ESVCP – ECVCP Congress
Athen 2018

MISTERY SLIDES SESSION

- Cytology -

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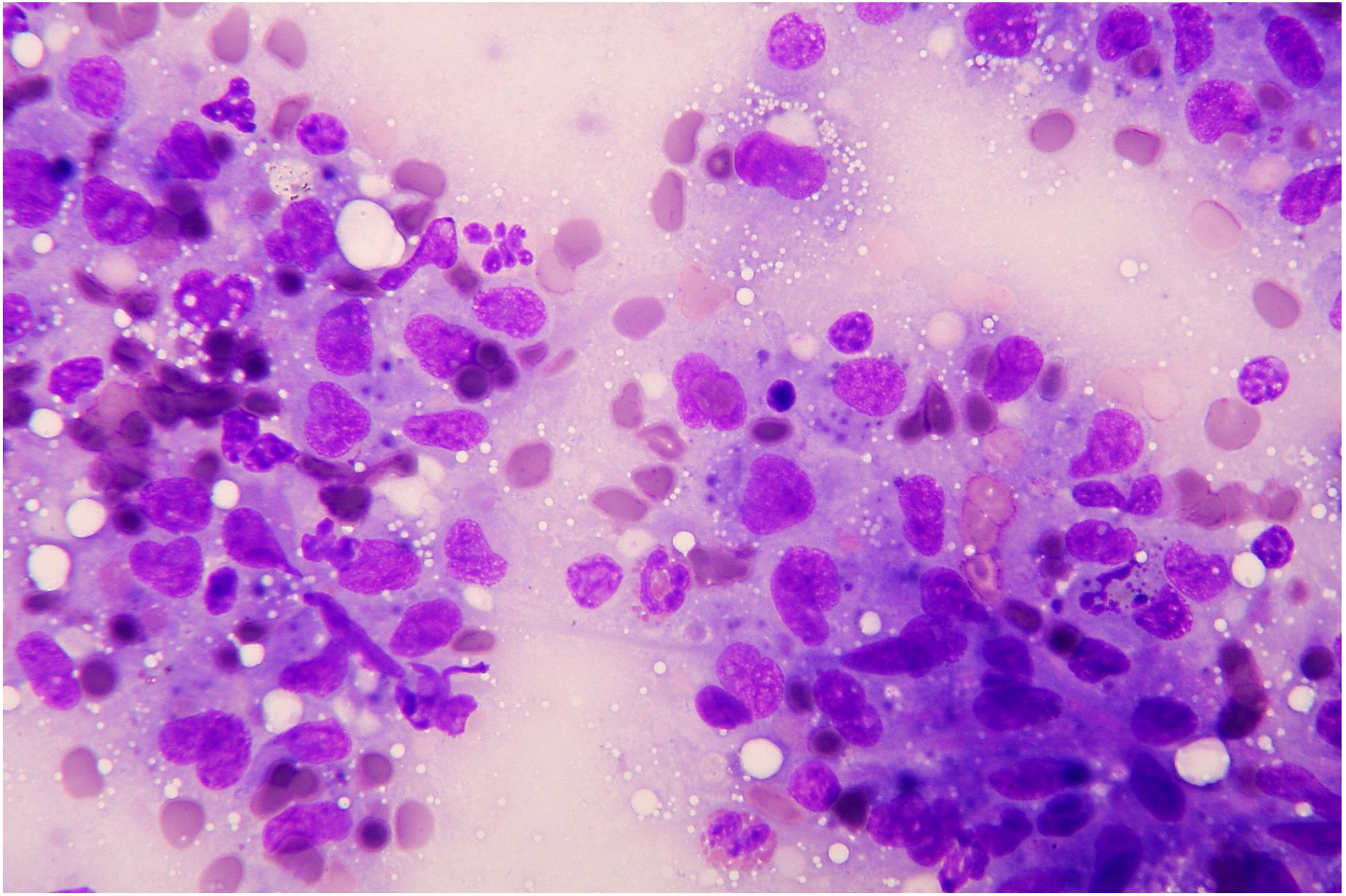
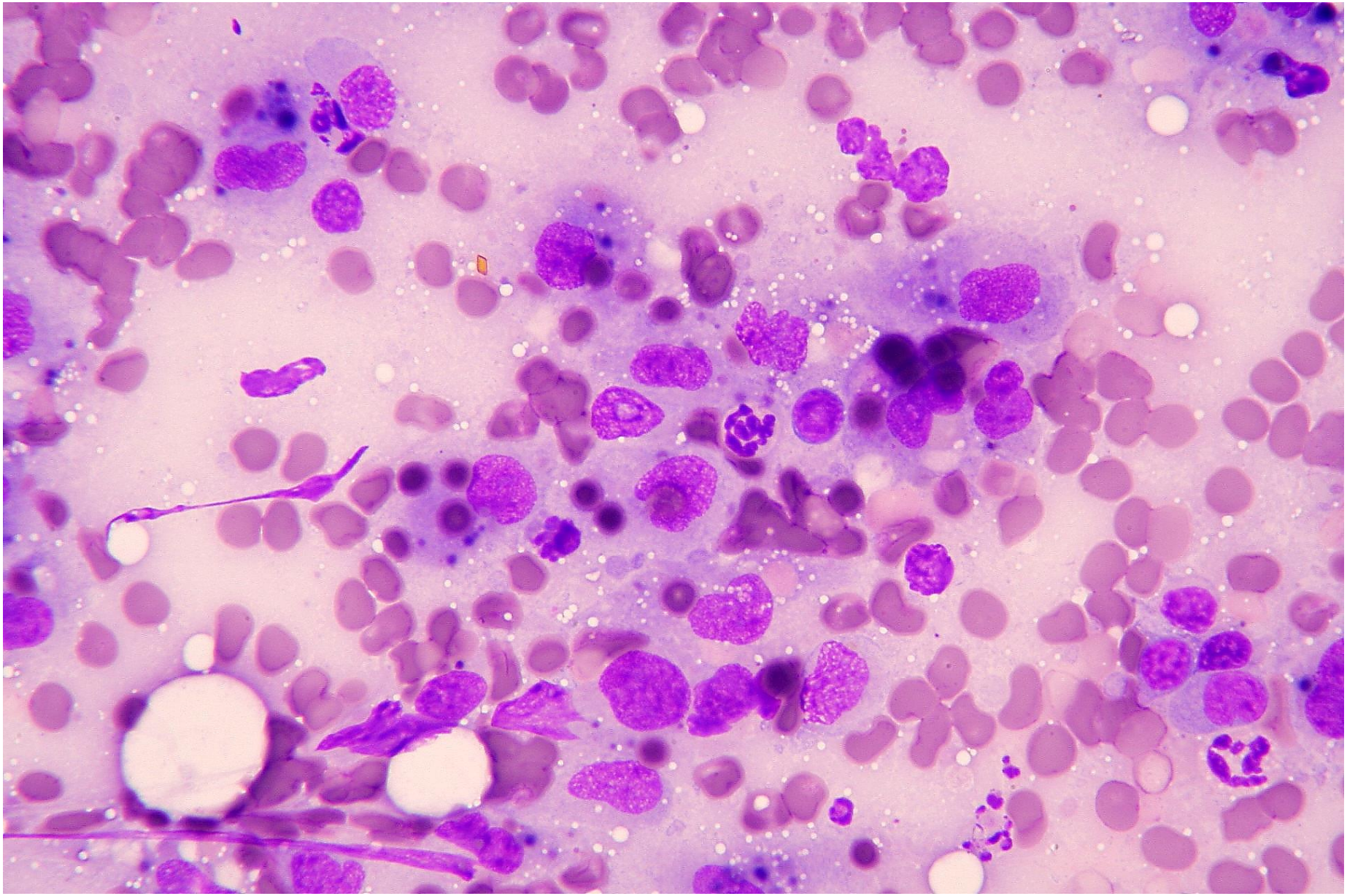
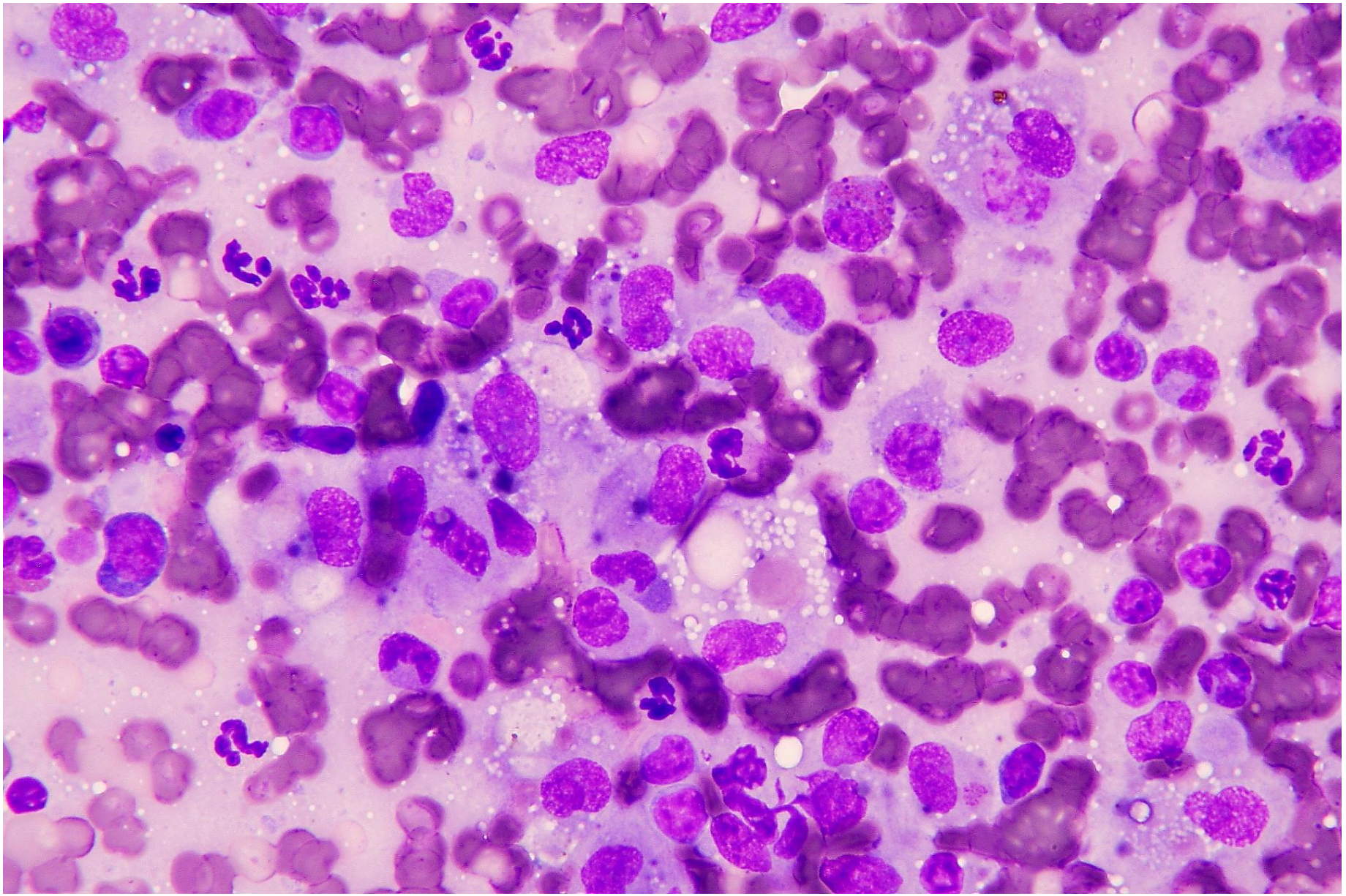
Case #1

