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Title page

IgG4-related sclerosing cholangitis and autoimmune pancreatitis: Histological assessment of biopsies from Vater's ampulla and the bile duct

Short running title: Endoscopic biopsy in IgG4-SC

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Abstract

Background and Aim. Autoimmune pancreatitis is commonly associated with immunoglobulin G4 (IgG4)-related sclerosing cholangitis. The discrimination between IgG4-SC and pancreatobiliary malignancies or primary sclerosing cholangitis (PSC) is now an important issue. This study was performed to examine the usefulness of endoscopic biopsies from Vater's ampulla and the bile duct to diagnose IgG4-SC.

Methods. This study included 29 IgG4-SC patients (26 with both pancreatitis and cholangitis, and 3 with cholangitis only), 6 PSC patients, and 27 pancreatobiliary carcinoma patients. All patients underwent endoscopic biopsies from Vater's ampulla and the common bile duct. Biopsied specimens were histologically examined using immunostaining for IgG4.

Results. For the ampullary and bile duct biopsies, the IgG4-SC samples had a significantly greater number of IgG4-positive plasma cells than the PSC or pancreatobiliary carcinoma specimens. In addition, bile duct biopsies from 5 patients (17%) with IgG4-SC showed diffuse inflammatory cell infiltration with irregular fibrosis corresponding to the histological features of lymphoplasmacytic sclerosing pancreatocholangitis. Based on the threshold of 10 IgG4-positive plasma cells per high power field, the diagnostic rates of the ampullar and bile duct biopsies were both 52% (15/29 cases). Twenty-one patients (72%) had more than 10 IgG4-positive plasma cells in at least either biopsy. The bile duct biopsy was significantly valuable for IgG4-SC patients with swelling of the pancreatic head.

Conclusions. This study suggested that ampullar and bile duct biopsies are useful for diagnosing IgG4-SC. In particular, bile duct biopsies can provide histological features that are more directly related to pancreatobiliary lesions.

Keywords:

Autoimmune pancreatitis

Immunoglobulin G4-related sclerosing cholangitis

Primary sclerosing cholangitis

Immunoglobulin G4

Endoscopic biopsy

Abbreviations

AIP, autoimmune pancreatitis; Ig, immunoglobulin; PSC, primary sclerosing cholangitis; IgG4-SC, Immunoglobulin G4-related sclerosing cholangitis; ERCP, endoscopic retrograde cholangiopancreatography; HPF, high power field.

Introduction

Autoimmune pancreatitis (AIP) is characterized by swelling of the pancreas, irregular stenosis of the pancreatic duct, high serum immunoglobulin (Ig) G4 concentrations, and steroid sensitivity [1,2]. IgG4-related diseases including AIP have characteristic pathologic features, such as diffuse lymphoplasmacytic infiltration, irregular fibrosis, occasional eosinophil infiltration, obliterative phlebitis, and many IgG4-positive plasma cells [3-5]. AIP is commonly associated with sclerosing cholangitis, which is also called IgG4-related sclerosing cholangitis or IgG4-associated cholangitis [6,7]. Much attention has focused on discriminating between AIP with cholangitis and primary sclerosing cholangitis (PSC) or pancreatobiliary malignancies, from both clinical and academic aspects [8-10]. Distinguishing between these two conditions is important because their therapeutic strategies are completely different [11]. In clinical situations, imaging and serological examinations, such as testing serum IgG4 levels, are performed to discriminate these two conditions. However, in some cases a pathological diagnosis is necessary for a definitive diagnosis.

The needle biopsy is one tool that can be used to pathologically examine the pancreas. However, interpreting the results is sometimes difficult due to the small specimen size and the heterogeneous distribution of inflammation in AIP [12,13]. Recently, several groups reported that IgG4 immunostaining of endoscopic biopsies from Vater's ampulla is useful for diagnosing AIP [14,15]. The number of IgG4-positive plasma cells in ampullar biopsies for AIP was significantly higher than those for PSC or pancreatobiliary malignancies. Sepehr A, *et al.* also suggested that ampullary biopsies might be useful for assessing IgG4-positive plasma cells based on pathological examinations of surgically resected specimens [16]. However, it seems

difficult to make a definitive pathological diagnosis of AIP based only on the number of

IgG4-positive cells, especially in a non-pancreatic tissue. We hypothesized that bile duct

biopsies might be more useful for diagnosing AIP and more closely reflect the histopathology of

the pancreas than ampullary biopsies because the bile duct is commonly involved in AIP.

Therefore, we performed a clinicopathological study to examine the usefulness of

endoscopic biopsies from Vater's ampulla and the bile duct for discriminating between AIP and

PSC or pancreatobiliary cancers.

Patients and methods

Patients

This study consisted of 26 AIP patients (all associated with cholangitis), 3 patients

with IgG4-related sclerosing cholangitis (without AIP), 6 PSC patients, and 27 pancreatobiliary

carcinoma patients. Patients with AIP or IgG4-related sclerosing cholangitis were examined in

single disease group named IgG4-related sclerosing cholangitis (IgG4-SC). All patients were

diagnosed and treated at Hokkaido University Hospital from April 2006 to February 2009. After

excluding 4 AIP patients without cholangitis, all patients diagnosed as AIP, IgG4-SC, and PSC

in our institute were included in this study. All pancreatobiliary carcinoma patients who

underwent endoscopic retrograde cholangiopancreatography (ERCP), and ampullary and bile

duct biopsies during this period were also included in this study. The average ages and

male/female ratios were as follows: IgG4-SC, 68 years, 23/6; PSC, 44 years, 1/5; and

pancreatobiliary carcinoma, 66 years, 22/5. Clinical presentations of patients with IgG4-SC

included obstructive jaundice (13/29, 45% of cases), mild abdominal pain (2/29, 7%), and body

weight loss (1/29, 3%). Two patients (7%) were found to have elevated biliary enzymes by the blood test. The remaining 11 patients (38%) did not have any subjective symptoms, and found to have abnormalities on the radiological examination for routine medical screening or follow-up for extra-pancreatobiliary diseases. PSC patients presented with serological liver dysfunction (4/6, 67%) and jaundice (2/6, 33%). Pancreatobiliary carcinoma patients showed obstructive jaundice (21/27, 77%), elevation of biliary enzymes (4/27, 15%), mild abdominal pain (1/21, 4%), and mild back pain (1/21, 4%). In accordance with the Declaration of Helsinki, written informed consent was obtained from each patient and their family members before ERCP or biopsies from Vater's ampulla and the bile duct. As this study comprised a retrospective review of cases, patient consent was required, and the institutional review board approved the study protocols.

Diagnosis

      IgG4-SC: AIP was diagnosed using the diagnostic criteria of the Japan Pancreas Society [17]. All 26 patients with AIP showed diffuse or localized narrowing of the main pancreatic duct with or without swelling of the pancreas in imaging studies. All AIP patients showed bile duct involvement in these images. IgG4-related sclerosing cholangitis without AIP (3 patients) was diagnosed based on cholangiopancreatographic findings. The bile duct showed segmental strictures, long strictures with prestenotic dilation, and strictures of the distal common bile duct [10]. No patients with IgG4-related sclerosing cholangitis showed characteristic pancreatographic findings and swelling of the pancreas like AIP without sclerosing cholangitis. In addition, all patients, including both AIP and sclerosing cholangitis

patients, had serological autoimmune abnormalities such as hyper γ-globulinemia (8/29 cases, 28% of cases, average 2.0 g/dL, range 1.3-6.1 g/dL), hyper IgG (18/29 cases, 62% of cases, average 2250 mg/dL, range 1265-6160 mg/dL), hyper IgG4 (27/29 cases, 93% of cases, average 549 mg/dL, 76-2970 mg/dL), or the presence of antinuclear antibodies (14/29 cases, 48% of cases). Two patients with normal levels of serum IgG4 showed characteristic findings on computed tomography and ERCP described above. One of them had antinuclear antibodies, and the other having retroperitoneal fibrosis. Swelling of Vater's ampulla was assessed using an endoscope in all patients according to previously reported criterion [18]. The distribution of pancreatic swelling was also examined by computed tomography.

