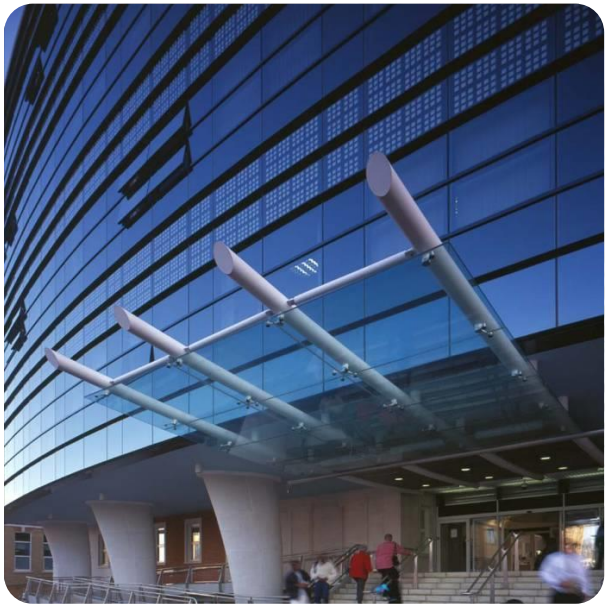
Pancreatico-biliary cytology: a practical approach to diagnosis

Corina Cotoi



Pancreatico-biliary lesions

- Solid:
  - Ductal adenocarcinoma
  - Cholangiocarcinoma
  - Acinar cell carcinoma
  - Neuroendocrine tumour / carcinoma
  - Inflammatory conditions: AIP, CP
  - Splenunculus
  - Lymphoma
  - Metastatic tumours
- Cystic
  - Pre-malignant: IPMN, MCN
  - Benign: serous cystadenoma
  - Low grade malignant potential: solid pseudopapillary neoplasm
  - Cystic transformation of NET
  - Lymphoepithelial cysts

Diagnosis of pancreatic lesions

- Clinical symptoms: ?painless jaundice
- Tumour markers (eg CA19.9)
- Imaging: US, CT, MRI, EUS, ERCP, MRCP, functional imaging…
- Cytology (brushings, FNAs)
- Cystic lesions
  - Fluid CEA >192 ug/ml (can be falsely elevated: lymphoepithelial cysts, mesothelial inclusion cysts)
  - Amylase: very high in pseudocysts (falsely elevated in other cystic lesions)
  - Molecular analysis: K-Ras (IPMN, MCN), GNAS (IPMN)
- Biopsies
- Diagnostic laparoscopy

Solid lesions

- Mass on imaging
- Stricture of CBD, ductal dilatation, double duct sign
- Raised tumour markers
- Biliary brushings or FNA for cytology
- Discussion at the MDM

Cystic lesions

- MRI characterisation of the cyst
- ? tumour markers
- EUS – ?relationship to the main pancreatic duct
- Worrisome features: eg mural nodule, thick wall
- Characterisation of fluid (?mucinous)
- Biochemistry: fluid CEA, amylase
- FNA for cytology
- Discussion at the MDM

Biliary brushings and FNAs

Brushings:

- For investigation of biliary strictures
- During ERCP

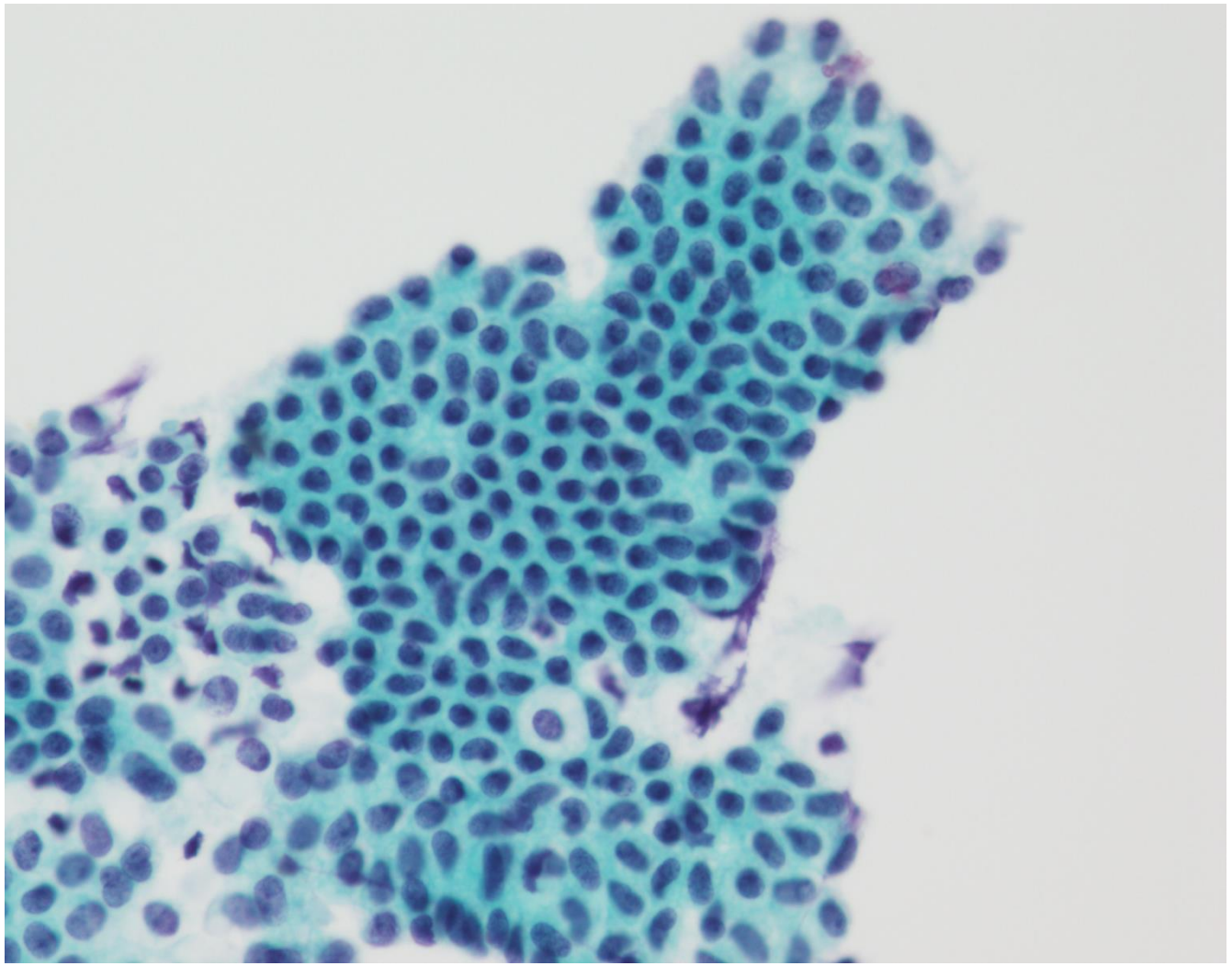
FNA:

- cystic lesions
- +/- solid lesions (TruCut biopsies)
- During EUS

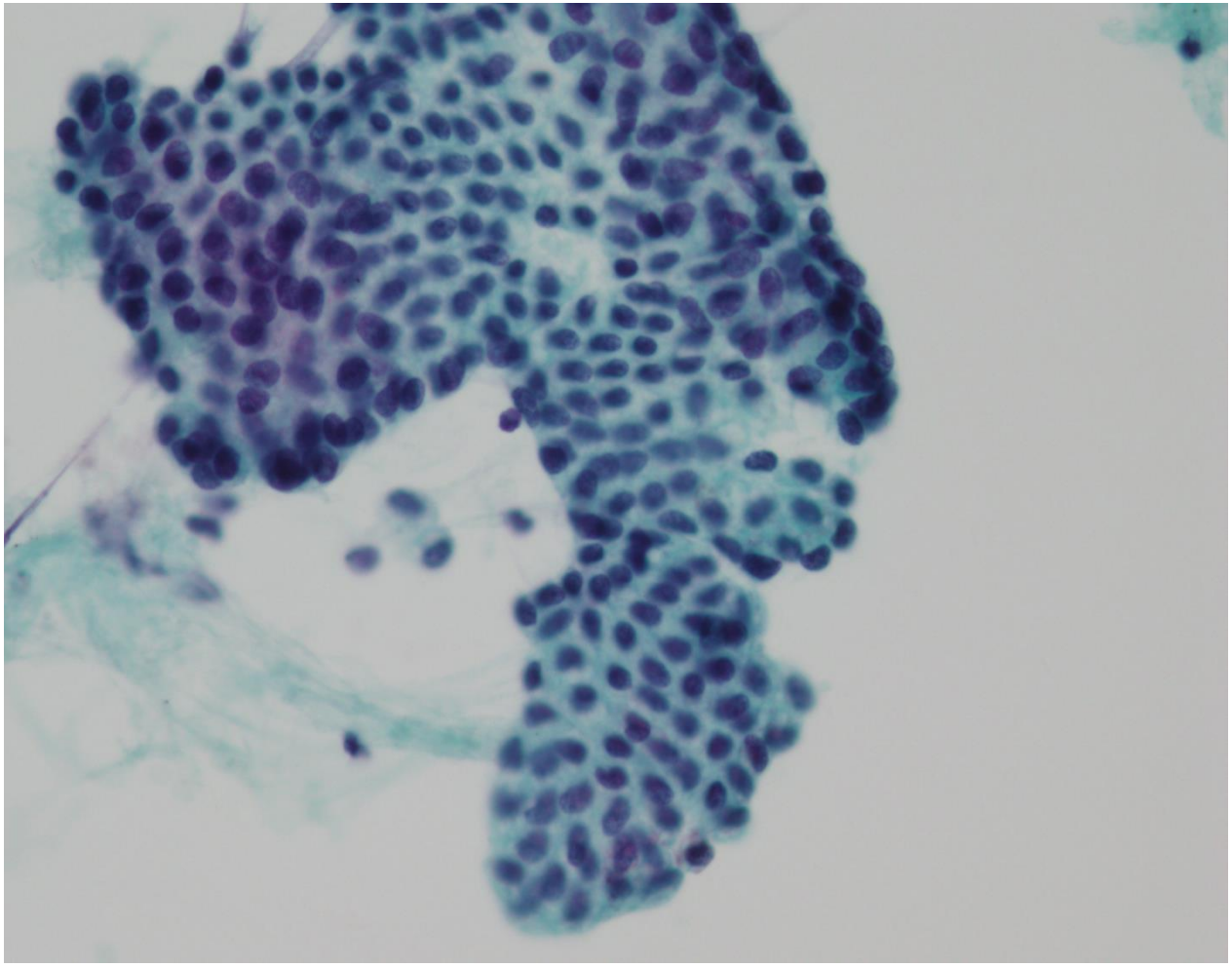
Approach to diagnosis (RCPath guidelines)

- immediate on-site assessment of adequacy
- direct air-dried smears are preferable
- collection in liquid medium (saline or other liquid media for LBC or cell block preparation)
- cytospins are generally suitable for cyst fluid
- Romanowsky staining, Papanicolaou staining, H&E (no benefit over PAP)
- Immunohistochemistry (on cell blocks, LBC samples, cytospins)
- No consensus on standardised reporting (?C1-C5)

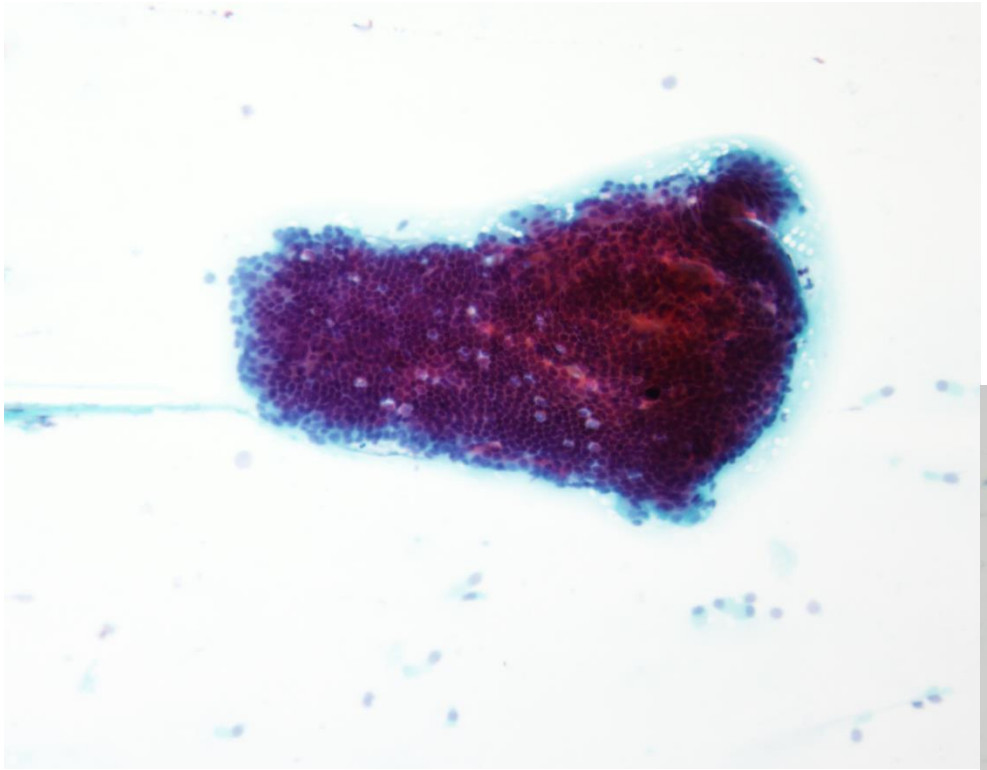
Pancreatic FNA - normal



Biliary brushing - normal

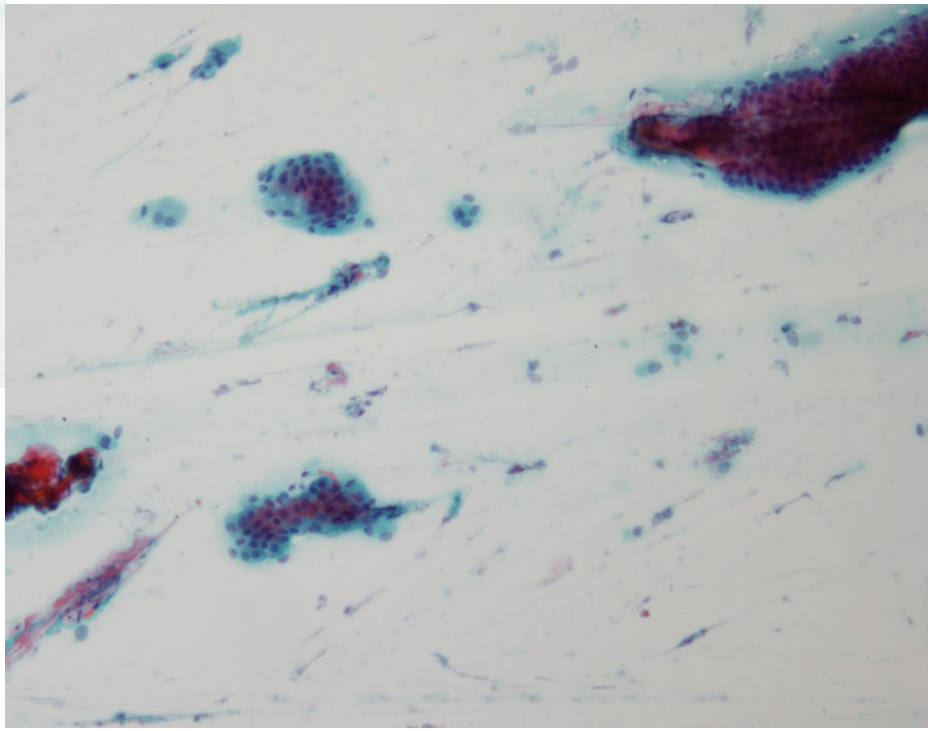


GI contaminant



Gastric:

- Mucin
- Stripped nuclei
- Nuclear grooves
- Cup shaped mucin



Duodenal:

- In general large sheets
- Brush border
- Goblet cells
- Thin mucin

Approach to diagnosis

- Clinical/radiological features are important
- ERCP/EUS report accompanies the cytology request form
- Fluid characterisation and CEA/amylase fluid analysis
- CytoSpin (FNA cyst) and recently ThinPrep (biliary brushings)
- PAP staining very useful in characterising the cellular nuclear features
- KRAS/GNAS mutation analysis for mucinous cystic lesions – not routine practice

Approach to diagnosis

- Adequacy: the context is important, presence of epithelial cells is not always a sign of non-diagnostic sample
- Background
  - Thick mucin
  - Inflammation
  - Necrosis
- Epithelial cells: ?double population, atypical single cells
  - Cytoplasm: mucinous, granular…
  - Nuclei: anisokaryosis (more that 1:3-1:4), nuclear crowding, irregular nuclear contours, prominent nucleoli, abnormal chromatin pattern, increased nuclear:cytoplasmic ratio…

Standardised reporting

- Standardized terminology and nomenclature for pancreatobiliary cytology: The Papanicolaou Society of Cytopathology Guidelines

Martha B. Pitman, Barbara A. Centeno, Syed Z. Ali, Muriel Genevay, Ed Stelow, MD,Mari Mino-Kenudson, Carlos Fernandez-del Castillo, C. Max Schmidt, William R. Brugge, and Lester J. Layfield

PROPOSED PANCREATOBILIARY TERMINOLOGY CLASSIFICATION SCHEME

- I Non-diagnostic
- II Negative (for malignancy)
- III Atypical
- IV Neoplastic: Benign or Other
- V Suspicious (for malignancy)
- VI Positive/malignant

Standardised reporting

CATEGORY I – Non-diagnostic

- **no diagnostic or useful information** about the solid or cystic lesion
- for example, an **acellular aspirate of a cyst without evidence of a mucinous etiology** (such as thick colloid-like mucus, elevated CEA or KRAS/GNAS mutation - see Category IV).
- Any cellular atypia precludes a non-diagnostic report

Standardised reporting

CATEGORY II Negative (for malignancy)

- **Descriptive,** without a diagnosis of a specific condition such as chronic pancreatitis or pseudocyst
- **not synonymous with a benign lesion**
- **adequate cellular and/or extracellular tissue** to evaluate or define a lesion that is identified on imaging.
- **should give a specific diagnosis when practical** including:
  - Benign pancreatobiliary tissue in the setting of vague fullness and no discrete mass
  - Acute pancreatitis
  - Chronic pancreatitis
  - Autoimmune pancreatitis
  - Pseudocyst
  - Lymphoepithelial cyst
  - Splenule/accessory spleen

Standardised reporting

CATEGORY III: ATYPICAL

- **reactive changes**
- **low cellularity**
- **premalignant changes** (dysplasia, PanIN)
- **observer caution in diagnosis**.

