Citopatologia Ecoendoscopica

XX Congreso de la Sociedad Espanola de Citologia

ZARAGOZA

Mayo 19, 2011

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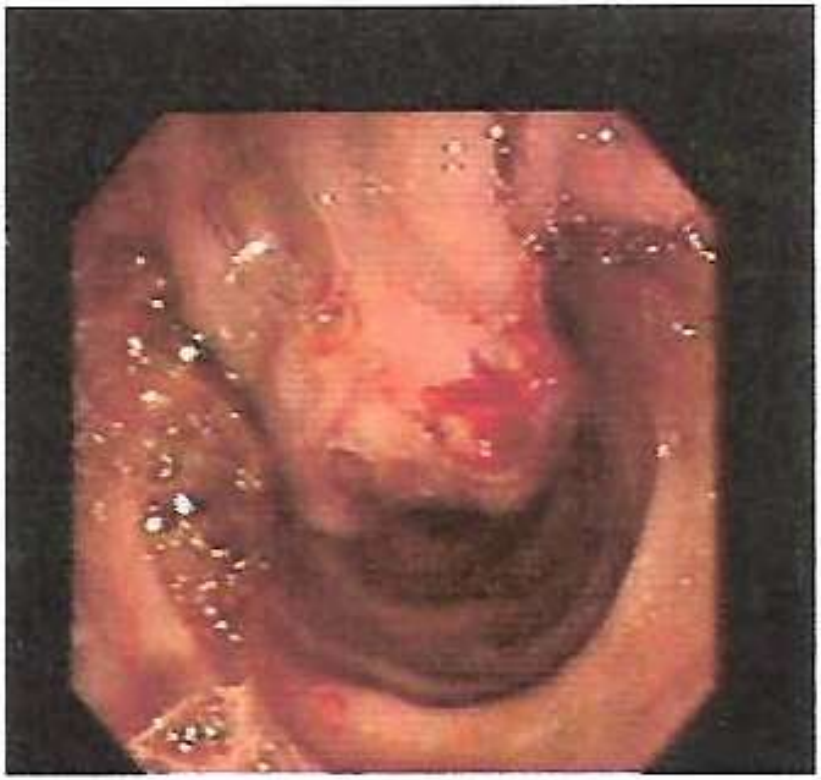
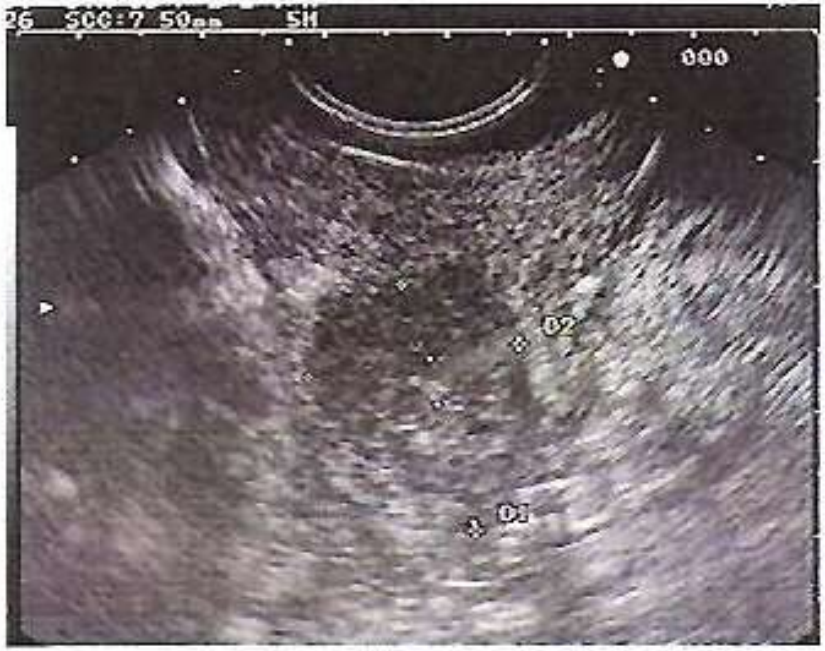
1 5:31PM

Case 1

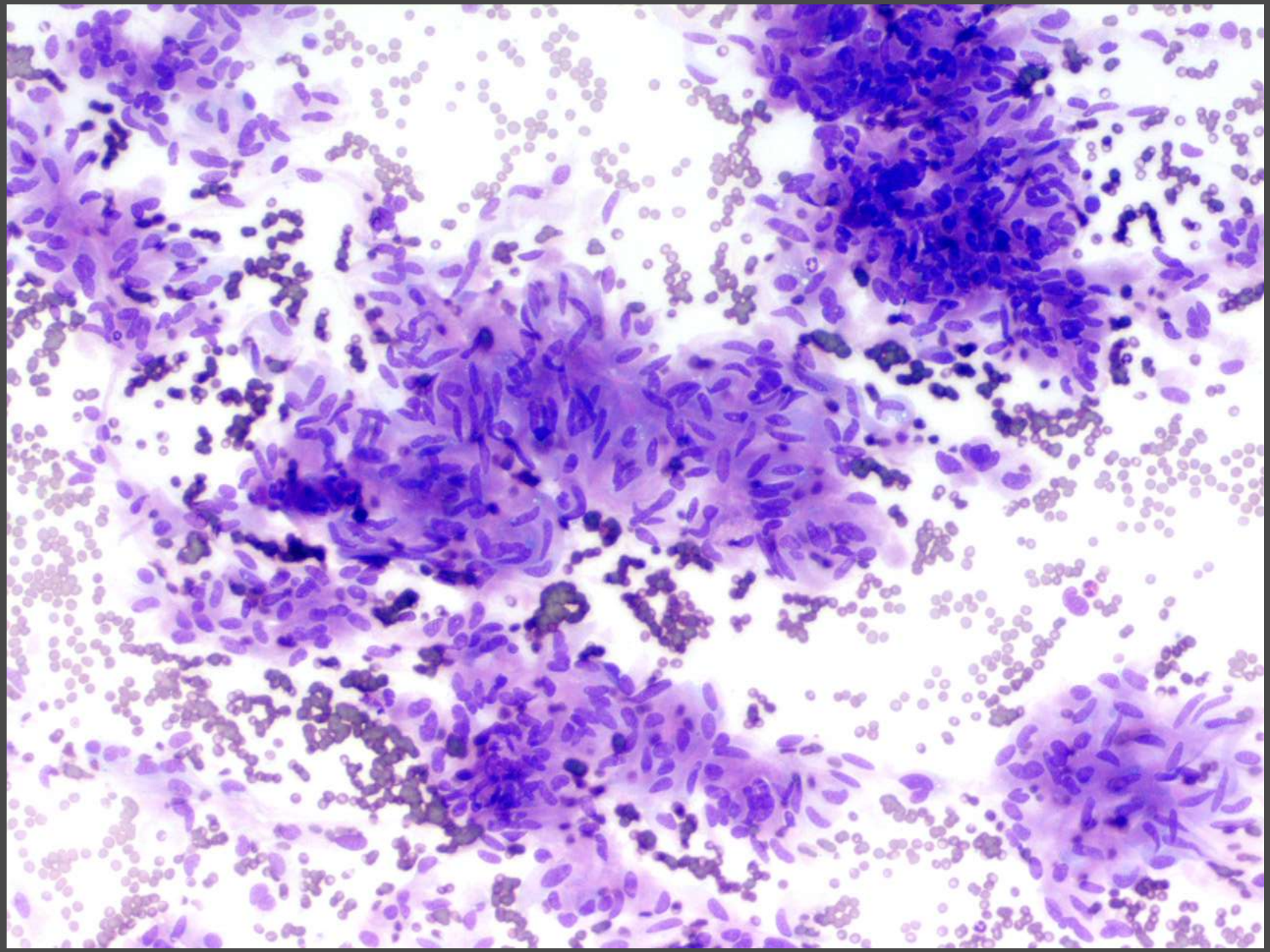
- 87 year-old woman presented with upper GI bleed
- EUS:
  - pedunculated ulcerated lesion 2nd portion duodenum.
  - Hypoechoic submucosal heterogeneous 21 mm x 18 mm.
  - No involvement of muscularis propria.
  - No regional lymphadenopathy.
- EUS-FNA with 25 gauge needle.

