanced phase is followed only by a combined nephrographic and excretory phase. During split-bolus, CT-urography the intravenous injection of contrast material is performed in two steps. First, 40 ml is injected at 2 ml/s and after 120 second from the beginning of the first injection, the remaining 80 ml is injected. This technique showed high sensitivity and specificity, for the detection of all proven cases of tumors of the upper urinary tract. The main objective with MDCT-urography is to detect all possible causes of hematuria while using the lowest possible radiation dose to the patient. As shown by the authors the split-bolus technique has the potential to reduce both radiation dose and the number of images generated by MDCT urography. In our opinion this protocol is ideal for patients submitted to previous cystoscopy since we might miss some small tumors within a fully distended and opacified bladder .As we have discussed previously in this journal (volume 33, number 3, pages 435-436), we consider "the bladder-wall phase" (scans at 60 or 70 seconds after intravenous injection of the total amount of contrast), essential for the detection of small bladder tumors. However, this "bladder phase wall" has the drawback of significant increase in the effective radiation dose to the patient (18 to 25 mGy).

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UROGENITAL TRAUMA

Selective nonoperative management of penetrating abdominal solid organ injuries

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Objective: To assess the feasibility and safety of selective nonoperative management in penetrating abdominal solid organ injuries.
Background: Nonoperative management of blunt abdominal solid organ injuries has become the standard of care. However, routine surgical exploration remains the standard practice for all penetrating solid organ injuries. The present study examines the role of nonoperative management in selected patients with penetrating injuries to abdominal solid organs.
Patients and Methods: Prospective, protocol-driven study, which included all penetrating abdominal solid organ (liver, spleen, kidney) injuries admitted to a level I trauma center, over a 20-month period. Patients with hemodynamic instability, peritonitis, or an unevaluable abdomen underwent an immediate laparotomy. Patients

who were hemodynamically stable and had no signs of peritonitis were selected for further CT scan evaluation. In the absence of CT scan findings suggestive of hollow viscus injury, the patients were observed with serial clinical examinations, hemoglobin levels, and white cell counts. Patients with left thoracoabdominal injuries underwent elective laparoscopy to rule out diaphragmatic injury. Outcome parameters included survival, complications, need for delayed laparotomy in observed patients, and length of hospital stay.
Results: During the study period, there were 152 patients with 185 penetrating solid organ injuries. Gunshot wounds accounted for 70.4% and stab wounds for 29.6% of injuries. Ninety-one patients (59.9%) met the criteria for immediate operation. The remaining 61 (40.1%) patients were selected for CT scan evaluation. Forty-three patients (28.3% of all patients) with 47 solid organ injuries who had no CT scan findings suspicious of hollow viscus injury were selected for clinical observation and additional laparoscopy in 2. Four patients with a "blush" on CT scan underwent angiographic embolization of the liver. Overall, 41 patients (27.0%), including 18 cases with grade III to V injuries, were successfully managed without a laparotomy and without any abdominal complication. Overall, 28.4% of all liver, 14.9% of kidney, and 3.5% of splenic injuries were successfully managed nonoperatively. Patients with isolated solid organ injuries treated nonoperatively had a significantly shorter hospital stay than patients treated operatively, even though the former group had more severe injuries. In 3 patients with failed nonoperative management and delayed laparotomy, there were no complications.
Conclusions: In the appropriate environment, selective nonoperative management of penetrating abdominal solid organ injuries has a high success rate and a low complication rate.

Editorial Comment

Most blunt solid organ injuries can successfully be managed nonoperatively. Stab wounds, in general can be managed nonoperatively about 50% of the time for anterior abdominal entrance wounds and 85% for retroperitoneal entrance. While traditionally teaching dictates that gunshot wounds of the abdomen were absolute indications for exploration, such concepts have been brought into question with multiple publications in the last few years, mostly championed by the trauma group from LA County Medical Center. In general the treatment algorithm for penetrating abdominal trauma is as follows: signs or symptoms of peritonitis, hemodynamic instability or an abdomen difficult to evaluate due to mental status change or body habitus, underwent intra-abdominal exploration. All other patients were imaged by CT with intravenous contrast. If the CT suggested a hollow organ viscus injury or a contrast "blush" with instability, the patient was explored. Contrast "bush" and stable patients underwent angiography. If there was no bowel injury and the penetrating wound was on the left and thoracoabdominal, a delayed diagnostic laparoscopy is performed to evaluate for a diaphragmatic injury. Any injury was repaired laparoscopically. The patient is then examined serially for the next 24 to 48 hours. Persistently asymptomatic patients were fed and discharged after 48 hours. 60% of penetrating injuries were explored immediately, 30% (27) of whom had with kidney injuries. Of the injured kidneys, 12 underwent renorrhaphy and 9 or (33%) nephrectomy. Of those kidney injuries managed conservatively, none of the Grade 1 and 2 injuries were explored while 1 of 3 of the Grade 3-5 injuries were explored. In conclusion, in the very select patient gunshot wound to the kidney patient, nonoperative management can be successful.

