



Published in final edited form as:

*Am J Surg Pathol.* 2020 December ; 44(12): 1649–1657. doi:10.1097/PAS.0000000000001603.

## MURAL INTRACHOLECYSTIC NEOPLASMS ARISING IN ADENOMYOMATOUS NODULES OF THE GALLBLADDER: AN ANALYSIS OF 19 EXAMPLES OF A CLINICOPATHOLOGICALLY DISTINCT ENTITY

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COI: The authors have no conflict of interest to declare.

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## Abstract

Intracholecystic neoplasms (pyloric gland adenomas and intracholecystic papillary neoplasms, collectively also called intracholecystic papillary/tubular neoplasms) form multifocal, extensive proliferations on the gallbladder mucosa and have a high propensity for invasion (> 50%). In this study, 19 examples of a poorly characterized phenomenon, mural papillary mucinous lesions that arise in adenomyomatous nodules and form localized intracholecystic neoplasms, were analyzed. Two of these were identified in 1750 consecutive cholecystectomies reviewed specifically for this purpose placing its incidence at 0.1%. Median age was 68 years. Unlike other gallbladder lesions, these were slightly more common in men (F/M=0.8) and 55% had documented cholelithiasis. All were characterized by a compact multilocular, demarcated, cystic lesion with papillary proliferations and mucinous epithelial lining. The lesions' architecture, distribution, location, and typical size were suggestive of evolution from an underlying adenomyomatous nodule. All had gastric/endocervical-like mucinous epithelium, but five also had a focal intestinal-like epithelium. Cytologic atypia was graded as 1 – 3 and defined as 1A: mucinous, without cytoarchitectural atypia (n=3), 1B: mild (n=7), 2: moderate (n=2), and as 3: severe atypia (n=7, 3 of which also had invasive carcinoma, 16%). Background gallbladder mucosal involvement was absent in all but two cases, both of which had multifocal papillary mucosal nodules. In conclusion, these cases highlight a distinct clinicopathologic entity; i.e. mural intracholecystic neoplasms arising in adenomyomatous nodules, which, by essentially sparing the “main” mucosa, not displaying “field-effect/defect” phenomenon, and only rarely (16%) showing carcinomatous transformation, are analogous to branch duct-type IPMNs.

## Keywords

Gallbladder; intracholecystic neoplasm; adenomyomatous nodule; dysplasia; IPMN; papillary; mucinous

## INTRODUCTION

Intraductal preinvasive mass-forming (tumoral intraepithelial) neoplasms are increasingly being characterized in the biliary tract and pancreas. Various entities have been recognized under this umbrella including pancreatic intraductal papillary mucinous neoplasms (also encompassing the group designated as pyloric gland adenomas), biliary intraductal papillary neoplasms, pancreatic/biliary intraductal tubulopapillary neoplasms, pancreatic/biliary intraductal oncocytic papillary neoplasms, and intraampullary papillary tubular neoplasms (1-7).

More recently, the gallbladder counterparts of these tumors (which for the purposes of this article will be referred to generically as intracholecystic neoplasms), have also attracted significant attention. In the 2019 WHO classification, two distinct forms of intracholecystic neoplasms are recognized, intracholecystic papillary neoplasms (ICPNs) (8) and pyloric gland adenomas (9). These have also been collectively referred as “intracholecystic papillary

tubular neoplasm” in some publications, (10-13) a term that embraces all tumoral intraepithelial neoplasms arising in this organ. Fundamentally, these lesions are collectively defined and characterized by polypoid/papillary dysplastic neoplasms that are, by default, growing on the (main) mucosal surface of the gallbladder and showing multifocal or diffuse growth. These lesions also have a high-rate of carcinomatous transformation and often show at least focal high-grade dysplasia, with > 50% having an associated invasive carcinoma (although a subset of these have proven to be more invasion resistant (14). Emerging evidence also indicates that intracholecystic neoplasms connote field-effect/field-defect risk for the biliary tract (15-18), with a not trivial number of such patients developing biliary cancers in long term follow-up.

An adenomyomatous nodule (also called adenomyoma or adenomyomatous hyperplasia) is a distinct localized lesion of the gallbladder wall characterized by a demarcated nodule composed of large cystic glands typically forming a 1-1.5 cm mural nodule in the fundus with variable amounts of muscle participation. It is a non-neoplastic and non-hyperplastic process that appears to be malformative in nature (19). It is distinct from Rokitansky-Aschoff sinuses, which are epithelial diverticula that occur secondary to injury, are typically multifocal, occur throughout the gallbladder. By virtue of their architecture and distribution, Rokitansky-Aschoff sinuses are different than adenomyomatous nodules. Although involvement of Rokitansky-Aschoff sinuses by dysplastic processes has been documented, is common, and in fact, is an adverse prognostic sign in early gallbladder carcinomas (GBCs) (20), dysplastic/carcinomatous transformation in adenomyomatous nodules appears to be far less common (19, 21, 22). The prevailing impression in the literature is that adenomyomatous nodules seldom show neoplastic change although recently some studies claim that a significant proportion of GBCs (26%) may in fact arise from adenomyomatous nodules (23). The true association of adenomyomatous nodules with neoplastic change remains to be determined.

