kV image (anterior view) showing Lipiodol contrast in the tumor bed in the anterior and right lateral bladder walls. (Page 193)
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Bladder Cancer: What we can do against this powerful enemy?

The March-April 2014 issue of the International Braz J Urol presents original contributions with a lot papers in different fields. The papers come from many different countries such as Brazil, USA, China, Italy, Japan, Chile and Turkey, and as usual the editor’s comment highlights some papers. In this number we can observe some papers about bladder cancer. The bladder cancer represents a clinical challenge for the urologist and in this number we had papers about the clinical diagnosis, prognostic markers, radiation therapy and the natural history of the high-grade T1 bladder cancer. The high-grade T1 bladder cancer is a very dangerous disease and the time of indication of radical cystectomy is very important in this treatment. We summarize the papers about bladder cancer below.

Doctor Canter and colleagues, from the Departments of Urology, from the University from Pennsylvania, USA performed on page 172 an interesting study Using two non-muscle invasive bladder cancer (NMIBC) databases, the authors re-examined the rate of progression of HG T1 bladder cancer in your bladder cancer populations. A total of 222 patients were identified and the authors found that the risk of progression at approximately four years was only 8.6%. This study could potentially serve as a starting point in re-examining the treatment algorithm for patients with HG T1 bladder cancer.

Doctor Zhao and colleagues from the Qingdao University, Qingdao, China performed on page 179 a elegant study about prognostic markers in bladder cancer. The goal of this study was to utilize long-term patient follow-up to determine whether epithelial-to-mesenchymal transition (EMT)-related markers can predict bladder cancer patient survival and progression of disease. The authors make the analysis of 121 bladder cancer patients and revealed that the frequency of E-cadherin expression was
59.5% (72/121), Twist was 54.5% (66/121), and Vimentin was 24.8% (30/121). In this analysis, grade and Vimentin expression were found to be significant prognostic factors in predicting progression-free survival in bladder cancer.

Doctor Freilich and colleagues from the Moffitt Cancer Center, Florida, USA performed on page 190 a study about the evaluation of Lipiodol as a liquid, radio-opaque fiducial marker for image-guided radiation therapy (IGRT) for bladder cancer. They studied 5 clinical T2a-T3b N0 M0 stage II-III bladder cancer patients were treated with maximal transurethral resection of a bladder tumor (TURBT) and image-guided radiation therapy (IGRT) to 64.8 Gy in 36 fractions ± concurrent weekly cisplatin-based or gemcitabine chemotherapy and concluded that Lipiodol constitutes a safe and effective fiducial marker that an urologist can use to demarcate a tumor bed immediately following maximal TURBT. Lipiodol decreases inter-observer variability in the definition of the extent and location of a tumor bed on a treatment planning CT scan for a radiation boost.

Doctor Dobbs and colleagues from the Emory University School of Medicine, Atlanta, USA performed on page 198 a interesting clinical study about the incidence of lower urinary tract symptoms (LUTS) as the sole presenting symptom for bladder cancer. The authors evaluated the prevalence and clinical characteristics of newly diagnosed bladder cancer patients who presented with LUTS in the absence of gross or microscopic hematuria. They studied 340 patients and observed that 4.1% of bladder cancer patients in our series presented solely with LUTS. In this cohort a small percentage of patients with newly diagnosed bladder cancer present with LUTS without gross or microscopic
hematuria. Despite a higher incidence of CIS compared to patients with other presenting symptoms, the majority of patients with LUTS presented with Ta lesions. This study suggests that urologists should have a low threshold for evaluating patients with unexplained LUTS for underlying bladder cancer.

Luciano A. Favorito, MD, PhD

Associated Professor of Urogenital Research Unit – State University from Rio de Janeiro - UERJ
Early stage prostate cancer: biochemical recurrence after treatment


Assis Gurgacz Medical School (DAZ, LWM); Department of Oncology, Cancer Hospital of Cascavel, UOPECCAN (RJA); Brazilian Society of Urology (EFP), Rio de Janeiro, RJ; Department of Statistics, Federal Technological University of Parana, UTFPR (RABA), Toledo; Department of Radiotherapy (VCFIM) and Department of Medical Physics (GMDS), Cancer Hospital Cascavel - UOPECCAN, Cascavel, PR

ABSTRACT

Objectives: To identify retrospectively through chart analysis the biochemical recurrence frequency of localized prostate cancer at diagnosis of patients submitted to surgery or radiotherapy; to correlate diagnostic characteristics associated with higher risk of biochemical recurrence.

Materials and Methods: Retrospective analysis of 483 patients treated in a single center, from March 2000 to December 2009 in order to verify factors associated with biochemical recurrence.

Results: Biochemical recurrence was more frequent in patients with higher initial PSA levels and those with higher risk disease. Recurrence was more frequent in patients with high risk (25.9%) than those with intermediate risk (10.7%) and low risk (5.5%). There was no significant statistical difference of biochemical recurrence between patients submitted to radiotherapy or radical prostatectomy. Biochemical recurrence was diagnosed in only 11 of 73 patients (15%) submitted to conformal radiotherapy using tridimensional technique.

Conclusion: Radiotherapy and radical prostatectomy have similar treatment results. Tridimensional conformal radiotherapy used nowadays is more efficient than earlier forms of radiation therapy (cobalt therapy and bidimensional linear accelerator therapy).

INTRODUCTION

Prostate cancer is the second more prevalent malignant tumor in men (skin cancer other than melanoma being the first); in 2008 it was estimated 900,000 cases and 258,000 deaths due to the disease (1).

In 2012 it was estimated nearly 60,000 new patients with prostate cancer in Brazil: 62 new cases for every 100,000 men (2). It is the most prevalent malignant tumor in the Southeast region (78/100,000); in Midwest 75/100,000, in South 68/100,000, Northeast 43/100,000 and in North region 30/100,000 (2).

Treatment options for localized disease include radical prostatectomy and radiotherapy, with similar results (3).

Biochemical recurrence is characterized by PSA elevation following primary treatment. It usually precedes often for many years, clinical recurrence and progression of the disease.
MATERIAL AND METHODS

From March 2000 to December 2009, 819 patients with prostate cancer were treated at Cancer Hospital of Cascavel - UPECCAN. From these, 483 had non-metastatic disease at diagnosis and were treated with curative purposes (radiotherapy or radical prostatectomy) and were followed in an out-patient basis at the institution.

The present study is a retrospective analysis of the evolution of these 483 patients, in order to identify biochemical recurrence and related factors.

Radical prostatectomy (RP) was performed by a trained group of urologists and radiotherapy (RT) evolved along the years, being divided in three phases: first phase, from March 2000 to September, 2004, it was used cobalt radiotherapy (RTCo). From October 2004 to August 2008, patients were submitted to bidimensional linear accelerator radiotherapy (RT2D) and from September 2008 on it was used conformal tridimensional RT linear accelerator (RT3D).

Patients were divided in three risk groups concerning biochemical recurrence: low risk (PSA < 10ng/mL and Gleason ≤ 6), intermediate (PSA between 10 and 20 ng/mL or Gleason = 7) and high risk (PSA > 20ng/mL or Gleason 8 to 10). The first group (low risk) encompasses patients with very low risk and low risk cited in previous studies (4).

Biochemical recurrence was considered when it was observed a rise of PSA following PR in two different occasions (> 0.2ng/mL).

Biochemical recurrence in patients submitted to RT was considered when the PSA level exceeded 2ng/mL from the lowest post-treatment value (nadir).

Diagnostic characteristics (PSA, Gleason score, risk categories) and treatment modalities (RP or RT) were submitted to statistical analysis in order to identify the risk of biochemical recurrence using the chi-square or Fisher tests (when the sample was small) and the t-Student test. Significance level was 5% (p = 0.05).

RESULTS

Four hundred eighty-three patients were treated with localized disease at diagnosis. Age varied from 41 to 90 years (median = 68 years). Time between diagnosis and treatment varied from two to 621 days (median = 54 days). Table-1 shows the distribution according to Gleason score, serum PSA and risk stratification.

Table 1 - Patients characteristic before treatment.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total patients (%)</th>
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<tr>
<td>Gleason &lt; 7</td>
<td>353 (73)</td>
</tr>
<tr>
<td>Gleason = 7</td>
<td>100 (21)</td>
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<tr>
<td>Gleason 8-10</td>
<td>030 (6)</td>
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<tr>
<td>PSA &lt; 10</td>
<td>200 (41.4)</td>
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<td>PSA 10 - 20</td>
<td>155 (32.1)</td>
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<td>PSA &gt; 20</td>
<td>128 (26.5)</td>
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<tr>
<td>Low risk</td>
<td>155 (32.1)</td>
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<tr>
<td>Intermediate risk</td>
<td>184 (38.1)</td>
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<tr>
<td>High risk</td>
<td>144 (29.8)</td>
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PSA = Prostatic Specific Antigen

Table-2 shows the classification of patients according to risk factors and treatment modality.

PSA was higher in patients submitted to RT than those submitted to RP (p < 0.05). There was also a higher number of high risk patients among those submitted to RT (p < 0.05). Table-3 shows the risk factors and biochemical recurrence prior treatment.

Biochemical recurrence was identified in 180 patients (37.3%). It was more frequent in patients with higher PSA, higher Gleason and high risk stratification. It was observed in 60% of patients with Gleason score 8-10, 50% of those with Gleason 7 and in 31.7% of patients with Gleason ≤ 6 (p < 0.05).

Recurrence was more frequent in patients with PSA higher than 20ng/mL (61.2% of patients), when compared to those with PSA 10-20ng/mL (30.3%) and below 10ng/mL (27%)(p < 0.05). More recurrence episodes were also observed in patients with high risk (61.1%) than those
Early stage prostate cancer

with intermediate risk (31%) and low risk (22%) p < 0.05.

The differences between recurrence levels of different treatment modalities are shown on Table-4 and Figure-1.

There was no statistical difference (p = 0.25) in recurrence between patients submitted to RP (34.4%) or RT (39.8%).

Median time of follow-up of patients submitted to RP (n = 227) was 1427 days (54 to 3431 days). Time between surgery and biochemical recurrence (n = 78, 34.4%) varied from 53 to 2451 days (median 502 days, Figure-2). There were more recurrence episodes in patients with Gleason score 8–10, higher initial PSA and those of high risk group.

Recurrence was more frequent in patients with Gleason 8–10 (90% versus 48% and 26%, p < 0.05), PSA > 20ng/mL (68% versus 30 and 29%, p < 0.05) and in high risk group (72% vs. 31 and 28%, p < 0.05) - Table-5.

The primary treatment of 256 patients was RT; the first 33 patients were treated with cobalt radiotherapy; 147 patients were treated with RT2D and 73 patients received RT3D.

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**Table 2 - Risk factors and modality of treatment.**

<table>
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<th>Characteristics</th>
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<th>RP (n = 227)</th>
<th>RT (n = 256)</th>
<th>P value</th>
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<td>Gleason = 7</td>
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</tr>
<tr>
<td>Low Risk</td>
<td>155</td>
<td>097</td>
<td>058</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Intermediate risk</td>
<td>184</td>
<td>094</td>
<td>090</td>
<td>0.16</td>
</tr>
<tr>
<td>High risk</td>
<td>144</td>
<td>036</td>
<td>108</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

**PSA** = Prostatic Specific Antigen; **RP** = Radical Prostatectomy; **RT** = Radiotherapy

**Table 3 - Pre-treatment risk factors and biochemical recurrence.**

<table>
<thead>
<tr>
<th>Treatment modality</th>
<th>Total of patients</th>
<th>Total of recurrences(%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTR</td>
<td>227</td>
<td>078 (34.4)</td>
<td>0.25</td>
</tr>
<tr>
<td>RT</td>
<td>256</td>
<td>102 (39.8)</td>
<td></td>
</tr>
<tr>
<td>RTCo</td>
<td>033</td>
<td>017 (51.5)</td>
<td></td>
</tr>
<tr>
<td>RT2D</td>
<td>150</td>
<td>074 (49.3)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>RT3D</td>
<td>073</td>
<td>011 (15.1)</td>
<td></td>
</tr>
</tbody>
</table>

**RP** = Radical Prostatectomy; **RT** = Radiotherapy; **RTCo** = Cobalt Radiotherapy; **RT2D** = Conformal Bidimensional Radiotherapy; **RT3D** = Tridimensional Conformal Radiotherapy
Table 4 - Biochemical recurrence according to treatment.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total of patients</th>
<th>Biochemical recurrence(%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gleason &lt; 7</td>
<td>353</td>
<td>112 (31.7)</td>
<td></td>
</tr>
<tr>
<td>Gleason = 7</td>
<td>100</td>
<td>050 (50)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Gleason 8-10</td>
<td>030</td>
<td>018 (60)</td>
<td></td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td></td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>PSA &lt; 10</td>
<td>200</td>
<td>054 (27)</td>
<td></td>
</tr>
<tr>
<td>PSA 10 - 20</td>
<td>155</td>
<td>047 (30.3)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>PSA &gt; 20</td>
<td>128</td>
<td>079 (61.2)</td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td></td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Low risk</td>
<td>155</td>
<td>034 (21.9)</td>
<td></td>
</tr>
<tr>
<td>Intermediate</td>
<td>184</td>
<td>058 (31.5)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>High risk</td>
<td>144</td>
<td>088 (61.1)</td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td></td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

RP = Radical Prostatectomy; RT = Radiotherapy

Mantel-Haenzel test between RP and RT

$X^2 = 9.5$, freedom grade=1 and p-value= 0.0021*

Figure 1 - Disease-free survival curves (Kaplan-Meier estimates) for RP and RT  
Figure 2 - Disease-free-survival curve (Kaplan-Meier estimates) for RP.

Cx = Prostatectomy; RxT: Radiotherapy.
Follow-up varied from 115 to 3638 days (median 1296 days). Median follow-up of patients treated with cobalt radiotherapy was 1811 days and those submitted to RT2D was 1421 days. As expected, median of follow-up of patients treated with RT3D was lower (1026 days).

There were 102 recurrences after RT (39.8%, Figure-3). Time between treatment and recurrence varied from 350 to 2532 days (median 685 days). Recurrence was identified in 17 patients treated with RTCo (51.5%), in 74 with RT2D (50.8%) and in 11 with RT3D (15.1%). These differences are significant (p < 0.05), Table-4 and Figure-4.

Table-6 shows the risk factors and biochemical recurrence after radiotherapy. The stratification of patients according to PSA level showed more recurrences in patients with PSA ≥ 20ng/mL and intermediate and high risk patients.

More recurrences were identified in patients treated with cobalt radiotherapy (55%) and

---

**Table 5 - Biochemical recurrence after RP.**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total</th>
<th>Recurrence</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Yes (%)</td>
<td>No (%)</td>
</tr>
<tr>
<td>Gleason &lt; 7</td>
<td>163</td>
<td>043 (26.3)</td>
<td>120 (73.6)</td>
</tr>
<tr>
<td>Gleason = 7</td>
<td>54</td>
<td>026 (48.1)</td>
<td>028 (51.8)</td>
</tr>
<tr>
<td>Gleason 8-10</td>
<td>10</td>
<td>009 (90)</td>
<td>001 (10)</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>PSA &lt; 10</td>
<td>123</td>
<td>036 (29.2)</td>
<td>087 (70.7)</td>
</tr>
<tr>
<td>PSA 10 - 20</td>
<td>76</td>
<td>023 (30.2)</td>
<td>053 (69.7)</td>
</tr>
<tr>
<td>PSA &gt; 20</td>
<td>28</td>
<td>019 (67.8)</td>
<td>009 (32.1)</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Median PSA</td>
<td></td>
<td>10.36</td>
<td>9.00</td>
</tr>
<tr>
<td>Low risk</td>
<td>97</td>
<td>023 (23.7)</td>
<td>074 (76.3)</td>
</tr>
<tr>
<td>Intermediate risk</td>
<td>94</td>
<td>029 (30.8)</td>
<td>065 (69.1)</td>
</tr>
<tr>
<td>High risk</td>
<td>36</td>
<td>026 (72.2)</td>
<td>010 (27.7)</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

*PSA = Prostatic Specific Antigen; RP = Radical Prostatectomy*
Early stage prostate cancer

There was no influence of risk factors (Gleason score, PSA and risk stratification) in biochemical recurrence incidence of patients treated with cobalt radiotherapy. Among patients treated with RT2D, recurrence rate was higher in patients with PSA ≥ 20ng/mL (43/56 patients, 77%) and intermediate risk (22/54 patients, 41%) and high risk (45/63 patients, 71%).

Biochemical recurrence was identified in 11 of 73 patients submitted to RT3D (15%). Analysis of pre-treatments characteristics did not show any statistical difference among patients classified as high risk (25.9%), intermediate (10.7%) and low risk (5.5%).

DISCUSSION

Prostate cancer is frequent and responsible for cancer associated morbidity and mortality. When treated initially, RP of RT are curative (1).
Table 7 - Biochemical recurrence according to RT modality of treatment.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>RTCo patients/ recurrence</th>
<th>RT2D patients/ recurrence</th>
<th>RT3D patients/ recurrence</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total of patients</td>
<td>33/17</td>
<td>150/74</td>
<td>73/11</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Gleason &lt;7</td>
<td>026/015</td>
<td>104/046</td>
<td>060/008</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Gleason =7</td>
<td>004/001</td>
<td>033/020</td>
<td>009/003</td>
<td>0.18 0.26</td>
</tr>
<tr>
<td>Gleason 8-10</td>
<td>003/001</td>
<td>013/008</td>
<td>004/00</td>
<td>0.08</td>
</tr>
<tr>
<td>p value</td>
<td>0.4 0.17 0.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSA &lt; 10</td>
<td>008/003</td>
<td>049/014</td>
<td>020/001</td>
<td>0.06</td>
</tr>
<tr>
<td>PSA 10-20</td>
<td>008/004</td>
<td>045/017</td>
<td>026/003</td>
<td>&lt; 0.05 0.48</td>
</tr>
<tr>
<td>PSA &gt; 20</td>
<td>017/010</td>
<td>056/043</td>
<td>027/007</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>p value</td>
<td>0.61 &lt; 0.05 0.11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median PSA</td>
<td>23.0 21.2 28.5 --</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low risk</td>
<td>007/003</td>
<td>033/007</td>
<td>018/001</td>
<td>0.09</td>
</tr>
<tr>
<td>Intermediate risk</td>
<td>008/004</td>
<td>054/022</td>
<td>028/003</td>
<td>&lt; 0.05 0.53</td>
</tr>
<tr>
<td>High risk</td>
<td>018/010</td>
<td>063/045</td>
<td>027/007</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>p value</td>
<td>0.84 &lt; 0.05 0.14</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PSA = Prostatic Specific Antigen; RTCo = Cobalt radiotherapy; RT2D = Bidimensional conformal radiotherapy; RT3D = Tridimensional conformal radiotherapy

Sustained elevation of PSA in any moment after treatment is related to the existence of viable prostatic tissue anywhere. Biochemical recurrence precedes the beginning of clinical disease in the majority of cases (5-7).

The concept of biochemical recurrence is not consensual in literature and varies according to the primary treatment (RP or RT) (8-10).

A widely accepted definition of biochemical recurrence after surgery is of a serum PSA greater than 0.2ng/mL in two different consecutive samples after treatment (11-13). Biochemical recurrence following radiotherapy is defined at present when serum PSA is equal or greater than 2ng/mL above the original lower level of PSA (nadir) following radiotherapy (14).

The present study analyzed a great number of patients with localized prostate cancer submitted to RP or RT in a single institution.

The criteria of biochemical recurrence after RP was PSA > 0.2. Although many authors consider PSA > 0.4, PSA > 0.2 is widely used in literature and probably more suitable due to more precise and sensitive detection methods (8-12).

Results showed that prognostic factors at diagnosis, PSA level and high risk stratification were associated with higher level of biochemical recurrence. These results are in accordance to literature (15-21).

Biochemical recurrence following RP was more frequent in patients with Gleason score 8-10, higher PSA level and those considered of high
risk, also in accordance to literature (5,7,22-25).

Three different radiotherapy techniques were used throughout the study: RTCo, RT2D and RT3D. The three groups of patients were compared and there were a lower number of recurrences in patients submitted to RT3D. The use of RT3D allows the use of higher doses associated to better therapeutic results (26-28).

Biochemical recurrence was identified in only 15% of patients submitted to RT3D. The low number of recurrences in this group of patients probably did not allow the identification of a relationship between recurrence level and associated risk factors.

The lower level of recurrence in patients submitted to RT3D is not explained by different pre-treatment characteristics among patients. They were uniformly distributed among the three modalities of radiotherapy. However, it is important to observe that the time of follow-up was lower in those patients than those treated with cobalt or RD2T radiotherapy; eventually during a longer follow-up more recurrence episodes will be identified.

The comparison of RT and RP did not show any statistical difference regarding biochemical recurrence. Available data in literature cannot conclude that one treatment is better than the other in any risk group of disease (3).

**CONCLUSIONS**

RT and RP have similar results. Today RT is more efficient than those previously used. Risk factors and treatment results are in accordance to literature data.

**CONFLICT OF INTEREST**

None declared.

**REFERENCES**


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Cascavel, PR, 85806-095, Brazil
Telephone: + 55 45 3321-3900
Email: daniellezanatta@gmail.com
Biochemical recurrence rates are similar for pT2-positive surgical margins and pT3a

Katia R. M. Leite, Carolina Hartmann, Sabrina T. Reis, Nayara Viana, Marcos F. Dall’Oglio, Alexandre C. Sant’Anna, Adriano Nesrallah, Luciano Nesrallah, Alberto A. Antunes, Luiz H. Camara-Lopes, Miguel Srougi

Laboratory of Medical Research, Urology - LIM 55, University of Sao Paulo Medical School (KRML, STR, NV, MFD, ACS, AN, AAA, MS) and Genoa Biotechnology (KRML, CH, LN, LHCL), Sao Paulo, Brazil

ABSTRACT

Objective: Histological details of positive surgical margins in radical prostatectomy specimens have been related to outcome after surgery in rare studies recently published. Our objective is to assess whether the status of surgical margins, the extent and the Gleason score of positive margins, and the extent of the extraprostatic extension are predictive of biochemical recurrence post-radical prostatectomy.

Materials and Methods: Three hundred sixty-five radical prostatectomy specimens were analyzed. The length of the positive surgical margin and extraprostatic extension and the Gleason score of the margin were recorded. Statistical analyses examined the predictive value of these variables for biochemical recurrence.

Results: 236 patients were stage pT2R0, 58 pT2R1, 25 pT3R0 and 46 pT3R1. Biochemical recurrence occurred in 11%, 31%, 20% and 45.7% of pT2R0, pT2R1, pT3R0 and pT3R1, respectively. The extent of the positive surgical margins and the Gleason score of the positive surgical margins were not associated with biochemical recurrence in univariate analysis in a mean follow up period of 35.9 months. In multivariate analyses, only the status of the surgical margins and the global Gleason score were associated with biochemical recurrence, with a risk of recurrence of 3.1 for positive surgical margins and of 3.8 for a Gleason score > 7.

Conclusion: Positive surgical margin and the global Gleason score are significant risk factors for biochemical recurrence post-radical prostatectomy, regardless of the extent of the surgical margin, the extent of the extraprostatic extension, or the local Gleason score of the positive surgical margin or extraprostatic tissue. pT2R1 disease behaves as pT3R0 and should be treated similarly.

INTRODUCTION

Between 10 and 40% of radical prostatectomy specimens will have a positive surgical margin, and margin status has long been predictive of clinical and biochemical recurrence of prostate cancer. However, only 10% to 40% of patients with positive surgical margins experience a recurrence (1,2). These numbers indicate that up to 90% of men who receive adjuvant treatment are overtreated. Some histological markers within the surgical margins and extraprostatic extension have been considered predictors of tumor behavior. Additionally, the International Society of Urological Pathology has recently recommended reporting the length of positive surgical margins in millimeters since there are evidences that it could be important in defining tumor behavior after surgery.
Positive surgical margins in Prostate cancer (3). However, few studies have addressed its ability to provide additional prognostic information. The use of the positive surgical margins Gleason score was also recently proposed to improve the accuracy of predictions of radical prostatectomy (4).

Extraprostatic extension has been considered an important prognostic factor related to biochemical recurrence and studies demonstrate that adjuvant radiotherapy could improve the outcome of patients after surgery (5-7). There are very few studies evaluating the predictive power of the extraprostatic extension length or Gleason score as a tool for risk stratification and or as an accurate indicator for adjuvant therapy in this group of patients.

Our objective was to explore the importance of histological details as predictors of biochemical recurrence in men treated for prostate cancer by open radical prostatectomy. Factors considered included the length and Gleason score of positive surgical margins and the length in millimeters and the Gleason score of extraprostatic extension.

MATERIALS AND METHODS

This is a retrospective study that reviewed the histology slides of surgical specimens from 365 men who consecutively underwent open radical prostatectomy by the same surgeon (MS) between January 2004 and December 2006. The demographic, laboratory and histological data are represented in Table-1. The surgical specimens were fixed in 10% buffered formalin, and the entire surgical margin was stained with India ink. The bladder neck margin was collected as a thinly shaved section. The most distal portion (5–6mm) of the prostate’s apex was amputated and then sectioned parallel to the urethra at 2–3mm intervals. The prostate’s left and right lobes were separated, 3mm transverse serial sections were taken from each lobe, and the entire gland was submitted for histological examination. The Gleason score was used for histological grading. The tumor’s volume was measured as previously described (8). Briefly, a grid was placed beneath slides in which the area comprising the tumor had been previously outlined. The percentage of the tumor on a slide was determined by dividing the number of grid squares overlapping the tumor by the number of squares overlapping the entire tissue section. Tumor volume was defined as the mean percentage of tumor in the prostate gland. Extraprostatic involvement was defined as tumor infiltration into the adipose tissue, the neurovascular plexus, or the parenchyma of the seminal vesicles. The TNM 2010 system was used for tumor staging. Patients were classified as pT2 when the tumor was confined to the prostate and pT3 when an extraprostatic extension was apparent or if the seminal vesicles were infiltrated by tumor. Positive surgical margins were considered when tumor glands were inked with India ink (Figure-1A). The length of the positive surgical margins and the extraprostatic extension of each subject was measured using a micrometer ruler (Figure-1B), and the higher radial

| Table 1 - Demographic laboratory and histological characteristics of 365 patients submitted to radical prostatectomy to treat prostate cancer. |
|-----------------|-------------|------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Age (Years)     | Gleason Score | % Gleason 4 or 5 | Tumor Volume (%) | PO PSA (ng/mL) | Follow-up (months) | PSA in BR (ng/mL) |
| Mean            | 60.7         | 7                | 47.3            | 14.1           | 6.6              | 35.9            | 1.7             |
| SD              | 7.9          | 0.9              | 36.7            | 11.4           | 4.2              | 23.1            | 5.2             |
| Median          | 61           | 7                | 46              | 12             | 5.4              | 36.1            | 0.6             |
| Minimum         | 42           | 7                | 4               | 0.1            | 0.5              | 1.2             | 0.2             |
| Maximum         | 82           | 10               | 100             | 80             | 29               | 78.7            | 42              |

PO = pre-operative; BR = Biochemical recurrence
extent was considered when an extraprostatic extension was present. Taking the end of fibromuscular tissue of the prostate at the initial point the tumor was present at the adipose extraprostatic tissue was measured. When multifocal, the sum of all numbers was considered for statistical analysis. For evaluation of the Gleason score in subjects with both a positive surgical margin and an extraprostatic extension, the higher Gleason score was recorded. Unilateral or bilateral involvement of surgical margins and extraprostatic extension were also recorded for evaluation. The goal was to define which variables were predictive of biochemical recurrence. There was no adjuvant treatment before biochemical recurrence. Statistical analysis was performed with SPSS 19.0 software, using T test tests for normally distributed data and the Mann-Whitney U test for data that were not normally distributed. Kaplan-Meier curves were used to analyze biochemical recurrence risk. The logistic regression was used for multivariate analyses.

RESULTS

Two-hundred-and-ninety-four (80.5%) patients were staged pT2. Fifty eight (19.7%) had a positive surgical margin, with bilateral involvement in 8 (13.8%). The mean length of the positive surgical margins was 4.0 mm (± 3.9mm). The median length was 3.0mm (0.1 to 15.0mm). The mean Gleason score of the positive surgical margins was 7.1 (± 0.9), and the median was 7(6 to 9).

Seventy-one (19.5%) patients were staged pT3, and 46 (64.8%) had a positive surgical margin. In 25 (35.2%) patients, there was involvement of the seminal vesicles and were staged pT3b, and one (1.4%) patient presented with lymph node metastasis and was staged pT3bN1. The extraprostatic extension was bilateral in 13 (18.3%) patients. The mean extraprostatic extension length was 19.5mm (± 18.5mm), and the median was 13.8mm (2.4 to 78mm). The mean Gleason score of the extraprostatic extension was 8.2 (± 0.7), and the median was 8 (6 to 10). In 13 (18.3%) patients, the positive surgical margin was bilateral. The mean length of the positive surgical margin was 5mm (± 6.8mm), and the median was 3mm, ranging from 0.2 to 32mm. The mean Gleason score of the positive surgical margin was 8 (± 1.1), with a median of 8 (6 to 10).

During a mean follow-up of 35.9 months (± 23.1m), 68(18.6%) patients experienced recurrence, and their mean PSA levels were 1.7 ng/mL (± 5.2ng/mL) (Table-2). There was clinical recurrence in 8 (11.7%) patients, with five local recurrence and two lung and one bone metastases. The
Table 2 - Univariate analysis relating biochemical recurrence with clinical and pathologic characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Yes (68-18.6%)</th>
<th>No (297-81.4%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pT and margin status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pT2-R0 (236)</td>
<td>10.6%</td>
<td>89.4%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pT2-R1 (58)</td>
<td>31.0%</td>
<td>69.0%</td>
<td></td>
</tr>
<tr>
<td>pT3-R0 (25)</td>
<td>20.0%</td>
<td>80.0%</td>
<td></td>
</tr>
<tr>
<td>pT3-R1 (46)</td>
<td>43.5%</td>
<td>56.5%</td>
<td></td>
</tr>
<tr>
<td><strong>Age (years old)</strong></td>
<td></td>
<td></td>
<td>0.539</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>61.3 (7.7)</td>
<td>61.0 (8.0)</td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>63 (45-77)</td>
<td>60 (42-82)</td>
<td></td>
</tr>
<tr>
<td><strong>Pre operatory PSA (ng/mL)</strong></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>8.5 (5.6)</td>
<td>6.0 (3.7)</td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>6 (3-29)</td>
<td>5 (1-27)</td>
<td></td>
</tr>
<tr>
<td><strong>Tumor volume (%)</strong></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>18.8 (12.6)</td>
<td>13.0 (10.8)</td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>15 (1-55)</td>
<td>11 (0.1-80)</td>
<td></td>
</tr>
<tr>
<td><strong>Gleason score</strong></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>7.6 (0.9)</td>
<td>7.0 (0.8)</td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>8 (6-10)</td>
<td>7 (4-10)</td>
<td></td>
</tr>
<tr>
<td><strong>%Gleason 4 or 5</strong></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>73.7 (29.2)</td>
<td>41.0 (35.4)</td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>80 (0-100)</td>
<td>38 (0-100)</td>
<td></td>
</tr>
<tr>
<td><strong>Gleason at PSM mean</strong></td>
<td></td>
<td></td>
<td>0.296</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>8.0 (1.0)</td>
<td>8.0 (1.0)</td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>8 (6-10)</td>
<td>8 (6-10)</td>
<td></td>
</tr>
<tr>
<td><strong>Length of PSM (mm)</strong></td>
<td></td>
<td></td>
<td>0.713</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>5.0 (5.4)</td>
<td>6.0 (7.6)</td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>3 (0.2-19)</td>
<td>5 (1-32)</td>
<td></td>
</tr>
<tr>
<td><strong>PSM bilateral</strong></td>
<td></td>
<td></td>
<td>0.208</td>
</tr>
<tr>
<td>Yes (%)</td>
<td>23.8</td>
<td>38.7</td>
<td></td>
</tr>
<tr>
<td><strong>Length of EPE (mm)</strong></td>
<td></td>
<td></td>
<td>0.008</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>14.3 (19.2)</td>
<td>7.0 (10.9)</td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>6 (0.1-78)</td>
<td>4 (0.1-78)</td>
<td></td>
</tr>
<tr>
<td><strong>EPE bilateral</strong></td>
<td></td>
<td></td>
<td>0.819</td>
</tr>
<tr>
<td>Yes (%)</td>
<td>26.1</td>
<td>28.6</td>
<td></td>
</tr>
</tbody>
</table>
multivariated analysis demonstrated that only the status of the margin (p < 0.001) and the global Gleason score (p = 0.008) were independently related to the biochemical recurrence. The Cox risk of biochemical recurrence was 3.1 for positive surgical margins and 3.8 for a Gleason score > 7. The Kaplan Meier curves (Figure-2) demonstrate higher survival rates in patients with negative surgical margins, regardless of tumor stage. The estimated recurrence-free survival time for pT2M(−) and M(+) patients was 70.4 and 59.4 months, respectively. For pT3M(−) and pT3M(+) patients, the recurrence-free survival time was 58.6 and 48.1 months, respectively.

DISCUSSION

Although 60 to 70% of prostate tumor patients will be cured with radical prostatectomy alone, adjuvant radiotherapy before biochemical recurrence has been recommended based on three randomized trials conducted by the Southwest Oncology Group, the European Organization for Research and Treatment of Cancer and the German Cancer Society (5-7). The pre-operative PSA levels, tumor stage, Gleason score, and positive surgical margins are all important predictors of tumor recurrence. However, histological details, such as the length of the positive surgical margins or extraprostatic extension and the Gleason score of these areas, have rarely been examined as potential predictors to identify patients more susceptible to recurrence. Following detailed evaluations of 365 surgical specimens, we showed that margin status, independent of extension in millimeters and the Gleason score of these areas, were independently related to tumor recurrence after radical prostatectomy. Risk of recurrence was 3.1 and 3.8 for positive surgical margins and for Gleason score higher than 7, respectively. Interestingly, we found that even in organ-confined tumors, when the margin was positive, the rate of biochemical recurrence was the same as in pT3 negative margin tumors. This finding illustrates the importance of a positive margin in prostate cancer outcomes. Similarly, Grossfeld et al. showed a risk of tumor recurrence of 2.6 for positive surgical margins independently of all
other prognostic factors (1). Adjuvant radiotherapy reduces metastasis and improves survival at 10 years, with no impact on quality of life (9). However, accurate selection of men who will benefit from a second treatment is crucial, and this problem has not yet been solved.

Randomized trials often use non-standardized methods to examine surgical specimens, so the true status of surgical margins is not always completely accurate. There are different methods of examination that have not been fully explored in the previous reports. Furthermore, inclusion of the whole gland in the analysis, which would guarantee adequate evaluation of the specimens, is never mentioned in the literature. This lack of information regarding the real tumor status makes the evaluation of results of adjuvant treatment difficult to judge. As a result, patients may be over treated.

Recently, the International Society of Urological Pathology has recommended reporting the length of positive surgical margins as a quantitative measure of the extent of a positive surgical margin (3). However, few studies have demonstrated the real power of these details to predict biochemical recurrence in prostate cancer. In our study, we have shown that some histological details, such as the extent of the positive surgical margin, are not accurate predictors of tumor outcomes. We have also shown that the simple identification of the tumor by India ink staining would be enough to determine whether a patient will benefit from adjuvant radiotherapy. Conversely, Cao et al. examined 294 similar specimens and reported that the length of positive surgical margins was independently related to biochemical recurrence in prostate cancer. In contrast, Lowe and Lieberman reported that the number of positive margins had an important impact on disease recurrence (15).

The Gleason score of the surgical margin has recently been reported as important in predicting biochemical recurrence (4,16). Conversely, we found no relationship between the Gleason score of positive surgical margins and biochemical recurrence. This is the third study evaluating the potential importance of the Gleason score of surgical margins, and future larger studies should be conducted to further examine the importance of this relationship in the prediction of prostate cancer outcomes.

Our results suggest that the length, the bilateral involvement, and the Gleason score of positive surgical margins have no significant impact on biochemical recurrence. Therefore, these values should not be used to indicate adjuvant treatment after radical prostatectomy. The same result was recently published by Udo et al. (17). We found that the status of surgical margins and the Gleason score were the main risk factors for biochemical recurrence, representing a risk of 3.1. This assessment agrees with the risk previously reported in the literature (2). Large patient cohort studies confirm that positive surgical margins are an independent predictor of cancer recurrence, regardless of other factors. Blute et al. examined more than 2,500 patients with a positive margin rate of 39%. The 5-year, disease-free survival rate was 67% vs. 84% for positive surgical margins, with a hazards ratio of 1.72 for biochemical recurrence (14). Positive surgical margins were recently included in a 10-year postoperative nomogram as independent predictors of biochemical recurrence (18).
We have shown that biochemical recurrence rates were not significantly different between patients with pT2 positive margins and pT3 negative surgical margins. It is important to note that positive surgical margins in lateral areas of the prostate gland were staged as pT2 and not pT3 because it is not possible to show tumor extension into extraprostatic tissue. It is recommended that this status be named pT2+ or pT2R1 meaning residual microscopic disease. Our data show that prostate cancer staged pT2 with a positive surgical margin had similar outcomes as pT3 margin-negative cancers, and thus should be managed as a pT3 disease.

We have shown that the status of the surgical margin is important, even in tumors not confined to the prostate. This finding is similar to other studies that also reported that margin status is important regardless of the tumor stage (19,20).

In conclusion, the status of surgical margins and the Gleason score are independently related to biochemical recurrence in prostate cancer, regardless of other details, such as the extension of positive surgical margins, the length of extraprostatic extension, or the Gleason score present in specific areas. Moreover, adenocarcinoma staged pT2R1 behaves as pT3R0 and should be treated similarly.

**CONFLICT OF INTEREST**

None declared.

**REFERENCES**


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Is active surveillance a safe alternative in the management of localized prostate cancer? Pathological features of radical prostatectomy specimens in potential candidates for active surveillance

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ABSTRACT

Introduction and objective: Active surveillance (AS) has become an accepted alternative for patients with low risk prostate cancer. The purpose of AS is to defer definitive therapy in these patients to avoid treatment-related complications. Our aim was to determine the pathological features of the surgical specimen from potential AS candidates that underwent radical prostatectomy (RP).

Materials and Methods: We retrospectively reviewed a group of patients submitted to RP who met criteria for AS: Gleason score (GS) ≤ 3+3 = 6, PSA ≤ 10ng/mL, T1c - T2a, < 1/3 of positive cores, < 50% of involvement in any core and PSA density < 0.15. We determined the concordance between GS in biopsy and RP specimen (RPS). Other pathological features of the RPS were also analyzed, including surgical margins, extracapsular extension, seminal vesicles and lymph node involvement.

Results: We identified 167 patients subjected to RP that met the criteria for AS. Fifty two patients (31.1%) had a GS > 6 in the RPS (GS 7 n = 49; GS 8 n = 3). Extracapsular extension, seminal vesicle and lymph node involvement was found in 6.1%, 3.1% and 1.2% of the specimens, respectively.

Conclusion: In this study a significant proportion of potential candidates for AS showed features of aggressive and/or high-risk tumors in the RPS. Therefore, before considering a patient for an AS protocol, a proper and strict selection must be performed, and informed consent is crucial for these patients.

Key words: Prostatic Neoplasms; Active surveillance; Prostatectomy

INTRODUCTION

Prostate cancer (PCa) is a significant public health problem in the occidental world and is one of the most important death causes in men over 50 years (1). The extension of the tumor at the moment of diagnosis is determinant of patient’s survival (2). The massive use of prostate specific antigen (PSA) has significantly increased the number of tumors diagnosed at early stages, but has also led to over-diagnosis and over-treatment of a considerable number of patients with
clinically insignificant PCa (3). Active surveillance (AS) is an accepted alternative for the management of patients with low risk PCa (4).

The purpose of AS is to defer definitive treatment with a strict follow-up including clinical visits every 3 months with digital rectal examination (DRE) and PSA measurements. An annual biopsy is also advised. Definitive treatment should be offered to those men with evidence of progression (5). The actual criteria for AS vary between different clinical centers. The most accepted criteria for including a patient with PCa to an AS protocol are: clinical stage T1c or T2, GS $\leq 3+3 = 6$, PSA $\leq 10$ng/mL, $\leq 2$ positive cores and $< 50\%$ involvement in each core (6).