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Proposed mechanisms of lower urinary tract injury in fractures of the pelvic ring

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Objective: To investigate whether the observation of particular pelvic fracture patterns enables the clinician to predict the presence and type of injuries to the lower urinary tract, as the mechanisms of injury to the lower urinary tract in association with fractures of the pelvic ring are unclear.
Patients and Methods: The case-notes and radiographs of 168 patients with either pelvic ring or acetabular fractures were reviewed; 108 pelvic ring fractures (81 men, 27 women) and 60 acetabular fractures (46 men, 14 women). The pelvic fractures were classified according to the system described by Tile and were correlated with the incidence and type of lower urinary tract injury (LUTI).
Results: Overall, of the 108 men and women with pelvic ring fractures, 27 (25%) had a LUTI documented either radiologically or as an intraoperative finding. Of the 81 men with pelvic ring fractures, 24 (30%) had a LUTI, of whom six (7%) had an isolated bladder laceration, 14 (17%) a partial urethral injury (PUI) and four (5%) a complete urethral disruption (CUD). Five of the 18 men with urethral injuries also had bladder injuries and in three of these, the bladder neck was also injured. Three of 27 women (11%) had a LUTI, all of whom had isolated bladder lacerations. Of the 46 men with an acetabular fracture, one (2%) had a CUD, and three (7%) had a PUI. One of 14 of women with an acetabular fracture sustained a bladder laceration. None of the three men with a Tile Type-A pelvic ring fracture sustained a LUTI. Of the 28 men with 'open-book' (Tile Type-B1) fractures, 21 (75%) had no associated LUTI and seven (25%) had a LUTI (five partial urethral injuries and two bladder lacerations). Of the 10 men with 'lateral compression' (Tile Type-B2) fractures, six had no LUTI and four had a LUTI (two partial urethral injuries and two bladder lacerations). Of the 40 men with 'vertical shear' (Tile Type-C) fractures, 27 (68%) had no LUTI and 13 (32%) a LUTI (four complete urethral disruptions, seven partial urethral injuries, and two bladder lacerations) including all of the combined bladder and urethral injuries and all of the bladder neck injuries.
Conclusion: The pelvic fracture pattern alone does not predict the presence of a LUTI. When it occurs, the type of LUTI appears to be related to the fracture mechanism. The pattern of injury to the soft tissue envelope and specifically to the ligaments supporting the lower urinary tract offers the best correlation with the observed LUTI. We propose a mechanism for this.

Editorial Comment

The above article by Mundy clearly deserves a closer look, particularly at the illustrative images and figures. Pelvic fractures are typically classified by fracture pattern and mechanism of injury. The two most commonly used schemas are the Young-Burgess and the Tile classifications. They divide the fracture patterns more by mechanism into Type A, anterior compression (AP) injury, Type B, lateral compression (LC) and Type C vertical shear and conformationally unstable. Bladder injuries in the male with pelvic fractures are primarily due to shearing forces and not to bladder penetration from a bony spicule. This is illustrated by publications from the SF General Group where half of bladder injuries occurred on the opposite side of the bony fracture. Intuitively, a pelvic fracture that results in the most shearing forces, then should also give the highest likelihood for bladder injury. Urethral injuries have been classically described by Turner Warwick as prostato-membranous disruption injuries. It is my observation, and that of others, most injuries to the urethra from pelvic fracture are at the bulbomembranous junction and not at the level of the prostate. Again, in the male, it appears that shearing forces are the cause of urethral injury and not direct

compression or penetration. In other words, the injury that causes the most shearing forces to the urethra should cause an injury.

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PATHOLOGY

The role of P501S and PSA in the diagnosis of metastatic adenocarcinoma of the prostate

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Background: Adenocarcinoma of the prostate can present as metastatic carcinoma with no known primary. Prostatic origin can be confirmed in most of these cases by immunohistochemistry for prostate-specific antigen (PSA) and prostate-specific acid phosphatase. In a small subset of high-grade prostate carcinomas, both markers are negative and therefore are not helpful for confirming prostatic origin. Recently, novel marker proteins that are preferentially expressed in prostate tissue were identified. One such marker is P501S or prostein, a 553-amino acid protein that is localized to the Golgi complex. It is expressed in both benign and neoplastic prostate tissues, but not in any other normal or malignant tissue examined to date. Owing to its apparent specificity, prostein may be a good marker to demonstrate prostatic origin in metastatic prostate cancer.
Design: Five-micron sections of a tissue microarray were subjected to immunohistochemistry with a monoclonal mouse anti-P501S (clone 10E3, Dako, Carpintera, CA) antibody and a monoclonal mouse anti-PSA (clone ER-PR8, Dako, Carpintera, CA) antibody. The tissue microarray contains 78 cases of metastatic prostatic adenocarcinoma, 20 cases of primary prostatic adenocarcinoma, and 20 cases of benign prostate tissue from the peripheral zone as well as samples of benign brain, pancreas, kidney, thyroid, testis, skeletal muscle, and fibroconnective tissue.
Results: Similar staining (intensity and extent) was identified for both markers in the majority of metastatic tumors (11 distant sites, 42 pelvic lymph nodes), in all 20 primary tumors and in all benign prostate and nonprostate tissues. The P501S stain had perinuclear cytoplasmic (Golgi) distribution even in poorly differentiated tumors and metastases. Two distant metastases were negative for PSA but retained focal weak positivity for P501S. Two other distant metastases were weakly PSA positive, but strongly P501S positive. Metastases in the pelvic lymph nodes were positive for both markers in 53 cases and 1 lymph node metastasis was strongly PSA positive but P501S negative. In summary, 67 of the 69 cases (97%) of metastatic prostate carcinomas were PSA positive, whereas 68 of the 69 cases showed at least focal weak reactivity for P501S (99%). None of the tumors were negative for both markers.
Conclusions: Immunohistochemistry for P501S is a sensitive and highly specific marker for identifying prostate tissue. The large majority of metastatic prostatic adenocarcinomas are P501S positive (99%). A small subset of metastatic prostatic adenocarcinoma shows significant differences in staining intensity and extent for PSA and P501S and, therefore, combined use of these markers may result in increased sensitivity for detecting prostatic origin.

