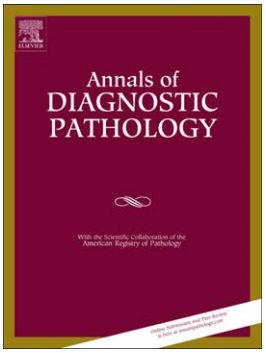

Seek and ye shall find: the importance of careful macroscopic examination and thorough sampling in 2522 cholecystectomy specimens

Brian D. Hayes BMedSc, MB FRCPath, Cian Muldoon MB FRCPath

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Seek and ye shall find: the importance of careful macroscopic examination and thorough sampling in 2522 cholecystectomy specimens

Brian D Hayes BMedSc MB FRCPath[1,2]
Cian Muldoon MB FRCPath[1]

1. Department of Histopathology, St James's Hospital, Dublin 8, Ireland
2. Department of Histopathology and Morbid Anatomy, Trinity College Dublin, Ireland

Corresponding Author:

Dr Brian Hayes
Email: dr.brian.hayes@gmail.com
Telephone: 00 353 86 3042707
Address:
Department of Histopathology,
Central Pathology Laboratory,
St James's Hospital,
James's Street,
Dublin 8,
Ireland

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ABSTRACT

Introduction:

Gallbladder dysplasia and carcinoma (GBDC) vary greatly in incidence worldwide. We aimed to determine their prevalence in an Irish population, to assess the influence of tissue sampling protocols upon GBDC diagnosis, and to correlate various macroscopic and microscopic features with GBDC.

Methods:

We retrospectively reviewed histology reports of cholecystectomy specimens accessioned from 2000 to 2013.

Results:

2522 cholecystectomy reports were reviewed, from 1860 female and 662 male patients. Male patients were significantly older (54.8 vs 46.8 years). There were 29 cases of dysplasia (1.15%) and 12 cases of carcinoma (0.48%), of which 10 were primary gallbladder cancers (0.4%). In 83.4% of cases there was pathological or radiological evidence of cholelithiasis. Histological findings included chronic (91.1%) or acute (15.4%) cholecystitis, cholesterosis (10.9%), adenomyomatous hyperplasia (2.1%), xanthogranulomatous inflammation (2.02%), and "porcelain" gallbladder (0.2%). Patients with GBDC were more likely to have a macroscopically identifiable lesion (29.4% vs 1.8%, PPV 18.18%, NPV 99.03%). GBDC patients also had larger gallstones (median 19 vs 12mm) and were more likely to have adenomyomatous hyperplasia (8.8% vs 2.05%). When cases with a macroscopically identifiable lesion or clinical details suggestive of a gallbladder tumour were excluded (n=2385), GBDC was significantly more frequently diagnosed if multiple tissue blocks had been sampled (2.91% vs 0.76%, RR 3.836).

Conclusions:

Rates of GBDC in Irish cholecystectomy specimens are low. The absence of a macroscopically identifiable lesion has a high (but not 100%) NPV for GBDC. Sampling with more than one block significantly increases pickup rates of GBDC in these cases.

Key Words

Gallbladder, Cancer, Dysplasia, Sampling, Adenomyoma, Cholecystectomy

INTRODUCTION

Cholecystectomy specimens are among the most frequently-accessioned specimens in general histopathology departments, and account for a significant portion of the workload. Examination of these specimens is primarily intended to rule out significant pathology, such as gallbladder dysplasia or carcinoma (GBDC), the incidence of which varies greatly worldwide. In areas with a low incidence of gallbladder cancer, such as Ireland and the United Kingdom, many non-neoplastic organs must be screened before a case of gallbladder cancer can be identified. There is general consensus that examination of a single tissue block with three sections of tissue to include the cystic duct margin represents the best balance between the necessity on the one hand to identify as many cases with significant pathology as possible, and on the other hand to exercise responsibility in expending scarce resources [1-3].

This study aims to determine the rates of dysplasia and carcinoma in a surgical series of cholecystectomy specimens from a large Irish teaching hospital, to assess to what extent a variety of macroscopic and microscopic findings are associated with these significant pathologies, and to assess the sampling protocols currently in use and their fitness for purpose.

MATERIALS AND METHODS

A computerised search was performed for all specimens reported in our department and assigned SNOMED code T57000 (gallbladder) between January 1st 2000 and October 24th 2013. The histopathology reports of all specimens were reviewed. Demographic and clinical data recorded included patient age and sex, date of surgery and clinical details (where available). Details of macroscopic examination of the specimen included length and integrity of gallbladder specimen, thickness of gallbladder wall, and presence or absence of macroscopic lesions (see Figure 1). The presence, colour, number and maximum size of gallstones was recorded. The initial number of tissue blocks sampled, the number of tissue pieces in the first block, whether further blocks were subsequently sampled, and the final number of tissue blocks sampled were recorded. On microscopic examination the presence and severity of chronic cholecystitis, presence of acute, resolving or gangrenous cholecystitis,

xanthogranulomatous inflammation, adenomyomatous hyperplasia (adenomyoma), severe mural fibrosis or calcification, lymphoid hyperplasia, empyema, mucocoele or intestinal metaplasia was recorded. Dysplasia grade and carcinoma type and pathological staging data were recorded where available.

