Cytopathology Study Day
16 April 2017

Guy's Hospital London

RCPath - BAC

Digital cytology: EUS FNA pancreas and head and neck

R. Dina MD, FIAC, FRCPath
Consultant Cyto/Histopathologist
Hon Sen Lecturer
Hammersmith Hospital
Imperial College NHS Trust

- **Advantages of whole slide imaging in cytopathology practice.** From :Patholog Res Int. 2011; 2011: 264683.
  Walid E. Khalbuss, [1, 2] * Liron Pantanowitz, [1, 2] and Anil V. Parwani [1]
- (1) Primary diagnosis (telecytology)
- (2) Remote second opinion consultation
- (3) Educational activity within the institution or remotely
- (4) Archiving interesting and legal cases (digital cytology slides replication)
- (5) Quality assurance
- (6) Educational conferences such as tumor boards (locally or remotely)
- (7) Online cytology proficiency testing
- (8) Online board exam or certification
- (9) Detailed image analysis and cytomorphometry
- (10) Annotation of various entities on the slides for teaching purpose
- (11) Easy acquisition of static images from whole-slide images
- (12) Provide cytopathology services to remote hospitals
- (13) Gains access to cytology subspecialty expertise
- (14) Remote on-site evaluation and triage
- (15) Synchronous consultation

Disadvantages of whole slide imaging in cytopathology practice

- (1) Costly: an expensive initial setup and storages
- (2) Limited focusing functions at present
- (3) Scanning time
- (4) Storage: large file size
- (5) Training requirements
- (6) Limited validation studies
- (7) Lack of standardization: multiple vendors, software, and lack of interoperability
- (8) Information technology infrastructure support (bandwidth limitation of networks)
- (9) Professional reluctance to adopt

Patholog Res Int. 2011; 2011: 264683.
Walid E. Khalbuss, [1, 2 *] Liron Pantanowitz, [1, 2] and Anil V. Parwani [1]
[1]Division of Pathology Informatics, Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA 15232, USA
[2]Division of Cytology, UPMC Shadyside Hospital, 5150 Centre Avenue, POB2, Suite 201, Pittsburgh, PA 15232, USA

Use of Digital Imaging

At Imperial College we have been using in the past fixed digital images for

- cytology tests during our MSc in Cytopathology
- Cytology mock exams during our Advanced Courses which prepare for the MRCPath examination
- WSI for research purposes and testing.
- We currently informally review WSI of cases but we do not issue a formal report on them. This is because although technology on the different platforms available on the market has markedly improved we do not feel that there is an agreed standardised practice for it.

Conclusions

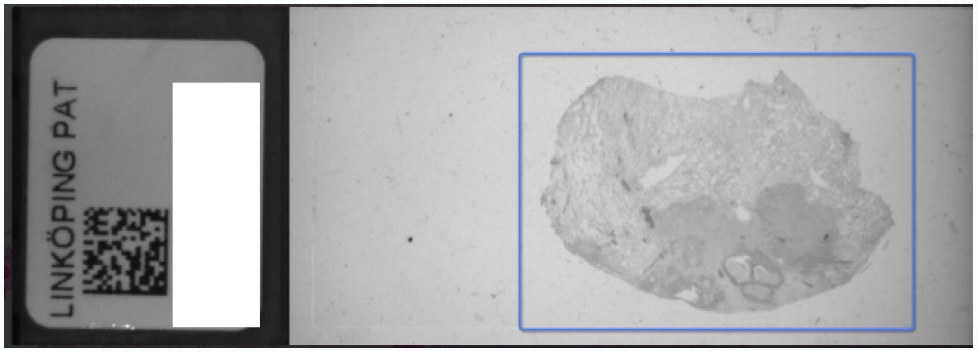
- WSI is here to stay and is fast improving and getting cheaper
- It is an important teaching and training tool
- It is used for EQA schemes and Quality Assurance
- It is used in MDT meetings (Tumour Boards)
- It helps retaining a screening component to all assessment tests
- BUT…… it is one of the many tools!

Digital Histology vs digital Cytology

- Digital Histology and digital Cytology need a different technical approach for many reasons

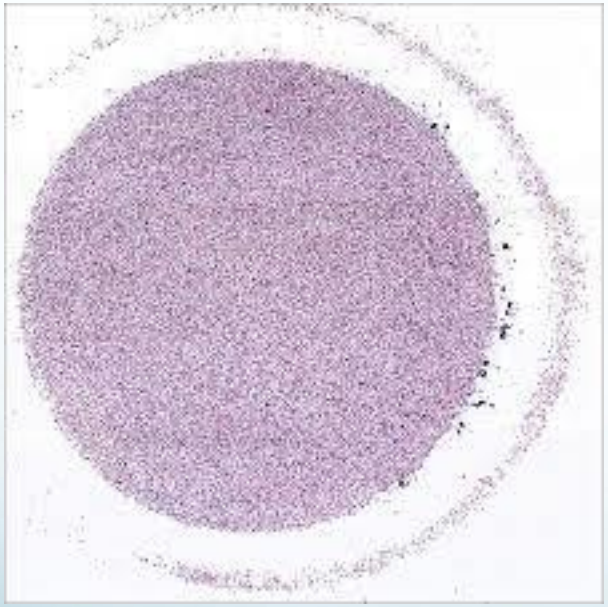
Digital Histology vs digital Cytology

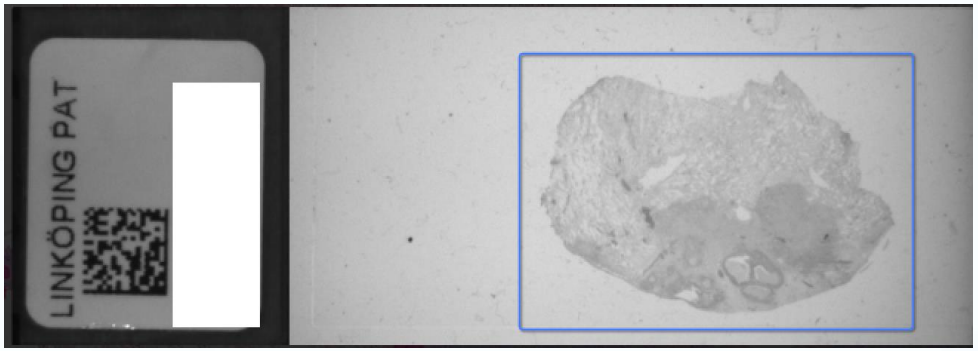
- Dimension:

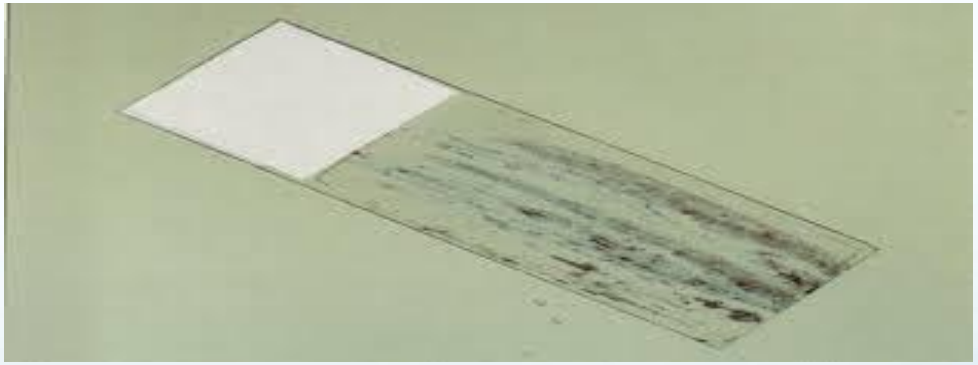


Digital Histology vs digital Cytology

- Dimension:



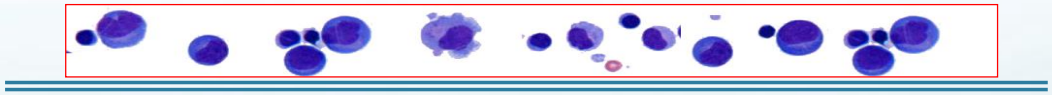




Digital Histology vs digital Cytology

- The nature of the material is different:
  - Histology

  - Cytology



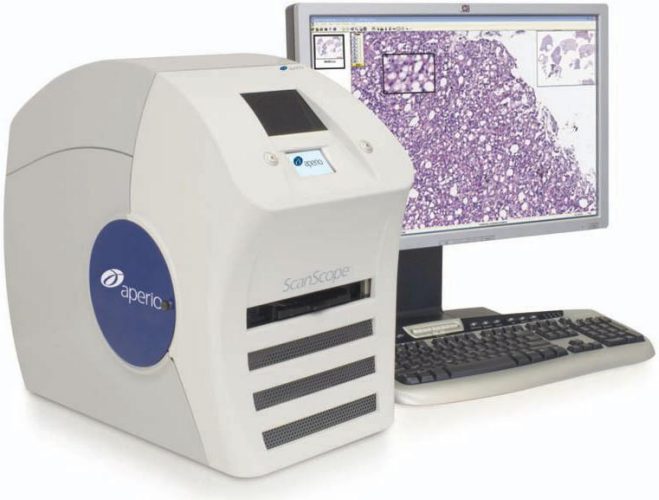
Digital Histology vs digital Cytology

- The microscopy is different:
  - A histological slide requires minimal focus adjustment
  - Micro focusing is the “essence” of cytological screening

Digital Histology vs digital Cytology

- The scanning technique is different:
  - If the scanner autofocus works well, a single layer virtual slide allows a high quality screen of a histological preparation.
  - A multi level scanning is compulsory to get an acceptable cytological virtual slide.

Digital Histology vs digital Cytology



Digital Histology vs digital Cytology

- In essence:
  - Digital Histology is two dimensional
  - Digital Cytology is three-dimensional.

This entails at least four problems.

Digital Cytology: a 3D problem

- The first:
  - How many levels are needed to define "acceptable" a virtual slide?

An immediate and seemingly logical answer is:

The more the better

Digital Cytology: a 3D problem

- The second problem:
  - Which is the optimal distance between each level?

Digital Cytology: a 3D problem

Strictly related to the first two parameters comes the third problem:

the size of the file.

Digital Cytology: a 3D problem

The relationship between file dimension and number of levels is linear.

Just for example:

- In four years in the Ljnkoeping University Hospital Pathology department (Sweden) about 1 000 000 histological slides have been scanned . The space occupied is 400TB.
- The same number of cytological cases scanned with just 5 levels would need

400 x 5 TB = 2000TB

Actually a huge amount of space!

Digital Cytology: a 3D problem

Finally the fourth problem: the time needed for a multi level scanning.

A 20x20 mm wide area can be scanned in about 50 seconds.