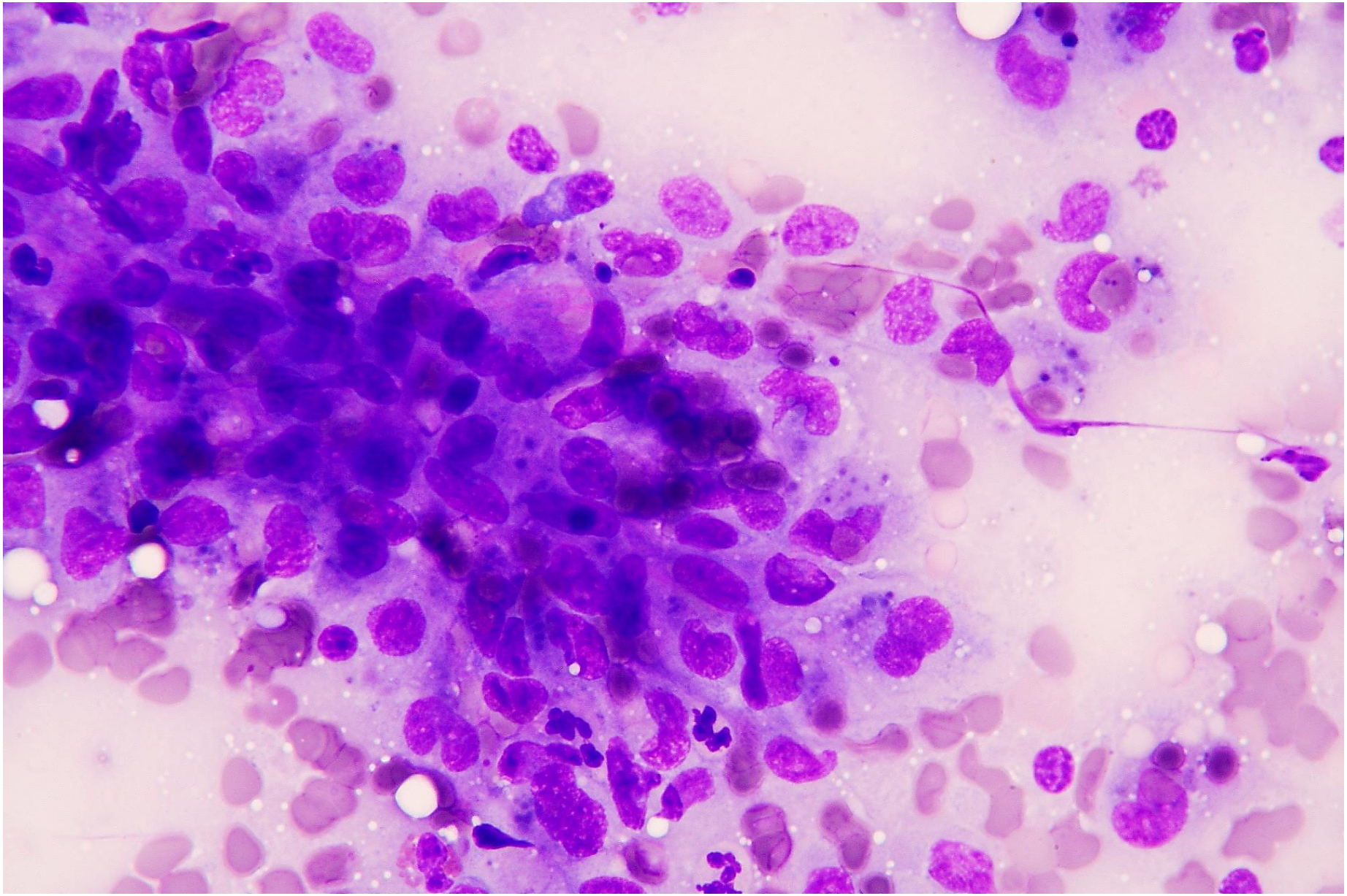
- Dog, Bernese mountain, 7-year-old, male
- Depression
- Anemia
- Splenic enlargement

- Sample: FNCS of spleen
- Stain: MGG









Cytologic findings

- Predominance of round cells
- Histiocytic appearance
- Medium-large size
- Eccentric, indented nucleus
- Erithrophagocytosis
- Presence of hemosiderin/hematoidin