Categorical variables were expressed as percentages, continuous variables as medians and means with standard deviations. Comparisons between categorical variables were effected by Fisher's exact test for smaller datasets and Chi square tests for larger datasets. Medians in continuous variables were compared using the unpaired t test (with or without Welch correction depending on differences in standard deviations) for parametric data and the Mann-Whitney U test for nonparametric data. Statistical significance was accepted as $p < 0.05$.

RESULTS

After exclusion of biopsies and erroneously coded cases, cholecystectomy specimens from 2522 patients were identified. There were 1860 (73.75%) female and 662 (26.25%) male patients with a mean age of 48.89±16.66 years (range 6 – 92). Male patients were significantly older than female patients (54.83 vs 46.78 years, $p < 0.0001$).

Some clinical details beyond the word "gallbladder" or "cholecystectomy" had been provided to the reporting pathologist by the surgeon in 1398 (55.43%) cases (see Table 1). The most common clinical presentations were "cholecystitis" (481, 34.41%), "stones" (406, 29.04%), "biliary colic" (264, 18.88%), and "pancreatitis" (104, 7.44%). In 42 (3%) cases the cholecystectomy was part of a larger oncological operation, most frequently a Whipple procedure or a gastrectomy. In 29 cases (2.07%) there was a radiological suspicion of a mass, polyp or cancer.

The specimen was fragmented in 26 (1.05%) cases. When intact, the mean gallbladder length was 75.1±17.71mm. This varied slightly between male (76.46±17.73mm) and female patients (74.61±17.68mm, p=0.0233). Mean wall thickness was 4.08±2.68mm. In 149 cases (5.91%), the gallbladder wall was described as "thickened" but a measurement was not provided, and in 1225 cases (48.57%) no comment was made

on the thickness of the wall. A mural or mucosal lesion was identified macroscopically in 55 (2.18%) cases.

Gallstones were present in 2014 (79.86%) specimens, although when clinical details are taken into account, the number of cases in which cholelithiasis or choledocholithiasis was pathologically or radiologically recorded was 2104 (83.43%). Of the cases with gallstones whose colour was recorded (1354), 870 were cholesterol stones (64.25%) and 484 were pigment stones (35.75%). The number of stones was recorded in 1930 cases – there was a single stone in 438 (22.69%) cases and multiple stones in 1492 (77.31%) cases. The mean diameter of the largest stone was 14.41±9.83mm (range 1 – 60mm).

The majority of specimens were sampled in a single block (87.57% - see Table 2). Of those specimens sampled in a single block, the modal number of pieces of tissue sampled was three (1666 – 77.34%). In 31 cases (1.23%) further blocks were sampled following initial histological examination.

Histological evidence of chronic cholecystitis was present in 2298 (91.12%) cases (see Table 3). A semiquantitative assessment of the severity of this was recorded in 705 cases – minimal in 100 (14.18%), mild in 407 (57.73%), moderate in 119 (16.88%), severe in 79 (11.21%). There was acute inflammation in 389 of the total 2522 specimens (15.42%), and gangrenous necrosis of the gallbladder wall in 31 cases (1.23%). Evidence of resolving or recent acute cholecystitis was present in 40 cases (1.59%).

Other findings were: xanthogranulomatous inflammation in 51 cases (2.02%), adenomyomatous hyperplasia or adenomyoma in 54 cases (2.14%), cholesterosis in 275 cases (10.9%), marked fibrosis in 110 cases (4.36%) with “porcelain” calcification in 5 cases (0.2%), lymphoid hyperplasia in 51 cases (2.02%), empyema in 9 cases (0.36%), mucocoele in 7 cases (0.28%) and intestinal metaplasia in 11 cases (0.44%).

Changes of dysplasia were present in 29 cases (1.15%), which was low grade (see Figure 2) in 19, high grade (see Figure 3) in 7, and of unrecorded grade in 3.

Invasive carcinoma was present in 12 gallbladders (0.48% - see Figure 4). In two cases this was a metastatic deposit from elsewhere (of renal and gynaecological origins), leaving ten primary gallbladder cancers (0.4%). Eight primary gallbladder cancer patients were female, two were male. Patients ranged in age from 38 to 74, with a mean of 54.6. In no case was there a clinical or radiological suspicion of cancer. A macroscopic lesion was identified in the gallbladder in five cases. The grades of the primary cancers were well-differentiated (1), moderately differentiated (4), poorly differentiated (1), unrecorded (4). The T-stages were pT2 (4), pT3 (3), pT4 (1), unrecorded (2). One cancer had extensive metastases to colon and peritoneum, and another involved 3 of 10 regional lymph nodes. Both of these metastatic cancers demonstrated prominent perineural invasion.