The same area scanned with 5 z-stack levels takes more than 4 minutes

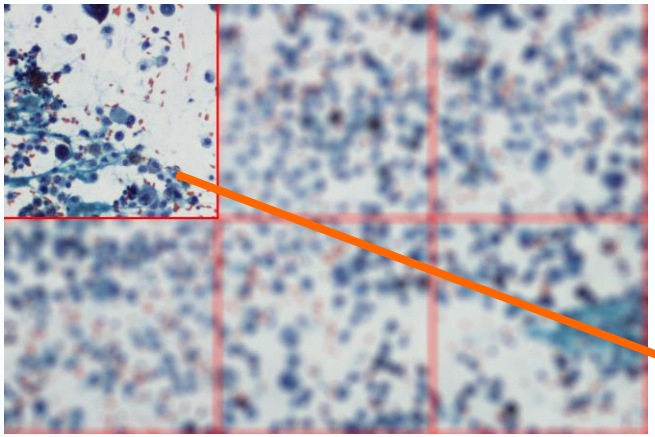
Digital Cytology: a 3D problem

This technique consists 3 steps:

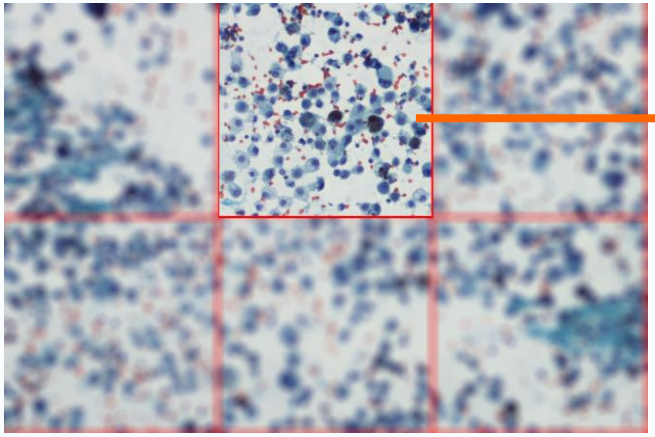
1. dividing in small areas (tiles) the image resulting from the scanning of each level

1. taking the best-focused tile from each layer

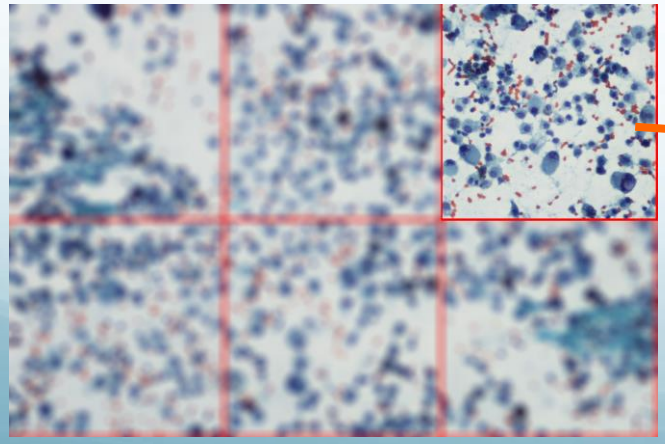
1. building a new virtual slide where all the objects result in focus



Level 1

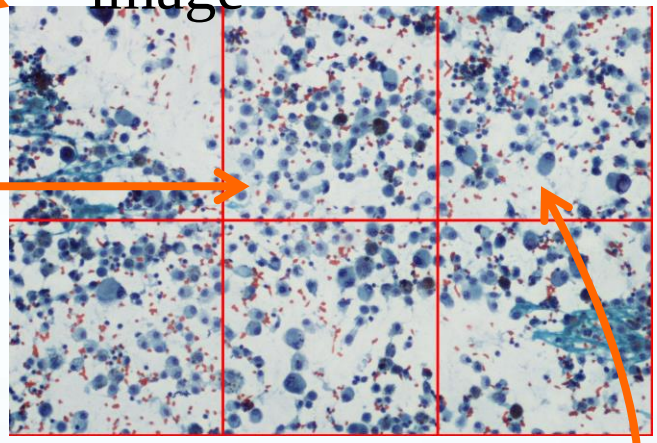


Level 2



Level 3

New single level image



Digital Cytology: a 3D problem

The final result is a single level virtual slides where all the tiles are perfectly in focus.

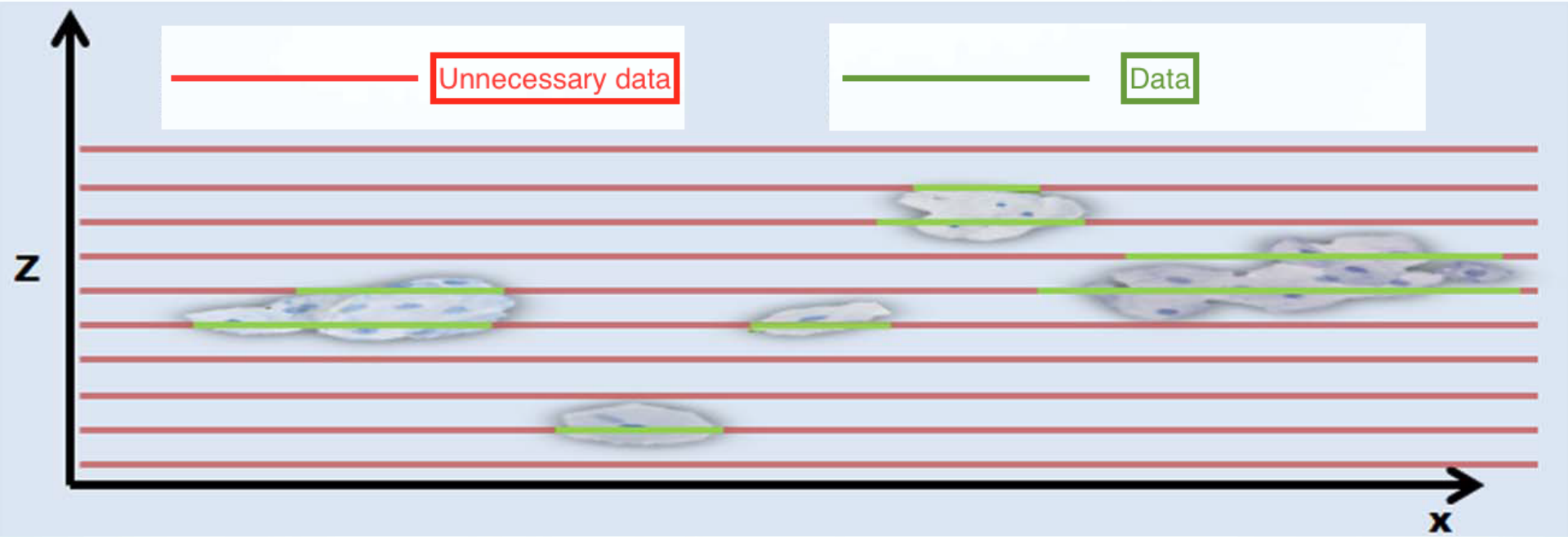
Pros: - small dimension of the file

- good “visual” results

Cons: - long processing time

- a lot of unnecessary data generated

Digital Cytology: a 3D problem



Digital Cytology: a 3D problem

A second interesting method is proposed in

OPEN ACCESS Freely available online

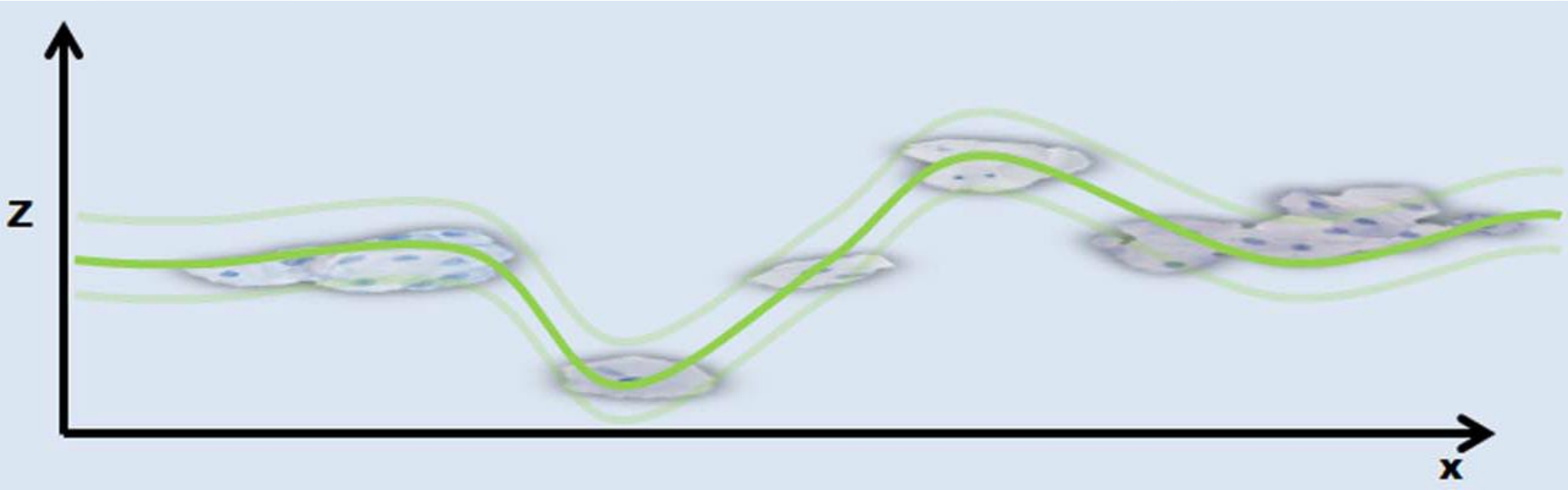
PLOS ONE

Semantic Focusing Allows Fully Automated Single-Layer Slide Scanning of Cervical Cytology Slides

Bernd Lahrmann[1,2,5], Nektarios A. Valous[1,2], Urs Eisenmann[4], Nicolas Wentzensen[3], Niels Grabe[1,2]*

1 Hamamatsu Tissue Imaging and Analysis Center (TIGA), BIOQUANT, University Heidelberg, Heidelberg, Germany, **2** National Center of Tumor Diseases, Medical Oncology, University Hospital Heidelberg University Heidelberg, Heidelberg, Germany, **3** Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland, United States of America, **4** Institute of Medical Biometry and Informatics, University Hospital Heidelberg, Heidelberg, Germany, **5** Institute of Pathology, University Hospital Heidelberg, Heidelberg, Germany

Digital Cytology: a 3D problem



A specific software generates during the scanning a three dimensional focus map of the cells in the slide.

Following this map the scanner takes only the images of the cells avoiding the generation of unnecessary and unwanted data.

How many web sites use digital cytology?

1) The research has used the *Google* search engine: www.google.com;

2) Searched nouns as keyword: nouns had to be the most concise as possible. The used keywords are: cytology web sites, cytology atlas, cytology and cytopathology journal, and cytology societies;

Criteria

1) Sponsor, scientific society, personal web page, academic institution or commercial site: whether a website is sponsored by a Society, a particular product or interest group, the owner of the web site. Personal web page web sites can list the author of the information and biographical information.

2) Society: the name of the involved Society.

3) Purpose: to provide educational information, professional advice, promoting the profession of cytologists, encouraging the science of cytology. Many web sites provide information on topics of interest to the owner, as well as tutorials or opinions.

4) Topic: FNA, gynaecologic or non-gynaecology cytology.

5) Target groups: whether the web site is recommended to cytologists, cytotechnologists, cytology trainees or students, laboratory personnel.

Criteria

6) Access: public, only registered members, any payment fees required.

7) Educational resources: each web site has been checked whether with or without educational purpose or to improve academic success.

8) Imaging: static or dynamic as virtual slides.

9) Passive or interactive: some web sites have just slides to look but no possibility to have an interactive approach. Other web sites allow the visitors to take quizzes or view solutions previously hidden, in order to test trainees or students.

Results

- The number of web sites is about 671,000 results for each keyword. Sites with only histopathology have been excluded.
- Based on the above mentioned criteria, the number of web sites considered adequate is 31.

Conclusions

- There are numerous web sites available
- Aims are different
- Few are available in multiple languages
- Cytology is notoriously more difficult to comprehensively scan
- Too few web sites are completely free to use
- Few offer interactive e-training
- However it is getting better all the time!

