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The names of the Authors of the paper "**Incidental urinary tract pathologies in the one-stop prostate cancer clinic**" *published in* **Archivio Italiano di Urologia e Andrologia vol. 82 n. 1, March 2010** *are as follows:* **Aza Mohammed, Iqbal S. Shergill, Muhammad T. Vandal, Sandeep S. Gujral.**

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REVIEW

Critical issues in chronic prostatitis.

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Summary

In the last decade, an impressive amount of clinical research data has shed new light on pathogenesis and management of the chronic prostatitis syndrome. A new classification and a validated symptom score have enabled urologists worldwide to speak a "common language", thus greatly improving the amount and quality of focused research in this field. In Europe, a large number of groups and experts have been actively involved in this research, and have developed in many cases a genuine view on prostatitis and chronic pelvic pain etiology, diagnosis and treatment. The present paper, written by a panel of researchers from Europe and Far East Russia, reviews the most recent findings, discusses the most controversial contemporary topics on prostatitis syndromes, and highlights a number of unresolved issues requiring further research and study.

KEY WORDS: *Prostatitis; Chronic Bacterial Prostatitis; Chronic Pelvic Pain Syndrome; NIH-CPSI; P/CPPS.*

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INTRODUCTION

The chronic prostatitis (CP) syndrome includes a group of diseases of bacterial and non-bacterial etiology, characterized by pelvic pain, voiding dysfunction and additional signs/symptoms (1).

The adoption of the NIH Chronic Prostatitis Symptom Index (NIH-CPSI) as an internationally-acknowledged symptom inventory, the use of low urinary tract segmented tests, the application of a novel classification system (1) and the administration of fluoroquinolones as first-line agents were major landmarks in research and clinical management of CP in the last decade. However, due to the complexity and heterogeneity of this syndrome, the knowledge about etiology, diagnosis and treatment of CP is continuously revised and discussed (2).

Rather than reviewing basic concepts already described in several comprehensive publications (3-7), this article focuses on critical, debated issues regarding classification, diagnosis and treatment of CP. The discussion of these topics is based on recently published evidence, on research experience and on the "real-life" practice of a panel of urologists and scientists from Europe and Far East Russia, who attended an international prostatitis meeting in the greater Milan Area in November 2008. Critical issues were thoroughly discussed and are presented here. Practical examples concerning the various diagnostic and therapeutic approaches to the various subclasses of prostatitis, adopted by different European groups are also provided.

CLASSIFICATION OF PROSTATITIS

The contemporary classification system for prostatitis was approved by a NIH-NIDDK workshop committee in 1999 (8), and represents today's standard for research:

Category I Acute Bacterial Prostatitis (ABP) is an acute prostate bacterial infection, in patients showing local

(pain, voiding disturbances, pyuria, bacteriuria) and systemic (fever, malaise, etc.) signs/symptoms;
Category II Chronic Bacterial Prostatitis (CBP) occurs when a patient undergoes recurrent episodes of urinary tract infection (UTI) caused by an uropathogen, and if the prostate is identified as the focus of these infections;
Category III Chronic Prostatitis/Chronic Pelvic Pain Syndrome (CP/CPPS) is mostly defined by exclusion criteria, being characterized by urological pain as major component. Patients with the inflammatory "a" subtype of CP/CPPS have leukocytes in their expressed prostatic secretions (EPS), post-massage urine, or semen. Patients with the noninflammatory "b" subtype have no apparent evidence of inflammation.
In *category IV Asymptomatic Inflammatory Prostatitis (AIP)*, no subjective symptoms are shown by subjects usually undergoing prostate biopsy or EPS analysis.

Chronic Bacterial Prostatitis

Category II CBP is traditionally characterized by recurrent episodes of UTI caused by acknowledged uropathogens. Between symptomatic UTI episodes, lower urinary tract (LUT) segmented cultures show an infected prostate as the focus of these infections (8).
As a consequence, the diagnosis of category III CP/CPPS is a diagnosis "of exclusion", made in symptomatic patients with "no demonstrable infection" of the prostate (9, 1), corresponding to the former categories of "abacterial prostatitis" and "prostatodynia". There is, however, contoversy between experts on how to classify patients with chronic prostatitis and pathogens localized to the prostate, but lacking a history of recurrent UTIs.
Some experts classify these patients into category III CP/CPPS by creating a new subcategory of "infectious/bacterial CP/CPPS" as proposed in the context of the recently published UPOINT phenotype classification (10,11). The idea of this classification is that category III CP/CPPS patients represent a very heterogenous group with urinary, psychosocial, organ specific symptoms, infection, neurologic symptoms and/or skeletal muscle tenderness (UPOINT). The composition and severity of these symptoms, however, vary widely between patients. The authors hypothesize that if patients could be stratified according to the composition of their symptoms and treated accordingly by a multimodal therapeutic approach, the final clinical outcome should be more favourable.
This hypothesis looks quite attractive, but needs to be substantiated by appropriate clinical studies. It is, however, not quite logical why a chronic infectious prostatitis caused by classical uropathogens, e.g. *E. coli, Enterobacteriaceae or Enterococcus*, with a history of recurrent symptomatic UTIs, and a chronic infectious prostatitis caused by the same classical uropathogens without a history of recurrent UTIs should be classified into two different categories. So far it is not known whether a difference can be found by clinical studies comparing the outcomes of antibacterial treatment in patients showing prostatic infection in the presence or absence of a history of recurrent UTIs.
If a more specific phenotyping is the reason for proposing the UPOINT system, then it would be more logical to differentiate between cat.II CBP on one side with subcategories according to the presence/absence of UTI history or to the type of pathogens, and cat.III CP/CPPS without an infectious component on the other side. In both categories patients could then also be phenotyped according to all the other UPOINT symptoms. Alternatively, symptomatic CBP and CP/CPPS patients could be lumped together and phenotyped according to the six UPOINT categories.
The panel of speakers at the Milan meeting, authoring the present article agreed that it is very important to stratify patients into those with an infectious component and those *without demonstrable infection of the prostate*. Therefore, they rather propose to classify the group with a chronic prostatic infection with and without a history of recurrent UTIs into category II and those without an infectious component into category III until more convincing arguments will be available. This does not represent a trivial diagnostic exercise, but is rather a key-issue, bearing major therapeutic implications, such *as the optionality, the aggressiveness and the duration* of antibacterial treatment and, ultimately, the success of therapy. This does not mean that an exact diagnosis of an infectious component is always easy. The mere laboratory evidence of the presence or absence of bacteria is often not sufficient to proof or exclude infection. Like in other chronic infectious diseases the final diagnosis is established by clinical and specific laboratory investigations and sometimes only by treatment outcome.

Chronic Prostatitis/Chronic Pelvic Pain Syndrome

A controversial issue in prostatitis classification is the usefulness and appropriateness of dividing cat.III CP/CPPS into inflammatory ("a") and non-inflammatory ("b") subclasses on the basis of the clinical presentation of patients, of the results of diagnostic tests and on the outcome of therapeutic interventions.
Leukocytes detected in EPS, post-massage urine or semen were considered markers of an inflammatory process likely caused by an undetected infection and triggering an immune response by lymphocytes, neutrophils, macrophages. Thus, despite assignment to the IIIa subcategory, the empirical administration of antibacterial agents was justified, as in cat.II CBP. However, the role of such hypothetic infection was never substantiated nor was a validated cutoff defined to differentiate between IIIa and IIIb subclasses of CP/CPPS, which would probably require different therapeutic approaches.
Non-inflammatory prostatitis, i.e. "an inflammatory condition without inflammation", may indeed represent an odd oxymoron. It is the opinion of the Milan panel that a "prostatitis without evidence of inflammation" most likely does not represent a static situation, but is rather a quiescent stage of a condition fluctuating between an active/productive phase, characterized by leukocytosis, and a repair/organization phase with mucosal oedema regression, increased blood circulation, decrease of infiltrating immune cells and activation of tissue repair mechanisms, accompanied by fibrosis and/or atrophy. Additionally, cellular signs of inflammation may not always be demonstrable, although inflammation may be present. Without new evidence of nature and progression of inflammation in CP/CPPS, the classification into IIIa or IIIb subclasses should not be modified, although there is consensus among the authors that the two subclasses widely overlap

regarding signs, symptoms and response to therapy. Moreover, there is extensive evidence that leukocyte counts do not correlate with symptom severity in CPPS patients (12). Although potentially useful for research purposes, the subclassification into IIIa and IIIb may not be relevant for routine clinical workup and therapeutic management. If a differential outcome of a specific therapeutic intervention will be demonstrated in the future (e.g., a positive response to antibacterial treatment in IIIa but not in IIIb), this classification system may be further supported.
Moreover, studies are needed to conclusively demonstrate the absence of infiltrating inflammatory cells in the prostatic tissue of patients with low or no leukocytes counts in prostatic secretions.

Etiology of chronic bacterial prostatitis - role of non-traditional uropathogens

Since its first consensus definition, CBP was acknowledged to be caused by "E. coli, other Gram-negative organisms, or Enterococcus spp." (8). However, the etiologic role of organisms other than these uropathogens has been recurrently proposed and debated. Several scientists retain the original conviction that Enterobacteria, Pseudomonas, Enterococci and few other genuses like Staphylococcus aureus are the causative agents of CBP (reviewed in: (1)). This is also based on studies reporting inconsistent findings of Gram-positive species in prostate-specific specimens (13).
Due to increasing evidence, other authors extend the spectrum of prostatitis pathogens to organisms involved in sexually-transmitted diseases, like *Chlamydia trachomatis, Mycoplasmata* and *Trichomonas vaginalis* (14-18). On the basis of clinical experience and of the outcome of trials showing comparable clinical/symptomatic response to antibacterial treatment, other researchers add to the spectrum of potential causative pathogens Gram-positive bacteria, like coagulase-negative Staphylococci, Streptococci, etc. (19, 20).
Although two recent studies from North America and Europe demonstrated a strong correlation between eradication and clinical remission rates in patients showing infection by either traditional uropathogens or nontraditional uropathogens (NTU) (21, 22), the issue is still controversial. Published data are still contradictory and further investigation is required. Indeed, with the exception of studies on *C. trachomatis* (14), reports describing the etiological hypothesis of NTU in CP have not focused on a single pathogen in a large patient population, but were based on patients showing prostatic localization of bacteria very different in terms of metabolic activity, virulence, and pathogenic potential. Placebo-controlled studies focusing on single pathogens will shed new light on this issue.

Diagnosis of Prostatitis Syndromes

Diagnostic Workup

All panel members recommend some form of LUT segmented test in symptomatic patients, since the main purpose of the prostatitis diagnostic workup remains to ascertain whether patients suffer from a treatable infectious disease or not. The basic diagnostic procedures adopted by the panel members include:

1. Detailed history;
2. Thorough urological/andrological visit with digital rectal examination;
3. NIH-CPSI questionnaire;
4. Uroflowmetry with residual urine assessment;
5. Urethral swab;
6. 4-glass test according to Meares/Stamey or 2-glass pre/post-massage test with culture and microscopy (leukocyte counts);
7. Semen analysis and culture;
8. Serum PSA;
9. Transrectal ultrasound.

Additional exams, adopted by some of the authors in their practice, include:

1. Additional questionnaires (IPSS, AMS, IIEF, CAS-XII, MSHQ);
2. Abdominal/pelvic ultrasound;
3. Hypoecoic periurethral zone volume measurement (23);
4. Analysis of inflammatory markers (IL-2, IL-6, IL-8, C-reactive protein) in serum or secretions;
5. Analysis of pathogen-specific IgAs in semen/secretions;
6. Analysis of Urinary Bladder Tumor Antigen;
7. Analysis of Sex hormone-binding globulin, Testosterone, LH in serum;
8. 5-glass test, consisting of sequential Meares/Stamey test followed by semen collection and analysis (24);
9. Intraprostatic Pressure Evaluation;
10. Cystoscopy;
11. Chlamydia, Herpesvirus, Cytomegalovirus, Hepatitis B and C virus antibodies in serum;
12. Prostatic biopsy (in specific, recurrent cases).

Interestingly, almost all panel members never administer the NIH-CPSI questionnaire alone in their routine diagnostic workup. The International Prostate Symptom Score (IPSS), the Aging Male Symptom (AMS), the Loran-Segal CAS XII (25) and the International Index of Erectile Function (IIEF) questionnaires are often added to the NIH-CPSI questionaire. This indicates that specialists need additional information to further phenotype the patients´ symptoms. Although the NIH-CPSI is unanimously seen as mandatory, it may be perceived as a starting rather than an arrival point in optimal prostatitis diagnostic management. Moreover, additional drawbacks and needs must be addressed for improvement of diagnostic workup:

1. Neuropathic pain patterns have not been sufficiently studied, and should be further investigated.
2. There is poor correlation between prostatitis symptoms and biopsy findings; further investigation is required.
3. Immune reaction in the prostate of CPPS patients is not realistically measured; validated tests are needed.
4. Algorithms describing the evaluation and processing of biopsies taken in prostatitis patients should be elaborated.
5. Inflammatory markers other than white blood cells need to be internationally validated.

Sexual dysfunction in prostatitis

Sexual dysfunction (SD) has a major impact on the quality of life of prostatitis patients (26-31). SD may be psychogenic or organic, or may be secondary to an inflammatory condition of the pelvic area. Clinical evidence showing improvement of SD parallel to cure/remission from CBP or CP/CPPS is lacking. The NIH-CPSI questionnaire does not address this major component of the prostatitis symptom array. Almost all authors routinely use questionnaires (e.g., IIEF; MSHQ) related to SD in clinical studies and in daily clinical practice. In some cases, questionnaires are also proposed to patients' sexual partners.

Because IIEF and MSHQ questionnaires address adequately all major issues related to sexual function, no new diagnostic tools in this field are needed. However, these questionnaires are not validated for prostatitis, and their inner coherence should be confirmed in CP patients. Additional diagnostic procedures for SD evaluation in CP patients may include penile doppler sonography, Rigiscan, local negative pressure diagnostics, lipid and hormone profile. The response to phosphodiesterase-5 inhibitors may also be used as a diagnostic tool for SD.

A detailed interview with the patient is extremely useful in ascertaining the nature of the sexual disturbances and in drawing a first psychosocial profile of the subject.

Therapeutic Options

Tables 1 and 2 illustrate a variety of therapeutic options, based on international guidelines (e.g., EAU guidelines), as well as on the clinical experience and practice of the co-authors.

Acute Bacterial Prostatitis - Cat. I

For ABP, a "switch-therapy" programme may represent the best therapeutic regime (Table 1). Initial empirical treatment with broad-spectrum parenteral antibacterial agents (beta-lactams with or without aminoglycosides, fluoroquinolones, if local resistance levels are low) can be switched to medium/long-term therapy with an oral fluoroquinolone, as soon as susceptibility testing is available and acute symptoms (acute pain, retention, fever) subside. Therapy may also include additional drugs like NSAIDS and alpha-blockers, targeting pain, inflammation or voiding disturbances (Table 1).

Chronic Bacterial Prostatitis - Cat. II

Oral fluoroquinolones, alone or combined with other drugs, are first-choice agents, and should be administered for 4-6 weeks, if causative bacteria are susceptible (Table 2). Antibacterial agents may be combined with alpha-adrenoceptor blockers to treat voiding symptoms, and to analgesic/anti-inflammatory agents. In some cases therapy may include herbal extracts and other supplements that may show a tropism to the prostatic tissue and may be beneficial in symptomatic relief.

Chronic Prostatitis/Chronic Pelvic pain Syndrome - Cat. III

A large variety of therapeutic options are described in Table 2 for therapy of CP/CPPS. This is probably due to the fact that CP/CPPS is the most complex and difficult-to-treat condition among prostatitis syndromes, because of the various clinical presentation patterns and of the differential responsiveness of patients to treatment.

Category IIIa

Almost all panel members administer alpha-adrenoceptor blockers to cat.IIIa CP/CPPS patients, combined or not with various drugs, herbal extracts and supplements. Alpha-blocker therapy is usually long-term, and the treatment regime should last at least 3-6 months.

The majority of the authors administer antibacterial agents (fluoroquinolones, macrolides, co-trimoxazole, doxycycline) empirically as monotherapy or in combination with other drugs, for a short time period. Antibacterial therapy may be continued only in case of positive response.

Examples of drugs and other therapeutic interventions for cat. IIIa CPPS are shown in Table 2.

Category IIIb

For therapy of cat.IIIb CP/CPPS, alpha-blockers may be administered as in cat.IIIa. Herbal extracts and supplements (*S.repens, P. africanum, Rye-grass* pollen extract, thioctic acid, sulbutiamine, vitamins) can also be administered. Empirical antibacterial agents are administered less frequently than in cat.IIIa, and for shorter periods (average: 2 weeks).

Examples of treatment strategies for category IIIb CP/CPPS - additional or alternative to the ones listed for cat.IIIa - are shown in Table 2.

Asymptomatic Inflammatory Prostatitis - Cat. IV

Due to its asymptomatic presentation, AIP is usually not treated. Under certain circumstances, therapy to asymptomatic subjects is based on positive microbiological or biochemical findings or elevation of serum PSA levels. Short-term fluoroquinolones or co-trimoxazole may be administered (average: 2 weeks), with or without anti-inflammatory agents. In these cases, monitoring of PSA levels and LUT segmented tests (to assess pathogen eradication) best follow the evolution of AIP.

Alpha-adrenoceptor blockers in CP

For many years, alpha-blockers have been included in the toolbox of urologists, and have been recommended for treatment of bacterial/abacterial prostatitis (5). Recommendations were based on evidence that alpha-blockers relieve voiding symptoms and counteract/delay exacerbation and infection relapses of chronic bacterial/abacterial prostatitis (32). Moreover, preclinical studies show that alpha-blockade may modulate neurogenic inflammatory/irritative responses and nociceptive signals in LUTs (33, 34), thus pointing to a pharmacological activity of alpha-blockers beyond their mere urodynamic effects. Alpha-blockers were shown to be beneficial either in randomized, placebo-controlled trials (35,36), or in open-label studies performed in "real-life" clinical settings (37). However, in 2004 the use of alpha-blockers in non-naive subjects has been discouraged by results of a NIH-CPCRN trial showing that 6-week tamsulosin versus placebo provided no additional bene?t in heavily pre-treated CP/CPPS patients (38). A second NIH-CPCRN randomized trial showed no benefit of alfuzosin

Table 1.

Therapy of acute and chronic bacterial prostatitis.

		Drugs	Route	Therapy Duration	Note
Cat. I - Acute Bacterial Prostatitis	**First-line Antibacterial Agents (AAs)**	3rd generation Cephalosporins	Parenteral	7-10 days or until acute symptoms subside	>May be combined with an aminoglycoside >Switch to oral fluoroquinolones for additional 2-4 weeks (e.g. Ciprofloxacin 1 g/day or Levofloxacin 500-750 mg/day)
		Ureidopenicillins combined with beta-lactamase inhibitors	Parenteral	7-10 days or until acute symptoms subside	>Switch to oral fluoroquinolones for additional 2-4 weeks (see above)
		Fluoroquinolones (e.g. ciprofloxacin 400-1200 mg/day, Levofloxacin 500 mg/day)	Parenteral	7-10 days or until acute symptoms subside	>Switch to oral fluoroquinolones for additional 2-4 weeks (see above)
		Fluoroquinolones (e.g. Ciprofloxacin 1000 mg/day, Levofloxacin 500-750 mg/day, Ofloxacin)	Oral	2-4 weeks	More suitable for subacute cases
	Second-line AAs (in case of chemoresistance or hypersensitivity to first- line AAs)	Carbapenems	Parenteral	7-10 days or until acute symptoms subside	>Switch to oral fluoroquinolones for additional 2-4 weeks (see above)
		Co-trimoxazole	Oral or parenteral	2-3 weeks	If parenteral: switch to oral co-trimoxazole when acute symptoms subside
		Aminopenicillins combined with beta-lactamase inhibitors	Parenteral	7-10 days or until acute symptoms subside	>Switch to same orally-administered agent or to oral fluoroquinolone for additional 2-4 weeks
	Other agents administered in combination	Alpha-adrenoceptor blockers	Oral	4 weeks	In case of high residual urine volumes consider insertion of sovrapubic catether
		NSAIDS	Oral	Depending on symptom sverity	/
		Steroid Anti-inflammatory agents	Oral	Depending on symptom severity	/
Cat. II - Chronic Bacterial Prostatitis	**First-line Antibacterial Agents (AAs)**	Fluoroquinolones (e.g. Ciprofloxacin 1000 mg/day, Levofloxacin 500-750 mg/day, Ofloxacin Moxifloxacin 400 mg/day, Prulifloxacin, 600 mg/day)	Oral	4-6 weeks	/
	Second-line AAs (in case of chemoresistance or hypersensitivity to first- line AAs)	Trimethoprim	Oral	4 weeks	/
		Co-trimoxazole	Oral	4-12 weeks	/
		Macrolides	Oral	4-6 weeks	May be administered in combination with Fluoroquinolones. Azithromycin (500 mg/day) is dosed only the first three days of each week of treatment (20).
		3rd generation cephalosporins	Oral	2-3 weeks	In case of chemoresistance to quinolones, also in combination with parenteral aminoglycosides
	Other agents administered in combination with AAs	Alpha-adrenoceptor blockers	Oral	4 weeks up to 3-6 months	/
		NSAIDS	Oral	2-4 weeks	/
		Steroid Anti-inflammatory agents	Oral	Depending on symptom severity	/
		Serenoa repens extracts with or without additional herbal extracts or supplements (e.g., lycopene, selenium, Urtica dioica, quercetin, curcumin, etc.)	Oral	4 weeks up to to 3-6 months	/
		Thioctic acid	Oral	Up to 8 weeks	/
		Peptide complexes extracted from cattle prostate tissue	Rectal	4 weeks	/
		Lyophilized bacterial lysates of Escherichia coli	Oral	4 months	/

Table 2.

Treatment of category III - chronic prostatitis/chronic pelvic pain syndrome.

CP/CPPS subtype	Drugs/Physical Therapy	Route	Therapy Duration	Note
IIIa	Alpha-adrenoceptor blockers (e.g., Alfuzosin ER, 10 mg/day; Tamsulosin 0.4 mg/day)	Oral	3-6 months or longer	
	Empirically administered antibacterial agents (Fluoroquinolones, co-trimoxazole, Doxycycline)	Oral	2-4 weeks	In case of symptom improve after 2 weeks, consider extending therapy up to 4 weeks
	NSAIDS	Oral	1-3 months	Some authors associate weekly prostatic massage
	Serenoa repens extracts	Oral	1-3 months	Alone or in various combined formulations with other herbal extracts or supplements (e.g., lycopene, selenium, *U. dioica*, quercetin, curcumin, etc.)
	Pygeum Africanum extracts	Oral	2 months	
	Cernitin pollen extracts	Oral	3 months	
	Sulbutiamine (400 mg/day)	Oral	4 weeks	
	Lyophilized bacterial lysates of *E. coli* (6 mg/day)	Oral	3 months	
	Prostatic massage (weekly)		1-3 months	
IIIb	Alpha-adrenoceptor blockers (e.g., Alfuzosin ER, 10 mg/day; Tamsulosin 0.4 mg/day)	Oral	3-6 months or longer	
	Empirically administered antibacterial agents (Fluoroquinolones, co-trimoxazole, Doxycycline)	Oral	2 weeks	
	Low-dose benzodiazepines (e.g., diazepam, 2 mg/day; clonazepam, 0.5 mg/day)	Oral	3-6 months	
	Antidepressant drugs (e.g., sertraline 5 mg/day)	Oral	4-8 weeks	
	5-alpha-reductase inhibitors	Oral	/	
	5-phosphodiesterase inhibitors	Oral	/	
	Bencyclane fumarate (100 mg/day)	Oral	2 months	
	NSAIDS	Oral	2-4 weeks	
	Behavioral treatments		According to specialist's protocol	
	Acupuncture		According to specialist's protocol	
	Prostatic massage (weekly)		1-3 months	
	Serenoa repens extracts	Oral	1-3 months	Alone or in various combined formulations with other herbal extracts or supplements (e.g., lycopene, selenium, *U. dioica*, quercetin, curcumin, etc.)
	Pygeum Africanum extracts	Oral	2 months	
	Cernitin pollen extracts	Oral	3 months	

compared to placebo also in drug-naive patients (39).
A possible explanation of the different results of the NIH-CPCRN studies vs. the Mehik trial can be found in the different length of the treatment course with alpha-blockers (CPCRN studies: 6-weeks (38) or 3 months (39); Mehik study (35): 6 months). Indeed, the discrepancies between these studies are probably apparent, because also in the Mehik study no short-term difference was observed between alfuzosin and placebo after 8 weeks of treatment. However, the difference became significant at week 16.
In this respect, the authors of the CPCRN studies admit that alfuzosin might have been beneficial in longer-term treatment or if patients had shown more clinically significant voiding symptoms (39). Based on published evidence and personal experience the Milan panel members agreed that long-term alpha-blockers may be beneficial in symptom relief/remission in CBP and CP/CPPS patients. These drugs should especially be administered in CP/CPPS patients showing or claiming voiding disturbances.