Cases in which dysplasia or primary carcinoma were identified (n=34) were compared with the remaining cases (n=2488, see Tables 4 and 5). Patients with GBDC were more likely to have at least some clinical details supplied with the laboratory request form (73.53 vs 55.18%, p = 0.0496). They were also significantly more likely to have a macroscopically identifiable lesion (29.41% vs 1.81%, RR 1.21, p < 0.0001). GBDC patients had a greater median diameter of largest gallstone (19 vs 12mm, p 0.0101) and a nonsignificant trend towards a higher proportion of solitary rather than multiple stones (40% vs 22.47%, RR 2.271, p = 0.0516). Gallbladders with GBDC were more frequently sampled initially in more than one block (47.06% vs 11.95%, RR 1.046, p < 0.0001) and more frequently subsequently had additional blocks sampled (70.59% vs 0.28%, RR 4.411, p < 0.0001). Chronic cholecystitis was reported more frequently in cases without GBDC (91.72% vs 47.06%, RR 0.9261, p < 0.0001). Adenomyoma was more often seen in cases with GBDC (8.82% vs 2.05%, RR 1.046, p 0.0345), as was intestinal metaplasia (17.65% vs 0.2%, RR 2.175, p < 0.0001). There were no significant differences between GBDC and non-GBDC cases with regard to sex, age, gallbladder length or wall thickness, presence or type of stones, the number of pieces of tissue sampled in single-block cases, or any other histological findings.

Of the 55 cases with a macroscopically identifiable lesion, 18.18% demonstrated GBDC, significantly more than the 2467 cases where no such lesion was evident (0.97%, p < 0.0001). Thus, a macroscopic lesion in a gallbladder has a sensivity for

GBDC of 29.41%, a specificity of 98.19%, a positive predictive value (PPV) of 18.18% and a negative predictive value (NPV) of 99.03%.

In order to assess the extent to which tissue sampling affects the diagnosis of GBDC, cases with macroscopically identifiable lesions, clinical details indicating a suspicion of carcinoma or lacking detail regarding numbers of blocks sampled were excluded from analysis. Of the remaining 2385 "low risk" cases 24 contained GBDC and 2361 did not. A greater proportion of GBDC cases (33.33%) in the low risk group had been sampled in more than one tissue block when compared with the non-GBDC cases (11.31%). Stated otherwise, significantly more cases sampled with more than one tissue block yielded GBDC than those sampled with only one block (2.91% vs 0.76%, RR 3.836, p = 0.0024). There was no significant difference in age profile (p = 0.7572) or in gallbladder wall thickness (p = 0.5637) between these two groups, and thus no feature which would have prompted the dissector to undertake more rigorous sampling. When low risk cases sampled in only a single tissue block were considered (n=2109), there was no significant difference in yield of GBDC when a cut-off of either two (p = 0.8128) or three (p = 0.8725) pieces of sampled tissue was used.

DISCUSSION

The current study demonstrates a rate of primary gallbladder carcinoma of 0.4%, and a rate of dysplasia of 1.15%, in an unselected large series of cholecystectomy specimens. As far as we are aware, it is the largest such series published in Ireland.

There is marked variation in the incidence of gallbladder carcinoma worldwide, with some of the highest reported incidence rates being in India (21.5/100,000), Pakistan (13.8/100,000) and Ecuador (12.9/100,000) [4]. The age-standardised incidence rate in Ireland is low (estimated at 2.5/100,000 for males and 3.5/100,000 for females) and in keeping with rates throughout the rest of Europe [5]. The two main morphological pathways to gallbladder carcinoma are through metaplasia and dysplasia in flat mucosa (the more common scenario), or arising within a macroscopically visible adenoma. There are certain differences at a molecular level between the pathways [6].

Gallstones are the most important recognised risk factor for gallbladder carcinoma, and increasing gallstone load appears to correlate with cancer risk, at least in high-incidence zones. However, the absolute risk for developing gallbladder cancer in the presence of gallstones is low. There is some debate as to whether gallstones are the cause or a cofactor in the development of gallbladder carcinoma. Given the poor prognosis of cancer developing at this site, prophylactic cholecystectomy for asymptomatic gallstones is often undertaken, particularly in high-incidence zones [7]. A Pakistani case-control study found that older age and stones greater than one centimetre in diameter are associated with risk of gallbladder carcinoma [8]. Stones in cases of carcinoma were of greater total weight and volume than in cases with cholecystitis, hyperplasia or metaplasia in an Indian study [9]. A Chilean study found similar results, finding for example that gallstone volumes of 6, 8 and 10ml had relative risks of cancer of 5, 7 and 11 respectively [10]. The current study, to our knowledge, is the first to attempt to correlate gallstone load on gallbladder cancer in a low-incidence zone. Our findings that dysplasia and carcinoma are associated with a greater diameter of largest gallstone are consistent with these geographically high-incidence studies, as this can be seen as a surrogate marker of greater gallstone load. Data regarding total weight and volume of gallstones were not available in the current study.

A detailed prospective study of 592 cholecystectomies found that patients with gallbladder carcinoma were more likely to have large stones, which tended to be multiple. These patients were also older and the authors speculate that the increased gallstone load in cancer patients may be related to age and duration of gallstone disease [11]. In support of this, a Greek study found that elderly patients most frequently presented with multiple gallstones, although they were usually small, and that severe inflammation was more likely to be found in younger patients and in those with solitary stones [12]. Alvi [8] found that solitary stones were more associated with gallbladder carcinoma. It is interesting that, in common with the Alvi study, there was a nonsignificant trend in the current study towards solitary rather than multiple gallstones being more closely associated with dysplasia and carcinoma.

Five gallbladders with mural calcification ("porcelain gallbladder") were identified in this series, but none showed evidence of dysplasia or carcinoma. In contrast, there

was no evidence of calcification in the 34 cases which did show dysplasia or carcinoma. These findings are in keeping with the recent observations that porcelain gallbladder, historically considered an important risk factor for gallbladder cancer, may not be as significant as previously thought [13].