PSC: PSC was diagnosed based on cholangiographic findings. The bile ducts showed multifocal stricturing and beading on ERCP. Extrahepatic and intrahepatic bile ducts were involved in all of the examined cases. All patients underwent a liver needle biopsy. All biospies revealed chronic cholangiopathic features, such as portal fibrosis, periductal fibrosis, portal inflammation, biliary epithelial damage, bile duct loss, or accumulation of copper-binding protein in periportal hepatocytes. Only a few IgG4-positive plasma cells were present in portal tracts on IgG4 immunostaining. In terms of exclusion criteria, no patients had any medical history for biliary surgery, trauma or choledocholithiasis. Biliary malignancy has not been identified in any patients during the medical follow up until now. Two patients underwent liver transplantation, and explanted livers showed chronic duct destructive cholangitis consistent with PSC. The serum IgG4 concentrations were within the normal range for all 6 patients.

Pancreatobiliary carcinoma: The diagnosis of hepatobiliary carcinomas was made based on radiologic and pathologic findings. Radiologically, 18 patients had

cholangiocarcinoma in their extrahepatic or hilar bile ducts, and 9 patients were diagnosed with pancreatic head cancer. A pathological diagnosis was also made for all 27 patients based on surgical or biopsied specimens. All 27 patients had serum IgG4 concentrations within the normal range.

ERCP and endoscopic biopsies from Vater's ampulla and the bile duct

All ERCP and endoscopic biopsies were performed during hospital stay. ERCP was performed using a duodenoscope (JF-240, TJF-240, TJF-260V; Olympus Medical Systems, Tokyo, Japan). A 1.7-mm-diameter cannula (PR-V416Q; Olympus) was inserted into the main pancreatic duct and bile ducts, cholangiopancreatograms were obtained, and the location of stricture was carefully studied. After documenting the stricture, a 0.035-inch hydrophilic guidewire (stiff-type Jagwire; Boston Scientific Japan, Tokyo, Japan) was advanced to the tip of the cannula, through the stricture, and into the bile duct beyond the stricture. After performing ERCP, all patients underwent endoscopic biopsies using side-opening biopsy forceps (FB-45Q-1; Olympus) from Vater's ampulla and the common bile duct in the same session. The guidewire was left in place and the biliary biopsy forceps were passed alongside and into the bile duct. Bile duct biopsies were taken from the lower and intra-pancreatic bile ducts or other stenotic portions in IgG4-SC patients, the extrahepatic bile duct in PSC patients, and the involved bile duct in pancreatobiliary malignancy patients under fluoroscopic guidance. In all 29 IgG4-SC patients, biopsies were obtained from Vater's ampulla and the common bile duct before corticosteroid therapy. After performing the bile duct biopsies, Vater's ampulla biopsies were taken from the orifice of the common bile duct near the guidewire, and were not taken near

the orifice of pancreatic duct to avoid acute pancreatitis from resultant edema and reduced ductal flow. Procedures were finished without placement of a pancreatic stent. All endoscopic procedures were performed by the same experienced endoscopist (H.K.) while the patient was under conscious sedation with intraveneous pethidine hydrochloride and diazepam. After ERCP-related procedures, 50,000 units of urinastatin were drip-infused twice (day of surgery and the next morning) over a period of 1-2 h. Through a side tube, antibiotic agent was drip-infused twice (once after ERCP-related procedures and once the next morning).

Histological examinations

Histological examination was performed by a pathologist (Y.Z.) blinded to clinical information. The biopsied specimens were fixed in neutral formalin and embedded in paraffin. Sections (4 µm) were cut from each paraffin block and stained with haematoxylin & eosin or examined by immunohistochemistry. The following histological features were assessed for both biopsies: inflammatory cell infiltration (1+, mild; 2+, moderate; 3+, severe) [6] (Figures 1 and 2), plasma cell infiltration (>20 cells/high power filed [HPF]), and eosinophil infiltration (>20 cells/HPF). Mild inflammatory cell infiltration was defined by a small number of inflammatory cells that were scattered throughout the HPF. In severe inflammation, many diffuse inflammatory cells were observed. Samples were considered to have moderate inflammation when they showed between mild and severe inflammation. Although obliterative phlebitis is a characteristic histological feature of IgG4-SC, it was not assessed in this study because it usually occurs in relatively large veins that were not sampled by the endoscopic biopsies.

Immunohistochemistry

IgG4 immunostaining was performed with an autostainer (HX System Benchmark, Ventana Medical Systems, Tucson, AZ) following the manufacturer's instructions. The primary antibody was an anti-IgG4 mouse monoclonal antibody (ZYMED Laboratory Inc., San Francisco, CA). Before incubating with the primary antibodies, the sections were pretreated with proteinase. IgG4-positive plasma cells were counted in the most inflammed HPF ($10\times$ eyepiece and $40\times$ lens) in both the Vater's ampulla and bile duct biopsies.

Statistical analysis

The degrees of inflammatory cell infiltration (mild/moderate/severe), plasma cells, eosinophils, and the numbers of IgG4-positive plasma cells on ampullary and bile duct biopsies were compared between three groups by using one-way analysis of variance or Kruskal-Wallis multiple comparison test. When a significant difference was present, the Tukey or Games/Howell pairwise comparison test for multiple comparisons was used. The numbers of IgG4-positive plasma cells on ampullary and bile duct biopsies and clinical characteristics (swelling of Vater's ampulla or the pancreatic head) in patients with IgG4-SC were also analyzed by using Chi square test and Fisher's test. A probability of $p<0.05$ was considered to be statistically significant. All the analyses were performed by using SPSS II for windows, Version 8.0.1. J (SPSS Inc.,Chicago, Illinois, USA).

Results

Biopsies from Vater's ampulla

The results of the histological examination of the ampullary biopsies are summarized in Table 1. There were no significant differences in the degrees of inflammatory cell infiltration, plasma cell infiltration (>20 cells/HPF), and eosinophil infiltration (>20 cells/HPF) among three disease groups. Three IgG4-SC cases had severe inflammatory cell infiltration (Figure 1C), although they were not associated with the irregular fibrosis that is typically observed in surgical AIP or IgG4-SC specimens.

Biopsies from the common bile duct

The bile duct biopsies showed inflammatory cell infiltration, fibrosis, and stromal edema to varying degrees in each case of IgG4-SC. Of the 29 IgG4-SC patients, 10 (34%) had plasma cell infiltrations greater than 20 cells per HPF, although this increase was not significantly greater than that observed in samples from PSC and pancreatobiliary carcinoma patients (Table 1). Interestingly, 5 of these cases (17% of all IgG4-SC patients, 4 with AIP and one with cholangitis only) showed lymphoplasmacytic infiltration intermixed with irregular fibrosis, which was corresponding to lymphoplasmacytic sclerosing pancreatitis and cholangitis (a pathological term of AIP/IgG4-SC) (Figures 1F and 2A). In addition, eosinophil infiltration (>20/HPF), a characteristic feature of AIP, was only observed in patients with IgG4-SC ($p < 0.05$ vs pancreatobiliary malignancies) (Figure 2B). Carcinoma tissues could be identified on the bile duct biopsy in 25 patients (93%) with pancreatobiliary malignancies.