Editorial Comment

In 2001 (1), Xu et al. identified P501S or prostein, a novel prostate-specific protein expressed in normal and malignant prostate tissues. Characterization of the prostein gene showed that prostein cDNA encodes a 553-amino acid protein. The protein is predicted to be a type IIIa plasma membrane protein with a cleavable signal peptide and 11 transmembrane-spanning regions. Prostein gene is located on chromosome 1 at the WI-9641 locus between q32 and q42. Prostein mRNA is shown to be uniquely expressed in normal and cancerous prostate tissues using Northern blot, eDNA microarray, and real-time PCR analysis. Furthermore, prostein mRNA expression does not appear to be prostate tumor grade related and is restricted exclusively to prostate cell lines. Immunohistochemical staining using a mouse monoclonal antibody generated against prostein demonstrates that this protein is specifically detected in prostate tissues both at the plasma membrane and in the cytoplasm.

P501S or prostein should not be confounded with P504S (alpha-methylacyl coenzyme A racemase or AMACR). In 2000, Xu et al. (2) using cDNA library substraction in conjunction with high throughput microarray screening, identified 3 genes: P503S, P504S and P510S that showed differential expression in malignant and benign prostate glands. It was demonstrated AMACR (P504S) immunoreactivity in prostatic adenocarcinoma but not in benign prostatic glands, while P503S immunoreactivity was present in both malignant and benign glands. Furthermore, it was found AMACR overexpression in colorectal, ovarian, beast, bladder, lung, and renal cell carcinomas, as well as lymphomas and melanomas (3). This findings makes AMACR unsuitable for the diagnosis of metastatic adenocarcinoma of the prostate.

In the study surveyed, P501S or prostein showed that is a good marker in metastatic adenocarcinoma of the prostate. The authors found that 67 of the 69 cases (97%) of metastatic prostate carcinomas were PSA positive, whereas 68 of the 69 cases showed at least focal weak reactivity for P501S (99%). None of the tumors were negative for both markers. They conclude that Immunohistochemistry for P501S or prostein is a sensitive and highly specific marker for identifying prostate tissue. The large majority of metastatic prostatic adenocarcinomas are P501S positive (99%).

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Renal medullary carcinoma: report of seven cases from Brazil

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We report seven cases of renal medullary carcinoma collected from several institutions in Brazil. In spite of a relatively high incidence of sickle cell trait in Brazil, this is a rare tumor. All patients were males between the ages of 8 and 69 years (mean 22 years). From the collected information, the most frequent presenting symptoms were gross hematuria and flank or abdominal pain. The duration of symptoms ranged from 1 week to 5 months. Most of the tumors were poorly circumscribed arising centrally in the renal medulla. Size ranged from 4 to 12 cm (mean 7 cm) and hemorrhage and necrosis were common findings. All seven cases described showed sickled red blood cells in the tissue and six patients were confirmed to have sickle cell trait. All cases disclosed the characteristic reticular pattern consisting of tumor cell aggregates forming spaces of varied size, reminiscent of yolk sac testicular tumors of reticular type. Other findings included microcystic, tubular, trabecular, solid and adenoid-cystic patterns, rhabdoid-like cells and stromal desmoplasia. A peculiar feature was suppurative necrosis typically resembling microabscesses within epithelial aggregates. The medullary carcinoma of the 69-year-old patient was associated with a conventional clear cell carcinoma. To our knowledge, this association has not been previously reported and the patient is the oldest in the literature. The survival after diagnosis or admission ranged from 4 days to 9 months. The 8-year-old African-Brazilian patient with a circumscribed mass is alive and free of recurrence 8 years after diagnosis. This case raises the question whether a periodic search for renal medullary carcinoma in young patients who have known abnormalities of the hemoglobin gene and hematuria could result in an early diagnosis and a better survival.

Editorial Comment

Renal medullary carcinoma is a rare, rapidly growing tumor that affects young individuals with sickle cell trait. This tumor was described in 1995 by Davis et al. (1), which considered it the seventh sickle cell nephropathy. The six sickle cell nephropathies previously described by Berman (2), in 1974, are gross hematuria, papillary necrosis, nephrotic syndrome, renal infarction, inability to concentrate the urine and pyelonephritis. All of them are to a certain extent related to the obstruction of blood vessels and tissue hypoxia resulting from red blood cell sickling. The renal medulla is particularly susceptible to damage in sickle cell disease due to its unique environment characterized by anoxia, hyperosmolarity and low pH that tend to promote hemoglobin S polymerization and red blood cell sickling. Over a period of 22 years, the Armed Forces Institute of Pathology had collected only 34 cases (1) and over the next 5 years, only 15 more had been described (3).

The incidence of sickle cell trait in Brazil is 6.7% in African-Brazilians, 5.4% in Mulattos (persons with mixed White and African-Brazilian ancestry) and 0.21% in Whites (4). Considering the large population at risk, the tumor is, in fact, very rare suggesting that additional factors are likely necessary. This is the first report from Brazil as a result of the collaboration of several pathologists that searched for cases of renal medullary carcinoma in their institution's files.

Renal medullary carcinoma is typically seen in young patients with the sickle cell trait and exceptionally with sickle cell disease. All seven cases described in the study showed sickled red blood cells in the tissue and six patients were confirmed to have sickle cell trait. Renal medullary carcinoma shows a male predominance (2:1) and the mean age at presentation is approximately 22 years, with ages ranging from 5 to 40 years.

The most frequent presenting symptoms are gross hematuria and flank or abdominal pain. A palpable abdominal mass is often observed. Some patients may present with symptoms of metastatic disease. Spontaneous gross hematuria, the first sickle cell nephropathy, is usually unilateral and occurs at the same age range that renal medullary carcinoma. It is worth noting, however, that most of these spontaneous benign bleedings occur from the left kidney and most of the renal medullary carcinomas arise on the right kidney. The origin and pathogenesis of renal medullary carcinoma are not completely understood.

