Effects of barbed suture during robot-assisted radical prostatectomy on postoperative tissue damage and longitudinal changes in lower urinary tract outcome

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Abstract

Objective To compare the postoperative tissue damage and longitudinal changes in functional and patient-reported outcomes after vesicourethral anastomosis with barbed suture and nonbarbed suture in robot-assisted laparoscopic radical prostatectomy (RARP).

Materials and methods This was a prospective cohort study involving 88 consecutive patients who underwent RARP. These patients were categorized into the barbed suture group (n = 50) and the nonbarbed suture group (n = 38). Urethral and periurethral damages determined by magnetic resonance imaging at nine months after RARP were compared using generalized linear models. The International Prostate Symptom Score (IPSS), quality of life (QOL) index, uroflowmetry, and the 1-h pad test were measured at baseline and at 1, 3, 6, 9, and 12 months after RARP. The findings were analyzed using mixed-effects models. Confounding was adjusted for using propensity score covariate adjustment.

Results The likelihood of having Grade 2/3 urethral and periurethral damages was greater in the barbed suture group than in the nonbarbed suture group (adjusted risk ratios: 2.98 and 3.85, respectively). IPSS, QOL index, and urinary leakage transiently increased at one month after RARP in both groups. QOL index was higher in the barbed suture group than in the nonbarbed suture group at 1, 9, and 12 months (P = 0.023, P = 0.025, and P = 0.011, respectively). The barbed suture group had significantly more cases of urinary incontinence than the nonbarbed suture group at 3 months (P = 0.041). Other outcomes were comparable between the two groups at all time points.

Conclusions This cohort study showed that, after RARP, barbed sutures during VUA induced more severe tissue damage as determined by MRI and greater transient aggravation of QOL and continence function than nonbarbed sutures. The present findings suggest that using nonbarbed sutures during VUA may facilitate earlier acquisition of urinary QOL and urinary continence.

Keywords Robot-assisted laparoscopic radical prostatectomy · Vesicourethral anastomosis · Unidirectional barbed suture · Lower urinary tract symptom

Robot-assisted laparoscopic radical prostatectomy (RARP) is enjoying widespread and expanding use for patients with prostate cancer. A particularly challenging reconstruction step during RARP is vesico-urethral anastomosis (VUA), as running sutures during the VUA risks slippage and subsequent complications, such as urinary leakage. To facilitate running sutures and prevent suture slippage, a
unidirectional barbed self-anchoring suture (V-Loc Wound Closure Device; Covidien, Mansfield, MA, USA) is being used with increasing frequency. Indeed, a number of studies on RARP have shown that the use of barbed sutures is associated with a decreased VUA time and a similar proportion of urinary leakage compared with the use of nonbarbed sutures [1–4]. However, despite the benefits of barbed sutures on surgeon-dependent outcomes and perioperative patient outcomes, the effects of these sutures on the tissue damage and functional and patient-reported outcomes related to the lower urinary tract after RARP remain unclear.

The V-Loc is absorbable but consists of a polyglyconate strand with barbs evenly spaced throughout the strand. Hence, when the suture is tightly tied at the suture site, it can grasp the surrounding tissue and interrupt the blood flow to the anastomotic site after RARP, resulting in an increased risk of urethral and periurethral damage and aggravation of lower urinary tract function. Regarding tissue damage associated with barbed sutures, an increasing number of reports have cited a risk of small bowel obstruction after laparoscopic gastrointestinal tract surgery [5, 6]. However, no urological studies have evaluated the tissue damage at the VUA site. In addition, few studies have evaluated the postoperative longitudinal changes in functional and patient-reported outcomes associated with barbed sutures during RARP.

Here, to examine the negative aspects of barbed sutures for VUA during RARP compared with nonbarbed sutures, we conducted a cohort study and evaluated the tissue damage using imaging techniques and repeatedly measured functional and patient-reported outcomes.

Materials and methods

This prospective cohort study involved consecutive patients who underwent RARP at a university hospital between July 2013 and November 2014. The study protocols were approved by the ethics committee at our institution (No. 2334). Informed consent was obtained from all patients.