- Mature to immature lymphocytes
- Eosinophilic granulocytes

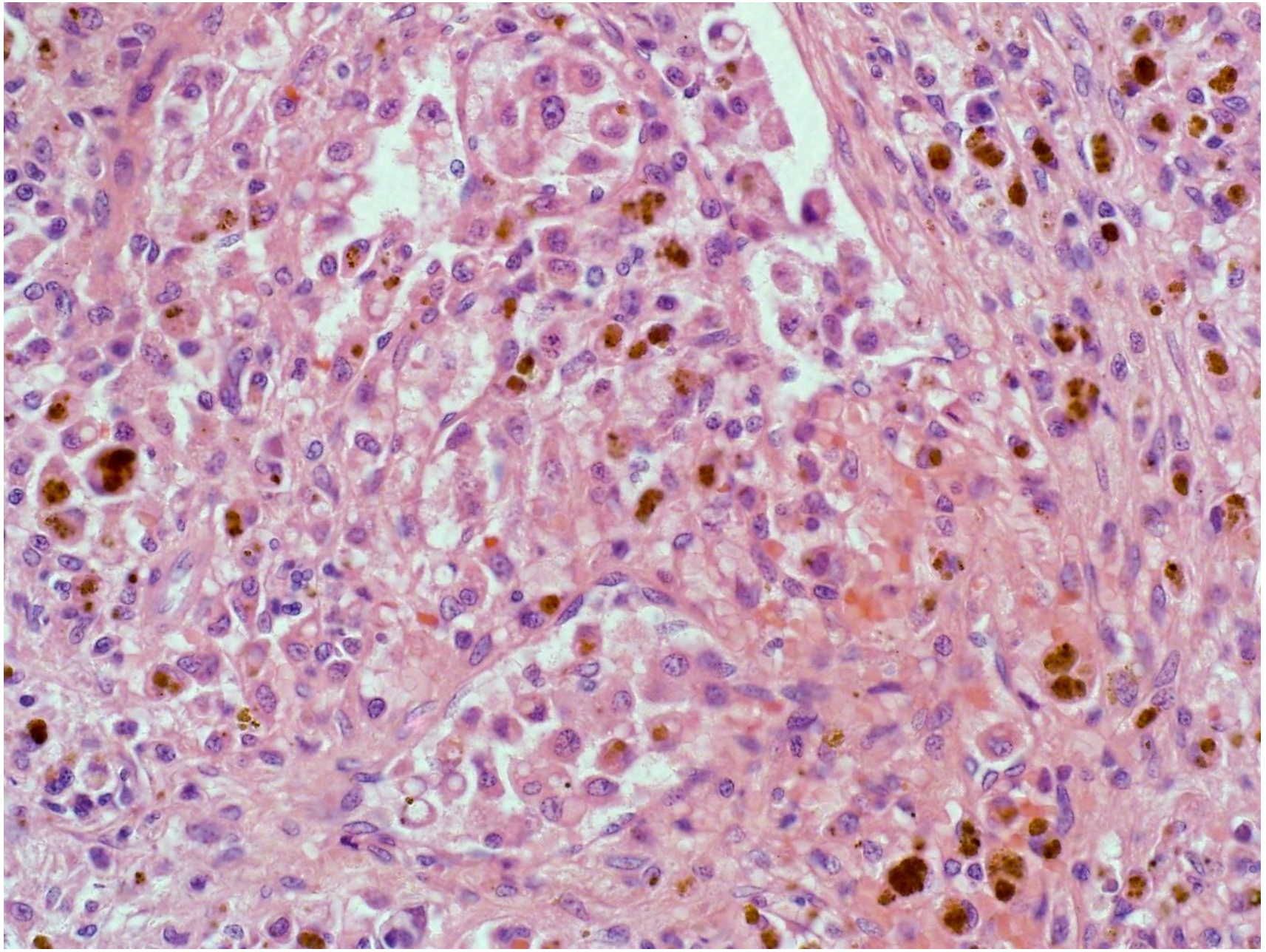
Diagnosis

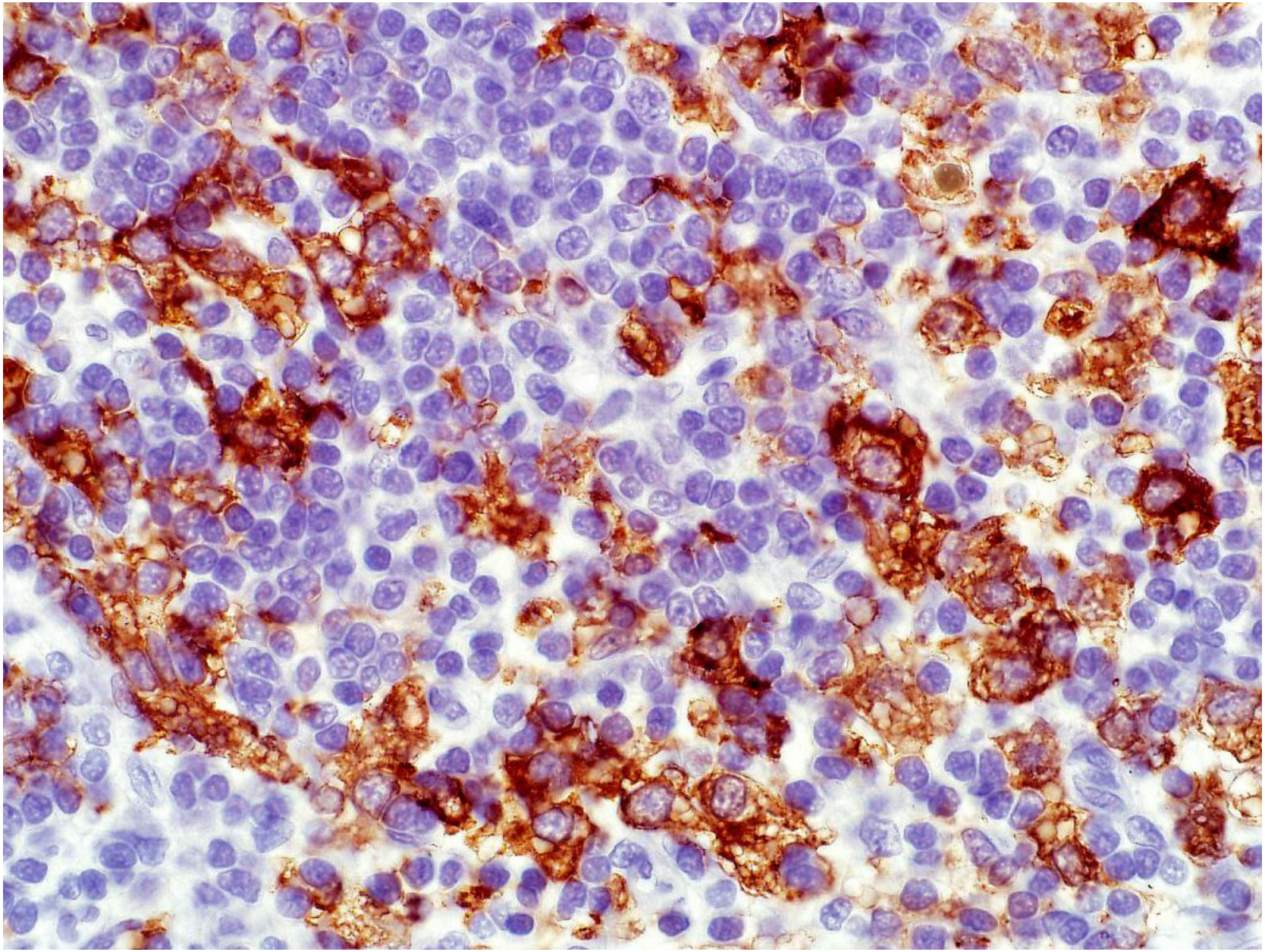
- Cytologic diagnosis: malignant round cells, with eritrhophagocytosis activity; haemophagocytic sarcoma
- Histological diagnosis: splenic hemophagocytic sarcoma
- IHC: splenic hemophagocytic sarcoma

Haemophagocytic histiocytic sarcoma

- This type of histiocytic sarcoma is represented by **neoplastic macrophages** of the splenic red pulp
- Diffuse hemophagocytic activity
- Frequently a severe secondary anemia is detectable, as consequence of massive RBC destruction
- Anatomical sites of onset:
  - Spleen
  - Bone marrow
  - Liver
- Phenotype: CD11d+, CD18+, CD11c-

