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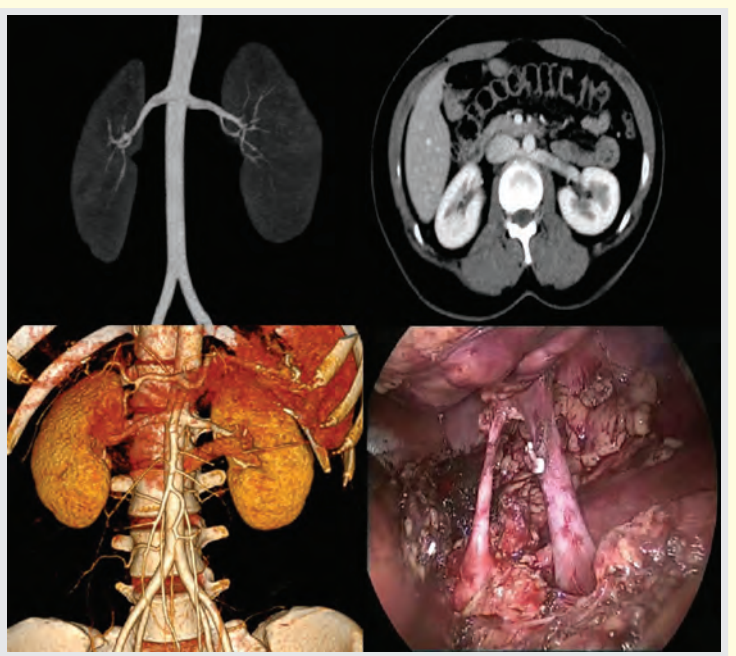

Figure 2- Type II retroaortic left renal vein with bilateral single renal arteries. (Page 673)

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The Middle Term

As it is been daily debated, the dilemmas on to treat or not to treat patients with localized prostate cancer are under the scope of the "Difference of opinion section": Between two extremes: the radical whole gland treatments versus the active surveillance protocols, the "middle term", probably will be arise from judicious and individualized patient selection. Using biomolecular markers, nomograms, modern magnetic resonance evaluations and the individual patient preferences and payment capacities, the urologists will be able to decide a personalized approach's, varying from traditional whole gland therapies, passing by the emerging focal treatments until exclusive surveillance. Concomitantly, in the future, less patients will be overdiagnosed and overtreated. Our readers will be able the get their self "middle term decisions", after reading the favorable arguments (Dell´Oglio and Sanchez Salas, from Paris) and contrary to treatment (Schulman and Polascyck, from North Carolina)

Another area of intense discussion regarding prostate cancer is the treatment of the primary tumors in the metastatic scenario, which was addressed in an extensive meta-analysis by Carneiro et al. (page 588).

A Turkish study showed that patients with non-muscle invasive bladder cancer are not correctly warrant about the importance of the smoking cessation and the risks of the disease progression. Authors reinforced that urologists, which are the closest help professionals in contact with these patients, can advice them and their relatives regarding the risks of tobacco exposure on urinary bladder neoplasms and the access to smoking cessation programs (page 607).

Karguzel et al. report a new renal cell carcinoma plasmatic biomarker, the SCUBE-1, that was compared to IX Carbonic Anidhrase and urokinase plasminogen activator receptor but in a limited case-control series (< 50 individuals), deserving future internal and external validations (page 638).

Moving for renal transplantation, the group from Bangalore, India, analyzed in 243 laparoscopic donor nephrectomies, their experience with patients presenting unusual venous anatomies (retro-aortic and/or surrounding aortic renal veins). Using angio CT in the surgical planning, the laparoscopic approach was secure.

Between the modern endourological approaches and the extracorporeal shockwave lithotripsy, the percutaneous nephrolithotomy (PCL) is well stablished and seems a "middle term" treatment that has maintained its role in the urinary stone management. Sometimes, the patient's body habitus, body masses index, and the extremes of age, can influence the PCL results as in prone or supine position. These questions were evaluated in three international studies in this issue of Int Braz J Urol (pages 679, 698 and 704).

EDITORIAL
IN THIS ISSUE

Greek researchers demonstrated that women with overactive bladder treated with long action teltoredine scored higher levels of sexual function (measured by the Female Sexual Function Index (FSFI)), after the medication in comparison with before the drug intake.

Ankylosing Spondylitis (AS) is a male prevalent rheumatic affection, that negatively impact the wellness and quality of life of much men. Interestingly, Santana et al., from Curitiba, Brazil detected through the use of diverse specific questionnaires, a high prevalence of erectile dysfunction in AS population. These data suggest to the urologists a proactive posture regarding erectile function during the anamneses of these rheumatic men.

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DIFFERENCE OF OPINION

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Most of patients with localized prostate cancer will be treated in the future? | *Opinion: Yes*

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Keywords: Prostatic Neoplasms; Patients; Epidemiology

INTRODUCTION

Localized prostate cancer (LPCa) is an heterogeneous disease extending from individuals who harbor indolent cancer, that are highly unlikely to develop metastases, to individuals with more aggressive disease, that have higher risk of metastatic burden. This would translates into different oncologic outcomes and have implications for disease management. Once the diagnosis of LPCa is established, remains challenging to identify those patients who may benefit from delayed or immediate treatment. Several options exist, from active surveillance (AS) to the whole-gland treatments (1). However, the optimal one is still unclear. To date, the percentage distribution of treatment for LPCa is around 8.4% for observation, 13.1% for ablative therapies, 28.1% for external beam radiotherapy (RT), 1.6% for brachytherapy, 45% for radical prostatectomy (RP) and 3.7% for primary androgen deprivation therapy (2).

Based on the recently findings of the PROTECT trial (3), we believe that the majority of patients with LPCa will be treated in the future. Below we analyzed several points to convince the reader of this statement.

With the introduction of PSA screening in the early 1990s, a sharp increase of earlier stage PCa at diagnosis was observed. However, the release of the United States Preventive Services Task Force statements against PSA-based screening combined with the discordant findings provided by ERSPC (4) and PLCO screening trials (5), might be the triggers of the decreased PSA testing rates observed in the medical community (6). This decrease in PCa screening might lead to a reverse stage migration towards more aggressive disease in the setting of LPCa with a consequently lower probability of deferred treatment.

To date is widely accepted that PSA has a suboptimal performance as a biomarker due to its low specificity. For this reason, several biomarkers were developed and will be developed to better identify LPCa patients at increasing risk of harboring clinically significant PCa at biopsy, who might benefit from early treatment. For example, the Prostate Health Index text (PHI), combining total, free and [-2]proPSA, was demonstrated

to outperform its individual components in predicting clinically significant PCa, also in biopsy naïve setting (7), reducing unnecessary biopsy. A panel of four kallikrein markers (total PSA, free PSA, intact PSA and human kallikrein 2) was also observed to improve the prediction of high-grade PCa relative to PSA testing and to reduce the number of unnecessary biopsy (43%) at the cost of missing few high-grade cancers (8). RNA biomarkers, such as PCA3 and TMPRSS2-ERG alone or combined, have shown to improve the performance of standard clinical criteria to predict high-grade PCa on biopsy (1, 9). Finally, tissue-based prognostic biomarkers, such as Prolaris and OncotypeDX, are commercially available and were observed to be strictly related in predicting adverse pathologic and oncologic outcomes (1). In conclusion, future evidence-based demonstrating superior performance of these novel promising biomarkers compared with existing standard of care is needed to allow their progressive clinical use and, consequently, more accurate identification of LPCa with aggressive disease that need immediate treatment.

Our ability to identify clinical significant PCa has dramatically improved during the last decades with the advances in imaging techniques. Multiparametric Magnetic Resonance Imaging (mpMRI) was demonstrated to have high accuracy in detecting clinically significant PCa (range: 44-87%) (10). Moreover, its high negative predictive value (range: 63-98%) could be used to rule out significant disease, sparing unnecessary biopsy (10). However, mpMRI despite promising results in biopsy naïve patients (11), is still not considered by urological guidelines in the primary biopsy setting (1). The PROMIS (11) recently provided evidence that mpMRI was more sensitive (93%) and less specific (41%) than TRUS-biopsy (48 and 96%, respectively), for detecting clinically significant PCa in biopsy naïve setting. Moreover, using mpMRI as a triage test before biopsy, will reduce unnecessary biopsy by a quarter (11). The future results of MRI-FIRST and PRECISION trials will help to define the added value of pre-biopsy MRI in biopsy-naïve setting. It is also of note to underline how mpMRI changed the biopsy paradigm opening the doors to MRI-targeted biopsy which provides higher rate of detection of clinically significant PCa (sensitivity: 91 vs. 76%, respectively) and lower rate of detection of insignificant PCa relative to TRUS-guided biopsy (sensitivity: 44 vs. 83%, respectively) (12). All these considerations suggest that mpMRI has and will have more and more high value as part of multivariable approach to early detection of clinically significant PCa.

According to the most updated urological international guidelines (1), AS is recommended for low-risk disease and a life expectancy (LE) of more than 10 years. The aim of AS is to achieve correct timing for curative treatment minimizing the treatment-related side effects without compromising oncological outcomes. Several studies reported excellent long-term oncological outcomes for patients enrolled in AS protocols (13, 14), suggesting that AS is a valid option for selected patients with LPCa. However despite AS protocols adopt stringent inclusion criteria, the treatment-free survival rates at 15 years of follow-up range from 34 to 55% (13, 14). These findings suggest that we are still far to select the optimal candidate with certainty and calls for novel biomarkers and genetic markers. Moreover, given the increasing of elderly patients, as well as the increasing LE worldwide, it might be reasonable to postulate that the future update of these AS studies will provide a trend towards higher shift into active treatment due to higher rate of disease reclassification. In consequence, identifying predictors of reclassification (e.g. PSA value at baseline, Gleason score on confirmatory biopsy) may help the physician in the daily clinical decision-making to shift into active treatment at the right time without compromising oncological outcomes. Moreover, it is of note that the risk of unfavorable pathological characteristics at RP (misclassification) is not negligible, also in those patients with very low-risk disease (15), and is higher relative to those who undergo immediate RP. Bearing in mind the predictors of unfavorable pathological characteristics in patients eligible for AS (e.g. older age, PSA density 10 ng/mL, number of positive cores) is fundamental to select the optimal candidate to immediate vs. delayed treatment. Furthermore, when we candidate a favorable intermediate-risk PCa patient to AS, we should remember that any grade pattern 4 is associated with 3-fold higher risk of metastases compared to gleason 6 (1).

The increase of low-risk and focal PCa afterwards the introduction of PSA screening combined with the well-known side effects related to whole-gland treatments, has led to the development and spreading of more conservative approaches, namely focal therapy (FT). FT is a treatment of specific

focus (targeted ablation) or limited defined region (quadrant ablation or hemiablation) (16), aiming to maintain the oncological benefit of active treatment, optimizing genito-urinary and gastrointestinal side-effects. Several studies, despite their short-term follow-up, reported excellent oncological outcomes (17) and improved postoperative preservation of sexual and urinary function relative to RP and RT with pad-free continence and potency preservation rates of 100 (IQR: 95-100) and 88.6% (IQR: 78.5-97.5) for HIFU and 100 (IQR: 100-100) and 81.5% (IQR: 69.3-88.2) for cryotherapy (17). Someone could argue that only a minority of patients, namely those with unifocal low-grade tumor, may be the real candidate to FT given the fact that around 86% of PCa patients harbor a multifocal or bilateral disease (18). However, if the multifocality is an exclusion criteria, why would we candidate patients to AS? According to the last consensus conference, also selected patients with multifocal PCa and a solitary clinically significant index lesion (16) should be considered. In this way the number of patients that could benefit from FT significantly increase. The rationale in considering also these patients stems in the natural history of PCa that seems to be linked to the index lesion that drives the spreading of metastatic PCa process in the majority of men, while low-grade lesions seem to have an indolent behavior (18). Moreover, evidence--based supported no differences in BCR between unifocal vs. multifocal in patients who underwent RP (18). According to a recent consensus conference (16), FT is an acceptable strategy up to and including Gleason 4+3. The ideal candidate for FT is a patient with good LE, with clinically LPCa and single lesion of Gleason 3+4 in a location/size favorable for FT (16). The advances in imaging and targeted biopsy allow an accurate selection of patients, that becomes mandatory to ensure the success of FT. mpMRI--TRUS fusion-guided biopsy is the modality of choice to proper select patients for FT (16) due to the high concordance between the index tumor location on biopsy and RP (18) and the reduce risk of missing clinically significant cancer relative to the TRUS-guided biopsy (12). Someone could argue that mpMRI alone is not sufficient to rule out all clinically significant PCa due to its intrinsic limitations. Again, the risk of non-detecting all clinically significant PCa is in common with prostate biopsy that allow to candidate a patient to AS. For this reason FT should not be considered a curative treatment and follow the treated patients over time is mandatory. Despite long-term oncological data are needed, given the improvement in proper patients selection, the promising results in terms of oncological outcomes, with a minimal or null impact on quality of life, as well as a shift towards extending the indication of FT for intermediate-risk PCa patients with limited targetable volume, we will expect a sharp increase of LPCa patients treated with FT.

Despite the well-known side effects and the consequently impact on quality of life, whole-gland therapies still have a dominant role in the management of LPCa patients (2) and represent the gold standard for this subset of PCa patients (1). While RT use is decreasing over time in this setting, RP remains the primary treatment of choice in contemporary patients diagnosed with LPCa (2). Moreover, the use of surgery is increasing across all risk groups with LPCa (2). One of the possible explanations is the recent spreading of the robot-assisted RP (RARP) that has largely replaced open RP as preferred approach for extirpative treatment for LPCa, due to better perioperative and functional outcomes. However, it is of note that in the most contemporary patients treated with RARP, up to 20% of patients with favorable characteristics experience urinary incontinence or erectile dysfunction (19). Moreover, the rate of postoperative complications may still reach 20% (20). In consequence, there is still need to improve and surgical expertise is one of the major determinants for this enhancement. Randomized controlled trials (RCT) have been conducted to provide insight into overall treatment strategies for LPCa. The SPCG-4 randomized study (21) compared RP vs. watchful waiting and provided evidence that overall survival, PCa survival and progression-free survival were higher in the treatment group at 18 years of follow--up. Despite the study enrolled men predominantly during the pre-PSA era, with a significant number of patients harboring palpable disease, it represents the RCT in LPCa with the longer follow-up available to date. The PIVOT trial (22) made a similar comparison relying on predominantly screen-detected LPCa patients and failed to observe a benefit in overall survival and PCa survival for treatment group, within a median follow-up of 10 years, except for patients with PSA>10 ng/ml or high-risk LPCa. However, it is reasonable to think that these findings were influenced by several factors. First, this study did not

meet the pre-specified enrollment targets and in consequence is underpowered to show treatment related differences. Second, 48% of patients died during the study period, of which around 85% died from other causes. These findings suggest that probably a significant number of patients enrolled into the PIVOT trial do not satisfy the 10-year LE benchmark as proposed by urological guidelines (1). Third, considering the fact that an overwhelmingly number of indolent cancers are included relative to the SPCG-4, the follow-up is quite short to evidence differences in survival between the two groups of treatments. The findings of the first RCT assessing effectiveness of RP vs. RT vs. AS were recently published (3). Despite the AS group did not undergo to a formal AS strategy (no systematic repeated biopsy/no imaging during follow-up), the PROTECT trial (3) failed to observe differences in PCa-specific and overall mortality at 10-years of follow-up between three randomized groups. However, the rate of disease progression was less than half in RP or RT groups relative to AS one. This calls for a longer follow-up to really verify the absence of benefit of immediate treatment, especially in the presence of high rate of T1c disease and low rate of Gleason 8-10 (76 and 2%, respectively).

In conclusion, our ability to identify and stratify clinically significant PCa has dramatically improved and it is likely to improve even further. First, because of the development of new biomarkers and genetic testing. Second, because mpMRI have definitely changed PCa pathway in the last few-years. Third, and most importantly, because the combination of these tools will positively lead to an accurate selection of LPCa patients who will undergo immediate treatment. The latter combined with the improvement of FT, the availability of long-term oncological data on FT, and the increasing surgical expertise with minimally invasive approach will lead to an increase of LPCa that will be safely treated in the future with more personalized approach starting from the assumption of the inter-patient and intra-glandular heterogeneity.

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DIFFERENCE OF OPINION

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Most of patients with localized prostate cancer will be treated in the future? | *Opinion: No*

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Keywords: Prostatic Neoplasms; Patients; Epidemiology

We are in the midst of a major shift in the diagnosis and management of localized prostate cancer. The prevailing approach of the 1980's and 1990's focused on widespread population-based prostate specific antigen (PSA) testing and curative-intent treatment for any detected cancer. The philosophical approach in the most recent decade is now defined by risk-adapted PSA screening, and integration of novel imaging techniques and biomarkers to increase the detection of clinically significant cancer. Concomitantly, we are witnessing the expanding utilization of active surveillance and partial ablation strategies to avoid overtreatment. We believe that continued development in each of these areas will continue to decrease the number of patients with localized disease treated with traditional whole gland surgery or radiation in the future.

Decreased screening practices

The incidence of prostate cancer rose steadily in the 1980's and exhibited a sharp increase in the early 1990's following the clinical integration of PSA as a screening test for prostate cancer (1). But subsequent concerns about the overdiagnosis of indolent disease and side effects associated with treatment raised questions about the benefit of widespread population screening. The United States Preventive Services Task Force (USPSTF) initially raised concerns about PSA screening in 2008 and in 2012 issued a recommendation against any PSA screening for prostate cancer (Grade D) (2). This recommendation was based in great part on results from the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial that did not show a cancer-specific mortality benefit in PSA screened men. It should be noted that subsequent detailed analysis of the trial has demonstrated that over 80% of patients in the 'control' group underwent ≥2 PSA tests within 3 years before entry into the trial contaminating the study findings (3). Nevertheless, the USPSTF recommendation has had a significant impact on screening practices in the United States.

Jemal et al. found meaningful decreases in both prostate cancer screening practices and incidence rates in 18 Surveillance, Epidemiology, and End Results (SEER) registries from 2005 through 2012 (4). The percentage of men 50 years old and over who reported PSA screening decreased from 40.6% in 2008 to 30.8% in 2013 and the incidence of prostate cancer in men 50 and over declined from 540.8 per 100.000 men in 2008 to 416.2 per 100.000 men in 2012. At the same time, there

is some evidence suggesting that rates of metastatic disease at diagnosis are increasing following decreased screening. Hu et al. reviewed SEER data from 2004 to 2013 and noted an increase in both the proportion of men presenting with intermediate and high risk disease (46.3% to 56.4%, p <0.1) as well as an increase in men presenting with distant metastases from 2.7% to 4.0% across this time period (5). It appears that changing practices are also impacting curative treatments with a 16.2% decrease in urologist radical prostatectomy volume between 2009 and 2016 (6).

Thus, recent controversies surrounding screening practices, at least in the United States, have had a negative impact on the proportion of men being screened, the proportion of men diagnosed with localized disease and the number of men undergoing radical prostatectomy. One corollary to this phenomenon is that men with low risk disease identified through screening will likely not be subjected to radical treatment, as has been the standard practice in the past.

Improved risk stratification

The last decade has also been notable for the proliferation of biomarkers and the integration of multiparametric magnetic resonance imaging (mpMRI) into the initial evaluation and management of men with localized prostate cancer.

Ahmed et al. have recently reported results from the Prostate MR Imaging Study (PROMIS) that compared the performance of mpMRI and transrectal ultrasound (TRUS) guided 12-core biopsy against a reference transperineal template mapping (TPM) biopsy in biopsy-naïve men with clinical suspicion of prostate cancer (7). In the analysis of 567 men, mpMRI was significantly more sensitive than TRUS biopsy (93% versus 48%, p <0.0001) for detection of clinically significant cancer. The negative predictive value of mpMRI was 89.2% compared to 73.7% for TRUS biopsy. Based on these findings the authors suggested that pre-biopsy mpMRI significantly decreases the diagnosis of clinically insignificant prostate cancer and may be considered as a triage test in selecting men for biopsy. The integration of image-guided fusion biopsy also improves the histologic detection of clinically significant prostate cancer. Valerio et al. performed a systematic review of the efficacy of mpMRI targeted biopsies and found that targeted biopsies detected significantly more clinically significant cancers compared to standard TRUS biopsy (33.3% versus 23.6%). Thus, recent evidence suggests that mpMRI is improving the visual and histologic detection of clinically significant cancer, while likely decreasing the diagnosis of clinically insignificant cancer. The current obstacle to widespread adoption of mpMRI and fusion biopsy is cost, but with time, expenses tend to decline enabling these technologies to become more readily available. Further, we expect that with progress, additional imaging technologies will be introduced that provide both anatomical and functional characterization of cancers, further distinguishing tumors that do not require treatment.

There have also been meaningful advances in the role of biomarkers to optimize screening and prostate cancer detection. Multiple blood, urine and tissue assays are now available to guide both screening and treatment recommendations. The serum based Prostate Health Index® (PHI) and 4Kscore® have both demonstrated pre-biopsy efficacy in predicting men who harbor aggressive prostate (8, 9). After biopsy, current National Comprehensive Cancer Network (NCCN) Guidelines recognize the potential value of the Oncotype Dx® Prostate and Promark® tissue-based tests for predicting the risk of Gleason grade 4 or non-organ confined disease at radical prostatectomy while the Prolaris® test improves the prediction of prostate-cancer specific mortality for men on active surveillance and the risk of biochemical recurrence after surgery or radiation (10). Thus, these biomarkers and genomic classifiers have an increasingly important role to select men for active surveillance versus definitive therapy. It is anticipated that novel and more informative biomarkers will be developed for clinical practice.

As the clinical integration of mpMRI and biomarkers continues to be refined and more widely used, there will be improved discrimination of patients who benefit from curative-intent surgery or radiation. In the future, it is likely that a smaller number of men will be treated for localized disease, but those receiving treatment will derive greater benefit from therapy.

Non-traditional management strategies

A third major theme of the last decade that is likely to continue to evolve is the expansion of the role of both active surveillance and partial gland ablation strategies for management of localized prostate cancer. While we recognize that partial ablation is a form of treatment, we would argue that it avoids the main therapeutic extensions of whole gland therapy and should be considered independently from traditional surgery or radiation.

Level I evidence is now available supporting the use of active surveillance to monitor indolent disease. Hamdy et al. noted equivalent 10-year cancer specific mortality of less than 1.5% after active monitoring compared to surgery or radiotherapy in the randomized Prostate Testing for Cancer and Treatment (Protec T) trial (11). Similarly, promising outcomes have been noted in other prospective active surveillance registries (12). Furthermore, there is increasing interest in expanding active surveillance criteria in properly selected patients who will be monitored closely (13). Active surveillance is now recognized by the NCCN as a viable management strategy for very low risk, low risk and favorable intermediate-risk prostate cancer (10). In the coming years, it is likely that active surveillance protocols will become better standardized and further expanded outside of academic centers leading to an overall decline in patients requiring treatment.

Significant academic and clinical interest in partial gland ablation strategies have also had a meaningful impact on management of localized prostate cancer and we anticipate growth in the coming years. While there is a range of emerging ablative modalities, cryotherapy has the most robust focal ablative data. Analysis of the Cryo Online Data (COLD) registry demonstrated that a cohort of 317 men with low-risk prostate cancer undergoing focal therapy had comparable rates of 60-month biochemical recurrence but improved 24-month erectile function when compared to similar men who underwent whole gland ablation (14). We have also noted that focal ablation is particularly promising for solitary anterior lesions as there is less risk of collateral damage to the urinary sphincter and neurovascular bundles during treatment (15). More recently, there has been growing interest in the use of high intensity focused ultrasound (HIFU) as an ablative medium following United States Food and Drug Administration (FDA) approval as a class II device for ablation of prostate tissue (16). Emerging evidence on focal HIFU supports particularly high rates of continence and potency preservation (17).

In conclusion, we believe that there will be a meaningful decline in the treatment of localized prostate cancer, particularly by traditional surgery or whole gland radiation therapy. Negative sentiments surrounding screening are likely to decrease the number of men diagnosed with localized disease and early evidence suggests that there will be an upward stage migration to non-organ confined disease at the time of diagnosis. At the same time, we are optimistic about the integration of both mpMRI and markers to improve candidate selection for screening, biopsy and optimizing management strategies. Ultimately, these tools will improve detection of clinically significant disease and decrease detection and treatment of non-lethal disease. Finally, we believe the expansion of both active surveillance and partial ablative strategies have the potential to safely reduce the number of men requiring traditional treatment, particularly if they are applied in properly selected patients and performed by well-trained practitioners.

CONFLICT OF INTEREST

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REVIEW ARTICLE

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Impact of local treatment on overall survival of patients with metastatic prostate cancer: systematic review and meta-analysis

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ABSTRACT

Context: Currently, standard treatment of metastatic prostatic cancer (MPCa) is androgen-deprivation therapy (ADT). Recent studies suggested that local treatment of MPCa is related to increase of survival of those patients, as observed in other tumors.
Objective: To evaluate the impact of local treatment on overall survival and cancer specific survival in 3 and 5 years in patients with MPCa.
Materials and Methods: Systematic review and meta-analysis of population studies published at PubMed, Scielo, Lilacs, Cochrane and EMBASE databases until June 2016. Several large cohorts and Post-Roc studies were included, that evaluated patients with MPCa submitted to local treatment (LT) using radiotherapy (RDT), surgery (RP) or brachytherapy (BCT) or not submitted to local treatment (NLT).
Results: 34.338 patients were analyzed in six included papers, 31.653 submitted to NLT and 2.685 to LT. Overall survival in three years was significantly higher in patients submitted to LT versus NLT (64.2% vs. 44.5%; RD 0.19, 95% CI, 0.17-0.21; p<0.00001; I^2=0%), as well as in five years (51.9% vs. 23.6%; RD 0.30, 95% CI, 0.11-0.49; p<0.00001; I^2=97%). Sensitive analysis according to type of local treatment showed that surgery (78.2% and 45.0%; RD 0.31, 95% CI, 0.26-0.35; p<0.00001; I^2=50%) and radiotherapy (60.4% and 44.5%; RD 0.17, 95% CI, 0.12-0.22; p<0.00001; I^2=67%) presented better outcomes.
Conclusion: LT using RDT, RP or BCT seems to significantly improve overall survival and cancer-specific survival of patients with metastatic prostatic cancer. Prospective and randomized studies must be performed in order to confirm our results.

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Prostate; Survival; Radiation Oncology; Prostatic Neoplasms

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INTRODUCTION

Radical prostatectomy (RP) has been reserved for patients with localized disease, and recently, its use was expanded to treat patients with locally advanced disease (1-3). Nowadays, it is discussed the impact of local treatment (LT) also for metastatic prostatic cancer (MPCa) in order to improve survival and time of response to androgen - deprivation therapy (ADT) and systemic pro-

gression of the disease (4-8). Standard treatment of patients with MPCa is single ADT, that has a overall survival of 42 months (9).

The treatment of the primary tumor of patients with metastatic disease has been stablished for some types of tumors. Two prospective and randomized studies showed a significant improvement of survival with cytoreductive nephrectomy associated to systemic treatment of patients with renal cell carcinoma (10, 11).

Also, current data show this benefit in relation to other tumors (ovary, gastrointestinal, among others) (12-14). However, until now, there is no study with evidence level 1 that demonstrates such benefit in relation to treatment of primary tumor in patients with MPCa. Recently published retrospective studies showed controversial results in relation to the benefits of LT associated to ADT on overall survival and cancer-specific survival (6, 15-18).

We decided to perform a systematic review and a meta-analysis in order to clarify the role of local treatment on overall survival in 3 and 5 years, as well on cancer-specific survival of patients with MPCa.

MATERIALS AND METHODS

Inclusion and Exclusion Criteria

We included case-control studies, big cohorts or clinical trials in English, Portuguese and Spanish, that presented data of patients with metastatic prostate cancer treated with LT (BCT and/or RDT and/or RP) or without LT (NLT) associated or not with ADT. The following aspects were analyzed: overall survival in 3 and 5 years, cancer-specific survival in 3 years and quality of life. Studies that did not separate results of treatment of high risk tumors and metastatic tumors were excluded.

Databases

Search was performed at MedLine, Lilacs and Embase until June, 26th, 2016. The terms included were: "((prostate OR prostatic) AND (cancer OR carcinoma OR tumour OR tumor OR neoplasm) AND (metastatic OR metastasis OR advanced OR "high risk" OR "lymph node" OR nodal)) OR (metastatic prostate cancer OR mPCa) AND ("local therapy" OR cytoreductive OR cytoreduction OR surgery OR prostatectomy OR "radiation therapy" OR radiotherapy OR Brachytherapy) AND (Castration OR Orchiectomy OR "Androgen-deprivation therapy" OR Androgen-deprivation OR "Gonadotropin-Releasing Hormone Agonists" OR "GnRHa treatment" OR "hormone therapy" OR "hormonal therapy" OR "Androgen deprivation" OR "chemohormonal therapy") OR (Outcomes OR "Perioperative Outcome" OR "Survival Rate" OR "Neoplasm Recurrence") AND ("prognosis/broad" [Filter] OR "therapy/broad" [Filter] OR "prognosis /narrow" [Filter])".

Selection

Selection Process

Two authors singly performed the selection of articles according to title. If the theme was adequate to previous stablished criteria or if there was any doubt as the possibility of inclusion, the summary was read. Abstracts were analyzed by three authors and if considered adequate by at least two researchers, the whole article was obtained (19).

Checklist

SIGN checklists were used for comparative studies, using cohort and case-control studies.

Critic evaluation

Biases

For cohort studies, the analyzed biases included selection biases, performance biases, detection biases, and memory biases. In case-control studies it was analyzed selection biases, detection biases and memory biases.

Extraction of Results

Selected disclosure was overall survival in 3 and 5 years, and cancer-specific survival in 3 years. Sensitive analysis, when adequate, was performed for patients submitted to LT with or without ADT.

ANALYSIS

For meta-analysis, RevMan 5.3 software from Cochrane Library was used. For cathegoric variables it was used Cochran-Mantel-Haenszel test and for continuous the reverse variation test. Results were demonstrated by Forest Plot. Hetero-

geneity was considered acceptable when $i^2<50\%$, and in those cases it was used a fixed model. Heterogeneity was considered elevated when $i^2\geq50\%$, and in those cases it was used the randomic model. In case of two meta-analysis being analyzed, it was performed the Egger's test, demonstrated by Funnel Plot. Studies that caused heterogeneity were removed and submitted to a new analysis.

RESULTS

Studies Selection

Our search was performed in June, 2016, and identified 19.958 articles, being 9 of grey area (using references of included articles). After the exclusion of 14.994 duplicate articles, 5.014 were selected for detailed analysis of summary, and 5.001 were excluded since they did not fulfill the inclusion criteria. After that, it was performed a detailed analysis of the remaining 28 articles and 16 were excluded due to inclusion criteria and 4 that did not include complete data of one of the targeted population.

In resume, 8 studies were included for systematic review and 6 for meta-analysis, with a total of 34.338 patients (Figure-1).

Studies characteristics

Seven cohorts were included from the articles (two were not included at meta-analysis since they did not provide adequate data) (20, 21) and one case-control study. No prospective and randomized study was identified (Table-1). For each study we made a detailed analysis of bias (Appendix 1).

Figure 1 - Studies Selection.

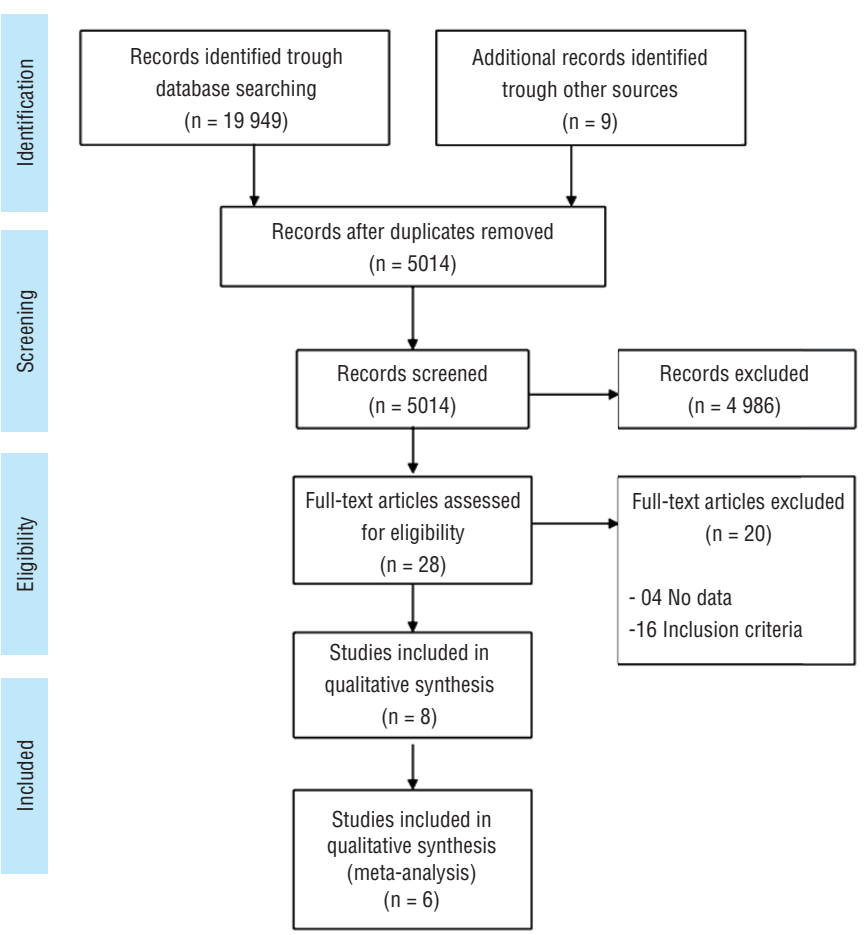


Table 1 - Characteristics of Studies.

Article	Type of Study	Age - I/C (years)	PSA - I/C (mg/dL)	Staging	Intervention	Comparison	Follow-up	N° I	N° C	Outcome
Culp 2014(7)	CR	64/72	Interval	M1a,b,c	RP or BT	NLT	5 years	8185	8185	OS, CSS
Antwi 2014(19)*	CR	Cutoff	Interval	M1a,b,c	RP or BT	NLT	3 years	7858	7858	-
Fossati 2015(20)*	CR	65/71	16/61	M1a,b,c	RP or BT	NLT	3 years	8197	8197	-
Satkunasivam 2015(21)	CR	74/78	246.4/588.4	M1a,b,c	RP or RT	NLT	3 years	4069	4069	OS, CSS
Heidenreich 2015(22)	CC	61/64	135.2/105.9	M1b	RP	NLT	3 years	61	61	OS, CSS
Cho 2016(23)	CR	69	190	M1b,c	RT	NLT	3 years	140	140	OS
Löppenberg 2016(24)	CR	65/69	16/46.7	M1a,b,c	RP, BT or RT	NLT	3 years	38929	15501	OS
Rusthoven 2016(25)	CR	66/69	Interval	-	RT	NLT	5 years	6382	6382	OS

CR = cohort retrospective; **M1a** = metastasis in pelvic lymph nodes; **M1b** = bone metastasis; **M1c** = visceral metastasis; **RP** = radical prostatectomy; **RT** = radiotherapy; **BT** = brachytherapy; **NLT** = not submitted to local treatment; **OS** = overall survival; **CSS** = cancer survival specific.

* Studies included just in the sistematic review.

Synthesis of results

Association Between Overall Survival and Local Treatment in 3 and 5 Years Five studies showed higher overall survival in three years in patients with MPCa submitted to LT in relation to those treated by NLT with or without ADT (64.2% vs. 44.5%; RD 0.19, 95% CI, 0.17-0.21; p<0.00001; I^2=0%) (Figure-2A). At sub-analysis, when we considered only patients submitted to ADT, we observed the same benefit on overall survival in three years of patients submitted to LT (63.6% vs. 43.1%; RD 0.19, 95% CI, 0.15-0.23; p<0.00001; I^2=0%) (Figure-2B).

During the analysis of 5-year survival, the results of two studies showed benefits of LT in relation to NLT (51.9% vs. 23.6%; RD 0.30, 95% CI, 0.11-0.49; p<0.00001; I^2=97%) (Figure-2C).

Association of Cancer-specific Survival and Local Treatment in 3 Years Analysis of two studies showed higher cancer-specific survival of patients with MPCa submitted to LT in comparison with NLT (69.1% vs. 46.3%; RD 0.16, 95% CI, 0.02-0.29; p=0.02; I^2=65%) (Figure-3).

Global and Cancer-specific Survival According to Modality of Local Treatment

When we considered local treatment with RP, the results of three studies showed higher overall survival in three years of patients treated with RP and LT in relation to NLT (78.2% and 45.0%; RD 0.30, 95% CI, 0.20-0.39; p<0.00001; I^2=50%) (Figure-4A).

During analysis of cancer-specific survival in three years it was not observed differences among groups of both included articles (84.3% vs. 46.3%; RD 0.20, 95% CI, -0.06-0.47; p=0.14; I^2=87%) (Figure-4B). After analysis of three studies, cancer-specific survival was higher in patient submitted to RP (77.6% vs. 47.9%; RD 0.23, 95% CI, 0.12-0.35; p=0.0001; I^2=74%) (Figure-5A). Again, at sensitivity analysis, LT was favored (76.1% vs. 47.8%; RD 0.28, 95% CI, 0.23-0.33; p<0.00001; I^2=0%) (Figures 5 B and 5C).

Four studies analyzed patients submitted to radiotherapy (BQT or RDT) and also showed benefit of LT in overall survival in 3 years (60.4% and 44.5%; RD 0.17, 95% CI, 0.12-0.22; p<0.00001; I^2=67%) (Figure-6A). During subanalysis, considering only patients submitted to ADT, overall survival in 3 years showed benefit in the group of patients submitted to BQT or RDT (62.5% vs. 43.0%; RD 0.20, 95% CI, 0.15-0.24; p<0.00001; I^2=0) (Figure-6B). Two studies showed higher cancer-specific survival after 3 years for the first group in relation to control (62.6% vs. 47.8%; RD 0.16, 95% CI, 0.10-0.21; p<0.00001; I^2=0%) (Figure-6C).

Figure 2 - (A) Forest Plot - overall survival in 3 years of patients submitted to LT in relation to those treated with NLT with or without; (B) Forest Plot - sub-analysis of overall survival in 3 years of patients submitted to LT in relation to those treated with NLT with ADT; (C) Forest Plot - overall survival in 5 years of patients submitted to LT in relation to those treated with NLT.

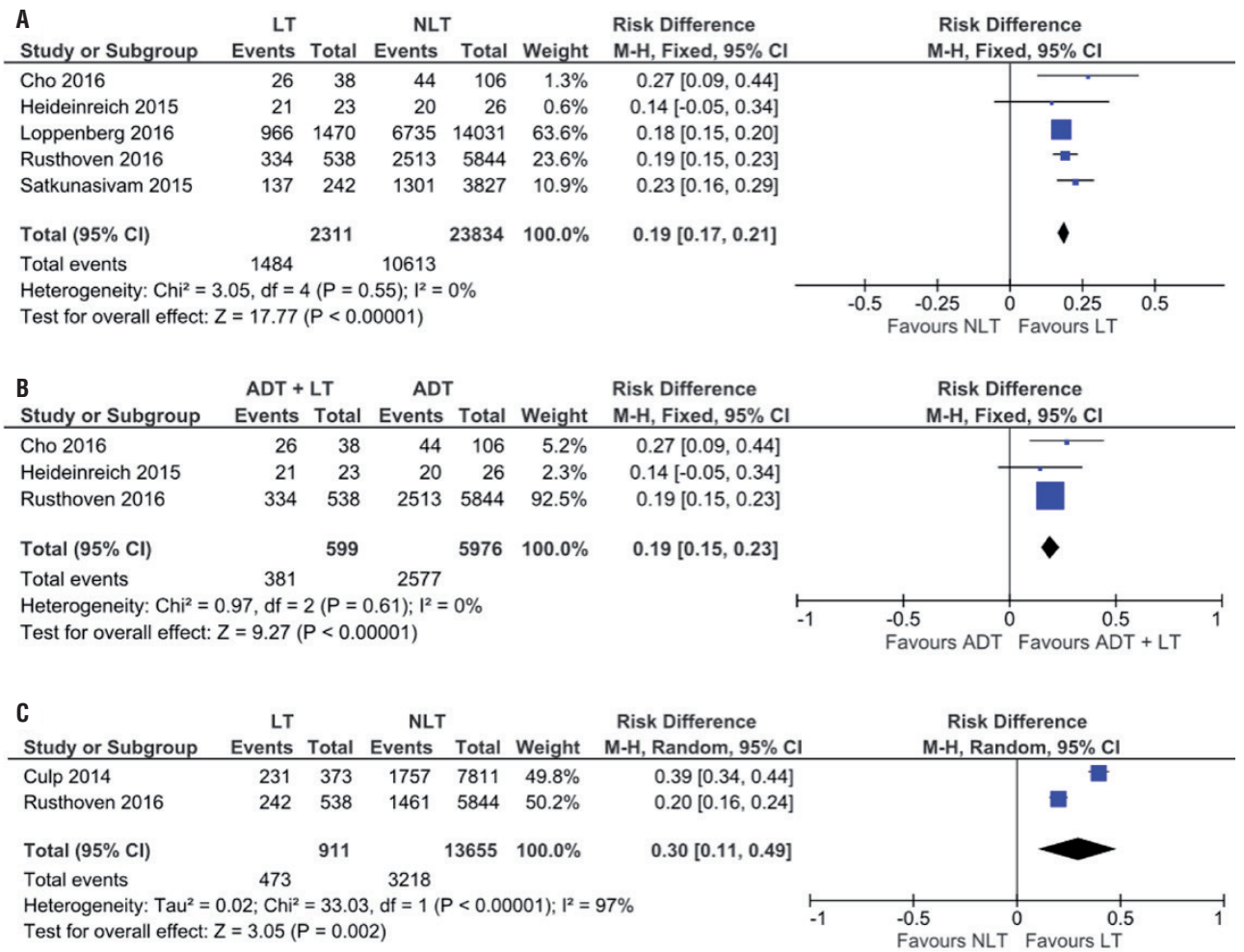


Figure 3 - Forest Plot - cancer-specific survival in 5 years of patients submitted to LT in relation to those treated with NLT.

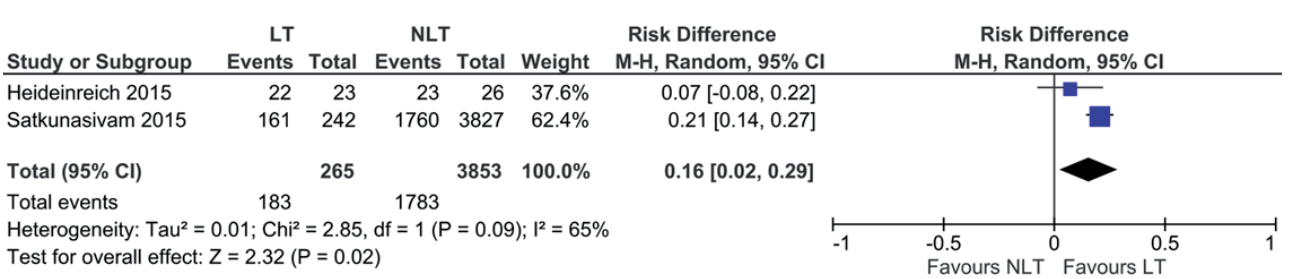
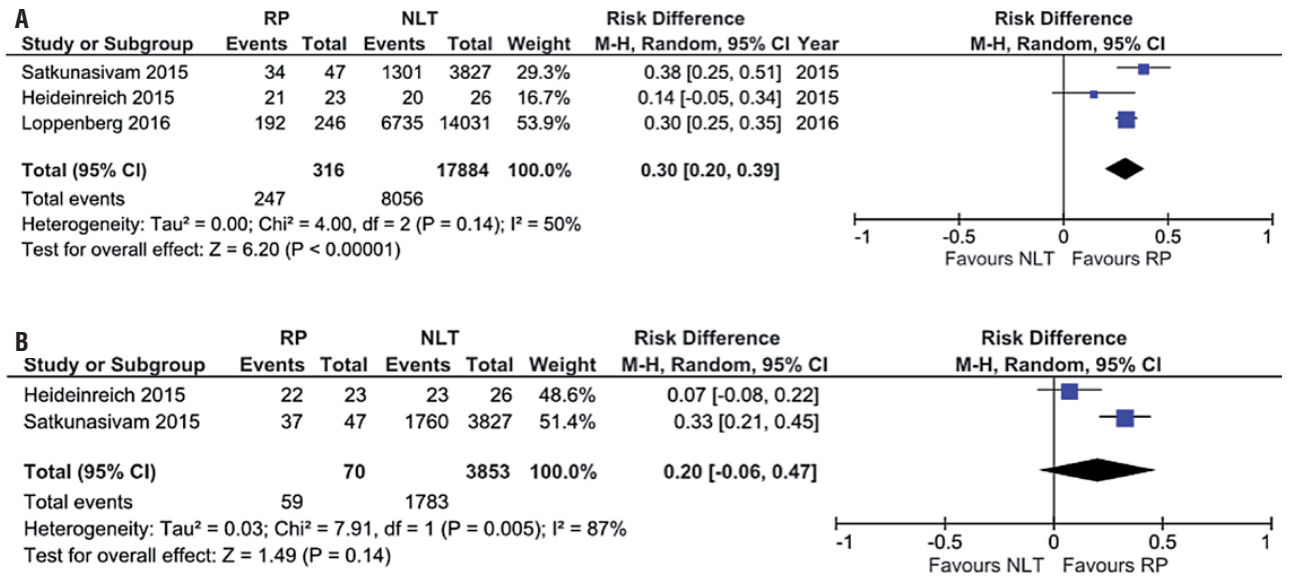


Figure 4 - (A) Forest Plot - overall survival in 3 years of patients submitted to LT with RP in relation to NLT group; (B) Forest Plot - cancer-specific survival in 3 years of patients submitted to LT and RP in relation to NLT group.



DISCUSSION

We are living a moment of transition in the profile of patients submitted to RP. In the 90's, the great majority of those patients were those with low risk prostate cancer. Those with high risk disease were initially submitted to pelvic lymphadenectomy that would stop RP in the presence of a compromised lymph node. In the present, it is recommended to avoid unnecessary treatment of patients with low-risk PCa and for locally advanced disease, even with positive lymph nodes, multimodal treatment with RP and RDT can heal the great majority of patients, and these are the patients who would benefit more with a more radical approach (22).

Until now, there are no level 1 studies that prove the benefit of local treatment of patients with MPCa. Our meta-analysis included big retrospective population studies, among which all five papers (26.145 patients with a median follow-up of 36 months) that evaluated overall survival in 3 years showed benefits with local treatment of patients with MPCa. In 2014, Culp et al. (7), using data collected from SEER, showed higher overall survival in 5 years of patients submitted to RP or BQT in comparison to NLT group. Two other studies extended those benefits for cancer-specific survival of patients with MPCa (23, 24). These series highlight the fact that some men have been treated with RP or RDT even in the absence of clear indication by literature (25).

Frequently, patients submitted to LT were younger, with better clinical conditions and more favorable in relation to GS and PSA at diagnosis, and the NLT group was not homogeneous in relation to the use of ADT. These facts highlight the discussion of the real difference of cancer-specific survival and mortality reached in every respective result. A series published by Rusthovem and cols (26) tried to eliminate these biases making a paired analysis with the same profile of patients in both groups, and also find a positive significance with the addition of local treatment for patients with MPCa.

It is known that the population of patients with MPCa is extremely heterogeneous and it is not possible to extrapolate the indication of local treatment to all patients. In order to try to identify the ideal candidate, Culp et al. (2014) (7) demonstrated an independent association among some variables (age higher than

Figure 5 - (A) Forest Plot - cancer-specific survival after 3 years of patients submitted to LT and RP in relation to NLT group; (B) Funnel Plot - analysis of sensitivity of cancer-specific survival after 3 years of patients submitted to LT and RP in relation to NLT group; (C) Forest Plot - analysis of sensitivity of cancer-specific survival after 3 years of patients submitted to LT and RP in relation to NLT group.

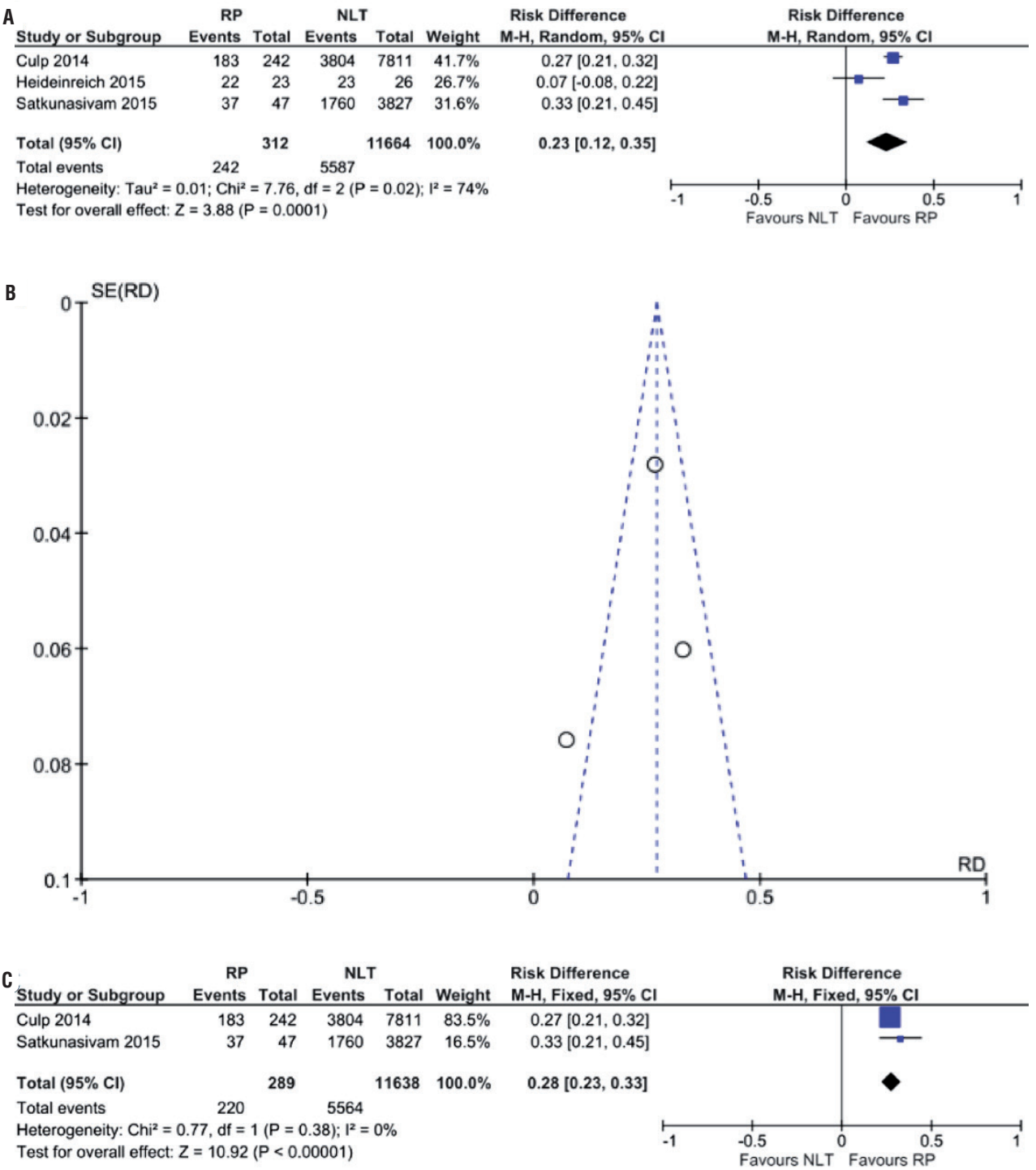
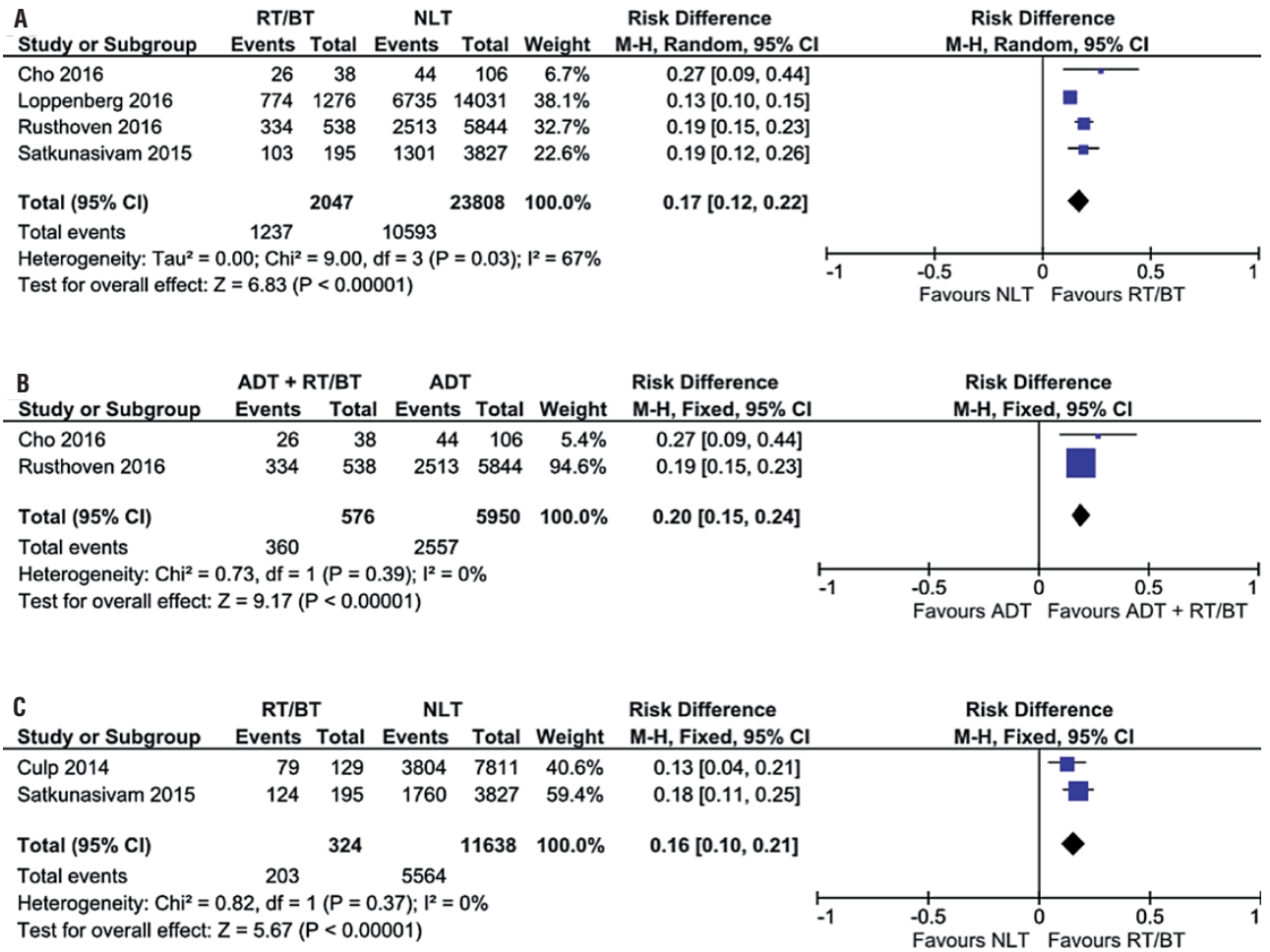


Figure 6 - (A) Forest Plot - overall survival in 3 years of patients submitted to LT with RDT or BQT in relation to NLT group with or without ADT; (B) Forest Plot - sub-analysis of overall survival in 3 years of patients submitted to LT in relation to those treated with NLT and ADT; (C) Forest Plot - sub-analysis of cancer-specific survival after 3 years of patients submitted to LT with RDT or BQT in relation to those treated with NLT.



70 years, Ct4 TNM disease, PSA>20ng/ML, high histological grade and pelvic lymphadenopathy) and an increase of cancer-specific mortality. Antwi et al. (2014) (21) showed that patients with MPCa with low differentiate tumors had a 46% higher risk of death due to all causes and 71% higher in relation to death due to PCa. In relation to the extension of the disease, mortality due to all causes was 52% higher for bone-restricted disease (M1b) and 88% higher for visceral disease (M1c) compared to the single involvement of non-pelvic lymph nodes (M1a). There was an increase of 70% of death due to PCa for M1b disease and a twice higher risk for M1c disease in relation to M1a.

Satkunasivam et al. (2015) (23) showed that advanced age, high levels of PSA, more aggressive high tumor, elevated CCI and bone irradiation less than 6 months of diagnosis are independent factors for the increase of cancer-specific mortality of MPCa patients, and those submitted to RP showed lower mortality when PSA≤20ng/ml. Fossati et al. (2015) (20) found benefits of local treatment in patients with cancer-specific mortality in 3 years predicted to be up to 40%. NNT (number needed to treat) was constant in the

interval between 10% and 30%, and rose exponentially when the risk was above 40%. Löppemberg et al. (2016) (27), based on some variables (age, initial PSA, CCI, Gleason Score-Gs and TNM AJCC) also developed a calculus in order to predict global mortality in 3 years of those patients, and concluded that risk over 70% did not add no time to survival with local treatment.

Several models of stratification of metastatic disease have been proposed and all consider visceral and lymph node metastasis important prognostic factors, highlighting the impact of the volume of the metastatic disease. According to SWOG (28), any metastatic lesion other than in the bones, regardless the number of lesions, must be considered high volume disease. Another criteria is the one adopted by the CHARTERED study (29), that considers high volume disease patients with visceral metastasis or >3 bone lesions of extra-axial bone lesion. This study showed that the combined treatment of QT and ADT was benefic in only patients with high volume disease (49 vs. 32.2 months). Cho et al. (30) also showed better prognosis of patients with metastasis restricted to bones in comparison to those with visceral disease. ECOG performance status, local of metastasis, extension of the disease and local therapy with RDT were related to increase of overall survival (ECOG PS 0-1 vs. 2-3, 3-yr OS 65% vs. 23%, p=0.004; M1b vs. other metastasis, 3-yr OS 52% vs. 3%, p=0.005; extension of the disease, single metastasis vs 2-4 metastasis vs. 5 metastasis 3-yr OS 57% vs. 41% vs. 28%, respectively, p=0.007). Therefore, the best candidate to local treatment is the young patient, without significant co-morbidities, and PSA lower than 20ng/ml and low volume metastatic disease (maybe restricted to bones).

Our analysis demonstrates the positive impact of local treatment on survival of patients with MPCa. Literature data show that more than one third of patients without local treatment will present severe local complications due to progression of primary tumor such as: number of hospitalizations, surgical procedures and consequently higher morbidity, with worsening of quality of life of patients (31-33). A case-control study suggests that RP lowers complication rates of urinary tract related to the progression of the disease, while one third of patients of control group presented lower urinary tract obstruction, hematuria or anemia (24). Morbidity and sequelae of local treatment still limit its indication in this scenario of no documented benefit. However, with evolution of technology, including robotic surgery, and more precise modalities of radiotherapy, the morbidity is being significantly reduced.

Surgical treatment of primary tumor is safe in locally advanced PCa (34, 35) and recent papers reproduced these results for metastatic disease. In the study of Cho et al. (2016) (30), 71% of patients treated with RDT received modulated intensity with the aid of helical tomography and none presented severe gastrointestinal or genital-urinary toxicity (grade 3-Radiation Therapy Oncology Group and EORTC criteria). Ten per cent of the RDT group presented hematologic complications grade 3 (Common Terminology Criteria for Adverse Events version 4.0).

However, Sooriakumaran et al. (2016) (25) published a multi-center study with 106 patients with MPCa submitted to RP (open or robotic). In their series, these therapeutic modalities were feasibly and safely performed in selected patients with MPCa, with general and peri-surgical specific complication rates similar to those with localized disease and locally advanced disease. Heidenreich et al. (2015) (24) showed no difference in the follow-up of patients submitted to RP (urinary incontinence and other post-surgical complications) in relation to those with high risk PCa. Complications due to local progression of disease with the necessity of surgical procedures correlated to GS at diagnosis (GS 8-10: 11 of 23, 47.8% vs. GS 7: 0 of 15, p=0.03).

Our work has several limitations. Firstly, our systematic review and meta-analysis were based on retrospective population studies. Secondly, the few studies available in literature are heterogeneous (design, end-points) and they do not allow us to conclude adequately. In third place, not all NLT patients were treated with ADT, but at sub-analysis it was possible to compare LT+ADT vs. ADT alone. And, lastly, local treatment was performed in few patients, that frequently were in better conditions. Anyway, this article presents the best evidence on the subject at the moment. Some big centers already perform prospective and randomized trials in

order to evaluate the impact of local treatment on survival. However, it would be very important to also include quality of life analysis objectively with validated question forms (36). Such studies will be fundamental for the evaluation of the real benefit of LT of MPCa and which are the best candidates for such treatment.

CONCLUSIONS

Local treatment with RDT, RP or BQT seems to contribute significantly to increase overall survival of patients with MPCa. However, prospective and randomized studies are needed to corroborate our data and to identify which patient with MPCa is the ideal candidate for local treatment in a multimode approach.

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CONFLICT OF INTEREST

None declared.

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APPENDIX 1

SIGN Checklist for Cohort Studies	CHO 2016	CULP 2014	SATKUNASIVAM 2015	LÖPPENBERG 2016	RUSTHOVEN 2016	ANTWI 2014	SIGN Checklist for Caso Control Studies	HEIDENREICH 2015
Section 1: Internal Validity **1.1.** The study addresses an appropriate and clearly focused question.	Y	Y	Y	Y	Y	Y	**Section 1: Internal Validity** **1.1.** The study addresses an appropriate and clearly focused question.	Y
SELECTION OF SUBJECTS: **1.2.** The two groups being studied are selected from source populations that are comparable in all respects other than the factor under investigation.	Y	N	Y	N	N	N	**1.2.** The cases and controls are taken from comparable populations.	Y
1.3. The study indicates how many of the people asked to take part did so, in each of the groups being studied.	DNA	DNA	DNA	DNA	DNA	DNA	**1.3.** The same exclusion criteria are used for both cases and controls.	Y
1.4. The likelihood that some eligible subjects might have the outcome at the time of enrolment is assessed and taken into account in the analysis.	Y	Y	Y	Y	Y	Y	**1.4.** What percentage of each group (cases and controls) participated in the study?	47 % x 53 %
1.5. What percentage of individuals or clusters recruited into each arm of the study dropped out before the study was completed.	DNA	DNA	DNA	DNA	DNA	DNA	**1.5.** Comparison is made between participants and non-participants to establish their similarities or differences.	Y
1.6. Comparison is made between full participants and those lost to follow up, by exposure status.	DNA	DNA	DNA	DNA	DNA	DNA		
ASSESSMENT: **1.7.** The outcomes are clearly defined.	Y	Y	Y	Y	Y	Y	**1.6.** Cases are clearly defined and differentiated from controls.	Y
1.8. The assessment of outcome is made blind to exposure status. If the study is retrospective this may not be applicable.	DNA	DNA	DNA	DNA	DNA	DNA	**1.7.** It is clearly established that controls are non-cases.	Y
1.9. Where blinding was not possible, there is some recognition that knowledge of exposure status could have influenced the assessment of outcome.	Y	Y	Y	Y	Y	Y	ASSESSMENT: **1.8.** Measures will have been taken to prevent knowledge of primary exposure influencing case ascertainment.	CS
1.10. The method of assessment of exposure is reliable.	Y	Y	Y	Y	Y	Y		
1.11. Evidence from other sources is used to demonstrate that the method of outcome assessment is valid and reliable.	Y	Y	Y	Y	Y	Y	**1.9.** Exposure status is measured in a standard, valid and reliable way.	Y
1.12. Exposure level or prognostic factor is assessed more than once.	DNA	DNA	DNA	DNA	DNA	DNA	CONFOUNDING: **1.10.** The main potential confounders are identified and taken into account in the design and analysis.	Y
CONFOUNDING: **1.13.** The main potential confounders are identified and taken into account in the design and analysis.	Y	Y	Y	Y	Y	Y		
STATISTICAL ANALYSIS: **1.14.** Have confidence intervals been provided?	Y	Y	Y	Y	Y	Y	STATISTICAL ANALYSIS: **1.11.** Confidence intervals are provided.	Y
Sections 2: Overrall Assessment of the Study **2.1.** How well was the study done to minimise the risk of bias or confounding?	+	+	+	+	+	+	**Sections 2: Overrall Assessment of the Study** **2.1.** How well was the study done to minimise the risk of bias?	+

ORIGINAL ARTICLE

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High cancer detection rate using cognitive fusion – targeted transperineal prostate biopsies

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ABSTRACT

Objective MRI of the prostate improves diagnostic accuracy of prostate cancer. Different fusion approaches with transrectal ultrasound images are employed. Objective: To determine detection rate of prostate cancer in men undergoing transperineal MRI-based cognitive fusion biopsy.

Materials and Methods: One hundred and sixty-four consecutive men underwent a multiple-core prostate transperineal biopsy. Univariable and multivariable logistic regression analyses were used to address the relationship between clinical parameters and prostate cancer detection rate.

Results: One hundred and fourteen patients underwent mpMRI prior to the transperineal biopsy, 52 (45%) were diagnosed with prostate cancer, of them, 36 had Gleason score ≥7 (69%). Among these 114 patients, 82 had suspicious lesions on MRI, and 43 of them were diagnosed with cancer (52%). On multivariate analysis, the most significant independent predictive factors were PSA density (P<0.001) and suspicious MRI lesion (P=0.006). Men with a PSA density of more than 0.22 and a suspicious lesion on MRI had a detection rate of 78%. Detection rate among 50 patients with no MRI study prior to this biopsy was 26%.

Conclusions: This study showed that among a group of mostly multi-biopsied patients, the presence of mpMRI lesions and high PSA density values helped to detect clinically significant prostate cancer using cognitive MRI/TRUS fusion biopsies.

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INTRODUCTION

Tools to enhance accurate detection of clinically significant prostate cancer are frequently developed. These tools are supposed to help avoiding the shortcomings of conventional biopsy such as false-negative results or under diagnosis of aggressive cancer as well as overdiagnosis of insignificant disease.

Until recently, saturation (at least 24 cores) biopsy was considered a method of choice to improve prostate cancer-detection rate after previous negative biopsy series (1-3). Bott et al. (4, 5) developed the brachytherapy template-guided transperineal technique, which increased the detection rate of significant tumors located especially in the anterior zone of the prostate gland. This procedure still has a role in defining disease previously missed or under-diagnosed (6). Transperineal saturation biopsies require increased resources in comparison with standard local anesthetic transrectal biopsies. In contrast to the high rate of infections caused by the transrectal approach (up to 5%), the transperineal approach is more prone to acute urinary retention. Most importantly, it seems most would agree that the transperineal approach

enables the examiner to get samples from prostate areas that are difficult, if not impossible, to sample by the transrectal approach (7).

Multiparametric magnetic resonance imaging (mpMRI) of the prostate has become recently a promising tool being increasingly used to improve the accuracy of prostate cancer detection. Suspicious lesions on mpMRI can guide targeted biopsy and allow better detection of clinically significant tumors and avoiding unnecessary repeated random biopsies (8-10).

Different approaches to the use of MRI in performing biopsies are currently employed. The most promoted one is via the use of dedicated hardware and algorithm-based fusion software (11-13). One can also perform the fusion based on cognitive appraisal of the location of the suspicious lesion seen on the different MR images on TRUS without any additional equipment (14, 15).

We hypothesized that the use of cognitive MRI/TRUS fusion transperineal template-guided biopsy enables better detection of clinically significant prostate cancer. In this cohort, most patients underwent multiple negative biopsies before they were referred to this biopsy.

In the present study, we evaluated the detection rate of cancer in the prostate gland in these men as well as the predictive factors for prostate cancer detection.

MATERIALS AND METHODS

The Institutional Review Board approved this study and waived informed consent requirements. Between the years 2011-2015, 164 consecutive men underwent transperineal template-guided biopsy from six regional locations, multiple core biopsies from each region, extending from the base, mid-gland and apex (16, 17). Biopsies were taken randomly from all regions; otherwise there were suspicious lesions on MRI, biopsies were directed first to those lesions. For 20 patients whose lesions on MRI were characterized using the first version of Prostate Imaging - Reporting and Data System (PIRADS, (18)) methodology, all PIRADS $\geq$3 were considered suspicious.

Biopsies were performed by two senior urologists (HM and NM) with previous extensive experience in transperineal prostate saturation biopsies as well as transperineal brachytherapy implant in the operating room under general anesthesia with the patient in the dorsal lithotomy position (17, 19). All men received perioperative antibiotics and an enema. The setup was the same as that used by us for brachytherapy. Before biopsy, the prostate gland was scanned from the level of the proximal seminal vesicles/base of the prostate gland to the apex and prostate volume was determined and the region of interest (suspicious lesions) was projected (cognitively) according to the MRI study.

This cohort of men had a history of pre-study biopsies with 113 men with at least 2 prior biopsies (2-8 biopsies, 69%), 45 men underwent 1 biopsy, (27%) and 6 men with no prior biopsy (4%).

One-hundred fourteen patients performed mpMRI prior to the current biopsy. All MRI scans were performed with either 1.5 Tesla with endorectal coil (n=80) or 3 Tesla without endorectal coil (n=34). Senior radiologists evaluated suspicious lesions using T2-weighted imaging, diffusion-weighted imaging (DWI), and dynamic contrast-enhanced MRI.