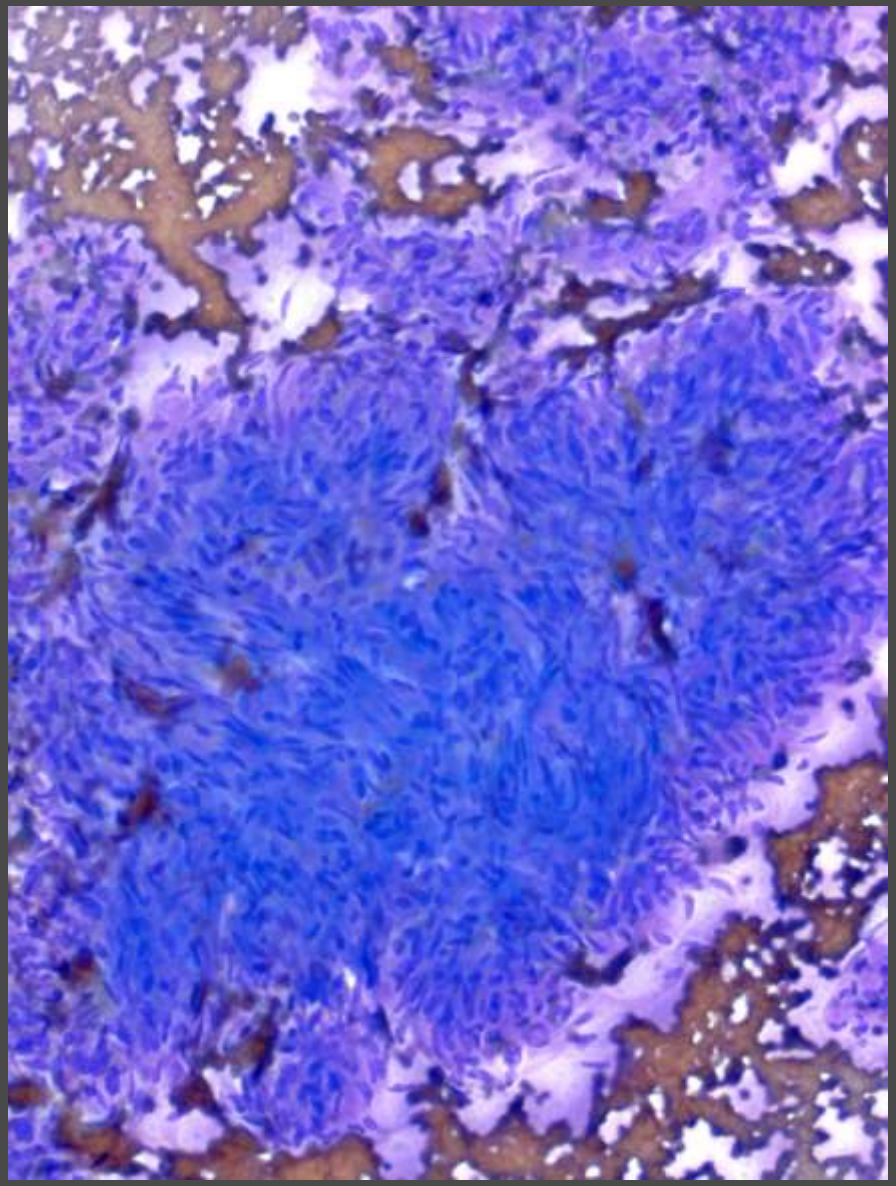


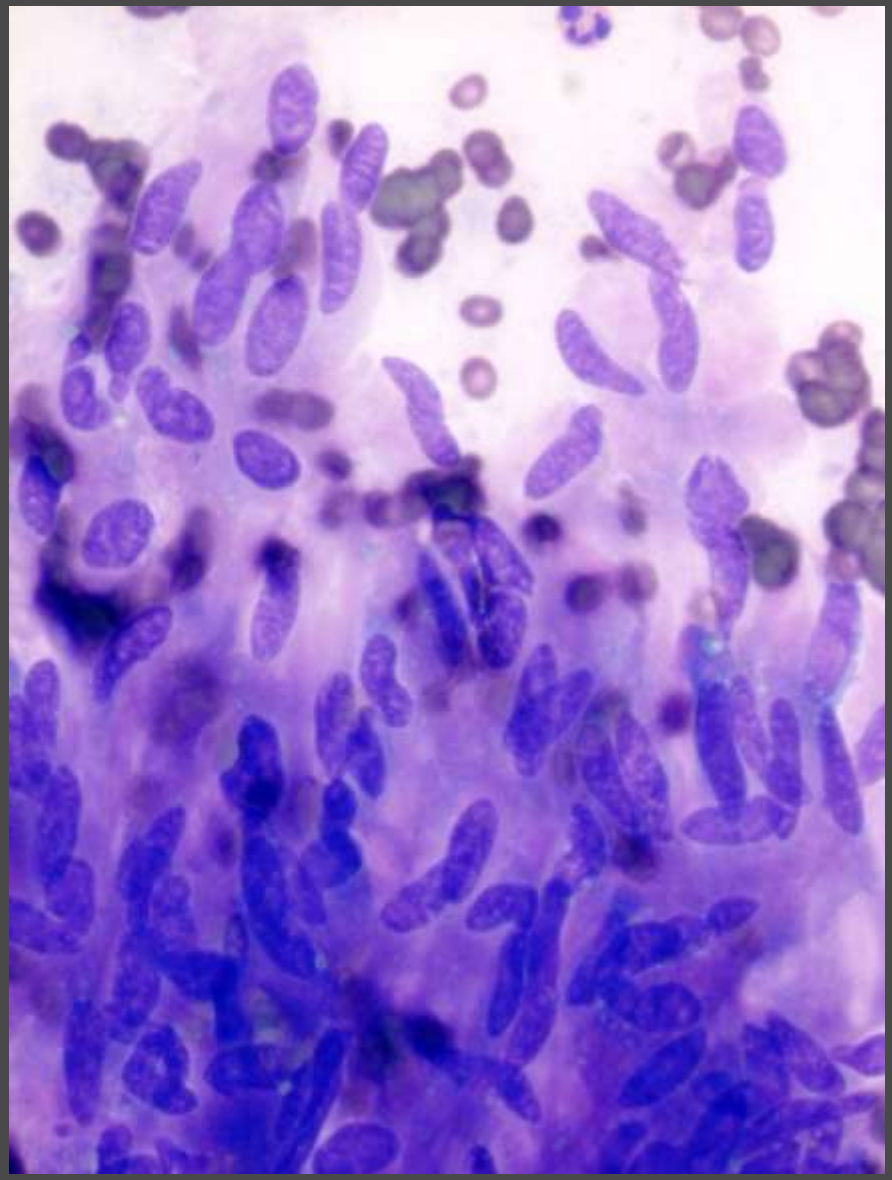
2 2nd Portion of the Duodenum
- ulcerated raised lesion