- **raises the possibility of a neoplasm**
- may be suggestive of a low-grade neoplasm, but the cytological findings are **insufficient to be suspicious for a high-grade malignancy / tissue is insufficient for confirmation of a specific diagnosis**

- **cytoplasmic, nuclear, or architectural features** that are **not** consistent with **normal or reactive** cellular changes of the pancreas or bile ducts and are **insufficient to classify them as a neoplasm or suspicious for a high-grade malignancy**
- insufficient to explain the lesion seen on imaging
- **Follow-up evaluation is warranted**.

Standardised reporting

CATEGORY IV: NEOPLASTIC

Category IVA: Neoplastic: Benign

- cytological specimen sufficiently cellular and representative
- with or without the context of clinical, imaging and ancillary studies
- **diagnostic of a benign neoplasm**

Standardised reporting

CATEGORY IV: NEOPLASTIC

Category IVB: Neoplastic: Other

- pre-invasive, **premalignant neoplasms** (IPMN and MCN with low, intermediate or high grade dysplasia)
- **low-grade malignant behaviour**
  - pancreatic neuroendocrine tumor (pNET)
  - solid-pseudopapillary neoplasm (SPN) that
- warrant distinction from aggressive, high-grade malignancies

Standardised reporting

CATEGORY V: SUSPICIOUS (FOR MALIGNANCY)

- ! “**Suspicious for**” is **NOT “diagnostic of**”
- Correlation with the clinical and radiological findings
- **insufficient number of the typical features of a specific malignant neoplasm**
- **qualitatively and/or quantitatively insufficient for a conclusive diagnosis of malignancy**
- **tissue is not present for ancillary studies** to define a specific neoplasm
- **malignancy is considered more probable than not**

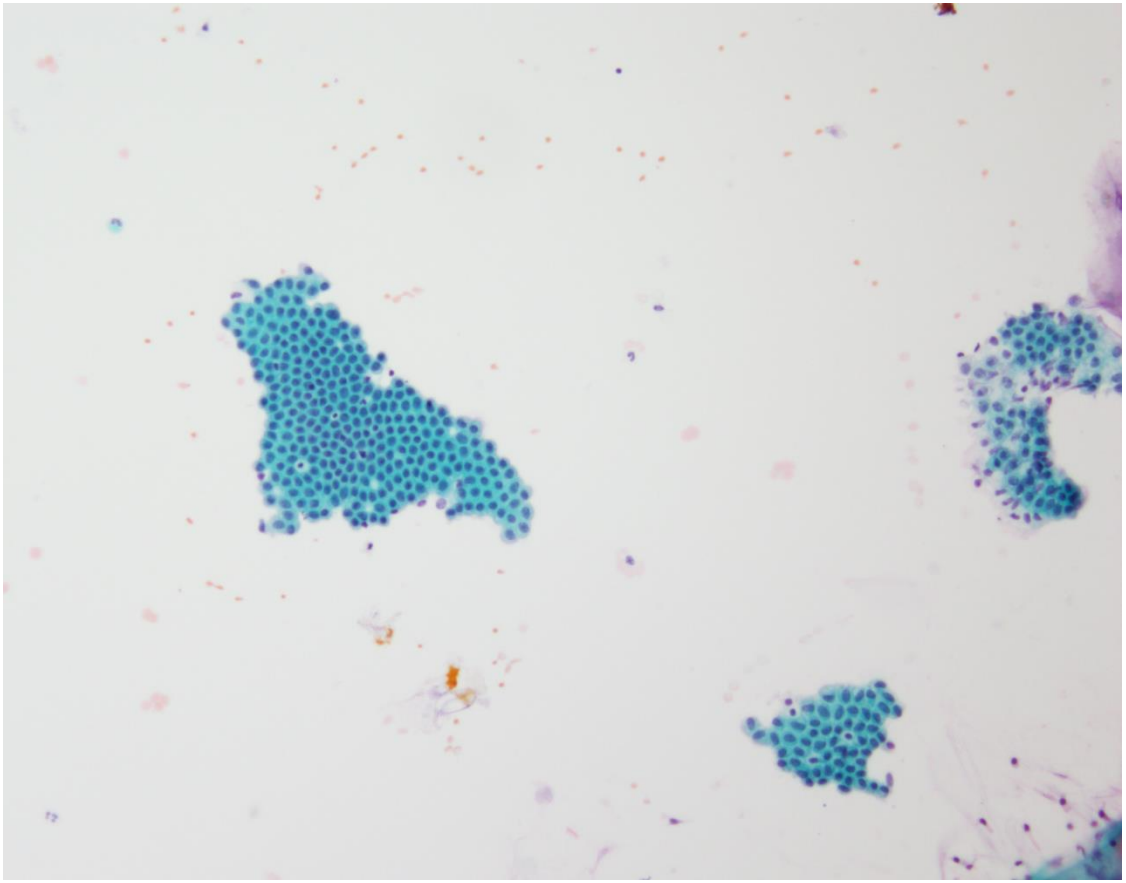
Standardised reporting

CATEGORY VI: POSITIVE OR MALIGNANT

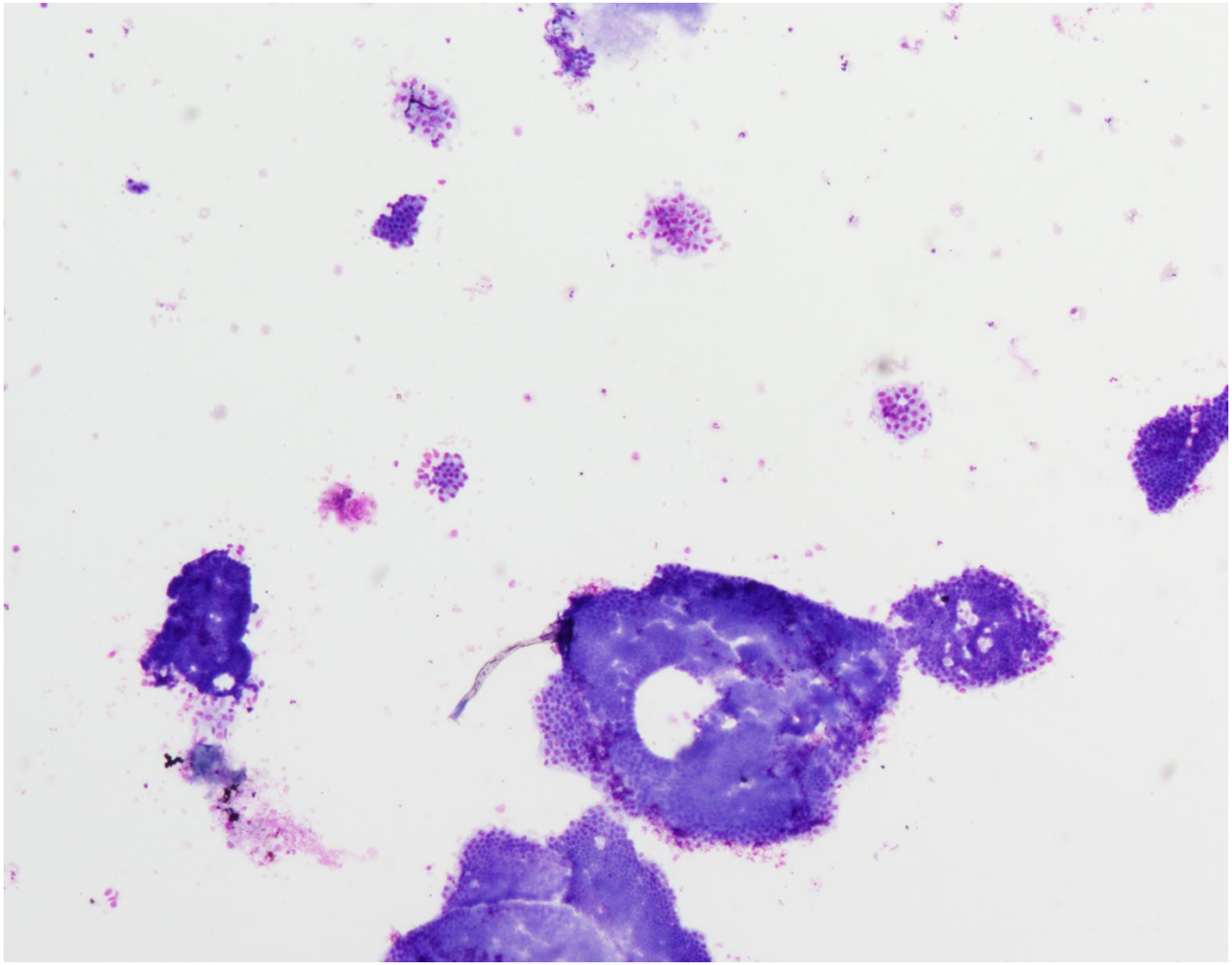
- **ductal adenocarcinoma and other high-grade malignancies**
- unequivocally display malignant cytologic characteristics
- includes **PDAC** and its variants, **cholangiocarcinoma**, **acinar cell carcinoma**, **high-grade neuroendocrine carcinoma** (small cell and large cell), **pancreatoblastoma**, **lymphomas**, **sarcomas** and **metastases** to the pancreas

Case 1 - cytology

80 y.o. female,
Hilar stricture ? malignancy.



Case 1 - cytology

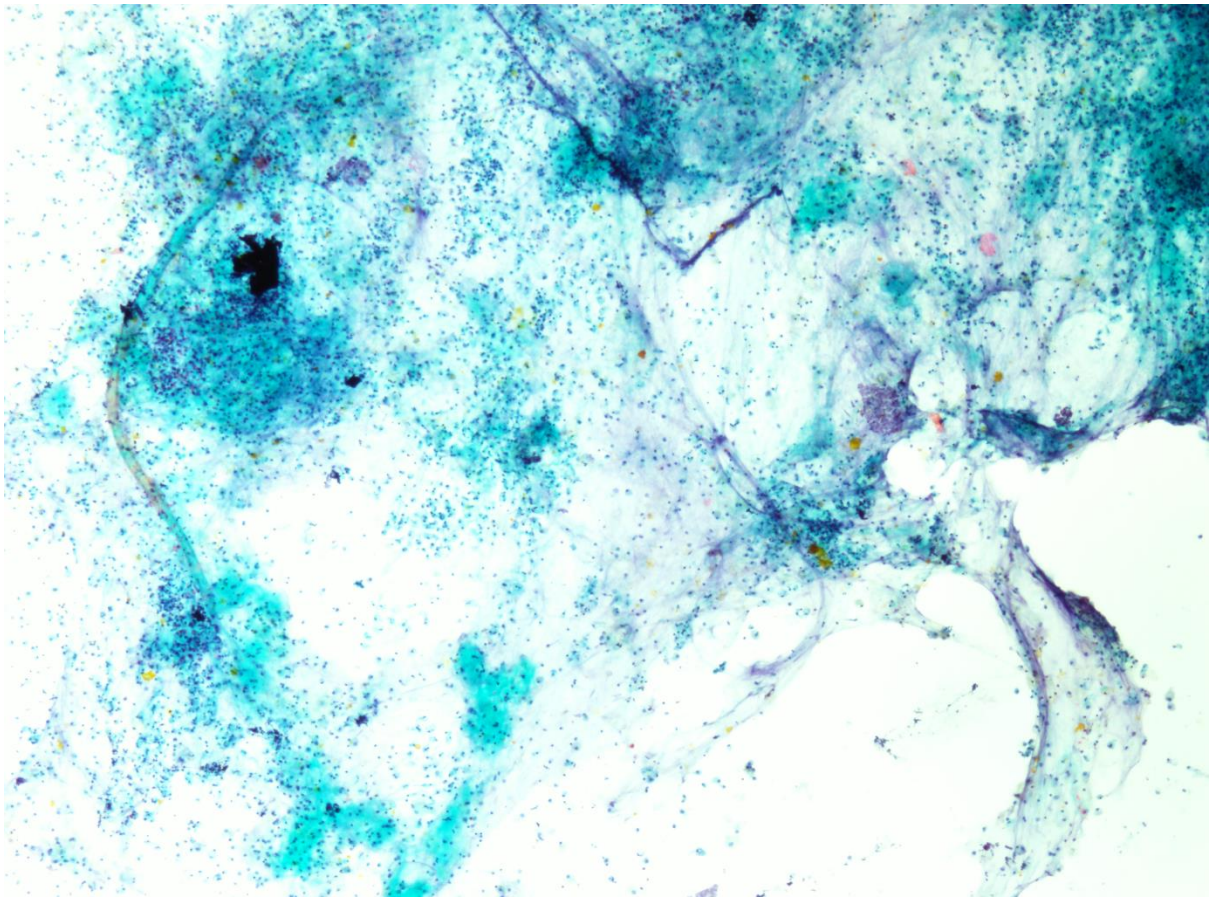


Case 1 - diagnosis

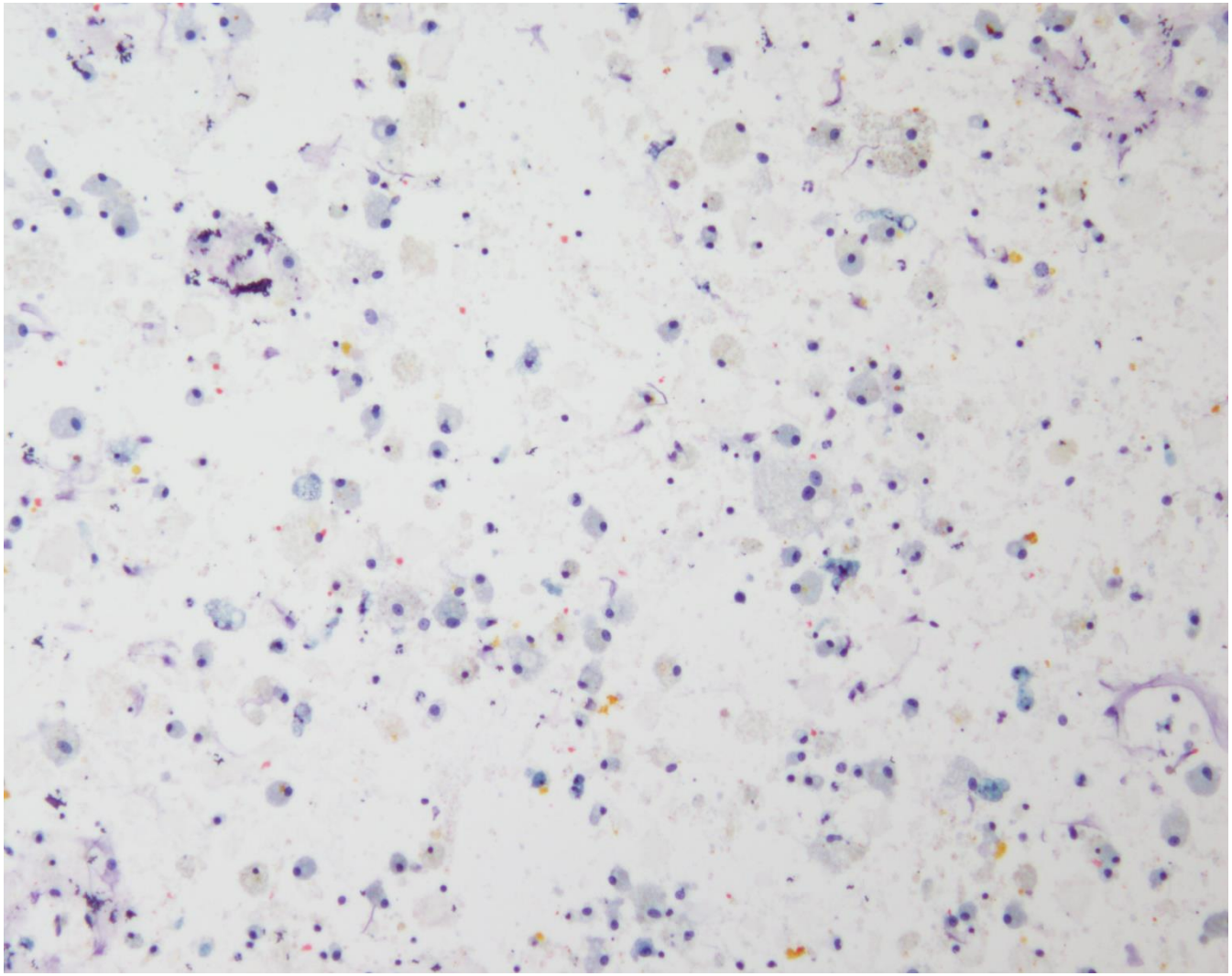
Cellular sample, satisfactory for evaluation.
Negative for malignancy (*reporting category II**).

Case 2 - cytology

53 y.o male,
EUS: cystic structure with features of pseudocyst
fluid amylase of 27000IU/L



Case 2 - cytology



Case 2 - diagnosis

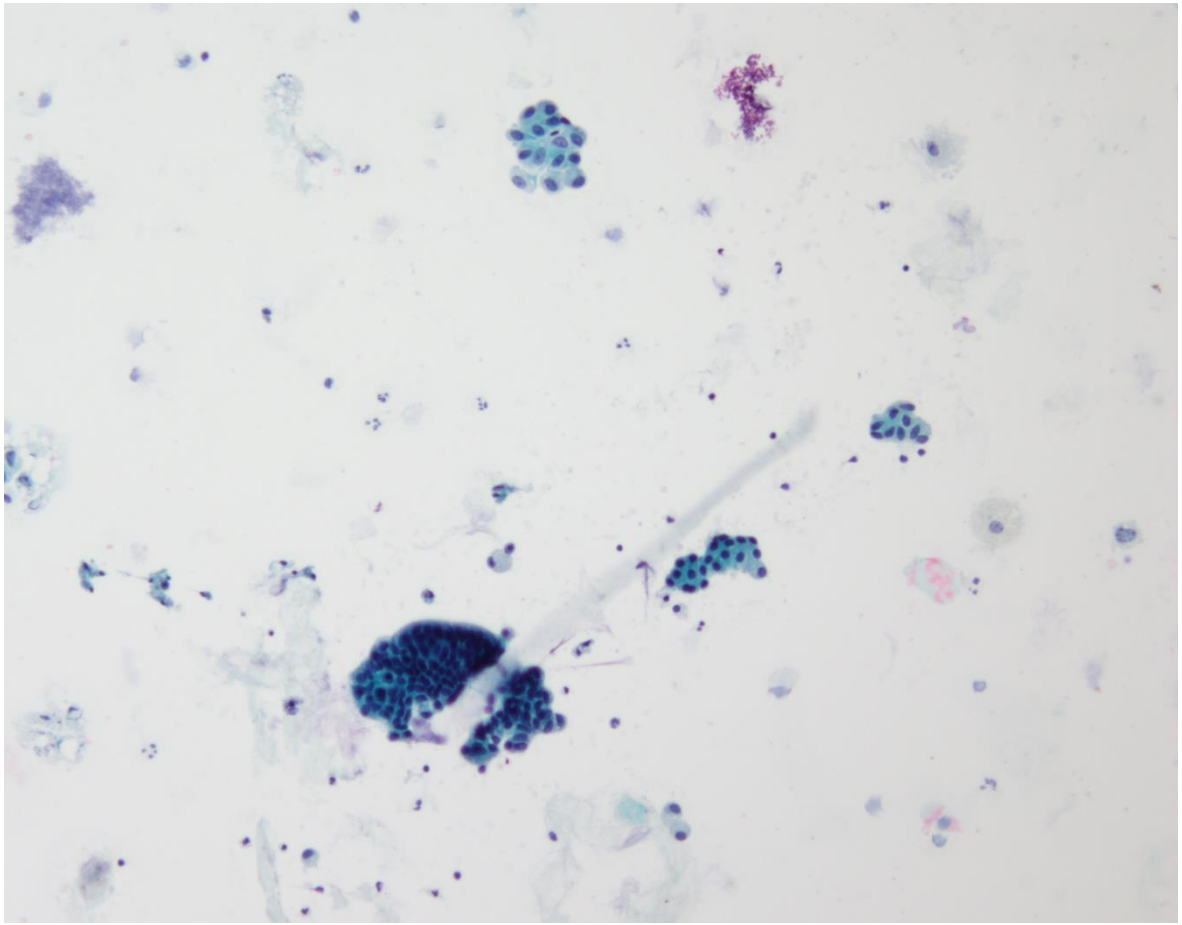
Satisfactory for evaluation.