These studies were reviewed before and during the procedure to help direct the biopsies on a cognitive basis to the suspicious MRI lesions and extra random cores were taken from all over the prostate gland as described above.

All patients who underwent prior biopsies had negative results except for 9 patients who were referred to this biopsy as part of their active surveillance program. All 9 had a Gleason score 6 prostate cancers.

The biopsy procedure was performed after thorough study of the mpMRI when available, with 18G, 20cm long Pro MagTM biopsy needles (Angiotech, Medical Device Technologies, Inc., Gainesville, FL, USA), which were placed transperineally through template apertures to correspond with the regional biopsy locations as described by Bott et al. (4). At least five biopsy cores, depending on prostate size, were obtained for each of six regional biopsy locations extending from the apex toward the base. Patients who underwent mpMRI prior to the biopsy had several targeted biopsies

taken on a cognitive basis and only then the random saturation biopsies were obtained. Patients were instructed to continue oral antibiotics for another 72 hours.

Descriptive statistics of the study sample were used to summarize participant characteristics. The Student's t-test was used for comparison of two means. Fisher's exact test was used for two proportions. Backward likelihood ratio multivariate logistic regression analysis was used to identify predictors of prostate cancer and to build the prediction model. Classification and regression tree and χ^2 automatic interaction detection (CHAID) methods were used to divide the predictors into categories on the basis of the cancer detection status (20). All tests were two-tailed and statistical significance was defined as a P <0.05.

RESULTS

All 164 consecutive men who had transperineal template-guided biopsy were included in the study. Table-1 summarizes the clinical features of the evaluated men.

Adenocarcinoma was diagnosed in 65 men (40%). Of them, 42 (65%) had Gleason score ≥7. One hundred and fourteen patients underwent mpMRI prior to this biopsy, 52 (45%) were diagnosed with prostate cancer; of them, 36 had Gleason score ≥7 (69%). Among these 114 patients, 82 had suspicious lesions on MRI, 43 of them were diagnosed with cancer (52%). Only 9 patients out of the 32 patients with normal MRI findings were diagnosed with cancer (28%, P=0.02).

Among 82 men with suspicious MRI lesion who underwent cognitive fusion biopsy 29 patients (35%) had clinically significant disease (Figure-1). On the other hand, only 13 patients

Figure 1 - Cognitive fusion biopsy results of 82 men who had suspicious prostate Lesions on MRI.

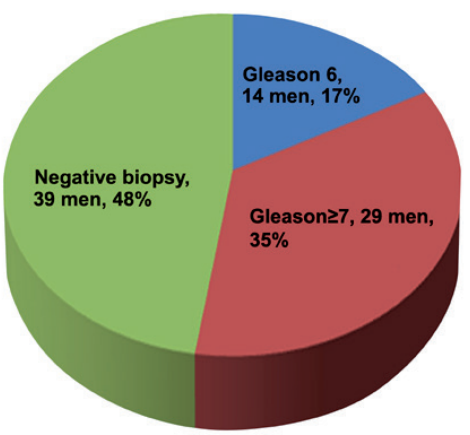


Table 1 - Clinical features of 164 men who underwent prostate biopsies.

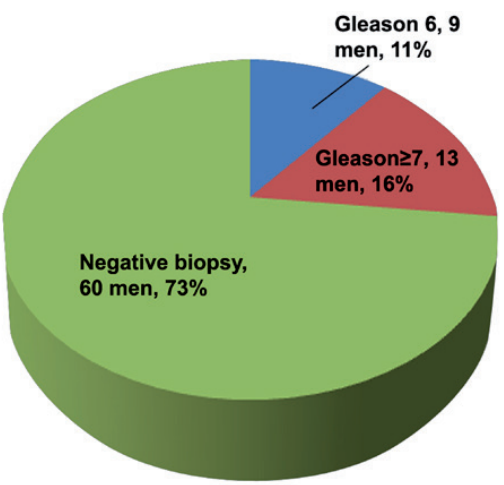
Characteristics	Total sample	Men performed MRI	Men did not perform MRI	P value*
No.	164	114	50	
Age (yrs); mean (SD, median)	65.18; (6.5, 65)	65.5 (6.8, 65)	64.3 (5.6, 64)	0.27
PSA (ng/mL); mean (SD, median)	14.3; (14.2, 11.2)	15.9; (16.3, 12)	10.6; (5.8, 9.6)	0.02
No. of prior biopsies mean; (SD, median)	2.1; (1, 2.2)	2.19; (1.2, 2)	1.87; (1.03, 2)	0.12
No. of cores taken during biopsy; mean (SD, median)	37.6; (4.9, 36)	37.7; (4.6, 36)	37.4; (5.5, 37)	0.7
Suspicious DRE; (No.,%)	23; (14)	18; (16)	5; (10)	0.46
Prostate volume (mL); mean (SD, median)	63; (32.1, 60)	60.8; (35, 57)	67.8; (23.5, 67)	0.2
PSA density**; mean (SD, median)	0.26; (0.18, 0.26)	0.3; (0.22, 0.3)	0.16; (0.07, 0.15)	0.001

PSA = prostate-specific antigen; **DRE =** digital rectal examination; **SD =** standard deviation.
* comparison between men who performed and who did not perform an MRI study.
** ng/mL/prostate volume (mL)

(16%) had significant disease out of the 82 men with normal MRI and men who did not perform an MRI study (Figure-2, P=0.007).

Prostate cancer detection rate among patients with no mpMRI study prior to this biopsy was 26% (13 patients out of 50); of them 6 had Gleason score ≥7 (46%).

Figure 2 - Transperineal biopsy results of 82 men without suspicious prostate Lesions on MRI.



Cognitive MR fusion-targeted biopsies detected 82% of cancers in the region of interest whereas the rest of cancer were detected in the same side of the region of interest.

Logistic regression models were constructed to identify significant independent predictors of prostate cancer among 114 patients who underwent mpMRI prior to the biopsy. Factors that were evaluated as related to prostate cancer were age, PSA, PSA density, number of previous biopsies, number of cores taken during the biopsy, suspicious MRI lesion, prostate volume, suspicious digital examination. Because PSA density, PSA and prostate volume were highly correlated, the last two were not included in the regression model. The model identified significant association between PSA density, suspicious MRI lesion and prostate cancer (Table-2). The number of previous biopsies was nearly insignificant.

We next used the CHAID methodology to create a decision tree and found that patients with PSA density higher than 0.22 have 60.2% chance to be diagnosed with prostate cancer. When combining the existence of a suspicious MRI lesion, the detection rate increased to 78.4% (Table-3).

DISCUSSION

In this study, cognitive MRI/TRUS fusion targeted-biopsy enabled better detection rate of prostate adenocarcinoma and specifically clinically significant cancers in a highly pre-study biopsied population. Detection rate among patients with no MRI study prior to this biopsy was 26%, similar to already published data from other centers (16) as well as by ourselves (17). It is important to note that all patients were referred to us from other centers and the decision whether to perform MRI prior to the biopsy was not ours.

Men with a suspicious lesion on MRI had a detection rate of 52%. The presence of a suspicious lesion on mpMRI and PSA density were significant independent predictors for cancer detection on our multivariate regression analyses. Interestingly, men with a PSA density more than 0.22 and a suspicious lesion on MRI had a detection rate of 78% (Table-3).

Several studies evaluated the use of cognitive fusion transperineal biopsies. Kasivisvanathan

Table 2 - Factors associated with prostate cancer detection among men who performed MRI.

P value	OR (95% CI)	Factor
< 0.001	1.104** (1.055-1.155)	PSA density*
0.047	0.625 (0.393-0.995)	No. of prior biopsies
0.006	4.986 (1.601-15.527)	Suspicious MRI lesion

*, ng/mL/prostate volume (mL)
**, per 0.01 increments of PSA density.

Table 3: CHAIDS decision tree

	No. (%)
All men	164 (100)
No cancer	99 (60.4)
Cancer	65 (39.6)

PSA density, *P* < 0.001

PSA density ≤ 0.221

	No. (%)
No cancer	73 (74.5)
Cancer	25 (25.5)
Total	98 (59.8)*

PSA density > 0.221

	No. (%)
No cancer	26 (39.4)
Cancer	40 (60.6)
Total	66 (40.2)*

Suspicious MRI lesion, *P* = 0.003

Yes

	No. (%)
No cancer	8 (21.6)
Cancer	29 (78.4)
Total	37 (22.6)*

No

	No. (%)
No cancer	18 (62.1)
Cancer	11 (37.9)
Total	29 (17.7)*

*, percent relates to total number of patients (164).

et al. examined 182 men with a lesion suspicious for cancer on MRI and found that transperineal prostate biopsy cognitively targeted to these lesions detected clinically significant cancer in 57% (21).

Valerio et al. compared software vs. cognitive based targeted transperineal prostate biopsies and found that both methods were almost comparable with only slightly and not statistically significant better results using the software based approach (64% vs. 68% detection rate, respectively) (22).

Radtke et al. compared systematic transperineal saturation biopsies to magnetic resonance imaging targeted biopsy and showed that while detecting similar amounts of Gleason score 7 or greater tumors, the use of mpMRI/TRUS fusion mitigated the detection of lower grade disease (23).

The present study has several limitations. First, this is a retrospective study without any randomization and therefore, inherently contains biases regarding patient selection data. However, the fact that this is a consecutive series and none of the men were excluded strengthens our conclusions and their applicability in daily urological practice. Second, the fact that the two groups (with and without prior mpMRI) had significantly different PSA density values (although almost identical in other measures) did not allow us to use statistical tests when comparing these two groups. Third, unfortunately, PIRADS score was not constantly described in the MRI reports and the existence of suspicious lesions was analyzed by the radiologist in a binary manner. Last, comparison was not established against software based MRI-US fusion technique.

In conclusion, this study showed that among this group of mostly multi-biopsied patients, the presence of mpMRI lesions and high PSA density values helped to detect clinically significant prostate cancer using cognitive MRI/TRUS fusion biopsies.

In this era, software-based fusion technologies are getting more and more popular among urologists worldwide. Our study shows that urologists who are experienced with biopsies and brachytherapy using the transperineal approach can consider the cognitive fusion approach as a feasible and promising technique for increasing the detection of significant prostate cancer. Although cost effectiveness was not evaluated in this report, one can assume that obviating the need for high--tech equipment will also reduce costs.

CONFLICT OF INTEREST

None declared.

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The awareness of patients with non – muscle invasive bladder cancer regarding the importance of smoking cessation and their access to smoking cessation programs

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ABSTRACT

Objectives: Smoking is the most important risk factor for bladder cancer and smoking cessation is associated with reduced risk of tumor recurrence and progression. The aim of this study is to assess the awareness of non-muscle invasive bladder cancer (NMIBC) patients regarding the importance of smoking cessation, determine their access to smoking cessation programs and the effects of smoking cessation on recurrence rates of NMIBC.

Materials and Methods: NMIBC patients who were followed with cystoscopy were included in the study. Their demographic properties were recorded, along with their smoking habits, awareness regarding the effects of smoking on bladder cancer and previous attempts for smoking cessation. Moreover, the patients were asked whether they applied for a smoking cessation program. Recurrence of bladder cancer during the follow-up period was also noted.

Results: A total of 187 patients were included in the study. The mean age was 64.68±12.05 (range: 15-90) and the male to female ratio was 167/20. At the time of diagnosis, 114 patients (61.0%) were active smokers, 35 patients (18.7%) were ex-smokers and 38 patients (20.3%) had never smoked before. After the diagnosis, 83.3% of the actively smoking patients were advised to quit smoking and 57.9% of them quit smoking. At the time of the study, 46.52% of the NMIBC patients were aware of the link between smoking and bladder cancer, whereas only 4.1% of the smoking patients were referred to smoking cessation programs. After a mean follow-up of 32.28±11.42 months, 84 patients (44.91%) had recurrence; however, current smoking status or awareness of the causative role of smoking on NMIBC did not affect the recurrence.

Conclusion: In our study group, the majority of the NMIBC patients were not aware of the association between smoking and bladder cancer. Although most of the physicians advised patients to quit smoking, a significant amount of the patients were still active smokers during follow-up. Only a small proportion of patients were referred to smoking cessation programs. Urologists should take a more active role in the battle against smoking and refer those patients to smoking cessation programs. Larger study populations with longer follow-up periods are needed to better demonstrate the beneficial effects of smoking cessation on recurrence rates.

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INTRODUCTION

Based on the data from International Agency for Research on Cancer, bladder cancer is the 6th most commonly diagnosed cancer of males and the 9th leading cause of cancer deaths worldwide, with an estimated 330.400 new cases and 123.100 deaths for 2012 (1). Moreover, with 2.9 billion health care costs, bladder cancer is accounted for 3% of all cancer costs in the European Union in 2012 (2).

Epidemiologic studies clearly show that cigarette smoking is strongly associated with an increased risk of bladder cancer (3-6). Moreover, smoking increases the risk of bladder cancer as the second smoking-associated cancers among survivors of kidney, head and neck, and stage I lung cancers (7). In a case control study, Howe et al. noted that elimination of smoking from the entire population would result in a 61% decrease of bladder cancer cases in men and 26% in women (8). Furthermore, a large population based bladder cancer study revealed that the risk of having a bladder cancer was significantly higher (OR=3.08; CI 95%, 1.16-8.22) among female lifelong non-smokers exposed to tobacco smoke at home during childhood (9).

Despite the establishment of public awareness regarding the association between smoking and cancer along with the vigorous efforts of global or local smoking cessation organizations, no significant changes in prevalence or cessation of smoking among Medicare Advantage participants between 2005 and 2012 were observed (10).

Patients with non-muscle invasive bladder cancer (NMIBC) are under great risk of recurrence and progression and thus require close surveillance programs. It has been documented that patients with bladder cancer made more than half a million outpatient visits to urology clinics in 2000 (11). Despite this invaluable opportunity to inform patients regarding the importance of cessation of smoking, the traditional role of urologists in treatment of bladder cancer is mainly toward treatment rather than prevention of the disease.

This study aims to assess the awareness of NMIBC patients referred to a tertiary reference center regarding the importance of smoking cessation, determine their access to smoking cessation programs and the effects of smoking cessation on recurrence rates of NMIBC.

MATERIAL AND METHODS

Study Design

Following the Institutional Review Board approval, data of patients who were under cystoscopy surveillance program (12) in our tertiary referral center were retrospectively collected. Patient demographics along with smoking habits were recorded. Only patients with pathologically confirmed bladder cancer and who were native Turkish speakers were enrolled. Patients who were referred to radical cystectomy or radiotherapy/chemotherapy because of muscle invasive disease or uncontrollable bleeding were not included. The data of cystoscopic surveillance of the patients together with the presence of recurrence disease were also recorded. The association of smoking habits and recurrence were evaluated.

Data Acquisition

A urology resident applied an anonymous, 19-item, multiple-choice questionnaire upon arrival to the clinic for cystoscopic evaluation between November 2013 - March 2014. After obtaining patient demographics, the questionnaire collected data on employment history, socioeconomic status, monthly income, awareness of the association between bladder cancer and smoking and smoking status and history. Moreover, the patients were asked whether they received cessation advice from their urologists or other physicians or applied for a smoking cessation program.

Besides documentation of smoking habits of the patients, the recurrence pattern of the disease was also recorded. The recurrence rates of the smokers, ex-smokers and never-smokers were compared.

Statistical analysis

Statistical analyses were performed using Number Cruncher Statistical System 2007 statistical software package program (NCSS, LLC, Kaysville, UT, USA). In addition to descriptive sta-

tistics (mean, standard deviation, median, interquartile range), data were-evaluated by independent t test for variables with normal distribution, by Fisher exact test and chi square test for comparison of qualitative data. Statistical significance was set as P <0.05.

RESULTS

A total of 212 patients were invited to participate in the current study, 187 (88.2%) of which completed the survey and were included into the study. Table-1 summarizes the patient's demographic characteristics and course of the disease.

The smoking habits of the patients as well as their awareness on the possible causative factors of the bladder cancer are provided in Table-2. While 46.52% of the NMIBC patients were aware of the link between smoking and bladder cancer, 88 (47.05%) patients had no idea about the possible causes of their disease (Table-2).

At the time of their diagnosis, 114 (61.0%) were active smokers, 35 (18.7%) were ex-smokers and 38 (20.3%) never smoked before. Of the actively smoking patients, 76.3% were advised to quit smoking and 66 (57.9%) of them quit smoking. Overall 76 (66.7%) actively smoking patients reported that their urologists advised them to stop smoking. Only 4.1% of the patients who were current or ex-smokers were referred to smoking cessation programs. Of these patients, a prescription medicine was recommended only to 5 (2.7%) patients to help stop smoking.

Unfortunately, 41 (62.12%) of the patients who quit smoking had started to smoke again.

After a mean follow-up of 32.28±11.42 months, 84 (44.91%) patients had recurrence. Patient's smoking habits (p=0.370), awareness of the causative role of smoking in NMIBC (p=0.316) did not affect the recurrence (Table-2).

DISCUSSION

Besides its causative role in development of various heart and lung diseases, it has also been confirmed that smoking increases the risk of recurrence and progression of NMIBC (13, 14). As the relationship between smoking and the course of the disease is well documented, the NMIBC is now accepted as a preventable condition (15, 16).

In an attempt to develop a model for predicting the outcomes of bladder cancer, Mitra et al. (17) combined molecular alterations involved in apoptosis, regulation of cell cycle, inflammation, angiogenesis and tumor invasion and smoking intensity. They showed that duration of smoking and number of cigarettes smoked daily were associated with COX-2 alterations and Bax expressions, respectively which in turn resulted in worse prognosis. Although previous reports demonstrated that the lowest risk of NMIBC is recorded in patients who never smoked before, cessation of smoking at time of diagnosis is also an important step in primary prevention of the disease (18, 19). Chen et al. evaluated the effects of cessation of smoking on the outcome of NMIBC using a post hoc questionnaire and interview (20). The 3-years recurrence free survival of continued smokers, non-smokers, ex smokers and patients who quit smoking within a year before and 3 months after the diagnosis was 45%, 57%, 62% and 70%, respectively. They also demonstrated that patients with bladder cancer who did not stop smoking had a 2.2-fold increased risk of disease recurrence when compared to patients who quit smoking (20). Recently, Al-Zalabani et al. conducted a systemic review of all meta-analyses, which evaluated the effects of modifiable risk factors on development of primary bladder cancer (21). Of the meta-analyses included, the one which has been performed by Van Osch et al. (13) reported the most comprehensive review regarding to smoking. The authors demonstrated that increasing duration since cessation of smoking reduced the development of bladder cancer, although former smokers had a 50% increased risk, even after more than 20 years of cessation. Moreover, Shiels et al. demonstrated that continuation of smoking might even result in development of a second malignant lesion in bladder (HR=3.67; 95% CI, 2.25 to 5.99) and kidney (HR=5.33; 95% CI, 2.55 to 11.1) among patients who survive from a previous smoking related cancer (7). Contrary to the previously published papers, our results failed to demonstrate any association between the recurrence rates and smoking cessation (p=0.370), a fact that can be explained by the low number

Table 1 - Demographic characteristics and course of the disease of patients who completed the survey and enrolled into the study.

Variable	Overall Patients (%)	Without Recurrence (%)	With Recurrence (%)	P
	n=187	n=103	n=84	
Mean age (year)	64.68±12.05	62.8±12.18	64.56±10.39	0.460
Gender				**0.316**
Female	20 (10.7)	18 (9.62)	2 (1.06)	
Male	167 (89.3)	85 (45.45)	82 (43.85)	
Employment				**0.354**
Working	69 (36.89)	37 (19.78)	32 (17.11)	
Retired	118 (63.10)	66 (35.29)	52 (27.80)	
Education				**0.173**
Illiterate	52 (27.8)	21 (11.22)	31 (16.57)	
Primary school	113 (60.4)	67 (35.82)	46 (24.59)	
High School	15 (8.0)	9 (4.81)	6 (3.20)	
College	7 (3.7)	6 (3.20)	1 (0.53)	
Monthly Income				**0.023**
<1000$	25 (13.36)	14 (7.48)	11 (5.88)	
1000-1500$	157 (83.95)	85 (45.45)	72 (38.50)	
>1500$	5 (2.67)	4 (2.113)	1 (0.53)	
Tumor grade*				**0.756**
Low Grade	112 (59.89)	77 (41.17)	35 (18.71)	
High Grade	75 (40.10)	26 (13.90)	49 (26.20)	
Tumor stage*				**0.420**
Ta	94 (50.26)	72 (38.50)	22 (11.76)	
T1	93 (49.73)	31 (17.11)	62 (22.15)	
Intravesical therapy				**0.046**
None	100 (53.47)	61 (32.62)	39 (20.85)	
Chemotherapy§	80 (42.78)	41 (21.92)	39 (20.85)	
BCG	7 (3.74)	1 (0.53)	6 (3.20)	

BCG = Bacillus-Calmette-Guérin

* Tumor grade and stage at time of diagnosis; § Epirubicin or Mitomycin-C

Table 2 - Smoking habits, awareness of the relationship between smoking and bladder cancer and their relationship with the recurrence of the disease.

Variable	Overall Patients (%)	Without Recurrence (%)	With Recurrence (%)	P
	n=187	n=103	n=84	
Current smoking status				**0.370**
Active smoker	48 (25.66)	18 (9.62)	30 (16.04)	
Ex-smoker	101 (54.01)	55 (29.41)	46 (24.59)	
Never smoked	38 (20.32)	30 (16.04)	8 (4.27)	
Smoking status at time of diagnosis				**0.460**
Active smoker	114 (61)	54 (28.87)	60 (32.08)	
Ex-smoker	35 (18.7)	19 (10.16)	16 (8.55)	
Never smoked	38 (20.3)	30 (16.04)	8 (4.27)	
Awareness of the relationship between smoking and BC				**0.316**
Yes	87 (46.52)	57 (30.48)	30 (16.04)	
Do not know	88 (47.05)	43 (22.99)	45 (24.06)	
No	12 (6.41)	3 (1.60)	9 (4.81)	
Smoking is the major risk factor for BC				**0.354**
Yes	76 (40.64)	41 (22.45)	35 (18.71)	
Do not know	97 (51.9)	57 (30.48)	40 (21.39)	
No	14 (7.48)	5 (2.67)	9 (4.81)	
Duration of smoking (years)	34.34±11.42	35.26±11.15	33.2±11.64	0.423
Cigarette packages smoked per day	1.21±0.76	1.3±0.76	1.16±0.74	0.428
Smoking cessation attempts				0.682
Yes	110 (58.82)	81 (43.31)	29 (15.50)	
No	39 (20.85)	24 (12.83)	15 (8.02)	
Duration since cessation smoking (years)	8.92±12.03	8.01±11.09	11.84±14.22	0.239
Advised to quit smoking				**0.420**
Yes	87 (46.52)	68 (36.36)	19 (10.16)	
No	62 (33.15)	35 (18.71)	27 (14.43)	
Who advised to quit smoking				**0.046**
Urologist	76 (40.64)	58 (31.01)	18 (9.62)	
Other physician	11 (5.85)	8 (4.27)	3 (1.60)	
Friends	8 (4.27)	7 (3.74)	1 (0.54)	
Internet/Social media	6 (3.20)	4 (2.13)	2 (1.06)	

BC = Bladder cancer

of patients enrolled into the study. Furthermore, being a current smoker during major oncological surgeries had higher odds of overall, pulmonary, wound and septic complications compared with non-smokers (22). Although the risk persisted in former smokers, the odds of experiencing surgical complications were significantly lower than that of current smokers (22). Considering the high possibility of undergoing repetitive surgical operations, cessation of smoking also has an utmost importance in overall patient's well being.

Nevertheless, almost half of the patients who were active smokers upon diagnosis of urothelial carcinoma did not quit smoking (23). In the current study, 42.1% of the patients who were smoking at time of diagnosis did not quit smoking. Besides highly addictive nature of nicotine, the patient's limited awareness of the causative role of smoking in the development of bladder cancer may contribute to this finding. In a survey conducted among patients presenting to a urology clinic, only 36% of the sample reported that smoking was a risk factor for bladder cancer (24). In a similar prospective cross sectional study, Johnson et al. assessed the knowledge regarding the association between smoking and various diseases (25). Although 45.2% of the respondents were aware of the association between smoking and bladder cancer, this rate was much lower than that of respondents who were aware of the association between smoking and lung cancer (97.4%). Guzzo et al., on the other hand, reported greater awareness rates compared to previous studies (16). They demonstrated that despite having a bladder cancer, the ratio of the patients who were aware of the association between smoking and bladder cancer was lower than that lung cancer (86% and 100%, respectively). Authors explained these high rates for being a tertiary referral center. In our study population, only 46.52% of the patients recognize that smoking was a risk factor for bladder cancer while 40.64% addressed smoking as the major risk factor. Although the current study was also conducted in a tertiary referral center, the awareness of our patients was not as high as the previously published series, which may be explained with relatively worse educational and socioeconomic level of the study population.

Given the benefits of cessation of smoking on both the outcome of NMIBC and overall health of the patients, all physicians should counsel smokers to quit and offer cessation assistance. It has been shown that patients diagnosed with a cancer are much more motivated in smoking cessation when compared to smokers without a cancer (26). This effect is more pronounced within the first 3 months of the diagnosis of cancer (26). It seems that although urologists have the unique advantage of seeing their patients every 3 months during their cystoscopy controls, they do not handle this opportunity effectively. Within the actively smoking population of this study, 76.3% were advised to quit smoking and 66 (57.9%) of them quit smoking. Of these physicians, 66.7% were the urologists. We think that every urologist must pay more attention to establish patient awareness and should counsel their patients regarding cessation of smoking.

Although it has been demonstrated that a smoking cessation program including a strong physician message, behavior modification and medications may increase the smoking cessation rates from 5.4% to 21.7% (27), urologists also fail to refer their patients to smoking cessation programs. Guzzo et al. reported that only 10.4% of all the respondents in the study population were offered specific cessation aids by their urologist in particular (16). In our study, only 4.1% of the patients who were advised to quit smoking were referred to smoking cessation programs which were organized by the Ministry of Health and consisted of the combination of counseling and medications (nicotine patch, bupropion SR and varenicline) (28). In order to encourage and influence the urologists to engage in bladder cancer prevention and smoking cessation, The World Urologic Oncology Forum organized a global initiative (29). The results of this initiative may change the behavior of the urologists in the near future.

The study has several limitations. First of all, the study population was small and follow-up period was relatively short. The limited number of the patients and short follow-up period might be responsible for the inability of our results to demonstrate the beneficial effects of smoking cessation on recurrence rates of NMIBC. Second, our

institute is a tertiary referral center and thus may not reflect the overall NMIBC population. Most of our patients come from rural parts of the country with lower socioeconomic status. Also, the rate of illiterates is relatively higher than that of the whole country, a limitation that may affect the accuracy of the responses of the patients. Considering the potential risk of recall bias due to the low socioeconomic status of our patients, we did not collect data regarding the exact time interval between the surgical procedure and the recurrence of the disease. Therefore, we could not perform Cox proportional hazards, which is a statistical method for assessing the impact of variables upon the time a specified event takes to happen. Another limitation of the study is the administration of a semi-structured interview, which bears a risk of obtaining misleading information from the respondents. However, such a risk exists in all kinds of interviews regardless of being structured or non-structured. In order to overcome this limitation, the questions in the survey were carefully selected not only to cover but also to secure the main objective of the study. In the current study, the questionnaires were conducted by the help of urology residents in order to prevent any misunderstandings. Also, a neutral and non-offensive language was used to avoid biased answers. Finally, the retrospectively collected data relies on self-reported questionnaires that were not validated yet. In spite of these limitations, the awareness of NMIBC patients regarding the importance of smoking cessation has not been well studied in a developing country thus our results may be beneficial for health policy makers and planners.

CONCLUSIONS

The majority of the NMIBC patients were not aware of the association between smoking and bladder cancer in our study group. Although most of the physicians advised to quit smoking, significant amount of the patients were still active smokers during follow-up. Only a small proportion of the patients were referred to smoking cessation programs. Urologists must take a more active role in the battle against smoking and refer those patients to smoking cessation programs as needed. On the other hand, larger study populations with longer follow-up periods are needed to demonstrate the beneficial effects of smoking cessation on recurrence rates.

CONFLICT OF INTEREST

None declared.

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Generation of potent cytotoxic T lymphocytes against in male patients with non-muscle invasive bladder cancer by dendritic cells loaded with dying T24 bladder cancer cells

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ABSTRACT

Background: In order to induce a potent cytotoxic T lymphocyte (CTL) response in dendritic cell (DC)-based immunotherapy for bladder cancer, various tumor antigens can be loaded onto DCs.
Objective: The aim of this study was to establish a method of immunotherapy for male patients with non-muscle invasive bladder cancer (NMIBC), using bladder cancer-specific CTLs generated in vitro by DCs.
Materials and Methods: Monocyte-derived DCs from bladder cancer patients were induced to mature in a standard cytokine cocktail (IL-1β, TNF-α, IL-6, and PGE_2: standard DCs, sDCs) or anα-type 1-polarized DC (αDC1) cocktail (IL-1β, TNF-α, IFN-α, IFN-γ, and polyinosinic:polycytidylic acid) and loaded with the UVB-irradiated bladder cancer cell line, T24. Antigen-loaded αDC1s were evaluated by morphological and functional assays, and the bladder cancer-specific CTL response was analyzed by cytotoxic assay.
Results: The αDC1s significantly increased the expression of several molecules pertaining to DC maturation, regardless of whether or not the αDC1s were loaded with tumor antigens, relative to sDCs. The αDC1s demonstrated increased production of interleukin-12 both during maturation and after subsequent stimulation with CD40L that was not significantly affected by loading with tumor antigens as compared to that of sDCs. Bladder cancer-specific CTLs targeting autologous bladder cancer cells were successfully induced by αDC1s loaded with dying T24 cells.
Conclusion: Autologous αDC1s loaded with an allogeneic bladder cancer cell line resulted in increased bladder cancer-specific CTL responses as compared to that with sDCs, and therefore, may provide a novel source of DC-based vaccines that canbe used in immunotherapy for male patients with NMIBC.

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INTRODUCTION

Urothelial carcinoma (UC) can be defined as neoplasms that arise from the epithelial lining of the urinary tract, from the minor calyces to the urinary bladder and even to the prostatic urethra. Based on histological evidence, majority of UC is bladder cancer (1). UC accounts for 90% of bladder

tumors, of which approximately 70% are confined to layers above the muscularis propria; these comprise the so-called non-muscle invasive bladder cancer (NMIBC). These tumors (previously termed "superficial bladder tumors") include stages Ta, T1, and Tis, which occur in 70, 20, and 10% of NMIBC cases, respectively (2). Standard primary treatment for NMIBC is transurethral resection; however, a problem in the management of NMIBC is its high intravesical recurrence rate, which ranges from 30 to nearly 80%, depending on the risk profile. Several mechanisms for intravesical recurrence have been proposed, including microscopic persistence of tumor, cancer cell implantation, and new tumor formation (1). More importantly, NMIBC may progress to muscle-invasive cancer during repeated episodes of intravesical recurrence. High rates of recurrence and progression of NMIBC have prompted investigation into a myriad of treatments attempting to decrease the burden of this tumor. Typically, treatment for high-risk NMIBC involves transurethral resection of the bladder tumor, and subsequent adjuvant therapy with Bacillus Calmette-Guerin (BCG) as one of feasible choices. The precise immunological mechanism of BCG has not been determined, but it is assumed that BCG is dependent on T cells. The role of Th1-mediated immunity, including CD4+ T cells and CD8+ cytotoxic T lymphocytes (CTLs), is well known. Ratliff et al.(3) showed that athymic nude mice did not undergo BGC-mediated antitumor activity. BCG treatment can reduce the risk of recurrence and progression of NMIBC, and is regarded as the most successful immunotherapy to date (4). However, 30-45% of patients are BCG failures, and its use is limited by its adverse effect profile and an intolerance that occurs in 20% of patients (5). Thus, new effective bladder-sparing treatments are needed in patients with NMIBC following BCG failure.

Dendritic cells (DCs) have the unique capacity to establish a primary immune response against tumor-associated antigens (TAA). This essential role of DCs in cellular immunity has led to the development of feasible and effective DC-based vaccines against tumor antigens to eliminate tumor cells (6). As a result, clinical trials using DC-based immunotherapeutic targeting of tumors are now underway (7).

Previously, Jonuleit et al.(8) introduced sDCs induced by a cytokine cocktail containing tumor necrosis factor (TNF), α/interleukin (IL)-1β/IL-6, and prostaglandin E_2 (PGE_2),which have been used in several clinical studies (9). However, the main disadvantage of sDCs is the absence of IL-12p70 secretion(10).This is important for the induction of effective Th1 and cytotoxic T lymphocyte (CTL) responses, which are assumed to be essential to cancer vaccination therapy. In an attempt to increase the potency of DCs, αDC1s were developed through the use of cytokine combinations. The αDC1s are induced to mature by the addition of an αDC1-polarizing cytokine cocktail containing IL-1β, TNF-α, IFN-α, IFN-γ, and polyinosinic:polycytidylic acid [poly(I:C)]. Compared to sDCs, αDC1s generate stronger and more functional CTLs for several diseases (11).

Effective tumor antigens are another important consideration of DC-based immunotherapy. Several studies have demonstrated that DCs pulsed with peptide and genescan generate potent bladder cancer-specific CTLs (12,13). The use of whole tumor cells rather than single antigens has the advantage of targeting multiple tumor antigens at once, which should avoid antigen escape mechanisms and allow for effective targeting of the majority of tumor cells within a growing tumor. However, this approach is technically limited, because harvesting tumor cells and generating a vaccine line that expresses a standardized amount of cytokine is not always feasible. It can be expensive, time-consuming, and unsuitable for those with a lower tumor burden status (e.g., carcinoma in situ).

To overcome this limitation, allogeneic tumor cells or established cancer cell lines from various tumors have been used as an alternative source of tumor-relevant antigens (14). Vaccines made from allogeneic cells circumvent the issue of individualizing each patient's therapy, and by using several cell lines derived from different tumors in the vaccine, there is an increased likelihood that the patient's tumor will share antigens expressed by the vaccine cells, including important tumor antigens that are frequently overexpressed or mutated in that particular cancer (15).

In the present study, we investigated the feasibility of DC-based immunotherapy for male patients with NMIBC. To achieve this purpose, αDC1s were loaded with UVB-irradiated allogeneic bladder cancer cells as tumor antigens. Their ability to elicit specific immune responses mediated by CTLs in vitro relative to sDCs loaded with tumor antigens was then assessed by IFN-γ enzyme-linked immunospot (ELISPOT) assay.

MATERIALS AND METHODS

Patient characteristics

A total of five patients with initial NMIBC treated by transurethral resection were enrolled for blood and tumor cell sampling. Patient demographics are shown in Table-1.

The study protocol was approved by the institutional review board at the Chonnam National University Hwasun Hospital (IRB registration number-2010-88; Hwasun, Korea). Informed consent was obtained from each patient.

Generation of αDC1s from patients with bladder cancer

Peripheral blood mononuclear cells were collected from bladder cancer patients. CD14+monocytes were isolated by positive selection using the magnetic-activated cell sorting system (MACS; Miltenyi Biotec, Auburn, CA, USA). The purity of the CD14+ cells was >90%. To generate immature DCs (iDCs), CD14+monocytes were cultured in IMDM (Gibco-BRL, Seoul, Korea) with 10% heat-inactivated FBS (Hyclone) and 1% penicillin/streptomycin (Gibco-BRL) for 6 d in 24-well plates with 5×10^5 cells per well in the presence of 50ng/mL granulocyte-macrophage colony-stimulating factor (GM-CSF) (PEPROTECH, Rocky Hill, NJ) and 20ng/mL IL-4 (PEPROTECH). On day 6, the iDCs were matured with either conventional cytokine cocktail composed of IL-1ß (25ng/mL, PEPROTECH), TNF-α (50ng/mL, PEPROTECH), IL-6 (1.000units/mL, PEPROTECH), and PGE_2(106M/L, Sigma-Aldrich, St Louis, MO, USA) to produce sDCs(8), or αDC1-polarizing cytokine cocktail composed of IL-1ß (25ng/mL), TNF-α (50ng/mL), IFN-α (3.000IU/mL, Intron-A-IFN-α-2b, Schering-Plough International, Kenilworth, NJ, USA), IFN-γ (1.000units/mL, Strathmann Biotech GmbH, Hannover, Germany) and poly(I:C) (20μg/mL, Sigma-Aldrich) to produce αDC1s (11). The DCs were then loaded with the UVB-irradiated T24bladder cancer cell line at a ratio of 2:1 at 2h after the addition of the maturation cytokinesas described previously (16). The matured DCs loaded with dying T24 tumor cells were harvested on day 8, washed, and analyzed by functional assay.

Preparation of the UVB-irradiated tumor cells as a source of tumor antigen

To load the tumor cells ontoDCs, T24 cells were irradiated with UVB ($30mJ/cm^2$) (International Light, Newburyport, MA, USA), cultured overnight in RPMI-1640 (Gibco-BRL) supplemented with FBS to induce apoptosis, and then thoroughly washed. The irradiated tumor cells were loaded onto DCs 2h after the addition of maturation cytokines, according to the previous reports (16). The irradiated dying cells were immediately confirmed using annexin-V and propidium iodide (PI).

Table 1 - Patient demographics.

	Sex	Age	Tumor size	Histologic examination
Patient 1	Male	72	1.5cm	T1 high grade, CIS
Patient 2	Male	74	2cm	T1 low grade
Patient 3	Male	77	2.5cm	T1 high grade
Patient 4	Male	74	1.5cm	Ta high grade
Patient 5	Male	55	1.5cm	T1 low grade

Preparation of bladder cancer cells from patients

Tumor tissues obtained from bladder cancer patients by transurethral resection were minced and lysed for 2-4h at 37°C in AIM-V medium containing 0.4% collagenase type III. The mononuclear cells were separated by density gradient centrifugation with Ficoll-Hypaque (Lymphoprep) and cryopreserved until their use as target cells in the cytotoxic assay.

Tumor antigen uptake by DCs

To measure tumor antigen uptake by the DCs, T24 cells were labeled with PKH67-GL- fluorescein isothiocyanate (FITC) (Sigma-Aldrich) before UVB irradiation. After the loading of tumor antigen onto DCs at a ratio of 1:2 on day 6, the αDC1s loaded with dying T24 tumor cells were stained with CD11c-phycoerythrin (PE) and analyzed by flow cytometry for tumor antigen uptake ($CD11c^+/PKH67^+$).

Immunophenotyping of DCs

To characterize the cell surface phenotypes on DCs, flow cytometry was performed using a FACSAria cell sorter (Becton Dickinson, San Jose, CA, USA) after labeling of the cells with CD86-PE, CD83-FITC, CCR7-FITC (PharMingen, San Diego, CA, USA), and the relevant isotype controls (mouse IgG1 and IgG2a, PharMingen). Cell debris was eliminated from the analysis by forward and side-scatter gating, and the data were analyzed with WinMDI Version 2.9 software (Biology Software Net).

Cytokine analyses by enzyme-linked immunosorbent assay (ELISA)

The levels of IL-12p70 and IL-10 in the primary culture supernatants of the DCs were measured using Quantikine Immunoassay Kits (R&D Systems, Minneapolis, MN, USA). Additionally, DCs harvested on day 8 were plated in 96-well plates at 2×104 cells/well and stimulated to secrete IL-12 with CD40 ligand (CD40L)-transfected J558 cells (as an analogue of CD40L-expressing Th cells; a gift from Dr. P. Lane, University of Birmingham, UK) at a density of 5×10^4cells/well. After 24h, the supernatant was harvested and the production of IL-12p70 determined by ELISA (R&D Systems).

Induction of bladder cancer-specific CTLs

Autologous $CD3^+$ (purity >90%) cells were positively isolated from the lymphocyte fraction after Percoll isolation using MACS (Miltenyi Biotec). T cells (1×10^6cells) were sensitized by autologous αDC1s (1×10^5 cells) loaded with dying T24 tumor cells. On day 3, rhuIL-2 (5ng/mL, R&D Systems) and IL-7 (10ng/mL, R&D Systems) were added. The CTLs were re-stimulated with the same DCs on day 10. On day 20, the number of antigen-specific T cells was analyzed by IFN-γ enzyme-linked immune spot (ELISPOT) assay. T24 and autologous bladder cancer cells from bladder cancer patients were used as target cells. MHC class I- and II-restricted recognition of the prostate cancer-specific CTLs was analyzed using MHC class I- and II-specific mAbs (clone W6/32 and clone CR3/43, respectively). The ELISPOT data were expressed as the mean number of spots (±SD) per $0.5\text{-}2\times10^5$ T cells. CTL alone was used as the control.

Statistical analysis

Data presented are mean plus or minus SD. The statistical significance of differences was assessed using the unpaired t-test. P values <0.05 were considered significant. All statistical analyses were performed with SPSS 17.0 for Windows software (SPSS Inc., Chicago, IL, USA).

RESULTS

Preparation of dying T24 cells as tumor antigens and antigen uptake by αDC1s. About 43.4% of T24 cells were shown as dying cells after UVB irradiation (Figure-1A). As shown in Figure--1B, αDC1s efficiently incorporated the dying T24 tumor antigen (65.8±14.9%; n=5) as measured by flow cytometry ($CD11c^+$/PKH-67). The efficacy of antigen uptake ofthe αDC1s and sDCs was similar (data not shown).

Characteristics of DCs generated from bladder cancer patients

The αDC1s showed typical morphology, with large and branching structures aggregated among the cells (not shown). Phenotypically, αDC1s exhibited higher expression of the co-stimulatory

molecule (CD86, p=0.001). αDC1s showed comparable expression of the maturation marker (CD83) and predictive marker of migratory ability (CCR7) to the sDCs (Figures2A and 2B).

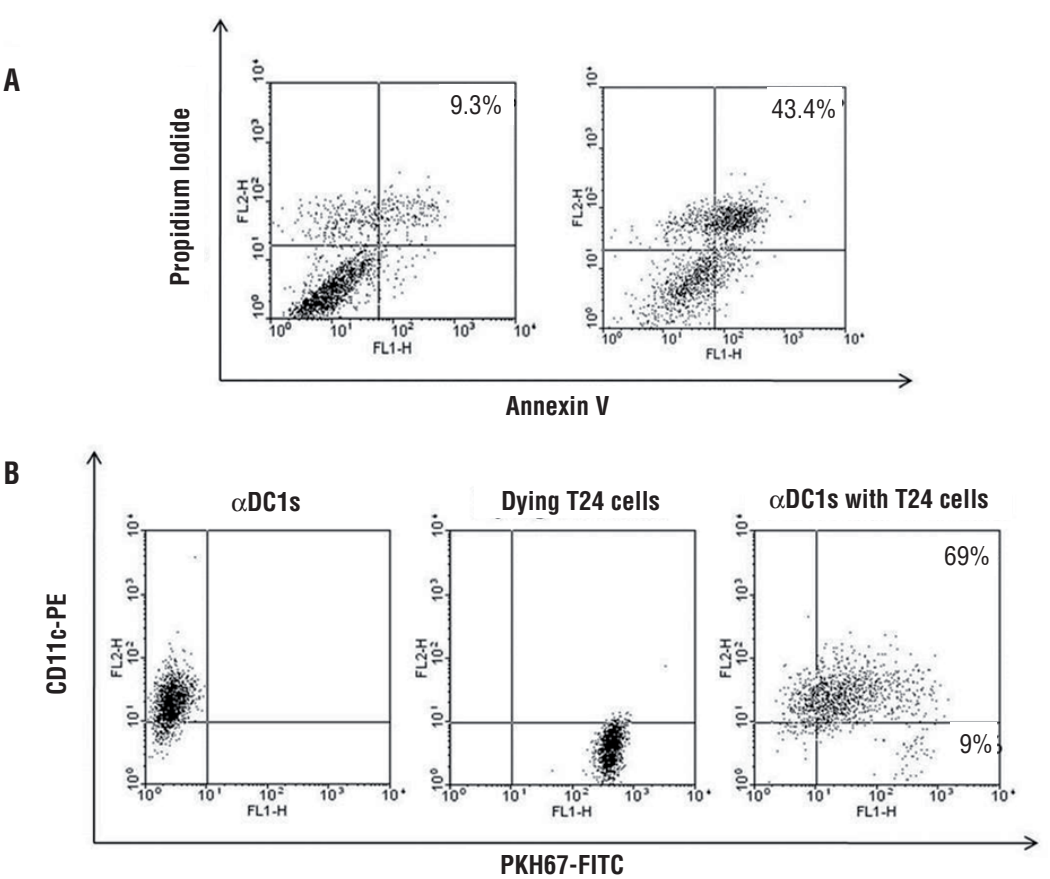
IL-12 production of DCs generated from bladder cancer patients

One of the best ways of determining DC function is to examine cytokine secretion. IL--12p70 is an important cytokine for stimulating naive T cells for Th1 polarization to benefit cancer treatment, but IL-10 is the main inhibitory cytokine for cancer treatment. As shown in Figure-3A, the αDC1s showed higher IL-12p70 levels, as measured in the primary culture supernatant collected at DC harvest (during maturation), than the sDCs did (p=0.001). Furthermore, the αDC1s showed higher production of IL-12p70 after subsequent stimulation with CD40L-transfected J558 cells as compared to the sDCs (p=0.001, Figure-3B). This cytokine secretory capacity of αDC1s was not significantly suppressed by loading tumor antigen. In contrast, production of the inhibitory cytokine IL-10 by αDC1s was not significant (Figure-3C).

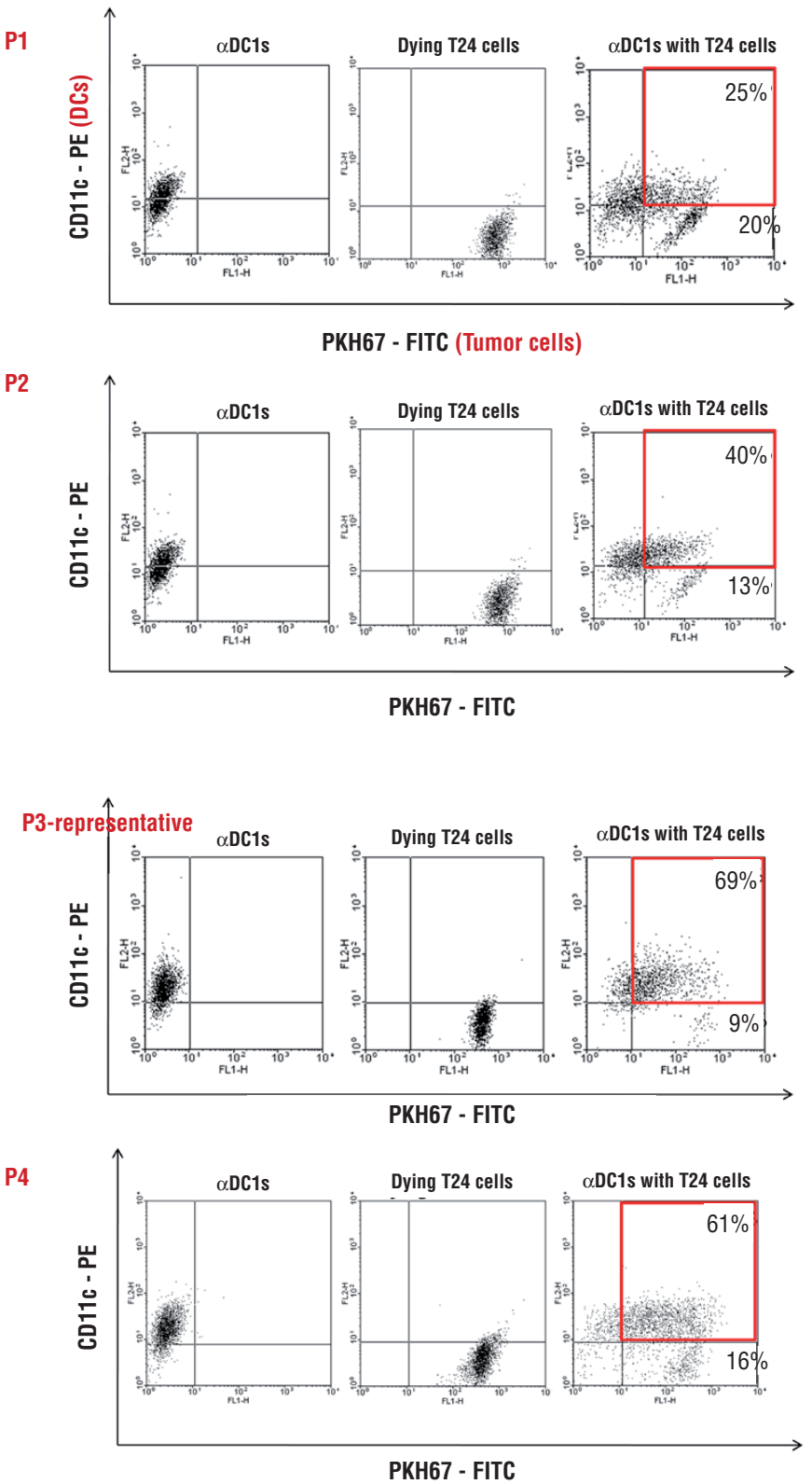
Generation of potent bladder cancer-specific CTLs by autologous DCs loaded with dying tumor cells

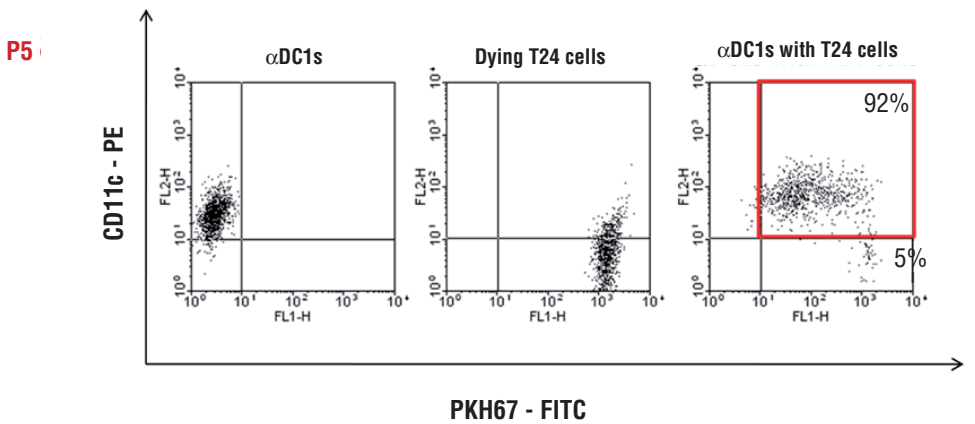
We next determined the tumor-specific generation of CTLs by DCs. The secretion of IFN-γ by the CTLs was measured in three independent experiments using ELISPOT assay. Even though those results represented the preliminary data in 5

Figure 1- A)Both Annexin-V positive/PI negative cells and Annexin-V/PI double-positive cells were considered to be dying cells;B) After co-culturing, the tumor antigen uptake of the αDC1s was measured by the percentage of double-positive cells. Data are from one representative experiment out of five independent experiments (Data from all cases are provided in supplemental Figures 1 Asupl, Bsupl and Csupl).



Data from all cases are provided in supplemental Figures Asupl, Bsupl and Csupl





cancer patients, consistent with their high ability to produce IL-12p70, primed $CD3^+$ T cells generated by the αDC1s loaded with dying T24 bladder cancer cells showed a larger number of IFN-γ-producing cells against T24 bladder cancer cells (p=0.001, Figure-4A) and autologous bladder cancer cells (NMIBC) obtained from bladder cancer patients than the sDCs did (p=0.002, Figure-4B). The MHC class I- and II-restricted recognition of the CTL response was confirmed using MHC class I- and II-specific mAbs, respectively.

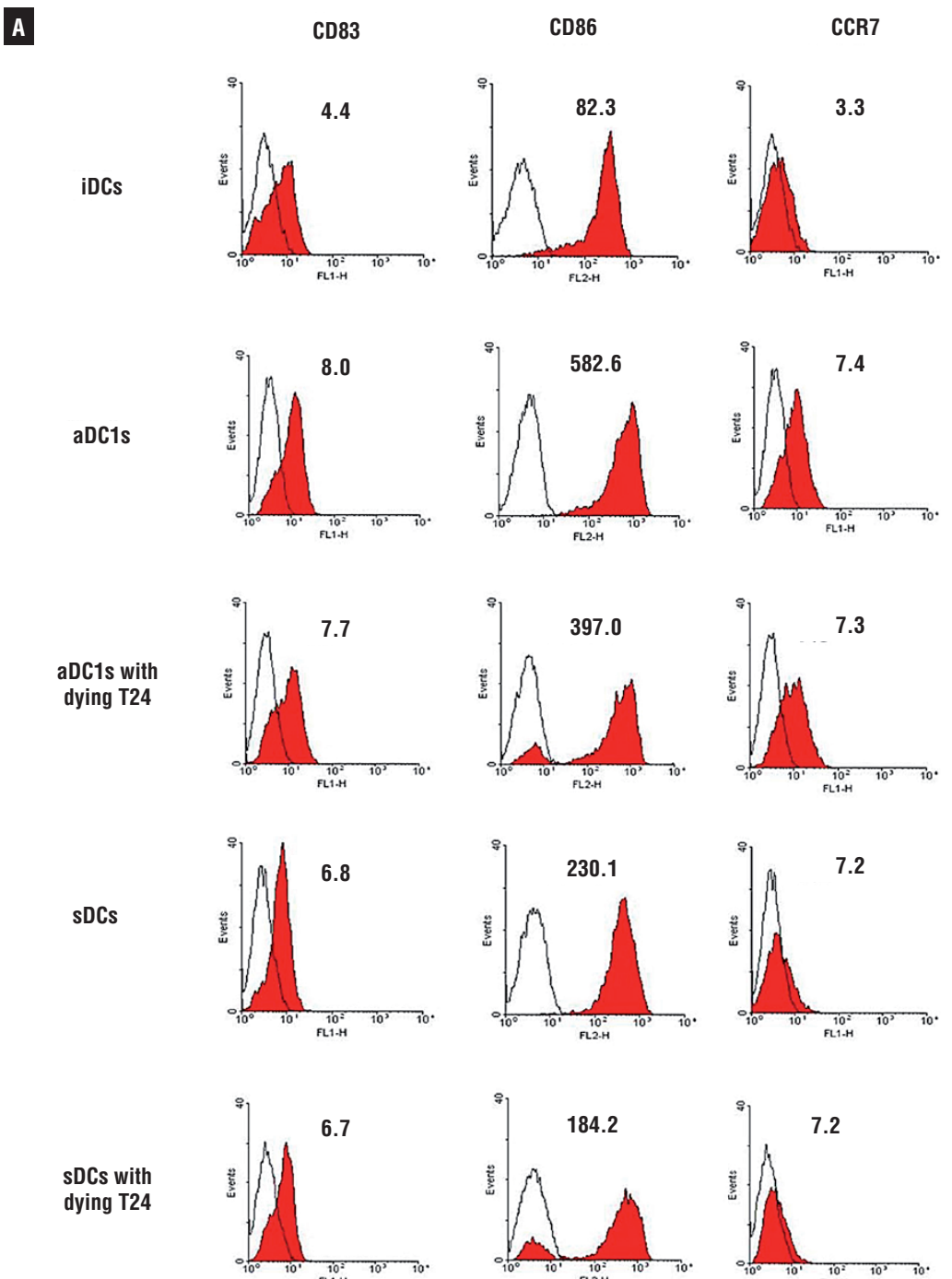
DISCUSSION

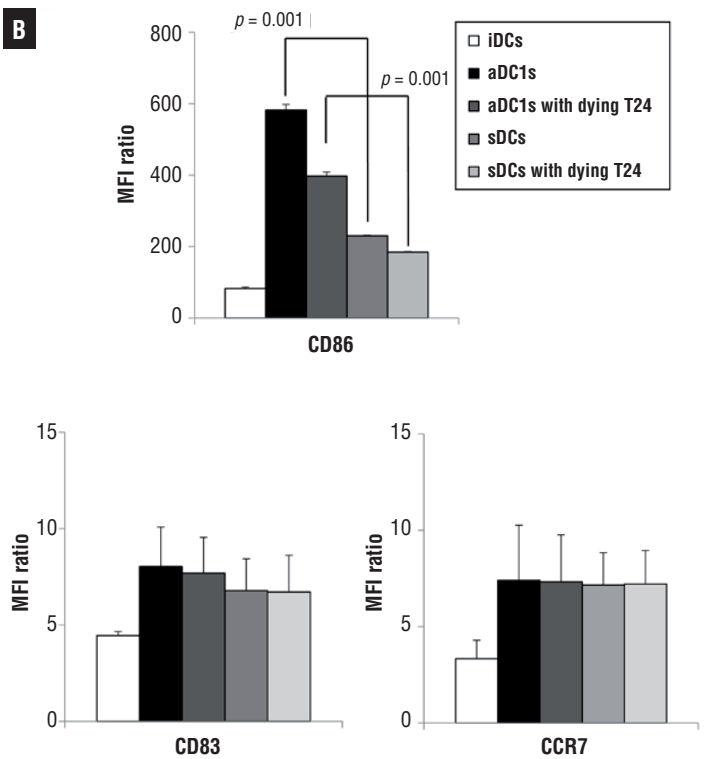
In this study, we investigated the feasibility of cellular immunotherapy using autologous DCs loaded with allogeneic dying bladder cancer cells that could generate potent bladder cancer-specific CTLs against the autologous bladder cancer cells of patients. The αDC1s were successfully generated and significantly increased the expression of several costimulatory molecules by the loading of tumor antigens. Furthermore, αDC1s showed a higher production of IL-12, without significant suppression by tumor antigen loading. In addition, potent bladder cancer-specific CTLs against autologous bladder cancer cells from patients were elicited by autologous αDC1s loaded with dying T24 cells, which was consistent withthe results of previous studies on other cancers (11, 16).

A majority of initial cancer immunotherapy trials have been performed in end-stage cancer patients, and the results of such trials have been disappointing (17). In recent years, there has been a paradigm shift away from administering cancer vaccines to advanced-stage patients and a move toward using cancer vaccines to treat earlier stages of carcinogenesis, before tumor- and treatment-mediated immunosuppressive environments can be established and before the accumulation of mutations that activate redundant pathways for tumor proliferation. Preliminary application of this strategy has yielded promising results. In a transgenic murine model of prostate adenocarcinoma, therapeutic vaccination directed against two different prostate cancer-associated antigens at the earliest stage of carcinogenesis elicited long--term protection against spontaneous prostate cancer development (18). Vaccination of premalignant cervical intraepithelial neoplasia lesions can cause their complete eradication or partial regression to a lower-grade lesion (19). DC--based immunotherapy therapy for NMIBC may fit this modelsince most cases of NMIBC have a reduced tumor burden due to TUR, and a recent study indicated that higher $CD3^+$ cell infiltration in NMIBC indicates better cancer-specific survival rates (20). All enrolled male patients in our study were diagnosed withNMIBC.

As mentioned previously, DCs are critical for the presentation of tumor antigens. However, although tumor-infiltrating dendritic cells (TIDC) are present in virtually all human cancers and

Figure 2- The expression of CD86 was higher in αDC1s than in sDCs (p <0.05). However, there was no difference in the expression of CD83 or CCR7 between the sDCs and αDC1s; A) X axis indicates mean fluorescence intensity (MFI) of FL1 (FITC) or FL2 (PE), and Y axis represent events (strength of fluorescence intensity), respectively;B) shows average MFI values from 3 independent experiments with SD).



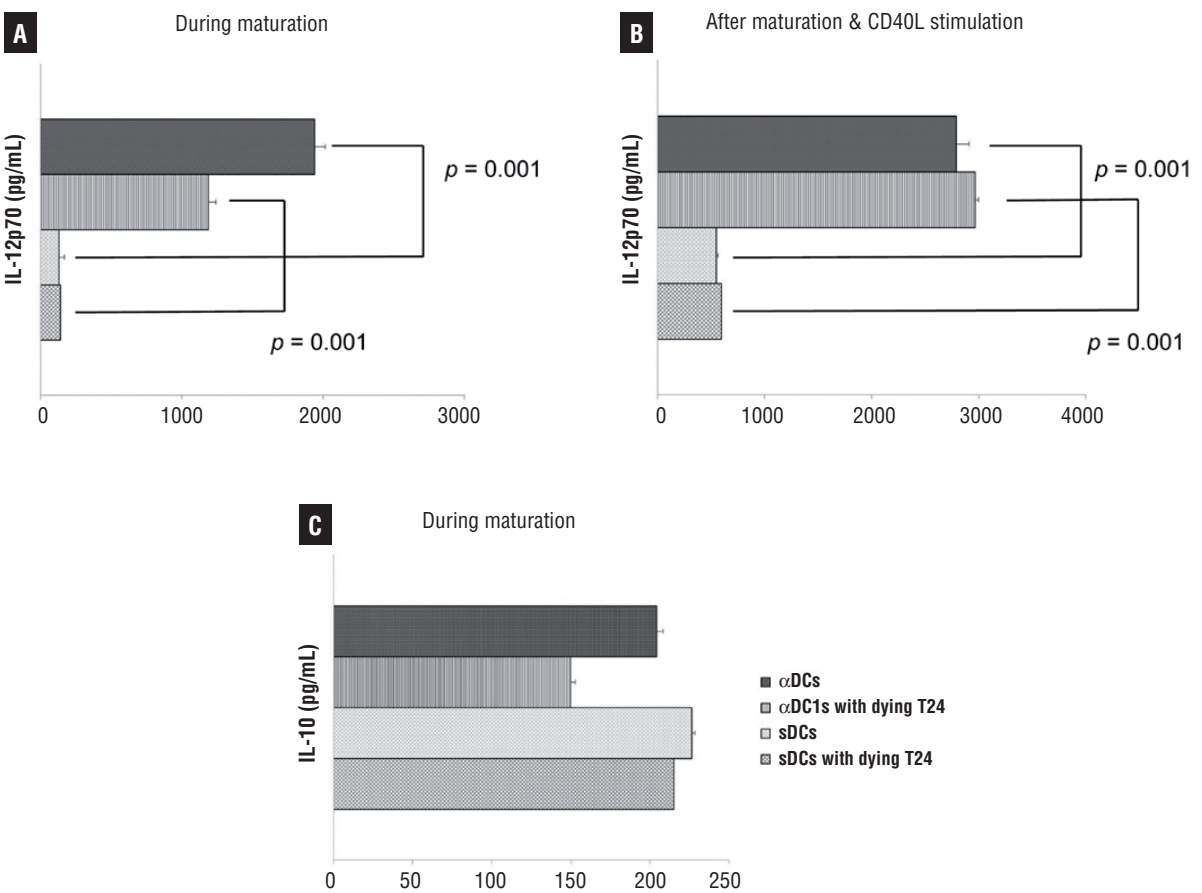


experimental tumor models, the tumor microenvironment compromises their differentiation, maturation, and survival (21). Any impairment of their normal function may represent a mechanism of tumor immune escape. Several studies demonstrated that DC generation and maturation are inhibited in patients with bladder cancer (22,23). According to Troy et al.(22), a very small proportion of DC in bladder cancer was found to express the activation antigen CD83. In addition, expression of the important costimulatory molecules CD80 and CD86, which are required for activation of T lymphocytes, was restricted. Similarly, Beatty et al.(23) reported that DCs from bladder cancer patient's urine and tumor tissues showed minimal expression of CD83 and CD86. Therefore, it is reasonable to expect that transplanted functional DCs engineered to overexpress and present specific antigens from bladder tumor cells may be effective in over comingthese dysfunctional autologous DCs. Aside from DC function, identifying effective immunogenic antigens is one of biggest challenges in DC-based vaccine development.

Fry et al.(24) showed that DCs loaded with irradiated apoptotic tumor cells elicited stronger immune responses than tumor cell RNA and tumor lysate. Its major advantage relies on the use of whole tumor proteome, encompassing that way multiple TAAs. Immunogenic cell death induced by UVB-irradiation stimulated the immune system through the release of damage-associated molecular patterns (DAMPs), including the exposure of calreticulins and release of ATP, HMGB1 and heat shock proteins (gp96, HSP70 and HSP90) (25).Based on their results, we used an irradiated dying allogeneic tumor cell line as a tumor antigen. This technique offers the advantages of requiring no knowledge of specific antigens, presenting no obstacles to the preparation of tumor cells, and possessing no need for HLA typing (26).

To enhance DC's capacity to induce an immune response, a number of immunotherapeutic approaches are being studied at the experimental level. DCs obtained from mice transfected with the Ag85A gene [which has been shown to induce substantial Th cell proliferation and vigorous Th1

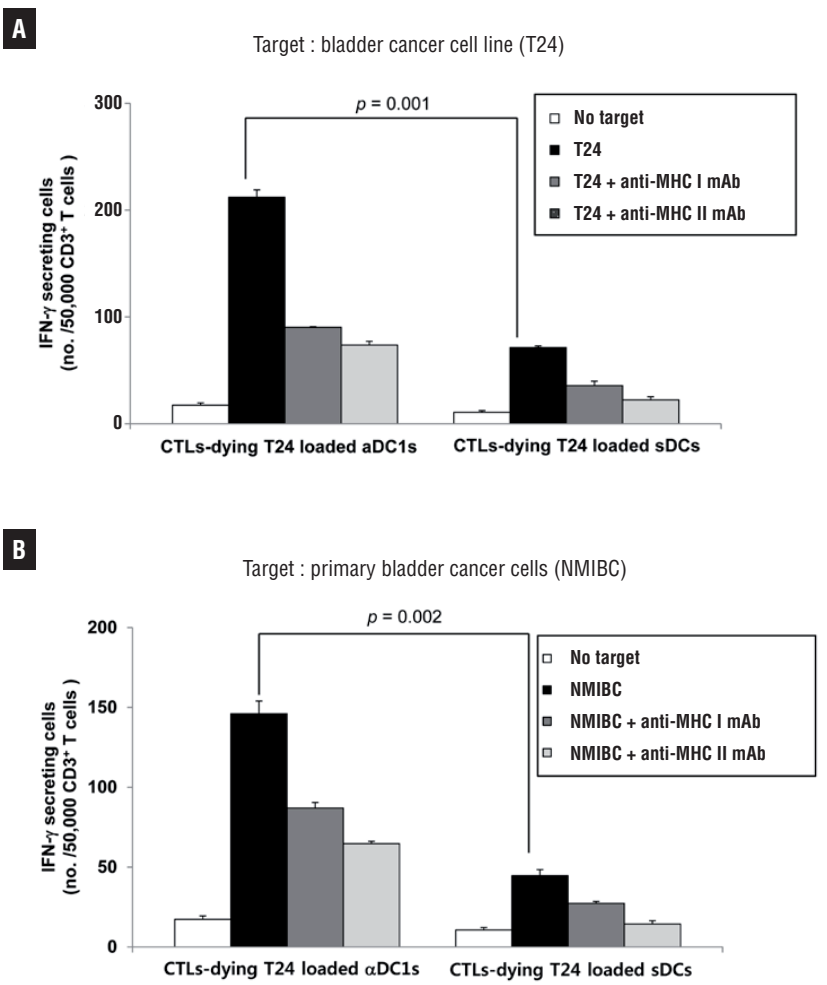
Figure3- Comparison of cytokine production by DCs loaded with or without tumor cells in A) primary culture supernatant during generation of DCs, and B) after stimulation with CD40L-transfected J558 cells. The αDC1s showed significantly higher production of IL-12p70 during maturation and after stimulation with CD40L-transfected J558 cellsthan the sDCsdid ($p<0.05$). In addition, production of the inhibitory cytokine IL-10 by αDC1s was not significant.C)Results, expressed as mean (pg/mL) ±SD of triplicate cultures, are representative of five independent experiments.



cytokine production in humans and mice infected with mycobacterial species, including individuals vaccinated with BCG (27)], which uses murine bladder tumor cell lysate as the source of antigen, exerted enhanced anti-tumor immunity against murine bladder cancer (13). In another study, DC obtained from human umbilical cord blood transfected with secondary lymphoid-tissue chemokine and the interleukin-2 gene, which used established bladder cancer celllines as the source of antigen, enhanced cytotoxicity against same bladder cancer cell lines (28). In addition, Nishiyama et al. (12) reported that autologous DCs pulsed with MAGE-3 peptide-induced autologous CTL in vitro showed tumor regression in pilot clinical trials for advanced bladder cancer.

Our study differs from the above in several important respects. We used DCs primed with dying cells from allogeneic cell lines. In addition to the induction of increased number of tumor--specific T cells, the use of Th1-polarized αDC1s with selectively elevated ability to attract effector (rather than regulatory T cells) and to induce the IL-12-dependent effector (CTL) pathway of

Figure 4- Comparison of bladder cancer-specific CTL induction in vitro with DCs loaded with T24 cells against A)the T24 cell line andB)autologous bladder cancer cells (NMIBC). CD8+ T cells primed by T24 cell-loaded αDC1s showed a larger number of IFN-γ releases than those stimulated by sDCs (p <0.05). ELISPOT data are the mean (±SD) number of IFN-γ-secreting cells of triplicate cultures in three independent experiments.



differentiation in CD8+ T cells may help to induce tumor-specific immunity of a more desirable pattern than that induced by whole tumor cells or standard non-polarized sDCs (29,30). Additionally, unlikethe Nishiyama study (12), we tested the feasibility of employing αDC1-based vaccines for the treatment of male NMIBC patients with autologous tumor tissue.