Most of the criteria accepted for including a patient into an AS protocol are based in pathological characteristics of preoperative transrectal biopsy. However, different studies report a GS up-grade in 24-39% of the surgical specimens when compared to preoperative biopsy (5). This means that a proportion of patients selected for an AS protocol may carry a higher risk disease not detected in the transrectal biopsy specimen. It is known that by increasing the number of cores in the transrectal biopsy, the correlation between the preoperative and postoperative findings improves (7). We do not know what is the percentage of Gleason score undergrading in our center, so without this data, active surveillance would not be a safe alternative management. The aim of our study was to determine the pathological features of the surgical specimen from potential AS candidates that underwent radical prostatectomy and compare them with the results of their pre-operative transrectal biopsies, and thus answer the question if active surveillance is a safe management alternative for prostate cancer.

MATERIALS AND METHODS

Patients

We conducted a retrospective study that included 167 Latin-American patients from a group of 623 subjected to RP at two institutions from 2008 to 2011. These patients could have been candidates for AS since they fulfilled the following criteria: GS $\leq 3+3 = 6$, T1c – T2a, $< n = 4$ positive. Less than 1/3 of cores and/or no more than n = 3 cores involved, $< 50\%$ of compromise in any core of the transrectal biopsy, PSA level $\leq 10$ng/mL and PSA density $< 0.15$ (8). PSA density was calculated by dividing the absolute PSA value by the prostatic weight assessed by transrectal ultrasound. Informed consent was obtained from each patient.

Histological study

Pre-surgical prostatic biopsy and radical prostatectomy specimen (RPS) samples from the 167 patients were analyzed by different pathologists in two medical centers. Surgical specimens were evaluated using the Gleason grading system. Extraprostatic extension, when present, was subclassified as being either focal or extensive. Surgical resection margins were designated as being positive or negative. Seminal vesicle involvement was considered to be present upon penetration of the tumor into the muscular coat of the seminal vesicle.

Statistical analysis

The percent of upgrading and downgrading of the surgical specimen was determined by comparing GS of biopsy and RPS. The data were tabulated and analyzed using the SPSS v17.0 software (IBM, USA). A chi square (X2) statistic was used to investigate whether distributions of categorical variables differ from one another considering a p value $< 0.05$ statistically significant.

RESULTS

Patients

Of 623 patients subjected to RP between 2008 and 2011, 167 fulfilled criteria and therefore could have been candidates for AS. Patient characteristics are showed in Table-1. The mean age in this group was 62.3 years (40-75) and the mean PSA value was 5.9ng/mL (0.59-10). Regarding to clinical stage, 74.2\% (n = 124) corresponded to T1c and 25.7\% (n = 43) to T2a.

All patients had preoperative biopsy that was performed 4-8 weeks before surgery. All patients included in the study had GS $\leq 6$ in pre-operative biopsy. From these, most patients (89.2\%) had a GS of 6. In the surgical specimen, the num-
Table 1 - Pre-operative clinical and pathological features.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age in years (range)</td>
<td>62.3 (40 - 75)</td>
</tr>
<tr>
<td>Mean pre-operative PSA in ng/mL (range)</td>
<td>5.9 (0.59 - 10)</td>
</tr>
<tr>
<td>Average PSA Density (range)</td>
<td>0.146 (0.07 - 0.149)</td>
</tr>
<tr>
<td>Clinical Stage (%)</td>
<td></td>
</tr>
<tr>
<td>T1c</td>
<td>124 (74.3%)</td>
</tr>
<tr>
<td>T2a</td>
<td>43 (25.7%)</td>
</tr>
<tr>
<td>Transrectal Biopsy Characteristics</td>
<td></td>
</tr>
<tr>
<td>Mean number of cores (range)</td>
<td>17.1 (8 - 24)</td>
</tr>
<tr>
<td>Mean Percent (%) of core involvement (range)</td>
<td>12% (2 - 49%)</td>
</tr>
<tr>
<td>Preoperative biopsy Gleason score</td>
<td>n (%)</td>
</tr>
<tr>
<td>3</td>
<td>1 (0.6)</td>
</tr>
<tr>
<td>4</td>
<td>10 (6)</td>
</tr>
<tr>
<td>5</td>
<td>7 (4.2)</td>
</tr>
<tr>
<td>6</td>
<td>149 (89.2)</td>
</tr>
<tr>
<td>Postoperative Gleason score n(%)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1 (0.6)</td>
</tr>
<tr>
<td>4</td>
<td>5 (3)</td>
</tr>
<tr>
<td>5</td>
<td>1 (0.6)</td>
</tr>
<tr>
<td>6</td>
<td>101 (60.5)</td>
</tr>
<tr>
<td>7</td>
<td>49 (29.3)</td>
</tr>
<tr>
<td>(3+4)</td>
<td>40 (23.9)</td>
</tr>
<tr>
<td>(4+3)</td>
<td>9 (5.4)</td>
</tr>
<tr>
<td>8</td>
<td>3 (1.8)</td>
</tr>
<tr>
<td>High grade PIN</td>
<td>5 (3)</td>
</tr>
<tr>
<td>Benign Prostatic Hyperplasia</td>
<td>2 (1.2)</td>
</tr>
<tr>
<td>Upgrading in the RPS</td>
<td>52 (31.1)</td>
</tr>
</tbody>
</table>

Table 2 summarizes pathological features of the surgical specimen. Positive surgical margins, extracapsular extension, seminal vesicle and lymph node involvement were reported in 30.7%, 6.1%, 3.1% and 1.2%, respectively. Table-3 shows a comparison between patients with clinical stage T1c and T2a regarding to pathological features of the surgical specimen. Thirty percent of patients with a clinical stage T1c had a GS > 6 in the surgical specimen, compared to a 58.1% of patients with a clinical stage T2a (p < 0.001). No other pathological differences (tumor volume, extracapsular extension, positive surgical margins, seminal vesicles involvement and lymph node involvement) were found. By using Epstein criteria for insignificant cancer (tumor volume < 0.2 cm³, organ confined disease and no Gleason pattern 4 or 5), only 16 (9.6%) patients were classified as such.

**DISCUSSION**

PCa is a significant public health problem in the occidental world, and one of the most important death causes in men over 50 years (1). In Chile, the mortality from PCa has steadily increased in the last decades reaching a rate of 20.9 per 100,000 men in year 2009, ranking in the second place of cancer death causes in men, producing approximately 1,753 deaths per year (9). The extension of the tumor at the time of diagnosis is determinant of patient’s survival (2). Independent of the controversies, PSA screening programs lead to an early detection of PCa in men, thereby substantially reducing their morbidity and mortality as showed by the Göteborg study and the European Randomized Study
of Screening for Prostate Cancer (ERSPC) (10,11). The massive use of PSA in the clinical practice has significantly increased the number of tumors diagnosed in early stages, leading to an over-diagnosis and over-treatment of clinically insignificant PCa (3). Recent studies have demonstrated that to save one life from prostate cancer is necessary to treat 48 men with a follow-up of at least 9 years (12).

The basis of AS is a careful selection of patients that can defer immediate treatment as they have a localized, well-differentiated and therefore low-risk PCa (13). Some centers recommend a 3-monthly follow-up with DRE and PSA, and a transrectal prostate biopsy every one or two years. In those patients in whom PCa progression is detected, definitive therapy has to be offered (5).

The actual criteria for the selection of patients for AS vary according to the urological center. A recent review of Conti et al. showed that those institutions with more strict inclusion criteria (including estimation of tumor volume based on the number and percent of cores compromised) had less adverse results in the surgical specimen (less percent of seminal vesicle involvement and/or extracapsular extension) (14).

Thaxton et al. published a study showing the pathological features of the surgical specimen from patients that fulfilled criteria for AS but otherwise were subjected to RP. From these patients 4% had a GS between 8 and 10, 5% had seminal vesicle involvement and up to 1% had lymph node involvement. This study concluded that the most important predictor of unfavorable outcome was a GS > 6 (15). Beauval JB et al. demonstrated that despite of a stringent selection of patients with low-risk prostate cancer, active surveillance definition included a significant proportion of patients with upstaged (about 12%) and upgraded (about one-third) disease at diagnosis (16). In this same sense Iremashvili studied the ability of contemporary AS criteria to identify patients with certain pathologic tumor features based on the results of an extended transrectal prostate biopsy. The authors concluded that significant variations exist in the ability of contemporary AS criteria to predict pathologically insignificant prostate cancer at radical prostatectomy (17).

In our experience, a high proportion of patients (31.1%) that were candidates for AS had a GS > 6 and also had pathological features of unfavorable outcome: extracapsular extension (6.1%), seminal vesicle (3.1%) or lymph node involvement.

<table>
<thead>
<tr>
<th>Table 2 - Anatomopathological features of the surgical specimen.</th>
</tr>
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<tbody>
<tr>
<td><strong>Frequency (%)</strong></td>
</tr>
<tr>
<td>Median Tumor Volume (cc)</td>
</tr>
<tr>
<td>Extracapsular extension</td>
</tr>
<tr>
<td>Seminal Vesicle Involvement</td>
</tr>
<tr>
<td>Lymph Node Involvement</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3 - Anatomopathological features of the surgical specimen. Comparison between T1c and T2a.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical Stage</strong></td>
</tr>
<tr>
<td>% of Patients with GS &gt; 6 in RPS</td>
</tr>
<tr>
<td>Mean tumor volume (cc)</td>
</tr>
<tr>
<td>Extracapsular Extension</td>
</tr>
<tr>
<td>Positive Surgical Margins</td>
</tr>
<tr>
<td>Seminal Vesicle Involvement</td>
</tr>
<tr>
<td>Lymph Node Involvement</td>
</tr>
</tbody>
</table>
The proportion of patients with GS > 6 was significantly higher in patients with clinical stage T2a compared to T1c (P < 0.001). No other differences were found in relation to clinical stage. Several factors can influence the likelihood that the biopsy GS underestimates the prostatectomy score, including the PSA level, the level of pathologist expertise, the patient’s age, the results of the digital rectal examination, the prostate gland volume, the percentage of cancer cells in the biopsy sample and the number of biopsies obtained (18,19).

The main limitations of the study were the retrospective design and that both biopsy and prostatectomy specimens were examined by different pathologists.

Another reason why the under-grading can occur is the presence of an anterior prostate cancer, which are less likely to be palpable and required more prostate biopsies than posteriorly located prostate cancers (PPCs) (20).

These results suggest that the selection criteria for AS have to be discussed and adjusted to the reality of each center. In our opinion, reliability of pathological findings in the pre-operative biopsy is of striking importance and efforts should be made to improve a correct pathological diagnosis. Until now, AS programs needs longer follow-up to prove that they are an appropriate and safe alternative for the management of patients with prostate cancer.

CONCLUSIONS

Patients with clinical T1c tumors were significantly less under-graded than T2a. However, a significant proportion of patients that could have been considered for AS, had tumors with unfavorable features (high GS, extracapsular extension, seminal vesicle and lymph-node involvement). In these cases, delay of definitive therapy could determine a lower survival rate. Therefore, before considering a patient for an AS protocol, a proper and strict selection must be performed, and informed consent is crucial for these patients.

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

The concept of Active Surveillance in patients with Prostate Cancer still deserves much thought, but the consensus is that the choice of any conduct should be personalized and non-transferable. The safety of this decision is based on the rigor of the selection of these patients. What is the ideal patient to undergo Active Surveillance? A definitive answer to this question is yet to come! I congratulate the authors of this work as call into relevant question and show with a clear methodology, the choice of Active Surveillance, based on clinical and morphological parameters may not be the ideal way and inclusion criteria for Active Surveillance should have extreme rigor. Are the known limitations of Gleason score, making it an inaccurate predictor based on needle biopsy. This finding was observed by other authors, who described the need for treatment in over 80% of patients with stage T1c and only 16% had insignificant tumor when compared to data from biopsy and surgical specimens (1). In this present study the authors found a rate of 31.1% of patients with Gleason score of the surgical specimen greater than obtained in needle biopsy. It is a significant data and should be valued in decision making, especially when the population studied was composed of relatively young patients, with a mean age of 62.3 years. In summary, the Active surveillance requires conditions that go beyond medical criteria as to their applicability is also required a high degree of patient information, as well as favorable psychological profile and above all ensuring access to health services, with full coverage costs. This is not easy!

REFERENCE


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External Validation of a Brazilian Predictive Nomogram for Pathologic Outcomes Following Radical Prostatectomy in Tertiary Teaching Institutions: the USP Nomograms

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Urology Division, University of São Paulo Medical School, São Paulo, Brazil and Cancer Institute of São Paulo, São Paulo, Brazil

ABSTRACT

Purposes: (a) To externally validate the Crippa and colleagues’ nomograms combining PSA, percentage of positive biopsy cores (PPBC) and biopsy Gleason score to predict organ-confined disease (OCD) in a contemporary sample of patients treated at a tertiary teaching institution. (b) To adjust such variables, resulting in predictive nomograms for OCD and seminal vesicle invasion (SVI): the USP nomograms.

Materials and Methods: The accuracy of Crippa and colleagues’ nomograms for OCD prediction was examined in 1002 men submitted to radical prostatectomy between 2005 and 2010 at the University of São Paulo (USP). ROC-derived area under the curve (AUC) and Brier scores were used to assess the discriminant properties of nomograms for OCD. Nomograms performance was explored graphically with LOESS smoothing plots. Furthermore, univariate analysis and logistic regression models targeted OCD and SVI. Variables consisted of PSA, PPBC, biopsy Gleason score and clinical stage. The resulted predictive nomograms for OCD and SVI were internally validated with bootstrapping and the same abovementioned procedures.

Results: Crippa and colleagues’ nomograms for OCD showed ROC AUC = 0.68 (CI: 0.65-0.70), Brier score = 0.17 and overestimation in LOESS plots. USP nomograms for OCD and SVI showed ROC AUC of 0.73 (CI: 0.70-0.76) and 0.77 (CI: 0.73-0.79), respectively, and Brier scores of 0.16 and 0.08, respectively. The LOESS plots showed excellent calibration for OCD and underestimation for SVI.

Conclusions: Crippa and colleagues’ nomograms showed moderate discrimination and considerable OCD overestimation. USP nomograms showed good discrimination for OCD and SVI, as well as excellent calibration for OCD and SVI underestimation.

INTRODUCTION

Prostate cancer (PCa) is the second most prevalent malignancy among Brazil’s male population. Its estimated incidence was 53.84 per 100,000 men in 2010 (1). The pathologic stage of PCa is critical for the success of treatment. Extra-prostatic extension and seminal vesicle invasion influence treatment choices, cure rates and decisions regarding preservation of the neurovascular bundles responsible for erectile function (2). In 1993, Partin’s pioneer study (3) estimated the risk of extra-capsular ex-
tension, seminal vesicle invasion (SVI) and lymph node status based on levels of PSA, clinical stage and Gleason score from prostate biopsy. The number of mathematical models used to predict the pathological stage has increased over the past 10 years. One systematic review identified 16 predictive and 22 prognostic models suitable for clinical use, most of them requiring external validation (4). According to Touijer and Scardino (5), there is a large degree of uncertainty when assessing the prognosis and predicting the outcomes in PCa management.

In 2006, Crippa et al. (6) published the first population-based study in Brazil aimed to predict organ-confined disease (OCD). PSA levels, Gleason score from prostate biopsies and percentages of positive biopsy cores (PPBC) were used as predictor variables. The model was constructed and internally validated on 898 private-practice patients submitted to radical retropubic prostatectomy (RRP) performed by one surgeon. The corresponding surgical specimens were examined by the same pathologist. The resulting nomograms correctly estimated OCD in 91.1% of patients. The main limitations of predictive nomograms included lack of external validation and of periodic updates to accommodate for changes occurring over time in populations, diseases and diagnostic methods [4]. Prediction tools become increasingly robust as they are successively validated in distinct environments because the variability improves the accuracy (calibration and discrimination) and the generalizability of the model (7). As a consequence, adjustment is strongly indicated when applying a prediction model to populations with distinct characteristics or when temporal changes in disease or variable behavior are suspected (8).

The University Hospital of the University of São Paulo Medical School and the Cancer Institute of the State of São Paulo are public reference centers for PCa in Brazil. As tertiary centers, their population of patients is quite heterogeneous, as most patients have their biopsies performed at their original institutions, while surgical procedures and pathological examinations are performed by supervised residents at distinct levels of training. We hypothesized that such heterogeneity could (a) significantly challenge the generalizability and transportability of a nomogram constructed on a more homogenous population and (b) require adjustments of the predictive nomogram to accommodate the characteristics of the population assisted at public tertiary centers.

The objectives of this study were (a) to perform the external validation of Crippa and colleagues’ nomograms and (b) to develop an adjusted nomogram for prediction of organ-confined disease and seminal vesicle invasion based on the population assisted at the abovementioned public tertiary institutions (USP nomograms).

**MATERIALS AND METHODS**

This study was approved by the Institutional Review Board. The patients’ informed consent was waived. Electronic medical records of 1,094 consecutive prostate cancer patients who underwent RRP by the Walsh technique (9) as modified by Srougi (10) between January 2005 and December 2010 were retrospectively reviewed. All surgeries were performed by a urology resident assisted by an experienced urologist assistant. The following data were extracted: (a) clinical staging based on the 2002 TNM classification (11). The T class was based on rectal examinations performed by urology residents and confirmed by a faculty urologist; (b) preoperative PSA levels, which were updated within the institution if measured more than 90 days from the preoperative consultation; (c) prostate biopsy findings, including total number of specimens obtained, number of positive fragments, and Gleason scores (12) stratified on primary and secondary components, and total scores; (d) TNM pathologic staging (11) and Gleason histological classification based on electronic reports of standardized pathological examinations of surgical specimens consisting of prostate, seminal vesicles and, eventually, the removed lymph nodes. Organ-confined disease was defined as the absence of tumoral cells in periprostatic adipose tissue and/or in neurovascular bundles. Seminal vesicle invasion was characterized by the infiltration of tumoral cells not limited to the adventitia.

**Statistical analysis**

The sample size was based on a 34% reported prevalence of non-OCD (6) and four predictor variables, with 10 and 25 events per variable, which required 118 and 294 subjects, respectively (13).
Accordingly, the available 1002 subject sample was considered suitable for the study.

This study was based on the premise that the current sample would differ in significant aspects from that of Crippa and colleagues’ original study (6). To test this hypothesis, demographic data, including the clinical stage, PSA values, and pathological findings presented in Table-1 of the original study were compared to data from the sample of the current study by two-sided unpaired t-tests and z-tests for proportions, as appropriate. The outcomes of interest were organ-confined disease and seminal vesicle invasion.

External validation of Crippa and colleagues’ nomograms

Crippa and colleagues’ nomograms were designed to predict OCD. For external validation, the probability of OCD was estimated for each patient in the validating sample as the average probability predicted by nomograms 1 and 2 of the original study (6), based on the respective ranges of PSA levels (0-4, 4.1-10, 10.1-20, and above 20ng.mL−1), of Gleason scores (2-6, 7 and 8-10 in nomogram 1, and 2-6 and 7-10 in nomogram 2) and PPBC (0-25%, 25.1-50%, 50.1-75% and 75.1-100%). Receiver operating characteristic (ROC) curves and the respective areas under the curves (AUC) accessed the discriminatory capability of the nomograms. Brier scores estimated the predictive performance of the nomograms based on mean squared deviations between predicted and observed outcomes and varied from 0 (perfect) to 0.25, which indicates that the model lacked any predictive capability. The extent of nomogram over- or underestimation was explored graphically within LOESS calibration plots (14). Coincidence of curves best fitted to scatterplots of predicted and observed outcomes with the diagonal lines on the plots indicates good model calibration along the ranges of prediction.

Construction of the USP nomograms

The USP nomograms aimed to predict both OCD and SVI based on ranges of PSA levels, of clinical stages, of Gleason scores and of PPBC.

For each predictor variable, cases were classified as follows: (a) PSA levels were categorized as 0-4, 4.1-10, 10.1-20, or above 20ng.mL−1. To increase the number of cases in extreme categories, three categories were rebuilt by collapsing the 0-4 and the 4.1-10ng.mL−1 ranges. (b) Clinical stage was classified according to the T component of the TNM class. (c) Gleason scores were grouped in three categories: 2-6, 7 and 8-10. To increase the number of cases at the extremes, two categories were rebuilt: 2-6 and 7-10. (d) PPBC were initially grouped into four categories: 0-25%, 25.1-50%, 50.1-75% and 75.1-100%. The categories 25.1-50% and 50.1-75% were collapsed to increase the representativeness of cases at the extremes.

Chi-squared tests were used to assess the association between predictor variables and binary outcomes (OCD and SVI). Significantly associated variables were entered into stepwise logistic regression analyses to identify independent predictors of the respective outcomes. Final coefficients and odds ratios and the respective 95% confidence intervals were obtained from 1000 bootstrap resampling procedures (15). Hosmer and Lemeshow tests were used to assure the adequacy of the models. ROC curves were constructed. Areas under the curves, positive and negative predictive values of each model assessed discriminatory capabilities. Brier scores and LOESS plots were also constructed, as described above.

Statistical analyses were performed on Stata v.10 (StataCorp LP, College Station). The significance level (alpha) was set at 0.05.

RESULTS

Of the total of 1,094 patients, seventy-seven incomplete records, thirteen records of patients who received neoadjuvant hormonal therapy and two records of patients diagnosed following endoscopic resection of the prostate were excluded, resulting in 1,002 patients.

External validation of nomograms from Crippa et al.

Table-1 shows the demographic, clinical and pathological data of patients in this study sample compared with the data from Crippa and colleagues’ study. Significant differences were observed with respect to age, clinical stage, pathological stage, Gleason score (7 and 8-10 categories), number of total and positive cores and PPBC.
Table 1 – Demographic, clinical and pathological data.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>USP</th>
<th>Crippa et al.</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N patients Age (years)</strong></td>
<td>1,002</td>
<td>898</td>
<td>0.00</td>
</tr>
<tr>
<td>Mean (median)</td>
<td>64.7</td>
<td>(65)</td>
<td>62.9</td>
</tr>
<tr>
<td>Min-max</td>
<td>44 - 81</td>
<td>40 - 83</td>
<td></td>
</tr>
<tr>
<td><strong>Clinical stage</strong></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>T&lt;sub&gt;1c&lt;/sub&gt;</td>
<td>627</td>
<td>62.5%</td>
<td>432</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>371</td>
<td>37.0%</td>
<td>459</td>
</tr>
<tr>
<td>-T&lt;sub&gt;2a&lt;/sub&gt;</td>
<td>248</td>
<td>24.7%</td>
<td>-</td>
</tr>
<tr>
<td>-T&lt;sub&gt;2b&lt;/sub&gt;</td>
<td>97</td>
<td>9.7%</td>
<td>-</td>
</tr>
<tr>
<td>-T&lt;sub&gt;2c&lt;/sub&gt;</td>
<td>26</td>
<td>2.6%</td>
<td>-</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>4</td>
<td>0.4%</td>
<td>7</td>
</tr>
<tr>
<td><strong>Pathologic stage</strong></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>16</td>
<td>1.6%</td>
<td>-</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>722</td>
<td>72.0%</td>
<td>599</td>
</tr>
<tr>
<td>-T&lt;sub&gt;2a&lt;/sub&gt;</td>
<td>156</td>
<td>15.5%</td>
<td>-</td>
</tr>
<tr>
<td>-T&lt;sub&gt;2b&lt;/sub&gt;</td>
<td>40</td>
<td>4.0%</td>
<td>-</td>
</tr>
<tr>
<td>-T&lt;sub&gt;2c&lt;/sub&gt;</td>
<td>526</td>
<td>52.5%</td>
<td>-</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>259</td>
<td>25.8%</td>
<td>296</td>
</tr>
<tr>
<td>-T&lt;sub&gt;3a&lt;/sub&gt;</td>
<td>157</td>
<td>15.6%</td>
<td>-</td>
</tr>
<tr>
<td>-T&lt;sub&gt;3b&lt;/sub&gt;</td>
<td>102</td>
<td>10.2%</td>
<td>-</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>5</td>
<td>0.5%</td>
<td>3</td>
</tr>
<tr>
<td><strong>PSA</strong></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Mean (median)</td>
<td>10.36</td>
<td>(9.90)</td>
<td>10.1</td>
</tr>
<tr>
<td>Min-max</td>
<td>0.1 - 61.2</td>
<td>0.3 - 63.5</td>
<td></td>
</tr>
<tr>
<td>0 - 4</td>
<td>111</td>
<td>11.1%</td>
<td>84</td>
</tr>
<tr>
<td>4.1 - 10</td>
<td>510</td>
<td>50.9%</td>
<td>512</td>
</tr>
<tr>
<td>10.1 - 20</td>
<td>295</td>
<td>29.4%</td>
<td>236</td>
</tr>
<tr>
<td>&gt;20</td>
<td>86</td>
<td>8.6%</td>
<td>66</td>
</tr>
<tr>
<td><strong>Gleason</strong></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>2 - 6</td>
<td>711</td>
<td>71.0%</td>
<td>635</td>
</tr>
<tr>
<td>7</td>
<td>226</td>
<td>22.6%</td>
<td>165</td>
</tr>
<tr>
<td>8 - 10</td>
<td>65</td>
<td>6.5%</td>
<td>80</td>
</tr>
<tr>
<td><strong>N Total cores</strong></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Mean (median)</td>
<td>11.6</td>
<td>(12.0)</td>
<td>8.1</td>
</tr>
<tr>
<td>Min-Max</td>
<td>2 – 30</td>
<td>2 - 22</td>
<td></td>
</tr>
<tr>
<td><strong>N + cores</strong></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Mean (median)</td>
<td>3.9</td>
<td>(3.0)</td>
<td>3.2</td>
</tr>
<tr>
<td>Min-Max</td>
<td>1 - 22</td>
<td>1 - 20</td>
<td></td>
</tr>
<tr>
<td><strong>PPBC</strong></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Mean (median)</td>
<td>34.50%</td>
<td>(33.3%)</td>
<td>41.20%</td>
</tr>
<tr>
<td>Min-max</td>
<td>3 – 100</td>
<td>5 - 100</td>
<td></td>
</tr>
</tbody>
</table>
ROC curves of predictions based on Crippa and colleagues’ nomograms on the observed outcomes of patients in the validation sample. AUC and the respective 95% confidence limits for the predictions based on nomograms 1 and 2 were 0.68 (0.65-0.70) and 0.68 (0.65-0.71). Both nomograms had Brier scores of 0.17 (Figure-1).

LOESS plots for predictions based on both nomograms. Considerable overestimation of OCD in all ranges of prediction is suggested (Figure-2).

USP nomograms

Table-2 shows the results of the chi-squared tests used in univariate analyses. No significant as-
Figure 2 - LOESS plots for predictions based on both nomograms. Considerable overestimation of OCD in all ranges of prediction is suggested.

Table 2 - Univariate analysis for OCD and SVI prediction.

<table>
<thead>
<tr>
<th>Variable</th>
<th>OCD</th>
<th>SVI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\chi^2$</td>
<td>p</td>
</tr>
<tr>
<td>Age</td>
<td>28.3</td>
<td>0.81</td>
</tr>
<tr>
<td>PSA</td>
<td>67.8</td>
<td>0.00</td>
</tr>
<tr>
<td>Clinical stage</td>
<td>34.68</td>
<td>0.00</td>
</tr>
<tr>
<td>N total cores</td>
<td>20.9</td>
<td>0.58</td>
</tr>
<tr>
<td>PPBC</td>
<td>59.1</td>
<td>0.00</td>
</tr>
<tr>
<td>Gleason score</td>
<td>48.4</td>
<td>0.00</td>
</tr>
</tbody>
</table>
associations with either OCD or SVI were found for the total number of fragments in prostate biopsies or patient’s age. PSA levels, Gleason scores, PPBC and clinical stage categories were significantly associated with both outcomes.

Table-3 summarizes the final logistic models, with bootstrap odds ratio 95% confidence limits. The final categories of PSA levels and Gleason scores that best fitted the model resulted from the collapsed ranges. Clinical stage was rejected from the final logistic models (Figure-3).

The areas under the curves were 0.73 (0.70-0.76) and 0.77 (0.73-0.79) for OCD and SVI, respectively. Brier scores were 0.16 and 0.08, respectively.

The LOESS plots in Figure-4 depict the calibration of USP nomograms. Visual inspection revealed that the OCD curve was mostly coincident with the diagonal line, suggesting good calibration in all segments except for the 15% through 30% range, where underestimation occurred. LOESS plots for SVI predictions suggest underestimation of SVI in all ranges of prediction above 2%.

<table>
<thead>
<tr>
<th>Table 3 - Multivariate analysis for prediction OCD and IVS.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organ Confined Disease</td>
</tr>
<tr>
<td>coef</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td><strong>PSA</strong></td>
</tr>
<tr>
<td>10.1-20 versus 0-4</td>
</tr>
<tr>
<td>&gt;20 versus 0-4</td>
</tr>
<tr>
<td><strong>Gleason 7-10 versus 4-6</strong></td>
</tr>
<tr>
<td>% positive cores</td>
</tr>
<tr>
<td>25.1-75 versus 0-25</td>
</tr>
<tr>
<td>75.1-100 versus 0-25</td>
</tr>
<tr>
<td><strong>Constant</strong></td>
</tr>
<tr>
<td>2.14</td>
</tr>
</tbody>
</table>

Positive predictive value: 77.03%; Negative predictive value: 59.49%; Hosmer and Lemeshow: $\chi^2 = 7.9; \text{df} = 6; p = 0.24$

<table>
<thead>
<tr>
<th>Table 3 - Multivariate analysis for prediction OCD and IVS.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organ Confined Disease</td>
</tr>
<tr>
<td>coef</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td><strong>PSA</strong></td>
</tr>
<tr>
<td>10.1-20 versus 0-4</td>
</tr>
<tr>
<td>&gt; 20 versus 0-4</td>
</tr>
<tr>
<td><strong>Gleason 7-10 versus 4-6</strong></td>
</tr>
<tr>
<td>% positive cores</td>
</tr>
<tr>
<td>25.1-75 versus 0-25</td>
</tr>
<tr>
<td>75.1-100 versus 0-25</td>
</tr>
<tr>
<td><strong>Constant</strong></td>
</tr>
<tr>
<td>-3.70</td>
</tr>
</tbody>
</table>

Positive predictive value: 42.86%; Negative predictive value: 89.53%; Hosmer and Lemeshow: $\chi^2 = 3.9; \text{df} = 6; p = 0.67$
DISCUSSION

This study has shown moderate discriminative power and considerable OCD overestimation bias of Crippa and colleagues' nomograms. Conversely, USP nomogram exhibited good discriminative power and calibration for prediction of OCD. In contrast, USP nomogram for prediction of SVI considerably underestimated that outcome.

Superoptimistic behavior of predictive models in external validation processes is a common and widely acknowledged phenomenon (16). The wide and persistent use of Partin tables is based more on its clinical usefulness than on its statistical performance (17). Similarly, in spite of the modest predictive accuracy and informative performance of Crippa and colleagues' nomograms, by incorporating relevant independent predictors of OCD, they
may contribute valuable prognostic information. In doing so, and considering their limitations, they may be used as a better alternative to clinical staging. The moderate predictive performance of such nomograms may have resulted from sample biases. Such a finding supports the original hypothesis of this study, according to which the heterogeneity of the tertiary teaching center population might disclose eventual weaknesses of nomograms developed in a more homogeneous population. The next logical step in the study was to create new nomograms based on the same variables but originating from and validated in the population of our teaching institutions, including a prediction model for SVI.

The USP predictive models exhibited consistency, as confidence intervals of the original sample coincided with those obtained by bootstrap, and adequate predictive performance, as assessed by the tests of Hosmer and Lemeshow. Areas under the ROC curves greater than 0.7 and overall percentage of correct classification equal to 73% (OCD) and 77% (SVI) suggest a moderate to high discriminatory ability of both models. Similarly, calibration of the OCD predictive model was robust. In contrast, LOESS diagrams for the SVI predictive model showed underestimation of the outcome in all ranges of prediction over 2%.

It has been suggested that proper calibration of a nomogram is more clinically useful than is its
discriminatory capability (7). Accordingly, USP nomograms for predicting OCD can be clinically useful. Conversely, given its poor calibration, the SVI nomogram demands extensive external validations and variable adjustments to improve its accuracy. Partin tables have demonstrated good discriminating capability (AUC = 0.74) (18). A recent validation in the Surveillance, Epidemiology and End Results (SEER) dataset showed appropriate discrimination of the Partin tables, but the study did not report on their calibrations (18). In contrast, a European validation study failed to confirm their accuracy (17). Reasons for these conflicting results may include the fact that at the time of its construction, in 1993, only 39% of patients had a non-palpable tumor at diagnosis (19). An increasing prevalence of T1c tumors at diagnosis has occurred over recent decades (20). In Crippa and colleagues’ study, clinical stage T1c was present in 48.1% of patients diagnosed between 1988 and 2002, while in our sample, 62.7% of patients were T1c. These findings justify repeated adjustment and revalidation procedures to accommodate disease and population changes over time.

The superiority of PPBC associated with PSA levels and Gleason scores over the clinical stage in predicting extra-prostatic disease has been demonstrated (6,21). This study confirmed these findings.

The use of only a few variables is desirable in nomograms to increase utility in busy practices (16). Clinically useful nomograms should be applicable to individual patients and provide this information as percentages of outcome likelihood (22). The nomograms in this study fulfilled the abovementioned requirements.

Seminal vesicle preservation during RRP may improve erectile function and urinary continence (23). Seminal vesicle involvement demands a wider radiation field during radiotherapy (24) and is associated with higher rates of biochemical recurrence and worse prognosis (25). Such practical issues stress the importance of accurate prediction of organ-confined disease.

The growing number of low-risk PCa patients managed by active surveillance continues to generate controversy about the concepts of indolent disease, the criteria for treatment and the impact on patient survival compared to treated patients (26). Nomograms for predicting indolent disease (27) are in use, but require extensive external validation.

This study included 419 (41.1%) low-risk patients who were treated surgically. Of these, 86.4% had OCD. The USP nomogram predicted 86.1% of OCD in low-risk cases. Active surveillance requires periodic measurements of PSA and repeated prostate biopsies (28). The availability of such data allows sequential recalculations of OCD likelihood in USP nomograms.

The retrospective nature of this study and the impossibility of reviewing prostate biopsies derived from several centers may have biased our data. Furthermore, clinical staging did not include imaging examinations. Inter-examiner bias may have caused eventual misclassifications of clinical stage and of pathological examinations of surgical specimens (29).

The abovementioned bias-inducing factors are inherent to retrospectively collected data from referral teaching centers and were acknowledged during planning. This study shares these features with other major validation studies.

Predictive values of further models may increase by the inclusion of additional variables as angiolympathic or perineural invasion and novel cellular, molecular, and genetic biomarkers (30).

CONCLUSIONS

USP nomograms showed good discrimination for OCD and SVI, as well as excellent calibration for OCD and SVI underestimation.

CONFLICT OF INTEREST

None declared.

REFERENCES


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São Paulo, SP, 01308-000, Brazil
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E-mail: marcosdalllogliouro@terra.com.br
Re-examination of the Natural History of High-grade T1 Bladder Cancer using a Large Contemporary Cohort

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ABSTRACT

Introduction: High-grade T1 (HGT1) bladder cancer represents a clinical challenge in that the urologist must balance the risk of disease progression against the morbidity and potential mortality of early radical cystectomy and urinary diversion. Using two non-muscle invasive bladder cancer (NMIBC) databases, we re-examined the rate of progression of HG T1 bladder cancer in our bladder cancer populations.

Materials and Methods: We queried the NMIBC databases that have been established independently at the Atlanta Veterans Affairs Medical Center (AVAMC) and the University of Pennsylvania to identify patients initially diagnosed with HGT1 bladder cancer. Demographic, clinical, and pathologic variables were examined as well as rates of recurrence and progression.

Results: A total of 222 patients were identified; 198 (89.1%) and 199 (89.6%) of whom were male and non-African American, respectively. Mean patient age was 66.5 years. 191 (86.0%) of the patients presented with isolated HG T1 disease while 31 (14.0%) patients presented with HGT1 disease and CIS. Induction BCG was utilized in 175 (78.8%) patients. Recurrence occurred in 112 (50.5%) patients with progression occurring in only 19 (8.6%) patients. At a mean follow-up of 51 months, overall survival was 76.6%. Fifty two patients died, of whom only 13 (25%) patient deaths were bladder cancer related.

Conclusions: In our large cohort of patients, we found that the risk of progression at approximately four years was only 8.6%. While limited by its retrospective nature, this study could potentially serve as a starting point in re-examining the treatment algorithm for patients with HG T1 bladder cancer.

INTRODUCTION

High-grade T1 bladder cancer (HGT1) represents a clinical challenge in that the urologist must balance the threat of progression from non-muscle to muscle invasive disease against the morbidity and mortality of early radical cystectomy (RC) with urinary diversion. Current AUA clinical guidelines for patients with HGT1 disease recommend induction BCG to limit the risk of recurrence and, more importantly, progression after an adequate re-resection (1). Despite high rates of...
progression and the associated increase in mortality, the AUA guidelines only state that radical cystectomy as a first-line treatment choice for HGT1 can be considered an option, advising the physician to weigh the likelihood of cure without invasive surgery against the associated morbidity and mortality of radical surgery (1).

Previous studies have reported that HGT1 bladder cancer will progress to muscle invasive disease in a significant number of patients, making early RC a reasonable course of action to consider (2). In one of the earliest series regarding HGT1 bladder cancer, the authors reported a 53% progression rate at 15 years of follow-up with 34% of patients ultimately dying of urothelial carcinoma (3). Other series have demonstrated progression rates ranging from 25 - 56% (4-7).

Understanding the natural history of HGT1 bladder cancer is essential to guiding therapy and creating treatment algorithms that incorporate bladder-sparing protocols along with RC. HGT1 bladder cancer represents a heterogeneous disease with varying phenotypes and outcomes, and currently no test or validated scoring system exists to predict which patients would benefit most from an early RC (8). Thus, this study re-examines the natural history of HGT1 bladder cancer by analyzing the recurrence and progression rates of a large cohort of patients initially diagnosed with HGT1 disease to determine if, in fact, the high incidence of disease progression and death due to bladder cancer is still witnessed in a more contemporary treatment era.

METHODS AND MATERIALS

To identify patients with HGT1 or under the older classification, Grade 3 T1 bladder cancer, two independent databases of non-muscle invasive bladder cancer patients were queried, one at the Atlanta Veteran Affairs Medical Center (AVAMC), the other at the University of Pennsylvania. Institutional Review Board approval was obtained at each institution. Patients identified were diagnosed and treated from 1980 - 2012. Patient demographic, clinical, and pathological variables were examined. Patients were excluded if they had muscle-invasive disease, pure CIS, Ta, or low-grade disease. This produced a total of 222 patients with HGT1 disease to be reviewed, 151 from the University of Pennsylvania database and 71 from the AVAMC.

Biopsies of the bladder distant to the tumor were done at the surgeon’s discretion and pathology was uniformly reported. Tumor characteristics such as tumor size, location, and multifocality were not uniformly reported and therefore were not included in our analysis. Patients were followed with endoscopic surveillance every 3 months for 2 years, 6 months until 5 years, and annually thereafter. Primary outcomes measured were recurrence and progression to muscle invasive disease. Recurrence was defined as any tumor present after initial complete resection at any surveillance point. Stage progression was defined as muscle invasive pathology at any surveillance point. However, patients with MIBC on restaging resection were considered to have MIBC at the time of their initial TUR; they were not considered to represent progression of HGT1 disease and were excluded from the analysis. Data was analyzed with Stata® software and statistics were described with Kaplan Meier curves.

All patients with HGT1 disease were reported in the analysis, including those who received definitive surgery with radical cystectomy prior to the occurrence of muscle invasion. This becomes important during the discussion of these patients being a cohort exposed to a more contemporary treatment era.

