Annals of Diagnostic Pathology 48 (2020) 151606

Contents lists available at ScienceDirect

Annals of Diagnostic Pathology

journal homepage: www.elsevier.com/locate/anndiagpath

Original Contribution

Macroscopic and microscopic characteristics of low grade appendiceal mucinous neoplasms (LAMN) on appendectomy specimens and correlations with pseudomyxoma peritonei development risk

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ARTICLE INFO

Keywords:
Appendiceal mucinous neoplasm
Low grade mucinous appendiceal neoplasm (LAMN)
Pseudomyxoma peritonei (PMP)
Appendiceal diverticula

ABSTRACT

Low grade appendiceal mucinous neoplasm (LAMN) is the primary source of pseudomyxoma peritonei (PMP). PMP may develop after seemingly complete resection of primary tumor by appendectomy, which is unpredictable due to lack of reliable prognostic indicators. We retrospectively reviewed 154 surgically resected LAMNs to explore if any of the macroscopic and microscopic characteristics may be associated with increasing risk of PMP development. Our major findings include: (1) As compared to those without PMP, the cases that developed PMP were more frequent to have (a) smaller luminal diameter (< 1 cm) and thicker wall, separate mucin aggregations, and microscopic perforation/rupture, all suggestive of luminal mucin leakage; (b) microscopic acellular mucin presenting on serosal surface and not being confined to mucosa; and (c) neoplastic epithelium dissecting outward beyond mucosa, however, with similar frequency of neoplastic cells being present in muscularis propria. (2) Involvement of neoplastic cells or/and acellular mucin at surgical margin did not necessarily lead to tumor recurrence or subsequent PMP, and clear margin did not absolutely prevent PMP development. (3) Coexisting diverticulum, resulted from neoplastic or non-neoplastic mucosa being herniated through muscle-lacking vascular hiatus of appendiceal wall, was seen in a quarter of LAMN cases, regardless of PMP. The diverticular portion of tumor involvement was often the weakest point where rupture occurred. In conclusion, proper evaluation of surgical specimens with search for mucin and neoplastic cells on serosa and for microscopic perforation, which are of prognostic significance, should be emphasized.

1. Introduction

Mucinous neoplasm of vermiform appendix, particularly the low-grade appendiceal mucinous neoplasm (LAMN), is the primary cause of the so-called pseudomyxoma peritonei (PMP), a clinically problematic condition in which widespread mucinous tumor disseminates along abdomino-pelvic peritoneum causing mucinous ascites and predominantly “ground-covering” fashion of tumor metastasis. In considerable number of cases, the primary appendiceal mucinous tumor is found incidentally in patients presenting clinically as acute appendicitis treated by appendectomy. In some of these patients, PMP may develops later, months to years after tumor resection by appendectomy, even when the appendix/tumor appears to be intact and entirely removed. This sequela is always worrisome to the surgeons for such patients, and it is somewhat unpredictable due to lack of reliable prognostic indicators up to date.

The possibility of disease recurrence and/or subsequent development of PMP motivates the exploration into the macroscopic and microscopic characteristics of appendiceal mucinous neoplasms and their prognostic significance. The bulk of current literature on the subject concentrates on the diagnostic classification and nomenclature of mucinous neoplasms of appendix but little on the prognostic factors. When considering the pathological evaluation of the appendectomy specimens, several findings, including the surgical margin involvement (by neoplasm or/and mucin), perforation of appendiceal wall, and presence of mucin on serosal surface, have been naturally taken into clinical

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https://doi.org/10.1016/j.anndiagpath.2020.151606

consideration. A few studies have demonstrated that tumors confined to appendiceal mucosa with negative resection margins have lower incidence of disease recurrence or PMP development. Yantiss et al. reported that a third of patients with neoplastic epithelium being present outside appendix and rare patients with periappendiceal acellular mucin developed PMP during postoperative follow-up, whereas those without extraappendiceal neoplastic epithelium were all disease free [1]. Fournier et al. demonstrated that patients with negative surgical margins and normal level of tumor markers at the time of appendectomy had a decreased risk of recurrence or PMP progression [2]. Roxburgh et al. recently found that in LAMN patients with remote mucinous deposits on periappendiceal tissue or local peritoneum, only those with cellular mucinous deposits (containing neoplastic cells) but not acellular mucinous deposits (containing no neoplastic cells) were associated with adverse outcome [3]. However, the study findings were not always in agreement and the exact significance of exhaustive macroscopic and microscopic characteristics associated with LAMN with regard to the patients' outcome has never been systematically studied.

The present study retrospectively reviewed 154 cases of LAMN that were initially treated by, or incidentally found in appendectomy, with regard to the initial macroscopic and microscopic findings and the patients' subsequent outcome (*i.e.*, be cured *versus* development of PMP), and aimed to explore if some of the macroscopic and microscopic characteristics may be associated with the increasing risk of disease remission and/or development of PMP and thus of prognostic significance.

2. Materials and methods

2.1. Study subjects

All cases of appendiceal mucinous neoplasms (AMN) diagnosed on appendectomy and extended resection specimens over the 20-year period of 1997 to 2017 in Calgary region were retrieved by searching the anatomic pathology records of the Calgary Laboratory Services (CLS, now a part of Alberta Precision Laboratories), a centralized pathology laboratory serving 5 general hospitals in the region. Various diagnostic terminology of LAMN used in the past were used as key words for the search, including mucocele, retention cyst, mucinous cystadenoma, mucinous adenoma, mucinous neoplasm of low or unknown malignant potential, and low-grade appendiceal mucinous neoplasm (LAMN). Only those cases that met all of the following criteria were included for the study: (1) the mucinous tumor was clinically proven to be appendiceal primary, (2) first time diagnosis, (3) tumor demonstrating diagnostic features of LAMN as described in the currently widely accepted diagnostic criteria according to the PSOGI classification as well as the WHO Classification of Tumors of Digestive System (4th edition) [4,5], in which LAMN was defined by the low-grade mucinous neoplastic epithelium expansion with loss (atrophy and fibrosis) of lamina propria and with a pushing front against muscularis mucosae and even penetrating into muscularis propria and beyond in the absence of high-grade cytoarchitectural features and destructive invasion. Cases with features of mucinous carcinoma and HAMN were excluded.

The initial total number of cases with suspected LAMN within the parameters of the 20-year data set was 177. Adjusting for those cases having unobtainable histology slides for review and those not warranting a LAMN diagnosis upon histologic evaluation, 154 LAMN cases were included in the study.

