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ORIGINAL ARTICLE

Peritoneal perforation is less a complication than an expected event during transanal endoscopic microsurgery: experience from 194 consecutive cases

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Abstract
Background Indications for transanal endoscopic microsurgery (TEM) have been extended to technically challenging tumors, which may be associated with an increased risk of peritoneal perforation (PP). The aim of the present study was to investigate the occurrence, management and outcome of PP in patients having TEM.
Methods All the patients who had TEM for rectal adenoma or adenocarcinoma in our unit were included. Patients in whom PP occurred (Group A) were compared to those without PP (Group B).
Results From 2007 to 2015, 194 TEM (116 men, median age 66 [range 21–100] years) were divided into Groups A ($n = 28$, 14%) and B ($n = 166$). The latter group included four patients, in whom a laparoscopy did not confirm suspicion of PP made during TEM. In 2 of 28 patients (7%), the diagnosis of PP was made postoperatively during reoperation for peritonitis. For the 26 other patients (93%), routine exploratory laparoscopy was performed with suture of the peritoneal defect on the pouch of Douglas in 24 cases and a rectal suture alone in 2 cases. Independent predictive factors for PP were: distance from the anal verge >10 cm (OR = 3.6), circumferential tumor (OR = 3.0) and anterior location (OR = 2.7). Hospital stay was significantly longer in Group A (7.5 [3–31] days) than in Group B (4 [1–38] days; $p < 0.0001$), whereas there was no significant difference regarding postoperative morbidity and recurrence rate.
Conclusions Our results suggested that PP is not a very rare event during TEM, especially in anterior, circumferential and/or high rectal tumors. Laparoscopic treatment of PP is feasible and safe. The occurrence of PP is not associated with poor oncologic results.

Keywords Transanal endoscopic microsurgery · Peritoneal perforation · Rectal adenoma · Rectal adenocarcinoma

Introduction

Local excision of selected rectal tumors has been proposed for many years as an alternative to radical total mesorectal excision. It is associated with low morbidity and mortality rates and very low risk of long-term functional disorders [1–3]. Nowadays, local excision of rectal tumors is safely proposed for benign villous adenomas and for early rectal cancer staged T1N0 on preoperative ultrasonography and T1sm1 or sm2, well differentiated and without lymphovascular invasion on pathological examination [4]. The gold standard is transanal endoscopic microsurgery (TEM), developed by Buess in 1983 [5]. TEM allows the full-thickness excision of lesions located up to approximately 10–15 cm from the anal verge, providing the advantages of excellent visualization of the rectum and larger operative fields (due to rectal insufflation) compared to standard transanal resection [3, 6–8]. We and other authors have progressively extended the indications for TEM to include technically challenging tumors, larger than 5 cm, involving more than 50% of the rectal circumference or located in the upper third of the rectum [9, 10]. However, the extension of indications for TEM could potentially be associated with an increased number of complication results such as

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peritoneal perforation (PP), piecemeal resection or incomplete resection. The occurrence of PP during TEM could be considered as a real complication that may require conversion to open surgery for adequate repair of the defect and may cause abdominal sepsis. Furthermore, it could potentially affect oncologic outcomes through implantation of cancer cells in the peritoneal cavity. Few authors have reported their experience of PP during TEM [11–16]. In these series, PP was always managed by an endorectal approach through TEM, whereas we prefer abdominal laparoscopic exploration in order to confirm the PP and then suturing of the peritoneal defect. The aim of the current study was to investigate the occurrence, management and outcomes of PP in patients undergoing TEM.

Materials and methods

Study population

All patients who had TEM for rectal adenoma or adenocarcinoma between October 2007 and October 2015 were identified from our prospective single-center institutional review board-approved database.

Two groups of patients were constituted according to the occurrence of PP during TEM:

- Group A: Patients with confirmed PP.
- Group B: Patients without PP, even if PP was suspected (but not confirmed) during TEM.

A comparative study was performed between Groups A and B for the following findings: patient features (gender, age, body mass index [BMI], comorbidities); tumor features (size, location, distance from the anal verge, pathological type, preoperative treatment, neoadjuvant radiochemotherapy, prior endoscopic or local resection); intraoperative features (piecemeal resection, intraoperative incident and associated procedures such as endoscopic, laparoscopic or open suture, diverting stoma); postoperative outcomes (length of hospital stay, in-hospital and 30-day postoperative morbidity and mortality); pathological results (size, resection margin involvement, full-thickness resection, staging according to the American Joint Committee on Cancer classification, 7th edition); and long-term follow-up (including reoperation, medical treatment and long-term oncologic outcomes).