Research Priorities and Ongoing Investigations

New frontiers and research priorities

The transition from the traditional spectrum of research themes concerning CBP and CP/CPPS towards new bio-psycho-social approaches, to identify and modulate maladaptive, cognitive and behavioral responses to pain is interesting (40).
Because a perturbed psychological profile – causative or

secondary to their condition – is frequently shown by CP/CPPS patients, cognitive/behavioral interventions can positively influence symptom perception through *control of pain and internal tension*.
Additional research priorities include:

1. the genesis of pain in CP/CPPS patients, with emphasis on a possible neuropathic component of this symptom;
2. the causative role of *Herpes simplex* virus in prostatitis or in other pelvic conditions;
3. new strategies targeting skin reflexogenic zones, responsible for the harmonic work of small pelvis organs, including the prostate;
4. new tools (questionnaires?) to evaluate the subjective influence on pain perception and personal reactivity to pain;
5. the mechanisms causing rapid worsening of pelvic/prostatic health after age 40 in men;
6. the relationship between atypical small acinar proliferation and prostate cancer;
7. the mechanisms of action of antioxidants like the thioctic salt of alpha-lipoic acid;
8. the role of immune reaction in prostatic inflammation;
9. new diagnostic tests for classifying CP/CPPS, given the limited accuracy of the Meares/Stamey or 2-glass procedures;
10. new prostatitis preclinical models.

Ongoing investigations

A number of research projects on CBP and CP/CPPS are performed in the authors' institutions, in Europe and in Far-East Russia, both at basic science and clinical levels. Both etiology and therapy are investigated, with prevalence of the pathogenic determinants of CP/CPPS:

1. a multinational European epidemiological prevalence survey on CP/CPPS, 2008-2009,
2. influence of patient lifestyle on manifestation of CP/CPPS,
3. role of atypical microorganisms in CBP,
4. role of *Chlamydia trachomatis* in CP/CPPS,
5. PK/PD, optimal dosage and duration of fluoroquinolone therapy, and other antibacterial regimens in CBP,
6. role and effect of antibacterial therapy in CP/CPPS,
7. new multimodal therapeutic algorithms in CP/CPPS,
8. relationship between prostatitis and cancer onset,
9. microbiological characterization of prostatic secretions and biopsies in the inflammatory reaction to pathogens
10. hormonal influence on triggering inflammation of the prostate and on the intensity of clinical symptoms in CP/CPPS,
11. relationship between histological and EPS/VB3 detection of white blood cells in CP/CPPS,
12. optimization of prostatitis therapy and drug safety profile by pharmacogenetic/pharmacogenomic testing,
13. application of Extracting microarray gene expression Patterns and Identifying co-expressed Genes to CP/CPPS research,
14. analysis of prostate tissue hypoxia levels,
15. new diagnostic markers related to prostatic tissue immunity disturbances,
16. activity of 5-phosphodiesterase agents on pain and sexual disturbances in CP/CPPS,
17. efficacy and tolerability of treatment with *Serenoa repens*, lycopene and selenium in cat. IIIb CP/CPPS,
18. role and significance of calcifications in prostatitis syndromes,
19. new diagnositic procedures for CP/CPPS

Conclusions

In the last decade, impressive amounts of clinical and preclinical research data have shed light on the pathogenesis and management of chronic prostatitis syndromes. NIH-sponsored programs in North America as well as independent research worldwide have opened the path to research in this field. A number of European groups have been actively involved in this research effort, and have in many cases developed an original view on prostatitis and chronic pelvic pain. The variety of diagnostic and therapeutic options summarized in this paper, and the number of ongoing studies presented herewith, demonstrate the richness and originality of European and Eurasian research, and may open new possibilities of international collaborative interactions.

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Paediatric urolithiasis in central coast region of Tunisia: Clinical characteristics.

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Summary

Objective: *To show an outline of the clinical and biological characteristics of paediatric urolithiasis among Tunisian children in the coast region.*
Patients and methods: *This retrospective study included 168 children under the age of 16 years presented with urinary stones (100 boys and 68 girls). Patients were reviewed in a multi-centric study with regard to age at diagnosis, sex, history, and physical, laboratory, and radiologic findings. The physical and chemical analysis of stones was carried out respectively by a stereomicroscope and by infra-red spectroscopy.*
Results: *The sex ratio was 1.47. The clinical presentation of this pathology was dominated by abdominal pain (28%), hematuria (25.6%), dysuria (16.7%) and urinary tract infection (14.3%). Stones were located in the upper urinary tract in 75.6% of cases. Of the urine cultures, 14.3% were positive. Whewellite was more frequent in children stones than in infants ($p < 0.05$) and was the main component in 46.4% of stone section and 55.4% in stone surface.*
Conclusion: *The male prevalence of paediatric urolithiasis is progressively decreasing in Tunisia. The epidemiological profile of renal stones in our country has changed towards a predominance of calcium oxalate stone and upper tract location.*

KEY WORDS: *Stone; Children; Infrared spectroscopy; Tunisia.*

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INTRODUCTION

Urolithiasis in children is a frequent disease characterized by its varied pathophysiological background (1). Clinical investigations of a patient with urolithiasis include a careful history, radiological and biochemical evaluation. It's always important to define the cause of urinary calculi disease among children to prevent reccurence and possible impairing of renal function (2). A number of publications have previously reported the high prevalence and particular patterns of stone disease among children in developing countries (2).
Our study is interested in the urolithiasis of the child in the area of the Tunisian Coast. By confronting the clinical data and the results from the stone analysis, we present an outline on the current state of the renal lithiasis of the child on the coast of Tunisia.

PATIENTS AND METHODS

Between June 1996 and February 2008, 168 children (68 females, 100 males; mean age 7.3 years; age range 0.25-16 years) suffering from urinary calculi and evaluated in our department were enrolled in the study. In each case age and sex of the patient, stone location, and circumstances of discovery were recorded. Various biochemical parameters indicative of renal functional status (serum calcium, phosphorus, alkaline phosphatase, creatinine, uric acid and electrolyte levels) were evaluated in 126 cases.
Stone analysis: the structure of each calculus was established using stereomicroscope to define the morphology of the stone and to select its representative parts (nucleus or core, internal section, and external surface), in order to determine its molecular and crystalline composition by infrared spectroscopy. From 0.5 to 2 mg of powder, of each stone part, was pulverised within an inert powdered support (dried potassium bromide) in a proportion of 0.5 to 2% in an agate mortar. This mixture was transferred into an appropriate die and pressed at 10t/cm2 to form a transparent pellet 13 mm in diameter. The spectral region investigated was from 4000 to 400 cm-1. Reference spectra were pure potassium bromide (KBr) pellets. Spectra were recorded by means of a Bruker IFS25 Fourier transform infrared spectrometer.

The various compounds were identified by comparison with previously published reference spectra. The results were expressed according to the main crystalline phase found in the stones and named as follows: whewellite (calcium oxalate monohydrate), weddellite (calcium oxalate dihydrate), carbapatite (carbonated calcium phosphate crystallized in the hexagonal system), struvite (magnesium ammonium phosphate hexahydrate), and calcite (anhydrous calcium carbonate). The stone component was considered as main component if it exceeded 70% of the total composition of calculus.
Statistical analysis of these data was carried out using software SPSS 11.0 for Windows.

Results

Children were aged between 3 months and 16 years (mean age 7.3 years). 15.47% of the cases were infants, aged between 3 months and 2 years. Urolithiasis was more frequent among boys. The sex ratio was 1.47 (100 boys and 68 girls). This ratio was 2.71 among infant patients. The most frequent initial symptoms were abdominal pain (28.0%) (Table 1), which was prevalent in school age children (30.3% in children *vs* 15.4% in infants) ($p < 0.05$), hematuria in 25.6% of cases and dysuria in 16.7%. Infants seem to be most frequently affected by urinary tract infection than children (26.9% *vs* 12.0%) ($p < 0.05$). A family history of urolithiasis was recorded for 19 patients (8.9%). Thirteen patients (7.7%) had an underlying anatomic abnormality, including vesicoureteral reflux in 4 cases, uretero-pelvic junction obstruction in 8 cases and valves of the posterior urethra in one case. Urinary calculi were more frequently located in the upper urinary tract (75.6%) than in the lower urinary tract (Figure 1). Bladder stones were more frequent in boys than girls (29.0% *vs* 7.4%) ($p < 0.05$). Multiple stones were found in 108 patients (64.2 %).
Evaluation of metabolic risk factors in 24-h urine samples revealed 22 children (13.1%) with a single risk factor, among which hyperoxaluria (3 cases), hypercalciuria (11 cases), cystinuria (2 cases) and hypocitraturia (6 cases) seemed to be the commonest. Among the possible predisposing factors, 24 patients (14.3%) had urinary tract infection (UTI) at the time of diagnosis and were treated with appropriate medication. The bacteria isolated were Proteus in 12 cases, Escherichia coli in 8, Klebsiella pneumoniae in 2, and Streptococcus and Staphylococcus aureus in 1 case each. 167 children were treated by open surgery and one by endoscopy.
The stone composition was homogeneous (> 90-95% of stone composition) in 37.5% of cases and calcium oxalate represented the more common component (60.0%). Purine stones were pure in 24.6% of cases.
As shown in table 2, the main component of the urinary stones – determined by infrared spectroscopy – was whewellite in 89 cases (53.3%). Struvite stones were associated with urinary tract infection in 66.6% of cases. The nucleus of stone was examined (found) in 6 cases (3.5%). The main component of the nucleus was ammonium urate in 33.3% of cases.

Table 1.
Clinical presentation of Tunisian children with urolithiasis according to age.

Clinical presentation	Infants		Children			TOTAL	
	Number	%	Number	%	p	Number	%
Abdominal pain	4	15.4	43	30.3	$p < 0.05$	47	28
Hematuria	9	34.6	34	23.9	NS	43	25.6
Dysuria	1	3.8	27	19	$p < 0.05$	28	16.7
Urinary tract nfection	7	26.9	17	12	$p < 0.05$	24	14.3
Colic	0	0	11	7.7	NS	11	6,5
Anuria	3	11.5	4	2.8	$p < 0.05$	7	4.2
Fever	2	7.7	4	2.8	NS	6	3.6
Accidental finding	0	0	2	1.4	NS	2	1.2

Table 2.
Main stone composition.*

Main stone component	Total	
	Number	%
Whewellite	89	53.3
Ammonium urate	23	13.8
Carbapatite	18	10.2
Weddellite	16	9.6
Struvite	13	7.8
Uric acid anhydrous	7	4.2
Cystine	2	1.2
Total	168	100

*Present in more than 70% of the total stone composition.

Staghorn stones were noted in 14 cases. Their composition was dominated by whewellite (53.8%), struvite and carbapatite (38.5% each).

Discussion

The epidemiological data related to the North African pediatric urolithiasis are very heterogeneous and not easily comparable to those observed in industrialised countries (2, 3).
The well-known male preponderance has been confirmed in our study, however this predominance is progressively decreasing compared to results previously published since the end of sixties (4, 5). According to some publications urolithiasis mainly affects babies (age

Figure 1.
Stone location.

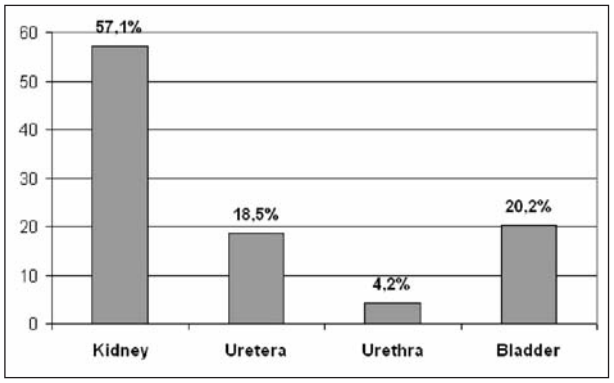
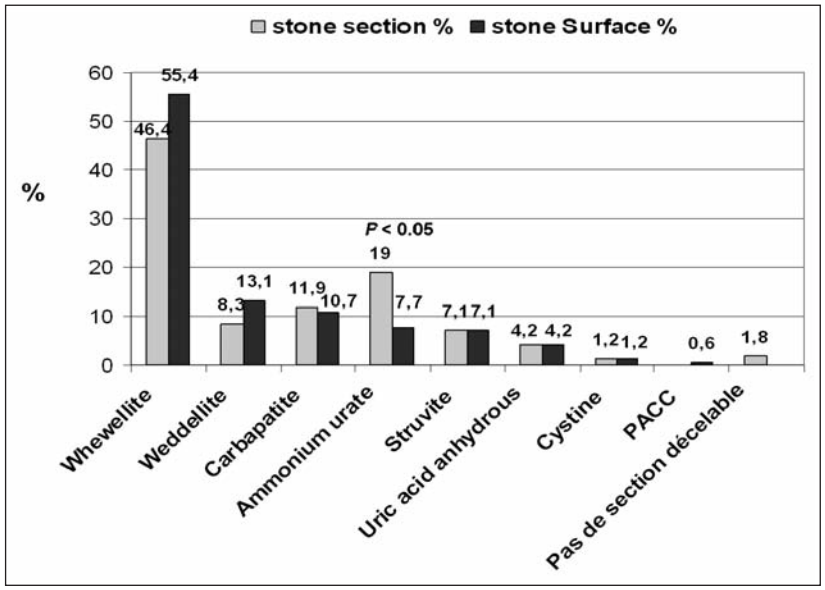


Figure 2.
Main stone section and surface component.



< 2 years) than children (6-8). In Tunisia, the baby urolithiasis was reported for the first time by *Nahlovsky* in the area of Sousse (4), where a frequency of 31.4% was recorded. Ten years later, in the same area, *Najjar et al.* (5) reported a frequency of 37.8%. During the past 25 years, this frequency changed in the central coast of Tunisia and it currently accounts for 15.47%.

Several studies noted a strong association between urolithiasis and urinary tract infections (6, 9) although it is not always easy to determine if the infection is the cause or the consequence of lithiasis. The frequency of the urinary tract infections was about 15 to 57% of the lithiasic patients in the years 1970-1980 (6, 10) and does not exceed at present in Europe the 2% (10). In our series, infection characterised 14.3% of cases comparable with that found in North America (8%) (11). This result was lower than that observed in England (30%) (12), in Kuwait (29%) (13) and in the Northern region of Tunisia (30%) (10), but it shows a decrease of the infection rate with regard to our first study in 1986 where we found a 57% of cases (5). The rate of metabolic abnormalities in our series (13.1%) is lower than that detected in Kuwait (13) where it reached 83% of the cases: this can be explained by the low level of biological investigation in our study or by the high level of consanguinity in Kuwait.

In Europe, urinary stones are mainly located in the upper urinary tract, and the proportion of bladder calculi does not exceed 14% (6, 14). It is even absent in other industrialised countries such as the United States of America (15). Bladder stones were only observed in the rural areas of the developing countries (51.1% in Morocco (16) and 71% in Cameroon (1), however, in our series, they were found in only 20.2% of cases (*Najjar et al.* 36.4%) (5), (*Kamoun et al*. 24%) (2).

Calcium oxalate is the most frequent chemical compound in the urolithiasis; it is present in approximately 80% of stones and represents the majority component in 70% of them (17). These data agree with our results that show that calcium oxalate was the main component in 105 cases (62.5%). It was present in 54.7% of the stone sections and 70.2% of the surfaces. These values were lower than those described in Pakistan (surface 85%, section 90%) (18) but comparable with those observed in Morocco (19), in Cameroon (1), and in our precedents studies (20, 21). In our study we observed a considerable modification of the stone composition of Tunisian children since the Seventies, where uric stone represented 83% (4). These results confirm those observed by *Daudon et al.* (22) which place the current profile of the paediatric urolithiasis in Tunisia between the developing countries and the industrialized ones.

Calcium oxalate monohydrate (whewellite) remains the most frequent component in Tunisian children and infants stones (20, 21, 23) even if its frequency was slightly decreased during the last 12 years period (20). In our study, whewellite was the main component in 53.3% of stones, and its frequency was higher on the level of the stone surface.

The presence of ammonium urate is especially considered as a marker of endemic urolithiasis when it is pure or associated with calcium oxalate and it constitutes the core of stones (16). It is particularly rare in the industrialized countries where it reaches 4.7% in France (24) whereas it is relatively frequent in childhood stones from the developing countries of Africa such as Cameroon where it reaches 57.1% (1). The ammonium urate was the main component in only 13.8 % of cases, and in 19% of the stones sections in our series. According to our results, purines (ammonium urate + uric acid anhydrous) stones were more frequent in infants than children. Metabolic (genetic or acquired) disorders can be mentioned to explain this change. However, we believe that tubular immaturity in infants associated to a lack of reabsorption of uric acid is the main reason in this case (16).

Struvite remains the best marker of urinary tract infections by urease producing bacteria (6, 10); 54% of struvite-containing stones are associated with a clinical infection (24). These stones, can in some cases reach a considerable burden in the excretory cavities of the kidney, from that the name of coralliform or staghorn stones. The frequency of staghorn stones varies from 19 to 54% in childhood urolithiasis (25); we found a 8.3% rate and an association to urinary tract infections in 38.5% of cases. The presence of whewellite in 53.8% of staghorn stones, is similar to the results described by several authors who found an associated primary metabolic cause, mainly hypercalciuria (26), which was not confirmed by our analyses.

In children with renal calculi, the aim of management should be complete clearance of stones, preservation of renal function and prevention of recurrence. In 167 cases, stone removal was achieved by open surgery. Our opinion is that open surgical procedures still provide an opportunity to clear stones in complex situations. In our country, open surgery still remains an important option due to the prevalence of large stones and calculi, and basically to the scarcity of equipment for lithotripsy and endourology in most paediatric surgical units (23).

Conclusion

The male prevalence of paediatric urolithiasis is progressively decreasing in Tunisia. The epidemiological profile of renal stones in our country has changed towards a predominance of calcium oxalate stone and upper tract location. The increase of calcium oxalate stones in school age children and the decrease of struvite and purines stones confirm the change on the aetiology of urolithiasis according to age. The persistence of urate stones reflects particular eating habits and infectious risk factors specific of the rural population.

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ORIGINAL PAPER

Pyrrolidine dithiocarbamate treatment prevents ethylene glycol-induced urolithiasis through inhibition of NF-kB and p38-MAPK signaling pathways in rat kidney.

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Summary

The aim of this study was to evaluate the role of the inducible nitric oxide synthase (iNOS), selective nuclear factor-kB (NF-kB) and p38-mitogene-activated protein kinase (p38-MAPK) on oxalate-induced crystal deposition in renal tubules. The rats were divided into three groups; group 1; control group, group 2; ethylene glycol (EG) group, group 3; EG + pyrrolidine dithiocarbamate (PDTC) group. Rats were sacrified on 7, 15 and 45th days. The iNOS expression, p65/NF-kB and p38/MAPK activity, and oxidative stress markers were evaluated in the kidney. Crystal depositions were evident on day 7, mild and severe cyrystallization were observed on day 15 and 45 in EG group, respectively. There was limited or no cyrstal formation in the EG + PDTC group. While EG stimulates iNOS, p65/NF-kB and p38/MAPK activity in renal tubules, PDTC inhibited it. PDTC prevents crystal depositions in renal tubules by reducing oxidative stress, iNOS, NF-kB, and p38-MAPK expression.

KEY WORDS: Pyrrolidine dithiocarbamate; Inducible nitric oxide synthatase (iNOS); Nuclear factor kappa B (NF-kB); p38-MAPK (mitogene–activated protein kinase); Lipid peroxidation; Oxidative stress; Calcium oxalate crystals

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INTRODUCTION

Stone formation in the kidney is one of the oldest and most wide spread diseases known to man. Calcium-containing stones, especially calcium oxalate monohydrate, calcium oxalate dihydrate and basic calcium phosphate are the most commonly occuring ones to an extent of 75-90% (1). Oxalate, a common constituent of kidney stones, is normally excreted by the kidney. Hyperoxaluric states including primary oxalosis and secondary hyperoxaluria can lead to renal tubulointerstitial damage. Hyperoxaluria is one of the major risk factors in human idiopathic calcium oxalate (CaOx) stone disease, and ethylene glycol (EG) can be used to produce CaOx urolithiasis in rats (2).

Convincing evidence indicates that both oxalate and CaOx crystals are harmful to renal epithelial cells and induces lipid peroxidation by unknown mechanism which causes disruption of the structural integrity of the membranes in vivo as well as in cell cultures (3-5). Reactive oxygen species (ROS) such as superoxide radicals, hydroxyl free radicals and hydrogen peroxide act as mediators of nuclear factor kapa-B (NF-kB) activation (6). NF-kB is one of the ubiquitos transcriptional factor of the inducible expression of many genes, including iNOS, that encode proteins involved in the modulation of inflammatory and host defense process (7). NF-kB family includes p50, p52, RelA (p65), Rel B, c-Rel, v-Rel and dorsal and Dif proteins (8). Normally these are squestered in the cytoplasm of cells through its binding with its inhibitors, p 105 and inhibitor kappa B (I-kB)-like proteins (8). Activation of NF-kB by external stimuli such as cytokins or ROS causes the degradation of its inhibitor Ik-B-alpha or proteolytic cleavage of p105. Free NF-kB dimers translocates to nucleus and activates the target genes, such as iNOS (9-11).

Mitogen-activated protein kinases (MAPK) are important mediators involved in the intracellular network of interaction proteins that transduce extracellular stimuli to intra-

cellular responses (12). Three distinct MAPK pathways have been described; extracellular signal-regulated kinase, c-Jun-N-terminal kinase, and p38-MAPK (13). p38-MAPK is a ubiquitous, highly conserved protein kinase that plays an important role in the inflammatory response and in the apoptosis process (14, 15). p38-MAPK is activated by cytokines and cellular stress, and its activation results in increased production of inflammatory cytokine genes including interleukin-1β and tumor necrosis factor-α (TNF- α) (15, 16). It has also been reported that p38-MAPK may be involved in NF-kB activation (17).
Nitric oxide (NO) acts as an intercellular messenger and regulates cellular functions such as vasorelaxation and inflammation. NO has an important role in the elimination of pathogens and tumor cells; however, overproduced NO is oxidized to ROS and result in the disruption of cell signaling and uncontrolled systemic inflammation (18, 19).
Pyrrolidine dithiocarbamate (PDTC) is an antioxidant. Recent studies have suggested that the pro-oxidant and metal-chelating properties of PDTC could also be involved in its ability to inhibit NF-kB (20-22). Some studies showed that PDTC may inhibit NF-kB activation via stabilization of the IkB-α (23), or likely via inhibition of the ubiquitin-proteasome pathway (24). There are a lots of studies concerning the selective NF-kB inhibitor properties of PDTC (25).
The aim of this study was to evaluate the role of iNOS expression, and p38-MAPK and NF-kB systems activation in the pathogenesis of EG-induced urolithiasis, and to investigate the possible inhibitory effects of PDTC known as a selective NF-kB inhibitor against the stone formation.

Materials and methods

Animals

In this study, 54 healthy, male Spraque-Dawley rats (240-250 g) were used. The animals were kept under standard laboratory conditions (12 hours lightness, 12 hours darkness, 26-28°C) for at least one week before the experiment and those conditions were preserved till the end of the experiment. Animal cages were kept clean, feed and water were given regularly every day. All experiments in this study were performed in accordance with the guidelines for animal research from the National Institutes of Health and were approved by the Local Committee on Animal Research.

Treatment and experimental design

The rats were randomly divided into three groups consisting of six animals each. In group 1 (control group), the rats received distilled drinking water. In group 2 (EG group), they received hyperoxaluria-inducing 0.75% ethylene glycol in distilled water. In group 3 (EG + PDTC group), they were injected intraperitoneally (i.p.) with PDTC (Sigma-Aldrich Chemical Corp, MO, USA) and received 0.75% ethylene glycol in distilled water. PDTC dissolved in distilled water and injected i.p. at the dose of 100 mg/kg body weight. The doses of PDTC were selected based on the results of recent studies where the antioxidant and anti-inflammatory action of this agent was apparent (26, 27).
Each of the major groups was then divided into three groups according to the experimental sampling periods; i.e., 7, 15, 45 days. After the last enjection, rats were placed in metabolic cages to determine 24-hour urine output, urinary pH and total urinary protein and oxalate concentrations. At 24 hours after the last enjection, all rats were killed with a high dose ketamine. The kidneys were quickly removed and separated from surrounding tissues and washed twice with cold saline solution, and one of the kidneys were stored at -80 C° to determination the level of renal malondialdehyde (MDA), glutathione (GSH) and nitric oxide (NO). The other kidney was stored in formaldehide solution for histopathological and immunohistochemical examination.

Histopathological examination

Paraffin embedded specimens were cut into 6 (μm) thickness and stained hematoxylin and eosin for light microscopic examination (Olympus, BH-2, Tokyo, Japan). The kidney sections were analysed semi-quantitatively using the technique of Houghton et al. (28).

