Low-grade appendiceal mucinous neoplasm mimicking an adnexal mass

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Abstract
We present a rare case of malignant epithelial neoplasm of the appendix, an uncommon disorder encountered in clinical practice, which poses a variety of diagnostic and therapeutic challenges. We report a particular case in which the appendix was abnormally located in the pelvis, mimicking an adnexal mass. Therefore, it was difficult to make the preoperative diagnosis on clinical examination, imaging studies and laboratory tests and we discovered the lesion during the diagnostic laparoscopy. No lymphadenopathy or mucinous ascites were found. The case was completely handled via the laparoscopic approach keeping the appendix intact during the operation. The frozen section, the detailed histopathology overview as well as multiple immunostaining with a complex panel of markers report diagnosed a low-grade appendiceal mucinous neoplasm (LAMN) with no invasion of the wall. No adjuvant therapy was considered needed. At a one-year follow-up oncological assessment, the patient was free of disease. In women with cystic mass in the right iliac fossa an appendiceal mucocele should be considered in the differential diagnosis. Laparoscopic appendectomy can represent an adequate operation for the appendiceal mucinous neoplasm if the histological report is clear and surgical precautionary measures are taken.

Keywords: low-grade mucinous appendiceal neoplasm, mucocele, laparoscopy.

Introduction
Appendical mucocele is a rare pathological entity that occurs in only 0.1–0.3% of all appendectomies. More than 70% of these tumors are discovered incidentally during laparotomy or laparoscopy. The term is purely descriptive and it characterizes the cystic dilatation of the appendiceal lumen and accumulation of mucin within the lumen [1–4]. This rare entity represents one of the most enigmatic areas in gastrointestinal pathology. The origin of these tumors and the nomenclature has been a source of controversy over the time. Most of the tumors are asymptomatic and found incidentally at laparotomy or laparoscopy performed for other reason. In women with cystic mass in the right iliac fossa, an appendiceal mucocele should be considered in the differential diagnosis [2, 3, 5]. The role of the laparoscopic approach in the management of an appendiceal mucocele, incidentally diagnosed during surgery, is not defined clearly because of the risk of rupture and peritoneal extension of the mucin (pseudo-myxoma peritonei). The risk is higher in laparoscopic surgery due to the greater possibility of inappropriate manipulation. There are few case reports in the literature, in which laparoscopy was accepted as a treatment modality for the appendiceal mucocele [1, 4, 6].

We report an interesting case of a particular low-grade mucinous appendiceal neoplasm manifesting as an adnexal mass, treated successfully by laparoscopy.

Case report
A 61-year-old Caucasian woman was admitted to our department with a history of right lower abdominal pain for the past two months. The pain was moderate in intensity and non-radiating. Her clinical and past history was unremarkable. The blood tests including tumor markers were in normal range. The gynecological exam revealed a firm adnexal mass, treated successfully by laparoscopy. She underwent an exploratory laparoscopy. We performed the operation in the French position, the trocars being placed at the umbilicus (00 videoscope) and on the left and right midclavicular line – at the level of umbilicus (non-traumatic instruments). After exploration of the entire peritoneal cavity, the patient was placed in a Trendelenburg position. Laparoscopically, we identified a mucocele involving the appendix. No mucinuous
ascites or lymphadenopathy was found and both adnexa were normal. The intact base was non-traumatically handled (bowel-holding graspers) and cut between two Roeder’s knots after the mesoappendix was coagulated using a LigaSure device (Figure 3). The specimen was extracted using a non-permeable bag (Figures 4 and 5).

On macroscopic examination, the specimen measured 12/4 cm and the weight was about 20 g. By sectioning it, we noted a thick-walled mucin-filled cystic architecture with a diameter of approximately 3.5 cm. Jelly-like mucoid content was sterile (mucus). The frozen section analysis at the time of surgery indicated a LAMN with a clear appendiceal base.

Postoperative course was unremarkable and she was discharged home on the 3rd postoperative day in good condition. Histopathologically, a low-grade appendiceal mucinous neoplasm (LAMN) with negative margins was confirmed. Figure 6 (a–d) shows that there is no lamina propria and the neoplastic epithelium rests on fibrous stroma, epithelial denudation and lakes of mucin. Immunohistochemical stains were performed on the paraffin-embedded tissue and were positive for CDX2, CK20 and occasionally immunoreactive to Ki67 antibody (Figure 7, a–c). No adjuvant oncological therapy was considered needed. At a one-year follow-up oncological assessment, the patient was free of disease.

Figure 1 – Abdominal US reveals a tubular-cystic mass located on the right of urinary bladder (arrow).

Figure 2 – CT scan shows a cystic mass in the right adnexal area (arrow).