Surgical procedure

All TEM procedures were performed according to the technique previously described [9, 17] using the TEO® platform (Karl Storz, Tuttlingen, Germany). All procedures were performed under general anesthesia, and the patient was routinely placed in the lithotomy position, in order to facilitate an eventual abdominal laparoscopy in case of suspicion of PP during the procedure. Antibiotic prophylaxis, based on metronidazole, was systematically administered during 2 days postoperatively.

In case of suspicion of PP, our policy was to perform a systematic laparoscopic exploration of the abdomen rather than an endorectal suture through TEM. Laparoscopic abdominal exploration (using three trocars) confirmed and located the perforation (at the Douglas pouch) and allowed us to suture the peritoneal defect with interrupted sutures (Prolene 2/0, Ethicon, Cincinnati, OH, USA). A transanal air and fluid leak test were always performed after suturing a PP. A pelvic suction drain was left in place in the pelvis at the surgeon's discretion. A diverting ileostomy was only constructed in case of large PP or a positive leak test.

Outcome measures

PP was suspected during TEM in case of pneumoperitoneum or direct visualization of the abdomen during TEM. Furthermore, it was also suspected in case of increased CO_2 pressure and/or impossibility to continue TEM because of the loss of the pneumorectum. Postoperative delayed diagnosis of PP was suspected in the presence of sepsis, abdominal pain and systemic inflammation syndrome, and always confirmed by computed tomography (CT) scan.

Postoperative morbidity was defined as any complication occurring during the hospital stay or within 30 days after surgery and classified according to Dindo et al. [18].

Additional treatment (i.e., salvage proctectomy) was indicated in case of rectal adenocarcinoma beyond T1sm2 at pathological examination. For these patients, a standardized follow-up was performed, as recommended for colorectal cancer.

For the patients with benign adenoma or early rectal cancer [19], a postoperative examination was performed at 6 months and a colonoscopy was performed 1 year after surgery. Local recurrence was always confirmed by histologic examination of biopsy material.

Statistical analysis

Quantitative data were reported as the median and range, and qualitative data were reported as the number of patients (percentage of patients). Normally distributed quantitative data were analyzed with Student's t test, and the Mann–Whitney U test was used otherwise. Qualitative data were compared using Pearson's Chi-square test or Fisher's exact test, as appropriate. Multivariate analysis of PP during TEM was performed according to a logistic regression model, which included all variables with a p value of less

than 0.2 in univariate analysis. All tests were two-sided, with a level of significance set at p value of less than 0.05. All analyses were performed using the GraphPad Prism software (San Diego, CA, USA) and the Statistical Package for the Social Sciences (SPSS) software (SPSS Inc., version 22.0, Chicago, IL, USA). This study was conducted according to the ethical standards of the Committee on Human Experimentation of our institution and reported according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [20].

Results

Patient characteristics

From 2007 to 2015, 194 TEMs were performed for rectal adenoma or adenocarcinoma, in 190 patients (116 men, median age of 66 [range 21–100] years), at our institution. Four patients underwent TEM twice. Twenty-eight PP occurred during TEM (14%, Group A). In 166 TEM, no PP was reported, including 4 cases in which exploratory laparoscopy eliminated a suspicion of PP (Group B). Patient and tumor characteristics are listed in Table 1. There were no differences between the two groups regarding gender, age, American Society of Anesthesiologists (ASA) grade and comorbidities. However, patients in Group A had a lower BMI (24 [17–34]) than those in Group B (25 [18–53], $p = 0.01$).

The rectal lesion was more frequently located anteriorly in Group A ($n = 15$, 53%) than in Group B ($n = 50$, 31%; $p = 0.02$) and more circumferential in Group A ($n = 4$, 14%) than in Group B ($n = 6$, 4%; $p = 0.02$). The median distance from the anal verge was greater in Group A (8 [2–14] cm) than in Group B (5 [1–16] cm, $p = 0.001$). There were significantly more lesions located beyond 10 cm from anal verge in Group A ($n = 10$, 36%) than in Group B (20/143, 14%, $p = 0.01$). No difference was observed between groups in surgical indication, tumor size, preoperative treatment or associated colonic resection.