Immunohistochemical studies

For imminohistochemical avaluation, specimens were processed for light microscopy and sections incubated at 60 ºC overnight and then de-waxed in xylene for 30 minutes. After rehydrating in a decreasing series of ethanol, sections were washed with distilled water and PBS for 10 minutes. Sections were then treated with 2% trypsin in 50 mM Tris buffer (pH 7.5) at 37 ºC for 15 minutes and washed with PBS.
Sections were delineated with a Dako pen (Dako, Glostrup, Denmark) and incubated in a solution of 3% H_2O_2 for 15 min to inhibit endogenous peroxidase activity. Then, sections were incubated with NF-kB/p65 (Rel A) Ab-1 (R-B-1638-R7, Neomarkers, Labvision, Fremont, CA, USA), MAPK/p38 (Vector Laboratories, Burlingame, CA, USA), and iNOS Ab-1 (R-B-1605-R7, Neomarkers) antibodies. The Ultra-vision (Labvision) horseradish peroxidase /3-amino-9-ethylcarbazole staining protocol were used at this stage.
Sections prepared for each case were examined by light microscopy. Sections of rat lung were used as the control for immunohistochemical staining specifitiy, according to data provided by the antibody manufacturer.
According to the diffuseness of the staining, sections were graded as 0= no staining, 1= staining < 25%; 2= staining between 25% and 50%; 3= staining between 50% and 75%; or 4= staining > 75%. According to staining intensity, sections were graded as 0= no staining; 1= weak but detectable staining; 2, distinct; 3= intense staining. Immunohistochemical values were obtained by adding the diffuseness and intensity scores.

Transmission electron microscopy

Tissues were prefixed in 1% glutaraldehide and 4% formaldehide in PBS for 2 hours. Then, the tissues were postfixed in phosphate-buffered osmium tetraoxide for 1 hour (pH 7.2), dehydrated in a graded series of ethanol, and embedded in Epon. Ultrathin sections were cut

using a Reinheirt Om U3 ultramicrotome with glass knives, stained with acetate and lead citrate, and examined using a carl Zeiss EM 9 S-2. Tissue sections were scored on a four-point scale by two histologist unaware of the treatment given and applying the scale to both the medulla and the cortex. The scoring was as follows: 0= no oxalate crystals in any field; 1= no more than two crystals in any field; 2= more than two crystals in any field; and 3= multiple collections of crystals in all fields.

Biochemical assesments

Nitric Oxide (NO) level

Total nitrite (NOx) was quantified by the Griess reaction (29) after incubating the supernatant with Escherichia coli nitrate reductase to convert NO_3 to NO_2. Griess reagent (1 mL 1 % sulfanilamide, 0.1% naphtyl-ethyl-enediamine hydrochloride, and 2.5 % phosphoric acid; Sigma Chemical Co., St. Louis, MO, USA) was then added to 1 mL of supernatant. The absorbance was read at 545 nm after a 30-min incubation. The absorbance was compared with the standard graph of $NaNO_2$, obtained from the reduction of $NaNO_3$ (1-100 µmol/L). The accuracy of the assay was checked in two ways; the inter-and intra- assay coefficients of variation were 7.52% and 4.61%, respectively. To check conversion of nitrate to nitrite (recovery rate), known amounts of nitrate were added to control plasma samples; these samples were deproteinised and reduced as above.

MDA level determination

Kidney tissue (300 mg) was homojenized in ice-cold tamponade containing 150 mM KCL for determination of MDA. MDA levels were assayed for products of lipid peroxidation. MDA referred to as thiobarbituric acid reactive substance, was measured with thiobarbituric acid at 532 nm in a spectrofluorometer, as described previously (30).

GSH level determination

GSH was determined by the spectrophotometric method, which was based on the use of Ellman's reagent (31).

Blood samples were also taken to asses the serum concentrations of urea, creatinine blood urea nitrogen (BUN), Na+and K.+ All biochemical variables were determined using an Olympus Autoanalyser (Olympus Instruments, Tokyo, Japan).

Statistical analysis

Statistical analyses of the histopathologic and immunohistochemical evaluation of the groups were carried out by the chi-square test and analyses of the biochemical data by the Mann Whitney U- test. Results of all groups were shown as mean values ± standard deviation (SD). $P < 0.05$ was accepted as statistically significant value.

RESULTS

Biochemical variables in urine, serum and tissue

There was no significant differences in BUN, serum urea and creatinine, Na+ and K+ concentrations 7, 15 or 45 days after administration in any of the groups (Table 1). There were no marked changes between the control and experimental groups in terms of daily urine output or total urinary protein. The urinary pH was significantly higher in the EG groups compared with the control and EG + PDTC groups at all time points ($p < 0.05$, Table 1). The 24-hour urinary oxalate excretion was significantly higher in EG group than in the control and EG + PDTC groups 7, 15 and 45 days after administration ($p < 0.01$). In the EG + PDTC groups, 24-hour urinary oxalate

Table 1.

Serum and Urine variables in control, EG, and EG + PDTC rats.

Group/Days	Urine Output (mL/24 h)	Urine pH	Total protein (g/24 h)	Oxalate (mg/24 h)	Creatinine (mg/dL)	BUN (mg/dL)	Na^+ (mmol/L	K^+ (mmol/L)
Control								
7	18.7 ± 2.1	6.9 ± 0.2	18.4 ± 2.8	1.7 ± 0.4	0.21 ± 0.04	17.9 ± 2.1	138.2 ± 2.6	4.0 ± 0.3
15	20.1 ± 1.7	6.7 ± 0.2	19.3 ± 2.5	1.8 ± 0.4	0.21 ± 0.05	17.7 ± 1.9	141.2 ± 2.2	4.0 ± 0.3
45	18.6 ± 1.9	6.7 ± 0.1	18.9 ± 2.6	1.9 ± 0.4	0.21 ± 0.04	18.2 ± 1.5	139.4 ± 2.1	3.9 ± 0.4
EG								
7	17.6 ± 1.8$^{a\#}$	7.8 ± 0.3^{a*}	19.4 ± 2.4$^{a\#}$	2.9 ± 0.9^{a*}	0.23 ± 0.06$^{a\#}$	18.9 ± 1.9$^{a\#}$	141 ± 1.9$^{a\#}$	4.3 ± 0.4$^{a\#}$
15	19.2 ± 1.9$^{a\#}$	8.0 ± 0.4^{a*}	19.6 ± 1.9$^{a\#}$	3.7 ± 1.2^{a**}	0.24 ± 0.05$^{a\#}$	19.4 ± 1.4$^{a\#}$	140 ± 1.5$^{a\#}$	4.3 ± 0.4$^{a\#}$
45	19.8 ± 2.4$^{a\#}$	8.1 ± 0.3^{a*}	18.8 ± 3.1$^{a\#}$	3.9 ± 0.8^{a**}	0.24 ± 0.03$^{a\#}$	20.1 ± 2.2$^{a\#}$	142 ± 2.7$^{a\#}$	4.1 ± 0.3$^{a\#}$
EG+PDTC								
7	19.6 ± 1.8$^{b\#}$	6.8 ± 0.3^{b*}	17.9 ± 3.6$^{b\#}$	1.8 ± 0.4^{b*}	0.20 ± 0.05$^{b\#}$	17.5 ± 2.2$^{b\#}$	142.6 ± 2.4$^{b\#}$	4.1 ± 0.4$^{b\#}$
15	18.4 ± 2.1$^{b\#}$	6.9 ± 0.2^{b*}	18.8 ± 3.2$^{b\#}$	1.9 ± 0.5^{b**}	0.22 ± 0.03$^{b\#}$	18.2 ± 1.7$^{b\#}$	137.8 ± 3.1$^{b\#}$	4.0 ± 0.3$^{b\#}$
45	21.4 ± 2.1$^{b\#}$	7.0 ± 0.2^{b*}	19.2 ± 2.7$^{b\#}$	1.8 ± 0.4^{b**}	0.22 ± 0.03$^{b\#}$	19.3 ± 2.7$^{b\#}$	139.9 ± 2.1$^{b\#}$	4.0 ± 0.2$^{b\#}$

Values are expressed as mean ± S.D. for six rats in each group.
Groups: Control, EG (Ethylene Glycol), and EG + PDTC (Pyrrolidine Dithiocarbamate)
"a" compared with control group and "b" compared with EG group.
#P > 0,05
**P < 0,05*
***P < 0,01*

Table 2.

The GSH, MDA and NO level in control, EG, and EG + PDTC groups.

Group/ Days	MDA (nanomol/g wet tissue)	NO (nanomol/g wet tissue)	GSH (micromol/g wet tissue)
Control			
7	44.3 ± 9.6	42.2 ± 10.5	1.7 ± 0.2
15	49.1 ± 8.9	46.3 ± 9.6	1.8 ± 0.2
45	47.6 ± 10.1	47.6 ± 10.1	1.7 ± 0.3
EG			
7	84.4 ± 12.4 a**	74.5 ± 10.6 a**	1.2 ± 0.3 a*
15	122.3 ± 16.3 a**	82.8 ± 6.5 a**	1.1 ± 0.2 a*
45	148.8 ± 18.4 a**	90.8 ± 13.1 a**	1.0 ± 0.3 a*
EG+PDTC			
7	51.6 ± 9.2 b**	49.8 ± 11.2 b**	1.5 ± 0.2 b*
15	53.1 ± 11.3 b**	51.2 ± 8.5 b**	1.5 ± 0.2 b*
45	54.5 ± 12.3 b**	55.4 ± 12.3 b**	1.4 ± 0.2 b*

Values are expressed as mean ± S.D. for six rats in each group.
Groups: Control, EG (Ethylene Glycol), PDTC (Pyrrolidine Dithiocarbamate)
"a" compared with control group and "b" compared with EG group.
**P < 0,05*
***P < 0,01*

excretion increased gradually after administration of the substance began. The excretion in this group was higher than in the controls, but the differences were not significant ($p > 0.05$, Table 1).
The MDA and NO levels were found to be significantly lower ($p < 0.01$) and the GSH level was higher ($p < 0.05$) in the renal tissues of the EG + PDTC groups compared with the EG groups at all time points (Table 2).

Transmission electron microscopy

Transmission electron microscopy clearly showed calcium oxalate deposits in the kidneys of the EG groups 7, 15 and 45 days after administration Crystals deposits were found in proximal tubules of the cortex. In the kidney sections of the rats treated with EG only on day 7, oxalate particles were found in the renal cortex. Only rats in EG groups calcium crystals in the renal sections, with diffirent cyrstal amounts being found on day 15. Cyrstals were found in all the renal cortex tips in all the rats in the EG group on day 45. No crystal deposits were detected in control groups at any time. There was limited or no crystal deposition in the rats treated with PDTC on day 7, 15 and 45 (Table 3).

Histological examination

Light microscopy of renal cortex and medulla showed

Table 3.

Crystallization rates of groups.

	Day 7			Day 15			Day 45		
Crystallization Rates	Control 7	EG 7	EG+PDTC 7	Control 15	EG 15	EG+PDTC 15	Control 45	EG 45	EG+PDTC 45
0	6	-	4	6	-	3	6	-	4
1	-	3	2	-	-	3	-	-	2
2	-	3	-	-	3	-	-	1	-
3	-	-	-	-	3	-	-	5	-

Transmission electron microscopy clearly showed calcium oxalate deposits in the kidneys of the EG, EG+PDTC groups 7, 15 and 45 days after administration Crystals deposits were found in proximal tubules of the cortex

Table 4.

The immunohistochemical staining score in control, EG, and EG+PDTC groups.

	Control			EG			EG + PDTC		
IHC Staining Score	7 Days	15 Days	45 Days	7 Days	15 Days	45 Days	7 Days	15 Days	45 Days
iNOS									
0	2	3	2	-	-	-	-	-	-
1	3	3	4	-	-	-	-	-	-
2	1	-	-	-	-	-	2	-	-
3	-	-	-	4	-	-	3	4	2
4	-	-	-	2	-	-	1	2	3
5	-	-	-	-	1	-	-	-	1
6	-	-	-	-	5	3	-	-	-
7	-	-	-	-	-	3	-	-	-
p65									
0	-	1	3	-	-	-	-	-	
1	2	4	2	-	-	-	-	-	
2	4	1	1	1	-	-	-	-	3
3	-	-	-	4	2	-	4	3	1
4	-	-	-	1	3	-	2	3	2
5	-	-	-	-	1	-	-	-	-
6	-	-	-	-	-	1	-	-	-
7	-	-	-	-	-	5	-	-	-
p38									
0	4	-	3	-	-	-	-	-	-
1	2	2	2	-	-	-	1	-	-
2	-	4	1	-	-	-	3	-	-
3	-	-	-	3	-	-	1	1	-
4	-	-	-	3	2	-	1	5	4
5	-	-	-	-	4	-	-	-	2
6	-	-	-	-	-	2	-	-	-
7	-	-	-	-	-	4	-	-	-

According to the diffuseness of the staining, sections were graded as 0= no staining, 1= staining < 25%; 2= staining between 25% and 50%; 3= staining between 50% and 75%; or 4= staining > 75%. According to staining intensity, sections were graded as 0= no staining; 1= weak but detectable staining; 2, distinct; 3= intense staining. Immunohistochemical values were obtained by adding the diffuseness and intensity scores.

tubular epithelial cell degenaration, granular material in the lumens of some tubules and mononuclear inflammatory cells infiltrating the interstitium.

These changes were more apparent in rats treated with EG only than the others and were especially more prominent in on day 45.

Imminohistochemical studies

On immunohistochemical avaluation, there were more intense expressions of iNOS, p38 MAPK and p65 NF-kB in rats treated with EG alone compared with control at all three times (Figure 1, Table 4). There were poor or slight expressions of iNOS, p38 and p65 in the control and the EG plus PDTC groups at all three times compared with the EG groups Immunohistochemical scores of the rats treated with EG alone on day 45 were the highest, and on day 15 were hihger than those on day 7, but there was no significant difference between the last two groups.

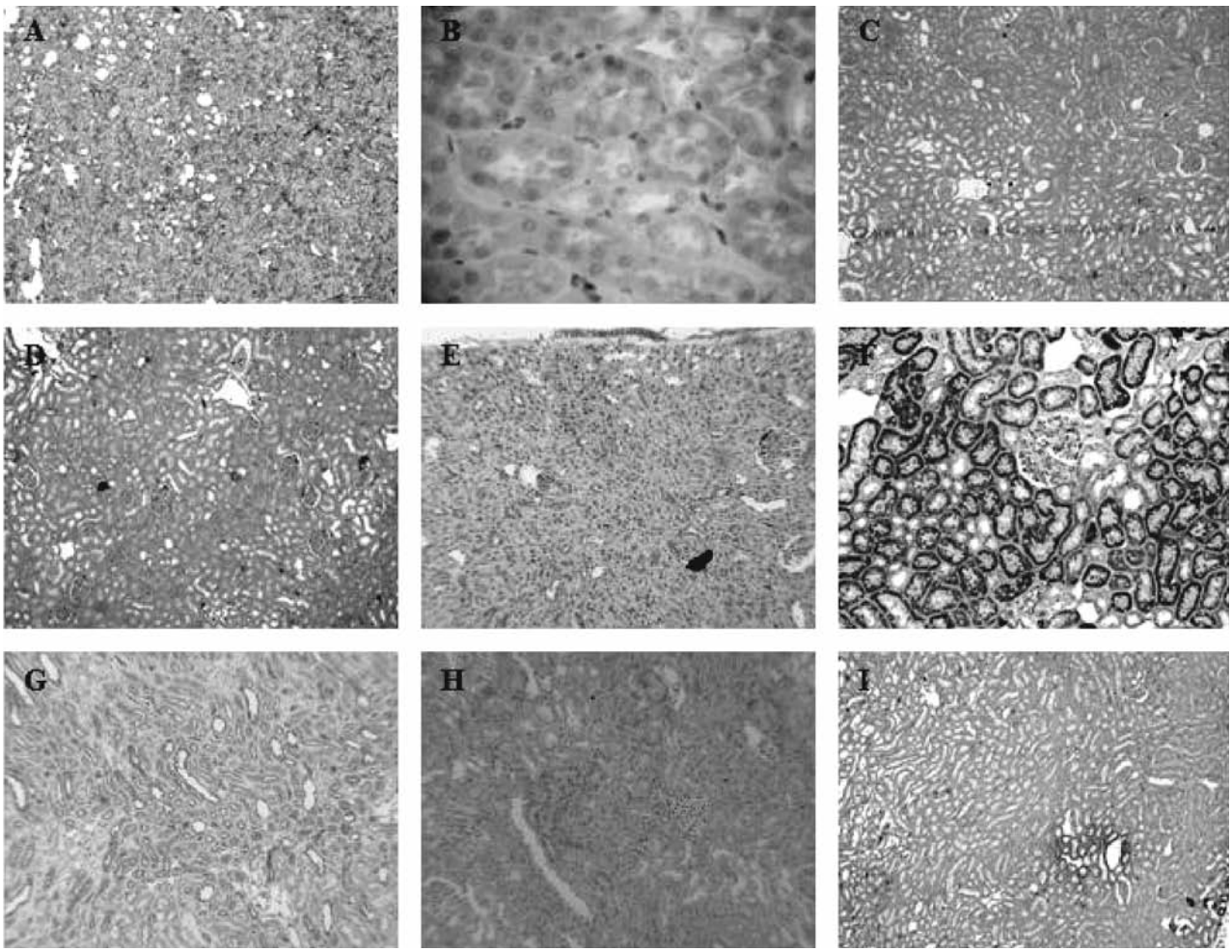
Discussion

Urolithiasis is a complex process that is a consequence of an imbalance between promoters and inhibitors in the kidneys (32). Considerable evidence has implicated ROS in the pathophysiology of a wide spectrum of disorders, including atherosclerosis, ischemia-reperfusion injury, inflammatory disorders, cancer and aging (33). Similarly association of urolithiasis and free radicals has been reported (4).

Many experimental models have been developed to investigate the mechanisms involved in the formation of urinary stones, and to ascertain the effect of various therapeutic agents on the development and progression of the disease (34-36). Rats are the most frequently used animals in models of CaOx deposition in the kidneys, a process that mimics the etiology of kidney stone formation in humans (37). Rats models of CaOx urolithiasis induced by EG alone or in combination with other

Figure 1.

Immunohistochemical staining showing iNOS, p65/NF-kB and p38/MAPK expression.



A) Focal mild staining (score 1) with iNOS in control (x 100);
B) Low NFkB/p65 positivity (score 2) in control (x 400);
C) Low MAPK/p38 positivity (score 2) in control (x 100);
D) Mild iNOS staining (score 3) in 7. Days EG (x 100);
E) Diffuse, intensive NFkB/p65 positivity (score 7) in 45. Days EG group (x 100);
F) Diffuse, intensive MAPK/p38 positivity (score 7) in 45. Days EG group (x 200);
G) Mild iNOS staining (score 3) in 15.Days EG+PDTC Group (x 100);
H) Low NFkB/p65 positivity (score 3) in 45.Days EG+PDTC Group (x 100);
I) Low MAPK/p38 positivity (score 3) in 15 Days EG+PDTC Group (x 100).

drugs, are often used to study the pathogenesis of kidney crystal deposition (2, 35).

A lots of experimental evidence has suggested the role of ROS in the pathophysiology of EG- induced urolithiasis in rats, and several antioxidant agents have been used to prevent CaOx crystal deposition in the kidney (38, 39). Here we measured the MDA, GSH, and total nitrite, a stable product of nitric oxide (NO), as a means of oxidative stress. The MDA and NO levels were found to be significantly lower and the GSH level was higher in the renal tissues of the EG + PDTC groups compared with the EG groups at all time points. PDTC may prevent CaOx crystal deposition in the kidney by preventing hyperoxaluria-induced peroxidative damage to renal tubular membrane surface, which in turn can prevent CaOx crystal attachment and subsequent development of kidney stones. In adition of these oxidative stress parameters we also evaluated the expression of redox sensitive transcription factors, NF-kB and MAPK, by immunohistochemistry.

Reverse transcriptase-polymerase chain reaction or Western blotting analyses are functional assays by which to measure the actual activity of iNOS. Western blotting provides a more quantitative way of measuring iNOS and p65 subunit activity. Therefore, it may be a limitation of this study that Western blotting analyses were not performed. However, there are a number of studies in the literature that have made use of immunohistochemical grading of iNOS to evaluate NO activity (40).

Increasing evidence suggests that PDTC have not only metal-chelating effect but also have thiol-modifying and oxygen radical-scavenging antioxidative properties mediate the inhibition of NF-kB (23). Since there is evi-

dence that both the MAPK and NF-kB systems can be activated by oxidative stress, we investigated the expression of p38-MAPK and NF-kB in the kidney in rats treated with EG alone or EG + PDTC. In present study, there were more intense expressions of p38-MAPK and p65-NF-kB in rats treated with EG alone compared with control, and there were poor or slight expressions of p38 and p65 in the control and the EG + PDTC groups at all three times compared with the EG groups.

It has been reported that p38-MAPK and NFκB pathways are activated in a variety of experimental models of renal inflammatory disease, including antiglomerular basement membrane nephritis (42), ureteric obstruction (43), endotoxemia (44), and immun complex nephritis (45). Our study shows that this also occurs in the kidney of rats with nephrolithiasis induced by EG. We think that increased ROS secondary to EG therapy in rat kidney causes the degradation of its inhibitor Ik-B-alpha or proteolyticcleavage of p105 and free NF-kB dimers translocates to nucleus and activates the target genes, such as iNOS. The iNOS is one of the three NOS isoforms that is affected by NF-kB as a result of tissue damage. The iNOS-mediated NO production is significantly elavated when there is increased oxidative stress (19, 47), and excessive NO production secondary to elevated expression of iNOS may impose cytotoxic effects on various organs, including the kidney (48).

It has been reported that selective p38-MAPK inhibitors can block the production of inflammatory molecules, reducing the apoptotic cell death and ameliorating the acute renal injury observed in some animal models of renal disease such as anti-GBM glomerulonephritis and ischemia/reperfusion (13,14). Furthermore, there is evidence that the production of TNF post-renal injury is triggered by the locally produced ROS, which activate NF-kB through p38 MAPK (51). Activation of p38-MAPK can also induce NF-kB activation and subsequent transcription of inflammatory cytokines (52). TNF-α and ROS also activates NF-kB (53). In this study we can suggest that calcium crystals activate p38-MAPK signal transduction pathway in renal epithelial cells, and the increased p38-MAPK activity further activates NF-kB and plays essential role in innate inflammation.

Our data suggest that NF-kB and p38-MAPK signal transduction pathway might serve as novel target in the treatment of the inflammatory conditions involving calcium deposits. The blockade of NF-kB and MAPK activation by antioxidant could be an effective strategy for the treatment and prophylaxis of urolithiasis in conditions such as hyperoxaluric states including primary oxalosis or secondary hyperoxaluria can lead to renal tubulointerstitial damage. PDTC attenuates EG-induced nephrolithiais presumeably antioxydant as well as NF-kB inhibitor properties. PDTC also can be used in clical practice in hyperoxaluric patients who are in high risc group for nephrolithiasis, but further animal and clinical studies are needed to confirm our suggestion.

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ORIGINAL PAPER

Prostate cancer detection after one or more negative extended needle biopsy: Results of a multicenter case-findings protocol.

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Summary

Objectives: *To evaluate PCa incidence in patients with one or more negative extended prostate biopsy who underwent repeat biopsy or TURP.*

Material and methods: *From June 2003 to February 2008, 308 patients were submitted to repeat prostate biopsy (median 20.5 cores) and 120 patients underwent TURP after one or more 12 cores prostate biopsy. Indications for biopsy were: abnormal DRE; PSA > 10 ng/mL; PSA included between 4.1-10 or 2.6-4 ng/mL with free/total PSA ≤ 25% and ≤ 20%, respectively. 262 and 46 underwent a second and a third biopsy: 218 because for high levels of PSA, 40 and 50 patients for a previous diagnosis of HGPIN and ASAP, 28 had an abnormal DRE. PSA in patients who underwent TURP was 11.6 ng/mL (median); in all cases DRE was negative and only 76 patients referred LUTS.*

Results: *PCa incidence at repeat biopsy was 16.9%; 96.2% of cancers were diagnosed at a second biopsy and 3.8% at a third one. PCa incidence was higher in patients with previous ASAP (43.4% and 50%) vs patients with HGPIN (25% and 0%) or benign pathology (11.9% and 0%). PCa was diagnosed in 11.1% and 19% of patients who underwent TURP previously submitted to a first and a second biopsy, respectively.*

Conclusions: *In case of persistent suspicion of PCa after a repeated negative saturation biopsy, TURP should be proposed as part of the diagnostic procedure aside from LUTS, especially in patients with a life expectancy greater than 10 years.*

KEY WORDS: *Prostate cancer, Extended prostate biopsy, TURP, Saturation biopsy, Repeat prostate biopsy..*

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INTRODUCTION

The widespread use of PSA in clinical practice has led to an increased diagnosis of prostate cancer (PCa), although the specificity of PSA levels less than 10 ng/mL is rather poor (1). In order to improve the diagnostic accuracy, some determinants of PSA kinetics (free to total PSA ratio, PSA density and PSA velocity) along with extended biopsy protocols have been introduced (2). In spite of all, still some patients remain with a persistent suspicion of harbouring a cancer despite multiple negative biopsies. In selected cases, some Authors proposed a transurethral resection of prostate (TURP) as a part of diagnostic procedure, lower urinary tract symptoms (LUTS) aside (3).