RESULTS

A total of 222 patients with HGT1 bladder cancer were identified from the two databases. Patient clinical and demographic data are presented in Table-1. One hundred ninety-eight (89.1%) and 199 (89.6%) of the patients were male and Caucasian, respectively. Mean patient age and pack-years smoking were 66.5 years (range = 29-93 years) and 37.3 (0-125). One hundred ninety-one (86.0%) of the patients presented with isolated high-grade T1 disease, and 31 (14.0%) patients had high-grade T1 disease with concomitant CIS. Two hundred and twelve (95.5%) patients presented with pure urothelial histology while 10 (4.5%)
patients had histological variants including micropapillary, sarcomatoid, and squamous differentiation. Peri-operative mitomycin C was used for 41 patients (18.6%); induction BCG was utilized in 175 (78.8%) patients and the mean number of BCG treatments was 5.80 (range = 0-28). One patient received induction mitomycin C (Table-2).

At a mean follow-up of 50.8 months (median = 32.5 months, range = 2.2-261.2 months), recurrence occurred in 112 (50.5%) patients. The mean number of recurrences was 1.28 (range = 1-10 recurrences) with a mean and median time to recurrence of 28.8 and 12.9 months, respectively.

Progression to muscle-invasive disease occurred in only 19 (8.6%) patients with a mean and median time to progression of 16.6 and 17.2 months. Kaplan Meier curves for recurrence-free and progression-free survival estimates are displayed in Figures 1 and 2.

At last follow-up, the overall survival of the entire cohort was 76.6%, 170 patients. Only 52 (23.4%) patients had died, 13 (25.0% of deaths, 5.9% of entire cohort) of whose deaths were related to bladder cancer. The remaining 39 deaths (75.0% of deaths, 17.6% of entire cohort) were attributable to patient competing co-morbidities.

Table 1 - Clinical and Demographic Characteristics at Presentation of Patients with High-Grade T1 Bladder Cancer.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender (Men)</strong></td>
<td>198 (89)</td>
</tr>
<tr>
<td><strong>Mean (median) age ± SD, (range)</strong></td>
<td>66.5 (66.8) ± 11.28, (29.2-93)</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>199 (89.6)</td>
</tr>
<tr>
<td>Other</td>
<td>23 (10.4)</td>
</tr>
<tr>
<td><strong>Mean (median) ± SD BMI, (range)</strong></td>
<td>27.5 (27.1) ± 5.3, (16.6 - 48.1)</td>
</tr>
<tr>
<td><strong>Mean (median) CCI</strong></td>
<td>2.63 (2)</td>
</tr>
<tr>
<td><strong>Mean/median pack-year smoking, ± SD (range)</strong></td>
<td>37.3 (35.5) ± 26.9 (0 - 125)</td>
</tr>
<tr>
<td><strong>Stage</strong></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>191 (86.0)</td>
</tr>
<tr>
<td>T1 + CIS</td>
<td>31 (14.0)</td>
</tr>
<tr>
<td><strong>Histology</strong></td>
<td></td>
</tr>
<tr>
<td>Urothelial</td>
<td>212 (95.5)</td>
</tr>
<tr>
<td>Other</td>
<td>10 (4.5)</td>
</tr>
<tr>
<td><strong>Intravesical Therapy</strong></td>
<td></td>
</tr>
<tr>
<td>Peri-operative mitomycin C (1 dose)</td>
<td>41 (18.6)</td>
</tr>
<tr>
<td>Induction BCG</td>
<td>175 (78.8)</td>
</tr>
<tr>
<td>Induction mitomycin C</td>
<td>1 (1.4)</td>
</tr>
<tr>
<td>Mean # BCG Treatments (range)</td>
<td>5.80 (0-28)</td>
</tr>
</tbody>
</table>
Table 2 - Recurrence, Progression, and Cause of Death.

<table>
<thead>
<tr>
<th>Event</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recurrence</td>
<td>112 (50.5%)</td>
</tr>
<tr>
<td>Mean No. of Recurrences</td>
<td>1.28 (Range = 1-10)</td>
</tr>
<tr>
<td>Mean/median time to recurrence</td>
<td>28.8 (12.9) months</td>
</tr>
<tr>
<td>Progression</td>
<td>19 (8.6%)</td>
</tr>
<tr>
<td>Mean/median time to progression</td>
<td>16.6 (17.2) months</td>
</tr>
<tr>
<td>Alive</td>
<td>170 (76.6%)</td>
</tr>
<tr>
<td>Dead</td>
<td>52 (23.4%)</td>
</tr>
<tr>
<td>Bladder Cancer-Related Death</td>
<td>13 (25.0%)</td>
</tr>
<tr>
<td>Other Cause of Death</td>
<td>39 (75.0%)</td>
</tr>
<tr>
<td>Mean Follow-up</td>
<td>50.8 months</td>
</tr>
<tr>
<td>Median Follow-up</td>
<td>32.5 months</td>
</tr>
<tr>
<td>Follow-up Range</td>
<td>2.2-261.2 months</td>
</tr>
</tbody>
</table>

DISCUSSION

Consistent with earlier reports demonstrating three to five year progression rates below 10% (9,10), our data shows an 8.6% rate of progression to muscle-invasive disease at a mean follow-up of over four years. This data contrasts the largest prospective studies, which report progression rates of 29% at five years, and 53% at 15 years (3,5). Notably, a majority of the patients who progressed in our study presented with variant histology. Additionally, in our large contemporary cohort, 13 patients (5.9%) died due to bladder cancer. This present data may argue for a more conservative approach for patients with HGT1 disease in the absence of high-risk features for progression such as variant histology and CIS.

The high rates of progression and mortality seen in past studies have spurred clinicians to advocate early definitive, radical surgery for patients with HGT1 disease, particularly if they have certain high-risk features (2,8,11,12). Despite no randomized trials, multiple retrospective studies have shown advantages for disease-specific and overall survivals in patients treated with RC before muscle invasion occurs (13-16). In these series, reasons to advocate for early RC include residual T1 disease at re-staging resection, extensive multifocal disease, large tumor volume, location making endoscopic management difficult, variant histology, and presence of CIS (2,5,11-13,17,18). Nevertheless, it is important to note that in these prior series, clinical understaging was prevalent in these patients treated with early RC (13,17), indicating, perhaps, that a portion of the survival benefit seen in patients undergoing early cystectomy is really a function of a poor clinical staging of bladder cancer.
To appreciate the lower progression rate seen in our series, the patient cohort must be viewed in the context of contemporary developments in the management of HGT1 bladder cancer. Increasingly, clinicians routinely perform a restaging TUR for patients with HGT1 bladder cancer followed by induction and maintenance BCG (1). High incidences of clinical understaging (34–64%) have been cited to support this recommendation (11,19-22). Restaging TUR and identification of patients with clinical T2 disease that should be treated with RC shows a substantial advantage for recurrence and progression free survival (23). Furthermore, restaging TUR before BCG has been shown to improve the response to the intravesical immunotherapy, lowering the observed recurrence and progression rates from 57% and 34% to 29% and 7%, respectively, at 3 year follow-up (9). Thus, the routine use of restaging TUR has two effects on the progression rate of patients with HGT1 disease: first, it selects out patients with clinical T2 disease, removing them from the HGT1 cohort and improving the measured progression rate. Secondly, restaging TUR should resect all remaining visible tumor and thereby improve the response to BCG therapy, further improving the progression rate for patients with HGT1 disease.

Our contemporary cohort also likely benefited from more sophisticated pathologic examinations as histologic variants with more aggressive phenotypes requiring more aggressive treatment have become recognized (24). For example, the presence of micropapillary histology, in particular, has been shown to be a poor prognostic factor for progression and disease free survival, and these patients deserve consideration for RC before HGT1 disease (25). The high incidence of these variant histologies was established with a recent large series showing variant histology was present in 19.9% of transurethral biopsies (24). It appears that these aggressive variant histologies are being identified more routinely, and patients are now more likely to be offered early RC and less likely to be managed with bladder sparing therapy, potentially improving progression rates.

This study has a number of limitations. It is a retrospective review open to significant selection bias. As previously discussed, restaging TUR, recognition of histologic variants, and an increased willingness of clinicians to manage worrisome high-risk patients with RC distorts this patient cohort and potentially makes it more an analysis of low-risk HGT1 patients. Importantly, this analysis was done with a relatively short follow up time compared to the prior report that established the natural history of HGT1 bladder cancer (3). At further follow-up, our reported progression rates may increase, nullifying these promising rates of prevention of progression to muscle-invasive disease. Furthermore, there are many tumor factors with important prognostic implications that are not accounted for in this study, making it difficult to characterize and compare the biology of this cohort with previous cohorts. Additionally, as restaging TUR has only recently become routine, our databases poorly captured which patients received this procedure, introducing uncertainty in the analysis of progression versus inadequate primary resection. Moreover, our database poorly captured which patients were treated with radical cystectomy, the time of surgical intervention, and also does not contain the pathologic outcomes of these surgeries.

Nevertheless, it is important to contextualize these results in that many patients diagnosed with HGT1 bladder cancer do not have a 15-year life expectancy. Thus, it is imperative to weigh the expected risk of disease progression against the morbidity and mortality of surgery in the context of a patient’s life expectancy, considering one’s co-morbidities and goals of care.

CONCLUSIONS

At approximately 4 years of follow-up, the progression rate to muscle-invasive disease in patients initially presenting with high-grade T1 bladder cancer is only 8.6%. Although potentially subject to selection biases, these results from a large, contemporary patient cohort appears promising, arguing against the routine use of early cystectomy. Clearly further follow-up is needed, however when one considers the potential morbidity and mortality of radical cystectomy, the progression rate reported here is acceptable, especially when risk-stratified against an older and co-morbid individual.
ABBREVIATIONS

HGT1 = High-grade T1
NMIBC = Non-muscle invasive bladder cancer
AVAMC = Atlanta Veterans’ Administration Medical Center
CIS = Carcinoma in situ
BCG = Bacillus Calmette-Guerin
RC = Radical cystectomy
TUR = Transurethral resection

CONFLICT OF INTEREST

None declared.

REFERENCES


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Prognostic significance of the epithelial-to-mesenchymal transition markers e-cadherin, vimentin and twist in bladder cancer

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ABSTRACT

Objective: The goal of this study was to utilize long-term patient follow-up to determine whether epithelial-to-mesenchymal transition (EMT)-related markers can predict bladder cancer patient survival and progression of disease.

Materials and Methods: This study included 121 patients with bladder cancer. Sixty-four of these patients presented with non-muscle invasive (NMI, stage T1) bladder cancer and 57 with muscle invasive (MI, stage T2, T3). The patients were diagnosed and treated between May 1998 and July 2012. The EMT markers E-cadherin, Twist, and Vimentin were detected via immunohistochemistry. Univariate and multivariate/Cox analyses were then utilized to determine whether these EMT markers could be useful prognostic markers for predicting bladder cancer patient outcomes.

Results: Analysis of the 121 bladder cancer patients in this study revealed that the frequency of E-cadherin expression was 59.5% (72/121), Twist was 54.5% (66/121), and Vimentin was 24.8% (30/121). Twist and Vimentin were found to have statistically significant correlations with grade, recurrence, and progression but not with stage, whereas E-cadherin was associated with stage but not with the other parameters. In the univariate analysis, grade (p = 0.02) was the only significant predictor for progression-free survival (PFS). Stage, grade, and expression of E-cadherin, Vimentin and Twist were included in the multivariate analysis of predicting PFS. In this analysis, grade (p = 0.01) and Vimentin expression (p = 0.001) were found to be significant prognostic factors in predicting PFS.

Conclusions: Grade and Vimentin are potential independent indicators in predicting bladder cancer progression and survival.

INTRODUCTION

Bladder cancer is the second most common malignancy of the urinary tract with 386,000 cases and 150,000 estimated deaths in the United States in 2011 (1). In Europe, approximately 36,500 men and 13,000 women die of this malignancy every year. Bladder cancer can originate in different parts of the bladder, and patient outcomes can be unpredictably variable. Initially, 70-80% of patients present with noninvasive (NI) (pTa) or superficially invasive (SI) (pT1) disease whereas 20-30% are diagnosed with muscle invasive (MI) bladder cancer (pT2-4) (2). Problematic
inconsistencies and errors exist in the diagnosis and treatment. Over-staging (40%) and under-staging (nearly 20%) of clinically localized lesions occur in the diagnosis of bladder cancer (3). Misdiagnosis can lead to improper treatment and an inaccurate prognosis. Therefore, more research is needed to determine how to accurately stage bladder cancer prior to radical cystectomy and to predict the prognosis.

Metastasis is the main cause of death from bladder cancer, and micrometastasis, as the first step of tumor metastasis, appears to be extremely important (4). Epithelial-to-mesenchymal transition (EMT) is a key step during embryonic development and epithelial tumor metastasis (5). EMT causes changes in cell-cell and cell-extracellular matrix interactions resulting in transmigration of cancer cells, thus leading to metastasis (6,7). EMT changes occur at the molecular level before changes in cancer morphology are observed, as has been shown in B-cell lymphoma (8) and melanoma (9); hence utilization of these early molecular changes may be advantageous in predicting the prognosis of bladder cancer (10).

Therefore, to ascertain new prognostic factors, a long-term follow-up study for patients with bladder cancer was conducted whereas the value of EMT biomarkers in predicting prognosis of this malignancy were assessed through the EMT indices E-cadherin, Twist, and Vimentin. E-cadherin is a cell-cell junction protein that is frequently downregulated or lost during EMT, whereas expression of Vimentin and Twist are acquired during this process.

**MATERIALS AND METHODS**

**Patients’ characteristics**

A retrospective review was performed for 121 bladder cancer patients who were diagnosed and treated in the Department of Urology at the Affiliated Hospital of Qingdao University Medical School (P. R. China) from May 1998 to July 2007. The cut-off for follow-up data was July 2012. Some patients with MI underwent radical cystectomy and those with superficially invasive bladder cancer underwent transurethral resection (TUR) followed by prophylactic intravesical chemotherapy. The operative method was selected according to the results of cystoscopy and biopsy in combination with the expressed desires of the patient. Fifty-eight low grade papillary carcinoma patients and 36 high grade papillary carcinoma patients underwent TUR-BT (transurethral resection of bladder tumor), six low grade papillary carcinoma patients and six high grade papillary carcinoma patients underwent partial cystectomy, and three low grade papillary carcinoma patients and 12 high grade papillary carcinoma patients underwent radical cystectomy (RC). Thirty-five of the patients (14 low grade; 21 high grade) who underwent TUR-BT, nine of the patients (four low grade; five high grade) who underwent partial cystectomy, and 13 of the patients (three low grade; 10 high grade) who underwent RC were certified as MI post-operation. Fifty-nine of the patients (44 low grade; 15 high grade) who underwent TUR-BT, three of the patients (two low grade; one high grade) who underwent partial cystectomy, and two of the patients (zero low grade; two high grade) who underwent RC were certified as NMI after operation. All of the patients who were diagnosed with MI bladder cancer after TUR-BT refused subsequent operations. The mean (range) age of the 121 patients was 67 (29-95) years-old. Table-1 shows the general characteristics of the patients. The tissues were analyzed via immunohistochemistry at the first operation. Tissue slides were reviewed by genito-urinary pathologists. Staging was determined according to the 2002 tumor, lymph nodes, metastasis (TNM) classification of the International Union Against Cancer, and grading was assessed following the 2004 World Health Organization/International Society of Urological Pathology standard.

**Immunohistochemistry**

A formalin-soaked, paraffin-encapsulated tissue block was cut into 4µm-thick sections, which were placed on slides. Some of the slides were stained with standard haematoxylin and eosin (H&E). For immunohistochemistry, the paraffin sections were first dewaxed in xylene and rehydrated in decreasing concentrations of alcohol. To restore the antigen, the slides were placed in 0.01mol/L citrate buffer (pH 6.0) and heated in
Table 1 - Clinical and pathological data of the bladder tumors.

<table>
<thead>
<tr>
<th></th>
<th>No. of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>31 (25.6)</td>
</tr>
<tr>
<td>Male</td>
<td>90 (74.4)</td>
</tr>
<tr>
<td>Multiplicity</td>
<td></td>
</tr>
<tr>
<td>Solitary</td>
<td>95 (78.5)</td>
</tr>
<tr>
<td>Multiple</td>
<td>26 (21.5)</td>
</tr>
<tr>
<td>Tumor size (diameter)</td>
<td></td>
</tr>
<tr>
<td>≤ 3cm</td>
<td>89 (73.6)</td>
</tr>
<tr>
<td>&gt; 3cm</td>
<td>32 (26.4)</td>
</tr>
<tr>
<td>Stage (TNM 2002)</td>
<td></td>
</tr>
<tr>
<td>pT1</td>
<td>64 (52.9)</td>
</tr>
<tr>
<td>pT2</td>
<td>46 (38)</td>
</tr>
<tr>
<td>pT3</td>
<td>11 (9.1)</td>
</tr>
<tr>
<td>Grade (WHO 2004)</td>
<td></td>
</tr>
<tr>
<td>Papillary carcinoma, low grade</td>
<td>65 (53.7)</td>
</tr>
<tr>
<td>Papillary carcinoma, high grade</td>
<td>56 (46.3)</td>
</tr>
<tr>
<td>Surgical procedure</td>
<td></td>
</tr>
<tr>
<td>TUR</td>
<td>94 (77.7)</td>
</tr>
<tr>
<td>Cystectomy</td>
<td>27 (12 partial, 15 total) (22.3)</td>
</tr>
<tr>
<td>Progression</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>41 (33.9)</td>
</tr>
<tr>
<td>pT1</td>
<td>22 (53.7)</td>
</tr>
<tr>
<td>pT2</td>
<td>14 (34.1)</td>
</tr>
<tr>
<td>pT3</td>
<td>5 (12.2)</td>
</tr>
<tr>
<td>Recurrence</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>49 (40.5)</td>
</tr>
<tr>
<td>pT1</td>
<td>18 (36.7)</td>
</tr>
<tr>
<td>pT2</td>
<td>22 (44.9)</td>
</tr>
<tr>
<td>pT3</td>
<td>9 (18.4)</td>
</tr>
<tr>
<td>Death</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>55 (45.5)</td>
</tr>
<tr>
<td>pT1</td>
<td>21 (38.2)</td>
</tr>
<tr>
<td>pT2</td>
<td>24 (43.6)</td>
</tr>
<tr>
<td>pT3</td>
<td>10 (18.2)</td>
</tr>
</tbody>
</table>

the microwave (100°C) twice for five min each time. Endogenous peroxidase activity was blocked by placing the slides in 3% hydrogen peroxide for 15 min. at room temperature. This was followed by three min washes with phosphate buffered saline (PBS). The tissue sections were placed in a 4°C humidified chamber with primary antibody. The rabbit polyclonal E-cadherin antibody, the goat polyclonal Twist antibody and the mouse monoclonal Vimentin antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were diluted 1:200, 1:100 and 1:100, respectively. After washing with PBS, the sections were incubated with secondary antibody (Zhongshan Golden Bridge Biotechnology, Beijing, China) for 30 min. and washed twice for five min with PBS. The sections were stained with diaminobenzidine tetrahydrochloride, then counterstained with haematoxylin, dehydrated, and covered with a cover slip. For the negative control, the primary antibody was replaced with PBS. The positive control was supplied with the kit (Santa Cruz Biotechnology).

Digital image analysis

All samples were reviewed by an independent investigator who was blinded to the clinical outcomes of the patients. The stained sections were reviewed using an Olympus microscope (Olympus Corporation, Tokyo, Japan) and digital images were captured and processed in TIFF format. Image Pro Plus 6.0 (Media Cybernetics Inc., Bethesda, MD, USA) was used to calculate the intensity of the detected molecules. Six microscopic fields within the tumor tissue sections (original magnification 40X) were randomly selected and the integral optical density (IOD) was calculated for each image. All IOD values were divided into four quartiles as follows: 0-25% negative expression, 25-50% weak expression, 50-75% moderate expression, and 75-100% strong expression. The sample was considered to be positive when the IOD ≥ 50% (11).

Statistical analysis

The survival time of patients who underwent TUR, partial cystectomy or RC for different-stage bladder cancer was analyzed. The time
points for TUR or partial cystectomy were from the last surgery to either the date of recurrence, date of progression or the date of final follow-up, whereas the time points for RC were from the operation date to either the date of death or the date of the last follow-up.

Overall survival (OS) was defined as from the day of surgery to either the day of death from any cause or censored at the time of the last follow-up, whereas progression-free survival (PFS) was defined as from the day of surgery to either the day of progression of the cancer or to the day of follow-up termination. Increases in T stage or in grade (from low to high) were considered indicators of mean progression. The Chi-squared test was used to determine the correlation between Vimentin, Twist and E-cadherin expression and sex, pathology, stage and grade. One-factor analysis was performed using the log-rank test. The Kaplan-Meier method was utilized and Kaplan-Meier curves were constructed. A multivariable analysis was performed using the Cox proportional hazard regression model. P < 0.05 was considered to be statistically significant. All of the tests were two-sided. Statistical Product and Service Solutions version 17.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis.

RESULTS

One-hundred-and-twenty-one patients were included in this study with a median follow-up time ranging from 60-180 months (median of 72 months). Fifty-five of the patients (45.5%) died during the follow-up period, 49 (40.5%) recurred, and 41 (33.9%) progressed (Table-1). Seventy-two (59.5%) of the 121 tissue specimens examined were positive for E-cadherin expression via immunohistochemical analysis, 66 (54.5%) were positive for Twist, and 30 (24.8%) were positive for Vimentin (Table-2 and Figure-1). The IOD of E-cadherin revealed higher expression in low grade papillary carcinoma (159105 ± 42036) than in high grade papillary carcinoma (15369 ± 4762), whereas Vimentin (17262 ± 5942 low grade, 71056 ± 27324 high grade) and Twist (24996 ± 7764 low grade, 86991 ± 19683 high grade) demonstrated the opposite expression pattern.

Three patients had previous TUR in a different hospital (3, 4 and 8 months, respectively) before admission to our hospital to undergo RC for recurrent bladder cancer. Their molecular profiles were E-cadherin negative, Twist positive, and Vimentin positive. These patients died at six, nine, and 10 months, respectively, after surgery in our hospital. These results are consistent with high grade papillary carcinoma having undergone EMT, thus losing expression of E-cadherin and gaining expression of the EMT markers Vimentin and Twist.

The Chi-squared test was used to evaluate the correlation of Vimentin, Twist, and E-cadherin expression with sex, pathology, stage, grade, recurrence and progression. The expression of both Twist and Vimentin were found to have statistically significant correlations with grade, recurrence and progression. In contrast, E-cadherin expression was found to have a statistically significant correlation with stage (Table-2, Figure-1). Among the patients diagnosed with pT2 bladder cancer, 22 patients recurred, 14 patients progressed, and 24 patients died. Sixty-eight-point-two percent (15/22) of the patients who recurred had tumors that stained positively for E-cadherin and 56.5% (13/23) were E-cadherin negative, 42.8% (6/14) of the patients who progressed had E-cadherin positive tumors compared to 68.8% (22/32) E-cadherin negative, and 58.3% (14/24) of the patients who died had E-cadherin positive tumors whereas 59.1% (13/22) were E-cadherin negative. Sixty-eight-point-two percent of the patients who recurred had tumors positive for Twist and 26.1% (6/23) were Twist negative, 92.9% (13/14) of the patients who experienced disease progression had Twist positive tumors whereas 46.9% (15/32) were Twist negative, and 66.7% (16/24) of patients who died had Twist positive tumors compared to 45.5% (10/22) that were negative. Finally, 36.4% (8/22) of patients who recurred had Vimentin positive tumors with 17.4% (4/23) being negative, 64.3% (9/14) of patients who progressed had tumors positive for Vimentin whereas 21.9% (7/32) were Vimentin negative, and 41.7% (10/24) patients who died had Vimentin positive tumors compared to 27.3% (6/22) that were Vimentin negative. E-cadherin, Twist and Vimentin expression exhibited statistically significant differences in the recurrence
Table 2 - Relationship of Twist, Vimentin, and E-cadherin expression with clinicopathological parameters.

<table>
<thead>
<tr>
<th></th>
<th>E-cadherin</th>
<th>Twist</th>
<th>Vimentin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive (%)</td>
<td>Negative (%)</td>
<td>p</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>56 (77.8)</td>
<td>34 (68)</td>
<td>0.3</td>
</tr>
<tr>
<td>Female</td>
<td>16 (22.2)</td>
<td>15 (32)</td>
<td></td>
</tr>
<tr>
<td><strong>Age in years</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 70</td>
<td>55 (76.4)</td>
<td>38 (77.6)</td>
<td>0.88</td>
</tr>
<tr>
<td>&gt; 70</td>
<td>17 (23.6)</td>
<td>11 (22.4)</td>
<td></td>
</tr>
<tr>
<td><strong>Stage, TNM 2002</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pT1</td>
<td>50 (69.4)</td>
<td>14 (28.6)</td>
<td></td>
</tr>
<tr>
<td>pT2</td>
<td>20 (27.8)</td>
<td>26 (53.0)</td>
<td></td>
</tr>
<tr>
<td>pT3</td>
<td>2 (2.8)</td>
<td>9 (18.4)</td>
<td>0.0007</td>
</tr>
<tr>
<td><strong>Grade, WHO 2004</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Papillary carcinoma, low grade</td>
<td>42 (58.3)</td>
<td>23 (46.9)</td>
<td></td>
</tr>
<tr>
<td>Papillary carcinoma, high grade</td>
<td>30 (41.7)</td>
<td>26 (53.1)</td>
<td>0.22</td>
</tr>
<tr>
<td><strong>Recurrence</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>YES</td>
<td>34 (47.2)</td>
<td>15 (32)</td>
<td>0.07</td>
</tr>
<tr>
<td>NO</td>
<td>38 (52.8)</td>
<td>34 (68)</td>
<td></td>
</tr>
<tr>
<td><strong>Progression</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YES</td>
<td>21 (29.2)</td>
<td>20 (40.8)</td>
<td>0.18</td>
</tr>
<tr>
<td>NO</td>
<td>51 (70.8)</td>
<td>29 (59.2)</td>
<td></td>
</tr>
<tr>
<td><strong>Death</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>YES</td>
<td>32 (44.4)</td>
<td>23 (46.9)</td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>40 (55.6)</td>
<td>26 (53.1)</td>
<td>0.79</td>
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<tr>
<td><strong>E-cadherin</strong></td>
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</tr>
<tr>
<td>Positive</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td><strong>Twist</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>–</td>
<td>–</td>
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</tr>
<tr>
<td>Negative</td>
<td>–</td>
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</tr>
</tbody>
</table>
versus non-recurrence, progression versus non-progression, and death versus no death groups, with the exception of E-cadherin expression in the progression versus non-progression group (Table-3).

In the univariate analysis, the time point for PFS/OS was set for 60 months and grade was found to be the only significant predictor for PFS. The others indices were not found to be significant predictors of PFS and OS (Table-4, Figure-1). To further investigate the relationship between survival and molecular profile and abate the inter-effect among the indices, Cox regression analysis was performed using the following parameters: stage, grade and expression of Twist, E-cadherin and Vimentin. Grade (p = 0.01) and Vimentin (p < 0.001) expression were found to be significantly correlated with PFS in this analysis.

**DISCUSSION**

Bladder cancer is a common malignancy with an estimated 70,530 new cases in the United...
Table 3 - Expression of E-cadherin, Twist and Vimentin in the recurrence, progression and death groups.

<table>
<thead>
<tr>
<th></th>
<th>Recurrence</th>
<th>Non-recurrence</th>
<th>p</th>
<th>Progression</th>
<th>Non-progression</th>
<th>p</th>
<th>Death</th>
<th>Non-death</th>
<th>p</th>
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<tbody>
<tr>
<td><strong>E-cadherin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>pT1</td>
<td>13</td>
<td>5</td>
<td>24</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>pT2</td>
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<td>7</td>
<td>13</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pT3</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0.037</td>
<td>1</td>
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<td></td>
</tr>
<tr>
<td>pT1</td>
<td>12</td>
<td>6</td>
<td>9</td>
<td>37</td>
<td></td>
<td>10</td>
<td>12</td>
<td>19</td>
<td>24</td>
</tr>
<tr>
<td>pT2</td>
<td>15</td>
<td>7</td>
<td>6</td>
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<td></td>
<td>13</td>
<td>1</td>
<td>15</td>
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<td>pT3</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0.024</td>
<td>5</td>
<td>0</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td><strong>Vimentin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pT1</td>
<td>3</td>
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<td>10</td>
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<td>18</td>
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<td>19</td>
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<td>5</td>
<td>7</td>
<td>25</td>
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<td>pT3</td>
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<td>0.010</td>
<td>3</td>
<td>2</td>
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Table 4 - Survival analyses of various clinicopathological parameters.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Kaplan-Meier analysis</th>
<th>Cox regression analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 - year survival (%)</td>
<td>5 - year survival (%)</td>
</tr>
<tr>
<td></td>
<td>n = 80</td>
<td>n = 76</td>
</tr>
<tr>
<td>Age in years</td>
<td></td>
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</tr>
<tr>
<td>≤ 70</td>
<td>61 (76.3)</td>
<td>58 (76.3)</td>
</tr>
<tr>
<td>&gt; 70</td>
<td>19 (23.7)</td>
<td>18 (23.7)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>62 (77.5)</td>
<td>61 (80.3)</td>
</tr>
<tr>
<td>Female</td>
<td>18 (22.5)</td>
<td>15 (19.7)</td>
</tr>
<tr>
<td>Stage, TNM 2002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pT1</td>
<td>42 (52.5)</td>
<td>43 (56.6)</td>
</tr>
<tr>
<td>pT2</td>
<td>32 (40.0)</td>
<td>29 (38.2)</td>
</tr>
<tr>
<td>pT3</td>
<td>6 (7.5)</td>
<td>4 (5.2)</td>
</tr>
<tr>
<td>Grade, WHO 2004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papillary carcinoma, low grade</td>
<td>47 (58.7)</td>
<td>45 (59.2)</td>
</tr>
<tr>
<td>Papillary carcinoma, high grade</td>
<td>33 (41.3)</td>
<td>31 (40.8)</td>
</tr>
<tr>
<td>E-cadherin</td>
<td></td>
<td></td>
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<tr>
<td>Positive</td>
<td>48 (60.0)</td>
<td>41 (53.9)</td>
</tr>
<tr>
<td>Negative</td>
<td>32 (40.0)</td>
<td>35 (46.1)</td>
</tr>
<tr>
<td>Twist</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>43 (53.8)</td>
<td>36 (47.4)</td>
</tr>
<tr>
<td>Negative</td>
<td>37 (46.2)</td>
<td>40 (52.6)</td>
</tr>
<tr>
<td>Vimentin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>19 (23.8)</td>
<td>9 (11.8)</td>
</tr>
<tr>
<td>Negative</td>
<td>61 (76.2)</td>
<td>67 (88.2)</td>
</tr>
</tbody>
</table>
States in 2010 (12). Most of the tumors initially present as NI or SI (i.e. pTa or pT1), so TUR is performed. Over 70% of these cases recur and require re-operation, of which, about 10-15% will advance to MI (13). Therefore, it is necessary to be able to accurately stage and predict the prognosis of this malignancy.

Molecular diagnostics are routine clinical practice in patients with lung, colon, and breast cancer. Bladder cancer, a heterogeneous disease with diverse genetic and environmental risk factors, currently lacks useful molecular biomarkers. Therefore, identifying biomarkers that can accurately predict recurrence, progression and outcome are needed. It is important that we find and validate prognostic molecular biomarkers that can help clinicians to identify patients in need of early, aggressive management (14,15).

Errors in clinical staging and pathologically grading of this malignancy frequently occur, thus procedures for staging and grading need to be improved (16). One problem that plays a role in bladder cancer staging is burning of the tissue during cauterization. Tissue burnt during electro-resection makes it very difficult to determine whether the stroma or superficial muscular layer of the bladder has been invaded, thus complicating staging of the cancer. To avoid this, energy during electrotomy should be kept to a minimum (17). To cure a patient with primary NMI bladder cancer, a high quality TUR that removes all (pre)malignant lesions must be performed (18). Over-staging typically occurs if muscle tissue is not included in the first-time excisional specimens of a T1 lesion (19). Computed tomography and magnetic resonance imaging cannot discriminate the various layers (lamina propria, superficial and deep muscle) of the bladder, therefore clinical classification of stages T1, T2a and T2b is nearly impossible (20). Due to the limitations of classic clinicopathological indices and imaging manifestations, other prognostic factors (carcinoma in situ, hydronephrosis) and molecular markers that are under investigation are promising as repeatable and predictive factors in the diagnosis of bladder cancer (21,22). Our data demonstrate that molecular changes in bladder cancer tissue are not significantly affected by outside factors (such as the method of operation or the experience of the operator) and more objectively reflect the characteristics of the cancer.

In the current study, stage, grade and expression of three EMT-related molecular markers were compared during the 60-180 month follow-up period. Grade was found to be more effective than stage in predicting progression and recurrence, and it correlates more efficiently with tumor markers than stage. This may be because diagnosis of stage is more prone to error due to cauterization of tissue than the diagnosis of grade.

EMT is a critical step during cancer progression in which downregulation or loss of E-cadherin expression (epithelial marker) occurs, making E-cadherin a potential biomarker. Downregulation or loss of E-cadherin is often accompanied by upregulation of N-cadherin, Twist, and Vimentin (mesenchymal markers) during EMT, thus making these factors attractive potential markers also (23). Various EMT related markers are used as prognostic markers in other types of cancers (7,10,24,25). In this study, the relationships between three EMT-related molecular markers and five year PFS and five year OS were investigated. Twist and Vimentin were significantly correlated...
with grade, progression, and recurrence, suggesting that these two factors would be useful biomarkers in the diagnosis and treatment of bladder cancer patients. Patients with NMI bladder cancer and expression of Twist or Vimentin would be treated with a more aggressive therapy regimen to prevent progression and recurrence.

Down-regulation or loss of E-cadherin is one of the initial molecular events in the process of EMT. This is followed by major changes in the cytoskeleton that enables cells to acquire a mesenchymal phenotype and subsequent increased motility and invasiveness. Cells undergoing EMT frequently acquire the expression of Vimentin and Twist. (26-28). The IOD of E-cadherin is higher in low grade papillary carcinoma than high grade papillary carcinoma, whereas Vimentin and Twist expression are lower in the low grade cancer and higher in the high grade papillary carcinoma. Khorrami et al. (29) examined E-cadherin expression in 180 patients with unifocal, superficial, low-grade, papillary transitional cell carcinoma of the bladder and followed these patients for 36 months after surgery. E-cadherin immunoexpression was negative in 101 (56%) and positive in 79 (44%) patients. Additionally, downregulation of E-cadherin was associated with disease recurrence in NMI bladder cancer. Our results show a similar trend whereby the IOD of E-cadherin in low grade papillary carcinoma was significantly higher than E-cadherin levels in high grade disease. In patients who experienced disease progression or died, E-cadherin expression was largely negative, while Twist and Vimentin expression was mostly positive. In the patients who recurred, no significant difference existed in E-cadherin expression; however, Twist and Vimentin remained primarily positive. These data support the idea that E-cadherin is down-regulated during EMT whereas Twist and Vimentin are up-regulated.

The limitations of the present study are that it is retrospective and immunohistochemical in nature. Clinical application of immunohistochemistry is limited by the following factors: discrepancies between different antibodies, diversity in interpretation and judgment, and inconsistency in specimen preparation and technical procedures. Another potential limitation of our study is that the different predictive value of staining between the nucleus and cytoplasm of E-cadherin, Twist, and Vimentin was not compared. Nonetheless, the results from this study indicate that these markers of EMT should be further investigated as markers to assist in the diagnosis, staging and treatment decisions for bladder cancer patients.

CONCLUSIONS

Bladder cancer is a multi-origin disease with variable outcomes. Despite the substantial advances made in biomedical research, the error of clinical grading and staging of bladder cancer still exists. Therefore, additional predictors such as molecular markers would be beneficial in improving the accuracy of staging and grading this malignancy. Our study shows that Vimentin has the potential to become an independent predictor for cancer progression and survival. In addition, we find that bladder tumor grade is better than stage at predicting progression and recurrence.

CONFLICT OF INTEREST

None declared.

REFERENCES


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Lipiodol as a Fiducial Marker for Image-Guided Radiation Therapy for Bladder Cancer


Department of Radiation Oncology (JMF, MCB, DCF, DCH, RBW); Department of Urology (PES); Department of Data Quality and Standards (EJS) and Department of Genitourinary Oncology (SG), Moffitt Cancer Center, Tampa, FL, USA

ABSTRACT

Purpose: To evaluate Lipiodol as a liquid, radio-opaque fiducial marker for image-guided radiation therapy (IGRT) for bladder cancer.

Materials and Methods: Between 2011 and 2012, 5 clinical T2a-T3b N0 M0 stage II–III bladder cancer patients were treated with maximal transurethral resection of a bladder tumor (TURBT) and image-guided radiation therapy (IGRT) to 64.8 Gy in 36 fractions ± concurrent weekly cisplatin-based or gemcitabine chemotherapy. Ten to 15mL Lipiodol, using 0.5mL per injection, was injected into bladder submucosa circumferentially around the entire periphery of the tumor bed immediately following maximal TURBT. The authors looked at inter-observer variability regarding the size and location of the tumor bed (CTV boost) on computed tomography scans with versus without Lipiodol.

Results: Median follow-up was 18 months. Lipiodol was visible on every orthogonal two-dimensional kV portal image throughout the entire, 7-week course of IGRT. There was a trend towards improved inter-observer agreement on the CTV boost with Lipiodol (p = 0.06). In 2 of 5 patients, the tumor bed based upon Lipiodol extended outside a planning target volume that would have been treated with a radiation boost based upon a cystoscopy report and an enhanced computed tomography (CT) scan for staging. There was no toxicity attributable to Lipiodol.

Conclusions: Lipiodol constitutes a safe and effective fiducial marker that an urologist can use to demarcate a tumor bed immediately following maximal TURBT. Lipiodol decreases inter-observer variability in the definition of the extent and location of a tumor bed on a treatment planning CT scan for a radiation boost.

INTRODUCTION

Maximal transurethral resection of a bladder tumor (TURBT) followed by external beam radiation therapy with concurrent chemotherapy constitutes a bladder-sparing treatment option for muscle-invasive bladder cancer. In terms of the radiation therapy, patients are typically treated to the whole bladder to 39.6–45.0 Gy followed by a boost to the tumor bed to a cumulative dose of 64.8–66.0 Gy using 1.8–2.0-Gy daily. In general, a bladder-sparing approach is performed in patients who are medically inoperable or elect not to undergo surgery. Reports show 47–87% complete
response rates with bladder-sparing treatment (1). Three-year to 5-year survival rates with an intact bladder have ranged from 37% to 66% (1).

Lipiodol (Lipiodol® Ultra-Fluide, Guerbet LLC, Bloomington, IN) consists of ethyl esters of iodized fatty acids of poppy seed oil. Lipiodol was the first iodinated contrast agent and has been in use since 1926. Possible side effects of Lipiodol include an allergic reaction, transient fever during the first few hours following injection, nausea, vomiting, or diarrhea.

Lipiodol may be injected into bladder submucosa circumferentially around the periphery of a tumor bed. The purpose of Lipiodol is to demarcate the tumor bed on a computed tomography (CT) scan to help with the planning of a radiation boost to part of the bladder.

Several groups have shown a high retention rate of Lipiodol in the bladder wall throughout a 6-week to 7-week course of radiotherapy and the feasibility of using Lipiodol as a radiopaque fiducial marker at sites throughout the bladder (2–5). Since Lipiodol is a liquid, it moves with bladder expansion and contraction. In contrast, Hulshof et al. (6) reported that half of the solid fiducial markers were lost a median of 11.5 days after implantation. Moreover, Mangar et al. (7) were not able to place gold fiducial seeds in the dome of the bladder.

A radiation oncologist normally creates a clinical target volume for a radiation boost (CTVboost) based upon information including a cystoscopy report and an enhanced CT scan of the pelvis that was obtained for staging (8). Jenkins et al. (9) have recommended that the CTVboost should include the tumor bed plus a 10-mm margin in patients with radiological evidence of extravesical disease. In patients with no evidence of extravesical disease, they have suggested that the CTVboost should include the tumor bed plus a 6-mm margin. Based upon these recommendations, the CTVboost would encompass microscopic disease extension in 90% of cases.