Peritoneal perforation

On multivariate analysis (Table 1), three predictive factors for PP were found: distance from anal verge superior to 10 cm (OR = 3.6, 95% CI 1.4–9.7; $p = 0.01$), circumferential tumor (OR = 3, 95% CI 1.1–8; $p = 0.026$) and anterior location of the tumor (OR = 2.7, 95% CI 1.1–6.6; $p = 0.028$).

Management of PP is shown in Table 2. Diagnosis of PP was made immediately in 26 patients (93%). An endorectal suture was placed in 2 of these patients (8%) at the beginning of our experience, the next four patients had an endorectal suture associated with exploratory laparoscopy (15%), and for the remaining patients with PP exploratory laparoscopy was routinely performed ($n = 20$, 77%). Exploration showed the perforation was always located at the same site, the peritoneal reflection on the pouch of Douglas. An abdominal suture was placed in 24 patients (92%), via a laparoscopic approach in all but one case, which was converted to open. Completion of rectal resection was performed via a laparoscopic approach in 2 cases (8%), because of a very large defect, making peritoneal sutures impossible. Transanal leak testing was performed in 16 patients (59%) and showed a persistent PP in 2/16 (12%) of them. A diverting stoma was created in these 2 cases, and in 2 other cases, because of the large size of the PP. A pelvic suction drain was placed in 16 patients (59%).

In four patients, despite suspicion of PP during TEM, PP was not confirmed by laparoscopy.

In 2 cases (7%), the diagnosis of PP was delayed (1 and 2 days), presenting with postoperative peritonitis. The two patients required open surgery because of emergent presentation, cardiac comorbidities and advanced age (80 years). One patient underwent a Hartmann procedure due to fecal peritonitis, and the second patient underwent a rectal suture with diverting stoma because of purulent peritonitis.

Surgical results

Median length of hospital stay was significantly longer in Group A (7.5 [3–31] days) than in Group B (4 [1–38] days; $p < 0.0001$), whereas there was no significant difference between the groups regarding overall, surgical, medical or major morbidity as shown in Table 3. Major morbidity (Dindo III–IV) occurred in two patients (7%) in Group A and consisted of rectal bleeding ($n = 1$) and peristomal evisceration ($n = 1$). In Group B, ten patients presented with major morbidity (6%): rectal bleeding ($n = 9$) and pneumothorax ($n = 1$).

No significant difference between groups was neither noted regarding pathological results. No residual tumor was observed in four patients in Group A (14%) and 19 in Group B (11%). No significant difference between the two groups was noted concerning tumor staging and additional treatment for infiltrant tumors in patients with adenocarcinoma (Table 3).

Rates of R1 resection were similar in Group A (4; 14%) and Group B (17; 10%; $p = 0.51$). Patients with adenocarcinoma and involved margins or those with an adenocarcinoma staged T1sm3 or more, underwent additional surgical treatment, except for one patient in Group A and 5 in Group B, due to serious comorbidities.

