Risk factors for bladder neck contracture after transurethral resection of the prostate

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Abstract

Introduction: Transurethral resection of the prostate (TURP) is the most frequently used treatment of benign prostate hyperplasia with a prostate volume of <80 mL. A long-term complication is bladder neck contracture (BNC). The aim of the present study was to identify the risk factors for BNC formation after TURP.

Methods: We conducted a retrospective analysis of all TURP primary procedures which were performed at one academic institution between 2013 and 2018. All patients were analyzed and compared with regard to postoperative formation of a BNC requiring further therapy. Uni- and multivariable logistic regression analyses (MVAs) were performed to identify possible risk factors for BNC development.

Results: We included 1368 patients in this analysis. Out of these, 88 patients (6.4%) developed BNC requiring further surgical therapy. The following factors showed a statistically significant association with BNC development: smaller preoperative prostate volume ($p = 0.001$), lower resected prostate weight ($p = 0.004$), lower preoperative levels of prostate-specific antigen (PSA, $p < 0.001$), shorter duration of the surgery ($p = 0.027$), secondary transurethral intervention (due to urinary retention or gross hematuria) during inpatient stay ($p = 0.018$), positive ($\geq 100$ CFU/mL) preoperative urine culture ($p = 0.010$), and urethral stricture (US) formation requiring direct visual internal urethrotomy (DVIU) postoperatively after TURP ($p < 0.001$), in particular membranous ($p = 0.046$) and bulbar ($p < 0.001$) strictures. Preoperative antibiotic treatment showed a protective effect ($p = 0.042$). Histopathological findings of prostate cancer (PCA) in the resected prostate tissue were more frequent among patients who did not develop BNC ($p = 0.049$). On MVA, smaller preoperative prostate volume ($p = 0.046$), positive preoperative urine culture ($p = 0.021$), and US requiring DVIU after TURP ($p < 0.001$) were identified as independent predictors for BNC development.
1 | INTRODUCTION

Benign prostatic hyperplasia (BPH) is a common condition in aging adult men. As the reason for therapy is not the size of the prostate itself, but the urethral obstruction and lower urinary tract syndrome (LUTS) it causes, the term benign prostatic obstruction (BPO) is now deemed more appropriate. Therapeutic options include pharmacological treatment with α1-adrenoceptor antagonists, 5α-reductase inhibitors, muscarinic receptor antagonists, or β3 agonists. For patients who do not respond sufficiently to pharmacological treatment or present with a decompensated BPO, surgical therapy is the recommended treatment. Transurethral resection of the prostate (TURP), either by monopolar or bipolar technique, is the surgical approach most frequently used and most thoroughly studied and is recommended by the European Association of Urology (EAU) for surgical treatment of moderately to severely symptomatic BPO patients with a prostate size of 30–80 mL.

Common early complications of TURP include blood loss requiring transfusion, TUR syndrome, gross hematuria with clot retention, urinary tract infection, and urinary retention, while late-onset complications range from iatrogenic stress incontinence to urethral strictures and bladder neck contractures (BNCs), which may require surgical re-treatment.

Several factors have been reported to be associated with BNC formation after TURP, including preoperative prostate-specific antigen (PSA) levels, larger prostate volume as well as smaller prostate volume, transitional zone volume, longer resection time, resected gland weight, and presence of prostatitis in the past medical history.

The aim of this study was to analyze the incidence of as well as possible risk factors for BNC after TURP in a large single-centre cohort.

2 | PATIENTS AND METHODS

2.1 | Study group and surgical technique

We conducted a retrospective analysis of all the patients who underwent TURP at our clinic from January 1, 2013 to December 31, 2018. After applying the exclusion criteria (only primary procedures were included), this resulted in a cohort of 1368 patients. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and had previously been approved by the institutional ethics committee (University of Regensburg, No. 21-2186-104).

TURP was conducted using either a monopolar or a bipolar resectoscope. In all patients, a preoperative urine culture was initiated. If a significant bacteriuria (≥10,000 CFU/mL) of pathogenic bacteria was found, an antibiotic treatment according to the antibiogram was applied for at least 1 day prior to the procedure. Patients received a transurethral catheter after TUR-P, which was left in place over the next 2 days (longer in case of persistent gross hematuria). Bladder irrigation was regularly omitted within 24 h after surgery. After removal of the catheter, a urine culture was routinely initiated and, if positive, an antibiotic treatment began.