According to the Radiation Therapy Oncology Group, one may create a planning target volume for a radiation boost, PTVboost, by adding a 20-mm margin on a tumor bed, assuming that Lipiodol was not used in order to help define the tumor bed. This margin accounts for factors such as daily setup error and variation in the position of the tumor bed based upon bladder and rectal filling (10,11).

**MATERIALS AND METHODS**

**Patients**

After obtaining institutional review board approval to prospectively study Lipiodol, the authors reviewed the medical records of all 5 bladder cancer patients who had undergone Lipiodol-based image-guided radiation therapy (IGRT) at their center between January 1, 2011 and June 30, 2012. All patients had high grade, clinical T2a–T3b N0 M0 stage II–III urothelial carcinomas. Patients with thyroid disease or a history of iodine allergy did not receive Lipiodol.

**Lipiodol demarcation of the tumor bed**

Lipiodol demarcation of the tumor bed was performed under general anesthesia immediately following maximal TURBT. Patients underwent rigid cystoscopy with a 22 French cystoscope by an experienced urologist. There was no difficulty accessing any site within the bladder, including the dome or trigone, with a rigid cystoscope. A 23-gage Chiba-tip needle with a retractable, flexible sheath (Injekt® Cysto Flexible Injection Needle, Cook Medical, Inc., Bloomington, IN) to protect the cystoscope was inserted through the working canal to the tip of the cystoscope. Lipiodol, 0.5mL per injection, was placed into bladder submucosa 2–3mm from the resection margin in order to outline the entire periphery of the tumor bed. A cumulative total of 10–15mL Lipiodol was used, depending on the size of the tumor bed. Roughly 20–30 separate injections were usually administered to demarcate the tumor bed. Fluoroscopic guidance helped to ensure that demarcation of the tumor bed was complete.

**Bladder-sparing treatment**

All 5 patients underwent maximal TURBT followed a median of 3 weeks later by intensity modulated radiation therapy (IMRT)/IGRT. IMRT was delivered in order to minimize radiation doses to the recto-sigmoid colon (1). Patients underwent...
IMRT to the whole bladder (clinical target volume) plus a 20-mm margin, which represented the planning target volume (PTV), to 39.6 Gy in 22 fractions over 4½ weeks. Patients were instructed to have an empty bladder for IMRT to the whole bladder. Patients then underwent an IMRT boost to part of their bladder to a cumulative total dose of 64.8 Gy in 36 fractions over approximately 7 weeks. The tumor bed that had been defined with the help of Lipiodol plus a 15-mm margin constituted the PTV boost. Patients were instructed to have a full bladder for the IMRT boost. There was no break between the whole bladder and partial bladder irradiation. Prior to each radiotherapy fraction, anterior and right lateral kV portal images were obtained to set the patient up for IGRT. Four patients received weekly cisplatin-based or gemcitabine chemotherapy concurrently with the IMRT. One patient with a poor performance status due to multiple co-morbidities including angina pectoris underwent IGRT without chemotherapy.

Statistics

Three radiation oncologists at our center created a CTV boost on a CT scan that had been obtained to plan a radiation boost for 2 paired study groups wherein each patient served as a matched control: 1) a no-Lipiodol group, where the CTV boost was based only on a cystoscopy report and an enhanced CT scan of the pelvis that had been obtained pre-Lipiodol for staging, i.e., Lipiodol on the CT scan was ignored; and 2) a Lipiodol group, where the CTV boost was also based on Lipiodol.

We assessed inter-observer variability in demarcation of the tumor bed by 3 different radiation oncologists based on mean percent volume overlap (PVO) of CTV boost. A similar approach has been used to examine the impact of fiducial markers on inter-observer variability in terms of demarcation of a lumpectomy bed on radiotherapy treatment planning CT scans in breast cancer patients (12,13). PVOs of the CTV boost between Radiation Oncologists 1 and 2, Radiation Oncologists 1 and 3, and Radiation Oncologists 2 and 3 were obtained and then averaged. The mathematic formulation of this is mean \(((V_1 \cap V_2)/(V_1 \cup V_2), (V_1 \cap V_3)/(V_1 \cup V_3), (V_2 \cap V_3)/(V_2 \cup V_3))\). A mean value for the PVO was calculated for each patient. A Wilcoxon signed-rank test was used to compare the PVOs between groups.

We calculated means and standard deviations for the left-right (LR), cranial-caudal (CC), and anterior-posterior (AP) patient shifts to account for inter-fraction bladder motion (2,5).

Assessment of toxicity

The Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 grading scheme was used to evaluate the severity of urinary and gastrointestinal toxicity due bladder-sparing therapy.

RESULTS

Median follow up was 18 months. Median age at diagnosis was 56 years (range, 39 - 65 years). There was one biopsy-proven local relapse in the tumor bed. To date, one patient has died of metastatic disease, one patient is alive with metastatic disease, and 3 patients are alive without evidence of disease.

Lipiodol diffused throughout the tumor bed. Lipiodol was visible on every digitally reconstructed radiograph (Figure 1-A) and every kV image (Figure 1-B) throughout the entire, 7-week course of IGRT. There were no adverse events associated with Lipiodol injections. In terms of treatment-related toxicity, one patient experienced CTCAE version 4.0 acute grade 2 bladder spasms that responded to antispasmodics (Table-1). There was no acute grade \(\geq 3\) toxicity or chronic grade \(\geq 2\) toxicity.

Mean daily patient shifts are presented in Table-2. There was a mean ± standard deviation absolute shift of 2 ± 2mm in the LR direction, 4 ± 3mm in the CC direction, and 2 ± 2mm in the AP direction. There was a maximum shift of 14 mm in the CC direction in one patient. Shifts greater than 5 mm were made in 4% (LR), 27% (CC), and 8% (AP) of the total number of daily radiotherapy fractions for all patients.

Figure-2 shows the CTV boost with versus without Lipiodol. Lipiodol made it easier to define the extent and location of the tumor bed on a treatment planning CT scan for the radiation boost. The mean PVO was 53% with Lipiodol versus 39%
without Lipiodol (Table-3; p = 0.06). In two of 5 (40%) patients, the CTV boost based upon Lipiodol extended outside a planning target volume that would have been treated with a radiation boost based upon cystoscopy reports and enhanced CT scans for staging.

**DISCUSSION**

The European Association of Urology recommends either an enhanced CT scan or magnetic resonance imaging scan for staging of muscle-invasive bladder cancer (14). Radiation therapy for bladder cancer is commonly planned using an enhanced CT scan of the pelvis.

Bladder cancer is an excellent candidate for IGRT because of substantial motion of the tumor bed during a 6-7 week course of radiotherapy. In this study, bladder motion was most pronounced in the CC dimension (Table-2). Similarly, Meijer et al. (15) found that bladder cancer set up uncertainties varied by direction with the CC dimension having the largest variation. Cranial movement was greater than caudal movement. Also, Fokdal et al. (10) found that bladder movement was most pronounced in the CC direction. Filling volumes of the bladder and rectum had a large impact on bladder movement.

Various studies have examined bladder motion and made recommendations regarding margins on a tumor bed for a PTV boost. Several groups (4,15,16) have suggested that one should provide a 20-25-mm margin on a tumor bed in order to create a PTV boost when no Lipiodol is used to help demarcate the tumor bed. In contrast, Sondergaard et al. (5) have suggested that one only needs a 10-15-mm margin on a tumor bed in order to accurately define a PTV boost when Lipiodol is used to help define the extent and location of the tumor bed. Furthermore, van Rooijen et al. (17) recommend only a 5-mm margin on a tumor bed in order to create a PTV boost when Lipiodol is used.
this study, the data were exponentially distributed. As a result, the mathematical formula to capture 95% of patient shifts in a margin on a tumor bed would be: margin = mean x ln 20. Based on the data in Table-2, the recommended margins on a tumor bed in order to create a PTV Boost when Lipiodol is used would be: LR = 6mm, CC = 12mm, and AP = 6mm.

Daily imaging for bladder cancer typically involves the acquisition of two-dimensional kV portal images or three-dimensional volumetric cone beam CT (CBCT) scans. IGRT is based simply upon
Lipiodol as a fiducial marker for bladder cancer

Bony structures in many radiation oncology departments. However, IGRT based upon bony structures does not take the substantial daily inter-fraction motion of the bladder into account.

In contrast to the approach taken in Denmark where 3-5 Lipiodol spots with a mean volume of 0.7cc were typically used to define the periphery of the tumor bed (2-5), Lipiodol was used to demarcate the entire tumor bed in this study. In this report, Lipiodol was visible on every digitally reconstructed radiograph (Figure-1A) and every kV image (Figure-1B) throughout the entire, 7-week course of IGRT. Similarly, in the study by Chai et al. (2), 34/37 (92%) Lipiodol deposits that were seen on digitally reconstructed radiographs remained visible on images throughout IGRT. Pos et al. (4) observed that there was a gradual loss of volume of the Lipiodol spots (“washout”) over time; however, all of the Lipiodol spots that were present on digitally reconstructed radiographs remained visible throughout radiation therapy. Similarly, Sondergaard et al. (5) observed a relative loss of 24% in Lipiodol volumes during a 6-week course of radiotherapy. Although there was Lipiodol washout, all Lipiodol spots remained visible throughout radiotherapy.

Reliable tumor bed demarcation on a treatment planning CT scan without Lipiodol is challenging. In many cases, a cystoscopy report and CT scan for staging do not allow a radiation on-

Table 3 - Mean percent volume overlap of CTV boost with versus without Lipiodol.

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Mean Percent Volume Overlap of CTV boost with Lipiodol</th>
<th>Mean Percent Volume Overlap of CTV boost without Lipiodol</th>
<th>P-Value for Patients 1-5</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>58%</td>
<td>35%</td>
<td>0.06</td>
</tr>
<tr>
<td>2</td>
<td>72%</td>
<td>55%</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>56%</td>
<td>46%</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>28%</td>
<td>14%</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>51%</td>
<td>45%</td>
<td></td>
</tr>
<tr>
<td>Mean:</td>
<td>53%</td>
<td>39%</td>
<td></td>
</tr>
</tbody>
</table>
coliologist to later accurately contour the tumor bed on a radiotherapy treatment planning CT scan. A preferred approach is for an urologist to visualize the tumor bed at the time of cystoscopy and to demarcate the tumor bed with Lipiodol immediately following maximal TURBT. In this report, Lipiodol helped to reduce inter-observer variability regarding the extent and location of the tumor bed on a treatment planning CT scan for a radiation boost (Table-3). In 40% of patients in this study, the CTV_{boost} based upon Lipiodol extended outside of a PTV_{boost} that would have been treated based upon cystoscopy reports and enhanced CT scans without Lipiodol. Similarly, Pos et al. (4) reported that quite often the extent, or sometimes even the location, of Lipiodol spots was different from what the radiation oncologists would have contoured based upon cystoscopy reports and CT scans without Lipiodol.

In accordance with reports by others (4,15), Lipiodol injections were well-tolerated in this study. There were no adverse events due to Lipiodol injections.

The main strength of this study is that it is the first one to look at inter-observer variability regarding the extent and location of the tumor bed on CT scans with versus without Lipiodol (Table-3). The main weakness of this study is its small sample size. This was due to the infrequent utilization of bladder-sparing therapy at our referral center due to patient treatment preference. Another weakness is that intra-fraction movement of the bladder was not assessed in this study.

CONCLUSIONS

Bladder motion between daily radiotherapy treatments occurs primarily in the CC dimension and is substantial. Lipiodol constitutes a safe and effective fiducial marker that helps one to define the extent and location of a tumor bed for a radiation boost. Consequently, Lipiodol may lead to better local control and progression-free survival through more accurate targeting of the tumor bed with radiotherapy. We recommend that future prospective trials involving radiotherapy for muscle-invasive bladder cancer should include Lipiodol as a fiducial marker to not only corroborate its safety but also to assess its potential to improve local control and progression-free survival.

ABBREVIATIONS

AP = Anterior-posterior
CC = Cranial-caudal
CTV_{boost} = Clinical target volume for a radiation boost
CTCAE = Common Terminology Criteria for Adverse Events
CT = Computed tomography
Gy = Gray
IGRT = Image-guided radiation therapy
IMRT = Intensity modulated radiation therapy
LR = left-right
TURBT = Maximal transurethral resection of a bladder tumor
PVO = Percent volume overlap
PTV_{boost} planning target volume for a radiation boost

CONFLICT OF INTEREST

None declared.

REFERENCES


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Incidence and Clinical Characteristics of Lower Urinary Tract Symptoms as a Presenting Symptom for Patients with Newly Diagnosed Bladder Cancer


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ABSTRACT

Purpose: The incidence of lower urinary tract symptoms (LUTS) as the sole presenting symptom for bladder cancer has traditionally been reported to be low. The objective of this study was to evaluate the prevalence and clinical characteristics of newly diagnosed bladder cancer patients who presented with LUTS in the absence of gross or microscopic hematuria.

Materials and Methods: We queried our database of bladder cancer patients at the Atlanta Veteran’s Affairs Medical Center (AVAMC) to identify patients who presented solely with LUTS and were subsequently diagnosed with bladder cancer. Demographic, clinical, and pathologic variables were examined.

Results: 4.1% (14/340) of bladder cancer patients in our series presented solely with LUTS. Mean age and Charlson Co-morbidity Index of these patients was 66.4 years (range = 52-83) and 3 (range = 0-7), respectively. Of the 14 patients in our cohort presenting with LUTS, 9 (64.3%), 4 (28.6%), and 1 (7.1%) patients presented with clinical stage Ta, carcinoma in Situ (CIS), and T2 disease. At a median follow-up of 3.79 years, recurrence occurred in 7 (50.0%) patients with progression occurring in 1 (7.1%) patient. 11 (78.6%) patients were alive and currently disease free, and 3 (21.4%) patients had died, with only one (7.1%) death attributable to bladder cancer.

Conclusions: Our database shows a 4.1% incidence of LUTS as the sole presenting symptom in patients with newly diagnosed bladder cancer. This study suggests that urologists should have a low threshold for evaluating patients with unexplained LUTS for underlying bladder cancer.

INTRODUCTION

In 2012, there will be an estimated 73,510 new diagnoses of bladder cancer with approximately 15,000 deaths attributable to this cancer in the United States (1). Bladder cancer is strongly associated with exposure to smoking, environmental toxins, and aging and peaks in prevalence and incidence in the 8th decade of life (2). Typically, the majority of patients with bladder cancer present with gross painless hematuria, with estimates of this presentation accounting for up to 85% of patients at initial diagnosis (3,4). Asymptomatic microhematuria is the next most common presenting symptom for bladder cancer.
cancer with up to 10% of patients evaluated for the presence of microscopic hematuria being diagnosed with bladder cancer (5). The remaining small subset of newly diagnosed bladder cancer patients will present with symptoms that mimic other common urological conditions, such as urinary tract infections and/or lower urinary tract symptoms (LUTS).

Refractory LUTS are a well-recognized initial presenting symptom in a small percentage of patients with newly diagnosed bladder cancer. Traditionally, patients who present with refractory LUTS are thought to have carcinoma in-situ (CIS) at diagnosis (6). Previous studies have shown that up to one fourth of patients with CIS will present with irritative voiding symptoms including frequency, urgency and dysuria (7).

In this study, we sought to examine the incidence of LUTS without hematuria as an initial presenting symptom for bladder cancer in a high risk population, namely an elderly (2), veteran (8), male (2) cohort with significant tobacco (9) and chemical/environmental (10) exposures. Further, we sought to compare the clinical and pathologic data between patients who presented with LUTS compared to those who presented with gross or microscopic hematuria.

**MATERIALS AND METHODS**

This study was approved by the institutional review board of the Atlanta Veteran’s Affairs Medical Center (AVAMC). Patients with bladder cancer at the AVAMC were identified through a comprehensive review of urologic surgical records, pathology records, International Classification of Diseases (ICD)-9 coding, and the AVAMC cancer registry from 2000-2012. Pertinent clinical and demographic data were retrospectively collected from patient records, including presenting symptomatology that prompted initial clinical workup. In the database, presenting symptomatology was divided into four categories based on the clinical records in patients’ charts: gross hematuria, microscopic hematuria, lower urinary tract symptoms (LUTS) and incidental detection. Other demographic and clinical variables collected included patient age, race, associated co-morbidities, tobacco use, relevant pre and post-operative laboratory values, histologic type, stage and grade based on transurethral resection/bladder biopsy of bladder lesion.

Patients in our bladder cancer database were classified as presenting with lower urinary tract symptoms based on their seeking/being referred for a urologic consultation for irritative and/or obstructive voiding symptoms in the absence of microscopic or gross hematuria. As a part of their initial and ongoing evaluation, these patients were further evaluated with the International Prostate Symptom Score (IPSS) questionnaire to assess the severity of their symptoms. Patient IPSS scores were calculated based on a series of questions assessing their urinary symptoms, scored from 0 (“not bothersome”) to 4 (“very bothersome”) for each question. The patient quality of life score was obtained from the final IPSS quality of life question regarding the patient’s overall satisfaction with their urinary symptoms, ranging from 0 “delighted” to 6 “terrible”. No specific numerical IPSS cut-off was utilized for inclusion of patients in the LUTS group - it was simply the presenting symptom for this subset of patients who were ultimately diagnosed with bladder cancer. Patients included in this analysis were all identified as a newly diagnosed with bladder cancer between 2000 and 2012. Patients with a previous history of bladder cancer or a diagnosis of bladder cancer from an outside facility were excluded from this analysis. Patients were classified as presenting with LUTS as long as they did not have a history of either gross or microscopic hematuria (≥ 3 RBC per HPF) or a history of bladder carcinoma. Categorical variables were analyzed using a chi-square or Fisher’s exact test when appropriate. Continuous variables were analyzed using either the Student’s t-test for comparing of means or the Wilcoxon rank sums test for comparing medians. All statistical tests were two-sided with the significance level α set to 0.05. Statistics were performed using SAS 9.3 (SAS Institute, Cary, NC).

**RESULTS**

A total of 340 patients were identified as presenting with a new diagnosis of bladder cancer to the AVAMC between 2000 and 2012. These patients were comprised of 337 male (99.1%) and 3 female (0.9%) patients with a mean/median age at diagnosis.
of 67.7 and 67 years (range = 30-93), respectively. The patient cohort consisted of 74 (21.8%) African-American patients and 263 (77.4%) non-African-American patients. 266 (78.2%), 40 (11.8%), 20 (5.9%), and 14 (4.1%) patients presented with gross hematuria, microhematuria, incidentally, and LUTS. The clinical and demographic information for the 14 patients presenting with LUTS are shown in Table-1 and compared to the remaining cohort of 326 patients. Both groups did not significantly vary in their composition by gender (p = 1.00), age (p = 0.63), BMI (p = 0.65), pack-years of smoking (p = 0.79) or Charlson Co Morbidity Index scores (p = 0.76). Patients presenting with LUTS did present with statistically significant worse IPSS (p < 0.001), Bother (p < 0.001), and QOL (p = 0.049) scores as compared to the other groups as shown in Table-1.

Mean and median follow-up for the entire cohort was 3.1 and 2.3 years respectively. Pathological characteristics of the two groups are presented in Table-2, patients presenting with LUTS were significantly more likely to present with CIS

### Table 1 - Demographic and clinical presentation for LUTS and Non-LUTS patient groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>LUTS (n=14)</th>
<th>Non-LUTS (n=326)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (Men)</td>
<td>14 (100%)</td>
<td>323 (99.1%)</td>
<td>1.00†</td>
</tr>
<tr>
<td>Mean Age ± SD, Range</td>
<td>66.4 ± 9.3, 52 - 83</td>
<td>67.8 ± 10.3, 30 - 93</td>
<td>0.63‡</td>
</tr>
<tr>
<td>Median BMI ± IQR, Range</td>
<td>28.2 ± 3.1, 21 - 51</td>
<td>30.0 ± 6.8, 17 - 51</td>
<td>0.65§</td>
</tr>
<tr>
<td>Median Pack Years ± IQR, Range</td>
<td>45.0 ± 57.0, 0 - 70</td>
<td>40.0 ± 44.0, 0 - 210</td>
<td>0.79§</td>
</tr>
<tr>
<td>Median CCI ± IQR, Range</td>
<td>3.5 ± 5.0, 0 - 7</td>
<td>3.0 ± 5.0, 0 - 16</td>
<td>0.76§</td>
</tr>
<tr>
<td>Mean IPSS ± SD</td>
<td>24.0 ± 4.6</td>
<td>13.0 ± 9.4</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Mean Bother ± SD</td>
<td>20.4 ± 3.8</td>
<td>10.7 ± 8.2</td>
<td>&lt;0.001‡</td>
</tr>
<tr>
<td>Mean QOL ± SD</td>
<td>4.2 ± 1.7</td>
<td>3.0 ± 1.8</td>
<td>0.049‡</td>
</tr>
</tbody>
</table>

† = Fisher’s exact test; ‡ = Student’s t-test; § = Wilcoxon rank sums test; SD = standard deviation; IQR = interquartile range

### Table 2 - Pathologic Characteristics for LUTS and Non-LUTS patient groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>LUTS (n=14)</th>
<th>Non-LUTS(n = 326)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIS (only)</td>
<td>3 (21.4%)</td>
<td>12 (3.7%)</td>
<td>0.02†</td>
</tr>
<tr>
<td>Ta</td>
<td>10 (71.4%)</td>
<td>184 (56.4%)</td>
<td>0.27‡</td>
</tr>
<tr>
<td>T1</td>
<td>0 (0.0%)</td>
<td>69 (21.2%)</td>
<td>0.08‡</td>
</tr>
<tr>
<td>Muscle Invasive (Stage ≥ T2)</td>
<td>1 (7.1%)</td>
<td>57 (17.7%)</td>
<td>0.48†</td>
</tr>
<tr>
<td>Grade (high)</td>
<td>7 (50.0%)</td>
<td>197 (60.4%)</td>
<td>0.44‡</td>
</tr>
<tr>
<td>Recurrence</td>
<td>7 (50.0%)</td>
<td>131 (40.2%)</td>
<td>0.46‡</td>
</tr>
<tr>
<td>Mean No. Recurrence</td>
<td>0.57</td>
<td>0.68</td>
<td>0.56§</td>
</tr>
<tr>
<td>Mean No. Recurrences (among those with a recurrence)</td>
<td>1.1</td>
<td>1.7</td>
<td>0.009§</td>
</tr>
<tr>
<td>No. Progression</td>
<td>1 (7.1%)</td>
<td>25 (7.7%)</td>
<td>1.00†</td>
</tr>
</tbody>
</table>

† = Fisher’s exact test; ‡ = chi square test; § = Student’s t-test
in the LUTS group compared to the non-LUTS group (21.4% versus 3.7%, p = 0.02). Conversely, patients who presented with non-LUTS were more likely to experience a disease recurrence than patients who presented with LUTS (1.7 mean recurrences versus 1.1, p = 0.009). At last follow-up, 11 (78.6%) of patients presenting with LUTS were alive without disease and 3 (21.4%) patients had died. Of the patients who died, one death (7.1%) was attributed to bladder cancer. This patient initially presented with CIS and declined further treatment despite multiple positive cytologies and evidence of progression of disease. Compared to patients that did not present with lower urinary tract symptoms, there were no significant differences in progression and bladder-cancer related death rates.

DISCUSSION

In our study, patients who presented solely with LUTS comprised 4.1% (14/340) of newly diagnosed bladder cancer patients. Within this group of patients, there was a statistically significant (p = 0.02) greater incidence of clinical CIS upon presentation as compared to the non-LUTS patients. While there was a significant portion of LUTS patients presenting with CIS, the majority of patients who presented with LUTS 71.4% (10/14) did not have CIS. These results indicate that while clinicians should have a strong clinical suspicion for potential CIS lesions, the majority of patients with LUTS who present with bladder cancer will have a papillary lesion.

Lower urinary tract symptoms (LUTS) comprise a constellation of storage, voiding and post-micturition symptoms that are common in both men and women and rise in prevalence with age (11). In men, LUTS are typically attributed to the presence of benign prostatic hyperplasia (BPH) although LUTS may occur independently of BPH (12). Like bladder cancer, LUTS and BPH are strongly associated with aging; large population studies estimate the overall prevalence of LUTS as 62.5% of men over the age of 40 and 80.7% of men over the age of 60 (13).

Estimates for the incidence of LUTS as a presenting symptom for bladder cancer varies widely due to heterogeneous patient populations and varied definitions for LUTS/voiding dysfunction. In a small patient cohort of 92 patients, voiding problems in conjunction with microhematuria was a presenting symptom in 18% of newly diagnosed bladder cancer patients (14). Similarly, in a larger study of 1,000 patients, frequency and dysuria (6.0%), difficult or poor stream (3.5%) and acute retention (4.0%) were presenting symptoms for bladder cancer albeit with significant co-existing (35-41%) hematuria in each of these groups (15).

In one study, 4 of 22 CIS patients were characterized as presenting with LUTS without hematuria (16). Also, for 481 newly presenting patients, 97.0% of patients presented with some form of hematuria with frequency and dysuria comprising the next most common presentation (17). In another study, “cystitis” was implicated as the primary symptom for patients with newly diagnosed bladder cancer in 12% of patients (12/100) and “obstructive symptoms” were implicated for 3% of patients (3/100) (18). While these studies note that many bladder cancer patients present with irritative or obstructive symptoms, they do not evaluate the oncological outcomes of patients who present solely with LUTS. Furthermore, the irritative and obstructive presentations are often contaminated with either microscopic or gross hematuria. Our study provides new data by estimating the incidence of bladder cancer patients who presented with isolated LUTS as 4.1% as well as specifically reporting these patients’ oncological outcomes.

The United States Preventative Services Task Force (USPSTF) current recommendations are that there is insufficient evidence to assess the harms and benefits of screening for bladder cancer in asymptomatic patients (19). Similarly, no major organization including the American Association of Family Physicians (AAFP), European Association of Urology (EAU) or The American Cancer Society (ACS) currently recommends screening asymptomatic patients for bladder cancer (19). Our patient cohort represents a unique patient population where screening is clinically indicated; namely, this patient population has a number of risk factors that have been associated with an increased risk of developing bladder cancer: tobacco use, chemical exposures, etc. As such, it appears reasonable for urologists to have a low threshold
for performing cystoscopy for LUTS, especially in a high-risk patient population.

A potential concern from our study is that the use of non-invasive urine cytology may be preferential to flexible cystoscopy for the surveillance and identification of potential bladder cancer patients presenting with LUTS. Previous studies have indicated that the yield of urine cytology in the workup for patients with LUTS without hematuria has been low (20). One of the major problems with urine cytology is that this test lacks sensitivity for low and intermediate grade malignancies that comprise the majority of bladder cancer patients as well as the majority of patients in our study.

Another potential weakness of our study was an inability to evaluate for the number of cystoscopies that may have been performed to identify our patient cohort. The value of cystoscopy for evaluation of LUTS symptoms has also been evaluated in a number of previous studies. Goldberg et al. evaluated 1,584 women undergoing cystoscopy for LUTS and noted that 10 patients (0.63%) were identified with bladder cancer (21). Of note, 60% (6/10) of the bladder cancer patients initially had a normal dipstick evaluation for hematuria (21). In a study by Weiss et al. evaluating patients for refractory overactive bladder without hematuria, 8 patients were identified with bladder cancer from a total of 1,420 patients undergoing cystoscopy for a diagnostic yield of 0.6% (22). Despite all these concerns, this study does provide some insight into the incidence and clinical course of newly diagnosed bladder cancer patients who present solely with LUTS.

CONCLUSIONS

In our patient cohort, a small percentage of patients with newly diagnosed bladder cancer present with LUTS without gross or microscopic hematuria. Despite a higher incidence of CIS compared to patients with other presenting symptoms, the majority of patients with LUTS presented with Ta lesions. In high risk populations with significant tobacco exposure and other risk factors, clinicians should have a low threshold for performing an endoscopic evaluation of patients’ lower urinary tracts when significant, difficult to treat LUTS exist.

ABBREVIATIONS

AVAMC = Atlanta Veteran Affairs Medical Center
AUA = American Urological Association
RBC = Red Blood Cell
LUTS = Lower Urinary Tract Symptoms
AA = African American
IQR = Interquartile Range
SD = Standard Deviation

CONFLICT OF INTEREST

None declared.

REFERENCES


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Tubeless percutaneous nephrolithotomy: outcomes with expanded indications

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Glickman Urological & Kidney Institute, The Cleveland Clinic, Cleveland, USA

INTRODUCTION

Percutaneous nephrolithotomy (PCNL) was introduced in 1976 (1), and rapidly evolved into the gold standard for treatment of large and complex renal stones. The procedure continues to evolve with an emphasis on maintaining a high success rate of stone treatment while improving patient outcome with decreased morbidity (2–5). Classically the procedure concludes with the placement of one or more nephrostomy tubes (PNT) based on the number of access used. A proposed advantage for the placement of the nephrostomy tube is to tamponade the percutaneous tract; however, typically the tube is significantly smaller than the diameter of the tract (6). Other advantages include providing drainage of the kidney and allowing an access to the renal collecting system for secondary procedures (6).
Tubeless percutaneous nephrolithotomy was introduced in 1997 (7). Subsequently, several groups reported their results with tubeless PCNL in selected patients (8-10), however all studies excluded patients with concerns regarding bleeding, perforation, or residual stones requiring a second-look nephroscopy. The benefits shown in these select patients included decreased postoperative pain and hospital stay.

In parallel with the evolution in the conservative management of renal trauma over the last decade, we thought it appropriate to re-evaluate the exclusionary criteria of significant bleeding and intraoperative extravasation as contraindications of a tubeless approach.

Our objective was to evaluate the use of a tubeless approach in all patients undergoing PCNL. Specifically, we sought to evaluate outcomes and complications with tubeless PCNL in expanded indications, including patients with intraoperative findings of bleeding or perforation.

**MATERIALS AND METHODS**

After IRB approval, a retrospective review of the charts of patients who underwent PCNL at our institute from 7/2010 to 2/2012 was conducted. One hundred and fifty nine consecutive patients were included in the study. Patients were assigned to one of two high-volume endourologists. One of the surgeons uses tubeless procedure as a standard technique for all patients undergoing PCNL, irrespective of length of surgery, presence of residual calculi that cannot be accessed, significant bleeding or collecting system perforation. The second surgeon practices the placement of a nephrostomy tube for drainage on regular basis. Patients who underwent bilateral procedures were excluded from the study. Patient demographics and preoperative parameters including age, sex, laterality, BMI, stone size, location and maximum diameter of stones, number of calyces involved by the stones, intra-operative parameters including the number of renal access used, the use of intra-corporeal lithotripsy, reporting of intraoperative bleeding, and post-operative parameters including residual stones visual analog pain score (0-10) as 1st recorded post operative day one and morphine narcotic equivalence while inpatient, operative and postoperative complications were recorded.

As intraoperative estimation of blood loss is difficult during endoscopic procedures involving high-volume irrigant, significant intraoperative blood loss was defined as a HB drop of 1gm or more from the preoperative hemoglobin to the HB value immediately post-operative in the recovery room. All post-operative HB in the post-anesthesia care unit (PACU) was obtained within one hour of completion of the surgery. The ability of the nephrostomy tube to tamponade bleeding in the face of significant intraoperative bleeding was evaluated specifically in this group.

**SURGICAL TECHNIQUE**

All patients underwent a non-contrast computerized tomography to evaluate stone burden, location, and location of pleura and adjacent organs. All patients received 24 hours of intravenous perioperative antibiotics starting on the day of surgery (cefazolin or ciprofloxacin). The patient was placed in a prone position, and access achieved through an endoscopic guided (47%) or fluoroscopic guided approach (53%). The percutaneous tract was dilated with a balloon dilator (15cm, 30F, Bard X-force, Bard Urological, Covington GA). Gravity irrigation was used at a height of 30cm from the table; pressurized irrigation was used (100mmHg) if bleeding obscured the view. The Amplatz sheath was advanced over the balloon dilator and rigid and flexible nephroscopy was performed. The Cyberwand (Olympus-ACMI, Southborough MA) was utilized for stone fragmentation when needed and Perc-circle (Cook Urological, Spencer IN) and the Uronet (US Endoscopy, Mentor OH) were utilized for stone retrieval.

The absence of residual stones > 4mm in size was confirmed using a complete inspection of the collecting system endoscopically (flexible nephroscopy and antegrade flexible ureteroscopy in all patients) in conjunction with high magnification rotational fluoroscopy (11).

TUBELESS was concluded by the placement of an indwelling double-J ureteral stent (placed either antegrade or retrograde using a split-leg prone positioning). The nephrostomy sheath was
removed without instillation of any hemostatic agents and nephrostomy site was closed with a single vertical mattress suture. PNT was concluded by the placement of a 5Fr. Nephro-ureteral stent along with an 8Fr. pigtail nephrostomy tube under fluoroscopic guidance. For patients who had more than one access tract, a nephrostomy tube was placed in each tract. The nephrostomy tube/s was removed when the patient was deemed stone free.

Data acquisition and Statistical analysis: The primary outcome used for evaluation was length of hospital stay. The secondary outcomes were postoperative pain control as measured by the visual pain analog score on postoperative day 1 and morphine equivalents utilized in the postoperative inpatient stay. Continuous measures were described as means, standard deviations, and percentiles. Categorical measures were summarized using frequencies and percentiles. The two sample T-test was used to evaluate the differences between PNT groups for continuous variables. The Pearson’s chi-square test or Fisher’s exact test was used to assess the differences between PNT groups for categorical variables. For the association involving ordinal variables, Mantel-Haenszel chi-square test was used. General linear regression or logistic regression was performed to assess the association between the primary or secondary outcomes of PNT as compared to TUBELESS, after adjusting for other covariates. All tests were performed at a significance level of 0.05. SAS 9.3 software (SAS Institute, Cary, NC) was used for all analyses.

RESULTS

Patient demographics are presented in Table-1. One hundred and fifty nine patients underwent either PNT (76 patients) or TUBELESS (83 patients). There was no difference between groups (Table-1) regarding age in years (54.2 vs. 55.5, p = 0.6), female gender (56% vs. 51.8%, p = 0.54), ASA score (2.4 vs. 2.6, p = 0.08), number of stones (2.9 vs. 3.5, p = 0.27), maximum cumulative stone diameter (39.2 vs. 37.7, p = 0.7), number of calyces involved with stones (2.7 vs. 2.2, p = 0.2), stone density as measured as (HU) (p = 0.58), laterality (p = 0.8), previous treatment of stones (p = 0.57), use of preoperative narcotics (p = 0.57).

Table 1 - Pre-operative demographics.

<table>
<thead>
<tr>
<th></th>
<th>PNT (76)</th>
<th>Tubeless (83)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years, mean (SD)</td>
<td>54.2(14.5)</td>
<td>55.5(16.4)</td>
<td>0.6</td>
</tr>
<tr>
<td>Sex (female) n (%)</td>
<td>43(56)</td>
<td>43(51.8)</td>
<td>0.54</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.6(7.4)</td>
<td>33.5(9.1)</td>
<td>0.03*</td>
</tr>
<tr>
<td>ASA</td>
<td>2.4 (0.5)</td>
<td>2.6 (0.5)</td>
<td>0.08</td>
</tr>
<tr>
<td>Laterality- right</td>
<td>37(48.6)</td>
<td>39(46.9)</td>
<td>0.8</td>
</tr>
<tr>
<td>Number of stones</td>
<td>2.9(2.5)</td>
<td>3.5(4)</td>
<td>0.27</td>
</tr>
<tr>
<td>Stone density (HU)</td>
<td>963 (312)</td>
<td>928(341)</td>
<td>0.58</td>
</tr>
<tr>
<td>Number of calyces</td>
<td>2.6(2.3)</td>
<td>2.1(1.8)</td>
<td>0.13</td>
</tr>
<tr>
<td>Staghorn stone</td>
<td>24(32.4)</td>
<td>15(18.2)</td>
<td>0.04</td>
</tr>
<tr>
<td>Stones maximum diameter</td>
<td>39.2(28)</td>
<td>37.7(20.7)</td>
<td>0.7</td>
</tr>
<tr>
<td>Previous treatment of stones</td>
<td>40(52.6)</td>
<td>40 (48.19)</td>
<td>0.57</td>
</tr>
<tr>
<td>Preoperative narcotics</td>
<td>26 (34.6)</td>
<td>32(39.02)</td>
<td>0.57</td>
</tr>
</tbody>
</table>

* Two sample t test for continuous variables and Pearson’s chi-square test for categorical variables
Patients who had tubeless PCNL had higher body-mass-index (BMI 33.5 vs. 30.6, p = 0.03) while number of access tracts (1.3 ± 0.6 vs. 1 ± 0.1, p ≤ 0.001) and staghorn stones (p = 0.008) were more common in the PNT group. Staghorn stones were more common in the PNT group, (32 vs. 18.2%, p = 0.04), and this was adjusted for in our multivariable analysis. Intraoperative perforation of the collecting system with moderate extravasation was noted on intraoperative antegrade nephrogram in 2 patients in each group (2.5%).

Peri-operative outcomes are presented in Table-2. There was no difference regarding operative time (p = 0.16). Hospital stay was shorter in the TUBELESS group versus the PNT (1.7 days vs. 3.0 days, p = 0.001). There was no difference in blood loss as estimated by change of hemoglobin between both groups, either in the immediate PACU evaluation (-0.9 vs. -1, p = 0.8) and as measured at the postoperative day 1 (-1.4 vs. -0.9, p = 0.09). Twenty four patients in the PNT group and 30 patients in the TUBELESS had a change in hemoglobin of 1gm or more from their baseline HGB to immediately post-operative in the PACU; constituting the “significant intraoperative bleeding” subgroup. In these patients there was no significant difference in the change in hemoglobin from immediate PACU HGB to postoperative day one (-0.89gm in the PNT group compared to -1.05gm in the tubeless group, p = 0.75).

Using a multivariable analysis, the LOS (hospital stay) for TUBELESS groups was 0.95 times (standard error = 0.30) that of the LOS for PNT group, after adjusting for age, BMI, culture, pre-op narcotics, access number, staghorn stones and renal access location (Table-3). The highest pain score recorded on the first postoperative day was less in the TUBELESS at 2.7 vs. 4.3, (p = 0.014), while postoperative narcotic use was less in the TUBELESS group at 70mg morphine equivalents vs. 149 (p ≤ 0.001). Using multivariable analysis, higher pain score and increased usage of analgesia was reported when a nephrostomy tube was placed for drainage, controlling for pre-operative use of narcotics, access number and access location (Tables 4 and 5). The other factor that correlated with intensity of postoperative pain was preoperative use of narcotics (p = 0.002).

### Table 2 - Peri-operative outcomes.

<table>
<thead>
<tr>
<th></th>
<th>PNT (76)</th>
<th>Tubeless (83)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operative time median (IQR)</td>
<td>114(55-160)</td>
<td>90 (65-115)</td>
<td>0.16*</td>
</tr>
<tr>
<td>Hospital stay (days)</td>
<td>3(1.6)</td>
<td>1.7(1.1)</td>
<td>0.001</td>
</tr>
<tr>
<td>PNT duration (days)</td>
<td>2.2(2.1)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Visual Pain Analog score (0-10)</td>
<td>4.3 (3)</td>
<td>2.7(3)</td>
<td>0.014</td>
</tr>
<tr>
<td>Post-operative narcotics (morphine equivalents)</td>
<td>149.7(167)</td>
<td>70 (102)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Change in Hemoglobin (preop to PACU)</td>
<td>-1.4 (2.2-0.5)</td>
<td>-0.9(1.7-0.4)</td>
<td>0.09</td>
</tr>
<tr>
<td>Change in Hemoglobin (PACU to POD #1)</td>
<td>-0.9 (1.65-0.4)</td>
<td>-1 (1.5-0.57)</td>
<td>0.86</td>
</tr>
</tbody>
</table>

* Two sample t test for continuous variables.

### Complications (Clavien)

For the PNT group, 5 patients suffered urosepsis with 2 patients admitted to surgical intensive care unit (ICU) (3-II + 2 -IVa), as compared to 2 patients (II, IVa) in the TUBELESS group, requiring admission to the surgical ICU. One of these patients in the TUBELESS succumbed to multi-system organ failure due to an untreated preoperative urinary tract infection.