The cases were further divided into 3 subgroups based on whether or not the tumor was complicated by concurrent PMP upon diagnosis or subsequent development of PMP during post-resection follow-up from the date of surgical resection to the end of the study, ranging from 2 months to 20 years (90 months on average). Group 1 - LAMN with no PMP/*LAMN-nPMP*, 131 cases: LAMN only, with no clinical presentation or development of PMP at the time of appendectomy and thereafter; Group 2 - LAMN with concurrent PMP/*LAMN-cPMP*, 18 cases: patients presented with PMP at the time of LAMN diagnosis on surgical resection; and Group 3 - LAMN with secondary PMP/*LAMN-sPMP*, 5 cases: LAMN patients who developed PMP after appendectomy up to the end of the study.

2.2. Macroscopic and microscopic characteristics review

For each case, the original gross description in the pathology report was reviewed to document the original gross features and measurements including the length, external diameter and maximum lumen diameter of the appendix, tumor location, presence or absence of adhesion, perforation, calcification, distension, mucin on serosal surface, and mucin within lumen upon sectioning.

The original histology slides were acquired and reviewed to confirm the diagnosis as well as to identify and record the following microscopic characteristics on a data requisition template: (1) the maximum lumen diameter and minimal thickness of appendiceal wall measured on glass slides, (2) the presence or absence of acellular mucin or neoplastic epithelium/cells throughout the appendiceal mucosa, submucosa, muscularis propria, serosa, and mesoappendix, (3) the surgical resection margin status, (4) the presence or absence of microscopic perforations/ruptures, as defined by tiny opening/defect (absence of covering) of the outer surface of involved (and destructed) appendiceal wall through which the mucin is in contiguous to the luminal or intramural mucin, and (5) any other concurring appendiceal pathologies such as calcifications, diverticulosis, acute appendicitis, and others. The documentation of tumor location and maximum lumen diameter took both macroscopic gross description and microscopic observation into consideration although was only recorded and discussed under the macroscopic characteristics to avoid redundancy. The serosal mucin deposits were determined with caution to exclude mucin spillage contamination during specimen handling, and it was accepted only if the mucin deposits were covered by ink or adhered to serosal surface with tissue reaction, such as fibrinous or/and fibrous band, inflammatory or foreign body-type reaction, or even neovascularization.

Also reviewed were additional subsequent pathology reports, when they were available in some patients, in search of pelvic washings or peritoneal fluid cytology, tumor recurrence and/or development of PMP. Our regional unified healthcare system and the centralized pathology service made such a follow-up study possible.

The study was approved by the University of Calgary Conjoint Health Research Ethics Board (CHREB) (approval ID: HREBA.CC-17-0353).

2.3. Statistics

Brown-Forsythe, Fischer's and Tamhane *post hoc* analysis were utilized in the determination of probability significance taking into consideration of the three target groups and their differing sample sizes. Results were considered statistically significant with a probability value equal to or less than 0.05.

3. Results

3.1. Patient demographics

The patient demographics are summarized in Table 1. 57% (88/154) of the patients were female with the exception being the LAMN-sPMP group in which 80% (4/5) were male. The mean age of LAMN diagnoses was 54 years. Appendectomy was the most common surgical resection procedure (×122), followed by appendectomy with cecum resection (×15), ileocecectomy (×11), and right hemicolectomy (×6).

LAMN was incidentally diagnosed in nearly 40% of the total cases and in the cases with no PMP and in more than 60% of those with PMP

Table 1
Patient demographic data and clinical features.

	LAMN-nPMP (n = 131)	LAMN with PMP			Total (n = 154)
		LAMN-cPMP (n = 18)	LAMN-sPMP (n = 5)	Subtotal (n = 23)	
Gender (M/F)	56/75	6/12	4/1	10/13	66/88
Age (years), mean (range)	54 (13–93)	56 (34–88)	45 (32–56)	54 (32–88)	54 (13–93)
Resection procedures (n) (%)					
Appendectomy	107 (82)	12 (67)	3 (60)	15 (65)	122 (79)
Appendectomy and cecectomy	12 (9)	1 (6)	2 (40)	3 (13)	15 (10)
Right ileocolectomy	7 (5)	4 (22)	0	4 (17)	11 (7)
Right hemicolectomy	5 (3)	1 (6)	0	1 (4)	6 (4)
Submitted *in toto*	64(49)	11 (61)	4 (80)	15 (65)	79 (51)

developed later, mostly in patients with acute appendicitis or with ovarian tumor. The frequency of incidental finding was lower in patients with concurrent PMP (16.7%).

The appendix was submitted *in toto* (SIT) for histological evaluation in 51% (79/154) of the total cases, including 49% (64/131) of LAMN-nPMP, 61% (11/18) of LAMN-cPMP, and 80% (4/5) of LAMN-sPMP cases. For the cases with appendices submitted in partial (SIP), on average approximately 65% of the appendices were submitted based on the average number of tissue blocks recorded in the original pathology reports.

3.2. Macroscopic characteristics of LAMN

The macroscopic characteristics are summarized in Table 2, and examples of appendectomy specimens harboring LAMN are shown in Fig. 1.

In 43% (66) of the cases the appendix was grossly distended. The mean measurements were 6.9 cm in length, 2.1 cm in maximum diameter, and 1.5 cm in maximum lumen diameter. The average maximum lumen diameter of LAMN-nPMP was significantly larger than that of LAMN-sPMP (1.55 cm *versus* 0.96 cm, $p = 0.006$).

The distinct tumor location was identified in only 93 cases, with 70% being located distally, 20% in mid-appendix, and 10% proximally. 10% of the specimens showed presence of mucin deposits on appendiceal serosal surface, including 10% of LAMN-nPMP, 17% of LAMN-cPMP and none of LAMN-sPMP cases. 71% had a mucinous lumen upon sectioning. Gross perforations were identified in 18% of all cases, including 15% of the cases without PMP and 35% of cases with PMP, for which the difference was significant ($p = 0.038$). Serosal adhesion was seen in 21% of all cases and seemingly more common in those with PMP (35%) than in those without PMP (18%), with a marginal statistical significance ($p = 0.056$). Gross calcification was seen in 4% cases, with no significant difference existed between the three groups, except no gross calcification recorded in the LAMN-sPMP cases. Aggregations of mucin separated from the main specimen were identified in 5% of all cases, which was significantly more common in the cases with PMP, including 17% of LAMN-cPMP and 20% of LAMN-sPMP cases ($p = 0.01$, as compared to LAMN-nPMP).

3.3. Microscopic characteristics of LAMN

The microscopic characteristics are summarized in Table 3. The average minimum wall thickness was 0.78 mm on microscopic measurements, as exampled in Fig. 2-E/F, with the thinnest wall in LAMN-nPMP cases although not being statistically significantly different from those associated with PMP.

Microperforation/rupture, as exampled in Fig. 3, was identified in 29% cases, which was significantly more frequent in LAMN-cPMP (61%) and LAMN-sPMP (40%) than in LAMN-nPMP cases (24%) ($p = 0.004$).