Currently, most gallbladder specimens in our department are processed according to the generally accepted guideline of three pieces in a single block [1-3]. Several studies have considered whether histological evaluation of some cholecystectomy specimens may be safely omitted, for example when the specimen appears macroscopically normal. Gallbladder cancer has a very poor prognosis, even when of early stage and potentially not macroscopically evident. For this reason, a selective approach to gallbladder histology should only be considered in regions of very low incidence, if at all [14]. All ten cases of primary gallbladder carcinoma in the current study presented without radiological suspicion of malignancy and most had typical gallstone-related clinical syndromes such as biliary colic. Although the NPV of macroscopic lesions for GBDC in the current study is high (99.03%), this is a function of the rarity of the disease in question, and it is sobering to note that 50% of the invasive cancers presented with no macroscopic findings. In fact, the current recommendations of the Royal College of Pathologists (which are generally followed in Irish histopathology departments) are that all resected gallbladders should be examined histologically, as significant disease may be present in spite of minimal or absent macroscopic abnormality [15].

Even if broad consensus exists that gallbladder microscopy should be omitted only in carefully selected situations, there remains controversy regarding optimal sampling of gallbladder specimens. Dysplasia and carcinoma may be focal, and can be missed if a suboptimal sampling protocol is used – a single random section will identify less than 30% of significant epithelial lesions [16]. When examination of initial tissue blocks reveals dysplasia, authors disagree as to whether the entire remainder of the organ or only selected sections from it need then be examined [17-19].

Following initial examination of a gallbladder in our department, further sampling is undertaken if dysplasia or carcinoma is identified or if the macroscopic appearances are unusual. A single initial block was employed in 87.57% of cases in the present

study. When cases with features which might prompt detailed histology in the first instance are excluded, such as a macroscopic lesion or radiological suspicion of tumour, there was a significantly higher pickup rate of GBDC when multiple blocks were taken. Similar age profiles and gallbladder wall thickness between the groups eliminate these as potential confounding factors. It is therefore possible that were a more extensive routine sampling procedure in place, more cases of dysplasia (or carcinoma) could have been identified. Such protocols might include sampling more pieces of tissue in a single block, sampling larger pieces of tissue in a single block (such as by the "jelly-roll" technique used for placental membranes), or sampling more blocks of tissue.

We found that adenomyomatous hyperplasia was associated with GBDC, but interestingly was not seen in any of the cases of primary gallbladder carcinoma. There are few data published regarding the malignant potential of adenomyoma of the gallbladder (or adenomyomatosis / adenomyomatous hyperplasia, as it is described when diffuse). Several case reports [20-22] describe dysplasia or cancer arising within an adenomyoma. A number of Japanese studies have found an association in large cholecystectomy series between gallbladder cancer and adenomyomatosis of the segmental (as opposed to fundal or diffuse) type [23,24]. In the Nabatame study, the association was more pronounced among patients over the age of 60 years. High grade dysplasia or carcinoma in situ arising in the exaggerated and deeply penetrating Rokitansky-Aschoff sinuses of adenomyomatous hyperplasia may give rise to diagnostic difficulty for the pathologist, and raise the possibility of invasive carcinoma.

This study has several weaknesses. Owing to the large number of cases the results are based on review of reports rather than detailed review by a pathologist of every glass slide. This means that some of the data is incomplete and the effect is to bias some of the results relating histological abnormalities considered unimportant by the reporting pathologist. For example, the rate of chronic cholecystitis in GBDC cases appears to be less than in the control group – but this is probably because the reporting pathologist did not consider the inflammation to be of any significance when compared with a neoplastic disease, and so did not record it. In spite of these shortcomings, the data concerning the main purposes of this study are of good quality.

In conclusion, the rates of GBDC in this Irish series of cholecystectomy specimens is low, and in keeping with the rates seen in other geographically low-incidence zones. Gallbladder carcinoma is often macroscopically occult and even in low incidence zones such as Ireland cases of dysplasia and carcinoma are more readily identified when the specimen is more extensively examined at initial microscopy. More detailed sampling protocols may be useful in certain circumstances.

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TABLES

Table 1: Clinical details supplied with specimen (n=1398)

Cholecystitis	481	34.41%
Gallstones	406	29.04%
Biliary colic	264	18.88%
Pancreatitis	104	7.44%
Part of cancer operation	42	3%
Empyaema	31	2.22%
Mass / polyp / ? cancer	29	2.07%
Mucocoele	25	1.79%
Other	78	5.58%

Table 2: Sampling of specimens

Number of initial blocks taken	(n=2461)	
1	2155	87.57%
2	191	7.76%
3	77	3.13%
4	19	0.77%
5 or more	19	0.77%
Further blocks sampled following initial examination?	(n=2522)	
Yes	31	1.23%
No	2491	98.77%
Total number of blocks examined	(n=2461)	
1	2132	86.63%
2	190	7.72%
3	74	3.01%
4	24	0.98%
5 or more	41	1.67%
Number of tissue fragments in single-block cases	(n=2154)	
1	34	1.58%
2	210	9.75%
3	1666	77.34%
4	215	9.98%
5 or more	29	1.35%