Four of the PNT patients suffered bleeding (2 II, 2IIa) two of them were treated with blood transfusion, while two were treated with angio-
embolization. No patients in the TUBELESS group required transfusion or embolization, and none of the patients developed a post-operative symptomatic urinoma. Though staghorn calculi were more common in the PNT group, there was no statistical difference of intraoperative (p = 0.12) and postoperative (p = 0.07) complications between patients with staghorn stones as compared to patients with non-staghorn stones.

### DISCUSSION

Percutaneous nephrolithotomy remains the mainstay for treatment of large, complex kidney stones (3,12,13). In an effort to shorten hospital stay and decrease postoperative pain and analgesia, the use of smaller caliber nephrostomy tubes (14,15) and tubeless PCNL were evaluated by several groups (7,16,17). Bellman et al. studied tubeless
PCNL in a selected group of patients who underwent the procedure for management of stones or antegrade endopyelotomy; in the initial series a ureteral stent was placed alongside the council tip nephrostomy tube and later a ureteral stent alone was utilized (7). The group reported shorter hospital stays and improved pain management. Patients with excessive bleeding, prolonged surgery, residual stones, and surgery for urothelial tumors were excluded from the study (7). Since this report, several other investigators have confirmed that tubeless PCNL leads to shorter hospital stays and less postoperative pain (16,18,19) and decreased cost as compared to nephrostomy tube drainage (7,20). While most studies have utilized a ureteral stent in patients undergoing a tubeless PCNL, other investigators have recommended a “totally tubeless” approach (21,22).

Tubeless PCNL was proven to be safe in patients with chronic anti-platelet therapy and liver cirrhosis (23), geriatric patients (24), chronic kidney disease (25), patients with solitary kidneys, bilateral procedures and supracostal access or multiple renal access (9). However, all these studies have excluded patients with intraoperative bleeding or urinary extravasation. In this study we confirm the advantages of tubeless PCNL as regarding shorter hospital stay, less postoperative pain and analgesia, but also expand the indications for tubeless PCNL to all patients as a standard procedure with no intraoperative exclusionary criteria.

Others have reported that factors influencing hospital stay included stone burden, number of access and tubeless PCNL, of them tubeless PCNL was the most significant factor (26). In our study, we concluded that a tubeless PCNL impacted hospital stay, pain scores and narcotic requirements, with preoperative narcotic usage being the only additional variable impacting narcotic use.

There is no validated measure for blood loss during PCNL - all attempts are confounded by the use of irrigation fluid which complicates the ability to measure blood loss by traditional means (suction, sponge weight). Post-operative hemoglobin drop is the standard in the literature; we utilized the immediate post-operative drop to identify those who may have had more significant intraoperative bleeding, and as such may have been at greater risk of post-operative bleeding to evaluate the impact of a tube for “tamponade”.

The role of the nephrostomy tube placement after PCNL for hemostasis was challenged by several reports (27,28). These studies reported no difference in the hemoglobin change and development of perinephric hematoma or urinoma using a tubeless approach. Of note, these studies excluded patients with complete staghorn, supracostal access and chronic kidney disease from the study; in contrast we included all such patients in our study. In this study we evaluated specifically the “tamponade” effect in patients with significant intraoperative bleeding, hypothesizing that these would be the patients where a tamponade effect would be most critical. There was no difference in the change of hemoglobin as measured at postoperative day one and compared to the PACU hemoglobin indicating that the tubeless approach is safe in patients with moderate intraoperative bleeding.

One limitation of our study is that the rate of collecting system perforation is low. However, the urologic trauma literature supports the use of a ureteral stent in the management of a collecting system injury, suggesting that such an approach would be appropriate also after PCNL (29). The retrospective nature of our study carries inherent risk for selection bias; prospective randomized trials would more definitively address the questions posed. More patients in our PNT group had staghorn calculi and multiple accesses; we controlled for these potential confounders by performing a multivariable analysis. One might argue that a small-bore nephrostomy tube is not the standard for a complicated PCNL; indeed a recent report suggests that a large bore nephrostomy tube may reduce bleeding and overall complication rates; however this was also not a randomized trial and has significant risk for selection bias (30).

**CONCLUSIONS**

Tubeless PCNL is safe irrespective of the presence of significant bleeding or collecting system perforation. Tubeless PCNL leads to shor-
ter hospital stays and less postoperative pain. We suggest the only indication for placement of a nephrostomy tube post PCNL is if a significant residual stone burden can be addressed by a second-look PCNL through the existing tract.

**CONFLICT OF INTEREST**

None declared.

**REFERENCES**


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The skin-to-calyx distance measured by renal ct scan and ultrasound

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ABSTRACT

Purpose: We developed a stereotactic device to guide the puncture for percutaneous nephrolithotripsy, which uses the distance from the target calyx to its perpendicular point on skin (SCD) to calculate the needle’s entry angle. This study seeks to validate the use of measurements obtained by ultrasound (US) and computerized tomography (CT) for needle’s entry angle calculation and to study factors that may interfere in this procedure.

Materials and Methods: Height, weight, abdominal circumference, CT of the urinary tract in dorsal decubitus (DD) and ventral decubitus (VD), and US of the kidneys in VD were obtained from thirty-five renal calculi patients. SCD obtained were compared and correlated with body-mass index (BMI).

Results: BMI was 28.66 ± 4.6 Kg/m². SCD on CT in DD was 8.40 ± 2.06cm, in VD was 8.32 ± 1.95cm, and US was 6.74 ± 1.68cm. SCD measured by US and CT were statistically different (p < 0.001), whereas between CT in DD and VD were not. SCD of the lower calyx presented moderate correlation with BMI.

Conclusion: SCD obtained by CT in ventral and dorsal decubitus may be used for calculation of the needle’s entry angle. SCD obtained by US cannot be used. A rule for the correlation between BMI and the SCD could not be determined.

Key words: Urolithiasis; Nephrostomy, Percutaneous; Radiosurgery; Body Mass Index

INTRODUCTION

Surgical treatment of renal stones presented considerable advances during recent decades. Before the 70s, retained stones were removed surgically by means of large incisions in the skin. After that decade, the treatment of calculi came to be undertaken by extracorporeal shockwave lithotripsy (ESWL) (1), percutaneous nephrolithotripsy (PCNL) (2-4) and endoscopic ureterolithotripsy. Today PCNL is the gold standard treatment for renal stones bigger than 2cm (5).

PCNL begins with an ultrasound or fluoroscopy guided puncture directed toward the desired renal calyx for removal of the stone. In some cases various attempts are necessary before the calyx is successfully reached, even by experienced surgeons, as the guidance given by fluoroscopy is made on a single plane (6). In up to 9% of cases it is impossible to undertake the procedure as a result of inability to attain the calyx (7). Only 27% of the urologists trained in PCNL during their medical internship continue to carry out the procedure of renal access by percutaneous approach.
The main reason is the technical difficulties of the method (8). An adequate puncture that penetrates the calyx's fornix is fundamental, as it diminishes the lesion of vessels and increases the chances of the patient's becoming stone-free (9,10). For the purpose of accessing the renal calyx more easily, we have been developing a stereotactic localization system, an apparatus called the "Renal Puncturometer". With this device it is possible to determine the exact position of the calyx and to guide the needle directly towards the desired calyx, based on the distance between the calyx and the point on the skin perpendicular to the calyx to be punctured. We have called this measurement the Skin-to-Calyx Distance (SCD). The measurement of this distance has not been standardized and there is no data regarding if this distance measured by computerized tomography (CT) or ultrasound (US) is the same.

Our purposes were to study the factors which may alter the skin-to-calyx distance, particularly the decubitus position and the body mass index (BMI) and to evaluate the feasibility of using computerized tomography or ultrasound to estimate the Skin-to-calyx Distance (SCD).

**MATERIALS AND METHODS**

This study was approved by the Ethics Committee for the Analysis of Research Projects of the University of Sao Paulo Medical School, under n° 1348/09. After consultation with the urologist, patients who agreed to participate in the study gave written informed consent. Between May 2010 and April 2011, thirty five patients diagnosed with renal calculi were included in the project. Age ranged from 18 to 65 years old. Pregnant women and patients with one kidney were excluded from the study.

Measurements of height, weight, abdominal circumference at the xiphoid appendix and at the umbilical scar were obtained. Non-contrast enhanced helicoidal CT was obtained for each patient in the dorsal decubitus (DD) and ventral decubitus (VD) and US of the kidneys in the ventral decubitus (VD) was also obtained. To perform the VD exams, 10cm diameters bolsters were placed at the level of the shoulder joints and at the abdomen. In order to reduce radiation exposure, the CT in VD was restricted to the kidney region.

The working principle of the device is to obtain a right-angle triangle (Figure-1) formed by the target calyx (C), the point on the skin in the lumbar region which is the perpendicular projection of the target calyx (S) and the projection of the needle’s entry point on the skin (E) on S’ plane (E projection). In a right-angle triangle, when we know the length of the two sides, we can calculate the angles and the hypotenuse (CE projection). One of the two sides is the distance between the projection of the point on the skin at which the needle entry on the S’ plan (E projection) and the point on the skin perpendicular to the target calyx (SE). The other side is the distance between the target calyx and the point on the skin which is its perpendicular projection (SC), which is the distance under study here. For the purpose of clarity we will call it skin-to-calyx distance (SCD).

Figure-2 shows the smaller triangle in red, which is derived from the bigger triangle in green. The smaller triangle (in red) allows us to calculate the needle’s entry angle. The identification of these triangles is cornerstone to the utilization of the device we are developing. We plan to validate that our calculations allow accurate targeting of the calyx in our next study by applying this process in PCNL.

The distances between the posterior calyx and the skin on the lumbar region perpendicular to the calyx (SCD) were obtained from the exams undertaken (US in VD and CT in VD and DD). We compared the distances obtained by the three exams and their correlation with the body-mass index (BMI) and abdominal circumference.

**Statistical analysis**

The values obtained were expressed in absolute values, average and standard deviation. Measures were compared using Analysis of Variance for repeated measurements (11,12). A level of significance of 5% (p < 0.05) was employed for this assessment.

Correlation between the distances and the measurements of the abdominal circumference and body-mass index (BMI) were undertaken by means of Pearson’s correlation coefficient (11,12).
Figure 1 - Drawing of the right-angle triangle: SC (Skin to Calix Distance) - Distance from the target calyx (C) to its perpendicular projection on the skin (S). SE - Distance between the projection of the point at which the needle enters the skin on S plane (E projection) and the point on the skin perpendicular to the target calyx (S). CE projection - Distance from the projection of the point at which the needle enters the skin on S plane (E projection) to the target calyx (C).

Figure 2 - Drawing of the smaller right-angle triangle in red: SC-N - Distance from the target calyx (C) to its perpendicular projection on the skin (S) minus the distance between E plane and S plane. SE - Distance between the needle’s entry point on the skin (E) and SC line, forming with it a right angle. EC - Distance from the point at which the needle enters the skin to the target calyx.
RESULTS

The mean Skin-to-Calyx Distance obtained by dorsal decubitus CT was 8.40 (± 2.06cm), by ventral decubitus CT was 8.32 (± 1.95cm) and by ventral decubitus US was 6.74(± 1.68cm) (Table-1). These measures showed a significant difference (p < 0.001). Multiple comparisons tests showed that this difference occurred between the US and dorsal CT and US and ventral CT. The comparison between the CT groups (CT in DD versus CT in VD) did not show a statistically significant difference, except in the lower posterior right calyx (p = 0.002). The results obtained are represented in Figure-3.

The BMI was 28.66(± 4.66) Kg/m², abdominal circumference at the level of the xiphoid process was 96.09(± 12.32cm) and at the level of the umbilicus scar was 100.79(± 14.66cm) (Table-2).

Table 1 - Skin-to-Calix Distance (SCD) with mean values and standard deviations for CT and US measurements. The last line corresponds to all the calices together.

<table>
<thead>
<tr>
<th>Posterior calyx</th>
<th>Dorsal CT</th>
<th>Ventral CT</th>
<th>Ventral US</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Standard deviation</td>
<td>Mean Standard deviation</td>
<td>Mean Standard deviation</td>
<td></td>
</tr>
<tr>
<td>Upper R</td>
<td>7.85 1.77</td>
<td>8.08 1.83</td>
<td>6.05 1.49</td>
</tr>
<tr>
<td>Intermed. R</td>
<td>8.23 1.84</td>
<td>7.96 1.90</td>
<td>6.42 1.46</td>
</tr>
<tr>
<td>Lower R</td>
<td>9.32 2.07</td>
<td>8.65 2.02</td>
<td>6.87 1.47</td>
</tr>
<tr>
<td>Upper L</td>
<td>7.79 1.92</td>
<td>7.94 1.80</td>
<td>6.58 1.70</td>
</tr>
<tr>
<td>Intermed. L</td>
<td>8.32 1.96</td>
<td>8.15 1.91</td>
<td>6.96 1.69</td>
</tr>
<tr>
<td>Lower L</td>
<td>9.25 2.30</td>
<td>9.13 2.06</td>
<td>7.55 1.90</td>
</tr>
<tr>
<td>All</td>
<td>8.40 2.06</td>
<td>8.32 1.95</td>
<td>6.74 1.68</td>
</tr>
</tbody>
</table>

DISCUSSION

Despite the improvement in the general health and social conditions of the population as well as the advances made in preventive medicine over recent decades, we still find people with large renal calculi. The best treatment for these stones is PCNL (2,5). The success of this surgery, with complete removal of the stones and small risk of complications, depends, among other factors, on the correct choice of the calyx for the puncture and on the correct puncture on the center of the calyx chosen. The correct percutaneous access used to attain the appropriate access to the calculus continues to constitute a challenge for urologists, because the guidance used for the puncture is given by fluoroscopy in one single plane. The chance of getting it right depends much more on the surgeon’s experience than on rational calculation. Various devices have been created to meet this challenge, from the robot system devised by Cadeddu et al. (13) and modified by John Bauer et al. (14) for remote-controlled surgery, to simple manual plates (15). All of them use a system of acquisition of the angle and depth of penetration of the puncture needle based on two image acquisitions from the C arm of the fluoroscopy at the moment of the surgery: 0 and 30 degrees or 0 and 90 degrees.

The renal puncturometer, which is the device we have developed, uses the distance of the

Table: 215
depth from the skin to the target calyx (SCD). There are various studies using CT in the dorsal and ventral positions for the localization of intraperitoneal organs, especially the colon. Ball et al. (16) studied the position of the internal organs by CT in both dorsal and ventral positions, in order to mark them out for radiotherapy. Those authors observed that when patients are in the ventral position, the kidney is ventrally displaced by 1 to 5 cm. We have found no study which has attempted to access this distance with the patient in position for percutaneous nephrolithotripsy.
Table 2 - Mean, standard deviation, minimum and maximum of BMI and abdominal circumference.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI Kg/m²</td>
<td>28.66</td>
<td>4.66</td>
<td>19.02</td>
<td>41.06</td>
</tr>
<tr>
<td>Xiphoid appendix (cm)</td>
<td>96.09</td>
<td>12.32</td>
<td>63.00</td>
<td>130.00</td>
</tr>
<tr>
<td>Umbilical scar (cm)</td>
<td>100.79</td>
<td>14.66</td>
<td>62.00</td>
<td>132.00</td>
</tr>
</tbody>
</table>

Figure 4 - Correlation of BMI with the abdominal circumference at the level of the xiphoid process (A) and at the level of the umbilicus (B).

Figure 5 - Correlation of BMI with the skin-to-calyx distance of the lower calices of the right (A) and left kidneys (B).

R = Right; L = left.
Azhar et al. (17), in a study using tomography in the dorsal and ventral position to assess the relationship between the internal organs in relation to the trajectory of the needle in calyx puncture, observed that in the ventral position, the distance from the lower calyx to the skin at the point at which the needle enters is reduced from 9 to 7.6 cm. Duty et al. (18) performed a similar study without the use of bolster. Comparing prone to ventral position, he observed a reduction in the distance of 2.57 cm in the right kidney and 1.83 cm in the left kidney. These assessments were of an oblique line, represented by the hypotenuse of our triangle (Figure-1). In our study it is a perpendicular line, represented by the vertical side of our triangle (Figure-1). This measurement in the CT exam varied very little between the dorsal and ventral decubitus positions. In the upper calyx this distance increased, on average, 0.24 cm in the right kidney and 0.17 cm in the left kidney, while in the lower calyx it diminished, on average, 0.77 cm in the right kidney and 0.22 cm in the left kidney. By virtue of the law of gravity it is expected that in the ventral decubitus position the kidney should move away from the spine, thus increasing the distance between the kidney and the dorsal wall, as was in fact found in the study of Ball et al. (16). The reduction of this distance, in the lower calyx, in our study, is probably due to the bolster placed on the abdomen, as happens in percutaneous nephrolithotripsy procedures. There was no statistical difference in the comparison of these distances under these conditions, except for the right lower calyx. For the right lower calyx we were not able to determine which CT exam (DD or VD) provides the most appropriate measurement. We hope to be able to answer that question after further studies.

There was a significant difference of the SCD in the CT both in the dorsal and ventral decubitus as compared with the US exam in the ventral decubitus, which may be due to the technical differences between the methods employed, or even to occasional pressure exercised by the ultrasonography operator on the skin. The distance obtained on the US should not, therefore, be used for the calculation of the angle of the needle’s entry. Although normally there is a correlation between BMI and the abdominal circumferences, there are people whose measurements are far from standard. As the correlation between BMI and the skin-to-calyx distance is less precise, we do not recommend its use in the determination of SCD.

CONCLUSIONS

The skin-to-calyx distances obtained by CT in the ventral and dorsal decubitus positions present no significant differences and both positions may be used indifferently for the calculation of the angle of entry of the needle during percutaneous surgery, in all calices, except for the lower calyx of the right kidney.

The skin-to-calyx distance obtained by the US exam cannot be used for the calculation of the angle of entry of the needle in percutaneous surgery. A rule for the correlation between BMI and the skin to calyx distance could not be determined.

ABBREVIATIONS

Kg = milogram
m = meter
cm = centimeter
% = percent
p = probability
r = rho(Pearson’s correlation coefficient)
ESWL = extracorporeal shockwave lithotripsy
PCNL = percutaneous nephrolithotripsy
SCD = skin-to-calyx distance
CT = computerized tomography
BMI = body mass index
US = ultrasound
DD = dorsal decubitus
VD = ventral decubitus

CONFLICT OF INTEREST

None declared.

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Application of Self-retaining Bidirectional Barbed Absorbable Suture in Retroperitoneoscopic Partial Nephrectomy

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ABSTRACT

Objective: To investigate the safety and feasibility of self-retaining bidirectional barbed absorbable suture application in retroperitoneoscopic partial nephrectomy.

Materials and Methods: From Sep 2011 and Aug 2012, 76 cases of retroperitoneoscopic partial nephrectomy were performed at our hospital. The patients were divided into two groups: self-retaining barbed suture (SRBS) group (n = 36) and non-SRBS group (n = 40). There was no significant difference in age, sex, tumor size and location between the two groups. Clinical data and outcomes were analyzed retrospectively.

Results: All 76 cases of retroperitoneoscopic partial nephrectomy were successfully performed, without conversion to open surgery or serious intraoperative complications. In the SRBS group, the suture time, warm ischemia time and operation blood loss were significantly shorter than that of non-SRBS group (p < 0.01), and operation time and hospital stay were shorter than that of non-SRBS group (p < 0.05).

Conclusions: The application of self-retaining bidirectional barbed absorbable suture in retroperitoneoscopic partial nephrectomy could shorten suture time and warm ischemia time, with good safety and feasibility, worthy of being used in clinic.

INTRODUCTION

With the fast development of laparoscopic technique, laparoscopic partial nephrectomy (LPN) became a new way to treat T1 renal cell carcinoma (RCC) (1). Compared with open partial nephrectomy (OPN), LPN has many advances such as less postoperative pain therapy, shorter hospital stay time and quicker recovery (2-4). But it has an increased complication rate and longer warm ischemia time (5,6). Quill SRS bidirectional barbed suture (Quill Self-Retaining System; Angiotech Pharmaceuticals, Vancouver, British Columbia, Canada) consists of a delayed-absorbable material (polydioxanone) cut with barbs that prevents slippage through tissue and avoids to knot, increases efficiency, and shortens suture time. Quill SRS has been described for use in LPN and can decrease suture time and warm ischemia time (WIT). From September 2011 to August 2012, 76 cases of retroperitoneoscopic partial nephrectomy (RPN) were performed at our hospital, and Quill SRS was used in 36 cases of them. Clinical data and outcomes were analyzed retrospectively.

MATERIALS AND METHODS

A total of 76 patient records were reviewed; all patients were diagnosed with renal carcinoma by CT or MRI before operation, all patients were randomly divided into two groups: self-retaining barbed suture (SRBS) group (n =
36) and non-SRBS (n = 40) group. There were no significant differences in age, sex, tumor size and location between the two groups (Table-1). All cases were in stage T1N0M0 according to AJCC. Prior to the study, the protocol was approved by our local institutional ethics committee, and in accordance to the ethical guidelines of the 1975 Helsinki Declaration. Written, informed consent was obtained from all of the subjects.

Retroperitoneoscopic Partial Nephrectomy Procedure (left)

The patient was placed in the right lateral position. Port A (posterior axillary line under the 12th rib) was created using a home-made balloon and 500-800 mL of CO₂ was inflated. Port B (anterior axillary line under the 11th rib) was created and digitally guided. Port C (median axillary line, 1-2 cm above the iliac crest) was created and a 10 mm trocar was inserted. A 12 mm trocar was inserted in port A. Initially the lumbar fascia was sutured and next the skin and muscle were sutured. After the access of the peritoneal cavity the extraperitoneal and perirrenal fascias were separated using an ultrasonic scissor from up to down and from anterior to posterior location, and the peritoneal reflection and the Gerota fascia were clearly identified. Gerota fascia was dissected close to the peritoneal reflection, beyond the renal superior pole and 3-4 cm below the inferior kidney pole. At this site, the dissection must be careful in order to identify the ureter. The renal pedicle was dissected and a bulldog clamp was used to clamp the renal artery. The mass was excised using a laparoscopic scissors maintaining a 0.5 - 1.0 cm margin. For SRBS group, a single barbed bidirectional suture 1-PDO 14x14 cm 1/2 was used to suture the kidney (Figure-1). One needle entered first through kidney surface and stopped at the middle of the whole suture. Continuous suture was used to close renal pelvis or calices; then the needle went out through contralateral surface of the kidney,

<table>
<thead>
<tr>
<th>Variable</th>
<th>SRBS group (n = 36)</th>
<th>Non-SRBS group (n = 40)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(y)</td>
<td>51.3 ± 10.1</td>
<td>50.8 ± 11.2</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Sex (m/f)</td>
<td>21/15</td>
<td>24/16</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Tumor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Size (X ± s) cm</td>
<td>3.1 ± 1.2</td>
<td>3.0 ± 1.4</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Left/right(n)</td>
<td>16/20</td>
<td>17/23</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Location (upper pole/middle part/ lower pole) (n)</td>
<td>14/5/17</td>
<td>17/4/19</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Suture time (X ± s) min</td>
<td>10.4 ± 3.2</td>
<td>19.4 ± 6.7</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Warm ischemia time (X ± s) min</td>
<td>15.2 ± 4.2</td>
<td>24.1 ± 5.6</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Operation time (X ± s) min</td>
<td>78.5 ± 15.4</td>
<td>90.3 ± 18.1</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Blood loss (X ± s) mL</td>
<td>60.5 ± 21.2</td>
<td>110.4 ± 21.1</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Blood transfusion(n)</td>
<td>0</td>
<td>2</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Urine leak (n)</td>
<td>0</td>
<td>1</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Blood urine(n)</td>
<td>1</td>
<td>4</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Hospital stay (X ± s) (d)</td>
<td>5.9 ± 2.1</td>
<td>6.8 ± 2.3</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>
and continuous suture was used again to close the kidney. Another needle was used to close the left part. After the suture, the left quill line was used to tie a knot, or a Hem-o-lock was used to close at the end of suture (Figure-2).

For the non-SRBS group, the tumor bed and the collecting system were sutured with a continuous 3-0 polyglactin suture then superficial renorrhaphy was performed with running 3-0 polyglactin line intermittently, with Hem-o-lock clip for every suture. The bulldog clamp was removed and there was no bleeding in the surgical field and the tumor was removed through the Port A.

Statistical analysis

Statistical analyses were performed with SPSS software for Windows (Statistical Product and Service Solutions, version 10.0, SSPS Inc, Chicago, IL, USA). Categorical variables were compared with the chi-square test; continuous variables were compared with the Mann-Whitney U test. A value for P < 0.05 was considered statistically significant.

RESULTS

All 76 cases of retroperitoneoscopic partial nephrectomies were successfully performed, without conversion to open surgery or important intraoperative complications. All patients were followed for 1~11 months, without local recurrence and distant metastasis. In the SRBS group, the suture time, warm ischemia time and operation blood loss were shorter than that of non-SRBS group significantly; operation time and hospital stay were also shorter than that of non-SRBS group (Table-1).

DISCUSSION

With the widespread application of B ultrasound, CT and MRI exams, incidental renal cell carcinoma increased generally recently, which has characteristics of small size, low stage, slow growth and low potential for metastasis, with better prognosis than symptomatic renal cell carcinoma; the operation is the gold standard treatment for most T1 RCC currently (7).

Partial nephrectomy (PN) has been a new treatment for T1a renal cell carcinoma(RCC). Some studies show that chronic kidney disease (CKD) has relations with cardiovascular diseases (8), and when GFR < 60mL/min, the risks of death and in hospital treatment increase (9). RN is considered as a risk factor for the genesis and development of CKD; PN treatment keeps more kidney units left and decreases those affected (10,11). RN is a risk factor for the genesis and worsening of CKD; the studies showed that RN could increase the death rate and renal failure of RCC patients (12,13), PN can get the same outcomes with RN in histology, and it can maintain the kidney and cardiovascular function better in a long term follow-up (11,14).

PN includes open partial nephrectomy (OPN), laparoscopic partial nephrectomy (LPN), and robot-assisted partial nephrectomy (RAPN). LPN has gained increased acceptance with equivalent results at oncological and renal function
outcomes as OPN, with many advances such as less postoperative pain therapy, shorter hospital stay, and quick recovery (2-4).

LPN includes transperitoneal approach and retroperitoneoscopic approaches. Gill described the first retroperitoneoscopic partial nephrectomy in 1994 (15), Winfield finished the first retroperitoneoscopic partial nephrectomy in 1993 (16). The retroperitoneoscopic approach has advantages of easier controlling of kidney vessel, less disturbance of internal organs, and disadvantages of smaller operation field, less anatomic landmarks. Anatomic, programmed and standard operation could make up the disadvantages of the retroperitoneoscopic approach (1).

Even though with more advantages, LPN keeps some challenge for many urologists, resulting in more intraoperative complications (blood and urine leak etc.) and longer WIT. The WIT is closely related with kidney function, while the WIT > 30 minutes, the kidney function was affected more than 3-5 times (17,18). Sutting was the best way to keep kidney and to avoid urine leak, but it had great challenges (19,20). The good suturing techniques could decrease the rate of complications and shorten suturing time (3). Hem-o-lok substitution for knots was valid and safe (20,21), and could shorten suturing time and reduce WIT, but renal closures was still not tighter enough.

Bidirectional barbed sutures are manufactured from monofilament fibers via a micromachining technique that cuts barbs into the suture around the circumference in a helical pattern. The barbs are separated from one another by a distance of 0.88 to 0.98 mm and are divided into 2 groups that face each other in opposing directions from the suture midpoint. The use of knotless, barbed suture can securely suture tissues with less time, to close multiple layers tissues at the same time, and to decrease operation blood loss (22). Our study showed that Quill SRS barbed suture could improve efficiency in LPN, simplifying the suturing procedure, shortening suturing time and WIT, decreasing blood loss, with a tighten renal closure, and decrease of the incidence of urine leaks, hemorrhage, or other complications. Quill SRS consists of a delayed-absorbable material (polydioxanone) cut with barbs, which could prevent slippage through tissue and strengthen the suture, decreasing the chance of blood loss.

The application of Quill SRS bidirectional barbed absorbable suture in retroperitoneoscopic partial nephrectomy could shorten suturing time and warm ischemia time, with good safety and feasibility, worthy of being used generally in clinic.

CONFLICT OF INTEREST

None declared.

REFERENCES


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A Single-Institution Experience with Metallic Ureteral Stents: A Cost-Effective Method of Managing Deficiencies in Ureteral Drainage

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ABSTRACT

Introduction: The limitations of traditional ureteral stents in patients with deficiencies in ureteral drainage have resulted in frequent stent exchanges. The implementation of metallic stents was introduced to improve the patency rates of patients with chronic upper urinary tract obstruction, obviating the need for frequent stent exchanges. We report our clinical experiences with the use of metallic ureteral stents in the management of poor ureteral drainage.

Materials and Methods: Fifty patients underwent metallic ureteral stent placement from 2009 to 2012. Stent failure was defined as an unplanned stent exchange, need for nephrostomy tube placement, increasing hydronephrosis with stent in place, or an elevation in serum creatinine. Stent life was analyzed using the Kaplan-Meier methodology, as this was a time dependent continuous variable. A cost analysis was similarly conducted.

Results: A total of 97 metallic stents were placed among our cohort of patients: 63 in cases of malignant obstruction, 33 in the setting of cutaneous ureterostomies, and 1 in an ileal conduit urinary diversion. Overall, stent failure occurred in 8.2% of the stents placed. Median stent life was 288.4 days (95% CI: 277.4-321.2 days). The estimated annual cost for traditional polymer stents (exchanged every 90 days) was $9,648-$13,128, while the estimated cost for metallic stents was $4,211-$5,313.

Conclusion: Our results indicate that metallic ureteral stent placement is a technically feasible procedure with minimal complications and is well tolerated among patients. Metallic stents can be left in situ for longer durations and provide a significant financial benefit when compared to traditional polymer stents.

ARTICLE INFO

Key words: Costs and Cost Analysis; Metals; Stents; Ureteral Obstruction; Ureterostomy


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INTRODUCTION

Long-term ureteral patency often presents a difficult therapeutic challenge in patients with chronic ureteral obstruction. Traditional polymer ureteral stents have been the mainstay of therapy; however, primary patency of polymer ureteral stents has been suboptimal due to tumor compression and encrustation (1,2). Additionally, standard of care recommends regular stent replacement at 3-month intervals to prevent failure (3,4).

Methods of managing poor ureteral drainage include percutaneous nephrostomy tube placement and internal drainage with double pigtail stent insertion. Many of these patients, however, experience recurrent urinary tract infections, tube
migration, bladder irritation, local urinary symptoms (e.g. frequency, urgency, dysuria, etc.), or require daily care of a nephrostomy tube site, causing reduced quality of life (5).

Metallic ureteral stents consist of spirally coiled metal constructed to optimize compressive and radial strengths. Their ability to resist encrustation allows them to remain in situ for up to 12 consecutive months (6). The implementation of metallic stents was introduced to improve technical feasibility and patency rates for management of patients with upper urinary tract obstruction, obviating the need for frequent stent exchanges.

Prior literature on metallic stents has reported equivocal results. Most retrospective studies have been somewhat limited by their small study populations, as metallic stenting is a relatively recent procedure. Failure rates anywhere from 7% - 66% have been reported in the few retrospective series currently available (5,7-11).

We report our clinical experience with the use of metallic ureteral stents in the management of poor ureteral drainage. We also present a comparative cost analysis of patients managed with such stents, as opposed to patients treated with traditional polymer ureteral stents.

**MATERIALS AND METHODS**

After institutional review board approval was obtained, 50 patients were retrospectively identified to have who had undergone metallic ureteral stent placements in the management of ureteral drainage deficiencies at two of our academic facilities in Tampa, Florida (Moffitt Cancer Center and Tampa General Hospital). A total of 97 metallic stents were placed between January 2009 and September 2012. The same two surgeons placed these stents at both hospitals. All stents had a diameter of 6 French and length, ranging from 20 to 30cm. All patients who had metallic ureteral stents placed, had chronic ureteral obstruction in the context of a malignancy or required chronic ureteral stenting in the setting of a cutaneous ureterostomy or ileal conduit. Exclusion criteria included patients that had a previous ureteral balloon dilation or retrograde/antegrade endopyelotomy. Covariates assessed in our Cox univariate/multivariate analysis of potential predictors of metallic ureteral stent failure included patient age at diagnosis, gender, body mass index, underlying malignancy, cancer stage, site of ureteral obstruction, prior radiation therapy, and serum creatinine/creatinine clearance (calculated by the Cockcroft-Gault formula).

The Resonance Metal Stent® (RMS; Cook Urological®, Bloomington, IN) was designed to provide long-term drainage of chronic upper urinary tract obstruction. All metallic ureteral stents were placed while the patient was under general anesthesia, in a retrograde manner, with both fluoroscopic and cystoscopic guidance in patients with an intact bladder. Patients undergoing a metallic ureteral stent placement, in the management of chronic ureteral obstruction, had an initial stenting using a polymer ureteral stent to ensure the ureteral obstruction was in fact chronic in etiology, and that patient tolerated internal ureteral stenting with minimal urinary symptoms, thus making this a feasible long-term treatment option. In the patients undergoing chronic ureteral stent placement in the context of a cystectomy and urinary diversion, consisting of either a cutaneous ureterostomy or ileal conduit, ureteral stents were placed under local, regional, or general anesthesia with the assistance of fluoroscopy. Once a retrograde pyelogram was performed, a guidewire was successfully placed into the collecting system, the cylindrical outer sheath was passed into the renal pelvis, and the wire was removed. The proximal stent was uncurled, and then advanced through the sheath using a pusher. Under fluoroscopy, a push-pull technique was used to overly advance the sheath while placing the stent. The proximal stent curl was noted in the renal pelvis, and the outer sheath was removed, causing the distal curl to uncurl. At the completion of the procedure, the final fluoroscopy image was shot and saved to confirm proper placement of the stent. Medical agents were not used to alleviate irritative or voiding symptoms unless symptoms were severe, in which case, oral anticholinergic medications were prescribed. Patients were seen at 6 months post-metallc stent placement to assess symptomatology, as well as a serum creatinine. If there were no issues at that visit, patients were scheduled for
metallic stent exchange between 9-12 months. If patients were symptomatic (i.e. flank pain, rising serum creatinine), a KUB and renal ultrasound were obtained to rule out stent migration, encrustation and non-functional stents, which would be suspected based on new or worsening hydronephrosis. If this was seen, patients then underwent an earlier stent exchange (within 1-2 weeks of that visit). In equivocal cases of a possible obstructed stent, a MAG-3 renal scan was obtained.

Stent failure was defined as: 1) an unplanned stent exchange, 2) the need for nephrostomy tube placement, 3) increasing hydronephrosis with metallic ureteral stent in place, or 4) a deteriorating renal function, as determined by serum creatinine or worsening creatinine clearance, suspected to be post-renal in nature. In patients tolerating the metallic ureteral stents, stent exchanges were scheduled at 9 to 12 months-time intervals to optimize stent function/drainage and decrease the likelihood of stent encrustation. Median stent life was calculated from date of stent placement to date of stent exchange using the Kaplan-Meier method. Stent exchanges were treated as separate, individual events in this statistical analysis. Predictors of stent failure were assessed using Cox regression univariate/multivariate modeling with a robust covariance matrix estimator.

The present cost analysis accounted for stent cost, mean operating room fees (billed 1 hour), mean anesthesia costs, and the annual stent exchange rate. The annual exchange rate for metallic stents was derived from data collected from this retrospective study, and the annual exchange rate from conventional polymer stents was extrapolated from previously published data (3,4). The cost analysis does not include any other direct or indirect cost, with the exception of assigning an economic loss to the patient for missed work. Economic loss was calculated based on Florida’s Bureau of Labor Services mean daily wage of $157 US dollars.

**RESULTS**

A total of 97 metallic stents were placed in 50 patients (27 men, 23 women) during the 45-month accrual period. The mean patient age at diagnosis was 63.0 years (22-88 years). 37 patients (74%) had stents placed due to malignant ureteral obstruction, 12 patients (24%) had stents placed in the setting of cutaneous ureterostomies, and 1 patient (2%) had stent placement in the context of an ileal conduit. 14 patients (28%) had stents placed for genitourinary malignancies, 7 patients (14%) had stents placed for gastrointestinal malignancies, and 16 patients (32%) had stents placed for other malignancies, including lymphoma and sarcoma. A full breakdown of the indications for chronic ureteral stent placements is reported in Table-1. 13 patients (10 men, 3 women) had stents placed in the context of a cystectomy and urinary diversion. A total of 19 patients died with stents in situ. The patient characteristics of our study population are shown in Table-2.

At a mean patient follow-up of 303.2 days, stent failure occurred in 8 of the 97 stents placed among 16% (N = 8) of the total patients. The most common signs of stent failure were hydroureteronephrosis (N = 3, 37.5%) and recurrent urinary tract infection (N = 3, 37.5%). 1 stent failure (12.5%) was attributed to deteriorating renal function suspected to be post-renal in etiology, and 1 stent (12.5%) failed due to stent migration in a patient with a cutaneous ureterostomy diversion. Median time to stent failure was 68 days. Stent failures were managed by placing new metallic stents in 3 patients (37.5%), placement of a nephrostomy tube in 3 patients (37.5%), and exchange to a conventional polymer ureteral

**Table 1 - Indications for Stent Placement.**

<table>
<thead>
<tr>
<th>Reason for Stent</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>GU Malignancy</td>
<td>28%</td>
</tr>
<tr>
<td>GI Malignancy</td>
<td>14%</td>
</tr>
<tr>
<td>Ureteral Stricture</td>
<td>8%</td>
</tr>
<tr>
<td>Other Malignancy *</td>
<td>24%</td>
</tr>
<tr>
<td>Cutaneous Ureterostomy</td>
<td>24%</td>
</tr>
<tr>
<td>Ileal Conduit</td>
<td>2%</td>
</tr>
</tbody>
</table>

*Consists of sarcoma, lymphoma, small cell carcinoma, malignant breast cancer, primary peritoneal cancer, hemangiopericytoma
stent in 2 patients, who appeared not tolerate the composition of the metallic stents (25%).

Eighteen of the 50 patients (36%) had stents exchanged during the study period of 45 months, with a median stent life of 288.4 days (95% CI: 277.4-321.2 days). Kaplan Meier analysis of stent life is shown in Figure-1. This analysis takes into account those stents that failed prematurely from the anticipated time of exchange.

The Cox univariate and multivariate analysis of potential predictors of metallic stent failure did not yield any endpoints of statistical significance including gender, age at diagnosis, body mass index, prior external radiation therapy, site of ureteral obstruction, and underlying malignancy (Table-3).

In our cost analysis, we determined that the mean cost for a single traditional polymer ureteral stent exchange is between $2,255 and $3,125 US dollars, while the mean cost for a single metallic ureteral stent exchange is between $3,170 and $4,040 US dollars, with their only difference being the cost of the actual stent being placed. The estimated annual cost for traditional polymer stents (exchanged every 90 days (3,4)) is between $9,648 and $13,128 US dollars, while the esti-

Table 2 - Patient Characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Number of Patients</th>
<th>Age</th>
<th>BMI</th>
<th>Length of Follow-Up (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Women</td>
<td>Men</td>
<td>Mean</td>
</tr>
<tr>
<td>All Metallic Stents</td>
<td>50</td>
<td>23</td>
<td>27</td>
<td>62.96</td>
</tr>
<tr>
<td>For Obstruction</td>
<td>37</td>
<td>20</td>
<td>17</td>
<td>61.41</td>
</tr>
<tr>
<td>For Patients with No Bladder</td>
<td>13</td>
<td>3</td>
<td>10</td>
<td>67.38</td>
</tr>
</tbody>
</table>

Figure 1 - Kaplan-Meir Analysis Median stent life calculated by Kaplan-Meier: 288.4 days (95% CI= 277.4-321.2 days).
Table 3 - Predictive factors of metallic stent failure with p-values.

<table>
<thead>
<tr>
<th>Variable</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis</td>
<td>0.50</td>
</tr>
<tr>
<td>BMI</td>
<td>0.23</td>
</tr>
<tr>
<td>Malignancy Stage</td>
<td>0.38</td>
</tr>
<tr>
<td>Prior XRT</td>
<td>0.18</td>
</tr>
<tr>
<td>Sex</td>
<td>0.32</td>
</tr>
<tr>
<td>Site of Obstruction</td>
<td>0.94</td>
</tr>
<tr>
<td>Underlying malignancy</td>
<td>0.62</td>
</tr>
</tbody>
</table>

BMI = body mass index; XRT = radiotherapy

Table 4 - Cost Analysis.