Formation of tumoral intraepithelial neoplasms akin to the intracholecystic neoplasms (of the main gallbladder mucosa) in adenomyomatous nodules as a distinct mural nodule on the gallbladder wall has been documented in only a limited fashion. In 1995, Lauwers et al. reported a case of this phenomenon under the heading of “papillary mucinous adenoma arising in adenomyomatous hyperplasia of the gallbladder” (24). Similar cases have been presented in an abstract as IPMN-like mucin-producing papillary lesions of the gallbladder (gallbladder intramural papillary mucinous neoplasm, “GB-IPMN”) (25) but without any specific reference to their association with adenomyomatous nodules. In another study by Albores-Saavedra et al. (26), papillary proliferations have been described in two cases with in situ carcinoma arising in adenomyomatous hyperplasia. The case reported by Muranushi et al. (27) as “intracholecystic papillary neoplasm resembling a submucosal tumor” also appears to be an example of this entity without specific reference to its origin in adenomyomatous nodule. The clinicopathologic characteristics of this process have yet to be established.

In this study, we analyzed the clinicopathologic features of this peculiar entity characterized by IPMN-like papillary mucinous proliferations developing in adenomyomatous nodules and forming intracholecystic mural neoplasms.

## MATERIALS AND METHODS

### Definitions

For the purposes of this article, in order to simplify the presentation and discussions, the term intracholecystic neoplasm was chosen to refer to all tumoral intraepithelial neoplasms occurring in the gallbladder mucosa including the entities recognized as intracholecystic papillary neoplasms (8) and pyloric gland adenoma (9) in the WHO-2019, which are also referred to collectively as intracholecystic papillary tubular neoplasms in other publications (10-13).

The study cohort was defined as follows: A well-defined demarcated mural lesion composed of cystically dilated glands filled with variable amounts of papillary proliferations lined by mucinous and/or overtly dysplastic epithelium and with the underlying structure showing all the defining features of adenomyomatous nodule. These cases were regarded as adenomyomatous nodule-associated intracholecystic neoplasm (AM-ICN). In qualifying a case for inclusion in this category, a purist's approach was employed and only the cases with florid papillary architecture (Figures 1-5), mucinous lining (Figures 1-3) and/or overtly dysplastic epithelium (Figure 4) qualified for inclusion. In essence, the morphologic criteria utilized in the remainder of the pancreatobiliary tract were used.

Excluded from analysis were intracholecystic neoplasms of the main gallbladder mucosa or flat (non-tumoral) mucosal dysplasia which is typically extensive and often extends into Rokitsky-Aschoff sinuses. AM-ICNs were distinguished especially by the overall architecture of the lesion being that of an adenomyomatous nodule and their localized nature among other characteristics.

### Case Identification

1750 consecutive cholecystectomy specimens were grossed prospectively by the authors themselves. Separately, 203 gallbladders were submitted entirely for microscopic examination and evaluated by the authors. Additionally, 2929 archival cholecystectomy specimens (including 582 with neoplasms) from the authors' institutional and consultation files were retrieved and reviewed. A search of the pathology departments' laboratory information system was also performed to identify gallbladders with a diagnosis of "adenomyoma", "adenomyomatous hyperplasia" or "adenomyomatous nodule".

Through these analyses, 19 examples of this entity were identified, along with 191 adenomyomatous nodules and 123 intracholecystic neoplasms of the main gallbladder mucosa.

### Clinical Data and Comparative Analysis with Other Gallbladder Pathologies

Clinical information was obtained from the patients' charts or from their primary physicians. The clinical characteristics and pathologic findings were compared with those of 191 gallbladder adenomyomatous nodules without papillary mucinous neoplasia on which adequate information was available, 459 ordinary GBCs, 1190 non-neoplastic

cholecystectomies (Table 1), and 123 intracholecystic neoplasms, which were previously published (10) (Table 2).

### Histopathological Analysis

All available material on these 19 cases was reviewed. A mean of 6.3 slides per case (range, 1-20) were available for histopathological examination. The type of epithelial lining or cell lineage of the AM-ICN was assessed morphologically and classified as gastric foveolar-type, intestinal-type, or biliary-type as was originally described for pancreatic IPMNs (28, 29) and now adopted for the entire pancreatobiliary tract. Dysplasia was graded per the 3-tiered grading system previously used for biliary intraepithelial neoplasia (BilIN) and for pancreatic intraepithelial neoplasia (PanIN) (30, 31): 1A (mucinous epithelium with minimal/no atypia), 1B (mild atypia), 2 moderate atypia (convincing dysplasia); 3 high-grade dysplasia (HGD). A three-tiered classification system was chosen instead of the two-tiered, in order to better capture and analyze the spectrum of histologic changes. However, the results were also presented according to the two-tiered system used in some studies for intracholecystic neoplasms of the gallbladder (10), and for BilIN and PanIN (32-34). The gallbladder surface mucosa adjacent to the lesion, as well as away from the lesion, was also carefully evaluated for metaplastic and dysplastic changes. The background gallbladder was also assessed for cholecystitis and other pathologic changes.

Follicular cholecystitis was defined as greater than or equal to three lymphoid follicles per centimeter of gallbladder mucosa (35). Any distinct and relatively round cluster of lymphoid cells was regarded as a follicle (with or without germinal center formation).