In this study, we showed that autologous αDC1s loaded with dying T24 cells could prime T cells to generate potent bladder cancer-specific CTLs thatcould kill bladder cancer cells in maleNMIBC patients. The inhibition of MHC-class molecules by the use of an anti-MHC class antibody prevented the development of CTL responses specific to autologous bladder cancer cells in patients. Thus, the use of an allogeneic tumor cell line as a source of tumor antigens could generate MHC class-restricted T cell responses against autologous bladder cancer cells. However, despite the strong immune data presented in our study, further in vivo and preclinical studies are needed for the development of immunotherapy using αDC1s as a novel treatment for NMIBC.

CONCLUSIONS

The present study showed that autologous αDC1s loaded with allogeneic dying bladder cancer cells could generate strong bladder cancer-specific CTLs against autologous bladder cancer cells. This technique may offer a highly feasible and effective method for DC-based cancer immunotherapy in male NMIBC patients. Further studies are necessary to enhance the in vivo anti-tumor effect of bladder cancer-specific CTLs generated by autologous dying bladder cancer cells against bladder cancer before clinical application.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Quality of Life after post-prostatectomy intensity modulated radiation therapy to the prostate bed with or without the use of gold fiducial markers for image guidance or higher total radiotherapy doses

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ABSTRACT

Purpose: To evaluate quality of life (QoL) after post-prostatectomy intensity modulated radiation therapy (IMRT) in the "adjuvant" setting starting within 4 months of radical prostatectomy for adverse features; and "salvage" setting for a PSA≥0.2ng/mL.
Materials and Methods: Retrospective review of 130 patients who underwent IMRT to the prostate bed±gold fiducial marker placement for image guidance to 64.8-72.0Gy (median, 70.2Gy) between 2004 and 2013. Higher doses were defined as 70.2-72.0Gy and lower doses were defined as 64.8-68.4Gy. Androgen deprivation therapy (ADT) was given to 4/48 (8%) adjuvant patients and 9/82 (11%) salvage patients. International Prostate Symptom Score (IPSS), Sexual Health Inventory for Men (SHIM), and Expanded Prostate Cancer Index Composite-26-bowel (EPIC-26-bowel) questionnaires were used to assess urinary, sexual, and bowel QoL, respectively.
Results: Median follow-up was 46 months. There were better urinary (p=0.03) and sexual (p=0.002) QoL scores with adjuvant IMRT relative to salvage IMRT. The use of prostate bed fiducial markers did not significantly affect urinary, sexual, or bowel QoL (p=0.39, p=0.49, and p=0.40, respectively). Higher total radiotherapy doses did not significantly affect urinary, sexual, or bowel QoL (p=0.21, p=0.61, and p=0.36, respectively).
Conclusions: There was no significant change in urinary, sexual, and bowel sexual QoL with post-prostatectomy IMRT regardless of whether prostate bed fiducial markers or higher total radiotherapy doses were used. QoL with IMRT in the present study compares favorably with prior reports for three-dimensional conformal radiation therapy.

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INTRODUCTION

Three Phase III studies have shown a benefit to post-prostatectomy radiation therapy (1-4). There is no consensus on the definitions of post-prostatectomy "adjuvant" radiotherapy versus "salvage" radiotherapy. Similar to the Tasman Radiation Oncology Group Radiotherapy and Androgen Deprivation In Combination After Local Surgery (RAVES) trial (5, 6), this study defines adjuvant radiotherapy as treatment starting within 4 months of radical prostatectomy for positive surgical margins, extraprostatic extension, or seminal vesicle invasion and salvage radiotherapy

as treatment for a post-prostatectomy prostate-specific antigen (PSA) measurement≥0.2ng/mL.

Physicians under-estimate worsening and over-estimate improvement in symptoms relative to patients (7). As a result, it is important to measure patient-reported outcomes when assessing quality of life (QoL). The primary aim of this retrospective observational study is to assess urinary, sexual, and bowel QoL prior to and following the delivery of post-prostatectomy intensity modulated radiation therapy (IMRT) to the prostate bed. QoL is compared in patients who received adjuvant IMRT versus salvage IMRT. Also, QoL is examined in patients who did or did not undergo placement of gold fiducial markers in their prostate bed for image guidance or received higher versus lower total radiotherapy doses.

MATERIALS AND METHODS

Patient Characteristics

After obtaining institutional review board approval, the authors reviewed the medical records of 130 prostatectomy patients who did not have evidence of regional or distant metastases on computed tomography scans of the abdomen and pelvis and bone scans prior to the initiation of IMRT. Patients underwent radiotherapy at a single institution between 2004 and 2013. Patients were included in this study if they underwent IMRT to the prostate bed alone. Patients were excluded if they underwent elective pelvic lymph node irradiation since it can result in worse acute urinary and bowel QoL (8). Twenty-eight percent of radical prostatectomy patients had positive surgical margins, extraprostatic extension, or seminal vesicle invasion and were referred within one month of surgery for consultation regarding the option of adjuvant radiotherapy beginning within 4 months of surgery.

Urinary, Sexual, and Bowel Quality of Life Questionnaires

When QoL data started to be collected in 2004 at our center, the International Prostate Symptom Score (IPSS) questionnaire was a popular method of assessing urinary QoL (9). As a result, this survey was adopted by the Department of Radiation Oncology and Department of Urology to assess urinary QoL. IPSS scores range from 0 to 35 with lower scores indicating a higher urinary QoL. Similarly, the SHIM questionnaire was commonly used in 2004 to assess sexual QoL (8). Consequently, this survey was adopted by the Department of Radiation Oncology and Department of Urology to grade sexual QoL and used in this report. SHIM scores range from 1 to 25 with higher scores indicating better sexual QoL. The Expanded Prostate Cancer Index Composite-26 (EPIC-26) instrument is a research tool used for capturing patient-reported QoL outcomes related to the domains of bladder, sexual, and bowel functioning for men undergoing treatment for prostate cancer (10). In this study, EPIC-26 was used to assess bowel (EPIC-26-bowel) QoL. The bowel summary score can range from 0 to 100, with a higher score indicating a better QoL. The urinary and sexual domains of the EPIC-26 form were not given to patients because IPSS and Sexual Health Inventory for Men (SHIM) forms were used to assess urinary and sexual QoL, respectively. In 2015, the International Consortium for Health Outcomes Measurement recommended EPIC-26 as the preferred method for measuring QoL in men with localized prostate cancer (11). As a result, we presently use EPIC-26 to determine urinary, sexual, and bowel QoL. The IPSS, SHIM, and EPIC-26 questionnaires are validated measures of QoL.

IPSS, SHIM, and EPIC-26-bowel questionnaires were given to patients prior to IMRT. Also, the questionnaires were given to patients after IMRT every 3 months during the first year, every 6 months during the second through third years, and annually during the fourth through seventh years of follow-up.

Intensity Modulated Radiation Therapy

Adjuvant IMRT started within 4 months of radical prostatectomy once any operative side effects had improved. In contrast, salvage IMRT started a median of 25 months postprostatectomy. There was more frequent delivery of tamsulosin hydrochloride in the adjuvant IMRT subgroup. Patients were simulated with an empty rectum using a pelvic CT scan using 3mm cuts. An urethrogram was performed, and 40mL saline mixed with 10mL

non-ionic contrast was injected into the bladder at the time of simulation. The European Organization for Research and Treatment of Cancer (EORTC) Radiation Oncology Group guidelines (12) were used to define the post-prostatectomy clinical target volume (CTV) and planning target volume (PTV). These guidelines allow for escalation of the total radiotherapy dose (13). Prescribed total doses were 64.8-72.0Gy using daily 1.8-Gy fractions (2). The median total dose was 70.2Gy using 1.8-Gy daily. The minimum allowable dose delivered to the PTV was 93% of the prescribed dose, and the maximum allowable dose delivered to the PTV was 115% of the prescribed dose. At least 98% of the PTV received ≥95% of the prescribed dose (14). The dosimetric goals for organs at risk were that no more than 25% of bladder or rectal volumes should receive >60Gy.

Image-Guided Radiation Therapy

Between 2009 and 2013, two of the radiation oncologists who specialize in the treatment of prostate cancer at our center inserted prostate bed fiducials in 45 patients. The other two radiation oncologists who specialize in the treatment of prostate cancer did not use prostate bed fiducials. Three gold fiducial markers were transrectally inserted under local anesthesia at the prior site of the seminal vesicles, right mid lateral prostate, and prostatic apex. The markers made it possible to determine the location of the prostate bed using electronic portal imaging immediately prior to each IMRT treatment. In order to account for inter-fraction organ motion, the patient's IMRT setup was adjusted each day based on the location of the markers.

Androgen Deprivation Therapy

Androgen deprivation therapy (ADT) always consisted of a gonadotropin-releasing hormone agonist. The median duration of a gonadotropin-releasing hormone agonist was 6 months. In some cases, ADT also included an anti-androgen. The median duration of an antiandrogen was one month starting two weeks prior to the gonadotropin-releasing hormone agonist. ADT was given at the discretion of the treating physician to 4/48 (8%) adjuvant IMRT patients and 9/82 (11%) salvage IMRT patients. ADT tended to be used in patients with extraprostatic extension and/or seminal vesicle invasion (pT3 disease). In the adjuvant IMRT subgroup, the median PSA was 0.1ng/mL at the start of radiotherapy in those who did and did not receive ADT. In contrast, in the salvage IMRT subgroup, the median PSA was 0.4ng/mL at the start of radiotherapy in those who did and did not receive ADT. In both the adjuvant and salvage subgroups, the median Gleason score on the prostatectomy specimen was 3+4=7 in those who did and did not receive ADT.

Phosphodiesterase-5 inhibitors

When used at the discretion of a patient and his physician, a phosphodiesterase-5 inhibitor typically started within the first 3 post-operative months (15). Sildenafil citrate was offered to men with erectile dysfunction, defined as a SHIM score <22, at an initial dose of 50mg two to three times per week. The dose was titrated to 100mg/day if there was no response at 50mg. Alternatively, vardenafil hydrochloride was prescribed at a starting dose of 10mg two to three times/week. This dose was titrated to 20mg up to three times weekly if needed. Men were encouraged to take up to 12 doses per month. Patients were advised to continue taking either medication for at least six doses before considering the drug to be a treatment failure. If one particular oral phosphodiesterase-5 inhibitor failed, the patient was offered an alternative oral agent. Adjuvant IMRT patients elected to use phosphodiesterase-5 inhibitors more often than salvage IMRT patients.

Definition of Recurrent Disease Post-IMRT

In the adjuvant IMRT group, recurrent disease post-irradiation was defined as a PSA ≥0.2ng/mL with a second confirmatory PSA ≥0.2ng/mL (2). In the salvage IMRT group, progressive disease was defined as a PSA ≥0.2ng/mL above the post-radiotherapy nadir followed by another higher value, a continued rise in PSA despite IMRT, initiation of systemic therapy after IMRT, or clinical progression (16).

Statistics

Statistical analysis was performed using Statistical Analysis System 9.3 (SAS Institute Inc., Cary, NC, USA). A two-sided t-test was used to calculate the difference in means. A means procedure was used to compute descriptive statistics. A mixed model for repeated measurements was used to compare QoL scores over time amongst subgroups. An α (type I) error <0.05 was considered statistically significant.

RESULTS

Median follow-up was 46 months (range, 3-116 months). Characteristics of the adjuvant and salvage radiotherapy patients are presented in Table-1. More patients (58%) in the adjuvant IMRT group had positive surgical margins than patients in the salvage IMRT group (37%) (p=0.02). However, there was no significant difference in the age of the adjuvant IMRT (mean±standard deviation: 61±7 years) versus salvage IMRT (mean±standard deviation: 63±8 years) patients (p=0.16). Similarly, there was no significant difference in the baseline SHIM scores in the adjuvant IMRT (mean±standard deviation: 5±7) versus salvage IMRT (mean±standard deviation: 6±8) patients (p=0.70). Patient compliance with questionnaire completion was 76% over the first 3 years post-irradiation and decreased thereafter. There were significantly better urinary QoL scores in the adjuvant IMRT relative to the salvage IMRT group (p=0.03, Figure-1). Similarly, there were significantly better sexual QoL scores in the adjuvant IMRT relative to salvage IMRT group (p=0.002, Figure-2). Bowel QoL scores did not change significantly after adjuvant or salvage IMRT (p=0.43, Figure-3). The use of prostate bed fiducial markers was not associated with urinary, sexual, or bowel QoL (p=0.39, p=0.49, and p=0.40, respectively). Higher total radiotherapy doses (70.2-72.0Gy versus 64.8-68.4Gy) did not significantly affect urinary, sexual, or bowel QoL (p=0.21, p=0.61, and p=0.36, respectively, Figure-4).

DISCUSSION

Few studies have focused on QoL following post-prostatectomy radiotherapy (17). This study adds to the small body of literature assessing QoL in patients who underwent modern post- prostatectomy radiation using IMRT to the prostate bed (18, 19). Advantages of this study are that modern radiotherapy techniques were used with relatively uniform planning target volumes. Disadvantages are that it is a retrospective study with the resulting potential for selection bias in subgroups. For example, there was an imbalance in the use of Tamsulosin hydrochloride and phosphodiesterase-5 inhibitors. Also, the study has a limited number of patients (n=130). In addition, QoL trajectory after surgery and before adjuvant or salvage IMRT was not examined. Moreover, only 76% of patients completed QoL questionnaires over the first 3 years post-irradiation, with worsening compliance thereafter. Similarly, other groups have reported 36-78% patient compliance rates with questionnaire completion (20, 21).

A key concern of clinicians and patients is that post-prostatectomy radiotherapy will cause deterioration in urinary, sexual, and bowel QoL (17). As a result, more than three quarters of North American prostatectomy patients with either adverse pathological features or an early rise in their postoperative PSA do not undergo post-prostatectomy radiation therapy (17, 22). IMRT is the preferred technique in the United States for post-prostatectomy radiotherapy since it can result in less acute toxicity (17, 23) and better urinary and bowel QoL compared with three-dimensional conformal radiation therapy (3DCRT) (18, 19). Consequently, this QoL report was limited to patients who underwent post-prostatectomy IMRT. In accordance with the findings of others (18, 24) urinary (Figure-1) and bowel (Figure-3) QoL following post-prostatectomy IMRT compare favorably with prior reports on QoL after post-prostatectomy 3DCRT.

In this study, adjuvant IMRT was associated with better urinary QoL than salvage IMRT (p=0.03, Figure-1). This may be related to more frequent use of tamsulosin hydrochloride in the adjuvant IMRT group. Also, this may be due to more rapid recovery of urinary QoL over the first year after radical prostatectomy (25), as is commonly seen in younger patients with few comorbities (26). Adjuvant IMRT started within 4 months of prostatectomy whereas salvage IMRT started a

Table 1 - Characteristics of patients treated with adjuvant and salvage radiotherapy.

Variable	Level	N (%)			P Value
		Total	Adjuvant (<0.2) (n=48)	Salvage (≥0.2) (n=82)	
Gleason Score on Prostatectomy Specimen	6	26 (20%)	6 (13%)	20 (25%)	0.16
	7	91 (70%)	36 (75%)	55 (67%)	.
	8	9 (7%)	3 (6%)	6 (7%)	.
	9	4 (3%)	3 (6%)	1 (1%)	.
	Total	**130**	**48 (100%)**	**82 (100%)**	.
Pathologic T Stage	T2a	26 (20%)	7 (15%)	19 (23%)	0.25
	T2b	2 (2%)	0 (0%)	2 (2%)	.
	T2c	61 (47%)	24 (50%)	37 (45%)	.
	T3a	27 (21%)	11 (23%)	16 (20%)	.
	T3b	14 (11%)	6 (13%)	8 (10%)	.
	Total	**130**	48 (100%)	**82 (100%)**	.
Pathologic N stage	N0	61 (47%)	22 (46%)	39 (48%)	0.85
	NX	69 (53%)	26 (54%)	43 (52%)	.
	Total	**130**	**48 (100%)**	**82 (100%)**	.
M stage	M0	61 (47%)	21 (44%)	40 (49%)	0.58
	MX	69 (53%)	27 (56%)	42 (51%)	.
	Total	**130**	**48 (100%)**	**82 (100%)**	.
AJCC stage	I	7 (5%)	1 (2%)	6 (7%)	0.37
	II	83 (64%)	30 (62%)	53 (65%)	.
	III	40 (31%)	17 (35%)	23 (28%)	.
	Total	**130**	**48 (100%)**	**82 (100%)**	.
Extraprostatic Extension	No	95 (73%)	33 (69%)	62 (76%)	0.41
	Yes	35 (27%)	15 (31%)	20 (24%)	.
	Total	**130**	**48 (100%)**	**82 (100%)**	.
Seminal vesicles invasion	No	116 (89%)	42 (87.5%)	74 (90%)	0.77
	Yes	14 (11%)	6 (12.5%)	8 (10%)	.
	Total	**130**	**48 (100%)**	**82 (100%)**	.
Positive Surgical Margins	No	72 (55%)	20 (42%)	52 (63%)	0.02
	Yes	58 (45%)	28 (58%)	30 (37%)	.
	Total	**130**	**48 (100%)**	**82 (100%)**	.
Total Dose (Gy) - Median	64.8-68.4	56 (43%)	22 (46%)	34 (41.5%)	0.71
	70.2-72.0	74 (57%)	26 (54%)	48 (58.5%)	.
	Total	**130**	**48 (100%)**	**82 (100%)**	.
Androgen Deprivation Therapy	No	117 (90%)	44 (92%)	73 (89%)	0.77
	Yes	13 (10%)	4 (8%)	9 (11%)	.
	Total	**130**	**48 (100%)**	**82 (100%)**	.
Fiducials placed in Prostate Bed	No	88 (68%)	31 (65%)	57 (69.5%)	0.6940
	Yes	42 (32%)	17 (35%)	25 (30.5%)	.
	Total	**130**	**48 (100%)**	**82 (100%)**	.

Figure 1 - Mean IPSS (urinary QoL) scores with standard error bars in patients who received adjuvant IMRT (—) or salvage IMRT (...) (p=0.03).

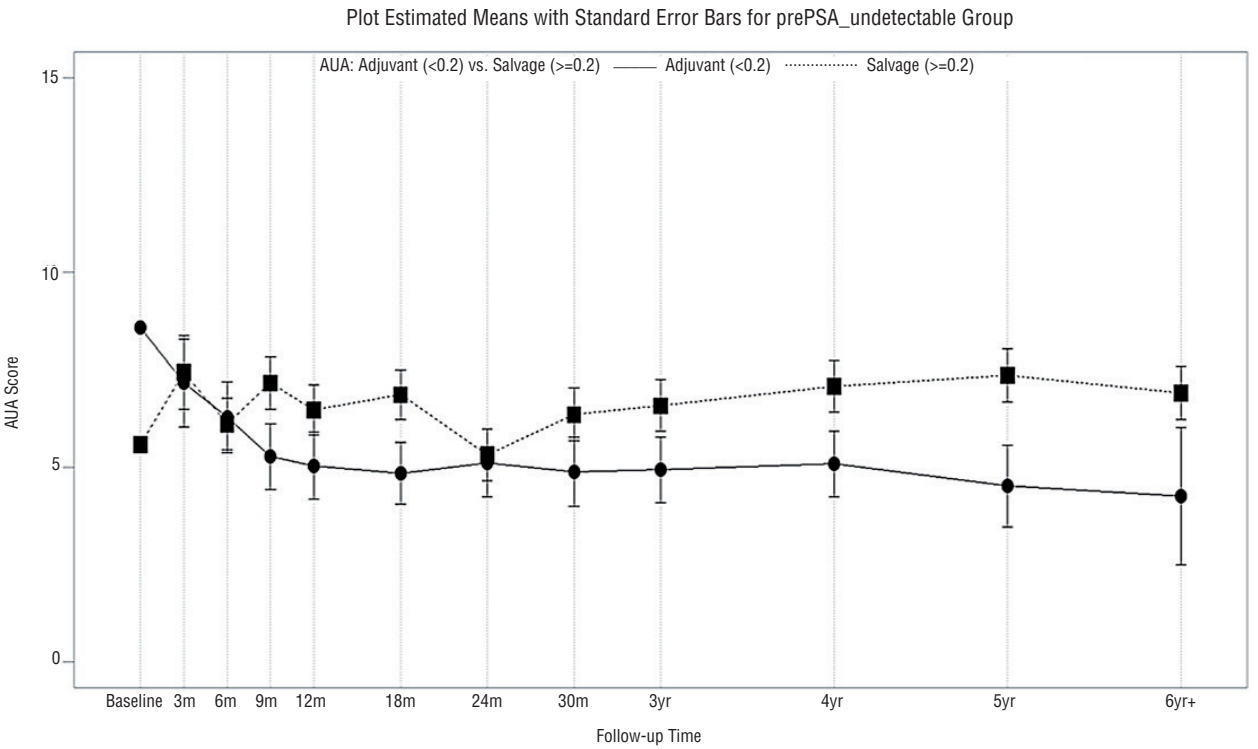


Figure 2 - Mean SHIM (sexual QoL) scores with standard error bars in patients who received adjuvant IMRT (—) or salvage IMRT (...) (p=0.002).

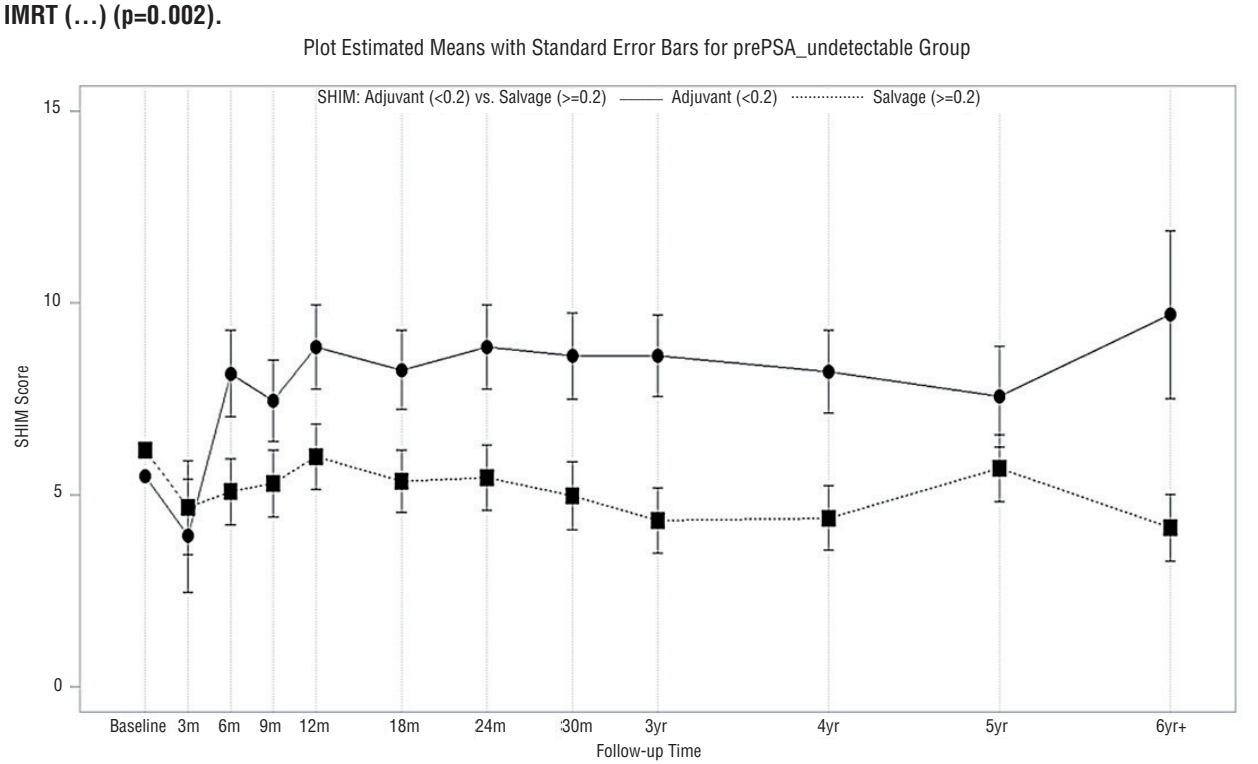


Figure 3 - Mean EPIC-26-bowel QoL scores with standard error bars in patients without fiducial markers (—) or with fiducial markers (...) in their prostate bed (p=0.40).

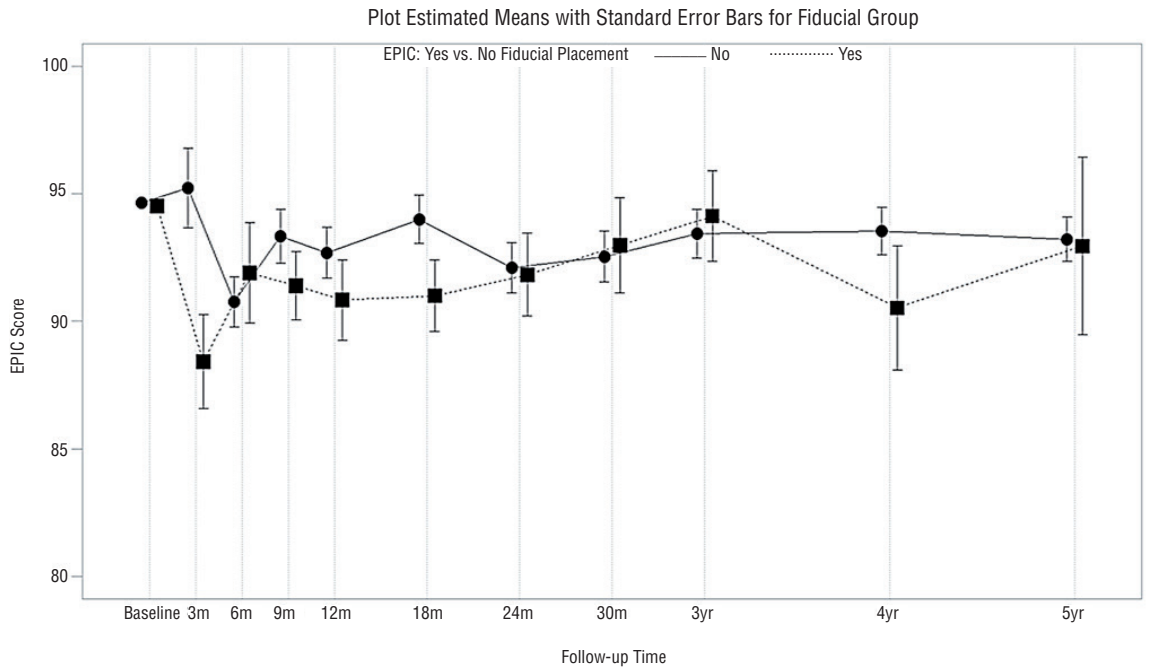
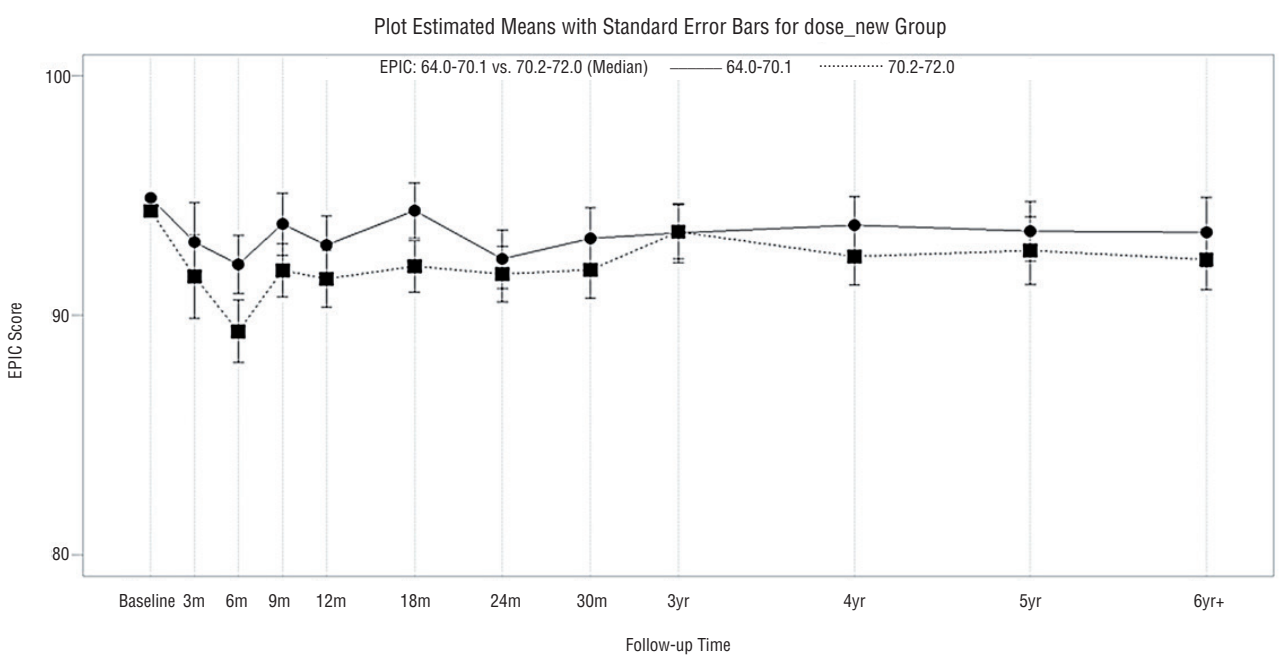


Figure 4 - Mean EPIC-26-bowel QoL scores with standard error bars in patients who received total radiotherapy doses of 64.8-68.4Gy (—) or 70.2-72.0Gy (...) (p=0.36).



median of 25 months post-prostatectomy. There was no significant difference (p=0.70) in baseline SHIM scores in the adjuvant IMRT versus salvage IMRT groups. However, adjuvant IMRT patients used phosphodiesterase-5 inhibitors more often than salvage IMRT patients. Adjuvant IMRT patients typically started to use phosphodiesterase-5 inhibitors within the first 3 postoperative months. This may help to explain why better sexual QoL was observed in adjuvant IMRT compared with salvage IMRT patients, particular over the first year post-irradiation (Figure-2). The use of early intervention or prophylactic phosphodiesterase-5 inhibitors resulted in improvement in overall sexual function in patients with intact prostate treated with IMRT or brachytherapy (15, 27). Also, it is possible that confounding factors for sexual QoL post-prostatectomy such as prostate size, educational level, or income could have been different between the adjuvant IMRT and salvage IMRT groups (28). Schiffner et al. (29) examined 10 patients who were treated with postoperative radiotherapy and had radio-opaque markers implanted transrectally into the prostate bed using ultrasound guidance. Although the motion of the prostate bed was less than that of an intact prostate, positioning errors exceeded 5mm in many treatment fractions. Therefore, they recommend using daily, image-guided, soft tissue verification with fiducial markers to improve the inter-fraction targeting of the prostate bed. By transrectally placing 3 fiducial markers in the prostate bed under ultrasound guidance, one can improve the accuracy of external beam radiotherapy compared with the use of radical prostatectomy clips (30). However, in the current study, placement of fiducial markers in the prostate bed was not associated with improved urinary, sexual, or bowel QoL (Figure-3).

The National Comprehensive Cancer Network Clinical Practice Guidelines for Prostate Cancer Version 2.2016 recommend adjuvant/salvage post-prostatectomy total radiotherapy doses of 64-72Gy in standard fractionation. In accordance with these guidelines, some groups have recommended higher total radiotherapy doses, i.e., 70-72Gy, in the adjuvant (31, 32) or salvage (33, 34) setting. In the present study, higher total radiotherapy doses did not significantly affect urinary, sexual, or bowel QoL (p=0.21, p=0.61, and p=0.36, respectively, Figure-4). The RAVES (6), Medical Research Council (UK) and National Cancer Institute of Canada Clinical Trials Group Radiotherapy and Androgen Deprivation In Combination After Local Surgery (RADICALS) (35), and French Groupe d'E´tude des Tumeurs Uro-Genitales (GETUG)-17 (36). Phase III studies will clarify whether adjuvant radiotherapy is equivalent to observation with early salvage radiotherapy for those who relapse (5). Moreover, the RADICALS (35), GETUG-16, and radiation Therapy Oncology Group 9601 and 0534 Phase III studies will determine if there is a benefit to adding either 4-6 months or 2 years of ADT to salvage radiotherapy.

In conclusion, there was no significant change in urinary, sexual, and bowel QoL with postprostatectomy IMRT regardless of whether prostate bed fiducial markers or higher total radiotherapy doses were used. QoL with IMRT in the current study compares favorably with prior reports for three-dimensional conformal radiation therapy.

CONFLICT OF INTEREST

None declared.

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SCUBE1: a promising biomarker in renal cell cancer

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ABSTRACT

Purpose: To investigate the efficacy of signal peptide-CUB-EGF domain-containing protein 1 (SCUBE-1) as a novel biomarker of renal tumors.
Materials and Methods: 48 individuals were included in the study. The patient group (Group-1) consisted of 23 subjects diagnosed with renal tumor, and the control group (Group-2) of 25 healthy individuals. Patients diagnosed with renal tumor received surgical treatment consisting of radical or partial nephrectomy. Blood specimens were collected following overnight fasting. Signal peptide-CUB-EGF domain-containing protein 1 (SCUBE-1), soluble urokinase plasminogen activator receptor (suPAR) and carbonic anhydrase IX (CA IX) levels were measured from plasma samples. Patients in groups 1 and 2 were compared in terms of these biochemical parameters.
Results: The 23-member renal tumor group was made up of 17 (73.91%) male and 6 (26.08%) female patients with a mean age of 58.5±15.7 years (range 25 to 80). The 24-member healthy control group was made up of 16 (64%) male and 9 (36%) female subjects with a mean age of 52.4±9.12 years (range 40 to 67). Analysis revealed significant elevation in SCUBE-1 levels in the renal tumor group (p=0.005). No significant differences were detected between the groups with regard to CA IX or suPAR measurements (p=0.062 vs. p=0.176).
Conclusions: SCUBE-1 appears to represent a promising biomarker in the diagnosis and follow-up of patients with renal tumor.

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INTRODUCTION

Renal cell carcinoma represents 2-3% of all adult malignancy neoplasms and is the most lethal urological cancer (1). Clear cell renal cell carcinoma (CCRCC) constitutes approximately 80% of renal cell carcinomas and follows a more aggressive course than papillary and chromophobe carcinoma, other pathological subtypes. Metastasis is present at diagnosis in approximate 1/3 patients, and there is a 30-40% risk of metastasis developing after surgery in subjects with localized disease at time of diagnosis (2). Radical or partial nephrectomy is the current gold standard in the treatment of localized disease.

Clinical, anatomical and histopathological criteria are used to determine prognosis of renal cancer. However, research aimed at identifying a biomarker that can be used in diagnosis, monitoring and determining tumors with a high risk of

recurrence and that can show the need for early adjuvant and advanced treatment in metastatic patients is still ongoing.

Transmembrane carbonic anhydrase IX (CAIX) is one component of the carbonic anhydrase family. It is an efficient catalyst of the reversible hydration of carbon dioxide into bicarbonate and a proton. This permits tumor cells to preserve a neutral pH in the presence of an acidic microenvironment. Expression of CAIX does not occur in healthy kidney tissue. However, it does occur in the majority of CCRCCs. This occurs by way of HIF-1a overexpression caused by hypoxia and inactivation of the von Hippel-Landau gene (3). Some studies have described CA IX as a prognostic marker in patients with metastatic clear-cell renal cell carcinoma (mccRCC) (4). CAIX, one of the most studied biomarker in CCRCC, is considered promising.

Soluble urokinase plasminogen activator receptor (suPAR) is a glycosylphosphatidylinositol (GPI) membrane protein that binds to urokinase-type plasminogen activator receptor (uPAR) in soluble form (5) suPAR is produced by various types of cells, such as vascular endothelial cells, neutrophils and monocytes, and is thought to be associated with chronic inflammatory conditions (6). Studies have shown that suPAR is correlated with poor prognosis in some types of cancer (7, 8).

Signal peptide-CUB-EGF domain-containing protein 1 (SCUBE-1) is a cell surface glycoprotein. This novel biochemical marker is expressed and secreted in early embryogenesis and is present in platelets and endothelial cells (9). The SCUBE gene family contains three different isoforms, including SCUBE-1 (SCUBE 1-3) (10). SCUBE-1, a cell surface protein, has been investigated in various types of cancer and non-cancer diseases.

Based on the objective of developing a biomarker capable of use in renal tumors, we investigated SCUBE-1, a marker that has not previously been studied in patients with renal tumor. We compared SCUBE-1, a potentially novel marker, with CA IX and suPAR, previously investigated markers in renal tumors.

MATERIAL AND METHODS

Study population

Forty-eight individuals were included in the study, 23 patients diagnosed with renal tumor (Group-1) and a control group of 25 healthy subjects (Group-2). All members of both groups provided informed consent. The Karadeniz Technical University Medical Faculty Ethical Committee approved the study. All of the patients were evaluated clinically and they were also previously biochemical and radiologically investigated. Surgical treatment in the form of radical or partial nephrectomy was performed in all cases of diagnosed renal tumor.

Blood samples

Blood samples were collected from patients following overnight fasting. These were taken from the peripheral vein and stored at 4°C. Plasma specimens were obtained by centrifuging the blood samples at 3000rpm for 10 min. Plasma specimens were then stored at -80°C until biochemical analysis.

Biochemical measurements

Measurement of signal peptide-CUB--EGF domain-containing protein 1 (SCUBE-1) levels.

Levels of SCUBE-1 were determined using an enzyme-linked immunosorbent assay kit (Cusabio Biotech Co., Catalog No. CSB-E15005h, P.R. China) in line with the manufacturer's instructions. The absorbance of samples was measured at 450nm using a VersaMax tunable microplate reader (designed by Molecular Devices in California, USA). The results were expressed as ng/mL. The minimum detectable level of human SCUBE-1 is generally lower than 0.16ng/mL.

Measurement of soluble urokinase plasminogen activator receptor (suPAR) levels.

Levels of suPAR were determined using an enzyme-linked immunosorbent assay kit (ViroGates A/S., Denmark) following the manufacturer's protocols. The absorbance of samples was measured at 450nm using a VersaMax tunable microplate reader (designed by Molecular Devices in California, USA). The results were expressed as ng/mL. The estimated detection limit was 0.1ng/mL.

Measurement of carbonic anhydrase IX (CA IX) levels.

Serum levels of human CA IX were determined using an enzyme-linked immunosorbent assay kit (R&D systems, Catalog No. DCA 900, P.R. China) in line with the manufacturer's protocols. The absorbance of samples was measured at 450nm using a VersaMax tunable microplate reader (Designed by Molecular Devices in California, USA). The results were expressed as pg/mL. The minimum detectable dose of human CA IX is generally lower than 2.28pg/mL.

Statistical analysis

Statistical analyses were performed using computer software (SPSS version 13.0 software, Chicago, Illinois, USA). Data were expressed as mean±standard deviation. The Mann-Whitney U test and t-test were used for statistical analyses. Spearman correlation analysis was used to determine the correlation between biochemical parameters in the groups. Statistical significance was set at $p<0.05$.

RESULTS

Forty-eight patients were enrolled in the study. The renal tumor group consisted of 23 patients, 17 (73.91%) of whom were male and 6 (26.08%) female, with a mean age of 58.5±15.7 (range 25 to 80). The healthy control group consisted of 24 subjects, 16 (64%) male and 9 (36%) female, with a mean age of 52.4±9.12 (range 40 to 67). Tumors were removed by the methods of radical nephrectomy and partial nephrectomy (nephron sparing surgery) in 18 patients (78.3%) and 5 patients (21.7%), respectively. The pathological distribution of the tumors (pathological type, Fuhrman's nuclear grade, pathological stage) in patients is shown in Table-1.

Table 1 - The pathological distribution of the tumors in patients.

	n (%)
Pathological type	
Clear Cell RCC	17 (73.9)
Papillary RCC	4 (17.3)
Chromophobe RCC	2 (8.6)
Fuhrman's nuclear grade	
Grade 1	7 (30.4)
Grade 2	11 (47.8)
Grade 3	3 (13.0)
Grade 4	2 (8.6)
Pathological stage	
pT1a	10 (43.4)
pT1b	7 (30.4)
pT2a	3 (13.0)
pT2b	2 (8.6)
pT3a	1 (4.3)

RCC = Renal cell cancer

Distribution of biochemical parameters in the two groups is shown in Table-2. Comparison of groups 1 and 2 revealed significantly elevated SCUBE-1 levels in the patients with renal tumor ($p=0.005$). No significant differences were observed between groups 1 and 2 in terms of CA IX or suPAR values ($p=0.062$ vs. $p=0.176$). There were also no significant differences between groups in terms of pathological type and stage and the Fuhrman's grade ($p>0.05$).

Table 2 - Comparison of biochemical parameters in the patient and control groups.

Biochemical parameters	Group 1 Patient group	Group 2 Control group	P value (p<0.05)
SCUBE1 (ng/mL)	14.80 ± 3.17	8.60 ± 5.22	0.005
CAIX (pg/mL)	59.06 ± 61.38	43.39 ± 61.73	0.176
suPAR (ng/mL)	7.54 ± 6.31	4.29 ± 5.23	0.062

SCUBE-1 = Signal peptide-CUB-EGF domain-containing protein 1; **CA IX =** Carbonic anhydraseIX; **suPAR =** Soluble urokinase plasminogen activator receptor

Spearman correlation analysis results of SCUBE-1, CAIX and suPAR in patient, control group and total sample is shown in Table-3. There was no correlation between biochemical parameters in patient, control group and total sample.

DISCUSSION

Considerable advances have been made in recent years in the diagnosis of renal cancers, methods of treatment and prognosis. However, there is still a need for a marker with high sensitivity and specificity capable of use in the diagnosis and in determining prognosis of renal cancers.

Table 3 - Correlation analysis of biochemical parameters in patient, control group and total sample.

Biochemical parameters	Group 1 Patient group	Group 2 Control group	Total sample
	r - p	r - p	r - p
SCUBE-1 (ng/mL)- CAIX (pg/mL)	0.090 - 0.683	0.083 – 0.692	0.112 - 0.450
SCUBE-1 - suPAR (ng/mL)	0.152 – 0.487	0.173 – 0.408	0.131 – 0.375
CAIX (pg/mL) - suPAR (ng/mL)	0.058 – 0.792	0.115 – 0.585	0.153 – 0.299

SCUBE-1 = Signal peptide-CUB-EGF domain-containing protein 1; **CA IX =** Carbonic anhydraseIX; **suPAR =** Soluble urokinase plasminogen activator receptor

r = represents pearson coefficient of correlation; **p =** represents significance of coefficient of correlation

Several studies have been performed with the aim of developing a biomarker with a high predictive value in renal tumors (11). CAIX is one biomarker that has been investigated for this purpose. CAIX was first tested as a predictive biomarker in a phase II study (SELECT trail), but the results were unsuccessful. CAIX was also investigated in mccRCC diagnosed patients using sorafenib, but no predictive or prognostic value was observed (12). Zhang et al. also concluded that CAIX is not an independent prognostic marker in patients diagnosed with CCRCC (13).

suPAR is a cell surface glycoprotein. Increased serum levels have been shown in several types of cancer. Elevated serum levels are closely correlated with poor prognosis (14, 15). In their study of patients with prostate cancer, Wach et al. revealed that high suPAR levels are a poor prognostic factor correlated with disease-specific survival (7). In a study of patients with gastrointestinal cancer, Zubkiewicz et al. suggested that suPAR is a significant prognostic marker in deciding on treatment in these cases (8).

SCUBE-1 is a member of the SCUBE gene family. Three different isoforms occur in mammals, SCUBE-1, -2 and -3. SCUBE-1 is a cell surface protein present in platelets and endothelial cells that is expressed and secreted in early embryogenesis (9). SCUBE-1 molecules are stored in alpha granules in inactive platelets. While thrombin is activated by platelets, SCUBE-1 is expressed on the platelet surface as a result of surface expression of the adhesion molecule P-selectin (9). It is released in the form of small, soluble particles incorporated into thrombus (10). In humans, it has robustly been shown in platelets and in fibrin-rich areas in organized thrombus (16).

SCUBE-1 has been investigated as a marker in non-cancer diseases (17-21). Türkmen et al. described SCUBE-1 as a potential marker capable of use in the early stage of acute mesenteric ischemia, in the specific diagnosis of pulmonary embolism and in the early diagnosis of acute ischemic stroke (17-19). SCUBE-1 has also been described as a valuable biomarker in determining severity and prognosis of disease in patients with Crimean-Congo hemorrhagic fever (20). Ozkan et al. determined high SCUBE-1 levels in patients with hypertension and suggested that SCUBE-1 may be an early biomarker of potential thrombotic complications occurring in association with hypertension (21).

Various studies have investigated and shown a close association between cancers and thrombosis (22-24). Some tumors trigger the coagulation cascade and procoagulant substances

and initiate the inflammatory process. Procoagulant substances are released from tumor cells with the inflammatory process (25). One in vitro study determined a decrease in SCUBE-1 concentrations with interleukin-1-β and TNF-α therapies, a finding implicating SCUBE-1 in the inflammatory process (10). Expression of SCUBE-1 transcripts in prostate cancer stromal cells was encountered in a series analysis of prostate mesenchymal cell gene expressions (26). Mentese et al. concluded that SCUBE-1 is a useful marker in determining recurrences that may occur after treatment in patients with stomach cancer (27). Topcu et al. reported that SCUBE-1 can be effective in determining the risk of thrombosis and in screening patients to receive anti-thrombotic therapy as a marker of hypercoagulability in patients with breast cancer (28). In the light of these studies, SCUBE-1 may be of significant value as a biomarker in renal cancer, with widespread angiogenesis and thrombosis. We also determined significantly higher SCUBE-1 values in patients with renal cell cancer. But there was no correlation between SCUBE-1 values and the pathological parameters like type, grade and stage. On the other hand, when we investigated the distribution of renal tumor stages in patients, there was only one patient with grade T3a renal tumor. Almost all of the patients were diagnosed and treated in early stages of the disease in our patient group. This could be a significant advantage for SCUBE-1 as an early cancer detection biomarker considering the distribution of the patients in the group.

The most important limitation of this study is the low number of patients with renal tumor and control cases enrolled. Further multicenter studies with larger patient series are now needed on this subject.

CONCLUSIONS

Research is continuing into different biomarkers in the diagnosis and prognosis and determination of response to treatment in renal tumors. In our study, SCUBE-1 levels were significantly elevated in cases of renal tumor. SCUBE-1 is a promising biomarker in the diagnosis and monitoring of patients with renal tumor, and further research with high number of cases is required.

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CONFLICT OF INTEREST

None declared.

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Antioxidant enzyme profile and lipid peroxidation products in semen samples of testicular germ cell tumor patients submitted to orchiectomy

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ABSTRACT

Purpose: To determine enzymatic antioxidant and lipid peroxidation levels in seminal plasma of patients orchiectomized for testicular tumors.
Materials and Methods: The study included 52 patients: 26 control men and 26 orchiectomized patients for testicular tumor, of which 12 men had seminoma tumor and 14 men non-seminoma tumor. After semen analysis performed according to the WHO guidelines, an aliquot of semen was centrifuged and the seminal plasma was collected. Lipid peroxidation was performed by thiobarbituric acid reactive substances (TBARS) assay and antioxidant profile was assessed by analyzing catalase, glutathione peroxidase (GPx) and superoxide anion (SOD) activities using colorimetric assays with a standard spectrophotometer. Data were tested for normality and compared using one-way ANOVA ($p<0.05$).
Results: Seminoma and non-seminoma groups presented lower sperm concentration and morphology when compared to control group ($p=0.0001$). Both study groups (seminoma and non-seminoma) presented higher TBARS levels when compared to control group ($p=0.0000013$). No differences were observed for SOD ($p=0.646$) andGPx ($p=0.328$). It was not possible to access the enzymatic activity of catalase in any group.
Conclusion: Patients with testicular tumor present increased semen oxidative stress, but no differences were observed in antioxidant levels, even after orchiectomy. This indicates that most likely an increased generation of oxidative products takes place in these patients.

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INTRODUCTION

Testicular tumors account for about 1 to 2% of all kinds of tumors that affect men, causing approximately 0.1% of deaths from cancers in men (1-3). In Brazil, in 2013, the National Institute of Cancer (INCA) estimated approximately 343 deaths caused by testicular cancer (4).

Histologically, testicular tumors are divided into germ cell (95% of cases) and non-germ cell tumors (5% of cases) (5). Germ cell tumors are further divided into seminoma (60% of cases) and non-seminoma (40% of cases) (5-7). Seminomas are more frequent in men about 40 years old, while non-seminomas are more frequent in men between 20-35 years old (5, 8, 9).

The treatment for patients with a testicular tumor is orchiectomy of the affected testis via inguinal surgery, followed, in most cases, by adjuvant treatments such as chemotherapy, and/or radiotherapy, and/or retroperitoneal lymphadenectomy (10, 11). Although unilateral orchiectomy does not result directly in infertility, because the remaining testicle may present normal spermatogenesis, potential side effects are disruption of retroperitoneal sympathetic nerves, which may result in retrograde ejaculation (12), and a high risk of hypogonadotropic hypogonadismdue to the reduction in testosterone production (13).

Regardless of their classification, studies have reported the association of testicular tumors with testicular oxidative imbalance (14, 15) which can compromise sperm motility, concentration, and morphology, as well as interfere with sperm capacitation and fertilization (16-18). The seminal plasma, however, represents the main protection system, which includes a chain of enzymatic antioxidants, composed by superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), which are capable of neutralizing ROS, thus preventing damage caused by oxidative stress (19).

Although it is well known that testicular tumors are harmful to the sperm membrane (20), it still remains to be clarified if orchiectomized patients return to a normal state after the surgery, or if oxidative stress is still present. Therefore, this study set out to investigate the enzymatic antioxidant profile and lipid peroxidation products in seminal samples of patients with testicular germ cell tumors after orchiectomy.

MATERIALS AND METHODS

Study design and patients

A cross-sectional study was performed with 52 men between 18-45 years old. The study groups comprised patients referred for semen cryopreservation at the Cell and Germinative Tissue Bank in the Federal University of São Paulo. The study groups included 12 patients orchiectomized for a unilateral seminoma testicular germ cell tumor (study group S) and 14 patients orchiectomized for a unilateral non-seminoma testicular germ cell tumor (study group NS), which did not present post-operative complications after the surgery. The semen collection was realized one month after surgery, before the beginning of any adjuvant therapy. The control group was composed of 26 healthy men without testicular germ cell tumor and semen quality within the 95% fertile range of the 2010 World Health Organization (WHO) guidelines (21), seeking the Human Reproduction Sector, Division of Urology, Sao Paulo Federal University Andrology Laboratory for semen analysis. Patients referring fever in the 90 days period prior to semen analysis, with evidence of urogenital infection, and patients with a history of cancer or endocrinophaties (and their treatments), smokers, individuals with BMI ≥30 and individuals who had not yet been submitted to orchiectomy were excluded from the study. Institutional Review Board approval was obtained from the Sao Paulo Federal University Research Ethics Committee.

Semen analysis

Semen samples were obtained by masturbation after 2 to 5 days of ejaculatory abstinence. After liquefaction, semen analysis was conducted according to the WHO guidelines (21). Immediately after the semen analysis, 0.5mL was centrifuged at 16.000*g* for 1h at 4°C to remove all the cellular debris. The supernatant seminal plasma was then collected and stored at -20°C until the time of the analyses. All of the remaining semen volume was transported to the Germ Cell and Tissue Bank of the Human Reproduction Sector / Division of Urology of the Sao Paulo Federal University for cryopreservation (22).

Lipid Peroxidation assessment

Lipid peroxidation was determined with the method described by Ohkawa et al. (23), which is based on the determination of its products, mainly the malondialdehyde (MDA) levels, due to its reaction with thiobarbituric acid (TBA). To precipitate proteins, 500µL of seminal plasma and 1000µL of a 10% solution (v:v) of trichloroacetic acid (TCA 10%) were mixed and centrifuged (18.000 x g for 15 min at 15°C). After centrifugation, 500µL of the supernatant and 500µL of 1% (v:v) thiobarbituric acid (TBA, 1%), in 0.05N

sodium hydroxide in glass tubes were placed into a boiling water bath (100ºC) for 10 min, and subsequently cooled in an ice bath (0ºC) to stop the chemical reaction. The TBARS were then quantified using a spectrophotometer (UV-vis Spectrophotometer Ultrospec 3300 Pro; BiochromLtd., Cambridge, UK) at a wave length of 532nm. The results were compared with a previously prepared standard curve with a standard solution of malondialdehyde (Sigma-10.838-3-St Louis, USA). Lipid peroxidation levels were described in nanograms of TBARS/mL of seminal plasma. TBARS levels were then normalized to sperm concentration (TBARS/sperm).

Antioxidant seminal profile

Catalase activity was determined indirectly through monitoring hydrogen peroxide consumption (H_2O_2). The reaction solution contained 10µL of seminal plasma added to 90µL of Tris(hydroxymethyl)-amino-methane/EDTA buffer solution (50 and 250mM, respectively) and 900µL of H_2O_2 (9.0mM). The reaction was allowed to take place at pH 8.0, 30ºC, for 8 min, and then the enzymatic activity was measured using a spectrophotometer (wavelength, 230nm). The absorbance was measured every 5 seconds, generating a curve of H_2O_2 consumption that was compared with a blank sample. The calculations considered the value 0.071M-1 x cm-1 as a molar extinction coefficient of H_2O_2. The activity of the enzyme catalase was calculated based on the formula [catalase activity=(initial absorbance-final absorbance)/0.071 x dilution] and the result expressed in UI/mL (24).

The enzymatic activity of the Glutathione peroxidase(GPx) was determined indirectly by measuring the consumption of reduced nicotinamide adenine dinucleotide phosphate (NADPH) (24). The assay mixture consisted of NADPH (0.12mM, 1mL), GSH (1mM, 100mL), GSSGr (0.25U/mL, 20mL), and sodium azide (0.25mM, 20mL). A volume of 100µL of seminal plasma was used. The spectrophotometer cell was brought up to a volume of 1.9mL with phosphate buffer 143mM, EDTA 6.3mM, (pH 7.5), that was also used to dissolve the NADPH. The GSH was dissolved in 5% metaphosphoric acid. Sodium azide was used to inhibit the action of catalase. This reaction was initiated with the addition of 1.2mM of tert-butyl hydro peroxide (TBHP, 100mL), and the consumption of NADPH was detected at a wavelength of 340nm, for 10 min at 37ºC (measurements performed every 5 seconds). The results of GSH-Px were expressed as units of GSH-Px/mL of semen, and calculations used 6.22mM-1 x cm-1 as the extinction coefficient of NADPH (24).

The SOD activity was measured indirectly by means of the reduction rate of cytochrome C by superoxide anion (O_2-) controlled every 5 min. The xanthine-xanthine oxidase system was used as a continuous generated of O_2- that, in turn, causes the reduction of cytochrome C. The total SOD in seminal sample competes with cytochrome C and superoxide anion (O_2-). The total SOD activity was measured indirectly by determining the decrease rate in the reduction of cytochrome C (Floré & Otting, 1984). The assay mixture consisted of 10µL of seminal plasma, 835µL of a solution containing cytochrome C (1mM) and xanthine (50mM), and 155µL of xanthine oxidase diluted in sodium phosphate/EDTA buffer (50 and 100mM, respectively, pH 7.8). The concentration of xanthine oxidase was calculated to generate the optimum amount of O_2 with a consequent reduction of cytochrome C that was calculated as the rate of cytochrome C reduction of 0.025 units of absorbance/min (at 550nm of wavelength); the basis of this calculation is that 1 unit of total SOD activity corresponded to 50% of this value. Therefore, SOD activity in the sample decreased the rate of cytochrome reduction when compared to the blank.

Statistical analysis

The data were analyzed in the SAS System for Windowssoftware (SAS, 2000). The applicative Guided Data Analysis was used to test the data for residue normality (normal distribution) and homogeneity of variances. If normality was not observed, variables were transformed into their logarithmic or square root values. If after transformation normality was not observed, a non-parametric analysis was carried out using theNPAR1WAY procedure. For data with normal distribution, the ANOVA test followed bya Least

Significant Differences (LSD) post-hoc test was used to compare the groups. An α of 5% was considered for all the analyses.

RESULTS

Individual characteristics and semen quality results are presented in Table-1. No statistically significant differences were observed in age, ejaculate volume, sperm motility, and round cells and neutrophil concentration between the three groups. The seminoma and non-seminoma presented lower sperm concentration, morphology, and total sperm count when compared to the control group.

The variables TBARS, GPx, SOD and TBARS/sperm of the three groups (seminoma, non-seminoma, and control) are shown in Table-2. The seminoma and non-seminoma groups presented higher TBARS levels when compared to controls. No statistically significant differences were observed for SOD and GPx activities. No reading levels were achieved for catalase enzyme activity by the Beutler method in any group.

DISCUSSION

Whichever the histological origin of the testicular germ cell tumor (seminoma or non-seminoma), orchiectomy is the initial therapeutic approach, usually followed by gonadotoxic adjuvant therapies, such as chemotherapy and radiotherapy (25). However, even before any treatment for the cancer begins, patients may display reduced fertility potential because of alterations to the testicular environment due to the disease itself (14, 15).

To our knowledge, there is no study in the literature that evaluates the role of oxidative stress and antioxidants in the seminal plasma of testicular germ cell tumor patients after orchiectomy. Thus, this study aimed to verify the activity of the main seminal antioxidant enzymes SOD, GPx, and catalase as well as the oxidative by-product malondialdehyde. The seminal non-enzymatic antioxidant milieu consists of GPx, SOD and catalase to physiologically control the balance between ROS production and neutralization (26). In this study, catalase levels were undetected. However, determining catalase activity in seminal plasma remains a matter of debate, because the presence of this antioxidant in the semen is usually due mainly to the presence of neutrophils (24).Because the semen of our patients presented low concentration of these cells (under 0.5million/mL in either group), this could explain why catalase was not detected in this study.

GPx protects sperm membrane from oxidative stress and is involved in redox regulation (27). In addition, SOD is responsible for dismutation of superoxide radicals (O_2-)into H_2O_2 and O_2 (28). Regarding the seminoma and non-seminoma groups,when compared to healthy control men, no differences were observed in the SOD and GPx activity, suggesting that antioxidant mechanisms were not altered in the presence of a testicular germ cell tumor followed by orchiectomy. This may stem from the fact that most of the seminal antioxidant levels is supported by secretions from the prostate and seminal vesicles (27, 28), which are apparently unaffected by the presence of a testicular cancer, at least in terms of their production of enzymatic antioxidants. It could be argued that epididymal antioxidants also contribute to the seminal antioxidant capacity, but their contribution is generally described as much lower (29), and it is still not known whether epididymal secretion of antioxidants is affected by the tumor.

However, even with unchanged levels of antioxidants, sperm from patients of the seminoma and non-seminoma groups showed greatly increased sensibility to oxidative damage - demonstrated by increased TBARS levels normalized to sperm concentration -when compared to controls. Besides the mean looks different by the observer, this study did not observe statistical difference in TBARS/sperm of seminoma vs. non--seminoma patients.The cancer itself causes an increase in ROS levels (30), which may be mainly due to: (i) increased metabolic activity and energy produced by mitochondria (30) and (ii) chronic inflammation and cytokine releasing (31).Moreover, the presence of a testicular cancer may produce an inflammatory state in the contralateral testis (31), which in turn may lead to delayed spermatogenesis, thus increasing the production of immature sperm (20). This sperm-centered oxidative

Table 1 - Semen analysis of orquiectomized men and healthy control men. Groups were compared by ANOVA followed by LSD post-hoc test.

	Control Group (n= 26)	Seminoma Group (n= 12)	Non-Seminona Group (n= 14)	p
Age (years)				
Mean; SD	29.8; 3.27	28.4; 6.02	26.7; 6.17	0.165
95% CI	[28.56; 31.21]	[24.59; 32.24]	[23.22; 30.35]	
Abstinence (days)				
Mean; SD	4.4; 3.47	6.7; 7.44	5.3; 6.00	0.993
95% CI	[3.06; 5.87]	[2.02; 11.48]	[1.89; 8.83]	
Volume (mL)				
Mean; SD	3.6; 0.97	3.8; 1.73	3.2; 1.30	0.478
95% CI	[3.23; 4.02]	[2.76; 4.96]	[2.45; 4.02]	
Progressive motility (%)				
Mean; SD	56.6; 7.05	63.1; 6.43	58.2; 9.80	0.098
95% CI	[53.84; 59.54]	[58.49; 67.70]	[52.63; 63.94]	
Non-progressive motility (%)				
Mean; SD	4.5; 1.98	3.8; 2.35	5.0; 2.09	0.340
95% CI	[3.70; 5.30]	[2.02; 5.40]	[3.86; 6.28]	
Sperm concentration ($X10^6$/mL)				
Mean; SD	82.4; 53.48[b]	18.1; 23.06[a]	16.7; 17.26[a]	**<0.0001***
95% CI	[60.82; 104.02]	[2.69; 33.68]	[6.79; 26.73]	
Total sperm count ($X10^6$)				
Mean; SD	295.7; 213.03[b]	59.6; 72.82[a]	69.6; 85.70[a]	**<0.0001***
95% CI	[209.73; 381.82]	[13.36; 105.89]	[20.14; 119.11]	
Morphology (% normal)				
Mean; SD	14.4; 0.85[b]	5.0; 3.22[a]	5.4; 2.06[a]	**<0.0001***
95% CI	[14.11; 14.81]	[2.97; 7.06]	[4.24; 6.62]	
Round cells ($X10^6$/mL)				
Mean; SD	0.9; 0.74	0.7; 1.08	1.3; 0.97	0.304
95% CI	[0.68; 1.28]	[0.07; 1.45]	[0.74; 1.86]	
Neutrophils ($X10^6$/mL)				
Mean; SD	0.1; 0.17	0.1; 0.18	0.2; 0.50	0.577
95% CI	[0.04; 0.18]	[-0.02; 0.22]	[-0.09; 0.50]	

SD=Standard deviation
95% CI =Confidence interval of 95% of the mean
* – significant difference
Different Letters in the same row indicate significant difference (*post-hoc* LSD test – $p<0.05$).

Table 2- TBARS, Glutathione Peroxidase (GPx) and Superoxide Dismutase (SOD) levels from orquiectomized men and healthy control men. Groups were compared by ANOVA followed by LSD *post-hoc* test.

	Control Group (n= 26)	Seminoma Group (n=12)	Non-seminoma Group (n=14)	p
TBARS/sperm				
Mean; SD	3.2; 2.59[b]	53.2; 127.19[a]	22.7; 25.95[a]	**<0.001***
95% CI	[2.16; 4.26]	[-27.62; 134.01]	[7.74; 37.71]	
GPx (UI/mL)				
Mean; SD	65.0; 20.02	59.9; 19.45	67.5; 23.05	0.646
95% CI	[56.97; 73.15]	[47.63; 72.35]	[54.21; 80.83]	
SOD (UI/mL)				
Mean; SD	63.9; 59.84	34.3; 30.64	50.0; 27.49	0.328
95% CI	[39.79; 88.13]	[14.88; 53.83]	[34.16; 65.92]	

SD=Standard deviation
95% CI=Confidence interval of 95% of the mean
* – significant difference
Different Letters in the same row indicate significant difference (*post-hoc* LSD test – $p<0.05$).

stress (as demonstrated by our results) would explain why lipid peroxidation levels would depend on sperm concentration - the latter acting as a substrate for the former (32). Given that,in our study, testicular germ cell tumor patients collected semen samples on average one month after orchiectomy,the inflammatory state would not have had time to be fully resolved, as one full cycle of spermatogenesis would not have yet occurred. This is further supported by the fact that the study group presented decreased sperm concentration (quantity) and morphology (quality). The findings corroborate with Tavilani et al. (33) study, which observed antioxidant profile and oxidative stress in asthenozoospermic men and only observed a significant difference in TBARS/sperm, indicating that the mechanism of oxidative stress occurs similarly in infertile men and testicular germ-cell tumor patients.

Besides, this study is limited to the fact that the patients have a short period to perform a sample collection, because after they are submitted to orchiectomy, the adjuvant therapy is required. This way, it is not possible to observe antioxidant profile difference after a complete spermatogenesis cycle. In addition, the number of patients included in the study could not be enough to demonstrate difference in some studied parameters. Moreover, a further path for future research would be increase the number of patients and include pre-operative samples, which would add information regarding how the antioxidants and lipid peroxidation act in a tumor milieu.

CONCLUSIONS

In short, we verified that there is an imbalance on lipid peroxidation in patients with testicular germ cell tumors when compared with healthy individuals. Therefore, patients with seminoma and non-seminoma tumors demonstrated an increased seminal oxidative stress. Also, there was no difference in antioxidant levels, after orchiectomy. This seminal increase in oxidative stress is attributed to increased ROS generation caused by the tumor itself. In conclusion, orchiectomized patients, when compared to healthy controls, do not differ in terms of enzymatic antioxidant levels, but present lower semen quality and increased sperm-centered oxidative stress.

CONFLICT OF INTEREST

None declared.

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EDITORIAL
COMMENT

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Editorial Comment: Antioxidant enzyme profile and lipid peroxidation products in semen samples of testicular germ cell tumor patients submitted to orchiectomy

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In this issue of the *Int Braz J Urol*, Sposito and colleagues, in a collaborative effort between the Human Reproduction Unit of Federal University of São Paulo and the Animal Reproduction Department of University of São Paulo, provide interesting data concerning antioxidants and oxidants in the semen of testicular germ cell tumor (TGCT) patients (1).

The authors measured lipid peroxidation, a marker of oxidative stress (OS), and the levels of enzymatic antioxidants (catalase, glutathione peroxidase, and superoxide dismutase) in the semen of 26 men with TGTC (12 seminomas and 14 non-seminomas) subjected to unilateral orchiectomy. Measurements were carried out one month after surgery and before initiation of adjuvant therapy (if required). Twenty-six healthy men with semen analysis within normal ranges (WHO 2010 criteria) served as controls. Patients and controls were matched by age and ejaculatory abstinence. The testicular cancer patients had lower sperm count than controls, which is expected as spermatogenesis is reduced by half in adult men with solitary testis due to various causes, including orchiectomy for testicular cancer (2, 3). More importantly, both cancer groups had higher oxidative stress markers than controls, albeit not different between seminoma and non-seminoma. But notably, seminal antioxidant levels were similar between controls and orchiectomized TGCT patients.

Why is this study being editorialized? First, for the novelty; it is to my knowledge the first report to investigate oxidative markers and antioxidant levels in semen of orchiectomized testicular cancer patients. Second, the authors' results add to the discussion about the optimal time for sperm cryopreservation. Third, Sposito and colleagues set the path for future research.

Reactive oxygen species (ROS) are formed during normal cellular metabolism and are involved in many physiological processes, including the activation of the immune system. Examples of ROS include superoxide anion ($\bullet O_2-$), hydrogen peroxide (H_2O_2), the extremely reactive hydroxyl radical ($\bullet OH$), and the peroxyl radical ($\bullet HO_2-$) (4). An increase in ROS levels that exceeds their physiological threshold can induce cellular damage due to deleterious effects on proteins, lipids, and DNA. Indeed, ROS production in the male reproductive tract has become a real concern because of their potential toxic effects on sperm quality and function (4, 5). The extent of damage due to ROS depends on several factors, including intracellular and extracellular levels of ROS and extent of anti-oxidation in the environment. Abnormal spermatozoa, polymorphonuclear granulocytes or both, are primary sources of excessive ROS. The seminal plasma contains natural antioxidants (AOX), such as vitamins C and E, superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase, which counteract the adverse effects of ROS (6). An imbalance between ROS production and antioxidant defenses leads to oxidative stress (OS). Lipid peroxidation (LPO) is one end product of OS that causes oxidation of cell membranes, thus impairing its function. The other is sperm DNA fragmentation (SDF). The likely result of SDF is infertility (7), but it has been suggested that offspring generated from such defective sperm are at an increased risk of imprinting disorders

and cancer (8). Oxidative stress is measured by direct and indirect methods. The direct assays measure ROS levels directly and include chemiluminescence, nitroblue tetrazolium test, cytochrome C reduction, to cite a few. Indirect methods measure the oxidized products or their effect at the molecular level and include myeloperoxidase test, redox potential, lipid peroxidation levels, total antioxidant capacity, SDF testing, among others. The assays principle, methodology, clinical utility, and drawbacks can be found elsewhere (5).

Although the literature is rich in studies examining the role of OS and antioxidants in male infertility, the study of Sposito and colleagues is the first to measure lipid peroxidation and antioxidant levels in the neat semen of TGCT patients subjected to orchiectomy (1). Before their study, LPO was investigated only in cryopreserved semen samples of testicular or non-testicular cancer patients. In frozen-thawed semen from TGCT men, LPO levels were not different than that of controls (9). The present study adds to the literature by demonstrating an oxidative imbalance among patients with TGCT, even after tumor removal.