Table 3: Histological findings

N=	2522	
Chronic cholecystitis	2298	91.12%
n=	705	
Minimal	100	14.18%
Mild	407	57.73%
Moderate	119	16.88%
Severe	79	11.21%
Acute cholecystitis	389	15.42%
Gangrenous cholecystitis	31	1.23%
Resolving acute cholecystitis	40	1.59%
Xanthogranulomatous inflammation	51	2.02%
Lymphoid hyperplasia / follicular cholecystitis	51	2.02%
Cholesterosis	275	10.9%
Adenomyoma	54	2.14%
Severe fibrosis	110	4.36%
Porcelain gallbladder	5	0.2%
Empyaema	9	0.36%
Mucocoele	7	0.28%
Intestinal metaplasia	11	0.44%
Dysplasia	29	1.15%
Primary gallbladder carcinoma	10	0.4%

Table 4: Comparison of GBDC with non-GBDC cases (categorical variables)

		Not GBDC		*GBDC*		*p*	*RR (95% CI)*
Sex	Male	1833	73.67%	27	79.41%		
	Female	655	26.33%	7	20.59%	0.5761	0.996 (0.9865 – 1.006)
Age	<48	1215	48.85%	14	41.18%		
	≥48	1272	51.15%	20	58.82%	0.4735	1.004 (0.9951 – 1.013)
Clinical details supplied	Yes	1373	55.18%	25	73.53%		
	No	1115	44.82%	9	26.47%	**0.0496**	**0.99 (0.9814 – 0.9988)**
Lesion	Present	45	1.81%	10	29.41%		
	Absent	2443	98.19%	24	70.59%	<0.0001	18.689 (9.395 – 37.139)
Gallstones	Present	1988	79.9%	26	76.47%		
	Absent	500	20.1%	8	23.53%	0.7791	1.003 (0.9908 – 1.015)
Stone type	Cholesterol	859	64.3%	11	61.11%		
	Pigment	477	35.7%	7	38.89%	0.807*	1.002 (0.9888 – 1.015)
Stone number	Solitary	428	22.47%	10	40%		
	Multiple	1477	77.53%	15	60%	0.0516*	0.9871 (0.9722 – 1.002)
Initial blocks	1	2137	88.05%	18	52.94%		
	>1	290	11.95%	16	47.06%	**<0.0001**	**1.046 (1.019 – 1.075)**
Further blocks sampled	No	2481	99.72%	10	29.41%		
	Yes	7	0.28%	24	70.59%	**<0.0001**	**4.411 (2.298 – 8.466)**
Total blocks	1	2131	87.8%	1	2.94%		
	>1	296	12.1%	33	97.06%	**<0.0001**	**1.111 (1.072 – 1.152)**
Chronic cholecystitis	Absent	206	8.28%	18	52.94%		
	Present	2282	91.72%	16	47.06%	**<0.0001**	**0.9261 (0.8908 – 0.9628)**
Acute cholecystitis	Absent	2101	84.45%	32	94.12%		
	Present	387	15.55%	2	5.88%	0.1895	0.9901 (0.9814 – 0.9989)
Gangrenous inflammation	Absent	2457	98.75%	34	100%		
	Present	31	1.25%	0	0%	0.5125	0.9864 (0.9818 – 0.9909)
Resolving acute cholecystitis	Absent	2448	98.39%	34	100%		
	Present	40	1.61%	0	0%	0.9567	0.9863 (0.9817 – 0.9909)
Xanthogranulomatous inflammation	Absent	2437	97.95%	34	100%		
	Present	51	2.05%	0	0%	0.818	0.9862 (0.9817 – 0.9908)
Lymphoid hyperplasia	Absent	2437	97.95%	34	100%		
	Present	51	2.05%	0	0%	0.818	0.9862 (0.9817 – 0.9908)
Cholesterosis	Absent	2215	89.03%	32	94.12%		
	Present	273	10.97%	2	5.88%	0.5036	0.993 (0.9818 – 1.004)
Adenomyoma	Absent	2437	97.95%	31	91.18%		
	Present	51	2.05%	3	8.82%	**0.0345**	**1.046 (0.9799 – 1.116)**
Severe fibrosis	Absent	2378	95.58%	34	100%		
	Present	110	4.42%	0	0%	0.406	0.9859 (0.9812 – 0.9906)
Porcelain gallbladder	Absent	2483	99.8%	34	100%		
	Present	5	0.2%	0	0%	0.7936	0.9865 (0.982 – 0.991)
Empyema	Absent	2479	99.64%	34	100%		
	Present	9	0.36%	0	0%	0.7253	0.9865 (0.982 – 0.991)
Mucocoele	Absent	2481	99.72%	34	100%		
	Present	7	0.28%	0	0%	0.7568	0.9865 (0.982 – 0.991)
Intestinal metaplasia	Absent	2483	99.8%	28	82.35%		
	Present	5	0.2%	6	17.65%	**<0.0001**	**2.175 (1.139 – 4.157)**

All p-values are derived from Chi Square tests, except for those marked with an asterisk, which are derived from Fisher's Exact tests.