### Immunohistochemical Analysis

Blocks from only 3 cases were available to the authors. Additional blocks could not be obtained despite repeated attempts. Nevertheless, in order to glean as much information as possible, immunohistochemical analysis was performed with cell lineage markers that are differentially expressed in different components of the gastrointestinal tract and have been used for classification of other pancreatobiliary lesions. These were (MUC1 (*Clone Ma695, 1:160; Novocastra*), MUC2 (*Ccp58, 1:100; Novocastra*), MUC5AC (*CLH2, 1:200; Leica*), MUC6 (*CLH5, 1:80; Leica*), and CDX-2 (*CDX2-88, 1:200; Biogenex*).

## RESULTS

### Clinical Findings

In total, 19 cases were identified. Two of these were identified among 1750 consecutive cholecystectomy specimens specifically processed and examined grossly by the authors for this specific purpose, placing the incidence of this lesion at 0.1%.

Detailed clinical information was available for 18/19 patients. The majority of cases occurred in older patients (median age, 68 years; range, 47 - 85), slightly older than the patients with intracholecystic neoplasms (median age, 62 years) and ordinary gallbladder adenocarcinomas (median age, 64 years); a decade older than patients with adenomyomatous nodules without papillary proliferations (median age, 56 years); and much

older than the conventional cholecystitis cohort (median age, 50 years) (Table 1). Unlike all other gallbladder pathologies, which are significantly more common in women, AM-ICN did not show any female predominance (female:male ratio, 0.8) (Table 1). Follow-up information was available in three patients with invasive carcinoma. The median follow-up time was 64±29 months (range 22-78 months), and one patient died of disease within 22 months, while the other two were still alive 5 years after cholecystectomy.

### Macroscopic findings

The cases were characterized by demarcated cystic nodular tumors occurring on the wall of the gallbladder (Figure 1) with preservation of the surface (main) mucosa. The mean size was 1.8 cm (median, 1.75 cm, range, 0.8 - 3.5 cm). In all the cases in which the location was specified, the lesion was localized in the fundus. All cases had a cystic component with a mean largest cyst diameter of 0.9 cm (median, 0.8 cm, range, 0.3 - 2.2 cm). The presence of gallstones was documented in 11 cases and cholelithiasis was present in 55%.

### Microscopic Findings

By definition, all cases formed a mural lesion containing variable amounts of papillary structures and cystic elements (Figure 2). The location and the findings were indicative that these lesions arose within an adenomyomatous nodule (Figures 1 and 2). Papillae ranged from short to well-defined and elongated. In some cases, florid papillary nodules filled the cyst lumen (Figures 1-3), and in two cases, the papillae partially projected into the gallbladder lumen (Figure 1). In fifteen cases, the lining of the cyst in the adenomyomatous nodule was predominantly composed of gastric/endocervical-like mucinous epithelium (Figure 3), some with pyloric gland features; seven cases also revealed foci of biliary-type epithelium, and five cases revealed foci of intestinal-type epithelium (Figure 4). Three cases had grade 1A dysplasia, seven had grade 1B, two had grade 2, and four had grade 3 (Figure 4). Using the recently adopted two-tiered system used for PanINs (32) and intracholecystic neoplasms (10), twelve cases had low-grade dysplasia, and four had high-grade dysplasia (also called “carcinoma in-situ” in some regions). Another three cases had both high-grade dysplasia and a component of invasive carcinoma (Figure 5 and 6).

The size of the invasive component in the three invasive carcinomas was 0.2 cm, 0.8 cm, and 2.2 cm. All invasive components were ordinary gallbladder adenocarcinomas and were confined to the gallbladder (stage T2 or lower). None of the cases showed neuroendocrine, squamous or other differentiation, which is slightly more frequent in intracholecystic neoplasm associated GBCs (10) than in GBCs arising without an accompanying intracholecystic neoplasm.

The lesion was confined entirely to the adenomyomatous nodule (within the wall of the gallbladder) without any surface mucosal involvement in 16 of 19 cases. In the two cases with florid papillary nodule in the adenomyomatous nodule, the papillary/polypoid growth also protruded from the adenomyomatous nodule onto the gallbladder mucosa (Figure 1); however, there was no pagetoid spread of dysplastic cells to the adjacent mucosa, and the mucosa away from this region was also free of dysplasia. One case showed high-grade dysplasia in the immediately adjacent mucosa but not away from the lesion. In nine cases,

the surface mucosa of the gallbladder was unremarkable, and in the remaining seven there were some signs of injury.

Follicular cholecystitis was present in 26% of AM-ICN cases. Follicular cholecystitis was less frequent in cases of adenomyomatous nodule without AM-ICN (16%). Follicular cholecystitis was present in 2.8% of ordinary GBCs without associated adenomyoma and in 2.8% of non-neoplastic cholecystectomies (36) (Table 1). Follicular cholecystitis was also less common in ICNs (11%, Table 2). Although there was stromal cellularity in some cases, it did not show the characteristics of ovarian-type stroma of mucinous cystic neoplasms.

### Immunohistochemical Findings

In the limited immunohistochemical analysis that could be performed (due to unavailability of tissue), the two cases with only gastric epithelium were positive for MUC5AC and MUC6 but negative for MUC2. The case with mixed gastric, biliary, and intestinal epithelium was positive for MUC5AC, MUC6, and MUC2, but negative for CDX2.