Equally important is to discuss the clinical implications of Sposito's et al. findings for testicular cancer patients banking their semen for fertility preservation. Foremost among all is perhaps the issue of when to freeze, before or after orchiectomy. On the one hand, some authors suggest cryopreservation is optimal before orchiectomy because sperm concentration decreases after surgery (10). On the other hand, others advocate sperm banking after orchiectomy, as a significant proportion of TGCT men have high SDF at diagnosis (11). Since cancer induces an overall inflammatory state with the release of cytokines and other products, it is possible that OS, including DNA damage, could be mitigated after orchiectomy. In the study of Sposito et al., although AOX levels were similar between TGCT patients and controls, LPO levels were higher in the former, thus indicating that the existing AOX could not fully protect sperm from the detrimental effect of ROS. Unfortunately, measurements of oxidation and AOX before orchiectomy were not available, thus precluding conclusion regarding the optimal time for cryopreservation. Notwithstanding, others have found that TGCT *per se* does not increase SDF, and suggest that sperm freezing done either before or after orchiectomy are equally valid (12, 13). In a recent study evaluating SDF rates among men with various diagnoses, we found SDF to be elevated in frozen-thawed semen of men with testicular cancer (14). Still, Spano et al. showed that SDF increases after both radiotherapy and chemotherapy, irrespective of the type of testicular cancer, an effect that persists for five years (15). Altogether, the existing evidence suggests OS is contributory to deterioration of semen quality in TGCT patients. The optimal time for freezing such specimens, before or after orchiectomy, is yet to be determined, but it should be carried out, unquestionably, before adjuvant therapy starts.

Lastly, Sposito's et al. intriguing findings open the possibility for future research. Measurement of oxidants and AOX before and after orchiectomy could be very informative, as would be the investigation of AOX added to the freezing media, as a means to overcome any deleterious effect OS post-thawing, as previously suggested (16-18). While awaiting for these results, it seems sound to offer sperm banking both before and after orchiectomy, coupled with the determination of oxidative stress status (if available) or SDF testing, which is now commonplace (19). Based on the levels of such markers obtained at the time of cryopreservation, it may be decided later which specimen is safer to use for Assisted Reproductive Technology.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Histopathological analysis of the non – tumour parenchyma following radical nephrectomy: can it predict renal functional outcome?

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ABSTRACT

Introduction: Radical nephrectomy (RN), a recommended treatment option for patients with Renal cell carcinoma (RCC) leads to an inevitable decline in global renal function. Pathological changes in the non-tumour parenchyma of the kidney may help predict the function of the remaining kidney.

Materials and Methods: Aim of this prospective, observational study was to find histopathological factors in the non-tumor renal parenchyma that could predict the decline in global renal function postoperatively and its association with co-morbidities like diabetes (DM). Data of consecutive patients undergoing RN from December-2013 to January-2015 was collected. Non-tumor parenchyma of the specimen was reported by a dedicated histopathologist. eGFR was calculated using Cockcroft-Gault formula before the surgery and at last follow up of at least 12 months.

Results: 73 RN specimens were analyzed. Mean follow up was 12.3 months. The mean decrease in eGFR was 22% (p=.0001). Percent decrease in eGFR did not show association with any of the histopathological parameters studied. DM was significantly associated with decrease in percent eGFR ($p<0.05$) and increase in arteriolar hyalinosis (p=0.004), Glomerulosclerosis (p=0.03) and Interstitial fibrosis/ Tubular atrophy (p=.0001). Maximum size of the tumor showed a negative correlation with percentage change in eGFR (p=.028).

Conclusion: Histological parameters in the non-tumour portion of the RN specimen may not be able to predict renal function outcome over a short follow up. However, presence of DM was associated with adverse pathological changes and significant decrease in renal function postoperatively.

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Keywords:
Nephrectomy; Arteriosclerosis; Glomerulosclerosis, Focal Segmental

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INTRODUCTION

Radical nephrectomy (RN) is one of the recommended treatment options for patients with renal cell carcinoma (RCC). However, loss of a functioning kidney leads to adaptive hyperfiltration in the remaining kidney (1) resulting in glomerulosclerosis (2, 3). This insult to the kidney is further exacerbated by conditions like diabetes mellitus (DM) and hypertension (HTN). These changes are reflected in patient's global renal function which shows a significant decline in 13% to 36% of patients (4). The pathological changes in the non-tumour parenchyma of the kidney may

be helpful in predicting the clinical outcome in terms of renal function of the remaining kidney. An early detection of these factors and its timely management can delay the onset of kidney damage and the consequent patient morbidity. The aim of this study was to prospectively look at histological parameters in the non-tumour parenchyma of RN specimens and correlate it with the change in the patient's eGFR over a period of at least 6 months. We also looked at the effect of DM and HTN on the non-tumour parenchyma and change in global renal function postoperatively.

MATERIALS AND METHODS

This prospective, observational study was carried out in the departments of Urology and Pathology, from Dec 2013 till Jan 2015. Institutional review board and ethics committee approval was obtained. All patients >18 years of age undergoing RN were consented and enrolled. Their clinical data was collected preoperatively, within 1 month of surgery and at last follow-up. A nephrectomy specimen in which the whole kidney was replaced by tumour was excluded. Sections of the non-tumour renal parenchyma for evaluation were taken at least 2cm away from the tumour margin to avoid any effect of tumour on the tissue. The biopsy specimen apart from haematoxylin and eosin (H&E) was also stained with periodic-acid Schiff (PAS). The specimen was reported by a dedicated histopathologist who was blinded to the patient's clinical details. The histological factors evaluated were vascular, glomerular and tubule-interstitial based on the Oxford classification for IgA nephropathy (5) (Figure-1).

Arteriosclerosis (AS) was scored based on most severe lesions involving the interlobular arteries. It was graded based on the intimal and medial thickening leading to narrowing of the vascular lumen: none, intima less than media and intima more than media. Arteriolar hyalinosis (AH) was noted as proportion of arterioles affected: none; mild- ≤25%; moderate- 26% to 50% and severe- >50%. For statistical analysis it was grouped into two groups as less than 25% and more. Glomerulosclerosis (GS) was graded as Diffuse: a lesion involving most (≥50%) glomeruli; Focal: a lesion involving <50% of glomeruli; Global: a lesion involving more than half of the glomerular tuft; Segmental: a lesion involving less than half of the glomerular tuft. Glomerulosclerosis and Interstitial fibrosis/Tubular atrophy (IF/TA) was evaluated as percentage estimated to the nearest of 5%. For statistical analysis GS was graded into less than 10% and more and IF/TA was graded into 5% and more. Preoperative calculation of eGFR was done by the Cockcroft-Gault formula: **[eGFR= {140-age (year)}×weight(kg)/ {72×serum creatinine (mg/dl)}×0.85 if female].** Post operatively eGFR was calculated again at 6 months or at last follow-up whichever was later and percentage change in eGFR calculated.

Sample size: Gautam et al. (6) reported that the change in eGFR from the baseline was nearly 31%. The standard deviation was 28.4mL/min. An assumption was made that greater decline in eGFR occurred in patients with more glomerulosclerosis, and change in those with least glomerulosclerosis would be as low as 10% to 15%. Keeping alpha and beta error at 5% and 10% respectively; and the power of the study at 90, the sample size calculated was 73.

Statistical analysis

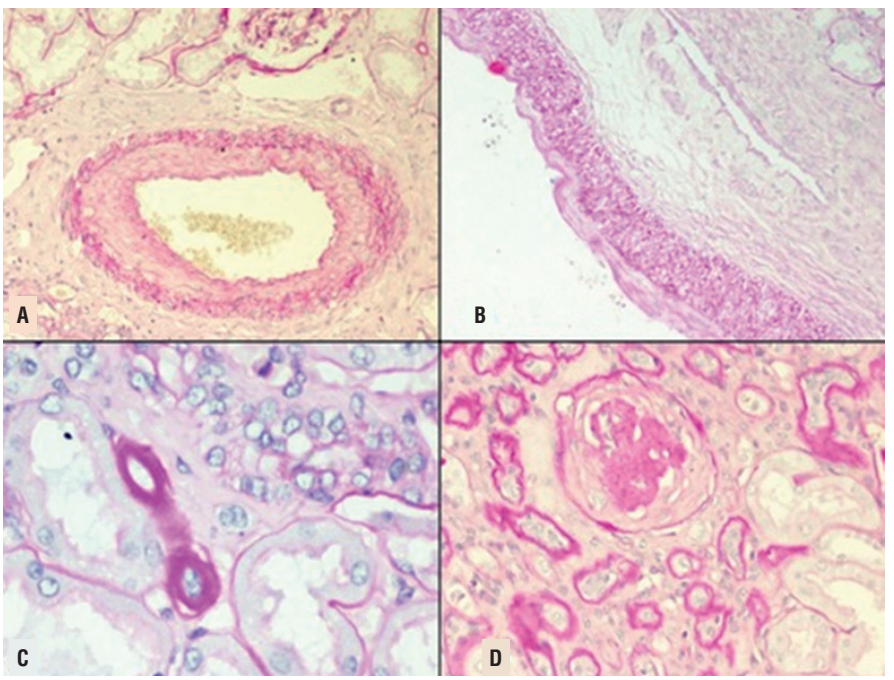
Data was entered using EPIDATA software version 3.1 and analysed using SPSS version 16. For preoperative characteristics of patients, p values were determined by the Mann-Whitney test for continuous variables and by $\chi 2$ test for categorical variables. As we had different categories of histological types to study the differences we used ANOVA test. Multivariable regression analyses having percent change in eGFR as dependent variable was done to control the effect of baseline variables such as DM, HTN etc.

RESULTS

Eighty-six RNs were carried out from December 2013 to January 2015 of which, 73 were available for analysis. The base line characteristics of the patients and tumours are summarised in Table-1.

The mean age of patients was 55.4 years with majority being male. They were followed up

Figure 1 - Histopathological factors (H&E): 1A) Arteriosclerosis- Intima more than media; 1B) Arteriosclerosis: Intima less than media; 1C) Arteriolar hyalinosis and 1D) Globally sclerosed glomeruli with tubular atrophy.



for an average period of 12.3 months. Hypertension was the most common co-morbidity. The histopathological findings are summarised in Table-2.

As expected, clear cell carcinoma was the most common pathology. Average size of tumour was 7.3cm. T1 tumours accounted for 50% of the RNs, of which majority were stage T1b.

The mean eGFR in preoperative period was 81.8±29.6mL/min and postoperative period was 63.5±20.9mL/min. The mean decrease in eGFR was 22% (p=0001) 16 (22%) patients in the preoperative period had eGFR less than 60mL/min compared to 36 (49.3%) postoperatively 21 (28.8%) patient's eGFR fell below 60mL/min compared to eGFR in the preoperative period. The mean fall in this group was 28.25±9.97mL/min (p=015). On linear regression analysis the percentage change in eGFR was not significantly affected by AS (p=0.7), AH (p=0.8), GS (p=0.4) or IF/TA (p=0.5). However, linear regression analysis showed that the percentage change in eGFR was significant in those affected by DM (p=05). Patients who had both DM and HTN also showed a significant decrease in eGFR compared to those who had either one or none (p=0.04). Maximum size of the tumour (sotl) showed a negative correlation with percentage change in eGFR change (p=03). In other words, smaller the tumour size greater the change in percentage eGFR. Other parameters like sex (p=0.9), HTN alone (p=0.8), grade of tumour (p=0.3), pre-

Table 1 - Baseline characteristics of patients.

Age (years)	55.4±10.7 *
Sex (%)	
Male	56 (76.7)
Female	17 (23.3)
BMI	24.36±3.5 *
Follow up (months)	12.3±2.6 *
Comorbidities (%)	
DM	23 (31.5)
HTN	44 (60.3)
DM+HTN	18 (24.6)
Type of Surgery (%)	
Open	25 (34.2)
Laparoscopic	48 (65.8)

*mean ± standard deviation

Table 2 - Histopathological findings.

Histopathological factors	n (%)
Glomeruloscleosis (GS)	
≤10%	68(93.2)
>10%	5(6.8)
Arteriolar hylinosis (AH)	
None	7(9.6)
≤25%	42(57.5)
>25%-≤50%	15(20.5)
>50%	9(12.3)
Arteriosclerosis (AS)	
Intima<Media	4(5.5)
Intima>Media	69(94.5)
Interstitial fibrosis/ Tubular atrophy (IF/TA)	
≤5%	32(43.8)
5-≤10%	19(26.0)
>10-≤50%	13(17.8)
>50%	9(12.3)

operative ESR (p=0.6) did not significantly affect the percentage eGFR change.

DM had a significant association with presence of AH (p=0.004), GS (p=0.03) and IF/TA (p=0001). Presence of both DM and HTN also had a highly significant impact on arteriolar hyalinosis (p=004), GS (p=0.03) and IF/TA (p=0.001). However, HTN alone had no significant effect on AS (p=0.4), GS (p=0.72) and IF/TA (p=0.90). This was so because most of the patients with diabetes also had hypertension.

On multivariate analysis, the only factor which significantly affected the percentage change in eGFR was DM (Table-3).

DISCUSSION

Pathological changes in the non-tumour parenchyma of the kidney may be helpful in predicting the clinical outcome in terms of renal function of the remaining kidney. The basis of this is the presumption that the changes in the non-tumour parenchyma of the nephrectomy specimen will be reflection of the parenchyma of the remaining kidney which is left with the patient (4). The studies till date which have tried to address

Table 3 - Univariate and Multivariate analysis with Percentage Change in eGFR as constant.

Risk Variables	Univariate		Multivariate	
	β	p-value	β	p-value
Age	- 0.25	0.22	- 0.36	0.16
Sex	0.37	0.94	0.64	0.92
BMI	0.26	0.68	- 0.05	0.95
Size of tumor	- 1.92	0.03	- 2.66	0.03
Arteriolar hyalinosis	- 4.32	0.35	- 6.88	0.24
Glomerulosclerosis	5.10	0.55	0.02	0.99
IF/TA	- 2.59	0.59	- 1.38	0.82
Arteriosclerosis	3.11	0.74	4.01	0.69
Diabetes Mellitus	- 9.25	0.04	- 14.78	0.02
Hypertension	- 0.88	0.84	3.56	0.52
Type of surgery	4.01	0.38	- 6.49	0.35
Blood loss	- 0.003	0.76	-0.005	0.64

BMI = Body mass index; **IF/TA =** Interstitial fibrosis/ Tubular atrophy; **Size of tumour =** Size of tumour in maximum dimension, Type of surgery (lap vs. open).

this possibility are fraught with limitations such as small numbers (6) and retrospective design (4). We evaluated the non-tumour portion of the RN specimens based on standard protocol to report medical renal disease (5). The renal function was measured using CG formula, as according to Kim et al., the CG model based on actual weight was 1 of 5 models that accurately estimates renal function in patients with a kidney tumour (7). The idea was to accurately document the parameters so that they can be objectively measured. We looked at the AH, AS, GS and IF/TA and did not find any significant association with the percent eGFR change. Gautam et al. in a retrospective study have shown that GS extent was associated with decrease in eGFR over a mean follow-up of 19.7 months (6). But, our study did not find the same. This can be because our follow-up period was short (mean of 12.3 months). This also could be because we used a different system of reporting the non-tumour parenchyma histology (5). Also, the studies done by Gautam et al. did not mention the type of staining used for slides. We used PAS stain for slides as recommended by the College of American Pathologists for the reporting of the surgically resected specimens of renal cell carcinoma (8).

Huang et al. reported that 26% of the patients in their cohort had pre-existing chronic kidney disease (CKD), defined as eGFR <60mL/min, before nephrectomy (9) and 70% of patients developed new onset CKD after RN over a mean follow-up of 19 months. In our study, 28.8% [21] patient's eGFR fell below 60mL/min compared to pre-nephrectomy values with a mean fall of 20.34±8.6mL/min over a mean follow-up of 12.3 months. Bijol et al. found that patients with severe histopathological findings like parenchymal scarring >20%, global glomerulosclerosis and advanced diffuse diabetic glomerulosclerosis showed a significant change in serum creatinine from the preoperative period to 6 months after the RN (10). In our study, we found that patients with DM showed a significant change in percent eGFR over a period of 6 months. Diabetic patients showed significant adverse renal parenchymal changes in terms of AH, GS and IF/TA compared to non-diabetics. This could explain the significant change in post nephrectomy renal function of diabetic patients although no straight association between parenchymal parameters and percentage eGFR was found. HTN failed to show any significant association with percent change in eGFR.

Size of the tumour in maximum dimension showed a significant negative correlation with percent change in eGFR. This could be explained by the fact that in kidneys with smaller tumours, the normal functioning renal parenchymal loss is higher when compared to large tumours where the normal functioning parenchyma is considerably replaced by tumour. Our findings support the fact that tumours of size up to 7cm (T1) if amenable must be dealt with nephron sparing surgery. And, the presence of DM should strongly tilt the decision towards nephron sparing surgery in such patients.

The renal parenchymal characteristics in our study did not appear to significantly impact functional outcomes at one year of follow-up. It may be due to the fact that many of the patients had normal kidney function pre-operatively or did not have significant parenchymal damage. Only 5 patients in our study cohort had significant GS and this could have had a bearing on the interpretation of results. We did not do immunofluorescence and electron microscopic examination, to which diagnostic kidney biopsies are routinely subjected. Hence, our pick-up rate of medical renal disease may have been lower. Nevertheless, reporting of the non-tumour portion of the renal parenchyma should preferably be carried out routinely as it would give us an insight regarding the "quality" of the remaining renal mass and its long-term behaviour. Incorporating other methods of testing renal quality such as looking at the presence of proteinuria, imaging characteristics etc. may improve the probability of predicting renal functional outcome more accurately.

CONCLUSIONS

Global renal function preservation should be aimed for in all the patients undergoing renal ablation surgery. Histological parameters in the non-tumour portion of the RN specimen may not be able to predict renal function outcome over

a short follow-up. However, presence of DM was associated with adverse pathological changes and significant decrease in renal function postoperatively.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Towards development and validation of an intraoperative assessment tool for robot-assisted radical prostatectomy training: results of a Delphi study

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ABSTRACT

Introduction: As urology training shifts toward competency-based frameworks, the need for tools for high stakes assessment of trainees is crucial. Validated assessment metrics are lacking for many robot-assisted radical prostatectomy (RARP). As it is quickly becoming the gold standard for treatment of localized prostate cancer, the development and validation of a RARP assessment tool for training is timely.

Materials and methods: We recruited 13 expert RARP surgeons from the United States and Canada to serve as our Delphi panel. Using an initial inventory developed via a modified Delphi process with urology residents, fellows, and staff at our institution, panelists iteratively rated each step and sub-step on a 5-point Likert scale of agreement for inclusion in the final assessment tool. Qualitative feedback was elicited for each item to determine proper step placement, wording, and suggestions.

Results: Panelist's responses were compiled and the inventory was edited through three iterations, after which 100% consensus was achieved. The initial inventory steps were decreased by 13% and a skip pattern was incorporated. The final RARP stepwise inventory was comprised of 13 critical steps with 52 sub-steps. There was no attrition throughout the Delphi process.

Conclusions: Our Delphi study resulted in a comprehensive inventory of intraoperative RARP steps with excellent consensus. This final inventory will be used to develop a valid and psychometrically sound intraoperative assessment tool for use during RARP training and evaluation, with the aim of increasing competency of all trainees.

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Delphi Technique; Prostatectomy; Robotic Surgical Procedures

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INTRODUCTION

Surgical education has recently undergone a paradigm shift towards competency-based frameworks for surgical training and evaluation. A need for improved training, certification, and recertification in Urology has been recognized. As such, health care regulatory bodies in the United States, Canada, and Europe are revising curricula with a new focus on what trainees should know in order to be deemed competent (1-3). With this shift, the need for valid, reliable, and feasible assessment tools exists; however, there is a paucity in many surgical specialities. Robot-assisted urologic surgery (RUS) is rapidly gaining in accessibility and popularity, with robot-assisted radical prostatectomy (RARP) now considered the frontline treatment for clinically localized prostate cancer. Yet, no standardized training or evaluation models have been developed for RARP. While its anatomic technique has been well- described by Menon and colleagues since its initiation in 2000,

with their most recent report in 2012 (4), reliable objective metrics for trainee evaluation of RARP have not been established.

The daVinci Surgical System® (Intuitive Surgical, Sunnyvale, CA) boasts multiple technical advantages over traditional laparoscopic and open radical prostatectomy, and affords a more practicable and ergonomic environment to enhance the learning curve (5, 6). Evidence suggests that RARP produces more favorable patient outcomes when compared to its traditional counterparts (7, 8). As RARP is the most commonly performed robotic procedure worldwide (9), and RUSs such as radical and partial nephrectomy and pyeloplasty are becoming more prevalent, proposed best practices, training and credentialing criteria, standard operating practices (SOPs), and frameworks for effective incorporation of robotic surgical programs into institutions is timely. The American Urological Association (AUA) has recently proposed SOPs for robotic surgery that include minimum requirements for granting urologic robotic privileges (5); however, consensus has not yet been reached for a standardized curriculum and credentialing system. Several academic centers have published their own guidelines for RUS credentialing; but again, a lack of universal consensus exists (10). Regarding RARP credentialing, Zorn and colleagues recently published recommendations on behalf of the Society of Urologic Robotic Surgery (SURS) (11), and McDougall and colleagues have established a successful mini-residency for RARP (12) that provides a framework for postgraduate teaching. More recently, best practices for RARP have been proposed (13); despite the efforts of numerous organizations, a consensus for training, credentialing, and assessment of competency for RUS, including RARP, have not yet been achieved (5).

Inanimate and virtual reality (VR) simulation has played a significant role in the training of robotic surgery. Clear benefits of simulation have been established in the literature, suggesting shorter operative time and fewer medical errors when skills transfer to the high-stakes environment of the operating room (OR) (14, 15). Further, the validity of the da Vinci Skills Simulator (Mimic™ VR software) has recently been established (16-18), providing support for an effective training platform, especially when surgical training time with the da Vinci is extremely limited. Despite preparing trainees for the robotic environment, it has not yet been unequivocally demonstrated that these simulated robotic skills can indeed transfer to the OR. Further, procedure-specific VR programs for RARP are not yet widely available.

Based on a clear need for a standardized intraoperative assessment tool for RARP to measure and establish competency during training, we set out to design a step-wise clinical assessment tool for the RARP procedure. We report on a study to address the first stage in this process, which comprises the development of an inventory of procedural RARP steps and sub-steps as defined by a panel of expert RARP surgeons via a modified Delphi process.

MATERIALS AND METHODS

Study Design and Population

A modified Delphi process was employed to achieve consensus of the items that expert RARP surgeons believe ought to comprise the assessment tool. The Delphi technique is an iterative structured group communication method to solicit expert opinion about new or complex problems, conducted through a series of questionnaires (typically three to four rounds) with controlled feedback each round (19). The Delphi process goal is to achieve expert consensus using qualitative and quantitative methodology. The feedback process allows and encourages participants to reassess their initial judgements and revise them throughout the iterations (20). Anonymity and confidentiality are maintained for each panel member. The controlled feedback process eliminates biases that often occur during group consensus approaches like panel meetings and focus groups. Further, Delphi statistical analysis ensures that each member's opinions are well represented in the final iteration, as it allows for objective and impartial analysis when summarizing the data (20). We first employed a modified Delphi study with urology staff surgeons, fellows, and residents at our institution to develop a preliminary inventory of RARP steps. Following the internal process, we recruited a panel of expert RARP surgeons external to our institution to participate in a Delphi study

to evaluate and edit the initial inventory, with the goal of developing a final inventory of steps and sub-steps for RARP.

Internal Delphi Process

Following ethics approval, we recruited seven participants at our institution to serve as a Delphi panel. This included two RARP experts (performed >300 RARP cases), one urology fellow, and four senior urology residents. The fellow and residents all had extensive experience as a RARP bedside assistant and the majority had some intraoperative da Vinci console experience. Our rationale for employing the modified internal Delphi technique was to create a RARP procedural inventory from scratch, in accordance with Delphi methodology. We provided each member of the group with a RARP video from our case database and asked members to create a list of the critical steps and sub-steps of the entire procedure, referring to the video as necessary. This began by using an open-ended format followed by a checklist system for inclusion criteria. Qualitative comments were encouraged and modifications were made. Four iterations were conducted until 100% consensus was reached. It was then circulated to all members for final approval.

External Delphi Process

The expert panel was recruited via email by two expert RARP surgeons at our institution. Twenty-nine expert RARP surgeons from Canada (17) and the United States (12) were asked to participate. Potential participants were provided with a comprehensive background of the study process, and those who chose to participate provided informed consent via by email, with the final panel totaling thirteen participants. The literature recommends that a total of ten to eighteen panel members is sufficient for consensus if the sample is homogenous (11, 21). An advanced version of the web-based SurveyMonkey® software (Palo Alto, CA) was used to create and submit each of the survey iterations. This email-based system provided us with controlled, quantitative and qualitative feedback, and allowed for analysis of data through Excel, SPSS, graphical formats, and data summaries.

Four iterations were conducted. The first round's survey was derived from the internal Delphi's inventory of steps. A 5-point Likert scale with a neutral option was used for each response option and panel members were instructed to rank the importance, in terms of agreement, of whether each primary step and sub-step ought to be included in a RARP assessment tool. They were also encouraged to provide feedback for each item, and overall comments at the end of the survey. Panel members had the option to anonymously contact the study team with questions during the process, and for reference, all members were provided a link to the same RARP video distributed during the internal Delphi study. The initial survey was piloted with two urologic surgeons at our institution. Following the first survey distribution, three reminders were sent at predetermined weekly intervals until all responses were received. Results of the first round were analyzed and edits to the inventory items were made based on feedback, while some sub-steps were moved in the sequence and/or eliminated based on score consensus. Each item's consensus was based on Ulschak's (22) criteria, whereby 80 percent of subject's votes fall within two categories on a 5-point scale. If items fell beneath a mean of 3.0, they were either deleted from the inventory or modified as suggested by panelists. Even when consensus was reached to keep the items, several required modifications based on feedback. The final iteration's methodology was modified per Delphi protocol to adjust to a 4-point Likert scale, eliminating the neutral response option to minimize satisficing. For consensus to be achieved on a 4-point scale, it is recommended that at least 70 percent of Delphi subjects need to rate a mean of 3.25 or higher on each item (20). Descriptive statistics were analyzed in SPSS v22® for each round of the Delphi process.

RESULTS

Detailed demographics of the expert Delphi panel participants are described in Table-1. All thirteen participants were male and the majority were fellowship trained in robotic surgery. At the time of the study, each panelist was performing RARPs at high volume academic tertiary care centers within the U.S. or Canada.

Table 1 - Demographics of External Delphi Panel.

	N (%)
Sex	
Male	13 (100)
Age	
35-44	10 (77)
45-54	3 (23)
Practice Setting	
Tertiary Care	13 (100)
Community	0 (0)
Practice Location	
Canada	9 (69)
USA	4 (31)
Years in Practice	
1-5	3 (23)
6-10	5 (38.5)
10-15	4 (30.8)
>15	1 (7.7)
Fellowship	
Yes	11 (84.6)
No	2 (15.4)
Years Performing RARP	
1-2	4 (30.8)
3-4	4 (30.8)
5-7	4 (30.8)
8-10	1 (7.6)
RARP per month	
1-5	10 (76.9)
6-10	2 (15.4)
10-15	1 (7.7)
>15	0 (0)

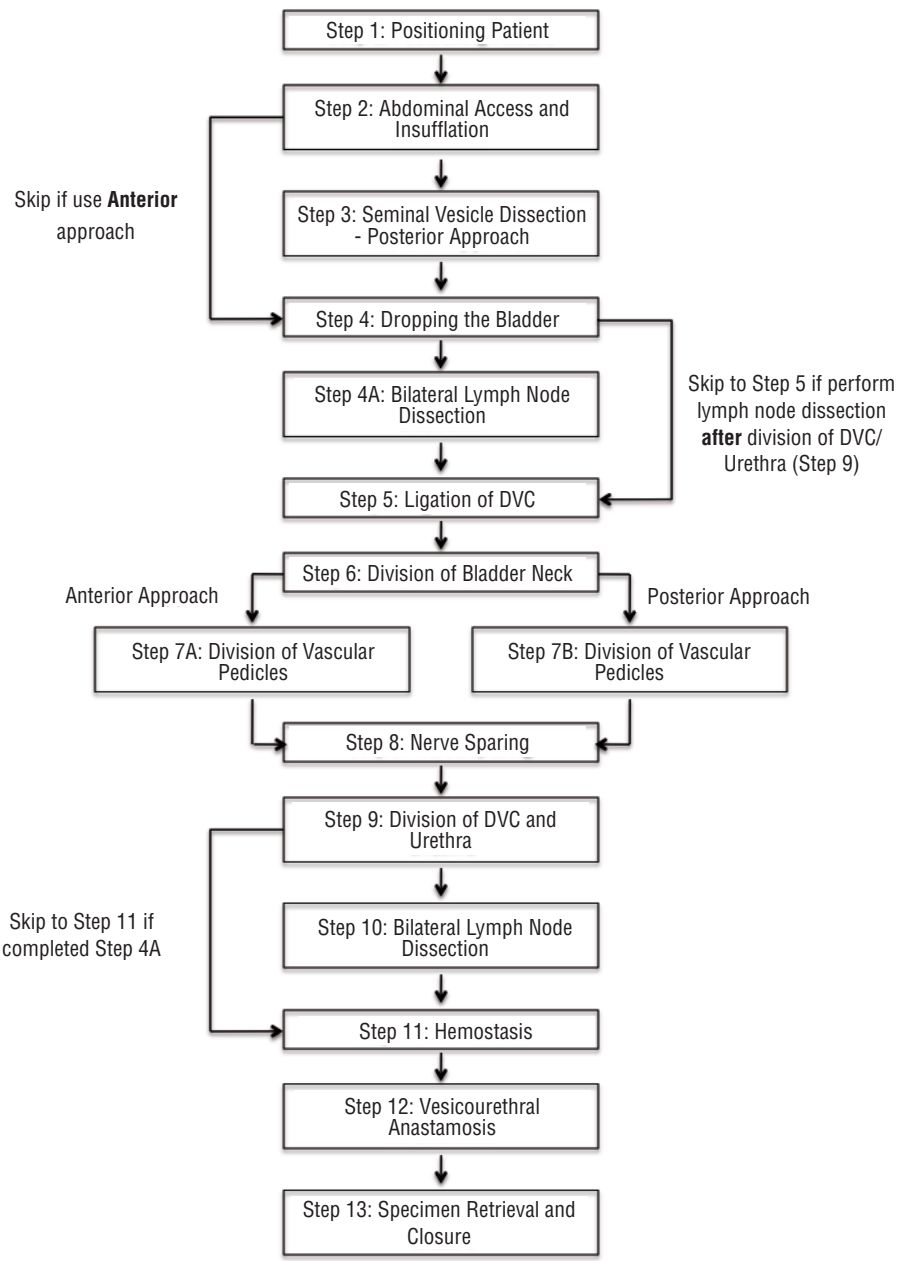
Results of the internal Delphi process included an overall reduction of inventory steps and sub-steps of 18% over a total of three iterations, with a fourth iteration serving to determine final agreement. The final inventory received 100% consensus and consisted of 13 critical steps and 60 sub-steps in total.

This initial inventory was used as the framework for the external Delphi study. After the first round, there was a 58% consensus of the RARP steps and sub-steps. Qualitative and quantitative feedback led to the removal of 4 sub-steps and required the addition of 2 skip patterns in the algorithm, specifically with regard to the approach to the prostate (anterior versus posterior) and timing of the lymph node dissection (Figure-1). The second iteration reached 75% consensus and further reduced the number of sub-steps to a total of 52. The third round reached 100% consensus. The fourth round served to determine whether the inventory was indeed the final version based on agreement by panel members (Figure-2). The internal and external Delphi processes are outlined in Figure-3.

DISCUSSION

Despite the widespread use of the da Vinci Surgical System, relatively little attention has been paid to robotic curricula and assessment metrics for training (5, 10, 14, 15). Currently, there are no standardized guidelines for teaching, evaluating, and credentialing robotic surgery. In 2014, Smith and colleagues (23) reported on several consensus conferences that took place with fourteen societies in an effort to develop a standardized process for certifying the skills of robotic surgeons. This has been termed Fundamentals of Robotic Surgery (FRS), which was modeled after the Society of American Gastrointestinal and Endoscopic Surgeon's (SAGES) validated and widely used Fundamentals of Laparoscopic Surgery (FLS) curriculum (23). However, this is generically designed for all types of robotic surgery, and therefore cannot be applied to specific procedures without first tailoring it to the procedure.

Figure 1 - Critical steps for RARP algorithm.



The current surgical education landscape favors global rating scales (GRSs) over checklists, as GRSs have been shown to improve validity and reliability. The recently published and validated Global Evaluative Assessment for Robotic Skills (GEARS) assessment tool (24), which was derived from the validated Global Operative Assessment of Laparoscopic Skills (GOALS) tool for laparoscopic surgery (25), expanded on the innovative work of Martin et al. to create a generalized assessment tool for robotic surgery (26). However, GEARS is not task-specific; therefore when used, it must be tailored to the task being evaluated. An example of a task-specific GRS is the recently proposed Robotic Anastomosis Competency Evaluation (RACE) that purports to assess the technical skills of per-

Figure 2 - Final inventory of steps and sub-steps.

Step 1: Positioning Patient
a) Padding and taping – arms tucked
b) Prep, drape, and insert Foley catheter
c) Trendelenberg positioning
d) Mark patient (incision markings)

Step 2: Abdominal Acess and Insuflation
a) Veress, Direct Optiview, or Hassan technique for access at umbilicus
b) Insuflation
c) Camera port placement
d) Laparoscopic-guided port placement using landmarks
e) Dock robot and insert robotic instruments under direct vision

Step 3: Posterior Approach to Seminal Vesicles
a) Retract bowel from pelvis and lysis of adhesions as necessary
b) Incise peritoneum in Pouch of Douglas, develop retrovesicle space, and identify seminal vesicles and vas deferens
c) Mobilization and visiono of vas deferens
d) Dissect seminal vesicle with care to avoid ureters

Step 4: Dropping the Bladder
a) Divide median and medial umbilical ligaments and develop the space of Retzius
b) De-fat the prostate
c) Divide the superficial DV, incise the endopelvic fascia, sweep levator muscle off prostate, and skeletonize apex
d) Identify acessory pudendal vessels if present, preserve if possible
e) Divide puboprostatic ligaments-optional

Step 4a/10: Bilateral Lymph Node Dissection
a) Identify external iliac artery
b) Incise fibroareolar tissue over external iliac vein
c) Identify and preserve the obturator nerve
d) Distal dissection to Cloquet's node and division once secured witch clip
e) Disset out the obturator lymph node packet; extended node dissection as indicated
f) Remove lymph node packets individually using graspers

Step 5: Ligation of Dorsal Venous Complex
a) Secure DVC with suture ligature or stapler
b) Secure back bleeding with additional suture ligature or with cautery (optional-depending on personal technique)
Step 6: Division of Bladder Neck
a) Retract bladder, identify bladder neck, and identify prostatovesical junction
b) Division of anterior bladder neck and identify Foley catheter at midline use monopolar cautery
c) Deflate ballon and deliver Foley tip and dissect remainder of anterior bladder neck with dissection curving toward contour of the prostate base
d) Division of posterior bladder neck and retrotrigonal fascia; identification of previously dissected vas and seminal vesicles
e) Satisfactory dissection of median lobe

Step 7A: Ligation and Division of Vascular Pedicles (Anterior Approach)
a) Identify vas deferens in the midline
b) Mobilize seminal vesicles bilaterally
c) Clip, cauterize, or suture and divide the vas deferens, blood supply to seminal vesicles, and vascular pedicles of the prostate

Step 7B: Ligation and Division of Vascular Pedicles (Posterior Approach)
a) Identify and develop pedicles to the prostate, use suture or clip and divide
b) Dissect below Denonvillier's fascia if indicated

Step 8: Bilateral or Unilateral (Full or Partial) Nerve Sparing or Resection
a) Incision of periprostatic fascia (if indicated)

b) Release of neurovascular bundle off prostate, high release of nerves (if indicated)
c) Release continued to apex of prostate
Step 9: Division of Dorsal Vein and Division of Urethra
a) Divide DVC (if not already stapled)
b) Complete the apical dissection with sharp dissection
c) Expose the urethra
Step 4A/10: As above
Step 11: Hemostasis
a) Careful hemostasis, decrease pneumoperitoneum and examine for bleeding
Step 12: Vesicourethral Anastamosis
a) Reconstruct bladder neck if necessary
b) Perform vesicourethral anastomosis using running suture line and place final catheter
c) Test anastomosis by filling bladder and check for watertight closure (fill bladder), repair leaks where appropriate
Step 13: Extraction of Specimen and Closure
a) Undock the robot
b) Remove speciment with endocatch bag via extension of midline port site
c) Introduce and position Jackson Pratt(JP) drain-optional
d) Desufflate the abdomen
e) Fascial closure of extraction site
f) Port site closure
g) Inject local anesthetic subcutaneously in excision port sites (can be performed when incisions are first made, at the end, or both)

forming a urethrovesical anastomosis during RARP (27). Metrics for assessing the competency of the entire RARP procedure are lacking; thus, our development of a consensus-based inventory of RARP steps is the first attempt at creating a valid and reliable stepwise RARP assessment tool for use in training. Research by Ali et al. and Schreuder et al. (28, 29) and Rashid et al. (30) have demonstrated that a proficiency-based stepwise approach to learning robotic training is both feasible and safe. Thus, instead of relying on a single GRS, our proposed assessment tool will allow surgical educators to rate trainees on each step of the RARP procedure as they progress through the learning curve.

The use of a modified Delphi methodology via survey software was ideal for gaining expert consensus on the critical main steps and the sub-steps of the RARP procedure, as it offered a systematic process for data collection. Firm timelines were used and regularly scheduled reminders were sent out during each round. We minimized attrition by selecting participants with a high interest in RARP training and by informing participants of the processes and goals of the study at the outset and by maintaining regular two-way communication. Anonymity was also preserved, allowing participants to overcome any communication barriers inherent in face-to-face interaction and focus groups, and participants were able to modify their views without the element of social pressure. Furthermore, the technique allowed for time flexibility, as respondents were able to complete their surveys on their own time. The process also afforded the respondents controlled feedback whereby they were able to see the inventory develop with each iteration, allowing them to observe that their input was leading to tangible results.

Limitations of the process included selection bias, as respondents were known to the two recruiting surgeons; however, maintaining anonymity throughout the process helped to control for this bias. Still, potential respondents may have felt social pressure to participate. Furthermore, inherent to all Delphi studies, the judgments were those of a select group of people and may not necessarily have been representative of all RARP surgeons. Additionally, Delphi methodology inherently limits or excludes outliers on a scale of an item and forces a more middle of the road consensus. This was mediated by including space for qualitative feedback for each item evaluated.

We have begun to develop an assessment tool based on the stepwise inventory, with evaluation metrics built in for each step. We have maintained a prospective database of RARP cases that will be used for rating each step.

Figure 3 - Outline of the Delphi process.

Internal Delphi process

1. Participant recruitment
   a) Internal to our institution
   b) N = 7 (2 RARP experts, 1 urology fellow, 4 senior urology residents)
2. Panel members were asked to create an inventory of RARP steps and sub-steps
   a) RARP procedure video provided for reference
3. After first iteration, the draft of the steps and sub-steps was emailed to each panel member
   a) Checklist created for each step to determine inclusion/exclusion
   b) Qualitative comments encouraged throughout the process
4. Four iterations conducted until 100% consensus reached
   a) Modifications to inventory made during each round
   b) The final inventory was circulated to each panel member for approval

External Delphi process

1. Participant recruitment
   a) External to our institution
   b) 29 expert surgeons within North America (17 from Canada, 12 from United States) were emailed an invitation to participate
   c) Based on agreement to participate, 13 RARP surgeons comprised the final Delphi panel
   d) All participants remained anonymous throughout the entire process
2. First iteration
   a) Based on the internal Delphi panel's final inventory
   b) Survey was created using SurveyMonkey® and included a 5-point Likert scale of agreement as to whether each primary step and sub-step should be included in the final inventory that would eventually be used to develop and validate a RARP procedural assessment tool
   c) Pilot survey was conducted with two urologic surgeons within our institution before being sent to the Delphi panel (edits were made based on feedback)
   d) Survey was sent out to all panel members with detailed instructions for completion
   e) Space for comments was provided for each primary step and sub-step
   f) Three reminders were sent at predetermined weekly intervals as required (through the SurveyMonkey® program) until all responses were received
   g) Results were analyzed and edits made based on consensus and qualitative feedback
3. Second iteration
   a) Conducted following the same protocol as the first iteration
4. Third iteration
   a) Conducted following the same protocol as the previous two iterations
   b) The Likert scale of agreement was modified via Delphi protocol to a 4-point scale, thus eliminating the "neutral" response option to minimize satisficing
5. Fourth and final iteration
   a) All panel members were sent the final inventory and asked to state whether they approved of it
   b) Ensured that 100% agreement was achieved, concluding the Delphi process

Experts will be recruited and asked to rate the endoscopic videos of resident (PGY3-5) and expert cases using the GEARS tool to assess each step. Experts will be blinded to level of training. To minimize time burden, participants will be asked to evaluate only two steps at a time until each step has been assessed. Access to the videos will be provided by a secure link sent via email. Our goal is to develop and validate a reliable stepwise RARP assessment tool based on our inventory of steps acquired during this Delphi process. This assessment tool may eventually be incorporated into residency and/or fellowship curricula for use during intraoperative RARP training. The potential for changes to the RARP inventory is indeed possible as we receive additional feedback on the evaluation tool, especially with regard to alternate means of techniques during the steps, with the potential to include issues specific to plausible patient outcomes.

CONCLUSIONS

Our team has successfully developed an inventory of crucial steps and sub-steps for RARP based on expert consensus using Delphi methodology. We aim to develop and validate a reliable assessment tool that will be based on this stepwise inventory and can be used during intraoperative RARP training to improve competency of trainees as they learn RARP.

ABBREVIATIONS

RARP = robot-assisted radical prostatectomy
RUS = robotic urologic surgery
SOP = standard operating practices
AUA = American Urological Association
SURS = Society of Urologic Robotic Surgery
VR = virtual reality
OR = operating room
FRS = Fundamentals of Robotic Surgery
SAGES = Society of American Gastrointestinal and Endoscopic Surgeons
FLS = Fundamentals of laparoscopic Surgery
GEARS = Global Evaluative Assessment for Robotic Skills
GRS = Global Rating Scale
GOALS = Global Operative Assessment of Laparoscopic Skills
RACE = Robotic Anastomosis Competency Evaluation

CONFLICT OF INTEREST

None declared.

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Laparoscopic donor nephrectomy in unusual venous anatomy – donor and recepient implications

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ABSTRACT

Objectives: Laparoscopic donor nephrectomy is now a commonly performed procedure in most of renal transplantation centers. However, the suitability of laparoscopy for donors with abnormal venous anatomy is still a subject of debate.

Materials and methods: Between August 2007 and August 2014, 243 laparoscopic donor nephrectomies were performed in our institution. All donors were evaluated with preoperative three-dimensional spiral computed tomography (CT) angiography Thirteen (5.35%) donors had a left renal vein anomaly. A retrospective analysis was performed to collect donor and recipient demographics and perioperative data.

Results: Four donors had a type I retroaortic vein, seven had type II retroaortic vein and a circumaortic vein was seen in three donors. The mean operative time was 114±11 minutes and mean warm ischemia time was 202±12 seconds. The mean blood loss was 52.7±18.4mL and no donor required blood transfusion. Mean recipient creatinine at the time of discharge was 1.15±0.18mg/dL, and creatinine at six months and one year follow-up was 1.12±0.13mg/dL and 1.2±0.14mg/dL, respectively. There were no significant differences in operative time, blood loss, warm ischemia time, donor hospital stay or recipient creatinine at 6 months follow-up, following laparoscopic donor nephrectomy in patients with or without left renal vein anomalies.

Conclusion: Preoperative delineation of venous anatomy using CT angiography is as important as arterial anatomy. Laparoscopic donor nephrectomy is safe and feasible in patients with retroaortic or circumaortic renal vein with good recipient outcome.

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INTRODUCTION

With the advent of laparoscopic live donor nephrectomy, there has been an increase in number of donors for kidney transplantation. The first laparoscopic donor nephrectomy was performed by Ratner et al. in 1995 (1). Since then laparoscopic donor nephrectomy has become the standard of care in most transplant centers around the world. Compared with open nephrectomy, it is associated with less postoperative pain, shorter length of hospital stay, and faster return to work (2-6).

However, the suitability of laparoscopy for donors with abnormal vascular anatomy is still a subject of debate. There is sparse literature regarding the impact of left renal vein anomaly on the overall outcome of renal transplantation. The objective of this study was to describe the safety and feasibility of laparoscopic donor nephrectomy in donors with renal vein anomalies and analyze the outcome of renal transplantation in recipients of such kidneys.

MATERIALS AND METHODS

Between August 2007 and August 2014, 243 laparoscopic donor nephrectomies were performed in our institution. A retrospective analysis was performed to collect donor and recipient demographics and perioperative data. All donors underwent standard preoperative evaluation including medical, surgical, psychological and immunological evaluation, and detailed informed consent. Three-dimensional spiral CT angiography was used to define the renal vascular anatomy and a renal isotope scan was performed to determine the choice of kidney for nephrectomy.

Left renal vein abnormalities are categorized into four types (7). Types I, II and IV are "retroaortic" left renal veins, while type III is considered as "circumaortic" vein. Type I retroaortic left renal vein typically joins the IVC in the orthotopic position, while type II retroaortic left renal vein joins the IVC at level L4-5. The type IV retroaortic vein joins the left common iliac vein. The circumaortic or type III left renal vein anomaly has both a pre-aortic as well as a retroaortic component (Figure-1).Information on donor age, gender, body mass index (BMI), relation to the recipient and type of renal vein anomaly was collected. Of the 243 donors, 13 (5.35%) donors had a left renal vein anomaly. Among these, four had a type I retroaortic vein, seven had type II retroaortic vein and three donors had a circumaortic vein. Among these 13 patients with left renal vein anomalies, 11 patients had a single left renal artery (Figure-2) while one patient with type I retroaortic vein had bilateral two renal arteries (Figure-3) and one patient had bilateral two renal arteries with right two renal veins and left circumaortic vein (Figure-4). Laparoscopic right donor nephrectomy was performed in 38 patients hence they were excluded from the study. Remaining 192 patients without any left renal vein anomaly had undergone laparoscopic left donor nephrectomy. Of these 192 patients, 20 patients had multiple left renal arteries and left kidney was selected due to anomalous right renal vein/artery. Surgical data included operative time, warm ischemia time, estimated blood loss, complications, and nadir serum creatinine.

The laparoscopic procedure was performed transperitoneally. All donor nephrectomies were performed by either one of two surgeons: HKN/TDJ, and all renal transplants were performed by a single recipient team. Briefly, dissection was started at the

Figure 1- Types of left renal vein anomalies: Type I Retroaortic Left renal vein (RLRV) join the IVC in the orthotopic position, Type II RLRV join the IVC at level L4-5, Type III - circumaortic left renal vein, Type IV renal vein joins the left common iliac vein.

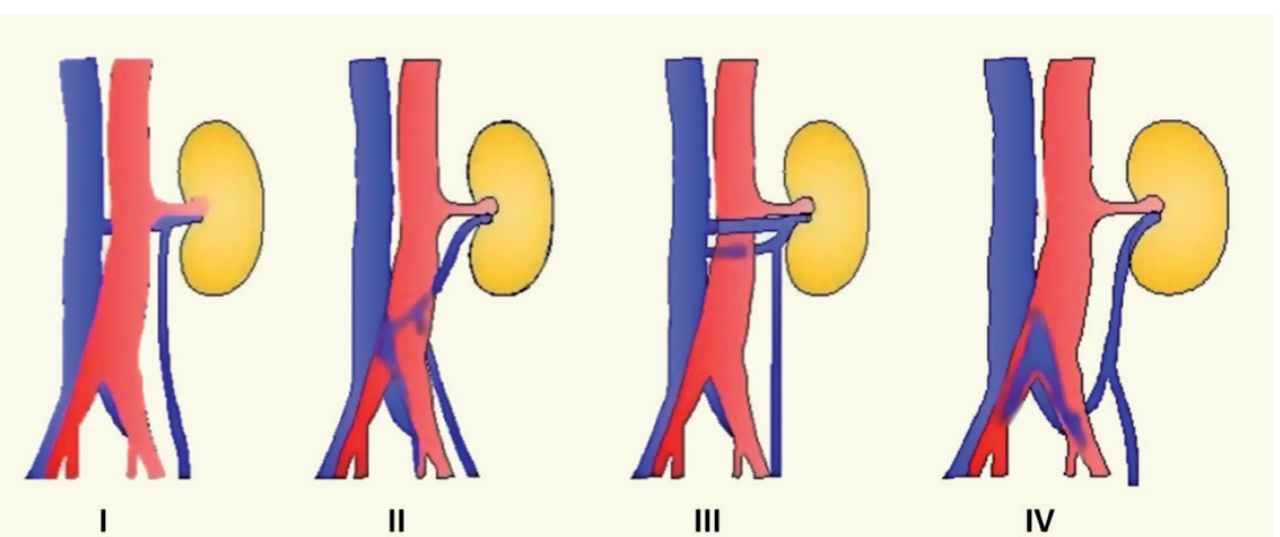


Figure 2- Type II retroaortic left renal vein with bilateral single renal arteries.

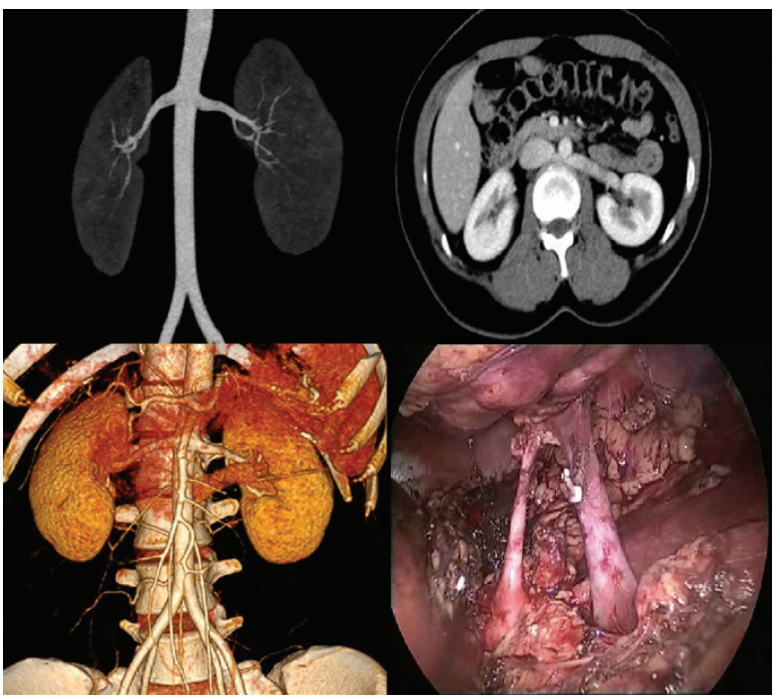


Figure 3- Type I retroaortic left renal vein with bilateral two renal arteries.

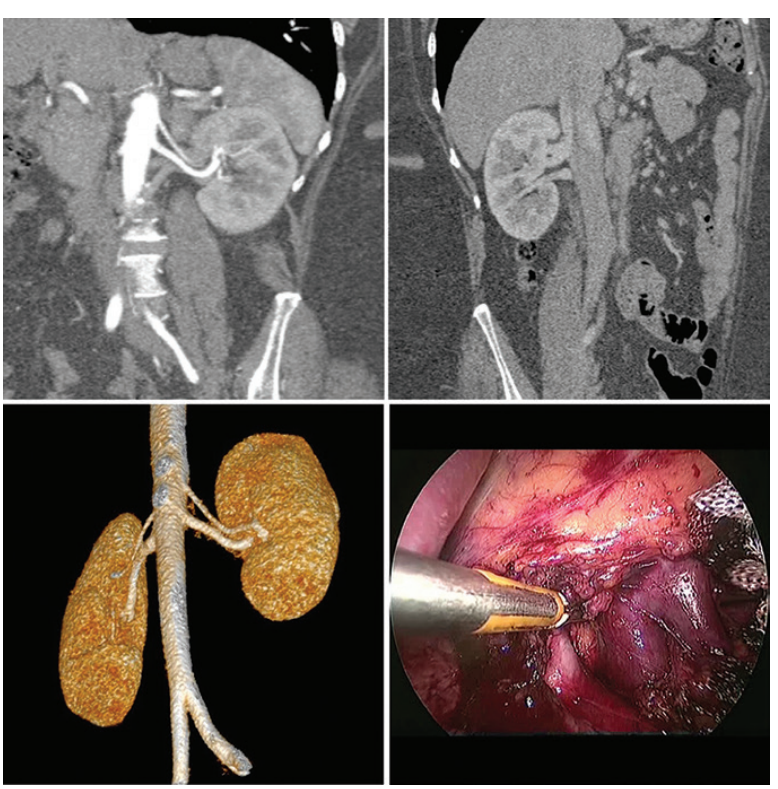
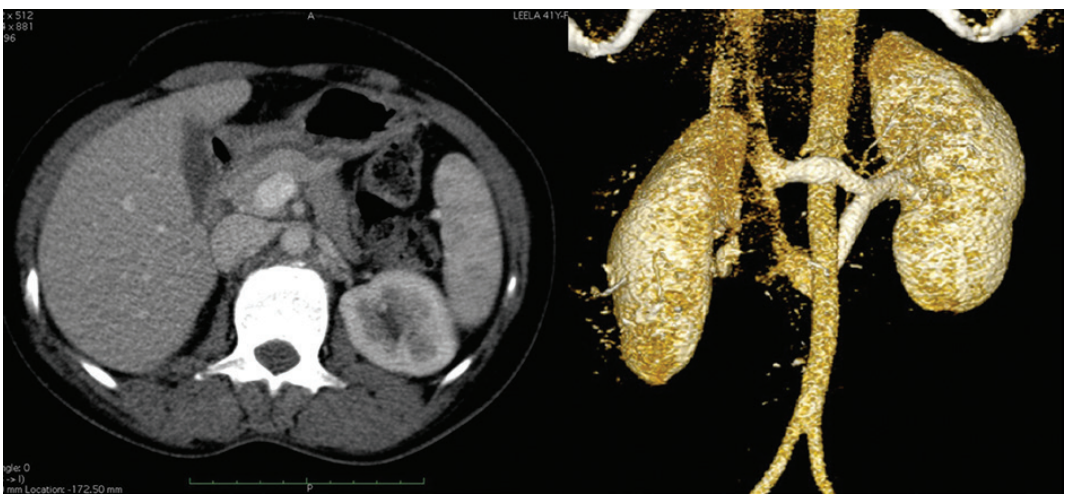


Figure 4- Circumaortic left renal vein.



lower pole of kidney and the ureter and gonadal vein were dissected. The gonadal vein was traced towards the left renal vein. The adrenal vein, gonadal vein and lumbar vein (if present) were identified, clipped and transected. In case of retroaortic vein, renal vein was dissected up to lateral border of aorta and clipped using two Hemolock clips (Weck® clips) and one metallic clip at the donor side. Renal artery was clipped using two Hemolock clips and one metallic clip. In two patients with circumaortic veins, smaller caliber retroaortic component was sacrificed. Kidney was delivered out through Pfannenstiel incision.

Operative time was defined as the time from the initial skin incision to delivery of the kidney to the recipient transplant team. Warm ischemia time was calculated as the time from renal artery ligation to immersion of the kidney in ice slush. Information was also collected on recipient allograft function, including serum creatinine at the time of discharge, at six months and at one year follow-up, as well as complications. Delayed graft function was defined as the patient requiring dialysis postoperatively.

Statistical analysis was performed using Instat® software (GraphPad Software, San Diego California USA). Both discrete and continuous variables were analyzed with the Student's t-test. A P value of less than 0.05 was considered statistically significant.

RESULTS

Donor demographic data, including age, gender, BMI, type of left renal vein anomaly, operative time, warm ischemia time and blood loss for 13 donors who underwent transperitoneal laparoscopic donor nephrectomy are listed in Table-1. There were two (15%) men and 11 (85%) women, with a mean age of 44.5±7.1 years. The mean operative time was 114±11 minutes and mean warm ischemia time was 202±12seconds.There were no intraoperative complications. The mean blood loss was 52.7±18.4mL,and no donor required blood transfusion. Donors were discharged on the third postoperative day and no donor required readmission. Mean donor creatinine preoperatively and at discharge was 1.0±0.14mg/dL and 1.27±0.18mg/dL, respectively.

Recipient outcomes are shown in Table-2. Mean recipient age was 45.2±7.3 years. The mean cold ischemia time was 50.2±8 minutes. The mean recipient blood loss was 317±39mL and mean hospital stay was 11.3±2.4 days. One patient required temporary dialysis after surgery due to delayed graft function due to acute tubular necrosis. Mean recipient creatinine at the time of discharge was 1.15±0.18mg/dL, and creatinine at six months and one year follow-up was 1.12±0.13mg/dL and 1.2±0.14mg/dL, respectively. We routinely used ureteral stents

Table 1 - Donor characteristics.

Sl. No.	Donor Age (Years)	Sex	BMI (Kg/m²)	Type of renal vein anomaly	Number of renal arteries	Operative time (minutes)	Warm ischemia time (seconds)	Blood loss (mL)	Complications	Serum Creatinine (mg/dL)	
										Before surgery	At discharge
1	46	F	26.2	Retroaortic type II	1	118	212	45	Nil	1.1	1.3
2	30	F	22.3	Retroaortic type II	1	112	194	60	Nil	0.9	1.2
3	41	F	24.6	Retroaortic type I	1	109	190	25	Nil	1	1.2
4	52	M	27.2	Retroaortic type II	1	103	210	40	Nil	1.1	1.4
5	41	F	24.5	Circumaortic	2	133	205	85	Nil	0.9	1
6	47	F	26.8	Retroaortic type II	1	112	188	70	Nil	0.8	1.1
7	53	F	27	Retroaortic type I	1	114	217	65	Nil	1.3	1.6
8	36	M	23.4	Retroaortic type I	1	108	196	80	Nil	0.8	1
9	43	F	24.1	Retroaortic type II	1	98	208	45	Nil	1	1.3
10	39	F	25.3	Retroaortic type II	1	115	220	55	Nil	0.9	1.3
11	46	F	27.4	Circumaortic	1	124	185	40	Nil	1.1	1.4
12	54	F	28.5	Retroaortic type I	2	136	208	45	Nil	1	1.3
13	50	F	29.2	Retroaortic type II	1	103	190	30	Nil	1.1	1.5

M= Male; **F=** Female; **BMI =** Body mass index

Table 2 - Recipient outcomes.

Sl. No.	Recepient Age (years)	Sex	Cold ischemia time (minutes)	Blood loss (mL)	Hospital stay (days)	Graft related complications	Serum Creatinine (mg/dL)		
							At discharge	At 1 month	At 6 months
1	50	M	52	280	11	Nil	1.3	1.2	1.3
2	33	M	48	310	12	Nil	1	1.1	1.1
3	38	M	45	350	9	Nil	1.2	1.2	1.3
4	46	F	53	290	13	Nil	1.4	1.3	1.4
5	45	M	67	285	11	Nil	0.9	1	1
6	51	M	43	370	10	Nil	1	1	1.2
7	45	M	40	335	8	Nil	1.3	1.3	1.2
8	40	M	53	390	12	Nil	0.9	1	1.1
9	35	M	41	325	17	Delayed graft function	1.2	1	1
10	44	M	47	290	11	Nil	1.2	1.3	1.4
11	49	M	54	265	14	Nil	1.4	1.2	1.3
12	58	M	63	340	9	Nil	1	1	1.1
13	54	M	47	285	10	Nil	1.1	1	1.2

M= Male; **F=** Female

in all uretero-vesical anastomosis and these stents were removed after 21 days.

These results were compared to the outcomes of 192 patients without left renal vein anomaly (Table-3). Incidences of multiple left renal arteries were comparable in both the groups. There was no statistically significant difference in donor outcomes(operative time, blood loss, warm ischemia time) and recipient outcomes (serum creatinine at 6 months follow-up).

DISCUSSION

Incidence of end stage renal disease and number of kidneys available for renal transplant has always been a major medical concern. With the advent of laparoscopic donor nephrectomy, there has been increase in live donor pool over last decade (8). Laparoscopic donor nephrectomy offers low morbidity, shorter length of hospitalization, less pain medication requirements, and reduced convalescence as compared to open donor nephrectomy (9).

The left kidney is favored for laparoscopic nephrectomy because it provides a graft with a longer renal vein (2,3). Traditionally, right open donor nephrectomy is chosen when the left kidney has multiple renal arteries or veins or other vascular anomalies. Major concernin right-sided laparoscopic donor nephrectomy is short length right renal vein which is further shortened by use of vascular clips. There is increased risk of vasospasm and iatrogenic vascular injury during laparoscopic right donor nephrectomy as the right renal artery is located directly posterior to short right renal vein. Some authors have reported a higher potential for vascular complications with eventual graft loss with laparoscopic right donor nephrectomy (10,11).

The most common renal venous anomaly is the occurrence of dual renal veins, accounting for 15%-30%, frequently on the right side (12-15).

Table 3 - Comparison of demographics and outcomes in patients with and without left renal vein anomaly.

		Donors with left renal vein anomalies (n=13)	Donors without left renal vein anomalies (n=192)	P value
Donor age (years), mean±SD		44.5±7	48.4±8.1	>0.05
Recipient age (years), mean±SD		45.2±7.3	40.1±6.9	<0.05
Multiple left renal arteries (%)		2 (15.4)	20 (10.4)	
Donor BMI (Kg/m2), mean±SD		25.9±2.0	25.5±3.7	>0.05
Operative Details	Operative time (minutes), mean±SD	114±11	109±17	>0.05
	Blood loss (mL), mean±SD	53±18	64±23	>0.05
	Warm ischemia time (seconds), mean±SD	202±12	211±18	>0.05
Hospital stay (Donors)		60 Hours	60 Hours	
Recipient outcomes	Delayed graft function (%)	1 (7.7)	11 (5.7)	
	Cold ischemia time (minutes), mean±SD	50.2±8	54.6±11	>0.05
	Serum Creatinine at 6 months (mg/dL), mean±SD	1.12±0.13	1.21±0.2	>0.05

SD= Standard deviation; **BMI=** Body mass index

Circumaortic and retroaortic variants constitute the most common anomalies of the left renal vein with incidence of 6.2%-14% (10,16,17).Because of the higher risk of vascular injury, the presence of a circumaortic or retroaortic renal vein has previously been considered a relative contraindication for left donor nephrectomy by some surgeons (18). Some authors have reported that there was no significant difference regarding parameters such as operative time, warm ischemia time, length of allograft vessels, and estimated blood loss in patients with circumaortic or retroaortic renal vein when compared to control group (19,20).

In this retrospective study, we analyzed safety and feasibility of laparoscopic donor nephrectomy in patients with left renal vein anomaly and we compared donor and recipient outcomes with group of patients without left renal vein anomaly. The use of CT angiography allows preoperative identification of venous anomalies (21). In our hospital, 243 patients underwent laparoscopic donor nephrectomy. On preoperative evaluation with three-dimensional spiral CT angiography, 13 patients (5.35%) were diagnosed to have left renal vein anomaly in form of retroaortic vein (11) or circumaortic vein (2). Retroaortic vein will have an abnormal course posterior to aorta. Adrenal, gonadal, and lumbar veins may enter the renal vein at abnormal position. Hence, after meticulous dissection and control of these tributaries, retroaotic vein can be clipped at the level of lateral border of aorta. In case of circumaortic vein, usually retroaortic component will have smaller caliber and it can be safely sacrificed. Preaortic component can be clipped at the opening into IVC. Careful preoperative radiological evaluation of vascular anatomy is mandatory and intraoperative potential variation in vascular anatomy has to be kept in mind. In our experience, operative time and warm ischemia time were not prolonged. The mean warm ischemia time was 202±12seconds and mean operative time was 114±18 minutes. One-year graft survival was 100%.

CONCLUSIONS

Preoperative delineation of venous anatomy using CT angiography is as important as arterial anatomy. Laparoscopic donor nephrectomy is safe and feasible in patients with retroaortic or circumaortic renal vein with good recipient outcome.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Skin to calyx distance is not a predictive factor for miniaturized percutaneous nephrolithotomy outcomes

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ABSTRACT

Objective: To evaluate the predictive value of the distance from skin to calyx (SCD) on the outcome and complication rates of patients undergoing mPNL.
Materials and Methods: Patient's charts, who had undergone mPNL between June 2012 and June 2015, were analyzed retrospectively. Patients who had a preoperative computerized tomography (CT) were enrolled into the study. Two separateurologists evaluated the CT scans and calculated the SCD defined as the distance between the skin and surface/lateral edge of the calyx, which was the preferred site of entry for percutaneous access. The average value of the two measurements was included inthe final analysis to avoid bias. The mean SCD was 75mm. According to the median SCD value, patients were divided into two groups: group 1 (SCD ≤75) and group 2 (SCD >75).
Results: A total of 140 patients and 130 patients were enrolled in groups 1 and 2, respectively. The mean operation time and the mean fluoroscopy time was significantly longer in group 2 (p:0.004 vs. p:0.021). The rate of blood transfusion was significantly higher in group 1 (6 patients). None of patientsin group 2required blood transfusion (p:0.017). Stone-free status after a single session of mPNL was 67.1% in group 1 and 75.4% in group 2 (p:0.112). After additional procedures, stone-free rates increased to 84.3% and 85.4% in group 1 and group 2, respectively (p:0.802).
Conclusion: Our study demonstrated that longer SCD was not a predictive factor for stone-free rates after mPNL. However, SCD over 75mm was associated with longer operation time and fluoroscopy time with lower rates of transfusion.

ARTICLE INFO

Keywords:
Obesity; Nephrostomy, Percutaneous; Kidney Calculi

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INTRODUCTION

Percutaneous nephrolithotomy (PNL) is a well-established surgical optionfor renal stone(s) larger than 2cm and staghorn calculi. Satisfactory stone-free rates can be achieved with PNL. Yet, the procedure itself bears some serious potential complications including bleeding,which may require blood transfusion, adjacent organ injury and septicemia (1). Conventional PNL is performed using a 24F to 30F nephrostomy tract and the use of larger size instruments have been associated with unfavorable outcomes (2). Recent advances in technology have enabled the design of instruments with smaller diameters to use in PNL. Miniaturized percutaneous nephrolithotomy (mPNL) is defined asPNL performed by using an instrument with an access sheath of 12-20F diameter(3).

Factors influencing the outcome of PNL including stone burden, renal abnormalities, surgeon experience and obesity had been clearly described (4). In obese patients, access to the *pelvi-calyceal system*and the appropriate dilatation of the tract presents a challenge for the surgeon. Mo-

reover, in obese patients, the inadequate length of the working sheath and working instrumentshave an adverse effect on PNL outcomes. Taking into consideration the differences in body types and body fat distribution among people and races, some authors suggested that the distance from skin to calyx (SCD) is amore predictive factor than body mass index on PNL outcomes (5).

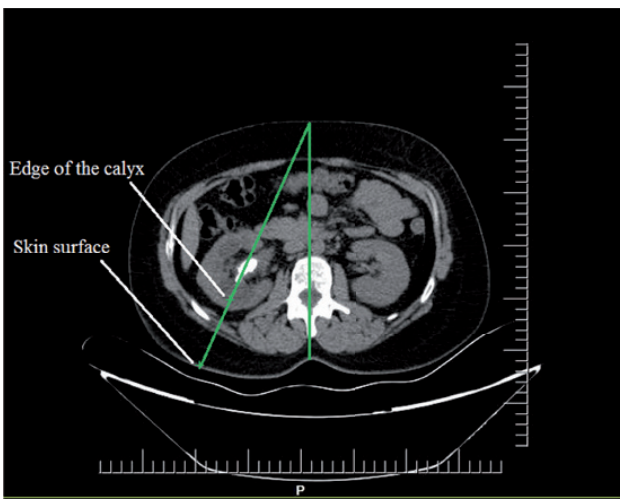
Factors affecting the mPNL outcomes are still being investigated and the role of SCD has not been previously evaluated. To our knowledge, this is the first study that investigates the effect of SCD onthe outcome and complication rates of patients undergoing mPNL.

MATERIALS AND METHODS

After approval from the ethics committee, a retrospective*chart*review of*a consecutive series of patients*undergoing mPNL in a tertiary academic center between June 2012 and June 2015 was analyzed. Every patient included in the study had undergone a preoperative computerized tomography (CT) scan and a follow-up imaging 3 months after the mPNL operation. Patients under 18 years of age, withkidney abnormalities, with no available preoperative CT scans and with a history of PNL procedurewith multiple accesses were excluded from the study.

Kidney and stone characteristics of every patient were evaluated with non-contrast computerized tomography, preoperatively. Complex stones were defined as those located in the pelvis and in at least one calyx or multiple calyces.For each patient, stone size and SCD were measured by two separate urologists to avoid bias. The average value between the two measurements was taken for final analysis. The distance was calculated by drawing a vertical line from the spinous process to the anterior abdominal wall and a second line starting from the anterior abdominal wall and traversing the point of the calyceal edge. The SCD was defined as the distance between the skin and surface/lateral edge of the calyx, which was the preferred site of entry for percutaneous access (Figure-1). While calculating the SCD, Hounsfield unit difference between renal parenchyma and pelvicaliceal system was taken into account to identify the exact point of differentiation between calyx and parenchyma. In our study, the calculated mean SCD was 75mm. According to the median SCD value, patients were stratified into two groups: group 1 (SCD ≤75) and group 2 (SCD >75).

Figure 1- Example of an axial image from a CT scan, demonstrating the way 'Skin to calyx distance'calculated.