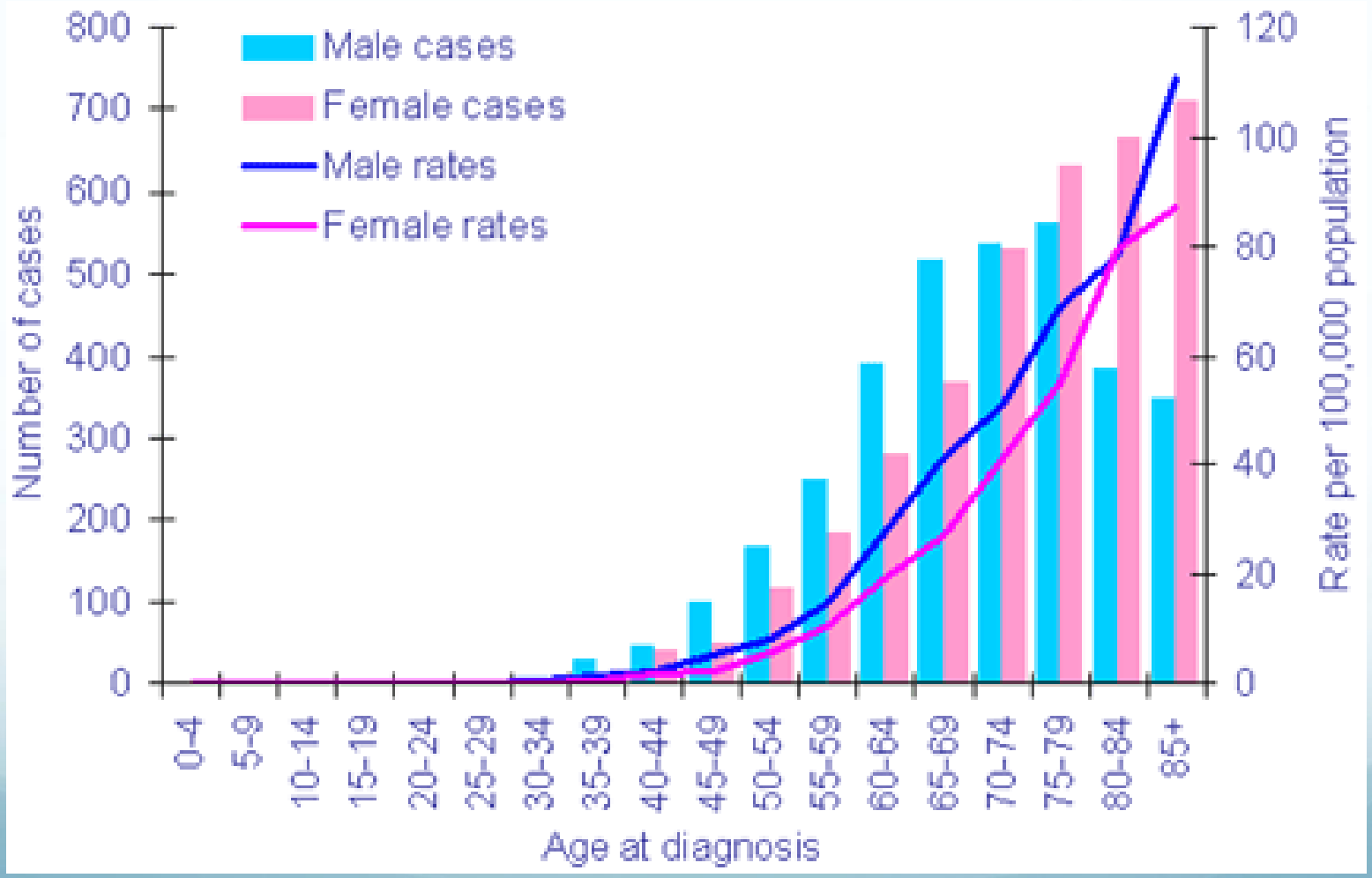
Incidence of Pancreatic Tumours

- Ductal adenocarcinoma - 80%
  - include all the variants, then 90%
- Other tumours - 10%
  - MCN - 2%
  - PET - 2%
  - IPMN - 1%
  - Acinar carcinoma - 1%
  - Serous cystadenoma - 1%
  - SPPT - 1%
  - Pancreatoblastoma

Ductal Adenocarcinoma of the Pancreas

- **85% of all pancreatic malignancies**
- **Increasing incidence 4-5000pa in UK**
- **M1.6:1F**
- **55-75 years (average 60)**
- **2% < 40 years**

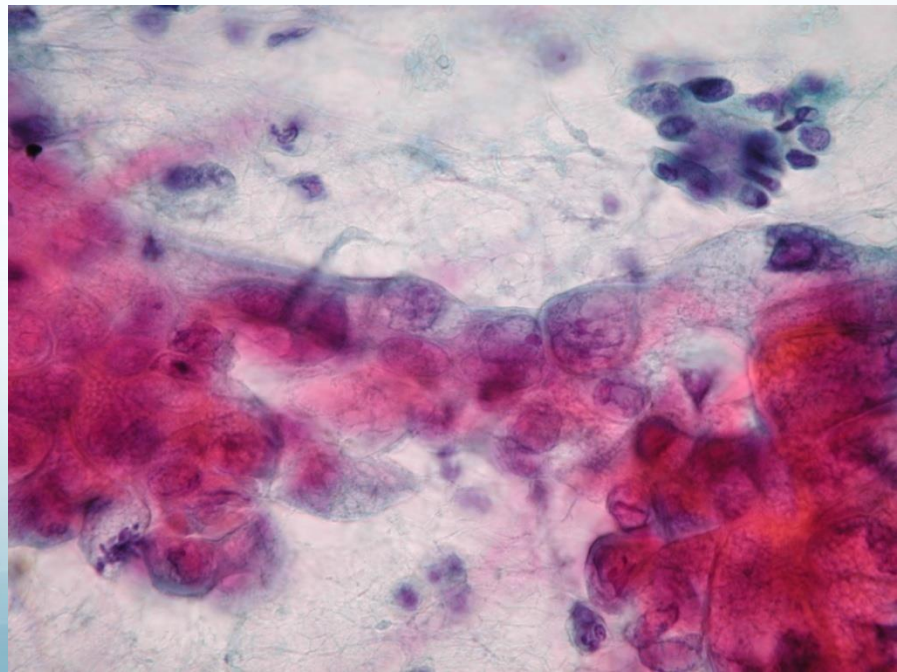
Incidence of Pancreatic Cancer



Ductal Adenocarcinoma of the Pancreas- Investigations

- CA19.9 >70IU/mL

- Biopsy -
  - Core needle (histology)
  - FNA
  - Biliary brushings



Why cytology?

- Resectable - just take it out?
  - Medical-legal issues related to a bad outcome with benign disease
    - 10% of jaundiced patients with an “obvious” malignant mass prove to have a benign lesion
  - Potential for lymphoma diagnosis, a non-surgical disease
  - Cystic lesions
  - Patient compliance

Why cytology?

- Unresectable, just leave it in?
  - Not all large masses that appear unresectable are ductal adenocarcinoma
  - advances in surgical and anaesthetic practices have improved surgical outcomes even in older, less fit patients
  - a positive tissue diagnosis is mandatory before chemotherapy or radiation therapy can be instituted

Pancreatic Mass: Solid or Cystic?

- Solid Pancreatic masses

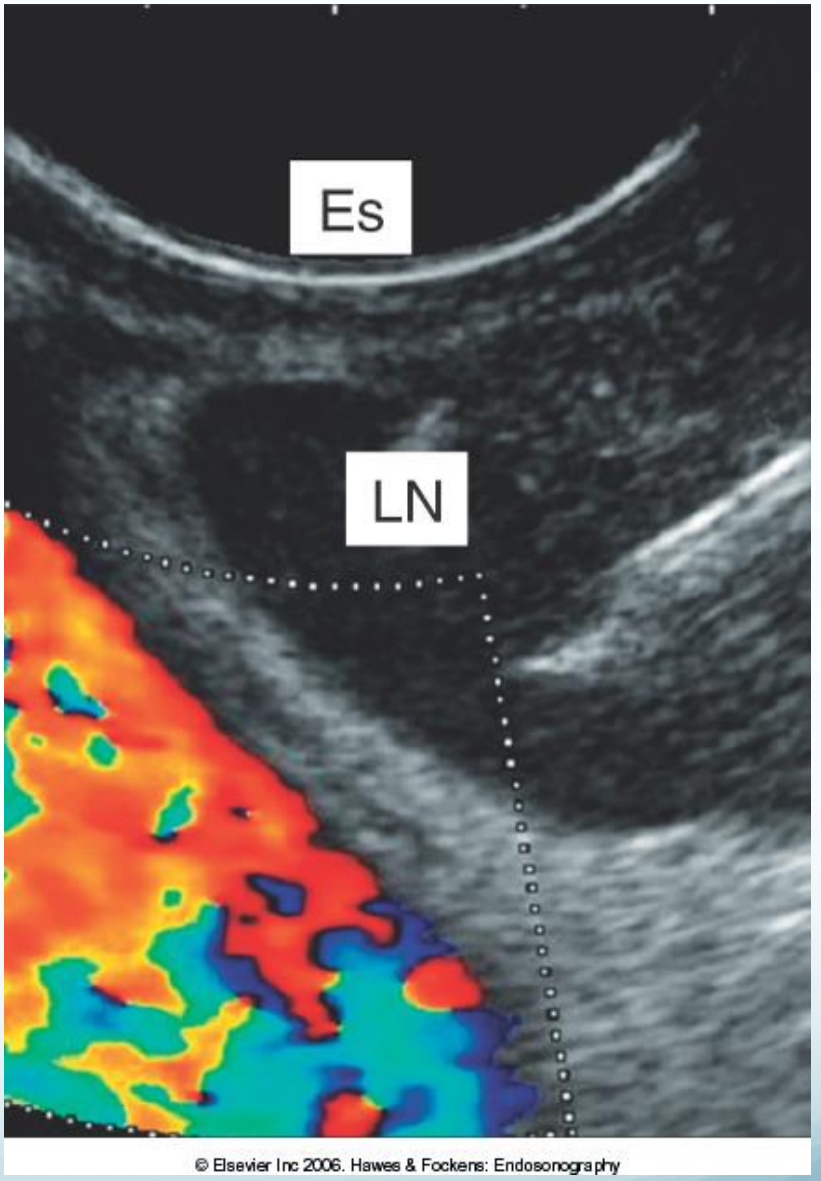
- ductal adenocarcinoma
  - typical
  - variant
- chronic pancreatitis
- Acinar cell carcinoma
- pancreatic endocrine tumour
- pancreatoblastoma

- Cystic pancreatic masses

- pseudocyst
- serous cystadenoma
- solid pseudopapillary tumour
- mucinous cyst
  - MCN
  - IPMN

Endosonography

High frequency miniature ultrasound transducer is incorporated into the tip of a conventional endoscope resulting in enhanced resolution of the GI wall and structures with close proximity to the GI wall



USS advantages

- High intrinsic spatial resolution
- No ionizing radiation
- Inexpensive and easily portable

USS Disadvantages

- Gas and bone impede the passage of USS waves
- As good as the operator

Types of Echoendoscopes

- Radial
- Linear
- Miniprobes

Advantages of EUS and EUS Guided FNAB

- Biopsy
  - Not percutaneous FNAB
    - no reported cases of needle tract seeding with EUS FNAB
  - Small trajectory to target compared to percutaneous method
  - More sensitive than CT for small masses (0.5 cm vs 2cm)
  - cost effective relative to CT biopsy
- Staging/determining resectability
  - distant metastases or SMA invasion=unresectable
  - peripancreatic nodes and accessible liver lesions can be biopsied during the same procedure

Disadvantages to EUS and EUS Guided FNAB

- Expensive equipment
- Technically difficult and requires significant expertise
  - low tissue yield with inexperience
- Currently no good core biopsy method
- GI contamination of cytology specimens
  - particularly a problem with cystic lesions