Immunostaining of IgG4

The numbers of IgG4-positive plasma cells are shown in Table 1 and Figure 3. The

number of IgG4-positive plasma cells in the ampullary biopsies from IgG4-SC patients was

significantly higher than those of patients with PSC and pancreatobiliary carcinomas (p<0.01)

(Figure 4A). The bile duct biopsies also showed a greater number of IgG4-positive plasma cells

in IgG4-SC patients. The number of IgG4-positive plasma cells in the bile duct biopsies from

IgG4-SC patients was significantly higher than those of patients with PSC and pancreatobiliary

carcinomas (p<0.05 and p<0.01, respectively). When 10 IgG4-positive cells/HPF was set as the

cutoff threshold according to previous reports [14,15], the diagnostic rates of the ampullar and

bile duct biopsies were both 52% (15/29 cases). IgG4-positivity was found in both biopsies in 9

patients (31%), and either of them in 12 patients (41%). In total, 21 patients (72%) showed more

than 10 cells/HPF in at least either biopsy (Table 2). The diagnostic sensitivity and specificity of

biopsies were as follows: Vater's ampulla biopsy, sensitivity 52%, specificity 89%; bile duct

biopsy, sensitivity 52%, specificity 96%. All 5 cases showing characteristic lymphoplasmacytic

sclerosing inflammation in the bile duct biopsy had more than 10 IgG4-positive plasma cells in

the bile duct biopsy (Figure 4B). Among non IgG4-SC patients, 4 patients (3 pancreatobiliary

carcinoma and one PSC) showed more than 10 positive cells/HPF in biopsies from Vater's

ampulla or the bile duct, but none of them had more than 20. The false-positive rates for the

ampullary and bile duct biopsies were 9% (3/33 cases) and 3% (1/33 cases), respectively.

Comparison between the number of IgG4-positive cells and clinical characteristics

Swelling of Vater's ampulla was identified in 16 of 29 patients (55%) with IgG4-SC

by the endoscopic examination. Compared between IgG4-SC patients with and without

ampullary swelling, the numbers of IgG4-positive plasma cells were not different in the both

ampullary and bile duct biopsies (Table 3).

Next, the number of IgG4-positive plasma cells was compared between AIP patients

with and without swelling of the pancreatic head. Among 29 IgG4-SC cases, 17 showed

parenchymal swelling in the head of the pancreas by radiological examinations. The swelling in

7 patients also involved the body and tail of the pancreas (diffuse swelling). In the remaining 12

patients, 9 had pancreatic swelling in the body or tail without involvement of the pancreatic

head, and 3 had sclerosing cholangitis only without pancreatic swelling. Interestingly, 13 of the

17 patients (76%) with pancreatic head swelling had more than 10 IgG4-positive plasma cells

per HPF in their bile duct biopsies, which was significantly higher than that observed in patients

without pancreatic head swelling ($p<0.01$) (Table 3). In contrast, there was no significant

difference in the number of IgG4-positive cells in the ampullary biopsies between patients with

and without pancreatic head swelling.

Complications of ERCP and endoscopic biopsies from Vater's ampulla and the bile duct

There were no complications related to ERCP or the biopsy, such as post-ERCP

pancreatitis or cholangitis and bile duct perforation, in any of the patients.

Discussion

The results obtained from this study can be summarized as follows: (1) Out of 29

patients with IgG4-SC, 21 (72%) had more than 10 IgG4-positive plasma cells per HPF in at

least either their ampullary or bile duct biopsy. (2) Compared between biopsies from Vater's

ampulla and the bile duct, the increased number of IgG4-positive plasma cells was the only

histological feature that could be examined to diagnose IgG4-SC in ampullar biopsies. In contrast, eosinophil infiltration, as well as the number of IgG4-positive plasma cells, is a useful feature in bile duct biopsies. (3) In the bile duct biopsies, 10 patients had a large number of plasma cells (>20/HPF). Five of these patients also had lymphoplasmacytic infiltration intermixed with irregular fibrosis, which are histological features that presumably correspond to lymphoplasmacytic sclerosing pancreatitis and cholangitis. (4) The bile duct biopsies were especially useful for IgG4-SC patients with pancreatic head swelling.

Similar to previous reports, this study also revealed that AIP patients had a significantly higher number of IgG4-positive plasma cells in their ampullary biopsies than patients with PSC and pancreatobiliary carcinomas. Kubota *et al.* reported that 18 of 27 patients (67%) with AIP had more than 10 IgG4-positive plasma cells in their ampullary biopsies [14]. Similarly, Kamisawa *et al.* reported that 8 of 10 patients (80%) were highly IgG4-positive (≥10 cells/HPF) [15]. Although the diagnostic rate of the current study was the lowest among three endoscopic studies, 62% (41/66) of the total patients of three studies had more than 10 IgG4-positive cells in the three studies. AIP is typically diagnosed based on a combination of serological, radiological, and pathological examinations [19]. As much data have accumulated in this field, most AIP cases can be diagnosed based on serum IgG4 concentrations and characteristic radiological features [20-22]. However, in some cases it is still difficult to differentiate AIP from pancreatic malignancies. For such cases, IgG4 immunostaining of ampullary biopsies may be one tool to facilitate the diagnosis of IgG4-SC or AIP.

For pathologists, it is not difficult to count the number of IgG4-positive cells in biopsied specimens, although it may be harder to interpret the number of positive cells. A

pathological diagnosis is usually based on haematoxylin & eosin-stained specimens. Therefore,

pathologists might hesitate to make a definitive pathological diagnosis of IgG4-SC or AIP based

only on the number of IgG4-positive plasma cells in ampullary biopsies. For this reason, a bile

duct biopsy might have an advantage compared to the standard ampullary biopsy. Five cases

(17%) in this study had a large number of plasma cells intermixed with irregular fibrosis. This

sclerosing inflammation is a characteristic histological feature of IgG4-related diseases

including AIP, and presumably might be biopsied from the main lesion of the pancreatobiliary

system. Indeed, the histological features of lymphoplasmacytic sclerosing pancreatitis are the

most important characteristics for diagnosing AIP [19]. In addition, all of those cases showed

more than 10 IgG4-positive plasma cells per HPF in their bile duct biopsies. In those cases, a

definitive diagnosis of AIP or IgG4-SC might be possible from the pathologic aspect.

The ability to discriminate between IgG4-SC and PSC is currently an important issue

[23]. Similar to previous reports, this study revealed that IgG4 immunostaining of the ampullar

biopsy is useful for making this distinction. Although a bile duct biopsy is also useful, it should

be noted that more than 10 IgG4-positive plasma cells might be rarely observed in bile duct

biopsies from PSC patients. The pathologic features of IgG4-related sclerosing cholangitis and

PSC are different [6]. IgG4-cholangiopathy inflammation shows a transmural and homogeneous

distribution within the bile duct wall. Erosions or intraluminal proliferation of

xanthogranulatious tissue is rare [6]. IgG4-cholangiopathy is sometimes associated with an

exuberant pseudotumorous inflammatory reaction. In contrast, inflammation is more

pronounced on the luminal side with erosions or ulcerations in patients diagnosed with classical

PSC. Before starting this study, we speculated that these features might be useful for assessing

bile duct biopsies. However, it was difficult to examine the distribution of inflammation on biopsied specimens because only luminal tissues were typically biopsied.

There is limitation in this study. The number of PSC patients is not enough. Further studies are necessary to conclude the usefulness of bile duct biopsies for the discrimination between IgG4-SC and PSC. Another issue is technical difficulty of bile duct biopsy. Biopsy samples are sometimes small in size or with artificial degeneration. The quality of the biopsy samples considerably depends on the experience of endoscopists. Someone might argue that the diagnostic rate for pancreatobiliary carcinoma by the bile duct biopsy seems too high in this study. The possible explanation is that we have actively performed that procedure for the patients suspicious for the malignancy, and have accumulated technical experiences.

Conclusion

This study revealed the usefulness of Vater's ampulla and bile duct biopsies for assessing the number of IgG4-positive plasma cells. Notably, the bile duct biopsy can indicate not only the number of IgG4-positive cells but also the histological features that are directly related to IgG4-SC or AIP.