In this prospective study we report the incidence of PCa in 428 patients, enrolled in a multicenter case-findings protocol, who, after one or more negative extended prostate biopsy, underwent repeat biopsy or TURP because of a persistent clinical suspicion of cancer.

Material and methods

From June 2003 to February 2008, among 428 Caucasians patients, who previously underwent one or more 12 cores prostate biopsy according to Ravery's scheme (2), an extended US-guided prostate biopsy was performed in 308 of them (age range: 45-76 years; median 62.4) and a TURP in the remaining 120 patients (age range: 53-78 years; median 68.4). Patients accepted to be enrolled in a multicenter case-finding protocol for PCa detection, signing an informed consensus form which, in addition to the biopsy-related complications, explicitely reported the risk of diagnosing a clinically non significant PCa for low PSA levels and/or when a greater number of cores are taken.

Indications for biopsy were: abnormal digital rectal examination (DRE); total PSA (tPSA) greater than 10 ng/mL; tPSA between 4.1 and 10 ng/mL with percent free PSA (%-fPSA) 25% or less; tPSA between 2.6 and 4 ng/mL with %fPSA 20% or less (4).

Among the 308 patients who were considered eligible for an extended biopsy protocol, 262 (85%) and 46 (15%) underwent a second and a third procedure: in 218 (70.8%) because of persistent high levels of tPSA and/or an abnormal %-fPSA; while 40 (13%) and 50 (16.2%) patients had a previous diagnosis of HGPIN and ASAP, respectively. Overall, in these patients the median tPSA was 9 ng/mL (range 2.9-36): 7.3 ng/mL in those with a negative primary biopsy, 6.2 ng/mL in subjects with previous HGPIN and 13.5 ng/mL in patients with previous ASAP. Twenty-eight out of 308 (9.1%) had an abnormal DRE (Table 1). Prostate biopsy was accomplished in a transperineal way supplied with a biplanar transrectal probe with a tru-cut 18 G needle (Bard; Covington, GA) under local anesthesia and antibiotic prophylaxis; a median of 18 cores (range 12-23) were taken from the peripheral region of the gland (apex, med and base) and 2-4 cores (median 2.5) in the transition zone.

Repeat biopsy was performed after an interval of 15.2 (second biopsy) and 16.5 (third biopsy) months in patients with previous benign findings (chronic prostatitis or normal parenchyma) and approximately after 7 months in those who had a diagnosis of ASAP or HGPIN. Three pathologists reviewed all cases.

To evaluate the role of TURP in diagnosing PCa after a negative biopsy, a TURP was performed in 120 patients with persistent elevated or rising PSA values: 36 (30%) and 84 (70%) of them previously had one or two negative extended prostate biopsy, respectively. The median tPSA was 11.6 ng/mL (range 2.7-36 ng/mL) and in all cases DRE was negative; only 76 of these 120 men (63.4%) complained LUTS (Table 1).

For statistical analysis the t Student's-test was used; a p value < 0.05 was considered statistically significant.

Table 1.

Clinical parameters in patients who underwent repeat biopsy or TURP.

	2nd biopsy	3rd biopsy	TURP
Race	**Caucasians 262 (85%) pts**	**Caucasians 46 (15%) pts**	**Caucasians 120 pts**
Age (median)	45-76 years (63.2)	49-73 years (60.2)	53-78 years (68.4)
PSA 2.6-4.0 ng/mL	4 (1.5%)	-	4 (3.3%)
PSA 4.1-10 ng/mL	228 (87%)	20 (43.4%)	56 (46.7%)
PSA > 10 ng/mL	30 (11.5%)	26 (46.6%)	60 (50.0%)
Abnormal DRE	24 (9.2%)	4 (8.5%)	0
Estimated median prostate weight (grams)	52 (31-100)	58 (42-86)	46 (30-90)
HGPIN	31 (11.8%)	9 (19.5%)	0
ASAP	44 (16.8%)	6 (13%)	0
LUTS	190 (72.5%)	18 (39.2%)	76 (63.4%)
Qmax < 10 ml/sec	-	-	51 (42.5%)
Post-void urinary residual > 100 ml	-	-	59 (49.2%)

Results

The median number of cores (peripheral + transition zone) at repeat biopsy was 20.5 (range 14-26), whereas 35 grams (range 20-73) of tissue were removed by TURP.

Biopsy-related complications were: hematuria in 32 cases (10.4%), acute urinary retention in 26 cases (8.5%), hemospermia in 42 cases (13.6%), UTI in 19 cases (6.28%), orchiepididymitis in 2 cases (0.6%). No patients who underwent repeat biopsy required hospitalization; in all patients submitted to TURP the postoperative course was uneventful.

Fifty-two cancers (16.9%) were found among 308 men after repeat biopsy, most of them (50 PCa) detected at a second biopsy and only 2 (3.8%) at a third one. Normal histology and chronic prostatitis was found in 189 (61.4%) and 67 (21.7%) patients, respectively. The median Gleason score (GS) was 6.4 (range 6-8) in men at a second biopsy (more precisely, 30 patients had a GS equal to 6, in 20 the GS was 7 and in 2 patients the GS was 8); in both cancers detected at the third biopsy the GS was 6. After the second and the third biopsy, the incidence of PCa was higher in patients with a previous ASAP (43.4% and 50%, respectively) than in those with previous HGPIN (25% and 0%; p = 0.005) and benign pathology (11.9% and 0%; p = 0.0001) (Table 2). The median number of positive cores was 2.8 (range 1-5) and 2 cores at second and third biopsy, respectively. In two patients the cancer was localized only in the transition zone (third biopsy).

PCa was diagnosed in 20 out of 120 men (16.7%) who underwent TURP: 4 cases (11.1%) among 36 men who had a negative primary biopsy and 16 (19%) among the 84 patients who previously

Table 2.

Clinical parameters and PCa incidence at second and third biopsy.

Indications to biopsy	pts	PSA ng/mL (median)	PCa incidence at repeat biopsy second (262 pts)	third (46 pts)
tPSA or %-fPSA	208	7.2	8.7% (16)	0%
Abnormal DRE + PSA	10	9.2	3.2% (6)	0%
HGPIN	34	6.1	18.8% (6)	0%
Abnormal DRE + HGPIN	6	6.8	6.2% (2)	0%
ASAP	38	13.0	28.2% (13)	25% (1)
Abnormal DRE + ASAP	12	15.0	15.2% (7)	25% (1)

underwent two negative biopsy sets. The median Gleason score was 6 (range 4-7) in both groups.

In the remaining 100 patients, 85 (70.8%) and 15 (12.5%) had a diagnosis of BPH and prostatitis together with BPH, respectively. The clinical stage of cancers found by TURP was T1a in 18 cases (90%) and T1b in 2 cases (10%). Thirty months (range 6-48) after TURP, in the patients with clinical stage T1a PCa, enrolled in an watchful waiting programme, median tPSA was 0.9 ng/mL (range 0.4-1.3 ng/ml) whereas median tPSA in the patients with benign findings was 1.5 (0.8-3.4 ng/mL). The two patient with clinical stage T1b PCa underwent radical prostatectomy and surgical specimens showed a pT3aNo stage, a GS of 6 and 7 (3 + 4) and negative surgical margins. Among the 20 patients with PCa found by TURP, only 11 (55%) complained preoperatively of LUTS.

Discussion

The transrectal US-guided sextant prostate biopsy, proposed by Hodge (5) in 1989, was considered the gold standard in the diagnosis of PCa until it was demonstrated that about 15-20 % of clinical significant cancers were missed (6), leading to the necessity of practicing several biopsy sets in a consistent number of patients. Today an extended TRUS-guided biopsy scheme with an increased number of cores on the peripheral portion and on the lateral margins of the gland is the only suitable method to improve the detection rate of PCa (7). The incidence of cancer at repeat biopsy after an extended primary prostate biopsy is lower than after a sextant biopsy (8); however, the saturation prostate biopsy (SPBx) (i.e., 24 or more cores), does not improve cancer detection rate in comparison with 12 or 18 cores biopsy as an initial prostate biopsy strategy (9,10). Some parameters (time passed from the previous biopsy; previous HGPIN or ASAP) have been proposed to predict positive repeat biopsy for cancer (11), although a previous diagnosis of ASAP seems to be the single risk factor that better relates with *PCa. Scattoni et al.* (12) showed a cancer in 39% of 105 patients with a diagnosis of ASAP who underwent a repeat biopsy reporting an even higher risk in men with concomitant HGPIN (50% of cases).

How many biopsy sets and/or others procedures such as TURP should be performed to rule out a cancer, especially in younger patients we suspect to harbour a PCa (persistently elevated or increasing PSA values, previous HGPIN or ASAP) is still under debate.

Djavan et al. (13) reported a PCa incidence of 10%, 5% and 4% in patients with negative DRE and PSA level of 4-10 ng/mL after a second, a third and a fourth sextant biopsy, respectively; *Philip et al.* (14) and *Singh et al.* (15) showed an incidence of PCa equal to 31.9% and 14% in 241 and 99 patients after a second 12 cores biopsy; *Satoh et al.* (16) found a PCa in 25.7% and 46.4% of 100 patients after a second and a third biopsy. *Kawakami et al.* (17) showed a PCa incidence of 37% in 235 patients using a 26 cores scheme with a three-dimensional TRUS guide and this result was achieved after 16 cores. *Eskicorapci et al.* (18) reported an incidence of cancer higher in patients previously submitted to a sextant biopsy (36.1%) than in men who underwent primary 10 cores biopsy and they found a PCa in the transition zone only in 3.7% of cases. SPBx increases the diagnosis of PCa during a repeated biopsy, but a greater number of cores increases the incidence of low volume PCa (a single positive core or a neoplastic microfocus) with the consequent risk of overdiagnosis/ overtreatment of clinically insignificant PCa, i.e., less than 0.5 cc in volume without Gleason grade 4 or 5 disease (19). Fleshner and Klotz (20) reported a PCa incidence of 13% in 37 selected patients submitted to SPBx after a negative second biopsy; *Walz et al.* (21) showed a high incidence of cancer (41%) detected by SPBx in 161 patients after previous negative biopsy sets; *Rabets et al.* (22) reported 41% and 24% of PCa in 116 men previously submitted to 6 and 10 cores biopsy schemes; *Rodriguez Alonso et al.* (23) and *Jones et al.* (24), using 24 cores extended biopsy, reported 40.8% and 33% of cancer, respectively. In our experience (10) a PCa detection rate of 22.6% and 10.9% in 75 and 73 patients submitted to SPBx and 18 core biopsy set after an initial negative extended biopsy was found.

A transition zone sampling is recommended at repeat biopsy, though the PCa detection is low. *Peizer et al.* (25) and *Miyake et al.* (26) reported a detection rate equal to 1.8% and 5.3% in 1475 and 788 patients, respectively. On the contrary, a consistent number of PCa not found by biopsy are subsequently diagnosed by TURP or open prostatectomy. Therefore many authors suggested to perform a TURP, after multiple negative biopsy series, even

in men not complaining LUTS. *Radhakrishnan et al.* (27), *Zigeuner et al.* (28) and *Puppo et al.* (3) after TURP reported a PCa detection of 21%, 7.9% and 42.8%, respectively. PCa diagnosed by TURP frequently originate in the anterior prostate, are clinically confined with a GS < 6 suggesting that tumours located in the transition zone have a less aggressive phenotype than those found in the peripheral zone (29) and PSA measured before and after TURP and GS are considered significant predictors of the presence of residual cancer at radical prostatectomy (30).
In our experience, all PCa were diagnosed in the peripheral area of the gland during a second biopsy and only two PCa (3.8%), located in the transition area, were detected at a third biopsy set. In patients submitted to repeat biopsy after a previous ASAP, the probability to find a PCa was significantly higher than in patients affected by HGPIN (p = 0.005) or with benign findings (p = 0.001).
In patients submitted to TURP, PCa incidence was 11.1% and 19% after one and two negative biopsies, respectively, showing an increasing risk of PCa detection in the transition area even after multiple negative biopsies. The extent of cancer in the tissue obtained by TURP allowed to distinguish 18 patients with a T1a cancer, who were enrolled in an watchful waiting programme, from 2 patients with a T1b cancer who underwent radical surgery. It is interesting that in all patients with clinical stage T1a PCa PSA was stable (median 0.9 ng/mL) after a median follow up of two years, suggesting that cancer was localized exclusively in the transition zone. In the majority of patients with benign findings after TURP, a significant reduction of tPSA values (1.5 ng/ml; range: 0.8-3.4 ng/ml) was observed: this allowed to distinguish patients with stable tPSA and normal DRE from those with increasing tPSA who are still eligible for a repeat biopsy. Specimen obtained by TURP allowed to stage PCa suggesting radical surgery only in patients with clinical T1b PCa; on the contrary, it is a difficult task to decide the best management, in presence of one or more positive biopsy cores found only in the transition zone, as prostatectomy could be an overtreatment.
In conclusion, in case of persistent suspicion of PCa after a repeated negative saturation biopsy, TURP should be proposed as part of the diagnostic procedure aside from LUTS, especially in patients with a life expectancy greater than 10 years.

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ORIGINAL PAPER

Is it possible to predict post-residual voided urine by bladder scan before uroflowmetry – a useful and timesaving test to reduce the number of non – evaluable uroflow measurements?

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Summary

Objectives: *Bladder-scan before uroflowmetry is useful to reduce non-evaluable Q_{max}-data. A significant problem is to receive an adequate voided volume in uroflow-measurements. Aims of this study were 1- to confirm if pre-voiding bladder scan can reduce the number of inadequate flow measurements, 2- to establish threshold values for pre-voiding bladderscan volumes before and after different treatments options 3- to study if it is possible to predict the post-residual voided volume.*

Material and methods: *121 patients performed 2 uroflowmetry before and after different treatments. Bladder volume was measured by transabdominal ultrasound when the patient had the sensation to void and after uroflowmetry to calculate residual urine. Same investigations were repeated after different treatments.*

Results: *21% of the patients had insufficient voided volume < 125 ml in 1st recording and 22% in 2nd ; while 28% of the patient had a volume voided < 150 ml in 1st recording and 33% in 2nd. There was a strong correlation between the pre-voiding measured volume and the voided volume ($r = 0.801$, $p < 0.0001$), linear regression analysis yielded 1st flow rate recording is Void-Vol = 32.703 + (0.637 * Pre-Vol) and 2nd flow rate recording is Void-Vol = 16.264 + (0.704 * Pre-Vol) ($r = 0.855$; $p < 0.0001$).*

Conclusions: *Bladder scanning before uroflowmetry reduces the number of non-evaluable Q_{max} data. If a voided volumes of > 125 ml (> 150 ml) is required a mandatory pre-voiding bladder scan volume should be > 200 ml (> 250 ml), so non elegible Q_{max} recordings will decrease from 21% to 5.8% (28% to 4.1%) in BPH patients who will undergo treatment and from 22% to 7.4% (33% to 5.8%) in BPH-treated patients. There is a difference between patients before and after treatment. It is not possible to predict the post residual voided volume by the bladder scan using the virtual calculation.*

KEY WORDS: *Bladder ultrasound scanning; Uroflowmetry; BPH studies; Minimum voided volume.*

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List of abbreviations:

LUTS: lower urinary tract symptoms.
BPH: benign prostatic hyperplasia.
Qmax: maximum flow rate obtained during free-flow measurement.
PSA: prostate specific antigen.
BPO: benign prostatic obstruction.
IPSS: international prostate symptoms score.
QoL: quality of life.
TRUS: transurethral utrasound.
Pre-Vol: pre-voiding measured volume.
Void-Vol: voided volume.

Introduction

The use of bladder scanning before uroflowmetry recording seems to be a useful test to reduce the number of non-evaluable Q_{max} data because of insufficient voided volume (1, 2). A significant problem in clinical studies of lower urinary tract symptoms (LUTS) due to bladder outlet obstruction (BOO) is to receive an adequate voided volume in uroflow measurements. Ultrasound bladder scanning is not a test performed just by radiologist to study the bladder; in fact it could be used in calculating the post residual voided volume by measurement of urinary bladder volume (3) by an urologist or a nurse and it could evaluate the prostatic intravesical protrusion correlated with uroflowmetry, this a new method to measure obstruction in patients with LUTS due to BOO without using Pressure/Flow studies (4). Benign prostatic hyperplasia and benign prostatic enlargement are one of the most common diseases in aging men which can lead to lower urinary tract symptoms (5). The clinical use of the urine flowmeters, simple non-invasive investigation, has become widespread to reveal abnormal voiding. Practice Guideline shows that correct uroflowmetry needs a voided volume > 125-150 ml and Q_{max} is better than Qmean and more specific to identify BPH patients (6). Uroflowmetry is a recommended investigation as a diagnostic test in the initial assessment of men with LUTS and should always be performed prior prostatic surgery. Post-void residual volume urine measurement is also recommended, Post-void residual volumes > 200 ml may indicate bladder dysfunction and predict a less favourable response to treatment (5).

The aims of this study were 1- to confirm if pre-voiding bladderscan can reduce the number of inadequate flow measurements in patients with LUTS due to BOO, 2- to establish threshold values for pre voiding bladder scan volumes before and after different treatments options 3- to study if it is possible to predict the post residual voided volume calculating the virtual post residual volume.

Material and methods

A total of 121 BPH patients with a mean age of 69.2 + 8.7 SD (range 46-88) years entered this study performing two flow rates before and after different BPH treatments. Initial patient evaluation consisted in medical history, urological visit including digital rectal examination and history of the patient taken from his clinical journal. Routine blood chemistry included PSA, hemoglobin and creatinine.

Prostate size was assessed by transrectal ultrasonography (probe of 7.5 MHz) and the prostatic volume calculated using the formula considering length, width and height and adjusting with $\pi/6$.

Bladder volume was measured by transabdominal ultrasound (3.5 MHz probe) when the patient had the sensation of need to void and after the uroflowmetry to calculate and measure residual urine. Bladder volume calculation was performed using the ellipsoid formula (A x B x C x 0.52) as simple as possible just marking with the cursor the peripheral area of the largest sagittal bladder image and the maximal diameter. We have used this method because the transverse diameter is the most difficult to measure and formulas that use transverse diameter show great inaccuracy in inexperienced hands (1, 3). Thus after assessment the patients were treated according to the severity of the disease: watchful waiting, medical therapy, surgical management and minimally invasive treatment.

Post residual voided volume was calculated by bladder scan volume after uroflowmetry and also subtracting the bladder scan before uroflowmetry to the voided volume at uroflowmetry.

Conventional statistical methods were used to calculate the mean and standard deviation using computer software and parameter analyses were calculated using simple linear regression test.

Results

All the 121 patients concluded the study. A baseline prior to treatment mean IPSS was of 17.0 + 9.2 (range 1-35) points, a mean QoL of 3.2 + 1.8 (range 0-5) points, a serum PSA of 4.3 + 3.9 (range 0.4-20) ng/ml and a serum creatinine of 112.8 + 19.0 (range 80-196), a TRUS volume of 50.0 + 25.4 (18 to 166) cc After the different

Table 1.

121 patients were divided into 4 groups (> 125 ml, > 150 ml, > 200 ml, > 250 ml) according to pre-voiding measured volumes (Pre-Vol). Voided volumes (Void-Vol) were then assessed in two different times (1st flow rate recording and 2nd flow rate recording). Patients with insufficient voided volume (< 125 ml and < 150 ml) in each group was calculated.

	1st flow rate recording		2nd flow rate recording	
Pre-Vol	Void-Vol < 125	Void-Vol < 150	Void-Vol < 125	Void-Vol < 150
///	25/121 = 21%	34/121 = 28%	26/121 = 22%	40/121 = 33%
> 125 ml	15/121 = 12%	///	16/121 = 13%	///
> 150 ml	12/121 = 10%	20/121 = 17%	12/121 = 10%	24/121 = 20%
> 200 ml	7/121 = 5,8%	10/121 = 8.3%	9/121 = 7.4%	19/121 = 15.7%
> 250 ml	4/121 = 3,3%	5/121 = 4.1%	5/121 = 4.1%	7/121 = 5.8%

Figure 1.
*Linear regression analysis yielded 1st flow rate recording is Void-Vol = 32.703 + (0.637 * Pre-Vol) (r = 0.801; p < 0.0001).*

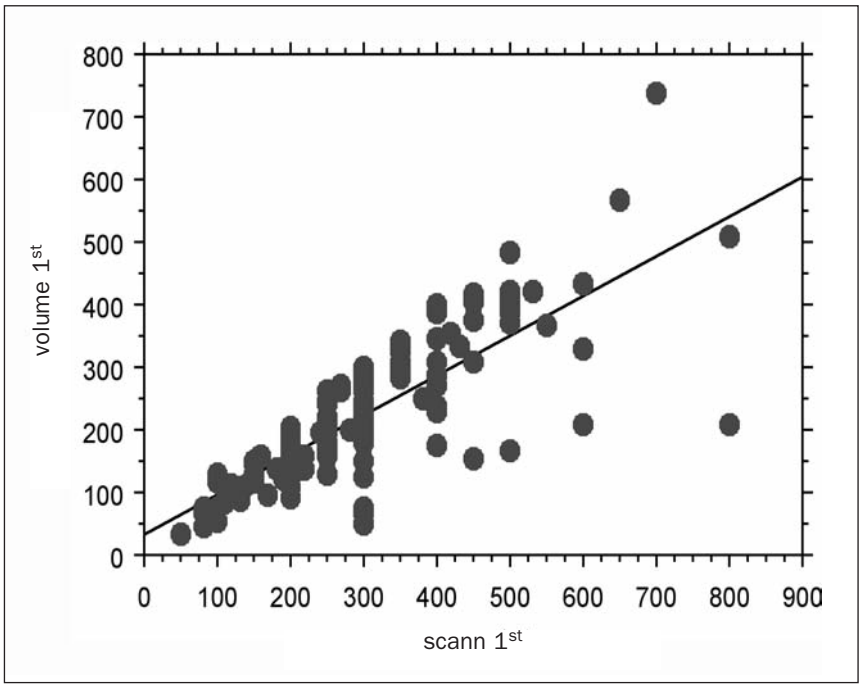
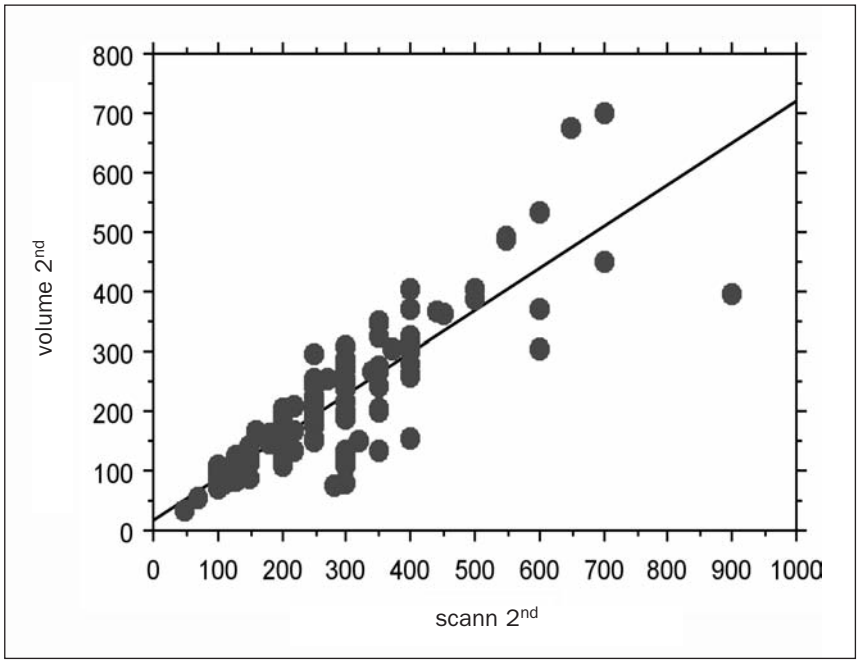


Figure 2.
*Linear regression analysis yielded 2nd flow rate recording is Void-Vol = 16.264 + (0.704 * Pre-Vol) (r = 0.855; p < 0.0001).*



treatments mean IPSS was 10.8 + 7.5 (range 0-31) points, mean QoL was 1.8 + 1.6 (range 0-5) points, mean Q_{max} 12.3 + 5.7 (range 1-35) ml/s, mean PVR 74 + 75 (range 0-510) cc. We had responders and non responders to the different treatments options.
In our records, see table 1, 21% of the patients had insufficient voided volume < 125 ml in the first flow rate recording and 22% in the second; while 28% of the patient had a volume voided < 150 ml in the first flow rate recording and 33% in the second measurement.
We have divided the pre-voiding measured volume (Pre-Vol) (see Table 1) into 4 categories: > 125 ml, > 150 ml, > 200 ml, > 250 ml and we have shown the relationship with the voided volume. Then we have counted the number of measurements with voided volume < 125 ml and < 150 ml in the first flow rate recording and in the second recording after the treatment.
There was a strong correlation between the pre-voiding measured volume (Pre-Vol) and the voided volume (Void-Vol) at 1st uroflowmetry (r = 0.801, p < 0.0001), Linear regression analysis yielded 1st flow rate recording is Void-Vol = 32.703 + (0.637 * Pre-Vol) (Figure 1). A good correlation (r = 0.855; p < 0.0001) has been found also between the pre-voiding measured volume (Pre-Vol) and the voided volume (Void-Vol) at 2nd uroflowmetry, Linear regression analysis yielded 2nd flow rate recording is Void-Vol = 16.264 + (0.704 * Pre-Vol) (Figure 2).
As we can see in the same table not useful flow rate (voided volume < 125 ml) in the first flow rate recording decreases from 21% to 12% and from 22% to 13% in the 2nd record if Pre-Vol was > 125 ml; if Pre-Vol was > 150 not useful Q_{max} decreased to 10% in both the 1st and 2nd records and decreased to 5.8% and 7,4% in the 1st and 2nd record respectively if Pre-Vol was > 200 ml.
Finally not useful flow rate decreased to 3,3% and to 4.1% in the 1st and 2nd record respectively if Pre-Vol was > 250 ml. Important percentage reduction has been obtained considering not useful flow rate with voided volume < 150 ml from 28% to 17%, 8.3% 4.1% in the 1st record and from 33% to 20%, 15.7%, 5.8% in the 2nd flow rate recording if Pre-Vol was > 125, 150, 200 and 250 ml respectively.
Mean bladder scan volume in the first measurement was 300 + 150 (range 50-800) ml, in the second measurement was 282 + 143 (range 50-900) ml. Mean post voided residual volume calculated by ultrasound was in the

first measurement 81 + 91 (range 0-600) ml and in the second measurement was 74 + 75 (range 0-510) ml. Mean voided urine volume calculated at uroflowmetry was in the first measurement 224 + 119 (range 33-737) ml and in the second measurement was 215 + 118 (range 33-698) ml. Mean virtual post voided residual volume calculated by mathematical substraction between bladder scan prior to uroflowmetry and voided volume was in the first measurement 76 + 89 (range -37-593) ml and in the second measurement was 67 + 74 (range -45-506) ml. There was statistical significant difference according to the Paired t-test between Post residual voided volume calculated by bladder scan after uroflowmetry and subtracting the bladder scan volume before uroflowmetry to the voided volume at uroflowmetry, $p < 0.0046$ in the first measurement and $p < 0.0002$.