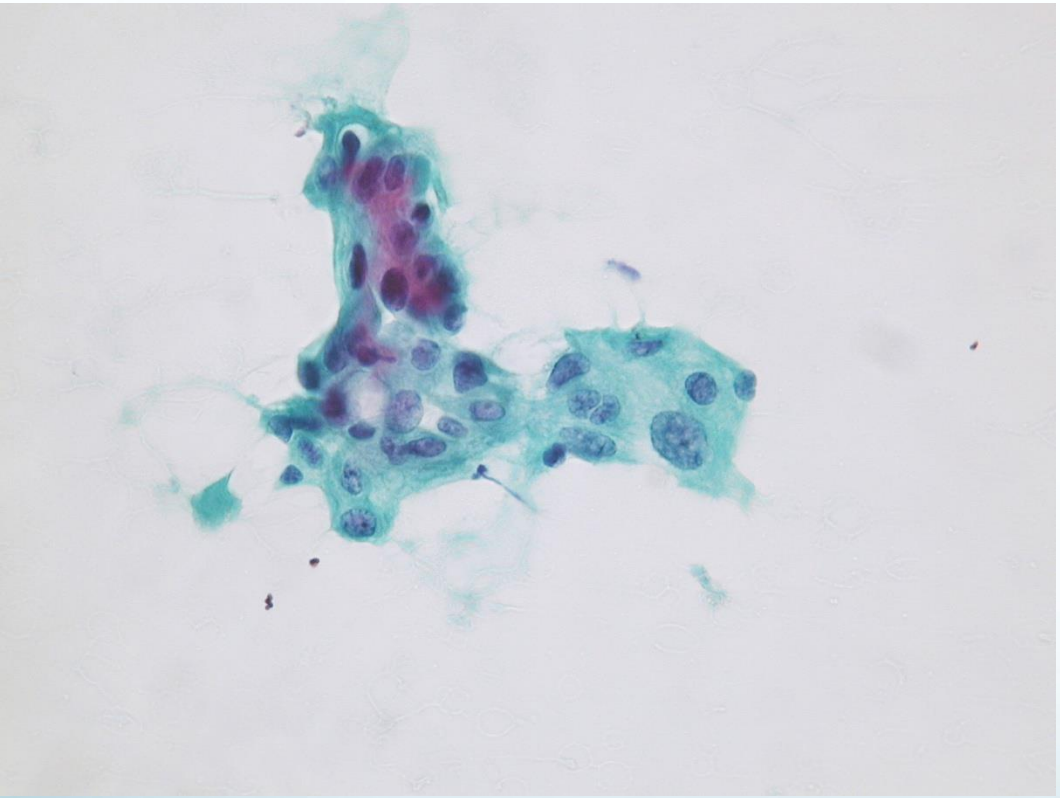
EUS-guided FNA for diagnosis of solid pancreatic neoplasms

- False –ve results up to 20-40 %
- False positive very rare

Optimizing diagnostic yield from EUS-FNA. Cytopathology June 2013

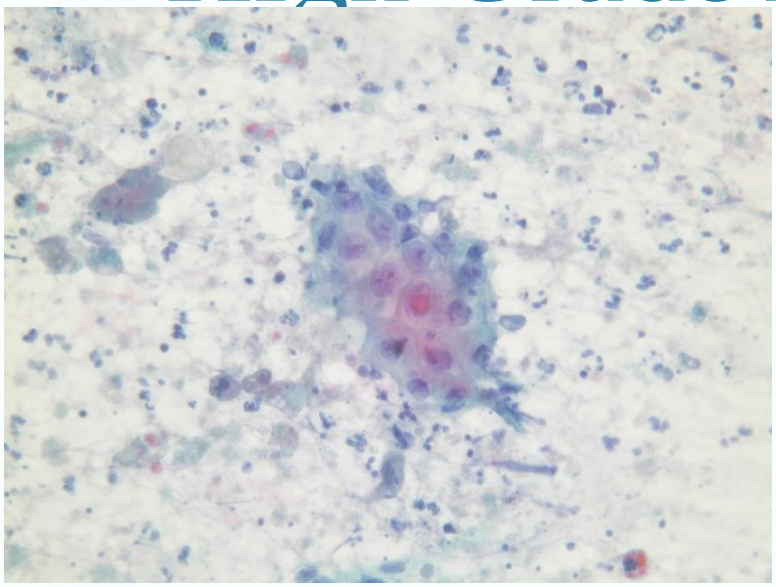
- ROSE increases diagnostic sensitivity and accuracy of FNA for solid pancreatic masses by up to 10-15 %
- Meta-analysis of 34 studies with 3644 patients : ROSE : p=0.001 for accuracy

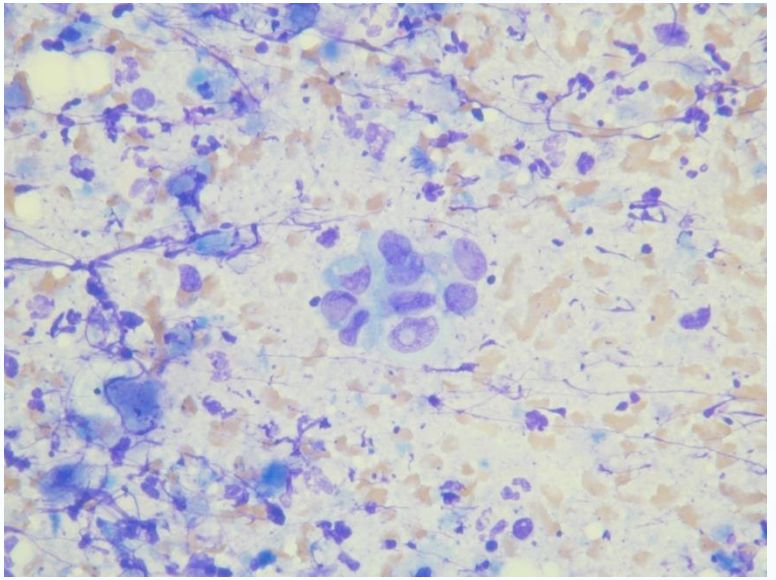
High Grade Adenocarcinoma

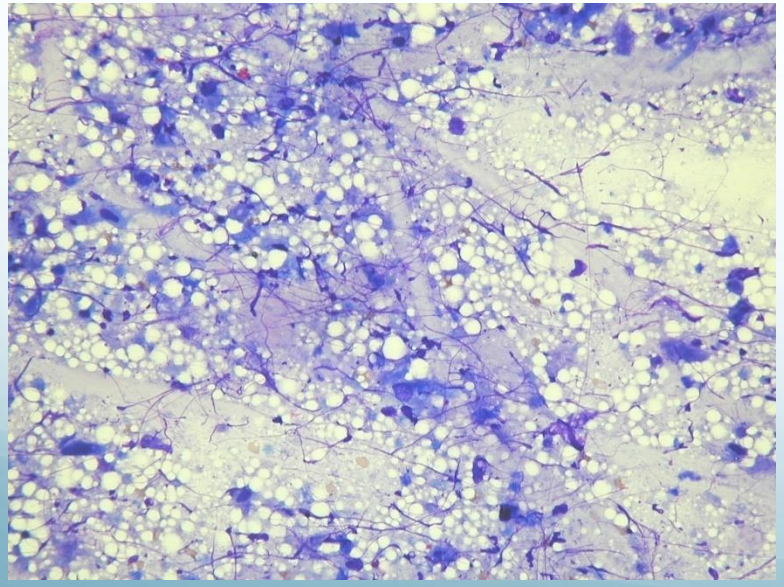


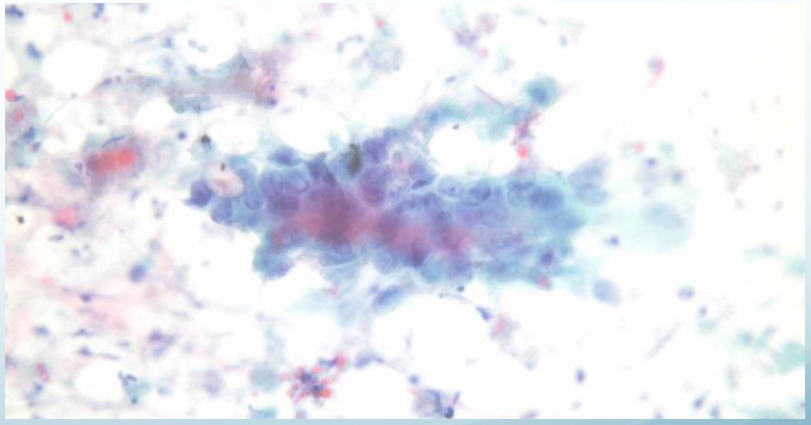
- Marked nuclear
  - atypia
  - hyperchromasia
  - pleomorphism
  - overlapping
- Prominent nucleoli
- Single atypical cells
- Mitoses
- Coagulative Necrosis

High Grade Adenocarcinoma





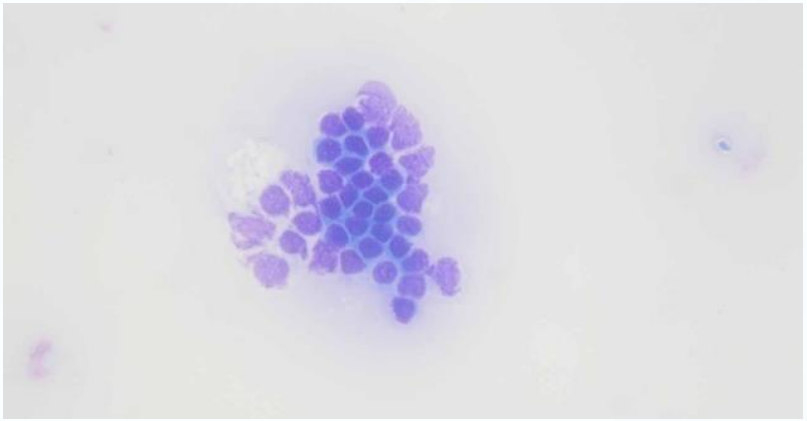


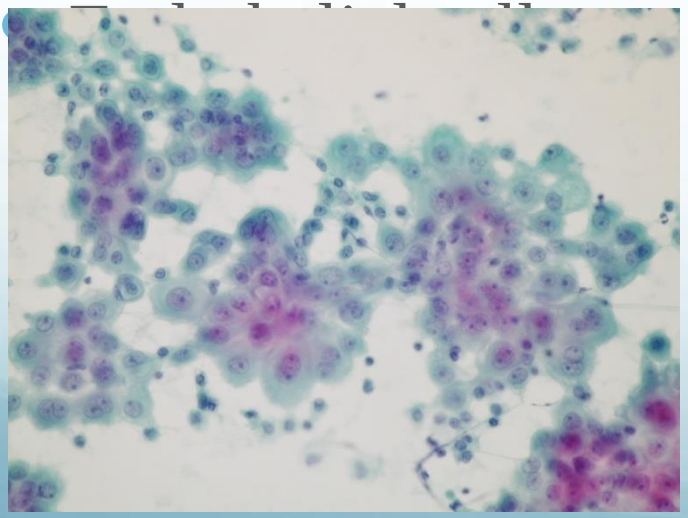


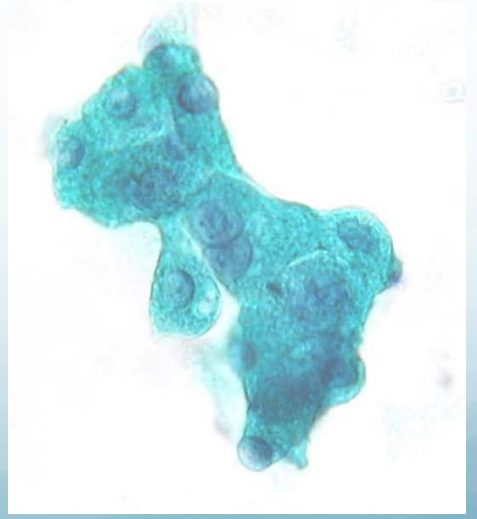
win.eurocytology.eu/virtualslides/git-eus/vs-064