Definition of suture groups as exposure

We used two suture methods for VUA during this study period: barbed and nonbarbed. Fifty patients received the polyglyconate, unidirectional barbed synthetic absorbable suture (barbed suture group), and 40 received nonbarbed sutures with polydioxanone (PDS II) (nonbarbed suture group). After exclusion of 2 patients who received nonbarbed sutures due to lost to follow-up right after the discharge, our analysis set comprised 50 patients in the barbed suture group and 38 patients in the nonbarbed suture group.

Operative technique

All cases underwent surgery using the 4-arm Da Vinci Si surgical system (Intuitive Surgical, Sunnyvale, CA, USA). Our VUA technique is similar to that described by Sammon et al. [4]. Briefly, in the V-Loc group, the VUA was performed similarly to the anastomosis between the urethra and bladder, using bidirectional, barbed 3-0 polyglyconate sutures (V-Loc®180). The VUA started at the 6 o’clock position in a clockwise fashion until the 11 o’clock position was reached. The other arm of the suture was performed from the 5 o’clock position to the 11 o’clock position. The VUA in the nonbarbed suture group was performed using a 3-0 polydioxanone suture (PDS II), which was prepared by tying together the ends of threads that had been adjusted to 15 cm.

RARP was performed by three well-trained surgeons (T.Y., N.H., and Y.K.). Through December 2013, all RARP procedures at our hospital were performed with barbed sutures. Starting in January 2014, we switched from barbed sutures to nonbarbed sutures for RARP. However, in February 2014, we started re-using barbed sutures and have since continued using both suture methods for RARP procedures, with the type of suture used left up to the surgeon’s preference.

Magnetic resonance imaging (MRI) of postoperative urethral and periurethral tissue damage as an outcome

To evaluate the damage in the urethra and periurethral tissue, MRI was performed at 9 months after RARP with a 1.5-T whole-body magnetic resonance scanner (Signa; GE Medical Systems, Milwaukee, WI, USA), as V-Loc180 is completely absorbed by the body by 6 months, [7] and at least 4–6 months are required for fibrosis and collagen remodeling during the period of inflammation induced by surgery. The presence of postoperative tissue damage was defined based on the observation of a T2-weighted low-signal-intensity (black) area in and around the urethra [8, 9]. The normal membranous urethra was identified as an intermediate-signal-intensity (gray) ring on axial T2-weighted images, and the normal periurethral tissue was identified as a high-signal-intensity (white) area around the membranous urethra. Grading of postoperative tissue damage was evaluated by a board-certified radiologist (O.H) and determined using axial T2-weighted images according to the classification by Paparel et al. [8] (Fig. 1). Representative MRI images are shown in Fig. 2.
Measurement of lower urinary tract symptoms (LUTS) and lower urinary tract function as outcomes

LUTS were evaluated using the International Prostate Symptom Score (IPSS) and a quality of life (QOL) index (higher score indicates poorer QOL). Further, the IPSS subscores, including the voiding symptom score and the storage symptom score, were also separately assessed [10]. Lower urinary tract function was evaluated based on the maximum flow rate and voided volume determined by uroflowmetry (UFM) and the post-void residual urine volume (PVR) determined by ultrasonography. Urinary incontinence was evaluated by a 1-h pad test [11]. LUTS and lower urinary tract function were assessed just before RARP and then again at 1, 3, 6, 9, and 12 months later.

Statistical analysis

Baseline patient characteristics and perioperative data were described as mean (standard deviation) and median (interquartile ranges) for continuous variables and number and percent for categorical variables. The differences in these characteristics between the two suture methods were evaluated using the nonpaired \( t \) test or Mann–Whitney U test for continuous variables and the Chi squared test or Fisher’s exact test for categorical variables.

To address confounding by indication, we used propensity score adjustment with the covariate adjustment method [12]. The propensity to use the barbed versus nonbarbed sutures was modeled using a logistic regression model with the following explanatory variables determined before the VUA procedure: age, D’Amico risk classification, body mass index, nerve preservation, prostate volume (as a proxy for preoperative estimated volume estimated by imaging), log-transformed PSA, preoperative IPSS total score, and preoperative QOL index. The differences in the baseline covariates used in the propensity score analysis between the two suture groups are shown in Supplementary Table 1. The findings for the appropriateness and discrimination of the logistic model are shown in Supplementary Table 2. The propensity score estimated from the logistic regression model was used as the covariate in the subsequent statistical models.