<table>
<thead>
<tr>
<th></th>
<th>Metallic Stent</th>
<th>Polymer Stent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stent Cost</td>
<td>$1,040</td>
<td>$125</td>
</tr>
<tr>
<td>Anesthesia Costs (Medicare)</td>
<td>$130</td>
<td>$130</td>
</tr>
<tr>
<td>Operating Room Fees</td>
<td>$2,000</td>
<td>$2,000</td>
</tr>
<tr>
<td>Average Lost Wages ($/day) a</td>
<td>$157</td>
<td>$157</td>
</tr>
<tr>
<td>Total Cost Per Stent Insertion</td>
<td>$3,327</td>
<td>$2,412</td>
</tr>
<tr>
<td>Stent Life (in years)</td>
<td>0.79</td>
<td>0.25 b</td>
</tr>
<tr>
<td>Total Cost Per Year</td>
<td>$4,211</td>
<td>$9,648</td>
</tr>
</tbody>
</table>

aFrom Florida’s Bureau of Labor Services
bFrom previously published literature (3,4)

DISCUSSION

Deficiencies in upper tract drainage are a frequent problem encountered in routine urologic practice today. Conventional approaches in the management of chronic ureteral obstruction have been to place percutaneous nephrostomy drainage, which significantly decreases quality of life of the patient ailing from their malignancy (2). In addition, polymer ureteral stents have been used but have had disappointing results due to the frequency of stent exchanges (approximately every 3 months), stent encrustation, and pelvic tumor compression (1,2,12). Failure rates for traditional polymer stents in the setting of malignant ureteral obstruction are estimated to be between 40% and 60% (6,13). The use of metallic ureteral stents in the setting of deficient ureteral drainage obviates the need for an external urinary drainage bag, as well as decreasing the frequency of stent exchanges.

Metallic ureteral stents have been studied in a limited number of retrospective studies. Overall failure rates of metallic ureteral stents have ranged from 7% - 66% (5,7-11); however, most studies have been limited by low statistical power, with study populations as low as 14 patients. The present study is one of the largest single-institution studies, encompassing 50 patients, undergoing placement of 97 metallic ureteral stents. Our results show a failure rate of these stents of only 8.2%,...
exemplifying their clear benefit. Currently, metallic ureteral stents are indicated that they can be left in situ for up to 12 months (6). Our data shows a median stent life of 288.4 days before necessitating exchange. This is over three times longer than the average polymer stent life (3,4). In addition, although stents were elected to be exchanged between 9 to 12 months, we are now changing subsequent stents at 12 months with no additional sequelae (e.g. encrustation, decreased function, etc.). Longer stent in situ durations lead to less frequent trips to the operating room, decreased patient morbidity, decreased healthcare costs, and improved overall quality of life for the patient.

In addition, metallic ureteral stent placement procedures had minimal complications and were well tolerated by patients. Some patients complained of mild flank pain and/or dysuria directly after stent placement. This phenomenon was usually self-limiting, and probably due to expanding forces of the endoprosthesis (5). Goldsmith et al. described subcapsular hematoma formation following metallic stent placement in 12% of their study cohort. They argued that this was likely “related to the excessive length of the inner cannula relative to the outer sheath in the supplied introduced system” (8). In our larger single institution series, we did not experience any such complications and recommend gentle manipulation of the upper tracts during stent placement to avoid this issue.

To help predict treatment success or failure, we based our research on previously published peer reviewed scientific literature. As previously shown in Ganatra et al., the type of underlying malignancy did not predict stent success or failure in our series (13). Ganatra et al. also reported that gross tumor invasion noted at cystoscopy was a significant risk factor for stent failure and requirement of percutaneous nephrostomy (p = 0.008) (13). In addition, Goldsmith et al. reported that prostate cancer invading the bladder was a risk factor for stent failure (8). Bladder invasion was not specifically assessed as a risk factor for stent failure in our study. Wang et al. also showed that patients who had received previous radiation therapy had a significantly lower stent patency rate than those who did not receive previous radiation therapy. They hypothesized that radiation therapy causes ureteral fibrosis and impairs ureteral peristalsis, ultimately leading to more encrustation and a smaller ureteral lumen (11). Other studies nevertheless, have shown no difference in stent patency rates whether or not patients had received radiation therapy (8,10). Previous radiation therapy did not appear to predict stent outcome in our present series (p = 0.18).

We provide the first documented experience of metallic ureteral stents in the setting of cutaneous ureterostomies. Recent literature established that long term stenting (defined as greater than 3 months) of cutaneous ureterostomies improves their clinical outcome, decreasing stenosis in the left crossover ureter in the urinary diversion (14). 12 patients in our single institution series had metallic ureteral stents in the setting of cutaneous ureterostomies. The failure rate in this cohort was 16.7%, with a mean exchange rate of 220.3 days. The use of metallic ureteral stents in the setting of cutaneous ureterostomies appears to be a cost effective management for chronic stent placement in this population.

Metallic ureteral stents, when used in managing poor ureteral drainage, not only improved quality of life, but also is a cost-saving service. Despite the initial higher cost of the individual metallic stent versus traditional polymer ureteral stents ($1040 versus $125 US dollars, respectively), we report fewer surgical procedures (i.e. stent exchange) needed, which accounted for this cost difference. The overall cost reduction was estimated to be between 56.4% and 59.5% per patient-year, not taking into account other cost savings, including reduced post-operative office visits, fewer follow-up imaging studies, and any unforeseen operative complications.

We recognize several limitations to the present study, including the retrospective constitution of this single institution study design. Although larger (multicenter) studies have been conducted, our sample size of only 97 stent placements made our univariate and multivariate Cox regression analyses somewhat limited. Additionally, our study did not look at bladder tumor invasion as a risk factor for premature stent failure and also did not specifically characterize AUA symptom scores pre- and post-stent placement.
within our study cohort. Furthermore, although our cost analysis examines the major variables of stent placement, it does not include data on failure and follow-up costs, such as repeat procedures, subsequent admissions, and necessary imaging. Cost analysis for patients with cutaneous ureterostomies and ileal conduits would differ slightly, however, these were not analyzed. Costs were calculated in patients with intact bladders. Lastly, the analysis took into account operating facility fees and anesthesia fees charged at our institutions, but these were not standardized across other institutions or regions.

CONCLUSIONS

In conclusion, this study highlights that metallic ureteral stents constitute a technically feasible solution for the management of deficiencies in ureteral drainage, while being well tolerated and imparting minimal complications to appropriately selected patients. Metallic ureteral stents can be left in situ for longer durations than traditional polymer ureteral stents and result in an estimated cost benefit of between 56.4% and 59.5%.

CONFLICT OF INTEREST

Dr. Philippe E. Spiess serves as a national lecturer for Cook Medical.

REFERENCES


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The comparison of urodynamic findings in women with various types of urinary incontinence

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ABSTRACT

Purpose: We aimed to determine the differences of the urodynamic findings of mixed urinary incontinence (MUI), urge urinary incontinence (UUI), and stress urinary incontinence (SUI), and to evaluate the urodynamic findings in different groups by using bladder sensitivity index (BSI).

Materials and Methods: The data of 99 patients who underwent urodynamic testing related to the suspicion of SUI, UUI or MUI were analysed. This analysis included a retrospective evaluation of patients’ cards, voiding diaries, and urodynamic reports. At filling cystometry, the parameters of first sensation of bladder filling (FSBF), first desire to void (FDV), strong desire to void (SDV), and bladder capacity (V_max), which were related to the bladder sensation, were determined. Subsequently, uroflowmetric findings were recorded during bladder emptying. BSI was defined as the ratio of V_max / FDV. These results were statistically compared among the groups.

Results: The sample included 35 (35.5%) MUI, 33 (33.3%) UUI, and 31 (31.1%) SUI. The mean ages were similar in all groups (P = 0.868). The mean FSBF, FDV, SDV and V_max values were significantly different among groups (p = 0.004, p < 0.001, p < 0.001, p < 0.001 respectively). Nevertheless, there was no statistically significant difference among the mean daily voiding accounts (P = 0.005). Although the mean maximum flow rate (Q_max) values were similar (P = 0.428), the mean maximum detrusor pressure (Pdet_max) values were significantly different (P = 0.021). The mean BSI values showed no significant differences (P = 0.097).

Conclusions: It was concluded that while the use of urodynamic testing could contribute to the management of urinary incontinence, the indexes including BSI require more detailed and comprehensive studies.

INTRODUCTION

Urinary incontinence (UI) is a disorder with various symptoms of different severities and influences the women in all ages. Although it is not a life-threatening disorder, it can significantly affect the physical, psychological and social conditions of patients (1). While a great deal of attention has been given to possible treatment options for incontinence, the clinicians have also made a great effort to standardize the evaluation of these patients. The determination of the type and severity of UI, and its impact on patients’ quality of life is an important issue, which affects the results of the treatment. Thus, it is a fact that the patients with UI must be evaluated by some diagnostic tools before treatment. Generally, these patients are evaluated by an initial detailed history, physical

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examination, routine urine test, and voiding diary. If required, non-invasive urodynamic testing and postvoiding residual urine measurement are performed. A multichannel urodynamic study is used in patients who are not diagnosed by previous analyses (2).

UI can be grouped according to etiological factors, as stress urinary incontinence (SUI), urge urinary incontinence (UUI) and mixed urinary incontinence (MUI). Although the routinely use of urodynamic testing at initial evaluation of UI is not suggested by the current guidelines, it is supposed that urodynamic testing will provide some contributions to the clinicians to understand these disorders (3). Currently, the index values associated with bladder filling phase are still on debate, and bladder sensitivity index (BSI) is one of the most important (4).

In this study, we aimed to analyze the presence of possible differences among the urodynammic findings of MUI, UUI and SUI, and to evaluate the urodynamic characteristics in these groups by using BSI.

MATERIAL AND METHODS

The data of 99 patients who underwent urodynamic testing between October 2009 and March 2011 as a result of the suspicion of SUI, UUI or MUI in Celal Bayar University, Faculty of Medicine, Department of Urology were analysed. This analysis included a retrospective evaluation of patients’ cards (that was completed previously to urodynamics), voiding diaries and urodynamic reports. The study protocol was approved by the Local Ethical Committee of Celal Bayar University, Faculty of Medicine.

The inclusion criteria to the study were:

1. The data of patients who underwent urodynamic investigation related to only the suspicion of SUI, UUI, or MUI.
2. The data of only the optimally performed urodynamic testing.
3. The previously recorded data of patients’ cards, voiding diaries, and urodynamic reports without any lacks.

The exclusion criteria to the study included:

1. The history of previous incontinence surgery.
2. The history of previous or ongoing anticholinergic treatment.
3. The patients with different types of urinary incontinence, such as overflow incontinence, except for SUI, UUI and MUI.
4. The data of patients who underwent urodynamic investigation related to other reasons than SUI, UUI and MUI.
5. The presence of an illness that can cause urinary system dysfunction, such as multiple sclerosis, spinal tumor.
6. The presence of urinary tract infection.
7. The presence of an urinary fistula or diverticulum.

The data of age, medical history, the complaint at the time of the application, the findings of physical examination, voiding diary and urodynamic testing were determined. All of the urodynamic studies have been peformed by using an “Aymed” model, multichannel urodynamic device. To avoid false urodynamic evaluation, all patients micturated before the urodynamic investigation to empty the bladder. If the residual urine was present, it was emptied after the insertion of the urodynamic catheter. Subsequently, a pressure-flow study was performed. Eight Fr, three-way cystometry catheter that measured the bladder pressure and rectal balloon catheter that measured the abdominal pressure were placed in. During the filling phase, the infusion rate of isotonic NACl solution was 40mL/sec. By filling of the bladder with isotonic NACl solution, the terms of first sensation of bladder filling (defined as the initially recognition of bladder filling, FSBF, mL), first desire to void (the time of voiding desire that can be voluntarily retarded, FDV, mL), strong desire to void (defined as the time of the presence of strong desire to void without any urges, SDV, mL), and maximum bladder capacity (V max) were determined. Subsequently, the findings were recorded during bladder emptying (5,6). The suggestions of
International Continence Society (ICS) were used for technique and terminology of urodynamics (7). At filling phase, the presence of an increased bladder pressure > 15cmH20 and uninhibited detrusor contractions (DO) were noted. BSI was defined as the ratio of maximum bladder capacity and the bladder capacity during the occurrence of FDV (V_max/FDV).

All statistical analyses were performed by using SPSS 11.0. A p-value < 0.05 was accepted as significant. A simplex variance analysis was used in triple group comparisons. The double group comparisons were done by using independent sample t-test for large sample and Mann-Whitney U test for small sample. Pearson correlation analysis was used for the correlation of parameters.

RESULTS

The study included the data of 99 patients: 33 (33.3%) patients with UUI, 31 (31.1%) patients with SUI and 35 (35.5%) patients with MUI. The mean age was 49.06 ± 15.9, 50.6 ± 11.1 and 50.3 ± 11.3 years, respectively. The mean ages were similar in all groups (P = 0.868) (Figure-1). The mean FSBF values of patients with MUI, UUI and SUI were 134.4 ± 31.7, 136.8 ± 33.8 and 112.9 ± 24.5mL, respectively. The mean FSBF values were significantly different (p = 0.004). While the mean FDV values were 232.6 ± 44.5, 195.2 ± 36.5 and 247.2 ± 56.5mL, the mean SDV values were 334.02 ± 64.8, 273.3 ± 61.5, and 340.8 ± 68.8mL, respectively. The mean FSBF and FDV values in all groups were statistically significantly different (p < 0.001 in all). The mean bladder capacity was 420.4 ± 58.6mL in MUI group, 331.7 ± 59.7mL in UUI group, and 453.9 ± 62.3mL in SUI group. These results were also significantly different (p < 0.001). Nevertheless, no statistically significant difference was found among the mean voiding accounts (10.9 ± 4.06, 12.2 ± 4.02, 9.1 ± 2.6) (p = 0.005). The emptying phase showed that the mean maximum flow rate (Q_max) was 25.3 ± 7.65mL in MUI group, 24.1 ± 5.49mL in UUI group, and 26.6 ± 9.3mL in SUI group. The mean value of maximum detrusor pressure (Pdet_max) was 33.5 ± 18.7, 32.6 ± 7.5, and 25.03 ± 9.9, respectively. Although no statistically significant difference was found in the mean Q_max values (p = 0.428), the mean Pdet_max values were significantly different (p = 0.021). The mean BSI values were 1.8 ± 0.3 in MUI group, 1.7 ± 0.2 in UUI group, and 1.9 ± 0.4 in SUI group. It was determined that there were no significant differences between the mean BSI values of 3 groups (p = 0.097). A detailed demonstration of these analyses is presented in Table-1.

In our sample, it was determined that 35 patients presented DO and 33 patients had no DO at urodynamic testing. When we looked at the distribution of these accounts according to the incontinence subgroups, it is observed that DO was present in 15 patients in MUI group and 20 patients in UUI group. However, 20 patients in MUI group and 13 patients in UUI group had no DO. We have not determined a significant correlation between the patient accounts with and without DO in MUI and UUI groups (P = 0.143). These results are clearly shown in Table-2. It has been determined that no significant difference was present among the BSI values in all three groups. However, we analyzed the relation between the BSI values of patients with and without DO in MUI and UUI groups. When we compared the BSI scores of patients with DO with the scores of patients without DO in MUI and UUI groups, it was also found that BSI scores had no statistically significant differences (p = 0.923, p = 0.686; respectively). The presence of DO had no impacts on the BSI scores of patients in MUI and UUI groups (Table-3). When the data were analyzed in paired groups, the comparison of daily voiding accounts between
### Table 1 - A detailed demonstration of the analysis of the parameters in all groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MUI (n = 35)</th>
<th>UUI (n = 33)</th>
<th>SUI (n = 31)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>50.3 (11.3)</td>
<td>49.06 (15.9)</td>
<td>50.6 (11.1)</td>
<td>0.868</td>
</tr>
<tr>
<td>Daily voiding account</td>
<td>10.9 (4.06)</td>
<td>12.2 (4.02)</td>
<td>9.1 (2.6)</td>
<td>0.005</td>
</tr>
<tr>
<td>First sensation of bladder filling</td>
<td>134.4 (31.7)</td>
<td>136.8 (33.8)</td>
<td>112.9 (24.5)</td>
<td>0.004</td>
</tr>
<tr>
<td>First desire to void</td>
<td>232.6 (44.5)</td>
<td>195.2 (36.5)</td>
<td>247.2 (56.5)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Strong desire to void</td>
<td>334.02 (64.8)</td>
<td>273.3 (61.5)</td>
<td>340.8 (68.8)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Bladder capacity</td>
<td>420.4 (58.6)</td>
<td>331.7 (59.7)</td>
<td>453.9 (62.3)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Qmax</td>
<td>25.3 (7.6)</td>
<td>24.1 (5.4)</td>
<td>26.6 (9.3)</td>
<td>0.428</td>
</tr>
<tr>
<td>Pdet max</td>
<td>33.5 (18.7)</td>
<td>32.6 (7.5)</td>
<td>25.03 (9.9)</td>
<td>0.021</td>
</tr>
<tr>
<td>BSI</td>
<td>1.8 (0.3)</td>
<td>1.7 (0.2)</td>
<td>1.91 (0.4)</td>
<td>0.097</td>
</tr>
</tbody>
</table>

### Table 2 - The distribution of patient accounts according to the presence of uninhibited detrusor contraction at urodynamic testing.

<table>
<thead>
<tr>
<th>Incontinence Type</th>
<th>MUI</th>
<th>%</th>
<th>UUI</th>
<th>%</th>
<th>Total</th>
<th>p - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No DO</td>
<td>20</td>
<td>60.6</td>
<td>13</td>
<td>39.4</td>
<td>33</td>
<td>0.143</td>
</tr>
<tr>
<td>DO</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DO present</td>
<td>15</td>
<td>42.9</td>
<td>20</td>
<td>57.1</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>35</td>
<td>51.5</td>
<td>33</td>
<td>48.5</td>
<td>68</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3 - The comparison of bladder sensitivity index scores of the patients in mix and urge urinary incontinence groups according to the presence of uninhibited detrusor contraction.

<table>
<thead>
<tr>
<th>DO</th>
<th>BSI</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>p - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MUI</td>
<td>BSI</td>
<td>no</td>
<td>20</td>
<td>1.8</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>present</td>
<td>15</td>
<td>1.8</td>
<td>0.3</td>
</tr>
<tr>
<td>UUI</td>
<td>BSI</td>
<td>no</td>
<td>13</td>
<td>1.7</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>present</td>
<td>20</td>
<td>1.7</td>
<td>0.2</td>
</tr>
</tbody>
</table>
MUI and UUI groups revealed that there were no statistically significant differences (p = 0.201). However, the comparison between SUI group and the groups of MUI and UUI showed that the daily voiding accounts in MUI and UUI groups were significantly higher than in SUI group (p = 0.037, p = 0.001; respectively). Although it was not determined a significant difference between the volumes of FSBF in MUI and UUI groups (p = 0.768), FSBF occurred in higher volumes in SUI group than in other two groups. The patients in SUI groups felt the FSBF in later volumes (p = 0.003, p = 0.002; respectively). Moreover, FDV and SDV values in SUI group were significantly lower than in SUI and MUI groups (p < 0.001 in all). Nevertheless, no significant difference was found between the FDV and SDV values of MUI and SUI groups (P FDV = 0.248, P SDV = 0.679; respectively). The comparison results of Vmax values among all groups showed that the Vmax in SUI group was the highest (453.9 ± 62.3mL), and Vmax in UUI group was the lowest (331.7 ± 5.7mL). The Vmax values were significantly different in each other (p < 0.001, p = 0.028, p < 0.001; respectively). The results of the comparison of all parameters in paired groups are clearly shown in Table-4.

DISCUSSION

Since the demonstration of the anatomy of female pelvis and its contents, the impact of the structure of female pelvic organs and the anatomical supplies on the continence mechanism have been discussed. It is currently possible to explain UI by urodynamic testing. In current practice, it is important to determine the changes in the anatomical structures with their functional interactions in the diagnosis and treatment planning of UI (8). The measurement of the intensity of desire for micturition has attracted great interest for the determination of the severity of symptoms and the evaluation of treatment results in many lower urinary tract disorders (9). In patients with overactive bladder (OAB), the development of an assessment scale and the evaluation of urgency, which is the most common complaint of OAB patients, is particularly important (10,11). Urodynamic study is the standard technique used for the evaluation of bladder sensation (7,12). Moreover, the voiding diaries, which are reliable and repeatable tests, can be used for the assessment of bladder sensation (13,14). In this study, we aimed to evaluate the presence of possible differences among the urodynamic findings of MUI, UUI and SUI, and to analyze the urodynamic findings in these groups by using BSI.

When Wiskind et al. compared the urodynamic findings of patients with MUI and UUI, they found that the Vmax was 308mL in UUI group and 396mL in MUI. The cystometric Vmax was significantly higher in patients with MUI (15). In another study, it was reported that the comparison of urodynamic findings between healthy volunteers and UUI patients showed that the patients with UUI felt the FSBF, FDV and SDV in lower volumes and earlier than control group (5). In our study, we analyzed the urodynamic findings in three subgroups of incontinence including MUI, UUI and SUI with the aim of finding possible alterations in bladder sensation related to the type of incontinence. Some significant differences were determined in terms of FSBF, FDV, SDV and Vmax among all three groups. Our study revealed that while the lowest FDV (195.2 ± 36.5mL) and SDV (273.3 ± 61.5mL) values were in UUI group, the highest FDV (247.2 ± 56.5mL) and SDV (340.8 ± 68.8mL) values were observed in SUI group. Nevertheless, contrary to the expectations, while the patients in UUI group had the highest FSBF value (136.8 ± 33.8mL), the lowest FSBF value (112.9 ± 24.5mL) was observed in SUI group. We thought that the sensitivity of FSBF was insufficient in the evaluation of bladder sensation. Thus, this unit should be analyzed by more detailed and prospective studies.

The comparison of the data in bladder diaries showed that daily voiding accounts in MUI, UUI, and SUI groups were significantly different. It was determined that the daily voiding account in UUI group had the highest result (12.2 ± 4.02). Nevertheless, the daily voiding account in SUI group was the lowest (9.1 ± 2.6). In a previous study, Chieh and et al. analyzed the urodynamic characteristics in patients with MUI and UUI. They reported that no significant difference was found between the daily voiding accounts of MUI and UUI groups (3). Furthermore, additional studies are present in the previous literature (9,17). On the other hand, when we looked at the urodynamic findings regarding the frequency of DO
Table 4 - The demonstration of the comparison results of all parameters in paired groups.

<table>
<thead>
<tr>
<th>Incontinence Type</th>
<th>N</th>
<th>Mean value (mL)</th>
<th>SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Daily voiding account</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUI</td>
<td>35</td>
<td>10.9</td>
<td>4.06</td>
<td>0.201</td>
</tr>
<tr>
<td>UUI</td>
<td>33</td>
<td>12.2</td>
<td>4.02</td>
<td></td>
</tr>
<tr>
<td>SUI</td>
<td>31</td>
<td>9.1</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>UUI</td>
<td>33</td>
<td>12.2</td>
<td>4.02</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>First sensation of bladder filling</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUI</td>
<td>35</td>
<td>134.4</td>
<td>31.7</td>
<td>0.768</td>
</tr>
<tr>
<td>UUI</td>
<td>33</td>
<td>136.8</td>
<td>33.8</td>
<td></td>
</tr>
<tr>
<td>MUI</td>
<td>35</td>
<td>134.4</td>
<td>31.7</td>
<td></td>
</tr>
<tr>
<td>SUI</td>
<td>31</td>
<td>112.9</td>
<td>24.5</td>
<td>0.003</td>
</tr>
<tr>
<td>UUI</td>
<td>33</td>
<td>136.8</td>
<td>33.8</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>First desire to void</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUI</td>
<td>35</td>
<td>232.6</td>
<td>44.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>UUI</td>
<td>33</td>
<td>195.2</td>
<td>36.5</td>
<td></td>
</tr>
<tr>
<td>MUI</td>
<td>35</td>
<td>232.6</td>
<td>44.5</td>
<td></td>
</tr>
<tr>
<td>SUI</td>
<td>31</td>
<td>247.2</td>
<td>56.5</td>
<td>0.248</td>
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<tr>
<td>UUI</td>
<td>33</td>
<td>195.2</td>
<td>36.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Strong desire to void</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUI</td>
<td>35</td>
<td>334.03</td>
<td>64.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>UUI</td>
<td>33</td>
<td>340.8</td>
<td>68.8</td>
<td>0.679</td>
</tr>
<tr>
<td>MUI</td>
<td>35</td>
<td>273.33</td>
<td>61.5</td>
<td></td>
</tr>
<tr>
<td><strong>Bladder capacity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUI</td>
<td>35</td>
<td>420.4</td>
<td>58.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>UUI</td>
<td>33</td>
<td>331.7</td>
<td>59.7</td>
<td></td>
</tr>
<tr>
<td>MUI</td>
<td>35</td>
<td>420.4</td>
<td>58.6</td>
<td></td>
</tr>
<tr>
<td>SUI</td>
<td>31</td>
<td>453.9</td>
<td>62.3</td>
<td>0.028</td>
</tr>
<tr>
<td>UUI</td>
<td>33</td>
<td>331.7</td>
<td>59.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SUI</td>
<td>31</td>
<td>453.9</td>
<td>62.3</td>
<td></td>
</tr>
</tbody>
</table>
in MUI and UUI groups, the frequency of DO in UUI group (57.1%) was higher than it was in MUI group (42.9%). But, this result was not found statistically significant. Similar results are present in the literature (16,18). Yong-Yau lin et al. reported that the parameters, which demonstrated the voiding function, such as maximum flow rate, average flow rate and maximum detrusor pressure were similar in the study groups (16). In our study, no significant difference was found in terms of $Q_{\text{max}}$ among all three groups ($p = 0.428$). Although $P_{\text{detmax}}$ values in 3 groups were significantly different ($p = 0.021$), the comparison of $P_{\text{detmax}}$ values between MUI and UUI groups showed no significant difference ($p = 0.807$). It was supposed that the presence of significant difference in terms of $P_{\text{detmax}}$ among all three groups was due to voiding in lower pressures of patients with SUI.

Currently, the nomograms such as Abram-Griffits and Schafer, and the indexes such as Bladder Outlet Obstruction Index and Bladder Contractility Index are used to objectively evaluate bladder emptying (19). Some studies also evaluate the filling phase of the bladder in an analytical plane. BSI, which was created by Al-Shukri et al. (4), is one of these studies. This index was proposed with the aim of increasing the accuracy of urodynamic diagnosis of patients with OAB (4). This term is calculated by the proportion of $V_{\text{max}}$ to the bladder volume at the time of the occurrence of FSBF ($V_{\text{max}}/\text{FSBF}$). This novel urodynamic parameter demonstrates the severity of voiding desire from FSBF to the squeeze. Low BSI value means that the duration from FSBF to reach $V_{\text{max}}$ is short. A previous study revealed that BSI was lower in male patients than in female patients. It was also lower in patients with DO than in patients without DO ($p = 0.001$). Moreover, it was reported that this parameter was lower in patients who had higher frequency of urgency (4). In our study, the overall comparison of BSI values among three groups and also the separate comparison of BSI values only between MUI and UUI groups revealed no significant differences.

**CONCLUSIONS**

Sensation is an essential prerequisite for voluntary control of the bladder. Urodynamic studies are the standard technique in the evaluation bladder sensation. At filling phase of cystometry, it has been previously shown that various groups of patients had voiding desire in different bladder volume and severity. In our study, it was found that FSBF, FDV, SDV and $V_{\text{max}}$, which were associated with bladder sensation, were significantly different in all three groups. Paired groups comparisons revealed that while the lowest FDV and SDV values were observed in UUI group, the highest FDV and SDV values were observed in SUI group. The presence of premature filling sensations and decreased bladder capacity in patients with MUI and UUI were demonstrated. Nevertheless, contrary to the expectations, while the patients in UUI group had the highest FSBF, the lowest FSBF value were seen in SUI group. On the other hand, no statistically significant difference was found among the BSI values and urodynamic data of patients in all three groups.

**ABBREVIATIONS**

UI = Urinary incontinence  
MUI = Mix urinary incontinence  
UUI = Urge urinary incontinence  
SUI = Stress urinary incontinence  
BSI = Bladder sensitivity index  
FSBF = First sensation of bladder filling  
FDV = First desire to void  
SDV = Strong desire to void  
$V_{\text{max}}$ = Maximum bladder capacity  
ICS = International Continence Society  
DO = Uninhibited detrusor contractions  
$P_{\text{detmax}}$ = Maximum detrusor pressure at maximum flow.  
$Q_{\text{max}}$ = Maximum flow rate  
OAB = Overactive bladder

**CONFLICT OF INTEREST**

None declared.

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Obesity may influence the relationship between sex hormones and lower urinary tract symptoms

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Division of Urology, University of Sao Paulo Medical School, SP, Brazil

ABSTRACT

Purpose: The effects of serum testosterone in the lower urinary tract symptoms (LUTS) in patients with benign prostatic hyperplasia (BPH) are not well established. The objective of the study is to evaluate the association of sex hormones with LUTS and control the results by patient weight.

Materials and Methods: The study comprised a cross-sectional analysis of 725 men included in a prostate cancer screening program at University of Sao Paulo Medical School. The serum concentrations of total testosterone (TT), free testosterone (FT) and sex hormone binding globulin (SHBG) were measured. Variables analyzed were age, American Urological Association (AUA) symptom score, storage symptoms, voiding symptoms, quality of life score, prostate specific antigen levels and prostate volume. Obesity was measured through the calculation of body mass index (BMI). A regression analysis model was performed.

Results: Median patient age was 65 years (48 to 94). A higher TT level was significantly associated with a severe AUA symptom score only among patients with a BMI ≥ 25. Median TT was 371, 370 and 427ng/dL (p = 0.017) in patients with mild, moderate and severe LUTS respectively. The multivariate regression analysis in patients with BMI ≥ 25 showed that only age, TT and sex score were related to LUTS.

Conclusions: A higher TT is associated with a severe AUA score symptom index only in obese patients. Further analysis are necessary to evaluate the mechanisms through which testosterone may influence LUTS in these patients.

ARTICLE INFO

Key words:
Prostatic Hyperplasia; Lower Urinary Tract Symptoms; Testosterone; Obesity


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INTRODUCTION

Benign prostatic hyperplasia (BPH) is the main cause of lower urinary tract symptoms (LUTS) in the aging man. About 90% of men in their 70s have some LUTS related to BPH and the main consequence is impairment in their health-related quality of life (1). The lifetime probability among men in their fifties to receive treatment for LUTS secondary to BPH is estimated to reach 35% of cases (2). Less commonly, LUTS related to BPH may also progress to acute urinary retention, need for surgery, urinary incontinence or recurrent urinary tract infection (3).

Despite these facts, the etiology of BPH is not fully elucidated and several etiologic factors have been proposed (4). Clinical and experimental evidence suggest that testosterone, estrogens and peptide growth factors (GFs) play important roles (5-7). It’s well recognized that sexual hormones are essential for normal prostate development and growth. Androgens are also important in the maintenance of BPH as demonstrated by the size reduction of an enlarged prostate with
androgen deprivation therapy (8). However, it is unclear whether altered serum concentrations of androgens are associated with increased risk of LUTS or clinical BPH.

Thus far, published studies regarding the association between androgens and LUTS or BPH have reported controversial results (9-12). Additionally, studies that have found positive associations have analyzed different hormones. These discrepancies may be attributed to small sample sizes, important selection bias, inadequate assessment of BPH, and failure to control by other potentially confounding factors that may influence serum testosterone levels, such as obesity (13,14).

As it’s well known, obesity in men is commonly accompanied by a decline of serum testosterone (TT) levels through different mechanisms (15,16). The objective of the study was to evaluate the association of sex hormones with LUTS and control the results by the body mass index (BMI).

**MATERIALS AND METHODS**

The study comprised a cross-sectional analysis of 725 men who participated in a prostate cancer screening program. Patients who were using 5-alpha reductase inhibitors, alpha adrenergic blockers, had history of prostatic surgery or endocrine diseases, were not considered for analysis. Patients with abnormal digital rectal examination (DRE) findings or an elevated serum PSA level (i.e. > 4.0ng/mL) were referred for prostate biopsy to exclude the possibility of prostate cancer.

Variables analyzed were age, American Urological Association (AUA) symptom score, AUA storage symptoms, AUA voiding symptoms, nocturia, AUA quality of life score (17), international index of erectile function (IIEF) score, prostate specific antigen levels and prostate volume. We measured the serum concentrations of total testosterone (TT), free testosterone (FT) and sex hormone binding globulin (SHBG) in all men. These hormones were measured through the fluorimetric, fluoroimmunoassay, and immunofluorimetric methods respectively (Wallac Perkin Elmer, Finland). We considered the following normal ranges: 271 to 965ng/dL for TT, 131 to 640pmol/L for FT and 12 to 75nmol/L for SHBG. Patients were submitted to DRE, which was performed only by urologists with experience in prostate examination. An enlarged prostate was defined as prostate volume > 30 grams. Obesity was defined through calculation of the BMI, which was calculated as weight in kilograms divided by the square of height in meters (Kg/m²) and categorized as underweight (< 18.5Kg/m²), normal weight (18.5 to 24.9Kg/m²), overweight (25 to 29.9Kg/m²) and obese (≥ 30Kg/m²). Waist circumference (WC) and hip circumference (HC) were also measured. Demographic characteristics of the men are described in Table-1. Median patient age was 65 years (48 to 94). Median serum PSA level was 1.9ng/mL (0.1 to 62.6) and median AUA symptom score was 10 (0 to 35). Median total testosterone, free testosterone and SHBG levels were 404.5, 236 and 54ng/dl respectively. Median BMI was 27 (15 to 48). TT, FT and SHBG were correlated with all clinical parameters. For analysis of obesity, patients were divided in two groups according to the BMI ≥ 25 or < 25. Sex hormones were correlated with all urinary symptoms among both groups.

For statistical analysis we used the Chi-square, ANOVA and Kruskal Wallis tests to correlate the sex hormones with clinical parameters. A multivariate regression analysis model was used. Statistical analysis was performed using the SPSS 12.0 for Windows software and significance was set as a p ≤ 0.05.

**RESULTS**

Table-2 shows correlation of patient characteristics with urinary symptoms. Patients with older age, higher PSA levels and lower IIEF presented worse LUTS with statistically significant results. Patients with a higher TT level and greater prostate weight also presented worse LUTS, but with marginally significant results. Mean TT among patients with mild, moderate and severe symptoms was 430.2, 430.3 and 468.0ng/dL respectively. When we analyzed filling symptoms, voiding symptoms and nocturia separately, no statistically significant associations were found.

TT didn’t show any significant correlation with the other clinical parameters but with obesity. Patients who were overweight and obese had
lower levels of TT ($p < 0.001$). Median FT levels were statistically lower among patients with 65 years or older (211 vs 254ng/dL) ($p < 0.001$), and with a higher BMI ($p = 0.006$). SHBG levels were statistically higher among patients with 65 years or older ($p < 0.001$) and statistically lower among patients with a higher BMI. These two hormones failed to show any significant correlation with urinary symptoms or other clinical parameters (data not shown).

Due to associations between serum hormone levels and surrogate measures of obesity,
we analyzed the associations between hormone levels and urinary symptoms among patients with BMI < 25 (underweight or normal weight) and ≥ 25 (overweight or obese) separately. The results showed that TT levels were significantly associated with AUA symptom score among patients with a BMI ≥ 25. Median TT was 371, 370 and 427 ng/dL (p = 0.017) in patients with mild, moderate and severe LUTS respectively. FT and SHBG were not associated with urinary symptoms in this BMI group (Table-3). Analysis of voiding symptoms, filling symptoms and nocturia separately failed to show any association with hormone levels according to this BMI category.

Table 3 - Logistic regression analysis for the occurrence of severe LUTS among patients with BMI ≥ 25.

<table>
<thead>
<tr>
<th>Variable (± SD)</th>
<th>OR</th>
<th>95%CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>1.03</td>
<td>1.00-1.06</td>
<td>0.02</td>
</tr>
<tr>
<td>Total PSA (ng/mL)</td>
<td>1.00</td>
<td>0.99-1.02</td>
<td>0.23</td>
</tr>
<tr>
<td>TT (ng/dL)</td>
<td>1.00</td>
<td>1.00-1.00</td>
<td>0.00</td>
</tr>
<tr>
<td>IIEF</td>
<td>0.95</td>
<td>0.93-0.98</td>
<td>0.00</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>0.90</td>
<td>0.34-2.38</td>
<td>0.83</td>
</tr>
<tr>
<td>Prostatic Weight (g)</td>
<td>1.00</td>
<td>0.99-1.01</td>
<td>0.64</td>
</tr>
</tbody>
</table>

No associations were found among patients with a BMI < 25. Median TT was 453, 450 and 467 ng/dL (p = 0.952) among patients with mild, moderate and severe LUTS respectively. FT and SHBG were not associated with urinary symptoms in this BMI group. Analysis of voiding symptoms, filling symptoms and nocturia separately failed to show any association.

Finally, we performed a multivariate regression analysis for the occurrence of severe LUTS among patients with a BMI ≥ 25 and < 25. An increased age, a higher TT level and a worse IIEF were independently associated with the presence of severe LUTS among patients from the former group. Only a worse IIEF was independently associated with LUTS among patients with a BMI < 25.

DISCUSSION

In the present study, we found that serum TT and FT levels were significantly lower, and SHBG levels were significantly higher in obese patients when compared to patients with lower weights. When the entire cohort was analyzed, TT levels were higher in patients with severe LUTS, however, these results reached only marginal statistical significance. When patients with a BMI ≥ 25 were analyzed, TT levels were significantly higher in patients with severe LUTS when compared to patients with moderate or mild LUTS. These findings suggest that sexual hormones may play a role in the pathogenesis of LUTS related to BPH. Multivariate analysis confirmed TT as an independent variable associated to a severe LUTS. No correlations were observed among patients with a BMI < 25.

The reasons why sex hormones may influence LUTS related to BPH are not well described. Despite the lack of knowledge regarding several aspects of BPH pathogenesis, its well recognized that the development of the histological features of the disease is dependent on the bioavailability of TT and its metabolite, dihydrotestosterone (18). A congenital lack of 5α-reductase results in a vestigial prostate gland (19) and castration in a man leads to glandular atrophy and regression of LUTS (20). It’s also reasonable to think that systemic effects of TT in bone and muscle mass, fat mass, body energy, and physical, sexual, and cognitive function may also be reflected on LUTS. Finally, there may be some direct effects of such hormones on bladder muscle function.

Despite these facts, most studies to date have reported inverted or no associations between sex hormones and LUTS. Trifiro et al. (10) evaluated baseline serum sex hormone concentrations and subsequent risk of LUTS with 20-year follow-up in a cohort of 158 community-dwelling older men. In opposition to our findings, they demonstrated that men with higher mid-life levels of testosterone/dihydrotestosterone and bioavailable testosterone had a decreased 20-year risk of LUTS. There were no significant associations of TT, oestradiol, testosterone/oestradiol, dihydrotestosterone, or dehydroepiandrosterone with LUTS.
Some limitations were the small sample size, the homogenous character of the community cohort (mostly white and upper middle class) limiting the external validity, and the 20-year gap between hormone measurement and LUTS assessment that precluded analysis of changes in serum sex steroid levels during this period. In another study, the authors performed a nested case-control analysis using the placebo arm of the Prostate Cancer Prevention Trial. The authors reported a decreased risk of incident BPH with higher baseline total testosterone and total testosterone : 17β-diolglucuronide (21). Litman et al. (9), analyzing data from the Boston Area Community Health (BACH) Survey, reported an inverse correlation of AUA-SI with both TT and bioavailable testosterone in non-age adjusted models and bioavailable testosterone in age-adjusted models. No significant association between LUTS and FT were found in the present analysis. Interestingly, some authors have also reported inverse relationships between LUTS and FT levels (22,23).