Figure 3 – Laparoscopic view: the lesion was resected without rupturing the tumor during manipulation. The base of the appendix is macroscopically clear.

Figure 4 – The specimen is intactly removed using a protective non-permeable bag.

Figure 5 – Macroscopic examination: after extraction, outflow of yellowish jelly-like mucus.

Figure 6 – LAMN histology, HE staining: (a) There is no lamina propria and the neoplastic epithelium rests on fibrous stroma (×200); (b) Epithelial denudation and lakes of mucin (×100).
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Discussion

Appendiceal mucocele is more frequently met in women over fifty years old [2, 3, 7]. Patient presentation is extremely variable and the symptoms are nonspecific including: moderate abdominal pain, nausea, vomiting, weight loss, changing in bowel habits or symptoms of an acute appendicitis [4, 5, 8]. In about 50% of cases, a palpable mass can be found at clinical exam [2, 5, 8]. The mucocele may mimic an adnexal mass if the appendix is abnormally located in the pelvis, which is commonly diagnosed at a routine gynecological examination. Appendiceal mucocele should be considered by all the gynecologists or general surgeons in the differential diagnosis of a right-sided pelvic mass [2, 3, 8, 9].

The preoperative diagnosis of mucocele is difficult because of its rarity and the non-specific imaging findings. There is no method to determine the diagnosis with certainty preoperatively. The differential diagnosis is made with cystic ovarian tumors, hydrosalpinx, periappendiceal abscess and mesenteric cysts [3–5]. Uncommonly, appendiceal mucinous neoplasm may coexist with other rare lesions, as for example intestinal endometriosis and, therefore, an appendiceal tumor and intestinal endometriosis should be excluded in patients with chronic abdominal pain.
Elevated carcinoembryonic antigen (CEA) levels have been described in neoplastic mucoceles [4, 5, 7]. Depending of the contents and the composition of the mucus, the ultrasound exam shows a well-encapsulated cystic lesion and sometimes the “onion skin pattern”, a condition that can be also found in mucinous cyst of the ovaries [3–5, 7]. Moreover, curvilinear mural calcifications are seen on CT examination in less than 50% of cases [2, 4, 5]. Colonoscopy can show associated colonic tumors, the elevation of the appendiceal orifice and mucous discharge through it. If an appendiceal mucocele is suspected, the endoscopic biopsy should be avoided due to the risk of appendiceal rupture at the time of surgery. Using a barium enema the appendix is filled only partially [1, 2, 5].

Histopathology and immunohistochemistry

According to the histopathological changes to the underlying epithelium of the appendix, the appendiceal mucocele is divided in retention cysts, mucosal hyperplasia, cystadenoma or cystadenocarcinoma [1, 4, 10]. The course and the prognosis of the disease are related to this classification. Based on certain features during histologic exam, the mucocele may be a benign or malignant process caused by epithelial proliferation. Inflammatory (ulcerative colitis) or obstructive (fecalith) causes can be involved in the etiology, but less frequently [10, 11]. Mucinous cystadenocarcinoma is rarely met in practice, cystadenoma being the most frequent occurrence, in both of cases the tumors size is up to 6 cm [4].

Retention cysts, in which there is no evidence of hyperplasia or neoplasia of the mucosa and the simple mucosal hyperplasia represents two subtypes of the appendiceal mucocele, which have no malignant potential [10]. Retention cysts are caused by the obstruction of the appendiceal lumen without any atypia of the mucosa. The mucosa is usually thinner than normal and contains non-branching crypts lined predominantly by mucus-secreting columnar cells. The muscularis mucosa is also thin and can be absent in many places. Absorptive cells are found in the luminal surface epithelium and the upper part of the crypts; a few epithelial endocrine cells are found at the base of the crypts. The mucosa still contains lymphoid follicles, many of them with germinal centers.

In the case of the spontaneous or iatrogenic rupture of this lesion, the mucin is typically confined to the area of right lower quadrant, does not contain any mucinous epithelial cell (acellular mucin) and does not continue to accumulate after appendectomy with mucin extraction [12]. The cysts are usually <2 cm and the recurrence is unusual after removal of extravasated acellular mucin [12, 13].

Cystadenomas are tumors that morphologically resemble adenomas in the colon, with architectural changes in the upper layer of the mucosa and intact muscularis mucosae (minimal dysplastic features). Being a non-invasive tumor, some authors consider cystadenoma to be benign and use this term to describe it [9, 13]. They make the difference between the spontaneous or iatrogenic rupture of a benign lesion of the appendix (“pushing invasion”) with secondary spreading of an acellular mucin, and the spillage of the mucin due to the infiltrative invasion of the appendiceal layers wall with subsequently true pseudomyxoma peritonei. In a dilated appendix, if there is evidence of epithelial dysplasia, papillary infoldings of the epithelium or a multiloculated appearance, the diagnosis should be mucinous cystadenoma at an early stage of development.