Disclosure/ Conflicts of interests

The authors declare that there is no conflict of interests.

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Table 1. Histologic features of ampullary and bile duct biopsies.

Biopsy from Vater's ampulla

		IgG4-SC (n=29)	PSC (n=6)	PB carcinoma (n=27)
Number of biopsies	(average and range)	1.4 (1-4)	1.2 (1-2)	1.1 (1-2)
Inflammation	(mild/moderate/severe)	3/23/3	1/5/0	13/12/2
Plasma cells	(>20/HPF)	7 (24%)	4 (67%)	14 (52%)
Eosinophils	(>20/HPF)	1 (3%)	0	0
IgG4-positive cells	(average and range/HPF)	27 (0-162)*	3 (0-5)	2 (0-20)

Biopsy from the bile duct

		IgG4-SC (n=29)	PSC (n=6)	PB carcinoma (n=27)
Number of biopsies	(average and range)	2.4 (1-7)	2.7 (1-7)	2.4 (1-12)
Inflammation	(mild/moderate/severe)	18/9/2	4/1/1	22/5/0
Plasma cells	(>20/HPF)	10 (34%)	2 (33%)	3 (11%)
Eosinophils	(>20/HPF)	5 (17%)**	0	0
IgG4-positive cells	(average and range/HPF)	21 (0-157)†	4 (0-12)	1 (0-4)

IgG4-SC, IgG4-related sclerosing cholangitis; PSC, primary sclerosing cholangitis; PB carcinoma, pancreatobiliary carcinoma; HPF, high power field; IgG4, immunoglobulin G4; *, $p<0.01$ vs. PSC and PB carcinoma; **, $p<0.05$ vs. PB carcinoma; †, $p<0.05$ vs. PSC and $p<0.01$ vs. PB carcinoma.

Table 2. Diagnostic rate of biopsies for IgG4-SC based on the threshold of 10

IgG4-positive cells/high power field.

		Bile duct	
		≤10 cells/HPF	>10 cell/HPF
Vater's ampulla	≤10 cells/HPF	8	6
	>10 cells/HPF	6	9

Diagnostic rate:

Biopsy of Vater's ampulla: 52% (15/29)

Bile duct biopsy: 52% (15/29)

Either ampullary or bile duct biopsy: 72% (21/29)

HPF, high power field

Table 3. Relationship between the number of IgG4-positive plasma cells in the biopsies and swelling of Vater's ampullary or the pancreatic head.

		The number of IgG4-positive plasma cells (/HPF)			
		Ampullary biopsy		Bile duct biopsy	
		≤10	>10	≤10	>10
Swelling of Vater's ampulla	(n=16)	7 (44%)	9 (56%)	6 (37%)	10 (63%)
No swelling of Vater's ampulla	(n=13)	7 (54%)	6 (46%)	8 (62%)	5 (38%)

		The number of IgG4-positive plasma cells (/HPF)			
		Ampullary biopsy		Bile duct biopsy	
		≤10	>10	≤10	>10
Swelling of pancreatic head	(n=17)	6 (35%)	11 (65%)	4 (24%)	13 (76%)*
No swelling of pancreatic head	(n=12)	8 (67%)	4 (33%)	10 (83%)	2 (17%)

IgG4, immunoglobulin G4; HPF, high power field; *, $p<0.01$ vs. patients with no swelling of the pancreatic head.

Figure legends

Figure 1. Degrees of inflammatory cell infiltration in Vater's ampulla and bile duct biopsies. Representative images of mild (A), moderate (B) and severe (C) inflammatory cell infiltration in Vater's ampulla biopsies. Original magnification ×200 (A, B and C). Representative images of mild (D), moderate (E) and severe (F) in bile duct biopsies. Severe inflammatory cell infiltration intermixed with irregular fibrosis is observed in F. Original magnification ×200 (D, E and F).

Figure 2. Histological features of a bile duct biopsy from patients with IgG4-SC. (A) Inflammatory cells consist of lymphocytes, plasma cells, and eosinophils. Original magnification ×400. (B) Numerous eosinophils infiltrate the bile duct wall. Original magnification ×400.

Figure 3. The number of IgG4-positive plasma cells in ampullary and bile duct biopsies. The number of IgG4-positive plasma cells is higher in patients with IgG4-SC than in patients with primary sclerosing cholangitis (PSC) or pancreatobiliary carcinomas. *, $p<0.01$, **, $p<0.05$.