## DISCUSSION

In this study, clinicopathologic features of a distinctive entity in the gallbladder, mural intracholecystic neoplasms arising in adenomyomatous nodules, are elucidated. This entity is characterized by papillary proliferations of variably dysplastic mucinous epithelium within a compact zone of aggregated cystically dilated ducts on the gallbladder wall forming a distinct mural nodule while mostly preserving the surface mucosa. As such, it is somewhat analogous to the branch-duct IPMNs of the pancreas. An example of this phenomenon seems to have been recognized first by Lauwers et al. in a case reported under the heading of “papillary mucinous adenoma arising in adenomyomatous hyperplasia of the gallbladder” (24). Later on, in an abstract, Nagata et al. described nine cases of “gallbladder intramural papillary mucinous neoplasm (GB-IPMN)” (25), which we believe are a very good way to view and describe these cases. The papillary proliferations which have been described by Albores-Saavedra et al. (26) in two cases with in situ carcinoma arising in adenomyomatous hyperplasia and the case reported by Muranushi et al. (27) as “intracholecystic papillary neoplasm resembling a submucosal tumor” also appear to be an example of this phenomenon. Sato et al. recently reported a case of what they classified as ICPN arising in a Rokitansky-Aschoff sinus of the gallbladder associated with a mucinous adenocarcinoma. This case may also be closely related to the lesions described here (37), although it is difficult to ascertain with certainty based on our review.

Adenomyomatous nodules are generally an incidental finding in cholecystectomies. The prevailing impression has been that it is an insignificant finding (21, 22) which only very rarely and incidentally shows dysplasia (24, 25, 38-41). However, recently, some observers have claimed adenomyomatous nodule to be more significantly associated with neoplastic transformation. Ootani et al. reviewed 3197 consecutive cholecystectomies and found that GBCs arose in 6.4% of segmental adenomyomatous nodule versus 3.1% of those without segmental adenomyomatous nodule (39). More significantly, Kai et al. recently claimed that 26% of GBCs arise in association with adenomyomatous nodules (23). Thus, the association of adenomyomatous nodules with neoplastic change remains a controversial issue. This

study illustrates that adenomyomatous nodules can form tumoral intraepithelial neoplasia (10, 42) leading to IPMN-like mural neoplasms, albeit relatively infrequently. We estimate the incidence of AM-ICN to be 0.1% in cholecystectomies since we identified only two examples in our cohort of 1750 consecutive cholecystectomies we sampled, specifically focusing on adenomyomatous nodules. In other words, this is a relatively rare occurrence. Considering there are 300,000 cholecystectomies in the US, it is expected that there will be 300 such cases annually in this country.

AM-ICNs analyzed here delineate a distinct entity, and some of its characteristics that were elucidated in this study bring new perspectives to the etiopathogenesis of GBCs in general. First, the mean age at which they are encountered is the upper 60s (a decade older than the typical cholecystectomy cohort, and slightly older than ordinary GBCs), which is similar to that of intracholecystic neoplasms (10) and intraductal neoplasms of the pancreatobiliary tract (2, 3, 43, 44). This suggests that aging may play a role in its pathogenesis as has been speculated for branch-duct IPMNs. The occurrence of follicular cholecystitis in 26% of AM-ICN cases (otherwise seen in <3% of cholecystectomies), an “elderly” disorder (speculated to result from immune derangement), is also noteworthy (35).

Additionally, while virtually all types of neoplastic gallbladder lesions are significantly more common in women (with a female/male ratio typically above 2), AM-ICNs appear to be at least as common in men, further highlighting their analogy to pancreatic IPMNs. It is believed that the preponderance of ordinary GBCs in women is beyond the association with gallstones and has something to do with other yet undiscovered carcinogenetic factors occurring more readily in women than men. This, however, does not seem to be valid for AM-ICNs as they are as common (if not more) in men. Of note, similar to branch duct-type pancreatic IPMNs, AM-ICNs are also typically of gastric-type and seldom show intestinal features. Also, the frequency of invasive carcinoma, 16%, is almost identical to that reported in branch-duct IPMNs (45, 46).

The dysplastic (neoplastic) process in these tumors is almost exclusively confined to the adenomyomatous nodule. This brings an interesting twist to gallbladder carcinogenesis, where most cancers otherwise appear to occur as a result of the intraluminal milieu alterations, involve the mucosa extensively, and/or are multifocal. Most GBCs are attributed to the irritant effects of gallstones, although for a smaller proportion (about 5%), other factors such as reflux of pancreatic enzymes into the gallbladder secondary to pancreatobiliary maljunction, also a mucosal-wide chemical exposure, have been implicated (15, 16). Regardless of the etiologic factor, cancers arising in these settings are commonly associated with widespread mucosal abnormalities including dysplasia or other background epithelial alterations (16), not only the entire gallbladder mucosa itself, but also the remainder of the biliary tract mucosa (47). All of these are also valid for surface intracholecystic neoplasms, which tend to be extensive and multifocal and exhibit field effect/defect in the biliary tract. In contrast, in the case of AM-ICN, there is no detectable mucosal dysplasia outside the adenomyomatous nodule, indicating that triggering factors leading to AM-ICN are confined to the adenomyomatous nodule itself, and do not show the field-effect/field-defect phenomenon that has been demonstrated in surface intracholecystic neoplasms. This difference also pertains to the importance of distinguishing AM-ICNs from

