Presurgical bladder wall thickness is a useful marker to predict the postsurgical improvement of symptoms in patients with pelvic organ prolapse-related overactive bladder

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Abstract

Objectives: Pelvic organ prolapse (POP) is a cause of overactive bladder (OAB), and transvaginal mesh (TVM) surgery can improve the symptoms. Bladder wall thickness (BWT) is a useful and safe marker to evaluate bladder function in urinary disorders. The main purpose of this study is to clarify the relationship between BWT and changes in the OAB symptom score (OABSS) after TVM operation in patients with POP.

Methods: BWT was measured by ultrasonography before and 6 months after surgery at three sites in the bladder: the anterior wall, trigone, and dome. Similarly, the OABSS was evaluated at the time of BWT measurement. Changes induced in BWT at each site and the mean BWT at all sites after TVM surgery were analyzed. Similarly, the relationship between presurgical BWT and the decrease in OABSS was investigated.

Results: TVM surgery improved OABSS in 30 patients (responders; 73.2%), while 11 patients were judged as nonresponders (26.8%). BWT at the anterior bladder wall and dome as well as the mean BWT at all three sites were significantly decreased by TVM surgery (P < .001). Similar trends were identified in OABSS responders; however, all markers showed no significant changes in OABSS nonresponders. All the BWT-related markers before surgery were significantly lower in OABSS responders than in OABSS nonresponders.

Conclusions: BWT at the bladder anterior wall and dome, but not the trigone, were decreased by TVM surgery. We conclude that presurgical BWT may be a useful marker to predict the improvement in OAB symptoms by TVM surgery in patients with POP-related OAB.

Keywords
bladder wall thickness, overactive bladder symptom score, pelvic organ prolapse, transvaginal mesh surgery
INTRODUCTION

Overactive bladder (OAB) is defined as urinary urgency, usually with daytime frequency and nocturia, with or without urgency urinary incontinence, in the absence of urinary tract infection or other obvious pathologies.1 Pelvic organ prolapse (POP) is a major health-care problem that impairs the quality of life in women, and many urologists support the opinion that patients with POP often experience OAB symptoms.2,4

Several reports revealed that transvaginal POP repairs, such as transvaginal mesh (TVM) surgery, are effective treatment tools, and they lead to the disappearance or improvement in OAB symptoms.3,4 Therefore, many investigators attempted to define predictive markers for the improvement in urinary conditions, including OAB symptoms, after surgery.2,4–7 Previous studies used the severities of preoperative subjective symptoms, including International Prostate Symptom Score, overactive bladder symptom score (OABSS), and urodynamic examination data, as useful preoperative predictors.2–4,7 However, since the examination exclusively based on subjective symptoms cannot evaluate objective findings, such as detrusor overactivity and bladder outlet obstruction, and urodynamic tests are invasive and carry the risk of urinary tract infections, noninvasive and effective predictors of surgery outcomes are needed.2,8

The measurement of bladder wall thickness (BWT) is supposedly a useful noninvasive parameter to evaluate the lower urinary tract function.9,10 In fact, BWT was significantly correlated with clinical parameters, symptom scores, and uroflowmetry results under obstructive bladder conditions.11,12 In addition to bladder outlet obstruction, BWT in women with OAB was greater than that in women with stress incontinence or normal urinary function.13–17 Thus, BWT has been hypothesized to reflect the bladder activity and urinary symptoms in various urinary disorders. Additionally, in recent years, sonographic parameters, including BWT, were reported to be useful predictors of outcomes in patients with lower urinary tract symptoms treated with an alpha-1 adrenoceptor antagonist.18 Similarly, BWT is considered a potentially useful parameter for monitoring the response to antimuscarinic treatment in patients with OAB.19,20 However, the predictive value of BWT for OAB symptoms in POP patients treated with TVM has not been studied. Therefore, this study mainly aimed to determine the usefulness of BWT as a predictive marker for the improvement in OAB symptoms after TVM surgery in POP patients.