Acute appendicitis, microcalcifications and diverticula were identified in 26%, 38% and 23% of the cases, respectfully. Additional concurring appendiceal pathologies were identified in 19% cases, including sessile serrated adenoma (10), neuroendocrine tumor (5), inclusion cysts (3), endometriosis (3), parasitic worms (2), cholesterol clefts (2), granuloma (1), necrosis (1), abscess (1), and pneumatosis coli (1); with two patients having multiple nonneoplastic pathologies.

Every histological slide was reviewed for evidence of acellular

Table 2
Macroscopic characteristics summary.

	LAMN-nPMP (n = 131)	LAMN with PMP		
		Subtotal (n = 23)	LAMN-cPMP (n = 18)	LAMN-sPMP (n = 5)
Length (cm), mean ± sd (range)	7.07 ± 2.12 (2.5–16.7)	6.09 ± 2.80 (1.6–14)	5.9 ± 2.83 (1.6–14)	6.7 ± 2.93 (3.5–11.5)
Maximum diameter (cm), mean ± sd (range)	2.07 ± 1.22 (0.4–6.0)	2.36 ± 1.45 (0.7–5.5)	2.45 ± 1.64 (0.7–5.5)	2.12 ± 0.87 (1.3–3.5)
Maximum lumen diameter (cm), mean ± sd (range)	1.55 ± 1.03 (0.2–6.0)	1.09 ± 0.64 (0.1–2.5)	1.15 ± 0.75 (0.1–2.5)	0.96 ± 0.27** (0.7–1.3)
Grossly distended, n (%)	60 (46)	6 (26)	4 (22)	2 (40)
Mucin on serosa, n (%)	13 (10)	3 (13)	3 (17)	0
Mucin in lumen, n (%)	95 (73)	14 (61)	9 (50)	5 (100)
Gross perforation, n (%)	20 (15)	8 (35)*	6 (33)*	2 (40)
Adhesion, n (%)	24 (18)	8 (35)	5 (28)	3 (60)
Aggregations of mucin, n (%)	3 (2)	4 (17)##	3 (17)#	1 (20)#
Gross calcification, n (%)	5 (4)	1 (4)	1 (6)	0
Tumor location, n (%)				
Distal	55 (67)	10 (91)	7 (88)	3 (100)
Middle	18 (22)	1 (9)	1 (13)	0
Proximal	9 (11)	0	0	0

$p = 0.010$, as compared to LAMN-nPMP.
* $p = 0.032$, as compared to LAMN-nPMP.
$p = 0.0013$, as compared to LAMN-nPMP.
** $p = 0.006$, as compared to LAMN-nPMP.

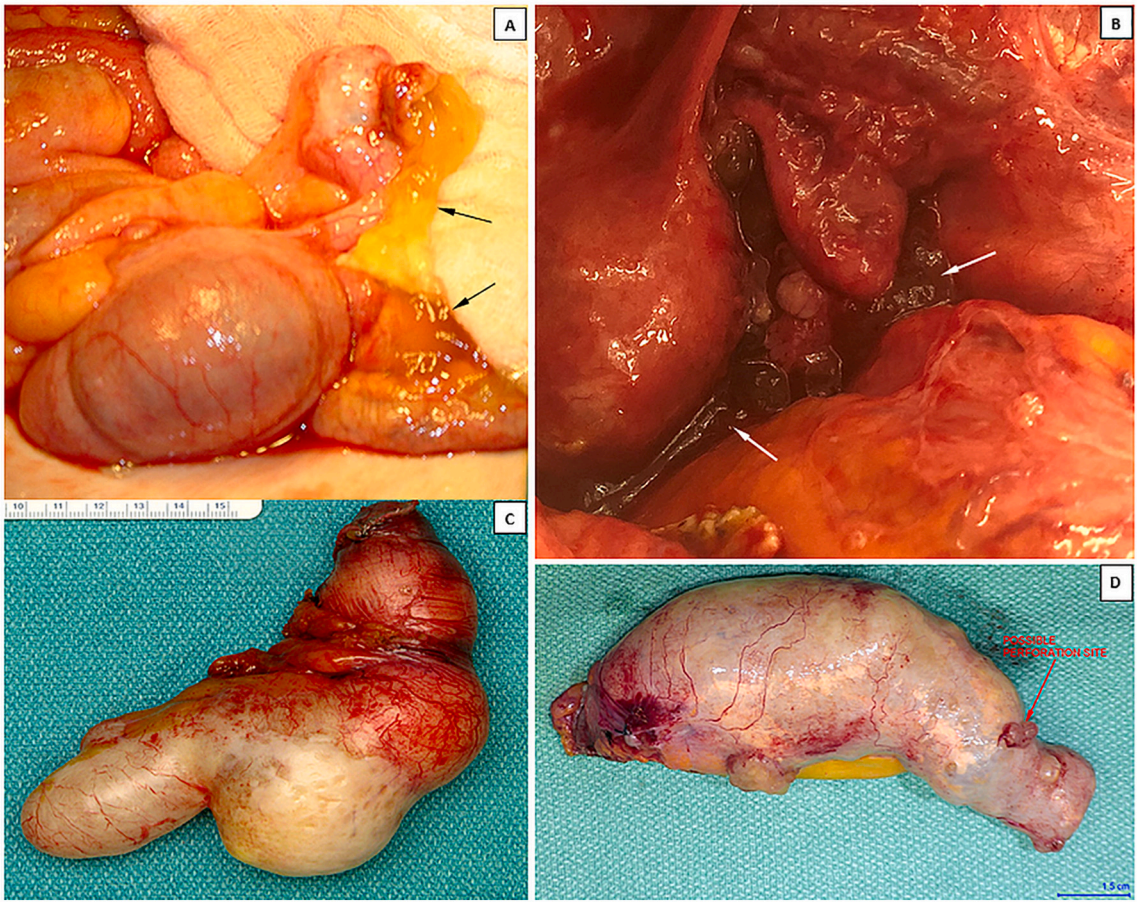

Fig. 1. Examples of appendiceal mucinous neoplasm and pseudomyxoma peritonei. A. Intraoperative view of a LAMN. B. Intraoperative view of abdominal peritoneum involved by widespread mucinous neoplasm (PMP). C/D. Appendectomy specimens harboring appendiceal mucinous neoplasms.

mucin and neoplastic epithelial cell extension, as exampled in Fig. 2. Acellular mucin was restricted to mucosa in 22% cases, of which none was seen in LAMN-sPMP cases. Acellular mucin was identified dissecting into submucosa, muscularis propria, mesoappendix and serosa in 18%, 55%, 29% and 43% of the cases, respectfully. Statistical significance with respect to acellular mucin on serosal surface was identified between the three groups: LAMN-nPMP (36%), LAMN-cPMP (83%) and LAMN-sPMP (80%) ($p = 0.001$). In other words, the presence of acellular mucin on serosa was seen in the majority (> 80%) of the cases with PMP development, either concurrent or subsequent, but less common in those with no PMP. On the other hand, in none of LAMN-sPMP cases the acellular mucin was confined to mucosa; and none of the cases with mucin confined to mucosal layer has developed PMP.