Surgical technique

In the lithotomy position under general anesthesia, a 5Fr ureteral catheter was inserted. In the prone position, the calyceal system was visualized using contrast media and access was performed to appropriate calyx using an 18G needle under the C-armed scopy unit. A 0.035-inch hydrophilic guide-wire was delivered into the pelvicalyceal system. The access tract was dilated using Amplatz dilatators, then an 18- or 20Fr Amplatz sheath was inserted into the pelvicalyceal system. Stone fragmentation was performed with laser or ultrasonic lithotripter and stone extraction forceps was used for stone removal. After completion of the procedure, nephrostomy tube was placed under fluoroscopy if necessary. Operation time was calculated as the time from access to the preferred calyx to the insertion of the nephrostomy tube.

On the first postoperative day, a kidney-ureter-bladder radiogram was performed to evaluate the success.On the 3rd postoperative month,

stone-free status was reassessed in the outpatient setting with non-contrast abdominal CT. Patients with complete stone clearence and patients with residual fragments under 4mm were accepted as stone free.

For statistical analysis, values were evaluated as numbers, means, percentages and intervals. Numbers and percentages were compared using the Chi-square test. Before the comparison of means of values, the values were evaluated for homogenity. Homogenously distributed values were compared using Student T test and heterogenously distributed values were compared using Mann Whitney U test.

RESULTS

According to the study design, 140 patients with SCD ≤75mm (range, 48-75mm) and 130 patients with SCD >75mm (range, 76-126mm) were enrolled into group 1 and group 2, respectively. Preoperative characteristics including gender, age, stone size and stone location were similar between the groups (p:0.823, p:0.129, p:0.143 and p:0.077, respectively). Also, the mean BMI was comparable between the groups (p:0.090). Preoperative charecteristics of the two groups are summarized in Table-1.

Table 1 - Comparison of preoperative demographics.

	Skin to calyx distance		
	≤75mm	>75mm	P value
Number	140	130	
Gender			**0.823**
Male	88	80	
Female	52	50	
Mean age (years)	43.09±12.5	45.4±12.4	0.129
Mean body mass index (kg/m^2)	25.7±3.5	26.6±5.3	0.090
Mean stone size (mm)	21.7±5.8	22.8±6.0	0.143
Stone location			**0.077**
Complex stone	60	57	
Pelvis	31	34	
Lower	40	30	
Middle	8	6	
Upper	1	3	
Operation side			**0.745**
Left	76	68	
Right	64	62	
Degree of hydronephrosis			**0.682**
0	6	4	
1	72	66	
2	54	54	
3	6	6	
4	2	0	
Previous renal stone treatment			**0.853**
SWL	22	21	
PNL	14	17	
Open renal stone surgery	11	13	

In both groups, the lower pole was the most preferred location for access. The mean operation time was 91.6±37.7min. in group 1 and 105.8±41.9 min. in group 2 (p:0.004). The mean fluoroscopy time was significantly longer in group 2 (p:0.021). The mean hemoglobin drop was higher in group 1 but the difference was not statistically significant (p:0.178). The mean duration of hospitalization time was comparable between the groups (p:0.404) (Table-2).

Complications as evaluated by the Clavien system were similar between the groups (p:0.155). Requirement of postoperative JJ insertion was the most common complication in both groups (9 patients in group 1 and 13 patients in group 2). Fever requiring antibiotic therapy was observed in 4 patients (2 patients in group 1 and 2 patients in group 2). When the complications were separately evaluated, the rate of bleeding requring blood transfusion was significantly higher in group 1 and occurred in 6 patients. In two of the 6 patients, angioembolisation was required. In group 2, no patients required blood transfusion (p:0.017). No pulmonary complications and Clavien grade 4 or 5 complications were encountered in both groups.

Stone-free status after a single session of mPNL was 67.1% and 75.4% in group 1 and group 2, respectively (p:0.112). Spontaneous passage of residual fragments occurred in 12 patients. Additional procedures including SWL, F-URS and mPNL were performed to 20 patients in group 1 and to 12 patients in group 2. After additional procedures, stone free-rates increased to84.3% and 85.4% in group 1 and group 2, respectively (p:0.802) (Table-3).

DISCUSSION

According to the World Health Organization, obesity is defined as body mass index (BMI) greater than or equal to 30kg/m^2(6). However, fat dispersion is not homogenous among patients with similar BMI. Different body types and thicker retroperitoneal fat tissue can become obstacles during percutaneous access, which is the most challenging part of the PNL procedure. Okhunov et al. developed the Size,Tract length (skin-to-stone distance), degree ofObstruction,Number of calyces involved and stoneEssence (density) (STONE) nephrolithometry scoring system for conventional PNL, which includes percutaneous tract length (PTL) and reported that STONE was predictive for stone-free status (7). In another study, Akhavein et al. demonstrated that higher residual fragments remained after conventional PNL in patients with PTL >100mm when compared with patients with PTL ≤100mm (8).

Previous studies that investigated the effect of BMI on PNL outcomes reported controversial results (9,10). When we divided patients with

Table 2 - Comparison of perioperative findings.

	Skin to calyx distance		
	≤75mm	>75mm	P value
Number	140	130	
Mean operation time (minutes)	119.6±39.1	131.8±43.1	**0.002**
Mean fluoroscopy time (minutes)	5.0±3.7	6.1±3.4	**0.021**
Access location			**0.051**
Lower	126	114	
Middle	14	10	
Upper	0	6	
Number of intercostal access	0	4	**0.037**
Mean hemoglobin drop (g/dL)	1.02±1.50	0.78±1.1	0.178
Mean hospitalization time (hours)	68.8±25.8	65.6±36.0	0.404

Table 3 - Comparison of postoperative results and complications.

	Skin to calyx distance		
	≤75mm	>75mm	P value
Number	140	130	
Post operative complications according to Clavien System			0.155
Grade 2			
UTI	2 (1.4%)	2 (1.5%)	
Transfusion requirement	6 (4.3%)	0	
Grade 3a			
Postoperative JJ insertion without anesthesia	7 (5%)	10 (7.6%)	
Grade 3b			
Postoperative JJ insertion with anesthesia	2 (1.4%)	3 (2.3%)	
Angioembolisation requirement	2 (1.4%)	0	
Stone free status	94 (67.1%)	98 (75.4%)	
Additional procedures			0.472
Spontane passage	6	6	
SWL	8	2	
URS/f-URS	4	4	
mPNL	8	6	
Stone free status after additonal procedures	118 (84.3%)	111 (85.4%)	0.802

different BMI subgroups according to the WHO criteria, we found no significant difference in operative and post operative results. We hypothesized that SCD may have a better predictive value in forecasting outcomes of mPNL because of the variances in fat deployment. To our knowledge, this is the first study to analyze the effect of SCD on intraoperative parameters, outcomes, and complication rates of patients undergoing mPNL.

Skin-to-stone distance (SSD) is a predictive value used to estimate outcomes after SWL. Gonulalan et al. studied the significance of SSD on PNL outcomes (11). In that study, the outcomes after PNL were compared between two groups that were stratified according to their median SSDs. Gonulalan et al. did not detect a significant relationship between longer SSD and PNL success. Once adequate access to the appropriate calyx is achieved, access to the renal pelvis is relatively easier, which decreases the importance of the skin-to-stone distance. In Gonulalan's study, nearly 45% of patients had a renal pelvis stone. Thus, we believe that SCD is more predictive than SSD in PNL procedures.

In a recent study, Cakmak et al. analyzed the effect of abdominal fat parameters on PNL outcomes (12). In a univariate analysis, they found that visceral fat area (VFA) and abdominal circumference on computerized tomography (ACCT) were predictive factors for estimating PNL success rates. Moreover, in a multivariate analysis, ACCT was found to be the only abdominal fat parameter to influence the stone-free rates. During PNL, access to the kidney is obtained through retroperitoneal fat tissue. We suggest that the skin-to-calyx distance is a more reliable parameter than the entire ACCT because patients who are obese tend to have different fat distribution patterns.

In our study, the stone-free rates for group 1 and group 2 were 82.9% and 83.1%, respectively. In the study by Knoll et al., which included patients with stones sizes similar to our study group (18 mm and 21.7mm in group 1 and 22.8mm in group 2), a stone free rate of 96% was reported following

mPNL. However, Knoll et al. study only included patients with solitary renal stones (13). In another study, Kirac et al. reported a stone-free rate of 96% after mPNL (14). In that study, the rate of multiple calyx stones was 32.4%, which was comparable with our complex stone rate (42.9% in group 1 vs. 43.8% in group 2). However, the mean stone size was smaller compared with our study (10.5 vs. 21.7 mm in group 1 and 22.8mm in group 2). The stone characteristics in our study may account for our lower stone-free rates compared with other studies. Also, we found no significant correlation between the length of SCD and stone-free rates.

We found that the mean operation time and mean fluoroscopy time were significantly longer in patients with longer SCD. Ortiz et al. reported that poor fluoroscopic visualization of the stone and proper calyx in the presence of increasing retroperitoneal fat tissue may bring about difficulties in obtaining access (15). Additionally, depth perception becomes harder with increasing SCD, which results in an increased number of access trials. In our study, the number of access trials was not reported because of missing data, which will be a subject of our future studies. Keheila et al. emphasized that dilatation and securing the tract in patients with a longer SCD was a challenging and time-consuming process (16).

Fuller et al. preferred to obtain subcostal access to avoid pulmonary complications in patients who were obese and thus more vulnerable to undesired respiratory events under general anesthesia in the prone position (17). However, our approach in selecting an access location is different. Upper kidney poles are closer to the back than the lower poles and this may shorten the SCD. We performed 6 upper pole accesses in group 2, but performed no upper pole access in group 1. There was a large difference with access locations between groups but it did not reach statistical significance (p=0.051). Additionally, upper pole access was performed through the 11th intercostal space in 4 patients; however, we encountered no pulmonary complications. Our results imply that using smaller caliber instruments may prevent pulmonary complications. Comparison of the efficiency and safety of upper pole access during conventional PNL and mPNL in patients who are obese may be the subject of another study.

Blood transfusion rate following conventional PNL has been reported between 0.8% and 45% (18). The incidence of blood transfusion significantly decreased with the use of miniaturized instruments. Cheng et al. reported a 1.4% blood transfusion rate in mPNL (19). Abdelhafez et al. demonstrated a 0.5% blood transfusion rate in 191 patients following mPNL (20). In our study population, blood transfusion was required in 6 (4.3%) patients in group 1, and no patients required blood transfusion in group 2. Kuntz et al. emphasized that thick perirenal fat tissue may have a protective role by providing external compression, thereby preventing hemorrhagic events intraoperatively and after removal of the nephrostomy tube, which may account for the lower transfusion rates in patients with SCD >75mm (21).

Our study, which to the best of our knowledge is the first to investigate the effect of SCD on mPNL outcomes, has some limitations. We are aware of the retrospective nature of our study. Additionally, SCD was measured preoperatively on CT scans from an axial plane, which would not match with the exact distance between the skin and the desired calyx for access. However, we measured SCD in a similar manner for every patient. Also, preoperative CT imaging was performed in the supine position and the PNL operation was performed in the prone position, which may have altered the exact SCD. Finally, our procedures were performed by different surgeons with different levels of experience.

To conclude, our study demonstrated that SCD was not a predictive factor for stone-free rates following mPNL. The SCD value >75mm was associated with a longer operation time, longer fluoroscopy screening time, and lower transfusion rates. Our findings need to be validated in further prospective, randomized studies with larger study populations.

ABBREVIATIONS

PNL= Percutaneous nephrolithotomy
SCD= Skin to calyx distance

BMI= Body mass index
CT= Computerized tomography
KUB= Kidney-ureter-bladder X-Ray
SWL= Shockwave lithotripsy
UTI= Urinary tract infection
JJ= Double J catheter
f-URS= Flexible ureteroscopy
PTL= Percutaneous tract length
mPNL= Miniaturized percutaneous nephrolithotomy

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Does index tumor predominant location influence prognostic factors in radical prostatectomies?

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ABSTRACT

Purpose: To find any influence on prognostic factors of index tumor according to predominant location.
Materials and Methods: Prostate surgical specimens from 499 patients submitted to radical retropubic prostatectomy were step-sectioned. Each transverse section was subdivided into 2 anterolateral and 2 posterolateral quadrants. Tumor extent was evaluated by a semi-quantitative point-count method. The index tumor (dominant nodule) was recorded as the maximal number of positive points of the most extensive tumor area from the quadrants and the predominant location was considered anterior (anterolateral quadrants), posterior (posterolateral quadrants), basal (quadrants in upper half of the prostate), apical (quadrants in lower half of the prostate), left (left quadrants) or right (right quadrants). Time to biochemical recurrence was analyzed by Kaplan-Meier product-limit analysis and prediction of shorter time to biochemical recurrence using univariate and multivariate Cox proportional hazards model.
Results: Index tumors with predominant posterior location were significantly associated with higher total tumor extent, needle and radical prostatectomy Gleason score, positive lymph nodes and preoperative prostate-specific antigen. Index tumors with predominant basal location were significantly associated with higher preoperative prostate-specific antigen, pathological stage higher than pT2, extra-prostatic extension, and seminal vesicle invasion. Index tumors with predominant basal location were significantly associated with time to biochemical recurrence in Kaplan-Meier estimates and significantly predicted shorter time to biochemical recurrence on univariate analysis but not on multivariate analysis.
Conclusions: The study suggests that index tumor predominant location is associated with prognosis in radical prostatectomies, however, in multivariate analysis do not offer advantage over other well-established prognostic factors.

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Keywords:
Neoplasms; Prostate; Prostatectomy; Prostate-Specific Antigen

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INTRODUCTION

In a previous study we showed that total and index tumor extent were significantly associated with higher preoperative prostate specific antigen (PSA), clinical stage T2, pathological stage greater than T2, positive surgical margin (PSM) and higher radical prostatectomy (RP) Gleason score (1).Total and index tumor extent predicted time to biochemical recurrence (TBCR) following

RP on univariate analysis. However, only dominant nodule (index tumor) extent was an independent predictor of TBCR on multivariate analysis. The study suggested that any type of tumor extent estimate in surgical specimens should be related to the dominant nodule (index tumor) and not to total tumor extent.

The aim of this study is to find any influence on prognostic factors related to location of index tumor.

MATERIALS AND METHODS

This retrospective study was based on 499 consecutive patients submitted to radical retropubic prostatectomy by one surgeon (UF). Several clinicopathological variables were studied.

After RP, serum PSA from all patients was drawn every 3 months during the first year, every 6 months during the second year, and annually thereafter. No patient of this series had radiotherapy or androgen manipulation before or after surgery until biochemical recurrence (BCR) was observed. Total serum PSA was measured utilizing previous validated Immulite® PSA kit. BCR following surgery was considered as PSA ≥0.2ng/mL with a second confirmatory level of PSA >0.2ng/mL according to recommendation of the American Urological Association (2). Patients without evidence of BCR were censored at last follow-up. The present study was approved by the Institutional Committee of Ethics of our Institution.

The surgical specimens were step-sectioned at 3 to 5mm intervals and totally embedded in paraffin. A mean of 32 paraffin blocks were processed and 6µm sections from each block were stained with hematoxylin and eosin. Each transverse section of the prostate was subdivided into 2 anterolateral and 2 posterolateral quadrants. Using the cone method, 8 sections from the bladder neck and 8 sections from the apex were obtained.

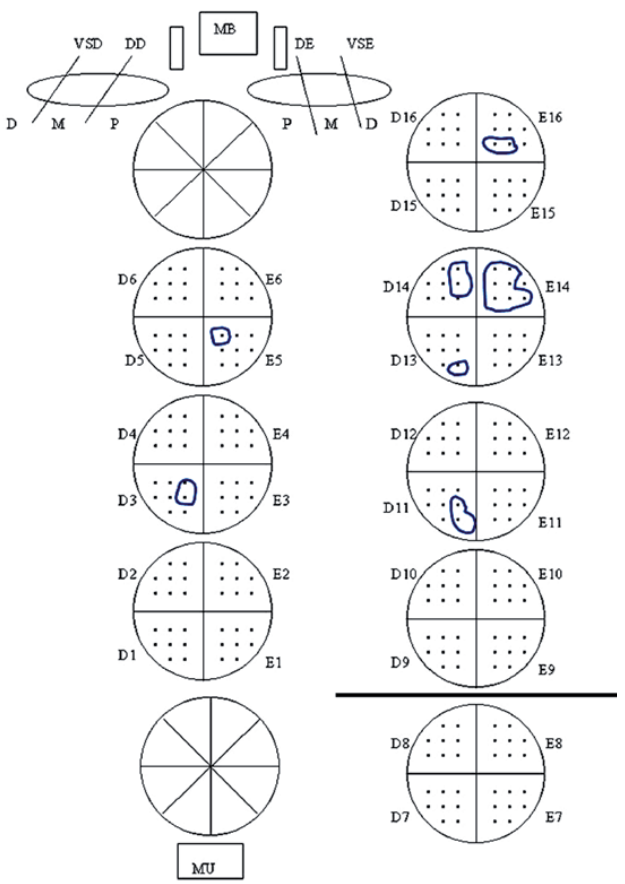
Gleason grading was considered from the overall tumor of the surgical specimen. PSM was defined as cancer cells in contact with the inked specimen surface. Extra-prostatic extension (EPE) was diagnosed whenever cancer was seen in adipose tissue and, in case of desmoplastic response, whenever a protuberance corresponding to extension of tumor into peri-prostatic tissue was seen. Seminal vesicle (SV) invasion occurred whenever there was involvement of the muscular coat. Tumor extent at RP was evaluated by a semi-quantitative point-count method previously described (3). Briefly, drawn on a sheet of paper, each quadrant of the transverse sections contained 8 equidistant points. During the microscopic examination of the slides, the tumor area was drawn on the correspondent quadrant seen on the paper. At the end of the examination the amount of positive points represented an estimate of the tumor extent. Total tumor extent was recorded as the total sum of positive points from all transverse quadrants. Index tumor extent (dominant nodule) was recorded as the maximum number of positive points from the most extensive area of cancer present in the quadrants.

From a total of 499 patients, index tumor was considered as predominantly anterior (located in anterolateral quadrants) in 110 prostates, posterior (located in posterolateral quadrants) in 235 prostates, basal (located in quadrants of the upper half of the prostate) in 117 prostates, apical (located in quadrants of the lower half of the prostate) in 279 prostates, left side of the prostate (located in left quadrants) in 155 prostates, and right side of the prostate (located in right quadrants) in 180 prostates. Index tumor was defined as the most extensive tumor area (largest nodule) in the surgical specimen. Total number of patients in each location group is not the same. The reason, for example, is that predominant right side index tumors may be located predominantly in different locations: basal or apical, and anterior or posterior. Extensive tumors equally distributed between the studied locations were excluded for analysis.

The clinicopathologic findings included: age, clinical staging (T1c, and T2), pathological staging (pT2, and pT3a/pT3b), preoperative PSA, prostate weight, PSA density, nodular hyperplasia, total tumor extent, needle Gleason score, RP Gleason score, PSM, EPE, SV invasion, and positive lymph nodes.

Figure-1 shows the drawing included in the pathology report with 8 equidistant points per quadrant. Total tumor extent was recorded as the total sum of the positive points of all transverse

Figure 1 - Semiquantitative point-count method to evaluate tumor extent. In this case total tumor extent was recorded as 17 positive points. Quadrant E14 shows largest single cancer focus or dominant nodule of all quadrants, recorded as 7 index tumor positive points. The tumor is predominantly basal (located in one quadrant of the upper half of the prostate). The horizontal line divides the prostate in quadrants located in upper and lower half of the prostate.



quadrants. Index tumor extent (dominant nodule) was recorded as the maximum number of positive points for the largest single focus of cancer in the quadrants. In this particular example, index tumor was in quadrant E14 and located predominantly at the base (upper half of the prostate).

Statistical analysis

The data were analyzed using the Chi--square and the Fisher exact test for comparison of proportions, the Mann-Whitney test for comparison of means, and the Kaplan-Meier product-limit analysis for the TBCR using the log-rank test for comparison between the groups. A univariate and multivariate Cox stepwise logistic regression model was used to identify significant predictors of shorter TBCR. The relative importance of the prognostic variables was measured by the Wald test. The P-values were two-sided at the significance level of <0.05. All statistical analyses were performed using the commercial available PASW Statistics (SPSS) 18.0.

RESULTS

Clinicopathological Findings

Index tumors with predominant posterior location were significantly associated with higher total tumor extent, needle and RP Gleason score, positive lymph nodes and preoperative PSA (the latter in the limit of significance) (Table-1).

Index tumors with predominant basal location were significantly associated with higher preoperative PSA, pathological stage higher than pT2, EPE, and SV invasion (Table-2).

Index tumors predominantly at right side were significantly associated with higher preoperative PSA and prostate weight (Table-3).

Time to biochemical recurrence

Index tumor with predominant anterior vs. posterior location

From a total of 345 patients following RP, 102 (29.6%) patients had BCR at a mean, median and range follow-up of 28, 15, and 1-158 months; 226 (65.5%) censored men remained at risk at a mean, median and range follow-up of 54, 44, and 1-169 months, respectively; and, 17 (4.9%) men had no serum PSA data.

At 5 years of follow-up, 74% of patients with predominantly anterior index tumor were free of BCR vs. 67% of patients with predominantly posterior index tumor (log-rank, p=0.208, Figure-2).

Index tumor with predominant basal vs. apical location

From a total of 396 patients following RP, 125 (31.6%) patients had BCR at a mean, median and range follow-up of 25, 10, and 1-158 months;

Table 1 - Clinicopathological features of 345 patients by index tumor predominant location.

Feature	Anterior (n=110)	Posterior (n=235)	p Value
Mean ± SD age/median (range)	63.63 ± 6.45/65 (45-75)	62.89 ± 6.73/64 (43-76)	0.290 (Mann-Whitney test)
No. race (%)			
Whites	86 (78.2)	188 (81%)	0.563 (Fisher exact test)
African-Brazilians	24 (21.8)	44 (19%)	
No. clinical stage (%)			
T1c	65 (60.7)	129 (55.8)	0.410 (Fisher exact test)
T2	42 (39.3)	102 (44.2)	
Mean ± SD pre-op PSA/ median (range)	8.03 ± 4.61/7.04 (0.6-22)	9.42 ± 5.64/8 (1.22-35)	0.050 (Mann-Whitney test)
Mean ± SD prostate weight/ median (range)	39.18 ± 21/35 (10-130)	40.42 ± 21.81/35 (15-190)	0.524 (Mann-Whiteny test)
Mean ± SD PSA density/ median (range)	0.24 ± 0.17/0.19 (0.02-.87)	0.35 ± 1.26/0.22 (0.04-19.25)	0.119 (Mann-Whitney test)
No. nodular hyperplasia (%)			
Neg	33 (30)	53 (22.9)	0.183 (Fisher exact test)
Pos	77 (70)	178 (77.1)	
Mean ± SD tumor extent/ median (range)	22.97 ± 19.62/19 (1-94)	29.26 ± 25.91/23 (1-147)	0.040 (Mann-Whitney test)
Mean ± SD needle Gleason score/median (range)	6.30 ± 0.64/6 (4-9)	6.51 ± 0.68/6 (6-9)	0.007 (Mann-Whitney test)
Mean ± SD RP Gleason score/median (range)	6.53 ± 0.57/7 (5-8)	6.82 ± 0.74/7 (4-9)	<0.001 (Mann-Whitney test)
No. surgical margin at any location (%)			
Neg	66 (60)	120 (51.3)	0.134 (Fisher exact test)
Pos	44 (40)	114 (48.7)	
No. Extra-prostatic extension (%)			
Neg	85 (77.3)	174 (74)	0.594 (Fisher exact test)
Pos	25 (22.7)	61 (26)	
No. seminal vesicle invasion (%)			
Neg	105 (96.3)	215 (93.1)	0.325 (Fisher exact test)
Pos	4 (3.7)	16 (6.9)	
No. pathological stage (%)			
pT2	85 (77.3)	172 (73.2)	0.508 (Fisher exact test)
pT3a/pT3b	25 (22.7)	63 (26.8)	
No. lymph nodes (%)			
Not resected	64 (58.2)	107 (45.5)	0.040 (Chi-square test)
Neg	46 (41.8)	123 (52.3)	
Pos	0 (0)	5 (2.2)	

Table 2 - Clinicopathological features of 396 patients by index tumor predominant location.

Feature	Basal (n=117)	Apical (n=279)	p Value
Mean ± SD age/median (range)	62.98 ± 6.40/64 (45-75)	62.96 ± 6.49/64 (42-76)	0.974 (Mann-Whitney test)
No. race (%)			
Whites	94 (81.0)	223 (80.5)	>0.999 (Fisher exact test)
African-Brazilians	22 (19.0)	54 (19.5)	
No. clinical stage (%)			
T1c	58 (50.9)	151 (54.7)	0.505 (Fisher exact test)
T2	56 (49.1)	125 (45.3)	
Mean ±SD pre-op PSA/median (range)	10.73±7.41/8.6 (0.60-51)	9.08±5.49/7.76 (0.28-33)	0.047 (Mann-Whitney test)
Mean ± SD prostate weight/ median (range)	40.94 ± 22.67/35 (11-130)	40.24 ± 28.44/35 (10-190)	0.985 (Mann-Whitney test)
Mean ± SD PSA density/ median (range)	0.30 ± 0.24/0.24 (0.03-1.38)	0.33 ± 1.16/0.21 (0.01-19.25)	0.133 Mann-Whitney test)
No. nodular hyperplasia (%)			
Neg	38 (32.8)	71 (26)	0.177 (Fisher exact test)
Pos	78 (67.2)	202 (74)	
Mean ± SD tumor extent/median (range)	35.12 ± 35.66/24.50 (1-225)	31.24 ± 27.18/26 (1-158)	0.775 (Mann-Whitney test)
Mean ± SD needle Gleason score/ median(range)	6.49 ± 0.77/6 (4-9)	6.49 ± 0.68/6 (5-9)	0.770 (Mann-Whitney test)
Mean ± SD RP Gleason score/median (range)	6.83 ± 0.87/7 (5-9)	6.76 ± 0.75/7 (5-9)	0.899 (Mann-Whitney test)
No. surgical margin at bladder neck (%)			
Neg	102 (90.3)	271 (97.8)	0.002 (Fisher exact test)
Pos	11 (9.7)	6 (2.2)	
No. surgical margin at apex (%)			
Neg	107 (94.7)	237 (85.3)	0.009 (Fisher exact test)
Pos	6 (5.3)	41 (14.7)	
No. extra-prostatic extension (%)			
Neg	76 (65)	217 (77.8)	0.012 (Fisher exact test)
Pos	41 (35)	12 (22.2)	
No. seminal vesicle invasion (%)			
Neg	96 (84.2)	265 (95.7)	<0.001 (Fisher exact test)
Pos	18 (15.8)	12 (4.3)	
No. pathological stage (%)			
pT2	76 (65)	215 (77.1)	0.017 (Fisher exact test)
pT3a/pT3b	41 (35)	64 (22.9)	
No. lymph nodes (%)			
Not resected	54 (46.2)	144 (51.6)	0.364 (Chi-square test)
Neg	58 (49.6)	129 (46.2)	
Pos	5 (4.3)	6 (2.2)	

Table 3 - Clinicopathological features of 335 patients by index tumor predominant location.

Feature	Left (n=155)	Right (n=180)	p Value
Mean ± SD age/median (range)	63.17 ± 6.83/64 (42-76)	63.02 ± 6.32/64 (46-76)	0.685 (Mann-Whitney test)
No. race (%)			
Whites	122 (78.7)	146 (82.0)	0.489 (Fisher exact test)
African-Brazilians	33 (21.3)	32 (18.0)	
No. clinical stage (%)			
T1c	75 (48.7)	104 (58.4)	0.079 (Fisher exact test)
T2	79 (51.3)	74 (41.6)	
Mean ± SD pre-op PSA/median (range)	8.44 ± 5.16/7.2 (0.28-35)	9.80 ± 5.99/8 (0.6-41)	0.028 (Mann-Whitney test)
Mean ± SD prostate weight/median (range)	35.86 ± 18.87/30 (10-190)	40.96 ± 22.5/35 (11-185)	0.017 (Mann-Whitney test)
Mean ± SD PSA density/median (range)	0.27 ± 0.19/0.22 (0.01-1.17)	0.28 ± 0.22/0.22 (0.03-1.38)	0.589 (Mann-Whitney test)
No. nodular hyperplasia			
Neg	47 (30.9)	45 (25.1)	0.269 (Fisher exact test)
Pos	105 (69.1)	134 (74.9)	
Mean ± SD tumor extent/median (range)	29.17 ± 26.03/22 (1-127)	28.88 ± 26.58/24 (1-151)	0.866 (Mann-Whitney test)
Mean ± SD needle Gleason score/ median (range)	6.49 ± 0.73/6 (5-9)	6.53 ± 0.69/6 (6-9)	0.435 (Mann-Whitney test)
Mean±SD RP Gleason score/median (range)	6.76 ± 0.69/7 (5-9)	6.78 ± 0.71/7 (4-9)	0.676 (Mann-Whitney test)
No. surgical margin at any location (%)			
Neg	91 (59.1)	91 (50.6)	0.124 (Fisher exact test)
Pos	63 (40.9)	89 (49.4)	
No. Extra-prostatic extension (%)			
Neg	110 (71)	129 (71.7)	0.904 (Fisher exact test)
Pos	45 (29)	51 (28.3)	
No. seminal vesicle invasion (%)			
Neg	143 (94.1)	165 (92.2)	0.525 (Fisher exact test)
Pos	9 (5.9)	14 (7.8)	
No. pathological stage (%)			
pT2	110 (71)	127 (70.6)	>0.999 (Fisher exact test)
pT3a/pT3b	45 (29)	53 (29.4)	
No. lymph nodes (%)			
Not resected	63 (40.6)	91 (50.6)	0.192 (Chi-square test)
Neg	88 (56.8)	85 (47.2)	
Pos	4 (2.6)	4 (2.2)	

Figure 2 - Kaplan-Meier product limit analysis shows time to PSA biochemical progression-free outcome by index tumor anterior vs posterior predominant location. Cum, cumulative.

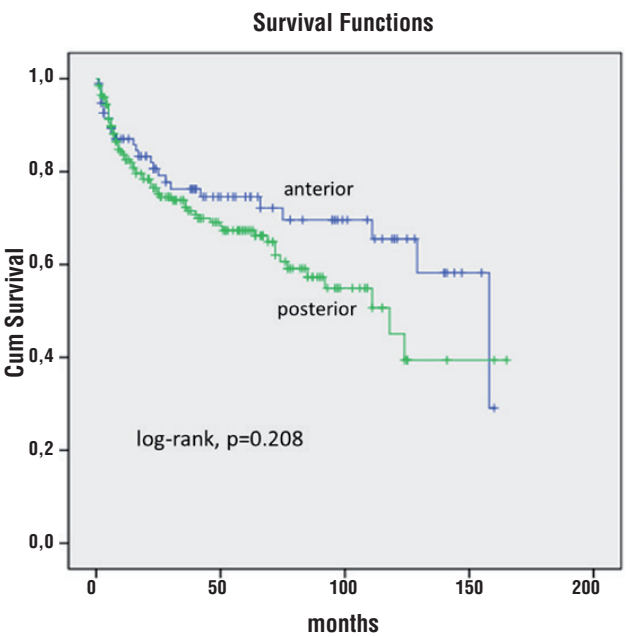
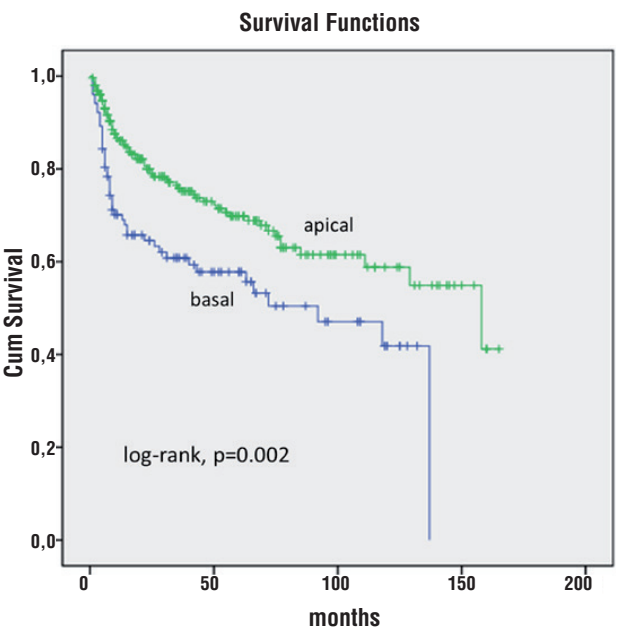


Figure 3 - Kaplan-Meier product limit analysis shows time to PSA biochemical progression-free outcome by index tumor basal vs apical predominant location. Cum, cumulative.



256 (64.6%) censored men remained at risk at a mean, median and range follow-up of 54, 43, and 1-169 months, respectively; and, 15 (3.8 %) men had no serum PSA data.

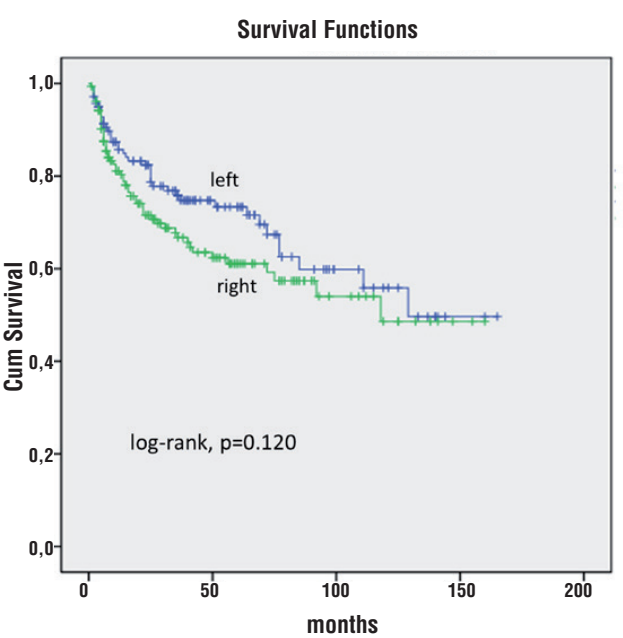
At 5 years of follow-up, 59% of patients with predominantly basal index tumor were free of BCR vs. 70% of patients with predominantly apical index tumor (log-rank, p=0.002, Figure-3).

Index tumor with predominant left vs. right location

From a total of 335 patients following RP, 103 (30.7 %) patients had BCR at a mean, median and range follow-up of 25, 13, and 1-129 months; 218 (65.1%) censored men remained at risk at a mean, median and range follow-up of 54, 43, and 1-169 months, respectively; and, 14 (4.2%) men had no serum PSA data.

At 5 years of follow-up, 79% of patients with predominantly left index tumor were free of BCR vs. 61% of patients with predominantly right index tumor (log-rank, p=0.120, Figure-4).

Figure 4 - Kaplan-Meier product limit analysis shows time to PSA biochemical progression-free outcome by index tumor left vs right predominant location. Cum, cumulative.



Risk of shorter time to biochemical recurrence

In univariate Cox regression analysis (Table-4), PSA density, needle Gleason score, preoperative PSA, predominant index tumor basal location, EPE, total tumor extent, pathological stage greater than T2, RP Gleason score, SV invasion, and PSM were significantly predictive of shorter TBCR.

In multivariate analysis (Table-4) including all significant predictors in univariate analysis, only SV invasion and PSM were independent predictors of shorter TBCR. In all models we used the backward stepwise logistic regression method.

DISCUSSION

Index tumors with predominant posterior location (posterolateral quadrants) comprise most part of the peripheral zone (PZ), and with predominant anterior location (anterolateral quadrants) most part of the transition zone (TZ). Index tumors with predominant posterior location were significantly associated with higher total tumor extent, needle and RP Gleason score, positive lymph nodes and preoperative serum PSA (the latter in the limit of significance).

Index tumors with predominant basal location were significantly associated with higher preoperative serum PSA, pathological stage higher than pT2, EPE, SV invasion, TBCR in Kaplan-Meier estimates and significantly predicted shorter TBCR on univariate analysis but not on multivariate analysis. There are several studies comparing index tumor in PZ location with index tumor with TZ location but to the best of our knowledge we did not find any mention to basal or apical location.

The 2009 ISUP (International Society of Urological Pathology) meeting failed to a consensus on the dominant pathological parameters of tumor extension or volume, Gleason score, or staging that define index tumor (4). However, most of the participants considered to be the largest nodule in multifocal disease. Moreover, in most of the cases, it corresponds also to the highest Gleason score in accordance with the global Gleason score.

Prostate cancer emerges as an evolutionary process often leading to multiple competing subclones within a single primary index tumor. This evolutionary process culminates in the formation of metastases. However, although the hypothesis that each metastasis originates from a single tumor cell is generally supported, several studies have supported the existence of polyclonal seeding from an interclonal cooperation between multiple subclones. These latter findings bring insights to find the "true" index lesion by looking on genetic, epigenetic and proteomic alterations (5, 6).

In Al-Ahmadie et al. (7) study in radical prostatectomies, 35.5% cancers were considered as originating from the TZ. This percentage is very similar to ours (31.9%). TZ tumors seem to be of lower degree of biologic aggressiveness (8). In radical prostatectomies, Grignon et al. (9) found that the mean Gleason score for the PZ and TZ tumors was 6.7 and 5.6, respectively ($p<0.001$). Gleason score also was higher in PZ cancers in the study by Lee et al. (10). In our study, the mean Gleason score in index tumors posteriorly located vs. anteriorly located was significantly higher in needle biopsies ($p=0.007$) and in RP ($p<0.001$).

In Lee et al. (10) study, 48% cancers originating in the PZ showed EPE, and 22% of cancers originating in the TZ. In our study, EPE was present in 26% and 22.7% of cancers located predominantly at posterior and anterior location, respectively ($p=0.594$). However, EPE was present in 35% and 22.2% of cancers located predominantly at basal and apical location, respectively ($p=0.012$). Basal tumor location was significantly associated with higher serum PSA (0.047) as well as index tumors with posterior location (the latter in the limit of significance, $p=0.050$). Interestingly, predominantly right side index tumors had significantly higher serum PSA ($p=0.028$) as well as higher prostate weight ($p=0.017$).

Greene et al. (8) found that SV invasion arose from 19% of the PZ but none of the TZ cancers. In our study, there was no significant difference in SV invasion comparing predominantly anterior with posterior located tumors ($p=0.325$). A very significant difference was found comparing basal with apical location. SV invasion was present in 15.8% of tumors located at the base and in 4.3% of tumors located at the apex ($p<0.001$).

Table 4 - Cox univariate and multivariate proportional hazard analysis of several clinicopathological factors predicting shorter time to biochemical recurrence after radical prostatectomy.

Predictors	HR (95% CI)	Wald test	p Value
	Univariate		
Age	0.997 (0.974-1.021)	0.063	0.802
Race	0.835 (0.551-1.264)	0.726	0.394
Clinical stage	1.174 (0.860-1.603)	1.021	0.312
Nodular hyperplasia	0.828 (0.594-1.154)	1.237	0.266
Index tumor: ant vs post	1.316 (0.855-2.025)	1.596	0.212
Index tumor: left vs right	1.361 (0.920-2.015)	2.377	0.123
Positive lymph nodes	2.002 (0.865-4.633)	2.631	0.105
Prostate weight	1.006 (1.000-1.013)	3.415	0.065
PSA density	1.812 (1.048-3.133)	4.530	0.033
Needle Gleason score	1.337 (1.077-1.659)	6.951	0.008
Pre-op PSA	1.026 (1.008-1.043)	8.605	0.003
Index tumor: basal vs apical	1.745 (1.218-2.500)	9.214	0.002
Extra-prostatic extension	1.708 (1.239-2.356)	10.674	0.001
Tumor extent	1.006 (1.003-1.010)	10.953	0.001
Pathological stage >T2	1.771 (1.287-2.438)	12.311	<0.001
RP Gleason score	1.422 (1.169-1.728)	12.471	<0.001
Seminal vesicle invasion	2.781 (1.832-4.223)	23.035	<0.001
Positive surgical margin	2.366 (1.709-3.275)	26.902	<0.001
	Multivariate		
Tumor extent	0.999 (0.992-1.006)	0.085	0.771
RP Gleason score	1.057 (0.784-1.426)	0.132	0.717
PSA density	0.802 (0.345-1.863)	0.263	0.608
Pathological stage >T2	0.438 (0.048-4.010)	0.533	0.465
Extra-prostatic extension	0.340 (0.770-0.451)	0.912	0.340
Pre-op PSA	1.029 (0.982-1.069)	1.268	0.260
Index tumor: basal vs apical	0.764 (0.512-1.139)	1.751	0.186
Needle Gleason score	1.293 (0.982-1703)	3.348	0.067
Seminal vesicle invasion	2.326 (1.314-4.120)	8.384	0.004
Positive surgical margin	2.150 (1.455-3.177)	14.761	<0.001

In Noguchi et al. (11) study, Kaplan-Meier curves showed that at 5 years of follow-up 49.2% of men with PZ cancer had undetectable PSA compared with 71.5% of those with TZ cancer (log rank, p=0.0002). Stamey et al. (12) reported a 5-year disease-free survival rate of 53% in men with PZ and 81% in those with TZ cancers. Sakai et al. (13) showed that there was no significant difference in biochemical recurrence-free survival between patients with TZ and PZ cancers. Augustin et al. (14) found that the location of prostate cancer in the TZ was associated with a greater overall biochemical cure rate after RP. However, they found that it was not an independent prognostic factor on multivariate analysis. Therefore, the authors concluded that knowledge about zonal location of prostate cancer offers no advantage over the well-established prognostic factors in predicting disease recurrence. Chun et al. (15) showed that in multivariate Cox models, the rate of BCR was not significantly different between TZ and PZ prostate cancers (p=0.4).

In our study, the Kaplan-Meier curves did not show any significant difference comparing anterior vs posterior index tumor location. At 5 years of follow-up, 74% of patients with predominantly anterior index tumor were free of BCR vs 67% of patients with predominantly posterior index tumor (log-rank, p=0.208, Figure-2). On the other hand, at 5 years of follow-up, 59% of patients with predominantly basal index tumor were free of BCR vs 70% of patients with predominantly apical index tumor (log-rank, p=0.002 Figure-3). In univariate analysis, predominantly basal tumor location had significantly shorter TBCR (p=0.002) but not in multivariate analysis (p=0.186). Only needle SV invasion (pT3b), and PSM were independent predictors of shorter TBCR.

Iremashvili et al. (16) found that the rates of PSM were similar in men with TZ and mixed tumors and were significantly higher than those with PZ tumors. In index tumors located at the TZ, Van de Voorde et al. (17) found that EPE, SV involvement, PSMs, and lymph node metastasis were seen in the TZ cancer group in 33%, 17%, 29%, and 4%, respectively versus 58%, 20%, 48%, and 6% in the PZ cancer group. In our cohort of patients who had lymph nodes resected, metastasis occurred in 2.2% of posteriorly located tumors and 0% anteriorly; 4.3% in basal tumors and 2.2% in apical located tumors.

Comparing anteriorly and posteriorly located tumors, Mygatt et al. (18) found that there was no difference between mean age, body mass index, racial distribution, family history, number of previous biopsies, clinical Gleason sum or pathological stage in the two groups. Lallas et al. (19) showed that patients with PSM were subsequently found to have higher risk of biochemical recurrence. O'Neil et al. (20) comparing TZ tumors with PZ tumors found that the formers were larger, more frequently lower grade, organ confined, and preferentially involved the bladder neck (49% vs 6%, p<0.001). Tumor zonality was not associated with BCR for the entire cohort. PSA recurrence in patients with histologically confirmed PSMs after RP was independent of the zonal location of the index tumor.

We did not find any racial difference considering all locations studied. Anterior vs posterior, and left vs right location did not show any statistical significant difference associated with PSM. However, in predominant basal location vs apical location the frequency of bladder neck PSM was 9.7% and 2.2% (p=0.002), respectively; and, apical PSM was 5.3% and 14.7% (p=0.009), respectively.

Predominant basal tumor location was significantly associated with higher pathologic stage. EPE was present in 35% of basal tumors vs 22.2% apical tumors (p=0.012), and SV invasion in 15.8% vs 4.3%, respectively (p<0.001). The finding of SV invasion in a RP specimen markedly diminishes the likelihood of cure. Possible routes of SV invasion are: 1) extension into soft tissue adjacent to the SV and then into the SV; 2) invasion via the sheath of the ejaculatory duct, penetrating the muscular wall of the ejaculatory duct, or extending up the ejaculatory duct wall into the SV muscle wall; 3) direct invasion of the SV; or 4) discontinuous metastases. There are conflicting studies as to whether the first or second method is most common (21-23). Metastases are the least common mode of spread.

Epstein et al. (23) reported the findings of 60 men who had undergone radical retropubic prostatectomy and whose tumors demonstrated isolated SV invasion. In their study the most frequent route of SV invasion (34/60 patients, 56.7%) was tumor extension out of the prostate at the base of the gland into the peri-seminal vesicle tissue, with subsequent invasion into the muscular wall of the SV. In favor of this finding is the fact that in unilateral invasion of the SV most frequently there is ipsilateral EPE and in bilateral invasion most frequently there is bilateral EPE (24). Besides the anatomic proximity, the finding in our study of a significant higher EPE in predominantly basal tumor location, favors that extension into soft tissue adjacent to the SV with subsequent invasion into the muscular wall is the most frequent route of SV invasion.

Some study limitations warrant discussion. Standard pathological evaluation of the index tumor may not be parallel to the axis and be a confounding location considering the tridimensional aspect of the lesion. Follow-up of the patients studied could be longer and the number of patients higher. If we had incorporated additional variables in the Cox model, such as tumor extent on biopsy, preoperative PSA velocity and others, results could have been different. Therefore, other studies are needed that incorporate these variables as well as studies that include basal and apical index tumor predominant location for the sake of comparison with our results.

CONCLUSIONS

Index tumors with predominant posterior location were significantly associated with higher total tumor extent, needle and RP Gleason score, positive lymph nodes and preoperative PSA. Index tumors with predominant basal location were significantly associated with higher preoperative PSA, pathological stage higher than pT2, EPE, SV invasion, TBCR in Kaplan-Meier estimates and significantly predicted shorter TBCR on univariate analysis but not on multivariate analysis. The study suggests that index tumor predominant location is associated with prognosis in radical prostatectomies, however, in multivariate analysis do not offer advantage over other well-established prognostic factors.

ABBREVIATIONS

PSA = Prostate specific antigen
RP = Radical prostatectomy
SD = Standard deviation
CI = Confidence interval
HR = Hazard
BCR = Biochemical recurrence
TBCR = Time to BCR
PSM = Positive surgical margin
EPE = Extra-prostatic extension
SV = Seminal vesicle
PZ = Peripheral zone
TZ = Transitional zone

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Effects of body mass index on the outcomes of percutaneous nephrolithotomy

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ABSTRACT

Objective: To examine the the effect of body mass index (BMI) on PNL results and complications with a large number of patients.
Materials and Methods: A total of 958 patients were included in the study, who underwent percutaneous nephrolithotomy in our clinic between 2008 and 2015. Patients were divided into 2 groups according to their body mass index. Patients with a BMI < 30 kg/m^2 were classified as group 1 (n:676) and patients with a BMI ≥ 30 kg/m^2 were classified as group 2 (n:282). Achieving stone-free status or having residual stones of ≤ 4 mm were considered as operational success.
Results: The mean age was 47.9 years for group 1 and 48.9 years for group 2 patients. At postoperative first month CT analysis, residual stone was not observed in 466 patients (69%) of group 1 and 20 (72%) patients of group 2. There was no significant difference between the groups in terms of stone-free status (p=0.348). There was no significant difference between two groups complications. Also, there was no difference between the groups for requiring additional intervention (p=0.924). No other complications were observed in the patients.
Conclusions: BMI does not affect the outcomes of percutaneous nephrolithotomy as well as complication rate.

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Body Mass Index; Nephrostomy, Percutaneous; Calculi

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INTRODUCTION

Obesity is a widespread health problem with an increasing frequency all over the World, due to inadequate diet and decreased physical activity (1). It is well known that urinary tract stone disease is more common in obese patients (2). Percutaneous nephrolithotomy (PNL) is accepted as the gold standard treatment method for renal stones greater than 2 cm with high stone-free and reasonable complication rates (3). However, the results of PNL in obese patients are controversial. There are studies in the literature, indicating that obesity negatively influences the outcome of the operation as well as studies stating that it has no effect (4, 5). In this study, we investigated the effect of body mass index (BMI) on PNL results and complications.

MATERIALS AND METHODS

We reviewed the data of 1150 patients who underwent PNL in our clinic between 2008 and 2015, retrospectively. Patients under the age of 18, who had an urinary system anomaly and underwent open stone surgery at the operation side were excluded from the study. A total of 958 patients with complete demographic, intraoperati-

ve, and postoperative data and having no exclusion criteria were included in the study. Patients were divided into two groups with respect to the body mass index. Those with a BMI < 30 kg/m^2 were classified as non-obese (Group 1), whereas those with a BMI ≥ 30 kg/m^2 were classified as obese (Group 2).

All patients were examined by non-contrast whole abdomen CT before the operation. In imaging studies, only the stones found in one calyx or only in the pelvis were named as simple stones while pelvic stones filling one calyx or more as well as staghorn stones were named as complicated stones (6).

The period, starting from the time of contrast agent administration to the patient at the prone position, up to the time of nephrostomy catheter insertion was recorded as the operation time. Duration of fluoroscopic imaging throughout the operation, the number of accesses, and the presence of intraoperative complications were also recorded. At the end of the operation, a 14 Fr re-entry Malecot catheter was placed in all patients and removed on postoperative day 1-3 and the patients without any complications were then discharged from the hospital. Furthermore, postoperative complications and additional interventions were noted for each patient. Complications were also classified according to Modified Clavien system.

All patients were reevaluated with non-contrast CT taken routinely at postoperative 1 month. The presence of residual fragments of ≤ 4 mm or no stone was considered as therapeutic success (7).

SURGICAL PROCEDURE

Complete blood count, biochemical tests, coagulation tests and urine culture were performed in all patients preoperatively. Appropriate antibiotic treatment was given to the patients with positive urinary cultures and they were operated when they had sterile urine cultures. The patient was placed in the prone position after an open-ended 6-Fr ureter catheter was inserted through the urethra via cystoscopy while in the lithotomy position. The procedure was performed under general anesthesia. Following the injection of an opaque agent from the ureter catheter, an access needle was introduced into the renal collecting system via the appropriate calyx under fluoroscopic guidance. After placement of the guiding catheter, the Amplatz dilator set was used in order to create the tract, first with a 6 Fr dilator followed by a 28-30 Fr dilator, using the single step method. The same technique was used for the patients with complex stones, where a second access was required. The stones were broken by using a 24 Fr nephroscope and an ultrasonic lithotripter. A 14 Fr malecot re-entry catheter was routinely inserted after the operation was terminated.

STATISTICAL ANALYSIS

The chi-square test was used to assess the categorical variables among the groups. The Mann-Whitney U test was used in order to compare the differences between the two independent groups. *P* value <0.05 was considered significant**.** IBM SPSS version 15.00 was used for analysis.

RESULTS

There were 676 patients in Group 1 and 282 patients in Group 2. Of the patients, 422 were male (62.4%) and 254 were female (37.6%) in group 1, while 150 were male (53.1%) and 132 were female (46.9%) in group 2. The groups were similar in terms of gender (p=0.557).

The mean age of the patients was 47.9±14.7 years in group 1 and 48.9±13.3 years in group 2. There was no statistically significant difference in terms of age between the two groups although the patients in the obese group were slightly older than others (p=0.065).

Among Group 1 patients, 387 (57.2%) had simple stones while 289 (42.8%) had complex stones. In group 2, 156 (55.3%) had simple stones and 126 (44.7%) had complex stones. There was no statistically significant difference between two groups in terms of complexity and simplicity of the stones (p=0.583).

The mean duration time of operation was 72.4±3.81 minutes in the non-obese group while 65.4±2.75 minutes in the obese group. The mean

duration of operation was not statistically significantly different between the two groups although it was relatively longer for group 1 patients (p=0.683).

The mean duration of fluoroscopy was 149.3±6.06 seconds in Group 1 patients and 144.4±9.74 seconds in group 2 patients, displaying no statistically significant difference between the two groups (p=0.803).

In the non-obese group, 580 patients had one, 83 had two, and 13 had three accesses. In the obese group however, 251 patients had one, 25 had two, and 6 had three accesses.Two groups were similar in terms of the number of accesses (p=0.311).

A total of 785 accesses were performed in 676 patients in Group 1. Of these, 709 (90.3%) were subcostal and 76 (9.7%) were intercostal access, through 11-12 intercostal space. In Group 2, totally 319 accesses were performed, of which 282 (88.4%) were subcostal and 37 (11.6%) were intercostal. There was no significant difference between the two groups in terms of the site of access (p=0.341) (Table-1).

Due to intraoperative hemorrhage resulting in hypotension, blood transfusion was given to 12 patients (1.7%) in group 1 and 4 patients (1.4%) in group 2. No significant difference was detected between the two groups, in terms of intraoperative blood transfusion (p=0.695). Except for hemorrhage, no other intraoperative complication was observed in the patients.

Blood transfusion was required due to postoperative hemoglobine loss and hemodynamic instability such as hypotension in 24 (3.5%) patients in group 1 and 7 patients (2.4%) in group 2. Postoperative transfusion was grade 2 according to the Modified Clavien Classification Sys-

Table 1 - Significant preoperative and intraoperative data of the patients.

	Group 1	Group 2	P value
	(n:676)	(n:282)	
Mean age (years)	47.9	48.9	0.065
Gender			
Male	422 (62.4)	150 (53.1)	0.557
Female	254 (37.6)	132 (46.9)	
Stone Load (%)			
Simple	387 (57.2)	156 (55.3)	0.583
Complex	289 (42.8)	126 (44.7)	
Operation time (min.)	72.4	65.4	0.683
Fluoroscopy time (sec)	144.4	149.3	0.803
Site of access (%)			
Subcostal	709 (90.3)	282 (88.6)	0.341
Intercostal	76 (9.7)	37 (11.4)	
Number of access			
One	580	251	0.311
Two	83	25	
Three	13	6	
Stone-free status (%)	466 (69)	203 (72)	0.348

tem and showed no difference between the groups (p=0.395). No patient needed more than 2 units of transfusion.

Fever (>38ºC) was determined before discharge in 27 patients of the obese group and 70 patients of the non-obese group, and they were appropriately treated before being discharged from the hospital. No significant difference was detected in terms of postoperative fever between the groups (p=0.715). None of the patients developed sepsis or died from operation-related complications. Fever was considered as grade 1 complication group according to the Modified Clavien Classification System.

When the groups were evaluated in terms of operation success, postoperative residual stone fragments were observed in 210 patients (31%) of group 1 and in 79 patients (28%) of group 2. There was no significant difference in operative performance between the groups (p=0.348).

The groups were also evaluated for additional interventions. Thirty-six patients of the non-obese group required additional interventions after their discharge from the hospital. Double-J stents (DJS) were inserted in twenty-six patients due to wound site discharge or severe colic pain. Ureteroscopy (URS) was performed in twenty-six patients for treatment of the ipsilateral ureteral stones determined by non-contrast abdominal CT. In group 2 however, a total of 15 patients required additional interventions. Eight patients were inserted DJS because of the wound site discharge. Six patients underwent URS due to ureteral stone. Due to postoperative persistent hematuria in one patient, selective angiography was applied and upon determination of arteriovenous fistula, eventually superselective embolisation was performed. No additional treatment was required after embolisation. The requirement for additional intervention was considered as Clavien grade 3 and there was no significant difference between two groups (p=0.924) (Table-2).

DISCUSSION

Obesity has become a major problem for both developed and developing countries as a result of reduced physical activity and increased calorie intake. Particularly high income countries display higher rates of increase in obesity in the last three decades (1). The incidence of health problems such as metabolic syndrome, cardiovascular disease, malignancy, and renal calculus also increased in the community with the increase in obesity (8).

Today, PNL is a widely used method in renal stone treatment in both obese and non-obese

Table 2 - Intraoperative and postoperative complications of the groups.

	Group 1	Group 2	p value
	(n:676)	(n:282)	
Intraoperative transfusion (%)	12 (1.7)	4 (1.4)	0.695
Postoperative transfusion (%)	24(3.5)	7 (2.4)	0.395
Postoperative fever (%)	70 (10.3)	27 (9.5)	0.715
Additional interventions			
URS	26	8	0.924
DJS	10	6	
Embolization	-	1	
Modified Clavien Classification System			
Grade 1	70	27	0.715
Grade 2	24	7	0.395
Grade 3	36	15	0.924

patients. However, anestesia-related problems can be seen in obese patients. Respiratory complications may occur, for example a decrease in total lung capacity, as a result of the prone position during operation. Again, entubation difficulties may also occur in obese patients (9). In some centers, PNL is performed in the supine position in order to minimize such complications (10). In our clinic however, PNL in the supine position is not performed due to the lack of experience, yet complications related to anesthesia have not been observed.

In obese patients, it may not be possible to reach the renal collecting system with Amplatz sheet or it may be difficult to reach the stone because of the thick subcutaneous fat tissue. Curtis et al. reported that they made an incision in the skin and adipose tissue and retracted the tissue and so gained extra distance (11). In our study, standard Amplatz dilatators were used in both groups and access was obtained in all patients.

Carson et al. compared 44 obese and 226 non-obese patients and found no significant difference between the groups in terms of operation time, stone-free status and complication rates (12). In their study conducted with 236 patients (57 obese, 279 non-obese), Pearle et al. found no significant difference between the groups in terms of operative success and complication rates whereas longer operation time and higher rates of blood transfusion in the obese group (13). El-Assmy et al. indicated the mean stone size as 2.5±0.85 cm and reported no correlation between BMI and operative success (14). Alyami et al. examined patients having renal stones smaller than 3 cm and determined no correlation between BMI and operative success (5).

Croes study group published the first prospective study including 3709 patients (15). Patients with PNL were sorted according to their BMI. In contrast to many publications, they indicated a decrease in the rate of stone-free status whereas an increase in the duration of the operation in parallel with the increased BMI. In a series of 530 patients, Fearber et al. determined significantly higher complication rates in obese patients than in patients with normal BMI (16). Meanwhile, Pearle et al. reported that the need for blood transfusion was higher in the group with obese patients (13). Koo et al. examined a series of 181 patients in 4 groups and determined no difference between them in terms of operation time and blood loss (4).

In our study with 958 patients (282 obese and 676 non-obese), no significant difference was determined between two groups in terms of operation time, duration of fluoroscopy, operational success, need for additional intervention and complication rates. Even when the complications were grouped according to Clavien classification, both groups showed no significant difference. In the obese group, arteriovenous fistula developed in one patient and it was successfully treated with selective embolization. None of the patients died due to complications.

As for the limitations of the study, we can mention its retrospective design and inability to group the Clavien 3 classification into 3A and 3B because of the missing information about if the patients with Double J stent were given anesthesia or not.

CONCLUSIONS

According to the results of our study, PNL is an effective method with high success rates, therefore it can be applied safely in obese patients as well as in non-obese patients.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Complications after prone PCNL in pediatric, adult and geriatric patients – a single center experience over 7 years

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ABSTRACT

Introduction: CROES-Clavien system (CCS) for grading complications in percutaneous nephrolithotomy (PCNL) is a step towards standardization of outcomes. We categorized complications based on CCS and predicted risk factors across the entire cohort and individually for pediatric (P: ≤18 years), adult (A: 19-65 years) and geriatric (G: ≥65 years) subgroups to assess the risk factors in each subset. We assessed association of complications with length of hospitalization (LOH) and operation time (OT).

Materials and Methods: Retrospective record review of unilateral PCNL performed between January 2009-September 2015 at a tertiary care center in India, performing around 150 PCNL per year.

Results: Out of 922 (P=61; A=794; G=67) PCNL, 259 (28.09%) complications occurred with CCS I, II, III and IV constituting 152 (16.49%), 72 (7.81%), 31 (3.36%) and 4 (0.43%) respectively and its distribution was similar across the subsets and majority (224; 24.3%) were minor (CCS-1, 2). Placement of a nephrostomy (47.4%; 18/38) in Group P, supracostal access, ≥2 punctures, higher GSS, nephrostomy, staghorn stones, ≥2 stones, stone size in Group A and hydronephrosis and prolonged OT in Group G were significantly associated with complications. On logistic regression, need of nephrostomy (adj. OR - 4.549), OT (adj. OR - 1.364) and supracostal access (adj. OR - 1.471) significantly contributed to complications in the study population. LOH was found to be significantly associated with complications (p<0.001).

Conclusions: Contrary to the belief that extremes of ages are associated with complications of prone PCNL, we found age does not alter the incidence or grade of complications and LOH.

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INTRODUCTION

Percutaneous nephrolithotomy (PCNL) is the standard of care for large and complex renal stones across all age Groups (1, 2). Though it is highly efficacious, it is associated with morbidity. Reported complication rates following PCNL range up to 83% and there is marked heterogeneity in reporting post-PCNL complications with underreporting of minor complications (3, 4). The modified Clavien-Dindo grading system for complications in urology has now been adapted for PCNL by the Clinical Research Office of the Endourological Society (CROES) Study Group, referred to as CROES-Clavien score (CCS) (4, 5). This score has been successfully used to report complications after pediatric PCNL (5).

Prone PCNL is the standard at most centres (6). Supine PCNL, introduced by Valdivia in late 1980s, is being adopted by experts due to the

proposed advantages which actually are the limitations of prone PCNL. These include anesthetic benefits like decreased cardiovascular and respiratory distress. From the endourologist's point of view, a more horizontal sheath position in supine PCNL improves the chances of spontaneous stone expulsion during the procedure and keeps the operator's hands away from the field of radiation (6, 7). Two recent meta-analyses showed that supine PCNL have similar LOH and complications but a significantly lesser stone free rates when compared to prone PCNL (8, 9). We routinely perform prone PCNL at our institute. We report the complications of prone PCNL in the entire cohort of PCNL patients using the CCS. We subdivided them into pediatric, adult and geriatric subgroups to assess the risk factors in each subset. We also intended to assess the association of complications with length of hospitalization (LOH) and operation time (OT).

MATERALS AND METHODS

This is a retrospective record review of unilateral PCNL performed between January 2009 and September 2015 at a tertiary care center in South India, performing around 150 PCNL per year. Institutional research review board approval was obtained.

Data collection

Being a teaching institute, all case records were prospectively filled in by resident urology trainees and these were checked by consultants. These data were then entered in Microsoft Excel 2010 spreadsheet by the authors. After checking for the accuracy of the data, it was transferred to SPSS version 20 (IBM Corp., Armonk, NY, USA) for statistical analysis.

Evaluation

Preoperative evaluation included hemoglobin, serum creatinine, serum electrolytes, urine analysis, urine culture and ultrasound of kidneys, ureter and bladder region (KUB), plain X-ray of KUB region (XRKUB) and intravenous urography or computed tomography KUB region. XRKUB and renal ultrasound were performed 48 hours after PCNL. Stone free status (SFS) was defined as absence of any residual stone >4mm on XRKUB (10).

Perioperative data

Patient factors recorded included age, gender, Charlson comorbidity index (CCI) and past surgical history for stone disease. Stone characteristics included stone size (mm), location, stone count, staghorn stones and Hounsfield units. Intraoperative data collected were supracostal/infracostal entry, calyces punctured, number of access tracts, tract size, OT and postoperative drainage. Postoperative outcomes assessed were SFS, residual stone, complications based on CCS, LOH and analgesic requirements. CCS grades 1 and 2 constitute minor complications and grades 3 and 4 have been grouped as major complications (4, 5). We also analyzed the outcomes based on OT cut-off of 75 minutes as defined by Smith et al. (10).

We used the Guy stone score as proposed by Thomas et al to predict the outcomes of PCNL (11). It comprises of 4 grades - grade I includes solitary renal calculus in mid/lower pole or solitary calculus in the renal pelvis with simple anatomy; grade II includes solitary calculus in the upper pole or multiple stones in a patient with simple anatomy or a solitary calculus in a patient with abnormal anatomy; grade III includes multiple calculi in a patient with abnormal anatomy, calyceal diverticular or partial staghorn calculi and grade IV includes staghorn calculus or any renal calculus in patients with spina bifida or spinal cord injury (11).

Surgical Technique

The procedure was performed under general anesthesia. In lithotomy position, an open--ended 5F/70cm ureteral catheter was placed on the side of stone through rigid cystoscope (Karl Storz Endoscopy, Tuttlingen, Germany). Patient was placed prone and retrograde pyelography was performed. Using the bull's eye technique, appropriate calyx was punctured by the urologist with an 18G/15cm diamond-shaped trocar needle under fluoroscopy. A 0.032" hydrophilic guidewire was inserted and tract dilation was done up to the desired size. The maximum tract size was up to 30F and a 17F, 22F or 26F nephroscope (Karl Storz Endoscopy, Tuttlingen, Germany) was used. Lithotripsy was done by pneumatic lithotripter (Nidhi Lith Digi, Nidhi Meditech Systems, India). Pos-

toperative drainage was based on intraoperative factors and surgeon choice. Ureteric catheter and nephrostomy tube were removed after 48 hours if the patient had no hematuria or fever. In case of hematuria or fever, these were removed 24 hours after hematuria and fever subsided. In patients with ureteric stents in situ, these were removed 4 weeks later.

Statistical analysis

Data was tabulated and statistical analysis was performed using SPSS version 20. Continuous variables were depicted as mean with standard deviation and categorical variables as median and interquartile range. For tabulation and analysis, the study population was divided into three subgroups - pediatric (Group P -≤18 years), adults (Group A - 19 - 65 years) and geriatric (Group G -≥65 years) (12, 13). Student t test (two tailed, independent) and one way ANOVA or Mann Whitney U test and Kruskal Wallis test as appropriate were used for continuous variables based on the normality of the distribution. Chi-square and Fisher exact test were used to compare parameters on categorical scale. Binomial logistic regression analysis was used to identify independent predictors for complications. A p value of <0.05 was considered statistically significant.

RESULTS

A total of 922 patients were eligible for analysis. Group P had 61 (6.62%) patients with a mean (±SD) age of 12.6±4.8 years, Group A had 794 (86.12%) patients with a mean age of 40.9±11.5 years and Group G had 67 (7.27%) patients with a mean age of 68.8±4.9 years. The mean (±SD) stone size was lower in Group P (19.3±7.1mm) and similar in Groups A (22.4±7.6mm) and G (22.7±8.0mm). Patient, stone characteristics and OT are depicted in Table-1. Across the three Groups, distribution of stone location, presence of staghorn stones, hydronephrosis, anomalous kidneys, side of stone and GSS were similar. A total of 259 (28.09%) complications occurred in the study cohort with CCS I, II, III and IV constituting 152 (16.49%), 72 (7.81%), 31 (3.36%) and 4 (0.43%) res-

Table 1 - Patient, stone characteristics and OT based on age groups.

Characteristics	Pediatric	Adult	Geriatric	P value
Age, years				<0.001
Mean±SD	12.56±4.8	40.91±11.5	68.78 ±4.9	
Stone size, mm				0.008
Mean±SD	19.31±7.13	22.41±7.59	22.69±7.98	
Staghorn stones				0.064
N(%)	7 (11.5)	99 (12.5)	15 (22.4)	
GSS N(%)				
I	39 (63.9)	467 (58.9)	39 (58.2)	0.414
II	12 (19.7)	164 (20.7)	11 (16.4)	
III	3 (4.9)	64 (8.1)	3 (4.5)	
IV	7 (11.5)	98 (12.4)	14 (20.9)	
CCI N(%)				
≤2	61 (100)	746 (94)	27 (40.3)	<0.001
>2	0	48 (6)	40 (59.7)	
OT, min				
Mean±SD	81.48±30.71	78.17±25.52	77.09±24.64	0.58

pectively. Majority of the complications were minor (224; 24.30%). Proportion of complications based on CCS was similar across all three age groups (Table-2).

Predictors of complications - overall

On univariate analysis, the factors contributing to complications included supracostal access (p=0.001), ≥2 punctures (p=0.002), nephrostomy insertion (p <0.001), increasing GSS class (p=0.042), increasing stone size (p=0.011), presence of staghorn calculi (p=0.008), multiple stones (p=0.022) and prolonged OT (p <0.001). On logistic regression, need of nephrostomy (adj. OR - 4.549), OT (adj. OR - 1.364) and supracostal access (adj. OR - 1.471) significantly contributed to complications (Table-3). When OT exceeded 75 minutes, a significantly higher proportion (36.5%; 134/367) of patients developed complications when compared to 23.2% (129/555) developing

Table 2 - Clavien-CROES complications – overall and based on age groups.

CCS grade	CCS grade	Description	N (%)	Pediatric	Adult	Geriatric	P value
Minor	**I**	Postoperative fever (38°C) without change of antibiotics	78 (8.5)				
		Bleeding without need for blood transfusion or required single episode of nephrostomy clamping or skin compression/ pressure dressing	54 (5.9)				
		Urine leakage - watchful waiting	20 (2.2)				
		Renal pelvic perforation - watchful waiting	5 (0.5)				
		Intestinal obstruction - without nasogastric decompression	2 (0.2)	18 (85.7)	193 (86.6)	13 (86.7)	
		Subcapsular hematoma - watchful waiting	1 (0.1)				
	II	Bleeding requiring blood transfusion	36 (3.9)				
		Postoperative fever (>38°C) with change of antibiotics	28 (3)				0.691
		Postoperative ileus - nasogastric decompression	4 (0.4)				
		Pulmonary edema – diuretics	3 (0.3)				
Major	**IIIA**	Ureteric stent without general anesthesia	13 (1.4)				
		Renal pelvic perforation - prolonged nephrostomy tube or postoperative ureteric stenting without general anesthesia	9 (1)				
		Hydrothorax managed by intercostal drainage under local anesthesia	9 (1)	3 (14.3)	30 (13.4)	2 (13.3)	
	IVA	Urosepsis - ICU management	2 (0.2)				
		Acute renal failure - ICU management	1(0.1)				
		Heart failure -ICU management	1 (0.1)				

complications when OT was <75 min (p <0.0001).