Negative for malignancy (*reporting category II**). See comment.

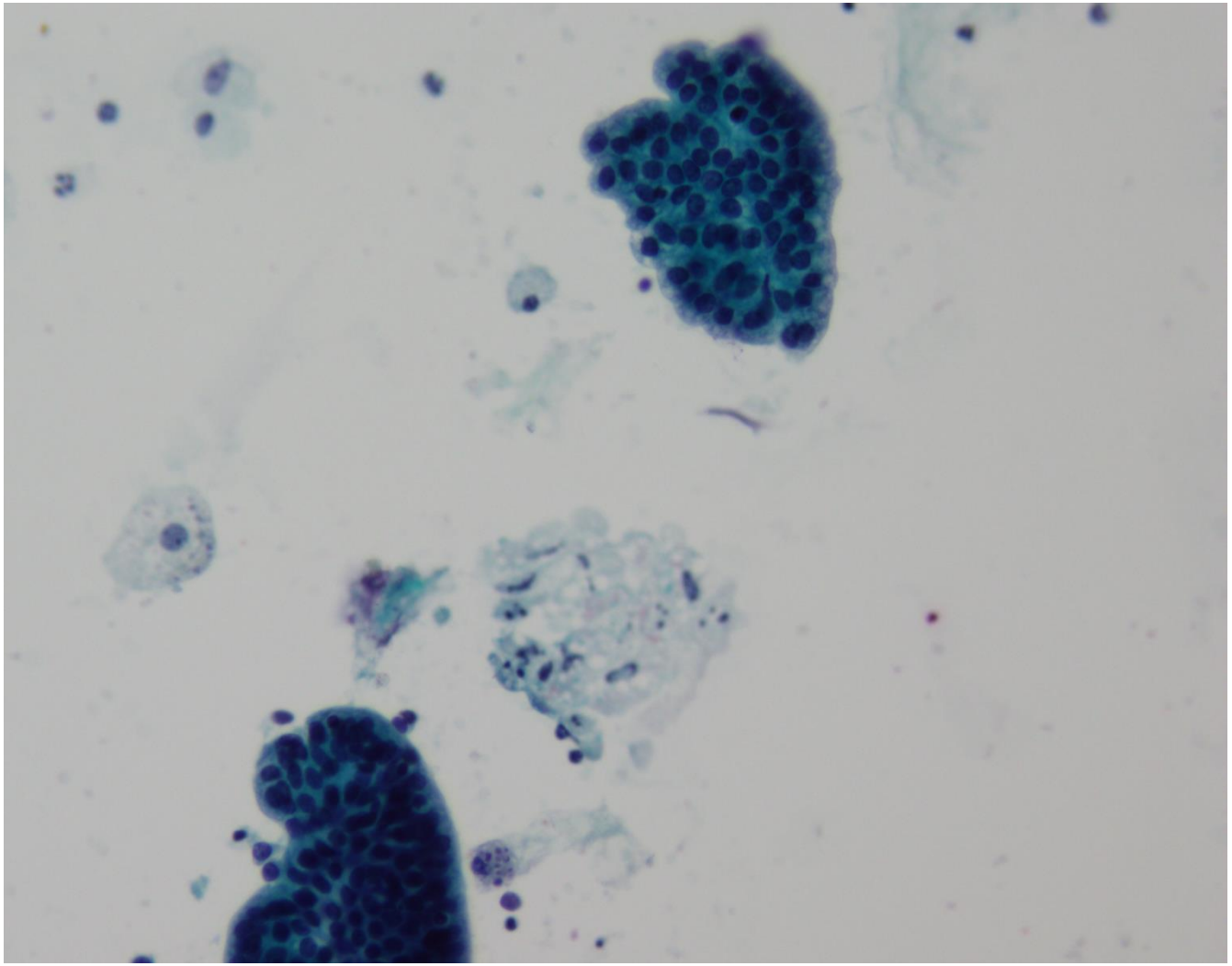
COMMENT: The findings are in keeping with the clinical impression of a pseudocyts (fluid amylase of 27000IU/L supports this diagnosis).

Case 3 - cytology

56 y.o. female
Known PSC with biliary stricture of the right hepatic duct. ERCP and biliary brushings.



Case 3 - cytology



Case 3 - diagnosis

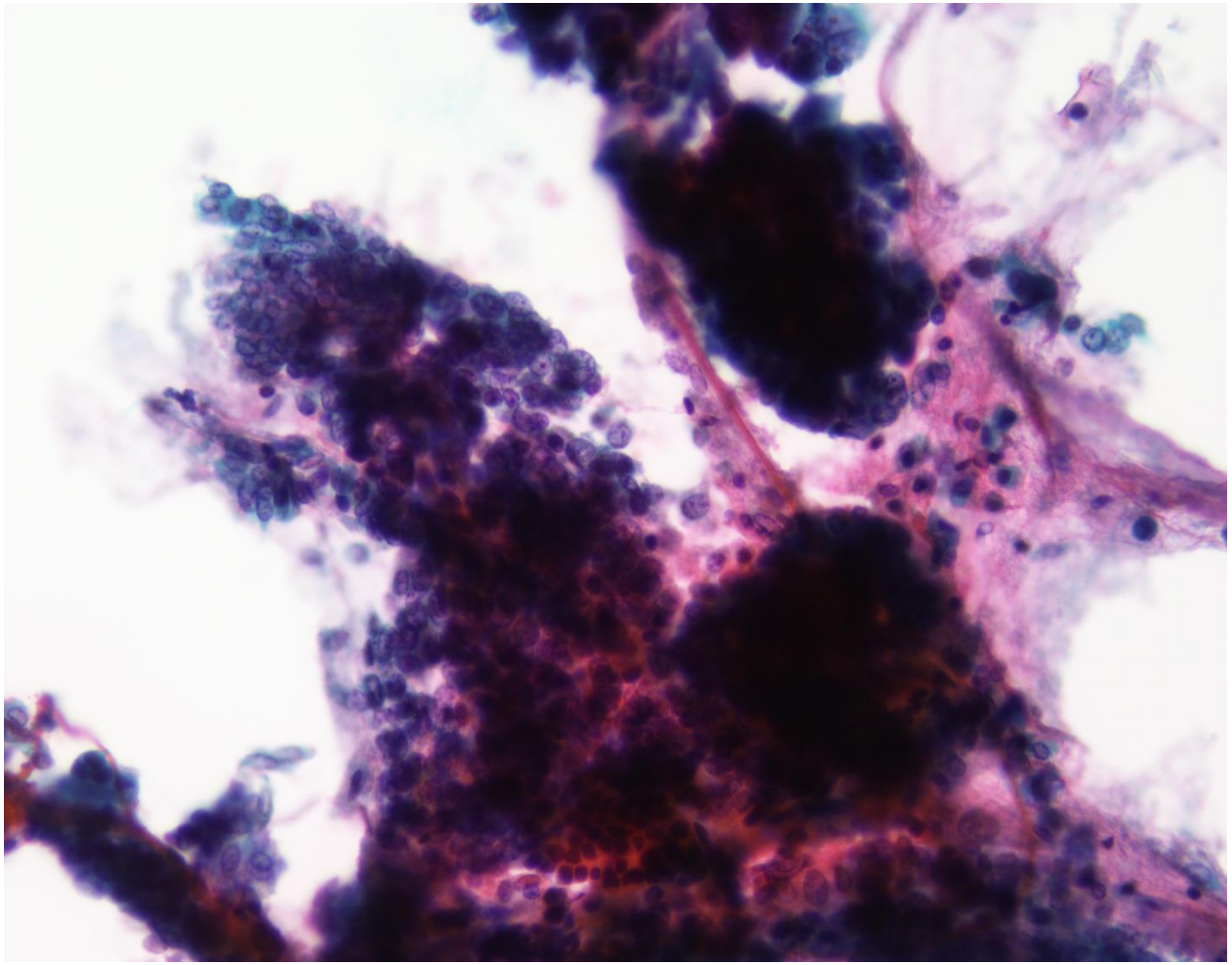
Cellular sample, satisfactory for cytological evaluation.
Atypical *(reporting category III*)*. See comment.

COMMENT: There is mild epithelial atypia, interpreted as reactive rather than neoplastic in the context of PSC

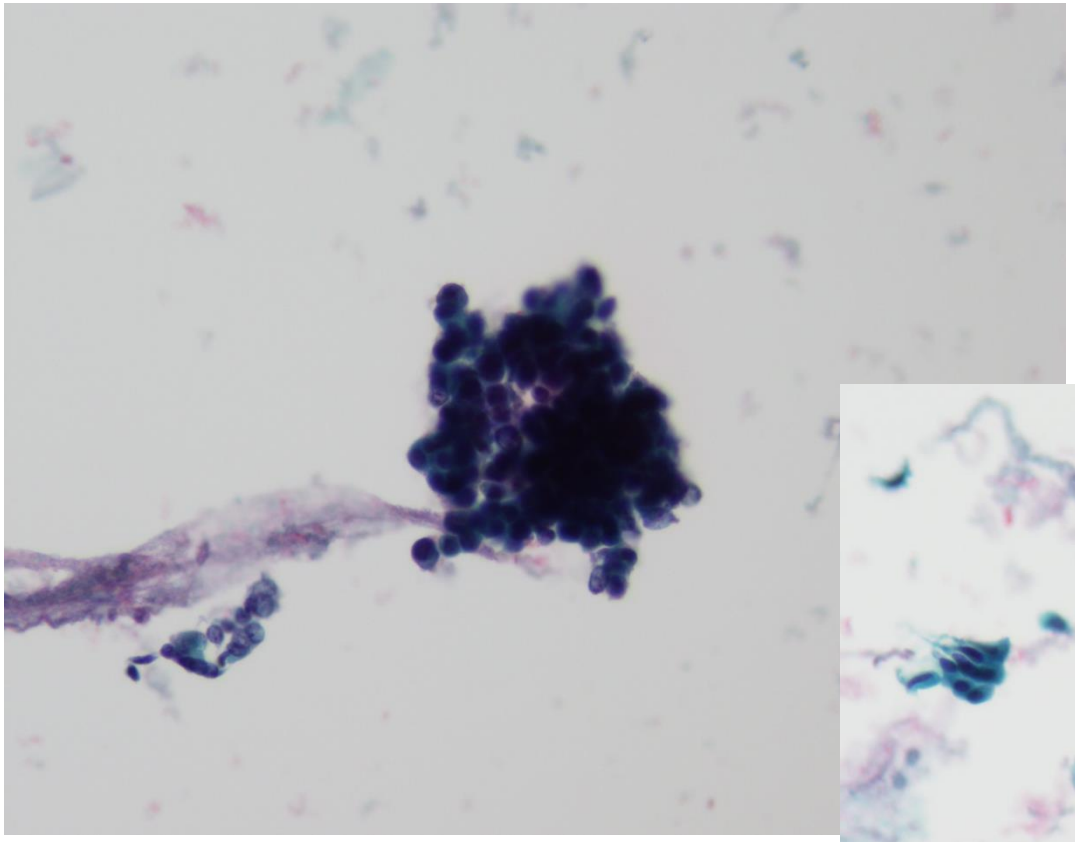
Please note that chronic cholangiopaties are inflammatory conditions that lead to severe reactive atypia, sometimes difficult to differentiate from neoplasia.
FISH, NGS: used in some centres, but not routine practice

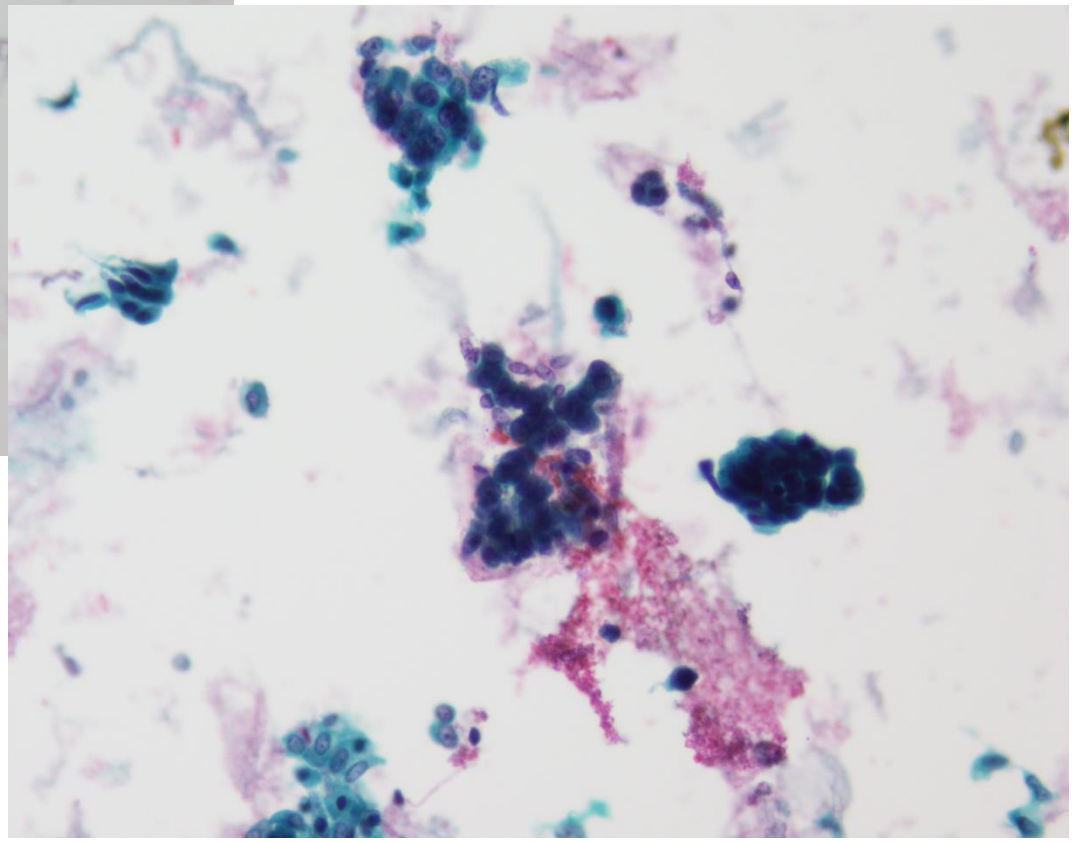
Case 4 - cytology

75 y.o male
Biliary obstruction and pancreatic mass. ERCP and brushings.