Affolter V., Moore P., Vernau W. 2006

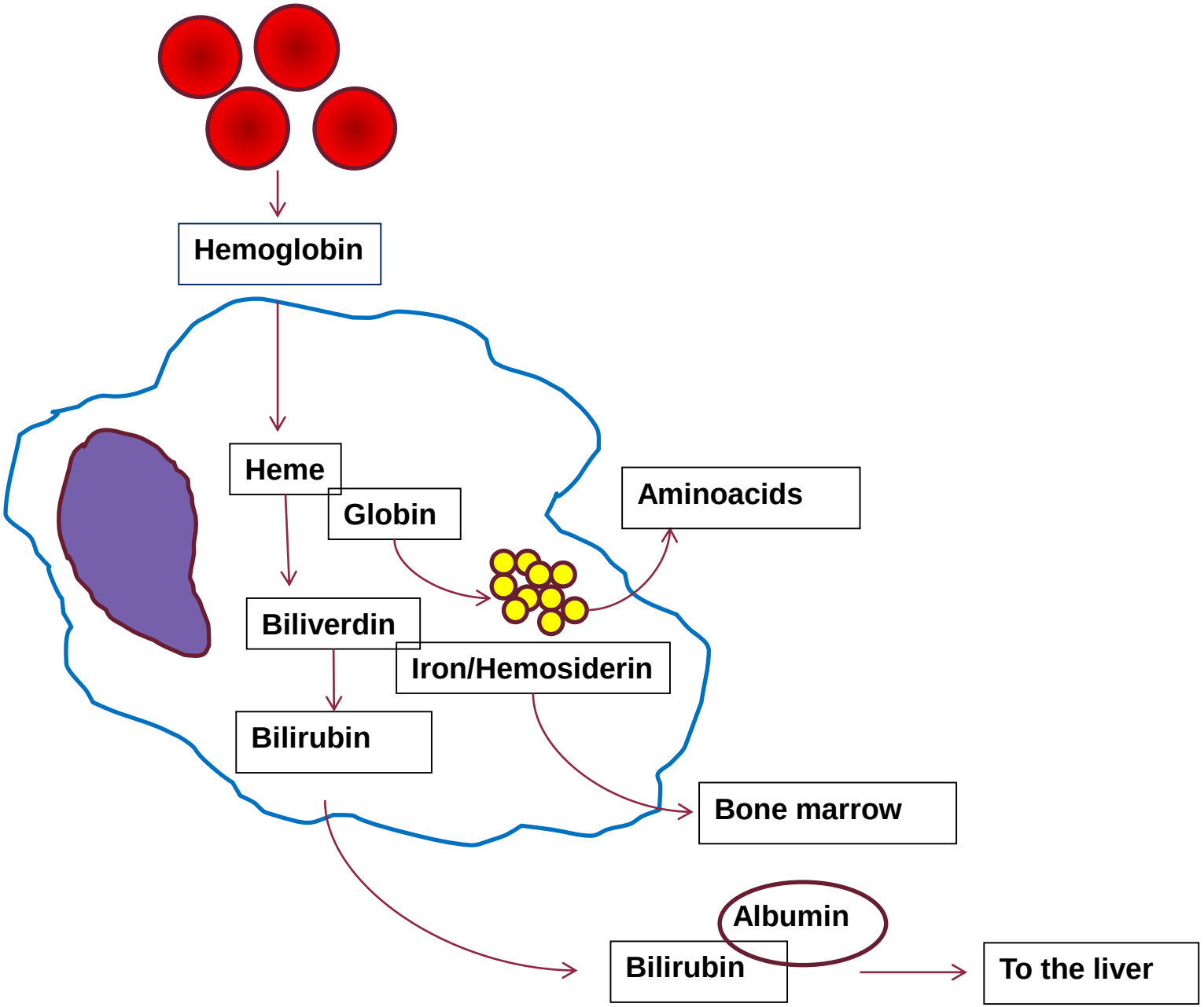


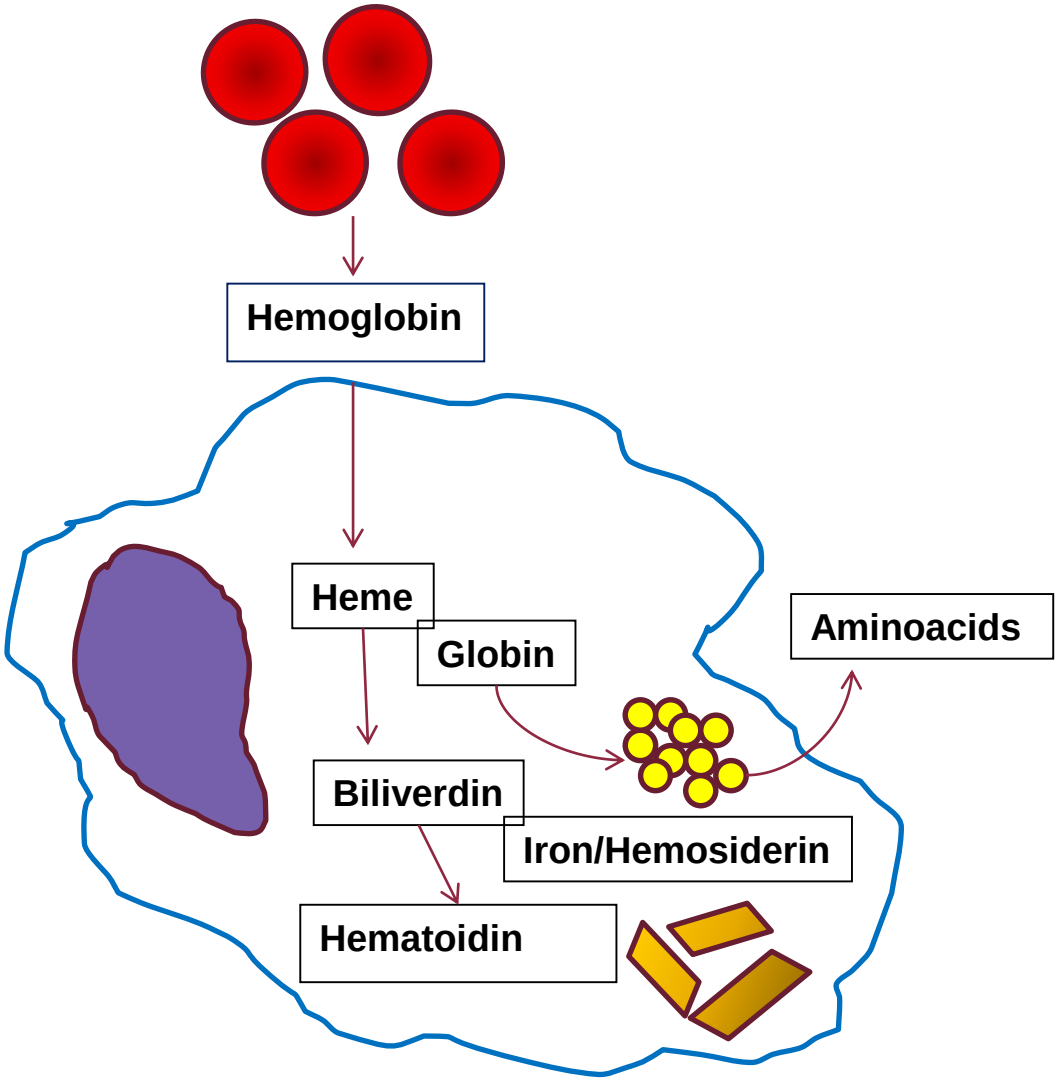


IHC: CD11d+

Discussion

- Do cytology alone allow a definitive diagnosis of this neoplasm?
- Are the neoplastic cells able to maintain their metabolic activities (erithrophagocytosis, metabolic degradation of heme group)?
- Clinically, what is the point of differentiating histiocytic sarcoma from hemophagocytic sarcoma?


Hemoglobin
Heme
Globin
Aminoacids
Biliverdin
Iron/Hemosiderin
Bilirubin
Bone marrow
Albumin
Bilirubin
To the liver