2.2 | Pre-, peri-, and postoperative criteria

The following patients’ characteristics were recorded and analyzed with regard to their effect on BNC development: patients’ age, height, weight, and body mass index (BMI), accompanying illnesses and regular anticoagulant, platelet inhibiting, or immunosuppressant medication. We also recorded patients’ most recent PSA levels, their preoperative prostate volume (measured by transrectal ultrasound), their preoperative hemoglobin levels, whether or not chronic prostatitis or prostate cancer (PCA) were known before TURP, prior treatment for non-muscle invasive bladder cancer with intravesical instillations (mitomycin or Bacillus Calmette-Guerin), previous pelvic radiotherapy, prior treatment with direct visual internal urethrotomy (DVIU) due to urethral stricture (US), whether or not the preoperative urine culture was sterile, and whether or not a preoperative antibiotic treatment was applied.

Regarding the perioperative criteria, we analyzed the duration of the procedure, the amount of prostate tissue resected (absolute, as well as compared to the preoperative prostate volume), periprostatic antibiotic treatment, whether or not a simultaneous DVIU was necessary, and the final histological result (PCA or chronic prostatitis [indicated by tissue infiltration of lymphocytes and plasma cells, interstitial edema or subepithelial hyperemia]).

Concerning the postoperative course of treatment, the duration of the inpatient stay was recorded, as well as patients’ postoperative hemoglobin levels, whether or not a secondary procedure due to gross hematuria or urinary retention was required during the
inpatient stay or later on, whether or not the postoperative urine culture was sterile and if postoperative antibiotic treatment was administered.

If patients presented again at our clinic with BNC requiring bladder neck resection (BNR), further parameters were recorded: whether pelvic radiotherapy had been performed, therapy with DVIU due to US (and stricture location), number of BNR, the time from first TURP to the first BNR, and whether or not a robotically assisted laparoscopic resection of the BNC had to be conducted after failed endoscopic BNR.

### 2.3 Statistical analysis

Continuous variables were reported as median and interquartile range (IQR), categorical endpoints as absolute and relative frequencies. Chi-squared test and Fisher’s exact test were used to describe the relation between categorical variables. Mann–Whitney U test was used to analyze the distribution of continuous variables.

In addition, univariable as well as multivariable logistic regression analysis (MVA) was performed on various criteria to evaluate their influence on postoperative BNC development. Due to the low number of events, the independent effect was analyzed using stepwise backwards elimination of the independent variables on the basis of the probability of the likelihood-ratio-statistics.

Statistical analysis was performed using IBM SPSS Statistics, version 25.0 (IBM Corp.). All the mentioned p values are two-tailed: the significance level was defined as p < 0.05.

### 3 RESULTS

A total of 1368 patients who had received primary TURP were included in this analysis. Out of these, 88 patients (6.4%) developed postoperative BNC requiring further surgical treatment.

Table 1 shows a detailed overview of the study population with a subdivision in patients with and without postoperative BNC. The two groups were balanced with regard to age and BMI at the time of TURP. Furthermore, no significant differences could be observed concerning pre- and postoperative hemoglobin levels, duration of the inpatient stay, regular medication with anticoagulant, platelet inhibiting or immunosuppressant agents, prevalence of diabetes mellitus type 2, preoperative intravesical instillations, and whether or not chronic prostatitis or PCA were known before TURP-P. Moreover, there were no differences with regard to pelvic radiotherapy (either before TURP-P or postoperatively).

However, patients who developed postoperative BNC had a statistically significant smaller preoperative prostate volume (p = 0.001), lower resected prostate weight (p = 0.004), lower preoperative PSA levels (p < 0.001), and shorter duration of the surgical procedure (p = 0.027). Surgical re-treatment due to gross hematuria with clot retention or due to urinary retention was also significantly associated with BNC development (p = 0.018). Furthermore, patients who did develop postoperative BNC more frequently showed a positive (>100 CFU/mL) preoperative urine culture (p = 0.010) than patients without postoperative BNC.