Table 5: Comparison of GBDC with non-GBDC cases (continuous variables)

	Not GBDC			*GBDC*			
	Mean	*St Dev*	*Range*	*Mean*	*St Dev*	*Range*	*p*
Gallbladder Length (mm)	75.04	17.56	10 - 160	79.12	26.47	20 - 148	0.3843
Maximum wall thickness (mm)	4.08	2.69	1 - 35	4.29	2.43	1 - 8	0.7731
Maximum stone diameter (mm)	12*		1 - 60	19*		8 - 40	**0.0101***
Initial blocks taken	1.18	0.61	1 - 9	2.79	2.88	1 - 13	**0.0026**

All p-values (except asterisked) are derived from Unpaired t-tests, with or without Welch correction depending on the presence or absence of a significant difference in standard deviations. Values provided for maximum stone diameter are medians and the p-value is derived from the Mann-Whitney U test for nonparametrically distributed data.

FIGURE LEGENDS

Figure 1

Formalin-fixed cholecystectomy specimen opened longitudinally to reveal a fleshy fundic mass, histologically confirmed as adenocarcinoma.

Figure 2

Intestinal-type adenoma of the gallbladder with low grade dysplasia. At scanning magnification (A) papillary fronds with fibrovascular cores are seen, some of which are oedematous. At high power (B) low grade dysplasia is apparent (lower part of image), with increased nuclear:cytoplasmic ratio, and early nuclear stratification and hyperchromasia. There is a sharp demarcation from adjacent nondysplastic epithelium.

Figure 3

High grade dysplasia. At medium power (A) marked architectural complexity with cribriform spaces is seen. Mitotic figures are prominent. At high power (B) there is a greater degree of cytological atypia than in Figure 2, with loss of polarity, some rounded nuclei and prominent nucleoli.

Figure 4

Adenocarcinoma. Moderately-differentiated adenocarcinoma at scanning magnification (A), invading deep into the gallbladder wall, best appreciated on a cytokeratin 7 stain (B). A separate case of poorly-differentiated adenocarcinoma with signet-ring forms (C) arises on a background of dyplasia and shows prominent lymphovascular invasion (D – inset D240 stain).