Discussion

Benign Prostatic Hyperplasia, Benign Prostatic Enlargment and Benign Prostatic Obstruction can be considered progressive disease based on published data on consequences and complications of the disease. With a changing demographic profile and an increasingly aging population in almost all societies it is estimated that one third of all men will need a treatment for relief of LUTS due to benign prostatic obstruction (BPO). These man should be investigated to identify patients at risk of progression and to initiate early preventive treatment (5).

Thus, research should be addressed also to improve accuracy and to optimize the recommended diagnostic test as uroflowmetry. To our knowledge few studies are available to provide evidence that pre-voiding transabdominal ultrasound bladder scanning is useful before the BPH patient undergoes free flow for BPH study before and after treatment (1, 2). Even if there is a widespread acceptance that a single free uroflow measurement is unsatisfactory, most clinicians and studies rely on this. We know that in the daily practice uroflowmetry test is time consuming both for the patient and for the nurse/doctor who is responsible for the test. Free uroflowmetry give evidence of urinary dysfunction although it has poor diagnostic specificity for BPO. Other methods are not reliable as home uroflowmetry in studies including large number of patients both for economical and organisation problems (7).

If we would have conducted a clinical assessment using our flow rate records we would have lost 21% of the patients at baseline because of insufficient voided volume < 125 ml for the first flow rate recording and the 22% in the first screening after the treatment; while 28% of the patients had a volume voided < 150 ml at baseline flow rate recording and 33% in the second measurement after the treatment to not lose so many patients we probably would have been asking to the patient to repeat the measurement. The patient would have lost time in the clinic and interest in the following visit of follow up with risk of losing the patient.

In comparison of *Roehrborn et al.* study and as confirmed in the previous study (1) we have achieved higher threshold volumes. Our patients data are, in our opinion, interesting because both at the first and at the second visit they represent a variety of BPH patients from obstructed to unobstructed ones and from responder to non responders with four times more measurements data. Using the threshold of *Roehrborn et al.* we would have lost too many patients (2).

It is not possible to predict the post residual voided volume in the way of measuring the bladder volume before uroflowmetry instead measuring the volume after the examination, in fact using a simple mathematical subtraction it is possible to calculate the virtual post residual voided volume but the result sometimes is negative with no possibility to have a clinical significance or sometimes the result has a significant difference between the reality the virtual calculation.

An explanation is that the volume obtained in this way is indirect and contains error of approximation. For this reason even if the calculation of the post voided residual urine by ultrasounds is a good method recommended by EAU BPH Guideline (5) it is not very precise as demonstrated by *Simforoosh N et al.* (8) For this reason nowadays several modern and technological methods exists and are proposed (9). It is not possible to avoid the measurement of the post voided urine volume after uroflowmetry. Determination of the residual urine volume could be done in different ways in the routine practice in urology. Calculation of the residual urine volume by catheterization, either transurethrally or sovrapubic, despite its accuracy, is an invasive procedure that it could have side effects and risk of infection. Radiological and isotopic methods for bladder volume calculation are inaccurate, may have radiation hazards and expensive. For these reason Ultrasound urinary bladder volume measurement is an easy calculation of bladder volume and it is relatively precise (3).

Time consuming is almost the same but with the opportunity to reduce significantly the number of nonevaluable Q_{max} we truly recommend bladder scan before uroflowmetry.

Bladder scanning is cost-effective and is also easy to perform, a well-instructed nurse is able to do the measurement. Time can also be saved both for the patient and for the clinical management.

Conclusion

The use of bladder scanning before uroflowmetry recording is a useful test to reduce the number of non-evaluable Q_{max} data. If a voided volumes of > 125 ml (> 150 ml) is required a mandatory pre-voiding bladder scan volume should be > 200 ml (> 250 ml), so the number of non eligible Q_{max} recordings will decrease from 21% to 5.8% (28% to 4.1%) in BPH patients who will undergo treatment and from 22% to 7.4% (33% to 5.8%) in BPH-treated patients. There is a difference between patients before and after treatment. Unfortunately it is not possible to predict the post residual voided volume by the bladder scan using the virtual calculation.

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ORIGINAL PAPER

Sarcoma of prostate: Case report and review of the literature.

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Summary

Objectives: *Prostate sarcomas are rare entity, the most common is leiomyosarcoma which account for 0.1% of all prostate malignancies. The presenting symptoms are mainly obstructive urinary symptoms. Surgery with chemo- or radiotherapy are the mainstay treatment options. The overall survival rate remains poor regardless of initial tumour size, grade or histological subtype. Immunohistochemistry reveals tumour cells diffusely positive for vimentin, smooth muscle actin, focally positive for progesterone receptor, whilst keratins are usually negative.*

Materials and Methods: *We describe a case of a patient affected by sarcoma of prostate. Furthermore, we reviewed the cases of prostate sarcomas available in literature to clarify the best therapeutic options to be applied.*

Results: *In the case described leiomyosarcoma diagnosed by an ultrasound guided biopsy was characterized by fascicles of spindle-shaped cells with a variable degree of nuclear atypia. The immunohistochemistry showed positive staining for smooth muscle actin, vimentin and focally for the S-100 protein. The patient was treated with radical retropubic prostatectomy and radiotherapy of the local recurrence, and chemotherapy at metastases onset.*

Conclusions: *Prostate sarcomas are highly aggressive, with limited therapeutic options. An early diagnosis and complete surgical excision with negative margins offer patients the long-term disease free survival.*

KEY WORDS: *Sarcoma; Prostate; Leiomyosarcoma.*

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INTRODUCTION

Sarcomas of soft tissues (STS) are a heterogeneous group of tumours arising from mesoderm. STSs of genitourinary tract (GU) are uncommon, accounting for 2% of STSs and representing just 1-2% of the urological malignant cancers (1). In the two largest series available, leiomyosarcoma followed by liposarcoma and rhabdomyosarcoma are reported to be the most common histological types (1, 2). Focusing on prostate, leiomyosarcoma represents less than 0.1% of all prostate malignancies and 38-52% of primary prostatic sarcomas, with a mean age at presentation of 61 years (range 41-78 years) (3-6). 89.4% of prostate sarcomas showed obstructive urinary symptoms as first manifestation followed by perineal or rectal pain in 25.6% of patients, burning on ejaculation and hematuria in 5.2% of cases (7, 8). In about 23.5% of patients the metastatic signs may be the presenting manifestations mainly involving lungs (17.6%), liver (11.7%), bone (5.8%) and brain (3.6%) (7).

Local recurrences (LR) of GU STS range from 21% to 32% (1, 9). The different LR rates may be accounted to biology, tumour size, patients selection, anatomical location. The biological aspects (grade, size, histology) are key factors for metastatic progression as well as age at presentation and histology are determinant in the metastases onset (1). Tumour histological grade, a well recognized adverse prognostic factor, is classified as low or high based on the number of mitoses, degree of cellularity, differentiation and nuclear pleomorphism (9-11). Predictors of disease specific survival are clinical local presentation, complete tumour resection, tumour grade, size, location and histological subtype. Patients with visceral and retroperitoneal sarcomas show a decreased sur-

vival in respect to the extremity sarcomas (10, 12). In addition, negative surgical margins are associated to freedom from LR and from metastases as well as to a prolonged survival (13). The reported disease specific survival at 5 years was variable in relation to the anatomical site ranging from 69 to 76% (1, 9, 10). Prostate sarcoma shows a worse disease free survival of 38% than the other STSs (10). The mainstay of STS treatment is complete surgical excision combined with radio- and chemotherapy. We describe the clinical characteristics of a leiomyosarcoma of the prostate presenting with unusual symptoms and normal serum prostate specific antigen (PSA). Moreover, we reviewed prostate sarcoma histological features and therapeutic options available.

Materials and Methods

A 62-years-old white man presented with frequent micturation, dysuria, poor urinary stream and nocturia of approximately ten months duration. He did not refer a family history of GU cancers. He was not a smoker or an abuser of alcohol. PSA was 1.60 ng/ml. Rectal examination (RE) revealed an enlarged prostate gland with a nodular mass extended to the right and left lobe which led to an ultrasound guided transrectal needle biopsy.

Results

The histological diagnosis was leiomyosarcoma. The tumour was characterized by areas of haemorrhage and necrosis, with focal bilateral extracapsular extension. At immunohistochemistry with H&E an high hypercellularity with interlacing fascicles of eosinophilic spindle-shaped cells with blunt-ended nuclei and a variable nuclear atypia with several mitosis were showed (Figure 1). Prostate cells expressed vimentin, smooth muscle actin and desmin as well as S-100 while immunostaining for keratins AE1-AE3 was negative (Figure 2). The bone scan, chest X-ray and total body computerized tomography (CT) were unremarkable. He underwent to a radical retropubic prostatectomy (RRP) with bilateral lymphadenectomy. The tumour was multifocal and bilateral, involving 76% of the prostate with surgical margins free from tumour and lymph nodes of the external, internal iliac and obturator area negative. 3 years after, he presented with acute urinary retention and an urethrocystoscopy visualized an irregular necrotic tissue growth. A permanent urinary catheter was placed. A magnetic resonance spectroscopy (MRS) showed a local recurrence and an enlarged left iliac lymph node. A palliative external-beam radiotherapy was recommended.

Figure 1.
Leiomyosarcoma (H&E). The tumour is composed of a dominant component of interlacing fascicles of spindle-shaped cells, with elongated, blunt ended and pleomorphic nuclei. Of note two benign entrapped prostatic glands (showed by the tip of the arrow).

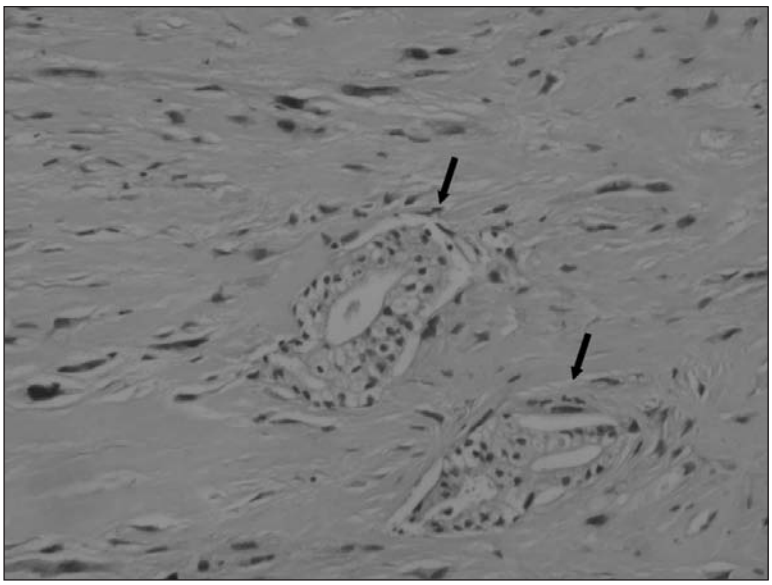
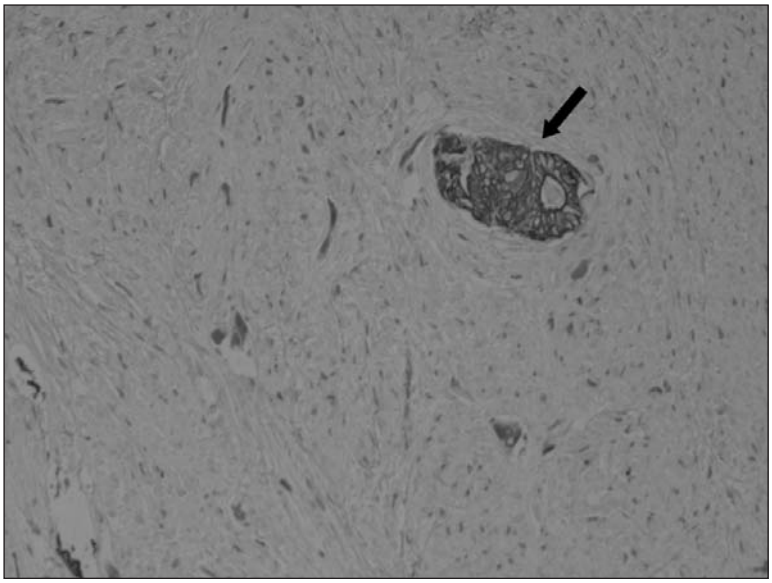


Figure 2.
Negative staining for keratins (AE1-AE3) which are expressed just in 25% of prostate sarcomas. Note that the benign gland on the upper right corner shows a positive staining.



The diagnosis of left lung metastasis led to a chemotherapy which was prematurely interrupted as his general status declined. He died 3 months later.

Discussion

Sarcoma of prostate arises from non-epithelial mesenchymal components with several theories concerning its origin from an incidental simultaneous development of both carcinoma and sarcoma from different areas of

the gland, a dissimilar differentiation from the same totipotent cell, a transformation of an adenocarcinoma in sarcoma or otherwise of a sarcoma in adenocarcinoma (14-19). Mostofi and Price defined carcinosarcoma as a prostatic adenocarcinoma with evidences of metaplastic cartilaginous or osseous sarcoma (20). More recently *Young et al.* described carcinosarcoma as a mixture of epithelial elements and spindle cells associated to heterogeneous tissues such as cartilage or skeletal muscle (21). Cytogenetic analysis of primary leiomyosarcoma revealed clonal chromosomal rearrangement involving chromosomes 2, 3, 9, 11, 19 (19). Stromal sarcoma expresses CD34, less frequently smooth muscle and keratin markers; sarcomatoid prostatic carcinoma is characterized by malignant epithelial cells with the appearance of spindle cells, expresses keratins and vimentin but no desmin and actin (20). Carcinosarcoma of prostate and other mixed-patterns arise from uncommitted stem cells which differentiate in mesenchymal or epithelial elements (21).

The most common presenting manifestations are the obstructive urinary symptoms: urgency, nocturia, hematuria, frequency, perineal or rectal pain and constipation. PSA is typically within normal limits and it is not surprising considering the non-epithelial origin of prostate sarcoma. The most frequent sarcoma of prostate is leiomyosarcoma in the adult whereas rhabdomyosarcoma is more common in children and adolescents (4, 6). Histological characteristics are high cellularity, bundles of eosinophilic spindle-shaped cells, increased mitotic activity and nuclear atypia, necrosis and cystic degeneration (5). Neoplastic cells exhibit vimentin, smooth muscle actin, and desmin (60%) while keratins are observed just in 25% of cases (3). Progesterone receptor should be also found whereas CD117 is absent; S-100 protein immunoreactivity can be seen focally (22).

In most patients diagnosis is made by transurethral resection (TURP) performed for obstructive urinary symptoms or acute urinary retention, however it may be missed if the sarcoma is not resected. A third of them show demonstrable metastases at presentation mainly located at the lung and liver (4, 6). MRS is characterised by a marked increase in the ratio of choline: citrate; this should be helpful in the differentiation with benign prostatic hyperplasia but unsuitable to differentiate prostate sarcoma from carcinoma as the MRI features are similar. At MRS adult prostate sarcoma typically appears as a large cystic and necrotic mass, with rapid growth, hypervascular, with a heterogeneous tissue enhancement (23). Lack of awareness of this rare tumour may responsible of a delayed diagnosis, mainly caused by the unrelated symptoms and normal serological markers. In the case reported, the symptoms suggestive of prostate benign enlargement and PSA normal value should be misleading; unlikely these are the most frequent prostate sarcoma presenting manifestations. Results related to tumour grade, size and stage as predictors of long-term survival are not homogeneous. Those differences are to be accounted to the period of enrolment with inadequate clinical staging due to inferior imaging capacities in earlier decades respect to more contemporary series and to the different selection criteria including all GU STS or just prostate sarcomas. An uniform agreement is related to the significantly improved disease-free survival of patients with negative surgical margins (2-4). Our patient showed at diagnosis a focal extracapsular extension and was free from metastases allowing to perform a RRP. A LR three years after, led to a combined radiochemotherapy. An hypothesized survival advantage may be offered by combining several treatments based on preoperative chemoradiation followed by surgical excision (24). A preoperative chemotherapy may be offered to patients at high risk of positive surgical margins (4, 25). Of note, the only predictive factors of long-term survival were absence of metastases and negative surgical margins (2, 4, 26, 27).

Conclusions

Prostate sarcoma is a very rare but highly aggressive cancer with limited therapeutic options: an early diagnosis is mandatory to offer patients the best chance for cure. A multidisciplinary approach including urological, radiotherapist and oncological consultations should be employed for an appropriate management. The awareness of its existence and of its clinical manifestations may achieve a correct diagnosis.

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ORIGINAL PAPER

Laparoscopic versus open radical retropubic prostatectomy: A case-control study at a single institution.

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Summary

We retrospectively compared 50 patients treated with open retropubic prostatectomy (RRP) with 50 patients treated with laparoscopic extraperitoneal radical prostatectomy (LRP) at our institution, in the same time period, with a follow-up up to 7 years. We focused on operative data, complications, pathological outcome and mid-term outcome and follow-up in terms of oncological results. The same surgeons performed both operations. The 2 groups were similar with respect to mean patient age, mean prostate specific antigen value, median Gleason score. No previous transurethral resection of the prostate nor neoadjuvant treatment, had been undertaken in both groups of pts. Mean operating time was significantly shorter after open surgery (126 minutes, range 90-185 minutes) [p = 0.03] compared to the laparoscopic group (188 minutes, range 130-250) but it did not differ significantly from the last 20 laparoscopic procedures, in which the time of procedure was reduced to a mean of 155 minutes group (range 140-184 minutes) [p = 0.1]. Mean blood loss (1,150 versus 800 cc) and transfusion rates (55.7% versus 19.6%) in the 2 groups significantly favored the laparoscopic group. Number of lymphnodes dissected during the procedures favoured, but not significantly, the RRP group: for RRP a mean 11 lymphnodes right side, 13 left side (ranges 2-20 and 2-19 respectively), while for LRP a mean of 9 lymphnodes right side, 11 left side (ranges 2-15 and 2-13 respectively) were collected. The complication rate was almost the same in both groups, with no major adverse events nor deaths, (19.2% versus 14.7%) but the spectrum differed. The laparoscopic group had a higher incidence of fever (1.8% versus 3.2% respectively) and subcutaneous or scrotal emphysema, whereas more lymphoceles (6.9% versus 0%), wound infection (2.3% versus 0.5%), embolism/pneumonia (2.3% versus 0.5%) and anastomotic strictures (15.9% versus 4%) occurred after open surgery. Median catheter time was longer after open retropubic prostatectomy (22 versus 8.9 days, respectively) but the continence rates (intended as complete continence with no use of pads) were similar in both groups at 12 months (90.3% versus 91.7%). The rate of positive margins did not differ significantly in groups, and was in all cases very low (8.2% versus 7.0%), prostate specific antigen biochemical recurrence was equivalent (10% vs 10%). Data regarding postoperative sexual function favoured the laparoscopic group, even if no statistical significance was recorded (55% vs 67%). No statistical differences were observed in terms of oncological results, with a 24 months mean follow-up. Laparoscopic radical prostatectomy is technically demanding, with an initially longer operative time and learning curve. The overall outcome in our series favours the laparoscopic approach regarding catheterization time, recover of continence and impotence , hospital stay, transfusion rate. The open approach is favoured for the still shorter time necessitating for the procedure. Consequently, at our institution laparoscopic radical prostatectomy is becoming the method of choice.

KEY WORDS: Laparoscopy; Radical prostatectomy; Prostate cancer.

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Introduction

There is an ongoing debate about the benefits of laparoscopic radical prostatectomy compared to the open retropubic approach. We retrospectively compared 50 patients treated with open retropubic prostatectomy (RRP) with 50 patients treated with laparoscopic extraperitoneal radical prostatectomy (LRP) at our institution, in the same time period, with a follow-up up to 7 years. We focused on operative data, complications, pathological outcome and mid-term outcome and follow-up in terms of oncological results.

Materials and methods

A total of 295 patients were treated with open prostatectomy (RRP) and pelvic lymph node dissection while 95 patients underwent LRP with pelvic lymph node dissection in the same time period. From the patients who underwent a RRP or LRP from December 2002 to November 2007 we recruited 50 consecutive patients for each technique to enter this case-control study. To reduce the bias due to the learning curve in LRP, the first 20 pts who had had undergone the procedure were not considered, such as the last 25 due to shorter follow-up period. The same surgeons performed both operations. The 2 groups were similar with respect to mean patient age, mean prostate specific antigen value, pathological stage, median Gleason score (Table 1).

No previous transurethral resection of the prostate nor neoadjuvant treatment, had been undertaken in both groups of patients.

Surgical Techniques

Open RRP was performed in the usual way, according to Walsh (1-3) nerve sparing technique. The limphnode template involved external iliac vessels and obturatorius fossa. 4 to 6 single stitches in Polyglicole were used for the urethrovesical anastomosys, with an indwelling Foley catheter.

LRP was conducted extraperitoneally, with antegrade bladder neck dissection until the identification of vas deferens and seminal vesicles. The template of lymphnode dissection was the same then in open procedure. The dorsal complex was secured, for the first 30 procedure, with Hem-O-Lok system but, due to the migration of the staples in the bladder, observed in 4 patients several months after the procedure, the actual technique went back to a single stitch (0 polyglicole, CT1 needle) passed after the dissection of the posterior and lateral faces of the prostate.

Prostatic pedicles and nerve-sparing procedure was, and still is, carried one with Hem-O-Lok staples, assuring a very good haemostasis with no use of termal sources.

Urethrovesical anastomosis has been carried out with 2 semicontinous running suture, according to Van Velthoven (4) technique.

Results

Mean operating time was significantly shorter for open surgery (126 minutes, range 90-185 minutes) [p = 0.03] compared to the early laparoscopic group (188 minutes, range 140-250) but it did not differ significantly from the last 20 laparoscopic procedures, in which the time of procedure was reduced to a mean of 155 minutes (range 130-184 minutes) [p = 0.1]. Mean blood loss (1.150 versus 800 cc) and transfusion rates (55.7% versus 19.6%) in the 2 groups significantly favoured the laparoscopic group.

Number of lymphnodes dissected during the procedures favoured, but not significantly, the RRP group: for RRP a mean 11 lymphnodes right side, 13 left side (ranges 2-20 and 2-19 respectively), while for LRP a mean of 9 lymphnodes right side, 11 left side (ranges 2-15 and 2-13 respectively) were collected (Table 2).

The complication rate was almost the same in both groups, with no major adverse events nor deaths, (19.2% versus 14.7%) but the spectrum differed. The laparo-

Table 1.
Patients data.

	RRP	LRP
Age years	68.3 (52-71)	66.6 (53-70)
PSA (Preop) (ng/ml)	6.38 (2.3-21)	5.8 (3-20)
Gleason Score (Postop)	6 (3+3)	6 (3+3)

Table 2.
Surgical outcome.

	RRP	LRP	P value
Mean Operating Time (min)	126 (90-185)	188 (140-250)	0.03
Last 20 LRP Op. Time (min)	155 (130-184)	0.1	
Blood loss (ml)	1150	800	0.03
Transf.rate	55.7%	19.6%	0.03
Number of LN right	11 (2-20)	9 (2-15)	> 0.5
Number of LN left	13 (2-19)	11 (2-13)	> 0.5

Table 3.
Complications.