BNC development was also significantly associated with US formation requiring DVIU postoperatively after TURP (p < 0.001). When analyzing the position of the US, a significant association could be shown for strictures in the area of the urethral sphincter (p = 0.046) as well as bulbar (p < 0.001) strictures, while strictures of the urethral meatus and penile strictures were not associated with BNC (p = 0.413 and p = 0.052, respectively). An additional statistical analysis of those patients who developed postoperative bulbar US showed no significant differences with regard to preoperative prostate volume (p = 0.534) or resected prostate weight (p = 0.609).

Of the 11 patients who developed both US and BNC after TURP, 6 patients showed US simultaneously with BNC. 2 patients developed US after BNC, and 3 patients developed US before BNC.

A statistically significant protective effect could be shown for preoperative (at least 1 day) antibiotic treatment according to the preoperative urine culture (p = 0.042). When analyzing the different antibiotic treatments, a significant association could only be shown for oral fluoroquinolones (p = 0.042).

Histopathological findings of PCA in the resected prostate tissue were more frequent among patients who did not develop BNC (p = 0.049).

On univariable logistic regression analysis, smaller preoperative prostate volume (p = 0.001), lower PSA levels (p = 0.001), lower resected prostate weight (p = 0.007), a positive (>100 CFU/mL) preoperative urine culture (p = 0.011), surgical re-treatment during the inpatient stay (p = 0.020), and urethral stricture requiring DVIU after TURP (p < 0.001) were significantly associated with BNC formation after TUR-P, while preoperative antibiotic treatment had a significant protective effect (p = 0.045). For the remaining criteria analyzed, no statistically significant effect was observed (Table 2).

On MVA (stepwise backwards elimination), the following parameters remained in the model until the last step and were identified as independent risk factors for BNC development: smaller preoperative prostate volume (confidence interval (CI) 0.970–1.000, p = 0.046), positive (>100 CFU/mL) preoperative urine culture (CI 0.281–0.903, p = 0.021), and US requiring DVIU after TURP (CI 0.54–0.323, p = 0.001) (Table 2).

Median time from TURP to BNR was 286.5 days (IQR: 154–498.25 days). Figure 1 shows the number of patients requiring BNR plotted against the number of days after initial TURP. In summary, 55 of the 88 patients requiring BNR after TURP (62.5%) required the procedure within the first year after TURP. Only 9 of these 88 patients (10.2%) required BNR more than 2 years after the initial TURP.