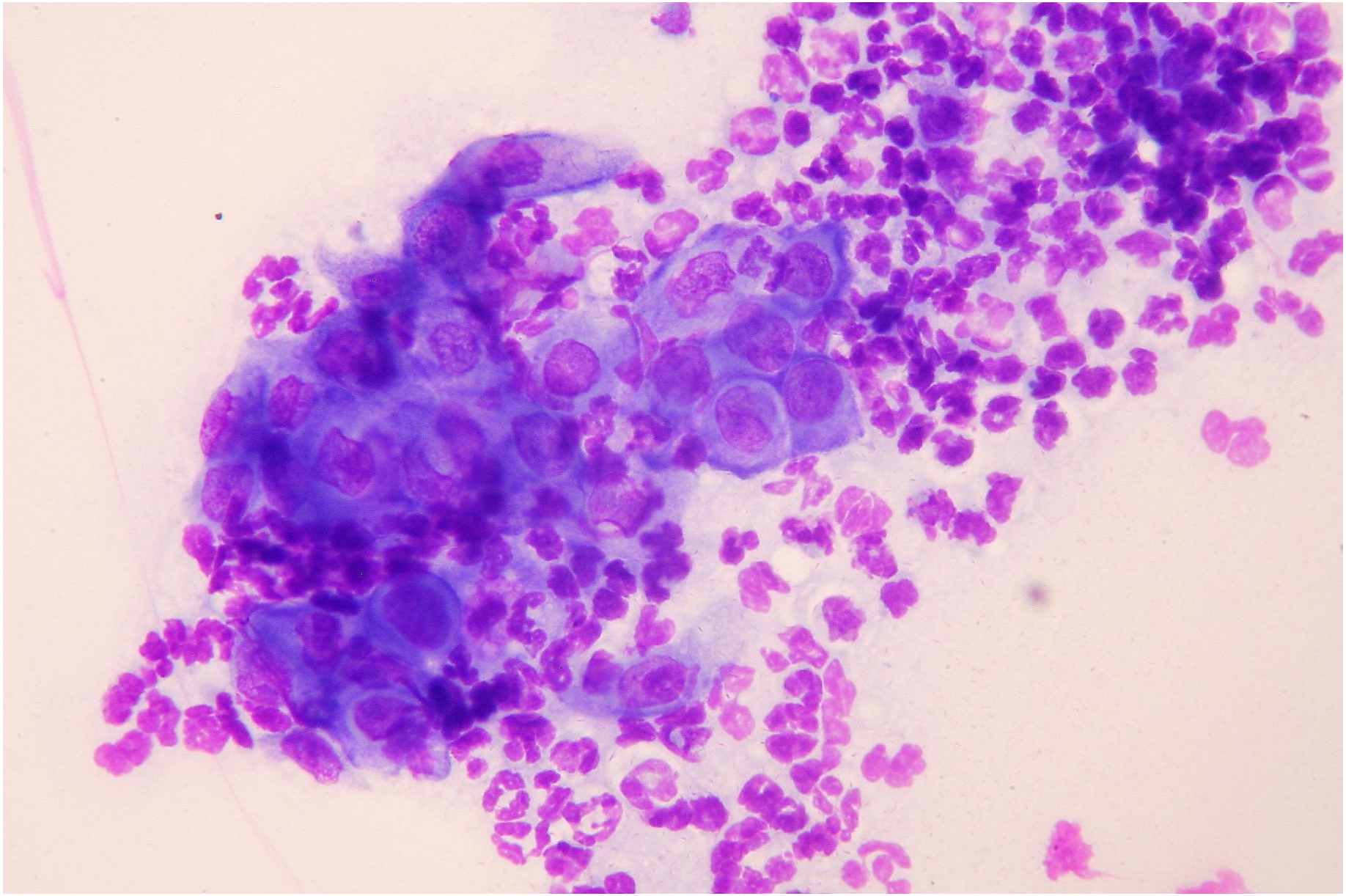
O_2

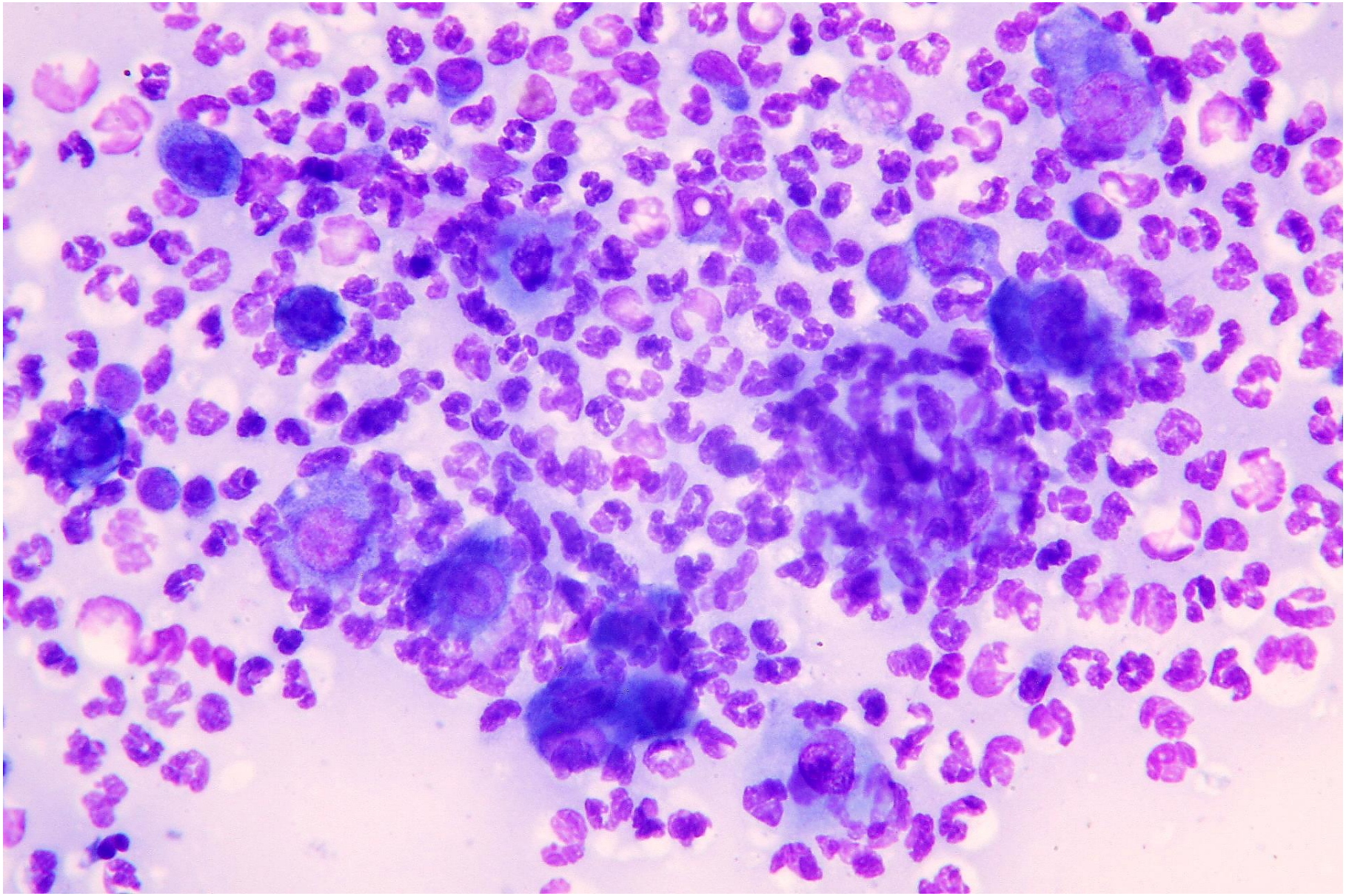
Low oxygen concentration
- Hematoma
- Cavitary effusions