Neoplastic epithelial cells were restricted to mucosa in 75% of all cases, and identified dissecting into submucosa, muscularis propria, mesoappendix and serosa in 1%, 12%, 5% and 9%, respectfully. With respect to the depth of neoplastic epithelial cell dissection, statistically significant difference existed between the three groups was seen. Neoplastic epithelial cells restricted to mucosa was identified in 79% of LAMN-nPMP cases, 50% of LAMN-cPMP, and 40% of LAMN-sPMP cases ($p = 0.005$). Neoplastic cell dissection into submucosa was identified in none of LAMN-nPMP and LAMN-sPMP cases but 11% of LAMN-cPMP cases ($p = 0.021$). Furthermore, neoplastic cells dissecting into mesoappendix, as exampled in Fig. 2-D, was identified in less than 2% of LAMN-nPMP, which was significantly less than that in LAMN-cPMP (22%) and in LAMN-sPMP cases (40%) ($p = 0.001$). Interestingly, 12% of LAMN-nPMP cases had neoplastic cells dissecting into muscularis propria, as exampled in Fig. 2-B, similar to the overall cases of LAMN with PMP (13%, $p > 0.05$).

The surgical transection margin of appendix was assessed for the presence of acellular mucin or/and neoplastic cells. Acellular mucin at surgical margin was identified in 5% of cases, including 5% of LAMN-nPMP (appendectomy margin) and 11% of LAMN-cPMP (all appendectomy margin). Neoplastic cells at surgical margin were identified in only 2 (1%) cases that were both LAMN-nPMP. All 5 LAMN-sPMP cases had surgical (appendectomy and cecectomy) margins being free of both acellular mucin and neoplastic cells.

The importance of submitting the entire appendix for microscopic examination was also investigated with respect to the difference of microscopic yields. Of the 131 LAMN-nPMP cases, 49% were submitted in total (SIT) and 51% were submitted in part (SIP). In the SIT cases, the detection rate of mucin within mesoappendix was significantly higher, as compared to the SIP cases (33% *vs* 11%, $p = 0.0018$), and in this regard it showed no significant difference between LAMN-nPMP and LAMN with PMP. The detection rate of neoplastic cells within mesoappendix seemed to be higher but did not reach statistical significance ($p > 0.05$), as shown in Table 3 (highlighted columns). No statistically significant difference in the detection rate of the other microscopic characteristics between SIT and SIP cases was appreciated. For the cases with concurrent PMP, between the SIT (n = 11) and SIP (n = 7) cases, the detection rates of all of the microscopic characteristics were not significantly different (data not shown).

Lastly, the patients' peritoneal fluid and pelvic floor washing cytology reports, which were available to all patients, were reviewed. Out of the total cases, 3% had positive cytology of which were 17% of LAMN-cPMP and 20% of LAMN-sPMP cases, while none was seen in LAMN-nPMP cases. The difference was statistically significant

Table 3
Microscopic characteristics summary.

	LAMN-nPMP			LAMN with PMP		
	Subtotal (n = 131)	SIT (n = 64)	SIP (n = 67)	Subtotal (n = 23)	LAMN-cPMP (n = 18)	LAMN-sPMP (n = 5)
Minimal wall thickness (mm), mean ± SD (range)	0.73 ± 0.69 (0.1–4.0)	0.69 ± 0.66 (0.1–3.0)	0.78 ± 0.71 (0.1–4.0)	1.11 ± 1.07 (0.3–4.5)	0.79 ± 0.45 (0.3–1.5)	1.96 ± 1.74 (0.3–4.5)
Microperforation, n (%)	31 (24)	19 (30)	12 (18)	13 (57)**	11 (61)**	2 (40)*
Appendicitis, n (%)	37 (28)	19 (30)	18 (27)	3 (13)	1 (6)	2 (40)
Calcification, n (%)	48 (37)	23 (36)	25 (37)	10 (44)	8 (44)	2 (40)
Diverticula, n (%)	32 (24)	20 (31)	12 (18)	3 (13)	2 (11)	1 (20)
Concurring pathology, n (%)	26 (20)	12 (19)	14 (21)	3 (13)	3 (17)	0
Positive pelvic cytology, n (%)	0	0	0	4 (17)	3 (17)	1 (20)
Surgical margin status, n (%)						
Acellular mucin	6 (5)	3 (5)	3 (5)	2 (9)	2 (11)	0
Neoplastic cells	2 (2)	1 (2)	1 (2)	0	0	0
Mucin dissection, n (%)						
Mucosa	33 (25)	16 (25)	17 (25)	1 (4)##	1 (6)	0
Submucosa	22 (17)	12 (19)	10 (15)	5 (22)	4 (22)	1 (20)
Muscularis propria	72 (55)	36 (56)	36 (54)	13 (57)	10 (56)	3 (60)
Mesoappendix	34 (26)	21 (33)&	7 (11)	11 (48)##	8 (44)	3 (60)
Serosa	47 (36)	26 (41)	21 (31)	19 (83)***	15 (83)**#	4 (80)##
Tumor cell extension, n (%)						
Mucosa	104 (79)	51 (80)	53 (79)	11 (48)**	9 (50)#	2 (40)##
Submucosa	0	0	0	2 (9)	2 (11)	0
Muscularis propria	15 (12)	8 (12)	7 (11)	3 (13)	3 (17)	0
Mesoappendix	2 (2)	2 (3)	0	6 (26)***	4 (22)***	2 (40)***
Serosa	9 (7)	4 (6)	5 (7)	5 (22)##	4 (22)##	1 (20)

$p \leq 0.04$, as compared to LAMN-nPMP (total).
$p = 0.006$, as compared to LAMN-nPMP (total).
* $p = 0.004$, as compared to LAMN-nPMP (total).
** $p = 0.001$, as compared to LAMN-nPMP (total).
**# $p = 0.0001$, as compared to LAMN-nPMP (total).
*** $p < 0.00003$, as compared to LAMN-nPMP (total).
& $p = 0.002$ between SIT and SIP.

($p = 0.001$).

3.4. Several noteworthy observations

During the review, we had several observations that are also worth describing here.