Outcomes – overall

A stone free rate of 78.31% (722/922) was achieved. Out of the remaining 200 patients (21.7%) with residual calculus, relook PCNL was performed for 78 (8.46%) patients and 58 (6.29%) required shock wave lithotripsy (SWL) for stone clearance. The mean (±SD) LOH was 4.56±3.15 days (Table-4). The mean LOH was higher (6.61±3.97 days) in patients with complications (vs. 3.74±2.29 days without complications; p <0.001). LOH increased proportionately with increasing CCS. The proportion of patients having prolonged LOH >3 days [CCS 1 - 120 (55.6%); 2 - 64 (29.6%); 3 - 27 (12.6%); 4 - 1.9%] was higher as CCS increased when compared to LOH ≤3 days

Table 3 - Logistic regression for predictors of complications.

Predictors	Adjusted OR	95% CI for Adjusted OR		P value
		Lower	Upper	
Supracostal	1.471	1.070	2.023	0.017
More than one puncture	1.283	.791	2.080	0.312
Nephrostomy	4.549	2.991	6.917	<0.001
Staghorn calculus	1.386	0.820	2.344	0.223
Stone size in mm	0.996	0.971	1.022	0.777
Duration of procedure >75min	1.364	0.986	1.887	0.048
Age group				
Pediatric	0.244	-	-	-
Adult	0.653	0.361	1.181	0.159
Geriatric	0.510	0.226	1.150	0.105
Stone in more than one location	0.874	0.586	1.305	0.511

Table 4 - Outcomes – overall and among three age groups.

Characteristics	Pediatric	Adult	Geriatric	P value	Overall
Stone free status					**722 (78.3)**
N (%)	50 (82)	623 (78.4)	49 (73.1)	0.718	
Residual stone					**200 (21.7)**
N (%)	11 (18)	171 (21.5)	18 (26.9)	0.713	
Complications					**259 (28.1)**
N (%)	21 (34.4)	221(28)	17 (25.4)	0.484	
Doses of analgesic					**7.29±3.02**
Mean±SD	6.67±3.19	7.3±2.98	7.72±3.35	0.144	
Relook PCNL					**78 (8.5)**
N (%)	3 (4.9)	69 (8.7)	6 (9)	0.588	
Length of hospitalization					**4.56±3.15**
Mean±SD	4.3±3.08	4.55±3.18	4.93±2.81	0.512	

[CCS 1 - 29 (67.4%); CCS 2 - 7 (16.3%); 3 - 4 (9.3%); 4 - nil]. However, it was not statistically insignificant (p=0.202).

The mean (±SD) number of analgesic demands after PCNL was 7.29±3.02 and mean analgesic requirements were similar across all three age groups (Table-4). Proportion of minor and major complications based on CCS was similar with respect to increasing GSS and OT and placement of a nephrostomy. The distribution of complications based on CCS was similar across the three age groups (Table-5).

Predictors of complications – Pediatric subgroup

In Group P, 21 (21/61; 34.4%) patients had complications with minor complications contributing to 85.7% (18/21). Placement of a nephrostomy after PCNL (47.4%; 18/38) was significantly associated with complications while only 13% (3/23) without nephrostomy developed complications (p=0.006). The mean (±SD) OT was 81.48 (30.71) min. In patients with OT >75 min, 40.7% (11/27) patients developed complications while only 29.4% (10/34) of patients with OT <75 min developed complications (p=0.355).

Predictors of complications – Adult subgroup

In adults, 224 (28.2%) patients had complications and 86.6% (194/224) of them were minor. Supracostal access (34.7% vs. infracostal access 23.7%; p=0.001), ≥2 punctures (40.7% vs. single 26.4%; p=0.005), higher GSS (I - 25.8%; II - 26.8%; III - 28.6%; IV - 40.2%; p=0.037), insertion of nephrostomy tube (36.8% vs. 11.3% without nephrostomy tube; p <0.001), presence of staghorn stones (39.8% vs. 26.3% without staghorn stones; p=0.005), stone count ≥2 (32.53% vs. 25.3% for single stones; p=0.013), stone size (23.59±8.11; p=0.007) and OT (85.11±27.75 min vs. 75.41±24.09 min in patients without complications; p <0.0001) had a significantly higher incidence of complications. With supracostal puncture, the proportion of severe complications increased (p <0.0001). In patients with OT >75 min, a significantly higher (36%; 113/314) proportion of patients developed complications while only 22.7% (108/476) of patients with OT <75 min developed complications (p <0.0001).

Predictors of complications – Geriatric subgroup

Among geriatric patients, 15 (15/67; 22.4%) had complications with Clavien I and II constituting 86.7% (13/15) of them. Presence of hydronephrosis (58.3% vs. 18.2% without hydronephrosis; p=0.008) and prolonged OT (88.82±31.35 min vs. 73.10±20.80 min in patients without complications; p <0.0001) were significant predictors of complications. The mean (±SD) OT was 77.09 (24.64) min. Patients with supracostal access had increasing CCS grade of complications, however it was not statistically significant (p=0.064). In patients with OT >75 min a higher (38.5%; 10/26) proportion of patients developed complications while only 17.1% (7/41) of patients with OT <75 min developed complications (p=0.082).

DISCUSSION

Complication rates for PCNL range from 20 - 83% (14). There is a need for standardized reporting of complication after PCNL (15). European Association of Urology (EAU) guidelines panel in 2012 highly recommended the use of CCS grading system as a uniform and standardized system to classify complications after PCNL. CCS is described based on the management of a given compli-

Table 5 - Complications based on CROES-Clavien score across different age groups.

Age group	CROES-Clavien score-1	CROES-Clavien score-2	CROES-Clavien score-3	CROES-Clavien score-4	P value
	N (%)	N (%)	N (%)	N (%)	
Pediatric	13(61.9)	4 (19)	3 (14.3)	0	
Adult	130(58.8)	60 (27.1)	26 (11.8)	4 (1.8)	0.691
Geriatric	6 (35.3)	7 (41.2)	2 (11.8)	0	

cation and it is not influenced by the potential risk to which a patient is exposed due to complication. Minor (grades 1 and 2) complications account for a high proportion and underreporting of low grade complications is not unusual (4).

Seitz et al. reported in their systemic review on complications of PCNL that fever is a common complication, with an overall incidence of 10.8% (16). The amount of irrigation fluid and operation duration influence postoperative infection. We observed fever as the most common complication in our study (106; 11.5%). It was graded as I in 8.46% (78) patients that could be managed without a change in antibiotics and as grade II in 3.04% (28) patients requiring a change in antibiotics based on urine culture and sensitivity report. Individual hospital protocol for perioperative antibiotic use could differ.

The second most common complication in our study was bleeding accounting for 9.76% (90) of patients. It was categorized as grade I in 5.86% (54) patients where bleeding was controlled by single episode of nephrostomy clamping, skin compression or pressure dressing and as grade II in 3.91% (36) patients who required blood transfusion. Seitz et al. reported that blood transfusion is required in 0 - 20% patients with an overall incidence of 7% (16.).

Ileus (0.22%, 2 patients), subcapsular hematoma (0.11%, 1 patient), urine leakage (2.17%, 20 patients) and renal pelvic perforation (0.54%, 5 patients) managed by watchful waiting needing no active intervention constituted grade I CCS complications. Ileus requiring nasogastric decompression (0.43%, 4 patients) and pulmonary edema requiring diuretics (0.33%, 3 patients) constituted grade II CCS complications.

Urine leakage (13 patients; 1.41%) and renal pelvic perforation (9 patients; 0.98%) managed by placement of nephrostomy tube or ureteric stents without general anesthesia were categorized as grade III-A. Hydrothorax (9 patients; 0.98%) managed by intercostal tube drainage under local anesthesia were also included in this Group. Lojanapiwat et al. reported 15.3% (26 patients) developed hydrothorax via supracostal puncture with only 5.3% (9 patients) requiring intercostal drainage (17). Patients requiring ICU care for urosepsis without multiorgan failure (2 patients; 0.22%), acute renal failure (1 patient; 0.11%) and heart failure (1 patient; 0.11%) were categorized as grade IV-A. None of our patients had grade III-B or IV-B complications.

De la Rosette et al. validated the modified Clavien-Dindo system for Urology on 5803 patients from 98 centers in 26 countries and proposed the CCS (4). In our study, complications based on CCS were observed in 28.1% patients. CCS I, II, IIIA and IVA constituted 152 (16.49%), 72 (7.81%), 31 (3.36%) and 4 (0.43%) patients respectively. The CROES global study Group reported an overall complication rate of 21.5% with low grade complications (grade I and II) accounting for 16.4%, grade III-a and III-b complications in 3.6% and grade IV in 0.5% patients (3).

Lee et al. reported that 15.4% of children and 17.9% of adults developed complications following PCNL (18). We observed that the distribution of complications across the pediatric, adult and geriatric cohorts are similar with minor complications accounting for 85.7%, 86.6% and 86.7%% respectively. Sahin et al. retrospectively compared the outcomes of 27 elderly patients of PCNL with 166 younger patients and reported similar stone-free rate, complications and length of stay between them (19).

Goyal et al. reported that OT was the only independent predictor of complications (5). In our study, we observed that in the pediatric subset, nephrostomy tube insertion was the only significantly predictor of complications. Though OT was higher in patients with complications, it could not achieve statistical significance. We noted that supracostal access, presence of nephrostomy tube and duration of procedure were the only significant predictors on multivariate analysis in the study population and adult cohorts. In the geriatric population also, OT was found as the only significant predictor of complication.

We further categorized our patients based on OT cut off of 75 min as proposed by de la Rosette et al. and found that the complication rates increased significantly in these patients (4). We also observed that the likelihood of hospital stay increased with increasing severity of complications. de la Rosette et al. used postoperative LOH as a surro-

gate measure for the severity of complications and showed that OT >75 min increased complications and also prolonged LOH (4). This could prove useful in counseling patients undergoing PCNL with an increased chance of complications. Prolonged OT and placement of nephrostomy may also indicate the greater level of difficulty for PCNL.

Our study has few merits. We have categorized complications based on CCS which was proposed as a step towards standardization of reporting of complications in PCNL. We noted that adults contributed to the majority of our patients and thus the overall outcomes were similar to that in adult subgroup. Most published studies report complications as a whole and do not sub-classify based on age and there is a dearth of reports on complications in pediatric and geriatric PCNL. To the best of our knowledge, ours is the first study stratifying patients into subgroups based on age. Though there are different predictors of complications in each subgroup, overall SFS, complications and LOH are similar. We observed that PCNL is safe in children and the geriatric population with SFS and complications similar to adults.

Being a retrospective study, we have taken adequate steps to avoid the inherent problems of this study design. However, it is possible that some bias could still exist. We have excluded patients with incomplete data. We used only XRKUB and US for assessing residual fragments. We could not perform CT KUB in every patient due to the additional costs involved. We hence defined stone free status as absence of any residual fragment >4mm. Our data includes complications of prone PCNL from a single center. They may need external validation.

CONCLUSIONS

Complications in prone PCNL are similar across pediatric, adult and geriatric subgroups. Age does not alter the incidence of complications or the grade of complications, stone free status and length of hospital stay, contrary to the belief that these patient populations are unique due to extremes of age and associated comorbidities. Need for nephrostomy in children and prolonged operation duration in geriatric patients were independent predictors of complications. In adults, supracostal access, complex renal stones, need of nephrostomy and prolonged operation duration predicted a higher complication rate.

CONFLICT OF INTEREST

None declared.

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The effect of extended release tolterodine used for overactive bladder treatment on female sexual function

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ABSTRACT

Introduction: Overactive bladder (OAB) is a common condition, especially in middle aged women, requiring long term therapy with anticholinergics to maintain symptoms relief. The aim of the study was to determine the effect of tolterodine extended release (ER) used for OAB treatment on the sexual function of women.

Materials and Methods: Between August 2010 and August 2014, 220 women with confirmed OAB, attended Urogynecology Outpatient Clinic and were prospectively enrolled in this study. 158 women were evaluated, with a comprehensive history, physical examination, urodynamic studies and Female Sexual Function Index (FSFI) questionnaire. 73 patients of group A (control group) received no treatment and 85 patients of group B received an anticholinergic regimen – tolterodine ER 4mg once daily. Data were evaluated again in accordance with FSFI after three months, using SPSS software.

Results: A statistically significant increase was noted in group B in domains of desire (pre-treatment 2.5±0.2 to 4.5±0.2 post-treatment), arousal (3.1±0.2 to 3.1±0.2 respectively), lubrication (3.4±0.3 to 4.3±0.3 respectively), orgasm (3.5±0.3 to 4.5±0.3 respectively), satisfaction (2.6±0.2 to 4.2±0.3 respectively) and pain (2.4±0.2 to 4.6±0.4 respectively) after three months treatment with tolterodine ER. In group A there were no statistically significant changes in pre and post treatment values ($p>0.05$). Total FSFI score for group B was significantly higher after tolterodine treatment (26.5±1.5) compared to pre-treatment values (17.4±1.4, $p<0.01$) and to control group A (17.7±1.2 and 17.9±1.5, $p>0,05$) respectively.

Conclusions: This preliminary study demonstrates that treatment of OAB with tolterodine ER was found to have positive effect on sexual function of patients with OAB.

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INTRODUCTION

Overactive bladder (OAB) is a symptom-driven condition characterized by urinary urgency with or without urge incontinence and is usually associated with increased daytime frequency and nocturia (1). It is a common condition, whose prevalence increases with advancing age and compromises health-related quality of life (2). Data from a large study of over 19.000 individuals in four countries across Europe, as well as Canada (the EPIC study), determined that OAB was present in 10.8% of men and 12.2% of women in the general population, becoming increasingly prevalent in individuals aged >40 years, at 13.1% and 14.6% men and women, respectively (3).

The most commonly employed methods for treating newly-diagnosed OAB are bladder training, anticholinergic therapy, beta-3 adrenergic agonists or a combination of them. Modalities

such as botulinum toxin injections, sacral neuromodulation and posterior tibial nerve stimulation are showing encouraging results in more refractory patients (4, 5).

Urinary incontinence is believed to contribute to the development of female sexual dysfunction (6). The impact of OAB symptoms on sexual function in women has been evaluated in a few studies (7-12). Kim et al.(8) conducted an Internet survey and found that women with OAB experienced a greater deterioration in sexual quality of life than women with urinary incontinence only.

Female sexual dysfunction is traditionally classified into disorders of desire, arousal, lubrication, orgasm and pain. While epidemiologic data are limited, the available estimates are that 43% of women complain of at least one sexual problem (13). Physiologic, iatrogenic and psychological factors place women at risk for developing female sexual dysfunction (FSD) while lower urinary tract symptoms are an independent risk factor for sexual dysfunction (13, 14).

Tolterodine is a potent, competitive and bladder selective muscarinic receptor antagonist, specifically developed for the treatment of OAB (3,15-17). Clinical trials have shown that tolterodine IR or ER is as effective as oxybutynin in the treatment of OAB, and that it is associated with a significantly lower frequency and severity of adverse events, most notably dry mouth (18). Tolterodine has also been shown to have a beneficial impact on health related quality of life in OAB patients (7).

The aim of the study is to describe the effect of an anticholinergic agent, tolterodine extended release, used for OAB treatment on sexual function of women using a validated questionnaire.

MATERIALS AND METHODS

Between August 2010 and August 2014, 220 sexual active women, with confirmed OAB, attended Urogynecology outpatient clinic and were prospectively enrolled in this study (19). OAB was defined using the International Continence Society (ICS) definition (19). That is, having urinary symptoms of urgency with or without urge incontinence, with frequency and nocturia.

All women reported having a urination frequency of 8 or more times per day, the presence of urge symptoms that may or may not accompany incontinence, symptom duration of 3 months or longer and no prior history of treatment for OAB. Subjects were excluded from participation if they had active urinary tract infection based on results from urine culture, clinically significant stress urinary incontinence, urinary retention or uncontrolled narrow-angle glaucoma or were at risk for these conditions. Women were also excluded if they had demonstrated hypersensitivity to tolterodine or other component of this product. The research project's protocol has been approved by the institutional Ethics Committee.

The inclusion criteria also integrated women over the age of 18 years in a sexually active relationship. All participants provided their written informed consent for being included in the study. Women who stated that they were not sexually active were excluded from further analysis. Furthermore, women who were afraid to receive tolterodine in terms of allergy or so, or considered having OAB as a result of aging, were included in control group.Patients were assessed by a comprehensive history, a detailed general and neurological complete physical examination and urodynamic evaluation. Urodynamic techniques and measurements, terms and diagnostic criteria conform to the recommendations of the ICS(19). Symptomatic diagnosis of OAB does not correlate with urodynamic diagnosis of detrusor overactivity (DO) and urodynamic studies are not necessary for the assessment of patients with OAB. The rational of performing themis the differential diagnosis from bladder outlet obstruction, occult neurogenic bladder or other underlying pathology that would weaken the strength of research.

All women were examined employing the pelvic organ prolapsed staging system recommended by the ICS to quantify loss of pelvic organ support (20). Women with more than stage I pelvic organ prolapsed, women who had previously undergone incontinence or prolapse surgery or with neurological diseases were excluded. Women with a history of pelvic muscles

training program were excluded because it is accepted that pelvic floor muscles exercises improve female sexual function (21).

The assessment of sexual function and sexual quality of life lends itself to evaluation by patient diary and patient-reported questionnaires (22). According to the latest report of the International Consultation on Sexual Medicine, the Female Sexual Function Index (FSFI) remains the gold standard assessment tool, has a level of evidence 1 and recommendation grade A, for evaluating female sexual dysfunction (23). Item selection and categories were based on the American Foundation for Urological Diseases classification system of female sexual dysfunction. All women were asked to complete the FSFI questionnaire which was previously linguistically validated its Greek version (unpublished data). The FSFI categorizes sexual dysfunction in the domains of (a) desire (b) arousal (c) lubrication (d) orgasm (e) satisfaction and (f) pain. A scoring system is developed to obtain individual domain scores, where higher scores indicate a more healthful condition. Wiegel et al. (24) found that a total FSFI score of 26.5 is the optimal cut score for differentiating women with and without sexual dysfunction.

To determine the eligible women all women were asked to answer the question: "Do you have sexual distress associated with sexual dysfunction?" and only women who gave a negative answer were finally available for analysis, since sexual distress needs special questionnaires to be evaluated. Patients were divided in two groups in terms of their decision to receive tolterodine treatment. In group A, which is defined as control group, 90 women with OAB did not wish to receive any therapy, while in group B, 110 patients with OAB were treated with tolterodine 4mg ER for 3 months.

Patients of group B completed the 3-day micturition diary prior and after the 3rd month of anticholinergic treatment. Patients of group A followed the same pattern prior and after the 3rd month, without anticholinergic therapy. For each episode of urinary symptoms, the patient recorded the date and time of each episode, whether or not they voided, the presence of urgency and/or incontinence, the volume voided, whether or not the episode disturbed the patient's sleep. 110 patients of group B received tolterodine ER tablets 4mg, once daily, for 3 months. In group A all women attended monthly office visits to ensure that they don't follow any pharmacotherapy or behavioural therapy for OAB. They were all informed about treatment modalities in their visits.

Voiding frequency, nocturia, urgency episodes, incontinent episodes, number of incontinence pads used and voided volume were measured after treatment using a 3-day micturition diary. Patients from both groups completed the FSFI questionnaire at the beginning and after the completion of the three month's period, to evaluate their pre and post-treatment sexual function in the case.

Our data were evaluated with the use of SPSS software, USA, release 13.0. The statistical analysis was done using the percentage, paired t-test. Statistical significance was accepted $P<0.05$. Data are presented as the mean ± standard deviation (range).

RESULTS

The study characteristics, including age, weight, symptom severity and duration, parity, presence and degree of urge incontinence are presented in Table-1. The incidence of DO was 62.7% in female OAB patients in group A and 64.1% in group B. There was an additional 19.2% in group A and 18.5% in group B that patients revealed DO after provocative maneuvers, such as posture change or coughing. 61% of women with urgency (OAB dry) had DO. Of the 220 women who reported sexual activity the last four weeks, 200 patients agreed to participate and completed the necessary FSFI questionnaires in order to evaluate their sexual function. There were 7 women in group A and 13 women in group B that denied completing the final FSFI questionnaire. Age, body weight, symptom duration, parity, educational level and occupation status were not significantly different between two groups (Table-1).

None of them stated being in menopause. In group B, throughout the study, 12 patients

Table 1- Demographic characteristics between two groups.

	Control Group A	Tolterodine Group B	P values
Age (year)	41±6.4years (range 18-48years)	43±8.4years (range 18-51years)	P>0.05
Body weight (kg)	58.5±8.9kg (range 49-78kg)	55.5±7.8kg (range 48-78kg)	P>0.05
Symptom duration (year)	4.3±3.1years (range 0.3-6years)	3.9±3.1years (range 0.3-6years)	P>0.05
Parity	2.1±1.2 (range 0-4)	2.0±1.3 (range 0-4)	P>0.05
Level of education	Educated: 70	Educated: 83	P>0.05
	Not educated: 3	Not educated: 2	
Occupation status	Occupied: 50	Occupied: 60	P>0.05
	Not occupied: 23	Not occupied: 25	

discontinued tolterodine medication for the following reasons: three for dry mouth, two for insufficient therapeutic response, three because of low treatment compliance and four because they realized that OAB requires a long term therapy. In Group A (control group), ten patients discontinued because during the first month office visit decided to follow a behavioral therapy and receive an anticholinergic regimen.

Mean total FSFI and its subscales were significantly different after three months between two groups. Repeated statistical analysis showed that mean FSFI and its subscales were significantly different before and after treatment in group B (P<0.01), but not in control group A (Table-2).

Thirty five women (41.7%) complained mainly for sexual pain disorders, 23 (27.4%) for hypoactive sexual desire, 13 (15.5%) for sexual arousal disorders and 13 (15.5%) for lubrication disorders and orgasmic deficiency.

After three months tolterodine treatment mean frequency, nocturia and incontinence episodes decreased statistically significant (Table-3) in comparison to control group, which showed no statistically significant differences.

Furthermore 53% of tolterodine patients who were incontinent at baseline became continent by the study endpoint. In accordance with the improvement in symptoms, other objective measurements such as the mean volume voided per micturition increased significantly during the study (35mL) compared to the women before treatment (p<0.010). The number of incontinence pads used by patients significantly reduced after treatment (p<0.001).

The total FSFI score was significant higher (26.5±1.5) compared to pre-treatment va-

Table 2- Mean FSFI (total and subscales) pre and post-treatment in Group B.

	Pre-treatment	Post-treatment	p values
Desire	2.5±0.2	4.5±0.2	P<0.01
Arousal	3.1±0.2	4.4±0.3	P<0.01
Lubrication	3.4±0.3	4.3±0.3	P<0.01
Orgasm	3.5±0.3	4.5±0.3	P<0.05
Satisfaction	2.6±0.2	4.2±0.3	P<0.01
Pain	2.4±0.2	4.6±0.4	P<0.01
Total FSFI score	**17.4±1.2**	**26.5±1.5**	**P<0.01**

Table 3- Evaluation of urinary parameters in the tolterodine-treated group.

	Pre-treatment	Post-treatment	P values
Frequency	11.93±2.58	8.99±1.54	P=0.041
Urgency episodes	6.78±3.58	4.38±2.14	P=0.032
Nocturia	1.43±1.04	0.6±0.4	P=0.045
Incontinence episodes	2.21±0.95	1.2±0.1	P=0.009
Incontinence pads	4.91±0.95	1.89±0.57	P<0.001
Voided volume (mL)	110±35	145±42	P=0.009

lues (17.4±1.4) (p<0.001) although there was a residual sexual dysfunction mainly in subscales of lubrication and orgasm (Table-2).On the other hand, in control group A there were no statistically significant changes after three month's observation. More specifically,pre and post-treatment values, expressed with median value ± standard deviation, were for desire 2.5±0.3 and 2.6±0.2 respectively,for arousal 3±0.3 and 3±0.4 respectively, for lubrication 3.6±0.3 and 3.4±0.3 respectively, for orgasm 3.4±0.1 and 3.5±0.2 respectively, for satisfaction 2.8±0.3 and 2.7±0.2 respectively and for pain 2.5±0.3 and 2.4±0.3 respectively. Moreover, the pre and post-treatment total FSFI score for group A was 17.7±1.2 and 17.9±1.5 respectively. All P values were >0.05 implying that there were no statistically significant changes during the three month's observation.

DISCUSSION

A significant amount of new information has been made available regarding the effects of OAB on female sexual function and quality of life (7, 8). Treatment of cases with OAB includes behavioral, pharmacological, surgical interventions and peripheral electrical stimulation. It seems logical that medical treatment of OAB improves sexual function of women with OAB. However, the amount of data, related on the effect of antimuscarinic agents, used for OAB treatment on sexual function of women is insufficient in the literature. This study aimed to describe the effect of tolterodine 4mg ER, specifically used for OAB treatment on sexual function of women.

Female sexual health is a dynamic and multifaceted phenomenon that is closely linked to a woman's overall quality of life. Sexual dysfunctions can interfere with intimacy, affect a marital relationship, and ultimately erode well--being and overall health. In contrast to the burgeoning data on men, clinical trials on sexual dysfunctions in women are few and also sexual dysfunctions are likely more common in women than in men.

Temml et al. (26) reported that 25% of incontinent women had some form of sexual dysfunction, and most of their subjects believed that incontinence during coitus was the most bothersome symptom. Other investigators (10, 27, 28) have found that detrusor overactivity has more impact on sexual function than urodynamic stress incontinence while others found that 1 to 4 women with OAB report some form of sexual impairment (20, 28). Yip et al. (10) found that women with urodynamic stress incontinence and detrusor overactivity had poorer quality of life and sexual satisfaction. Regression analysis revealed that poor sexual satisfaction correlated with worsening marital relationships in the incontinent women in their study. More than 60% of incontinent women that reported sexual pain disorders reported also recurrent bacterial cystitis that could be implicated with inflammation at the genitalia, flogosis, vaginal lubrication disturbance and higher incidence of pain disorders (29).

Some researchers believe that lower urinary tract symptoms are more important with

respect to sexual activity and sexual satisfaction than urine leakage during intercourse and that women with OAB complained mainly of repeated urgency or frequency during intercourse (8). According to the participants the incontinence associated with intercourse does not have the greatest impact but that urgency and frequency after coitus as well as the fear of leakage during stimulation and intercourse are quite detrimental to enjoying sexual relations.

The results of the current study revealed that tolterodine ER applied for OAB treatment improved female sexual dysfunction. The aspects of sexual life that improved in this study were pain, orgasm, sexual enjoyment, sexual desire, and even vaginal wetness. We believe that this improvement in the sexual quality of life comes from the major improvement described in bladder pain. This finding may be in favor of a causal relationship between urinary symptoms and sexual dysfunctions. These results may not be true for women with OAB who are not sexually active.

In OAB patients, pain could be derived from vaginal dryness and lack of lubrication caused by the urine presence in the vagina that affects the normal acidic pH of the vagina and the normal flora as well as hypertonicity of pelvic floor muscles due to fear of urine leakage(8). Sexual pain disorders may be due to long term effects of recurrent infection and inflammation of the genitalia. Furthermore in the arousal phase there is an increase in clitoral and vaginal blood flow(30). The decreased localized blood flow may facilitate reduced bladder wall resistance to bacteria and a loss of genitalia excitability (29).

In a previous study of Eftekhar et al. (4), fifty women facing OAB, were randomly assigned to PTNS (posterior tibial nerve stimulation) plus tolterodine or tolterodine alone treatment. The results showed no significant difference between two groups regarding FSFI score and its subscales. Hajebrahimi et al. (7) indicated an improved sexual function of women suffering from OAB, by administrating tolterodine IR, using the Arizona Sexual Experience Scale (ASEX).

The impact of OAB or urinary incontinence on sexual health is not a topic that patients freely initiate. Not only may women, who experience incontinence and sexual impairment, be embarrassed to approach a health professional, but health professionals may also be embarrassed to confront patients. The use of self-administered questionnaires provides a means of collecting information on sexual health while reducing potential embarrassment and response bias associated with interviewer-administered questionnaires.

In conclusion, according to our research, treatment of OAB with tolterodine ER improves sexual function of female patients with OAB. However, this study has some limitation. First, we were not able to observe whether adverse effects of the medication, such as dry mouth and constipation, affect sexual function because these patients were withdrawn and did not complete the final FSFI questionnaire. Second, two only patients presented total FSFI score after treatment worse than the introductory FSFI total score. The limited number of these patients did not permit us to conclude which are the factors for not having good response in sexual function.

Our study had a control group with women not taking anticholinergic treatment but no placebo group.Thus, there is no evaluation about the effect of placebo on sexual function. Furthermore, there was no power analysis for confirming the validity of our sample size. It is not possible to use a randomization method in our paper since the decision of female patients, to receive tolterodine or not, was the reason for being in group A or group B. Someone could claim that it is a random process of selection although this can by chance lead to disparities. In spite of these limitations, we believe that significant results have been obtained, which are of value for clinicians working in this field. Women with OAB should be evaluated also in terms of sexual function to provide better quality of life and the therapeutic use of tolterodine ER for OAB improved FSFI total scores of the patients.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Long-term response of different Botulinum toxins in refractory neurogenic detrusor overactivity due to spinal cord injury

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ABSTRACT

Purpose: To assess the response in spinal cord injured patients alternatively treated with different types and dosages of Botulinum neurotoxin type A (BoNT/A) over 15 years.
Material and methods: Patients who underwent first BoNT/A from 1999-2001 and practiced intermittent catheterization were included. Baseline 3-day bladder diary (BD) and urodynamics were collected. BoNT/A failure was defined when patients asked for re-injection ≤ 3 months post-treatment. Criteria for re-injection was at least one daily episode of urinary incontinence at BD. Before re-injection, patients were asked if they had reached 6 months of dryness without antimuscarinics (YES response).
Results: Overall, 32/60 (53.4%) "No failure" (NF) group; 16 (26.6%) "occasional failure" (OF) and 12 (20%) "consecutive failure" (CF) were included. A total of 822 BoNT/A infiltrations were performed. The mean interval from previous injection to treatment re-scheduling was 8 months. No significant differences between treatments were found within the three groups (p>0.05).
The percentage of YES responses increased from 19% (AboBoNT/A 500IU) to 29 % (OnaBoNT/A 300IU) in NF, and from 18% (AboBoNT/A 500IU) to 25% (OnaBoNT/A 300IU) for OF. Five NF cases (15.6%) maintained 6 months of dryness after each injection. Among the baseline variables, only low compliance (< 20mL/cmH_2O) was found as predictor for failure (p=0.006).
Conclusions: Long term BoNT/A for NDO did not increase failures, independent of the types of treatments and switching. Definition of failure and other criteria for continuing repetitive BoNT/A treatment is mandatory. CF was predictable for no response in earlier follow-up.

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INTRODUCTION

One of the major health problems in patients with spinal cord injury (SCI) is bladder dysfunction. Particularly, the presence of neurogenic detrusor overactivity (NDO) may be a threat to the upper urinary tract (1-3).

In 2000, Schurch et al. first published on detrusor botulinum neurotoxin type A (BoNT/A) injections to treat NDO in SCI individuals (4). Since then, literature reports a high percentage of patients who have gained clinical and urodynamic benefits after BoNT/A, achieving urinary continence, increasing bladder capacity, reducing detrusor pressures in patients refractory to antimuscarinics (5-8). Both BoNT/A injections, Abobotulinumtoxin (Dysport®, Ipsen Biopharm, Slough, UK) as well as Onabotulinumtoxin (Botox®, Aller-

gan, Irvine, CA, USA), have been proven to be safe with a positive impact on quality of life (9-15).

Other BoNT/A such as NT-201 (Xeomin®) or CBTX-A distributed under several different brand names in different countries (Prosigne®, Lanzox®, Lantox®, Liftox®, and Redux®) have been studied by some authors, but evidence in literature is still lower compared to the Abo and OnaBoNT/A (16).

Nevertheless, only OnaBoNT/A 200IU has been approved for NDO since 2013 in our country (17).

However, not much information is available on follow-up longer than 10 years, especially taking into account the switching of BoNT/A types (Dysport® vs. Botox® or vice versa) and/or different dosages in the same cohort of patients over time.

Aim of the study

In this retrospective study, we report the experience of our patients affected by NDO, treated with two types of BoNT/A (Botox® and/or Dysport®) at different dosages during a 15-year follow-up.

MATERIAL AND METHODS

Only adult SCI patients who had undergone the first BoNT/A injection for their NDO refractory and/or intolerant to antimuscarinics, and who were managing their bladder via intermittent catheterization were selected in our Italian Centre. Only NDO exclusively secondary to SCI were included. The study was conducted in accordance to the Declaration of Helsinki and the International Guidelines on Good Clinical Practice.

From December 1999 to October 2001 the first injection was administered with two different BoNT/A at various dosages: AboBoNT/A (Dysport®) 500 or 750IU and OnaBoNT/A (Botox®) 200 or 300IU.

Before the first injection and throughout the entire follow-up, a 3-day bladder diary (BD) was collected. At baseline, each subject was submitted to urodynamics as recommended by the International Continence Society (18).

The interval between the previous injection and scheduling the patient's following injection was also recorded. We evaluated the BD at the time of rescheduling for a new injection. If unable to attend our clinic, patients were advised to send us their BD by any means of communication (e.g, fax, post and/or mail).

BoNT/A "failure" was defined as patients, within 3 months post-injection, who reported at least one daily episode of urinary incontinence in their BD.

If ineffectual, the following repeated injection was offered with the same dosage and type of BoNT/A previously used. Only after two consecutive failed attempts, a different type of BoNT/A and/or higher dosage were chosen randomly.

All those responding to the other 3 treatments were switched to OnaBoNT/A 200IU after its approval in Italy in March 2013. Those patients continued with OnaBoNT/A (Botox®) 200IU unless they had two consecutive "failures".

The urodynamic parameters were: maximum detrusor pressure during involuntary contraction (Pdetmax), maximum cystometric capacity (MCC), and bladder compliance (Pdet at MCC).

After baseline, urodynamics pre re-injection was mandatory only when BoNT/A failure occurred.

Apart from failures, time for further urodynamic follow-ups was scheduled according to the International Guidelines depending on the individual's risk for upper urinary tract deterioration but never exceeding 2 years (19).

Individuals taking antimuscarinics were advised to progressively stop the dosages if dryness was achieved and then start again if urinary incontinence occurred.

Prior to each new BoNT/A, the number of YES responses concerning the question on whether patients were continuously dry for at least 6 months without oral drugs, was recorded.

Subjects showing three consecutive failures were advised to undergo major surgery. For those subjects, data were reported from the first baseline up to the last injection. No BoNT/A treatment was ever performed before 3 months had elapsed from previous injections.

Detrusor infiltrations were performed on 20-30 sites, trigone and bladder neck sparing,

with a 5mm 23 gauche needle and a rigid cystoscope only by experienced urologists.

The following baseline predictable variables were taken into account: ≥ 40 years old; SCI >3 years; traumatic aetiology; gender; tetraplegia; complete lesion; concomitant antimuscarinics; compliance <20mL/cmH_2O; urinary incontinence episodes ≥ 4 per day and mean bladder capacity > 250mL at the BD.

Statistical analysis

Statistical analyses were performed using the R software [R Core Team (2016)]. In particular, Kruskal-Wallis one-way analysis of variance was carried out to test differences in the duration of the efficacy in the three groups of patients defined, regardless of the treatment used (kruskal function of the R package 'agricolae' version 1.2). Similarly, differences in duration of efficacy across the four treatments here considered were tested for each group of patients. Moreover, the efficacy of the four treatments was evaluated using Linear Mixed-Effects Models with random intercept and the log-likelihood function (lme function of the 'nlme' R package version 3.1).

The predictable variables were assessed using a Multinomial Log-linear Model (multinom function of the R package 'nnet' version 7.3).

RESULTS

Patient population selection

From our database, a total of 72 SCI patients, of whom 19 female (26.4%), was initially identified. Twelve individuals (16.6%) were excluded: 4 due to missing data, 4 because of documented mixed urinary incontinence, 2 who were under 18 years old, and 2 who showed epilepsy as another neurological co-morbidity.

A total of 60 patients (83.3%) were included. Overall the SCI population had suprasacral lesion ≤ thoracic (T11) level and exclusively A or B degree lesions according to the American Spinal Injury Association Impairment Scale (AIS) (20).

All subjects reported at least 2 daily episodes of urinary incontinence in their BD at baseline. Individuals were sub-grouped in: "No Failure," group (NF) who never reported BoNT/A injection "ineffectiveness" according to our criteria; "Occasional Failure" (OF) who had at least one, but not successive failures; "Consecutive Failure" (CF) subjects with persistent BoNT/A ineffectiveness.

No failure group

Thirty-two out of 60 SCL patients (53.3%) were defined as No Failure (NF). The mean duration of efficacy in months for each BoNT/A treatment is reported in Figure-1.

Twenty-seven out of 32 subjects (84.4 %) underwent all four treatments at least once (Figure-2). Twenty-nine individuals (90.6%) were still on follow-up at the last visit, while 3 patients (9.3%) had stopped treatments because they desired a definitive solution for their NOD. All of them underwent bladder augmentation respectively after 5, 6 and 7 BoNT/A detrusor injections. At BD during follow-up, the mean bladder capacity at each catheterization ranged from 210-260mL, while mean episodes of daily incontinence varied from 2.39 to 2.96.

For any BoNT/A type of treatments, the percentage of YES responses went from 19% with aboBoNT/A 500IU to 29% with onaBoNT/A 300IU (Table-1).

Only five (15.6%) patients reported at least 6 months of dryness after overall injections despite the BoNT/A used.

Occasional failure group

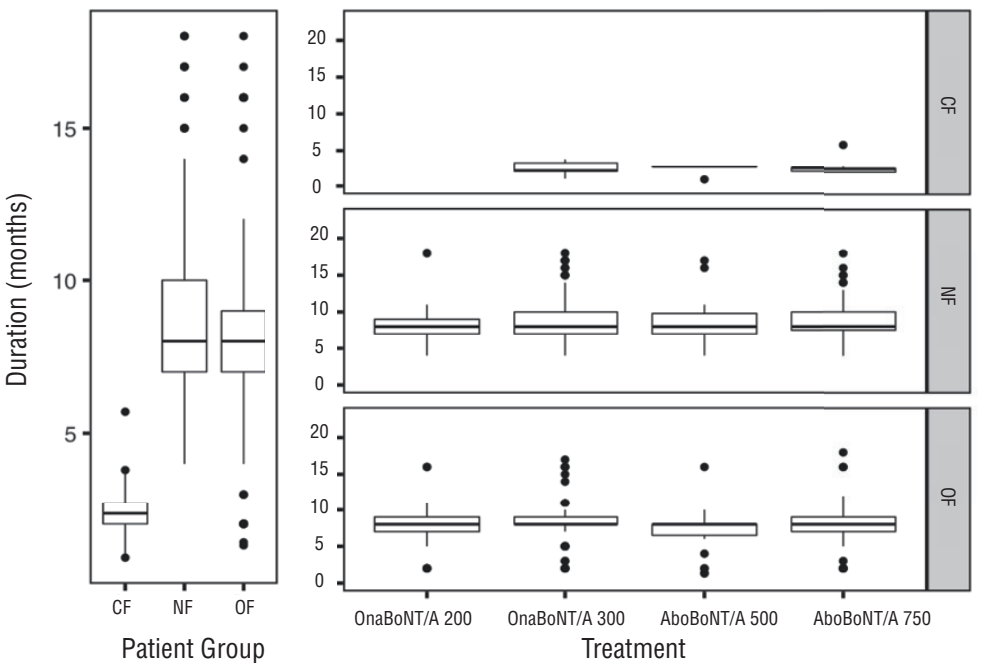
Sixteen out of 60 subjects (26.6%) were defined "occasional failure" (OF). The mean duration of efficacy in months for each BoNT/A treatment is reported in Figure-1.

Five and two out of 16 subjects failed twice and three times during follow-up, respectively.

Eleven (68.7%) were treated by overall types and dosages of BoNT/A (Figure-2). Twelve out of 16 (75%) were still in follow-ups.

Two out of 16 subjects (12.5%) stopped BoNT/A injections for non-urological complications. One required a permanent indwelling

Figure 1 - Boxplots reporting the efficacy of treatments in the three groups of patients (left panel), and across all treatments used (right panel).



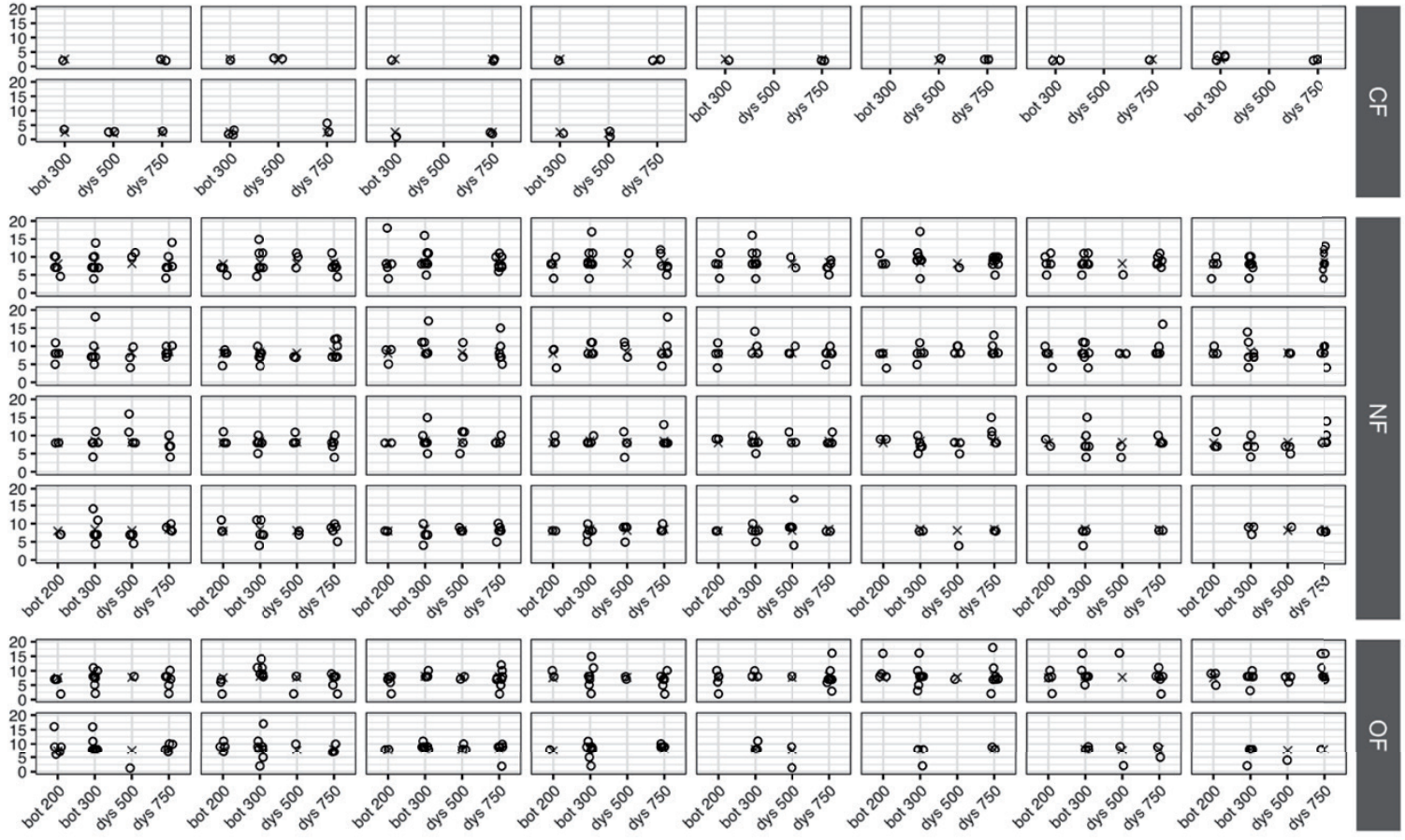
No Failure (NF)			Occasional Failure (OF)			Consecutive Failure (CF)		
Type of BoNT/A	Nº of injections	Mean (± SD)	Type of BoNT/A	N° of injections	Mean (± SD)	Type of BoNT/A	N° of injections	Mean (± SD)
OnaBoNT/A 300 IU	202	8.4 (±2.64)	OnaBoNT/A 300 IU	96	8.34 (±2.87)	OnaBoNT/A 300 IU	17	2.43 (± 0.83)
OnaBoNT/A 200 IU	96	7.98 (±2.09)	OnaBoNT/A 200 IU	42	7.62 (±2.98)	OnaBoNT/A 200 IU	0	-
AboBoNT/A 500 IU	79	8.27 (±2.36)	AboBoNT/A 500 IU	22	7.1 (±3.29)	AboBoNT/A 500 IU	7	2.47 (± 0.69)
AboBoNT/A 750 IU	162	8.50 (±2.24)	AboBoNT/A 750 IU	81	7.88 (±3.02)	AboBoNT/A 750 IU	18	2.5 (±0.84)
Total	**539**	**8.33 (±2.39)**	**Total**	**241**	**7.94 (±2.99)**	**Total**	**42**	**2.47 (±0.79)**

catheter for an unsolved sacral sore lesion. One became unable to perform self-catheterization due to the onset of cerebrovascular disease. Instead 2 patients (12.5%) quit treatment, requiring definitive alternatives for their NDO. As for the previous group, all these patients were submitted to enterocystoplasty.

At BD during follow-up the mean bladder capacity at each catheterization ranged from 230 to 265mL, whereas mean episodes of incontinence/day varied from 2.15 to 2.79.

Twenty-five urodynamics were carried out within a three-month interval post-injection (range 7-12 weeks). Mean MCC was 230mL

Figure 2 - Random intercept models for each patient included in the study (The x-axis reports the different treatment used in the study whereas the y-axis reports the duration of the treatment expressed in month. Crosses report the predicted efficacy for each treatment and for each patient, computed by using linear mixed-effects models).



CF = Consecutive Failure; **NF =** No Failure; **OF =** Occasional Failure; **Bot =** OnaBoNT/A; **Dys =** AboBoNT/A

Table 1 - Six months dryness (%) achieved without antimuscarinics.

	onaBoNT/A 300 IU	aboBoNT/A 750 IU	onaBoNT/A 200 IU	aboBoNT/A 500 IU
No Failure group				
Total number of injections	202	162	96	79
Number of injections 6-months dry (%)	58 (29%)	44 (27%)	21 (22%)	15 (19%)
Occasional Failure group				
Total number of injection	96	81	42	22
Number of injection 6-months dry (%)	24 (25%)	18 (22%)	8 (19%)	4 (18%)

(range 190-280mL), while mean Pdet max was 63.24cm$H_2$0 (range 35-90cm$H_2$0).

Low compliance was documented in the same five subjects (31.2%), both at baseline and during follow-ups.

The percentage of YES responses ranged from18% with Dysport 500IU to 25% with Botox 300IU (Table-1).

Consecutive failure group

Twelve out of 60 individuals (20%) stopped BoNT/A injections due to consecutive failures. Nine out of 12 subjects (75%) reported failure with the first injections. All these individuals quit the follow-up before March 2013 (max follow-up 22 months). Two subjects (12.5%) were not on antimuscarinics at baseline. Nine (75%) showed low compliance at urodynamics (Table-2).

The mean duration of efficacy in months for each BoNT/A treatment is reported in Figure-1.

Thirty-five urodynamics were performed within 3 months following injection (range 8-12 weeks).

Mean MCC was 213.14mL (range 170-240mL), while mean Pdet max was 64.74cm $H_2$0 (range 35-90cm$H_2$0). The same nine patients with documented low compliance at baseline showed comparable values (<20cm$H_2$0/mL) during follow-up.

The 10 patients using antimuscarinics at baseline never dropped their baseline dosage of oral treatment following each BoNT/A injection.

Statistical results

The CF group showed a pronounced difference in the duration of efficacy of the treatments with an average two-months duration against eight-months reported for the other two groups (Kruskal-Wallis test p-value <0.01) (Figure-1). No significant differences were found in the three groups of patients regarding the type of treatments considered with an average duration of efficacy per group equal to the one reported above (Kruskal-Wallis test p-values >0.05) (Figure-1). In addition, the efficacy of treatments in the three groups of patients was assessed through Linear Mixed-Effects Models with random intercept, reporting a different regression model for each patient (Figure-2). Results showed that the efficacy of the treatments drastically changes in the three groups of patients considered, regardless of the type of treatment (p-values >0.05). Considering the predictable variables, only low compliance at baseline had a significant impact on determining consecutive failures (95% confident interval [CI] 0.53-3.14; Odd Ratio [OR] 7.27; p value=0.006).

DISCUSSION

According to our results, long term response does not depend on the type of treatments. As a matter of fact, no significant differences in mean duration of efficacy between treatments were found in the overall sample. The mean clinical efficacy was around 8 months as reported in

Table 2 - Baseline characteristics of patients.

	No Failure	Occasional failure	Consecutive failures
Total numbers of patients	32	16	12
Age in years (mean ± SD)	39.97 ± 10.79	37.62 ± 10.61	39.50 ± 9.07
Duration in months of SCL pre-first BoNT/A (mean ± SD)	41.03 ± 16.87	39.25 ± 17.32	33.83 ± 18.21
Nº with traumatic aetiology of SCL (%)	27 (84.4)	13 (81.2)	11 (91.6)
Nº of males (%)	25 (78.1)	12 (75)	8 (66.6)
Nº of tetraplegics (%)	7 (21.8)	5 (31.2)	4 (33.3)
Nº with complete SCL (%)	22 (68.7)	9 (56.2)	7 (58.3)
Nº on antimuscarinics (%)	27 (84.3)	11 (68.7)	10 (83.3)
3-day Bladder Diary			
Bladder capacity in mL (mean ± SD)	203.12 ± 31.87	206.87 ± 34.89	193 ± 38
Episodes of daily urinary incontinence (mean ± SD)	4.11 ± 0.75	3.83 ± 0.76	4.19 ± 0.75
Urodynamics			
MCC in mL (mean ± SD)	190.62 ± 28.62	168.12 ± 11.06	177.50 ± 28.30
Pdetmax in cmH_20 (mean ± SD)	64.40 ± 10.71	63.51 ± 12.07	66.50 ± 10.50
Compliance in mL/H_20 (mean ± SD)	26.19 ± 13.44	24. 74 ± 14.17	17.67 ± 20.47

SD = Standard deviation; **Nº =** Number of patients; **BoNT/A =** Botulinum Toxin A; **SCI =** spinal cord lesion; **Pdetmax =** maximum detrusor pressure at involuntary contraction; **MCC =** Maximum Cystometric Capacity

literature in shorter follow-ups using only one type of BoNT/A at the same or different dosages (14-17).

Moreover, the switch option does not seem a valid strategy for failed patients. In the OF, patients responded to the same type and dosage of BoNT/A that had previously failed. Moreover, considering the CF, switching was not helpful to avoid a further "failure". Although, the number of CF patients was small, this group showed a higher percentage of low compliance at baseline urodynamics compared to others. This finding is similar to that reported in literature (15, 21) and it could explain why CF discontinued follow-up earlier and 75% of them reported failure at first injection.

Concerning the use of oral antimuscarinics, whether they are useful to extend the interval between injections is still widely debatable. As a matter of fact the main RCTs were designed without antimuscarinic washout (9, 11). In our study the percentage of YES responses was ≤30% in both OF and NF groups. Finazzi et al. reported a similar trend about the use of antimuscarinics during the first injection follow-up. In their prospective observational study on 105 patients, only 23.8% of patients discontinued oral therapy at 120 days, whereas almost 97% of patients were on antimuscarinics with a majority of them (52%) already having returned to oxybutinin t.i.d. within 6 months following 300IU BoNT/A treatment (22). Similarly, Alvares et al. showed that 18 out of 22 subjects (81.8%) continued to use anticholinergics to achieve continence (23).

In our sample, a high percentage of SCI patients (70.7%) has been in follow-up for

longer than 15 years. Seventeen out 60 patients (12 of whom were CF) asked for bladder augmentation (24, 25).

This result raises the question whether or not treating subjects with BoNT/A for longer than 15 years is the right approach.

Nowadays, although the alternative option for treating refractory NDO is bladder augmentation, over the last 10 years other lesser invasive treatments have been proposed to selected SCI patients, such as sacral neuromodulation, which may also have potential positive effects on other concomitant pelvic dysfunctions (26). Our findings (i.e. the need for continuative use of antimuscarinics, the time duration of dryness, the variability in responsiveness and possible occurrence of occasional BoNT/A ineffectiveness) ought to be shared and discussed with patients at the time of counselling and at follow-ups. Thus, patients should be well informed from the beginning about the opportunity for alternative long term solutions which could guarantee dryness.

We are aware that our study was designed retrospectively and we could not determine whether some criteria were used to switch treatment despite response. Considering this limit, our methodology requires further explanations. Firstly, at the time of inclusion in 1999, no literature was available (4). Our policy since the beginning of our experience was exclusively to repeat the same BoNT/A when failure occurred once. This approach was chosen by the fact that we did not know the real individual response to one BoNT/A rather than another, in order to definitively exclude one treatment before moving on to the next. Although our clinical behaviour and definition of non-responsiveness (CF) could be debatable, we think it was helpful to discriminate the occasional failure vs. non-responders and give stronger reasons for recommending major surgery. Again, to that end, we always objectively documented failure through urodynamics.

CONCLUSIONS

Long-term BoNT/A for NDO did not increase failures, independent of the types of treatments. Again, switching did not improve response (14, 27, 28). Non-responsiveness (CF) was found in short follow-ups despite switching, so patients should be promptly advised about other treatment options. The possibility of an indefinite treatment period using BoNT/A for other groups of patients does not seem impractical, but definition of long term response and/or criteria for discontinuing repetitive BoNT/A injections are urgent (29). Randomized prospective comparative studies are needed to confirm our results, especially in populations with a history of BoNT/A failures.

CONFLICT OF INTEREST

None declared.

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Erectile dysfunction in ankylosing spondylitis patients

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ABSTRACT

Background: Rheumatic diseases such as ankylosing spondylitis (AS) may be associated with sexual dysfunction.
Aim: To study erectile function of a group of Brazilian AS patients comparing them with controls.
Materials and Methods: This was a cross sectional study approved by the local Committee of Ethics in Research. The questionnaire IIEF (International Index of Erectile Function) was applied to 40 AS patients and 40 healthy controls. AS patients had determination of disease activity (through BASDAI or Bath Ankylosing Spondylitis Disease activity index), ASDAS (Ankylosing Spondylitis Disease Activity Score, MASES or Maastricht Ankylosing Spondylitis Score and SPARCC or Spondyloarthritis Research Consortium of Canada), function (through BASFI or Bath Ankylosing Spondylitis Functional Index and HAQ or Health Assessment Questionnaire) and BASMI (Bath Ankylosing Spondylitis Metrological Index).
Results: AS patients had a median score on IIEF of 22.0 (IQR=18-25) while controls had 29 (IQR=27-30) with p<0.0001 Only 17.5% of the AS patients had no erectile dysfunction, in opposite to 87.5% of controls (p<0.0001). IIEF scores had a negative association with BASDAI (p<0.0001), HAQ (p=0.05), body mass index (P=0.03), MASES (P=0.02) and SPARCC (P=0.02) in a univariate analysis. Multiple regression showed that BASDAI was the only variable independently associated with IIEF.
Conclusion: There is a high prevalence of erectile dysfunction among AS patients that is associated with disease activity measured by BASDAI.

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INTRODUCTION

Ankylosing spondylitis (AS) is a chronic rheumatic disease that affects mainly young males and belongs to the group of Spondyloarthritis (SpA) (1). The most distinguishing feature of this disease is inflammation of axial joints (starting at sacroiliac joints) causing inflammatory low back pain. Eventually, the axial disease causes joint fusion, which is associated with substantial functional impairment and with the appearance of the classical "skier posture". Patients may also present with asymmetric oligoarthritis (especially of the lower extremities), dactylitis (sausage digits), and enthesitis (inflammation at sites of ligamentous or tendon attachment to bone). Additional features include eye and bowel inflammation, an association with preceding or ongoing infectious disorders, and a strong association with the human leukocyte antigen (HLA)-B27 (1). The diagnosis of AS, as in others SpA, can be done by the ASAS (Assessment of SpondyloArthritis International Society) classification criteria that includes a set of clinical findings such as inflammatory back pain,

arthritis, enthesitis etc., presence of HLA B27 and image findings of sacroiliitis (1) (Table-1). The estimated prevalence of SpA in a Caucasian population is approximately 0.5 to 2 percent (1). Its treatment varies according to presenting clinical features but in patients with axial predominance it is based mainly on AINH (anti-inflammatory non-steroidal drugs) and biological medication such as anti-TNF alpha (TNF-α). When it affects peripheral joints or has an extra-articular involvement, sulphasalazine, methotrexate and leflunomide may be used (1).

Musculoskeletal chronic pain, stiffness, depression, loss of self-esteem by a disturbed perception of the own body image and fatigue are commonly seen in this disease and affect significantly the quality of life of these patients (1). Sexuality has an important role in the personal satisfaction and is associated to quality of life, being a complex aspect of human life and is more than the sexual act. Rheumatic diseases may affect all aspects of life including sexual functioning due to multifactorial disease-related factors as well as therapy. Some factors include pain, fatigue, stiffness, functional impairment, depression, anxiety, negative body image, reduced libido, hormonal imbalance and drug treatment (2). However, studies on sexual dysfunction among AS patients are contradictory; while some of them report a low level of sexual satisfaction (3-5), others do not (6, 7). A possible reason for those dissimilar outcomes is that many studies were of small size; another might be the presence of confounding factors such as duration and activity of the disease (8).

Several instruments using image and endocrinological resources have been used to identify erectile dysfunction; however, questionnaires of self-reported performance are considered an important tool to diagnose and classify this problem in clinical assays (7). The International Index of Erectile Function (IIEF) is one of such questionnaires and is considered to have high sensibility and specificity in this context (9).

In the present study, we have analyzed a sample of Brazilian AS patients aiming to know the influence of this rheumatic disease in their erectile function using the IIEF.

MATERIALS AND METHODS

This was a cross sectional study approved by the local Committee of Ethics in Research (CAAE number 50667215.0.0000.0103) and all participants signed consent. It was a convenience sample that embraced all SpA patients that attended regular consultations in a single Rheumatologic Unit from Evangelical University Hospital in Curitiba-Paraná, Brazil, during a period of six months and that agreed to participate in the study. This sample gave a power analysis of 92%.

The sample included 40 patients with AS that fulfilled the ASAS criteria for the disease (1), older than 18 years. ASAS criteria are shown in Table-1.

As comparison group, 40 voluntary healthy men, paired for age, tobacco exposure and comorbidities answered the questionnaire.

Table 1 - ASAS (Assessment of SpondyloArthritis international Society) Classification Criteria for Spondyloarthritis (1).

Lumbar pain >3 months in patients with age <35 years. +		
Sacroiliitis at imaging + one or more criteria bellow	or	HLA B27 + two or more criteria bellow
Inflammatory lumbar pain		Inflammatory bowel disease
Arthritis		Good response to NSAID
Enthesitis		Family history of Spondyloarthritis
Dactilytis		HLA B27
Psoriasis		Elevated C reactive protein

Data on disease duration, epidemiological and clinical profile, HLA-B27 positivity was collected in medical records of AS patients. They had also determination of overall disease activity through BASDAI (Bath Ankylosing Spondylitis Disease Activity Index) (1), ASDAS (Ankylosing Spondylitis disease Activity Score) (1), erythrocyte sedimentation rate (ESR) and C reactive protein (CRP); enthesitis activity (through MASES or Maastricht Ankylosing Spondylitis Score (10) and SPARCC or Spondyloarthritis Research Consortium of Canada) (11); function through BASFI (Bath Ankylosing Spondylitis Functional Index) (1), HAQ (Health Assessment Questionnaire) (12) and BASMI or Bath Ankylosing Spondylitis metrological index (1).

Patients and controls were submitted to the IIEF. About erectile function, this questionnaire has six closed questions that were answered by patients without interference from other person or researchers. Each question has a value ranging from 1 to 5, and responses with low values represent poor condition of the quality of erectile function and those with results lower than 6-10 points were considered to have severe erectile dysfunction; values between 11 and 16 with severe moderated, values ranging from 17 to 21 as moderate dysfunction, values of 22 to 25 as mild dysfunction and those with values higher than 26 as no erectile dysfunction (8, 13) (Table-2).

Data was organized in contingency and frequency Tables. Normality distribution was assessed by the Kolmogorov Smirnov test. Central tendency was expressed in mean and standard deviation (SD) in parametric samples and median and interquartile intervals (IIQ) in the non-parametric data. Association studies of nominal data were done through by Fisher Exact test and by Mann Whitney test, when data was numeric. Correlation analysis of IIEF with disease activity,

Table 2 - The International Index of Erectile Function (IIEF-5) Questionnaire (8, 13).

Over the past 6 months					
1 - How do you rate your confidence that you could get and keep an erection?	**Very low**	**Low**	**Moderate**	**High**	**Very high**
	(1)	(2)	(3)	(4)	(5)
2 - When you had erections with sexual stimulation, how often were your erections hard enough for penetration?	**Almost never/never**	**A few times (much less than half the time)**	**Sometimes (about half the time)**	**Most times (much more than half the time)**	**Almost always/always**
	(1)	(2)	(3)	(4)	(5)
3 - During sexual intercourse, how often were you able to maintain your erection after you had penetrated (entered) your partner?	**Almost never/never**	**A few times (much less than half the time)**	**Sometimes (about half the time)**	**Most times (much more than half the time)**	**Almost always/always**
	(1)	(2)	(3)	(4)	(5)
4 - During sexual intercourse, how difficult was it to maintain your erection to completion of intercourse?	**Extremely difficult**	**Very difficult**	**Difficult**	**Slightly difficult**	**Not difficult**
	(1)	(2)	(3)	(4)	(5)
5 - When you attempted sexual intercourse, how often was it satisfactory for you?	**Almost never/never**	**A few times (much less than half the time)**	**Sometimes (about half the time)**	**Most times (much more than half the time)**	**Almost always/always**
	(1)	(2)	(3)	(4)	(5)

functional, metrological indexes, BMI and disease duration were done by the Pearson test. All variables with P<0.1 were studied through multiple regression to verify its independency. The adopted significance was 5% (P≤0.05).

RESULTS

A) Comparison of AS patients with controls

In the AS group patients age ranged from 24 to 63 years old (mean 45.8±11.41); 7.6% were smokers and 7.6% had arterial hypertension. In the control group, age went from 24 to 63 years (mean 46.0±11.1) none was smoker or had hypertension. Pairing showed p=0.92 for age and 0.11 for smoking and 0.11 for arterial hypertension. None of participants (patients or controls) had diabetes mellitus. In the AS group, 90% had lumbar pain; 77.5% had peripheral arthritis; 45% had anterior uveitis, 37.5% had enthesitis; 12.5% had coxalgia; 12.5% had dactylitis; 70.5% were HLA B27 positive; 32.5% were on treatment with sulphasalazine; 7.5% were on non-steroidal anti-inflammatory drugs and 7.5% on anti-TNF medications.

b) Study of IIEF among AS patients

IIEF score in AS patients had median value of 22.0 points (IQR=18-25, range 14-30); controls had median value of 29 points (IQR=27-30, range 13-30); with p<0.0001 (Mann Whitney test).

Table-3 displays the scores of studied patients and controls according to IIEF classification and shows a significant difference between the two groups.

Data on inflammatory, functional, laboratory measurements as well as its correlation with IIEF values is shown in Table-4. In this table, it is possible to note a negative correlation of the IIEF score with BMI (body mass index), with several indexes of inflammatory activity such as BASDAI, MASES, SPARCC and with a functional index: the HAQ.

All data with p<0.1 in the univariate analysis were studied through multiple regression to evaluate their independency; only BASDAI kept its correlation with IIEF (p=0.007; t=-2.86).

DISCUSSION

Our results endorse the finding that AS male patients had worse erectile function than the general population. Most AS patients are young adults, in their active stage of sexual activity, so this finding may contribute significantly to loss of life quality in this population (14). However, despite of the fact that that only a small amount of patients (less than 20%) had normal sexual performance, most of health care providers neglect or are not prepared to discuss such problems with their patients in daily practice.

The present work also shows that inflammatory disease activity, measured by BASDAI but not by ESR and CRP is associated with sexual impairment. ESR and CRP are usually used to judge inflammatory activity in rheumatic diseases (1); nevertheless, it is well known that no serological marker is good enough to reflect the ongoing inflammation in the SpA such as AS (1) and this may justify our findings. BASDAI is a composite index that takes into account pain in spina, peripheral joints and enthesis, fatigue as well as degree and duration of morning stiffness. All of these domains are measured according to the patient's point of view (1). Fatigue and pain are well known to hamper sexual function in patients with rheumatic diseases (5). Pirildar et al. (15) found that

Table 3 - International Index of Erectile Function (IIEF) scores in patients with Ankylosing Spondylitis (AS) and healthy controls studied.

IIEF Classification (points)	AS PATIENTS n=40	HEALTHY CONTROLS n=40	P
No erectile dysfunction (>26)	7 (17.5%)	35 (87.5%)	<0.0001
Mild (22 to 25)	13 (32.5%)	4 (10.0%)	0.02
Moderate (11 to 21)	15 (37.5%)	1 (2.5%)	0.0001
Severe (<6 to 10)	5 (12.5%)	0	0.054

Table 4 - Data on clinical profile, inflammatory, functional and laboratory of Ankylosing Spondylitis patients and its correlation with IIEF (International Index of Erectile Function).

Variable	Sample Range	Correlation with IIEF		
		Pearson's Rho	95% CI	p
Age (years)	14 - 30 (mean=21.6±4.3)	-0.16	-0.46 to 0.16	0.31
BMI (kg/m^2)	17 - 43.7 (mean=7.8±5.2)	-0.34	-0.59 to -0.02	0.03
Disease duration (years)	2 - 26; (median 18.0; IQR=8.2-20.0)	0.25	-0.06 to 0.53	0.11
ESR (mm)	2 - 58 (mean=26.5±21.4)	0.12	-0.19 to 0.42	0.44
CRP (mg/dL)	0 - 58 (median=6.7;IQR=3.5-17.2)	0.07	-0.25 to 0.37	0.66
BASDAI	0-7.8 (median=2.4; IQR=1.4-4.0)	-0.61	-0.77 to -0.37	<0.0001
ASDAS CRP	0 - 4.7 (mean=2.1±1.0)	-0.30	-0.56 to 0.01	0.057
MASES	0 - 13.0 (median=0; IQR=0-2.0)	-0.10	-0.59 to -0.03	0.02
SPARCC	0 - 12.0; (median=0;IQR=0.0-1.5)	-0.34	-0.59 to -0.04	0.02
BASFI	0 - 9.2; (mean=3.5±2.7)	-0.21	-0.49 to 0.10	0.18
HAQ	0 - 2.4 (median=0.6;IQR=0.1-1.0)	-0.31	-0.57 to -0.0003	0.05
BASMI	0.7 - 7.2; (mean 4.0±1.9)	-0.10	-0.40 to 0.21	0.52

BMI = Body mass index; **ESR =** Erythrocyte sedimentation rate; **CRP =** C reactive protein; **BASDAI =** Bath Ankylosing Spondylitis index; **ASDAS =** Ankylosing Spondylitis disease Activity Score; **MASES =** Maastricht Ankylosing Spondylitis Score; **SPARCC =** Spondyloarthritis Research Consortium of Canada; **BASFI =** Bath Ankylosing Spondylitis Functional Index; **HAQ =** Health Assessment Questionnaire; **BASMI =** Bath Ankylosing Spondylitis metrological index.

the duration of morning stiffness was the only clinical feature related to erectile dysfunction in AS patients. Some authors have suggested that high levels of pro-inflammatory cytokines, specifically TNF-α, involved in the pathogenesis this disease, are related to fatigue. In this context, anti-TNF drugs could help improve fatigue (14, 16) and consequently sexual function. Corroborating this idea, Matos et al. (17), studying 383 males without rheumatic disease, found a significant association between high levels of serum TNF-α and erectile dysfunction. Hotston et al. (18) have observed that these drugs could inhibit the TNF-α upregulation of phosphodiesterase type 5 expression which, in turn, impairs nitricoxide-induced pro-erectile effects, resulting in dysfunction. Our sample of anti-TNF users (n=3) was too small to allow any conclusion of the effect of this drug on sexual performance, but all users had relatively high scores (20, 25 and 27 points). Dong et al. (14), comparing 22 AS patients on anti-TNF drugs with 20 without it, found that patients on this treatment had improvement in their score of sexual quality of life that was correlated with the degree of improvement in the BASDAI.