The prognosis of renal medullary carcinoma is very poor due to the highly aggressive behavior of this neoplasm and to its resistance to conventional chemotherapy. Metastases are both lymphatic and hematogenous with liver and lungs most often involved. The mean duration of life after surgery is about 15 weeks (5) and the

longest documented survival for renal medullary carcinoma was 15 months (6). Exceptionally, the 8-year-old African-Brazilian patient with a circumscribed mass described in the study is alive and free of recurrence 8 years after diagnosis. Chemotherapy has been known to prolong survival by few months but generally, neither chemotherapy nor radiotherapy has altered the course of the disease (7).

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INVESTIGATIVE UROLOGY

Structural organization of fibrous connective tissue in the periacinar region of the transitional zone from normal human prostates as revealed by scanning electron microscopy

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BJU Int. 2007; 100: 940-4

Objective: To analyse, using scanning electron microscopy (SEM), the organization of stromal fibrous components in the transitional zone (TZ) from normal human prostates; because of its association with disease, greater emphasis was placed upon the periacinar region of the stroma.
Materials and Methods: TZ specimens were obtained from normal prostates during autopsy of six men, aged 18-30 years, who had died from accidents. Tissue was fixed for SEM in a modified Karnovsky solution for 48 h at 4 degrees C, and to visualize the three-dimensional organization of the stroma, samples were treated to remove cells.
Results: In acellular preparations, narrow fibrous septa formed a dense and supportive scaffold for ducts and acini, and a smooth and homogeneous fibrous sheet, herein identified as pars fibroreticularis, lined the acinar

lumen. More internally, fibrous septa had a spongy organization with dense lamellae. Higher magnification showed that the smooth luminal sheet is made of 115-154-nm thick fibrils in a tight parallel arrangement. Just under this layer there was a meshwork of fibrils 77-115 nm thick that were orientated in less defined directions. Conclusion: In the TZ of the human prostate, dense stromal fibrous components around acini act as a barrier that might enhance local cellular responses and events that occur in disorders such as benign prostatic hyperplasia. The periacinar pars fibroreticularis supports the notion of high structural variability in this region of basement membranes.

Editorial Comment

The transitional zone (TZ) is particularly relevant for prostate pathology as it is thought to be the main region of the gland that enlarges in BPH.

The present findings show that in the TZ of the human non hyperplastic prostate, dense stromal fibrous components around acini may act as a diffusion barrier that might enhance local cellular responses and events that are known to occur in disorders such as BPH. The periacinar stroma also includes a distinct pars fibroreticularis, and this supports the notion of high structural variability in this region of basement membranes.

The normative findings on prostate TZ presented here will also serve as comparison for future findings of this region in patients with BPH.

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Relationship between adult dark spermatogonia and secretory capacity of Leydig cells in cryptorchidism

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Objective: To examine whether hormonal therapy before orchidopexy affects the histology of the testis and to assess the responsiveness of the Leydig cells, as it has been shown that although basal plasma testosterone levels are within the 'normal' range in cryptorchid boys there is an insufficient increase of testosterone after a human chorionic gonadotrophin (hCG) stimulation in approximately 30% of cryptorchid boys.
Patients and Methods: In all, 55 boys (aged 1-7 years) with a unilateral undescended testis were included in the study and divided into two groups. Group I (32 boys) received hormonal therapy before orchidopexy; 17 boys received a long-acting LHRH analogue (buserelin) administered as a nasal spray in doses of 20 microg/day for 28 days, followed by 1500 IU hCG intramuscularly (i.m.) once a week for 3 weeks, and the remaining 15 received 1500 IU hCG i.m. once a week for 3 weeks. Group II (33 boys) had orchidopexy alone. During orchidopexy biopsies were taken from the undescended and contralateral descended testes of the boys in both groups for histological analyses. Variations in the number of adult dark (Ad) spermatogonia per tubule (Ad/T) were assessed and testosterone levels were measured during the course of the hormonal therapy (before treatment, 14 days after initiation of buserelin administration, 24 h after each hCG injection, and 3 months after cessation of therapy).
Results: In group I, 17 boys (53%) had a 'normal' Ad/T after hormonal treatment vs only six (18%) in group II after orchidopexy alone (P = 0.019). In the hormonally treated boys (group I) we compared the testosterone

values 24 h after the second injection of hCG (when the response was most pronounced). Those with a normal Ad/T had a mean (sd) testosterone level of 199.5 (97.6) ng/dL vs 99.6 (85) ng/dL in those with an inadequate Ad/T response to hormonal therapy ($P < 0.003$).
Conclusion: We have confirmed that there are two subgroups of cryptorchid boys. Patients with a sufficient Leydig cell secretory capacity will have normal testicular histology and Ad spermatogonia count after hormonal treatment. While those with a suboptimal Leydig cell capacity will have a low Ad spermatogonia count and consequently poor prognosis for future fertility, despite successful surgery. As to whether different types and durations of the hormonal therapy in patients with impaired Leydig cell response could lead to improved testicular histology and consequently improved prognosis for future fertility, remains to be answered.

Editorial Comment
This paper presents new important insights on the understanding of cryptorchidism and its treatment and I will highlight some important points.

The authors demonstrated for the first time, that the transformation of gonocytes into Ad spermatogonia is a testosterone-dependent process. If an adequate increase in plasma testosterone follows hormonal stimulation, normal germ-cell maturation occurs. Patients that have an insufficient Leydig cell response to hormonal stimulation, resulting in an inadequate testosterone increase, will have poor testicular histology and a low Ad spermatogonia count.

Interesting, the authors concluded that appears to be two subgroups of cryptorchid boys; those with a sufficient Leydig cell secretory capacity and those with a suboptimal Leydig cell secretory capacity.