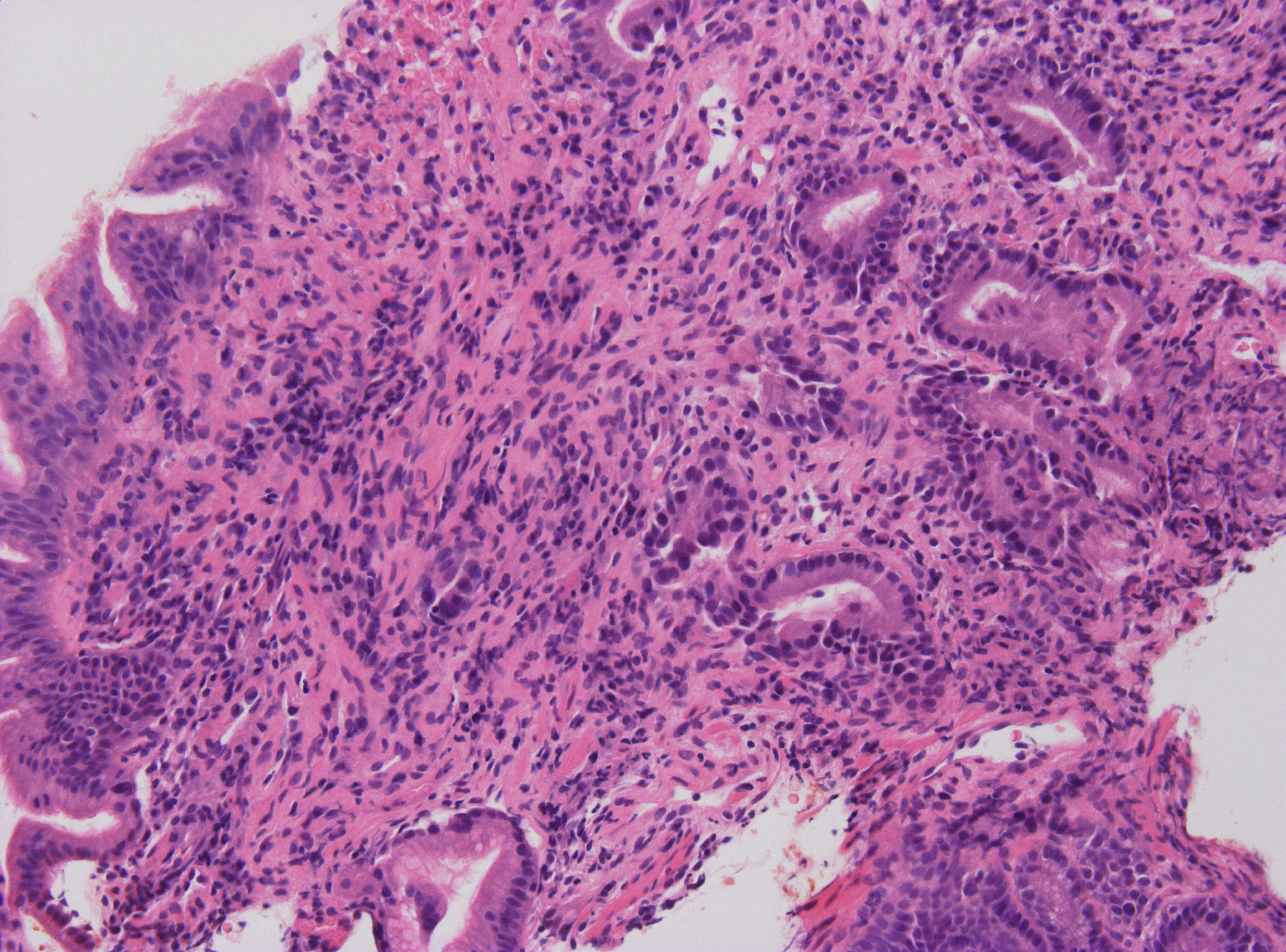**Figure 4. IgG4 immunohistochemistry of a Vater's ampullary or bile duct biopsy (IgG4-SC patients).** (A) Many IgG4-positive plasma cells