Pseudomyxoma peritonei represents a complication characterized by mucinous deposits throughout the peritoneum with multifocal peritoneal epithelial implants, and the appendix represents the primary source of mucin. Nowadays, this term is used to describe only for cases in which epithelial cells are present within the peritoneal mucin [12, 14]. On the other hand, due to the possibility of pushing invasion of the appendix and peritoneal spillage of mucin, Misdraji include cystadenoma in LAMN’s group even if the muscularis mucosae is intact [12].

On gross examination, we diagnosed the lesion under the general descriptive term of appendiceal mucocele. Macroscopically, there is an unremarkable difference between retention cyst, cystadenoma or LAMN, the appendiceal wall being fibrotic with calcification [12]. Microscopically, in LAMN a villous or flat mucinous epithelial proliferation replaces the mucosa [12, 15]. The morphology of the LAMNs can be serrated, villous or undulated but, as compared to the adenomas they rest on fibrous tissue rather than lamina propria, as we present in the Figure 6a [9]. The mucinous epithelial cells contain mucin, are columnar and display low-grade dysplasia. Also, the denudation of the epithelium is frequent in LAMN, as we faced in our case as well (Figure 6, a and b) [12, 16].

Fibrosis and hyalinization are common in the appendiceal wall and the epithelial proliferation grows on this modified tissue or dissects through the appendiceal wall, resembling diverticula, which seems to be a possible way of lesions’ rupture [9, 12, 16]. Due to the fibrosis of the appendiceal wall, it is difficult to discern its layers in order to appreciate the invasion status [17].

The process of developing pseudomyxoma peritonei in LAMN is through “the pushing invasion” process, a term that is more accepted by the pathologist than “the classical rupture” of the organ. In opposition, in invasive mucinous adenocarcinoma the neoplastic cells infiltrate the layers of the appendiceal wall in a conventional manner, beyond the muscularis mucosae, and disseminate to the peritoneal cavity [12, 18, 19]. In adenocarcinoma, pseudomyxoma peritonei is more cellular with greater architectural complexity of the epithelium and high-grade cytological atypia [12]. However, this risk is higher and has worse prognosis for malignant cases because the appendix ruptures more easily and the mucin seeding is more aggressive. Thus, the main difference between LAMN and mucinous adenocarcinoma is the manner of invasion and degree of atypia, adenocarcinoma being used for tumors with either high-grade cytology or destructive invasion [9, 10, 12, 13, 15, 20]. All low-grade mucinous tumors of the appendix with no destructive invasion of the appendiceal wall are included in LAMN [15].

The 7th edition of TNM classification and the 4th edition of World Health Organization (WHO) Classification of Tumors of the Digestive System try to homogenize the terms, proposing a uniform reporting system [9, 11].
They also indicate that LAMN grows slowly and will likely produce the clinical picture of low-grade pseudomyxoma peritonei, in which spread beyond peritoneum or nodal metastasis is rare but nevertheless possible [9, 11]. The high-grade mucinous carcinoma peritonei is caused by the mucinous adenocarcinoma [9].

Appendiceal mucinous neoplasms usually express CK7, CK20, CDX2 or β-catenin, an immunophenotype that is similar to that of other mucinous tumors in lower intestinal tract [9, 12, 21]. CK20 is principally expressed in epithelial cells of the gastrointestinal tract, as we encountered in our case as well. We noted also a CDX2-positive reaction in glandular epithelium, with strong and uniform staining, thus being a common occurrence in appendiceal mucinous lesions. The proportion of CK7-positive cells in mucinous neoplasm of the appendix is higher than in the colorectal ones but significantly lower than the ovary, pancreatic or upper gastrointestinal mucinous tumors [19, 21, 22]. MUC2 represents a universal molecular marker for appendiceal mucinous tumors and pseudomyxoma peritonei [12, 20–22]. MUC5A is positive in more than 80% of mucinous appendiceal neoplasms [22]. Its overexpression is associated with an intestinal origin for pseudomyxoma peritonei, especially if the appendiceal primary location was documented [9, 19, 20]. Leptin, MUC2, MUC5A, mTOR and ERK are more frequently immunopositive in mucinous adenocarcinomas as compared with LAMN and are involved in mucin-producing carcinogenesis; MUC-1 is negative in adenomas [12, 20]. We noted Ki67 “hot spot” area of the glandular epithelium, this antigen being used in order to predict the malignant degree and the biological behavior of the appendiceal mucinous lesion. The frequency of Ki67 antigen expression is higher in mucinous appendiceal adenocarcinoma as compared to adenoma and this occurrence can suggest appendiceal mucin tumor proliferation activity. Furthermore, this expression may differentiate adenocarcinoma from LAMN and from mucinous adenoma. On the other hand, in comparison with the mucinous colorectal adenocarcinoma, the appendiceal ones showed lower grade of Ki67 expression [17].