Pitfalls

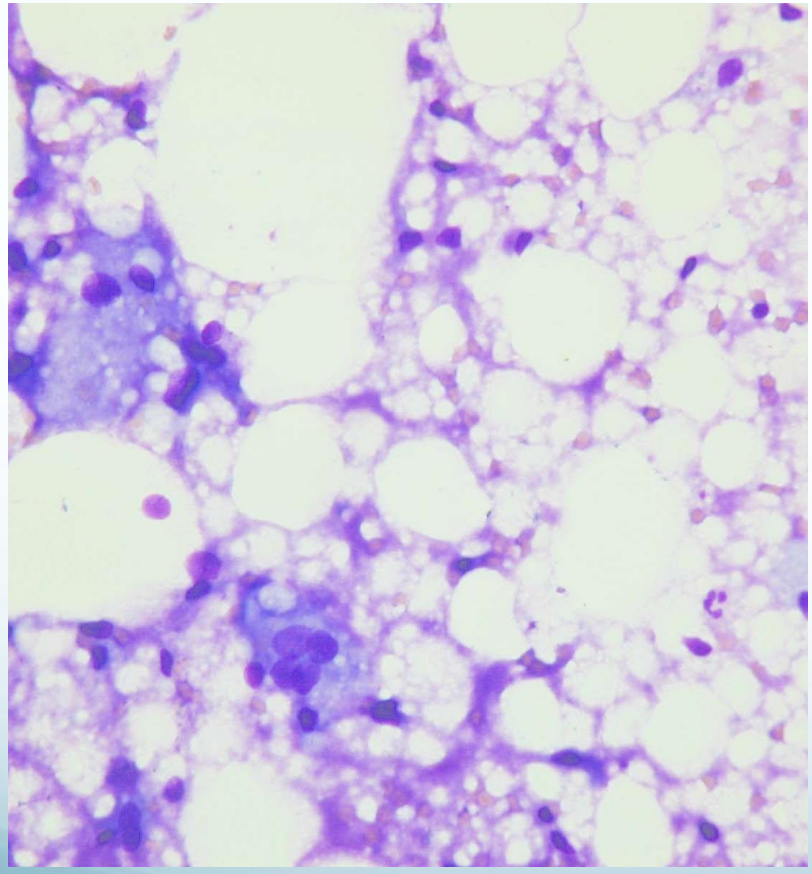
- Liver cells
- Intestinal cells
- Mesothelial cells







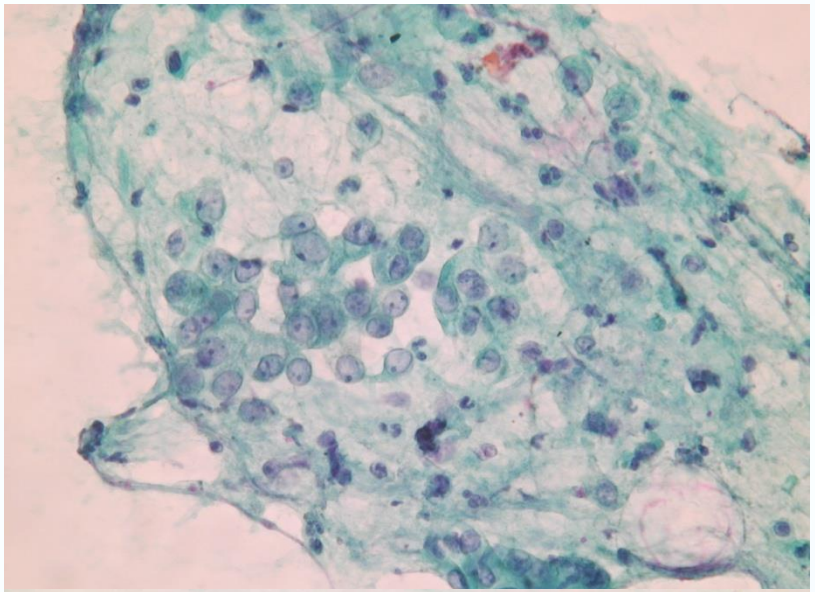
Early stages of Chronic active pancreatitis

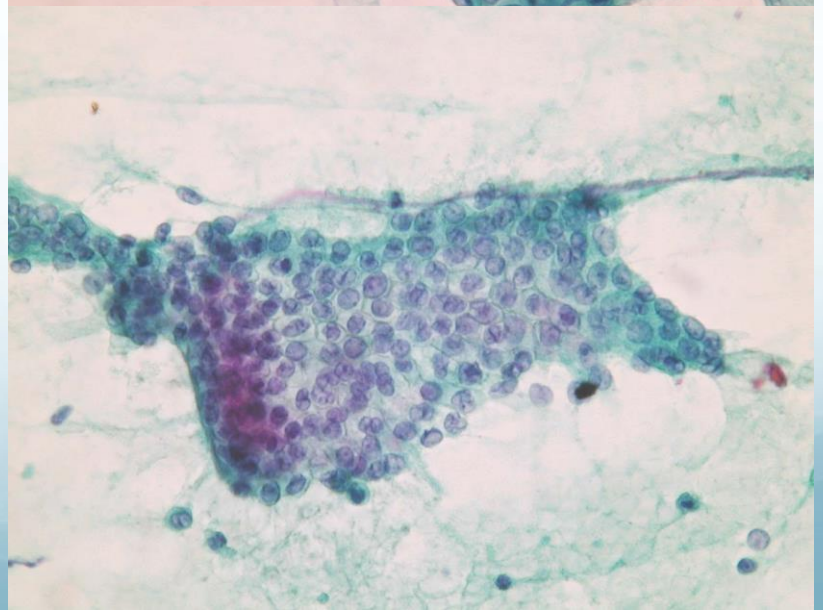


- Both ductal and acinar cells
- Background inflammation
- Granulation tissue
- Fat necrosis

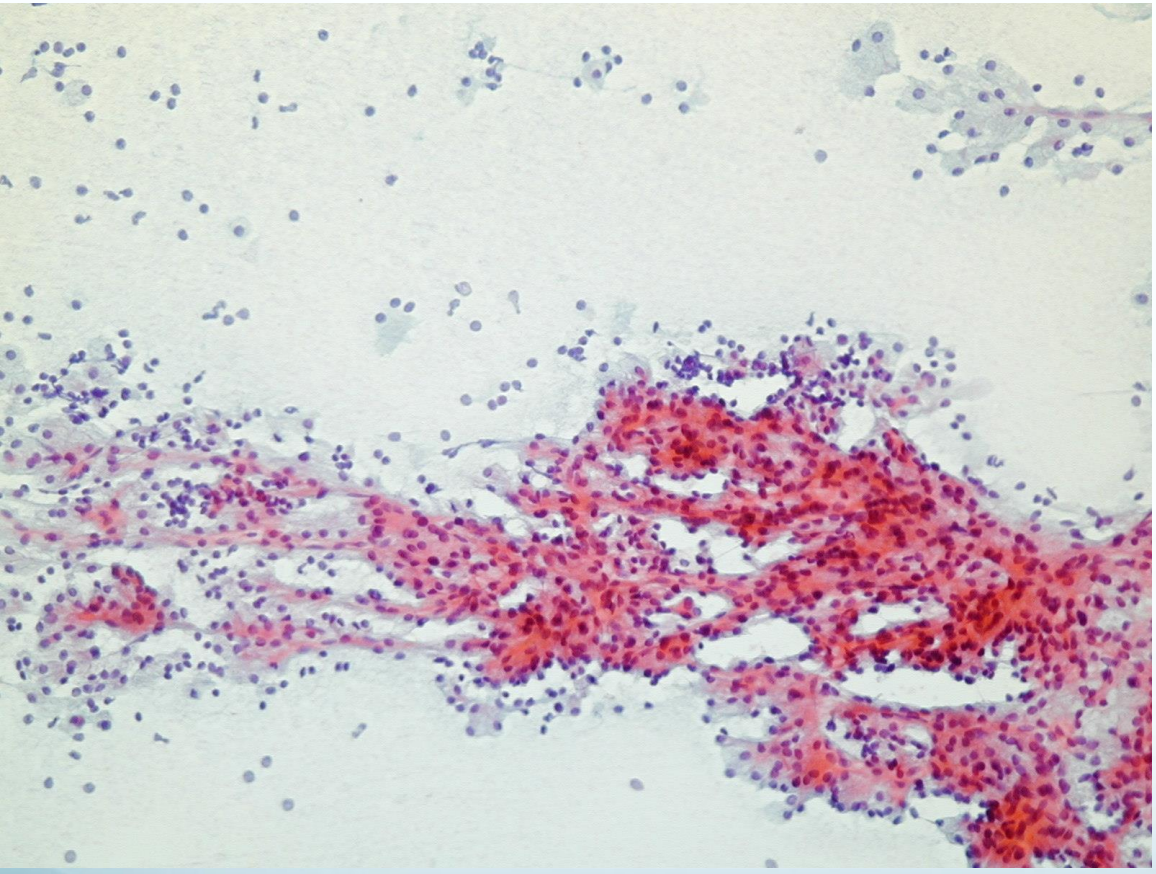
Late Chronic Pancreatitis

- mostly ductal cells
- few to no acinar cells
- some islet cells
- monolayered sheets
- cohesive, few single cells
- maintained polarity
- minimal nuclear overlap
- mild anisonucleosis
- smooth nuclear membranes
- rare/normal mitoses
- no coagulative necrosis





Acinic cell Carcinoma



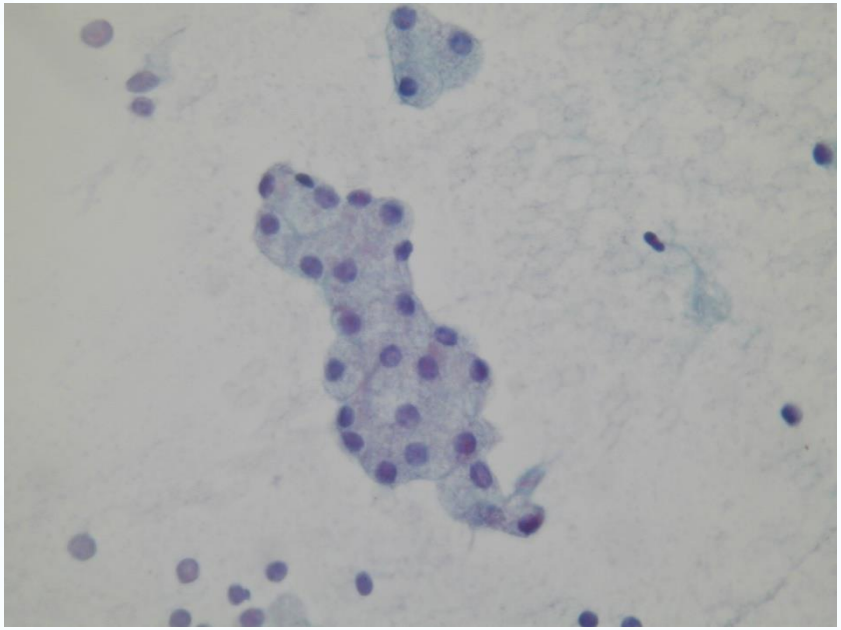
Rare primary tumour

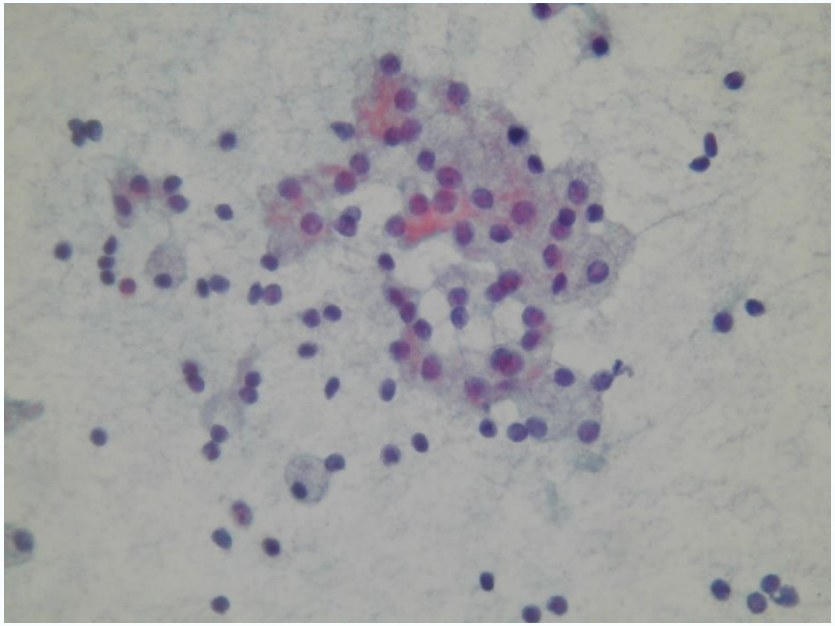
Highly aggressive but better 5 year survival than ductal carcinoma (50% vs. <10%)