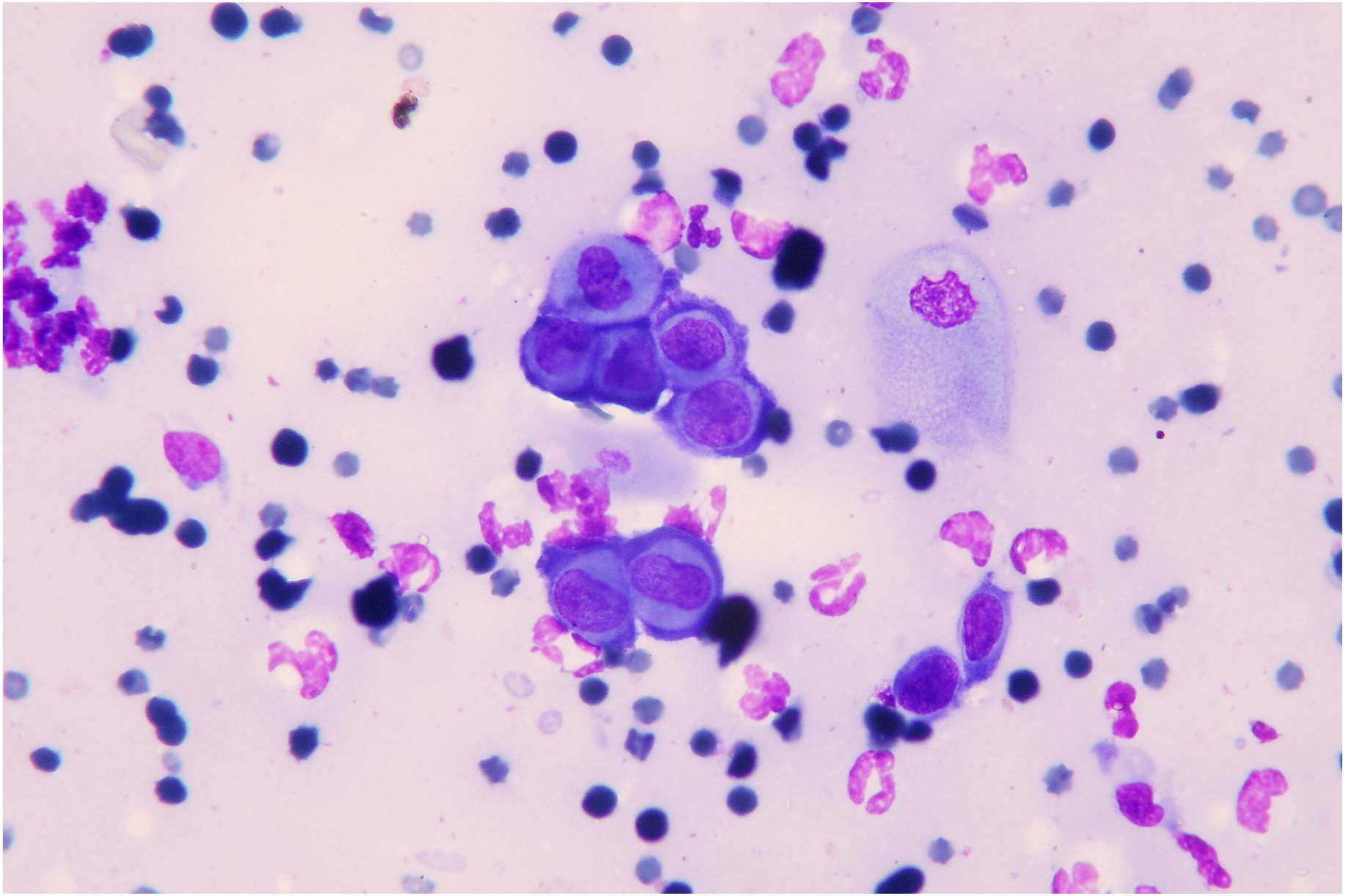
Case #2

- Cat, DSH, 3-year-old, female, neutered
- Crusting lesions on the face

- Sample: apposition of skin lesion after crusts removal
- Stain: MGG







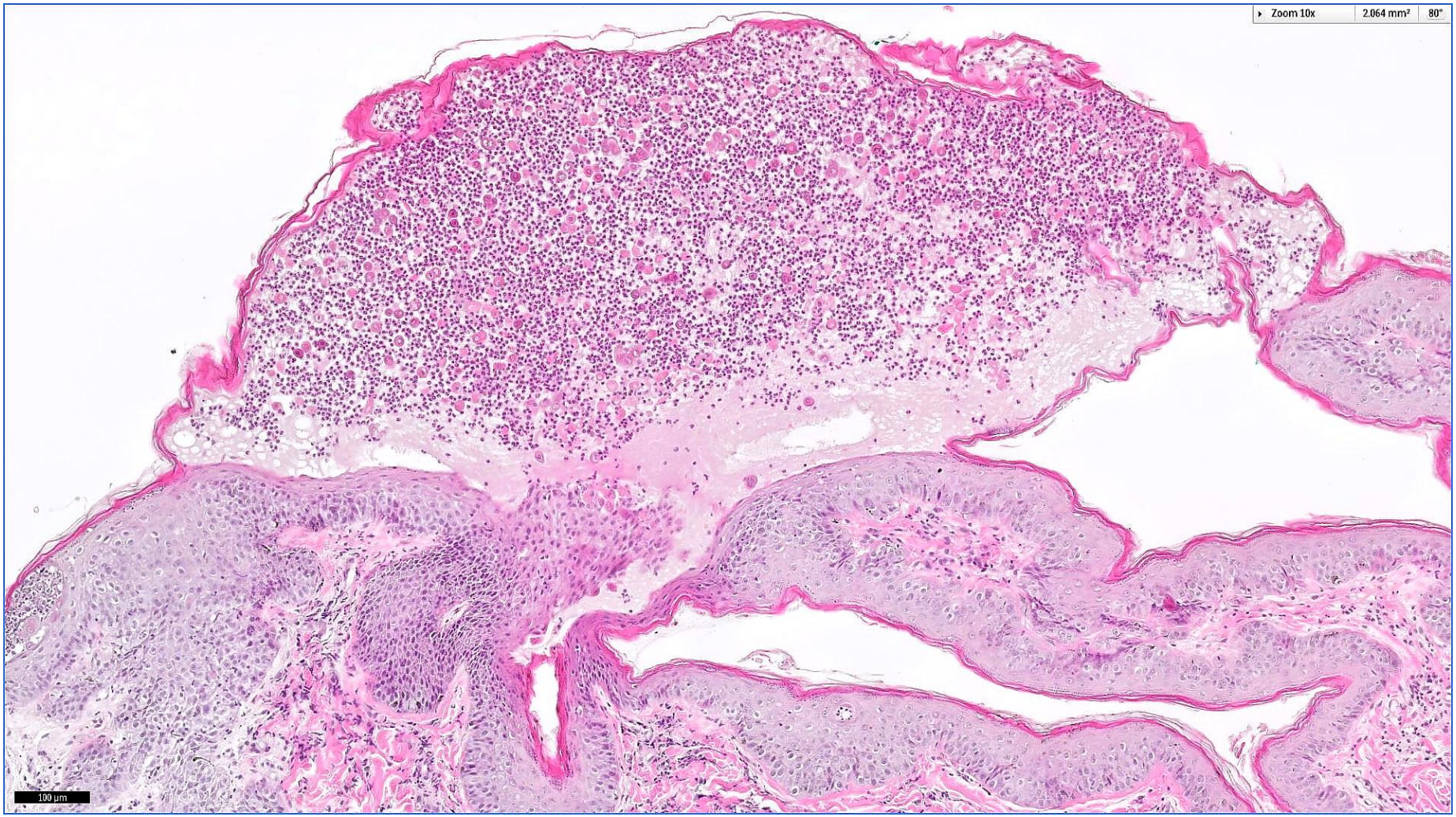
Cytologic findings

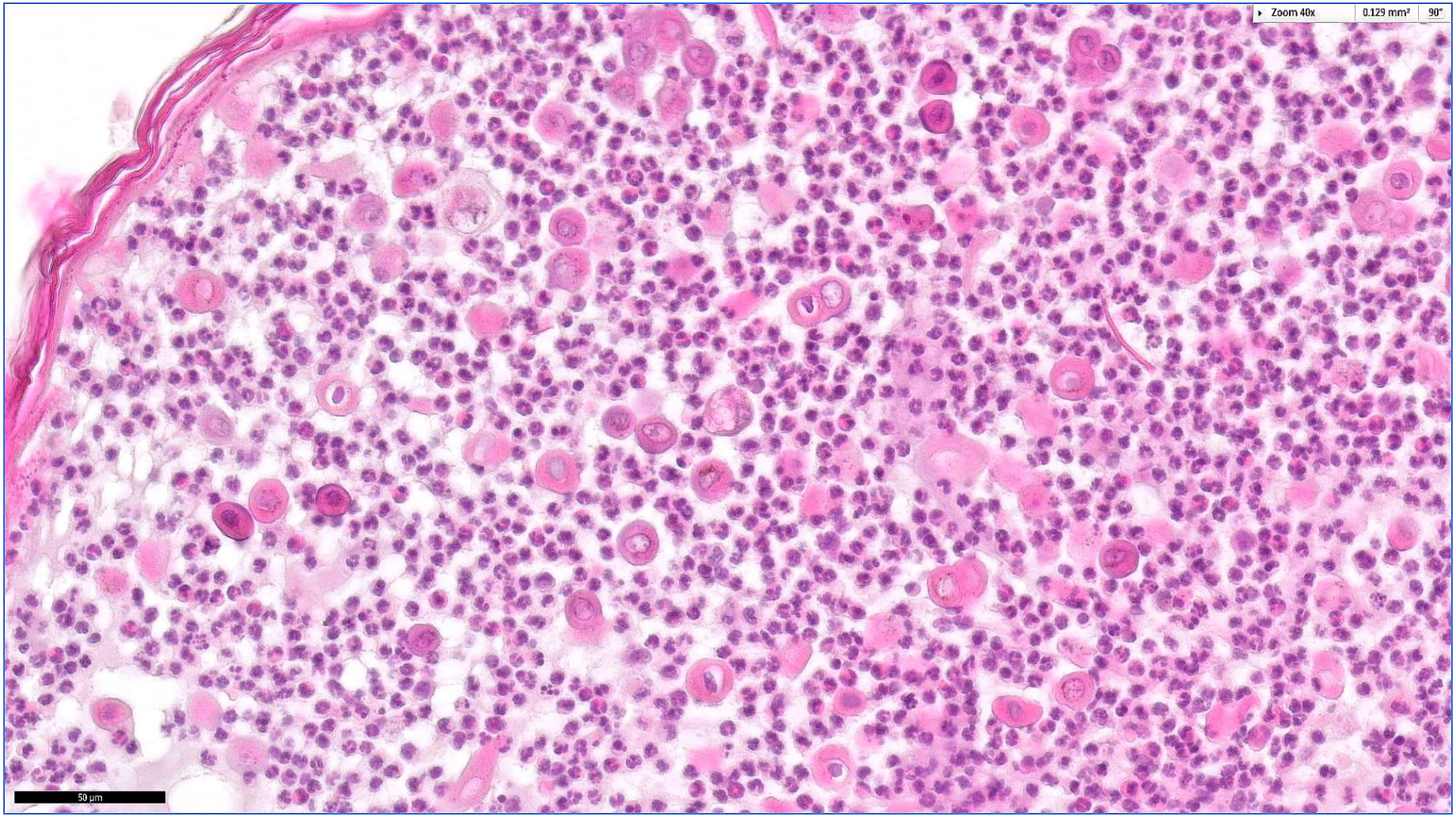
- Presence of many single epithelial cells
  - Round shape
  - Round nucleus with compact chromatin
  - Clear halo around the nucleus
- Inflammatory cells
  - Mostly neutrophils
  - Frequent arrangements of inflammatory cells around the epithelial cells

Diagnosis

- Cytologic diagnosis: acantholysis of epithelial cells with suppurative, non-septic inflammation; pemphigus complex