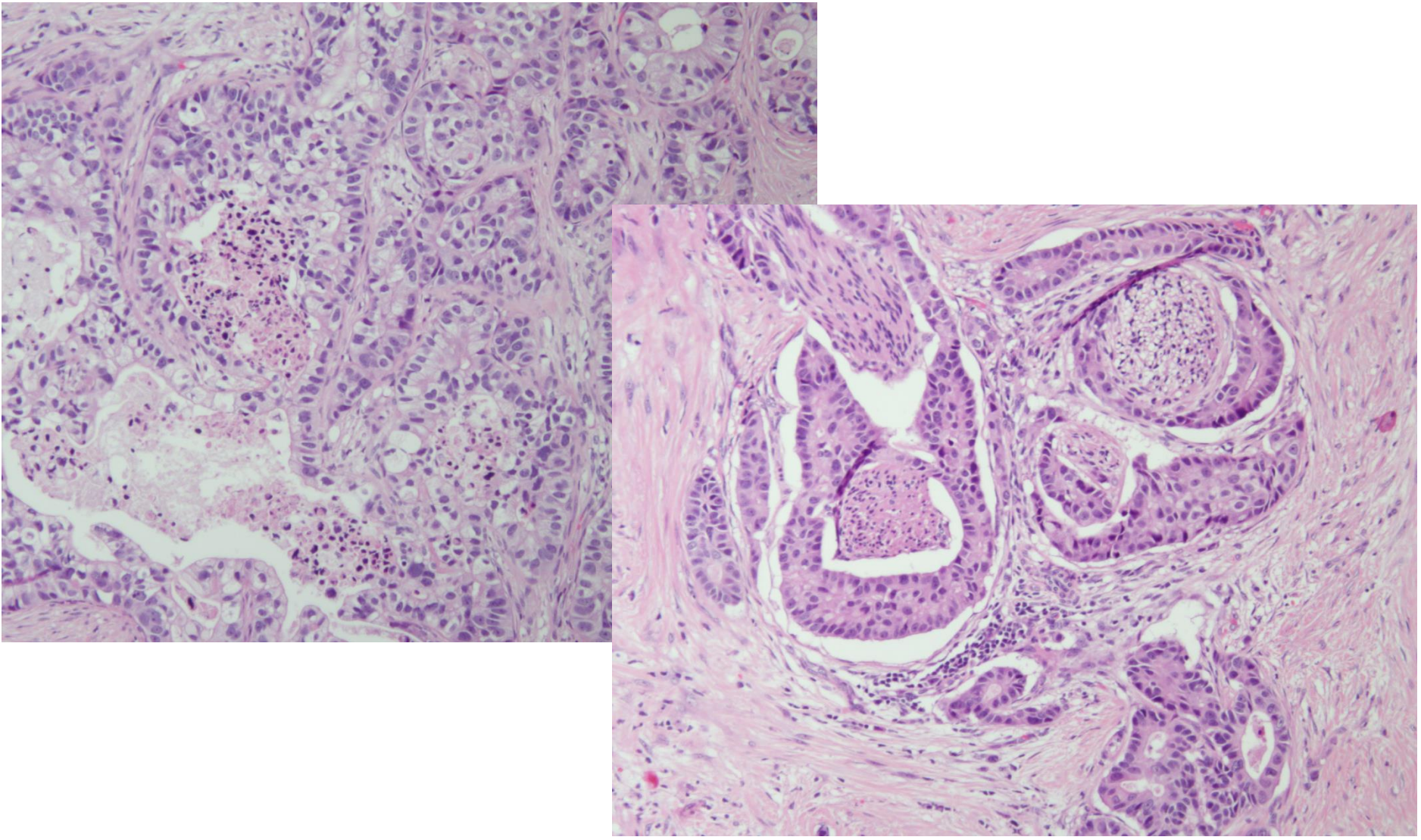
Case 4 - cytology





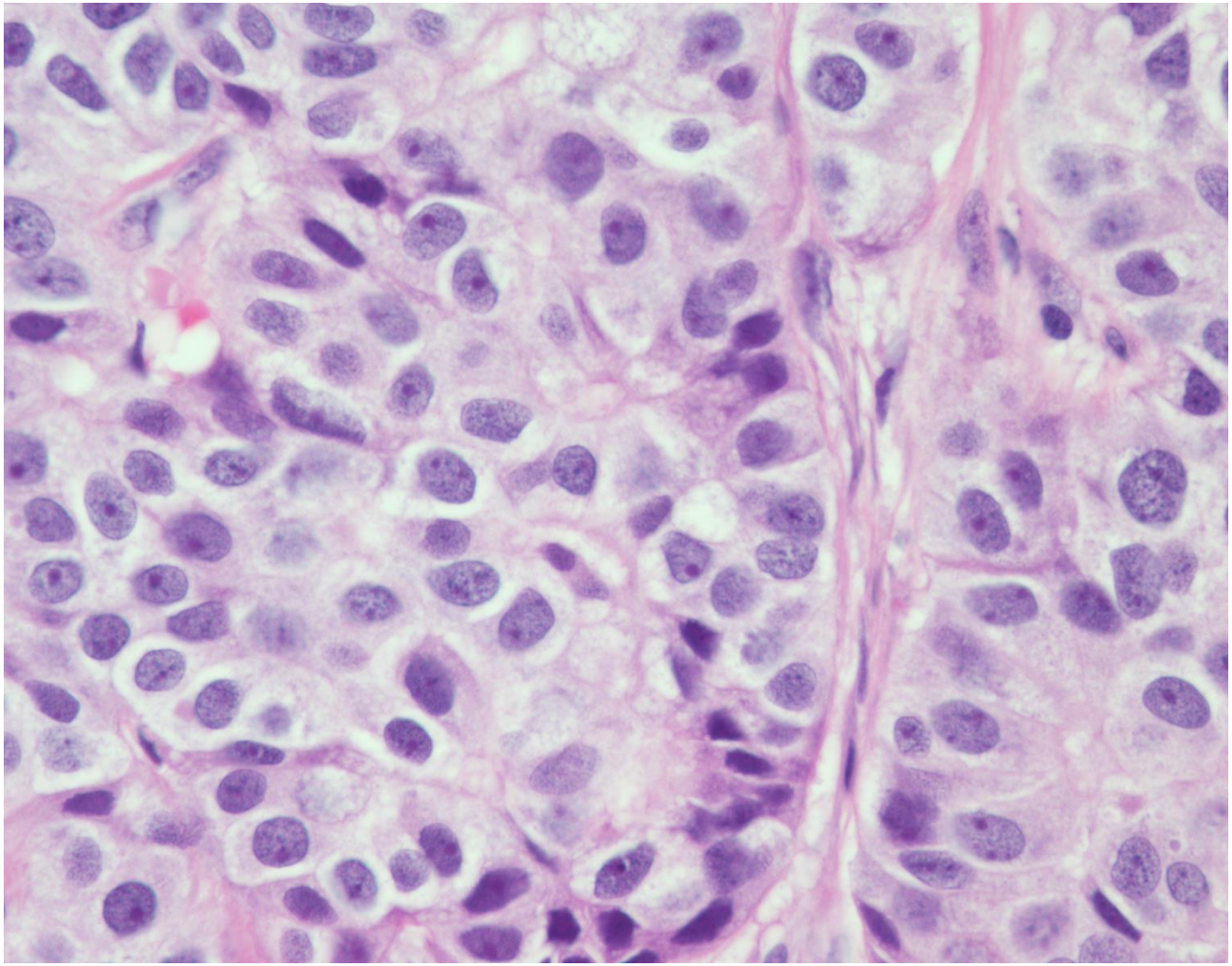
Case 4 - histology

Mixed ductal neuroendocrine carcinoma



Case 4 - histology

Mixed ductal neuroendocrine carcinoma

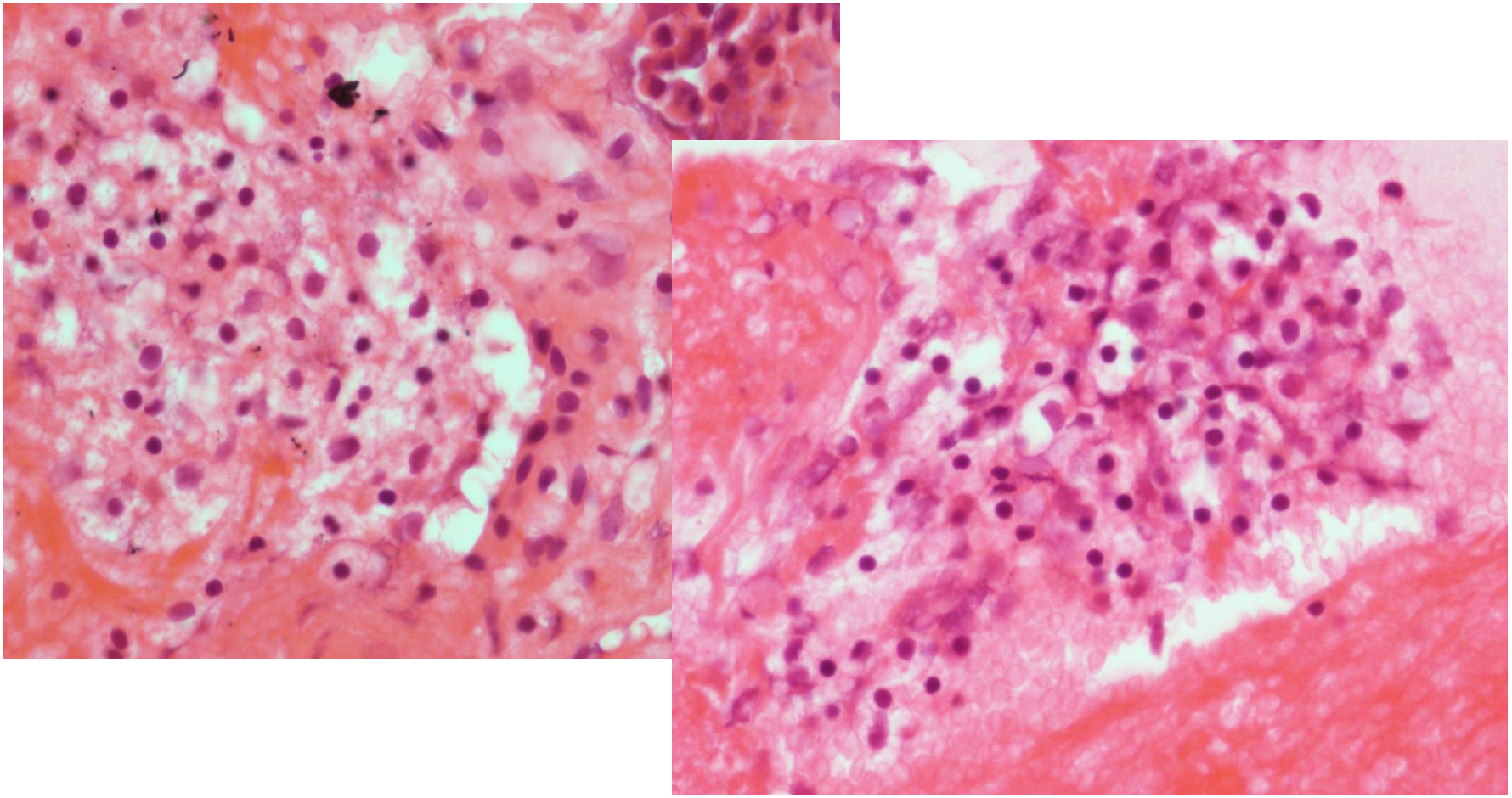


Case 5 - cytology

Pancreatic cyst FNA

Multi-cystic tumour in body of pancreas. Fluid amylase 1149 UI/l. CEA

<2. EUS DG Vascular tumour - large. Suitable for resection.



Case 5 - diagnosis

Cellular sample, satisfactory for cytological evaluation.

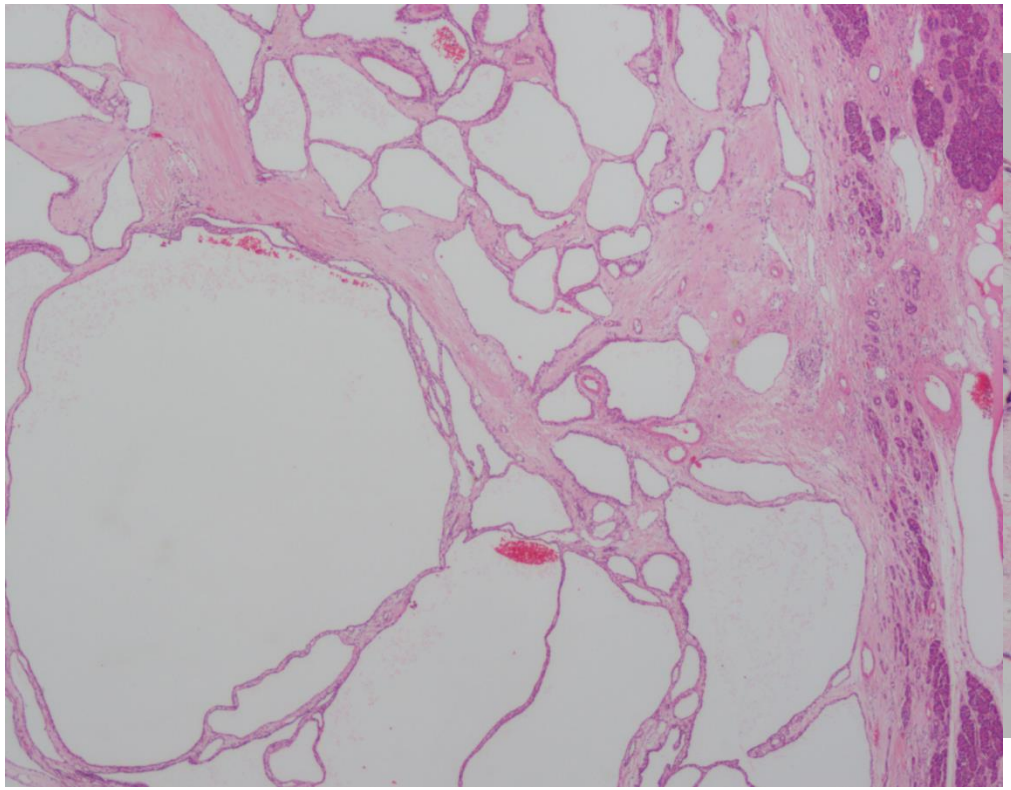
Neoplastic: Benign *(reporting category IVA*)*. See comment.

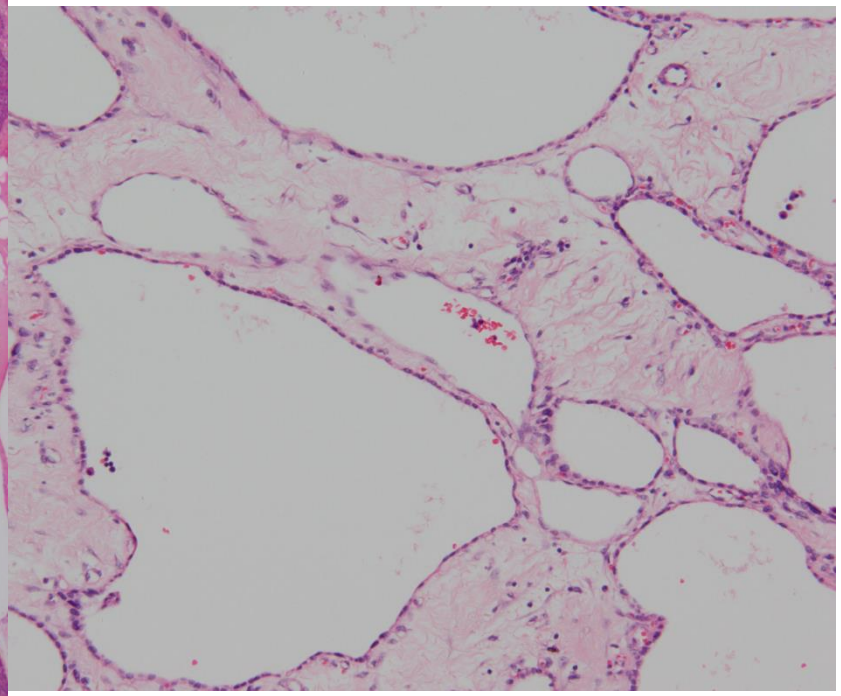
COMMENT: The findings are in keeping with a serous cystadenoma. Low fluid CEA, lack of thick mucus and the bland epithelial cells, in places with clear cytoplasm support the diagnosis.

Cytology slide circulation: 60% SCA (IVA), 40% SCA/NET (IVA-IVB)

Case 5 - histology

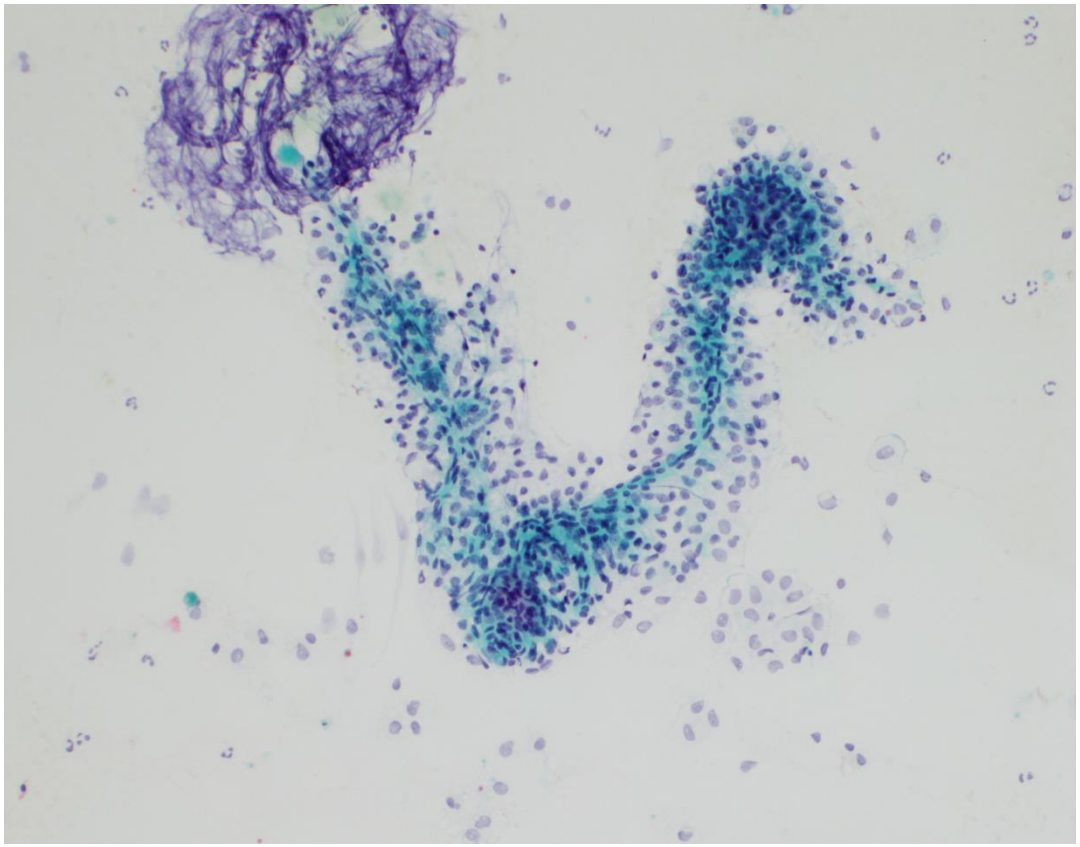
Distal pancreatectomy: Pancreatic serous cystic neoplasm (microcystic serous cystadenoma).