	RRP	LRP
Overall complication rate (%)	19.7	14.7
Fever (%)	1.8	3.2
Subcout./scrotal emphysema (%)	0	15
Lymphoceles (%)	6.9	0
Wound infection (%)	2.3	0.5
Embolism/pneumonia (%)	2.3	0.5
Anastomotic strictures (%)	15.9	4
Migration of Hem-O-lok (%)	0	8

Figure 1.
Kaplan Meier curves for biochemical progression.

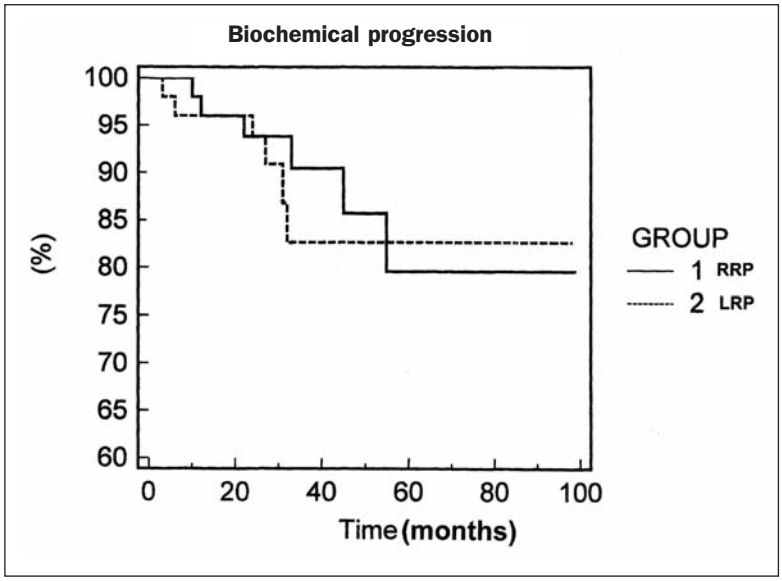

Table 4.
Comparison of KM curves for biochemical progression.

Endpoint observed n.	6	6
Expected n	6.8	5.2
Chi-square	0.2378	
DF	1	
Significance	p = 0.6258	
95% CI	0.2325 to 2.4051	

scopic group had a higher incidence of fever (1.8% versus 3.2% respectively) and subcutaneous or scrotal emphysema, whereas more lymphoceles (6.9% versus 0%), wound infections (2.3% versus 0.5%), episodes of embolism/pneumonia (2.3% versus 0.5%) and anastomotic strictures (15.9% versus 4%) occurred after open surgery.

In a small group of patients who underwent LRP and in which the Hem-O-lock system has been used for haemostasys of the dorsal complex, a migration of the staples has been observed in 4 pts (8%), necessitating of late endoscopic removal from the bladder (Table 3).

The amount of postoperative analgesia was significantly greater after open surgery (50.8 *versus* 33.8 mg respectively). Median catheter time was longer after open retropubic prostatectomy (22 *versus* 8.9 days, respectively) but the continence rates (intended as complete continence with no use of pads) were similar in both groups at 12 months (90.3% *versus* 91.7%).

Data regarding postoperative impotentia coeundi were favouring the laparoscopic group, even if no statistical significance was recorded (55% *vs* 67% of recover after surgery, with early rehabilitation carried out with oral agents). Actually most of the patients are satisfied for their sexual life, half of them still using daily vardenafil 5 mg.

The rate of positive margins did not differ significantly in groups, and was in all cases very low (8.2% *versus* 7.0%), prostate specific antigen biochemical recurrence was equivalent in the 2 groups (10% vs 10%). Actually, we had no death resulting from the progression of prostatic carcinoma (PCa), and biochemical progression rate, to date, with a mean follow-up of 38 months (24-84 months, for both groups) is reported in Figure 1.

Discussion

Since early eighties RRP has become the procedure of choice for the treatment of localized PCa. Anatomical studies by *Walsh et al.* (1-3) dramatically improved the outcome of the open procedure in terms of postoperative recover of continence and sexual function. Therefore, ameliorating the procedure by means of reduction of hospital stay, catheterization time, pain reduction and return has been the objective surgeons where looking for.

Laparoscopic RP was first performed on a large scale at the beginning of this century thanks to the standardization of the technique by *Vallancien* and *Guilloneau* (6.11, 12) that led to a worldwide spread of this technique. On the other hand LRP is a challenging procedure that needs a skilled laparoscopic surgeon and a well trained equipe (13). At our institution laparoscopy was began in the mid nineties with incontinence surgery (14) thus developing skilness to perform LRP. Analysis of our case-control study confirms the advantages of of LRP, in respect to open RP, almost in all aspects, except for time needed to perform the procedure, that, in any way, tends to reduce with further surgical experience.

From an oncological point of view it seems also from our series that no differences can be shown if LRP is performed correctly, following the same principles, in respect to open surgery (8, 9). Further follow-up may be needed to confirm these data.
Therefore we can conclude that laparoscopic radical prostatectomy is technically demanding, with an initially longer operative time and learning curve. The overall outcome in our series favours the laparoscopic approach regarding catheterization time, recover of continence and impotence, hospital stay, transfusion rate. The open approach is favoured for the still shorter time necessitating for the procedure. No statistical differences were observed in terms of oncological results, with a 24 months mean follow-up.
Consequently, at our institution laparoscopic radical prostatectomy is becoming the method of choice.

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CASE REPORT

Ureteral injury concomitant with kidney injury due to blunt trauma: Case report.

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Summary

A 27- year old man fell from seven meters high. A CT of abdomen and pelvis with contrast injection showed injury of right kidney, perirenal hematoma, and periureteral extravasation of contrast. Retrograde pyelography confirmed the diagnosis of partial transection of the right upper ureter. Conservative management of the case is discussed. A JJ internal ureteral stent was inserted successfully.

KEY WORDS: *Ureteral injury; Blunt trauma; Stent; Renal injury.*

Submitted 30 November 2008; 15 January 2009

INTRODUCTION

Traumatic ureteral injuries are less than 1% of all genitourinary injuries from external violence. Only 2% to 3% of gunshot wounds to the abdomen result in ureteral injury (1, 2). Blunt trauma is rarely causative and constitutes only 3.5% of traumatic ureteral injuries. Penetrating trauma is the cause of remaining 96.5% (3). Noniatrogenic ureteral injuries are uncommon because the ureter is well protected anatomically by the bony pelvis and psoas muscle. Thus, injuries to the ureters are associated with major organ injury (3). Iatrogenic ureteral injuries occurring in up to 1.1% of hysterectomies and in up to 4.7% of ureteroscopies (4).

CASE PRESENTATION

A 27-year old man was admitted after he had fallen from seven meters high. At admission he was alert, spontaneously breathing, Blood pressure was 120/70 mm Hg, and heart rate (HR) 70 bpm. Cardiopulmonary examination was normal. There was tenderness on the right upper abdomen, tenderness on the right flank without hematomas, swelling and tenderness on the right elbow. Genitalia and digital rectal examination were normal.
Laboratory evaluation demonstrated: Haemoglobin (Hb)13.1 g/dl, Hematocrit (Ht) 40%, White blood count (WBC) 20280/ul, platlets (PLT) 261000/ul, Serum Urea 34 mg/dl, Serum Creatinine 1.12 mg/dl. Urine examination showed microhematuria. Chest and pelvis X-Ray were normal. There were fractures of right radius and ulna. Computed Tomography (CT) of abdomen and pelvis with contrast injection and a kidney ureter bladder X- Ray scan showed multiple injuries of right kidney grade 4, perirenal hematoma, and periureteral extravasation of contrast (Figure 1, Figure 2). The patient was taken to the operating room and retrograde pyelography confirmed the diagnosis of partial transection of the right upper ureter close to ureteropelvic junction and extrava-

Figure 1.

CT of abdomen with contrast injection shows major right renal injury, perirenal hematoma (white arrow), and periureteral extravasation of contrast (black arrow).

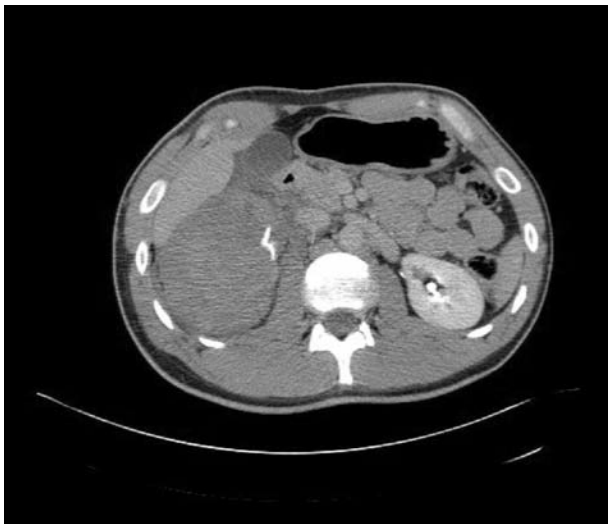


Figure 2.
KUB some minutes post the abdominal CT scan which shows contrast extravasation of the right upper ureter before internal stent insertion.

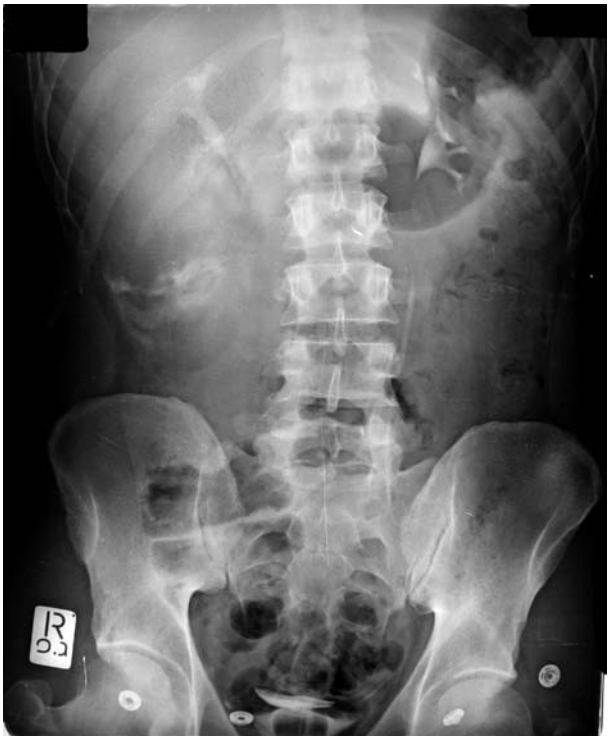
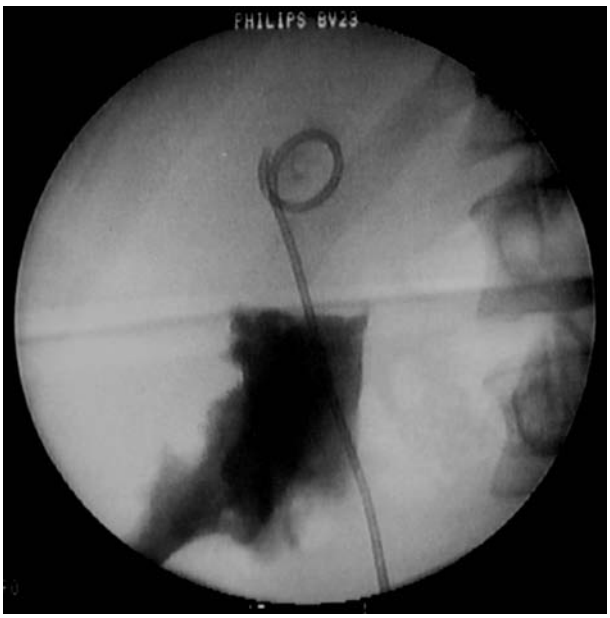
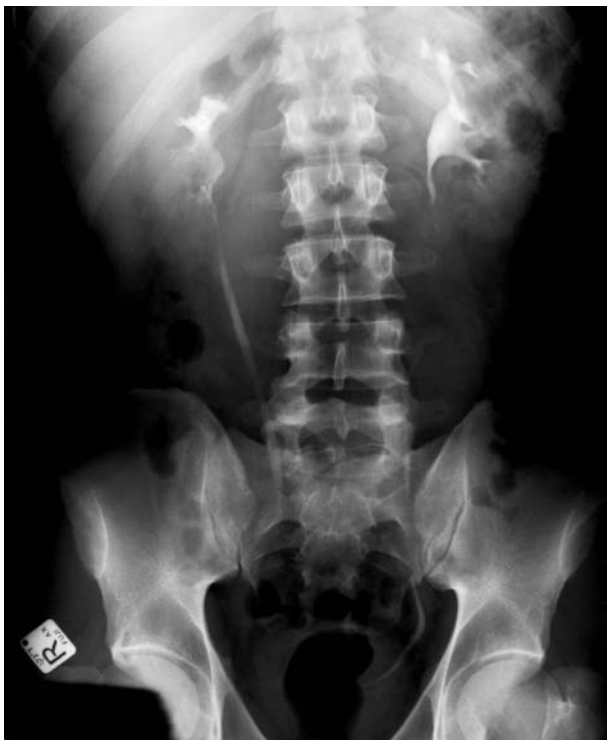


Figure 3.
Post ureteral stent insertion with the extravasation of contrast medium adjacent to the upper ureter.



sation from the lower pole of the kidney. A JJ stent 6 Fr was inserted successfully (Figure 3).
The patient was followed with bed rest, physical examination, complete blood count, renal function, electrolytes every day, and antibiotics. Two days post the admission there was a drop of Hb to 7.6 g/dl and four packed cell units were given. Hb rose to 12.2 g/dl. Another CT of abdomen with intravenous contrast injection revealed large right perirenal hematoma, stent in normal position, and normal left kidney. Twelve days after the admission the patient was operated by the orthopedic team because of the fractures of right radius and ulna.
Three weeks after the admission the patient was discharged and eight weeks later the ureteral stent was removed. In retrograde pyelography a minimal irregularity of the right upper ureter without obstruction and no extravasation of contrast medium was revealed. Intravenous pyelography (IVP) 3 months after the trauma showed no obstruction or ureteral stricture (Figure 4). The patient has not had any problems for 21 months of follow up.

Figure 4.
Intravenus pyelography twelve weeks post the injury after the ureteral stent was removed. There was no obstruction and no extravasation of contrast medium.



Discussion

Blunt ureteral injury is rare, most of the injuries were published as case reports (5-10). Bilateral ureteral rupture is extremely rare (6). Best et al reported 57 ureteral injuries in a 120 months study in which the mechanism of injury was penetrating in 96.5% and blunt in only 2 (3.5%) (11). Elliot and McAninch reported their 25 year experience with 38 traumatic ureteral injuries, blunt trauma was the cause in 5 patients (3). Avulsion of the ureteropelvic junction is by far the most common ureteral injury in children, usually occurring in those involved in motor vehicle acci-

dents or in falls from a height (12-14). Complete rupture occurs mostly 1 to 2 cm below the ureteropelvic junction, whereas only a few sites of rupture at a greater distance from the renal pelvis have been reported. Laceration without complete avulsion is far less common and predominantly involves adults (7, 14).

Diagnosis of the ureteral injuries can be difficult. Hematuria, which is the most consistent finding in renal trauma, is absent in approximately 30% of ureteral injuries. Fever, flank pain, expanding flank mass, urinoma and fistula formation, and sepsis usually confirm late diagnosis (3, 7, 13, 14). CT scan is the gold standard for staging of blunt abdominal and genitourinary trauma.

For the diagnosis of renal injury contrast enhanced CT with delayed scans and pyelography are indicated in all patients with blunt injury with macrohematuria, microhematuria with hypotension, or rapid deceleration injuries. A similar protocol can be used for ureteral injuries. Many ureteral injuries will be missed if hematuria and hypotension are absent (3). Best et al reported hematuria only in 17% of their 57 patients with ureteral injuries (11).

Therefore, to improve the detection of ureteral injuries the index of suspicion should increase to include imaging in patients with flank ecchymosis, tenderness or rapid deceleration trauma. CT is better than Intravenous pyelography (IVP) for the diagnosis of ureteral and associated injuries. IVP was positive in 65% in confirmed traumatic ureteral injuries (3).

In our case we decided to start with internal drainage and stenting instead of open exploration, repairing the renal injuries, debridement, and watertight tension free ureteroureteral anastomosis.

In conclusion, blunt ureteral injury requires a high index of clinical suspicion to establish the diagnosis. Contrast enhanced CT with delayed scans can help in the diagnosis. Conservative treatment with double J internal stent can be successful for patients with combined blunt renal and ureteral injury.

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CASE REPORT

Thirty years old man with a huge benign prostatic enlargement.

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Summary

We present a case of a thirty years old man who had suprapubic prostatectomy in our department. Patient's history started from the age of 25, when he experienced multiple urinary retention attacks. Imaging revealed an enormous prostatic mass. Combining this finding with elevated PSA values, lead us to prostatic biopsies which proved to be benign. Following our advice, the patient had children and afterwards he had his prostate removed. The suprapubic prostatectomy was extremely challenging with a lot of technical difficulties, considering that the net weight of the removed adenoma was 250gr. Pathological examination of the tissue proved that it was benign prostatic hyperplasia.

Our case is particularly interesting for two reasons: On one hand because of the unusual size of the prostate and on the other hand because of the young age of the patient. Epidemiological studies showed that prostatic hyperplasia has been pathologically proved only after the age of 40, while pathological signs of the disease could be found after the age of 30. Concerning the size of the adenoma, a search in the literature showed that only 4% of the removed glands weight more than 100 gr, and that has to do with men over 70 years of age. Concluding, our case seems to be extremely rare. Furthermore, our search through the literature could not reveal any similar case report.

KEY WORDS: *Prostatic adenoma; Benign prostatic hyperplasia; Prostatectomy.*

Submitted 2 April 2009; Accepted 30 June 2009

CASE REPORT

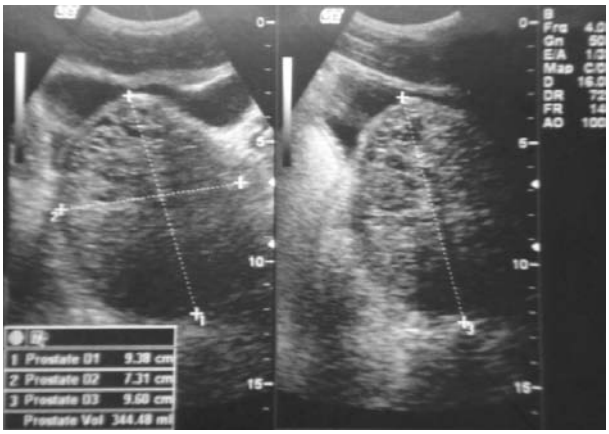
A thirty years old male was referred to our clinic because of recurrent urinary retention episodes and severe LUTS. His first urinary retention had occurred five years before (at the age of 25) when he had a knee surgery. Another couple of retention episodes took place up until three years before when LUTS had started. He complained for nocturia (1-2 times), frequency and dysuria. During the last three years, the frequency of retention had become higher, taking place every two to three months. Retention occurred after he had to postpone micturition or after consuming alcohol.

DRE revealed a huge prostate with edges that could not be reached to palpation. Normal prostatic texture was palpated with no hard nodules or any signs of malignancy. The patient's uroflowmetry had an obstructed pattern. Ultrasonography, CT scan, MRI, showed a very large, heterogeneous, well defined prostatic mass, 300ml in volume (Figure 1, 2, 3). PSA was 43, while hormonal profile of the patient was normal.

A needle biopsy was performed. 17 cores were examined and no sign of malignancy was found. BPH was the pathology diagnosis. Because of the recurrent urinary retention episodes and after a-blockers and finasteride have failed, surgery has been advised. Taking in mind the

Figure 1.

Ultrasound image of the adenoma.



risk of retrograde ejaculation, the patient postponed the surgery for two years and had two children.
Finally we performed an open suprapubic prostatectomy. The operation was extremely challenging. No post operative complications occurred and the patient had the catheter removed 8 days after surgery.
Pathology examination of the specimen measured a 250 gr prostatic adenoma (Figure 4) with 12 x 11 x 8 cm dimensions. The diagnosis was BPH again. Microscopic examination showed prevalence of glandular over stromal hyperplasia.

Figure 2.
CT scan.

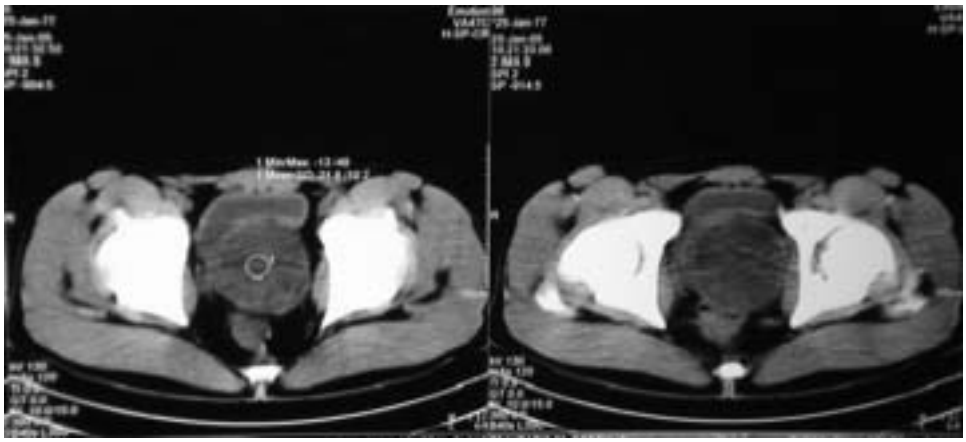


Figure 3.
MRI.

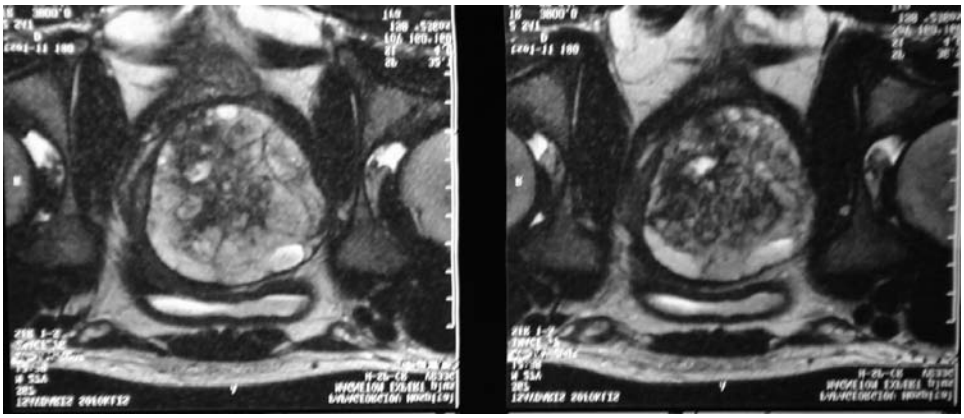
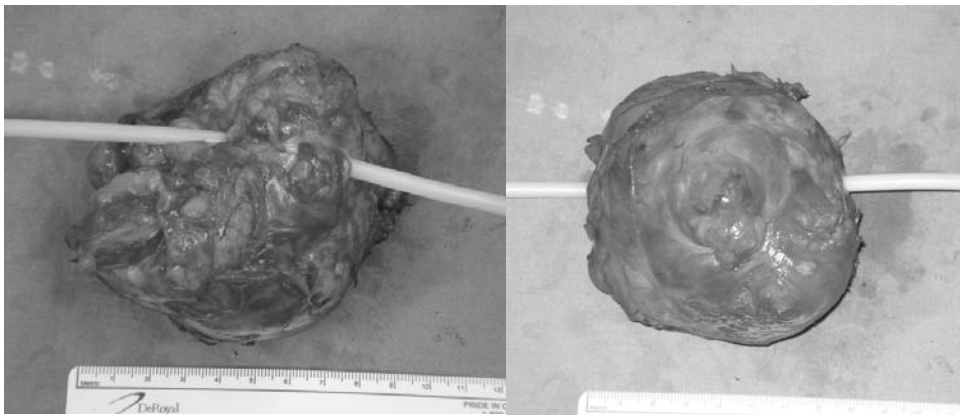


Figure 4.
The prostatic adenoma specimen.