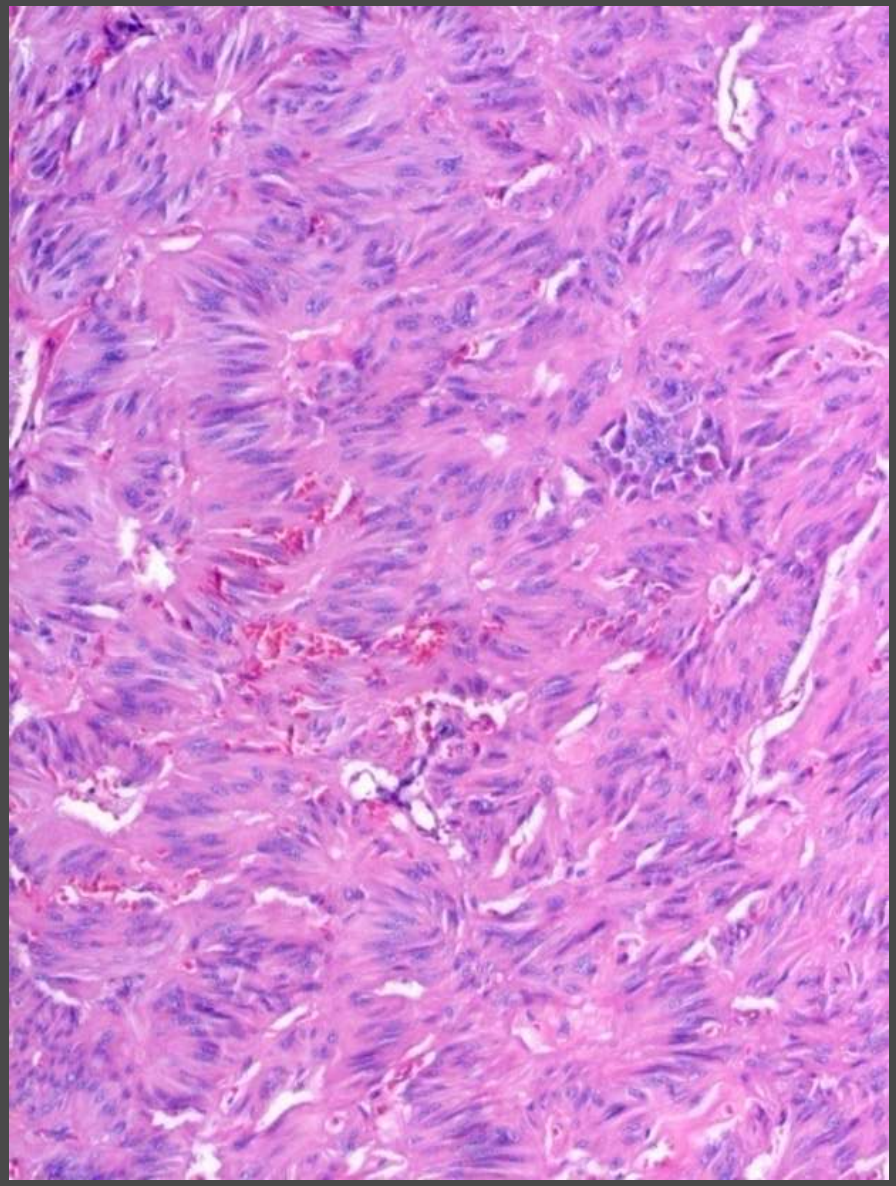


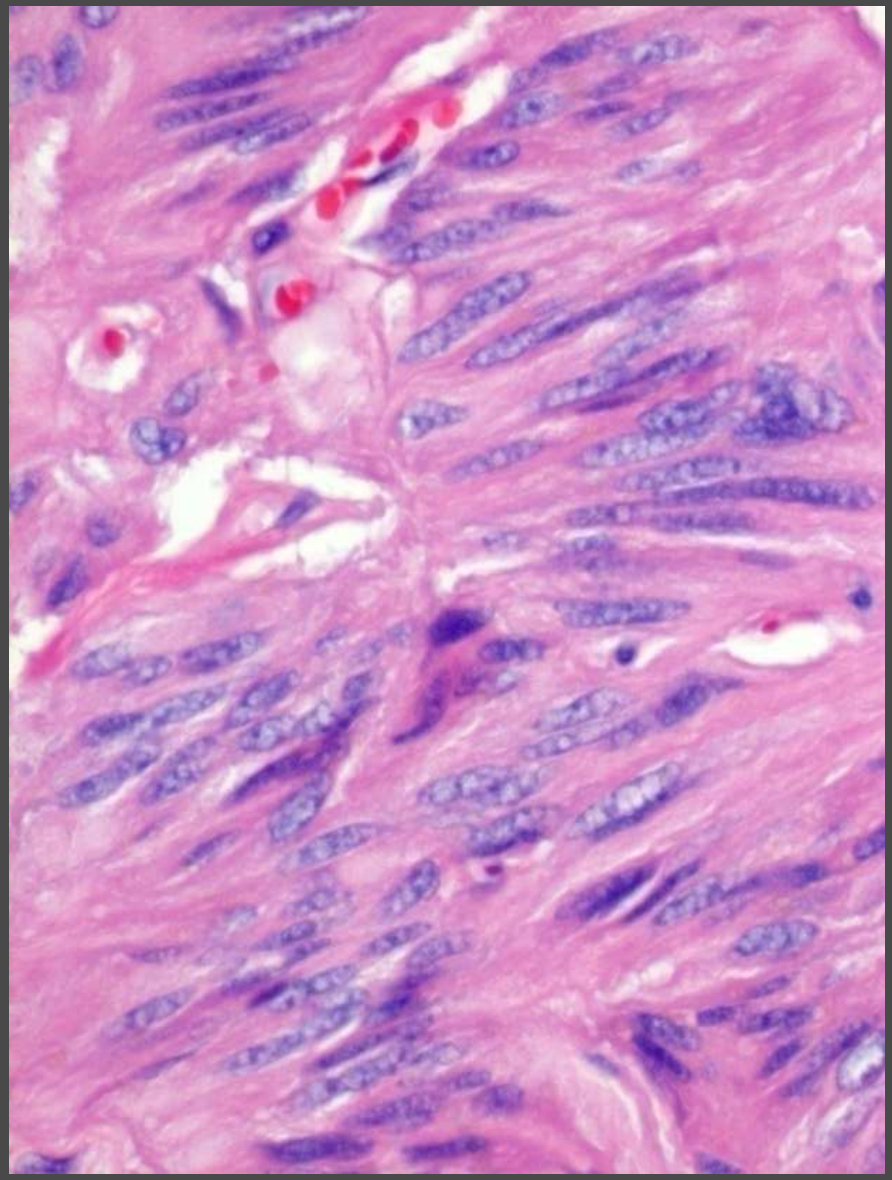
3 Duodenal lesion







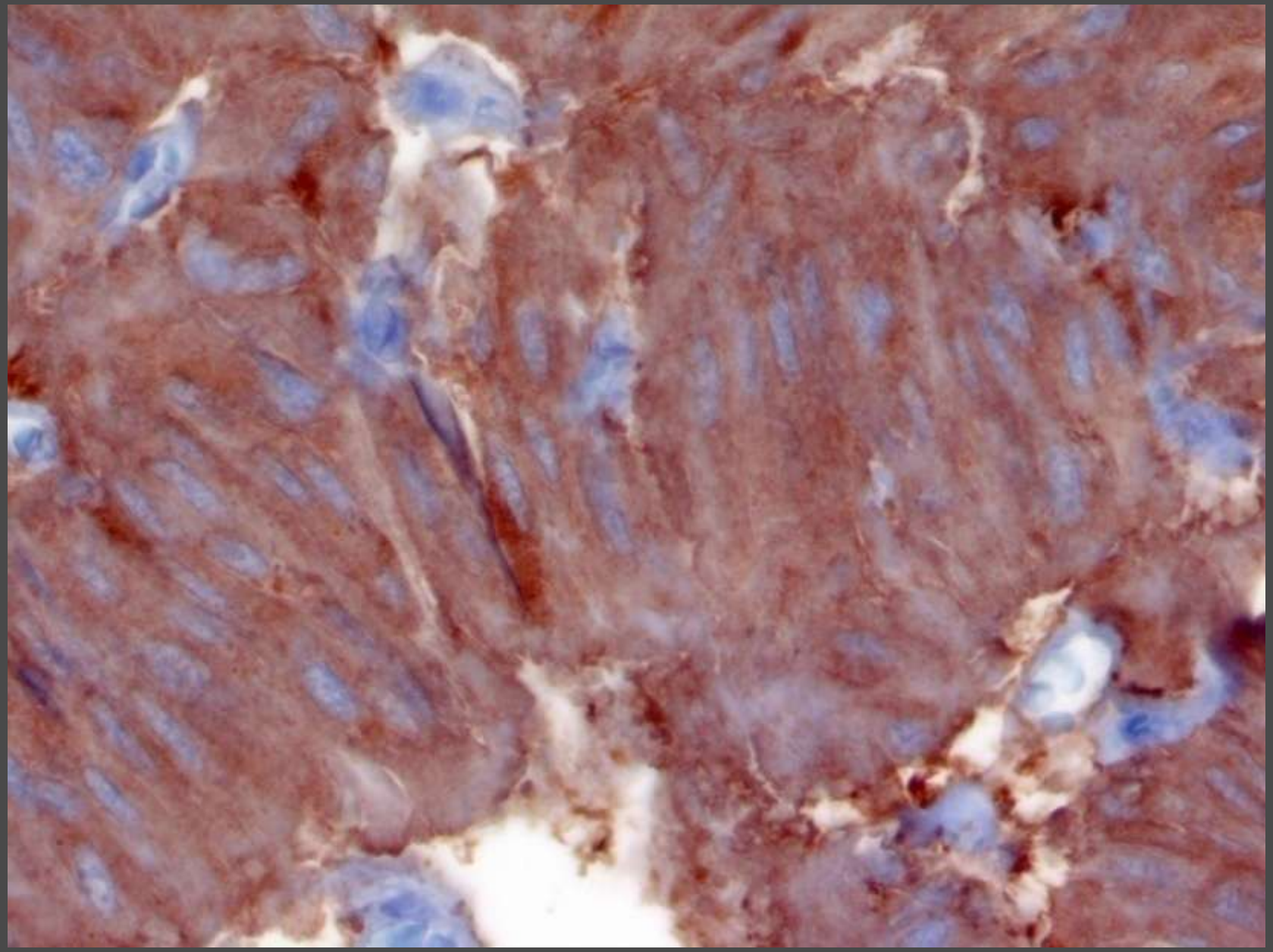




Requested Immunostains

- CD117 (-)
- CD34 (-)
- S100 protein (-)
- SMA (-)
- Could it be a CD117 (-) GIST??
- Any other IHC stain?

Synaptophysin



Follow-up

- Endoscopic polypectomy
- Final Dx: Well-differentiated NET (“carcinoid tumor”), spindle cell type (15 x 15 x 9 mm) completely excised.

The Spindle Cell Pattern

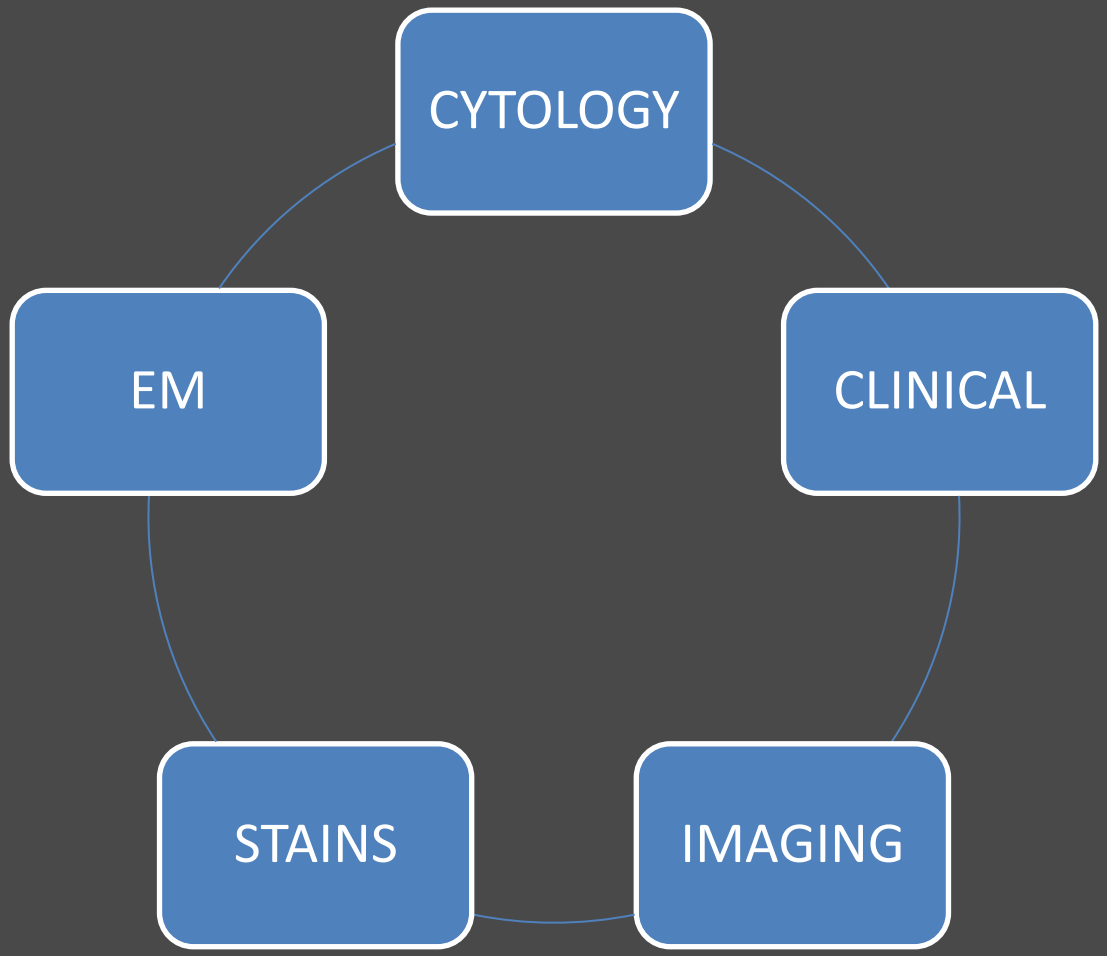
	GIST	LEIOMYOMA	SCHWANNOMA	NET
Cytology	Paranuclear vacuoles	Eosinophilia	Lymphocytes	Nesting at low power
CD117	+	-	-	-
CD34	+	-	-	-
S100 protein	-	-	+	-
Actin	-	-	-	+
Synaptophysin	-	-	-	+

GIST, gastrointestinal stromal tumor
NET, neuroendocrine tumor

How do we classify NET?

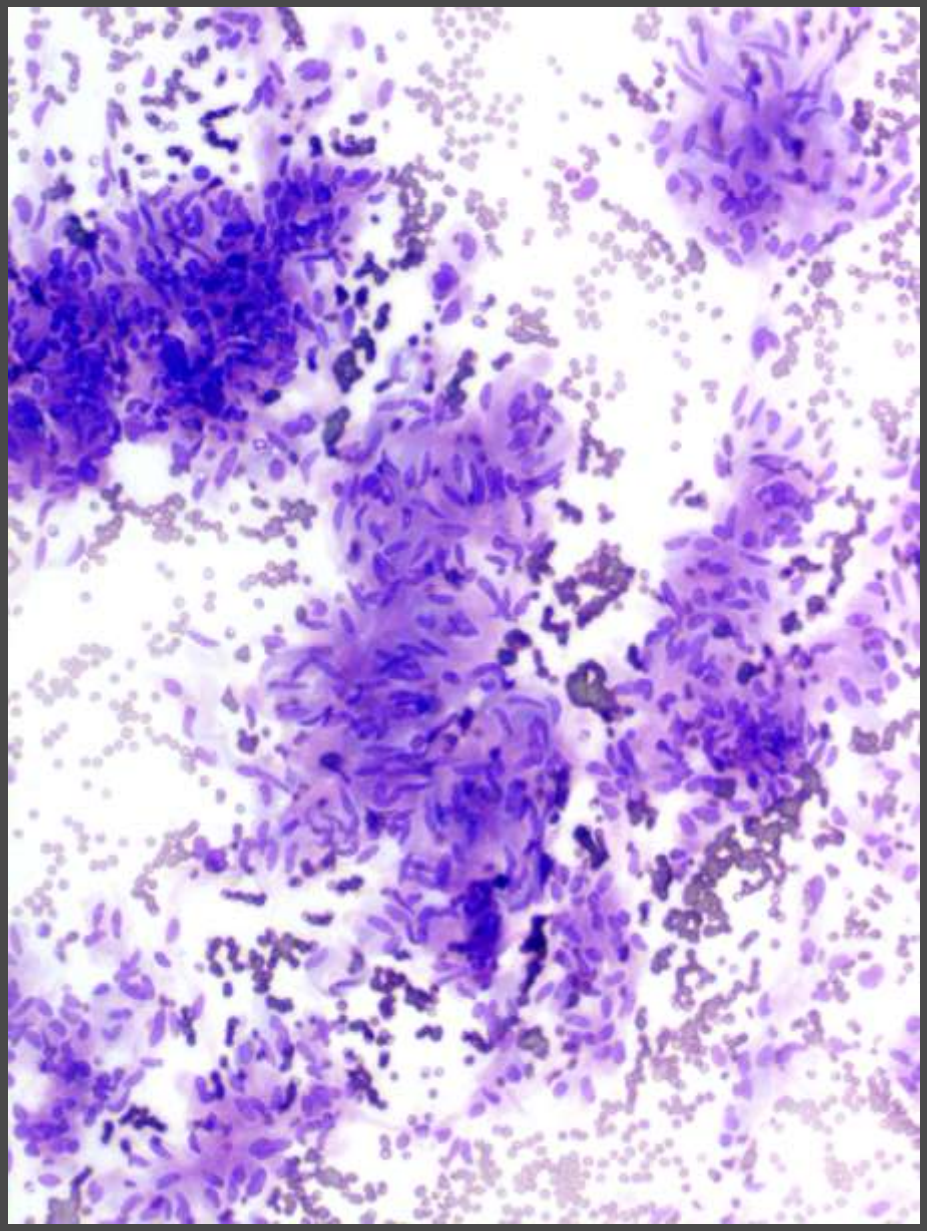
- GEP tract (the most frequent site)
  - GE: WD NET, WD NECa, PD NECa
  - Pancreas: PEN functioning & non-functioning
- Bronchopulmonary system
  - WD NET (typical carcinoid), WD NECa (atypical carcinoid), PD NECa (small and large cell NECa)
- Other
  - Medullary carcinoma of the thyroid, Merkel cell carcinoma, pheochromocytoma, extradrenal paraganglioma.
- Inherited tumor syndromes.