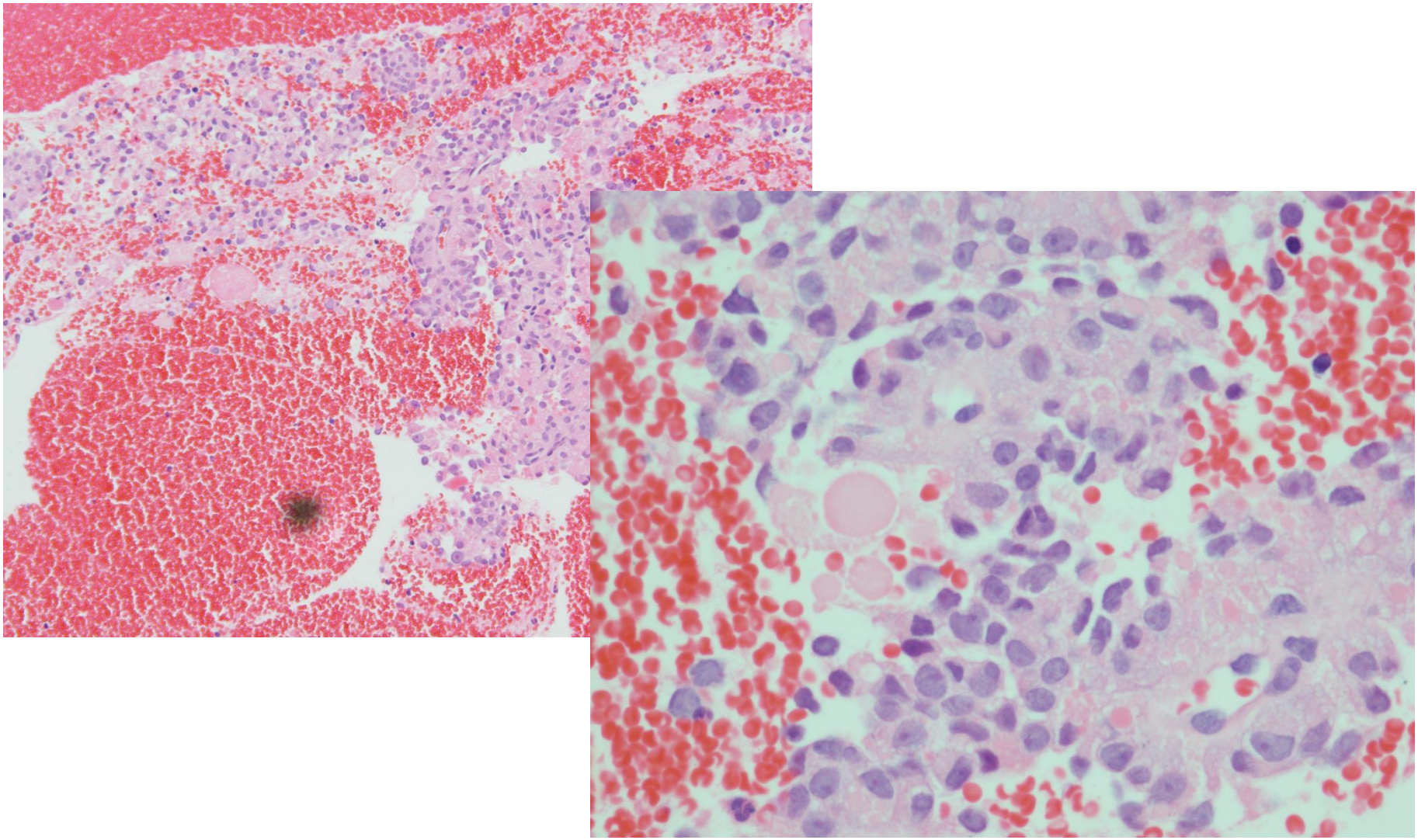


Case 6 - cytology

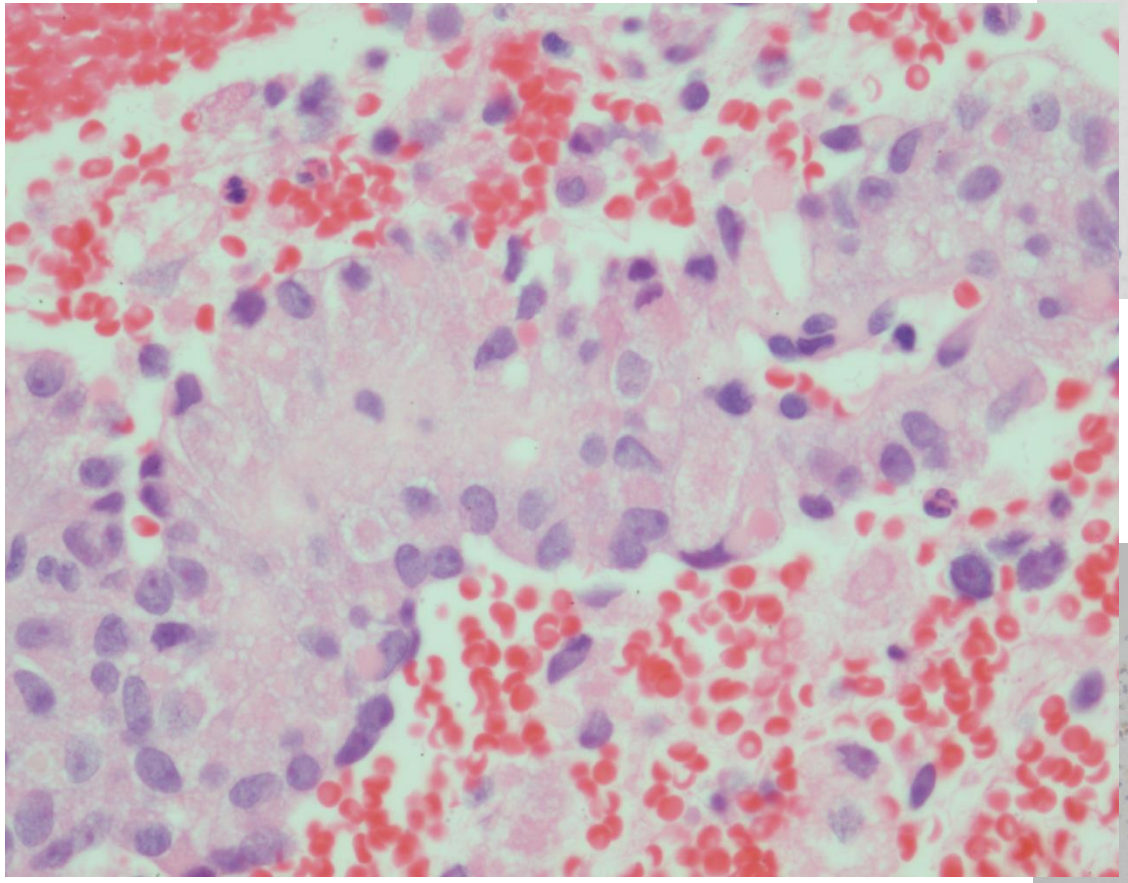
Pancreatic cyst, FNA, 16 y.o. female
Endoscopic ultrasounds. 2.6 cm pancreatic cyst ?debris within it ?solid component. Single pass, sent for histology and cytology (transgastric approach).
Fluid CEA < 2 ug/l, amylase 213 UI/l

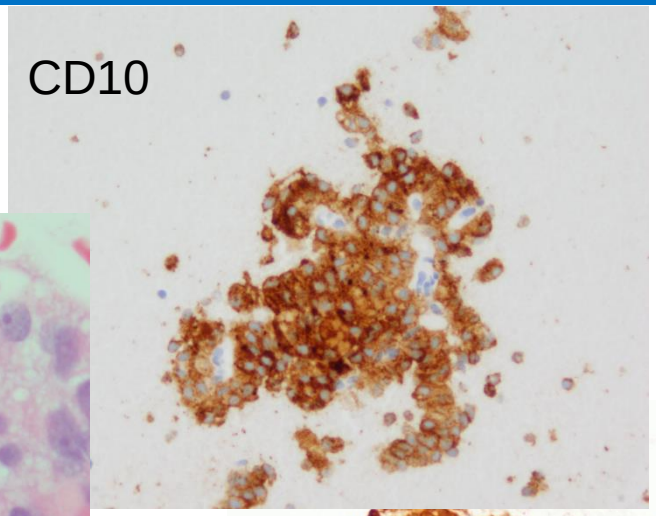


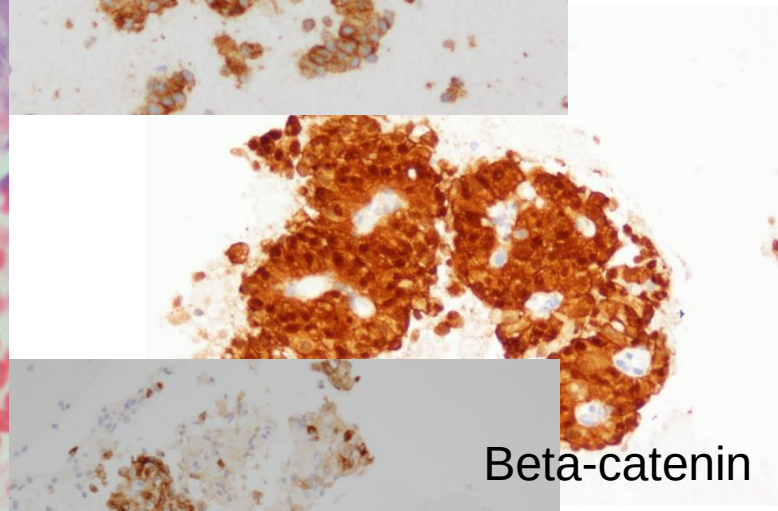
Case 6 - cytology

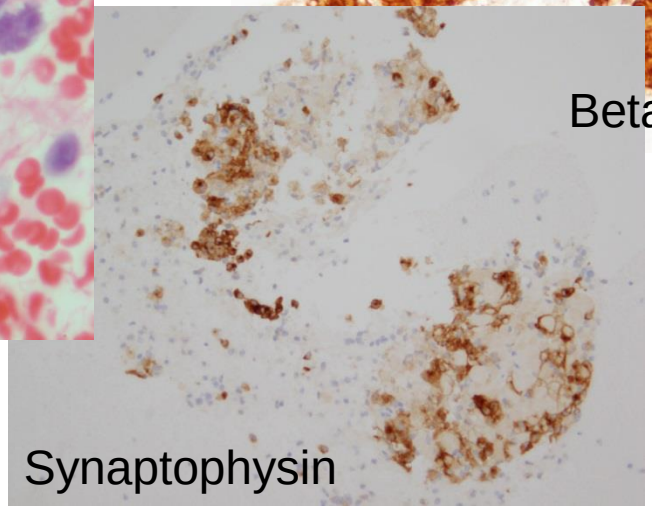


Case 6 - cytology







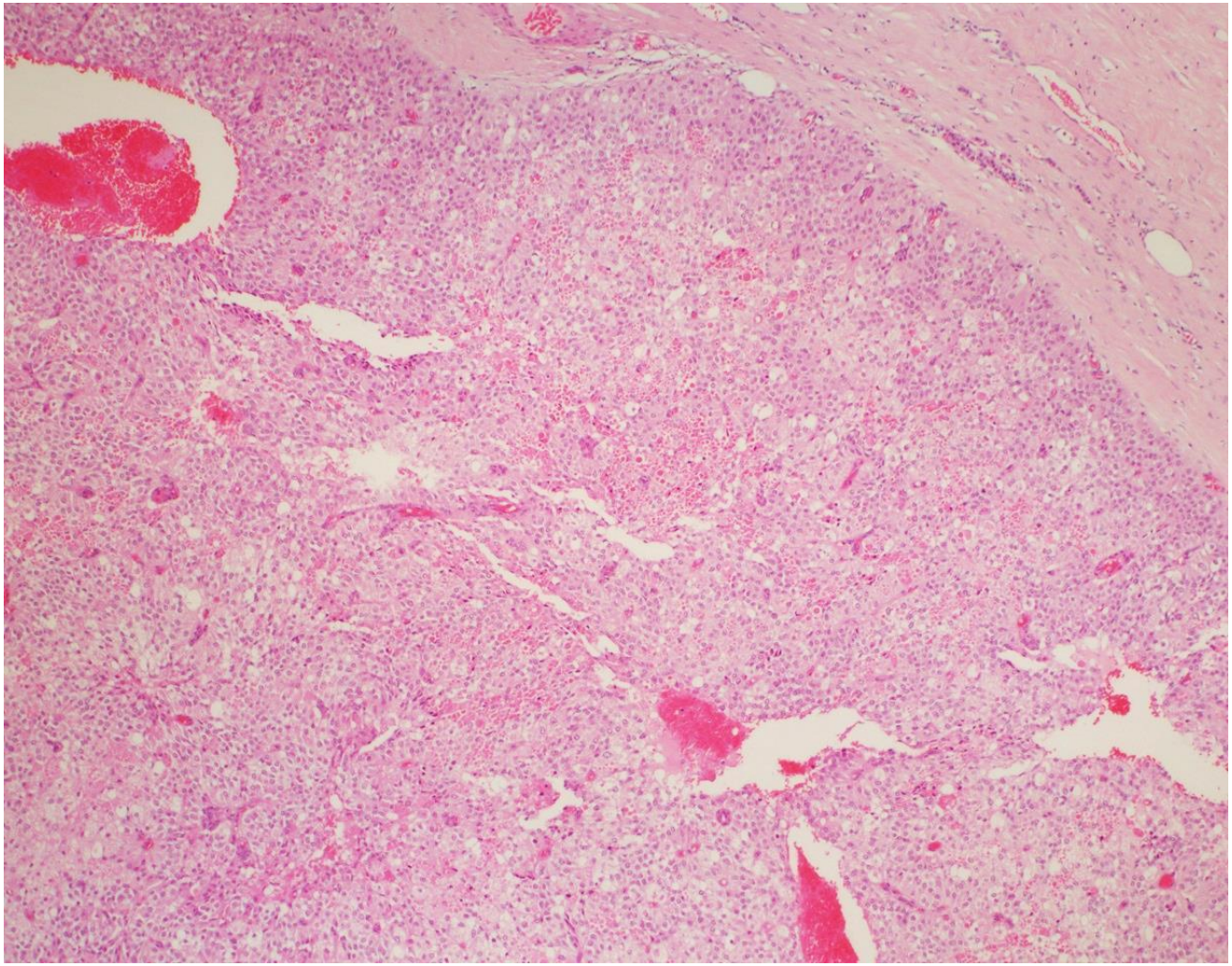


Case 6 - diagnosis

Cellular sample, satisfactory for cytological evaluation.
Neoplastic: Other *(reporting category IVB*)*. See comment.

COMMENT: The cytological and immunohistochemical findings are in keeping with a solid-pseudopapillary neoplasm .

Case 6 - histology



Case 6 – Solid-pseudopapillary tumour

Differential Diagnosis:

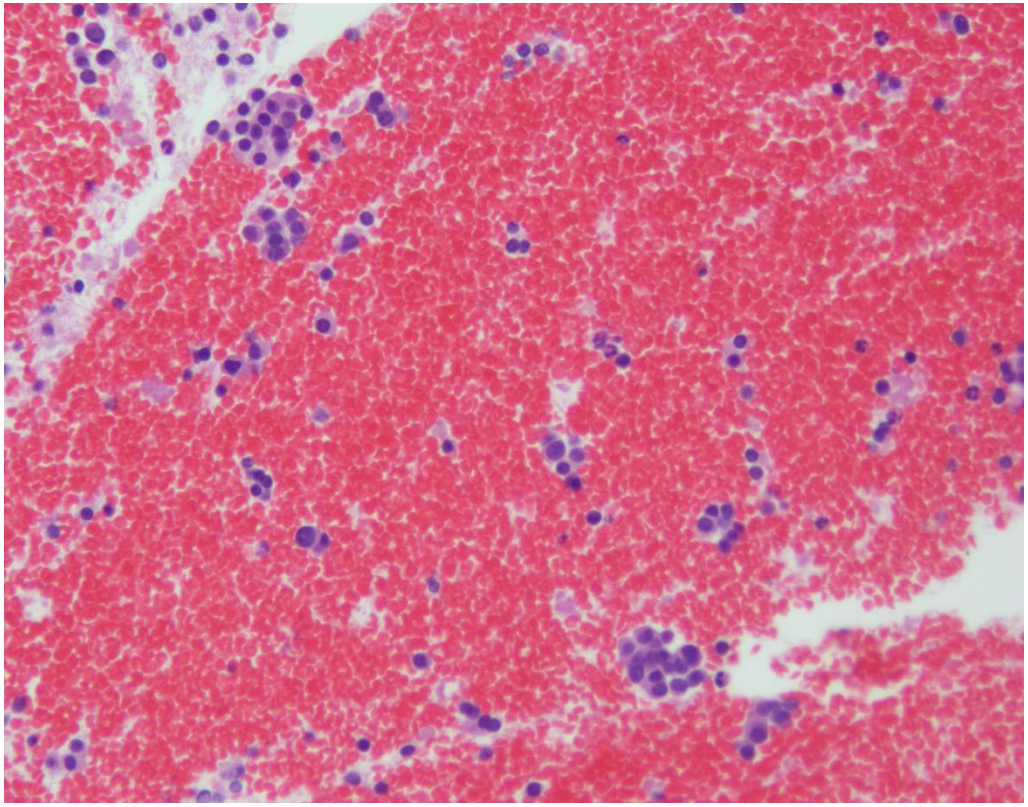
- Neuroendocrine tumour
- Acinar cell carcinoma
- Serous cystadenoma

Alpha 1 antitrypsin	positive
Alpha 1 antichymotrypsin	positive
Neuron specfic enolase	positive
Vimentin	positive
Progesterone receptor	positive
Beta-catenin	positive
CD10	positive
CD56	positive
Trypsin	negative
Chymotrypsin	negative
CD117	positive
Cytokeratins	variable
Synaptophysin	~ half of cases
CD34	negative
Estrogen receptor	negative
Chromogranin	negative