METHODS

2.1 Patients

The study group consisted of 41 women who underwent TVM surgery for POP and who met the diagnostic criteria for OAB using OABSS (urgency ≥2, total score ≥3)21 at Nagasaki University Hospital. Patients who underwent a sling procedure for stress incontinence at the time of the TVM surgery were excluded from the study. In addition, bladder or rectal cancers, urinary tract infection, additional perioperative treatment, including antimuscarinic drugs, or uncontrollable systemic disorder were the major exclusion criteria (Figure 1). We used a soft polypropylene mesh (Gynemesh PS; Ethicon, Somerville, New Jersey, or Polyform; Boston Scientific, Tokyo, Japan), and the surgical technique was similar to that of the French TVM group.22 Patients with anterior vaginal wall prolapse underwent the anterior TVM (A-TVM) procedure. The A-TVM procedure starts with anterior colpotomy after local infiltration. Repair of a cystocele required two arms of the transobturator mesh to be passed on both sides to suspend the cystocele. On either side, both arms of the mesh were passed into the paravesical region using a modified Emmet needle. The anterior subvesical strap was inserted into the tendinous arch of the pelvic fascia. Patients with both anterior and posterior vaginal wall prolapse underwent an anterior-posterior TVM (AP-TVM) procedure. The posterior subvesical strap was inserted into the tendinous arch 1 cm from the ischial spine using a gently curved needle. In the posterior TVM (P-TVM) procedure, posterior colpotomy was performed longitudinally, and the mesh was placed under the vaginal wall. On each side, one strap of the mesh was passed into the pararectal space through the sacrospinous ligament and exteriorized via incisions located outside and below the anus. Furthermore, patients without a uterus underwent total TVM (T-TVM), wherein one piece of a prosthetic mesh, consisting of two connected parts, is inserted into the anterior and posterior walls.22–24 All patients underwent the same surgical procedure using TVM by an expert surgeon (T.M.). Finally, 41 patients treated with TVM (anterior 24, posterior 0, anterior and posterior 12, and total 5) were enrolled in this study. Table 1 shows the baseline characteristics of the patients and the surgical procedures this study. This study was approved by the Nagasaki University Hospital Ethical Committee and was performed in accordance with the principles of the Declaration of Helsinki. All patients provided written informed consent.
2.2 Evaluation of BWT and urinary conditions

BWT was evaluated by ultrasonographic examination before and 6 months after the surgery at three sites in the bladder: the anterior wall, trigone, and dome, as reported earlier.25,26 All patients were examined by one urologist (T.M.). Ultrasonography was performed by a transvaginal approach using a convex probe (HI VISION Avius, 7.5 MHz B mode; Hitachi-Aloka Medical, Ltd, Tokyo, Japan). When the examination was performed, <50 mL of urine pooled in the bladder (Figure 2). All patients simultaneously filled in the self-reporting questionnaire for evaluating the OABSS. The validated Japanese language questionnaire was completed by patients in a separate and secluded space. Patients with lower OABSS after surgery than before operation were considered responders, while those with worsening or unchanged OABSS after operation were considered nonresponders.

### TABLE 1 Patients’ characteristics at the presurgical stage

|                        | Entire       | OABSS responder | OABSS nonresponder | P value |
|------------------------|--------------|-----------------|--------------------|---------|
| Number of patients     | 41           | 30              | 11                 |         |
| Age, y                 | 68.0 ± 7.3   | 69.0 ± 4.7      | 69.3 ± 8.1         | .539    |
| POP-Q stage (%)        |              |                 |                    | .545    |
| 0                      | 0 (0)        | 0               | 0                  |
| 1                      | 0 (0)        | 0               | 0                  |
| 2                      | 4 (9.8)      | 2               | 2                  |
| 3                      | 33 (80.5)    | 25              | 8                  |
| 4                      | 4 (9.8)      | 3               | 1                  |
| Postmenopausal status  | 39 (95.1)    | 29              | 10                 | .448    |
| Hormone replacement therapy (%) | 0 (0) | 0 (0) | 0 (0) |
| Parity                 | 2.1 ± 0.9    | 1.9 ± 0.8       | 2.2 ± 0.8          | .889    |
| Previous surgery (%)   |              |                 |                    |         |
| Hysterectomy           | 4 (9.8)      | 3 (10.0)        | 1 (9.1)            | .931    |
| Reconstructive surgery | 8 (19.5)     | 7 (23.3)        | 1 (9.1)            | .308    |
| Others                 | 0 (0)        | 0 (0)           | 0 (0)              | 1.000   |
| Surgical procedure (%) |              |                 |                    | .566    |
| Anterior               | 24 (58.5)    | 19 (63.3)       | 5 (45.5)           |         |
| Posterior              | 0 (0)        | 0 (0)           | 0 (0)              |         |
| Anterior and posterior | 12 (29.3)    | 8 (26.7)        | 4 (36.4)           |         |
| Total                  | 5 (12.2)     | 3 (10.0)        | 2 (13.6)           |         |
| OABSS                  |              |                 |                    |         |
| Q1 Daytime frequency   | 0.9 ± 0.7    | 1.1 ± 0.6       | 0.6 ± 0.7          | .048    |
| Q2 Nighttime frequency | 1.3 ± 0.8    | 1.3 ± 0.8       | 1.3 ± 0.9          | .915    |
| Q3 Urgency             | 2.0 ± 1.6    | 2.8 ± 1.1       | 2.3 ± 0.9          | .136    |
| Q4 Urgency incontinence | 1.0 ± 1.5   | 1.2 ± 1.7       | 0.4 ± 0.7          | .243    |
| Total score            | 5.1 ± 3.3    | 6.3 ± 3.1       | 4.6 ± 2.1          | .071    |
| Voided volume (mL)     | 199.6 ± 154.5| 167.5 ± 118.5   | 289.3 ± 209.4      | .097    |
| Qmax (mL/s)            | 16.7 ± 11.4  | 14.8 ± 9.8      | 21.9 ± 14.3        | .066    |
| PVR (mL)               | 65.4 ± 78.0  | 68.5 ± 80.9     | 56.9 ± 72.6        | .751    |