surface intracholecystic neoplasms. For the latter, extensive sampling of uninvolved mucosa is a must, and the patient needs to be investigated and followed for other lesions in the surrounding biliary tract, whereas extensive sampling of uninvolved mucosa and follow up does not seem to be indicated for AM-ICNs. This distinction further emphasizes the analogy that AM-ICNs are like the gallbladder counterpart of branch-duct IPMN of the pancreas, which are more innocuous, typically of gastric phenotype and progress to invasive carcinoma in only 15% of cases (45, 46). Whereas surface intracholecystic neoplasms are like main-duct counterpart of pancreatic IPMNs (or IPNBs), which tend to be much more complex and show high propensity for invasion (> 50%) and dissemination. Of note, it should be emphasized here that surface intracholecystic neoplasms and high-grade dysplasia of gallbladder commonly involve Rokitansky-Aschoff sinuses, and taken in isolation microscopically, there may be similarities. However, these can be distinguished from AM-ICNs by their localization, pattern, distribution and architecture (19, 20). This can be likened to a main duct-type IPMN spreading in a pagetoid manner to small branch ducts versus a true branch duct-type IPMN.

The recognition of AM-ICN also raises the question of whether an adenomyomatous nodule may in fact prove to be the culprit of more GBCs than we currently appreciate (as recently suggested by Kai et al. (23)), and whether with more careful sampling of the GBCs we may in fact find higher frequency of association of adenomyomatous nodule with GBCs. After all, most GBCs arise in the fundus, and virtually all adenomyomatous nodules are also in the fundus. Although AM-ICN appears to be a relatively uncommon occurrence (estimated to be in 0.1% of gallbladders, and some 300 cases in the US annually), nevertheless, it necessitates a more careful scrutiny of adenomyomatous nodules than currently afforded. If nothing else, adenomyomatous nodules certainly ought to be searched for, and examined carefully microscopically in a cholecystectomy.

It should also be noted here that, as is the case with IPMN-associated invasive carcinomas, staging of AM-ICN associated invasion, if present, may require more critical examination and nuance. Considering that the invasion, if present, is on the wall of adenomyomatous nodule, it is by default stage pT2 even if minute in size. In the gallbladder, 5-year survival of T2 GBCs is 45% (Manuscript submitted, under revision) although small superficial tumors may in fact have a very good prognosis (48) provided that they are not very close to the external surfaces (49). At the same time, it has been documented that when dysplasia/carcinoma involves Rokitansky-Aschoff sinuses, it appears to somehow confer more aggressive behavior even into mucosal spreading carcinomas (20, 50). Hypothetically, this may also be applicable to AM-ICNs. In this study, 3 of the invasive cases had to be classified as T2 by default, two were alive after 5 years and one died within 22 months. This is of course too limited to drive any reliable conclusion. Nevertheless, while these issues are further clarified, in an invasive AM-ICN, it is advisable to record the size of the carcinoma as well as its distance from the serosal/hepatic surface, which may have to be considered in the prognostic evaluation and management of these patients. Aggressive adjuvant therapy is offered to most T2 GBCs; however, aggressive therapy becomes a concern for patients with co-morbidities. For the minute invasive carcinomas discovered in AM-ICNs, treatment plan may have to be determined on a case-by-case basis.

In summary, AM-ICN is a distinctive type of tumoral intraepithelial neoplasm occurring in the gallbladder that is akin to branch-duct IPMNs of the pancreas as they both occur in elder patients, are localized (and do not show dysplastic spread to the main gallbladder mucosa) and have low incidence of carcinomatous change (16%). These features are in marked contradistinction with intracholecystic neoplasms of the main mucosa, which are widespread, connote field-effect/field-defect phenomenon for the remainder of the biliary tract, and have very high propensity for invasion (> 50%). The presence of invasive carcinoma in AM-ICN should be reported in more detail than in an ordinary gallbladder carcinoma and the treatment may have to be devised on a case-by-case basis. Until further studies, it may be warranted to pay more attention to adenomyomas of the gallbladder for potential neoplastic change.