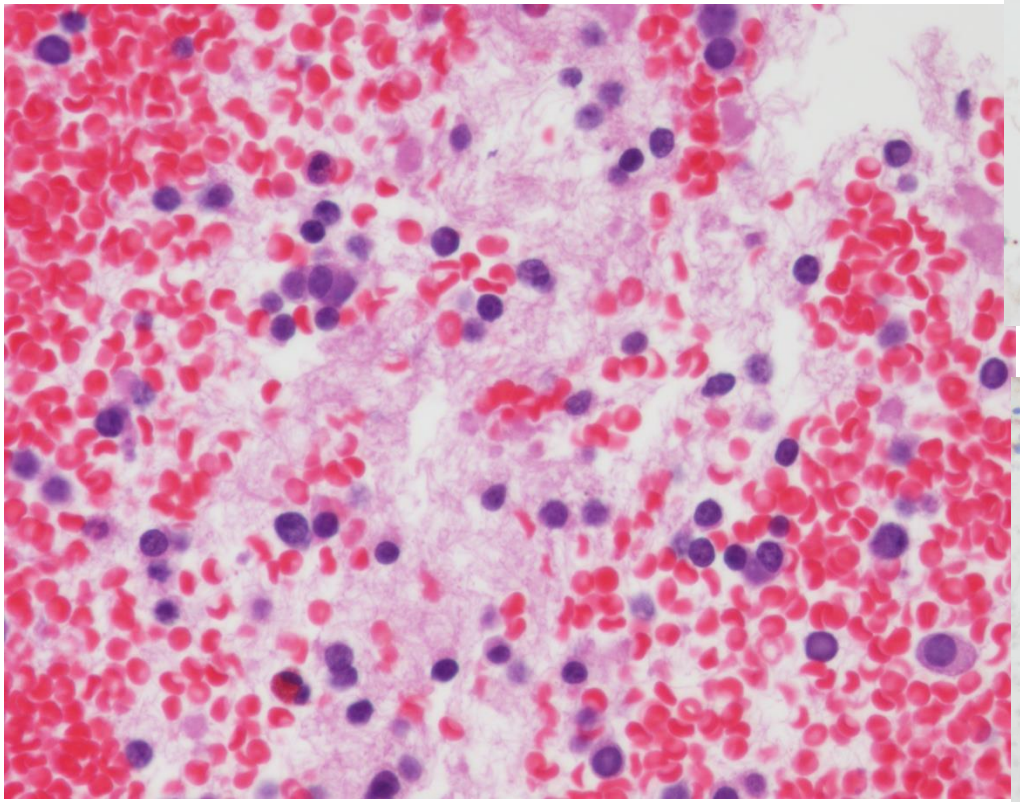
Case 7 - cytology

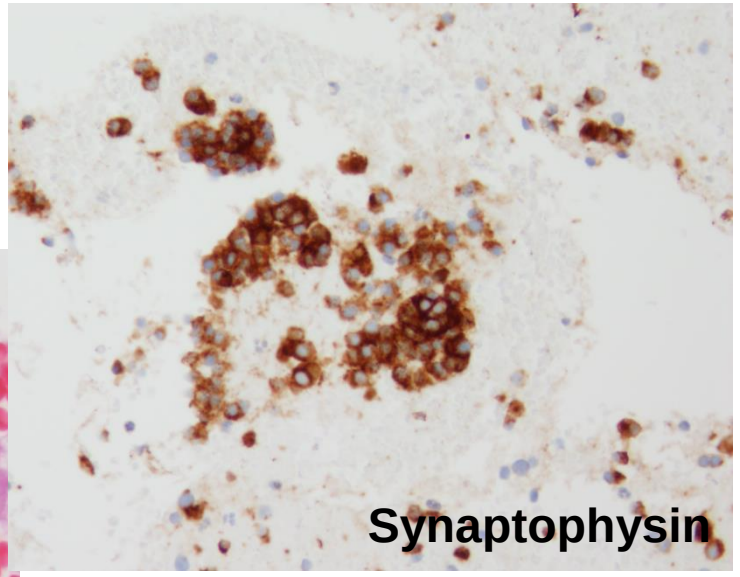
50 y.o. male
Pancreatic FNAB

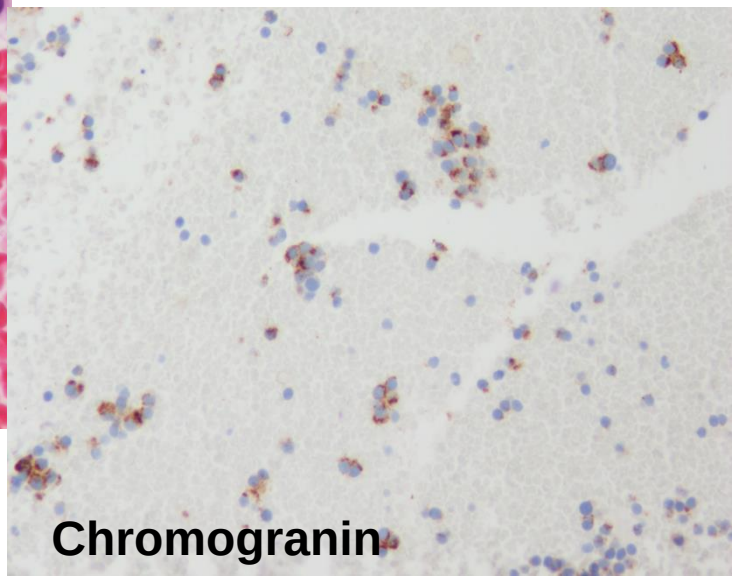
Uncinate process tumour, well demarcated.



Case 7 - cytology





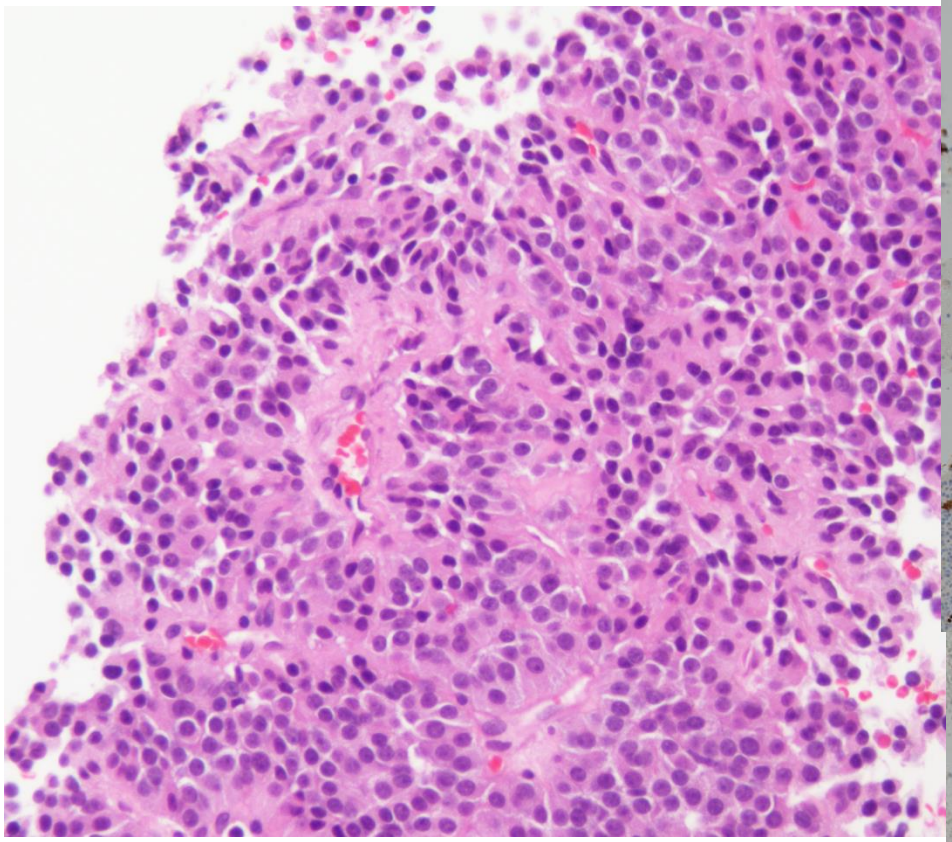


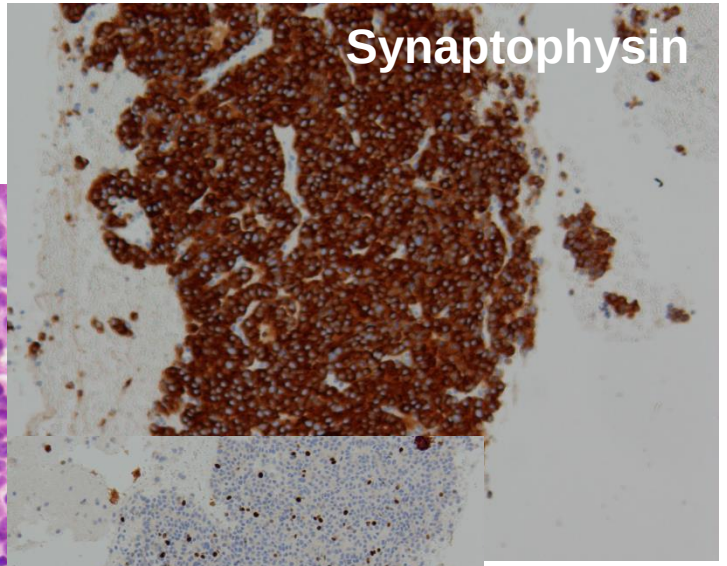
Case 7 - diagnosis

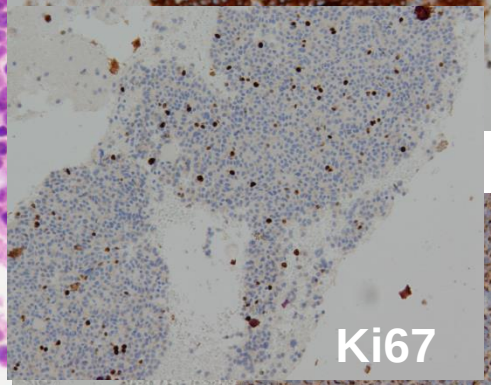
Cellular sample, satisfactory for cytological evaluation.
Neoplastic: Other *(reporting category IVB*)*. See comment.

COMMENT: The cytological and immunohistochemical findings are in keeping with a well differentiated neuroendcrine tumour.

Case 7 - histology

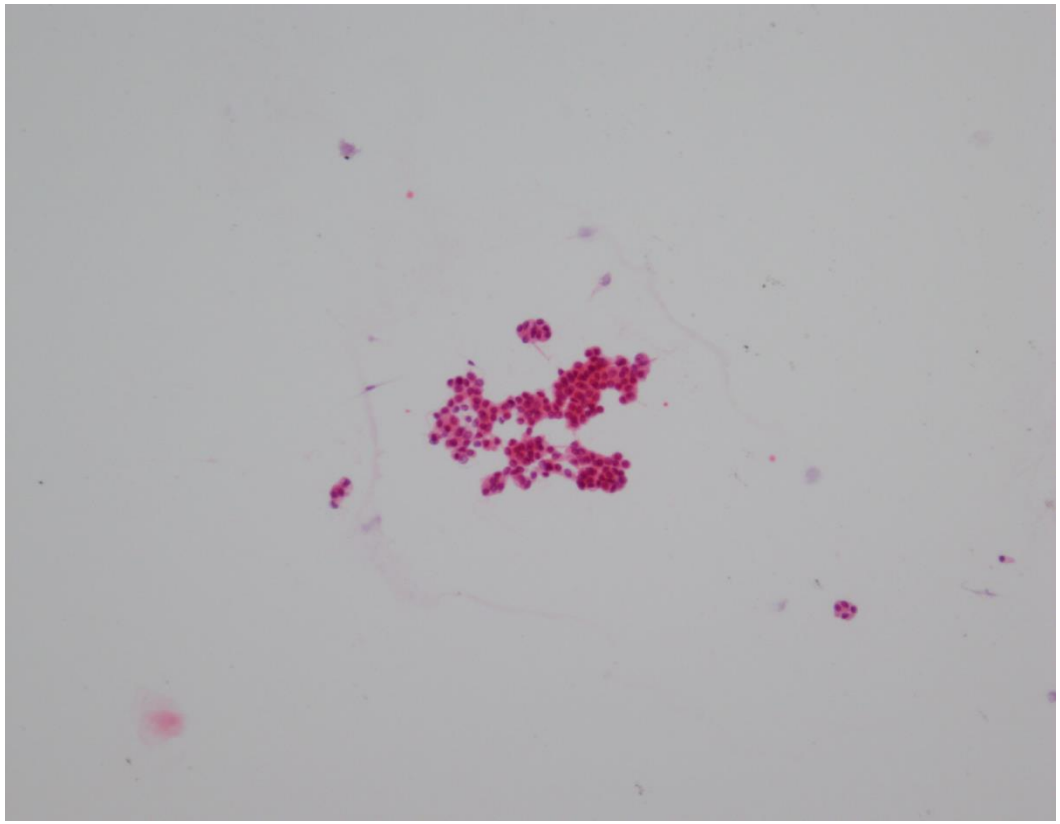




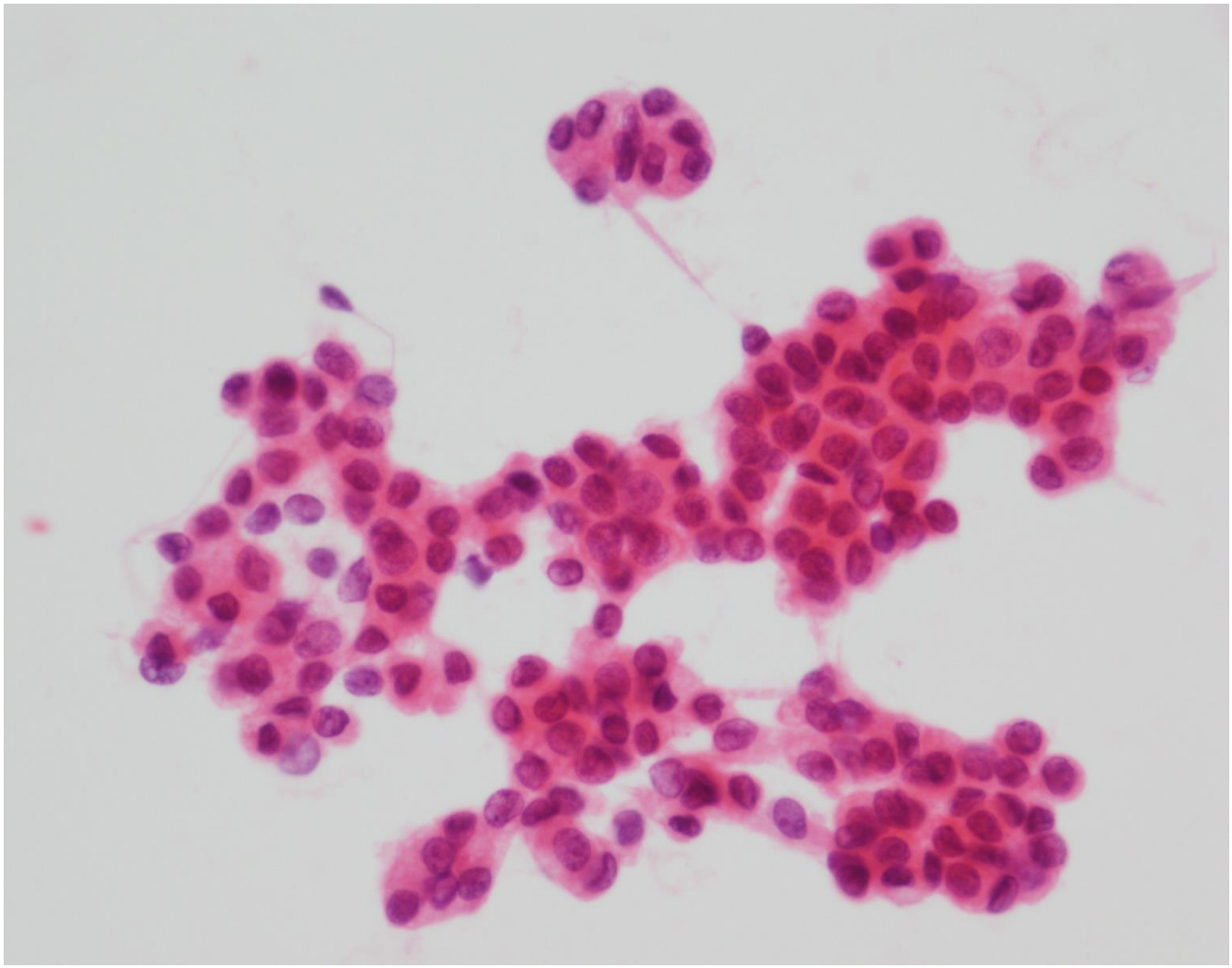


Case 8 - cytology

Pancreatic cyst fluid FNA
Query IPMN and dysplasia. CEA 5331 ug/l amylase 1879 UI/l, mucinous fluid aspirated



Case 8 - cytology



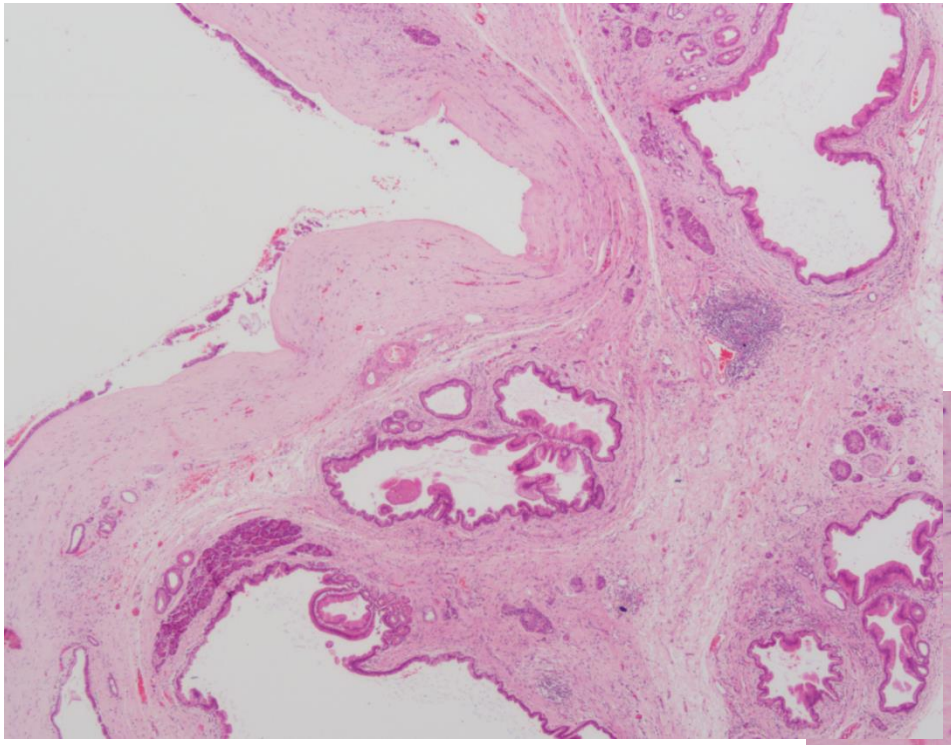
Case 8 - diagnosis

Paucicellular sample, limiting cytological evaluation.
Neoplastic: Other *(reporting category IVB*)*. See comment.