are observed in an ampullary biopsy. Original magnification ×400. (B) Numerous IgG4-positive plasma cells are observed in a bile duct biopsy. Original magnification ×200.
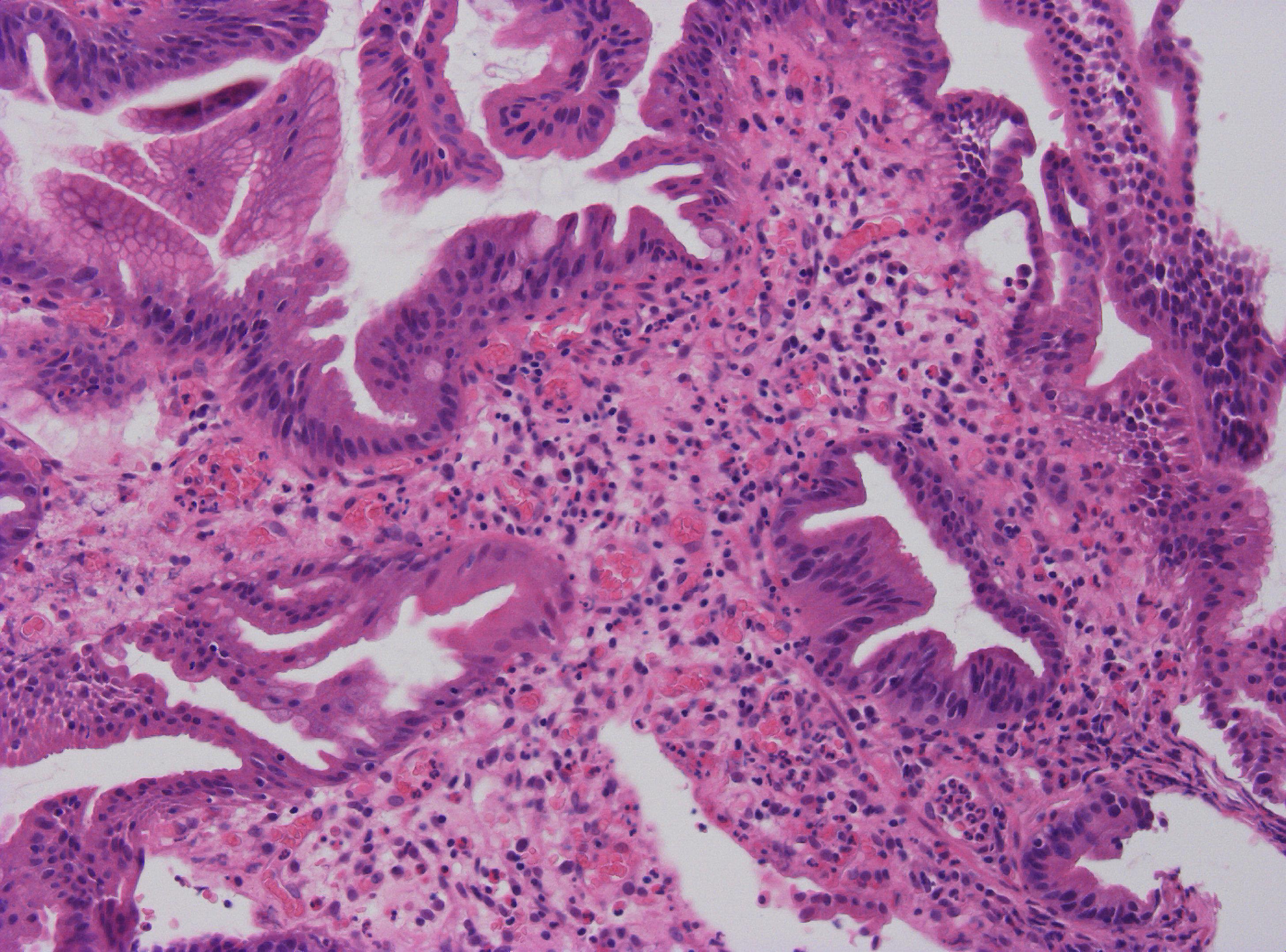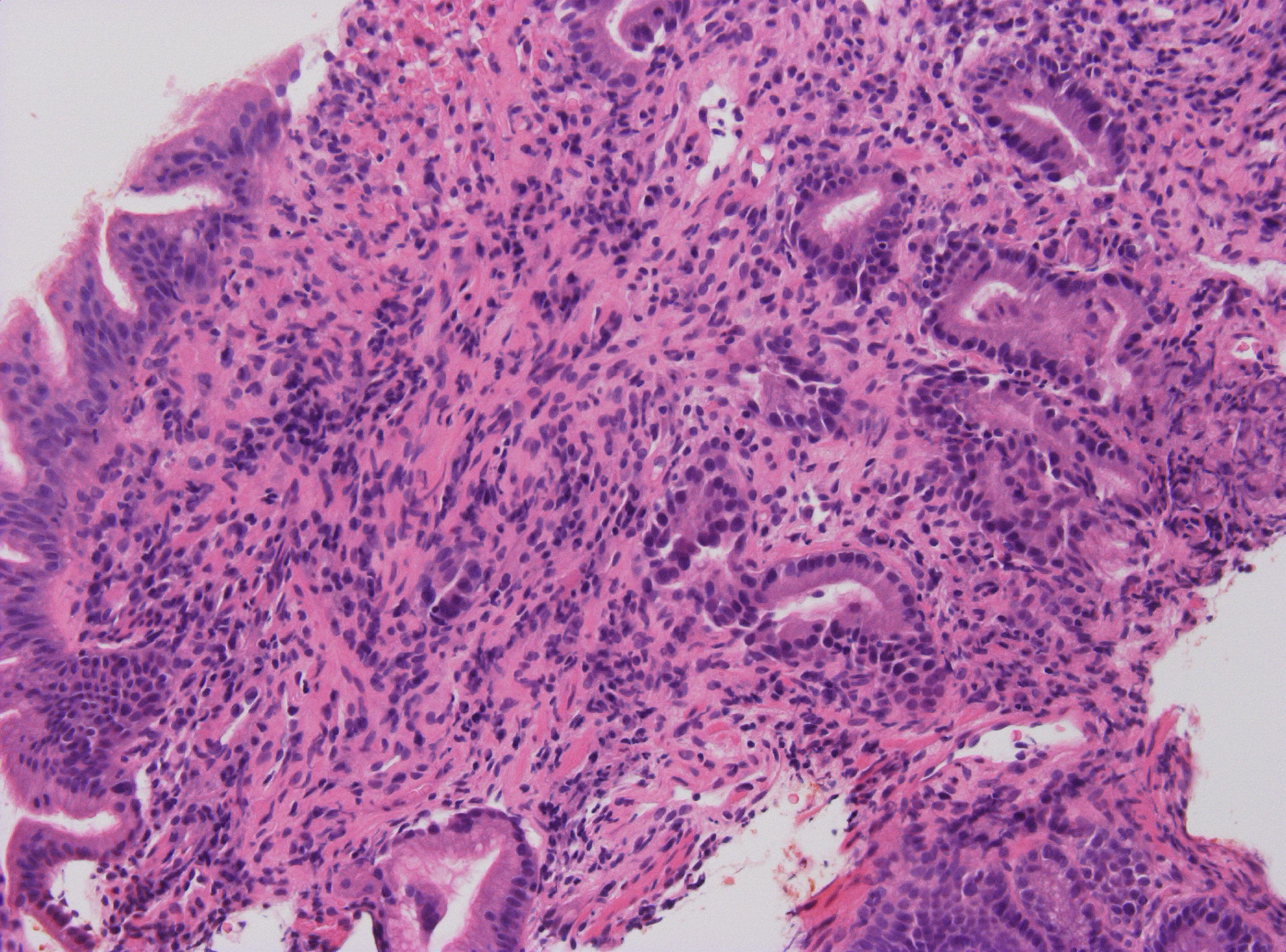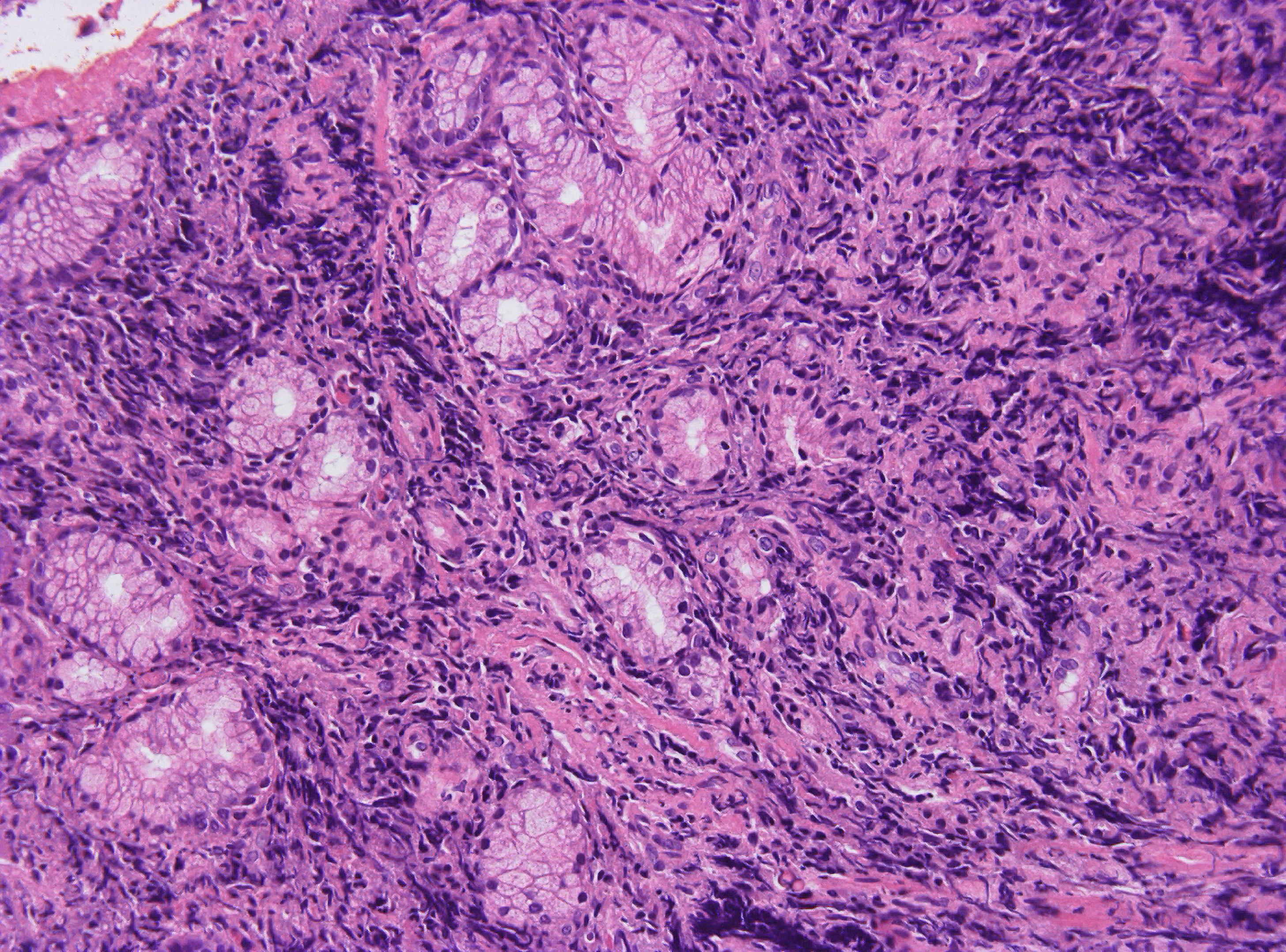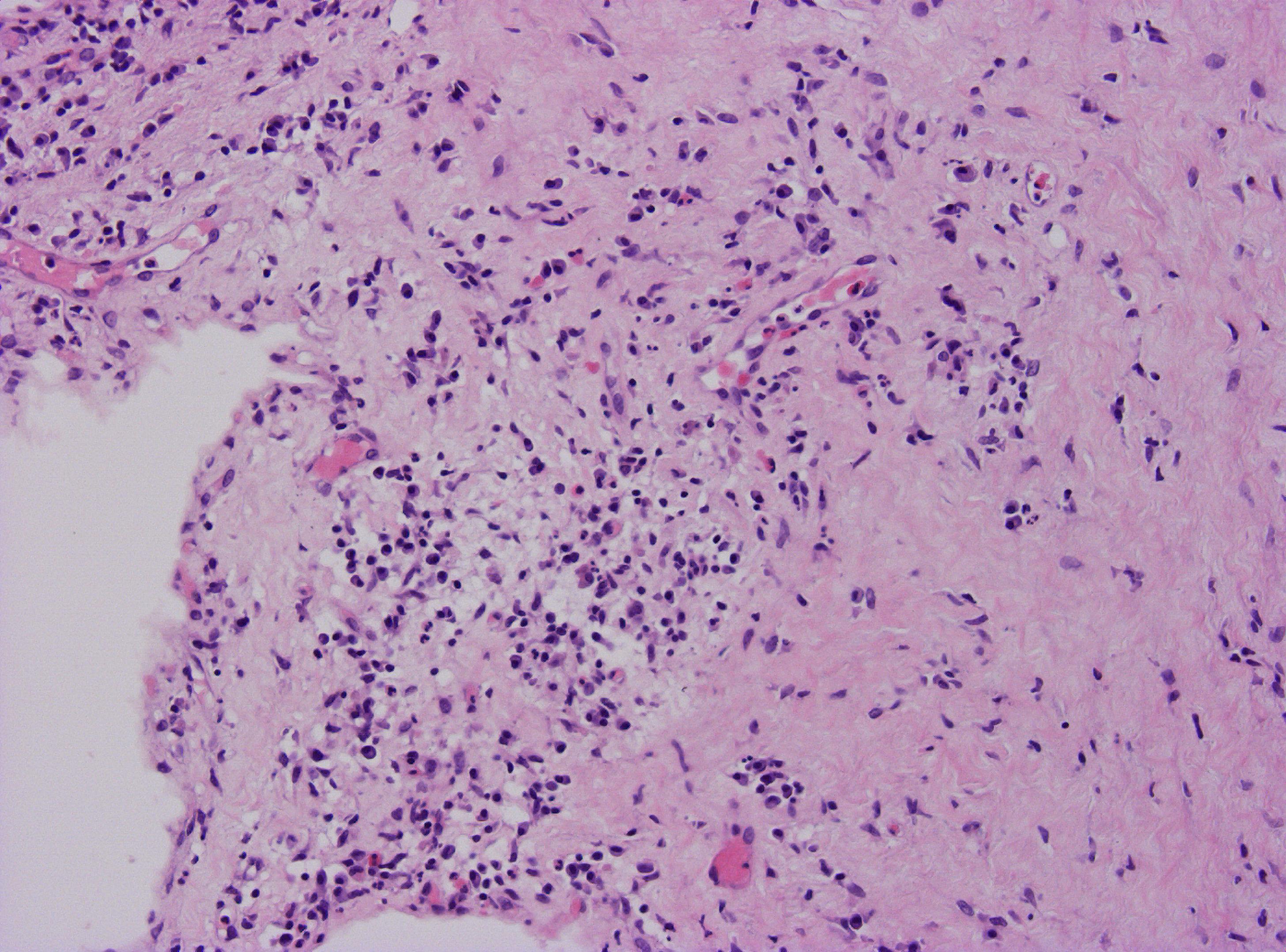
Vater's ampulla biopsy