One was the concurrent diverticula and diverticulum-like growth of LAMN. The out-of-lumen sac-like protrusion of LAMN through muscular layer of appendiceal wall was commonly seen in as many as a quarter of our cases, and it was similarly frequent in those with and without PMP development. This finding raises a question about whether these diverticulum-like structures were preexisting acquired or congenital diverticula involved by the mucinous neoplasia or they represent the diverticulum-like growth of LAMN, *i.e.*, acquired diverticular out-pouching of the neoplastic mucosa secondary to the increased intraluminal pressure due to the large amount of mucin production of LAMN. We noticed that the diverticulum-like structures were in two forms: one occurred with no underlying muscularis propria defect but only compressed the muscle by the tongue-shaped pushing front of tumor, as shown in Fig. 4-A; the other form occurred at a site where there was a focal abrupt absence of muscularis propria layer, forming a muscle-lacking gap in which there was only fibrous tissue or a penetrating artery passing through, as shown in Fig. 4-B. The latter form, the false and acquired diverticula by definition, was much more common and almost all occurred along the mesenteric border of mesoappendix. The localized muscle-lacking gap was also frequently seen in the part of appendiceal wall without LAMN involvement and without diverticular structure formation (Fig. 4-C/D/E). It was our impression that most of the diverticulum-like growth of LAMN were obviously the result of herniation of the neoplastic mucosa into the muscle-lacking gap site and therefore acquired pseudodiverticula by definition, as shown in Fig. 4-F, and the muscle-lacking defect point represents normal anatomic vascular hiatus of a penetrating artery along the mesenteric border, as previously suggested by some investigators [6-8].

The second observation was regarding how to determine if the presence of mucin deposits on serosal surface represents true transmural passage through adjacent microperforation or artifactural contamination during specimen grossing. In our review, we followed two criteria and determined that it was the true transmural mucin extension if it met one or two of the following criteria: One, if the serosal surface of appendix has been inked before cutting when it was intact and the mucin was covered on the outside by the ink. Two, there was a clear fibrous adhesion between the mucin deposits and serosa/peritoneum surface. Some of these are exampled in Fig. 5.

The third observation was in relation to microperforation, as exampled in Fig. 3, which was identified in nearly 30% of our cases and significantly more common in those with PMP both concurrently and developed later. The sites of microperforation frequently corresponded to the foci of serosal mucin deposits. Microperforations were determined microscopically when there was a focus of severe wall destruction with mucin dissection and with no intact overlying serosa. Some of the microperations appeared to be rupture of the tumor-associated diverticula (Fig. 3-A/B); however, in comparison between the cases with and without diverticula, the frequency of identifying microperforation in those with diverticula was slightly higher (37% *vs* 26%) but the difference was not statistically significant ($p > 0.05$).

4. Discussion

The present study has revealed several macroscopic and microscopic characteristics of LAMN resection specimens that are favorable or unfavorable with respect to increasing risk of PMP development, as summarized in Table 4.

Macroscopically, the cases with PMP development tended to be

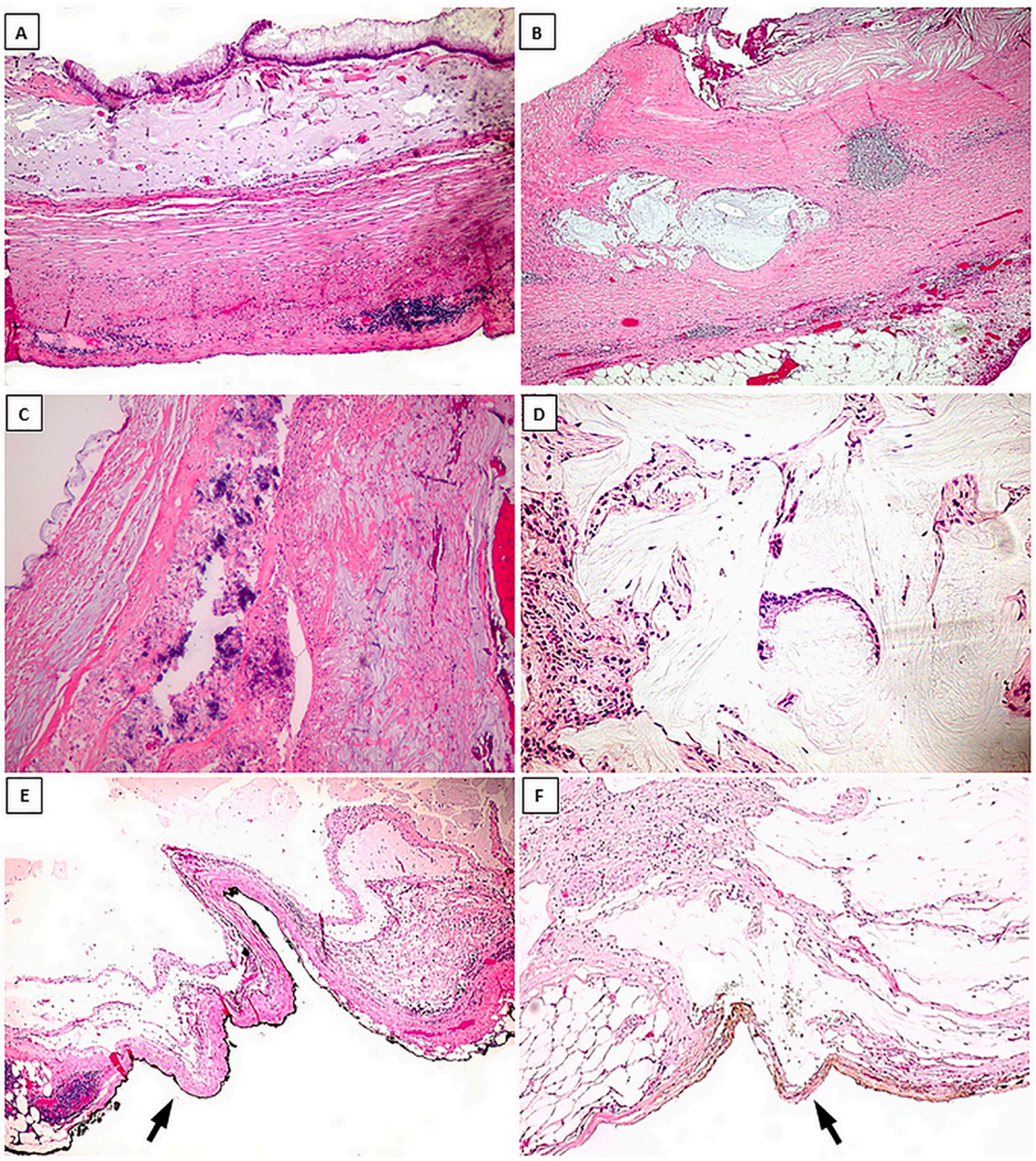

Fig. 2. Microscopic view of LAMN. A. LAMN with mucin dissection into submucosa. B. A part of appendiceal wall with the neoplastic mucosa denuded, while the neoplastic epithelium and mucin are embedded in muscularis propria. C. Mucin dissection through appendiceal wall and into mesoappendix. D. Mucin pool with floating neoplastic epithelium within mesoappendix. E/F. Mucin dissection into appendiceal wall (E) and mesoappendix (F), compressing the connective tissue and forming a thin wall remained on the outer surface.