Note: Data are presented as mean ± SD.

Abbreviations: OABSS, overactive bladder symptom score; POP-Q, Pelvic Organ Prolapse-Quantification; PVR, postvoid residual urine volume; Qmax, maximum urinary flow rate.
Briefly, OABSS responders in this study were defined as individuals with a decrease in the total score of OABSS by 1 point or more. In addition, the maximum urinary flow rate (Qmax) and voided volume (VV) were determined using free uroflowmetry, and postvoid residual urine volume (PVR) was measured using suprapubic ultrasonography.

### Statistical analysis

Data are presented as the number of patients (percentage, %) or mean ± SD. All statistical analyses were performed using the JMP14 software (SAS Institute Inc, Cary, North Carolina). Differences in the changes in parameters from baseline to 6 months in patients were examined using the paired Student’s t or Wilcoxon signed rank tests. Values of \( P < .05 \) were considered statistically significant.

### RESULTS

#### 3.1 Changes in urinary symptoms after surgery

Among the 41 patients evaluated, TVM surgery improved urinary condition in 30 patients, as evaluated by the OABSS (73.2%). However, the score deteriorated in 6 patients (14.6%) and was unchanged in 5 patients (12.2%).

There was no case of POP recurrence, especially during the follow-up period regarding surgery. Table 2 shows the changes observed in the OABSS and objective findings, including uroflowmetry, after TVM surgery. After the operation, the total OABSS of all patients decreased significantly (5.1 ± 3.3 to 2.9 ± 2.9; \( P < .001 \)). In OABSS responders, the total score improved from 6.3 ± 3.1 to 2.0 ± 2.0 (\( P < .001 \)). The individual questionnaires showed that the Q1 (Daytime frequency) and Q3 (Urgency) scores were significantly decreased by TVM surgery (0.9 ± 0.7 to 0.3 ± 0.5 and 2.0 ± 1.6 to 1.0 ± 1.6, respectively), whereas the changes in Q2 (Nighttime frequency) and Q4 (Urgency incontinence) were not significant (1.3 ± 0.8 to 1.1 ± 0.9 and 1.5 ± 1.0 to 0.5 ± 1.1, respectively) in patients overall. In OABSS nonresponders, total OABSS and all items in the OABSS did not improve after TVM operation. Finally, among the 41 patients, 30 and 11 were found to be OABSS responders and OABSS nonresponders, respectively (73.2% and 26.8%, respectively). In objective findings, the PVR of patients overall and OABSS responders improved after operation (overall, 65.4 ± 78.0 mL to 19.7 ± 33.7 mL, \( P < .001 \); OABSS responders, 68.5 ± 80.9 mL to 19.6 ± 35.8 mL, \( P < .001 \)). However, not all objective findings significantly improved after operation in OABSS nonresponders (Table 2).