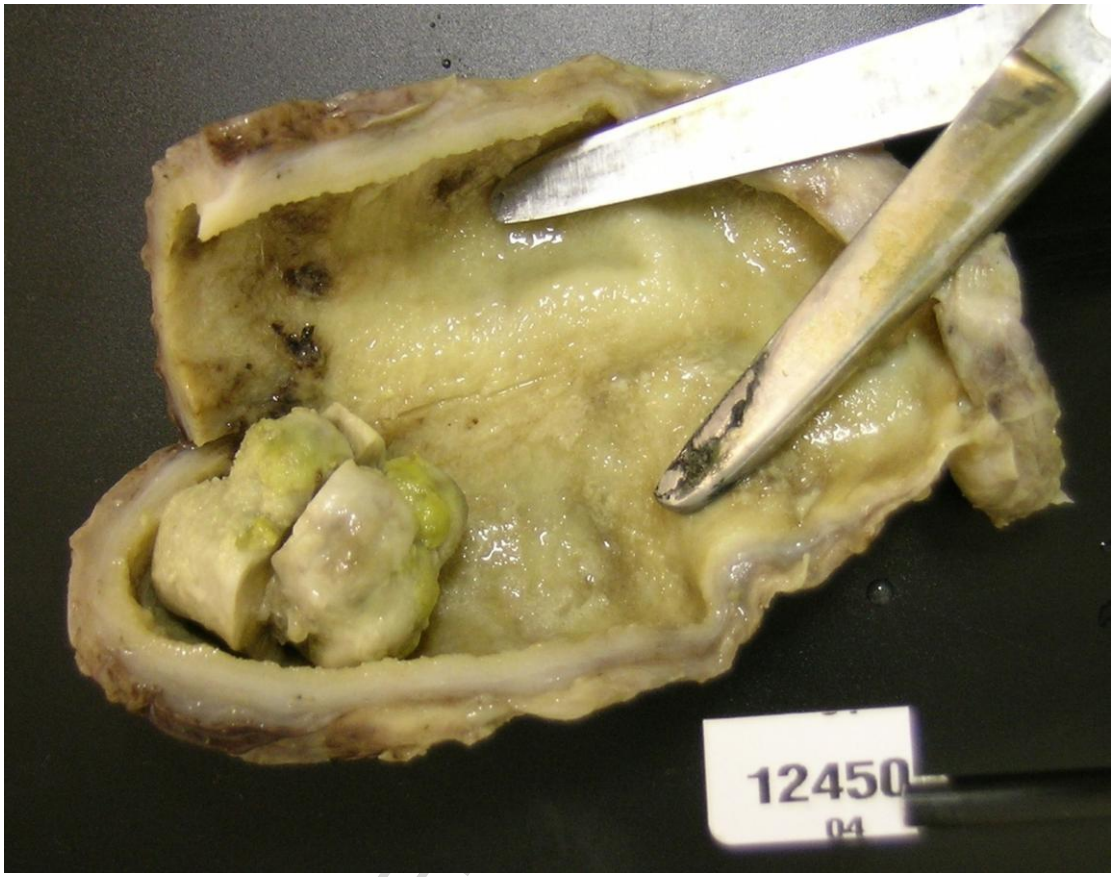

Fig. 1

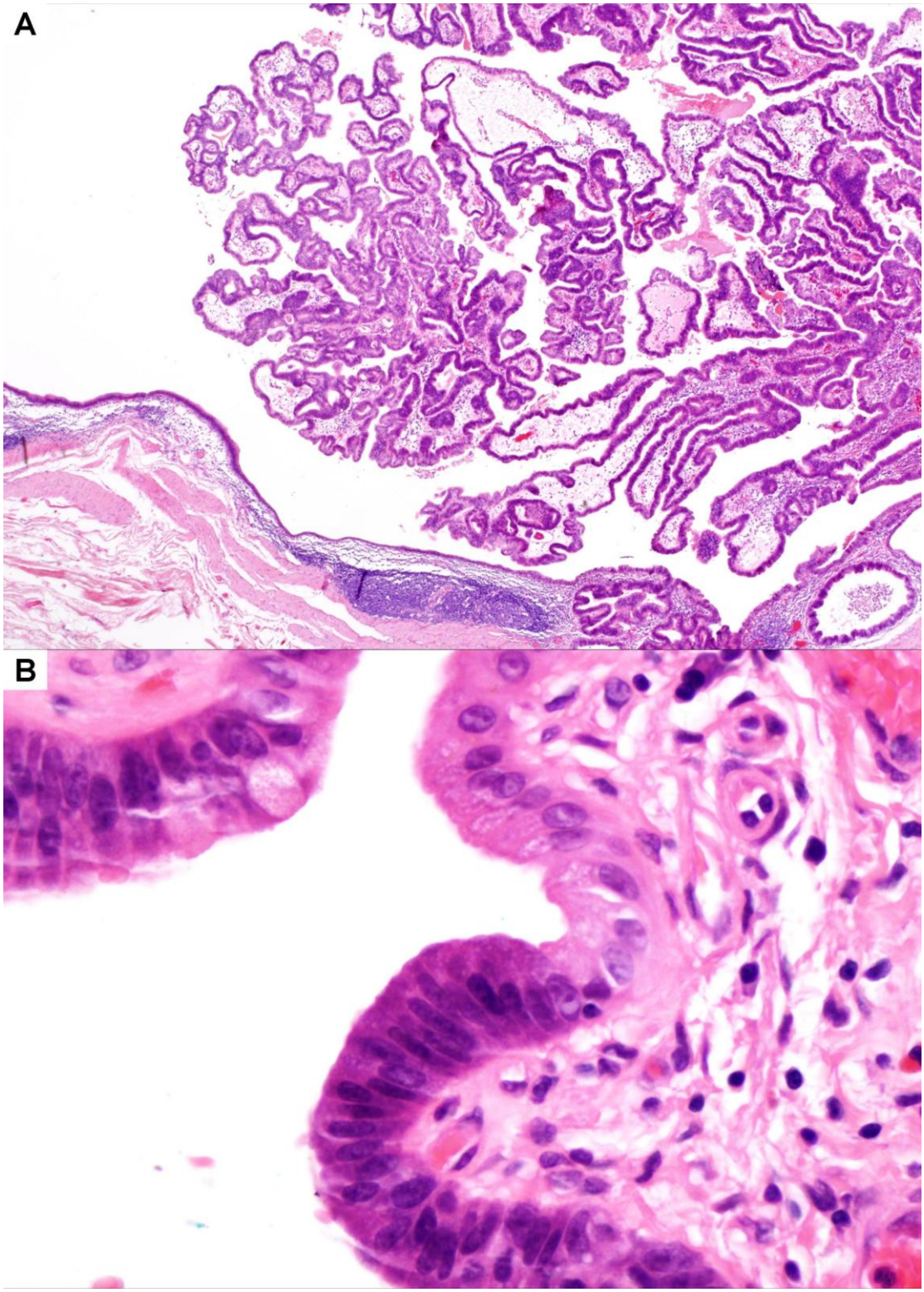

Fig. 2