smaller (< 1 cm) in appendiceal luminal diameter and were more frequent to have mucin aggregations received separate from the appendix/LAMN specimen itself. These interesting findings suggest that the cases of LAMN with higher risk of PMP development actually have a larger amount of mucin production and higher intralumanal pressure resulting in luminal mucin extravasating out of the lumen into peri- and extra-appendiceal tissue earlier and more prevalent, whereas those that did not lead to PMP development had less mucin extravasation and held more luminal contents resulting in a greater luminal diameter. On the other hand, surprisingly, serosal mucin deposits were also seen in 10% of those cases that did not develop PMP but observed in none of those cases that later developed PMP. In other words, the gross observation of serosal mucin deposits does not necessarily indicate risk of PMP. Furthermore, gross perforation was more common in those cases with PMP development than those with no PMP development, yet perforation was still present in 15% of the latter cases. Taken together, the gross findings of serosal mucin deposits and perforation are suggestive of a higher risk of, although not absolutely indicative of, PMP development.

Microscopically, several features are notable. First, the appendiceal wall seemed to be thinner in those cases with no PMP development but thicker in those who developed PMP, although the difference did not reach statistical significance. It was contrary to what we naturally expected. Second, microperforation was significantly more common in those cases with PMP development. These two findings support our aforementioned thoughts regarding the leakage of luminal mucin in these cases. To search for microperforation during microscopic examination, which may be a predictor of future PMP development,

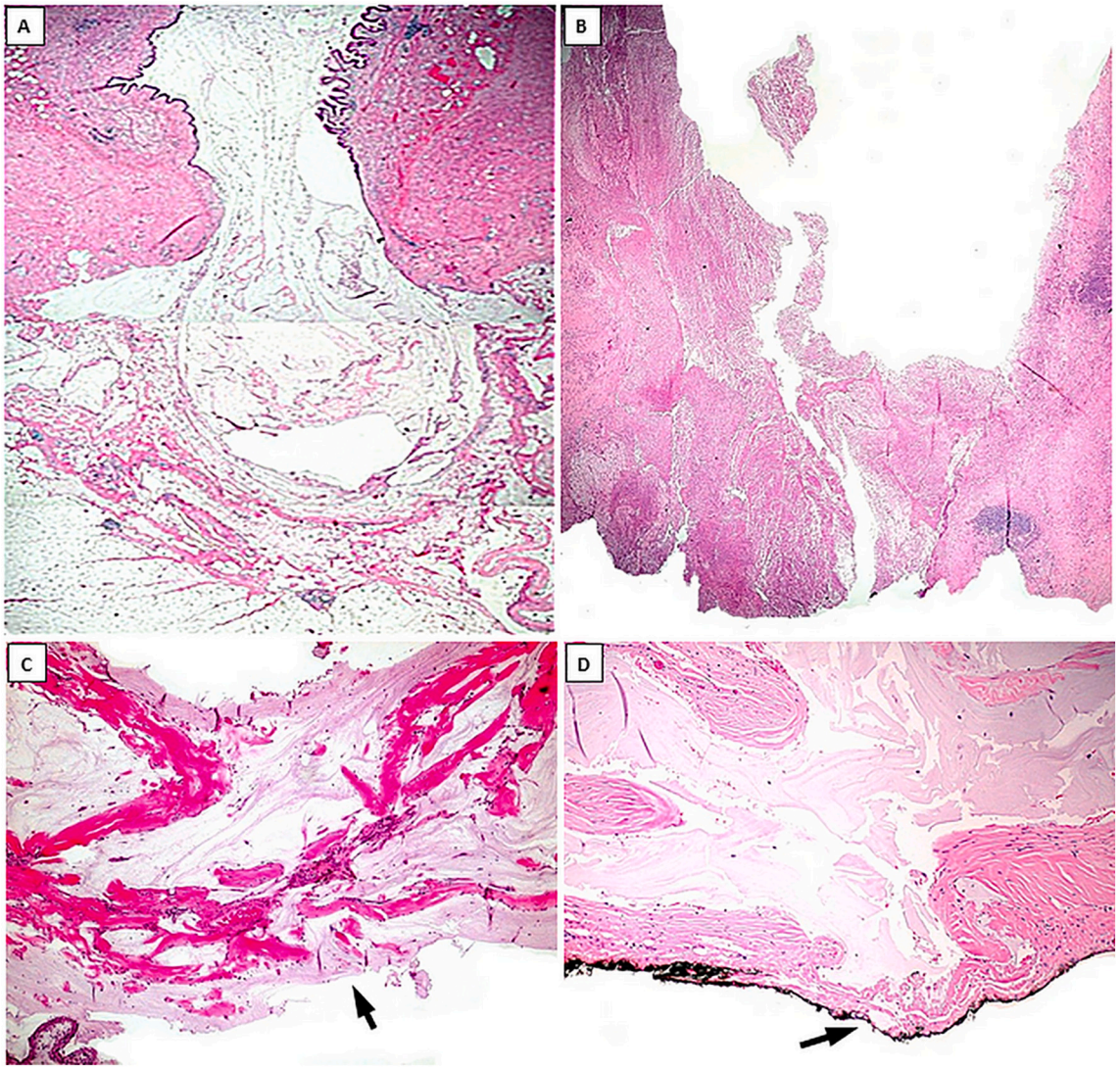

Fig. 3. Microscopic perforation/rupture of LAMN. A/B. Rupture through diverticular structure. C. Microscopic view of a serosal mucin deposit site. D. A tiny opening of the outer surface with mucin being in contiguous to the intramural mucin pool.

should be stressed. To our knowledge, these two significant features have not been described before.

Third, the extent of acellular mucin dissection is important. The presence of microscopic acellular mucin on serosal surface was identified in more than 80% of the cases with either concurrent or subsequent PMP development, which was two times more frequent than those cases without PMP development. This finding is also in line with the second point we made above. On the other hand, none of the cases that later developed PMP had acellular mucin being confined to mucosal layer, and none of the cases with mucin confined to mucosa have developed PMP later.