Table 1 Characteristics of 194 transanal endoscopic microsurgery procedures

	Group A	Group B	*p* value	Multivariate analysis	
	Perforation (*n* = 28)	No perforation (*n* = 166)		OR (95% CI)	*p* value
Gender			0.83		
Male	16 (57)[a]	100 (60)			
Female	12 (43)	66 (40)			
Age (years)	70 [42–93][b]	66 [21–100]	0.35		
BMI[c]	24 [17–34]	25 [18–53]*	**0.01**	0.3 (0.07–1.5)	0.144
ASA grade[d]**			0.38		
I	7 (25)	27/119 (23)			
II	14 (50)	74/119 (62)			
III	7 (25)	18/119 (15)			
Comorbidities	9 (30)	61 (37)	0.64		
Arteriopathy	2	8			
Cardiopathy	7	43			
Respiratory insufficiency	2	11			
Past colonic surgical history	–	11			
Indication					
Adenoma	22 (79)	101 (61)	0.12	0.7	0.7
Adenocarcinoma	6 (21)	51 (31)		(0.2–2.1)	
Other[e]	–	14 (8)			
Size (mm)	40 [10–100]	40 [10–130]**	0.22		
Distance from anal verge (cm)	8 [2–14]	5 [1–16]***	**0.001**		
Distance from anal verge			**0.01**	**3.6**	**0.01**
<10 cm	18 (64)	123/143 (86)		(**1.4–9.7**)	
≥10 cm	10 (36)	20/143 (14)			
Location			**0.02**	**2.7**	**0.028**
Anterior	15 (53)	50 (31)[$]	**0.03**	(**1.1–6.6**)	
Posterior	5 (18)	69 (43)	**0.01**		
Lateral	7 (25)	34 (21)	0.63		
Circumferential	4 (14)	6 (4)	**0.02**	**3** (**1.1–8**)	**0.026**
Preoperative treatment					
Radiochemotherapy	1 (4)	10 (6)	1.00		
Endoscopic resection	6 (21)	34 (20)	1.00		
Incomplete endoscopic resection	3/6	24/34			
Transanal resection	3 (11)	13 (8)	0.71		
Associated colonic resection	2 (7)	5 (3)	0.27		

Results from 157 patients; ** results from 148 patients; *** results from 138 patients

$p < 0.05$ was considered as significant (in bold)

OR odds ratio, *CI* confidence interval

[a] Number of patients (percentage)

[b] Median (range)

[c] Body mass index

[d] American Society of Anesthesiology grade

[e] Neuroendocrine tumor ($n = 10$), leiomyoma ($n = 1$), gastrointestinal stromal tumor ($n = 1$), lipoma ($n = 1$), rectal duplication ($n = 1$)

[$] Results from 159 patients

Table 2 Management of 28 patients undergoing peritoneal perforation during transanal endoscopic microsurgery

	Group A Perforation ($n = 28$)
Immediate diagnosis	26 (93)[a]
Endorectal suture	6 (23)
Laparoscopic exploration	26 (100)
Laparoscopic suture	24 (92)
Completion of rectal resection	2 (8)
Diverting stoma	4 (15)
Pelvic suction drain	16 (62)
Delayed diagnosis	2 (7)
Suture and diverting stoma	1
Hartmann's procedure	1

[a] Number of patients (percentage)

Long-term follow-up

Median follow-up was 11.5 [1–35] and 12 [1–62] months, in Groups A and B, respectively. At the end of follow-up, the long-term morbidity rate was significantly higher in Group A ($n = 9$, 32%) than in Group B ($n = 17$, 10%, $p = 0.004$). Long-term morbidity is shown in Table 4. The most frequent complications were rectal stenosis and motility disorders, which were significantly more frequent in Group A than in Group B ($n = 6$, 21 vs. $n = 8$, 5%, $p = 0.007$, and $n = 9$, 32% $n = 12$, 7%, $p = 0.0005$, respectively). No fecal incontinence was reported.

There was no significant difference between the group in recurrence rate and additional surgery. One case of recurrence occurred in Group A (1/28; 4%), after removal of an adenoma with positive margins, and required repeat TEM. In Group B, 10 local recurrences occurred (10/166; 6%), 8 of whom after excision of adenocarcinoma. Of the 10 recurrences 3 had positive margin at time of TEM. Among these, five patients underwent repeat TEM, two patients underwent a salvage proctectomy, and three patients were not reoperated on. No deaths occurred during follow-up.

Discussion

In this study, we reported that in treating PP that which occurred during TEM exploratory laparoscopy had several advantages. PP was not associated with a higher risk of postoperative morbidity, local recurrence or additional surgery. We consider PP as a part of the TEM procedure in challenging tumors (i.e., circumferential, on the high rectum) rather than a real complication.

PP occurred in 14% of TEM in our series. This is higher than the incidence rates reported in most studies (2–8.5%)

Table 3 Postoperative results in 194 transanal endoscopic microsurgery procedures