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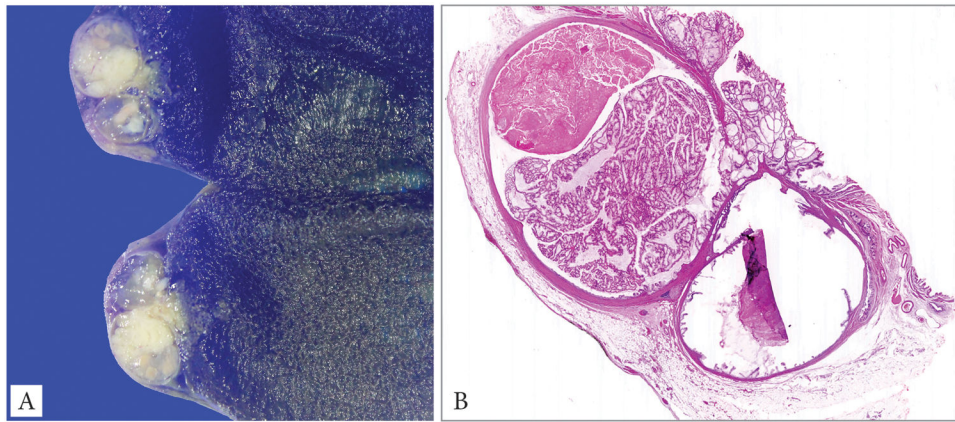
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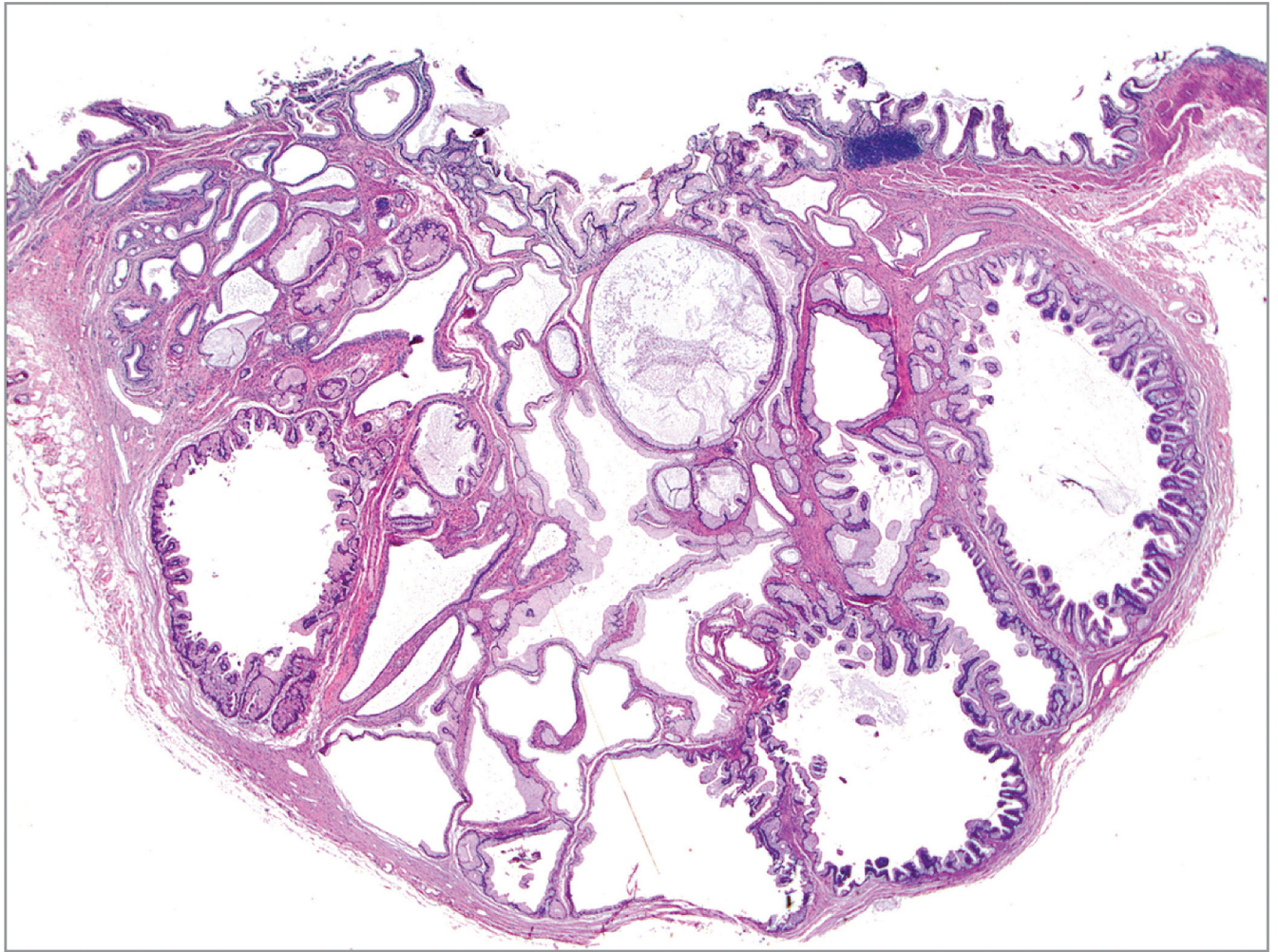
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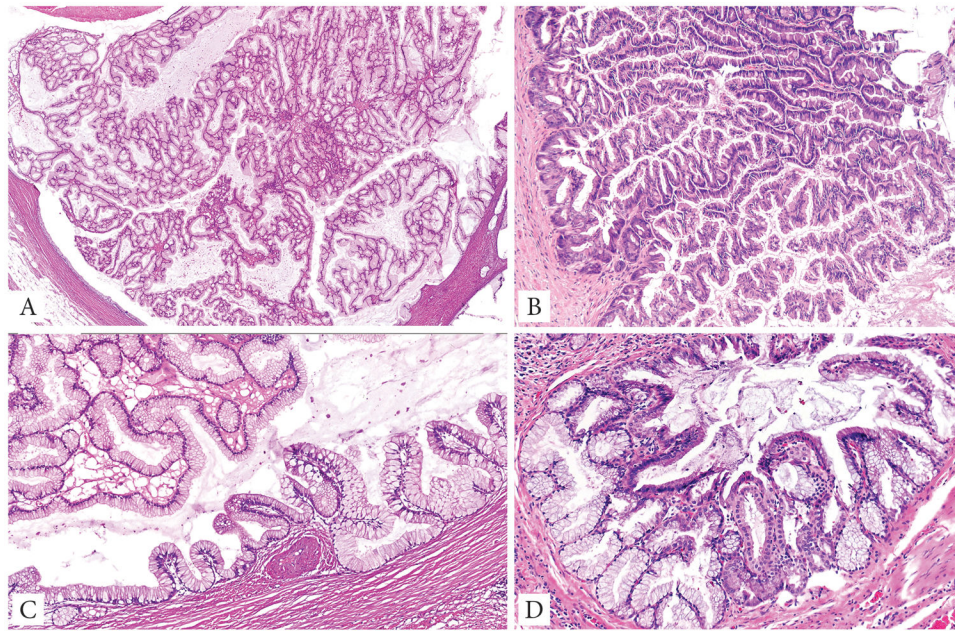
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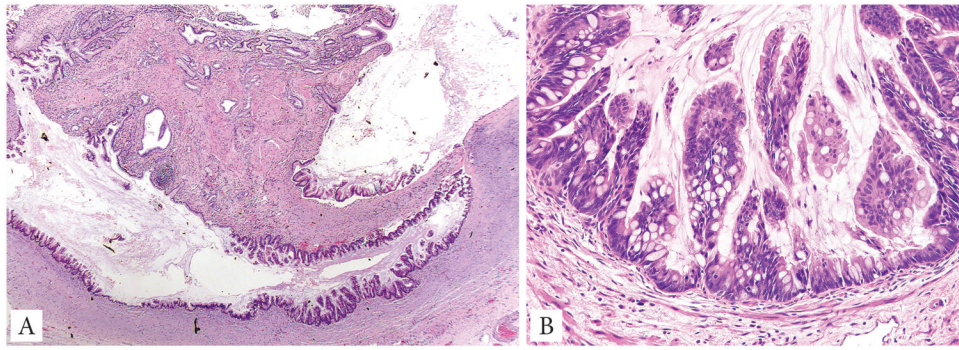
**Figure 1.** AM-ICN, gastric-type, A) gross and B) microscopic view (hematoxylin-eosin, original magnification x20). Cystic and solid lesion grows beneath normal appearing gallbladder mucosa or as in this case papillary changes may be seen in the overlying mucosa.