Interesting, age, a recognized variable associated with lower sexual function (19, 20) did not show any association in our study. When men aged 18-39 years were compared with those aged 60-69, they presented 2.2 higher risk of erectile dysfunction. The same was observed by other authors (5). It is possible that the low sexual performance of young AS patients due to disease activity blunted this connection. Ireland et al. (21) described erectile dysfunction as a rare side effect of sulphasalasine (<0.1%). In our study, among 13/40 (32.5%) in use of this drug, there was no significant difference in the scores of these patients compared with others (data not shown).

In addition, it is worthwhile to observe that Dincer et al. (3) found a relationship of sexual dysfunction with lower scores in questionnaires of function, as we did with the HAQ as observed in Table-4. However, in our study, this association did not sustain itself in the multi-variated analysis, suggesting

that the inflammatory components such as pain, fatigue and stiffness impair function and underlie this association. Nowadays, with the modern treatment strategies that propose aggressive treatment of inflammation, patients have a higher chance to preserve their mobility. So, loss of function due to loss of mobility seems not to exercise important influence.

In summary, we have found that AS patients have high levels of erectile dysfunction and that disease activity measured by BASDAI is associated with sexual impairment. Health care providers should be aware that sexual impairment is a consequence of AS disease activity and discuss this issue with their patients and provide referral to specialists when appropriate.

CONFLICT OF INTEREST

None declared.

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Novel penile circumcision suturing devices versus the shang ring for adult male circumcision: a prospective study

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ABSTRACT

Introduction: To evaluate the safety and efficacy of a novel penile circumcision suturing devices PCSD and Shang ring (SR) for circumcision in an adult population.
Materials and Methods: A total of 124 outpatients were randomly assigned to receive PCSD (n=62) or SR (n=62). Patient characteristics, operative time, blood loss, return to normal activities time (RNAT), visual analogue scale (VAS), scar width, wound healing time, cosmetic result, and complications were recorded.
Results: There were no significant differences in blood loss, RNAT, or complications between the two groups. There were no significant differences in the VAS scores at the operation, at 6 or 24 hours after surgery (P>0.05). The wound scar width was wider in the SR group than in the PCSD group (P<0.01). Patients in the SR group had significantly longer wound healing time compared with those in the PCSD group (P<0.01). Patients who underwent PCSD were significantly more satisfied with the cosmetic results (P<0.01).
Conclusions: SR and PCSD are safe and effective minimally invasive techniques for adult male circumcision. Compared with SRs, PCSDs have the advantages of faster postoperative incision healing and a good effect on wound cosmetics.

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INTRODUCTION

The results of 3 large-scale random control tests in Africa indicate that male circumcision (MC) reduces the risks of sexually-transmitted HIV infection by 50%-60% (1-3). The accumulated evidence also demonstrates that male circumcision is capable of preventing other sexually-transmitted infections (STIs), for example, circumcision reduces the possibility of males infecting or transmitting genital ulcer disease (GUD), trichomonads and gonococcus, and decreases the risks of infection with human papillomavirus (HPV) and herpes simplex virus-2 (HSV-2) (4-6). According to Wright et al., (7), male circumcision can reduce the risk of prostate cancer by 15%. Following circumcision in young adults, participants exhibit more erection confidence (8).

Clinically, the Shang ring (SR) is widely used across the world for circumcision and is associated with the advantages of a short operating time, an obvious effect and few complications. Additionally, the SR produces good long-term cosmetic results with no significant complications or adverse effects on sexual function (9). However, the SR still has disadvantages, such as postoperative pain and reduced postoperative incision healing (10). The data regarding the clinical effects of novel penile circumcision suturing devices (PCSD) for adult male circumcision are insufficient. This study will compare

the clinical effects and safety of two operation methods for male circumcision in an adult population through a randomized controlled trail.

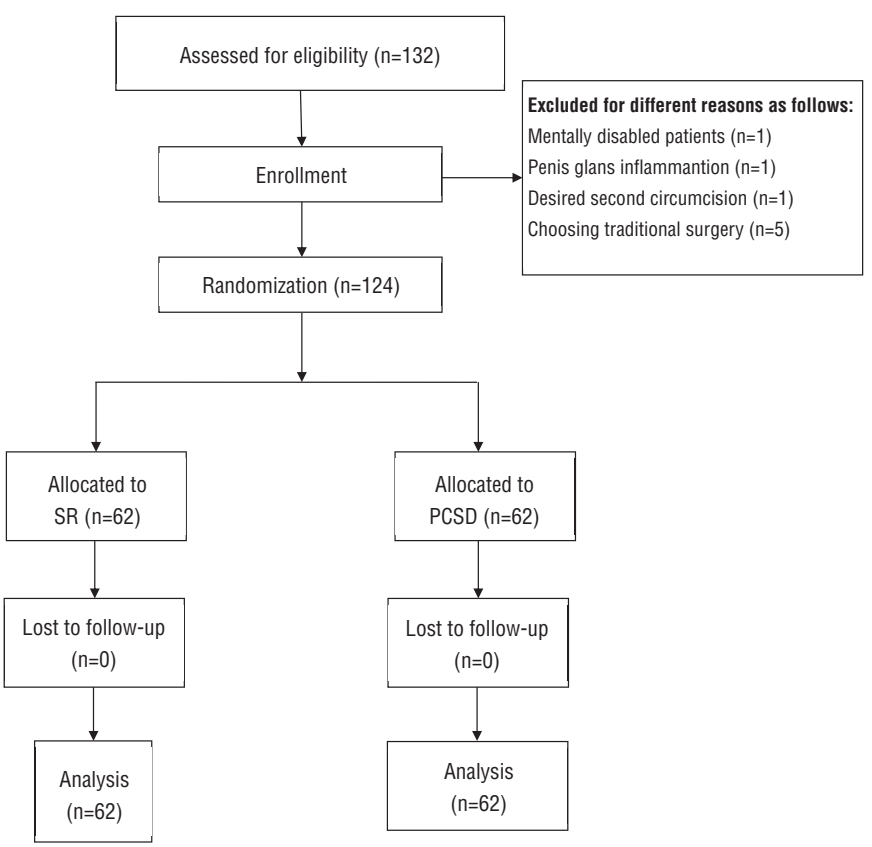
PATIENTS AND METHODS

Participants and Eligibility

Our study included all the patients with redundant prepuce or phimosis requiring penile circumcision. All the patients were >18 years of age and free of penile circumcision histories. The study was approved by the ethics committee of our hospital, and every participant provided written informed consent. The patients were randomly assigned to one of two groups: PCSD or SR. The randomization was performed using computer-generated simple random Tables. The inclusion criteria for the patients were the following: (1) patients with redundant prepuce or phimosis; (2) at least 18 years old and younger than 65 years who provided informed consent; (3) willing to undergo penile circumcision; and (4) willing to be randomly assigned to the SR or PCSD operation.

The excluding criteria were patients with the following: 1) penile malformations; 2) acute preputial balanitis; 3) HIV-positive status; 4) abnormal blood clotting function; 5) difficulty communicating, e.g., intellectual disabilities and/or low education levels; 6) unwillingness to be assigned to the SR or PCSD surgery; and 7) medically necessity for two penile circumcisions. All the selected patients conforming to all the inclusion criteria (without any of the exclusion criteria) were divided into two treatment groups and were subjected to post-operation follow-ups for at least 2 months. From February to October 2014, a total of 124 patients were invited to the study. Specific process flow charts were show in Figure-1.

Figure 1 - Consort diagram.

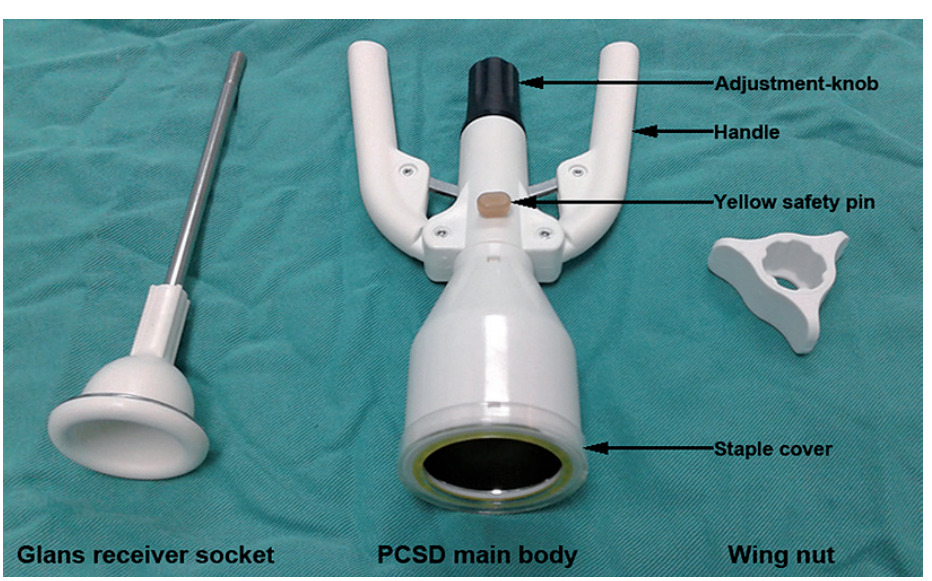


Surgical Procedures

For the patients in the SR group, a Shang ring (Wuhu Shengda Medical Treatment Appliance Technology Co.,Ltd. Wuhu City, Anhui Province, China) which is a disposable, single-use, minimally invasive device, was used. We utilized the no-flip technique that does not require the eversion of the foreskin as previously described (11). During the operation, 4 incisions were made in the incisal edge for decompression after the operation. The SR was removed with a flat plier. For the patients with phimosis, the dorsum of the penis was cut to enlarge the prepuce external orifice so that the inner ring could be easily placed between the glans and the inner preputial skin.

For the patients in the PCSD group, we used the one-time PCSD invented by Changshu Henry Medical Instrument Co., Ltd. in Jiangshu China, which includes 6 models of 11-, 11+, 15, 21, 27 and 33 based on the penis circumference (Figure-2). For the selection of the model of the PCSD, the principle of "larger rather than smaller" was followed. The details of the PCSD procedure were as follows: First, the patient was placed in supine position, and the appropriate PCSD was selected according to the penis circumference. Second, disinfection with povidone iodine was applied, and the base of the penis was locally narcotized with 1% lidocaine, The PCSD of the selected size was removed from the sterile pouch, and the adjustment-knob was turned counterclockwise until the glans receiver socket could be removed. Third, the prepuce was clamped with 2-3 mosquito forceps and lifted up to place the glans receiver socket on the glans at approximately 30° of incline relative to the dorsum of penis. Fourth, the lengths of the inner and outer skin were adjusted, and the prepuce was fixed with the forefinger and middle finger of the left hand to remove the mosquito forceps. Fifth, the assistant removed the staple cover from the main body, aligned the glans receiver socket black rib with the main body rib, inserted the glans receiver socket shaft into the main body, and turned the adjustment knob clockwise via a wing nut until it stopped at the right position so that the main body was snug onto the foreskin without cutting it. Regarding stopping at the right position, the finger can be used to touch the adjustment-knob end, and if it is in the same plane, the metel shaft of glans receicer socket can be turned together with the adjustment-knob Sixth, the yellow safety pin was removed to prepare for holding the PCSD handles and squeezing evenly on both sides. The handle was then pressed to

Figure 2 - Novel penile circumcision and suturing devices.



the end of its travel and held for 3-5 seconds, The handle was then released, the squeeze handle was pressed again to ensure complete cutting, and stapling was applied when necessary. Finally, the adjustment-knob was turned counterclockwise 5-8 turns to open the glans receiver socket with main body while maintaining a distance of approximately 5-6mm to determine whether foreskin adhesion was present between the glans receiver socket and main body, Pressing the foreskin with a finger cause the foreskin to fall out naturally. Because the PCSD was equivalent to a circular cutter with stapled anastomosis for circumcision (CCSAC) and a disposable circumcision suture device (DCSD), detailed descriptions of the surgical technique and figures explaining surgical procedures can be found in the studies of Yuan et al., (12), study and Lv et al., (13). For the patients with phimosis (types II, III, and IV), the dorsum was cut, and the veutro side of the penis was cut simultaneously so that the glans receiver socket could be easily placed between the glans and the inner preputial skin. The incision was sutured with 4-0 sutures to fix the foreskin on the glans receiver socket to prevent foreskin slippage and bleeding. For patients with prepuces that were not sufficiently long, absorbable sutures were used with purse-string suturing to reduce the external orifice of the prepuce so that it could be fixed on the PCSD glans receiver socket.

In both groups, preoperative, intraoperative, and postoperative parameters were determined, including age, the surgical indication for male circumcision, operative time, blood loss, the return to normal activities time (RNAT), the intraoperative and postoperative pain scores, scar width, inner plate length, time to the removal of the ring or nail, time spent removing the ring or nail, wound healing time, cosmetic results, total procedural cost, and complications were measured. The operative time was recorded from the initiation of the local anesthesia until the end of surgery. The intraoperative blood loss was calculated as follows: a completely soaked 5cm×5cm piece of gauze has an average carrying capacity of 3.25mL of blood (14).

Follow-Up and Data Collection

All patients were advised to attend subsequent visits after surgery at 2 days, 5 days, 1 to 2 weeks and 1 month; and oral antibiotics were administered for 3 days postoperatively. The complications and wound healing times were recorded upon reexamination at 2 months postoperatively.

The pain scores were calculated via an internationally accepted visual analogue scale (VAS) pain score at the time of surgery, 6 and 24 hours after the surgery, and upon the removal of the ring or nail. We evaluated the VAS scores before oral painkillers were administered in order to reduce the deviation.

The wound scar width and the inner plate length were measured with a ruler upon the removal of the ring or nails. The inner plate length of the prepuce was defined as the length of the penile dorsal coronary groove to the incision. The wound healing time was measured from the date of surgery to the date when the wound scar was completely gone and the surgical wound had completely healed.

All patients were asked to grade the cosmetic appearance of their incision after their wound finished healing. The cosmetic results were defined on a verbal response scale. The verbal response scale had the following four options: 1, bad; 2, acceptable; 3, satisfactory; and 4, very good. The total cost of the procedure included the operative cost and medical device costs. The postoperative complications were assessed and recorded at each follow-up. Photographs were taken in preoperatively, intraoperatively, and postoperatively to document patient's information.

Statistical analysis

The data and results area presented as the means±the standard deviations. Statistical analysis were performed using the SPSS 18.0 statistical software package. Student's t test, Pearson's chi-square test and a continuity-correctioned chi-square test were used as appropriate. P values<0.05 were accepted as statistically significant. The intraoperative and postoperative data were examined with intent-to-treat analyses.

RESULTS

Baseline characteristics

From February 2014 to October 2014, a total of 132 outpatients were included in this study, Eight patients did not meet the inclusion criteria based on preliminary assessments, These patients included 1 case who was ruled out because he was a mentally disabled patient, 1 case was rejected for penis glans inflammation, 1 patient desired a second circumcision, and 5 patients preferred traditional surgery rather than being randomly assigned to one of the two types of surgical equipment. Ultimately, sixty-two patients were randomized to the SR approach, and 62 were randomized to the PCSD approach.

All the procedures were performed by the same urologist. Two patients experienced failed procedures due to instrument hand fracture in the PCSD group, and these failures occurred in the first ten procedures. The mean patient ages were 27.1±7.3 years in the SR group and 29.4±8.4 years in the PCSD group (P=0.115).

There was no significant difference between the two groups in terms of the indication for male circumcision (P>0.05). The patient characteristics of the two groups are illustrated in Table-1. Phimosis was classified as follows and according to Hsieh, et al., (15), study: type I (normal), the entire glans penis was visible after the retraction of the foreskin; type II (adhesion of the prepuce), the urethral meatus and part of the glans penis were visible after the retraction of the foreskin; type III (partial phimosis), the urethral meatus was visible but not the glans penis after retraction of the foreskin; type IV (phimosis), the urethral meatus and glans penis were invisisble after foreskin retraction.

Intraoperative and postoperative outcomes

The intraoperative and postoperative data are provided in Table-2. There were no significant differences in blood loss during the operations (0.7±0.7 vs. 1.2±1.7mL, P=0.054), RNAT (1.8±1.3 vs. 2.0±1.3 days, P=0.447) or the time to the removal of the ring or nail (10.5±1.0 vs. 10.4±1.1 days, P=0.499) between the SR and PCSD groups. The patients in the SR group had a shorter median operation time than those in the PCSD group (6.7±1.3 vs. 8.9±5.8 min, P=0.004). There were no significant differences in the VAS scores at the operation, at 6 or 24 hours after surgery, or at the removal of the ring or nail between the two groups (P>0.05).

The wound scar width was wider in the SR group than in the PCSD group (2.8±0.4 vs. 0.9±0.5mm, P<0.01). The inner plate length of the prepuce was significantly shorter in the SR group than in the PCSD group (0.9±0.9 vs. 1.4±0.5cm, P=0.001). The patients in the SR group experienced significantly longer wound healing times that did those in the PCSD group (30.2±4.9 vs. 15.7±3.0 days, P<0.01). The times spent removing the rings or nail were significantly shorter in the SR group

Table 1 - Baseline characteristics.

	SR	PCSD
Number	62	62
Age(year),mean ± SD	27.1±7.3	29.4±8.4
Surgical indication(%(n))		
Type I (normal)	87.1(54/62)	90.3(56/62)
Type II (adhesion of prepuce)	4.8(3/62)	3.2(2/62)
Type III (partial phimosis)	4.8(3/62)	4.8(3/62)
Type IV (phimosis)	3.2(2/62)	1.6(1/62)

SR=Shang ring; **PCSD**=penile circumcision and suturing devices

Table 2 - Intraoperative and postoperative outcomes.

	SR	PCSD	P value
Number	62	62	
Operative time(min),mean ± SD	6.7±1.3	8.9±5.8	0.004[a]
Blood loss(mL), mean ± SD	0.7±0.7	1.2±1.7	0.054[a]
RNAT(days),mean ± SD	1.8±1.3	2.0±1.3	0.447[a]
VAS score, mean ± SD			
VAS in operation	1.1±1.5	1.0±1.7	0.782[a]
VAS 6h	2.7±1.8	2.3±1.6	0.162[a]
VAS 24h	1.4±1.6	1.6±1.7	0.541[a]
VAS in removal ring or nail	5.0±2.1	5.5±2.1	0.246[a]
Scar width(mm),mean ± SD	2.8±0.4	0.9±0.5	0.000[a]
Inner plate length(cm),mean ± SD	0.9±0.9	1.4±0.5	0.001[a]
Time to removal ring or nail(days),mean ± SD	10.5±1.0	10.4±1.1	0.499[a]
Time spent removing ring or nail(min),mean ± SD	5.6±1.4	27.8±12.8	0.000[a]
Wound healing time(days),mean ± SD	30.2±4.9	15.7±3.0	0.000[a]
Cosmetic result, mean ± SD	3.1±0.6	3.7±0.5	0.000[a]
Cost(Dollars),mean ± SD	259.6±3.8	267.6±8.4	0.000[a]
Complication(%(n))			
Edema or hematoma	16.1(10/62)	8.1(5/62)	0.169[b]
Incision errhysis	0.0(0/62)	6.5(4/62)	0.127[c]
Incision dehiscence	6.5(4/62)	8.1(5/62)	1.000[c]

[a] Calculated by student t test; [b] Pearson's chi-square test was used; [c] continuity correction chi-square test was used.
SD = Standard deviation; **VAS** = Visual analogue scale; **RNAT** = Return to normal activities time; **SR** = Shang ring; **PCSD** = Penile circumcision and suturing devices., **1RMB** = 0.1626 Dollar.

than in the PCSD group (5.6±1.4 vs. 27.8±12.8 min, P<0.01). The patients who underwent PCSD were significantly more satisfied with the cosmetic results as assessed with a verbal response scale (P<0.01). The cosmetic results regarding the wounds at approximately three weeks after the operations are provided in Figure-3.

The mean costs (US dollars) for the two groups were 259.6±3.8 and 267.6±8.4 (P<0.01). None of the patients in either group group experienced a wound infection. There were no significant differences in the rates of edema or hematoma, incision erhysis or incision dehiscence between the two groups. Although four incision dehiscence cases occurred at the ring removal after the operation in the SR group, none of the patients required suturing again after the operation. In contrast, 5 patients experienced partial wound dehiscence that required suturing during the operation in the PCSD group. There waswas no incision erhysis in the SR group, but 4 patients in the PCSD group experienced incision erhysis that could be alleviated by intermittent sutures or sterile gauze compression bandages during the operation. The complications among the patients in the PCSD group primarily occurred in the first 20 surgeries. Nearly every patient in the both groups exhibited involuntarily erect penises within 1 to 2 days after surgery, and this condition reduced quality of sleep at the follow-up.

DISCUSSION

In the both groups of patients, the surgeries completed with the exceptions of 2 cases in which hand fracture occurred and necessitated changes

Figure 3 - Wound healing after operation: (a1, a2) wound healing results about 3 weeks in PCSD, (b1, b2) wound healing results about 3 weeks in SR.

to that were submitted open surgery in the PCSD group. The median operative time in the SR group was shorter than that in the PCSD group. There were no significant differences in blood loss, the time to return to normal activities, the time to the removal of the ring or nail, the VAS scores at the operation or 6 and 24 hours after surgery, or complications between the SR and PCSD groups. The patients in the PCSD group were more satisfied with the cosmetic aspects of the wounds than were the patients in the SR group.

The advantages of SR are high patient and provider acceptability and rates of mild adverse events that compare favorably with WHO--recommended surgical approaches (10, 16-18). The PCSD is a novel circumcision device that is based on bowel anastomotic stapler principles. The PCSD is the equivalent of a product from a different manufacturer that involves a circular cutter with stapled anastomosis for circumcision (CCSAC) reported by Yuan et al., (12), with similar operating principles.

The PCSD includes the 11-, 11+, 15, 21, 27 and 33 models. Before the operations, the PCSD models were selected based on the penis circumference and in adherence to the principle of "larger rather than smaller". Our study indicated that the 21 and 27 models were mainly used for Chinese adults.

The median operation time in the SR group was 6.7±1.3 in our study, and this time is similar to previously described results (10, 13, 17, 18). The median operation time in the PCSD group was 8.9±5.8min, which is similar to with 7.6±4.5min reported in the study by Lv, et al., (13). In this paper, the duration of the operation equaled the

sum of duration of the anesthesia of the dorsal penile nerve and the operating time. In Lv et al., (13), study, no dorsal penile nerve block was applied to the patients using disposable circumcision suture device (DCSD) and SR, but 5% lidocaine cream was applied to the surface of the penis prior to the operation. The intraoperative pain levels of the DCSD and SR group wre1.9±1.3 and 5.8±2.1, respectively, which are higher than the 1.0±1.7 and 1.1±1.5, respectively, that were observed in the present study. Therefore, we believe that the effects of superficial anesthesia are reduced compared with those of the traditional dorsal penile nerve block.

The RNAT of the patients in the PCSD group were similar to those of the patients in SR group, which is consistent with a previous study (16). Additionally, neither of the two operation methods had any effect on the regular work of the patients following the operations. Therefore, operations with either method can be completed in outpatient clinics.

Regarding the management of postoperative pain, we found no significant differences in the pain scores at 6 or 24 hours after the operations with SRs or PCSDs. The most serious postoperative pain occurred during ring and nail removal. In order to reduce the pain and enhance comfort, the patients were advised to take a painkiller or apply some topical surface anesthetic cream before coming in for ring or nail removal.

The scar width and time required for complete wound healing were significantly superior in the PCSD group. The randomized control trails of several African centers indicated that the median time to complete wound healing is 43 days in SR groups (10). Nevertheless, complete wound healing at 4 weeks was observed in 84% of patients with the ring in Rakai, Uganda (18). The scars of the patients in the SR group were wide, and the time for healing was much longer because the surface skin required healing after necrosis due to the pressing action between the inner and outer rings. Although the lengths of the inner skins of the patients in the SR group were shorter than those of the patients in the PCSD group, which may have been related to the technical level, this difference had no influence on the effects of the operations. Additionally, this factor may also have been reflected during the assessments of the two groups regarding wound cosmetics. The patients in the PCSD group felt more satisfied with the appearances of the wounds.

There was no obvious significant difference in the times to the removal of the ring or nail between the patients of the two groups. Generally, it is advised that the ring or nail be removed at approximately 10 d after the operation. Regarding ring removal at 7d, 14d or 21d after the operation, one study highlights that removal time has little effect on healing (17). However, some scholars sugested that it is better to remove the SR at approximately 2weeks after the operation so that the pain caused by ring removal can be reduced (19). In PSCD arm patients had their surgery in a mean operative time of 8.9 min. However, the procedure for removing the nail was three times longer (27.8 min). Therefore, the patients must be aware that despite better cosmetic outcomes, they will be subjected to a longer “second” surgical procedure. Luckily, based on our communications with the manufacturer about the long time required for nail removal in the patients of the PCSD group, the current PCSD has been improved so that the suturing nail can fall off automatically 3-4weeks after the operation.

For reference, the cost of dorsal slit circumcision is $17.67 and using the SR the cost is $18.21 in Zambia (20). The main disadvantage of the PCSD is that it is a one-time non-reusable device with a higher cost. In this study, there was no significant cost difference between the SR and PCSD group, in spite of a statistic difference.

During the SR procedure, the foreskin is sandwiched between the inner and outer rings before the redundant foreskin is removed. Therefore, no hematomas or exudations occurred in the majority of the SR group patients. Regarding individual patients, edema may occur due to the obstruction of lymphatic return. However, hematoma or exudation occurred in the majority of the PCSD group patients, particularly during the early stage of the application of the technique. With increased experience, we began to bandage the wound with gauze immediately after the operation and advise the patients to press the wound forcefully

for approximately 5min. No wound dehiscence or bleeding occurred in any of the patients.

Wound dehiscence occurred in the SR group and PCSD groups at similar rates. Among patients in the SR group, wound dehiscence primarily occurred at ring removal after the operation, but no secondary suturing was required due to the capability for self-healing over a longer time. However, for the patients in the PCSD group, wound dehiscence primarily occurred during the operations. In this study, 5 patients exhibited partial wound dehiscences, which were discontinuously reinforced with absorbable sutures, and all the patients recovereded well after the operations. An analysis of the reason for wound dehiscence revealed that first, the adjustment-knob was not tightened during the operation, which prevented the suturing nail from completely penetrating the prepuce, and second, the wounds were was not bandaged immediately after the operations, which resulteded in hematomas and partial wound dehiscence. Among the patients in the two groups, no wound infections occurreded, which indicates that the two types of devices exhibit good bio--compatibilities with the human body.

Complications among the patients in the PCSD group primarily occurred in the early stage. Therefore, we believe these complications were strongly associated with the experience and operative skills of the surgeon. SRs and PCSDs can be used for patients with redundant prepuces and phimosis in addition to patients in whom the prepuce is not sufficiently long. During the operation, absorbable sutures can be applied with purse-string suturing to reduce the prepuce external orifice so that it can be fixed on the PCSD glans receiver socket.

The main limitations of our study are that sample size was not big enough in the PCSD group, and the follow-up was relatively short. The curative effects of PCSDs on adult patients require further clinical study for continuous confirmation. Furthermore, there is a lack of clinical studies in children patients, and this issue will be the target of our future study. In adult populations, the gold standard surgery for male circumcision is open surgery, therefore, future studies should compare PSCD with traditional surgery. Additionally, there are no comparisons on the curative effects of the PCSD devices from two Chinese companies.

CONCLUSIONS

Generally, SR and PCSD are safe and effective minimally invasive techniques for the treatment of adult patients with redundant prepuces and phimosis. Compared with SRs, PCSDs have the advantages of faster postoperative incision healing and a good effect on wound cosmetics. Larger samples and long-term follow-up studies are needed to ascertain the clinical efficacies of PCSD devices in the future.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Effect of platelet-rich plasma on polypropylene meshes implanted in the rabbit vagina: histological analysis

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ABSTRACT

Purpose: The polypropylene mesh (PPM) is used in many surgical interventions because of its good incorporation and accessibility. However, potential mesh-related complications are common. Platelet-rich plasma (PRP) improves the healing of wounds and is inexpensive. Thus, the purpose of this study was to analyze the effect of the PRP-gel coating of a PPM on inflammation, production of collagen, and smooth muscle in the rabbit vagina.

Materials and Methods: The intervention consisted of a 1.5cm incision and divulsion of the vaginal mucosa for the implantation of a PRP-coated PPM. The PRP-coated mesh was implanted in 15 rabbits, and in the second group, the same implant was used without the PRP coating. In the sham group, the intervention consisted of the incision, divulsion, and suture. The rabbits were euthanized at 7, 30 and 90 days, and full-thickness sagittal sections of the posterior vaginal wall and rectum were scored. The inflammatory infiltrate was evaluated using hematoxylin and eosin staining. The Sirius Red stain was used to examine deposition of collagen I and III, and Masson's trichrome staining was used to visualize the smooth muscle.

Results: The group with PRP-coated meshes had a lower inflammatory infiltrate count at 30 days. Deposition of collagen III increased with the use of PRP-coating at 90 days.

Conclusions: The area of inflammatory infiltrate was significantly increased in the group without the PRP-coated mesh at 30 days but not in the group with the PRP-coated mesh, indicating a less intense inflammatory response. In addition, a significant increase in collagen III occurred at 90 days.

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INTRODUCTION

The integration between meshes and vaginal tissue depends on the structure of the mesh and on factors such as tissue tropism, infection, and inflammation; these factors are also directly related to the risk of complications (1-4).

Rechberger et al. observed that the serum levels of cytokines are higher in patients with erosion slings than in those with proper healing (5). Similarly, Di Vita et al. demonstrated that the use of polypropylene for hernia repair is associated with high levels of interleukin-6 and interferon, when compared to traditional correction (6). Thus, chronic inflammation with a large foreign body reaction promotes the occurrence of complications.

Previous studies have evaluated coating meshes with materials known for their potential

to accelerate healing and attenuate the inflammatory response and downstream fibrosis and to modulate collagen deposition (7, 8). The composition of these coatings varies from synthetic materials including prophylactic antimicrobials and metal films to biologic materials such as collagen (7-10). Unfortunately, the results are inconsistent.

However, there are few studies on the use of PRP on meshes for the repair of hernias and in Urogynecology. An in vitro study performed using seven types of meshes with PRP showed a reduction in adhesions and improved biocompatibility after 6 weeks (11). In addition, in two experiments, using PRP in a biological loop resulted in less severe adhesions, increased angiogenesis, increased neovascularization, increased integrity of the fabric, and a decrease in the recurrence of hernia in the group with PRP (12).

Similarly, Gerullis et al. conducted an in vitro study comparing polypropylene-coated meshes coated with peripheral blood mononuclear cells, platelets, and plasma. They concluded that the autologous plasma promoted increased biocompatibility of fabrics, justifying in vivo studies (11).

Smooth muscle is often a minor contributor to resisting passive mechanical loading; however, it is extremely important in maintaining vaginal tone and actively resisting the forces of the surrounding connective tissues. Few studies have thoroughly investigated the impact of synthetic meshes on the smooth muscle, also known as functional properties (13).

To improve the understanding of the impact of meshes on the vagina, multiple mechanisms that could affect the properties of the vaginal tissue, like smooth muscle, should be investigated.

The PRP is acquired by centrifuging plasma in order to obtain a platelet and leukocyte concentration 2 to 3 times higher (on average) than the regular plasma and is a clinical option for accelerating the healing process in hernia correction with meshes (14, 15). PRP is easy to obtain at a low cost. Hence, we proposed coating the monofilament polypropylene mesh with PRP to study its effect on the inflammation process and collagen deposition in the vaginas of rabbits.

MATERIALS AND METHODS

The sample consisted of 45 sexually mature, pure-bred, female, white rabbits aged 40 weeks and weighing 4.5kg. A pilot project was performed to test the methods and define the sample numbers. Three groups of fifteen rabbits were randomly generated: sham, vaginal deployment of 1.0cm polypropylene mesh with pores of 1500µm, and the same mesh coated with PRP gel obtained after removal of a sample of 10mL of blood obtained by cardiac puncture (a large blood volume proportional to the animal's weight).

The blood sample was obtained during the implant procedure and was immediately taken to the laboratory for preparation using the same protocol as used for human samples (16). The specimens were anesthetized, and the blood was transferred to a sterile 1.8mL tube containing 0:10mL of citrate as an anticoagulant. The material was homogenized and centrifuged at 24ºC for 10 minutes. After centrifugation, it was possible to distinguish two distinct layers in the tube: the red blood cells at the bottom and the supernatant plasma. All plasma was removed with a pipette and placed in a sterile plastic tube. Additional centrifugation was performed at a speed of 1500rpm at 24°C. After centrifugation, the plasma around the top of the tube was removed, leaving only the portion to which 0.5mL 10% calcium gluconate was added. The solution was homogenized and allowed to stand for 30 minutes, acquiring a gel-like consistency.

Platelet counts were performed in 25% of the plasma samples chosen randomly before and after PRP preparation to confirm the increase in the number of platelets. The gel contained, on average, three times the platelet count of the peripheral blood. A 1–1.5cm vaginal incision was performed, and the implant was inserted, without fixation, to prevent tissue reactions. The mesh was inserted, in a standardized manner, between the vaginal epithelium and the rectovaginal fascia (17), and was coated with PRP gel so that the entire length and the interstice between the mesh pore was filled. The vaginal incision was closed with Vicryl. Penicillin was administered. The sham group underwent an operation consisting of the

same vaginal incision using the same protocol.

The animals were divided into three groups of fifteen animals per group and 5 were euthanized at 7, 30 and 90 days after implantation. All were anesthetized before lethal injection. The implant site was removed en bloc, including the vagina, mesh, and rectum. At each time point, the wounds were harvested and their histologic features were assessed in paraffin-embedded sections using hematoxylin and eosin staining. The Sirius Red stain was performed and samples were assessed using polarized light microscopy, a simple, sensitive, and specific method for quantification of collagen. It is particularly useful for examining the heterogeneity of collagen fibers in connective tissues, providing essential information in pathological studies (18).

One pathologist, who was blinded to the tissue type and time from wounding, evaluated all specimens. The slides were scanned under a microscope. Collagen I and III were assessed using polarized light, by density per micra. The inflammatory infiltrates (INI) and muscle tissue (micra^2) were counted in different fields. Four fragments of the material were placed on each slide.

All statistical analyses were carried out using the SPSS 20.0 system. We analyzed the hypothesis of normal distribution and homogeneity using the Shapiro and Levene tests. Because of a violation of normality, the data were analyzed using the Kruskal-Wallis test, followed by the Bonferroni test for comparisons between groups with and without PRP-coating and for comparisons between different time-points.

RESULTS

An extrusion of polypropylene mesh occurred in each of the groups (with and without PRP-coating); these animals were excluded from the study and replaced. None of the animals died during the observation period. Moreover, none of them presented signs of systemic compromise or procedure-related complications.

Table 1 shows the results (median and interquartile range) at the euthanasia times.

The amount of the inflammatory cells in the first seven days did not become elevated. However, at 30 days, the PRP-coated group had significantly lower levels of inflammatory cells than the group without PRP-coating (Figure-1). After 90 days, the inflammatory response between study groups was indistinguishable. The sham group had significantly lower levels of inflammatory cells at 30 and 90 days than the other groups.

In the group without PRP-coating, the concentration of collagen III did not vary between euthanasia times. In the group with PRP-coating, this value was significantly increased at 90 days (Figure-2).

The collagen I concentration did not vary with time and presence of PRP (Figure-3). The smooth muscle area showed a small increase; however, this was not significant (Figure-4).

DISCUSSION

To our knowledge, this is the first study coating meshes with PRP for vaginal implants. The local inflammatory reaction is an early event that occurs after mesh implantation, and a subsequent foreign body reaction caused by the implant was already established after 3 months and did not significantly change over a 24-month period (11).

In the present study, an acute inflammatory reaction occurred in rabbits implanted with meshes both with and without PRP-coating after seven days, suggesting that the use of PRP did not affect this initial inflammatory process. At 30 days, the PRP-coated group showed a significant reduction in inflammatory cells, suggesting that the PRP-coating shortened the time of the acute inflammatory response, leading to an early tissue repair proliferative phase.

In both in vitro and in vivo studies, Gerullis et al. also noted that the use of plasma on various materials did not influence the early inflammatory reaction (11, 19). However, three months after implantation, markers of tissue vascularization organization (invasion of myofibroblasts and endothelial cells) were detectable and there were differences between the three meshes investigated.

In addition, in the PRP-coated group, at 90 days post-implantation there was a significant increase in type III collagen fibers, the first to be produced in the presence of inflammatory cells.

Tabela 1 - The median and the interquartile range at the euthanasia times, groups with and without PRP. Quantification by cells number (INI) and micra² (collagen type I, type III and smooth muscle).

	Seven days		*p* Value
	Without PRP	**With PRP**	
INI	5.00 (1.00)	3.00 (3.00)	0.09
Ci	2874,01 (2140,42)	3455,19 (1040,62)	0.40
CIII	3060,48 (1094,56)	2398,66 (194,05)	0.26
Muscle	15522,15 (11707.01)	16829,06 (3085,59)	0.49
	30 Days		
INI	**141,00 (173,00) #**	**4,00 (0,00) #**	**0.0175#**
Ci	3463,84 (1836,11)	3846,56 (1614,01)	0.34
CIII	2613,64 (4687,18)	2543,25 (495,76)	0.17
Muscle	10216,80 (2361,56)	15085,41 (8758,95)	0.08
	90 days		
INI	20,00 (12,00)	19,00 (7,00)	0.6
C III	**2304,46 (1383,01) #**	**8617,72 (16671,74) #**	**0.022#**
CI	2247,62 (487,07)	1153,37 (18101,06)	0.098
Musc	6198,46 (1562,64)	10734,65 (9259,69)	0.061

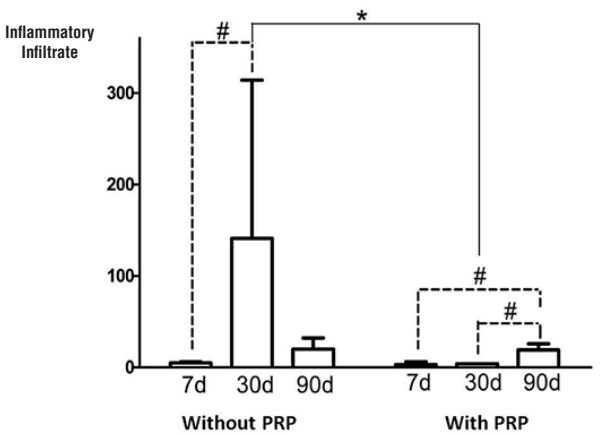
INI = Inflammatory infiltrate; **C III** = Colagen III; **C I** = Colagen I; **musc** = muscle
#*P*<0.05 without PRP x with PRP Mann-Whitney test;
**P*<0.05: Comparing days; Kruskall-Walis Test.

The higher presence of immature collagen (III) in the PRP-coated group at 90 days suggests that the wound was in the phase characterized by Schultz et al. as contraction and remodeling (20). It has been shown that premature type III collagen is predominantly synthesized in the early phases of wound healing and in the presence of inflammatory cells. Collagen III is then replaced by highly cross-linked and stable collagen type I later after implantation, and this slowly increases the tissue tensile strength.

Remodeling of the extracellular matrix is essential for implant integration, and the mesh-induced foreign body responses must be balanced to result in normal wound healing. Swift and adequate tissue ingrowth into the mesh results in superior biocompatibility and likely improves the clinical performance. Intense or prolonged inflammation and bad infiltration, resulting in scar plate formation, can be accompanied by shrinkage or deformation of the biomaterial, recurrence, adhesion, fistula, or erosion of nearby tissue (21).

The vagina is comprised of both passive (collagen) and functional (smooth muscle) components. To date, studies have thoroughly investigated the impact of synthetic meshes on the active properties of the vagina. Tissue degeneration was found to be in large part related to mesh stiffness (22). Liang et al. showed that following implantation with a stiffer mesh, the vagina demonstrated evidence of a maladaptive remodeling response (23). This is characterized by the thinning of the smooth muscle layer, increased cell apoptosis, increased collagenase activity, decreased collagen and elastin content, and increased glycosaminoglycan content. Furthermore, Jallah et al. obser-

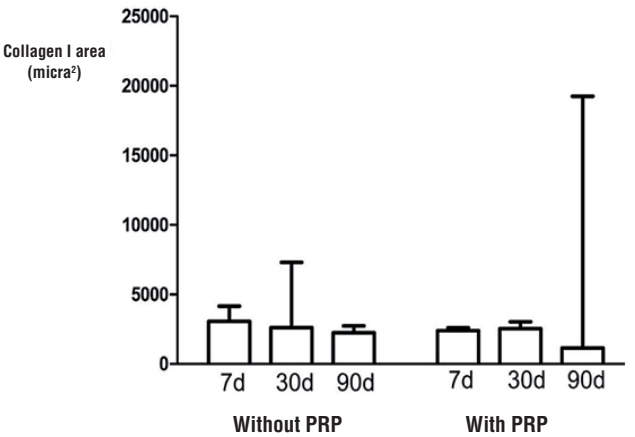
Figure 1 - Inflammatory infiltrate (INI) area at different time points in groups with and without PRP-coating.



*p<0.05 without PRP-coating versus with PRP-coating at 30 days (Mann-)

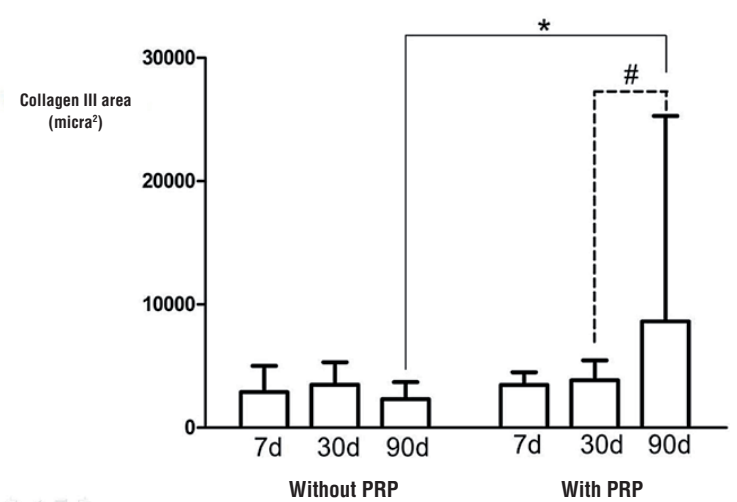
#p<0.05 without PRP-coating, comparisons between 7 and 30 days, Group with PRP-coating: comparisons between 7 and 30 days and 7 and 90 days (Kruskal)

Figure 3 - Comparisons of the median of collagen I area for the rabbits sacrificed at 7, 30 and 90 days.



PRP = Platelet-rich plasma.

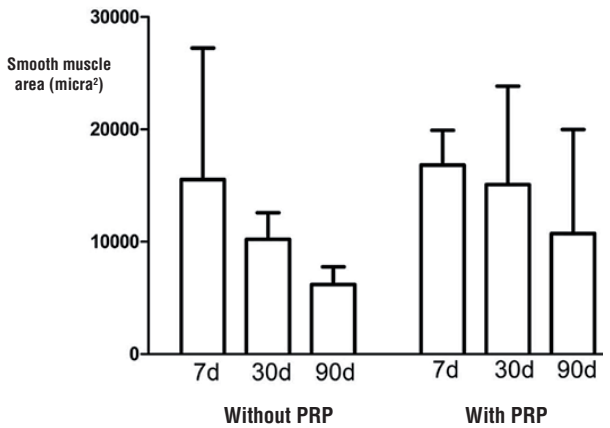
Figure 2 - Comparisons between the median collagen III areas in rabbits sacrificed at 7, 30 and 90 days.



ved that the mesh has an overall negative impact on vaginal smooth muscle function. In this study, unfortunately, the PRP-coating did not change the size of the vaginal smooth muscle area (13).

As with any animal research, the extrapolation of the results to clinical practice should be carefully considered, but the vaginal implant model is an advantage of this study (24). The inflammatory response and repair go far beyond the type of mesh deployed; each tissue and implant site responds differently to aggression. Abdominal implants, for example,

Figure 4 - Comparisons of the median of smooth muscle area for the rabbits sacrificed at 7, 30 and 90 days.



PRP = Platelet-rich plasma.

are placed in a sterile environment, with vastly different biomechanics than the vaginal implants, which are put into a potentially contaminated environment (25, 26). Thus, the use of implants in rabbits and vaginal mucosa are important points in our study.

The rabbit is considered a useful animal model for vaginal implants but is not a large primate mode. The rabbit's vagina has two portions; the inner is more akin to small intestinal histology, but the wider segment of the external vaginal wall makes a suitable model for histocompatibility studies (26).

A possible limitation in this study is the age of the rabbits; all were of reproductive age and had a good vaginal trophism. It is known that hipoestrogenic vaginal mucosa is less receptive to implantation meshes, increasing the rates of complications (27). Likewise, postoperative estrogen replacement for eight weeks in rabbits increased collagen deposition in the vaginal mesh implantation (28). However, there is data that suggests that the PRP would have an even more positive action in this type of animal. Abramov et al. observed in spayed rabbits that collagen production is diminished in the healing of the vaginal mucosa and there is increased inflammation (29).

Another limitation of this study was that only one mesh type was investigated. We chose a monofilament and macroporous polypropylene mesh because it is the most accepted design based on the literature and is used in surgeries (30). A study about the different structural weights and pore sizes is one possible continuation of this research. It is also necessary for better understanding the action of PRP on the enzymatic and immunological processes involved in mesh integration.

Moreover, before clinical implementation, it is necessary to conduct further studies evaluating the use of PRP-coating on mesh implants in vaginas of oophorectomized, older, and multiparous animals.

CONCLUSIONS

The inflammatory infiltrate area did not elevate in the group with platelet-rich plasma, at 30 days, indicating a less intense inflammatory response. Also, a significant increase of collagen type III occurred at 90 days of the study in the group with platelet-rich plasma.

CONFLICT OF INTEREST

None declared.

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ORIGINAL ARTICLE

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Penile alterations at early stage of type 1 diabetes in rats

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ABSTRACT

Objective: Diabetes affects the erectile function significantly. However, the penile alterations in the early stage of diabetes in experimental animal models have not been well studied. We examined the changes of the penis and its main erectile components in diabetic rats.

Materials and methods: Male Sprague-Dawley rats were divided into 2 groups: streptozotocin (STZ)-induced diabetics and age-matched controls. Three or nine weeks after diabetes induction, the penis was removed for immunohistochemical staining of smooth muscle and neuronal nitric oxide synthase (nNOS) in midshaft penile tissues. The cross-sectional areas of the whole midshaft penis and the corpora cavernosa were quantified. The smooth muscle in the corpora cavernosa and nNOS in the dorsal nerves were quantified.

Results: The weight, but not the length, of the penis was lower in diabetics. The cross-sectional areas of the total midshaft penis and the corpora cavernosa were lower in diabetic rats compared with controls 9 weeks, but not 3 weeks after diabetes induction. The cross-sectional area of smooth muscle in the corpora cavernosa as percentage of the overall area of the corpora cavernosa was lower in diabetic rats than in controls 9 weeks, but not 3 weeks after diabetes induction. Percentage change of nNOS in dorsal nerves was similar at 3 weeks, and has a decreased trend at 9 weeks in diabetic rats compared with controls.

Conclusions: Diabetes causes temporal alterations in the penis, and the significant changes in STZ rat model begin 3-9 weeks after induction. Further studies on the reversibility of the observed changes are warranted.

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INTRODUCTION

The U.S. Centers for Disease Control and Prevention estimated in 2010 that diabetes affects 25.8 million people in the United States, or 8.3% of the population (1). Erectile dysfunction (ED) is a significant complication for a large number of men with diabetes. Reports of the prevalence of ED in diabetic men range from 27.5% to 75%, depending on age and disease severity (2, 3). In the Massachusetts Male Aging Study sample, the prevalence of ED was three times higher in treated diabetic men than in the age-adjusted general population (28% versus 9.6%) (4). Diabetes and obesity account for more than 8 million ED cases in the US, and the annual cost of diabetes- and obesity-associated ED drug treatment has been estimated at more than $4 billion (5, 6). Moreover,

studies have shown that ED tends to be less responsive to treatment in diabetic patients than in non-diabetic individuals (4).

The onset of ED generally occurs at an earlier age in men with diabetes than in men in the general population (4), and its prevalence increases with disease duration, being approximately 15% at age 30 years and rising to 55% at age 60 (7). This suggests that the development and progression of diabetes-associated ED are time dependent. Further studies on temporal structural changes of the penis are needed to guide the development of new treatments.

The normal erection requires integrity of the intracavernous structures, including endothelium, smooth muscle, and nerve terminals. One important erectile mechanism is the release of nitric oxide (NO) from nNOS-immunoreactive nerve terminals or endothelial cells in the corpus cavernosum, leading to increased cyclic guanosine monophosphate and decreased cytosolic calcium concentrations in the smooth muscle cells, causing smooth muscle relaxation and ultimately penile tumescence (8). Previous studies of the corpora cavernosa in streptozotocin (STZ)-diabetic rats have shown significant decreases in the smooth muscle and endothelial cell densities 6 months after induction of diabetes (9), and decreased levels of endothelial nitric oxide synthase (eNOS) and nNOS isoforms 12 weeks after diabetes induction (10). However, the time window from no obvious alterations to the significant morphological changes of the penis and its main erectile components, including the corpora cavernosa, cavernous smooth muscle, and nNOS, during the diabetes progression is not well known. In the present study, we measured the temporal changes of morphology, corpus cavernosal smooth muscle, and dorsal nerve nNOS expression in cross sections of the midshaft penis at early stage of STZ-induced type 1 diabetes in a rat model.

MATERIALS AND METHODS

Experimental animals

Male Sprague-Dawley rats (280 to 310g, 10 weeks-old, Harlan) housed in a 12 hours light/dark facility with ad libitum access to food and water were used in this study. The animals were randomly allocated to two groups: diabetics (n=12) and age-matched controls (n=9). Diabetes was induced by a single intraperitoneal injection of STZ (60mg/kg dissolved in 0.1M citrate buffer, pH 4.5), and was confirmed by measurement of blood glucose (>300mg/dL) 72 hours after administration of STZ and at the time of euthanasia. The ACCU-CHEK Advantage blood glucose monitoring system (Roche Diagnostics Corporation, Indianapolis, IN) was used to measure the blood glucose levels. At 3 weeks (n=4 in control group, n=6 in diabetic group) or 9 weeks (n=5 in control group, n=6 in diabetic group) after injection, the rats were euthanized by a single intraperitoneal injection of pentobarbital (200mg/kg). The foreskin and shaft skin were removed. The penis was isolated and excised at the level of the ischial arch. The weight and the stretched length from the tip of the glans penis to the end were measured, and then the tissue was fixed with 10% phosphate buffered formalin solution for immunohistochemical staining. All procedures were approved by the Institutional Animal Care and Use Committee of our University (#08150).

Immunohistochemistry

After fixation, penile tissues were dehydrated and embedded in paraffin. Sections (5µm) from the middle of the body were used for immunohistochemical staining. In brief, the sections were dewaxed and rehydrated in graded ethanol. After heat-induced epitope retrieval with citrate buffer (Dako, Carpinteria, CA), slides were treated with 0.3% hydrogen peroxide in methanol to quench endogenous peroxidase. The sections were then incubated with blocking buffer for 30 min at room temperature. Primary antibody (rabbit anti-α-smooth muscle actin, Abcam, Cambridge, MA; or purified mouse anti-nNOS, BD Biosciences, San Jose, CA) in 1% BSA was applied overnight at 4°C. After rinsing 3×5 min., the sections were incubated with secondary antibody (biotinylated anti-rabbit or anti-mouse IgG H+L, Vector, Burlingame, CA) for 2 hours at room temperature. The Avidin--Biotin Complex (ABC) staining method was applied. The sections were then counterstained with hematoxylin. No primary antibody control was

used to support the specificity of the immunoreactive staining.

Image analysis

The stained slides were scanned (Leica SCN 400 Slide Scanner, Leica Microsystems, Buffalo Grove, IL) and digital images of whole cross sections of penile midshaft were saved for analysis using Visiopharm Image Analysis Software (Agern Alle 3, DK-2970 Hoersholm, Denmark), which can distinguish regions stained with different colors and accurately measure the areas by counting the pixels and converting the number of pixels to number of square micrometers. The immunohistochemically-stained α-smooth muscle actin images were used to measure the whole tissue and corpora cavernosa cross-sectional areas. The analysis method is illustrated in Figure-1. The figure includes representative images of immunohistochemically-stained α-smooth muscle actin (brown color) at low (Figure-1A) and high (Figure--1C) magnifications, and nNOS (brown color) at high magnification (Figure-1E). Figures-1B, 1D, and 1f are composite images based on the recognition of different colors by the software. The blue color in Figures-1B and 1d was produced automatically based on the recognition of deep brown color by the software and was used for measuring the α-smooth muscle actin-immunoreactive tissue area. The blue color in the Figure-1F was used for measuring the nNOS-immunoreactive tissue area. The green color in Figures-1B, 1D, and 1F was produced automatically based on the recognition of gray color by the software, indicating non-immunoreactive tissues. The total cross-sectional area of corpora cavernosa or penile dorsal nerves was calculated by adding the green- and blue-colored areas. The yellow colored areas indicate blank space, devoid of any tissue, and were not included in the calculations of tissue areas. The whole tissue cross-sectional area of the penile midshaft was measured using the same method. The percentages of α-smooth muscle actin immunoreactive area in the corpora cavernosa area and nNOS immunoreactive area in the penile dorsal nerve area were calculated. In every case, the processing of images was performed by the same investigator unaware of treatment group assignments.

Statistics

The data are presented as the mean±standard error of the mean (SEM) for each group. Statistical analysis was done by two-way analysis of variance with Tukey's multiple post hoc pair-wise comparisons, using Prism 4 (GraphPad, La Jolla, CA). P values <0.05 were considered to indicate statistical significance.

RESULTS

General characteristics

General weight and glycemic characteristics of the animals are shown in Table-1. The initial mean body weights of the rats in the diabetic and age-matched control groups were similar, but the diabetic rats weighed significantly less than the controls at 3 weeks and 9 weeks after induction of diabetes with STZ ($p<0.05$). The mean blood glucose levels of the diabetic rats at 3 and 9 weeks were 4.5 and 4.2 times higher, respectively, than in the control rats ($p<0.001$). Penis lengths were similar in the two groups at both time points, but penis weight was significantly lower in diabetic rats compared with controls at both 3 and 9 weeks after diabetes induction.

Morphological analysis

As shown in Figure-1A, the body of the penis comprises a pair of corpora cavernosa that are located dorsolateral to the urethra, a corpus spongiosus that surrounds the urethra, and the dorsal nerves and vessels. The cross-sectional areas of the whole tissue and the corpora cavernosa at the penile midshaft were significantly lower in diabetic rats compared with controls at 9 weeks, but not at 3 weeks, after diabetes induction (Table-2).

Smooth muscle in corpora cavernosa

As shown in Figure-2, smooth muscle cells are important components of corpora cavernosal sinusoids, lying adjacent to the endothelium that lines the sinusoidal space. The sinusoids are separated by dense bundles of connective tissues. There was diffuse positive α-smooth muscle actin immunoreactivity in the controls, whereas dia-

Figure 1 - Image analysis method. (A, C) positive immunohistochemical staining of α-smooth muscle actin (dark brown) in a penile midshaft specimen from a control rat. The area of the corpora cavernosa was circled manually in green color in (A). (B, D) software color segmentation performed on the images in (A) and (C) shows the blue-colored smooth muscle and green-colored non-immunoreactive tissue areas that were recognized and captured by the automated digital image analyzer for area measurements, as well as the yellow-colored blank spaces. (E) Positive immunohistochemical staining of nNOS (dark brown) in the dorsal nerve in a penile midshaft specimen from a control rat. (F) software color segmentation performed on the image in (E) shows the blue-colored nNOS and green-colored non-immunoreactive tissue areas that were recognized and captured by the automated digital image analyzer for area measurements.

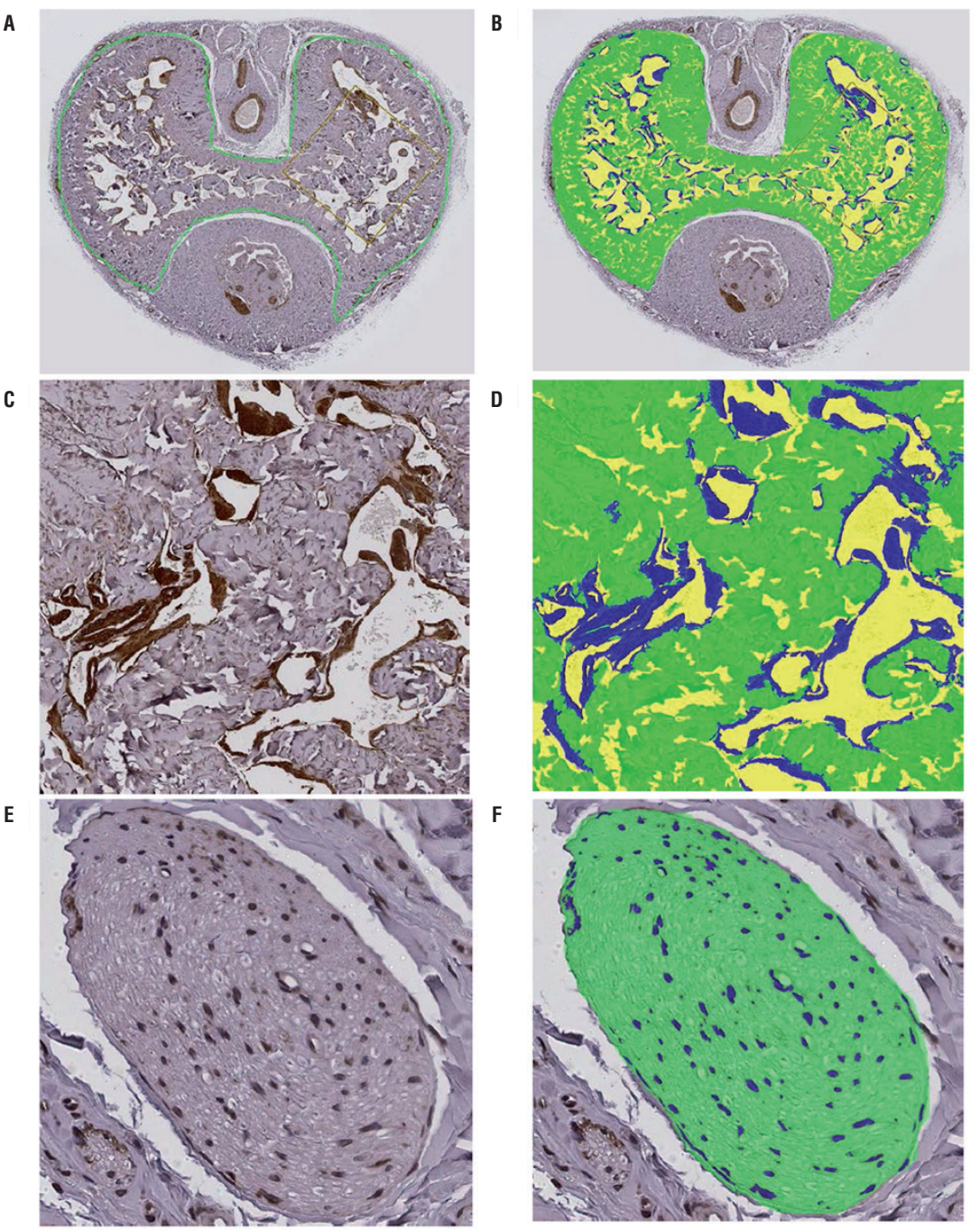


Table 1 - General characteristics of diabetic and age-matched control rats.

Time Point	Group	n	Initial Weight (g)	Final Weight (g)	Blood Glucose (mg/dL)	Penis Length (mm)	Penis Weight (mg)
3 weeks	Control	4	293.25±2.34	371.25±5.79	132.25±2.25	21.25±0.20	263.50±2.32
	Diabetic	6	298.67±2.53	251.83±9.92*	591.50±2.16*	19.00±0.26	226.17±4.02*
9 weeks	Control	5	293.50±6.13	454.75±14.31	138.50±6.80	22.60±0.22	292.40±11.60
	Diabetic	6	299.17±3.82	220.83±14.12*	576.50±8.54*	21.00±0.26	212.33±6.85*

Values are expressed as mean plus or minus SEM of 4 to 6 individual rats. *significantly different from corresponding value in control group ($p<0.01$).

Table 2 - Temporal changes of cross-sectional areas of total midshaft penis, corpora cavernosa, smooth muscle as a percentage of corpora cavernosa area, and nNOS as a percentage of dorsal nerves area in diabetic compared with age-matched control rats.

Time Point	Group	n	Area of total penis (mm^2)	Area of Corpora Cavernosa (mm^2)	%SMA (%)	%nNOS (%)
3 weeks	Control	4	5.37±0.26	3.93±0.21	10.6±1.4	3.7±0.6
	Diabetic	6	4.76±0.15	3.50±0.12	8.1±0.8	3.7±0.9
9 weeks	Control	5	5.55±0.26	3.89±0.20	9.8±0.5	3.5±0.6
	Diabetic	6	4.31±0.19*	3.17±0.16*	6.6±0.5*	2.3±0.3

Values are expressed as mean plus or minus SEM of 4 to 6 individual rats. *significantly different from corresponding value in control group ($p<0.05$).

betic rats exhibited less α-smooth muscle actin immunoreactivity. The cross-sectional area of the smooth muscle within the corpora cavernosa as a percentage of the overall corpora cavernosal area was significantly lower in diabetic rats compared with age-matched control rats at 9 weeks, but not at 3 weeks after induction of diabetes (Table-2).

nNOS expression in dorsal nerves

nNOS immunoreactive staining was observed in the dorsal nerves of diabetic and age-matched control rats at both time points (Figure-3). The cross-sectional area of nNOS immunoreactivity as a percentage of dorsal nerve area was similar in diabetic and age-matched control rats at 3 weeks after induction of diabetes, but was decreased at 9 weeks in diabetic rats compared with controls, although the difference was not significant (Table-2).

DISCUSSION

Studies in experimental animal models and diabetic patients have indicated that the pathogenesis of diabetes-related ED is multifactorial, probably related to central and peripheral neuropathy, endothelial dysfunction, impaired vasodilatory signaling, cavernosal hypercontractility, veno-occlusive dysfunction, hypogonadism, oxidative stress, proinflammatory changes, and psychogenic factors (11, 12). The present study showed that diabetes can cause changes in penile morphology, corpora cavernosal smooth muscle, and dorsal nerve nNOS expression over time.

Our data showed that, while the penis length remained similar between diabetic and control rats, diabetes caused a progressive reduction in total penis weight, from a 14% reduction 3 weeks after STZ injection to a 27.4% reduction at 9 weeks. The reduced weight was due to the decreased main components of the erectile tissue.

The body of the penis from both human being and rat consists of the paired corpora cavernosa, which occupy most of the shaft, are separated by blood vessels and nerves located dorsolaterally, and a corpus spongiosus located ventrally that surrounds the urethra (13). In rats, there is a

Figure 2 - Representative cross-sectional, large field-of-view images of α-smooth muscle actin immunoreactive tissue (dark brown) in penile midshaft sections from 3-weeks (A, B) and 9-weeks (E, F) control rats, and 3-weeks (C, D) and 9-weeks (G, H) diabetic rats, at low (4x) magnification (A, C, E, G) and high (20x) magnification (B, D, F, H).

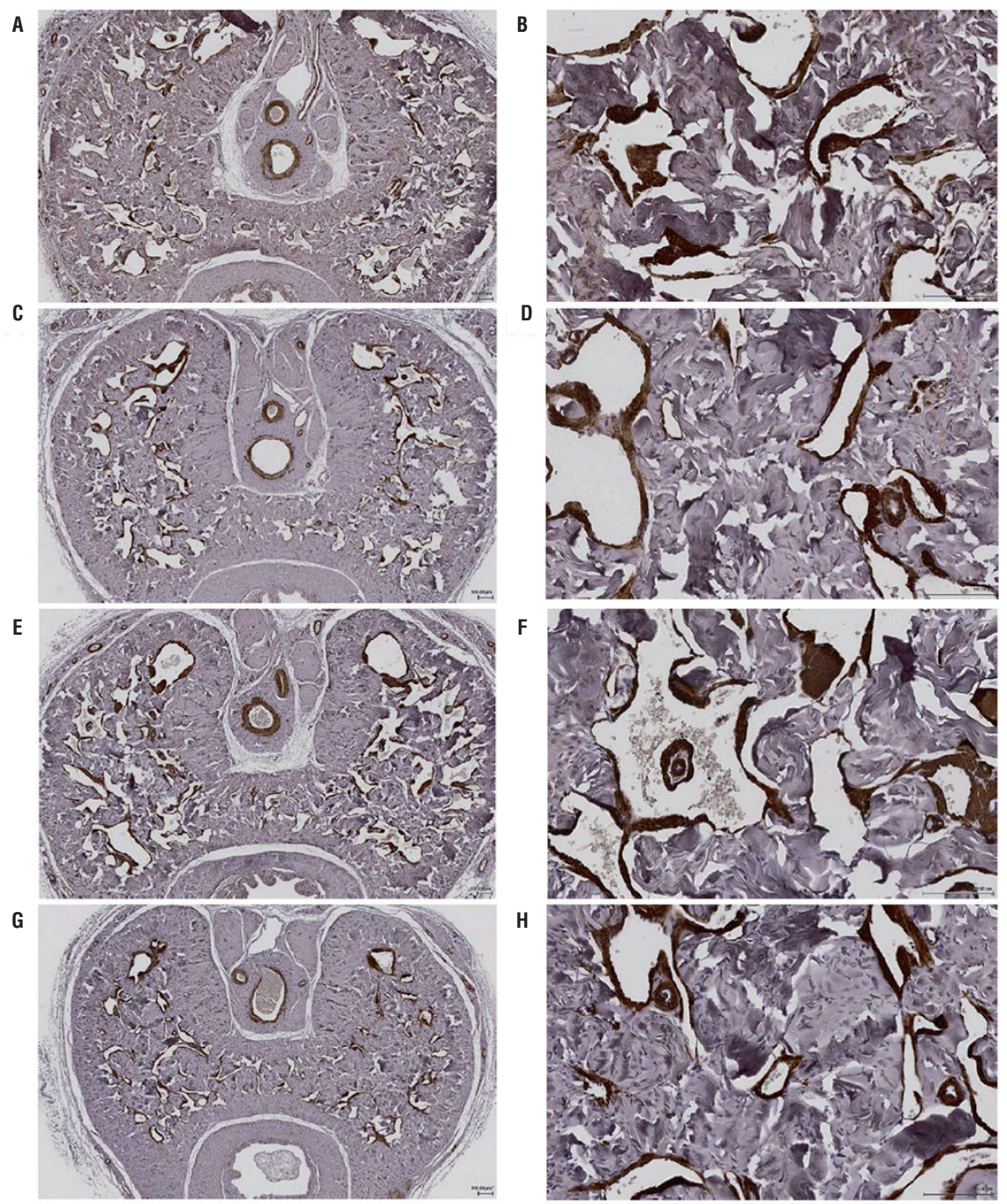
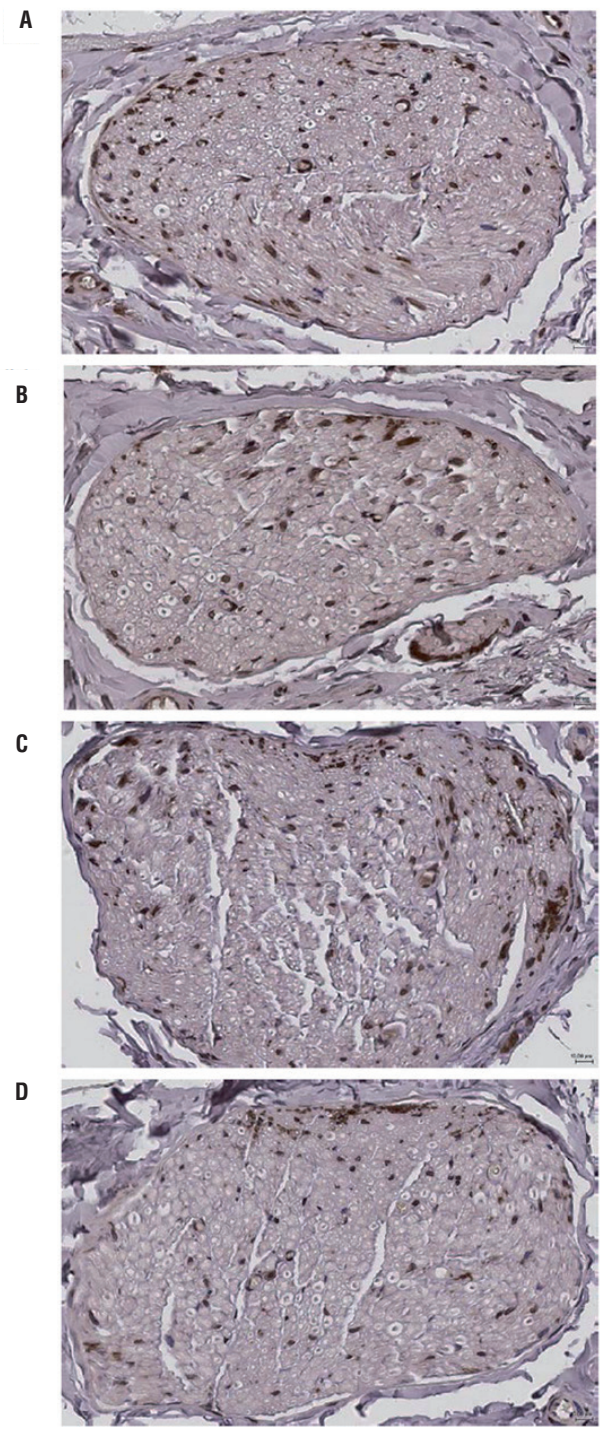


Figure 3 - Representative cross-sectional, large field-of-view images of nNOS immunoreactive dorsal nerves (dark brown) in penile midshaft sections from 3-weeks (A) and 9-weeks (C) control rats, and 3-weeks (B) and 9-weeks (D) diabetic rats.