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RECONSTRUCTIVE UROLOGY

Resurfacing and reconstruction of the glans penis

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Eur Urol. 2007; 52: 893-8

Objectives: To describe the techniques and results of surgical reconstruction of glans penis lesions.
Methods: Seventeen patients (mean age: 53.2 yr) were treated by resurfacing or reconstruction of the glans penis for benign, premalignant and malignant penile lesions. The aetiology of the lesions was one Zoon's balanitis, four lichen sclerosus, one carcinoma in situ, five squamous cell carcinomas, and six squamous cell carcinomas associated with lichen sclerosus. Five cases were treated by glans skinning and resurfacing; five cases by glans amputation and reconstruction of the neoglans, and seven cases by partial penile amputation and reconstruction of the neoglans. Glans resurfacing and reconstruction were performed with the use of a skin graft harvested from the thigh.
Results: The mean follow-up was 32 mo. All patients were free of local premalignant/malignant recurrence. Patients who underwent glans resurfacing reported glandular sensory restoration and complete sexual ability. Patients who underwent glansectomy or partial penectomy with neoglans reconstruction maintained sexual function and activity, although sensitivity was reduced as a consequence of glans/penile amputation.

Conclusions: In selected cases of benign, premalignant or malignant penile lesions, glans resurfacing or reconstruction can ensure a normal appearing and functional penis, without jeopardizing cancer control.

Distal urethral reconstruction of the glans for penile carcinoma: results of a novel technique at 1-year of followup

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Purpose: No satisfactory techniques are available to replace the anatomy and function of the penile glans after radical surgery for penile carcinoma. We report a new technique of glans reconstruction using distal urethra. We evaluated anatomical, physiological and esthetic features as well as short-term and long-term clinical outcomes.
Materials and Methods: A total of 14 patients with a mean age of 54 who had squamous penile carcinoma underwent glans reconstruction after simple glansectomy in 8 and after amputation of the distal third of the shaft in 6. Glans sensibility, erectile function, ejaculation, orgasm, penile length, local recurrence, patient and partner satisfaction, and quality of life were evaluated before and after the operation. Mean followup was 13 months.
Results: All patients noticed subjective and objective thermal and tactile epicritic sensibility in the area of the neoglans. Ten of 14 patients (71%) noticed spontaneous and/or induced rigid erections. Interestingly International Index of Erectile Function scores in the ejaculation and orgasm domains did not significantly change in the period before and after surgery. No local disease recurrence or penile retraction were reported at long-term followup.
Conclusions: Reconstructive glanuloplasty with distal urethra in penile tumor surgery is an innovative, easy and rapid surgical technique with appreciable functional and esthetic results.

Editorial Comment

Reasons for penile reconstruction may not only be neoplasia, but also trauma, inflammatory disease and congenital malformation. In many cases, careless even unnecessary amputations eliminate the possibility for a satisfactory glans reconstruction. Because penile anatomic reconstruction is often possible, the EAU has established treatment guidelines on penile cancer (1) which favor the use of conservative penile sparing techniques for the tumor entities of Ta-T1, G1-3 and select cases of T2 tumors.

Palmintteri et al. and Gulino et al. published their techniques, which appear to help in the reconstruction of the penile glans with a good cosmetic outcome (2,3). Palminteri et al. used a free split-thickness skin graft of the thigh. Gulino et al. investigated their functional outcome even further after using the distal urethra in the reconstructive approach. With a physical examination, the IIEF (erection, ejaculation, orgasm and libido domain score) and the Bigelow & Young scores, they evaluated an overall satisfying outcome with a minor additional surgical effort (mean 35 min). The advantage of using the distal urethra is the untroubled blood supply (including certain rigidity under erection) and the sensibility.

Both techniques can be performed for a distal penile reconstruction involving amputation up to one third of the penile length. In case of penile cancer, both oncological radicality and satisfactory body image can be achieved. It complies with compliance of EAU penile cancer guidelines and maximizes patients´ quality of life without compromising tumor survival.

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Nerve-sparing radical cystectomy and orthotopic bladder replacement in female patients

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Objectives: Orthotopic diversion, initially performed solely in men, has now become a viable option in women. Approximately 15 yr ago, at several centres, urethra-sparing cystectomy and orthotopic diversion were initiated in women with bladder cancer. Several studies have since addressed both the oncologic and functional outcomes of this procedure.
Methods: We describe our surgical technique of cystectomy and orthotopic urinary diversion in female patients, with an emphasis on how we preserve the neurovascular bundle.
Results and Conclusions: An improved understanding of the anatomic neurovascular and fascial planes related to the rhabdosphincter has facilitated identification of elements needed for orthotopic diversion in female patients. The technique of en bloc anterior exenteration includes the anterior portion of the vagina; however, preservation of the rhabdosphincter and its autonomic nerve supply necessitates specific modifications of the standard operation. The video provides a detailed description of our surgical technique with attention to anatomic details necessary to avoid damage to the proximal urethra and to preserve the autonomic innervation of the rhabdosphincter.
Autonomic nerve preservation reconfirmed

Editorial Comment

This is another detailed description and rationale for an orthotopic bladder substitution in female patients undergoing radical cystectomy. There is a plethora of literature now about the use of urinary continence diversion to the urethra in female patients. Whereas almost all contributions agree that such a diversion can and should be authored to female patients there is still no agreement whether better functional results can be achieved with a preservation of autonomic nerves running to the remnant isolated urethra. This contribution by well-known experts clearly favor preservation of autonomic nerves for two reasons: sexual activity, especially in younger women has been more or less neglected for many years but seems to be important (reference 16 and 17 in the manuscript), this group has formerly demonstrated that preservation of autonomic nerves also contributes to continence. It is therefore not only important to preserve autonomic nerves in the younger patients, although sexually active, but to preserve autonomic nerve in elderly patients as well, those patients that are in danger of having a borderline continence postoperatively. A better sensitivity of the remnant urethra will be better for the "first drop incontinence" due to better reaction of urine entering the urethra and resulting in reflex contraction in the pelvic floor but also will help in achieving better results with postoperative physical therapy for urinary incontinence (1).