In addition, we conducted an analysis of the postvoid residual after initial TUR of all 88 patients who later presented with BNC. We found that 16 patients (18.2%) were discharged with urinary catheter due to insufficient bladder voiding.
<table>
<thead>
<tr>
<th>Criteria</th>
<th>Whole study group (n = 1368)</th>
<th>Pts. without BNC (n = 1280)</th>
<th>Pts. with BNC (n = 88)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, median (IQR)</td>
<td>71 (64–77)</td>
<td>71 (64–77)</td>
<td>71 (64–76)</td>
<td>0.802</td>
</tr>
<tr>
<td>BMI, kg/m², median (IQR)</td>
<td>26.9 (24.6–29.5)</td>
<td>26.9 (24.6–29.4)</td>
<td>26.9 (24.7–30.6)</td>
<td>0.450</td>
</tr>
<tr>
<td>Preoperative prostate volume, mL, median (IQR)</td>
<td>48 (35–65)</td>
<td>49 (35–66)</td>
<td>40 (30–50)</td>
<td>0.001</td>
</tr>
<tr>
<td>PSA, ng/mL, median (IQR)</td>
<td>2.98 (1.46–5.52)</td>
<td>3.05 (1.55–5.72)</td>
<td>1.63 (0.80–3.81)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Surgery duration, min, median (IQR)</td>
<td>61 (45–77)</td>
<td>61 (46–78)</td>
<td>56 (40.5–70)</td>
<td>0.027</td>
</tr>
<tr>
<td>Resected prostate weight, g, median (IQR)</td>
<td>20 (15–32)</td>
<td>20 (15–32)</td>
<td>19 (12–25)</td>
<td>0.004</td>
</tr>
<tr>
<td>Resection ratio, %, median (IQR)</td>
<td>46.2 (35.1–57.7)</td>
<td>46.2 (35.1–58.1)</td>
<td>45.5 (35.0–57.1)</td>
<td>0.702</td>
</tr>
<tr>
<td>Preoperative intravesical instillation therapy, n (%)</td>
<td>19 (1.4)</td>
<td>17 (1.3)</td>
<td>2 (2.3)</td>
<td>0.464</td>
</tr>
<tr>
<td>Preoperative chronic prostatitis, n (%)</td>
<td>46 (3.4)</td>
<td>44 (3.4)</td>
<td>2 (2.3)</td>
<td>0.558</td>
</tr>
<tr>
<td>Preoperatively known PCA, n (%)</td>
<td>108 (7.9)</td>
<td>104 (8.1)</td>
<td>4 (4.5)</td>
<td>0.228</td>
</tr>
<tr>
<td>Preoperative urine culture positive (≥100 CFU/mL), n (%)</td>
<td>791 (57.8)</td>
<td>739 (57.7)</td>
<td>62 (70.5)</td>
<td>0.010</td>
</tr>
<tr>
<td>Preoperative antibiotic treatment, n (%)</td>
<td>415 (30.3)</td>
<td>397 (31.0)</td>
<td>18 (20.5)</td>
<td>0.042</td>
</tr>
<tr>
<td>Perioperative antibiotic treatment, n (%)</td>
<td>713 (52.1)</td>
<td>671 (52.4)</td>
<td>42 (47.7)</td>
<td>0.469</td>
</tr>
<tr>
<td>Postoperative antibiotic treatment, n (%)</td>
<td>630 (46.1)</td>
<td>594 (46.4)</td>
<td>36 (40.9)</td>
<td>0.317</td>
</tr>
<tr>
<td>Surgical re-treatment during inpatient stay, n (%)</td>
<td>146 (10.7)</td>
<td>130 (10.2)</td>
<td>16 (18.2)</td>
<td>0.018</td>
</tr>
<tr>
<td>Histopathological result PCA, n (%)</td>
<td>283 (20.7)</td>
<td>272 (21.3)</td>
<td>11 (12.5)</td>
<td>0.049</td>
</tr>
<tr>
<td>Histopathological result prostatitis, n (%)</td>
<td>777 (57.0)</td>
<td>726 (56.9)</td>
<td>51 (58.0)</td>
<td>0.846</td>
</tr>
<tr>
<td>Preoperative pelvic radiotherapy, n (%)</td>
<td>25 (1.8)</td>
<td>24 (1.9)</td>
<td>1 (1.1)</td>
<td>0.617</td>
</tr>
<tr>
<td>Postoperative pelvic radiotherapy, n (%)</td>
<td>39 (2.9)</td>
<td>38 (3.0)</td>
<td>1 (1.1)</td>
<td>0.266</td>
</tr>
<tr>
<td>Secondary TURP before BNC, n (%)</td>
<td>47 (3.4)</td>
<td>42 (3.3)</td>
<td>5 (5.7)</td>
<td>0.233</td>
</tr>
<tr>
<td>DVIU before TURP, n (%)</td>
<td>28 (2.0)</td>
<td>26 (2.0)</td>
<td>2 (2.3)</td>
<td>0.877</td>
</tr>
<tr>
<td>DVIU during TURP, n (%)</td>
<td>58 (4.2)</td>
<td>54 (4.2)</td>
<td>4 (4.5)</td>
<td>0.883</td>
</tr>
<tr>
<td>DVIU after TURP, n (%)</td>
<td>32 (2.3)</td>
<td>21 (1.6)</td>
<td>11 (12.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stricture positiona</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meatal, n (%)</td>
<td>8 (0.6)</td>
<td>7 (0.5)</td>
<td>1 (1.1)</td>
<td>0.413</td>
</tr>
<tr>
<td>Penile, n (%)</td>
<td>6 (0.4)</td>
<td>4 (0.3)</td>
<td>2 (2.3)</td>
<td>0.052</td>
</tr>
<tr>
<td>Bulbar, n (%)</td>
<td>8 (0.6)</td>
<td>3 (0.2)</td>
<td>5 (5.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Membranous, n (%)</td>
<td>13 (1.0)</td>
<td>10 (0.8)</td>
<td>3 (3.4)</td>
<td>0.046</td>
</tr>
</tbody>
</table>