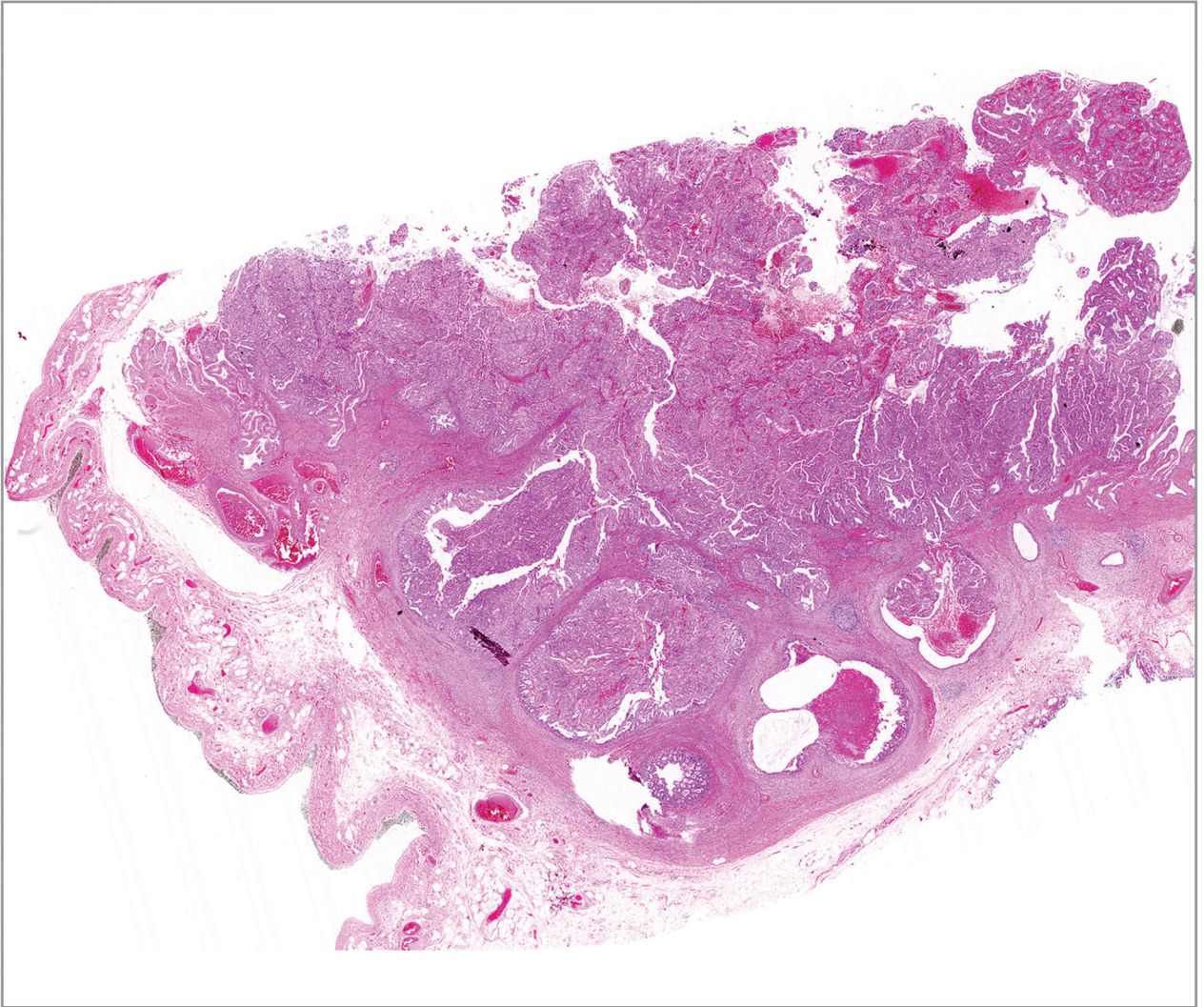
**Figure 2.** All cases arose within an adenomyoma and had at least some degree of cyst formation and papillae formation, hematoxylin-eosin, original magnification, x20.