The distribution of the urethral and periurethral damage incurred with each suture method was graphically described. The differences in the degree of damage between the two methods evaluated on MRI were compared using the Mann–Whitney U test. According to previous research [8, 9], the degree of damage was dichotomized (i.e., “Grade 0 or 1” and “Grade 2 or 3”), and likelihood of having Grade 2 or 3 was estimated using generalized linear models to estimate risk ratio (RR). RRs were estimated without any adjustment (unadjusted analysis) and with adjustment using propensity score as a covariate (adjusted analysis using propensity score).

The time course of the outcome data for the two suture methods were described graphically using box plots (Figs. 3, 4) and numerically using summary tables (Supplementary Tables 4–11). To examine how the outcome data changed over time and how the patterns of change differed between the methods, longitudinal mixed-effects
models were fitted [13]. The \( P \) values without adjustment for baseline variables (\( P_{\text{unadjusted}} \)) and those with adjustment using the propensity scores as covariates (\( P_{\text{adjusted}} \)) are shown in Figs. 3 and 4. The details of the analysis method are shown in Supplementary Table 3. \( P \) values <0.05 were set to indicate statistical significance. All statistical analyses were performed using Stata version 14 (Stata Corp., College Station, TX, USA).

Results

The baseline characteristics and perioperative outcomes are shown in Table 1. No significant differences were noted in the perioperative outcomes between the two groups. No urinary leakage at the vesicourethral anastomotic site was observed in either of the groups.

MRI results

The rate of Grade 2 or 3 urethral damage was 58.3% (28/48) in the barbed suture group and 21.6% (6/37) in the nonbarbed suture group. The rate of Grade 2 or 3 periurethral damage was 45.8% (22/48) in the barbed suture group and 18.9% (7/37) in the nonbarbed suture group. Postoperative urethral damage and periurethral damage were significantly more severe in the barbed suture group than in the nonbarbed suture group (\( P < 0.001 \) and \( P = 0.027 \), respectively). Compared with
the nonbarbed suture, after adjustment with the propensity score, use of the barbed suture was associated with a 2.98-fold increased risk of Grade 2/3 urethral damage (95% CI 1.39–6.38) and a 3.85-fold increased risk of Grade 2/3 periurethral damage (95% CI 1.17–12.66) (Table 2).

Fig. 3 Time course of patient-reported outcomes in two suture groups. V barbed suture group, P nonbarbed suture group, Pre preoperative period, mo month after surgery. A International Prostate Symptom Score (IPSS) total score. In the barbed suture group, compared with the preoperative period, the IPSS total score increased significantly after 1 and 3 months and decreased significantly after 9 and 12 months (All \( P_{\text{adj}} \leq 0.05 \)). No marked differences were noted between the two groups. B IPSS voiding symptom score. The voiding symptom score is the sum of the incomplete emptying score, intermittency score, weak stream score, and straining score of the IPSS. In the barbed suture group, compared with the preoperative period, the IPSS voiding symptom score increased significantly after 1 month but decreased significantly after 6, 9, and 12 months (All \( P_{\text{adj}} \leq 0.05 \)). Patients in the barbed suture group tended to have lower scores than those in the nonbarbed suture group in the preoperative period. However, the preoperative difference was not significant after propensity score adjustment (\( Pi_{\text{adj}} = 0.466 \)). Both groups had similar scores after surgery. C IPSS storage symptom score. The storage symptom score is the sum of the frequency score, urgency score, and nocturia score of the IPSS. In the barbed suture group, compared with the preoperative period, the IPSS storage symptom score increased significantly after 1 and 3 months but returned to the preoperative range after 6 months. No marked differences were noted between the two groups. D QOL index. In the barbed suture group, compared with the preoperative period, the QOL index increased significantly after 1 month but returned to the preoperative range after 6 months. Patients in the barbed suture group tended to have lower scores than those in the nonbarbed suture group in the preoperative period. However, the preoperative difference was not significant after propensity score adjustment (\( Pi_{\text{adj}} = 0.788 \)). Patients in the barbed suture group had significantly higher scores after 1, 9, and 12 months. Data were analyzed by longitudinal mixed-effects models. Indicators of statistical significance for longitudinal changes and group differences are represented by \( P \) and \( Pi \), respectively. Only significant \( P \) and \( Pi \) values are shown. \( P(i)_{\text{unadj}} \) was derived from 471 to 476 observations from 87 patients. \( P(i)_{\text{adj}} \) was derived from 446 to 449 observations from 82 patients. The actual numbers are shown in Supplementary Tables 4–7.
The total IPSS score, the voiding and storage symptom subscores of the IPSS, and QOL index increased transiently in both the barbed suture group and the nonbarbed suture group at 1 month after RARP but then decreased to or below their preoperative levels in both groups (Fig. 3A–D). Specifically, the total IPSS score and the voiding subscore at 12 months were lower than their preoperative levels ($P_{\text{adjusted}} = 0.007$ and $P_{\text{adjusted}} = 0.001$, respectively) (Fig. 3A, B). Regarding the group comparison, total IPSS, voiding and storage symptom subscores were similar throughout the study period (Fig. 3A–C). In contrast, the QOL index was significantly higher in the barbed suture group than in the nonbarbed suture group at 1, 9, and 12 months after RARP ($P_{\text{adjusted}} = 0.023$, $P_{\text{adjusted}} = 0.025$, and $P_{\text{adjusted}} = 0.011$, respectively) (Fig. 3D).