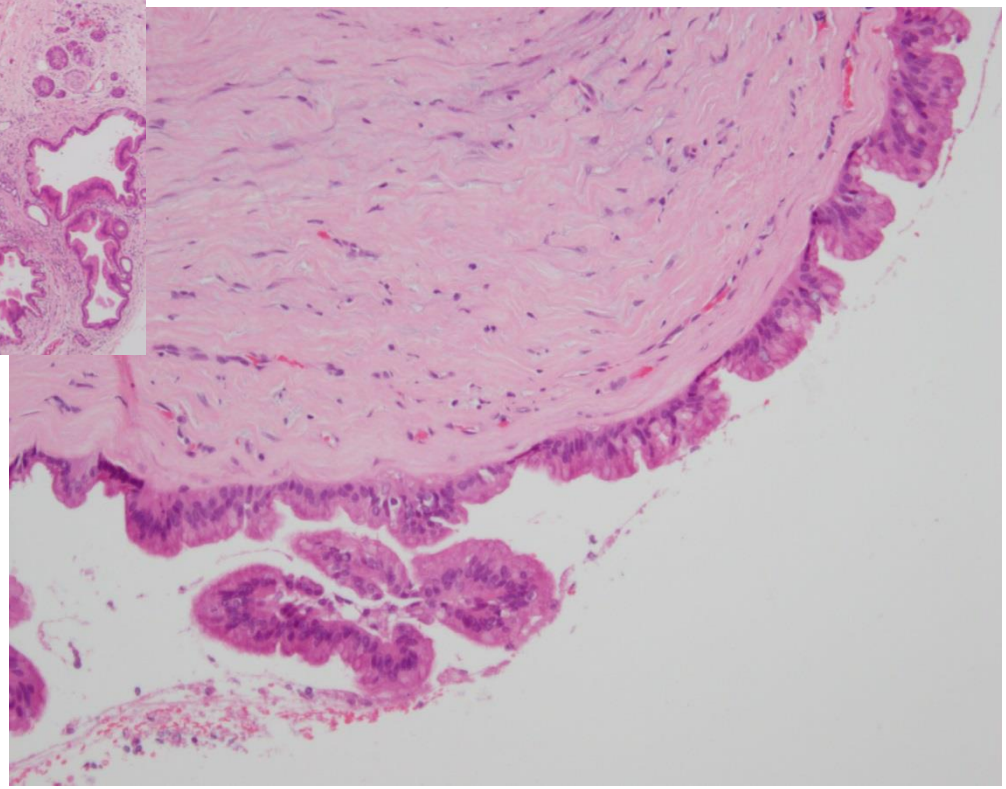
COMMENT: Although no thick mucus is seen, the high CEA and the aspirated mucinous fluid support a diagnosis of a mucinous cystic lesion. No high grade atypia is seen, but the sample is limited.

Cytology slide circulation: 100% agreement in diagnosis

Case 8 - histology



IPMN with low and intermediate grade dysplasia



Case 8 - histology

Cytological criteria of high-grade epithelial atypia in the cyst fluid of pancreatic intraductal papillary mucinous neoplasms.
Pitman MB, Centeno BA, Daglilar ES, Brugge WR, Mino-Kenudson M.
Cancer Cytopathology, 2014

Agreement between cytopathologists:

Significantly different between the LGD and HGD groups included:

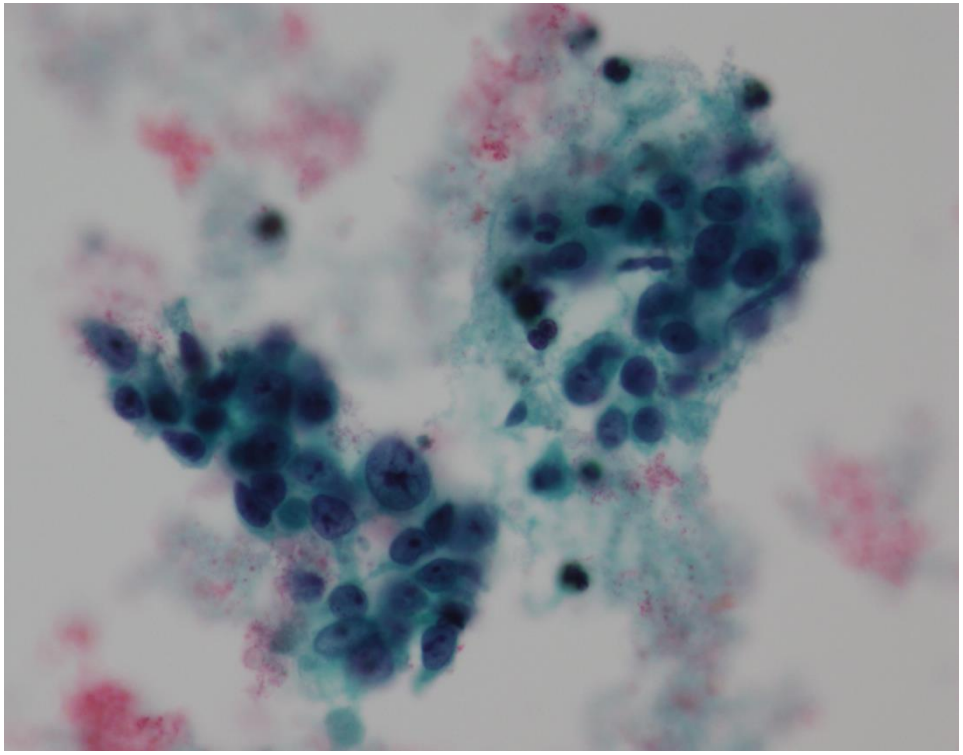
1) cell size < a 12-µm duodenal enterocyte for HGD and equal size for LGD
2) **increased nuclear-to-cytoplasmic (N/C) ratio**;
3) marked nuclear membrane abnormalities;
4) **abnormal chromatin pattern** (hypo or hyperchromasia)
5) **background necrosis**

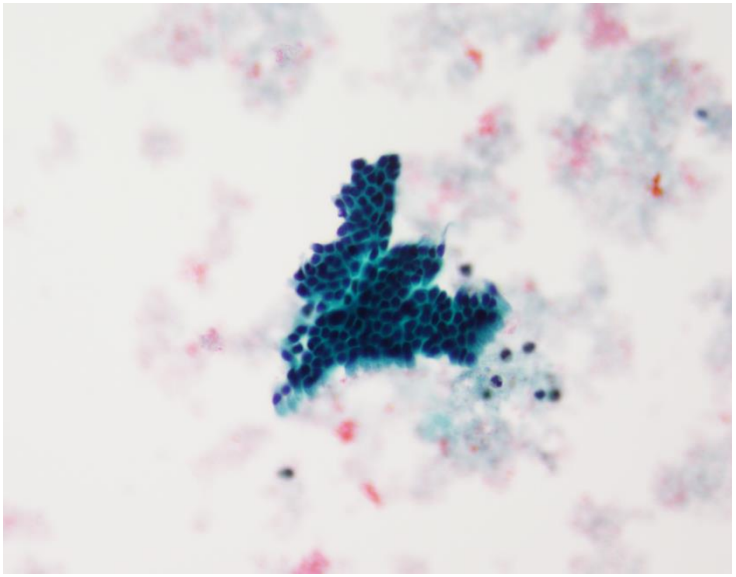
Case 9 - cytology

59 y.o. male

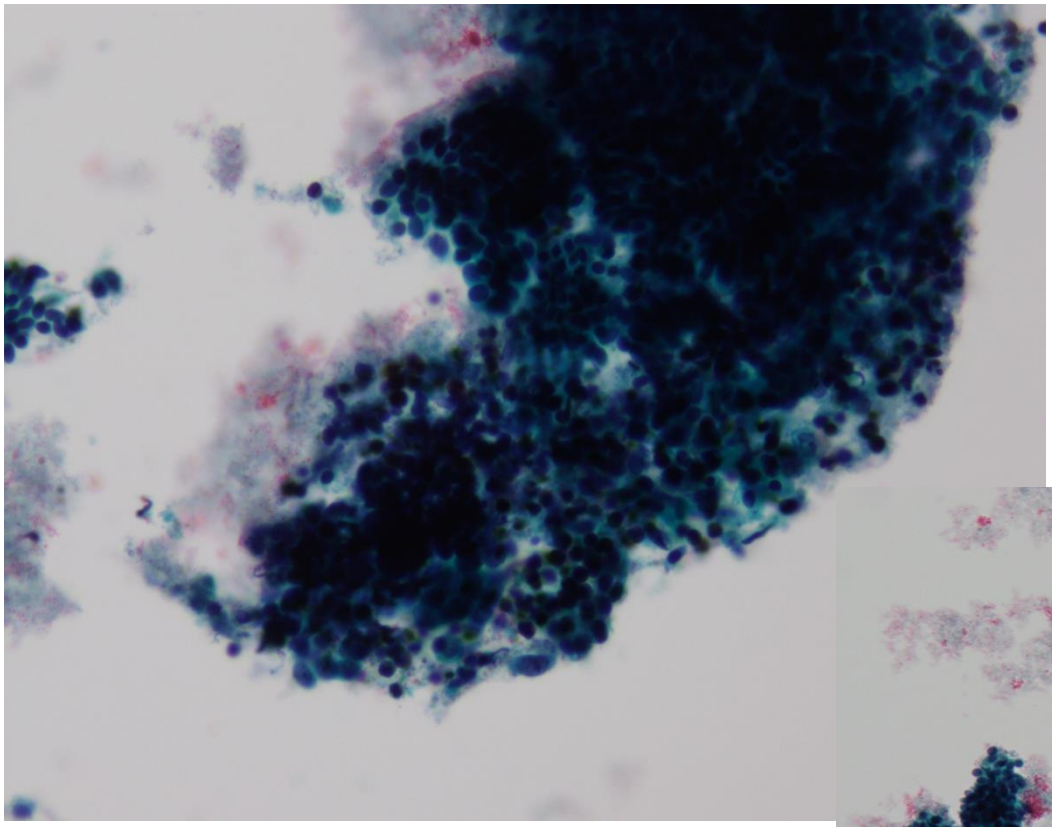
Imaging revealed dilated intraphepatic ducts and hilar mass infiltrating the liver. Biliary brushing