Fourth, similar to the mucin dissection, the depth of extension of neoplastic epithelial cells through dissection, resulted from displacement secondary to the distention-induced rupture or/and herniation of luminal neoplastic epithelium, either being alone or mixed with mucin extravasation, was associated with PMP development. As compared to those with PMP, most of LAMNs without PMP had neoplastic epithelium being confined to mucosa, and only very rare cases had neoplastic cells reaching mesoappendix. In summary, in those with PMP development, the presence of neoplastic epithelial cells dissecting beyond mucosa layer was significantly more common. Meanwhile, however, it is interesting to note that the frequency in which neoplastic cells dissecting into muscularis propria was similar between the cases with no PMP development and those with concurrent PMP, same as the finding regarding mucin extravasation. In other words, our data demonstrates that when it is confined to muscularis propria, the presence of mucin and neoplastic epithelium/cells are not indicative of a higher staged disease or a higher risk of PMP development. From this point of view, our data supports the latest AJCC staging strategy (the current 8th edition) for LAMN [9], in which the presence of acellular mucin or/and tumor cells in muscularis propria is staged as pTis, with T1 and T2 being no longer applicable to LAMN staging.

Fifth, we have placed much consideration on the histological evaluation of the surgical margin to predict disease recurrence and/or PMP development. Interestingly, of all cases that eventually developed PMP after surgery in our case series, no one had mucin or neoplastic epithelium involving surgical margin, yet a few cases who had mucin and/or tumor epithelium at margin did not develop PMP. Based on this finding, it is apparent that occasional involvement of neoplastic epithelial cells or/and mucin at surgical resection margin does not necessarily lead to LAMN recurrence or subsequent PMP development; on the other hand, clear margin does not absolutely prevent the later development of PMP. Our finding seems to argue against the common surgical practice recommendation [10-12] and is contrast to some previous reports [2]; however, it is in agreement with the finding reported by Misdraji's group in which of 16 LAMN patients with positive

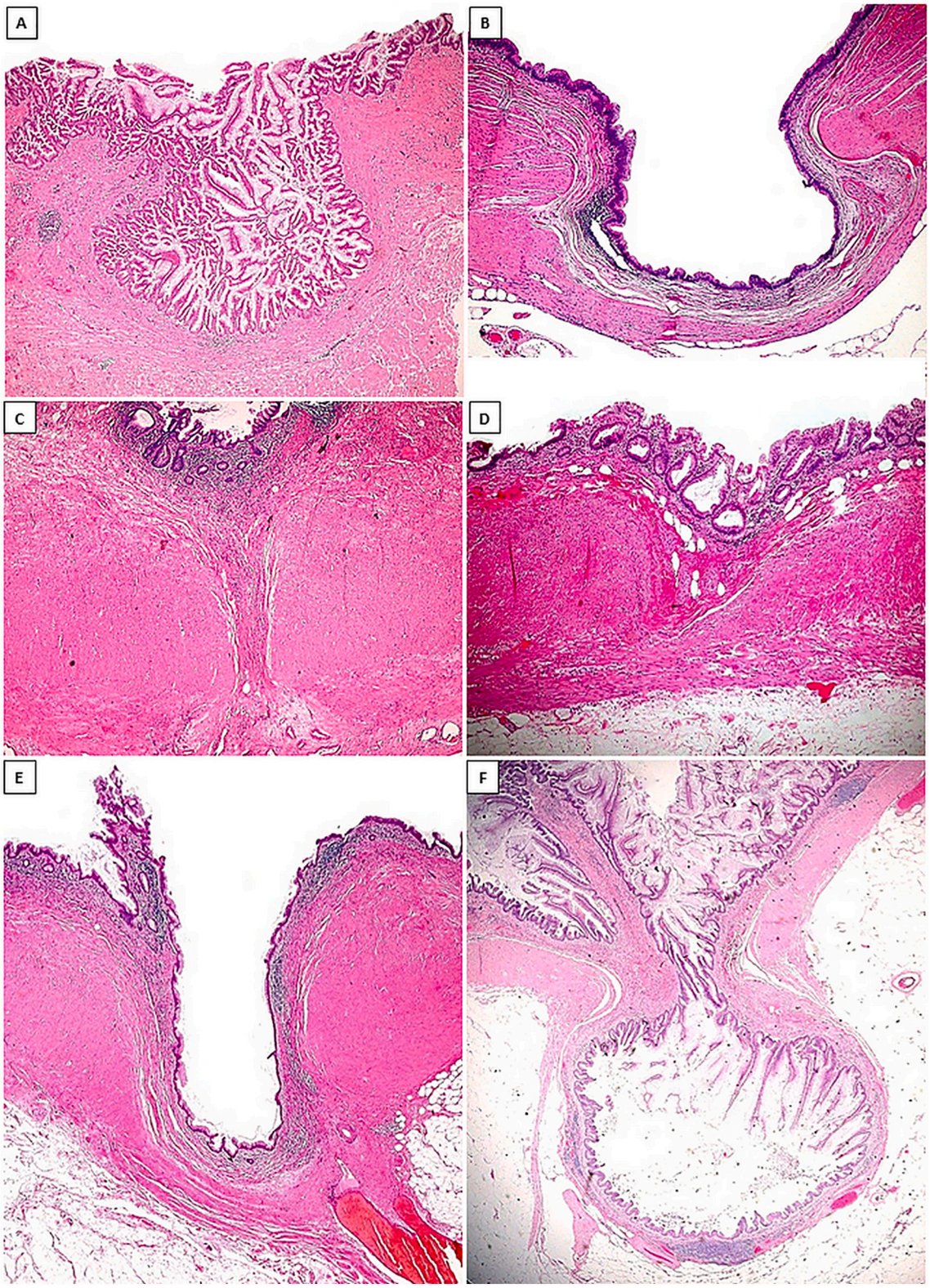

Fig. 4. Diverticulum-like growth of LAMN and diverticulum associated with LAMN. A. Diverticulum-like growth of LAMN, with broad front pushing into the appendiceal muscular wall. B. Neoplastic mucosa herniated through a weak point of appendiceal wall where there is a defect of muscular layer. C. A muscle-free gap in appendiceal wall, with normal overlying appendiceal mucosa. D. A muscle-free gap in appendiceal wall, with overlying appendiceal mucosa showing features of serrated lesion and being invaginated into the muscle defect site. E. Appendiceal diverticulum, with nonneoplastic mucosa herniated through the muscle-free gap of the appendiceal wall. F. A diverticulum lined entirely by LAMN, representing herniation of LAMN through the muscle defect site.

margin involved by neoplastic epithelium or mucin but none of them developed PMP or disease recurrence or was found to have residual tumor on subsequent cecum resection [13].