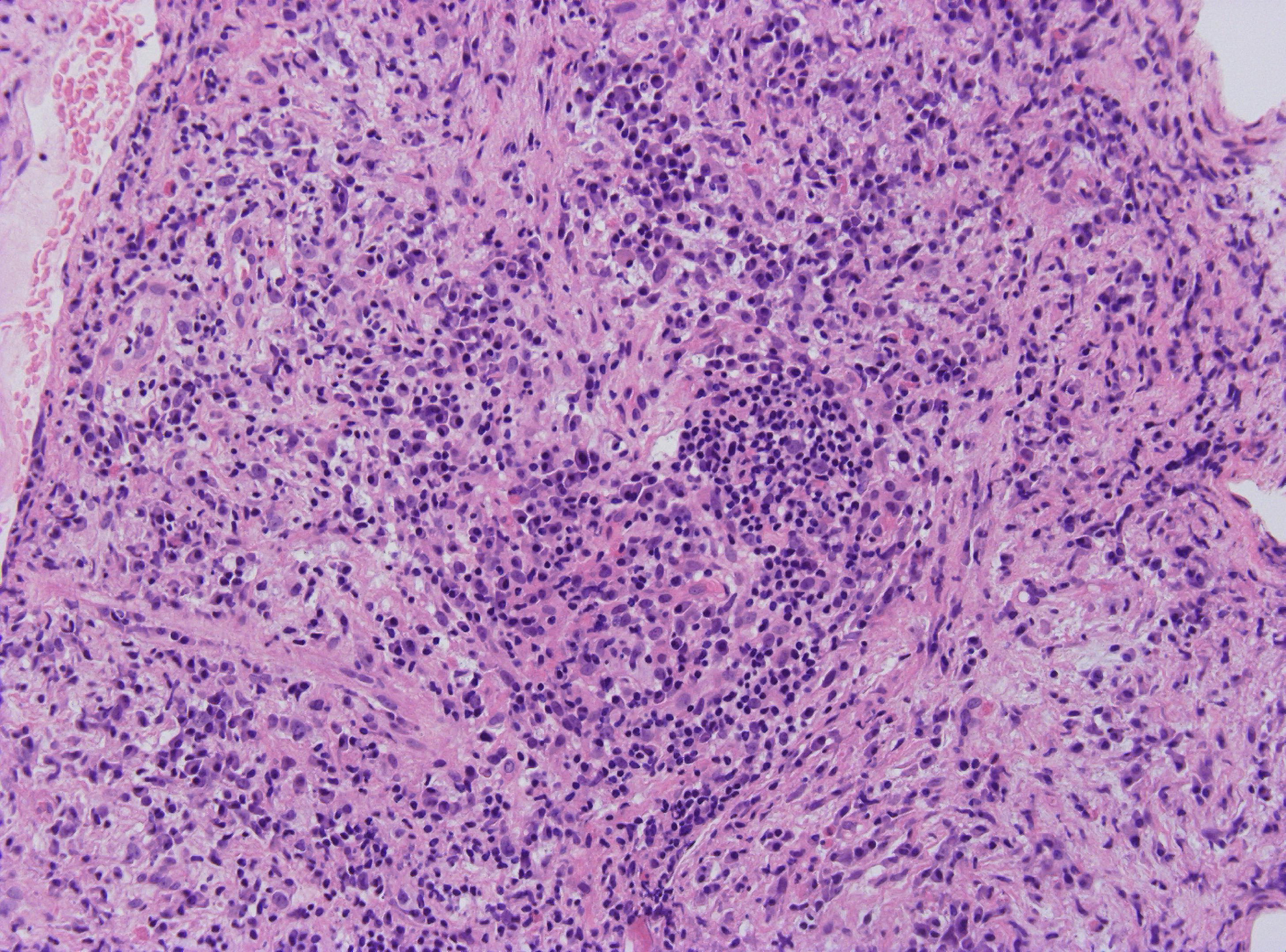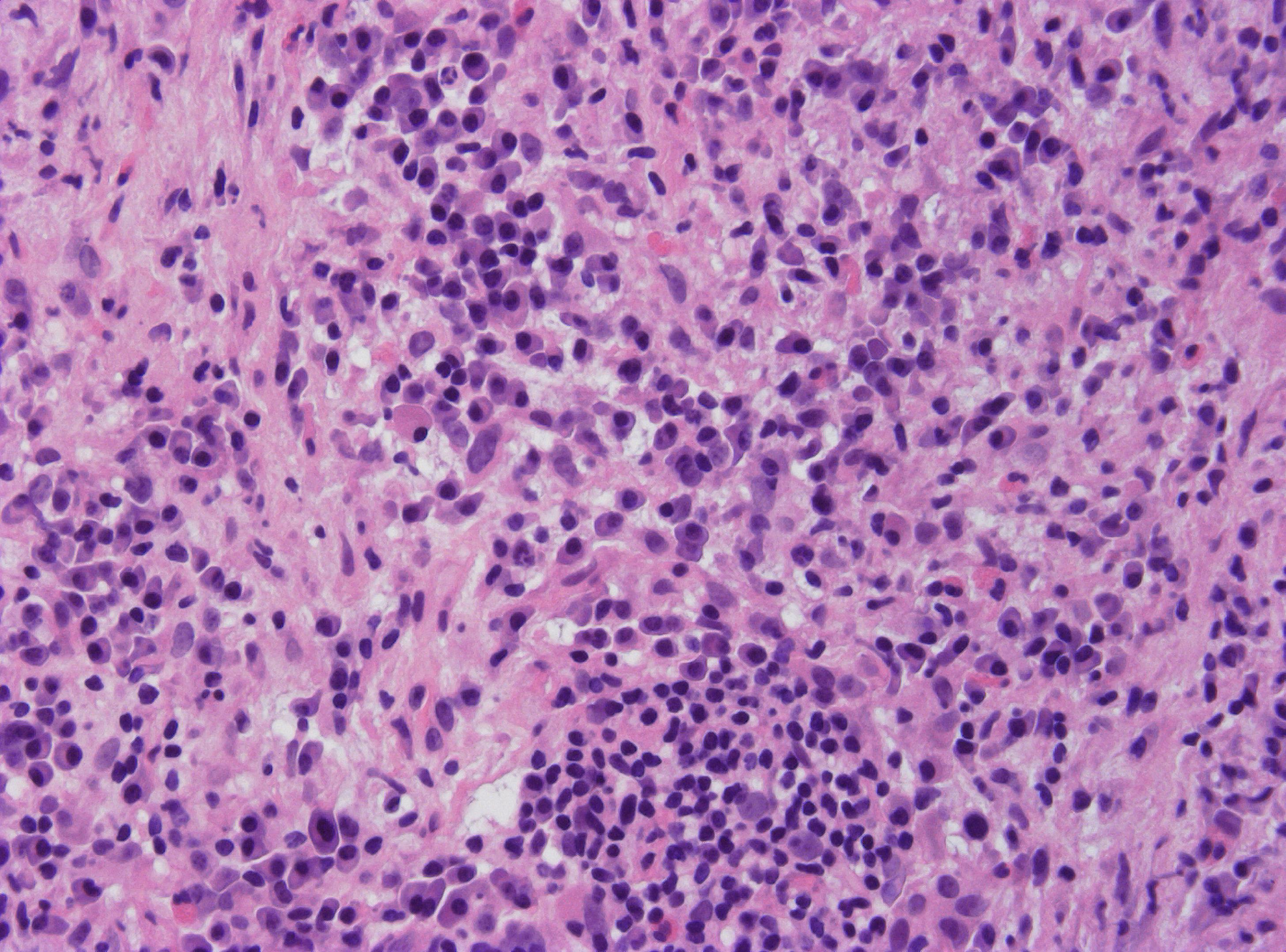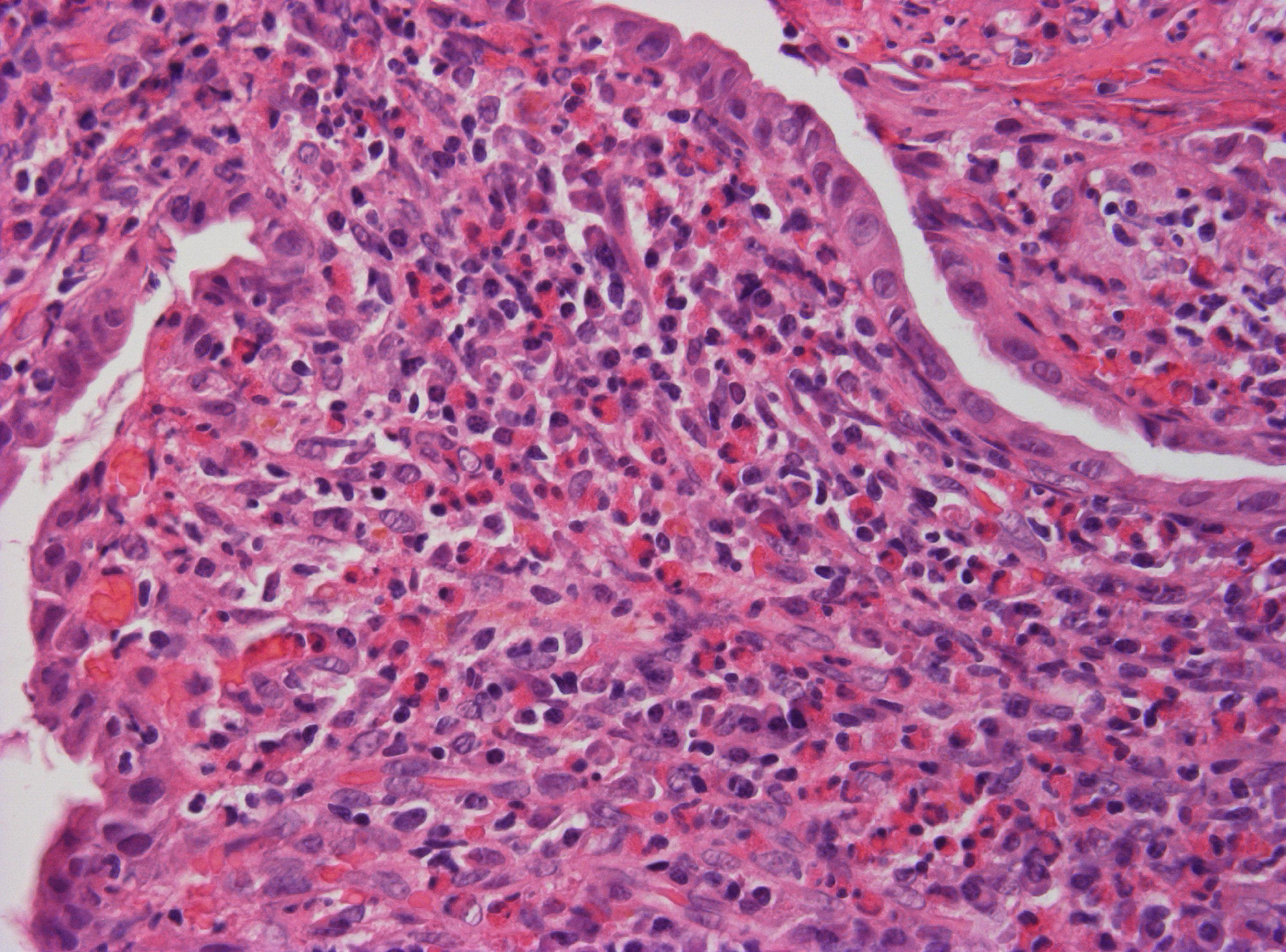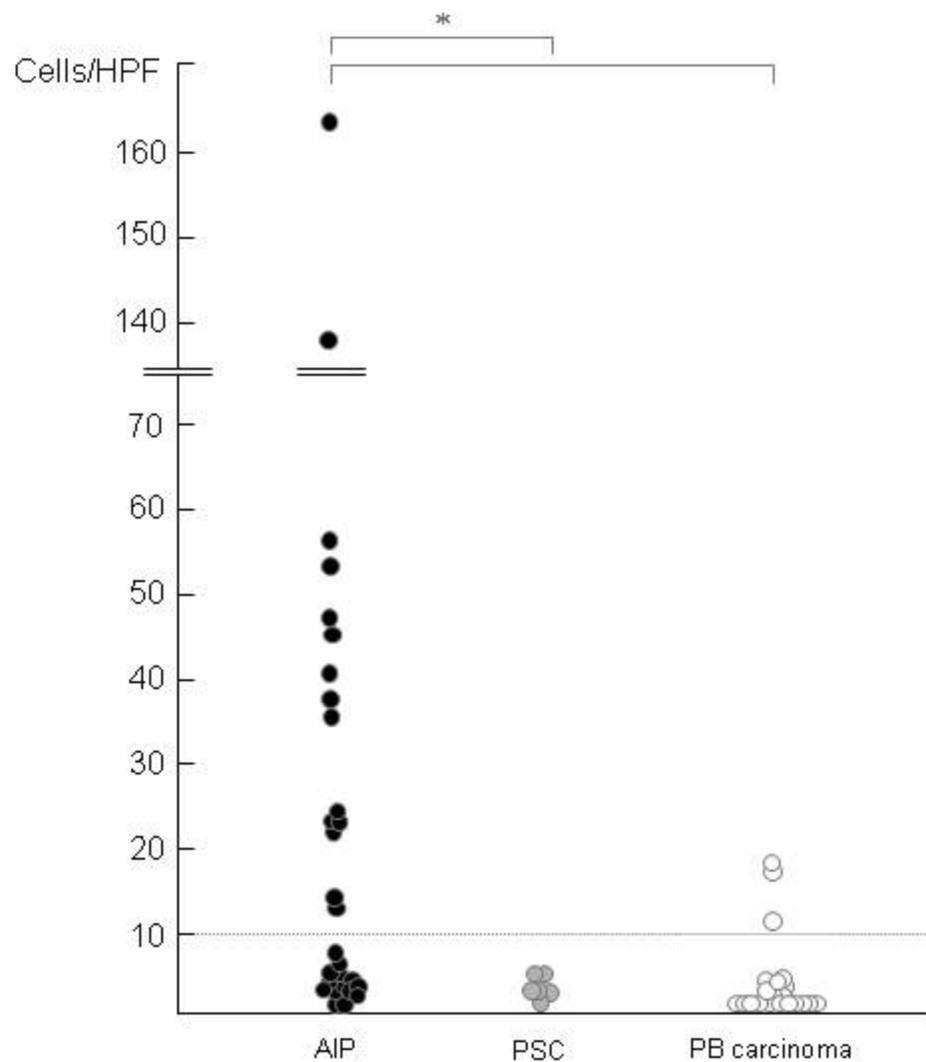
Bile duct biopsy