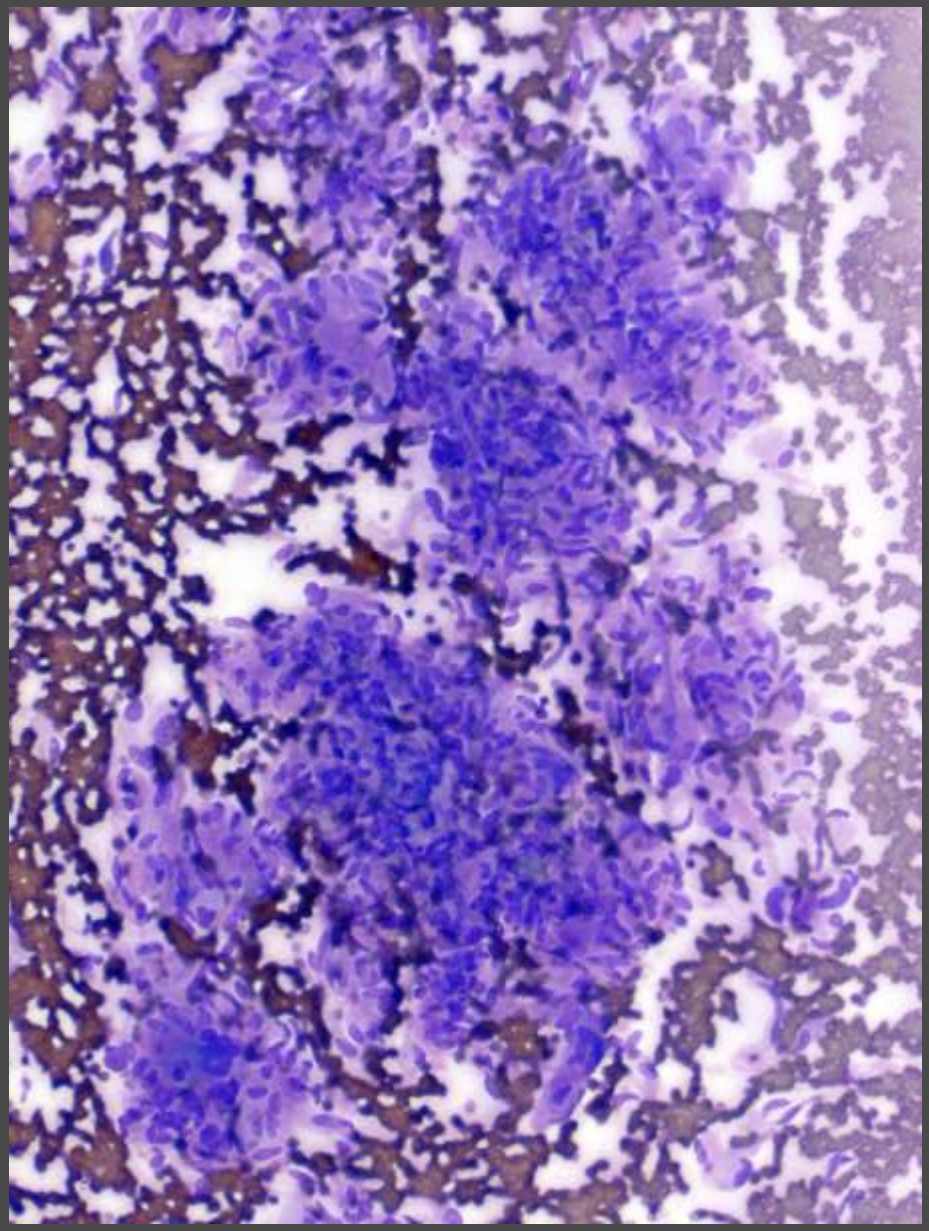
NET Diagnosis



Cytological Criteria: Low Power

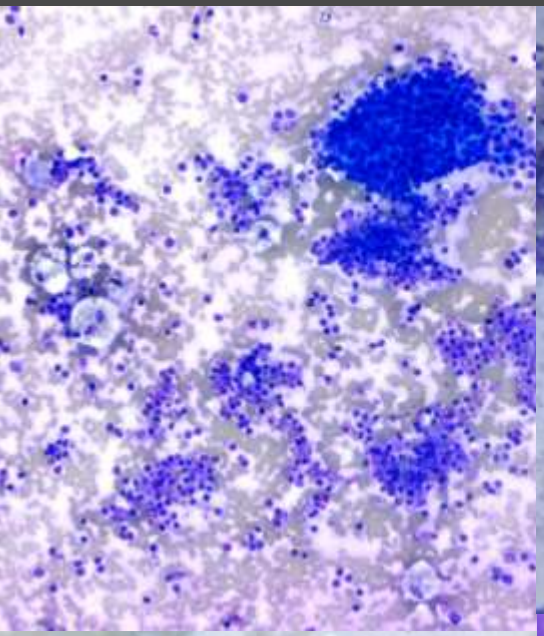
- Cellular pattern
- Predominance of cell aggregates
  - Organoid pattern
  - Nesting
  - Cords/trabeculae
- Predominance of isolated cells
  - High power evaluation

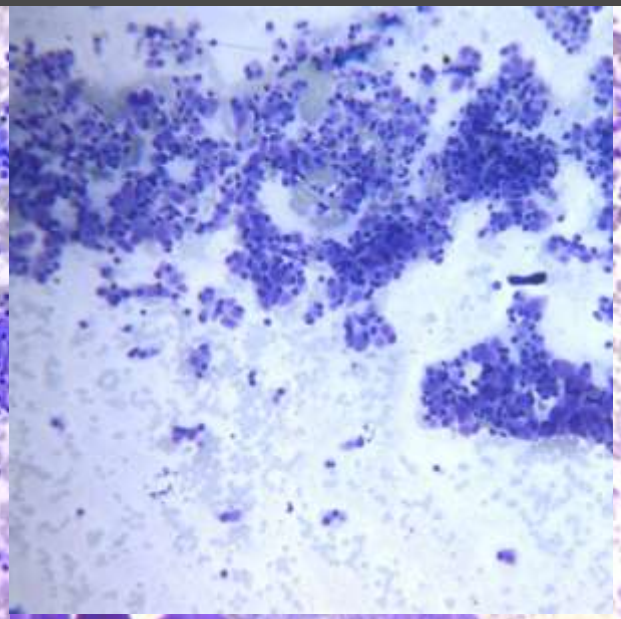


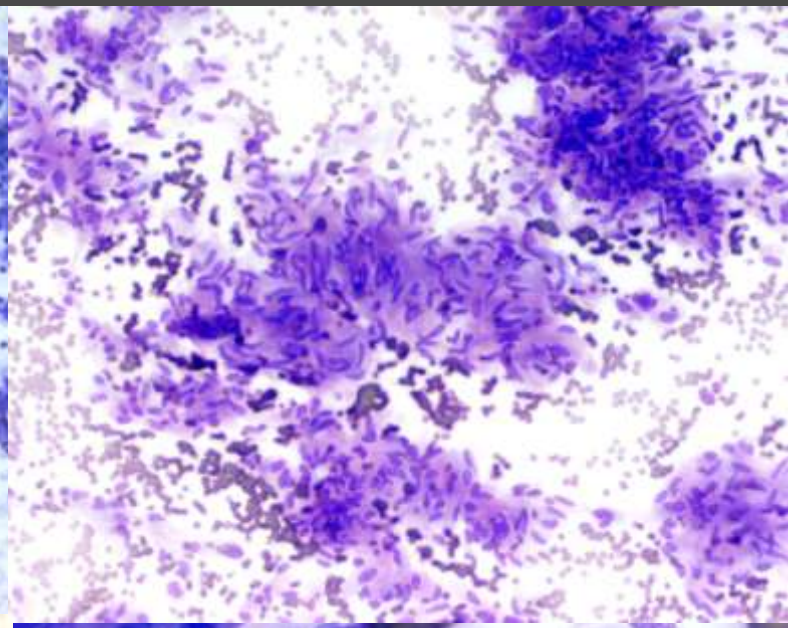


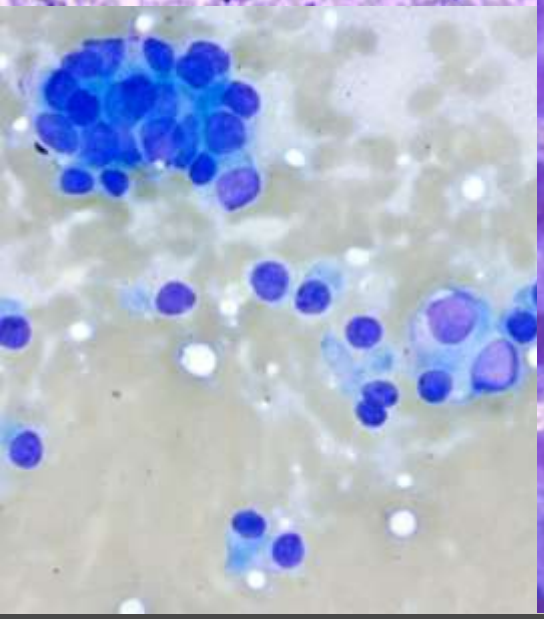
Cytological Criteria: High Power

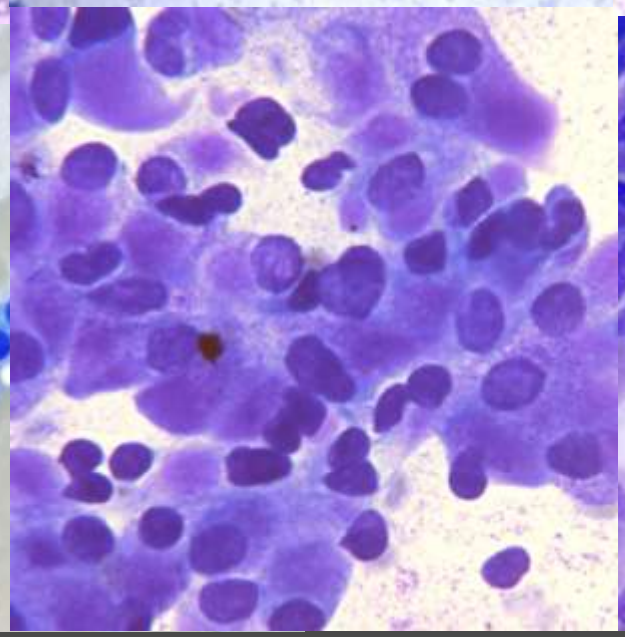
- Small to medium cell size. Large… rare
- Round/cuboidal, spindle shape.
- May have fine red cytoplasmic granules.
- Uniform, round, oval, elongated nuclei. Minimally pleomorphic. High NCR.
- “Salt & pepper” chromatin
- Absent or inconspicuous nucleoli
- Apoptosis, necrosis, mitosis may be present.