Lastly, we have again demonstrated the frequent concurrence of diverticula in patients with LAMN. In addition to the rare diverticulum-like pushing fashion of tumor growth, the diverticula were almost

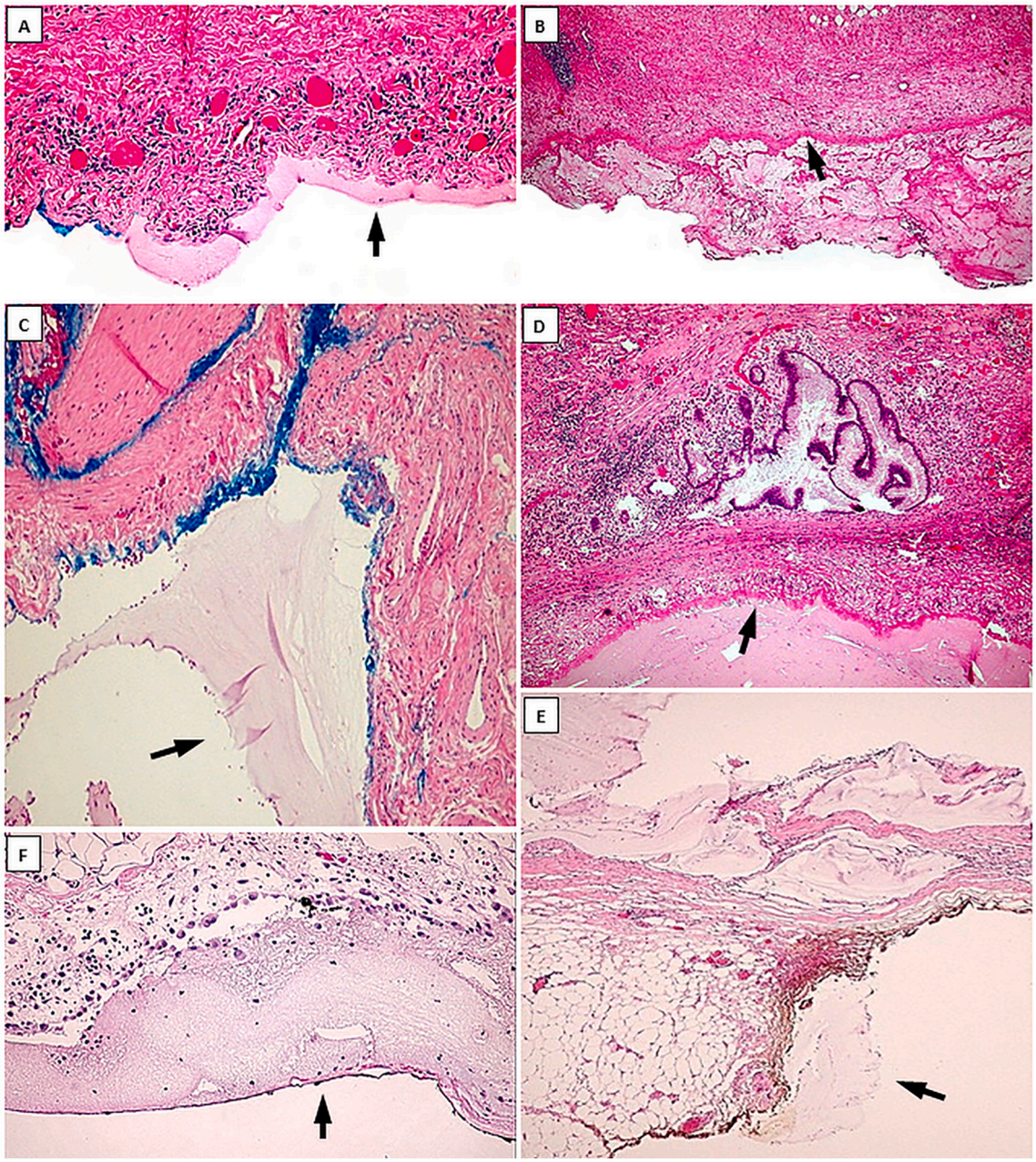

Fig. 5. Examples of true and false serosal mucin deposits. A/B/D. Mucin deposits on serosal surface with underlying tissue reaction (true serosal mucin deposits). C/E. Mucin present on the top of the inked serosal surface (false serosal mucin deposits), representing contaminated mucin during specimen grossing. F. Mucin present beneath the serosal surface ink with underlying foreign body-type giant cell reaction, indicative of true serosal mucin deposit.

Table 4
Favorable and unfavorable features.

Favorable	Unfavorable
No adhesions	Adhesions
No mucin aggregations	Mucin aggregations
No mucin on sectioning	Mucin on sectioning
Larger lumen diameter	Smaller lumen diameter
Mucin/neoplastic epithelium restricted to mucosa, muscularis	Mucin on serosa or neoplastic epithelium extends into mesoappendix/serosa
No perforation	Perforation
Negative pelvic wash cytology	Positive pelvic wash cytology

always the acquired pseudodiverticula that was the result of herniation of appendiceal mucosa through the preexisting muscle-lacking vascular hiatus where a transmural artery penetrating through muscularis propria. These acquired diverticula were present in three forms. One is lined by unremarkable or mildly hyperplastic mucosa and irrelevant to the mucinous neoplasm. The second form shows partial involvement of mucosa by the mucinous neoplasia at the shoulder of diverticulum. The third form was entirely herniation of the neoplastic mucosa, and this form seemed to be most common. These findings are in agreement with quite a few published reports that described a drastically increased prevalence of appendiceal diverticula in appendices harboring neoplasm (up to 33.3%) [14-18], particularly mucinous neoplasm, and similarly increased rate of neoplasia in appendiceal diverticula (up to 43.6%) [17], while in the general population the prevalence of appendiceal diverticular is less than 2% [14]. In our cases the pure complex appendiceal diverticula were excluded by using strict diagnostic criteria of LAMN and the differentiating features recommended by Hsu and Lowes et al. [19,20]. It is our impression that in LAMNs the

neoplastic mucosa is frequently herniated through the physiologic muscular defects in response to the intraluminal hypertension secondary to large amount of mucin production of tumor, and the diverticular portion of the tumor is often the weak point where rupture occurs. The high rate of co-existence of appendiceal diverticula and neoplasm, especially LAMN, may simply be the result of herniation of neoplasm through the appendiceal wall at the anatomic weak point which is otherwise normal and unnoticed.

In order to maximize the prognostic value of macroscopic and microscopic evaluation in the surgical resection specimens of appendiceal mucinous neoplasms, proper grossing and sampling is of pivotal importance. Our recommendation based on the study includes the followings: 1) painting the serosal surface of the distended appendix prior to cutting, 2) inking the site of possible perforation, 3) measuring the luminal diameter, 4) fixing and specimen and contents well before cutting to prevent or minimize mucin spillage which would contaminate the serosal surface of appendix, and 5) submitting the appendix *in toto* and in sequential order from one end to the other, which is more important to the cases without PMP at the time of surgical resection.

Source of support

This study was supported in part by an internal research fund (RS17-605, to the corresponding author) from Calgary Laboratory Services (now a part of Alberta Precision Laboratories).

Declaration of competing interest

All authors declare no conflicts of interest.

Acknowledgement

The authors like to thank Dr. Tak S. Fung, PhD at the Research Computing Services, University of Calgary for his assistance with statistical analysis.

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