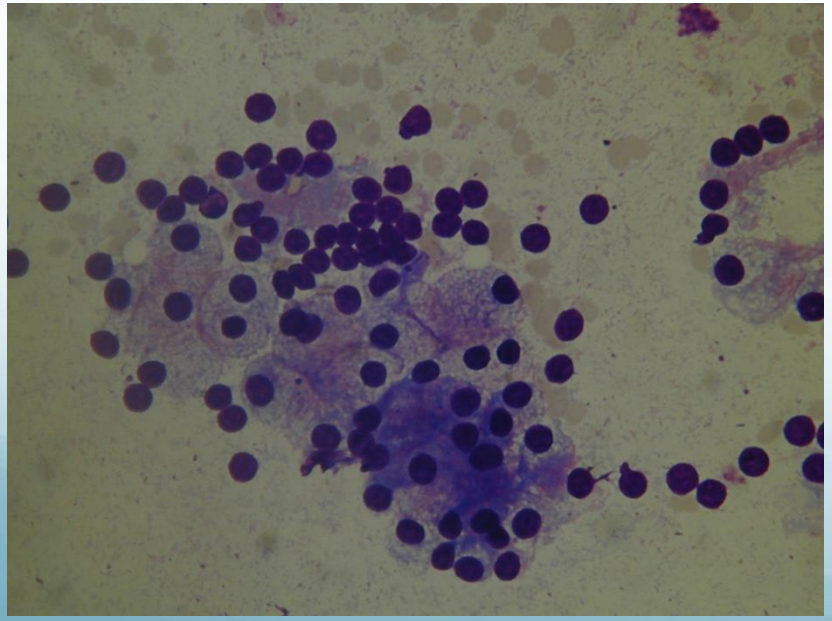
Mostly adult men but can be seen in children

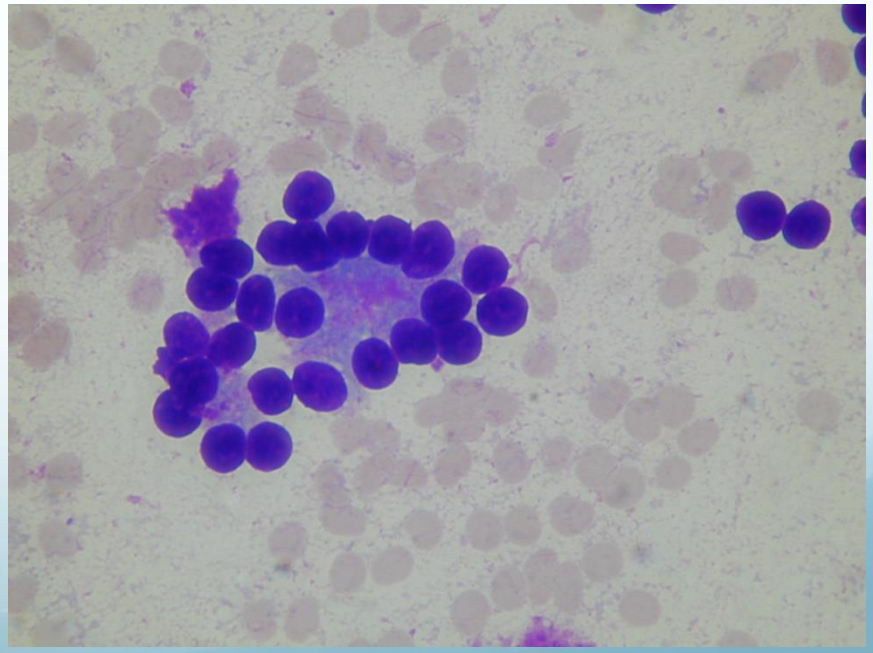
Presentation variable but generally non-jaundiced (in contrast to ductal ca.)

Small %- syndrome of disseminated fat necrosis/ polyarthralgia due to serum lipase secretion by tumour

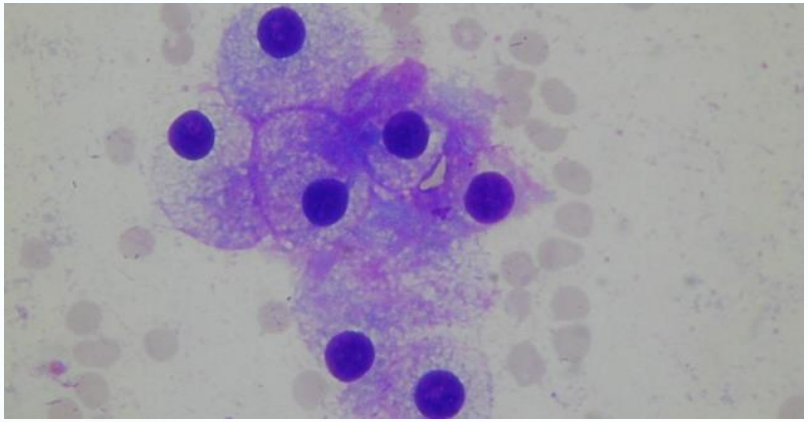


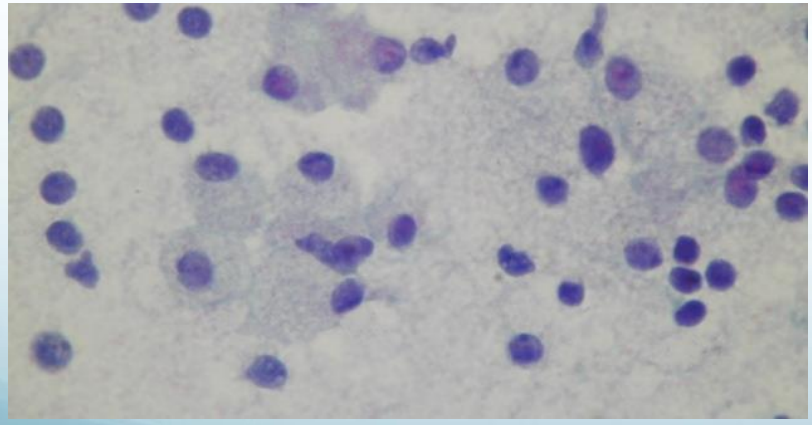






Acinic cell carcinoma

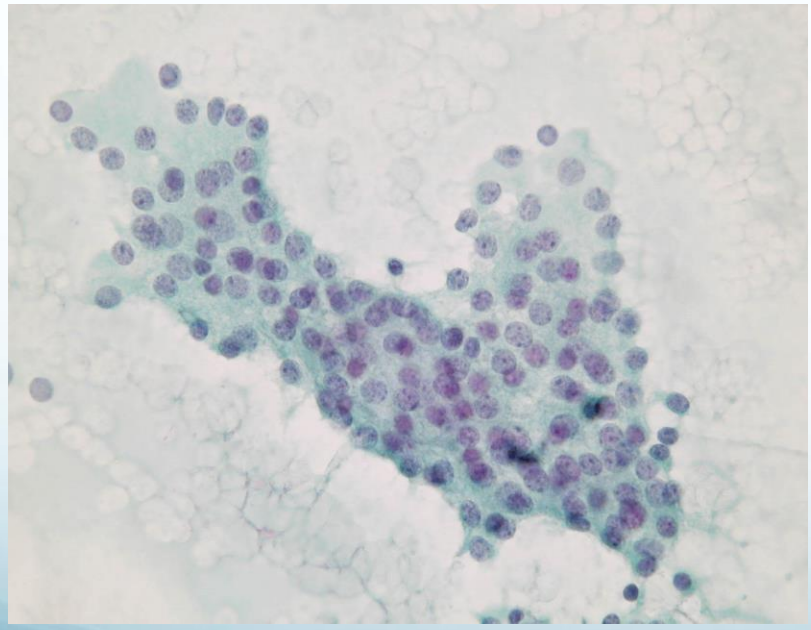


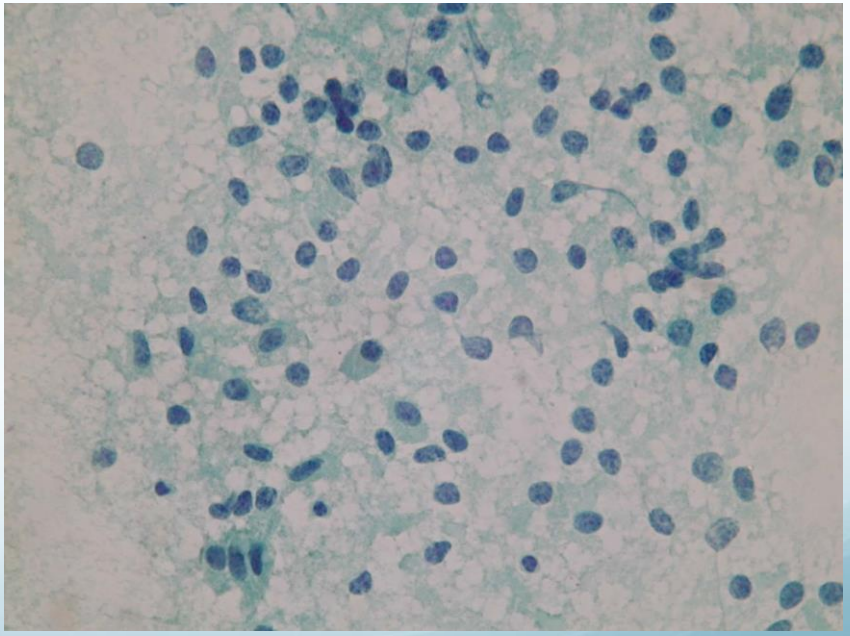


- High cellularity
- No ducts
- No fatty stroma
- Poorly formed acini
- Variable cells
- Atypia variable