The maximum flow rate after RARP did not change or differ markedly between groups (Fig. 4A). Both PVR and voided volume significantly decreased after RARP, but

Fig. 4 Time course of lower urinary tract function in two suture groups. V barbed suture group, P nonbarbed suture group, Pre preoperative period, mo month after surgery. A Maximum flow rate in uroflowmetry. In the barbed suture group, the maximum flow rate did not increase significantly after surgery during the observational period. No marked differences were noted between the two groups. B Voided volume in uroflowmetry. In the barbed suture group, compared with the preoperative period, the voided urine volume decreased significantly after surgery. Both groups had similar urine volumes after surgery. C Post-voided residual urine volume. In the barbed suture group, compared with the preoperative period, the post-voided residual urine volume decreased significantly after surgery. No marked differences were noted between the two groups. D 1-h pad test. In the barbed suture group, compared with the preoperative period, the 1-h pad test results increased significantly after 1 month but decreased significantly after 9 and 12 months. Patients in the barbed suture group had a higher volume than those in the nonbarbed suture group after 3 months. However, both groups had similar volumes after 6, 9, and 12 months. Data were analyzed by longitudinal mixed-effects models. Indicators of statistical significance for longitudinal changes and group differences are represented by $P$ and $P_i$, respectively. Only significant $P$ and $P_i$ values are shown. $P_{(i)}_{\text{unadjusted}}$ was derived from 480 to 487 observations from 87 patients. $P_{(i)}_{\text{adjusted}}$ was derived from 454 to 461 observations from 82 patients. The actual numbers are shown in Supplementary Tables 8–11.
with no significant differences noted between the groups (Fig. 4B, C). Urinary leakage measured by the 1-h pad test increased significantly at 1 month after RARP and then gradually decreased to below baseline levels at 9 and 12 months (Fig. 4D). The urinary leakage in the barbed suture group was nonsignificantly more than that in the nonbarbed suture group at 1 month and significantly more at 3 months ($P_{\text{adjusted}} = 0.041$) (Fig. 4D). The findings from the 1-h pad test were similar from 6 months to 12 months after RARP between the two groups (Fig. 4D).