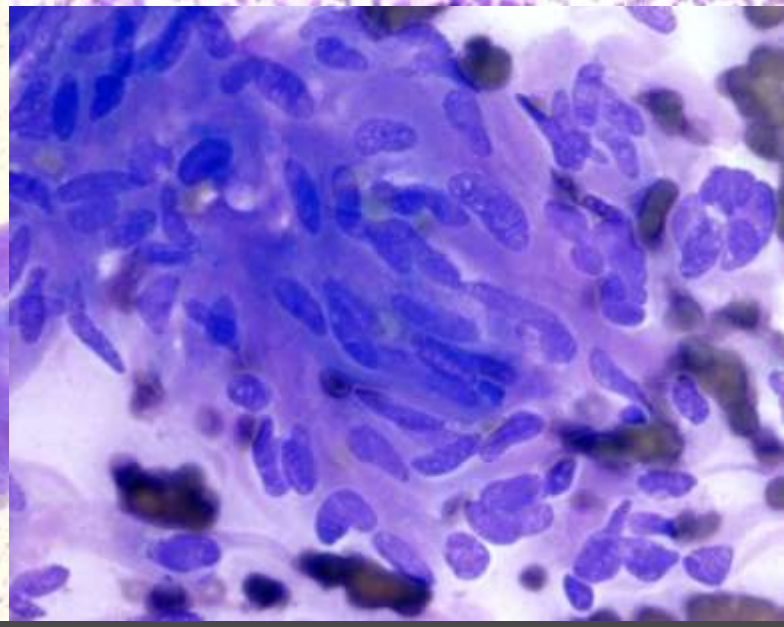








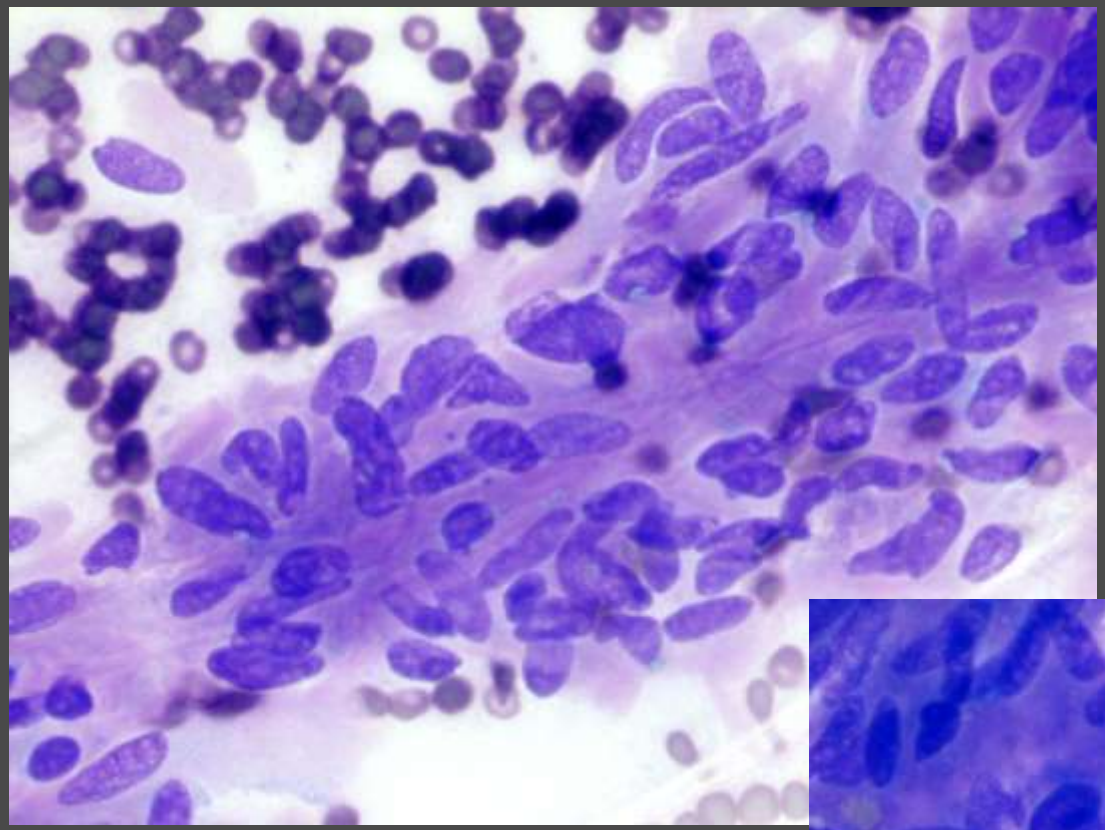
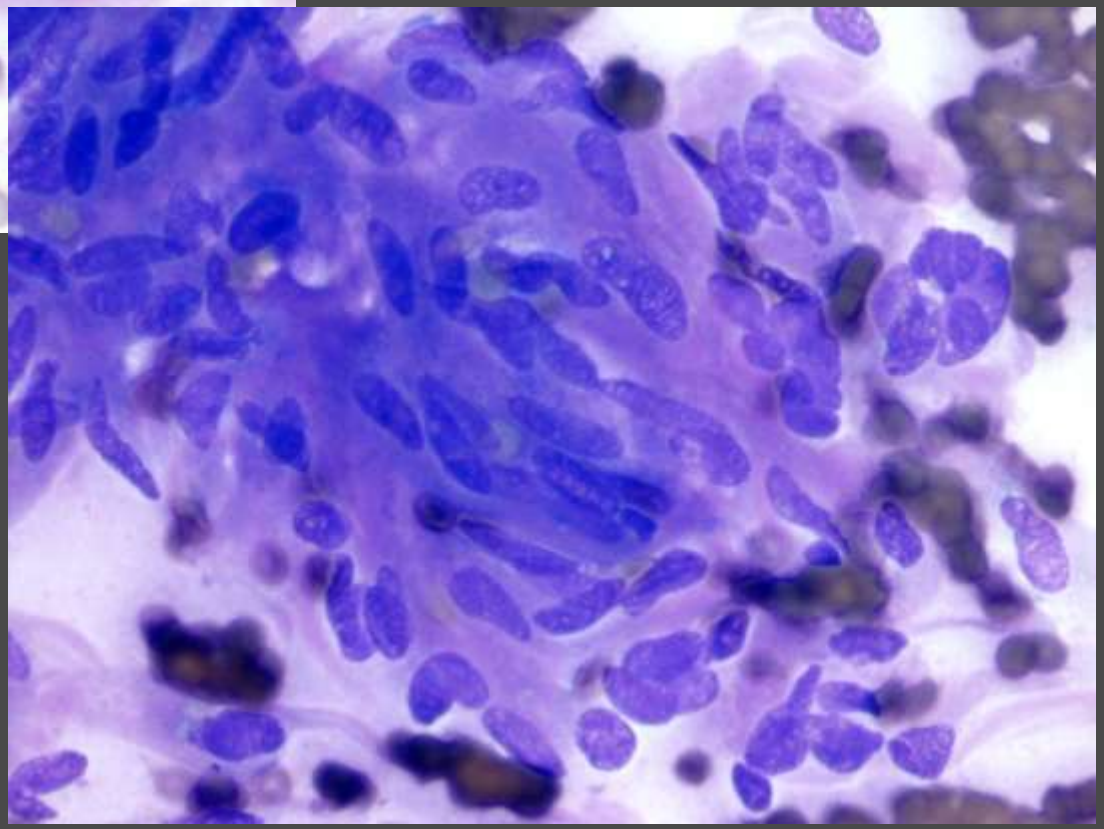




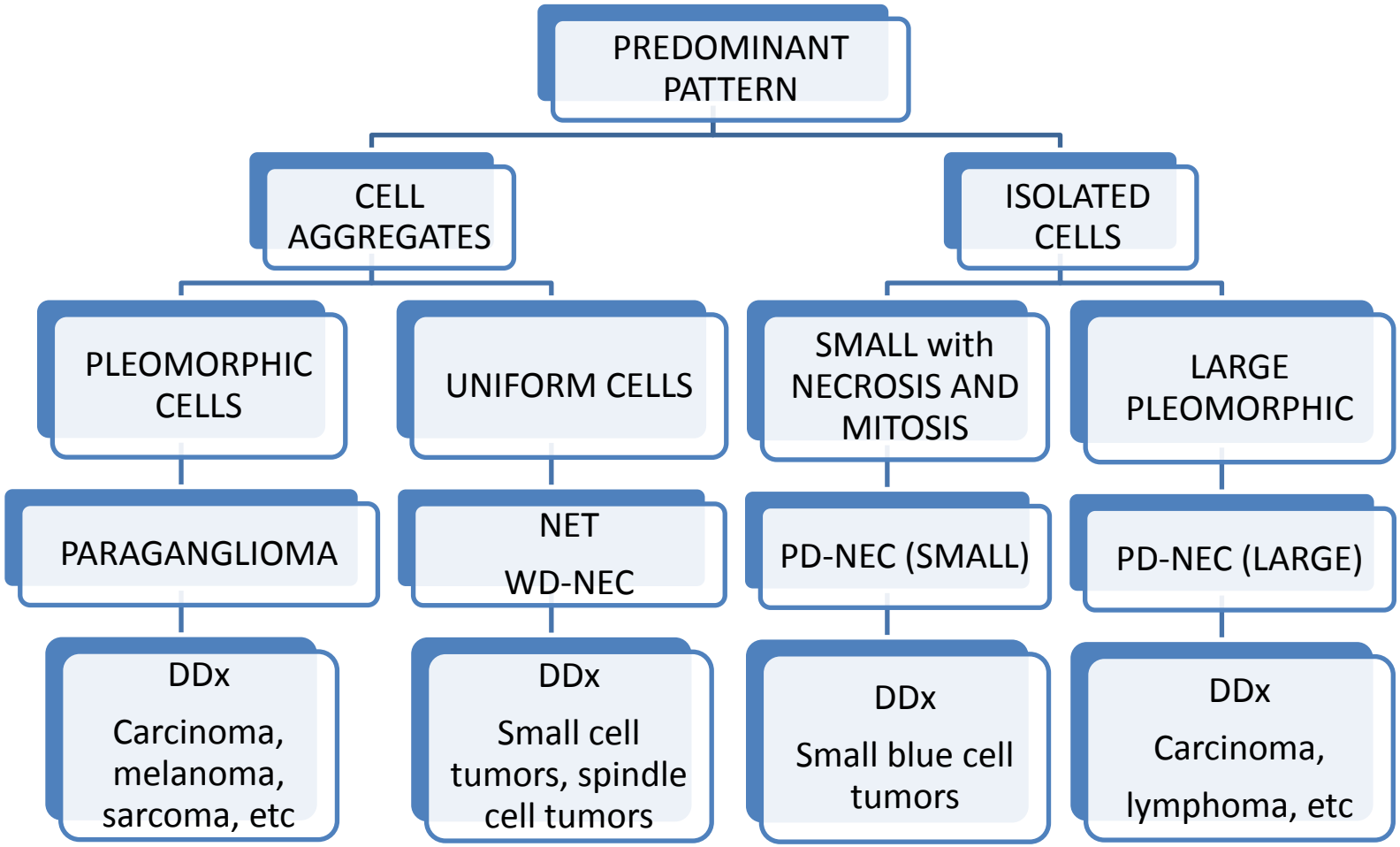
PEN

PEN

DUODENUM

Algorithm for NETs



NETs in the 21st C

- They are more prevalent
  - Increased reported incidence of NETs
  - Increased survival durations over time
- Pathologists need to
  - Transmit a clinically relevant terminology
    - The term “carcinoid” is a misnomer → NET
  - Be familiar with treatment modalities

Siegfried Oberndorfer

Fig. 4 First page of Siegfried Oberndorfer's famous publication on carcinoids in the Frankfurter Zeitschrift für Pathologie (1907)

Aus der Prosektur des städtischen Krankenhauses München
(Prosektor: Privatdozent Dr. Oberndorfer.)

Karzinoide Tumoren des Dünndarms.

Von

Siegfried Oberndorfer.

(Mit 2 Abbildungen auf Tafel XI.)