#### 3.2 Changes in BWT after surgery

Table 3 shows the measurements of BWT at three different sites and their mean values before and after TVM surgery. No significant differences in BWT were observed among the three sites before surgery. Postsurgical BWT levels at the anterior and dome regions were altered significantly, along with the mean levels at all three sites (\( P < .001 \)). However, BWT at the trigone region was not altered significantly (\( P = .741 \)).

#### 3.3 Correlation between BWT and urinary symptoms after surgery

Figure 3 shows the correlation between the changes observed in BWT and OABSS after surgery. In OABSS responders, BWT at the
anterior wall and dome was significantly decreased after TVM surgery (5.46 ± 1.32 mm to 4.47 ± 1.20 mm and 5.56 ± 1.06 mm to 4.65 ± 1.20 mm, respectively). In contrast, in OABSS nonresponders, these two parameters exhibited a decreasing trend but did not reach significance (6.56 ± 1.02 mm to 5.89 ± 1.53 mm and 6.62 ± 1.02 mm to 5.88 ± 1.55 mm, respectively; \( P = .126 \) and \( P = .093 \), respectively). BWT at the trigone region was unchanged after surgery in both responders and nonresponders (5.69 ± 1.27 mm to 5.69 ± 2.08 mm, \( P = .501 \), and 7.04 ± 0.89 mm to 7.53 ± 2.13 mm, \( P = .169 \), respectively). The mean BWT at all three sites before and after TVM surgery was significantly different among OABSS responders (5.57 ± 1.21 mm and 4.94 ± 1.63 mm, \( P < .001 \)), unlike among nonresponders (6.74 ± 0.92 mm to 6.43 ± 1.84 mm, \( P = .254 \)).

### 3.4 Prognostic roles of BWT for the efficacy of the surgery

Table 4 shows the relationship between lower OABSS and BWT before surgery. All measurements of BWT at the three sites in OABSS responders were significantly lower than those in OABSS nonresponders. In addition, the mean BWT at all three sites was also significantly different among the responders, and it showed the most significant difference among the parameters evaluated. In addition, when the postoperative BWT change between OAB responders and OAB nonresponders was examined, BWT decreased postoperatively in the OAB responder group at all sites. However, no statistically significant difference was observed (Table 5). Furthermore, we examined the correlation between the changes in bladder capacity (VV + PVR) and BWT before and after operation. Consequently, the increase in postoperative bladder capacity was statistically significantly inversely correlated with BWT (\( r = -0.4541, P = .005 \)). In contrast, improvement in OAB symptoms and BWT with receiver operating characteristic (ROC) curve showed an area under the curve (AUC) of 0.858 (95% CI, 0.713-1.003) and 0.868 (95% CI, 0.743-0.993) for anterior wall and dome, respectively. When the cutoff values for BWT were defined as 6.72 mm and 6.41 mm for the anterior wall and dome, the sensitivities were 77.8% and 77.8%, and the specificities were 79.3% and 75.9%, respectively, for the predicted improvement in OAB symptoms after TVM surgery (Figure 4A,B).

In addition, the unfit rate for the diagnosis of OAB was 61.0% (25/41). Furthermore, the deviation from diagnostic criteria for OAB and BWT with ROC showed an AUC of 0.698 (95% CI, 0.531-0.864) and 0.703 (95% CI, 0.510-0.895) for the anterior wall and dome, respectively. When the cutoff values for BWT were defined as 6.43 mm and 6.74 mm for the anterior wall and dome, the sensitivities were 80.0% and 84.0%, and the specificities were 56.3% and 50.0%, respectively, for predicting the deviation of the diagnosis for OAB after TVM surgery (Figure 5A,B).

### Table 3

| Bladder wall thickness (mm) | Transvaginal mesh surgery | \( P \) value |
|-----------------------------|---------------------------|--------------|
|                            | Before                   | After        |               |
| Anterior                   | 5.73 ± 1.25              | 4.75 ± 1.37  | <.001         |
| Trigone                    | 6.09 ± 1.28              | 6.26 ± 2.18  | .741          |
| Dome                       | 5.82 ± 1.09              | 4.98 ± 1.34  | <.001         |
| Mean of the three sites    | 5.88 ± 1.17              | 5.33 ± 1.53  | <.001         |