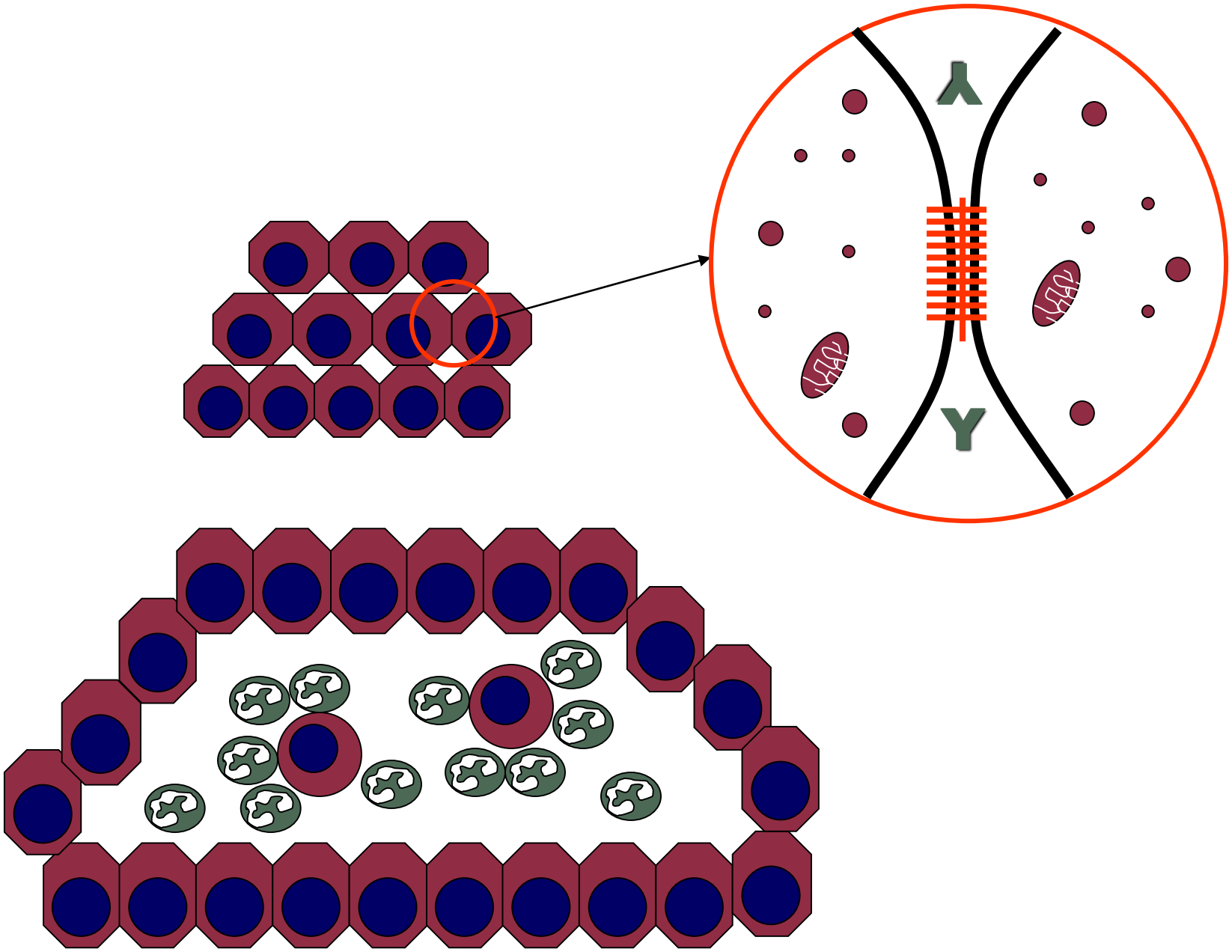
- Histological diagnosis: pemphigus foliaceus


Zoom 10x
2.064 mm²
80°
100 µm


Zoom 40x
0.129 mm²
90°
50 µm

Discussion

- The pathomechanism of PV hinges on autoantibodies damaging cell-cell cohesion and leading to cell-cell detachment (acantholysis) of the epidermis and Malpighian mucosae (mainly oral mucosa).
- A controversy exists about which subset of autoantibodies is primarily pathogenic:
  - Desmoglein 1-reactive antibodies
  - Antibodies directed against the acetylcholine receptors of the keratinocyte membrane



Case #3

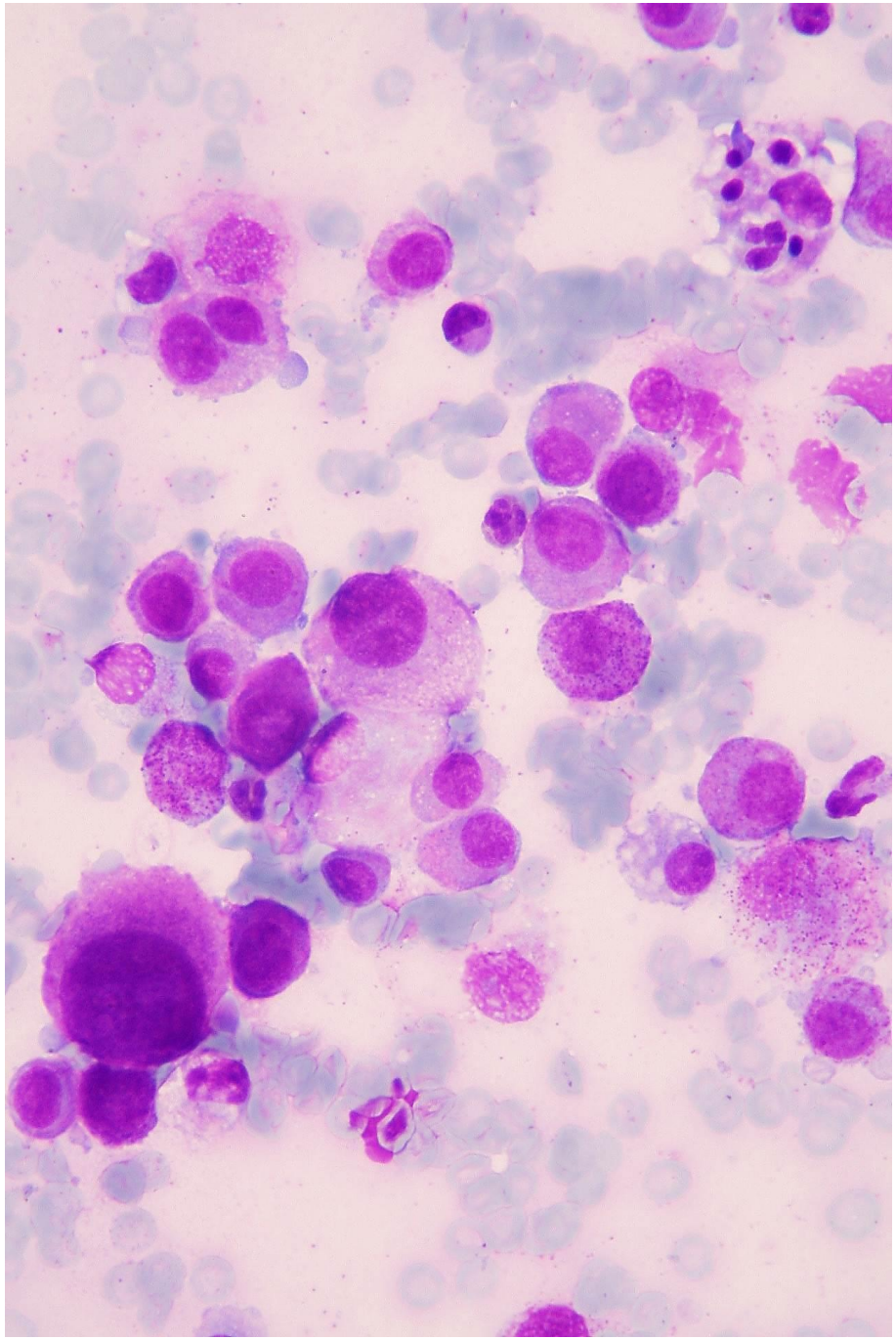
- Dog, mongrel, female neutered, 11-year-old
- Cutaneous mass on the back