By analyzing the immunochemistry for 24 markers divided in oncogenic, tumor suppressors and mucin proteins, Yoon et al. tried to identify differential immunoprofiling profiles of proteins in mucinous adenoma, LAMN and mucinous adenocarcinoma [17]. They establish a panel of nine markers-status to be representative for appendiceal mucinous adenocarcinoma: cyclin D1 positive, Ki67 high index, NF-κB positive, VEGF positive, E-cadherin loss, p53 overexpression, β-catenin loss, MUC2 positive, and MUC5A positive (more than 80%).

The alteration rates of β-catenin, cyclin D1, Ki67, E-cadherin, MUC2, and MUC5AC in LAMN approached those in mucinous adenoma, whereas frequencies of NF-κB, VEGF and p53 were comparable to those in mucinous adenocarcinoma. The authors demonstrated the statistically significance for the mean number of altered markers in mucinous adenoma, LAMN and mucinous adenocarcinoma, respectively 1.4, 2.6 and 5.5 [17]. In mucinous adenocarcinoma, p53 overexpression was related with shorter periods of disease-free survival and overall survival of patients. Apart from this, more than five altered markers and NF-κB positivity or β-catenin loss were associated with shorter periods of disease-free survival or overall survival [17].

**The relationship between histopathology, treatment and follow-up**

The therapy for appendiceal mucocele is fundamentally surgical and is connected with the histopathology report. The main purpose is to achieve negative margins, therefore, right hemicolectomy must be the therapy of choice only for tumors located at the base of the appendix. For the other tumors, even for mucinous adenocarcinoma, appendectomy can be an adequate operation if margins are clear and if there is no evidence of epithelial implants in the peritoneum (tumor confined to the appendix). Therefore, in order to demonstrate that margins are free of disease, frozen sections are mandatory [1, 2, 8, 11, 22].

If there is no mucin or neoplastic epithelium in the appendiceal base or per-appendiceal tissue (the disease is limited to the appendiceal lumen), laparoscopic appendectomy can represent an adequate operation for LAMN for experienced surgeons [1, 2, 8, 11]. This type of LAMN is confined to the appendix; therefore, the treatment is similar to adenomas. If there is no macroscopic mucinous fluid, cytology could be necessary to confirm the presence of the epithelial cells. Recent studies have revealed that in LAMN, right hemicolectomy provides no survival advantage (even those that have ruptured) and appendectomy or cecectomy with a negative margin is preferable [11, 12, 23, 24]. The laparoscopic approach involves precautionary measures to be taken in order to avoid rupture in the peritoneal cavity and dissemination of mucin [2–4, 11]. Conversion to the open surgery is recommended if the lesion must be grasped or if the tumor clearly extends beyond the appendix [2, 4, 7, 8, 12]. The surgeon must grasp the mesoappendix, the clear base or the cecum (“the macroscopically healthy tissue”). During the resection, the surgeon should search for any macroscopic mucinous deposits, especially in the right retrohepatic and left paracolic spaces, pelvis and omentum [2, 7, 11, 25]. All peritoneal implants should be removed and examined in order to detect any epithelial atypia. Patients with peritoneal deposits should undergo surgical debulking and heated intraperitoneal chemotherapy in selected cases [8, 20, 25, 26].

Due to association with neoplasms in other locations and the later risk of pseudomyxoma peritonei it seems reasonable to follow it up every six months for first two years after surgery and then yearly [2]. The risk of developing an adenocarcinoma of the colon is six times higher in patients with mucocele than in the general population [26]. Despite this, long-term follow up is mandatory in these patients, a recurrence at six-year after complete removal of a non-perforated mucocele being reported [4, 27].

**Conclusions**

We recommend that if an appendiceal mucocele (misdiagnosed as an adnexal mass in preop) is found incidentally by the gynecologist during laparoscopy, the surgical intervention should be continued by a surgeon with experience in minimally-invasive surgery, due to his
ability to safely handle viscera and adequately assess the peritoneal cavity for lesion’s extent and other seedings. Although LAMN has a low-grade malignant behavior, therefore complete excision and follow-up is advised. Use of the term mucocele, although widespread, does not reflect the neoplastic nature of the lesion. There is a need for a consensus between the pathologists and the clinicians on these terms and further studies should clarify this aspect. Immunohistochemistry is helpful especially in demonstrating the intestinal origin of the cells (CDX2, CK7, CK20, MUC2 and MUC5A positivity) and their low rate of proliferation (a low overall Ki67 index).

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

Author contribution
All authors contributed equally to the manuscript.

References

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