Note: Bold values indicate statistical significance.
Abbreviations: BMI, body mass index; BNC, bladder neck contracture; DVIU, direct visual internal urethrotomy; IQR, interquartile range; PCA, prostate cancer; PSA, prostate-specific antigen; TUR-P, transurethral resection of the prostate.

aMultiple stricture positions possible.
Of the 88 patients who received transurethral BNR, there were 59 patients who received no further treatment at our clinic after the first resection, 15 patients who required 2 resections, 8 patients who required 3 resections, 4 patients who required 4 resections, 1 patient who required 5 resections, and 1 patient who required 6 resections. Twelve patients (13.6% of all BNC patients, 0.9% of the entire study cohort) further required robotically assisted laparoscopic bladder neck reconstruction.

## DISCUSSION

BNC is a well-described late-onset complication following surgical interventions of the prostate such as TURP. Although the frequency of BNC after TURP is difficult to determine precisely (reports range from 0.3% to 12.3%), the condition's tendency towards recurrence makes it a burdening and challenging complication for patients as well as urologists.
Among the factors previously reported to be associated with BNC formation after TURP are patients’ PSA levels, larger or smaller preoperative prostate volume, longer resection time, resected gland weight as well as a larger resectoscope diameter.\(^4\)\(^{-6}\)\(^{10}\) Furthermore, patient characteristics such as smoking status, age, BMI, and diabetes mellitus type 2 have been reported as modifiable risk factors.\(^{10}\)

In our retrospective single-centre cohort of 1368 patients with primary TURP, we observed a postoperative BNC rate of 6.4%, which lies in the aforementioned range reported in the current literature.\(^3\)\(^{-7}\)\(^{9}\) We found smaller preoperative prostate volume to be an independent risk factor for postoperative BNC formation. This is in accordance with most of the literature on this topic, which suggests that the ratio between prostate volume and resectoscope diameter contributes significantly to BNC formation.\(^8\)\(^{-10}\)\(^{-12}\)

The exact mechanisms causing BNC are still not fully understood. Proposed predisposing factors are extensive resection of the bladder neck as well as excessive fulguration with high ablative energy in the bladder neck leading to scar tissue formation.\(^6\)\(^{-12}\) It can therefore be hypothesized that an unfavorable ratio between resectoscope and prostate (by either smaller prostate volume or larger resectoscope diameter) leads to higher heat development and consequently to more pronounced scar tissue formation. Another possible explanation is perforation of the prostate capsule, which has been described to occur in up to 7.1% of TURP.\(^{13}\) Consequently causing scarring of the bladder neck. Prostate capsule perforation is also more likely to occur in TURP of smaller prostates.

The risk of capsule perforation is even higher when performing surgical re-treatment by secondary TURP within short time after the initial surgery. Further fulguration in case of persistent gross hematuria or additional resection due to urinary retention will understandably cause further scar tissue formation. Surgical re-treatment, however, lost its statistical significance when included in MVA.

On the other hand, transurethral incision of the prostate (TUIP) is a procedure that involves deliberate incision of the prostatic tissue and the capsule,\(^{14}\) therefore also leading to urinary extravasation. However, TUIP has not been reported to lead to a higher rate of postoperative BNC formation than TURP.\(^{15}\)\(^{16}\) But, as TUIP is still a not widely used procedure,\(^{16}\)\(^{17}\) this association might therefore not have reached significance yet.

Among patients with postoperative BNC, we observed lower resected prostate weight, lower preoperative PSA levels, and shorter duration of the surgical procedure, again in accordance with current literature.\(^{11}\) As these parameters did not keep their significance when included in MVA, they can be attributed to the smaller preoperative prostate volume.

In our analysis, patients who developed BNC more frequently showed a positive (\(\geq 100\) CFU/mL) preoperative urine culture compared to patients without postoperative BNC. A positive preoperative urine culture could also be confirmed as an independent predictor for BNC in MVA. On univariable logistic regression analysis, preoperative antibiotic treatment had a significant protective effect (\(p = 0.045\)), which it lost in MVA. Therefore, as only in patients with significant bacteriuria (\(\geq 10,000\) CFU/mL) of pathogenic bacteria a preoperative antibiotic treatment was applied, while our analysis showed even an insignificant bacteriuria of \(>100\) CFU/mL to have a significant effect on BNC formation, preoperative antibiotic treatment even in patients with insignificant bacteriuria might be advisable.