### Table 1 Preoperative patient characteristics and perioperative outcomes

<table>
<thead>
<tr>
<th></th>
<th>Total $n = 88$</th>
<th>By suture methods</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>66.8 (5.0)</td>
<td>66.4 (4.6)</td>
<td>67.2 (5.6)</td>
</tr>
<tr>
<td>D’Amico risk classification, %</td>
<td>66.5 (63, 71)</td>
<td>66 (63, 70)</td>
<td>68 (63, 72)</td>
</tr>
<tr>
<td>Low</td>
<td>17 (19.3%)</td>
<td>6 (12.0%)</td>
<td>11 (29.0%)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>36 (40.9%)</td>
<td>22 (44.0%)</td>
<td>14 (36.8%)</td>
</tr>
<tr>
<td>High</td>
<td>35 (39.8%)</td>
<td>22 (44.0%)</td>
<td>13 (34.2%)</td>
</tr>
<tr>
<td>Body mass index, kg/m$^2$</td>
<td>23.7 (3.3)</td>
<td>23.3 (3.1)</td>
<td>24.3 (3.5)</td>
</tr>
<tr>
<td>PSA, ng/mL</td>
<td>9.4 (6.1)</td>
<td>10.9 (7.2)</td>
<td>7.4 (3.4)</td>
</tr>
<tr>
<td>Console duration, min</td>
<td>177 (28)</td>
<td>176 (27)</td>
<td>178 (29)</td>
</tr>
<tr>
<td>Estimated blood loss, mL</td>
<td>223 (186)</td>
<td>236 (195)</td>
<td>206 (175)</td>
</tr>
<tr>
<td>Nerve preservation, %</td>
<td>9 (10.2%)</td>
<td>4 (8.0%)</td>
<td>5 (13.2%)</td>
</tr>
<tr>
<td>Prostate weight, g</td>
<td>41.3 (13.9)</td>
<td>40.8 (12.6)</td>
<td>42.0 (15.7)</td>
</tr>
<tr>
<td>Duration of catheter placement, days</td>
<td>6.4 (0.9)</td>
<td>6.5 (1.1)</td>
<td>6.2 (0.5)</td>
</tr>
<tr>
<td>Pathological stage, %</td>
<td>68 (77.3%)</td>
<td>40 (80.0%)</td>
<td>28 (73.7%)</td>
</tr>
<tr>
<td>≥T3</td>
<td>20 (22.7%)</td>
<td>10 (20.0%)</td>
<td>10 (26.3%)</td>
</tr>
<tr>
<td>Vesicourethral anastomotic time, min</td>
<td>24.2 (7.3)</td>
<td>24.0 (7.7)</td>
<td>24.3 (6.8)</td>
</tr>
<tr>
<td>Anastomotic leakage, %</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Barbed barbed suture group, nonbarbed nonbarbed suture group
Continuous variables are shown as the mean (SD); median (interquartile range)
Categorical variables are shown as the number (percent)
a Nonpaired t-test
b Mann–Whitney test
c Fisher’s exact test
d Chi squared test

### Discussion
In the present study, MRI findings revealed that postoperative urethral and periurethral damage was greater in the barbed suture group than in the nonbarbed suture group. Regarding the patient’s reported and functional outcomes, QOL index was significantly higher in the barbed suture group than in the nonbarbed suture group at several time points, and urinary leakage was significantly greater in the
barbed suture group than in nonbarbed suture group in the early postoperative period.

Our clinical finding that urethral and periurethral tissue damage determined by MRI was related to the use of unidirectional barbed sutures was corroborated with an in vivo histopathological analysis using a porcine model, which showed that unidirectional barbed polydioxanone sutures at the VUA induced significantly greater fibrosis of the lamina propria and perianastomotic fat than nonbarbed polyglactin at 1 week after surgery [14]. As our imaging analysis was conducted at 9 months after surgery, the present findings suggest that histological damage may have been sustained for a period longer than previously reported. Thus, damage to the urethra and periurethral tissue associated with the barbed sutures may explain the transient aggravation in QOL and continence function after RARP observed in our study, possibly via decreased elasticity of the external urethral sphincter [8].

In our study, continence function determined by the 1-h pad test after RARP was worse in the barbed suture groups than in the nonbarbed suture group at 1 and 3 months. This finding was inconsistent with those of previous reports showing that urinary continence outcome did not differ markedly between the barbed and nonbarbed suture groups [1, 2, 4]. This discrepancy may have any of several explanations. First, we assessed continence function using an objective 1-h pad test for quantitative evaluation and compared the finding as a continuous outcome. In contrast, previous studies used pad count per day reported via questionnaire and compared the finding as a categorical outcome [2, 4, 15]. Therefore, our study is more likely to detect between-group differences than the previous studies.

Second, the longitudinal mixed-effects models used in the study might have increased power to detect group differences (Supplementary Table 3). Other studies used mere repetition of comparison tests to evaluate the differences at each observational point [2, 4, 15].