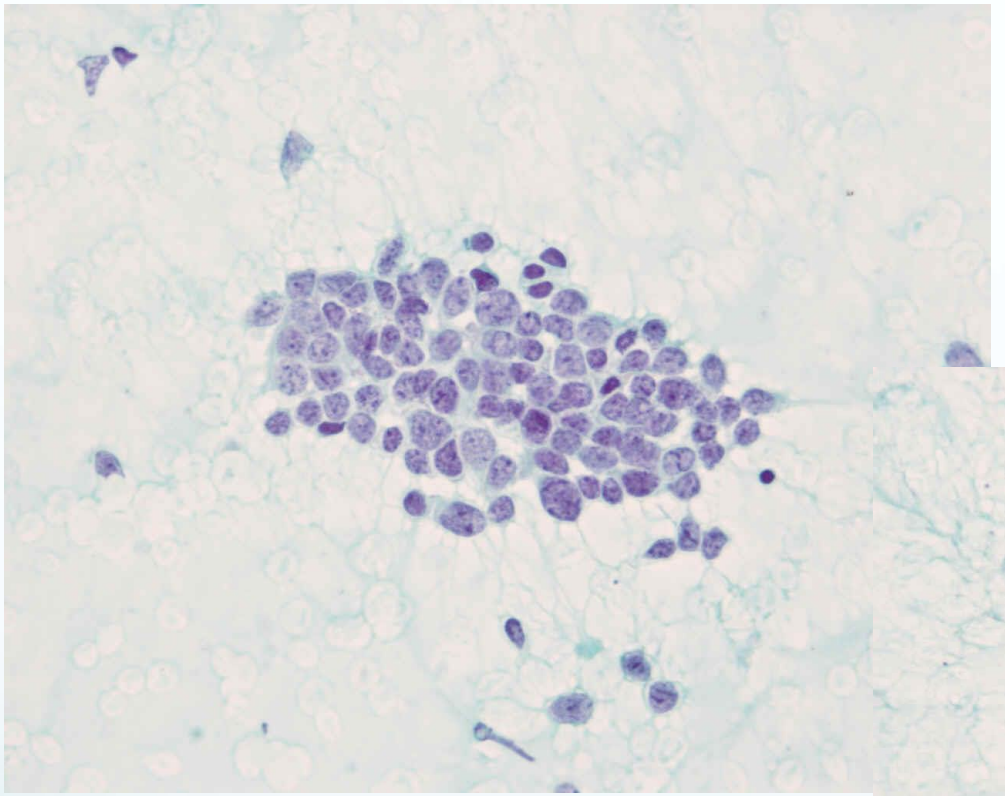
Pancreatic Endocrine Tumours

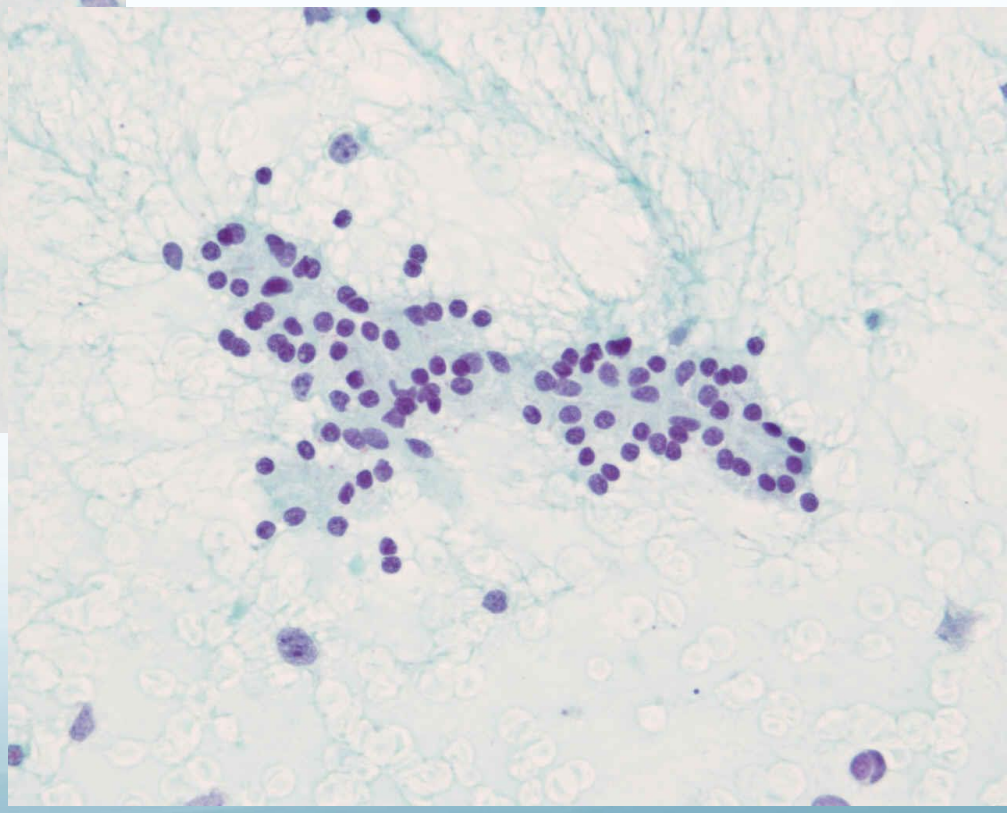
- PET can be cystic due to central necrosis
- PET, cystic or solid, located most commonly the body and tail
- Most cystic PET are non-functioning





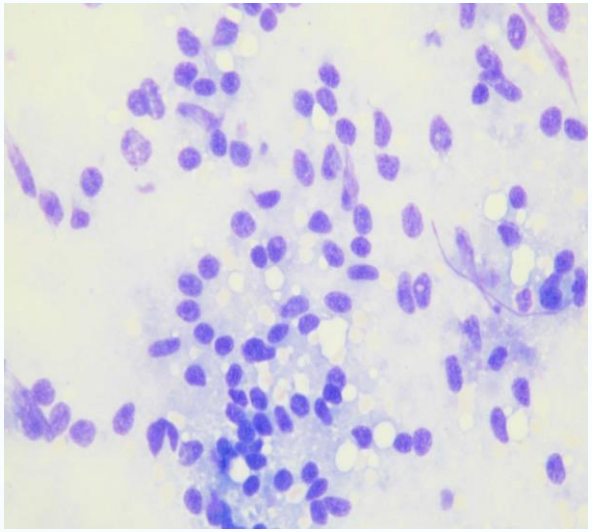
Neuroendocrine Cytology

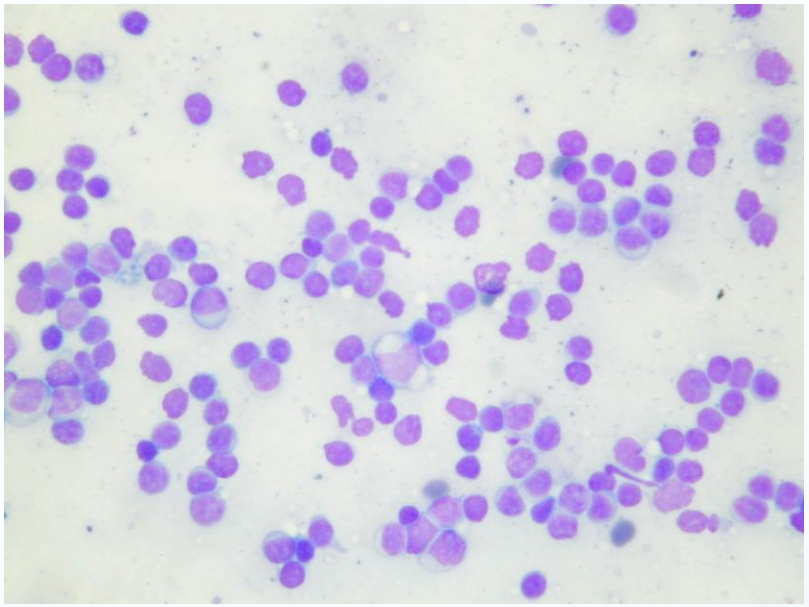




win.eurocytology.eu/virtualslides/git-eus/vs-052

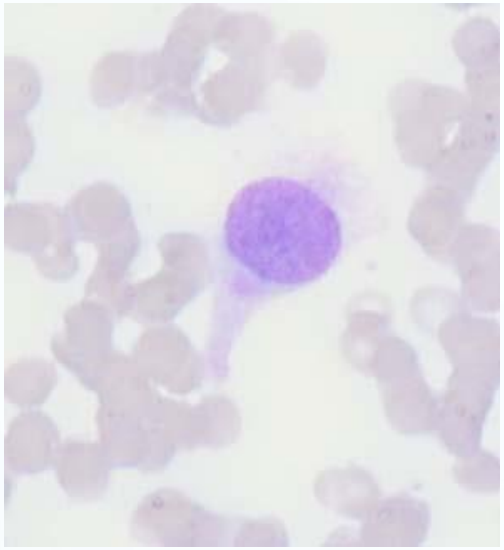
Pancreatic Endocrine Tumours

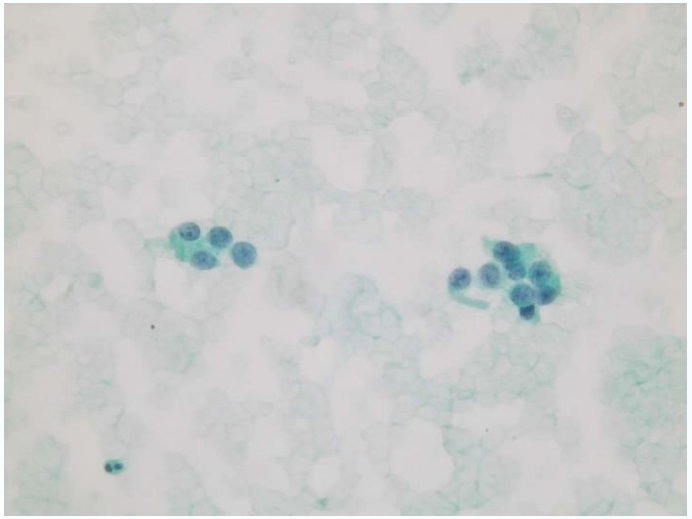


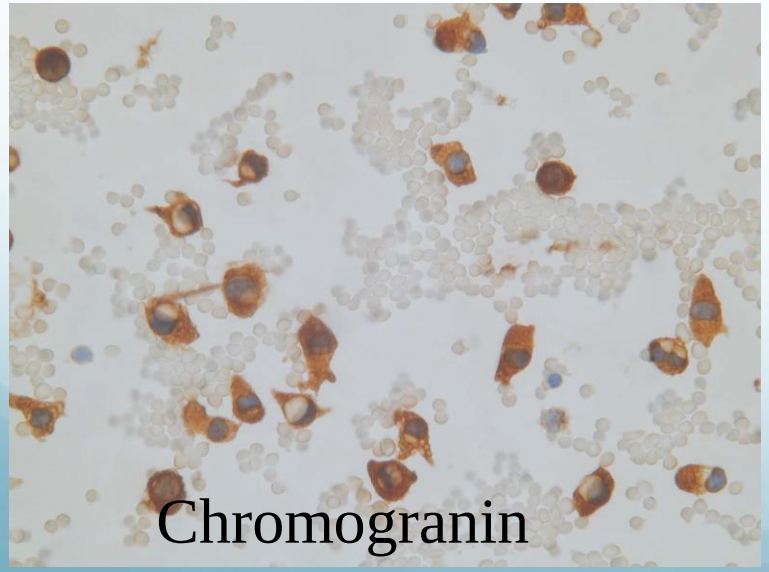


- homogenous small cell population
- loosely cohesive clusters and single cells
- plasmacytoid morphology not uncommon
- round to oval nuclei
- coarse, speckled chromatin
- nucleoli also not uncommon
- chromogranin should be positive