Im Laufe der letzten Jahre fand ich verschiedene Male kleine Tumoren im Dünndarm, zum Teil multipel, zum Teil isoliert, von kleiner Stecknadelkopf- bis etwas über Hanfkorngrösse, die grösstenteils mikroskopisch das Bild kleiner Karzinome boten, doch aber so viel Eigenartiges aufweisen, dass es der Mühe wert erscheint, sie einmal im Zusammenhang zu besprechen. Zwei der Fälle habe ich bereits früher veröffentlicht, es handelte sich um multiple Geschwulstbildung in beiden Fällen: in dem einen bei einer 48jährigen, an Knochentuberkulose zu-

Oberndorfer S (1907) Karzinoide Tumoren des Dünndarms. Frankf Z Pathol 1:425–432

(p. 426)

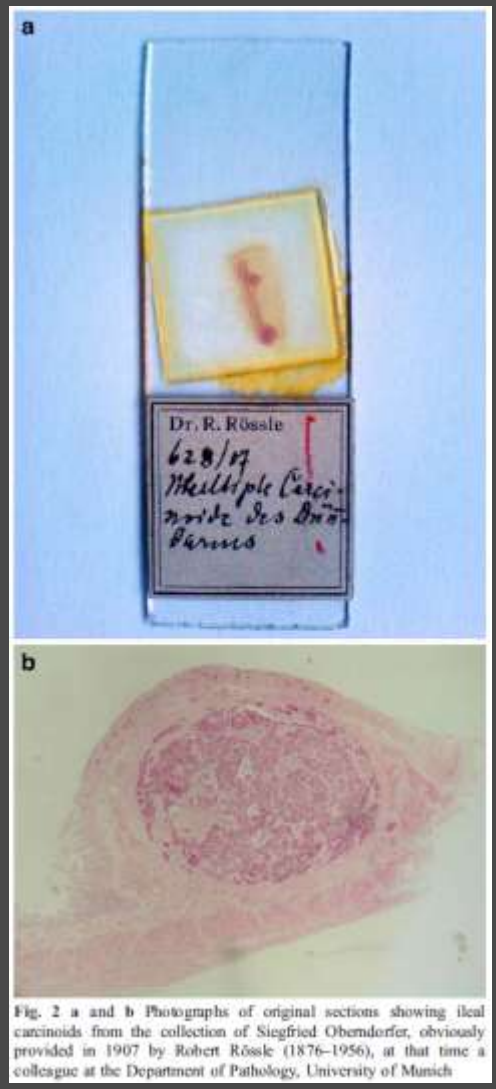


Fig. 2 a and b Photographs of original sections showing ileal carcinoids from the collection of Siegfried Oberndorfer, obviously provided in 1907 by Robert Rössle (1876–1956), at that time a colleague at the Department of Pathology, University of Munich

lesions in five statements. The main characteristics of these tumorlets are:

1. They are usually small and often multiple.
2. Their cells form undifferentiated formations, at most with slight indications of glands.
3. They are well defined and show no tendency to penetrate infiltratively into the surroundings.
4. They do not metastasize.
5. They appear to grow extremely slowly, do not reach any great size, and are thus apparently harmless in nature.

Virchows Arch (2007) 451 (Suppl 1):S3–S7

GEP-NET Targeted Therapy

Table 5 Results of studies of molecularly targeted agents in patients with neuroendocrine tumours[54,55]

Agent	Response rate (%)	PFS rate (%)/Duration
VEGF monoclonal antibody		
Bevacizumab[56]	18	95 at 18 wk
mTOR inhibitor		
RAD001 (everolimus)	13	71 at 24 wk
Temsirolimus[57]	5.6	50 at 6 mo
VEGF TKI		
Sunitinib	10	Median, 42 wk
Vatalanib	In progress	(time to progression)
Sorafenib	In progress	
Pazopanib	In progress	
PDGFR/Kit/Abl inhibitor		
Imatinib[58]	4	Median, 5.9 mo
EGFR inhibitor		
Gefitinib	4	61 (carcinoids) and 31 (pancreatic tumor) at 6 mo
Other		
Bortezomib[59]	0	Median, 3 mo (Time to treatment failure)

World J Gastroenterol 2008 September 21; 14(35): 5377-5384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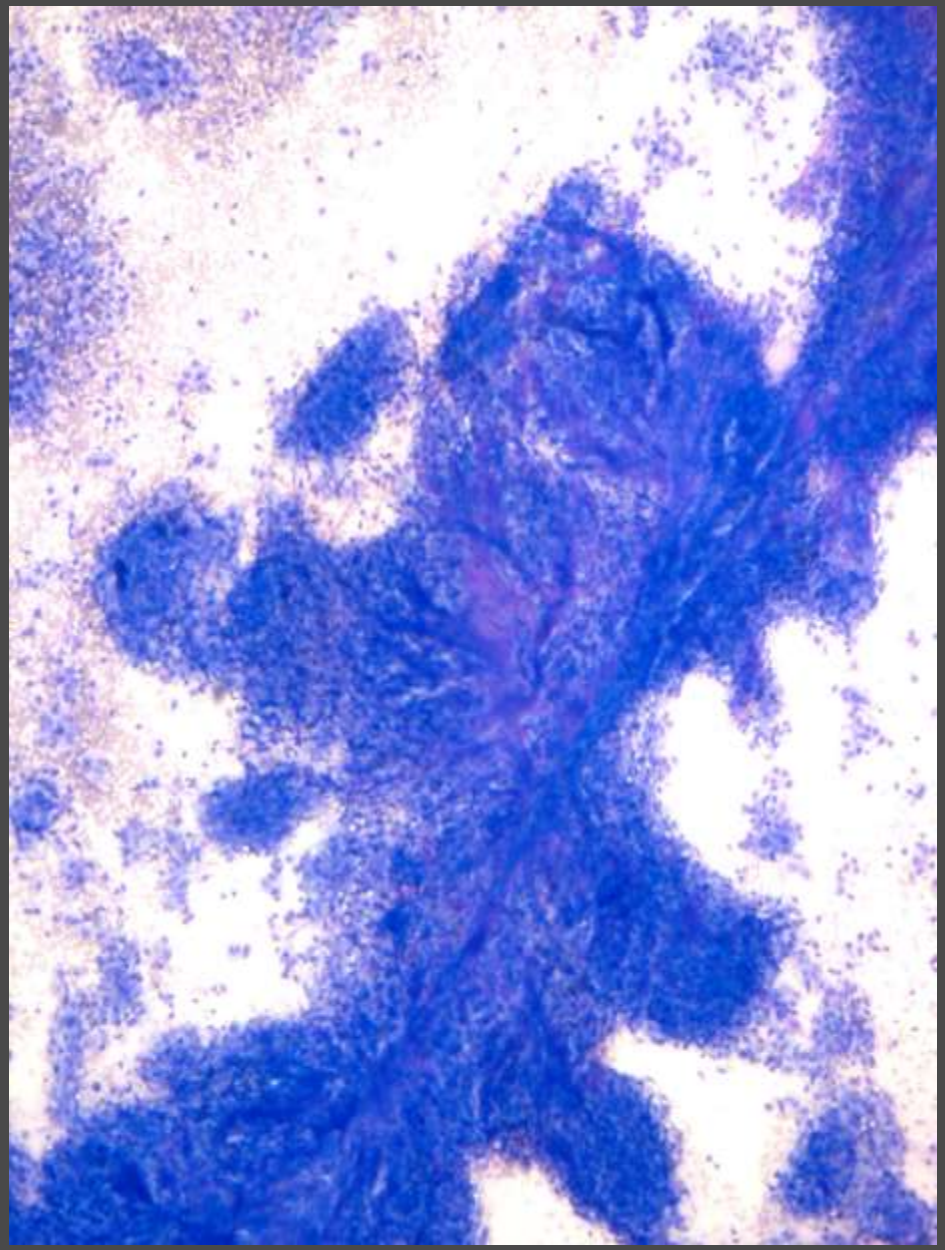
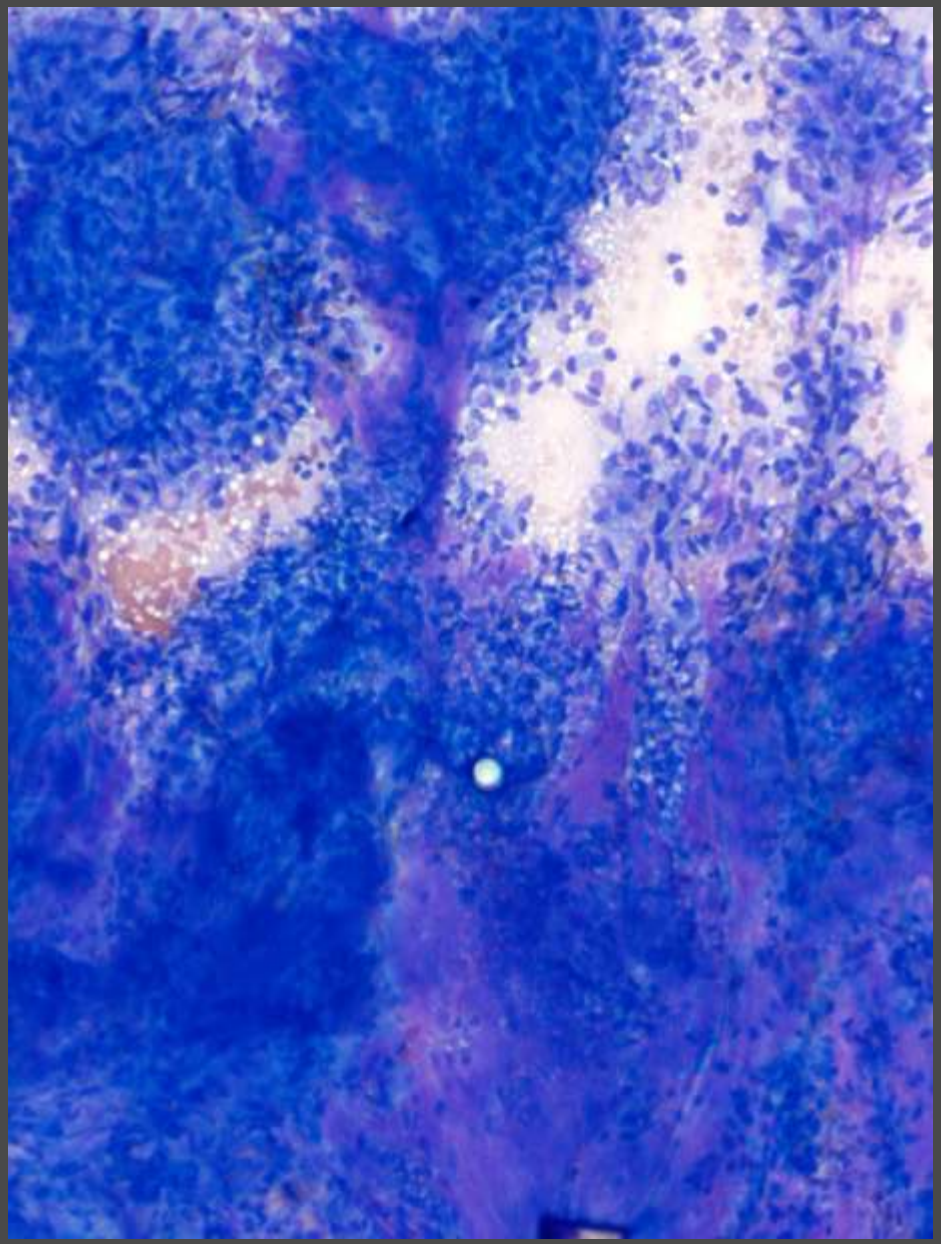
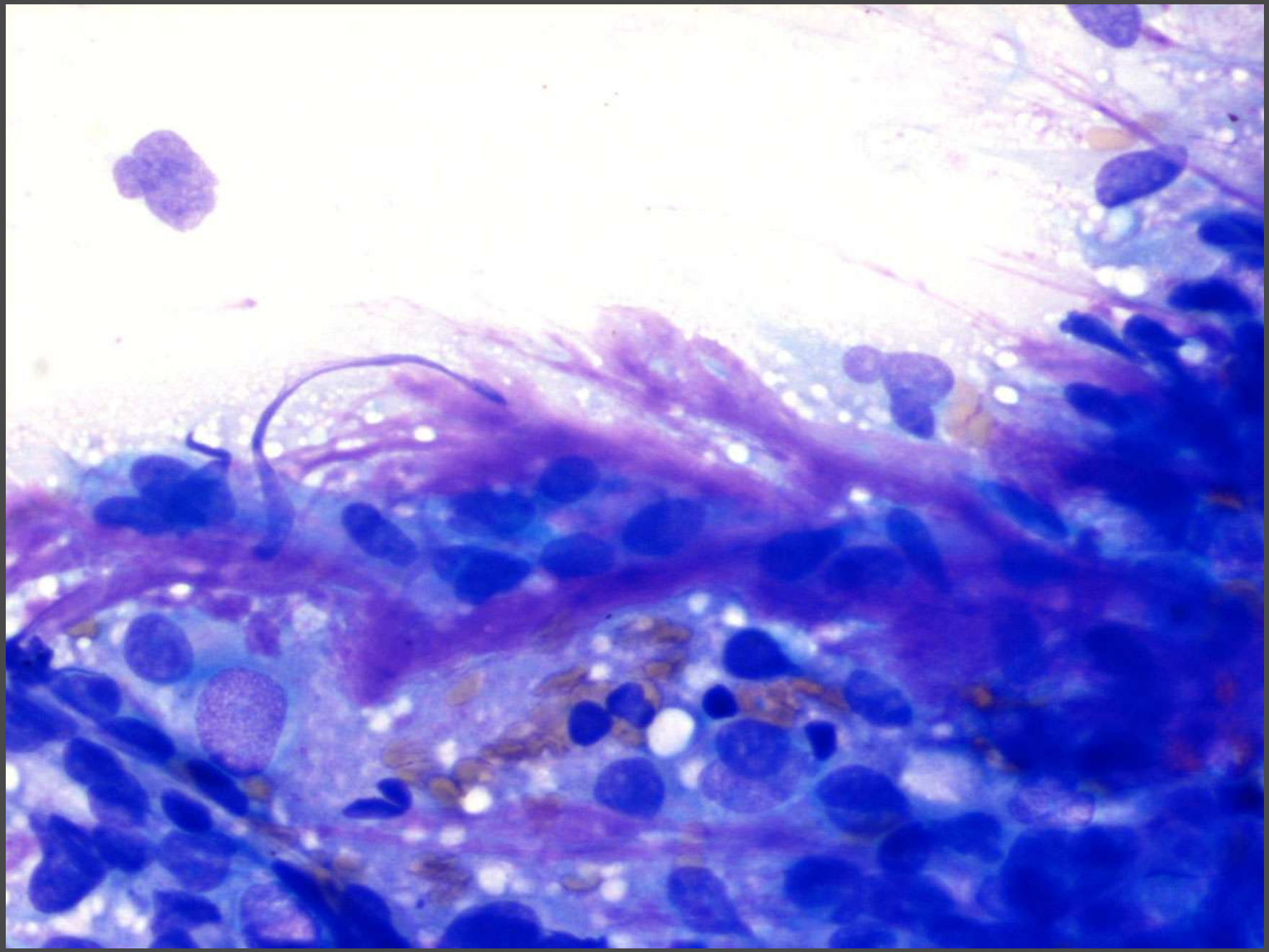
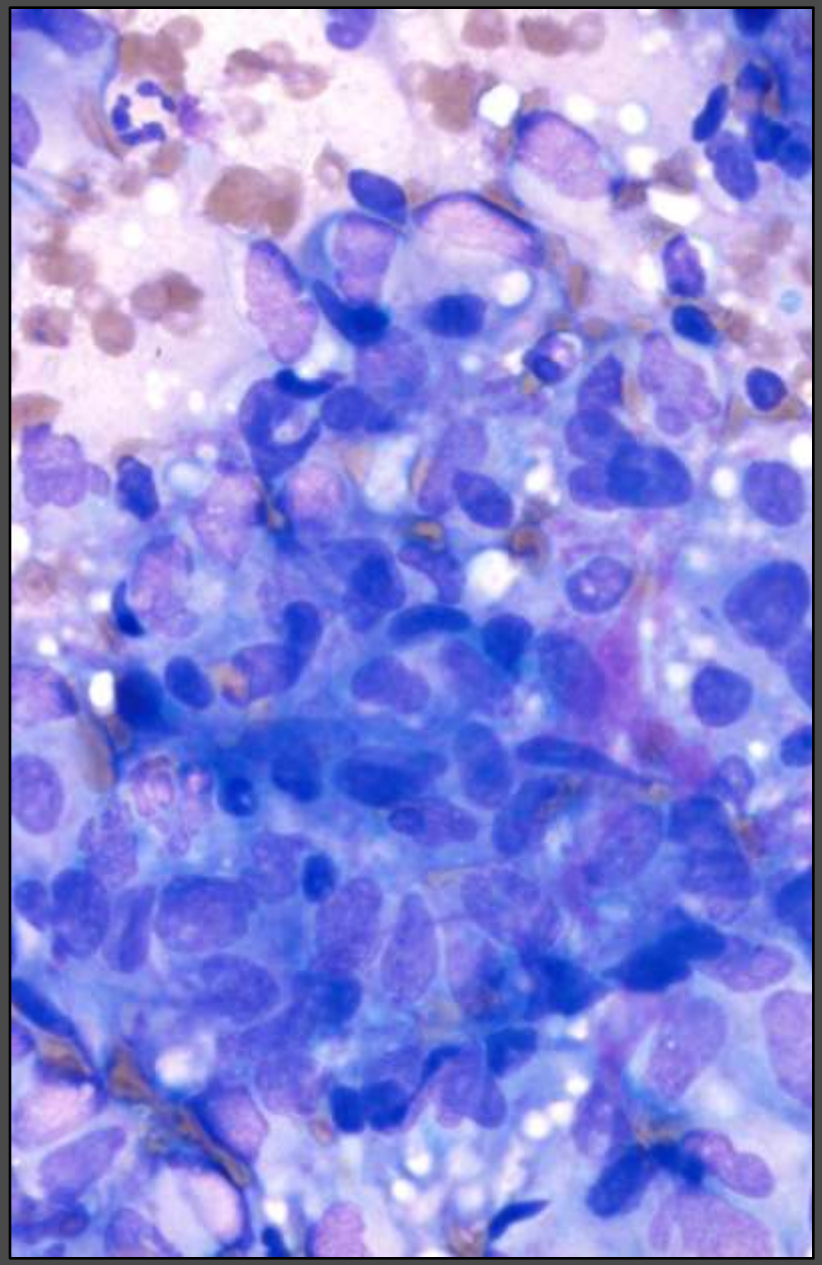
Case 2