Discussion

The natural history and development of BPH with age has always been an enigma for Urologists. A survey of the literature on the growth of human prostate and prevalence of histological recognizable benign prostatic hyperplasia identified 23 independent studies (1-23). These studies proved that normal prostate increases in size in association with age. The growth of most human prostates can be divided into rapid (subjects between 0 and 30 years old) and slow (men between 31 and > 90 years old) phases.
However, 3.7% of the prostates in men > 70 years old attain a large weight (> 100 gr) (24).
Analyzing 10 autopsy and prostatectomy studies, *Berry et al.* concludes that the first pathological sign of benign prostatic hyperplasia is seen in men between 31 and 40 years old but the prevalence is only 8%.
The amount of hyperplastic tissue removed at surgery increases with advancing age and the same review article *Berry et al.* concludes that "the initiation of growth of BPH probably begins in men < 30 years old. With the small doubling time of the human tumor the first appearance of a pathological nodule would not be noted for an additional 10 to 20 years."
Concerning the development of BPH with age Oesterling agrees that the initial development of BPH begins as early as 25 to 30 years of age. Furthermore, the time required for macroscopic BPH to develop from microscopic BPH

is approximately 5 years. The age specific prevalence of macroscopic BPH, as detected by DRE, increases with age. Concerning the large prostates, Oesterling found that among men who were older than 70 years, 3.5% had a prostate that weighted more than 100 gr. (25)
According to these studies, macroscopic BPH could not be found earlier than the age of 30. On the contrary, our case presents a huge prostatic enlargement found and digitally palpated on a 28 years old man.
At the age of 30 our patient had prostatectomy and the specimen weighted 250 gr. This size of prostate is very rare even between men > 70 years old.
The meticulous examination of the specimen proved only typical BPH pathology. Taking also in mind that the patient had no other diseases, no family history and his hormonal status was normal, BPH is the only clinical diagnosis that could be made for this young man.
As a conclusion, our case is extremely rare for two reasons: first because of the young age of the patient, and the second because of the very large size of the prostate. No similar case could be found through the literature.

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CASE REPORT

Metastasis to the renal hilum from malignant melanoma of the anterior trunk: An unusual finding.

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Summary

A retroperitoneal metastasis from malignant melanoma is an uncommon event and mostly secondary to a primary lesion of the posterior trunk. We report on a 38-year-old patient with malignant melanoma of the anterior trunk who presented a symptomatic metastatic mass of the left renal hilum not originating from the retroperitoneal lymph nodes of the renal hilum, surrounding and infiltrating the renal pelvis, treated with left nephrectomy, complete mass excision and regional lymph node dissection. The patient later developed also brain metastases and is now undergoing immunotherapy.

KEY WORDS: Skin melanoma; Metastasis; Retroperitoneum; Lymphatic drainage.

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INTRODUCTION

Clinically evident metastatic melanoma to the retroperitoneum is an uncommon event. Up to 50% of patients with a history of melanoma have multiple renal sub cortical micro metastases on autopsy (1). A review of the published papers showed several previous cases of solitary metastatic melanoma renal lesions (2) but only few reports were published about retroperitoneal metastasis from melanoma of the posterior trunk (3-4).
We report the case of a metastasis from malignant melanoma of the anterior trunk surrounding the left renal pelvis not originating from the retroperitoneal lymph nodes of the renal hilum. To Our knowledge, the location of this metastastatic disease has never been previously described.

CASE REPORT

A 38-year-old man first presented a malignant melanoma of the anterior trunk in the sternal area in August 2006. The patient underwent a wide local excision with an overdraw resection after 2 months. In December 2006 he underwent the left sentinel axillaries lymph node dissection that resulted positive for metastatic melanoma at histopathological examination. Subsequently, the patient underwent immunotherapy with interferon-alfa.
In December 2007 he underwent a new left axillaries lymph node dissection for a positive PET-TC but the histopathological examination was negative for metastatic melanoma.
In February 2008 the patient presented left lumbar pain, fever, without hematuria. The physical examination findings were unremarkable. The laboratory data from the blood and urine samples were within normal limits except for a mild anaemia. Urinary cytology was negative for neoplastic cells.
The radiologic workup with computed tomography (CT) disclosed a solid-inhomogeneous mass of 3 x 6 cm that completely filled the left renal hilum and partially occluded the renal pelvis. It determined an increase of density of the adipose perirenal tissue that catch up the wrap renal front (Figure 1).
Subsequently, the patient underwent left nephrectomy, complete excision of the mass surrounding the renal pelvis and a local lymph node dissection through a lombotomic approach. The surgeon described the presence of a neoformed solid weaving that encircled and seemed to infiltrate the left renal pelvis reaching the parietal peritoneum.
The patient's postoperative course was uncomplicated and he was discharged home on postoperative day 5.
The final pathologic examination demonstrated a 6 cm tumour, occupying the renal hilum. The mass was

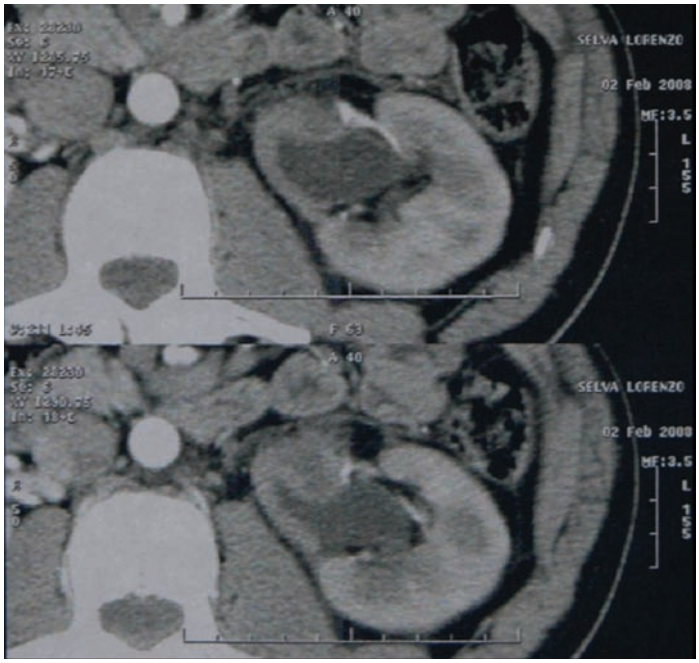

Figure 1.
Abdominal computed tomography (CT) showing a solid mass that completely filled the left renal hilum and partially occluded the renal pelvis.

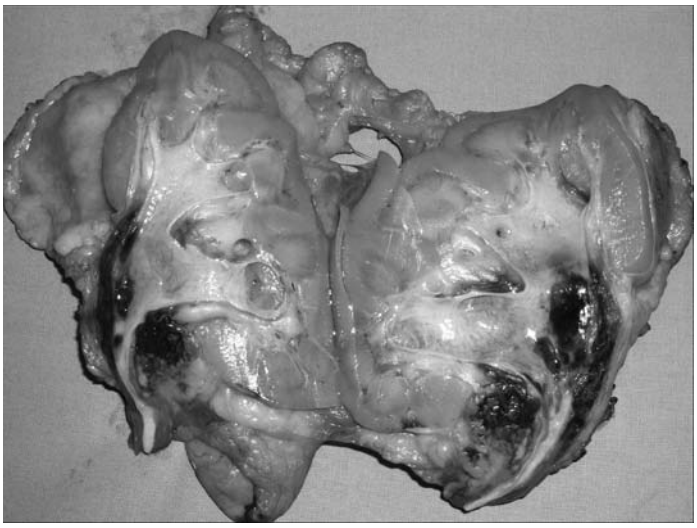

Figure 2.
Macroscopic appearance of the tumor encompassing the renal hilum.

multinodular and heterogeneously pigmented (Figure 2). Microscopic examination showed an ilar tumour that appeared to involve the peri-ilar adipose woven, the ureteral wall, the renal parenchyma and the pelvic wall with involvement in such zone of the muscular woven and the connecting tissue but with partial conservation of the transitional tissue. The tumour showed a pattern of solid growth and alveolar, with cancer elements with pleomorf nuclei, constantly nucleated, and eosinophils that sometimes included a large cytoplasm.

Immunohistochemical staining with positive melanoma antigen recognized by S-100, Melan-A, HMB-45 and negative for cytokeratin, confirmed the diagnosis of malignant melanoma (Figure 3). The surgical margins were negative. The tumor did not show characteristics that could lead back to a lymph node origin and, moreover, the ilar lymph nodes dissected were all free of tumor.
At three months follow-up, radiologic evaluation showed brain tumor metastases and the patient is now undergoing a new course of immunotherapy.

Comment

We report a case of a metastasis from a malignant melanoma of the anterior trunk involving the left renal hilum, surrounding the renal pelvis not originating from the retroperitoneal lymph nodes of the renal hilum.
Melanoma with any visceral metastasis confers a poor prognosis: 1-year and 2-year survival are approximately 50% and 25% respectively (5). The optimal treatment for melanoma is surgical resection. Many adjuvant therapies are available, including immunotherapy such as interferon-alpha and IL-2 and chemotherapy such as dacarbazine and fotemustine (6).
Immunotherapy and/or chemotherapy are the current treatment options for melanoma with retroperitoneal lesions or unresectable lesions.
Retroperitoneal metastases of malignant melanoma are rare findings. Abeshouse et al. reviewed 142 cases of melanoma in the genitourinary tract, including 56 primary and 86 secondary melanomas. Eighty percent of melanoma metastases to the kidney had the primary lesion arising from the skin (7).
Uren et al. observed many unusual lymphatic drainages pathways, including direct drainage through the body wall to retroperitoneal and paravertebral lymph nodes from the skin of the back (3). The Author found this type of lymphatic drainage occuring in 14 out of 542 patients (3).
Subsequently, *Roger et al.* demostrated that only lymphatic drainage for malignant melanomas of the posterior trunk can involve a direct passage through the posterior body wall into paravertebral, para-aortic or retroperitoneal areas, while melanomas of anterior trunk metastasize to axillaries nodes, clavicle or neck nodes using lymphatic drainage (4).
From a review of the literature, to Our knowledge, the location of this metastastatic disease has never been previously described. The presented case is a peculiar metastasis of the renal hilum from an anterior trunk malignant melanoma that is hardly explainable by the lymphatic drainage pathways widely described (3, 4) also because of the absence of histopathological features distinctive of a lymph node origin and the presence of multiple ilar lymph nodes dissected free of tumor.
A review of the literature demonstrated that metastasectomy followed by a new adjuvant treatment is now the

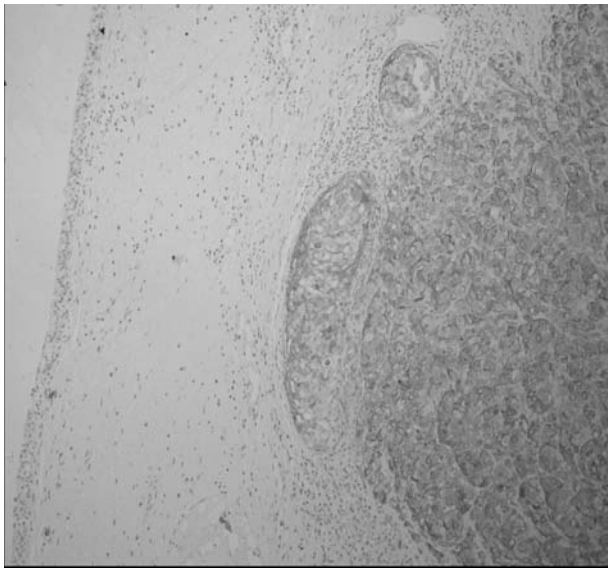

Figure 3.
Microscopic appearance (original magnification x 400) of the tumor: Immunohistochemical staining for MELAN-A.

ideal therapeutic approach for patients with singular or multiple distant metastases of melanoma (stage IV) and it can extend 5-year survival (8). Morton et al. showed with an international randomized, double-bind, phase 3 study, that the best therapeutic approach for patients with stage IV disease is not systemic chemotherapy or biological treatments but rather a complete metastasectomy if technically feasible (9).
In Our case the unusual and single localization of the mass at diagnosis could appear suspicious for a primary tumour. A percutaneus biopsy might be warranted to evaluate cases of suspected secondary metastatic lesions but in Our case resulted technically difficult for the tumor location in the renal hilum. Moreover, the results of a biopsy would not have affected the decision for nefrectomy because the patient was symptomatic, the mass was solitary and the brain metastases were not evident when the retroperitoneal mass was diagnosed.

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ORIGINAL PAPER

Interstitial cystitis with plasma cell bladder infiltration: Case report and literature review.

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Summary

We report the case of a 76ys-old woman with overactive bladder syndrome, determined by an histological exam of interstitial cystitis with plasma cell infiltration. To the best of our knowledge, in literature only a similar case has been described. The patient has been treated with corticosteroid therapy allowing a transitory benefit; despite this fact, after side effects have been shown, this therapy has been interrupted leading to the worsening of the previous sintomatology. Therefore the patient has undergone to radical cystectomy with orthotopic ileal neobladder. The phlogistic infiltration of the bladder wall is represented by the plasma cells for over 90% of the whole population. In addition, blood specimen was positive for perinuclear antineutrophil cytoplasmic antibodies (p-ANCA). All these elements could hint at a chronic cystitis due to autoimmune aetiology.

KEY WORDS: Plasma cell; Interstitial cystitis.

Submitted 23 February 2009; Accepted 20 April 2009

INTRODUCTION

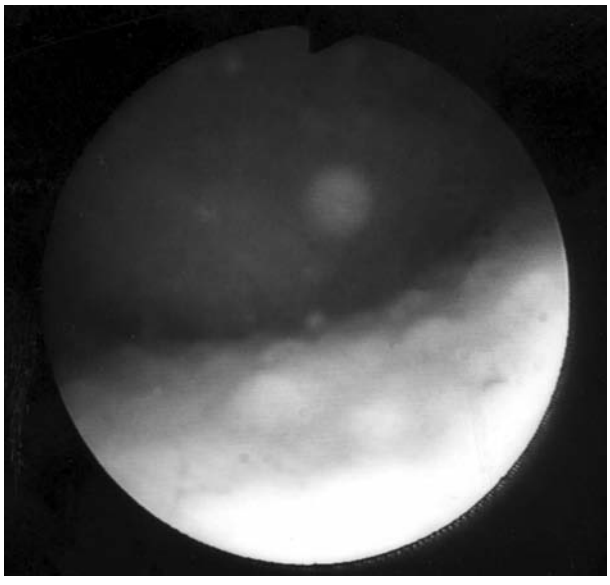
The presence of plasma cells in the bladder mucosa is frequent and observable in anatomo-pathological specimens of patients with any kind of chronic cystitis. We report the case of a patient with severe low urinary tract symptoms due to a chronic cystitis with massive plasmacells infiltration in the bladder mucosa.
Even if the patient showed clinical and pathologycal evidences in agreement with interstitial and eosinophilic cystitis, anatomo-patological response of a predominant plasmacellular infiltration of the bladder suggested a very rare nosological entity, described in literature only once so far (1).

CASE REPORT

A 76ys-old woman showed an overactive bladder syndrome getting worse and worse. The patient had been previously hit by two episodes of bacteric cystitis. After the second episode of cystitis, the storage symptoms only partially regressed, worsening again in the following weeks, despite the urine culture was negative.
Physical examination was negative. A cystoscopy showed a spread oedema of the bladder mucosa with a lot of fibrin in suspension. A biopsy of bladder mucosal revealed inflammation characterized by plasma cells infiltration which interested the deep layers of corion.
After that it was performed a systematic cold cup histologic mapping under general anaesthesia and the histological report confirmed the previously diagnosis with massive plasmacells infiltration of the bladder mucosa (Figure 1).

Figure 1.

Flexible cystoscopy: The mucosal surface of the bladder is diffusely edematous and erythematous.



No antinuclear antibodies were found in the blood while widely positive for p-ANCA. The citofluorometry determination of the lymphocytic under populations in the peripheral blood did not highlight significant alterations. The cytofluorometric immunophenotypical analysis on the urine leucocytes revealed a notable presence of CD13+ and CD16+ leucocytes. The plasma levels of C-protein and of beta 2 microglobulin turned out respectively increased by 30% and by 10% regarding the normal range. All the other inspections were normal.
She was given an oral therapy with Prednisone 25 mg/die for 15 days and a progressive reduction of the dosage for a month until 5 mg/die open-ended, with a weekly single dose of Fosfomycin (2-4). The urinary symptoms significantly decreased after two weeks from the beginning of the corticosteroid therapy with almost complete regression after one month.
After 60 days from the beginning of the corticosteroid therapy the blood analysis showed a normalization of the levels of C protein and a p-ANCA decrease; the values of beta 2 microglobulin remained steady.
After 4 months the storage symptoms reappeared.
The renewal of the storage urinary symptoms led the patient to assume increasing dosages of Prednisone without any therapeutic results. The patient experienced a pathological rise of glycemia and arterial pressure and besides a state of worsening osteoporosis. The patient went through a very bad quality of life with intervals between micturitions of about 15-20 minutes and stopped her public relations. In the hypothesis of an autoimmune disease of the bladder the patient was submitted to an empiric treatment with Cyclosporin (5-8), without results.
The patient was offered a cistectomy with a ortotoptic ileal neobladder. Histopathologic examination of the specimen confirmed the massive plasmacells infiltration of the bladder mucosa (Figures 2 and 3).

Discussion

Chronic cystitis with sterile urines in its several anatomopathological kinds (interstitial cystitis, eosinophilic cystitis, cistic cystitis) presents with the same symptoms of acute bacterial cystitis, but lasting in time: frequency, urgency, painful micturition, sometimes macrocospic haematuria, until the clinical manifestation of a pain pelvic syndrome. The chronic cystitis is an underhand disturb and it is often hard to accurately diagnose.
The presence of plasma cells in the bladder wall is frequent in chronic interstitial cystitis.
In our case report, the phlogistic infiltration of the bladder wall was characterized by one single cellular member, the plasma cells, for over 90% of the whole population. The discover of a policlonal phenotype for chains kappa and lamda in the histological examination allowed to exclude the possibility of a plasmocitoma infiltrating the bladder. According to our knowledge, in literature only a single case of plasma cells infiltration of the bladder mucosa has been previously described (1) but the Authors did not report if corticosteroidal therapy mantained the positive effects with time.
The ANCA are antibodies whose location plays an impor-

Figure 2.
Plasma cells predominance of the mucosal infiltrate is evident (E.E., 200 x).

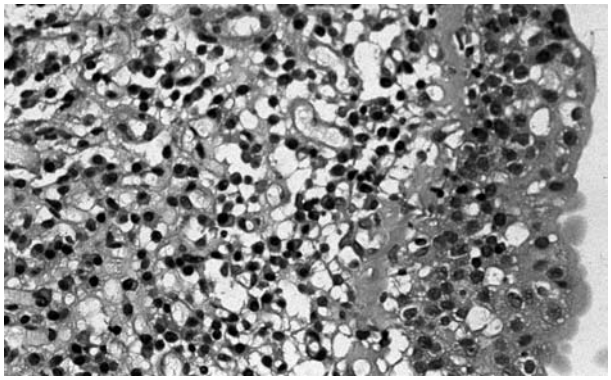


Figure 3.
Gross appearance of the bladder mucosa.

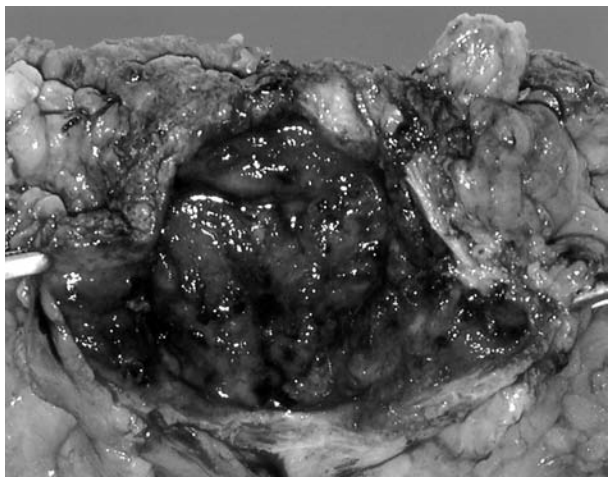
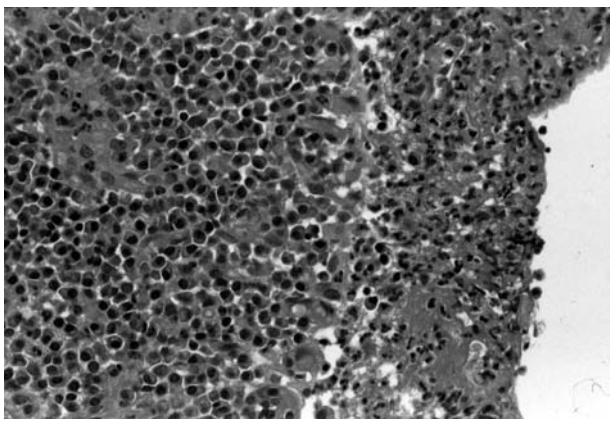


Figure 4.
The urothelium is denuded and lamina propria is extensively infiltrated by plasma cells. The phlogistic infiltration of the bladder wall was characterized by one single cellular member, the plasma cells, for over 90% of the whole population (E.E., 200 x).



tant diagnostic role in some sistemic vasculitises. An increased level of beta 2 microglobulin seems to be less relevant. Beta 2 microglobulin is one small molecule present in the serum, in the urines and in the cerebrospinal liquid. It is made of the antigens of HLA and it is present in high concentration on the surface of some cells of the immune system. Although it is also used as a marker for some tumours, it is worth to remember that increased values are not a clear evidence of neoplasia. In fact, its concentration may increase in a wide range of affections, among which the autoimmune sistemic diseases.

During the period of action of the corticosteroidal therapy, besides a meaningful regression of the urinary symptoms, we noticed the improvement of the cystoscopic features state and the reduction of the levels of p-ANCA. All these elements could hint at a chronic cystitis due to autoimmune aetiology, even if we haven't been technically able to characterize specific antibodies anti-bladder mucosa.

In addition, a very recent study (9) explored the specific autoimmune mechanism of urinary bladder developing a new animal model. Using a novel line of transgenic mice, *Wujiang et al.* were the first to demonstrate that the bladder epithelium actively presents self-Ag to the immune system and induces autoimmune response.

Therefore, it is likely that the cystitis with plasmocells infiltration is less rare than one can think when basing on the only two reported cases in literature: the spreading of the clinical practise of systematic cold cup histologic mapping of bladders with chronical cystitis could reveal a greater number of cases suffering from this clinical entity. From a therapeutic point of view, an extended treatment with corticosteroids seems not feasible for the side effects and the transient state of the benefits. The therapy with Cyclosporin could turn out effective, even if in our case it has not been possible to determine because of patient's refusal. Despite a total cystectomy with urinary diversion is an aggressive therapeutic option, it has undoubtly solved the symptoms of our patient who has regained a good quality of life and up to now she is greatly satisfied of her choice.

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ORIGINAL PAPER

Lidocaine spray administration during transrectal ultrasound guided prostate biopsy modified the discomfort and pain of the procedure: Results of a randomized clinical trial.

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Summary

Objectives: *We report the results of a study about the possible benefit of lidocaine spray perineal administration before transrectal ultrasound guided biopsy of the prostate. Many patients frequently report some kind of discomfort and (or) pain during this procedure, that when pain is severe, may be necessary to interrupt.*

Materials and Methods: *Between September 2007 and October 2009 372 consecutive male patients with elevate PSA and (or) abnormal digital rectal and (or) suspect TRUS were scheduled for prostate biopsy and divided in 3 groups. Group 1 (n = 98) underwent intrarectal instillation of a lidocaine/prilocaine cream (EMLA R), Group 2 (n = 126) of a 2,5% lidocaine gel, and Group 3 (n = 148) administration of a lidocaine spray (10 gr/100 ml) before the procedure. A verbal numerical pain score (VNS) from = 0 no discomfort to 10 = severe pain was admnistrated to the biopsied patients who were asked to evaluate separately the degree of pain associated with the insertion of the probe and the manoeuvres associated with it and the degree of pain associated with the biopsy.*

Results: *The mean value of pain VNS in patients of the first group was respectively 5.3 (2-8) for the insertion of the probe (first question) and 3.2 (2-7) for the biopsy by itself (second question), whereas in the second group it was 6.2 (4-9) and 3.8 (3-8), and in the third group 3.1 (1-6) and 2.8 (0-6).*

Conclusions: *Pain score results showed that the use of intrarectal lidocaine spray provided significantly better pain control than cream and anaesthetic gel. Our pain score data suggests that lidocaine spray provides efficient patient comfort during prostate biopsy by reducing pain both during probe insertion and insertion of the needle through the prostate gland.*

The use of lidocaine spray makes an excellent alternative, causing a reduction of anal sphincter tone with better patient compliance and tolerability to the ultrasound probe during biopsies with an optimization in terms of cost-effectiveness of the procedure.

KEY WORDS: *Prostate biopsy; Local anesthetic; Prostate innervation.*

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INTRODUCTION

Over the past twenty years, transrectal ultrasound has greatly simplified the procedure of prostate biopsy (BP), while increasing its accuracy and sensitivity. Transrectal ultrasound guided BP is now a routine outpatient procedure that is easy to learn and quick and simple to perform, but still associated to side effects including pain and discomfort.

Over 70% of patients feel pain and discomfort during the procedure that makes it difficult to continue and sometimes cause to stop procedure (1).

BP is today the only mean of obtaining an early diagnosis of prostate cancer in order to cure the disease. The efficacy of this procedure is dependent on the number of cores obtained, hence the need to make it as comfortable as possible for the patient, mantaining it outpatient and low cost.

The pain associated with BP originates mainly in the pseudo-capsule or stroma of the peripheral zone that is richly innervated by fibers from the S2-S5 spinal cord and from the ortho-sympathetic chain, through the postero-lateral neuro-vascular pedicles.

On the contrary, the anterior fibro-muscular prostate, is not significantly innervated (2, 3).