	Group A Perforation ($n = 28$)	Group B No ($n = 166$)	p value
Postoperative results			
Length of stay (days)	7.5 [3–31][a]	4 [1–38]	**<0.0001**
Overall morbidity	9 (32)[b]	39 (23)	0.34
Surgical morbidity	6 (21)[c]	19 (11)	0.21
Rectal bleeding	5	19	0.24
Profound abscess	1	–	0.24
Peristomal evisceration	1	–	0.24
Medical morbidity	3 (11)	24 (14)	0.77
Fever	1	8	1.00
Cardiac disorder	–	5	1.00
Urinary infection	1	4	0.47
Urinary blockage	1	5	0.54
Other[d]	–	2	1.00
Dindo classification			1.00
I–II	7 (78)	29 (74)	
III–IV	2 (22)	10 (26)	
Unplanned reoperation	2 (7)	9 (5)	0.39
Pathological results			
Type			0.67
No residual tumor	4 (14)	19 (11)	
Adenoma	11 (39)	59 (35)	
Adenocarcinoma	13 (47)	76 (46)	
Neuroendocrine tumor	–	6 (4)	
Other[e]	–	6 (4)	
Staging of adenocarcinoma			0.75
In situ	7 (54)	35 (46)	
T1sm1	3 (23)	16 (21)	
T1sm2	–	4 (5)	
T1sm3	–	5 (7)	
T2–3	3 (23)	16 (21)	
Resection			
Involved margin	4 (14)	17 (10)	0.51
Full thickness	28 (100)	163 (98)	1.00
Additional treatment	2 (7)	16 (10)	1.00
Proctectomy	1	14	
Radiochemotherapy	–	2	
Radiotherapy	–	1	
Chemotherapy	1	1	

$p < 0.05$ was considered as significant (in bold)

[a] Median (range)

[b] Number of patients (percentage)

[c] Some patients had several complications

[d] Functional bowel obstruction, pneumothorax

[e] Rectal duplication, leiomyoma, gastric heterotopy, gastrointestinal stromal tumor and myxoïd tumor

Table 4 Long-term results in 194 patients undergoing transanal endoscopic microsurgery

	Group A Perforation ($n = 28$)	Group B No perforation ($n = 166$)	p value
Follow-up (months)	11.5 [1–35][a]	12 [1–62]	0.69
Long-term morbidity	9 (32)[b]	17 (10)	**0.004**
Rectal stenosis	6 (21)	8 (5)	**0.007**
Rectal pain	1 (4)	3 (2)	0.46
Transit disorder	9 (32)	12 (7)	**0.0007**
Tumor recurrence	1 (4)	10 (6)	1.00

$p < 0.05$ was considered as significant (in bold)

[a] Median (range)

[b] Number of patients (percentage)

[12–16], except that by Molina et al. [21] who reported PP in 28.2% of their patients. The high incidence rate may be explained by the fact that TEM is the first surgical option for us in case of high and large villous adenoma. We have identified three independent predictive factors for PP: distance from the anal verge greater than 10 cm, circumferential tumor and anterior location. Morino et al. [14] reported that on multivariate analysis tumors located more than 7 cm from the anal verge were at higher risk of perforation. In another comparative series, median distance of the tumor from the anal verge was also significantly higher and the tumor was most frequently anterior in case of PP, but the authors did not perform a multivariate analysis [15, 21]. Ramwell et al. [12] reported 15 cases of PP during TEM, all in an anterior location. In our opinion, PP occurs more frequently than before, because the increase in indications means that more patients with larger, higher or more circumferential tumors undergo the procedure [9]. Molina et al. [21] reported that on univariate analysis PP occurred more frequently during TAMIS than during TEM and TEO procedures (67 vs. 25%, $p < 0.05$). We did not observe a significant difference in the incidence of PP according to the type of platform (TEO vs. TAMIS with disposable material) in a recent case-matched study [22].

What constitutes optimal treatment for PP is still a matter of debate PP. We believe, exploratory laparoscopy is the best method, because it allows: (1) confirmation (or elimination) of PP with visualization of the peritoneal defect, or in case of doubt, the possibility to test peritoneal integrity with air or Betadine; (2) an easily performed laparoscopic suture of the perforation; (3) the possibility to test the efficacy of the suture, avoiding an unnecessary diverting stoma; (4) the possibility to place a pelvic drain, if needed; (5) if an ileostomy was needed, laparoscopy allows easy identification of the distal ileum.