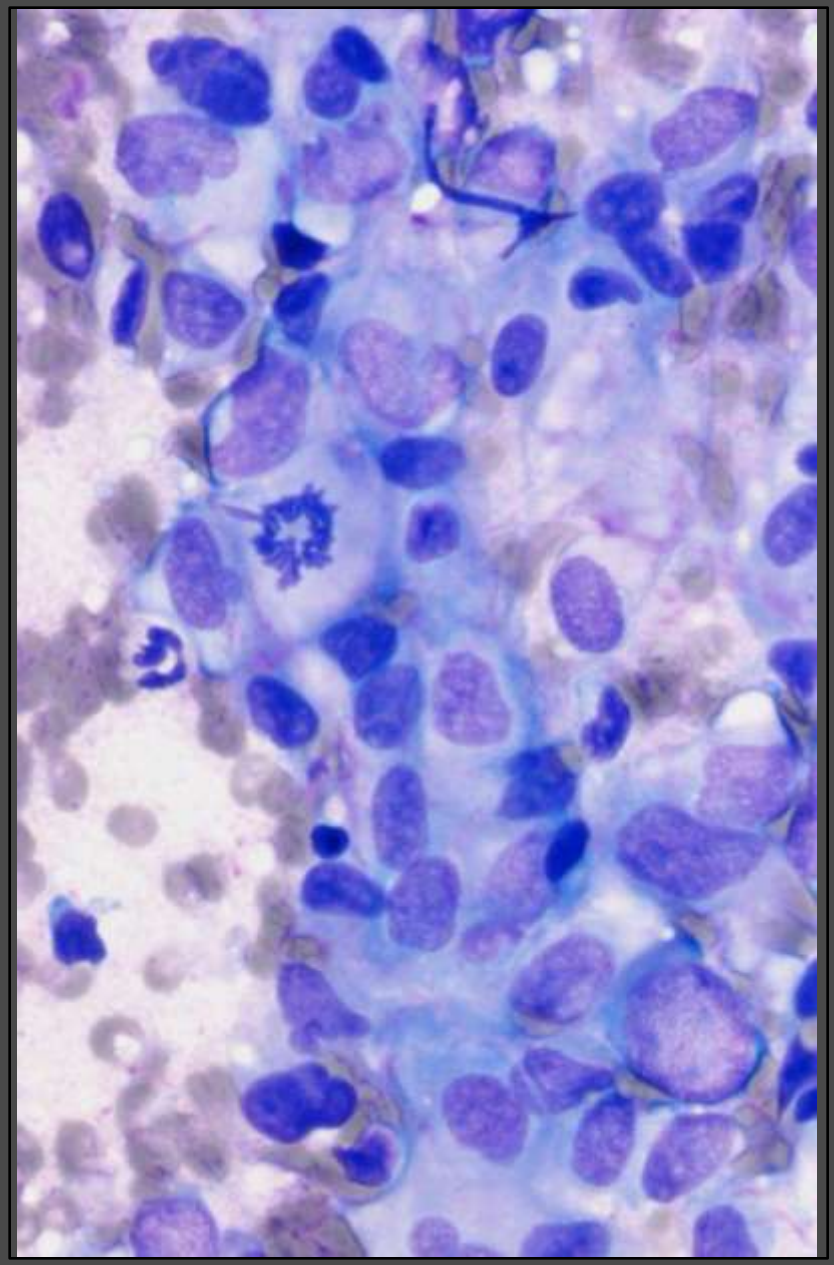
- 52 yo male presented with duodenal ulcer with obstruction and bleeding
- 11.6 x 8.5 cm vascular mass ulcerating into the duodenal mucosa
- ? GI or retroperitoneal ? Rt kidney
- Plan Whipple - close proximity to pancreas











Cytologic Findings

- Predominantly epithelioid pattern
- Numerous isolated cells
- Cellular pleomorphism
- Numerous abnormal mitoses
- Necrosis