Currently, the techniques most frequently proposed for local anesthesia during transrectal BP appear to be the local application of anesthetic gel and the periprostatic injection of lidocaine (4).
The rationale for the use of intrarectal application of anesthetic gel is related to the high capacity of absorption of drugs through the rectal mucosa and the presence of a rich innervation of the prostate in the space between rectum and prostate.
Periprostatic infiltration of anesthetic before BP may be directed to the space between prostate and seminal vescicles or at the prostatic apex (5).
The optimal schedule of injections is difficult to define such as the ideal dose of anesthetic to be injected, although the vast majority of authors use 5 or 10 ml of lidocaine (5, 6).
In our view, an important factor of the state of discomfort during the procedure is the tone of the anal sphincter and the presence of the ultrasound probe that increases pressure and causes stretching of muscle fibers and sensory nerve fibers.
The pain caused by biopsy itself contributes less significantly to the state of discomfort of the patient.
The presence of anal stenosis, haemorrhoidal prolapse or anal fissures increases the discomfort of the procedure.
In this study we aimed to assess the efficacy of a new method of application of local anesthetic during prostate biopsy using lidocaine spray in comparison with customary method with intrarectal lidocaine gel and lidocaine/prilocaine (EMLA R) anesthetic cream (7, 9, 15).

Material and methods

The criteria for prostate biopsy and patient enrolment in the study were:
– abnormal digital rectal examination;
– PSA > 4ng/ml;
– significant increase of PSA over time (PSA velocity > 0.75 ng/ml/year);
– PSA 2-4 ng/ml (patients with family history of prostate cancer and PSA ratio < 10%);
– hypoechoic lesions at prostatic transrectal ultrasound (TRUS);

Were excluded from the study patients with:
– PSA > 100 ng/ml with suspect digital rectal examination;
– radiological or scintigraphic evidence of metastases;
– positive histology after TURP;
– reduced life expectancy;
– concomitant severe pathologies.
Between September '07 and October '09 we selected 372 patients undergoing transrectal ultrasound guided prostate biopsy. For this procedure a G.E. ultrasound (LOGIQ 7) with "end-fire" multi-frequency convex probe and 18 Gauge tru-cut needle were used.
Biopsies were performed alternately by two operators with experience Rectal enema and antibiotic prophylaxis were administered to prevent infectious sequelae (11, 12) and patients were asked to empty the bladder prior to the procedure, because in our opinion the bladder repletion is an important element of discomfort during BP. Before the application of anesthetic a thorough disinfection of the skin of the perineum was carried out with the use of common solutions of povidone iodine or chlorexidine (10, 11).
The patients were divided into 3 groups.
Group 1 (n = 98) underwent intrarectal instillation of a 25 mg lidocaine/ 25 mg prilocaine cream (EMLA R) applied 5 minutes before the procedure, Group 2 (n = 126) was treated with 2,5% lidocaine gel applied 5 minutes before the procedure, and Group 3 (n = 148) was treated with the administration of a lidocaine spray (10gr/100ml) applied 2 minutes before the BP.
The first intention was to obtain 14 TRUS-guided cores in all the patients.
Pain was self evaluated by the patiens with the use of a simple rating scale of pain called Verbal Numerical Scale (VNS). This is a scale easily understood by the patient who chooses a number between 0 (no pain) to 10 (the greatest pain imaginable) to represent the level of pain. Immediately after the end of the procedure two scales VNS of pain were separately administered to measure the pain due to the insertion of the ultrasound probe and its movement during the procedure and the pain caused by biopsy itself.

Results

Only in 4 patiens we were unable to insert TRUS probe for the presence of fibrous anal in 3 and for severe haemorrhoidal prolapse in another.
The mean age of patients in the group treated with EMLA cream was 67 years (range 52-75), the value of the PSA 8.2 (range 4.5-12.2), total prostate volume 54 ml (36-102).
In the second group treated with use of 2.5% lidocaine gel the mean age was of 70 years (range 53-76), the value of the PSA 7.1 (range 2.5-14.2), total prostate volume 49 ml (range 32-120).
In the third group treated with use of lidocaine spray 10 gr/10 ml the mean age was 69 years (range 48-74), the value of the PSA was 9.2 (range 3.6-17.2), total prostate volume 56 ml (range 28-96).
We performed a mean of 11.2 (range 7-16), 10.8 (range 5-14), 12.4 (range 6-16) biopsies, respectively for first, second and third group.
The mean pain in the visual numerical scales in patients in the first group was respectively 5.3 (2-8) in the first questionnaire and 3.2 (2-7) in the second questionnaire, in the second group was of 6.2 (4-9) and 3.8 (3-8), in the third group was of 3.1 (1-6) and 2.8 (0-6).
A statistically significant difference was observed in the tolerability of the procedure according to the first questionnaire, not to the second questionnaire ($p < 0.001$).
Subjects aged > 70 years in all three groups of patients tolerated the procedure better according to both questionnaires (average pain VNS was respectively 2.8 and 1.9).
In the elderly there is a reduced perception of pain that can be related to several factors such as the decrease in the number of nociceptors and nociceptive afferents responsible for the threshold and tolerance of pain (8).

Discussion

Our study reveals that the use of lidocaine spray in the procedure of transrectal BP is a technique easier, cheaper and more effective than the traditional use of the anesthetic gel and the anesthetic cream EMLA.

Currently, the techniques most frequently proposed for local anesthesia during transrectal BP appear to be the application of gel or an injection of lidocaine around the prostate.

Since our study suggests that the main element of discomfort for the patient during the procedure results from the introduction of the ultrasound probe and its movements during the procedure than by the biopsy itself, we discarded the option of periprostatic injection of anesthetic.

This new technique of anesthetic application by lidocaine spray proved to be an excellent alternative to those currently practiced by most urologists, owing to the reduction of the anal sphincter tone with better patient compliance and tolerability to the ultrasound probe during the procedure and with optimization in terms of cost-effectiveness of the procedure.

A single spray dispenser of lidocaine (10 gr/100 ml) allows to handle a number of patients equal to that treated wth a double pack of 2.5% lidocaine gel and five times more than using a single pack of prilocaine/lidocaine cream.

Furthermore spray anesthetic administration reduces the time of the procedure of 2-5 minutes in comparison to the other two methods.

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ORIGINAL PAPER

Extracorporeal shock wave therapy in the treatment of Peyronie's disease: Long term results.

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Summary

Purpose: *Controversial data on ESWT (Extracorporeal Shock Wave Therapy) for the treatment of Peyronie's disease are controversial. This study was performed to access the efficacy, reliability and the side-effects of the ESWT.*

Materials and Methods: *From 2000 to 2004, 157 patients with an average age of 58 years and with Peyronie's disease were enrolled for a conservative treatment. All 150 eligible patients were treated with ESWT, using Dornier Compact Delta II UIMS® lithotripter. The median number of treatments per patient was 3.5 with the delivery of 2000 shock waves (SW) for each treatment. There was no use of anaesthesia and analgesy. An ultrasound study was made for each patient before treatment. We considered: plaque size, penis curvature, pain, penis rigidity and tumescence, sexual intercourse ability and side-effects. Median follow-up of the study was 36.9 months.*

Results: *Average duration of the treatment was 20 minutes without relevant side-effects. With reference to the curvature, we obtained a significative reduction in 33.3% of the patients, whereas the plaque size was not statistically reduced. Regarding the pain issue we achieved good results with a reduction in more than 90% of the patients and a complete relief in 6%. The quality of the intercourse was reported slightly enhanced. No significant difference was observed in penis tumescence and rigidity.*

Conclusions: *ESWT is a non-invasive treatment for the Peyronie's disease. Our study confirms that the best results are obtained regarding pain and less with the curvature. For the plaque size and quality of sexual intercourse the results are not satisfactory.*

KEY WORDS: *ESWT (Extracorporeal Shock Wave Therapy); Peyronie's disease; Penile recurvatum.*

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INTRODUCTION

Peyronie's disease is a connective tissue disorder and is also the most frequent cause of penile curvature, a distressing and sexually disabling disease; it tipically regards middle aged men. The tunica albuginea and the erectile tissue, that is adjacent, are affected by localized plaques. Early inflammatory stages show thickening of the tunica, while later on fibrotic, often calcified plaque is typical (1). Two phases are usually reported, one acute and one chronic. The acute phase involves an inflammation that can lead to pain experienced from the patient either in the soft (flaccid) state or during erection. The chronic phase is when the scar develops and penile deformity occurs, pain during this phase is usually limited to the erect state and doesn't respond to pharmacological treatments. In many cases, the pain will gradually settle down and disappear without treatment in a few months, but the bend in the penis may remain a problem, making sexual intercourse difficult. The predominantly dorsal deviation that is the main symptom can be combined with erectile dysfunction (2). The etiophathology of the disease remains unclear and a study has suggested a prevalence rate as high as 8.9% (3). Despite the innovative investigations in cell culture with identification of potential biomarkers (4), no causal therapy is yet available. Surgical procedures that are the standard choice of treatment in cases of severe sympomatic angulation (5, 6), include: plication procedures (attempt to straighten the penis by trying to shorten the longer side to match the shorter side), excision-grafting procedures (incision of the scar in the tunica albuginea and straightening of the penis followed by reconstruction of the resultant defect using a variety of graft material like vein, buccal mucosa, preputial skin, pericardial sac, small intestinal submucosa). However, these procedures have significant risks, such as penile shortening, reduced sensation, impo-

tence, new fibrotic reaction (7). There is no clear indication as to when semi-invasive and surgical procedures have to be initiated. It seems to be generally accepted that conservative therapy is required in the earlier inflammatory, painful stages (8). A wide range of conservative therapies have been proposed, including radiation, oral drugs such as Potaba, tamoxifen, colchicine and vitamin E and intralesional injections of verapamil and interferon. Extracorporeal shock wave therapy (ESWT) has been used since the 1980s and was introduced by Bellorofonte and associates (9) as a conservative option. The action mechanism of ESWT is unclear, although an improvement in vascularization with consecutive resorption of calcification has been discussed as one possible mechanism (10). Another possible action could be the creation of contralateral scarring of the penis resulting in "false" straightening (11). In connection to non-calcified diseases, a change in the milieu of the free radicals or a direct disturbance of the pain receptors could be a reason for the pain-relieving effect (12, 13). There are many studies regarding the Peyronie's disease treatment with ESWT (14, 21-26) (Table 5), and the authors report an improvement in symptoms of pain, deviation and sexual intercourse; the studies are different for the type of lithotripter, number of settings, number of impulses and the energy rate thus demonstrating that ESWT is still not standardized and must be considered as an interesting, but clinically experimental, form of therapy (14).
We evaluated, as objective of our study, changes in plaque size, pain relief, enhancement of curvature of the penis, restoration of normal sexual function, penis rigidity and tumescence and side-effects.

Material and methods

Patients. From January 2000 to September 2004 157 patients with Peyronie's disease and with an average age of 58 years (range 24-84 years), were treated by ESWT and were enlisted for a prospective design study. All the patients' characteristics are listed in Table 1. Of 150 eligible patients for the study, all with a plaque (calcified or not) and the deviation of the penis, none was given any adjuvant oral therapy, before and after the treatment, nor phosphodiesterase type 5 inhibitor and prostaglandine E1.
We obtained from each patient a signed consent form and all patients were informed with all the details about the therapeutic options for the disease, furthermore we asked every one a detailed sexual and medical detailed history. Of the initial group of 157 patients, 7 refused to give informed consent for the study. The majority of patients who gave the consent desired to avoid the surgical invasive procedures.

ESWT technique.
ESWT was performed with an ultrasound guidance using a Dornier Compact Delta II UIMS® lithotripter (*Dornier MedTec Europe GmbH, Wessling Germany*) delivering 2000 shock waves per treatment session, with an emission frequency of 120 shocks/min.
ESWT was started with a low energy flow density and the power was increased step by step according to the patient tolerance level, usually up to 0.16 mJ/mm^2. Even if the majority of the studies used 3000 SW for each application, we used a scheme of 2000 SW because in our initial experience, applying more shock-waves, we have had more side-effects presented by severe urethral bleeding and extended skin haematoma.
Initially we treated 20 patients with 3000 SW and everyone, eveyone presented skin haematoma and in 12 it was extended. In 9 patients we observed also urethral bleeding.
The average duration of the treatment was 20 minutes. If improvement in symptoms but no complete straighten-

Table 1.
Patients characteristic.

Patients, n	150	
Average age, years	58	(24-84)
Duration of disease, months	3.3	(1-6)
Average plaque size, mm^2	299.9	(50-508)
Calcification	60/150	(40%)
Patients with deviation	150/150	(100%)
Average deviation, degree	49.9	(15-88)
Suffering pain	78/150	(52%)
Quality of sexual intercourse	Table 3	

Table 2.
Plaque size and deviation before and after ESWT.

	Before treatment		After treatment		p value
Plaque size, mm^2	299.9	(50-508)	295.0	(10-555)	0.0854
Curvature, degree	49.9	(15-88)	25.2	(9.6-40.8)	0.044

Table 3.
Before and after ESWT.

	Sexual intercourse before treatment		Sexual intercourse after treatment	
Possible	15	(10%)	36	(24%)
Moderately restricted	103	(68.6%)	94	(62.6%)
Severely restricted	24	(16%)	12	(8%)
Impossible	8	(5.3%)	8	(5.3%)

Table 4.
Side effects.

	Side effects
Patients, n	150
Total side-effects	33 (22%)
Superficial skin haematoma	30 (20%)
Urethral bleeding	3 (2%)
Moderate local pain during treat.	69 (43%)
Strong local pain during treat.	6 (4%)

ing was obtained and also if no effect was seen, the treatment was repeated after 2 months. The treatment was halted to those patients that suffered strong pain during treatment and no more treatments were performed to those reporting side-effects like penis skin haematoma and urethral bleeding. The median number of treatment per patient was 3.5 (range 1-9). We didn't use any kind of anaesthesia and analgesia. Patients were arranged in a reclining position with a supportive stand to stabilize the penis and avoid injury to the testes. To obtain a precision delivery of the shock waves we localized the plaque using a 7.5-MHz inline linear ultrasound transducer. The ultrasound drill permitted also the evaluation of the calcification of the plaque, as an hyperechogenic image.
The median follow-up of the study was 36.9 months (range 33-41 months).

Standardized diagnostic evaluation.
The basal data before treatment of all patients are given in table 1.
The plaque size was obtained as a product of length and width using for the measurement a measuring tape and was evaluated before and after treatment. The curvature (degree), calculated before and after treatment, was measured using a goniometer and by photo documentation after artificial induction of erection using a vacuum device. Pain during erection was, instead, evaluated using a visual analog scale (VAS 0-5). Finally we asked all the patients a personal feedback regarding the frequency, the quality and the duration of the erection according to the Bahren and Stief (15) score system: E1 little tumescence, no rigidity, E2 moderate tumescence, no rigidity, E3 full tumescence, no rigidity, E4 full tumescence, moderate rigidity, E5 full tumescence, full rigidity. The quality of intercourses was evaluated using a score system by asking all patients if the coitus was possible, moderately restricted, severely restricted or impossible. The questionnaire was based on three main parameters that describe sexual intercourses difficulty: rigidity deficit, altered penile curvature and pain.
We finally evaluated the complication following the treatment.
Statistical analyses.
Statistical analyses were performed using a GraphPad InStant system, version 3.05 (*San Diego, CA USA*). P values were calculated and were considered significant with a $p < 0.05$.

Results

Side-effects. We didn't observe any relevant side effect during and after ESWT treatment. Of 150 evaluable patients only 33 (22%) reported side effects. In particular 30 (20%) had a superficial skin haematoma and 3 (2%) had a slight urethral bleeding. We also reported 69 (43%) patients with moderate local pain during the treatment and 6 (4%) with a strong pain during treatment. None of the patients reported a urethral stricture during our follow-up of 36.9 months (Table 4).
Plaque size. With the ESWT treatment we didn't observe a significant reduction in plaque size. In fact in 30 patients (20%) we observed a size reduction of the plaque, in 36 patients (24%) we saw a size increase of the plaque and in 84 (58%) there were no changes in the plaque size ($p = 0.0854$). All the characteristics regarding the plaque are listed in Table 2.
Regarding the 60 patients with the calcified plaque in 6 (10%) of them there were no longer ultrasound evidence of the calcification. In only 1 patient (0.75%) with a non-calcified plaque we saw a new calcification.
Curvature. Generally, the curvature did not change as we expected. Of the 150 patients 50 (33.3%) had a reduction of the curvature with a mean reduction of 25.2 degrees (+/-15.6 degrees) ($p = 0.044$), 96 (64%) had no enhancement of the curvature and 4 (2,66%) had an increase of the curvature with a mean increase of 12 degrees (7-17 degrees) (Table 2).

Table 5.
Results of previous studies of ESWT for Peyronie's disease (27).

References	No. pts.	Mean follow up (mo.)	No. decrease plaque size (%)	No. decrease penile curv. (%)	No. decrease pain (%)	No. improve sexual intercourse(%)
Abdel-Salam et al. (21)	24	3-9	14 (58)	14/24 (58)	17/24 (72)	14/24 (58)
Hamm et al. (22)	28	not avail.	not avail.	18/28 (64)	13/16 (81)	20/28 (71)
Hauck et al. (14)	20	9 (3-13)	2 (10)	10/20 (50)	5/9 (56)	3/20 (15)
Husain et al. (23)	34	8 (5-11)	not avail.	15/32 (47)	12/20 (60)	not avail.
Kiyota et al. (24)	4	< 1	1 (25)	0/4 (0)	4/4 (100)	not avail.
Manikandan et al. (25)	42	6 (2-18)	not avail.	22/38 (58)	21/25 (84)	5/42 (12)
Mirone et al. (26)	21	not avail.	11 (52)	11/14 (75)	16/21 (76)	9/12 (75)

Pain. With pain we obtained good results. Of the 150 patients 78 (52%) suffered pain. Of this 78 patients more than 90% reported good improvement in the sintomatology ($p < 0.01$); 9 (6%) of them reported a complete pain relief.
Sexual function. After ESWT treatment the quality of the intercourses are reported slightly enhanced. We didn't prescribe any PDE5 inhibitors or PGE1 drugs to avoid altered outcomes of the study. We observed also some cases of worsened sexual intercourses. Table 3 shows the detailed change in sexual activity before and after the treatment.
Penis tumescence and rigidity. No significant difference was observed before and after the treatment (average score before treatment 4.5 and average score after treatment 4.4), only 23 patients (15.3%) reported a difference.

Discussion

Peyronie's disease is a connective tissue disorder involving the growth of fibrous plaques in the soft tissue of the penis.
Specifically the process occurs in the tunica albuginea, a fibrous envelope surrounding the penile corpora cavernosa, causing an abnormal curvature of the penis (16). While the scar in the tunica albuginea is undergoing the process of remodelling, penile distortion may increase, remain static or resolve and disappear in younger spontaneously. In most patients the curvature remains static as the scar matures, although, in some patients, it becomes worse as fibrosis ensues and the scar contracts (17). In 25% of these patients the scarring process progresses to calcification, and in 25% of those it progresses to bone formation (18). We prospectively evaluated the efficacy of ESWT for Peyronie's disease in 150 patients. The indication for ESWT in Peyronie's disease is debated; it could be used both in non-calcified and calcified plaque with an important effect on the penis pain. First of all, before the treatment, we have to establish the goal of the ESWT therapy. The goal should be the reduction in plaque size with a consecutive reduction in the deviation angle, and a good improvement in the capacity to have regular sexual intercourse.
Glancing the literature we made a comparison with the results of our study (Table 5).
Regarding the pain we can say that it is more difficult to evaluate because is a subjective parameter and it is not infrequent a spontaneous regression of the pain particularly in early stages (19-20). Spontaneous regression of the disease has been seen in 13%, stable disease in 47% and progression in 40% of the cases (20). To treat the pain is also possible to apply a conservative treatment with analgesics and anti-inflammatory medication; this treatment is effective in particular in early inflammatory stages because is mainly is a symptom-directed approach (14), but is less effective in later stages when the pain is only during erection. With the ESWT it is possible in some cases to obtain a more lasting analgesia sometimes also in later stages. In regards to the ESWT mechanism of action it has been suggested an improvement in vascularization with consecutive resorption of calcification (10), as an alternative it has been proposed, in non-calcified plaques, a possible action on free radicals or pain receptors (12-13).
As in our patients no particular side-effects were observed in any study. Main side-effects, moderate and transient, noticed are the same in all studies and in our series; pain during treatment, skin haematoma and urethral bleeding (14, 21-26).
We didn't report a significant decrease in plaque size because we observed an overall reduction of the plaque in only 20% of our patients. We reported also a reduction of the size of the calcified plaques in 10%. In the literature are described reduction in plaque size in respectively 10% (22), 25% (24), 52% (26), and 58% (21). We remark that in all studies there isn't any note regarding the extent of the plaque size decrease nor any hint concerning the distinction between calcified and not calcified plaques.
Concerning the penis curvature we didn't report an important reduction, although 33.3% (50) of the patients had a significant reduction with a mean reduction of 25.2 degrees (+/-15.6 degrees). On the contrary 96 patients (64%) had no enhancement of the curvature and even more noticeably 4 patients (2,66%) reported a worsening of the curvature during our long term follow-up.
Other studies report a curvature reduction from 0% to 75% (14, 21-26). Only one study reports a significant statistical reduction (24). It is debatable if this statistical significant reduction is really of practical value for the patient. Only one series of the studies that we analysed did not show any worsening of the curvature (22).
Of 150 patients who signed up in our study 22 (14.6%) underwent surgery later, ESWT therapy not affecting in any way the surgical procedures. Other patients agreed with the simple conservative ESWT approach hoping to achieve an improvement in symptoms and pain without any invasive procedures. We can confirm that for curvature less than 30 degrees surgery seems not to be indicated.
The pain is the parameter that is more improved with more than 90% of the 78 patients suffering pain reporting good improvement of the sintomatology and 9 (6%) of them reporting a complete pain relief. In literature are also reported good improvements in pain: one study, but with only 4 patients, refers 100% of improving (24); others report respectively 56% (14), 60% (23), 72% (21), 76% (26), 81% (22), and 84% (25). What we can notice is that pain seems to decrease more rapidly with ESWT treatment than without any treatment. However we should still establish if the pain hault because of the treatment or because in the majority of the affected people the painful sintomatology resolves spontaneously with time (20).
Another important aspect is the sexual function. This parameter is very difficult to evaluate because there are many different way to measure it and because it is a subjective symptom. The problem is to evaluate this data without objective measurement of the quality of sexual intercourse. Anyhow 5 of the analyzed studies show an improvement of this aspect ranging from 12% to 75% (14, 21, 22, 25, 26).
Other 2 studies don't include a sexual evaluation (23-24). Clear data on changes in tumescence and rigidity are missing in all analyzed series. In our study there is an enhancement of sexual intercourse, in particular patients who referred a severe sexual dysfunction reported in many

cases an improvement such as those who referred a moderate dysfunction. Patients that referred to have impossible sexual intercourse remained unchanged. Regarding penis tumescence and rigidity only 23 patients (15.3%) reported a slight improvement although this data is purely subjective and not validated.
After analyzing all the outcomes of different studies it is important to observe that everyone used a different lithotripter with different modalities of application.
Our scheme was based on the administration of 2000 shock waves for treatment with an average number of treatment of 3.5, in other studies the number of shock wave for treatment ranges from 1000 to 4000 and the number of treatments from 1 to 5 per patient. All the series used a different energy (14, 21-26).
Furthermore we must acknowledge that different patients with also subjective evaluations results in different outcomes. Therefore, due to different technical aspects in different patient groups it is difficult to define the more appropriate protocol of ESWT for this specific disease. In the case calcified plaque ESWT could be the first approach for patients refusing an invasive approach such as surgery. Noncalcified plaques could be treated with ESWT after the drugs failure.

Conclusions

Our study is relevant due to the high number of patients involved (the highest reported to date) and to the long follow-up (36.9 months).
In consideration to the results obtained we can outline some important conclusion in relation to the modality of application of ESWT in Peyronie's disease:
Plaque size reduction. There is no significant reduction of the plaque size since it was reported in only 20% of patients and in only 10% of those with a calcified plaque.
Pain. In 90% of the patients there is a pain improvement although we are not able to differentiate if this result depend on the treatment because in the initial acute phase of the disease pain can dissolve by itself or be easily controlled with oral analgesics.
Curvature. A statistically significant reduction of the penis curvature was reported with a median improvement of 25.2 degrees.
Sexual function. A slight improvement of sexual function can be certainly ascribed to the reduction in penis curvature and penis pain.
Penis tumescence and rigidity. There is no significative difference between tumescence and rigidity before and after the treatment.
In conclusion the results obtained with the ESWT, a no-evidence based therapy, for the treatment of Peyronies's disease are not fully satisfactory.
The best outcomes have been achieved in the reduction of penis curvature and penis pain (both flaccid and rigid condition).
In our opinion ESWT should be proposed as a possible conservative treatment to those selected patients that refuse any surgery invasive approach.
Our schedule of procedure is safe and without relevant side-effects, therefore we believe that is certainly the most appropiate since others didn't reported better results. Owing to the high number of patients, the long follow-up, the high number of parameters evaluated the present study set up, at the moment, an important clinical evidence.

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