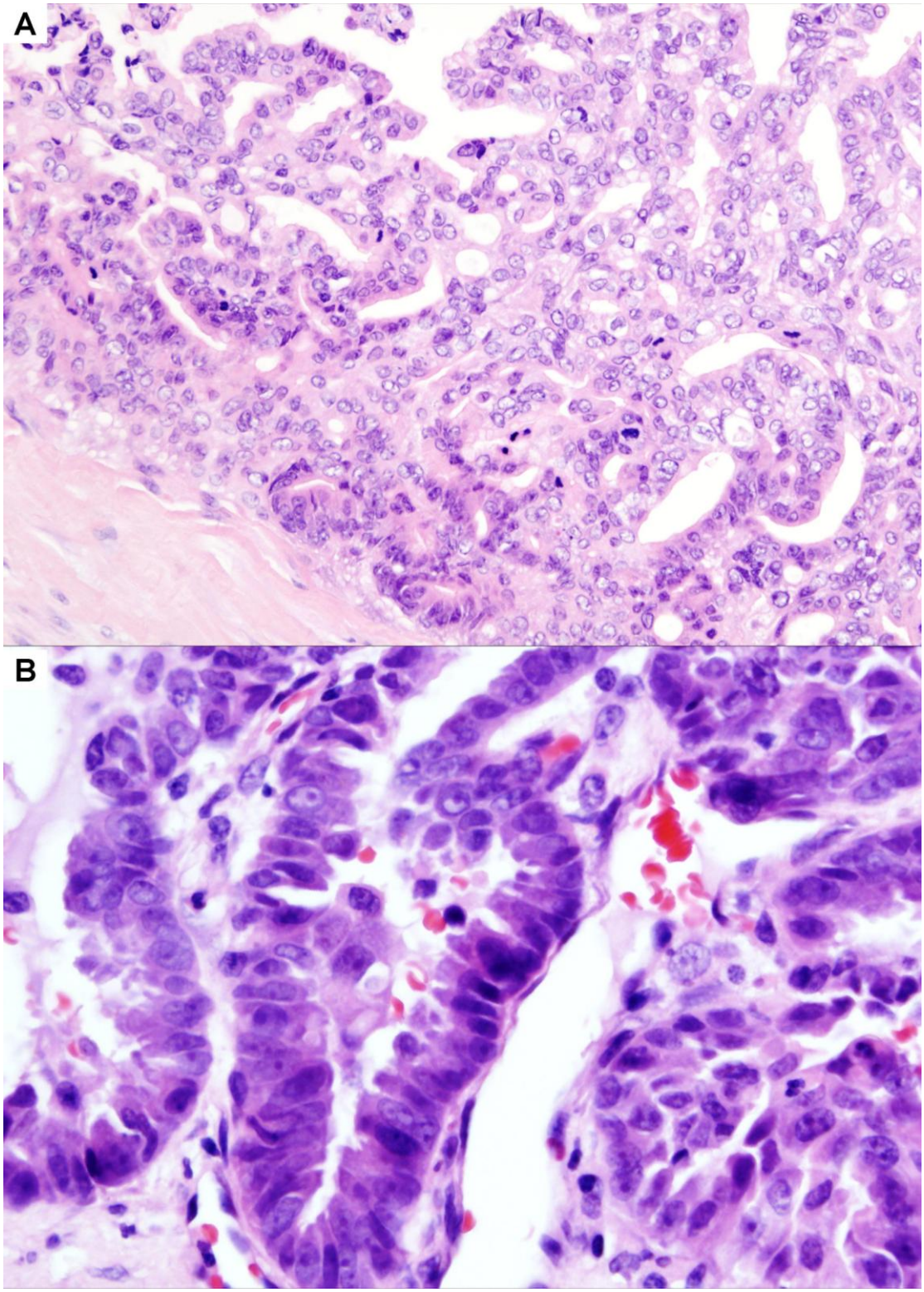

Fig. 3

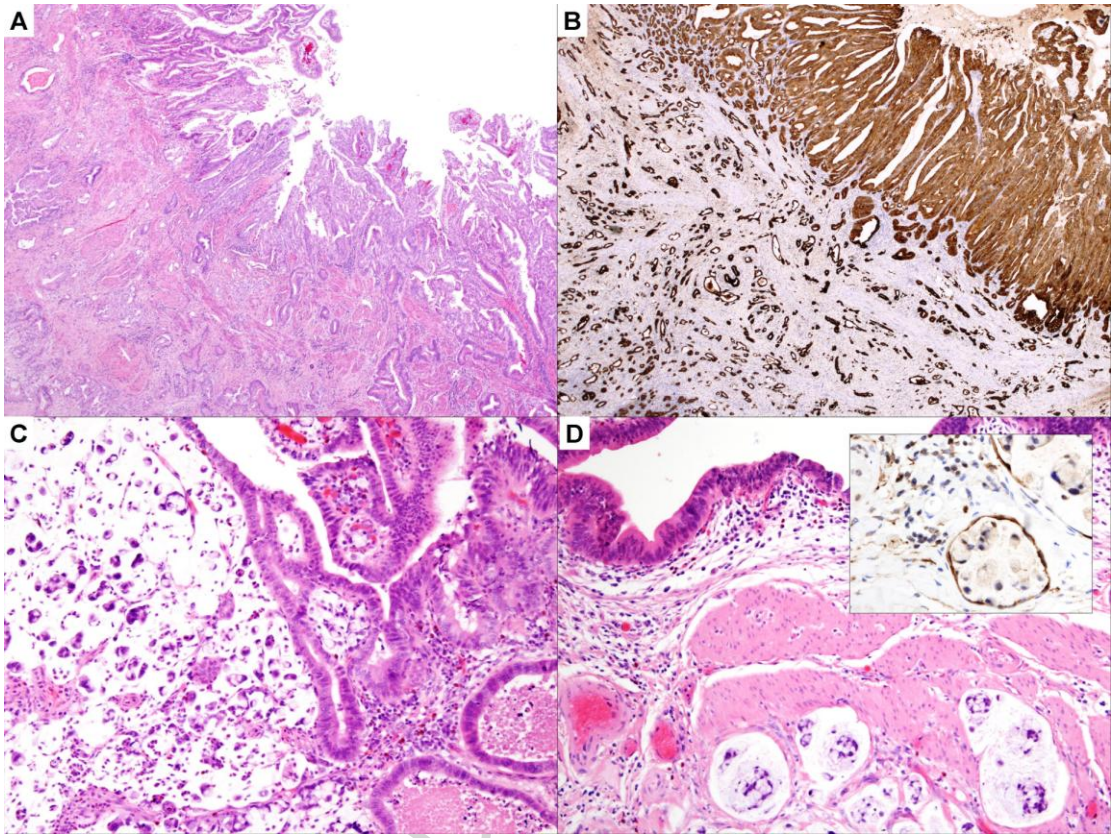

Fig. 4