**FIGURE 3** Changes in bladder wall thickness (BWT) before and after transvaginal mesh surgery in overactive bladder symptom score (OABSS) responders and OABSS nonresponders. In OABSS responders, BWT at the anterior wall and dome and mean BWT at all three sites after surgery was significantly lower than before surgery.
This study shows that presurgical measurement of BWT using ultrasound may be a useful predictor of lower OABSS after TVM surgery in patients with POP. In general, the most useful method to evaluate bladder activity is supposedly a conventional urodynamic test. In fact, in previous studies, urodynamic tests revealed useful predictive factors for improving OAB symptoms in POP after TVM surgery.3,4 However, this test is invasive, time-consuming, and presents various types of risks.8 Clinical parameters, such as age, body mass index, postmenopausal status, and smoking, have similarly been investigated as potential predictive factors for improved OAB symptoms after TVM repair, and some of them were identified as useful predictors.3 In this study, we focused on the measurement of BWT due to its noninvasive nature.

One of our interesting results is that all BWT parameters were significantly thinner in OABSS responders than in OABSS nonresponders. Based on our results regarding changes in BWT at individual sites, the findings that BWT at the anterior wall and dome regions can predict the improvement in OABSS after TVM surgery are expected. Similarly, the finding that the mean BWT of all three sites before surgery was associated with improvement in the OABSS is logically expected. However, surprisingly, presurgical BWT at the trigone region in OABSS responders was significantly lower than that in OABSS nonresponders, despite remaining unchanged before and after TVM surgery. Unfortunately, our study design cannot provide an answer to this phenomenon. However, we speculate that the higher BWT is attributable to the development of connective tissue and/or fibrosis of the bladder, resulting from mesh insertion, because the thickness was unchanged despite improvement in OABSS symptoms.

### TABLE 4

| Bladder wall thickness, presurgery (mm) | OABSS responder | OABSS nonresponder | P value |
|--------------------------------------|-----------------|--------------------|---------|
| Anterior; mm, mean ± SD              | 5.46 ± 1.32     | 6.56 ± 1.02        | .017    |
| Trigone                              | 5.69 ± 1.27     | 7.04 ± 0.89        | .003    |
| Dome                                 | 5.56 ± 1.06     | 6.62 ± 1.02        | .008    |
| Mean of the three sites              | 5.57 ± 1.12     | 6.77 ± 0.92        | <.001   |

Abbreviation: OABSS, overactive bladder symptom score.

### TABLE 5

| Changes in BWT by operation (mm) | OABSS responder | OABSS nonresponder | P value |
|-----------------------------------|-----------------|--------------------|---------|
| Anterior; mm, mean ± SD           | −1.03 ± 1.22    | −0.82 ± 1.54       | .676    |
| Trigone                           | −0.01 ± 1.86    | 0.61 ± 1.60        | .207    |
| Dome                              | −0.91 ± 1.32    | −0.82 ± 1.31       | .765    |
| Mean of the three sites           | −0.62 ± 1.18    | −0.32 ± 1.37       | .507    |

Abbreviations: BWT, bladder wall thickness; OABSS, overactive bladder symptom score.

![Figure 4](image-url)  
**FIGURE 4** Receiver operating characteristic (ROC) curves for the relationship between preoperative bladder wall thickness (BWT) and improvement in overactive bladder (OAB) symptoms. The black line shows the ROC curve in JMP software. The yellow line in the plot is a straight line at a 45-degree angle tangent to the ROC curve, and the contact point with the ROC curve shows the optimal cutoff value.
Therefore, the increase observed in BWT at the trigone region in patients with POP may be irreversible.

Several investigators have proposed that BWT may be a useful diagnostic tool for OAB.\textsuperscript{15,16,25,26} In addition, change in BWT was suggested to be a potential marker to predict the success of treatments in patients with OAB.\textsuperscript{20,27} On the other hand, Robinson et al\textsuperscript{17} reported no significant change in BWT after 12 weeks of administering solifenacin to female patients for OAB. However, we strongly emphasize that standardized methods and convincing criteria to measure BWT according to pathological conditions remain unestablished. Hence, we considered the following arguments to design our study and discuss the clinical significance of BWT based on previous reports.