Chronic prostatitis, however, did not have a significant effect on BNC formation. These findings are inconsistent with reports in the literature, in which prostatic inflammation was found to be a risk factor for BNC development.\(^{18}\) In their histopathological analysis of resected BNC tissue, Kaynar et al.\(^{12}\) found chronic inflammation in 51.7% and chronic active inflammation in 18.8%, while cystitis could only be found in 0.5%. As inflammatory processes in the tissue lead to scar tissue formation,\(^{19}\) these findings are to be expected. On the other hand, chronic inflammatory processes are believed to contribute to BPH\(^{20}\) in the first place, which is why the significance of the reported findings of inflammatory processes in BNC tissue can be questioned and why these chronic inflammatory processes may be less present in patients with smaller prostate volume, although our...
analysis showed no significant differences regarding the presence of chronic prostatitis either in patients' history or in the histopathological report. In addition, one has to bear in mind that the clinical syndrome of chronic prostatitis does not necessarily correlate with urinary tract infection or with histopathological findings of chronic inflammation in the prostatic tissue. Furthermore, as inflammatory processes vary in degrees which cannot be sufficiently differentiated by the histopathological results, a possible association between BNC formation and severe inflammation might be present, but undetected in this analysis.

Nonetheless, urinary tract infection (UTI) has so far not yet been reported as a risk factor for BNC development. Untreated UTI has, however, been reported as a risk factor for US formation after TURP.\(^\text{4,10}\)

Accordingly, our analysis showed US formation after TUR-P to be an independent predictor of BNC formation after TURP. This was particularly true for membranous and bulbar strictures. This association has been reported before\(^\text{4,5}\) and is to be expected, as similar histopathological processes are proposed to be responsible.\(^\text{21}\) As urethral mucosa rupture with the possibility of urinary extravasation has been reported as risk factors for US formation,\(^\text{4,21}\) this supports our hypothesis of perforation of the prostate capsule as a pathogenetically relevant mechanism in BNC development.

The association between pelvic radiotherapy and BNC formation is known and well described.\(^\text{7,22-25}\) The proposed mechanisms are progressive fibrosis and necrosis of the tissue as well as pubovesical fistula formation. Yet our analysis showed no significant effect of pre- or postoperative radiotherapy on BNC formation after TURP. BNC development after primary radiation, however, occurs several years after the initial treatment.\(^\text{18}\) Therefore, our follow-up might not be long enough to detect statistical differences. Due to the retrospective nature of our analysis, however, we refrain from drawing definitive conclusions.

Histopathological report of PCA in the resected prostate tissue was not associated with BNC formation and was in fact more frequent among patients who did not develop BNC, in accordance with current reports in the literature.\(^\text{12}\)

Another interesting observation is that among those patients who later presented with BNC a rate of 18.2% were discharged with indwelling urinary catheter due to insufficient bladder voiding after the initial TURP. One possible explanation for this association is that patients with a weak detrusor function have a higher risk for developing BCN because a beginning scar is not sufficiently dilated by hydraulic forces during micturition. This possible effect should be further evaluated in a prospective setting.

Several further patient characteristics such as age, BMI, and prevalence of diabetes mellitus type 2 have been reported as additional risk factors for BNC development.\(^\text{10,11}\) In our analysis, however, none of these parameters were associated with BNC development.

Although our analysis has the advantage of a large cohort, the main limitation is the inconsistent documentation of prostate capsule perforation and type of electrocoagulation (monopolar vs. bipolar) due to its retrospective design. Additionally, a possible bias might arise from the fact that patients who develop BNC after receiving TURP at our centre might choose to present to another urological clinic for further treatment.

## 5 CONCLUSION

BNC is a relevant long-term complication after TURP. In particular, patients with a smaller prostate should be thoroughly informed about this complication as well as US formation. If UTI is present in the preoperative urine culture, an antibiotic treatment should be administered preoperatively.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## REFERENCES


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