Not everything concerning the function of autonomic nerves with regards to clitoral and vaginal function, secretion of pelvic glands, function and long-term fate of urethral smooth musculature, and interaction with rhabdosphincter and pelvic floor musculature is known to date. However, with increasing knowledge we know that it is important to preserve at least part of the ganglions and nerve fibers of pelvic autonomic nerves to increase the quality of life for these patients in the long-term. In addition, it is the long-term quality of life where functional outcome is important contrary to oncological outcome, which in the first few years seems to be dominant as quality of life studies have shown.

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UROLOGICAL ONCOLOGY

Preoperative serum testosterone level as an independent predictor of treatment failure following radical prostatectomy

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Objectives: Preoperative low serum testosterone (TS) level has been reported to be associated with adverse pathologic results in patients with clinically localized prostate cancer (pCA) treated with radical prostatectomy (RP). However, prior studies failed to show prognostic impact of preoperative low TS in these patients. The aim of this study was to investigate the relationship between preoperative TS and prostate-specific antigen (PSA) failure in these patients.
Methods: Of 304 patients diagnosed with clinically localized pCA who had been treated with RP alone, 272 patients whose preoperative TS level had been measured were eligible for this analysis. Postoperative TS levels were also available in 222 of the 272 patients. Cox proportional hazard model was used to elucidate factors predictive for PSA failure.
Results: Of the 272 patients 49 had low (< 300 ng/dl) and 223 had normal preoperative TS level. In a stepwise multivariate analysis, preoperative TS ($p = 0.021$) was an independent and significant predictor of PSA failure along with RP Gleason score ($p = 0.006$), surgical margin status ($p = 0.0001$), and PSA ($p = 0.0001$). Five-year PSA failure-free survival rate of the patients with preoperative low TS (67.8%) was significantly worse than that with normal TS (84.9%) ($p=0.035$). Serum TS levels increased significantly after RP ($p < 0.0001$). The increment of TS level in preoperative low TS group was significantly greater than that in preoperative normal TS group ($p = 0.0003$).
Conclusions: The current results demonstrated that preoperative TS level is an independent and significant predictor of PSA failure after RP in patients with clinically localized pCA. European Association of Urology.

Editorial Comment

Testosterone levels and prostate cancer are a topic that attracts much attention and stirs controversy. This contribution from Tokyo, Japan adds to the multifaceted database. With a cut-off at 300 ng/dL total testosterone the authors found an inverse correlation of testosterone level and prostate cancer aggressivity as measured by Gleason score, positive surgical margins, PSA, and 5-year postoperative PSA failure status.

Most interestingly, testosterone levels increased after radical prostatectomy. The postoperative increment of testosterone levels was significantly higher in the group with preoperative low testosterone. Clearly, these data deserve confirmation from other groups and elucidation of the mechanisms involved in testosterone level variation after radical prostatectomy.

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Concomitant carcinoma in situ is a feature of aggressive disease in patients with organ-confined TCC at radical cystectomy.

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Objectives: Carcinoma in situ (CIS) is a nonpapillary, high-grade, potentially aggressive, and unpredictable manifestation of transitional cell carcinoma (TCC) of the bladder. The aim of this study was to assess whether presence of concomitant CIS has a detrimental effect on cancer control after radical cystectomy.
Methods: The records of 812 consecutive patients who underwent radical cystectomy and pelvic lymphadenectomy for bladder TCC at three US academic centres were reviewed. Ninety-nine of 812 (12%) patients had CIS only at radical cystectomy and were excluded from the analyses.
Results: Three hundred thirty of the 713 (46.3%) patients had concomitant CIS at radical cystectomy. Patients with TCC involvement of the urethra were more likely to have concomitant CIS than not (61% vs. 40%, p=0.018). Concomitant CIS was significantly more common in patients with lower cystectomy stages and higher tumour grades. In univariate, but not multivariate, analysis, patients with concomitant CIS versus those without were at increased risk of disease recurrence (p=0.0371). In patients with organ-confined disease, concomitant CIS was an independent predictor of disease recurrence (p=0.048 and p=0.012, respectively) but not bladder cancer-specific mortality (p=0.160 and p=0.408, respectively) after adjusting for the effects of standard postoperative features.
Conclusions: Concomitant CIS in the cystectomy specimen is common, and patients with concomitant CIS are at increased risk of urethral TCC involvement. The presence of concomitant CIS appears to confer a worse prognosis in patients with non-muscle-invasive TCC treated with radical cystectomy.

Editorial Comment

The outcome of patients with bladder cancer of any stage and concomitant CIS was analyzed in this retrospective study on 713 patients undergoing radical cystectomy. Several issues deserve comments: The percentage of

CIS present increased over the years from 33% in the eighties to 52% in period from 2001 to 2003. The majority of patients had grade 3 tumors (82.5%) and/or pT2 and pT3 disease (60.8%). Roughly, half of the patients had lymphovascular invasion, one-fourth (24.9%) had lymph node metastases. Accordingly, after 5 years, half of the patients with concomitant CIS had recurrences and half of the patients with concomitant CIS had died from bladder cancer. Patients without concomitant CIS fared better than those with concomitant CIS (7-year recurrence-free survival 58.1% and 41.5%, respectively). Interestingly, the incidence of concomitant CIS was highest in patients with organ-confined disease (pTa excluded) and higher in lower-stage and higher grade disease. Involvement of the urethra was more common in CIS patients.

The authors state correctly, that presence of concomitant CIS worsens the outcome significantly. In practical terms, early radical treatment should be considered if CIS is present.