First, we measured BWT at three different anatomical points of the bladder: the trigone, dome, and anterior wall, and calculated their mean. Previous reports used the mean values of measurements at these three sites as BWT for statistical analyses in women with OAB symptoms.\textsuperscript{17,20} In addition, there is the opinion that the average of results at more than a single site is better for the measurement of BWT.\textsuperscript{28} Our results show that the mean BWT at the three sites and the anterior wall and dome of the bladder were altered by TVM surgery, while BWT at the trigone region was not altered. In addition, interestingly, similar changes in BWT were observed in OABSS responders, unlike in nonresponders. Hence, we support the opinion that measurements at multiple sites are essential to accurately calculate BWT. Furthermore, we would like to emphasize that the measurement of BWT at the trigone region alone may lead to an erroneous evaluation of bladder function and understanding of the pathological role of BWT.

Second, in addition to the transvaginal approach, the transrectal or suprapubic approach has been used to measure BWT.\textsuperscript{12,29} However, several investigators reported that transvaginal ultrasound was the best method for measuring BWT in women regarding intraobserver and inter-observer reliability compared with the others.\textsuperscript{30,31} Therefore, we used a transvaginal probe in this study. Although we were uncertain initially about the suitability of a transvaginal approach for patients with POP, BWT could be measured easily in all patients. Similarly, we suggest that the transvaginal method is useful in patients with POP.

Subsequently, we evaluated the OABSS and BWT at 6 months after TVM surgery. However, consensus about the optimal post-TVM surgery duration to evaluate the subjective and objective parameters does not exist. Previous studies ranged from 1 month,\textsuperscript{32} 3 months,\textsuperscript{4} 6 months,\textsuperscript{3} to 12 months.\textsuperscript{33} In reality, although we performed the examinations 6 months post surgery due to past experience and the patients’ requests; it is not supported by medical rationale. Therefore, further studies are necessary to determine the optimal schedule for the evaluation of BWT. In addition, the optimal cutoff value to diagnose OAB and criteria to reflect the accurate pathological significance still need to be established.\textsuperscript{15,16,25}

The major limitation of our study is the relatively small number of patients. Although we defined the optimal cutoff value of BWT to predict the improvement in OAB symptoms by TVM surgery in this study, the accuracy of these indicators remains questionable. In addition, because the number of cases was small and many of the included patients had mild to moderate OAB based on the OABSS, it was impossible to conduct a comparative study based on the severity of the OAB symptoms. In particular, in this study, we did not conduct a pressure flow study and could not examine the relationship between BWT and detrusor pressure in detail. However, we highlight that BWT is a noninvasive parameter that can directly evaluate the bladder wall condition. Presently, we agree that BWT cannot replace urodynamic tests.\textsuperscript{15,26} In addition, we similarly agree that the

![Figure 5](image-url)

**FIGURE 5** Receiver operator characteristic (ROC) curves for the relationship between preoperative bladder wall thickness (BWT) and deviation for diagnosis of overactive bladder (OAB) using OAB symptom score (OABSS). The black line shows the ROC curve. The yellow line in the plot is a straight line at a 45-degree angle tangent to the ROC curve, and the contact point with the ROC curve shows the optimal cutoff value.
standardization of BWT measurements, such as the types of transducer, frequency of the ultrasonic waves, and zooming power, is essential for widespread adoption. However, this is the first report evaluating the relationship between the changes in OAB symptoms and BWT in POP patients before and after transvaginal operation. More standardized clinical trials using larger study populations are required to clarify the “real power” of BWT as a predictive marker for the improvement in symptoms after TVM surgery in patients with POP.

In conclusion, our results demonstrate that BWT at the anterior wall and dome and the mean BWT at all three sites were significantly lower after TVM surgery than before surgery in OABSS responders, unlike in OABSS nonresponders. On the other hand, all BWT-related parameters before the surgery were significantly associated with the improvement in OABSS by the surgery. Based on these results, we suggest that presurgical measurement of BWT may be a useful marker to predict the therapeutic effects evaluated by OAB symptoms after TVM surgery in patients with POP-related OAB.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS
Tomohiro Matsuo and Yasuyoshi Miyata were responsible for the design and the conceptualization of this study. Data acquisition was performed by Tomohiro Matsuo. The clinical studies were conducted by Asato Otsubo, Yuta Mukae, and Kojiro Ohba. Data analyses were performed by Tomohiro Matsuo, Yasuyoshi Miyata, and Kensuke Mitsunari. The draft manuscript was written by Asato Otsubo and Tomohiro Matsuo, and Yasuyoshi Miyata and Hideki Sakai edited the manuscript.

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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