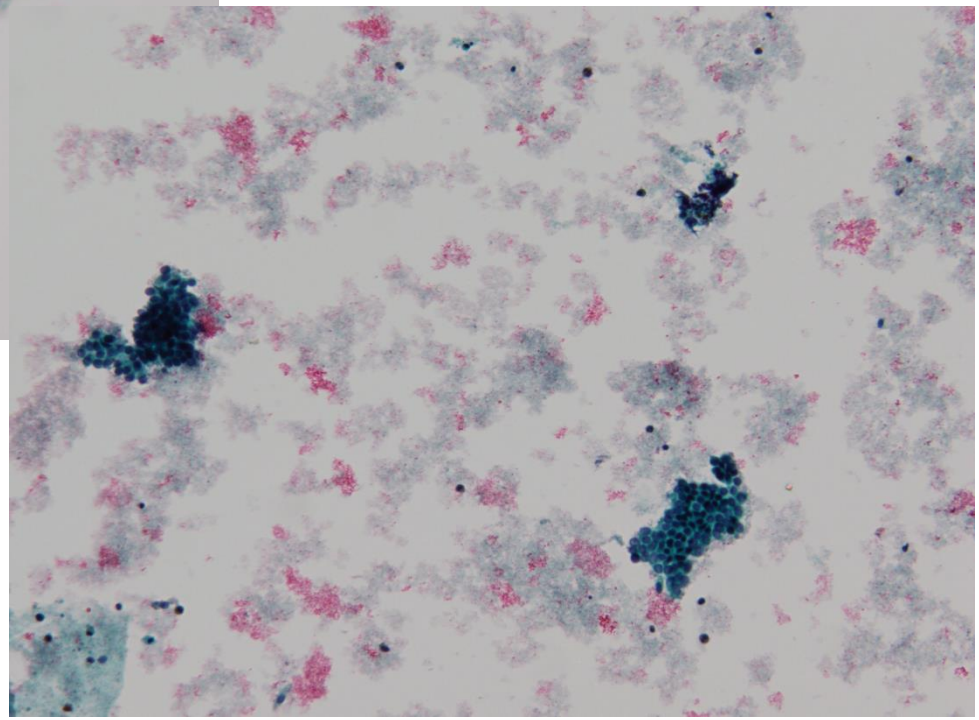
History of colon cancer



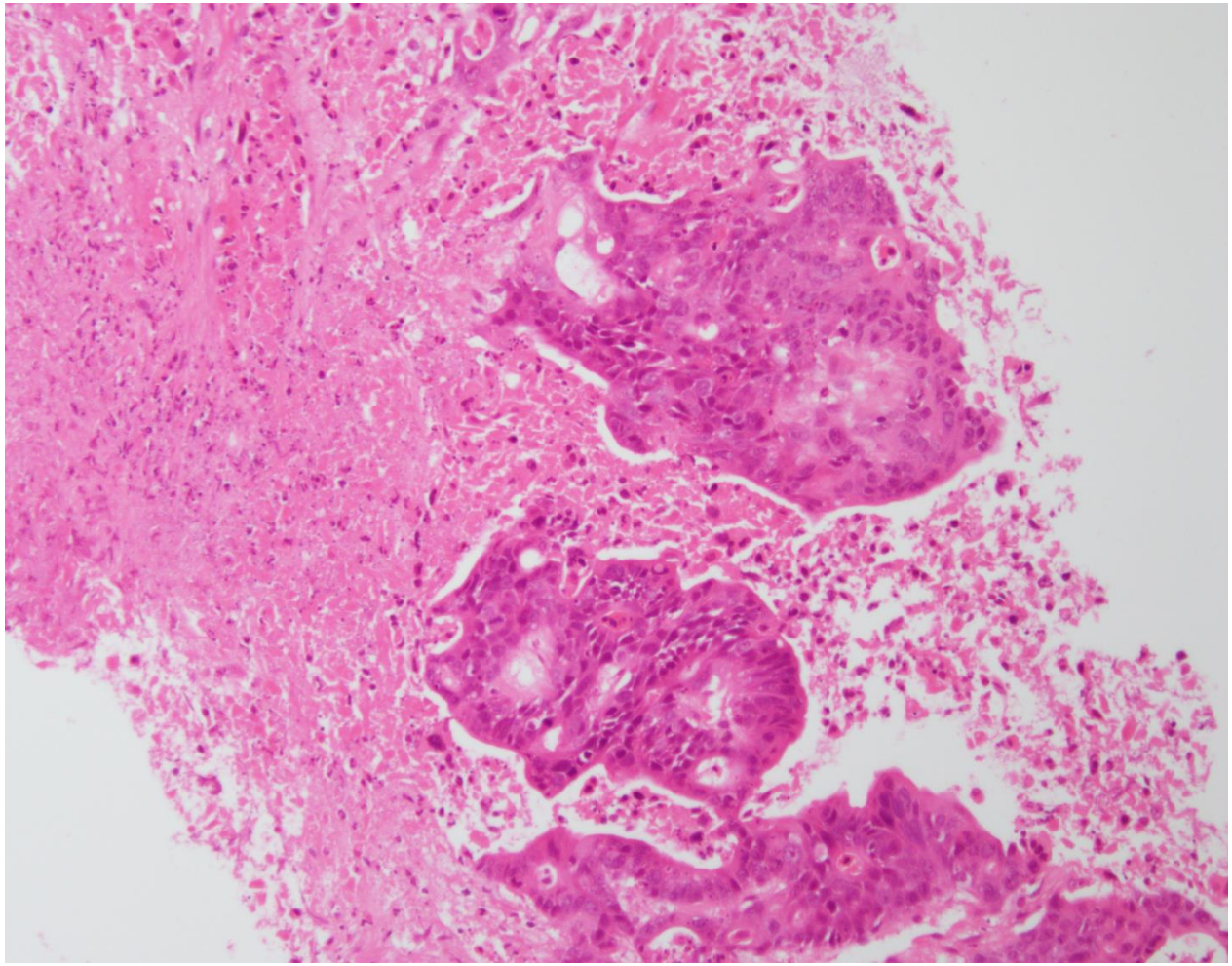


Case 9 - cytology

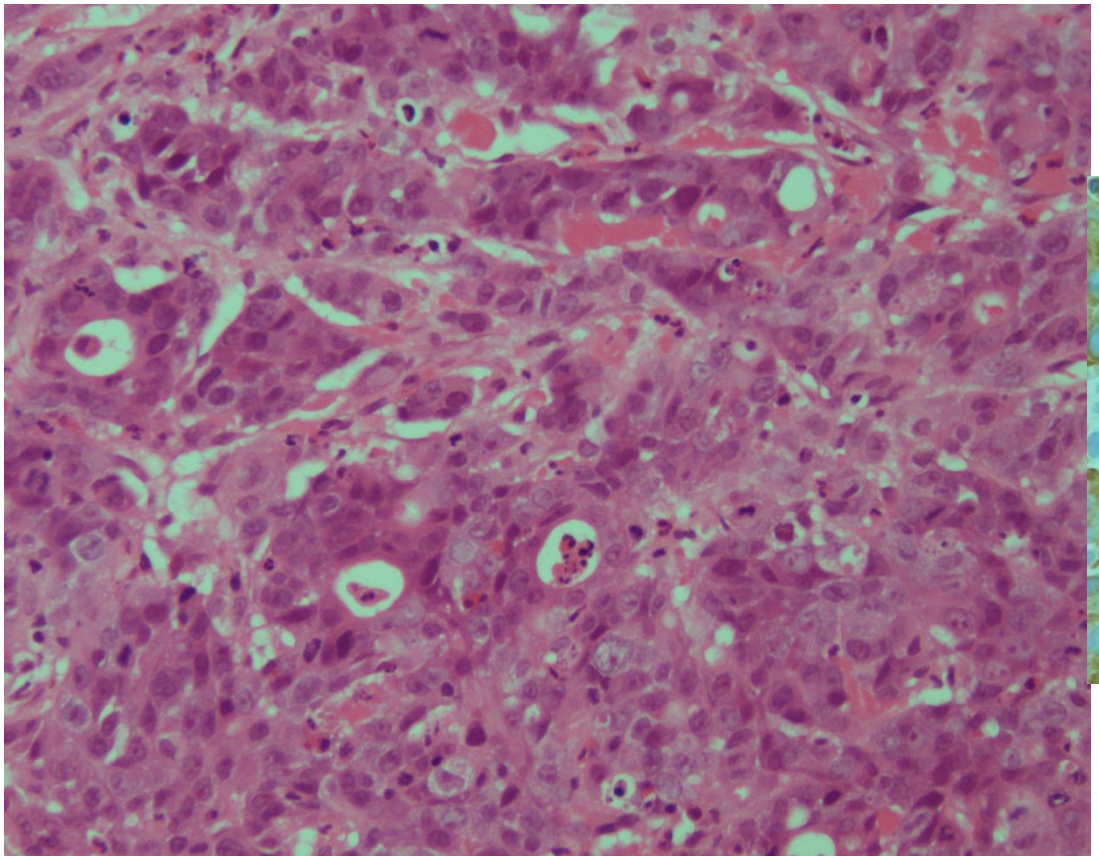


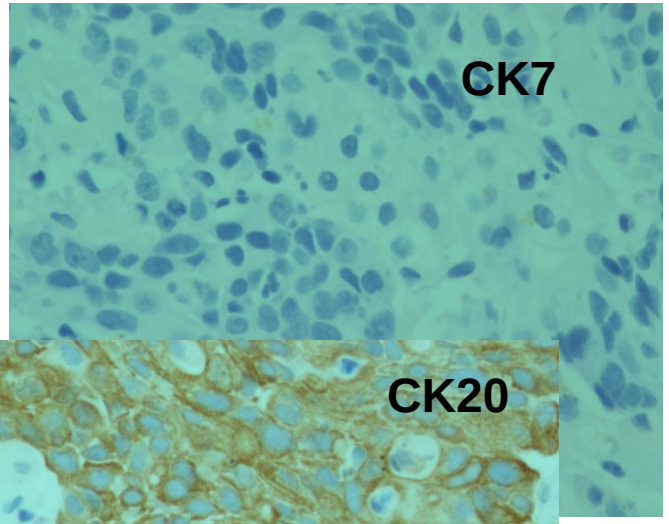


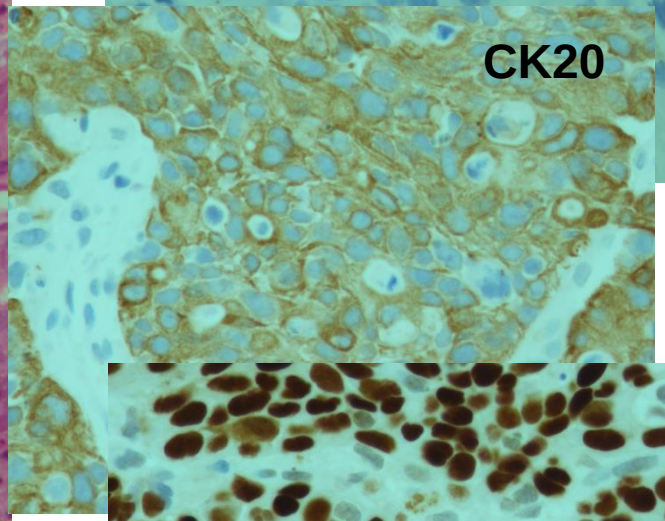
Case 9 - histology

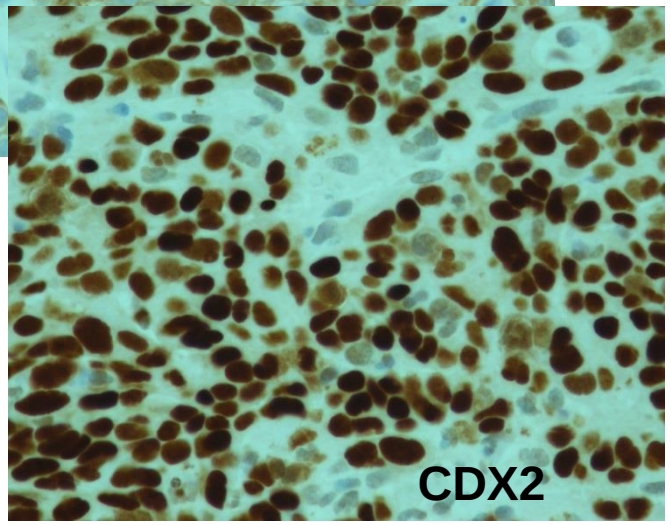


Case 9 - histology







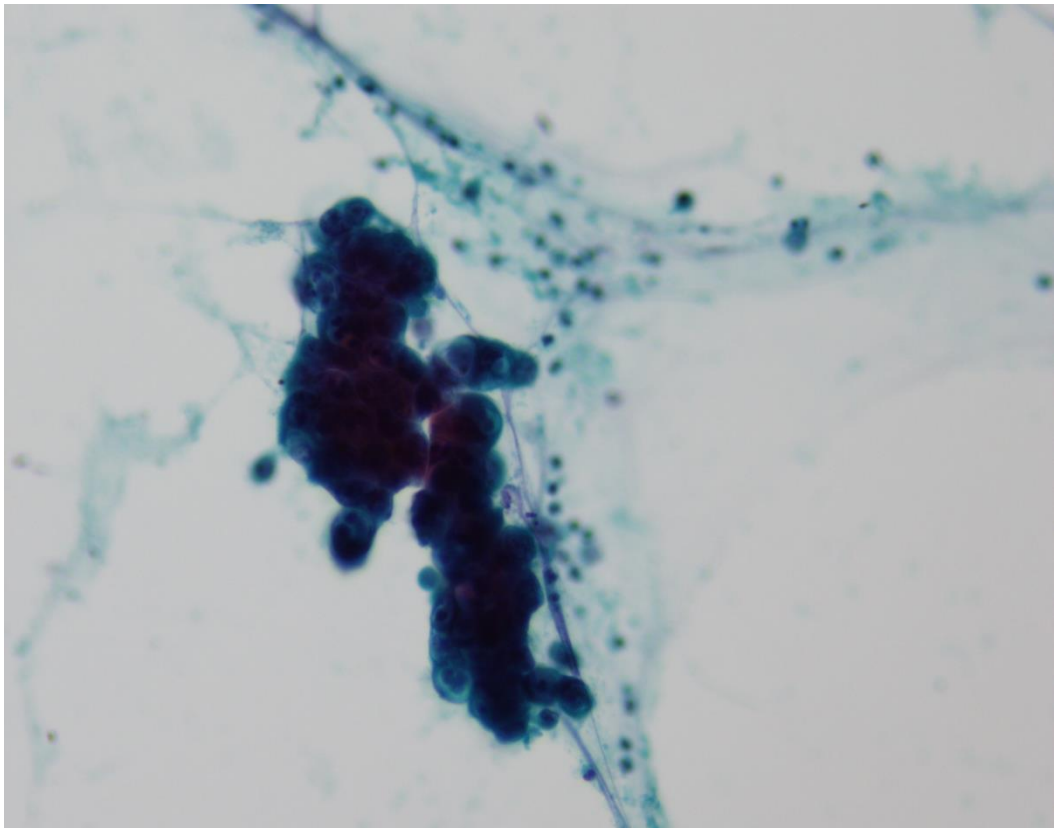


Intestinal-type adenocarcinoma in keeping with colo-rectal metastasis

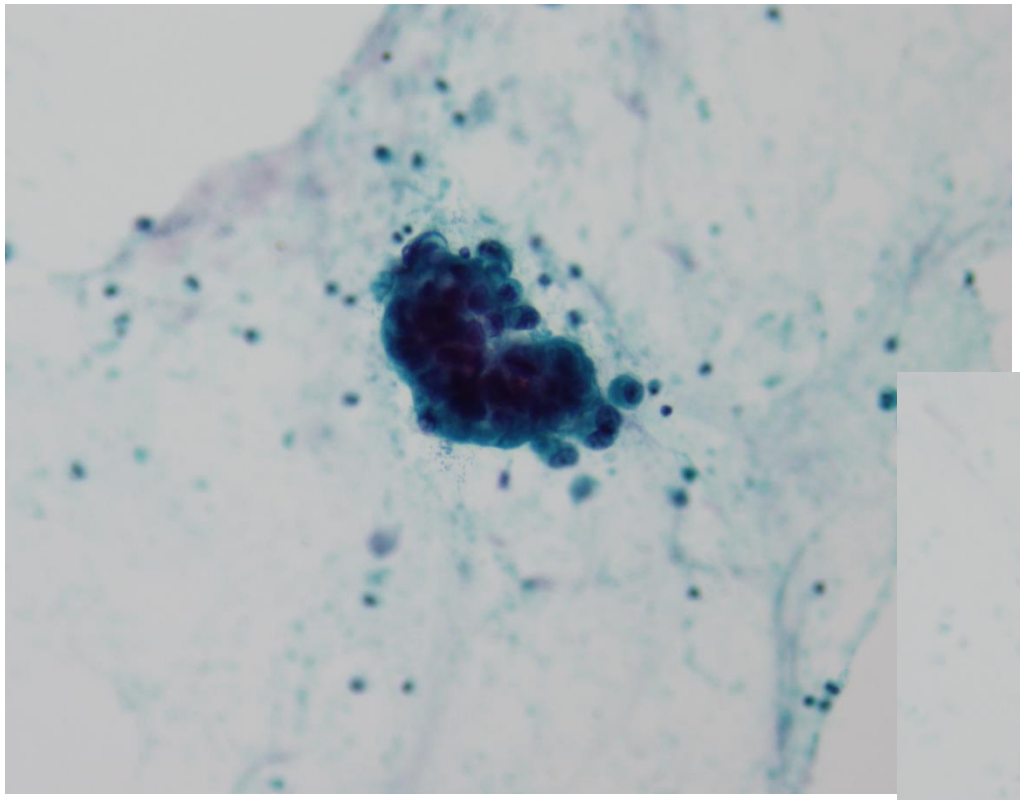
Case 10 - cytology

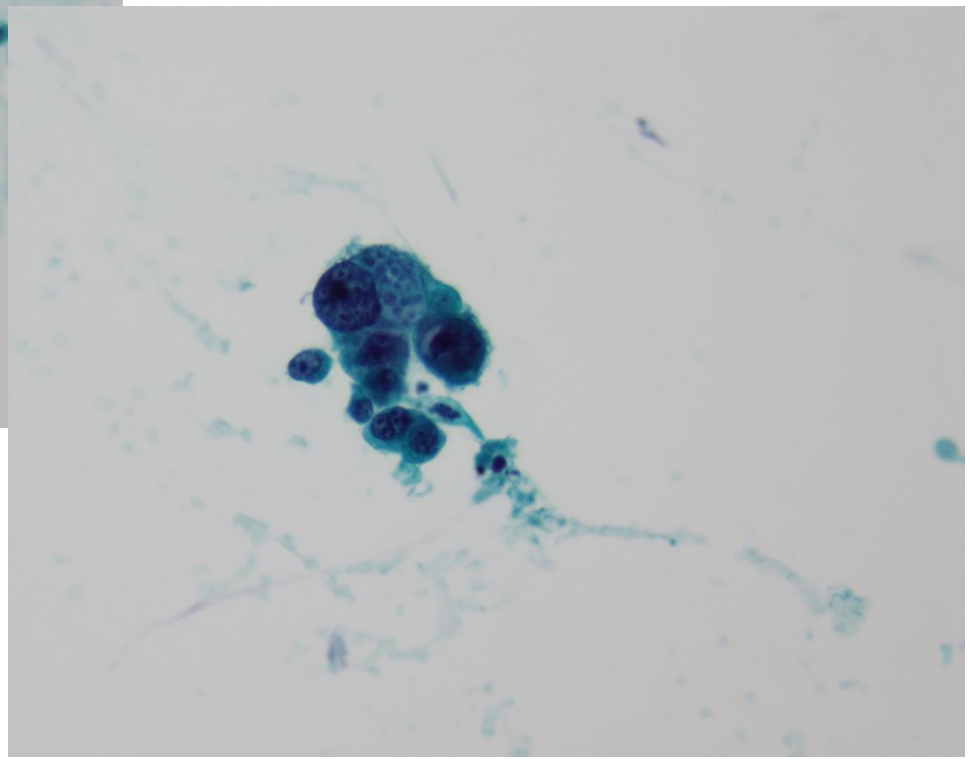
78 y.o, male

Jaundice. ?cholangiocarcinoma. ERCP: Mid CBD stricture. – biliary brushings



Case 10 - cytology



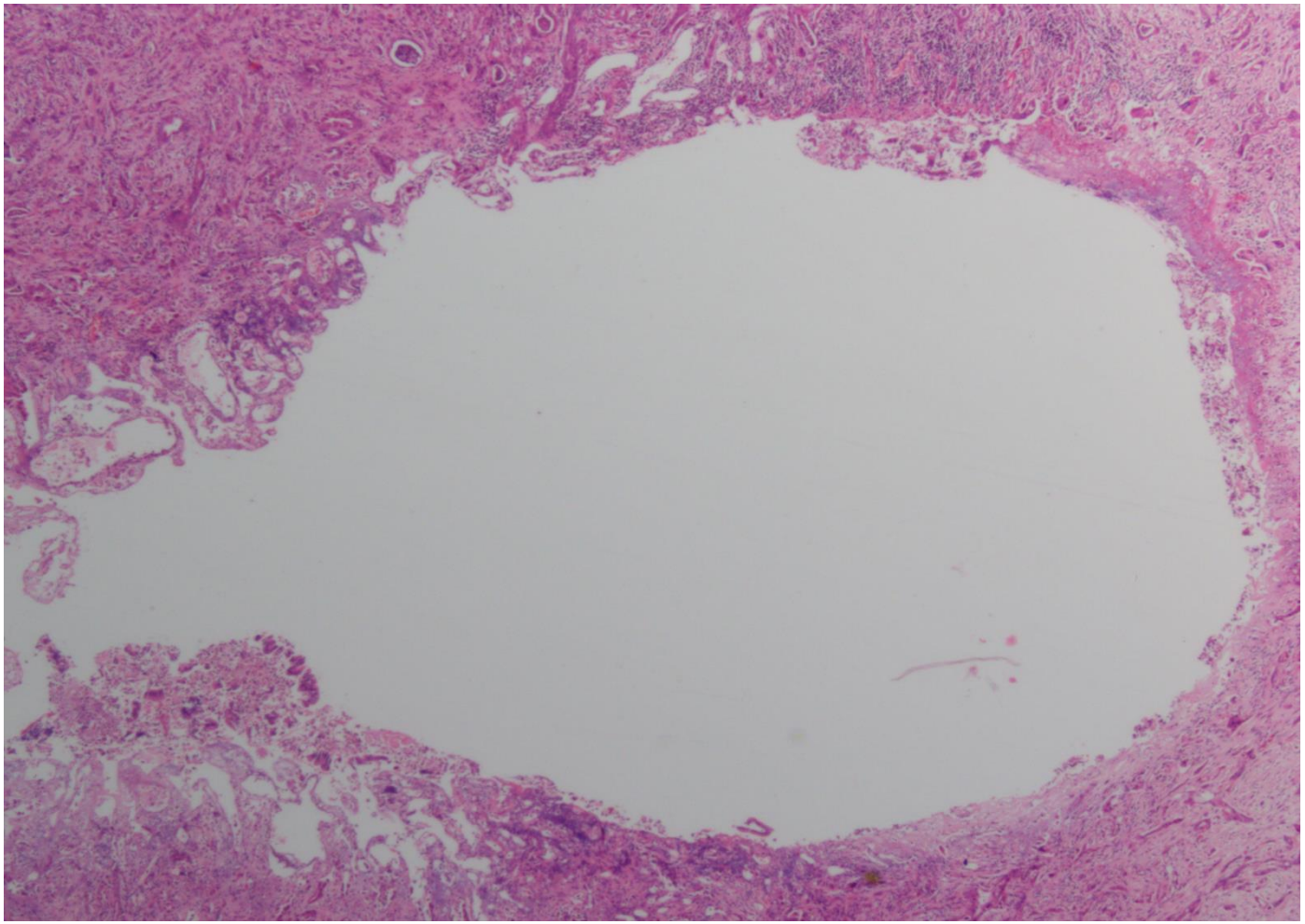


Case 10 - diagnosis

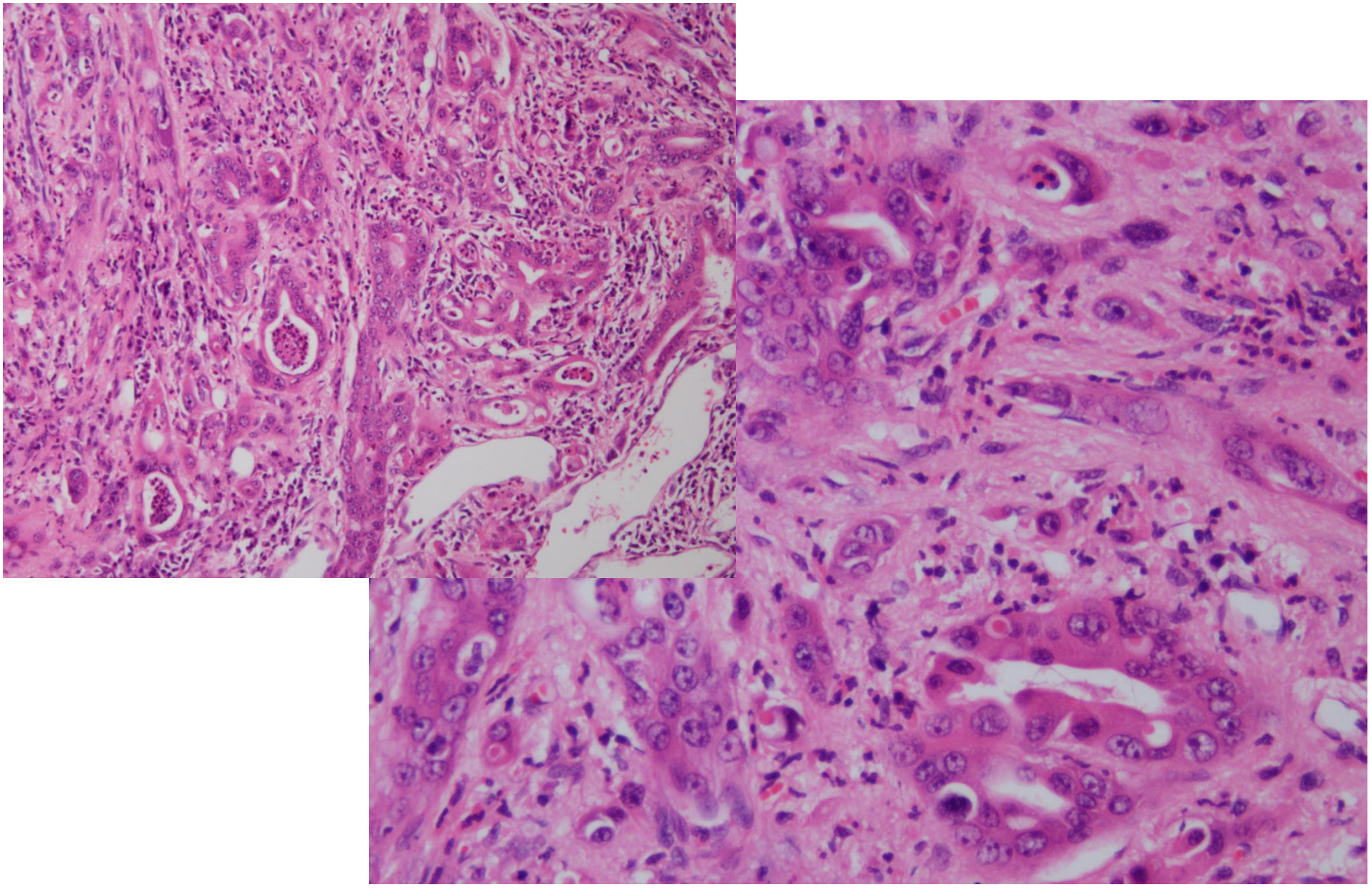
Cellular sample, satisfactory for cytological evaluation.
Positive/malignant *(reporting category VI*)*.

Case 10 - histology

Distal cholangiocarcinoma



Case 10 - histology



THANK YOU

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Dr Maesha Deheragoda - for clinical cover

Alessandra Pacheco - for preparing the cases for today and doing an excellent job in processing and staining our cytology samples