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NEUROUROLOGY & FEMALE UROLOGY

Urodynamic studies in women with stress urinary incontinence: Significant bacteriuria and risk factors

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Neurourol Urodyn. 2007; 26: 847-51

Aim: A prospective study was performed to determine the incidence of significant bacteriuria and to identify the risk factors for bacteriuria after urodynamic studies (UDSs) in women with urodynamic stress urinary incontinence (SUI).
Methods: A total of 225 women with urodynamic SUI were evaluated. All women were negative on double-screened urine cultures, in clean-catch midstream urine (MSU) specimens, before UDS. Another urine specimen was obtained for urinalysis and culture at 3-7 days after UDS. Urinary culture with 10(5) CFU/ml or more was regarded as significant bacteriuria. To identify the risk factors for significant bacteriuria, the clinical characteristics of all patients including age, BMI, parity, medical and operation history, degree of pelvic organ prolapse, results of urinalysis, and UDS were evaluated.
Results: The prevalence of significant bacteriuria was 6.2%. The most common identified microorganism was Escherichia coli (57.1%). Univariate analysis demonstrated that a history of recurrent urinary tract infection (UTI; P = 0.002) and urological surgery or procedure (P = 0.02) were significant predictors of significant bacteriuria. On multiple logistic regression analysis the past history of recurrent UTI was the only significant independent risk factor (OR = 28.5, 95% CI = 4.309-188.488, P = 0.009).
Conclusions: This study suggests that for most women with SUI it may be unnecessary to use preventive prophylactic antibiotics in UDS. However, our results suggest that in patients with a previous history of recurrent UTI or urologic surgery the risk for significant bacteriuria is increased and use of prophylactic antibiotics should be considered. Neurourol. Urodynam. 26:847-851, 2007. (c) 2007 Wiley-Liss, Inc.

Editorial Comment

Investigators performed a prospective study examining the prevalence of significant bacteriuria after urodynamic studies and to identify risk factors for same. It was noted that recurrent cystitis and previous urologic instrumentation or procedures were significant risk factors of bacteriuria. The authors obtained urine approximately one week before the urodynamics, at the time of the urodynamic studies, as well as 3-7 days after urodynamic studies were done. These investigators concluded that because the cultures were sterile for the procedure that all acquired infections within the week after the urodynamic studies were most likely due to the urodynamic studies. Of note is that the bacteriuria after the urodynamic studies was most likely non-nosocomial. It would have been or great interest if the authors had been able to query the patients on the frequency and intensity of coitus for the period immediately after the urodynamic studies to the time that the post-procedure urine studies were obtained. The existence of "honeymoon cystitis" is well known even in the mature or infirmed population.

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Outcomes following repeat mid urethral synthetic sling after failure of the initial sling procedure: rediscovery of the tension-free vaginal tape procedure

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J Urol. 2007; 178: 1370-4; discussion 1374

Purpose: We evaluated outcomes of the repeat mid urethral sling to treat recurrent or persistent stress urinary incontinence after failure of an initial mid urethral sling.
Materials and Methods: We retrospectively analyzed data on patients who underwent the repeat mid urethral sling procedure due to persistent or recurrent stress urinary incontinence. Repeat slings were placed without removal of the previous sling. All patients were followed at least 1 year after the second mid urethral sling.
Results: Of the 31 female patients with a repeat mid urethral sling 29 were followed, including 13 with a retropubic and 16 with a transobturator sling. For the first mid urethral sling 17 patients received a retropubic sling (tension-free vaginal tape) and 12 received a transobturator sling (6 inside out and 6 outside in procedures). Cure and improvement rates irrespective of the approach were 75.9% (22 of 29 patients) and 6.9% (2 of 29), respectively. Cure rates for the retropubic and transobturator slings were 92.3% (12 of 13 patients) and 62.5% (10 of 16), respectively, a difference that did not quite attain statistical significance ($p = 0.089$).
Conclusions: The repeat mid urethral sling for persistent or recurrent stress urinary incontinence has a lower cure rate than the initial sling. However, the retropubic approach tends to have a higher cure rate than the transobturator approach in repeat sling cases.

Editorial Comment

The authors review their very large experience with suburethral slings and report on patients who underwent a repeat suburethral sling. The study group included retropubic suburethral slings as well as the

transobturator approaches. The surgeons noted that their repeat suburethral sling procedure that was a re-do operation had a lower success rate than the initial operation success rate. This has been noted as well for patients undergoing re-do pubovaginal slings using autologous fascia for operative failures (1). The trend towards a lesser cure rate with a repeat transobturator procedure versus a retropubic approach could potentially be explained by both the urethral angle theory as discussed by the authors as well as the level of suburethral support that can be provided by the different techniques. The diminished efficacy of transobturator slings in patients with lower Valsalva leak point pressures is currently being explored in the literature (2).

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PEDIATRIC UROLOGY

Efficacy of combined anticholinergic treatment and behavioral modification as a first line treatment for nonneurogenic and nonanatomical voiding dysfunction in children: a randomized controlled trial

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Purpose: This randomized blinded clinical study was designed to compare the efficacy of tolterodine treatment combined with behavioral modification, behavioral modification alone and behavioral modification plus placebo in children with nonneurogenic, nonanatomical voiding dysfunction.
Materials and Methods: A total of 72 children meeting inclusion criteria were randomly allocated to 1 of 3 groups. One group received tolterodine (1 mg twice daily) along with behavioral modification, 1 received behavioral modification only and 1 received placebo with behavioral modification. A dysfunctional voiding scoring system questionnaire was completed for all patients at the beginning of the study, and at 1 and 3 months of treatment.
Results: A total of 71 patients were evaluated. The groups did not differ with respect to age, gender and symptom score before study enrollment ($p > 0.05$). Repeated calculations of symptom scores at 1 month of the treatment revealed a significant decrease in symptoms in all 3 groups, with a significant decrease in patients receiving tolterodine. In addition, at month 3 the symptom score of the tolterodine group was significantly lower compared to month 1, while scores remained steady in the behavioral modification and behavioral modification plus placebo groups.
Conclusions: Tolterodine combined with behavioral modification for voiding dysfunction in children without neurological or anatomical abnormality can be recommended as a first line treatment before invasive evaluation.