Conversely, other studies have reported positive relationships. Favilla et al. (2010) investigated a possible association between the severity of LUTS and the serum levels of sex hormones in 122 men with symptomatic BPH. In accordance to our results, on statistical analysis they found that the total IPSS was significantly associated with age and TT but not with free testosterone or the serum levels of the other sex hormones. While men with a IPSS > 19 presented a median testosterone of 425.6ng/mL, median testosterone level among men with a IPSS < 19 was 346.8ng/mL. However, the limitations of the study were again the small sample size and the fact that they only included men with severe LUTS who were candidates to surgery.

Reasons that may help to explain the lack of associations in some studies may be attributed to determination of sexual hormones levels at different points in the natural history of BPH. Additionally, it’s possible that plasma levels of these hormones are not representative of intra-prostatic levels. Regarding this fact, the dosage of sexual hormones metabolites may be more representative of the intra-prostatic environment. Platz et al. (12), demonstrated the association between an SHBG metabolite, androstanediol glucuronide (AAG), severe LUTS and the risk of BPH surgery. Patients with higher serum levels of AAG were at increased risk of having either BPH surgery or severe LUTS.

The unique strength of the present analysis is that we demonstrated a clear influence of BMI on the relationship between testosterone and LUTS, a variable that has been neglected by most studies. According to some reports, obesity may be associated with BPH. Giovannucci et al. (24), followed men enrolled in the Health Professionals Follow-up Study for the incidence of prostatectomy due to BPH and for the frequency and severity of symptoms of urinary obstruction. After adjustment for age, smoking, and BMI, only abdominal obesity showed a relationship with the rate of prostatectomy. Similar results were observed regarding frequency of urinary symptoms among those without prostatectomy (OR 2.0). However, these results should be analyzed carefully, since only a minority of patients with BPH undergoes surgical treatment for relieving LUTS.

Conversely, associations between obesity and BPH were not reproduced by other authors. Meigs et al. (25), defined risk factors for a clinical diagnosis of BPH among subjects of the population-based Massachusetts Male Aging Study. They found that BMI did not individually predict clinical BPH. More recently, in the study of 1,206 participants in the comparison arm of the Air Force Health Study with a median follow-up of 15.6 years, no relation was seen between weight or BMI and BPH (26). In another recent study of a cohort of healthy caucasian men aged 40–79 years randomly selected from the Olmsted County, Minnesota, few significant associations of anthropometric measures with the presence or progression of components of BPH were found (27).

Clearly, most of the controversy regarding relationship between BPH and anthropometric measures is due to the variable definitions of BPH used in studies. Technically, the diagnosis of BPH is made based on histological examination of a prostate specimen from biopsy, surgery or autopsy. However, as this approach gives limited insight into the incidence or progression of the disease, studies have considered surrogate measures to as-
sess BPH, including clinical (LUTS), physiologic (urinary flow rates), anatomic (prostatic volume) and biochemical (PSA levels) measures (28). For this reason, in the present study we investigated specifically the associations with LUTS.

Some advantages of the present study over the others should be mentioned. We analyzed a relative large series of patients who were not candidates for surgery, thus all LUTS categories could be analyzed. Additionally, patients were not using any placebo, which could have influenced LUTS. Limitations of the present study should also be noted. We didn’t analyze other important sex hormones such as dihydrotestosterone and oestradiol. Other clinical conditions that could influence sex hormone levels such as metabolic syndrome, diabetes or tobacco use were not included in the analysis.

Finally, if the results of the present series could be confirmed by further clinical analyses, there will be a role for the clinical use of serum testosterone, at least in obese patients with BPH. Basic research studies addressing the mechanisms of action of testosterone over the bladder and prostate tissues on a molecular basis are also necessary for the understanding of LUTS physiopathology and for the development of new therapeutic targets.

CONCLUSIONS

Serum TT and FT levels are significantly lower, and SHBG levels is significantly higher in obese patients when compared to patients with lower weights. A higher TT is independently associated with a worse AUA symptom index only in obese patients.

ABBREVIATIONS

LUTS = Lower Urinary Tract Symptoms
BPH = Benign Prostatic Hyperplasia
TT = Total Testosterone
FT = Free Testosterone
AUA = American Urological Association
BMI = Body Mass Index
GFs = Growth Factors
DRE = Digital Rectal Examination
SHBG = Sex Hormone Binding Globulin

CONFLICT OF INTEREST

None declared.

REFERENCES


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Human papillomavirus infection is not related with prostatitis-related symptoms: results from a case-control study

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ABSTRACT

Purpose: To investigate the relationship between human papillomavirus (HPV) infection and prostatitis-related symptoms.

Materials and Methods: All young heterosexual patients with prostatitis-related symptoms attending the same Center from January 2005 to December 2010 were eligible for this case-control study. Sexually active asymptomatic men were considered as the control group. All subjects underwent clinical examination, Meares-Stamey test and DNA-HPV test. Patients with prostatitis-related symptoms and asymptomatic men were compared in terms of HPV prevalence. Moreover, multivariable Cox proportional hazards regression analysis was performed to determine the association between HPV infection and prostatitis-related symptoms.

Results: Overall, 814 out of 2,938 patients (27.7%) and 292 out of 1,081 controls (27.0%) proved positive to HPV. The HPV genotype distribution was as follows: HR-HPV 478 (43.3%), PHR-HPV 77 (6.9%), LR-HPV 187 (16.9%) and PNG-HPV 364 (32.9%). The most common HPV genotypes were: 6, 11, 16, 26, 51, 53 and 81. No difference was found between the two groups in terms of HPV infection (OR 1.03; 95% CI 0.88-1.22; p = 0.66). We noted a statistically significant increase in HPV infection over the period 2005 to 2010 (p < 0.001) in both groups. Moreover, we found a statistically significant increase in HPV 16 frequency from 2005 to 2010 (p = 0.002).

Conclusions: This study highlights that prostatitis-like symptoms are unrelated to HPV infection. Secondary, we highlight the high prevalence of asymptomatic HPV infection among young heterosexual men.

INTRODUCTION

Human papillomavirus (HPV) infection is one of the most common sexually transmitted infections in both genders (1). HPV infection is the main cause of cervical cancer in women and is responsible for other cancers such as penile, oral-neck and anal cancer in men (2). Men are key to the transmission of HPV to women, but relatively little is known about the natural history of HPV infection in men (3). The prevalence of HPV in males ranges from 7% to 45% (4), but the majority
of studies have been conducted on homosexuals, HIV patients or infertile men (5,6). Recently, Klinglmair et al. found a high prevalence of HPV infection in a cohort of 250 young males, including children (0–10 years), indicating non-sexual transmission pathways (7). Genital infection is often asymptomatic and undiagnosed. In a recent study on 2,702 uncircumcised, HIV sero-negative males, Rositch et al. found that 51% of them presented occult HPV infection, of whom 57% with HPV multiple types (8). Thus, HPV prevalence data in men vary widely depending on the anatomical sites sampled, populations studied and analytical methods used for HPV detection (9). Several authors suggest that HPV tends to infect the prostatic epithelium (9), however, no correlation between symptoms from prostate pathology and HPV infection has ever been reported. On the basis of HPV DNA PCR findings, it has been suggested that the male genitourinary tract, including sperm cells, might act as a reservoir for HPV persistence and infection (9,10). On the other hand, prostatitis-related symptoms are recognized as an important socio-economical problem and several sexually transmitted infections are linked to the presence of prostatitis-related symptoms (11,12). We investigated HPV infection prevalence in young heterosexual males with prostatitis-related symptoms in order to find a possible relationship between HPV infection and prostatitis-related symptoms. The present case-control study aimed to determine whether HPV infection could be considered a risk factor of prostatitis-related symptoms in young heterosexual males.

**MATERIALS AND METHODS**

**Study design**

The study population consisted of two groups of young heterosexual men attending the same Sexually Transmitted Diseases Center from January 2005 to December 2010: Group A (cases), all consecutive patients with prostatitis-related symptoms, and Group B (controls), sexually active asymptomatic men whose female partners were infected with Chlamydia trachomatis. All patients and controls were screened for this study using the 4-glass Meares-Stamey test and and DNA-HPV test. All controls underwent 4-glass Meares-Stamey test and DNA-HPV test due to the fact that they were partners of female affected by Chlamydia trachomatis infection. This is the routinely practice in our STDs Centre. Clinical and laboratory findings for the two groups were compared. This case-control study aimed to determine whether exposure to HPV infection could be associated with clinical outcomes such as the presence of prostatitis-related symptoms. This study was conducted in line with the STROBE statement (http://www.strobe-statement.org). Italian law does not require authorization from the institutional review board (IRB), nor informed consent from the patients (http://www.agenziafarmaco.gov.it/it/content/linee-guida-studi-osservazionali). Nevertheless, our study was conducted in line with the Good Clinical Practice guidelines and with the ethical principles laid down in the latest version of the Declaration of Helsinki.

**Inclusion and exclusion criteria**

Group A patients were selected consecutively from a series of individuals suffering from chronic prostatitis-related symptoms for over 6 months, as defined in the latest version of the European Association of Urology (EAU) guidelines (13). In particular, the patients were selected and categorized according to Nickel’s criteria for perineal or ejaculatory pain. All enrolled patients must have had a pain score equal to or greater than 4, defined as “mild prostatitis” if the pain score ranged from 4 to 7 and “moderate or severe prostatitis” if the score was 8 or greater (14). Subjects under 18 and over 45 years of age, affected by major concomitant diseases, with known anatomical abnormalities of the urinary tract or with evidence of other urological diseases, and diagnosed with genital or anal warts, participating in an HPV vaccine study, were excluded as well as patients with suspected urothelial cell carcinoma at cytological urine analysis or who had previously undergone prostate surgery. Group B patients were included in the study only if asymptomatic and falling within the age range of 18 – 45 years, and with no known major concomitant diseases. All homosexuals were excluded from both study groups due to their increased risk of exposure to
HPV infection. Moreover, all patients have been asked about the sexual experience and the circumcision status. A detailed assessment about the sexual behavior has been carried out.

Study schedule and sample collection
We retrospectively collected anamnestic, clinical, laboratory and microbiological data for 6,743 patients from our database (Advanced PROSTATitis DataBase, Microsoft Access format). From these, we excluded 2,725 due to lack of data. Finally we considered 4,018 due to lack of data. We collected the validated Italian versions of the NIH Chronic Prostatitis Symptom Index (NIH-CPSI) (15) and the International Prostatic Symptom Score (IPSS) (16) for all patients and controls. All microbiological data were collected in accordance with indications described by Mazzoli et al. first void early morning urine (VB1), mid-stream urine (VB2), expressed prostatic secretion (EPS), post prostate massage urine (VB3) and total ejaculate (TE) (12). In order to evaluate the HPV infection in the urethra, a urethral swab (UR_SW) was taken from all subjects. Moreover, in our Centre we did not routinely performed the HPV specific antibodies.

Laboratory procedures and microbiological considerations
Microbiological cultures were performed in accordance with the methods described by Motrich et al. (17). DNA extraction and purification of all biological materials was performed using the DNeasy® Tissue Kit by QIAGEN Spa, Italy. 200µL of pellet was pre-incubated overnight with proteinase k and the next day extracted and purified following the manufacturer's instructions. All the biological material from the whole study population was tested for the presence of genital HPV using Alpha Watch HPV, Alphagenic-Diaco-Biotechnology, Trieste, Italy. All biological materials from our patients were analyzed by Inno-Lipa HPV Genotyping Extra (Innogenetics, Italy). Amplification of a

Figure 1 - The figure shows the Study flow-chart.
fragment of the b-globin gene served as an internal quality control for each specimen. In accordance to Munoz et al., we classified the following genotypes as high risk-HPV (HR-HPV): 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82; as probable high-risk (PHR-HPV): 26, 53, and 66; and as low risk (LR-HPV): 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 8118. HPV positive samples which did not hybridize with any of the type-specific probes were referred to as positive non genotype-able (PNG-HPV) (18). According to Giuliano et al., a participant was considered positive for “any HPV” if he tested HPV-positive by PCR or by genotyping (19). The category “any oncogenic type” included those who were positive for only oncogenic genotypes and those who were positive for both oncogenic and non-oncogenic types. Only single or multiple infections with non-oncogenic HPV types were classified as “any non-oncogenic type”. This STDs laboratory is registered by the United Kingdom National External Quality Assessment (NEQUAS) for microbiology for molecular detection of Ct (Quality Assurance Laboratory, Health Protection Agency Centre for Infection, 61 Colindale Avenue, London NW 95HT, United Kingdom). All microbiological analyses were performed blindly. Following Nickel et al., white blood cell (WBC) counts in all biological samples were carried out, though not considered in this study (20).

**Statistical analysis and considerations**

Pearson’s coefficient was adopted to evaluate the correlation between the different parameters in all patients, and Fisher’s exact test or Chi-square test ($\chi^2$) used to assess statistical significance with $p < 0.05$ accepted as significant. Multivariable Cox proportional hazards regression analysis was then performed to determine the association between HPV infection and prostatitis-related symptoms. The ANOVA test was applied to evaluate the difference between the two groups in terms of NIH-CPSI and IPSS questionnaire scores. The Bonferroni adjustment test was also used at the second stage of the analysis of variance. Odds ratio (OR) and 95% confidence intervals (CI) were calculated to determine the significance of differences. Statistical significance was set at $p < 0.05$. All reported $p$-values are two-sided. All statistical analyses were performed by using SPSS 11.0 for Apple-Macintosh (SPSS, Inc., Chicago, Illinois).

**RESULTS**

Data from 4,018 subjects were collected and analysed. A total of 2,938 patients were assigned to Group A (cases, prostatitis-related symptoms) while 1,080 to Group B (controls, asymptomatic subjects).

**Clinical and microbiological evaluation**

Detailed information about demographic and socioeconomic variables, medical history and clinical data at enrollment are given in Table-1. All cases revealed a mean symptom time of 15.4 months (range 10-26 months) but none presented genital or anal warts on physical examination. Overall, 814 out of the 2,938 patients (27.7%) and 292 out of the 1,081 controls (27.0%) proved positive to HPV (Odds ratio [OR] 1.03; 95% CI 0.88-1.22; $p = 0.66$). The HPV genotype distribution is as follows: HR-HPV 478 (43.3%), PHR-HPV 77 (6.9%), LR-HPV 187 (16.9%) and PNG-HPV 364 (32.9%). Data stratification according to age is given for both groups in Tables 2 and 3. In Group A, 28 out of 814 (3.4%) were also positive to Chlamydia trachomatis vs 31 out of 292 (10.6) in Group B, with a statistically significant difference ($p < 0.001$; Chi square 20.5; df = 1).

**HPV prevalence and genotype distribution**

**Patients with prostatitis-related symptoms (Group A)**

HPV genotype distribution was as follows: HR-HPV 417 (51.3%), PHR-HPV 70 (8.7%), LR-HPV 64 (7.7%) and PNG-HPV 263 (32.3%). The most common HPV genotypes were: 16 (18%), 31 (29%) and 33 (24%). HPV genotype distribution stratified per year is detailed in Table-2. HPV 16 and PNG-HPV frequencies were found to significantly increase over the period 2005 to 2010 ($p = 0.002$ and $p = 0.003$, respectively). No statistically significant increase in frequency was found for the other HPV types. In addition, there was a decrease in HR-HPV and PHR-HPV incidence in 2009.

**Asymptomatic patients (Group B)**

HPV genotype distribution for asymptomatic subjects was as follows: HR-HPV 116
Table 1 - Patient's sociodemographic anamnestic, clinical characteristics at enrolment time.

<table>
<thead>
<tr>
<th>No. of total enrolled subjects</th>
<th>4,019</th>
<th>Patients</th>
<th>Controls</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>Median age (± SD*)</td>
<td>35.7 ± 5.8</td>
<td>36.0 ± 5.9</td>
<td>0.14</td>
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<tr>
<td>Educational level</td>
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<tr>
<td>Primary school</td>
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<tr>
<td>Secondary school</td>
<td>2,001 (68.1)</td>
<td>716 (66.2)</td>
<td>0.25</td>
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</tr>
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<td>Post-secondary education</td>
<td>937 (31.9)</td>
<td>365 (33.8)</td>
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<td>Sexually active (past month)</td>
<td>2,899 (98.6)</td>
<td>1,070 (99.0)</td>
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<td>Sexual behavior</td>
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<tr>
<td>1 partner</td>
<td>2,376/2,899 (81.9)</td>
<td>881/1,070 (82.3)</td>
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<tr>
<td>&gt; 1 partners</td>
<td>523/2,899 (18.1)</td>
<td>189/1,070 (17.7)</td>
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<tr>
<td>Contraceptive use</td>
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<td>Condom</td>
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<td>693/1,070 (64.7)</td>
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<td>Coitus interruptus</td>
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<td>214/693 (29.8)</td>
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<td>Clinical presentation</td>
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<td>Urgency</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>Dysuria + Frequency</td>
<td>1,123 (38.2)</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Burning</td>
<td>981 (33.3)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perineal</td>
<td>1,341 (45.6)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Scrotal</td>
<td>742 (25.2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Suprapubic</td>
<td>311 (10.5)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lower Abdominal</td>
<td>214 (7.2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Start of CP# history (months)</td>
<td>15.4 ± 8.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Symptoms Score at baseline (mean) (range)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIH-CSPI§</td>
<td>18.9 (13-26)</td>
<td>2.8 (0-3)</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>IPSS†</td>
<td>17.3 (1-24)</td>
<td>2.2 (0-6)</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>

SD* = Standard Deviation; CP # = Chronic prostatitis; NIH-CSPI§ = NIH- Chronic Prostatitis Symptom Index; IPSS† = International Prostatic Symptom Score.
HPV symptoms in young sexually active men

The most common HPV genotypes were: 11 (17.2%) and 31 (30.2%). Table-3 shows HPV genotype distribution per year. There was no statistically significant increase in PCN-HPV prevalence from 2005 to 2010 (p = 0.68). As in Group A, we found a decrease in HR-HPV and PHR-HPV incidence in 2009. There was a statistically significant increase in HPV prevalence from 2005 to 2010 (p < 0.001) in both patients and controls (Figure-2).

On the other hand, there was no increase in HR-HPV infection prevalence from 2005 to 2010 (Figure-3).

HPV incidence and distribution per biological sample

We collected 18,351 samples from the all patients. We found 2,276 positive samples from 1,106 subjects (814 Group A and 292 Group B) (Table-4). There was no difference in HPV detection rate among VB1, TE and UR_SW were found (p = 0.30), nor be-

Table 2 - Summary results for grouped HPV type distribution by years (Patients).

<table>
<thead>
<tr>
<th>Year</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>584</td>
<td>446</td>
<td>523</td>
<td>547</td>
<td>427</td>
<td>411</td>
<td>2,938</td>
</tr>
<tr>
<td>HPV positive</td>
<td>86 (14.7)</td>
<td>123 (27.5)</td>
<td>112 (21.4)</td>
<td>183 (33.4)</td>
<td>134 (31.3)</td>
<td>176 (42.8)</td>
<td>814 (27.7)</td>
</tr>
</tbody>
</table>

Sub-analysis on HPV positive patients

<table>
<thead>
<tr>
<th>HPV type</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR-HPV</td>
<td>62 (72.0)</td>
<td>81 (65.8)</td>
<td>79 (70.6)</td>
<td>86 (46.9)</td>
<td>26 (19.4)</td>
<td>83 (47.1)</td>
<td>417 (51.2)</td>
</tr>
<tr>
<td>PHR-HPV</td>
<td>5 (5.8)</td>
<td>11 (8.9)</td>
<td>5 (4.4)</td>
<td>20 (10.9)</td>
<td>5 (3.8)</td>
<td>24 (13.6)</td>
<td>70 (8.6)</td>
</tr>
<tr>
<td>LR-HPV</td>
<td>4 (4.6)</td>
<td>7 (5.7)</td>
<td>8 (7.2)</td>
<td>12 (6.6)</td>
<td>24 (17.9)</td>
<td>9 (5.2)</td>
<td>64 (7.8)</td>
</tr>
<tr>
<td>PNG-HPV</td>
<td>15 (17.4)</td>
<td>24 (19.6)</td>
<td>20 (17.8)</td>
<td>65 (35.6)</td>
<td>79 (58.9)</td>
<td>60 (34.1)</td>
<td>263 (32.4)</td>
</tr>
</tbody>
</table>

HR-HPV = High-Risk HPV; PHR-HPV = Probable High-Risk HPV; LR-HPV = Low-Risk HPV; PNG-HPV = Positive Non Genotype-able HPV.

Table 3 - Summary results for grouped HPV type distribution by years (Controls).

<table>
<thead>
<tr>
<th>Year</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>205</td>
<td>171</td>
<td>189</td>
<td>165</td>
<td>134</td>
<td>217</td>
<td>1,081</td>
</tr>
<tr>
<td>HPV positive</td>
<td>33 (16.0)</td>
<td>42 (24.5)</td>
<td>37 (19.5)</td>
<td>51 (30.9)</td>
<td>39 (29.1)</td>
<td>90 (41.4)</td>
<td>292 (27.0)</td>
</tr>
</tbody>
</table>

Sub-analysis on HPV positive patients

<table>
<thead>
<tr>
<th>HPV type</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR-HPV</td>
<td>15 (45.3)</td>
<td>18 (42.8)</td>
<td>17 (45.9)</td>
<td>19 (37.2)</td>
<td>8 (20.5)</td>
<td>39 (43.3)</td>
<td>116 (39.7)</td>
</tr>
<tr>
<td>PHR-HPV</td>
<td>4 (12.1)</td>
<td>4 (9.5)</td>
<td>5 (13.5)</td>
<td>3 (5.9)</td>
<td>1 (2.6)</td>
<td>9 (10)</td>
<td>26 (8.9)</td>
</tr>
<tr>
<td>LR-HPV</td>
<td>2 (6.0)</td>
<td>5 (11.9)</td>
<td>3 (8.1)</td>
<td>9 (17.6)</td>
<td>2 (5.2)</td>
<td>5 (5.5)</td>
<td>26 (8.9)</td>
</tr>
<tr>
<td>PNG-HPV</td>
<td>12 (36.6)</td>
<td>15 (35.8)</td>
<td>12 (32.5)</td>
<td>20 (39.3)</td>
<td>28 (71.7)</td>
<td>37 (41.2)</td>
<td>124 (42.5)</td>
</tr>
</tbody>
</table>

HR-HPV = High-Risk HPV; PHR-HPV = Probable High-Risk HPV; LR-HPV = Low-Risk HPV; PNG-HPV = Positive Non Genotype-able HPV.
tween VB1 and UR_SW (p = 0.69). We also noted a statistically significant difference among VB1, VB2 and VB3 (p < 0.001) (Figure-4). We did not include the EPS data in the analysis due to the limited number of samples collected (81 out of 814 patients for Group A, 18 out of 292 controls for Group B).

HPV and other related risk factors
No significant difference in overall HPV prevalence was found in the different age categories in either group (Table-1). Finally, we found a higher HR-HPV prevalence in Group A compared with Group B patients (39.7% vs 51.2%) (p = 0.008).

Table 4 - HPV prevalence according to collected biological samples and years.

<table>
<thead>
<tr>
<th>Sample</th>
<th>VB1</th>
<th>VB2</th>
<th>VB3</th>
<th>TE</th>
<th>UR_SW</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>2005</td>
<td>2006</td>
<td>2007</td>
<td>2008</td>
<td>2009</td>
<td>2010</td>
</tr>
<tr>
<td>VB1</td>
<td>77</td>
<td>119</td>
<td>101</td>
<td>161</td>
<td>123</td>
<td>163</td>
</tr>
<tr>
<td>VB2</td>
<td>2</td>
<td>5</td>
<td>9</td>
<td>12</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>VB3</td>
<td>8</td>
<td>18</td>
<td>14</td>
<td>27</td>
<td>11</td>
<td>19</td>
</tr>
<tr>
<td>TE</td>
<td>69</td>
<td>112</td>
<td>108</td>
<td>115</td>
<td>99</td>
<td>121</td>
</tr>
<tr>
<td>UR_SW</td>
<td>81</td>
<td>118</td>
<td>105</td>
<td>169</td>
<td>129</td>
<td>170</td>
</tr>
<tr>
<td>Total</td>
<td>237</td>
<td>372</td>
<td>337</td>
<td>484</td>
<td>368</td>
<td>478</td>
</tr>
</tbody>
</table>

VB1 = First void early morning urine; VB2 = Mid-stream urine; VB3 = Expressed prostatic secretion; TE = Total ejaculate; EPS = Expressed prostatic secretion; UR_SW = Urethral swab.
There was no difference in terms of sexual behavior or use of contraceptives (Table-1).

**DISCUSSION**

HPV transmission normally occurs through sexual intercourse with commonly recognized infection distribution. In the absence of any obvious clinical manifestations, such as genital warts, a male is often considered as a healthy disease-carrier (21). Genital infection is often asymptomatic in both genders, although genital warts can generate local symptoms such as burning, itching and occasional bleeding (22). In the present study, no significant difference in prevalence of HPV infection-related symptoms was found between the two groups, although approximately 33% of Group A patients complained of burning during micturition. This symptom should be considered part of chronic pelvic pain syndrome, since all these patients had VB2 samples negative for bacteria at the 4-glass Meares-Stamey test. The cohort of young heterosexual males with prostatitis-related symptoms was selected on the basis of the recent literature on HPV reporting a high prevalence of infection (over 70% of cases) in men between 17 and 45 years of age. Similarly, the control group was selected from asymptomatic and sexually active males, because their female sexual partners were infected with Chlamydia trachomatis, thus more exposed to the risk of developing sexually transmitted infections. Homosexuals were excluded from the study due to their increased risk of developing HPV infection (23). Several authors have demonstrated the presence of HPV in the semen, although none of them has suggested any relationship with male infertility. Perino et al. showed that the abortion rate in male-infected couples is significantly higher (66.7%) than in normal couples (15%) (24). Indeed, viral bodies adhere to spermatozoa, subsequently reducing their motility (25). HPV could then be transmitted from men to women during sexual intercourse through sexual “contact” as well as through biological fluids. Moreover infection of the seminal tract (prostate, seminal vesicle, vas deferens) could also be hypothesized on the basis of HPV presence in the semen of asymptomatic patients. Our results partially contradict this hypothesis. The HPV prevalence was higher in VB1 and urethral swabs than in VB2 and VB3 samples. Several authors report HPV prevalence in the urethra ranging from 20 to 48%, although these data could be influenced by either the limited number of subjects evaluated, the wide age range of selected subjects or the difference in their sexual habits, activity and attitudes. On the other hand, for the same reasons, the prevalence of HPV infection in the semen has been investigated by various authors with conflicting results. Foresta et al. found 40.9% prevalence in subjects with infected sexual partners and 2.2% in fertile controls, while Nielsen et al. found 5.3% prevalence in 463 heterosexual men (26,27). Our study confirmed the high prevalence of HPV infection (27.7%) in a large cohort of young heterosexual men and an increasing positive trend of HPV infection over a 5 year period. No significant difference in prevalence was found between the group with prostatitis-related symptoms and the asymptomatic control group, although Group A patients exhibited an increased prevalence in HR-HPV genotypes. Despite the lack of differences in sexual behavior or contraceptive use between the two groups, the control group would have been expected to show a higher rate of HR-HPV infection due to their increased risk of developing Chlamydia.
trachomatis co-infection, commonly considered a sexually transmitted infection. HPV infection prevalence in the control group was independent from Chlamydia trachomatis co-morbidity (28). These results confirm that an increased risk of developing HPV infection in young heterosexual men is not related to the number of sexual partners but to the social diffusion of the disease in both genders. This is also supported by the increasing trend of HPV infection over the five-year period. The present study shows few limitations that should be taken into account. Firstly, the retrospective nature of this study. Secondly, the highly selected patient population due to the fact that we have enrolled patients attending a specific STDs Centre. Finally, the fact that we have no data about the impact of female vaccination on HPV male prevalence.

CONCLUSIONS

In conclusion, we found no correlation between the presence of prostatitis-related symptoms and HPV infection, highlighting an asymptomatic and increasing prevalence of HPV in young heterosexual males.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

None declared.

REFERENCES


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Qualitative analysis of the deposit of collagen in bladder suture of rats treated with tacrolimus combined with mycophenolate-mofetil

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Department of Clinical Surgery. Health Sciences Sector of the Federal University of Parana (UFPR), Curitiba, PR, Brazil

ABSTRACT

Purpose: To evaluate the synthesis of type I (mature) and type III (immature) collagen in bladder suture of rats treated with a combination of tacrolimus and mycophenolate mofetil for 15 days.

Materials and Methods: Thirty rats were divided into 3 groups: the sham, control and experimental groups. All the animals underwent laparotomy, cystotomy and bladder suture in two planes with surgical PDS 5-0 thread. The sham group did not receive treatment. The control group received saline solution, and the experimental group received 0.1mg/kg/day of tacrolimus with 20mg/kg/day of mycophenolate mofetil, for 15 days. From then on, the tacrolimus was dosed. The surgical specimens of the bladder suture area were processed so that the total type I and type III collagen could be measured by the picrosirius red technique.

Results: There was a predominance of type I collagen production in the sham and control groups compared to the experimental group, in which type III collagen was predominant. The production of total collagen did not change.

Conclusion: The association of tacrolimus and mycophenolate mofetil in animals qualitatively changes the production of collagen after 15 days with a predominance of type III collagen.

INTRODUCTION

Urological complications increase the morbidity and mortality of kidney transplantation by increasing the length of hospital stays and the need for re-surgery (1). The incidence of urological complications after kidney transplantation ranges from 2.5% to 14.7% (2). The main urological complications in kidney transplants are ureteral strictures and urinary fistulas (3). Most urinary fistulas appear early, within the first 90 days postoperatively (4).

After tissue damage, the process of restoring the tissue through a series of biochemical and physiological cellular processes begins (5). Collagen has a special feature. It is the main protein of connective tissue, responsible for the mechanical strength and resistance of the scar tissue. Regardless of the injured tissue, collagen is the most important component in tissue repair (6). Type I (mature) collagen is the most frequent. It is synthesized by fibroblasts and predominant in bones and tendons. Type III (immature) collagen is most commonly found in soft tissues, such
as blood vessels, dermis and fascia. The physical characteristic that best distinguishes type I collagen from type III is the interlacing of their fibers. The fibers of type I collagen are more intertwined and compacted than those of type III collagen, which has little interlacing, which results in lower tensile strength for scar tissue. The strength of a suture can be evaluated by the ratio of immature and mature collagen (7). These qualitative characteristics of deposited collagen are important for the structural support of an anastomosis. The maximum deposition of collagen in healing tissue is found on the fifteenth day (8). Every organ has a varying capacity for tissue repairs. The bladder has different characteristics when compared to gastrointestinal tract regeneration (9).

The picrosirius red staining technique stands out due to its greater selectivity for conjunctive tissue (10). This staining is specific for collagen, since there are no strong stains on the glycoprotein fibers (11). The less interwoven collagen fibers, representing type III collagen, are represented in green. The more interwoven fibers, aligned and with strong staining, representing type I collagen are orange-red (12). The calculation of the percentage of fibers, classified as type I or type III according to their color, enables a qualitative assessment of collagen fibers (13).

Among the various factors that may affect wound healing, immunosuppression is an important factor that hinders the healing process (14). There are various immunosuppressive regimens, and these drugs are based on calcineurin inhibitors, with cyclosporine and tacrolimus being the most commonly used. The most studied adjuvant drugs are mycophenolate mofetil and sirolimus. A combination of tacrolimus with mycophenolate mofetil is more commonly used nowadays (15).

This experimental study with rats aimed to verify the effect of the combination of Tacrolimus and Mycophenolate Mofetil on the synthesis of types I and III collagen in bladder wound healing.

MATERIALS AND METHODS

Animals and groups
We observed the ethical principles in animal experimentation established by the Brazilian School of Animal Experimentation (COBEA). Thirty Wistar rats, aged 120-140 days and weighing 265.34 ± 23.73 grams were used. They were divided into 3 groups of 10: the sham, control and experimental groups.

Surgical technique
The rats were weighed and submitted to inhalation of halothane sedation and anesthesia by intramuscular injection of ketamine and xylazine hydrochloride. A four-centimeter longitudinal midline incision was made in the following sequence: skin, subcutaneous tissue, rectus abdominis muscles and peritoneum. The isolated urinary bladder of the animal was subjected to a three-centimeter longitudinal cystotomy in the anterior bladder wall. The defect was closed with 5-0 polydioxanone suture in two planes. The closure of the abdominal cavity was done with Polyglactin 910 (3-0) thread, and the skin closure with simple colorless nylon (3-0) thread.

The animals in the sham group received no specific treatment after the surgery procedure. The animals in the control group were subjected to the same conditions of sedation and received daily subcutaneous injections and oral saline solution in volume proportional to their weight. The rats in the experimental group received daily treatment with tacrolimus and mycophenolate mofetil. The tacrolimus was administered subcutaneously on a daily basis at a dose of 0.1mg/kg/day for 15 days and mycophenolate mofetil daily dose of 20mg/kg/day for fifteen days, administered orally (4).

By the fifteenth day of evolution, all the rats were sedated and underwent cardiac puncture for blood collection. The blood samples were sent to the laboratory in order to perform a clinical analysis of tacrolimus (16). After the death of the animals, samples were collected from the bladder wall. The sample was then sent for the determination of total type I and type III collagen tissue by the histological technique of picrosirius red (17).

Optical microscopy
We assessed the area, density and the percentage of type I and III collagen. For identification of type III and type I collagen, the sections were analyzed by an Olympus® brand optical
microscope with 400 times magnification under polarized light. The images were captured by an optical system, frozen and scanned. This was performed by image analysis application using Image-Pro Plus version 4.5 for Windows (RGB). This program identifies the type of collagen-based colors. Red, yellow and orange correspond to type I collagen (mature), whereas green is equal to type III collagen (immature). Three fields were evaluated (upper, middle and lower), perpendicular to the suture bladder. The result was expressed as a percentage area.

A descriptive analysis of the data was applied to graphs and charts. The Student t and ANOVA parametric tests were used with the GraphPad application and a significance level of less than 5% (p < 0.05) was adopted.

In histopathological reviews concerning the histometric assessment of the areas of total collagen, when the values of the areas occupied by the total collagen of the groups were compared, there was no statistical difference, as shown in Figure-1. The control group had a mean of 22,728,734.89 ± 8,535,056.23μm², the sham group 20,280,575.18 ± 6,637,851.96μm², and the experimental group 20,467,537.37 ± 8,946,377.93μm² total collagen. There was no significant difference between the groups (p = 0.7558).

The histology assessment of the areas of type I collagen (Figure-2) showed that in the control group the average mature collagen detected was 95.94 ± 2.28%, in the sham group 94.76 ± 4.05%, and in the experimental group, 4.95 ± 3.97% of mature collagen (type I) in square microns x 1.000.000. There was no statistically significant difference between the control and sham groups (p = 0.4362). Comparing the sham and experimental groups, there was a statistically significant difference (p < 0.0001), and also between the control and experimental groups (p < 0.0001).

**RESULTS**

Regarding the dosage of tacrolimus it was observed that no serum levels of the drug were detected in the sham and control groups. In the experimental group an average of 11.3 ± 2.07ng/mL of tacrolimus was detected.
Concerning the histological evaluation of areas of type III collagen (Figure-2), in the control group the average immature collagen detected was 4.06 ± 2.28%, in the sham group, 5.23 ± 4.05% and in the experimental group, 95.04 ± 3.97%, in square microns x 1,000,000. Between the control and sham groups, there was no statistically significant difference (p = 0.3307). Comparing the sham and experimental groups, there was a statistically significant difference (p < 0.0001), as there was between the control and experimental groups (p < 0.0001).

Figure-3 shows the histological sections stained with Sirius red F3BA (40x). On the left picture, shown in red, type I collagen, and on the right picture in blue-green, type III collagen, under polarized light.

**DISCUSSION**

The main contribution of this study is that it demonstrates the qualitative change in the synthesis of collagen in a bladder wound in rats subjected to pre-defined immunosuppression drugs. There are several studies showing the complications and healing changes in the presence of immunosuppression, but few are prospective and well controlled. There have been many studies of other tissues such as the skin and gut, but little in urothelium (6). In this study, there was significant reduction in the production of type I collagen, using immunosuppression with tacrolimus and mycophenolate mofetil after fifteen days of the experiment. In the present study, the choice of immunosuppression was based on numerous studies that demonstrate the advantages of the combination of tacrolimus and mycophenolate mofetil (15). International study protocols (SWTC) see no statistical difference between immunosuppression with tacrolimus and cyclosporine as indices of acute rejection, but there is a trend of longer survival with the use of tacrolimus. The use of mycophenolate mofetil significantly reduces the incidence of rejection when compared with azathioprine (3). Tacrolimus should be monitored to prove the therapeutic concentration of the tacrolimus (16). In this study,
drugs were only detected in the samples of the experimental group, with all doses falling within the therapeutic range of the drug.

Many authors have reported the deleterious effect of immunosuppression on wound healing and most of these studies do not analyze type I and type III collagen separately. In practice, we observed that in tissue healing, type III collagen can indeed be a precursor of type I collagen as it has a lower quantity of fibers, less intertwined fibers and a lower quantity of local cellularity. Among the articles that studied tissue healing in non-urothelial tissue, one study that stands out is that of Kita et al. (18), who looked at the healing of the small intestine and colon of rats, observed that the tensile strength (bursting pressure of the anastomosis) of colonic anastomoses was less resistant in animals treated with tacrolimus at the end of seven days of treatment with tacrolimus for via intra-peritoneal. Furthermore, Schaffer et al. (19) studied the effects of tacrolimus in the healing of intestinal tissue and dermis and observed that the administration of 2mg/kg of tacrolimus led to a reduction in the healing dermis of animals. On the other hand, regarding the study of urothelial tissue, Ekici et al. (20) looked at the effects of immunosuppression with sirolimus in the healing of sutures in the bladder of rats and concluded that sirolimus affects all stages of healing of the bladder, including reducing the number of inflammatory cells, angiogenesis and the proliferation of myofibroblasts, thus delaying the healing process.

Some more recent works are using immunosuppressant drugs such as tacrolimus in the study of the treatment of diseases involving cellular proliferation disorders. Of these articles on cellular biology, one that deserves to be mentioned is that of Wu et al. (21). Concerning the behavior of keloid fibroblasts activated with tissue growth factors (TGF-β1), this study concluded that tacrolimus inhibits the growth factor action on the fibroblast in vitro. Inhibiting the proliferation of fibroblasts and their tissue migration, the entire protein synthesis of tissue collagen is impaired. Following the same line of research, Nankoong et al. (22) studied the effect of the topical tacrolimus therapy in the healing of cutaneous wounds in the backs of mice. They observed that after 3, 7 and 11 days of healing, there was no significant alteration in the healing of tissue between the groups under study, but the group treated with tacrolimus had slightly reduced levels of expression of mRNA of IL-1α and TGF-β.

Even topical therapy with tacrolimus appears to reduce local fibrosis. Ismailoglu et al.
observed that topical therapy with tacrolimus in the dura-mater of rats submitted to laminectomy reduced the occurrence of local fibrosis. The animals treated after thirty days with tacrolimus had a reduced amount local distribution of fibroblasts and reduced local fibrosis (23). But the most interesting study was certainly that in which Raptis et al. (24) observed that tacrolimus, when employed in healing the colons of rats, after 4 and 8 days of study reduced the occurrence of inflammatory reactions and the presence of local type I collagenase, although it increased the hydroxyproline concentration, neo-angiogenesis and the bursting pressure of anastomosis in the colons of the rats. Finally, Que et al., studying the regeneration of sciatic nerves in rats observed that tacrolimus reduces the formation of scar tissue in the area of the wound. These authors also observed that this reduction is associated with reduced proliferation and the apoptosis of fibroblasts induced by tacrolimus (25).

A joint analysis of our experiment with the literature shows that the immunosuppressive scheme that uses calcineurin inhibitors such as tacrolimus leads to a reduction in the proliferation of fibroblasts and the production of collagen. This reduces the amount of residual scar tissue. However, not all the studies that analyzed the bursting tension of anastomosis in these animals found that the animals treated with the immunosuppression scheme saw worsened bursting tension in their anastomosis, with some even noting increased bursting tension of anastomosis with the use of tacrolimus, in studies with a shorter trial period. More studies correlating the presence of type I collagen, type III collagen and tissue bursting pressure in the urothelium are required.