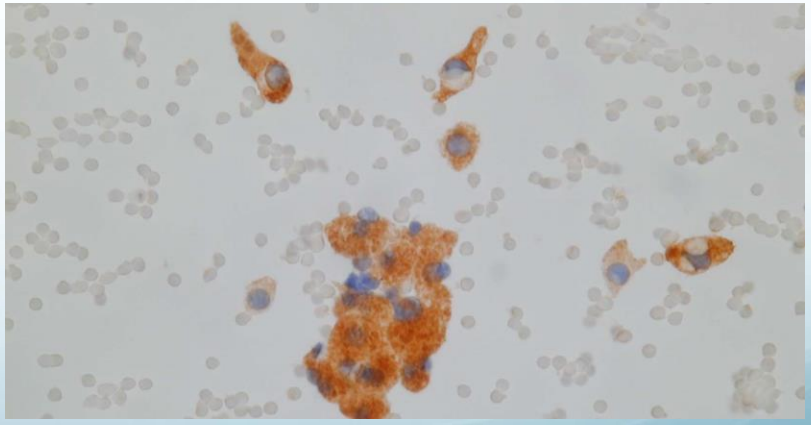
(Pancreatic?) Endocrine Tumours







Chromogranin



Calcitonin

Pancreatic cysts
(most common and clinically relevant)

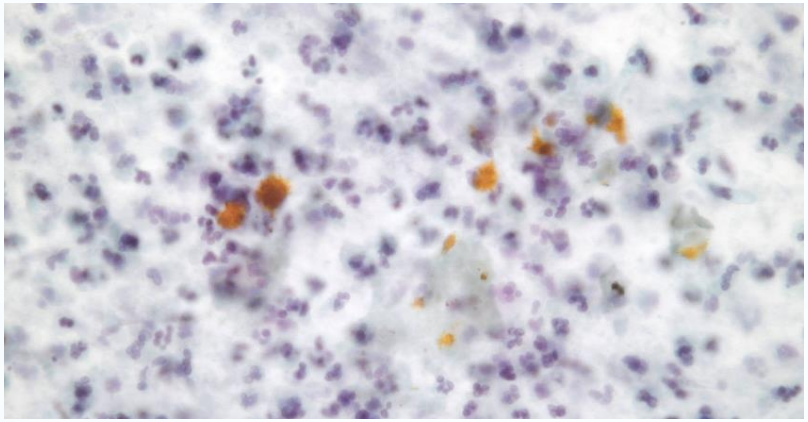
- Pseudocyst
- Serous cystadenoma
- Solid pseudopapillary tumour
- Mucinous cysts
  - mucinous cystic neoplasm
  - intraductal papillary mucinous neoplasm

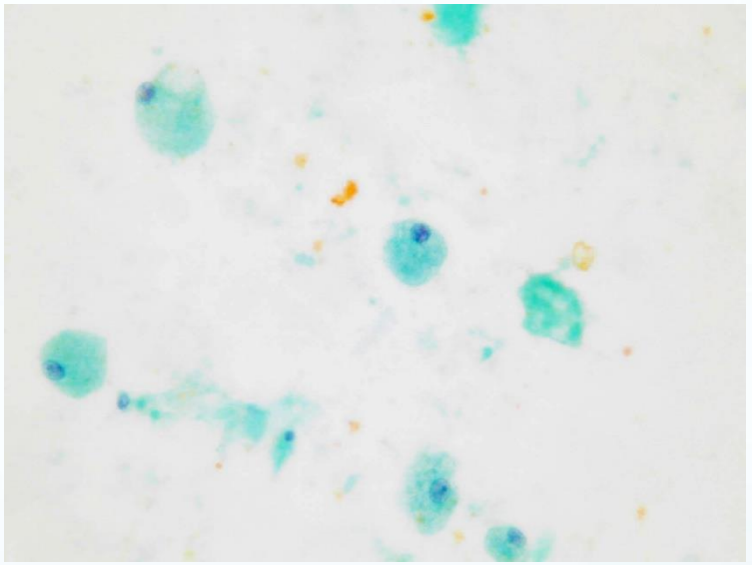
win.eurocytology.eu/virtualslides/git-eus/vs-097

Pancreatic Pseudocyst

- Most common cystic lesion in the pancreas (75-90%)
- Associated with pancreatitis, trauma, surgery
- Thick walled, unilocular, +/- communication with duct
- Fluid aspirated is often dark and not viscous

Pancreatic Pseudocyst
cytology





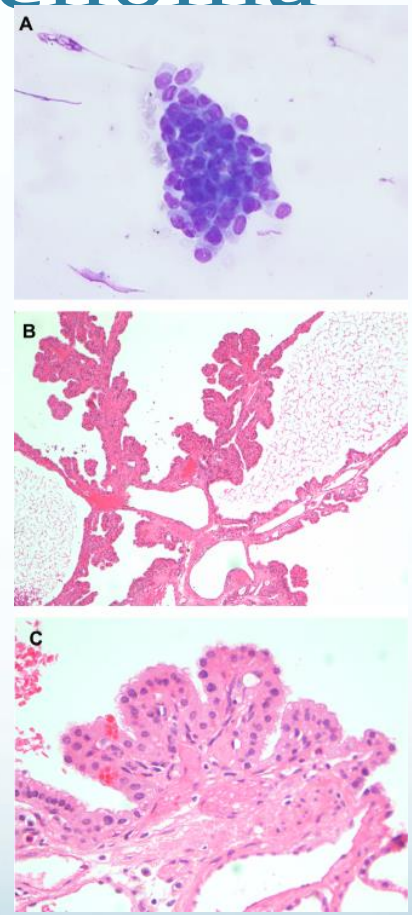
- Cyst debris with blood, proteinacous material and sometimes bile
- variable inflammation
- NO cyst lining epithelium (beware of contamination, mucin and epithelium)

Serous Cystadenoma

- benign neoplasm in the head and tail of elderly men and women
- star-burst calcifications within a central scar diagnostic on imaging when present, but this is rarely present
- most tumours are “microcystic” with multiple, <2cm cysts, but can be unilocular due to specific variant or due to haemorrhagic degeneration, causing problems with imaging diagnosis

Serous Cystadenoma

- Watery, non-mucinous fluid
- scant cellularity
- clean, proteinaceous or bloody background
- monolayered sheets or small, flat clusters
- bland, uniform, round nuclei
- scant but visible non-mucinous cytoplasm



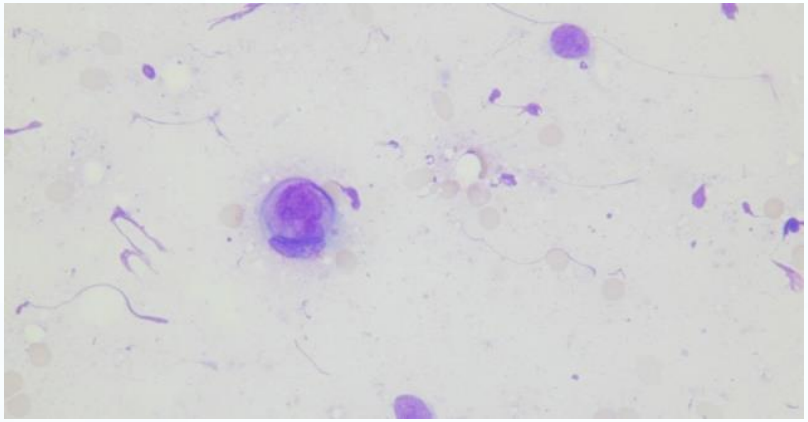
Mucinous Cysts of the Pancreas
WHO Classification

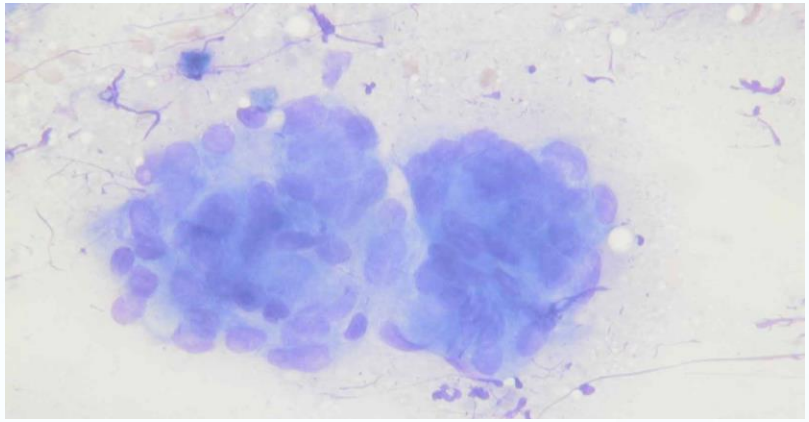
- Mucinous cystic neoplasm
  - Mucinous cystadenoma
  - Borderline mucinous cystic neoplasm
  - Mucinous cystadenocarcinoma
- Intraductal papillary mucinous neoplasm
  - Intraductal papillary mucinous adenoma
  - Intraductal papillary mucinous neoplasm of borderline malignancy
  - Intraductal papillary mucinous carcinoma
  - Intraductal papillary mucinous neoplasm with invasive carcinoma: tubular type or colloid carcinoma

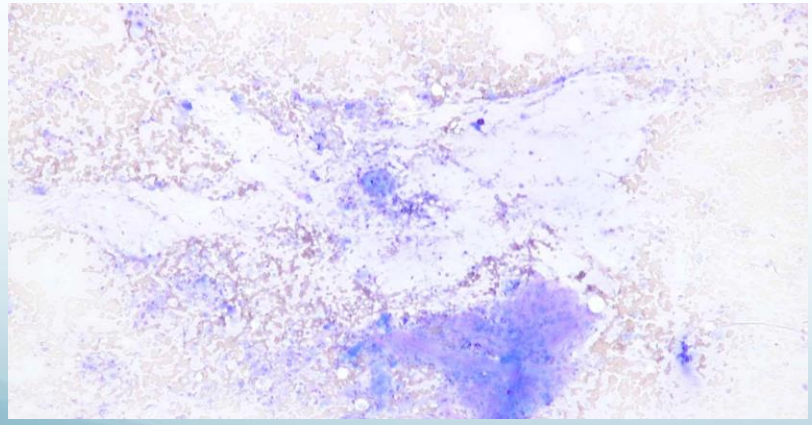
Mucinous Cystic Neoplasms (MCN)

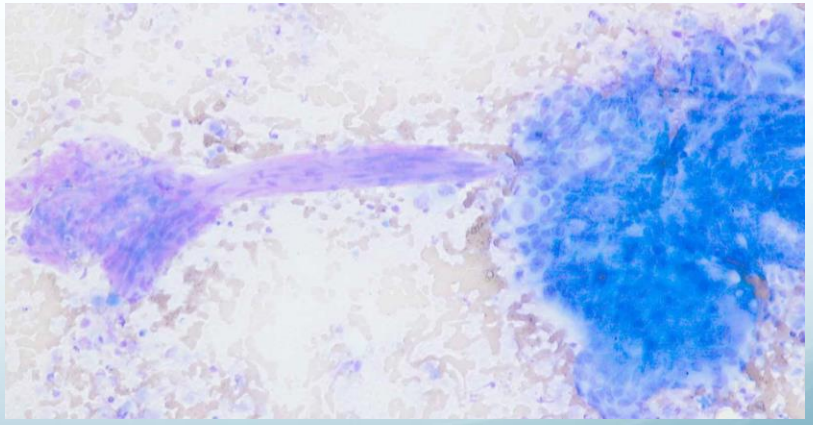
- Lined by mucinous, generally non-papillary epithelium, but can be focally papillary
- Associated with a subepithelial “ovarian-like stroma” (females)
- Predominantly in middle aged females
- Mostly in the pancreatic tail
- Cysts do not communicate with the pancreatic ductal system
- Thin septae

Mucinous Cystic Neoplasms (MCN)









Intraductal papillary mucinous tumour (IPMT)

- Main duct or branch duct types
- Macroscopic papillae or mucin
- Focal or diffuse > 1cm
- PanIN < 5mm
- M>F (Main Duct Equal)
- 60 years average

Thank you!