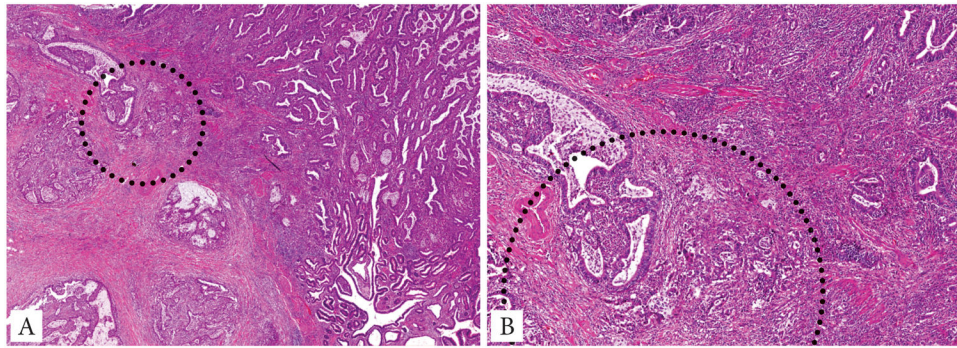
**Figure 3.**  
A-B) Florid papillary nodules that fill the cyst lumen, hematoxylin-eosin, original magnification x100). C-D) The mucosa of the cyst in the AM in all cases was composed predominantly of gastric/endocervical-like mucinous epithelium and focal pyloric gland features, with focal intestinal phenotype in some (B, upper right), hematoxylin-eosin, original magnification x200.



**Figure 4.** Case of AM-ICN showing high grade dysplasia and intestinal-type epithelium, hematoxylin-eosin, original magnification x40 (A) and x400 (B).



**Figure 5.** High grade dysplasia (“carcinoma in-situ”) arising in an AM-ICN. While most cases were exclusively mural lesions, confined to the wall, in three cases, one of which is illustrated here, the papillary proliferation protruded into the gallbladder lumen. However, even in these cases the normal mucosa on the edges of the lesion was noticeable, and the lesional tissue in fact stopped abruptly once it reached the surface mucosa, hematoxylin-eosin, original magnification x20.



**Figure 6.** Invasive carcinoma arising in an AM-ICN. A) The intracholecystic (non-invasive) component is depicted on the right. On the left and in B, the invasive component is illustrated. It is composed of both large and small invasive glandular units as well as non-glandular poorly differentiated elements (circled), hematoxylin-eosin, original magnification x20 (A) and X400 (B).

**Table 1.**

Comparison of adenomyomatous nodule-associated intracholecystic neoplasms (AM-ICN), pure adenomyomatous nodules, ordinary gallbladder carcinomas and non-neoplastic cholecystectomies.

	AM-ICN (n=19)	AM without ICN (n=191)	Ordinary Gallbladder Carcinoma (n=459)	Non-Neoplastic Cholecystectomies (n=1190)	P value ( $\chi^2$ test)
<b>Median age</b>	68 (47-85)	56 (21-95)	64 (22-94)	50 (14-94)	<0.001
<b>Female/male</b>	0.8	1.8	3.0	2.4	<0.001
<b>Follicular cholecystitis (%)</b>	26	16	2.8	2.8	<0.001
<b>Cholelithiasis (%)</b>	55	17	41 <sup>*</sup>	72	<0.001

\* Cholelithiasis was documented in only 10% of the cases with invasive carcinoma.

**Table 2.**

Clinicopathologic characteristics of adenomyomatous nodule-associated intracholecystic neoplasms (AM-ICN) and Intracholecystic Neoplasms.

	AM-ICN (n=19)	Intracholecystic Neoplasms (n=123 <sup>*</sup> )	P value ( $\chi^2$ test)
<b>Number of the cases per age groups</b>			
50 years	1	26	0.18
51-65 years	7	47	
65 years	10	44	
<b>Median age, years</b>	68 (47-85)	62 (20-94)	
<b>Female/male ratio</b>	0.8	2	
<b>Cholelithiasis (%)</b>	55	20 <sup>*</sup>	<b>0.044<sup>*</sup></b>
<b>Follicular cholecystitis (%)</b>	26	11	<b>0.049</b>
<b>Invasive carcinoma (%)</b>	3/19 (16)	68/123(53)	<b>0.001</b>
<b>Number of the cases with invasive carcinoma per age groups</b>			
50 years	1	13	>0.05
51-65 years	1	30	
65 years	1	21	
<b>Size of the invasive component, cm</b>	2.2, 0.8 and 0.2	Mean: 1.72 Median: 1 Range: 0.1-11.7	

\* Characteristics of these 123 intracholecystic neoplasms have been previously published (10). Cholelithiasis could not be documented for all cases.