However, we need to take into account that our study is experimental, conducted in rats and with a short time frame for evaluating the results. We do not know whether these alterations in qualitative production of collagen will be maintained beyond the fifteen days of this study. For the time being, we cannot consider these results directly for clinical practice on human beings, where the scenario tends to be more complex and involves some variables that were not evaluated during the present study.

CONCLUSIONS

The combination of immunosuppressant tacrolimus and mycophenolate mofetil qualitatively alters collagen synthesis, resulting in increased production of proportional type III collagen after fifteen days of treatment at the doses used in rats.

CONFLICT OF INTEREST

None declared.

REFERENCES


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EDITORIAL COMMENT

Nowadays the association of a calcineurin inhibitor (CNI) with Mycophenolate mofetil (MMF) represents the backbone of solid-organ transplant immunosuppression. Although CNIs [Cyclosporine A (CsA) and Tacrolimus (FK506)] remain the most effective and widely used immunosuppressive agents in organ transplantation, their prolonged use may result in renal toxicity, renal dysfunction and irreversible renal failure characterized by extensive tubulo-interstitial fibrosis. The immunosuppressive effect of CNIs depends on the formation of a complex with their cytoplasmic receptor that inhibits calcineurin and impairs the expression of several cytokine genes that promote T-cell activation such as IL-2, IL-4, INF-γ and TNF-α (1). Moreover CNIs induce the expression of TGF-β1, which contribute to IL-2 inhibition but it is the main responsible for the development of CNI-associated interstitial fibrosis. TGF-β1 is well recognized as the major inducer for tissue fibrosis due to its stimulatory effect on extracellular matrix (ECM) production and inhibitory effect on matrix metalloproteinases. Recently it has been suggested that epithelial-mesenchymal transition (EMT) could play a role in the progression and maintenance of fibrosis in many pathological conditions, including tubulo-interstitial fibrosis (2). EMT is defined as the acquisition by epithelial cells of the phenotypic and functional characteristics of mesenchymal cells, intermediate between fibroblast and smooth muscle cells. These myofibroblasts have the ability to produce and secrete the extracellular matrix components such as collagen I and III, fibronectin and express α-smooth muscle actin (α-SMA). It has been shown that long-term exposure to CsA, induces EMT in human proximal tubular cells and that this event is mediated by CsA-induced TGF-β1 secretion (2). Moreover it has been observed that Tacrolimus up-regulates the expression of TGF-β1 and Smad2 in renal graft, while MMF has opposite effects (3). In fact it has been reported that MMF can reduce transplant fibrosis in a rat model of chronic rejection possibly by reducing the expression of α-SMA, collagen and connective tissue growth factor (CTGF), a matricellular protein with an important role in fibrosis and EMT (3). In accordance with these findings, Jiang et al. showed that MMF treatment prevented the deterioration of renal function and interstitial fibrosis in a renal ischemia-reperfusion injury model (4). In particular MMF significantly reduced the macrophages infiltration and the tissue expression of TGF-β1 and MCP-1, a diagnostic marker of renal injury (5).

In this scenario Wu et al. investigated the effects of Tacrolimus in wound healing, especially in a particular pathological process characterized by aberrant fibroblast activity with development of keloids (6). These authors showed that Tacrolimus could inhibit the TGF-β1-stimulated cell proliferation, migration and type I collagen production in keloid fibroblasts via Smad-related pathways inhibition (6). A fundamental characteristic of tissue fibrosis is the deregulated deposition of ECM, especially type I and III collagen. The imbalance between matrix metalloproteinases (MMPs) and their specific inhibitors (TIMPs: tissue inhibitors of MMPs) may lead to ECM accumulation and tissue fibrosis. Lan et al. showed that the use of Tacrolimus increased MMPs production and decreased TIMPs, with abrogation of TGF-β1-induced type I collagen synthesis (7).

The present study provides new insights into biological effect of Tacrolimus-MMF combination on the collagen synthesis in bladder wound healing. Even if the specific effect of the single immunosuppressive drugs was not addressed separately, for the first time it has been clearly shown a qualitative alteration in collagen synthesis characterized by a switch from type I to type III deposition. Understanding the mechanisms involved in tissue fibrosis may lead to the development of novel strategies for the treatment of CNIs-associated nephrotoxicity with the aim to increase graft survival.

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Pure Conventional Laparoscopic Radical Nephrectomy with Level II Vena Cava Tumor Thrombectomy

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ABSTRACT

The surgical management with laparoscopic technique for renal cell carcinoma with inferior vena cava tumor thrombus (IVTT) remains challenging and technically demanding in urological oncology. We present two patients with level II IVTT that were managed with pure conventional laparoscopic radical nephrectomy and thrombectomy. Two patients were diagnosed with a renal tumor with level II IVTT from December 2011 to January 2012. They both underwent pure conventional laparoscopic radical nephrectomy with thrombectomy. During these operations, intraoperative laparoscopic ultrasonography was used to detect the thrombus and ensure complete removal. Two patients were operated through retroperitoneal approach for right renal tumor and transperitoneal approach for left renal tumor respectively. The demographics, perioperative and follow-up data were recorded for the study. Both operations were successfully performed without conversion. They both had no radiographic evidence of recurrence during follow-up. It is concluded that it is feasible to manage renal cell carcinoma with level II IVTT through pure conventional laparoscopic approach in carefully selected patients, which might expand the indication for laparoscopic surgery. The pure laparoscopic approach in the treatment of renal cell carcinoma with level II vena cava tumor thrombus is challenging and requires advanced laparoscopic skills. Multicenter prospective randomized control trials are needed to prove the benefits of this approach.

INTRODUCTION

Renal cell carcinoma (RCC) has a natural tendency for formation of tumor thrombus, which can extend into the inferior vena cava (IVC) in 4% to 10% of cases (1). The surgical management for RCC with IVC tumor thrombus (IVTT) is extremely challenging and technically demanding. Open surgery is still the preferred method to manage RCC with IVTT (2). With continued advances in laparoscopic surgical techniques and increase experiences among urologists, the indications of laparoscopic techniques have expanded to more complex renal tumors.

Since the first description of laparoscopic radical nephrectomy (LRN) with level I vena cava thrombectomy in 1996 (3), several medical centers presented laparoscopic approach to deal with RCC with level I thrombus with encouraging outcomes (4,5). One case of renal tumor with IVTT extended 3cm above the renal vein (level II tumor thrombus) managed with pure LRN with tumor thrombectomy was reported in 2006 (6). To the best of our knowledge, only limited data are available in English literature with regard to pure LRN with level II IVC tumor thrombectomy and no report of more extensive tumor thrombus treated with pure LRN has been described up to now.
In the present study, we aim to demonstrate two cases of renal tumor with level II IVTT managed by pure LRN and thrombectomy.

MATERIAL AND METHODS

From January 2011 to February 2012, a total of 75 radical nephrectomies (RN) were performed in our department of which one patient underwent open RN with level II tumor thrombectomy and three patient received LRN and tumor thrombectomy. Among the three patients, two patients underwent LRN with level II tumor thrombectomy. With Institutional Review Board approval, two patients undergoing LRN and IVC tumor thrombectomy for RCC with level II IVTT were identified and reviewed. Both patients had clinically localized disease without metastasis. The renal tumor thrombus was classified according to the Mayo classification (7). The RCC was classified based on American Joint Committee on Cancer 2010 TNM staging criteria, Fuhrman grading system and 2004 WHO classification. One surgical team leaded by a laparoscopic surgeon (N.Z.X) with high volume experiences performed both procedures.

Data including demographics, perioperative data, pathologic data and oncological outcomes were collected and analyzed. The comorbidity was classified according to Charlson’s index (CI) (8). Perioperative data involved tumor’s computed tomography-scan size, side and location, IVTT length (evaluated by abdominal vascular magnetic resonance imaging), operative time, estimated blood loss, and intraoperative and postoperative complications (Figure-1). Postoperative complications within 30 days were classified according to Clavien’s system (9).

SURGICAL TECHNIQUE

The transperitoneal laparoscopic approach was used for the patient 1 with left renal tumor and IVTT, while the retroperitoneal approach was used for the patient 2 with right renal tumor and IVTT.

Following general anesthesia and Foley catheter placement, patient 1 was positioned in a 60° modified lateral decubitus position. Five trocars were placed: one 10mm trocar at the umbilicus for the camera, one 5mm trocar lateral to the umbili-
cus at the left anterior axillary line and one 12mm trocar in the midline between the xiphoid and the umbilicus for dissection, and one 10mm trocar subxiphoid trocar and one 10mm trocar lateral to the umbilicus at the right anterior axillary line for clamping the IVC.

The colon was reflected medially and the renal hilum was identified. The left renal artery was clipped with Hem-o-lok clip and divided by LigaSure. After the left renal vein was mobilized the IVC was dissected circumferentially above the left renal vein as proximal as possible (Figure-2A). The intraoperative laparoscopic ultrasound probe (UST-5536-7.5 Ultrasound; Aloka) was utilized to identify the extent of the tumor thrombus. The right renal vein was identified and any lumbar tributaries were isolated and ligated. A laparoscopic Satinsky clamp was used to block the IVC distal to the thrombus. Since the dissection of IVC can not reach the tail of the tumor thrombus, we didn’t clamp the IVC above the thrombus. The left renal vein was incised circumferentially at its junction with the IVC, and the tumor thrombus was immediately extracted entirely (Figure-2B). Then another prepared laparoscopic Satinsky clamp was immediately introduced to clamp the IVC above the renal vein through the subxiphoid trocar. The IVC was closed with 5-0 polypropylene suture. The rest of the kidney and adrenal gland were dissected outside of Gerota’s fascia and were removed integrally with tumor thrombus using a retrieval bag (Figure-2C).

The patient 2 was firmly secured to the operating table in the lateral position. The right kidney bridge was elevated moderately, and the operating table was flexed somewhat to increase the space between the lowermost rib and the iliac crest. During LRN, four trocars were placed. In brief, the first 2cm incision was performed under the 12th rib in the posterior axillary line. A retroperitoneal space was created with the index finger followed by a balloon dilator inflated with 1000mL air. A 5mm port was placed in the subcostal region

Figure 2 - A) The IVC was recognized and carefully dissected. B) After clamping the IVC, the IVC was incised and the IVTT was identified. C) The tumor was 13*10*8 cm in size with 8cm tumor thrombus in length. IVC, inferior vena cava; IVTT, inferior vena caval tumor thrombus.
on anterior axillary line guided by the forefinger. Another two 10mm port were positioned on the midaxillary line above the iliac crest and 3cm anterior at the same level as this point respectively.

Gerota’s fascia was incised longitudinally in the general area of the renal hilum. Lifting the kidney, blunt and sharp dissection in this area was performed to identify renal arterial pulsations, which could indicate the location of the renal artery. After complete circumferential mobilization of the artery Hem-o-lok clips were applied and the artery was transected directly by LigaSure. Dissection continued toward the renal vein, which was lying anterior to the renal artery. As the renal vein was dilated by thrombus, it was easy to identify and dissect the renal vein, contralateral renal vein and IVC. Any luminal veins were clipped and severed. Then dissection continued upwards towards the adrenal gland as far as the diaphragm. The adrenal gland was isolated and the adrenal vein was clip-ligated and divided. After the kidney was fully mobilized, the IVC could be exposed with only connecting to renal vein in renal hilum area. After the IVC was isolated, intraoperative ultrasonography was performed to identify the extent of IVC thrombus. Tourniquet loops were placed and tightened around the IVC above and below the IVC tumor thrombus and the contralateral renal vein was clamped by bulldog clamp (Figure-3A). Then the renal vein was incised circumferentially at its junction with the IVC, and the tumor thrombus was extracted entirely (Figure-3B and C). The IVC was stitched with a running 5-0 polypropylene suture (Figure-3D). No tumor thrombus pieces were dislodged detected by intraoperative ultrasonography. No bleeding was noted when the tourniquet loops were removed.

RESULTS

Table-1 shows patient characteristics and perioperative data. Pure LRN with IVC thrombectomy was successfully performed in both patients with no conversion.

Figure 3 - A) The IVC was clamped by tourniquet loops. B) and C) The IVTT was exposed after incising the IVC. D) The IVC was repaired by 5-0 polypropylene suture. IVC, inferior vena cava; IVTT, inferior vena caval tumor thrombus.
Table 1 - Demographics, clinical/pathologic data, and perioperative variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patient 1</th>
<th>Patient 2</th>
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<tr>
<td>Age/sex</td>
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<td>71/Male</td>
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<tr>
<td>BMI (kg/m²)</td>
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</tr>
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<td>Date of surgery (yyyy/mm)</td>
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<td>2012/02</td>
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<tr>
<td>Tumor size (cm)</td>
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<td>8<em>5.7</em>5</td>
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<tr>
<td>Side</td>
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<td>Right</td>
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<td>IVTT length (cm)</td>
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<td>4</td>
</tr>
<tr>
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<td>T3bN0M0</td>
</tr>
<tr>
<td>IVTT Grade</td>
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<tr>
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<td>ECOG (n)</td>
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<td>1</td>
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<td>ASA score (n)</td>
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**Operative parameter**

- Operative time (min.) 400 180
- EBL (mL) 1600 200
- Transfusion (mL) 2000 0
- Intraoperative complications (n) 1 0
- Postoperative complications (n) 1 0

**Pathologic data**

- RCC type          Clear cell  Clear cell
- T stage           T3b        T3b
- Fuhrman Grade     90% II, 10% III III
- Preoperative SCr (mg/dL) 0.51 1.36
- Postoperative SCr (mg/dL) 0.79 1.50
- Follow-up (mon)   18 15

BMI = body mass index; IVTT = inferior vena cava tumor thrombus; ECOG = Eastern Cooperative Oncology Group Performance Status; ASA = American Society of Anesthesiologists score; EBL = estimated blood loss; RCC = renal cell carcinoma; SCr = serum creatinine.

The first patient was a 55 years old woman, whose BMI was 17.6kg/m2. She presented with the symptoms of anemia. Preoperatively, computed tomography (CT) indicated the tumor was left sided measuring 14*10*8cm (Figure-1A). Magnetic resonance imaging (MRI) were used to evaluate the extent of the tumor thrombus, showing the length of the thrombus in the IVC was 6.9cm below the hepatic vein, which was a level II tumor thrombus according to the Mayo classification (Figure-1B). The TNM staging of this renal tumor was T3bN0M0. The American Society of Anesthesiologists (ASA) score was 2, the Eastern Cooperative Oncology Group Performance Status

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(ECOG) was 2 and the weighted index of comorbidity and the combined condition and age-related scores were 2 and 3 according to Charlson's index. She underwent angiembolization 24 hours before surgery.

The estimated blood loss was 1600mL, and the operative time was 400 min. She received blood transfusion (2000mL) intraoperatively. The postoperative serum creatinine was 0.79mg/dL. A small amount of oral intake began at postoperative day 2 and she got out of bed that afternoon. The drainage tube was removed at postoperative day 8. Her final pathology was 13*10*8cm, 90% Fuhrman grade II and 10% grade III, clear cell carcinoma with tumor thrombus protruding 8cm into the IVC with negative surgical margin and no invasion of renal vein and IVC walls. The patient was discharged from the hospital on postoperative day 15. One week after discharge, the patient went to our department again because of two time convulsion at home. After laboratory and imaging examinations she was diagnosed with hypokalemia due to bad diet. Her electrolyte was corrected and intravenous nutrition therapy was given to improve her condition. The surgical complication was grade II according to Clavien classification system. The patient returned to full activity two months postoperatively and had no radiographic evidence of recurrence at 18 months follow-up.

Our second patient was a 71 years old man with BMI 20kg/m2. CT-scan indicated the renal tumor was right sided measuring 8*5.7*5cm and abdominal vascular MRI demonstrated the length of the tumor thrombus in IVC was 4cm, which was also a level II tumor thrombus (Figure 1C and D). The TNM staging of this renal tumor was also T3bN0M0. The patient had multiple comorbidities, including chronic obstructive pulmonary disease for 6 years, hypertension for 16 years and type II diabetes mellitus for 2 years, which placed the patient in the high risk category for surgical intervention. The ASA score was 2, the ECOG was 1 and the weighted index of comorbidity and the combined condition and age-related scores were 4 and 7.

The estimated blood loss was 200mL, and the operative time was 180 min. No serious intraoperative and postoperative complications occurred. The postoperative serum creatinine was 1.50mg/dL. He got out of bed and oral intake began at postoperative day 1. The drainage tube was withdrawn at postoperative day 7. He was discharged from the hospital on postoperative day 10. The final pathology was 7.5*6*4cm, Fuhrman grade III, clear cell carcinoma with tumor thrombus protruding 4cm into the IVC with surgical margins free of tumor and without renal vein and IVC walls invasion. The patient returned to full activity about one month postoperatively and had no radiographic evidence of recurrence at 15 months follow-up.

**DISCUSSION**

Since the first introduction of LRN in 1990, the indications for LRN have expanded (10,11). RCC with IVTT was once considered a relative contraindication to LRN (12). In 1996, McDougall et al. presented the first successful LRN with level I thrombectomy (3). During the following years, many case series studies involving LRN with level I thrombectomy were reported proving that the procedure was feasible (4,5). In this procedure, the tumor thrombus was “milked back” into the renal vein so as to block the vein with vascular clips or an endoscopic stapling device (13). Before Romero et al. (6) reported the first case of pure LRN with level II tumor thrombectomy, Fergany et al. (14) successfully performed laparoscopic radical nephrectomy with level II thrombectomy in a survival porcine model, which showed clinical application of this technique appeared possible. The report of conventional pure LRN with level II tumor thrombectomy was very rare.

Robotic technology has been introduced to facilitate such complex procedures in recent years. Abaza (15) reported the first series of robotic nephrectomy with IVC tumor thrombectomy, and thrombi protruded 1cm, 2cm, 4cm and 5cm into the IVC in five patients and 3cm and 2cm in a patient with two thrombi. The results were encouraging, and the mean estimated blood loss was 170mL (50-400mL), the mean operative time was 327 min. (240 - 411 min.), and no complications and transfusions were encountered. They used cross-clamping technique for level II tumor
thrombus which was similar to ours. They placed vessel loops around the IVC above and below the tumor thrombus and around the contralateral renal vein. Although the favorable outcomes reported in this first series, case-control studies are needed to evaluate whether a robotic approach is superior to pure laparoscopic and open technique.

Currently most authors agree that the presence of the thrombus itself has no specific prognostic significance if it can be removed successfully (16). With advances in immunotherapy and molecular targeted therapy with such agents as interferon and Sorafenib, control of distant metastases in patients with RCC extending into the IVC can be achieved, thus survival of these patients may increase if aggressive surgery including tumor thrombectomy is combined with immunotherapy and molecular targeted therapy. It appears worthwhile to perform thrombectomy even in patients in whom RCC thrombus extends to the level of the right atrium or the pulmonary artery (17).

In our study, we employed Neves system to surgically stage tumor thrombus. Four staging systems were proposed for RCC with thrombus in the literature, TNM (18), Neves (7), Novick (19) and Hinman (20) (Table-2). The Neves and Novick are currently the most widely used. However, differences should be noted among several staging systems. Since the surgical approach depends mainly on the tumor thrombus level, so a consistent surgical staging system is of utmost important for preoperative planning.

Similar to open surgery, pure LRN with level II thrombectomy has several important steps. (1) dissection and ligation of the renal artery; (2) vena cava, contralateral and ipsilateral renal vein exposure; (3) occlusion of the IVC under the thrombus, contralateral renal vein, and the IVC above the thrombus; (4) opening of the vena cava and thrombectomy; (5) stitching the IVC and release of the vascular clamps; (6) nephrectomy. Completely control of the IVC is a vital important step. In our first case, we didn’t clamp the IVC above the thrombus because the dissection of IVC could not reach the tail of the tumor thrombus. However, this late clamping technique has several drawbacks, mainly intraoperative massive hemorrhage. As the IVC above the thrombus wasn’t clamped, intraoperative massive hemorrhage occurred when we incised the IVC wall and drew out the tumor thrombus. However, after the tumor thrombus was dragged out, the IVC was immediately clamped by a prepared laparoscopic Satinsky clamp, which could avoid fatal hemorrhage. Secondly, pulmonary embolism. Patients may develop pulmonary embolism secondary to tumor thrombus detachment. Our first patient was stable intraoperatively and continued to have no evidence of disease.

In our cases, we employed flexible intraoperative ultrasonography to detect the extent of IVC thrombus. The tumor thrombus can’t be dislodged if we find the right place to block the IVC. Another important function of intraoperative ultrasonography is identification whether the IVC wall was invaded by the tumor thrombus. Conversion is required if the IVC wall is invaded by the thrombus.

With the present study, we demonstrated another report that pure LRN with level II IVTT thrombectomy might be safe for highly selective patients. This study had several shortcomings:
retrospective study design, the small number of patients, and relatively short length of follow-up. Only two cases were involved in our study. Additional reports and randomized studies are needed. Long-term oncologic outcomes are required to elucidate the safety and efficiency of this approach.

CONCLUSIONS

Pure LRN with level II IVC thrombectomy is feasible and can be performed in carefully selected patients. However, the laparoscopic approach in the treatment of RCC with level II IVTT is challenging and technically demanding.

ABBREVIATIONS

RCC = renal cell carcinoma
IVC = inferior vena cava
IVTT = inferior vena cava tumor thrombus
RN = radical nephrectomy
LRN = laparoscopic radical nephrectomy

CONFLICT OF INTEREST

None declared.

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Urothelial carcinoma in a pyelocaliceal diverticulum discovered by magnetic resonance urography

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ABSTRACT

Neither computed tomography (CT) nor intravenous pyelography (IVP) alone can diagnose tumors of renal pelvic diverticula, but magnetic resonance urography (MRU) can obtain accurate preoperative information.

CASE

A 78-year-old woman presented with a history of macrohematuria for several months. Cystoscopy showed no bleeding from the left and right ureteral orifices or the bladder wall.

Urine cytology was class V, and tumor markers including serum squamous cell carcinoma antigen and cyfra 21-1 were not elevated. The IVP, CT and MRU findings indicated a left renal pelvic diverticulum (Figure-1), left renal calcification (Figure-2) and a tumor in the pyelocaliceal diverticulum (Figure-3), respectively. Brain, chest, abdominal and pelvic CT did not detect distant metastasis. The tumor in the pyelocaliceal diverticulum was treated by left laparoscopic nephrouretero-cystectomy. The macroscopic findings showed a papillary tumor in

Figure 1 - Intravenous pyelography findings. Arrow shows left renal pelvic diverticulum.

Figure 2 - Computed tomography findings. Left renal calcification is evident.
the pyelocaliceal diverticulum of the left kidney (Figure-4). The pathological findings revealed papillary urothelial carcinoma with squamous metaplasia of the left renal pelvic diverticulum, G2 >> G3, pT1, INFb, and negative surgical margins. The patient has remained alive without recurrence for 14 months.

**DISCUSSION**

Pyelocaliceal diverticula are congenital, nonsecretory, urothelium-lined cavities within the renal parenchyma that communicate with the caliceal fornix through the diverticular neck. They occur at rates of 0.21% to 0.45% of excretory urograms (intravenous pyelograms) and calculi occur in 9.5% to 50% of all diverticula (1,2). However, urothelial carcinoma of a pyelocaliceal diverticulum is exceedingly rare; fewer than 20 patients have been described in the literature since 1960 (3).

Pyelocaliceal diverticula that communicate with the caliceal fornix through the diverticular neck must be proven before tumors of renal pelvic diverticula can be diagnosed.

We finally diagnosed urothelial carcinoma of a pyelocaliceal diverticulum in this patient based on the MRU and pathological findings that fulfilled the diagnostic criteria. The pathological features of pyelocaliceal diverticulum tumors tend to resemble those of high-grade urothelial carcinoma (4). Several reports have described treatment by radical nephrectomy because of difficulties associated with preoperative diagnoses using only CT and IVP. Preoperative diagnosis is important, because tumors of pyelocaliceal diverticula should be treated like upper urothelial cancer, that is, by nephroureterectomy. Strict follow-up is required because the long-term prognosis is unknown. To our knowledge, this is the first report of a urothelial carcinoma of the pyelocaliceal diverticulum discovered by MRU. Urothelial carcinoma of the pyelocaliceal diverticulum is rare and often presents a diagnostic and therapeutic challenge. Magnetic resonance urography is helpful for diagnosing urothelial carcinoma of a pyelocaliceal diverticulum.

**ABBREVIATIONS**

CT = Computed tomography
IVP = Intravenous pyelography
MRU = Magnetic resonance urography
REFERENCES


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Fracture of corpora cavernosa with massive cavernosal-venous shunts

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CASE DESCRIPTION

A 43 year old white male with no significant past medical history presented to our emergency department (ED) with worsening penile pain and difficulty urinating. Several hours prior to admission, the patient was involved in a motor vehicle accident in which he was an unrestrained driver. His pelvis struck the steering wheel, while he was sexually aroused. Over the next 16 hours, his penis became increasingly swollen and painful. He presented to our ED when he was unable to void.

On physical exam, the patient’s vitals were unremarkable. His chest was clear and abdomen was soft. The patient’s right thigh was discolored and swollen. His penis was enlarged and swollen with a very prominent and distended dorsal penile vein. Auscultation over the penis revealed a loud bruit.

Urinalysis was positive for 20-30 red blood cells and isolated small blood clots.

A retrograde urethrogram was attempted, revealing a concentrically compressed, pendulous urethra with incomplete filling of the bulbous urethra. There was no evidence of extravasation. However, the procedure was aborted secondary to intolerable pain.

A T2-weighted MRI revealed a transverse fracture across the corpora cavernosa (Figure-1, black arrows). Minimal extravasation of blood was noted on the right juxta-corporal area extending through the tunica albuginea, but contained within Buck’s fascia (Figure-1, white arrow). A massively dilated draining vein was identified, indicative of arteriovenous shunt (Figure-2, arrow).

Surgical exploration revealed two small arteriovenous fistulas, which were ligated without difficulty. The enveloping tunica was repaired and the patient recovered. He sustained no loss of erectile function.

DISCUSSION

Penile fracture is a rare urologic emergency that constitutes rupture of the corpus cavernosum and tunica albuginea secondary to trauma or a sudden increased intracorporeal pressure within an erect penis. Past imaging options included cavernosography and ultrasonography. However, newer options are an MRI, which offers the most accurate assessment when penile fracture is suspected and urethrogram if urethral injury is suspected (1). Contrast enhanced imaging offers no additional information.

The tunica albuginea shows low intensity on both T1 and T2-weighted sequences allowing for optimal evaluation of its integrity even in patients with severe pain and swelling of the penis (2). Tears of the tunica albuginea can appear as discontinuity of the low signal intensity structure (2). Other findings include intracavernosal and extratunical hematomas, as well as arteriovenous fistulas, as demonstrated
in our case. Additionally, the presence of urethral or spongiosal involvement is associated with higher complication rates and necessitates immediate surgical intervention (3).

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ABSTRACT

The Paraganglioma is the most common extra-adrenal pheochromocytoma arising from neural crest (1) (It will better to write: The paraganglioma is an extra-adrenal pheochromocytoma arising from the neural crest. 10% of pheochromocytomas are extra-adrenal and can arise form chromaffin tissue derived from primitive neuroectoderm). Minimally invasive techniques allow surgeons to perform the procedure without wide exposure and mobilization of intra abdominal organs. To our knowledge we present the third case of robotic excision of a retroperitoneal paraganglioma (2,3).

MATERIAL AND METHODS

A 31 years old male with incidental 3 x 3 cm retroperitoneal mass located between the left renal vein and aorta. Even if serum Neuron-Specific Enolase and chromogranine-A were negative an anti-hypertensive prophylaxis was administered. (Will be worthwhile to add if cathecolamines E.g. metanephrines where measured in urine as part of the most common lab tests used when pheochromocytoma is suspected. Also if possible the type of anti-hypertensive drug used). We preferred a retroperitoneal approach. The ureter and the spermatic vessels were identified and not resected. The peritoneum was incised at the level of the tumor and the mass was carefully dissected. The maneuver was difficult because the paraganglioma was characterized by many small vessels leading to the lesion, unlike adrenal pheochromocytoma where a definite pedicle may be identified. Robotic surgery allowed to identify the vascular branches and to clip them with 5mm Hem-o-Lok. The manipulation of the mass caused some hypertensive peaks up to 240/130mmHg. For this reason, we were forced to perform a step by step dissection. Finally, the tumor was inserted into an EndoBag and retrieved from the optical trocar.

RESULTS

Operative time was 120 minutes; estimated blood loss was less than 50cc. No complications occurred. After surgery the blood pressure was normal. Patient was discharged on postoperative day 2. Final histopathological exam showed paraganglioma (a short pathology description would be helpful to add a little more information to the video case report).

CONCLUSIONS

The robotic excision of paraganglioma is a safe (a comment on the extremely high blood pressure during surgery should be added in order to show that this could be a high risk surgery when precautions prior to surgery are not made) and efficacious surgical strategy.
REFERENCES


ARTICLE INFO

Available at: www.brazjurol.com.br/videos/march_april_2014/Giovanni_279_280video.htm


EDITORIAL COMMENT

In the video by Dr. Cochetti and colleagues, a robotic assisted laparoscopic excision of a paraganglioma is well illustrated constituting the third such reported case in the scientific literature. Paragangliomas are not common, they represent only 10% of the pheochromocytomas in adults. The diagnosis requires a high index of clinical suspicion. In this context, a very thorough history taking and physical examination to look for signs and symptoms of catecholamine excessive release in those patients with functional lesions is pivotal. This will be critical as the patient will need pre-operative antihypertension management in order to avoid potential elevations of blood pressure and tachycardia leading to potential catastrophic hemodynamic sequelae in the peri-operative period.

As with other surgical procedures, minimally invasive techniques offer not only cosmetic advantages but also a quicker recovery and less surgery related pain. In the case of paragangliomas, minimally invasive surgery will add improved optics and 3-dimensional visualization of the incipient lesion, with surgical magnification of important anatomical structures including feeding (arterial and venous) vessels whereby avoiding excessive manipulation of the paraganglioma that can have far reaching implications pertaining to patient outcome.

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Laparoscopic bladder diverticulectomy assisted by cystoscopic transillumination


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INTRODUCTION

Acquired bladder diverticula are herniations of the bladder mucosa through detrusor muscle. Due to the ineffective emptying of the bladder diverticulum, urine accumulation may lead to urinary tract infection, stone disease, and lower urinary tract malignancy in the diverticulum (1). The symptomatic bladder diverticula may require surgical treatment. Surgical approaches include open operation via an extravesical or a transvesical approach for large diverticula or endoscopically with transurethral fulguration for small diverticula (2).

Herein, we present a video of a Laparoscopic Bladder Diverticulectomy for recurrent urinary tract infection, aided by concurrent cystoscopy.

MATERIALS AND METHODS

Female patient, 37 years old, complaining of recurrent urinary tract infection for three years. A bladder diverticulum was found on ultrasonography. Cystoscopy revealed a posterior right-side diverticulum next to the ipsilateral ureteral ostium. A laparoscopic bladder diverticulectomy with the aid of intraoperative cystoscopy was proposed.

Surgical Technique

Under general anesthesia, the patient was placed in lithotomy and Trendelenburg position. An umbilical incision was used for pneumoperitoneum creation and insertion of a 10mm trocar. Three other 5mm trocars were inserted at positions equidistant between the navel and the pubis, and between the umbilicus and the iliac crests bilaterally. Concomitant cystoscopy was performed for location of the diverticulum by transillumination and help to identify the diverticular neck. The diverticulum was dissected both sharply and bluntly until the whole diverticulum was freed. After completion the resection, a catheter was inserted in the right ureter near the diverticulum to assess inadvertent lesions.

The mouth of the diverticulum was closed by 2-0 double-layered absorbable running suture and a suction drain was placed through a lateral 5mm port.

RESULTS

The surgery was uneventful. The operative time was 120 minutes with minimal blood loss. There was no postoperative leakage, the drain was removed after 24 hours and the patient discharged.

The indwelling catheter was removed after 7 days and the patient progresses without voiding complaints or new infectious episodes in a follow-up of 10 months.

CONCLUSIONS

Laparoscopic diverticulectomy is technically feasible and safe. The concomitant use of
cystoscopy facilitates the identification and location of the diverticulum, thereby minimizing dissection of the bladder and decreasing operative time. Cystoscopy may also be useful in the delineation of margins in cases of neoplasia within the diverticulum.

REFERENCES


EDITORIAL COMMENT

Dr. Rebouças and colleagues present an elegant video demonstrating a safe and efficacious means of diverticulectomy. This technique is performed under direct visualization both laparoscopically and cystoscopically. It is exciting to see how well and confidently this surgery can be performed by using the technique herein described. Important caveats to keep in mind are to consider the reason for the diverticulum and secondary causes should be addressed. Furthermore, any potential for malignancy should be assessed. When using this technique with the aforementioned precautions, a minimally invasive approach to addressing this problem can be performed while minimizing morbidity. Overall the authors should be congratulated for this innovative technique.

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Transluminal approaches to vesicorectal fistula repair


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INTRODUCTION

Vesicorectal fistula is a devastating postoperative complication after radical prostatectomy. Definitive treatment is difficult. Despite many options, currently there is not one universally accepted approach.

OBJECTIVES

We describe two new minimally invasive approaches for the repair of vesicorectal fistula.

METHODS

We treat two patients with vesicorectal fistula after radical prostatectomy. In the first case, we perform the repair using Transanal Minimally Invasive Surgery (TAMIS) with standard laparoscopic instrumentation. We use Alexis device for transanal access, one rigid 10mm port for 0 degrees endoscope and two minilap 3mm ports for surgical manipulation. The surgical steps were: Cystoscopy and implant of guide wire on fistula; Positioning; Transanal access; Identification of the fistula; Dissection; Closure of vesical wall; Injection of fibrin glue in the defect; Closure of rectal wall. In the second case, we perform the repair using Transvesicoscopic Surgery. We use one rigid 10mm port for 0 degrees endoscope and two 5mm ports for surgical manipulation. The surgical steps were: Positioning; Transvesical access; Identification of the fistula; Dissection; Closure of rectal wall; Closure of vesical wall.

RESULTS

Mean operation time was 225 minutes, with a time of surgery slightly higher in TAMIS. The time of dissection was similar (120 minutes). No perioperative complications and conversion were observed. Hospital stay was 2 days and the catheters were removed at 4 weeks. No recurrence was observed.

CONCLUSIONS

The greatest difficulties were maintaining luminal dilation, instrumental manipulation and suturing. Nevertheless, these new approaches are feasible, with low morbidity.
EDITORIAL COMMENT

The video by Machado and colleagues nicely depicts two different minimally invasive approaches for the treatment of rectovesical fistulae. Repair of these fistulae can be quite challenging, especially if the defect is large or if the tissues were previously irradiated. Transanal surgeries are becoming more widespread as equipment and surgeons’ experience has improved (1). Transvesical surgery has slowly been adopted for various conditions including simple prostatectomy, vesicovaginal fistula repair and ureteral reimplantation (2). Endoscopic treatment of a rectovesical fistula was reported in 2010 using small clips and tissue glue (3). The potential benefits of these less invasive approaches include less pain and shorter recovery. These new techniques must be compared to traditional surgery and we look forward to additional reports from the authors (4).

REFERENCES

To the editor,

Sir, the recent article on “PGC and PSMA in prostate cancer diagnosis: tissue analysis from biopsy samples (1)” is very interesting (1). Antunes et al. concluded that “PGC gene expression is significantly higher in prostatic tissue in men affected by PCa when compared to normal prostates (1).” This report repetitively confirmed a similar report by Antunes et al. in J Urol (2). There are some concerns on using PGC in diagnosis of prostate cancer. A recent report by Diamandis et al. showed “no correlation between prostate-specific antigen concentrations and concentrations of PGC in serum of prostate cancer patients (3)” . Diamandis et al. concluded that that PCG “is not useful for either diagnosing or monitoring prostatic carcinoma (3)” . Also, the diagnostic property of PCG should be discussed. The abnormal PCG value can be seen in several disorders including breast diseases, which can also be seen in male although it is uncommon. The immunoassay test also has its diagnostic limitation at 0.1 microgram/L. To implement PCG in clinical practice, further validation study is required.

REFERENCES


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REPLY BY THE AUTHORS

The search for a method that could overcome PSA limitations is a very hard field of work. The high concentrations of PGC in the seminal fluid drew our attentions to this molecule as a potential marker for prostate cancer diagnosis. Although the blood tests have not demonstrated usefulness, hopefully the tissue analysis may become an important way to differentiate benign from malignant prostatic cells and thus avoid unnecessary prostate biopsies in a large group of men. Now, prospective validations are necessary to confirm our results.

The authors
Re: A review of continuous vs intermittent androgen deprivation therapy: Redefining the gold standard in the treatment of advanced prostate cancer. Myths, facts and new data on a “perpetual dispute”

Zisis Kratiras, Charalampos Konstantinidis, Konstantinos Skriapas

Department of Urology, “Koutlibanio” General Hospital of Larisa (ZK, KS), Larissa, and Department of Urology and Neuro-urology, National Rehabilitation Center (CK), Athens, Greece


To the editor,

After mature randomized clinical trial, some criticisms on what we expect from intermittent androgen deprivation and how we have to administer IAD are still open. An extensive discussion on testosterone as ruler for retreatment should be opened.

I read the review by Zisis Kratiras et al. (1) with great interest and expectancy because intermittent androgen-deprivation (IAD) therapy, which is commonly used, is still in the ‘empirical’ stage. After many randomized clinical trials, who is the best candidates to IAD, what we expect from IAD and how we should administer IAD remain unknown.

Androgen-deprivation therapy (ADT) for the treatment of prostate cancer is old and it is based on the reduction of androgen hormones to a castration level. To effectively evaluate the response to surgical or chemical castration, we have to measure testosterone levels, which is the key point of the definition of castration-resistant prostate cancer.

The definition and strategy of IAD is to alternate androgen blockade (on-phases) with treatment cessation (off-phases), which allows for androgen recovery between treatment periods. The relevant clinical trials are summarized in the excellent review of Zisis Kratiras (1), as well as recently by Sciarrà et al. (2).

The hypothetical value of IAD comes from the original experiment of Akakura et al. (3), which hypothesized and demonstrated in vivo that the replacement of androgens at the end of a period of castration-induced, apoptotic regression might result in the regeneration of differentiated tumor cells with further apoptotic potential.

Consequently, we expect that testosterone levels are the primary consideration of all clinical trials, but this is not always the case.

Laurence Klotz (4) and Gustavo Franco Carvalhal in his editorial comment to current paper correctly poses some questions. The review paper (1) shows that the induction periods and the criteria for resuming treatment are extremely variable and that both are PSA-driven. The selection criteria for IAD was a variable reduction with respect to the baseline PSA levels, but it is not clear whether the inadequate drop in PSA levels was linked to the primary extent of disease or...
the incomplete response to hormonal therapy (testosterone > 20 ng/mL). Additionally, because the rationale of the ‘off’ phase is to permit testosterone recovery, a hypothetical trigger for retreatment should be the recovery of baseline testosterone, independent of the PSA levels. Moreover, testosterone recovery translates to a longer off phase and better quality of life.

Conversely, the recovery of the baseline testosterone level is the mainstay of IAD, which is otherwise an ‘intermittent drug administration’. Because a significant percentage of subjects in IAD studies did not recover to the baseline testosterone levels (5) and suddenly received active retreatment, many patients had to be considered as receiving continuous androgen deprivation therapy. Upon analyzing all trials, no study has verified the impact of testosterone recovery with a more complex analysis with respect to the primary endpoint.

Based on these key points, I suggest that in guidelines to IAD patients should have PSA and testosterone levels assayed at baseline and every 3 months during the ON and off-treatment interval. Moreover, I think that we need to discuss the use of testosterone levels as trigger point for retreatment.

REFERENCES


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Re: Long-term results of permanent memotherm urethral stent in the treatment of recurrent bulbar urethral strictures

Atesci Y. Z., Karakose A., Aydogdu O.

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To the editor,

We congratulate the authors for the wonderful results with permanent urethral stent placement in bulbous urethral strictures. The authors describe long-term results that are fairly good. Conventionally, urethral stents are placed in old patients who cannot undergo urethroplasty. However, the authors have placed these stents in relatively young men (mean age 48 years [range, 23 to 76]). These men are sexually active. However, the authors have not given comments on this issue.

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