Case Thoughts

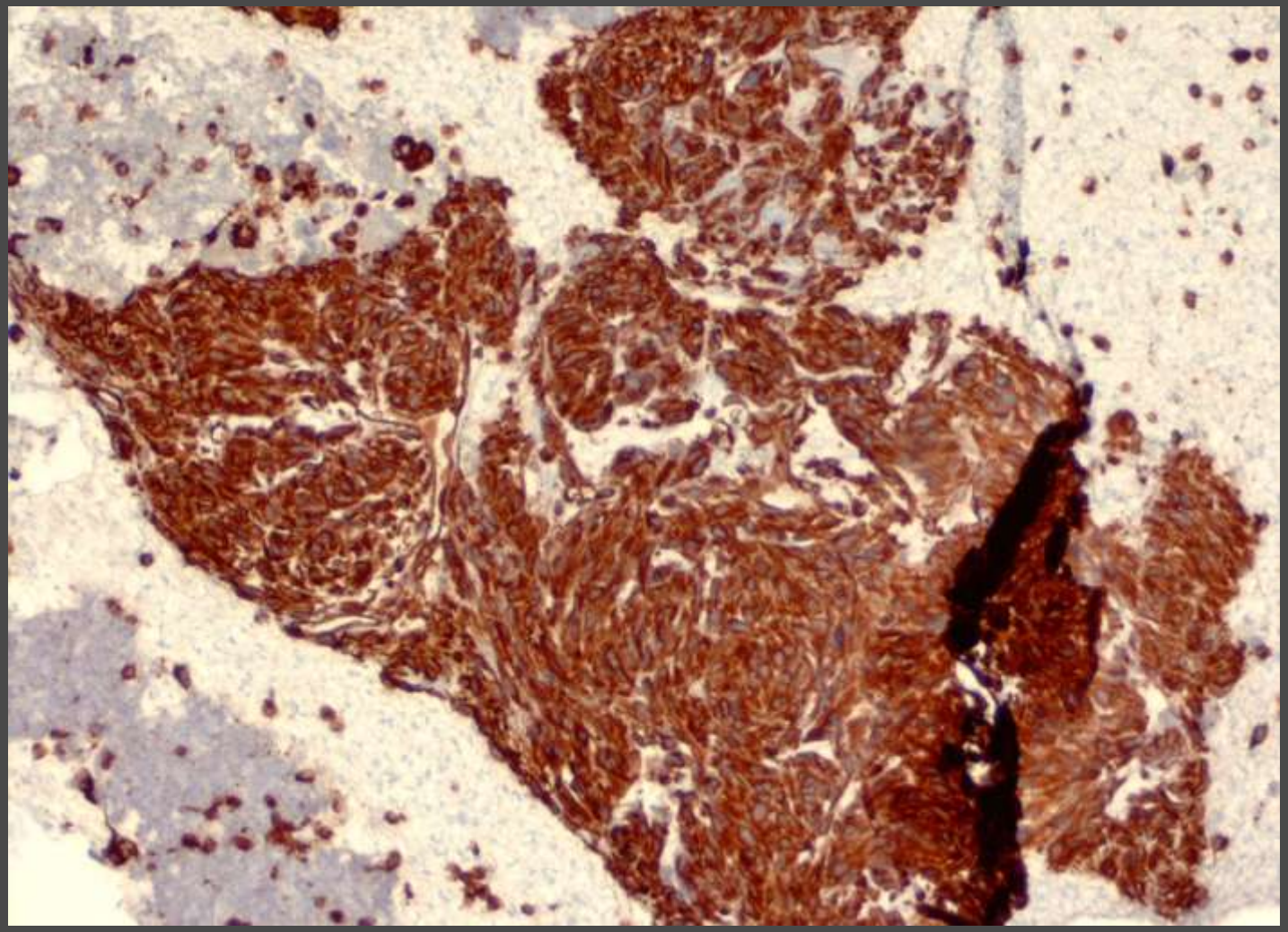
- Retroperitoneal
  - Liposarcoma
  - Malignant Fibrous Histiocytoma
  - Leiomyosarcoma
- Renal Cell Carcinoma – sarcomatoid type
- GI – Spindle cell neoplasm - malignant

Vimentin +

CD 117 +

S100 w+

(CD 34, desmin, CK, and SMA negative)



Diagnosis:

GIST – High risk!

GIST – Risk of Aggressive Behavior - Consensus Approach*

	Size (cm)	Mitotic Count per 50 HPF
Very low risk	<2	<5
Low risk	2-5	<5
Intermediate risk	<5 5-10	6-10 <5
High risk	>5 >10 Any size	>5 Any mitotic rate >10

*** NIH – April 2001**

Fletcher CDM, et al. Hum Pathol 2002;33:459.
Blay J-Y, et al. Ann Oncol 2005;16:566.

GIST: Predictors of Malignancy

- **Tumor size**
- **Cell proliferation** [Mitotic count
  Labeling index (Ki-67 >10%), p53]
- ***p16, *ezrin, low apoptosis, high telomerase, angiogenesis markers (MVD, *VEGF)**
- **Location**

- Great majority of very-low-risk, low-risk, and intermediate risk GISTs behave in a benign manner.
- There is an unpredictable subset (~10%) that behaves aggressively.

*****Over expression

Corless CL. AJCP 2004;122:11.
Fletcher CDM, et al. Hum Pathol 2002;33:459.
Steigen SE at al. Mod Pathol 2008;21:46.
Wang Q et al. World J Gastroenterol 2007;13:2626.
McAuliffe JCA et al. Clin Cancer Res 2007;13:6727.
Wei Y-C, et al. Mod Pathol 2009;22:1351.

RTK (mutually exclusive) Gene Mutations

- *KIT* – 75% - 85%
  - Exon 11 > 9 > 13* > 17*
- *PDGFRA* – 5% - 10%
  - Exon 18* > 12 > 14*

Primary

- KIT
  - Exon 13*, 14, 17*
- PDGFRA
  - Exon 18*

Secondary

***Do not generally respond to Imatinib**

Sunitinib

GIST – Treatment

- Surgery with good margins
- Imatinib: response dependent on the type of CD117 mutation:
  - Exon 11 (best); exon 9 (interm); wild (lowest)
- Recurrent tumors: secondary c-kit mutation – different histophenotype – sunitinib

THE MOLECULAR CLASSIFICATION OF GISTs PLAYS A CENTRAL ROLE BEFORE AND DURING THE TREATMENT WITH TYROSINE KINASE INHIBITORS.

Primary KIT mutation exon 11 – Imatinib 400 mg/d

Primary KIT mutation exon 9 – Imatinib 800 mg/d

Primary KIT wild type – Sunitinib

Secondary mutations KIT exons 13 or 14 – Sunitinib

Individual phenotype and clinical factors may help to maximize clinical benefit of RTK inhibitors in patients with GIST.

Walderman ER et al. Virchows Arch. 2007.
Reichardt P. Oncology 2010; 78:130-140

Technologies Applied to EUS-FNA Obtained Samples

- Examine genomic and proteomic changes in cancer cells
  - High-throughout sequencing (point mutations)
  - High-density single nucleotide polymorphism (amplifications, deletions)
  - Gene expression profiling (signatures)
  - Micro-RNA profiling
  - Mass spectrometry
  - IHC, ISH, FISH

Reichardt P, et al. Sem Oncol 2009;36:290-301

EUS-FNA & GISTs

- Diagnosis: reliable method.

Stelow EB, et al. Am J Clin Pathol 2003; 119:703.

- Behavior: necrosis and mitoses seem to correlate with malignant GIST.

Elliott, DD et al. Cancer 2006; 108:49.

- Molecular analysis: in CB (n = 33 EUS-FNA)
  - PCR amplification analysis of *c-Kit* and *PDGFRA* exons
  - c-KIT mutations: 57.5% exon 11, 3% exon 9
  - No PDGFRA mutations
  - More precise diagnosis and therapy decision for primary, recurrent, and/or metastatic GISTs.

Gomes AL, et al. Am J Clin Pathol 2007; 127:89.

Other CD117(+) Tumors

- Adenoid Cystic Carcinoma, Melanoma, Seminoma, AML, Mast Cell Lesions

- Most are stromal* and most do not occur in the gut:

  Synovial Sarcoma, MFH, DFSP, Fibromatoses, Angiosarcoma, Ewing's sarcoma, Liposarcoma, and Hemangiopericytoma

  Uterine & ovarian mesenchymal tumors??

* Most show *only cytoplasmic* CD117(+)

REVIEW ARTICLE

The Utility of Discovered on Gastrointestinal Stromal Tumor 1 (DOG1) Antibody in Surgical Pathology—the GIST of It

Cheng-Han Lee, MD, PhD, Cher-wei Liang, MD,† and Inigo Espinosa, MD‡*

Abstract: DOG1 (discovered on GIST 1), known also as *TMEM16A* and *ANO1*, has emerged in recent years as a promising biomarker for gastrointestinal stromal tumors (GIST). It was originally discovered through microarray expression profiling analysis as gene that is highly expressed in GIST, and subsequent immunohistochemical studies have shown its use in its diagnosis. The results from several series have shown a high overall sensitivity and specificity for DOG1 in the detection of GISTs and about 6% of GISTs overall exhibiting a DOG1+/KIT- immunoprofile. DOG1 antibodies are more sensitive than KIT antibodies in detecting tumors of gastric origin, tumors with epithelioid morphology, and tumors harboring *PDGFRA* mutation. Furthermore, DOG1 immunoreactivity is rarely observed in other mesenchymal and nonmesenchymal tumor types. These results support the use of DOG1 as a diagnostic biomarker for GIST. When used in combination with KIT, this panel of diagnostic biomarkers can help pathologists and clinicians to identify more patients who may benefit from targeted therapies.

Key Words: DOG1, *ANO1*, *TMEM16A*, gastrointestinal stromal tumor, GIST

(*Adv Anat Pathol* 2010;17:222–232)

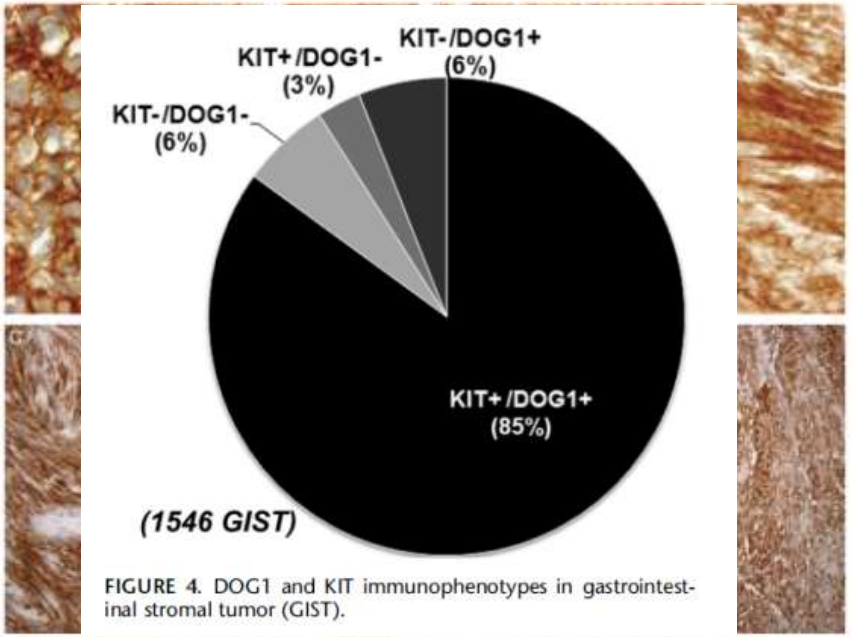


FIGURE 4. DOG1 and KIT immunophenotypes in gastrointestinal stromal tumor (GIST).

FIGURE 2. DOG1 staining patterns in gastrointestinal stromal tumor (GIST). (A). Predominantly membranous staining observed in GIST with epithelioid morphology (DOG1.1). (B). Predominantly cytoplasmic staining observed in GIST with spindle cell morphology (DOG1.1). (C). A DOG1-positive spindle cell GIST (clone K9) with negatively stained stromal vessels (D). A DOG1-positive spindle cell GIST and adjacent DOG1-negative normal muscularis propria (clone K9).

GIST: New IHC Markers

- Ezrin – over expressed in 2/3 of GISTs. Adverse prognostic indicator.
- p16 – over expression. Adverse prognostic indicator.
- DOG1 – not related to mutational status and may be useful to identify CD117 (-) GISTs
- Protein-kinase θ – Useful in CD117(-) GISTs. Although, 14% schwannomas (+).
- H-caldesmon – Useful in CD117(-) GISTs. Positive in smooth muscle tumors.

CONGRESO LATINOAMERICANO E IBEROAMERICANO DE CITOLOGIA

GRACIAS

Lima, Peru Junio 19-23, 2011