Editorial Comment

This is an interesting prospective randomized controlled trial, which relied primarily on a dysfunctional voiding scoring system from Toronto Children's to evaluate the outcome of the treatment. 72 children were selected with equal number of boys and girls, allocated into one of three groups. Voiding dysfunction that qualified them for the study was incontinence, frequency, urgency or obstructive symptoms with or without recurrent non-febrile urinary tract infections in the absence of obvious anatomical or neurogenic disease. Patients were between 4 and 12 years-of-age. Anatomic disease was evaluated by ultrasound and not VCUG, and the patients were not selected by any urodynamics or uroflow criteria. All patients were trained in behavior modification, including timed voiding, double-voiding and relaxation of the pelvic floor during voiding.

Group 1 patients were started on tolterodine 1 mg twice daily and were maintained for three months. Group 2 had no medications and received only behavior modification training. Group 3 were patients who had a placebo administered along with behavior modification training. Dysfunctional bowel was noted and treated in 30 of the 72 patients. The dysfunctional voiding questionnaire was given at the beginning of the study, at end of one month and again at the end of three months.

The results showed that initial dysfunctional voiding symptom scores were not significantly different. All three groups showed significant decrease after one month of treatment with a greater statistical significance in the Tolterodine group. Interestingly, the behavior modification group that did not receive placebo had lower symptom scores at one month and three months. Gender adjustment did not affect statistical results of the groups. 41 patients had a history of afebrile UTI's and 15 patients had afebrile urinary tract infections at enrollment during the study. Urine cultures were monitored monthly with new UTI's in 18 patients relatively equally spread over the three groups.

This is an interesting study because of its prospective randomized nature. Tolterodine was tolerated in all the patients except one, with statistically beneficial effects combined with behavior modification. The patients chosen were patients similar to an office practice and were not particularly well screened with urodynamics or uroflow studies, so that this represents an "all-comers" group with very good outcomes. It will be interesting to see if other studies use the dysfunctional voiding scoring system and if it stands up under the test of other investigators scrutiny.

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Quality assessment of hypospadias repair with emphasis on techniques used and experience of pediatric urologic surgeons

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Objectives: To assess outcomes in hypospadias repair at our institution, as compared with the literature, with repair technique and surgeon considered as risk factors.
Methods: The results of 299 primary hypospadias corrections were analyzed. All procedures were performed by three experienced pediatric urologists. Mean patient age at operation was 16.3 months. Follow-up was between 6 months and 5.5 years. Distal hypospadias repair was carried out in 242 patients, with tubularized incised plate reconstruction in 100 patients, advancement in 128, and the Mathieu technique in 14.

Results: During follow-up, complications occurred overall in 93 patients (31%). For distal hypospadias complications occurred in 59 patients (24%). The most common findings for distal hypospadias were urethral fistulas (14.4%). The complication rate depended on the severity of the anomaly (0 glanular, 28% pericoronal, and 63% proximal) and the chosen technique (16% advancement technique versus 60% tubular techniques). We found statistically significant differences in complication rates between operating surgeons.
Conclusions: Complications after hypospadias surgery are frequent. They are multifactorial and depend mainly on the type of the anomaly, the chosen technique, and the experience of the surgeon. More studies are needed to obtain an internationally accepted quality indicator for the outcome of hypospadias repair.

Editorial Comment

These authors reviewed 299 primary hypospadias repairs over a five year period with a special emphasis on the technique used and the experience of the pediatric urologic surgeon, with a mean follow up of 29 months and with a very critical eye for complications. The mean age of surgery was 16.3 months.

Tubularized incised plate technique was used in 133 patients and advancement techniques not requiring sutures in the urethra were used in 128 patients with 38 patients having miscellaneous techniques. Prophylactic antibiotics were given. Stenting was left to the choice of the individual surgeons. All procedures were performed with loupe magnification.

The groups were analyzed according to technique used and with respect to the three operating pediatric urologic surgeons. All glanular hypospadias patients did uniformly well. 93 patients, or 31% of the patients had a complication after surgery. 7% have recurrent problems that required more than one surgical intervention. 18% were fistulas, partial dehiscence of the wound or glans resulting in meatal retraction was 7.4% and urethral stenosis was 2%. Complication rates were higher the further away from the tip of the penis that the hypospadias meatus was, which is not surprising. Advancement techniques had a complication rate of 16%, while tubular reconstructions had a complication rate of 56%. Tubularized incised plate urethroplasties had a complication rate of 27%. When tubularized incised plate was used for hypospadias on the shaft of the penis, the complication rate was 66% and when it was used for distal hypospadias, it was 35%.

The pediatric urologist who had the most experience had a statistically significant better success rate for hypospadias repairs than the pediatric urologist with the least experience. 24% complication rate was noted in the hands of the most experienced surgeon and 40% complication rate in the least experienced surgeon.

At first glance this manuscript seems to have a high complication rate, however all patients that had a single-staged hypospadias reconstruction were included and complication rates are higher in the studies that include all patients rather than those that deal with a single technique. The authors should be congratulated on their attention to the detail of the complications and their honest reporting.

Of note for students of hypospadias, when the tubularized incised plate urethroplasty was used for mild hypospadias, it was very successful, however when it was extended to more severe hypospadias patients it was not.

It has always been my belief that hypospadias complications are directly proportional to the length of the repair and this study seems to validate that relationship also. There is some speculation in the study about the learning curve, since the newest member of the faculty member had a higher complication rate than those who had been there for 5 and 14 years. In some respects, it is encouraging to note that within 5 years the experience seems adequate to have very good results.

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