Several strengths to the present study warrant mention. First, our study is the first report showing tissue damage at the suture site after RARP among real-world clinical studies. Second, we focused on examining the longitudinal changes in postoperative functional and patient-reported outcomes, while previous studies have focused on surgical outcomes directly related to RARP (i.e., VUA time and urinary leakage at anastomotic site). Although RARP per se allows for the early recovery of urinary continence [16, 17], the challenge of new surgical techniques like the suture method is also of clinical relevance for further early acquisition of continence, as early-stage recovery of continence fosters social reintegration for patients after RARP. This notion was supported by earlier improvement in the QOL in the nonbarbed suture group than in the barbed suture group. We believe that both the minimization of urinary complications and preservation of the urinary QOL can function as secondary goals of RARP, given the increasing demand for patient-oriented outcomes. Indeed, in fields other than urology, an increasing number of studies are focusing on patient-oriented outcomes, which measure the impact on the QOL, after oncologic surgery [18, 19]. Thus, our study supports the use of nonbarbed sutures for VUA for early acquisition of urinary continence and improvement of QOL.

However, several limitations to the present study should also be mentioned. First, as with previous studies [2–4], our study was conducted in a single institution, and therefore

### Table 2 Associations between barbed suture use and Grade 2/3 urethral and periurethral damage

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted analysis</th>
<th>Adjusted analysis using propensity scorea</th>
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<tbody>
<tr>
<td></td>
<td>Risk ratio 95% CI</td>
<td>P value</td>
</tr>
<tr>
<td>Grade 2/3 urethral damage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barbed (vs. nonbarbed)</td>
<td>2.70 1.40–5.21</td>
<td>0.003 2.98 1.39–6.38 0.005</td>
</tr>
<tr>
<td>Grade 2/3 periurethral damage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barbed (vs. nonbarbed)</td>
<td>2.42 1.16–5.04 0.018</td>
<td>3.85 1.17–12.66 0.026</td>
</tr>
</tbody>
</table>

Analyzed using the generalized linear model to estimate the risk ratio

The numbers of Grade 0/1/2/3 of urethral damage in the barbed suture group are 4/16/17/11, respectively
The numbers of Grade 0/1/2/3 of urethral damage in the nonbarbed suture group are 11/18/6/2, respectively
The numbers of Grade 0/1/2/3 of periurethral damage in the barbed suture group are 13/13/9/13, respectively
The numbers of Grade 0/1/2/3 of periurethral damage in the nonbarbed suture group are 14/16/4/3, respectively

CI confidence interval, barbed barbed suture group; nonbarbed suture group
a Adjusted for propensity score estimated by logistic regression models with the following predictors: age, body mass index, D’Amico risk classification, adoption of nerve preservation, prostate volume, log-transformed PSA, IPSS total score, and quality of life index

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our findings may not be generalizable, since technique-specific factors might have also affected the present results (i.e., VUA time, operative duration). Second, the tissue damage determined by MRI might have been mixed with artifacts, as this imaging modality can be affected by even a small amount of metal, which is included in the dye tinting the string. Third, the results from our observational study suggest associations but not causation, due to confounding. However, we tried to adjust for confounding by indication derived from differences in the baseline characteristics using the propensity score method with covariate adjustment [12]. Fourth, the sample size of our study seems relatively small. However, based on the proportion of Grade 2 or 3 damage observed on MRI, the powers to detect statistical significance were 0.91 and 0.67 for postoperative urethral and perirethral damage, respectively. These findings allowed us to reasonably conclude that our study had enough power at least to assess the effect of barbed sutures on postoperative urethral damage, provided the association between barbed sutures and urethral damage was true.

Conclusions

This cohort study showed that, after RARP, barbed sutures during VUA induced more severe tissue damage as determined by MRI and greater transient aggravation of QOL and continence function than nonbarbed sutures. The present findings suggest that using nonbarbed sutures during VUA may facilitate earlier acquisition of urinary QOL and urinary continence.

Compliance with ethical standards

Disclosures Drs. Nobuhiro Haga, Noriaki Kurita, Tomohiko Yana-gida, Soichiro Ogawa, Michihiro Yabe, Hidenori Akaiahata, Junya Hata, Yuichi Sato, Kei Ishitashi, Osamu Hasegawa, and Yoshiyuki Kojima have no conflict of interest or financial ties to disclose.

References