Magnification 40x.

cylindrical bone that extends from the distal end of the penis body to the tip of the glans. However, it is known that the erection of the penis in mammals, including rats, mainly depends on the engorgement of cavernous spaces with blood and the relaxation of smooth muscle cells (14, 15). Therefore, the corporal smooth musculature plays an important role in the erectile process. Previous studies showed significant smooth muscle loss at 10 weeks (16), 12 weeks (17), 14 weeks (18), or 6 months (9) after diabetes induction. In 7 months old obese Zucker fa/fa rats, a type 2 diabetes mellitus model, Kovanecz et al. (19) found a considerable reduction in penile smooth muscle content. However, the time window from no obvious alterations to the significant morphological changes of the penis is not well investigated. Current study showed that the percentage of smooth muscle in the cross-sectional area of the corpora cavernosa was significantly lower at 9 weeks, but not at 3 weeks, indicating the significant loss of smooth muscle cells happened between 3 to 9 weeks after diabetes induction. The mechanisms of the loss of smooth muscle are not clear. The apoptosis may be one of the pathogenesis. Previous studies have shown significantly increased apoptosis in corporal components from type 1 and type 2 diabetic rat models (20, 21).

Nitric oxide (NO) serves as a principal neurotransmitter in the induction of smooth muscle relaxation (22). NO is generated by the catalytic conversion of L-arginine to L-citrulline by the enzyme nitric oxide synthase (NOS). The primary isoforms of NOS are neuronal (nNOS), endothelial (eNOS), and inducible (iNOS) nNOS and eNOS are constitutively expressed, while iNOS expression is induced by inflammatory stimuli and is associated with immune responses (23, 24). In the penis, eNOS typically is localized in the vascular and sinusoidal endothelium, whereas nNOS is mainly distributed in the non-adrenergic and non-cholinergic nitrergic nerve terminals (23, 24). We stained nNOS in the penile dorsal nerves, rather than in intracorporal tissue, because previous studies have shown that quantification of nNOS in the dorsal nerves is more reliable and is a suitable surrogate for corporal nNOS (25, 26). We found the percentage of nNOS immunoreactivity in the

cross-sectional area of the dorsal nerves was similar at 3 weeks and noticeably, but not significantly lower at 9 weeks in diabetic rats, indicating nNOS-enriched nerve fibers were not significant damaged by 9 weeks. However, previous reports have shown reduced nNOS levels in the penis in rats 12 weeks after STZ--induced diabetes (17, 27) and in 26-week old Zucker diabetic fatty rats (26). These results suggested the loss of nNOS and nNOS-enriched nerve fibers could have happened between 9 to 12 weeks. The reduction of nNOS may be caused by a deficiency in antegrade axonal transport of nNOS from the cell bodies, or by accumulation of advanced glycation end products that synergize with NO, leading to oxidative stress and apoptosis of nitrergic nerves (28).

The temporal diabetes-induced changes in the penis inevitably affect the erectile function. Uncovering the time course of these changes is important for the evaluations of drug treatment effects and prognosis. Our study showed that penile weight and morphological measurements, including the important erectile components corporal smooth muscle and nNOS, were not changed significantly at 3 weeks after induction of diabetes. Thus, the first 3 weeks of STZ-induced diabetes is an appropriate time window for observing the preventive effects of new treatments on diabetes-related penile changes. By 9 weeks of diabetes, the penile tissues had changed significantly, at which time the effects of new treatments on the reversibility of diabetes-related ED could be evaluated. Preventative treatments might be expected to have a higher probability of success than later treatments that require reversal of morphological changes. Cho et al. (29) found that phosphodiesterase type 5 inhibitors partially ameliorated ED in 10-week diabetic rats, but were much less effective at 12 and 14 weeks after induction of diabetes. Cellek et al. (30) demonstrated that nitrergic neurons innervating the penis and gastric pylorus lose some of their neuronal nitric oxide synthase content and function less than 8 weeks after diabetes induction, which is followed by nitrergic degeneration more than 12 weeks after diabetes induction. Insulin treatment before 8 weeks reversed the reduction of nitric oxide synthase, but the later nitrergic degeneration was irreversible.

The limitation of this study is that we did not investigate: 1) the erectile function of the rats at the different time points after diabetes induction; 2) if insulin treatment at 3 weeks after diabetes induction can fully prevent the structural changes of penile components. Further studies are needed to answer those questions.

CONCLUSIONS

Our results show that diabetes affects penile morphology and its components in a temporally progressive manner in STZ-induced diabetic rats, and the alterations are significant at 9 weeks after diabetes induction. Further studies on the reversibility of the observed temporal changes by insulin or new treatments are warranted.

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CONFLICT OF INTEREST

None declared.

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SURGICAL TECHNIQUE

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Use of the Uro Dyna-CT in endourology – the new frontier

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ABSTRACT

We describe the use of the Uro Dyna-CT, an imaging system used in the operating room that produces real-time three-dimensional (3D) imaging and cross-sectional image reconstructions similar to an intraoperative computerized tomography, during a percutaneous nephrolithotomy and a contralateral flexible ureteroscopy in a complete supine position. A 65 year-old female patient had an incomplete calyceal staghorn stone in the right kidney and a 10mm in the left one. The procedure was uneventful and the intraoperative use of the Uro Dyna-CT identified 2 residual stones that were not found by digital fluoroscopy and flexible nephroscopy at the end of surgery, helping us to render the patient stone-free in one procedure, which was confirmed by a postoperative CT scan. Prospective studies will define the real role of the Uro Dyna-CT for endourological procedures, but its use seems to be a very promising tool for improving stone free rates and decreasing auxiliary procedures, especially for complex cases.

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INTRODUCTION

Computerized tomography (CT) is the gold standard for evaluation of residual stones after any endourological procedure, but it is commonly performed after the surgery. The recent development of the Uro Dyna-CT (Siemens Healthcare Solutions, Erlangen, Germany) provides CT-like images in the endourological operating room, in addition to working as a digital fluoroscopy. The use of this new equipment in Urology was described by Ritter et cols. and is still very limited, but might have a major impact if it proves to increase the immediate stone free rates (1, 2). We have reported the first case of the use of the Uro Dyna-CT during a simultaneous percutaneous nephrolithotomy (PCNL) and contra-lateral flexible ureteroscopy (FURS) in a complete supine position.

SURGICAL TECHNIQUE

We evaluated a 65 year-old female patient, with recurrent urinary tract infections, bilateral lumbar pain for two years and a previous double J stent placement during a renal colic episode for a left proximal ureteral stone. Her body mass index was 36, she had mild hypertension and her ASA Score was 2. CT scan revealed an incomplete staghorn stone in the right kidney of low density (400 Hounsfield Units) (Guy's Stone Score III) (3) and a 10mm calyceal left kidney stone (380HU). A left ureteral stent was placed previously due to acute renal colic. A proposal of a single stage surgery with the use of the Uro Dyna-CT was done and the patient consented to the procedure. She had a 7-day treatment of 500mg bid ciprofloxacin before surgery and 1g of tranexamic acid at the beginning of the anesthesia.

We performed a right PCNL and left FURS in a complete supine position, as previously described (4) (Figure-1). Briefly, the patient was positioned with the head on the suspended part of the table, under general anesthesia. She was in a supine position, with no boosters under her back. The right side of her back was a few centimeters over the lateral border of the Table, her right leg was straight and her left leg was slightly bent. The surgical table had no option for lithotomy position, but our group is used to performing surgeries in a complete supine position.

The first step was to make a 3D image for initial evaluation of the stones. Image acquisition was performed using an Artis Zeego (Siemens Healthcare Solutions, Erlangen, Germany) with a standard 6s DCT Body protocol of the Uro Dyna-CT, which gives images of 0.5mm of each slice. We initiated the procedure with a rigid cystoscopy and right side insertion of a ureteral catheter 6Fr. Then, we removed the previous double J stent on the left side and inserted a hydrophilic guidewire inside it. The surgeon was standing all the time on the right side of the patient. A 35cm 12Fr ureteral sheath was inserted and the FURS was performed with a 270nm laser fiber set at 12Hz and 0.5J. Fragments were removed with a tipless nitinol basket and a new double J stent was inserted. At this point, another scan with the Uro Dyna-CT was done, to check the stone status on the left. A 20% diluted contrast agent was used and was still present inside the collector system. Then, with the patient in the same position, a right kidney puncture was done on the previously demarked area on the patient flank, guided by ultrasound. A 30Fr Amplatz sheath, a 26Fr rigid nephroscope and an ultrasonic device were used for treating the stone (Lithoclast Master, EMS, Swiss). After the removal of all fragments, a high resolution fluoroscopy and a flexible nephroscopy were performed and showed stone--free status. A 3D image with the Uro Dyna-CT showed the presence of 4 and 2mm residual stones that were found and removed with the rigid nephroscope, achieving the final stone-free status (Figure-2). Another 3D image was made for final stone-free status checking. A double J stent was retrogradely inserted at the right side and the procedure was tubeless.

Total surgical time, from the beginning of the cystoscopy until the removal of the Amplatz sheath was 175 minutes. The hemoglobin drop was 1.6g/dL and the patient was discharged on POD1. A CT scan at the POD2 confirmed no residual fragments on both sides (Figure-3). Stone analysis revealed a pure uric acid composition. The total radiation dose for this initial case was 1912.8mGy (541.9 for preoperative CT, 83.5 for digital fluoroscopy, 745.5 for five 3D images, and 541.9mGy for the final post operative CT).

COMMENTS

The use of Uro Dyna CT works like an intraoperative CT scan, in addition to producing fluoroscopic images. It gives 0.5mm cross-sectional images and has a high correlation with the real stone size (5). Its use in Urology has been described recently and its indication is not established yet. In our case, it correctly identified two residual stones that were not visualized by a high resolution fluoroscopy and the flexible nephroscopy, helping us to render the patient stone-free in one procedure. This result was very exciting and gives us the expectation that the routine use of the Uro Dyna-CT might improve the outcomes of endourological procedures. Particularly in this case, the stone was radiolucent, and potential residual fragments were very difficult to see. The residual stones images were not clearly seen on the screen as a normal CT and the presence of the guidewire causes some artifacts. The total dose of radiation certainly can be reduced, when we define the moment of making the acquisitions and the best customized protocol. Besides that, if the accuracy of the Uro Dyna-CT for residual stones proves to be similar to a CT scan, we can preclude this postoperative image, reducing the radiation exposure. We opted for not using the Uro Dyna-CT for puncture, because it was an easy puncture and its use could increase the radiation dose unnecessarily.

The procedure was performed in a complete supine position with no difficulties, perhaps because our group has a large experience operating in this position; actually, it was very comfortable for the surgeon and the assistant, because the screens were in the front of the team and no neck twisting was necessary. For those who are not used to this position, it can cause some diffi-

Figure 1 - A) Lateral view of the patient in complete supine position; B) Frontal view of the patient in complete supine position; C) Surgical team at the right side of patient performing flexible ureteroscopy and laser lithotripsy.

Figure 2 - A) Intraoperative image acquisition by Artis Zeego – Uro Dyna-CT; B) Intraoperative CT scan showing one residual stone (arrow); C) intraoperative CT showing a stone free right kidney.

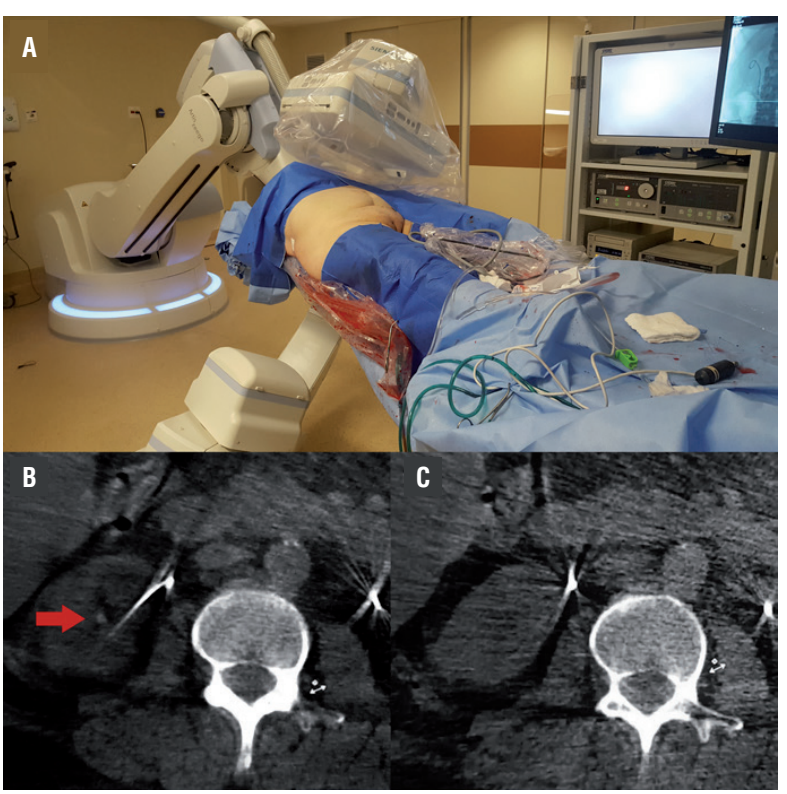
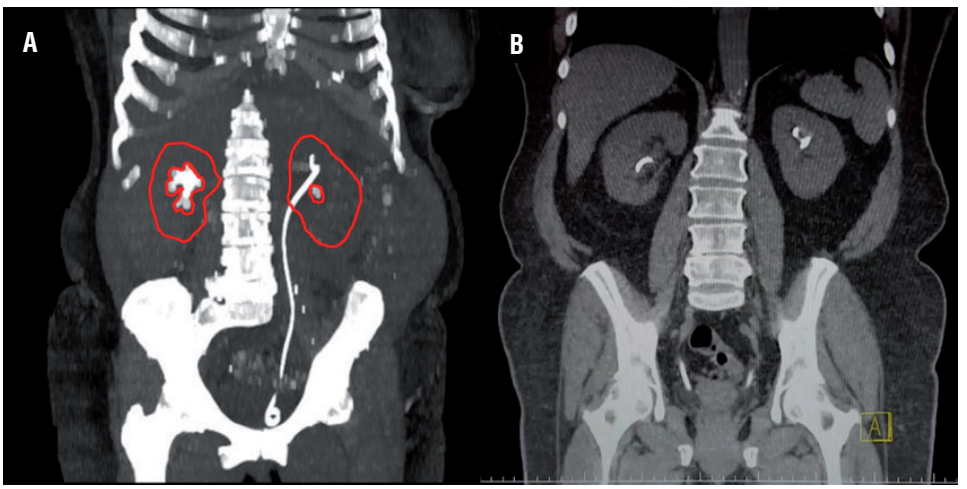


Figure 3 - A) preoperative CT scan showing an incomplete staghorn stone in the right kidney of low density and a 10mm calyceal left kidney stone; B) postoperative CT scan showing the stone free status and bilateral ureteral stents.



culties in the initial cases. However, if a semi-rigid ureteroscopy is necessary, the table may not be suitable for that. Ritter et cols. described the use of the Uro Dyna-CT for punctures for PCNL in prone position, but the results in terms of stone--free rates are still lacking (2).

Some questions need to be clarified for using this new technology:

1 - When should we use it? For all cases or just for complex ones?

2 - How many 3-D images do we need?

3 - Which is the best imaging protocol?

4 - What is the real accuracy for residual stones?

5 - Does it really improve the outcomes and reduce reoperations?

6 - Are the patients and staff exposed to more or less radiation?

7 - Is it cost-effective?

The Uro Dyna-CT in not available worldwide, but can be found in some institutions, mainly for angiographic studies. Prospective studies will verify the real role of the Uro Dyna-CT, but it's believed that it has a great potential of improving the immediate stone-free rates of complex endourological procedures, reducing the necessity of post operative CT scans and auxiliary procedures.

CONFLICT OF INTEREST

None declared.

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Vaginal evisceration related to genital prolapse in premenopausal woman

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ABSTRACT

Background: Vaginal evisceration is a rare problem, usually related to a previous hysterectomy. We report a case of spontaneous rupture of the cul-de-sac in a premenopausal woman under treatment with glucocorticoids to treat Systemic Lupus Erythematosus (SLE), with uterine prolapse that occurred during evacuation.
Case Report: A 40-year-old woman with SLE, using glucocorticoids, with uterine prolapse grade 4 (POP-Q), awaiting surgery presented at the emergency room with vaginal bleeding after Valsalva during defaction. Uterine prolapse associated with vaginal evisceration was identified. Under vaginal examination, we confirmed the bowel viability and performed a vaginal hysterectomy and sacrospinous fixation.
Case hypothesis: This case draws attention to the extreme risk of untreated uterine prolapse, as well as the importance of multidisciplinary care of patients with vaginal prolapse and chronic diseases.

ARTICLE INFO

Keywords:
Prolapse; Vagina; Lupus Erythematosus, Systemic

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Promising future implications:

Uterine prolapse may be related to serious complications when associated with other chronic diseases.

Vaginal evisceration may occur in premenopausal women, unrelated to vaginal trauma.

Scenario

Vaginal evisceration is a rare condition, usually related to genital trauma. In the literature reviewed, we found no reference to this occurrence related to genital prolapse without direct genital trauma, as in this case (1-3) (Table-1).

Pelvic-organ prolapse is the downward descent of female pelvic organs, which can affect the bladder, uterus, vaginal cuff (post-hysterectomy), and the small or large bowel. Prolapse development is multifactorial; the most consistent risk factors are vaginal delivery, advancing age, increasing body-mass index, and connective-tissue abnormalities (4).

Systemic lupus erythematosus (SLE) is an autoimmune connective-tissue disorder with a wide range of clinical features, which predominantly affects women. SLE can affect the skin, joints, kidneys, central nervous system, serous fluid, blood and immune system. Although glucocorticoids are important in the treatment of SLE,

Table 1 - Prior reports of vaginal evisceration.

First Author/ Year	Clinical cases
Gheewala U, 2015	Transvaginal bowel evisceration in a patient with prolapse, associated with perineal trauma after a fall from a chair.
Matthews C, 2014	Four cases of vaginal cuff evisceration after hysterectomy
Austin JM, 2013	Postcoital vaginal rupture in a 23-year-old woman

they are related to a number of adverse effects, which vary according to time and dose. The effects on the skin and soft tissues are most prominent (5).

We describe the case of a 40-year-old woman with complete uterine prolapse and SLE, who presented with spontaneous rupture of the vagina during defecation.

Case Report

A 40 year-old-woman, under treatment for systemic lupus erythematosus for the past 5 years, taking high doses of glucocorticoids without adequate medical supervision, was referred to our department with complete uterine prolapse associated with 2 vaginal ulcers. She had a body-mass index of 31.2kg/m^2, 3 previous spontaneous vaginal deliveries (last one 3 years before this incident), hypertension under treatment, Cushingoid appearance, and no previous surgeries. After preoperative evaluation, her surgery was scheduled. Fifteen days before the date planned, she presented at the emergency room describing significant vaginal bleeding after Valsalva during defecation. She denied any history of constipation. An examination identified spontaneous rupture of vagina, with evisceration of small bowel loops. The bowel loop herniated through a hernia ring which measured about 5cm, located on the posterior vaginal wall (Figure-1). We immediately covered the bowel with warm sterile gauzes and started antibiotic therapy (Cyprofloxacine and Methronidazole). The patient was taken to the operating room. Initially, the small bowel was assessed vaginally and carefully inspected for areas of devascularization (Figure-2). We performed a manual reduction of the loops and closed the vaginal rupture using unabsorbable sutures (polypropylene).

Figure 1 - Preoperative.

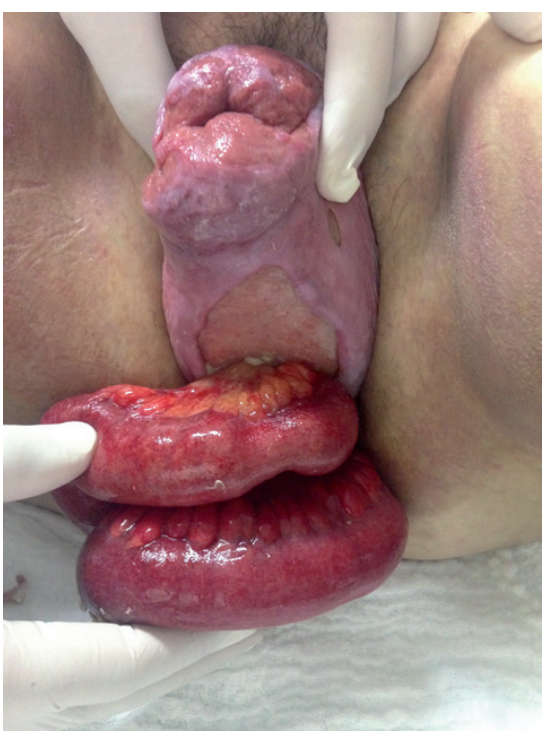
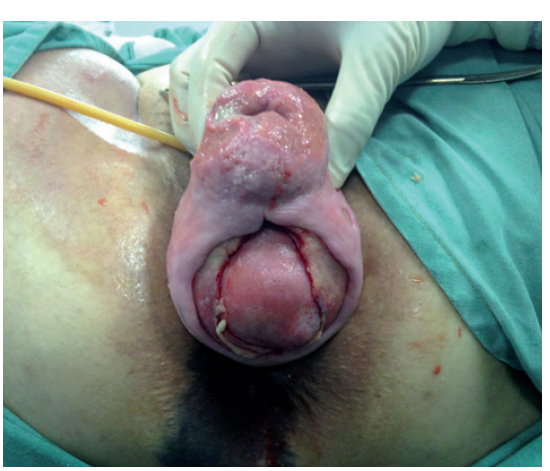


Figure 2 - Trans-operative.



At the same time, vaginal hysterectomy, anterior vaginal repair, posterior vaginal repair and sacrospinous fixation of the vaginal vault at the right sacrospinous ligament were performed. All procedures were performed through the vaginal route. During postoperative follow-up, the patient had good evolution, but she was only discharged seven days after the procedure as she has to compensate her SLE.

Discussion and future perspectives

This report describes an extremely rare but potentially catastrophic complication related to genital prolapse. We believe that lupus and irregular corticosteroid intake may be associated factors with the vaginal rupture.

Pelvic-organ prolapse is a benign disease, with no relationship to mortality. Most patients with pelvic-organ prolapse are asymptomatic. Seeing or feeling a bulge of tissue that protrudes to or past the vaginal opening is the most specific symptom. This condition may potentially affect millions of women worldwide. Currently, it is the most common non-cancer indication for hysterectomy in menopausal women in the United States (4).

Patients may present with complaints related to prolapse, including bladder, bowel, and pelvic symptoms; however, with the exception of vaginal bulging, none is specific to genital prolapse. Women with symptoms suggestive of prolapse should undergo a pelvic examination and review of medical history. When prolapse is symptomatic, options include observation, pessary use, and surgery (4).

Burge et al. studied the prevalence and pattern of mucosal involvement in 121 patients with lupus erythematosus. Twenty-one per cent of the patients with SLE had signs of mucosal involvement, which could contribute to vaginal weakness (6).

Glucocorticoids are estimated to be used long-term by 0.5–1% of the general population and up to 2.5% of older adults. Chronic use of corticosteroids is related to many side effects (7). Mean plasma estrone levels are significantly low in patients treated with a corticosteroid (8). We therefore hypothesized that the very low plasma estrone levels, which are secondary to pituitary suppression and consequent low androstenedione levels, may have contributed to the weakness of the vaginal mucosa in our patient.

All reports of vaginal evisceration are related to some direct trauma as the causal factor, such as coitus, obstetric, pessary use, or pelvic surgery (1-3). We hypothetized, based on our case, that evisceration can occur even without direct significant trauma, because other factors contribute to weakness of the mucosa.

Atrophy and menopausal status are considered risk factors for evisceration (1, 2). This patient was 40 years old and premenopausal, but she probably had clinically moderate atrophy secondary to corticosteroid use, which may have contributed to the evisceration.

As vaginal evisceration is a gynecological emergency, it is essential to treat this condition promptly, focusing on maintaining bowel viability and reducing the risk of infection though immediate surgical correction. When genital prolapse is present, concomitant management of it can be a good option, reducing the risk of recurrence of the evisceration.

This case illustrates the importance of effective team care to treat patients with chronic diseases. In a severe case like this, when an urgently schedule for surgical treatment is not possible due to clinical conditions, a pessary as intermediate intervention could have helped to prevent the evisceration. It also shows a possible association between SLE, chronic use of a glucocorticoid, and fragility of the vaginal mucosa, causing possible fatal complications in benign pathologies, such as uterine prolapse.

CONFLICT OF INTEREST

None declared.

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Ten cases with 46,XX testicular disorder of sex development: single center experience

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ABSTRACT

Objective: To present clinical, chromosomal and hormonal features of ten cases with SRY-positive 46,XX testicular disorder of sex development who were admitted to our infertility clinic.

Cases and Methods: Records of the cases who were admitted to our infertility clinic between 2004 and 2015 were investigated. Ten 46,XX testicular disorder of sex development cases were detected. Clinical, hormonal and chromosomal assessments were analized.

Results: Mean age at diagnosis was 30.4, mean body height was 166.9cm. Hormonal data indicated that the patients had a higher FSH, LH levels, lower TT level and normal E2, PRL levels. Karyotype analysis of all patients confirmed 46,XX karyotype, and FISH analysis showed that SRY gene was positive and translocated to Xp. The AZFa, AZFb and AZFc regions were absent in 8 cases. In one case AZFb and AZFc incomplete deletion and normal AZFa region was present. In the other one all AZF regions were present.

Conclusion: Gonadal development disorders such as SRY-positive 46,XX testicular disorder of sex development can be diagnosed in infertility clinics during infertility work-up. Although these cases had no chance of bearing a child, they should be protected from negative effects of testosterone deficiency by replacement therapies.

ARTICLE INFO

Keywords:
Chromosome Aberrations; Infertility; 46, XX Testicular Disorders of Sex Development

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INTRODUCTION

Forty Six,XX testicular disorder of sex development (DSD) is a rare clinical condition with a reported incidence of 1:20.000 in newborn males (1). It was first described by De la Chapelle et al. in 1964 (2) By 1996, only 150 patients with classical 46,XX testicular DSD syndrome have been reported (3); however, more than 100 cases were described in the next ten years (4).

The sex-determining region Y gene (SRY) located in Y chromosome plays a major role in encoding a testis determining factor (TDF) (5, 6). About 90% of 46,XX testicular DSD have Y chromosomal material including the SRY gene, that is usually translocated to the distal tip of the short arm of X chromosome or autosomal chromosomes. About 10% of 46,XX testicular DSD cases are negative for SRY gene, which could carry different degrees of masculinization (4, 7).

Cases with SRY-positive 46,XX testicular DSD are usually diagnosed after puberty when present with hypogonadism, gynecomastia and infertility (1). Short stature and normal mental development are the other clinical characteristics of these patients (4).

In this retrospective study, we analized clinical, chromosomal and hormonal features of ten cases with SRY-positive 46,XX testicular DSD who were admitted to our infertility clinic.

CASES AND METHODS

Records of the cases who were admitted to our infertility clinic between 2004 and 2015 were investigated. Ten 46,XX testicular DSD cases were detected.

Medical/family history and detailed physical examination records, body mass indexes, presence of parenteral consanguinity, records of each testicular volume measured with Prader orchidometer, serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), prolactin (PRL), total testosterone (TT), semen analysis results, karyotype and molecular analysis results and Dual-energy X-ray absorptiometry (DEXA) reports, if present, were assessed retrospectively.

Samples of peripheral blood (3mL) for chromosomal analysis were collected into 0.3mL heparin containing injectors. Conventional method was used on the lymphocyte cultures for karyotype analysis. By G band staining technique 550 level bands were obtained and twenty metaphases were counted.

The probe mix containing SRY gene, Yp11.31 locus specific probe, labelled in red, and control probes for the X centromere (DXZ1), labelled in blue, and for chromosome Y (DYZ1, the heterochromatic block at Yq12), labelled in green which was used for FISH analysis was purchased from Cytocell (Oxford Gene Technology, UK). After the harvest of cultured blood samples and FISH slide preparation, probe mix was applied according to the manufacturer's instructions, and materials were examined by using the Nikon, ECLIPSE E1000 fluorescent microscope (Tokyo, Japan) and analized with the CytoVision software (CytoVision, AB Imaging, Germany).

Samples of peripheral blood for sequence analysis were collected into commercially available EDTA-treated tubes. DNA was isolated from peripheral blood samples drawn from control, and study groups using High Pure Polymerase chain reaction (PCR) Template Preparation Kit (Roche Applied Science). After amplifying AZF regions by using PCR mixture containing 10xPCR Buffer 5µL, 2.5mM dNTP 3µL, 25mM MgCl 3µL, 10pmol Primer-1 3µL, 10pmol Primer-2 3µL, Taq DNA polymerase 0.5µL, DNA 5µL and completed to total volume of 50µL by adding water. Primers are specific to AZFa (sy81p1, sy81p2, sy82p1, sy82p2, sy84p1, sy84p2), AZFb (sy127p1, sy127p2, sy142p1, sy142p2, sy164p1, sy164p2, rbm1p1, rbm1p2), and AZFc (sy254p1, sy254p2, sy255p1, sy255p2, sy277p1, sy277p2, cdyp1, cdyp2, bpy2p1, bpy2p2) regions and were used separately in mixture. After 40 cycles of PCR by using "94°C 40 seconds, 58°C 40 seconds, 72°C 45 seconds" program, final product was loaded to 2% agarose gel. After electrophoresis, gel was examined by using UV transilluminator for absence or presence, location and, size of bands.

The medical ethics committee of Erciyes University approved this study and informed consent was obtained from all patients.

RESULTS

In our ten cases, mean age at diagnosis was 30.4, mean body height was 166.9cm (lower than general population), mean body weight was 72.6kg, mean BMI was 26.02. Semen analysis showed azoospermia and average semen volume was 1.68mL. All cases had small testicular volumes. Mean testicular volume was 3.1mL for right testes and 2.5mL for left testes. Detailed general characteristics of the cases are presented in Table-1.

Two of the patients (patients 3, 10) had prior orchiopexy operation in their medical history and one of them (patient 3) had parental consanguinity. All patients had no family history for genetic disorders.

Four patients (patients 1, 5, 9, 10) had decreased axillary and pubic hair and one patient (patient 5) had gynecomastia Tanner stage III.

Hormonal data indicated that the patients had a higher FSH, LH levels, lower TT level and normal E2, PRL levels (Table-2).

Three patients had dual-energy x-ray absorptiometry (DEXA) assessment. First one

Table 1 - Clinical data and semen volume analysis.

Cases	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m2)	TV (R/L) (mL)	EV (mL)
1	26	170	63	21.8	6/5	2.4
2	31	167	72	25.8	2/2	2.5
3	28	169	71	24.9	5/1	1.6
4	30	170	72	24.9	2/2	1.1
5	39	161	74	28.5	3/3	2
6	40	168	81	28.7	5/4	2.5
7	30	162	66	25.1	4/3	3.2
8	28	165	68	25	1/1	0.2
9	24	163	66	24.8	1/1	0.1
10	28	174	93	30.7	2/3	1.2
Mean	30.4	166.9	72.6	26.02	3.1/2.5	1.68

BMI = Body Mass Index; **TV =** Testicular Volume; **EV =** Ejaculate Volume; **R =** Right; **L =** Left

Table 2 - Hormonal status of the patients.

Cases	FSH (mIU/mL)	LH (mIU/mL)	E2 (pg/mL)	TT (ng/dL)	PRL (ng/mL)
1	58.1	42.2	52.0	183.0	5.6
2	36.0	16.8	57.9	376.0	3.5
3	35.4	9.5	48.6	94.4	8.1
4	17.9	10.3	24.6	272.7	7.9
5	37.5	16.3	24.5	96.6	6.9
6	36.8	9.8	53.8	290.0	5.6
7	57.3	16.9	49.2	153.6	6.4
8	50.5	11.3	39.1	103.0	9.0
9	43.1	17.9	21.2	242.0	8.6
10	31.3	23.5	27.6	203.0	9.0
Mean and 95% CI	40.4 (31.5-49.2)	17.4 (10.4-24.4)	39.8 (29.7-49.9)	201.4 (134.0-268.7)	7.1 (5.8-8.3)

FSH = Follicle-stimulating Hormone (1.5 -12.4 mIU/mL); **LH =** Luteinizing Hormone (1.7-8.6 mIU/mL); **E2 =** Estradiole (25.8-60.7 pg/mL); **PRL =** Prolactin (4.0-15.2 ng/mL); **TT =** Total Testosterone (280-800 ng/dL); **95% CI =** Confidence interval of mean References interval of hormones (FSH, LH, E2, PRL, TT) are given in parentheses

(Patient 5) was assessed osteopenic for lumbar vertebraes and femoral neck; second one (Patient 8) was assessed osteopenic for lumbar vertebraes and third one (Patient 7) was assessed osteoporotic for lumbar vertebraes and osteopenic for femoral neck.

Initial karyotyping analysis of all patients were considered as 46,XX with some hesitation because of derivative X chromosomes observed in metaphase fields. After fluorescence in situ hybridization (FISH) analysis, it was revealed that all patients were SRY positive. Next step which was the determination of the presence or the deletion of AZFa, AZFb, and AZFc regions revealed that all these regions were deleted in eight patients, in one patient (patient 9) none of the regions were deleted and in one patient (patient 2) AZFb and AZFc regions were deleted and AZFa was present.

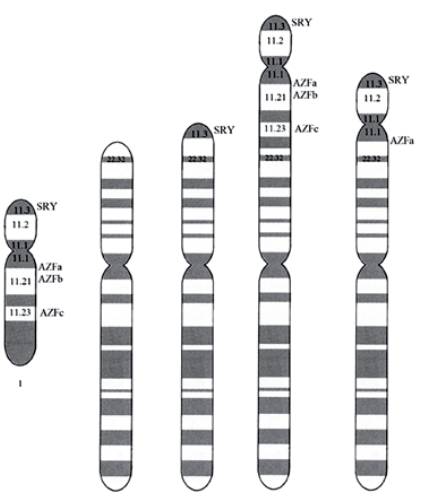
Final karyotype for patients was as 46,XX, final FISH report was written as 46,XX ish der (X) t(X;Y) (p22.3;p11.3) (SRY+) and AZF region deletion summarized in Table-3. Presumable Ideogram of the chromosomes is given in Figure-1.

Table 3 - AZF region analysis of the patients.

Patient	AZFa region	AZFb region	AZFc region
1	-	-	-
2	+	-	-
3	-	-	-
4	-	-	-
5	-	-	-
6	-	-	-
7	-	-	-
8	-	-	-
9	+	+	+
10	-	-	-

- deletion of gene region; + presence of gene region

Figure 1 - Presumable Ideogram of chromosomes.



1 - Normal Y Chromosome; **2 -** Normal X Chromosome; **3 -** Derivative X Chromosome of cases number 1, 3-8, and 10; **4 -** Derivative X Chromosome of case 2; **5 -** Derivative X Chromosome of case 9.

DISCUSSION

46,XX testicular DSD is a rare sex reversal syndrome characterized by a female karyotype in discordance with a male phenotype (4). Although 46,XX male DSD is frequently sporadic (8), familial cases have also been reported (9). All our patients were considered sporadic based on their family history.

46,XX testicular DSD patients can be classified into two groups according to presence or absence of SRY gene (10). Appearance of the external genitalia and masculinization are usually normal in 46,XX SRY-positive testicular DSD cases (4). Before puberty there is no clinical sign except for undescended testis and therefore 46,XX SRY-positive males are usually diagnosed in late adolescence or adulthood through chromosome analyses performed for infertility and/or small testis (11). Two patients had prior orchiopexy operation in their medical history, although, chromosomal analysis is not required for all patients with undescended testes. Possibility of chromosomal abnormality should be kept in mind for azoospermic patients who had prior orchiopexy history. Furthermore, it is a well-known fact that infertile men have 8 to 10 times more chromosomal anomalies than fertile men do, and many times, do not present with other phenotypic characteristics (12). Based on prevalence data, recommendation is made that routine karyotyping be requested of infertile men with deficient spermatogenesis and sperm concentrations lower than 10 million/mL before they are submitted to any assisted reproduction technique (13).

Vorona et al. stated that 46,XX men tend to be shorter than men with Klinefelter syndrome (7). In a previous study, Y chromosome growth-control gene had a possible impact on growth (14). Mean body height was relatively lower than normal population in our cases. Absence of specific growth genes in the Y chromosome may have some effects for this situation.

Classical 46,XX testicular DSD have normal testosterone level and free testosterone level during adolescence, but may decrease in adulthood, leading to hypergonadotropic hypogonadism (15). Testicular volumes are usually lower than 5mL in these cases. While testis morphology is normal in infancy, hyalinization of the seminiferous tubules in early childhood causes loss of spermatogonia (16, 17). Gunes et al. showed hyalinization of the seminiferous tubules by testicular biopsies of a patient with 46,XX testicular DSD (18). Low testicular volume and hypergonadotropic hypogonadism are constant findings in our patients. But testicular biopsies were not performed because these patients had no chance of bearing a child by assisted reproductive techniques.

Imaging of the pelvis is required to look for remnants of mullerian ducts that may cause morbidity in the form of repeated infections or hematuria and require surgical removal (19, 20). Differential diagnosis with other genetic conditions such as Persistent Mullerian Duct Syndrome in which virilization is achieved despite having low testosterone levels may be a challenging issue in some clinical presentations.

Recent epidemiologic studies have suggested that hypogonadal concentrations of TT are associated with an increased risk of fragility fracture (21, 22). Replacement therapies may protect these patients from bone fracture risk. Prior to initiating therapy, a baseline bone density scan should be performed to look for osteopenia or frank osteoporosis (23). Patients with a T score of <-1.0 would benefit from treatment with Vitamin D and calcium, bisphosphonates, or calcitonin, and require annual repeats of DEXA scan until results are normal (16). Our three patients had DEXA assay and all of them were assessed as osteopenic or osteoporotic. Bone mineral density measurements were absent in the records of our initial patients. We began to perform these measurements and apply replacement therapies as our clinical experience increased. Important part of individuals with SRY-positive 46,XX testicular DSD are diagnosed at adulthood in infertility clinics. But testosterone replacement should be given to patients which have clinical and/or laboratory signs of androgen deficiency in puberty.

During genetic counselling, it was advised to patients that this report must not affect their life and they must continue to live as before. While explaining inheritance it was stated that SRY-positive 46,XX testicular DSD is generally not inherited because of infertility of patients and de novo occurrence of Y and X chromosome translocation. But as there is always possibility of paternal balanced translocation or gonadal mosaicism, chromosome analyses were advised to father and brothers.

In conclusion, chromosomal abnormalities such as SRY-positive 46,XX testicular DSD can be diagnosed in infertility clinics during infertility work-up. Although these patients had no chance of bearing a child, they should be protected from negative effects of testosterone deficiency by replacement therapies.

CONFLICT OF INTEREST

None declared.

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RADIOLOGY PAGE

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Minimally Invasive Radiologic Uretero-calycostomy; a salvage procedure for late transplant rejection ureter necrosis

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Uretero-calycostomy is a time-honored procedure that has primarily been advocated for the management of failed pyeloplasties associated with long segment upper ureter strictures (1-3). We have expanded this concept to serve as salvage procedure in patients with necrosis of the transplant ureter as consequence of late rejection.

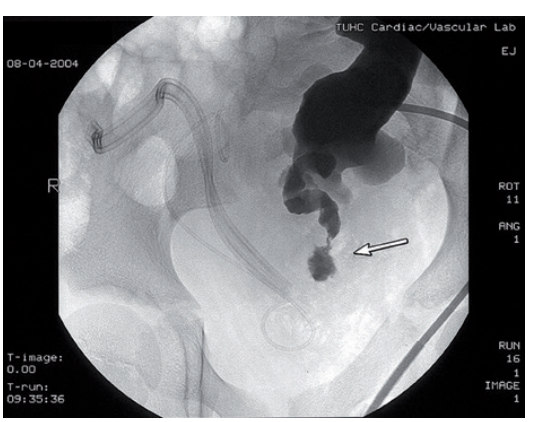
After satisfactory function of a cadaver transplant kidney for 4 years, this 36-year old female presented in the emergency room with evidence of rapidly progressing renal failure. Examination revealed 3+ edema of lower extremities, orthopnea, chest X ray bilateral pleural effusions, and laboratory findings: creatinine 14, BUN 52, K 4,8, urine output 240mL/qd, ultrasonogram showing hydronephrosis of the right transplant kidney.

A percutaneous antegrade nephro-ureterogram demonstrated hydronephrosis of the right transplant kidney and strictures as well as ulcerated segments of the right transplant ureter (Figure-1). Necrosis of the transplant ureter as sequel of late rejection was suggested.

Conservative management by percutaneous antegrade stent placement, anti-microbial therapy and corticosteroids failed to improve condition of the ureter (Figure-2). The uretero--neocystomy dehisced and a urinoma formed at the uretero-neocystostomy site.

Small bowel interposition to re-establish drainage or replacement of the transplant kidney by a new transplant were considered as remedial actions (4-6).

Figure 1- PA view: A percutaneous antegrade nephro-ureterogram demonstrates stenosis and ulcerations (arrow) in the dilated segment of the right transplant ureter. A double "J" stent is seen in the left transplant ureter, likewise an attempt to foster healing of this ulcerated ureter.

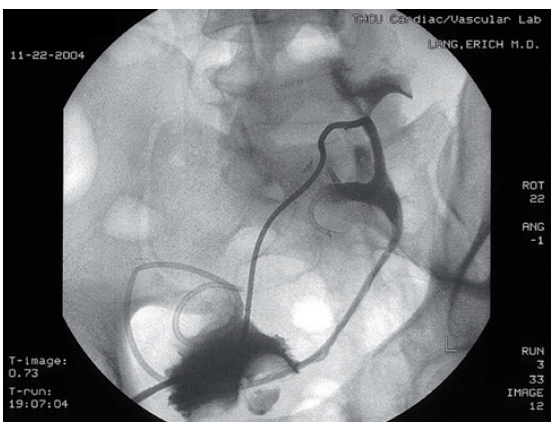


Avascular necrosis of ureter

Considering the dismal condition of the transplant ureter, we decided to modify the uretero-calycostomy procedure, by creating a fistula to the native right ureter, which was available since the native right kidney had been retained for erythropoetin production. Most importantly, the native ureter was not at risk of rejection.

A rigid ureteroscope was advanced under fluoroscopic guidance into the right native ureter, displacing the same toward the dilated superior hydrocalyx, which was then accessed by an 18 gauge needle advanced via the ureteroscope

Figure 2 - PA view: A stent has been seated via antegrade percutaneous approach from the transplant kidney pelvis into the bladder. The nephrostomy (arrow) is maintained to ensure ready access.



Double J stent seated and percutaneous nephrostomy seated

(Figure-3). A stiff Amplatz guide wire was then introduced into the kidney pelvis, over which a double "J" stent was placed, maintaining drainage from the kidney into the bladder (7) (Figure-4). The stent was maintained in position for 12 weeks. A solid fistula (uretero-calycostomy fistula) between calyx and native ureter resulted. Urine output of the transplant kidney stabilized; creatinine dropped to 2.6 in 4 weeks and remained stable at a level of 1.4 – 1.8 over the next 7 year follow-up, as did BUN at levels of 18 - 24.

Percutaneous radiologic antegrade uretero-neocalycostomy is recommended as a minimally invasive intervention that can manage the complex problem of ureter necrosis in otherwise well -functioning transplant kidneys.

Figure 3 - AP view: A rigid ureteroscope has been advanced in the native ureter under fluoroscopic guidance close to the dilated hydrocalyx of the right transplant kidney. The pelvis is accessed by needle-puncture through the ureteroscope and a guide wire introduced.

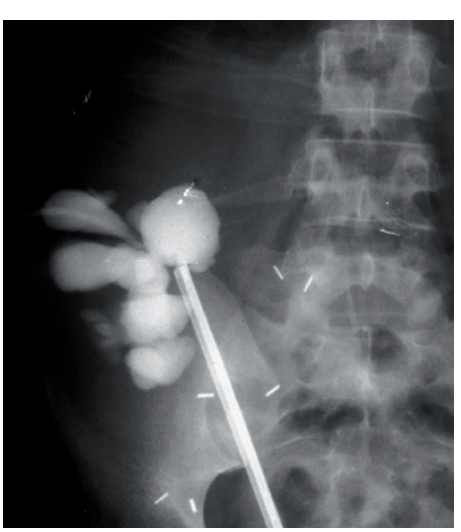
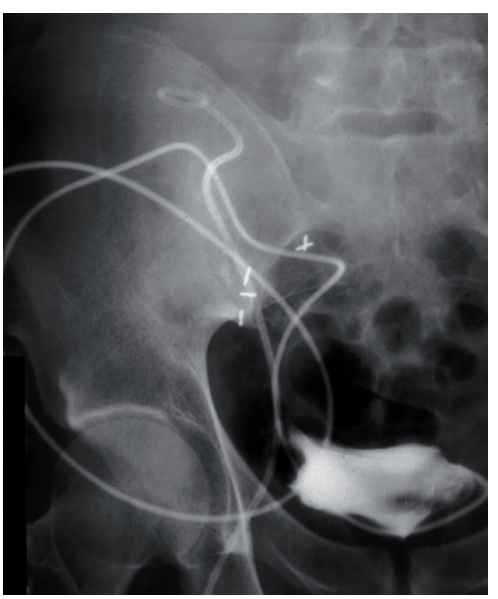


Figure 4 - AP view: A double "J" stent from transplant kidney pelvis to bladder has been seated over the guide wire in the native ureter. The nephrostomy has been maintained as safety measure to ensure ready access.



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VIDEO SECTION

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Feasibility of Robot – assisted Segmental Ureterectomy and Ureteroureterostomy in Patient with High Medical Comorbidity

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ABSTRACT

Introduction and objectives: Nephroureterectomy remains the gold standard treatment option for upper tract tumors. However, segmental ureterectomy may be another option in patients with single kidney, borderline renal function or high medical comorbidities. The aim of this video is to assess the feasibility of robotic surgery as a minimally invasive technique in treatment of a high comorbid patient with ureteric tumor.
Materials and Methods: Eighty-year old male patient, with a medical history of chronic hypertensive and uncontrolled Diabetes Mellitus, was referred to our department for treatment of ureteric tumor. Patient underwent robot-assisted radical prostatectomy 5 years ago. Patient's Charlson comorbidity index score was 9. Computed tomography showed a 2.5cm right ureteral luminal filling enhancing lesion at lower part of upper 1/3 ureter. We performed diagnostic flexible cystoscopy under local anesthesia to exclude associated lower urinary tract carcinoma, and bladder wash was negative for malignancy. Under general anesthesia patient underwent diagnostic flexible ureteroscopy to confirm mass location, and a retrograde pyelography to rule out additional tumors on the right collecting system. Then, the patient was placed in the full lateral flank position without Table flexion. Ports placement were inserted as follow: a "12mm" optical trocar at pararectal line superior and lateral to umbilicus, two "8mm" robotic trocars cranial and caudal to optical trocar (8cm distance), a "8mm" robotic trocar towards anterior superior ischial spine, and a "12mm" assistant trocar was inserted between umbilicus and pubic bone. The surgical steps are shown in the video.
Results: The procedure was performed easily. The total operative time and consol time were 100 and 60 minutes, respectively. Blood loss was 50ml. No reported intraoperative or postoperative complications. Notably, we took full precautions in case of intraoperative failure to complete the procedure successfully, nephroureterectomy was our second option. Postoperative serum creatinine was 1.2mg/dL and length of hospital stay was 2 days. The frozen biopsy showed that the tumor was resected with safe proximal and distal surgical margins. Final histopathology revealed high grade (G3) urothelial carcinoma (pT3), measures (1.3x1.2x0.2cm), associated with carcinoma in situ.
Conclusion: We affirm that robotic segmental ureterectomy and ureteroureterostomy could be offered safely as a minimally invasive treatment for patients with ureteric tumors and high-risk medical comorbidities. It provides excellent perioperative outcomes and early oncological safety with regard to surgical margins.

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

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Editorial Comment: Feasibility of Robot – assisted Segmental Ureterectomy and Ureteroureterostomy in Patient with High Medical Comorbidity

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In this video Mello et al. (1), demonstrate the case of a Robot assisted segmental ureterectomy for a patient with a solitary kidney with high medical co-morbidities that had a single localized ureteral lesion consistent with a urothelial neoplasm.

The surgical team carefully diagnosed and staged the ureteral tumor. A formal cystoscopy, bladder washing cytologies, retrograde pyelogram and ureteroscopy was performed to verify that this tumor was localized, single and that there were no other concomitant pathology such as carcinoma in situ of the upper collecting system.

Nephroureterectomy remains the gold standard for the surgical management of upper tract transitional cell carcinoma; However, recently, minimally invasive approaches including endourological (for non invasive disease) as well as laparoscopic or robotic assisted for segmental resections of the distal ureter with ureteral reimplantation has been reported with great oncological and clinical outcomes (for non invasive and invasive disease).

The authors clearly follow oncological principles of resection and anastomosis of clean ureteral margins. They demonstrate the importance of clipping the ureter above and below the tumor with a clear margin of resection. This will prevent spillage of neoplastic cells into the peritoneal cavity, preventing peritoneal carcinomatosis. In this case, the ureters were anastomosed without tension and the patient profitted from a less morbid approach to deal with a single ureteral tumor.

The final pathology revealed a pT3 high grade urothelial carcinoma with associated carcinoma in situ. The prognosis of these cases is poor, but the option of local control in a patient with high medical co-morbidities and a solitary kidney is clearly evident. Finally, we congratulate the authors for such a great demonstration of a Robotic assisted segmental resection of the ureter for a clinically localized ureteral tumor

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VIDEO SECTION

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Robot-assisted laparoscopic radical prostatectomy with early retrograde release of the neurovascular bundle and endopelvic fascia sparing

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ABSTRACT

Introduction: Robotic-assisted radical prostatectomy (RAP) is the dominant minimally invasive surgical treatment for patients with localized prostate cancer. The introduction of robotic assistance has the potential to improve surgical outcomes and reduce the steep learning curve associated with conventional laparoscopic radical prostatectomy. The purpose of this video is to demonstrate the early retrograde release of the neurovascular bundle without open the endopelvic fascia during RAP.
Materials and Methods: A 51-year old male, presenting histological diagnosis of prostate adenocarcinoma, Gleason 6 (3+3), in 4 cores of 12, with an initial PSA=3.41ng/dl and the digital rectal examination demonstrating a prostate with hardened nodule in the right lobe of the prostate base (clinical stage T2a). Surgical treatment with the robot-assisted technique was offered as initial therapeutic option and the critical technical point was the early retrograde release of the neurovascular bundle with endopelvic fascia preservation, during radical prostatectomy.
Results: The operative time was of 89 minutes, blood loss was 100ml. No drain was left in the peritoneal cavity. The patient was discharged within 24 hours. There were no intraoperative or immediate postoperative complications. The pathological evaluation revealed prostate adenocarcinoma, Gleason 6, with free surgical margins and seminal vesicles free of neoplastic involvement (pathologic stage T2a). At 3-month-follow-up, the patient lies with undetectable PSA, continent and potent.
Conclusion: This is a feasible technique combining the benefits of retrograde release of the neurovascular bundle, the preservation of the pubo-prostatic collar and the preservation of the antero-lateral cavernous nerves.

CONFLICT OF INTEREST

None declared.

ARTICLE INFO

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VIDEO SECTION

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Step-by-step Laparoscopic Vesiculectomy for Hemospermia

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ABSTRACT

Hemospermia has been considered as a benign and self-limiting condition. It usually has an inflammatory or infectious cause. However, recurrent or persistent hemospermia may indicate a more serious underlying pathology, especially over 40 years of age.

Biopsy or surgical excision is indicated in cases of suspicious findings during investigation, such as cysts or masses. Open surgery has been considered the definitive form of treatment, however, it can be associated with significant morbidity. With growing experience in laparoscopics, this approach is becoming the preferable way to access the seminal vesicles.

Our objective is to demonstrate a step-by-step operative technique for laparoscopic unilateral vesiculectomy approach in a man with hemospermia.

Case: A 61 year-old man presented with 1 year of hemospermia. He was treated empirically with a fluoroquinolone plus a nonsteroidal anti-inflammatory without resolution of symptoms. Ultrasonography and MRI showed a solid-cystic mass in the right seminal vesicle. The patient was submitted to a laparoscopic unilateral vesiculectomy. Histopathological analysis showed intraluminal dilatation with blood content. During follow-up, complete resolution of symptoms was seen.

Results: Three patients composed our cohort. Mean age was 53 years-old (range 45-61 years), the right side was more commonly affected (two unilateral on the right and bilateral). Mean operative time was 55 minutes (range 40-120min). One patient presented amyloidosis in the histopathological analysis. All cases presented complete resolution of symptoms.

Conclusions: Laparoscopic vesiculectomy is a safe and feasible approach in cases of hemospermia. This technique showed good outcomes and minimal morbidity.

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EDITORIAL
COMMENT

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Editorial Comment: Step-by-step Laparoscopic Vesiculectomy for Hemospermia

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In this video Mello et al. (1), the authors highlight the clinical merit of step-step laproscopic vesiculectomy for hemospermia. The authors adopt a robotic minimally invasive surgery to the realm of seminal vesiculectomy, which was first highlighted by Kavoussi et al. in 1993 (2). It depicts an easy step-by step approach and nicely demonstrates how to mange the vascular pedicle. The present video highlights that that this be accomplished to address an underlying clinical manifestation requiring surgical resection. In their series Mello et al. (1) pathological analysis showed amyloidosis, and transitional epithelium without atypia. With the advantage of combined 3D vision and wristed instrumentation, robotic excision of the seminal vesicles is feasible, safe and regarded as a natural continuity of conventional laparoscopy.

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LETTER TO THE EDITOR

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Image-guided percutaneous targeting of lymph nodes: a novel approach for salvage pelvic lymphadenectomy in recurrent prostate cancer

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To the editor,

Recently, Torricelli et al. (1) published a video showing a step by step technique for salvage lymph node dissection after radical prostatectomy. With the development of novel imaging techniques, the identification of PCa patients with a clinical lymphonode (LN) relapse has become feasible. Salvage LN dissection (SLND) represents a treatment option for patients with prostate cancer relapse limited to the LN, with a potential beneficial impact of pelvic LN dissection on survival in these patients (2, 3). Usually a template extended SLND is performed, however the properly identification of the compromised LN is still a challenge and may be related to the treatment fail (3). We present a case of successful de novo SLND with image-guided percutaneous targeting LN using colloidal charcoal for recurrence detected by ^{68}Ga-PSMA PET/CT following RP and previous salvage lymphadenectomy.

Our patient is a 52-year old man with PCa diagnosed by transrectal ultrasound guided biopsy (Gleason 4+3 in 2/14 cores and 3+3 in 3 cores) with PSA: 4.58ng/dL and negative CT and bone scan who underwent retropubic radical prostatectomy and limited LN dissection [Pathology: PCa Gleason 8 (4+4) and 7 negative LN]. One month post-operatively the urinary continence and erectile function were recovered with PSA: 0.18ng/dL and 0.22ng/dL after 3 months. ^{68}Ga-PSMA PET/CT revealed positive LN in the right obturatory region. Open SLDN was performed displaying 7 free LN. One month post-operatively the PSA was still elevated (0.82ng/dL). A new ^{68}Ga-PSMA PET/CT revealed the same suspected LN with higher SUV (Figure-1). De novo bilateral robotic SLDN was performed after percutaneous CT-guided targeting of ^{68}Ga-PSMA PET/CT scan positive LN. The lesion was identified and 3mL of 4% solution of colloidal charcoal and lipiodol was injected into LN using a extraperitoneal lateral approach 20G needle (Figure-2). The rationale is to dilute a small amount of activated carbon into a thick substance to stabilize the material and prevent migration to adjacent structures, which may be an oil (such as ethiodinzed oil-lipiodol®) or a tissue adhesive (such as n-butyl-2-cyanoacrylate-histoacryl®) as we have preferred and recent data have been published (5). We found an inflammatory and stuck tissue around the blood vessels and ureter related with the two previous surgeries (Figure-3). In the right side an extended LN dissection was performed identifying the target LN previously tattoed close to the hypogastric artery distal to the umbilical artery. In the left side, a classic extended LN dissection was performed.

Our operative time was just under 3 hours with an estimated blood loss of 150mL. JP drain was maintained until discharge on postoperative day 2. There were no intraoperative or postoperative complications. The final pathology revealed 1 of 4 positive LN on the right side. Three months follow-up revealed PSA<0.04ng/dL.

Figure 1 - ^{68}Ga-PSMA PET/CT (fusion images) revealed an increased uptake in gallium Ga 68 (68Ga) - labeled PSMA (SUV=45.8) in suspected LN with 1.0cm in the right obturator fossa.

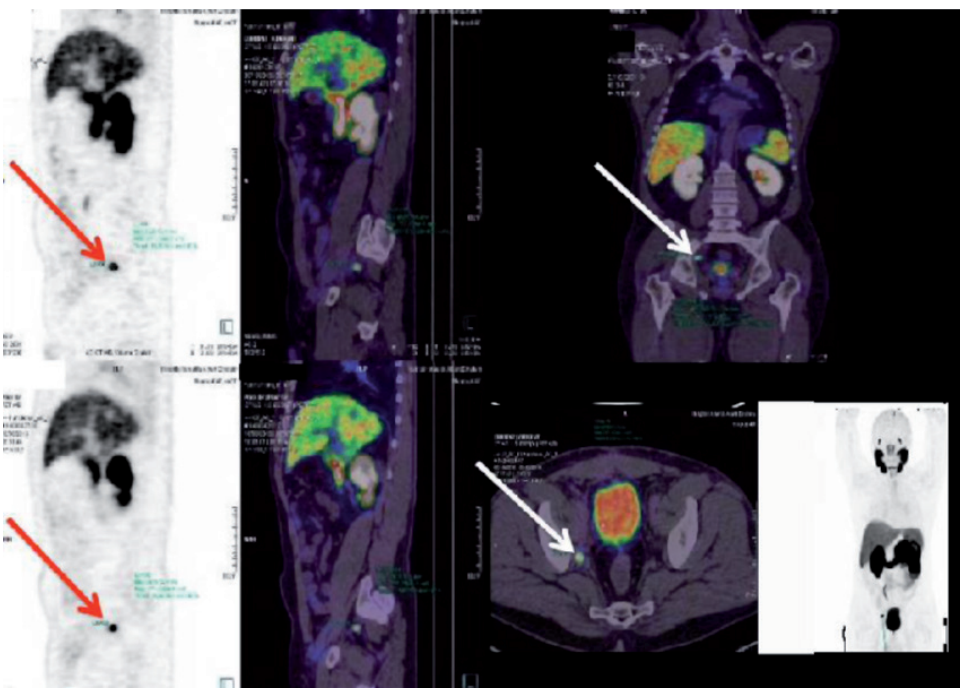

Figure 2 - CT-guided percutaneous puncture of the lymph node using a extraperitoneal lateral approach 20G needle and 3mL of solution of 4% colloidal charcoal and lipiodol was injected into the LN.

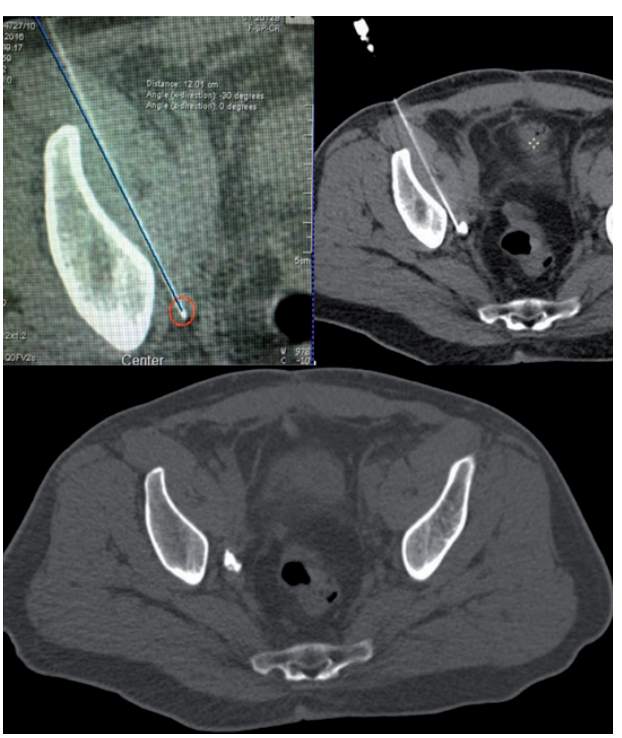

Figure 3 - Target LN previously tattoed close to the hypogastric artery distal to the umbilical artery.

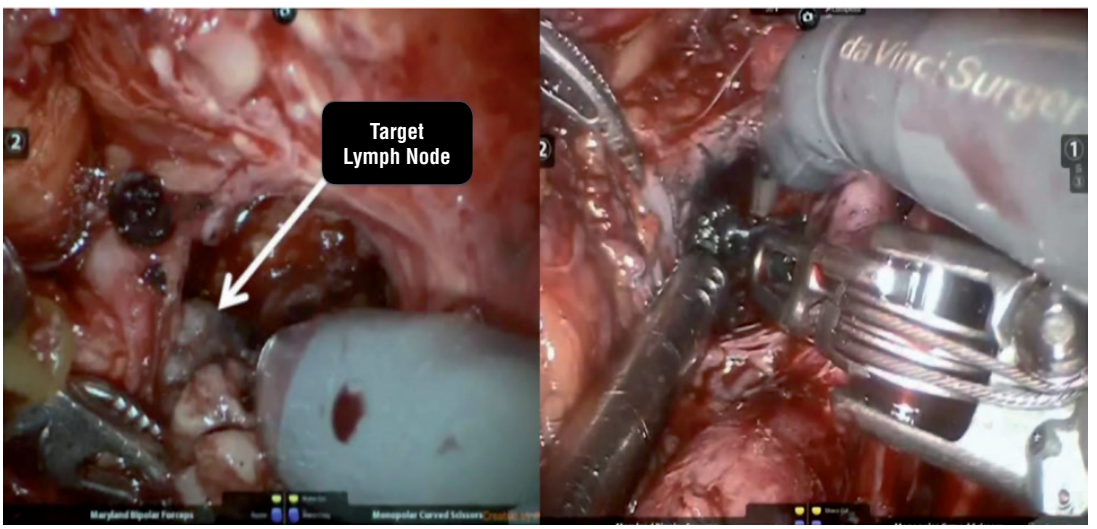

Image-guided percutaneous targeting of ^{68}Ga-PSMA PET scan positive LN is a safe, reasonable cost and useful technique in facilitating salvage pelvic lymphadenectomy for recurrence following radical prostatectomy, that may reduce cost by avoiding additional surgeries or radiotherapy. It helps to identify the target LN and may be related to the improvement of the outcomes in experienced hands.

CONFLICT OF INTEREST

None declared.

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LETTER TO THE EDITOR

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Nutcracker syndrome: how are we cracking the nuts and whose nuts are we cracking?

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To the editor,

The Nutcracker phenomenon refers to the compression of the left renal vein between the superior mesenteric artery and the aorta, and is often asymptomatic. It is not uncommon, and can be found in up to 10.9%-14% of asymptomatic adults (1, 2) and 33% of children with hematuria (3). The Nutcracker syndrome (NCS) comprises symptoms and findings such as varicocele, ovarian vein syndrome, hematuria, proteinuria and flank pain.

Computed tomography and magnetic resonance imaging can demonstrate the anatomic abnormality, and Doppler ultrasonography can help to measure pressure gradient and diameter differences between the left renal vein at the hilum and at the aortomesenteric level. Phlebography might be of value when there are doubts (4). Treatments include clinical management in most cases, and weight gain might be of benefit (5). Nephrectomy, reno-caval re-implantation or shunts (open, laparoscopic or robotic), external stents (open, laparoscopic or robotic) and endovascular venous stents have also been reported (4, 6, 7). Since surgical alternatives are invasive and outcomes are not outstanding (8), endovascular stents have emerged with the appeal of a less invasive procedure. However, some statements have to be reinforced.

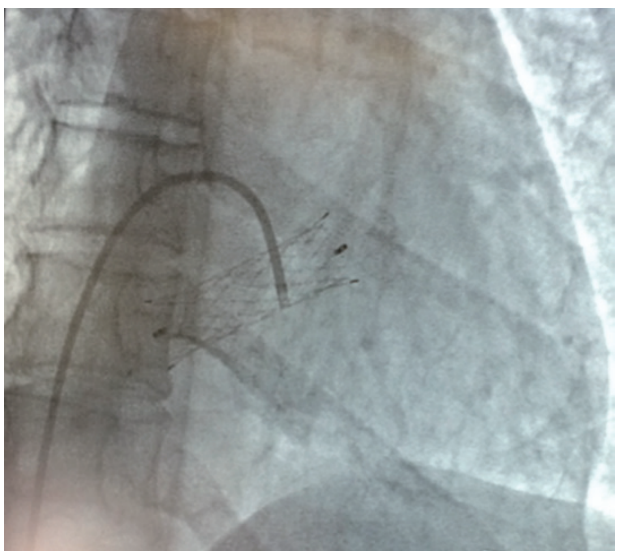
First, as procedures seems less invasive, there is an apparent increment in the number of indications. We have been seeing a significant increase in the number of cases of tomographic diagnosis of Nutcracker phenomenon. More than that, a significant number of patients have been treated with venous stents, even in cases of doubtful indications.

The Nutcracker syndrome is a benign and in most cases self-limited condition, that occurs almost commonly in young patients (resolve with time) (5). Treatment is reserved only for the severe cases.

Additionally, venous stents are currently poorly understood. Distinctively from the large acquired experience with arterial stenting, there is relatively little experience and no long term studies with venous stenting. A crucial difference between arterial and chronic venous disease is that the latter seldom poses a threat to life. The venous system might be particularly prone to some severe complications. Fibromuscular hyperplasia, which can lead to vascular occlusion, seems to be more common in veins than in arteries. Proximal embolization can occur, as had the experience with vena cava filter demonstrated (9).

Experience with inferior vena cava filters have demonstrated that there is a significant risk of long term complications including IVC thrombosis, perforation, penetration of adjacent viscera, device migration and deep vein thrombosis. These complications get more common according to dwell time (9). For these reason, temporary devices have been developed (9). Additionally, cur-

Figure 1 - Atrial migration of a venous stent rendered this 35-year-old woman with a severe valvar insufficiency.



rent stents are not ideally developed for the venous system (9). In the last years, an impressive increase in the number of complications of these procedures have been reported. We have reviewed the current literature and counted 816 cases of NCS reported. Of those, 354 were managed clinically (43%); 160 were managed through open, laparoscopic or robotic surgery (20%); and there are 224 reports of endovascular management of NCS (27%). From 2000 to 2005, 12 cases of stent placement for NCS were reported; from 2006 to 2010, 23 cases; and from 2011 to 2016, 189 cases have been reported. But impressively, there were also 21 reports of these venous stents used to treat NCS that have migrated, either immediately after placement or up to 12 months after surgery. Of the total of patients with NCS treated with stents reported in the literature, stent migration occurred in 9% of the cases. Stent migration is relatively common after endovascular stenting. In one of the largest series of endovascular stents, 75 young patients received venous stents for this benign condition. After a short term follow-up, stent migrated in five cases (7%). And unfortunately, there were not any anatomic or stent related factor that could predict this severe complication.

The authors of this study have concluded that venous stent migration in patients with NCS is much more common than believed (11). In other small series, migration occurred in 17%-20% of cases (8, 12). And almost all of these cases, migration occurred to the vena cava or to the heart, in many cases with serious complications requiring open heart surgery and even valvar replacement (11, 13, 14).

Moreover, long term venous stents patency is uncertain with stents. For this reason, experts in the field strongly recommend the maintenance of antiplatelet agents or anticoagulation following endovascular treatment of Nutcracker syndrome (15). But considering that we are treating young patients, mainly women with 20-40 years, future pregnancies can become risky (16) and lifetime anticoagulation can bring additional concerns.

It is very important to stress some points. Nutcracker syndrome is not always an easy diagnosis. Most patients should be treated conservatively because spontaneous remission does occur. It has been observed that 75% of patients younger than 18 years old will have complete resolution of hematuria within two years of presentation (4). Similarly, asymptomatic patients with incidental findings of Nutcracker phenomenom should be managed conservatively, as the natural history of such findings is not well delineated (1). Intervention shall only be indicated in severe lesions when there are disabling symptoms that do not respond to conservative management. When treatment is to be considered, care should be taken, and the best technique should be discussed with the patient or his/her parents. And venous stents don't seem to be a good alternative for this young population of patients with this generally benign disease. We have to take care about whose nuts are we cracking and how we are cracking those nuts.

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