Our laparoscopic management of PP differs from other methods, in which the peritoneal defect is always closed endoscopically through TEM [11–16, 21, 23, 24]. We believe that transanal suturing of the perforation is not only more difficult for optimal exposure of the defect, which is always anterior, but also makes air or fluid leak testing impossible. Indeed, in our study, an endorectal suture was performed in only six patients, at the beginning of our experience, but 4 out of these six patients also underwent exploratory laparoscopy. Morino et al. reported only 3 cases of abdominal laparoscopic ($n = 2$) or open ($n = 1$) conversions. These conversions occurred during their first 100 TEM, confirming the crucial role that experience plays in the management of PP [14]. On the other hand, Ramwell et al. reported the realization of defunctioning stoma in six patients with PP. For the authors, there was no evidence that increased experience reduced the need for a stoma [12]. Issa et al. [25] reported a similar experience to our own, with 13% of PP, treated at the beginning only by endorectal suture, and then, with routine exploratory laparoscopy. To date, there is no comparative study about the transanal versus the laparoscopic repair of PP. In our opinion, exploratory laparoscopy should not be considered as a sign of a lack of experience, but presents several advantages over endorectal repair.

We reported 2 cases of delayed diagnosis (7%) (on postoperative days 1 and 2) in the presence of postoperative peritonitis. These patients underwent a rectal suture with diverting stoma ($n = 1$) and a Hartmann procedure. Two cases of delayed diagnosis (11%) were also reported by Issa et al. [25] for which a conservative treatment and an exploratory laparotomy with loop ileostomy were performed. Eyvazzadeh et al. reported 1 case of delayed diagnosis (4%) on postoperative day 1, in the presence of leak on contrast enema. Abdominal exploration found minimal peritoneal fluid, no gross pus or feces and a sealed leak on insufflation testing. The rectal suture was reinforced and drained, and the patient was discharged home on postoperative day 11 [16]. Due to the presence of the 2 cases in our series, in whom PP was not diagnosed intraoperatively but only postoperatively, in case of patients at high risk of PP (i.e., with anterior, high or circumferential rectal tumors), we prefer to quickly perform exploratory laparoscopy if there is any suspicion of PP, even without strong evidence.

Even if length of stay was significantly longer in patients with PP [14, 15], morbidity was not significantly increased in our series and others [11, 14, 21]. Regarding late complications, we reported 6 cases of rectal stenosis (22% of those with PP), which were successfully managed by endoscopic or surgical dilatation. All these patients had large lesions occupying more than 50% of the rectal lumen. Only Ramwell et al. [12] reported a case of rectal stenosis treated by balloon dilatation (7%) and a case of stoma site hernia (7%).

Oncologic results after PP have rarely been reported. Baatrup et al. reported that negative margins were achieved in 17 out of 22 patients (77%) with PP. Local recurrence occurred in two patients (10%), successfully treated with curative resection. Distant metastases occurred in three patients, after a mean follow-up of 37 months, and all of them died [13]. For Morino et al., local recurrence developed only in 2 out of the 6 patients with pT2 and pT3 tumors (33%) who did not receive further treatment after TEM. Distant metastases occurred in two patients with pT2 or pT3 tumor (33%). For the authors, the occurrence of PP did not correlate with an increased risk of local recurrence and/or distant metastases [14]. Marks et al. [15] reported 2 cases of positive margins (7.7%) and 1 case of local recurrence (3.8%). In our study, we did not observe a significant difference between patients with or without PP, regarding involved margins and metastatic or local recurrence. One case of involved margin occurred in a patient with adenocarcinoma after PP, and was treated with proctectomy, with no residual tumor at the histologic analysis. One case of local recurrence (adenoma) and 1 case of distant metastases (pT3) occurred after PP, the latter requiring proctectomy and adjuvant chemotherapy. Therefore, PP does not seem to us to correlate with increased risk of local recurrence or distant metastases.

Study limitations

Limitations of the current study include its retrospective nature and the fact that the numbers of perforations in cancer patients was too small and the follow up was too short to draw firm conclusions about oncologic results.

Conclusions

Our results suggest that PP occurring during TEM is an expected event rather than a real complication. Patients should always be informed about the potential occurrence of PP during TEM, especially in case of large, anterior or highly located tumors. Management of perforation is feasible and safe through abdominal laparoscopy. Morbidity and oncologic results of TEM in case of PP do not seem to be influenced by the occurrence of PP.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was conducted according to the ethical standards of the Committee on Human Experimentation of our institution.

Informed consent Informed consent was obtained from all the individual participants included in the study.

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