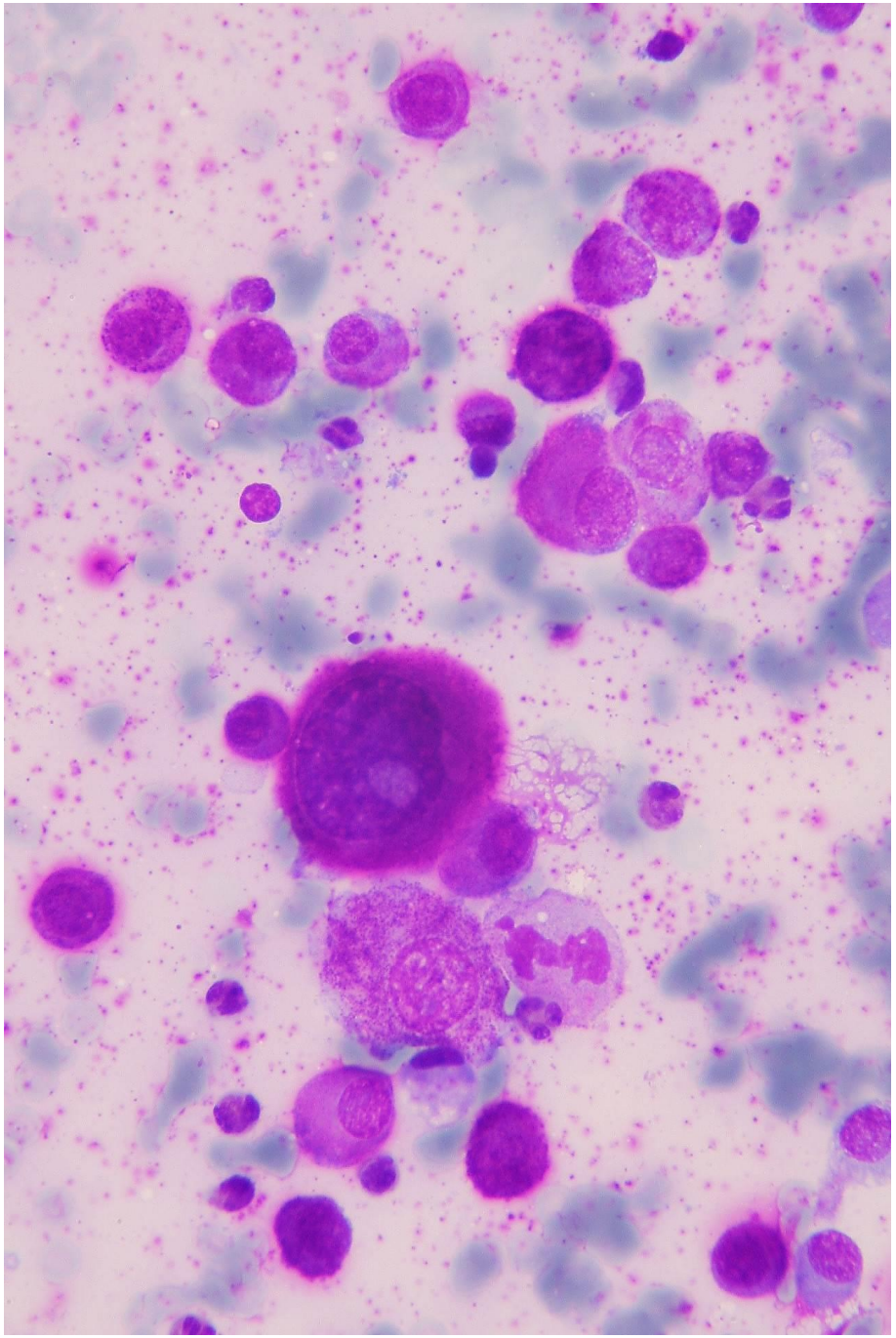
- Sample: FNCS of the mass
- Stain: MGG

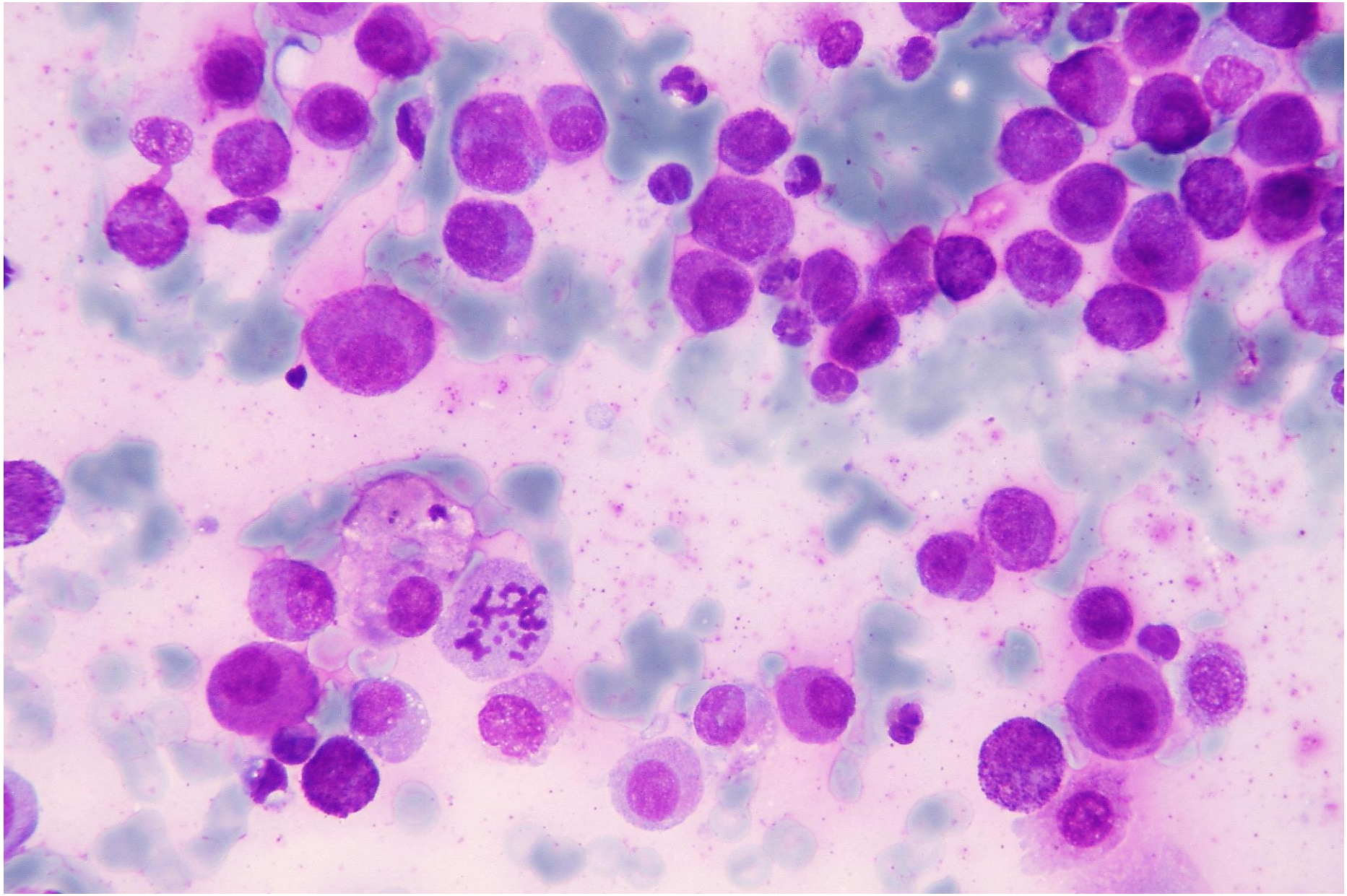
Cytologic findings

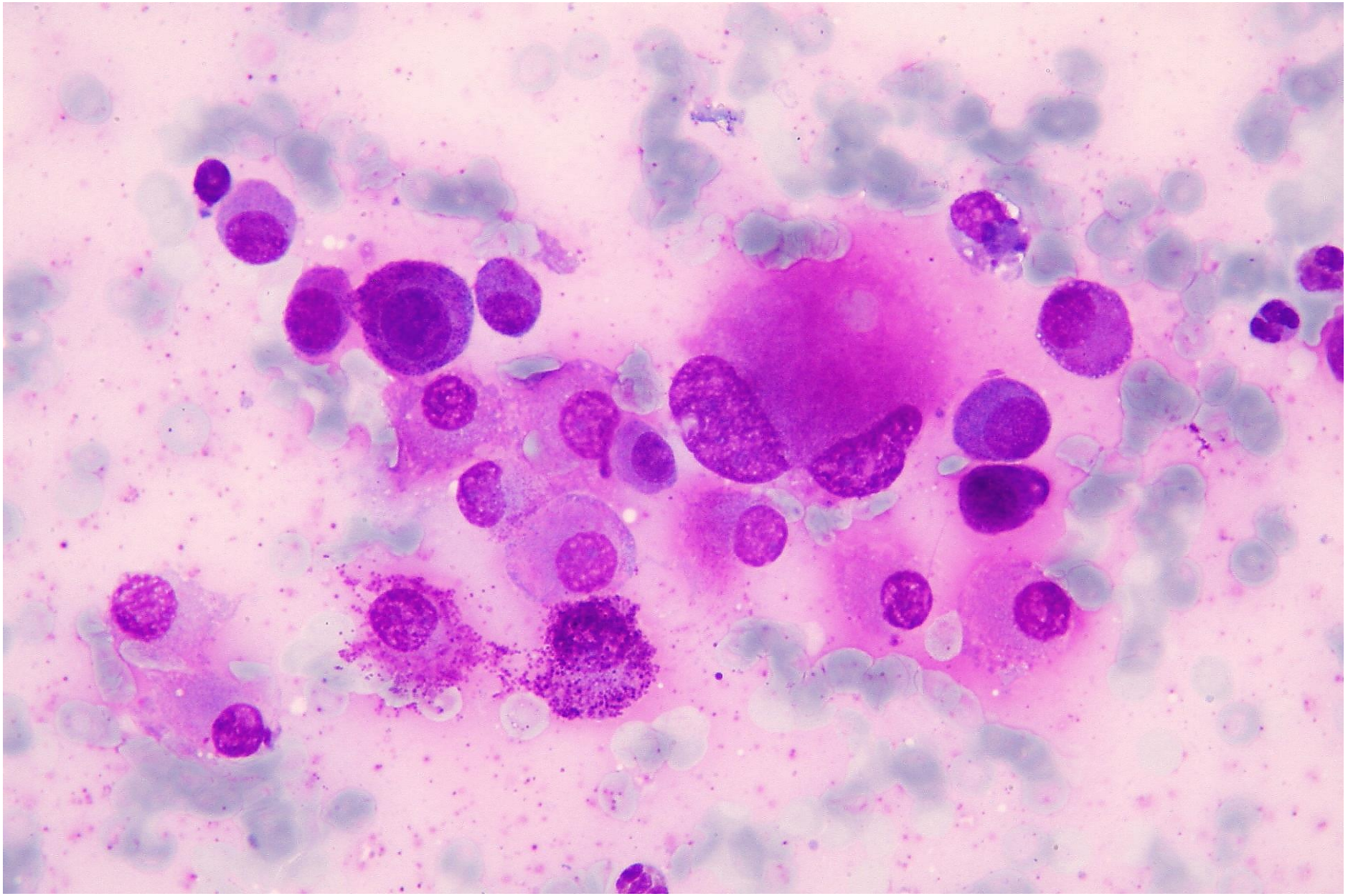
- Round cells
- Poorly granulated cytoplasm
- Anisokaryosis
- Double-triple nuclei
- Clumped chromatin
- Mitoses

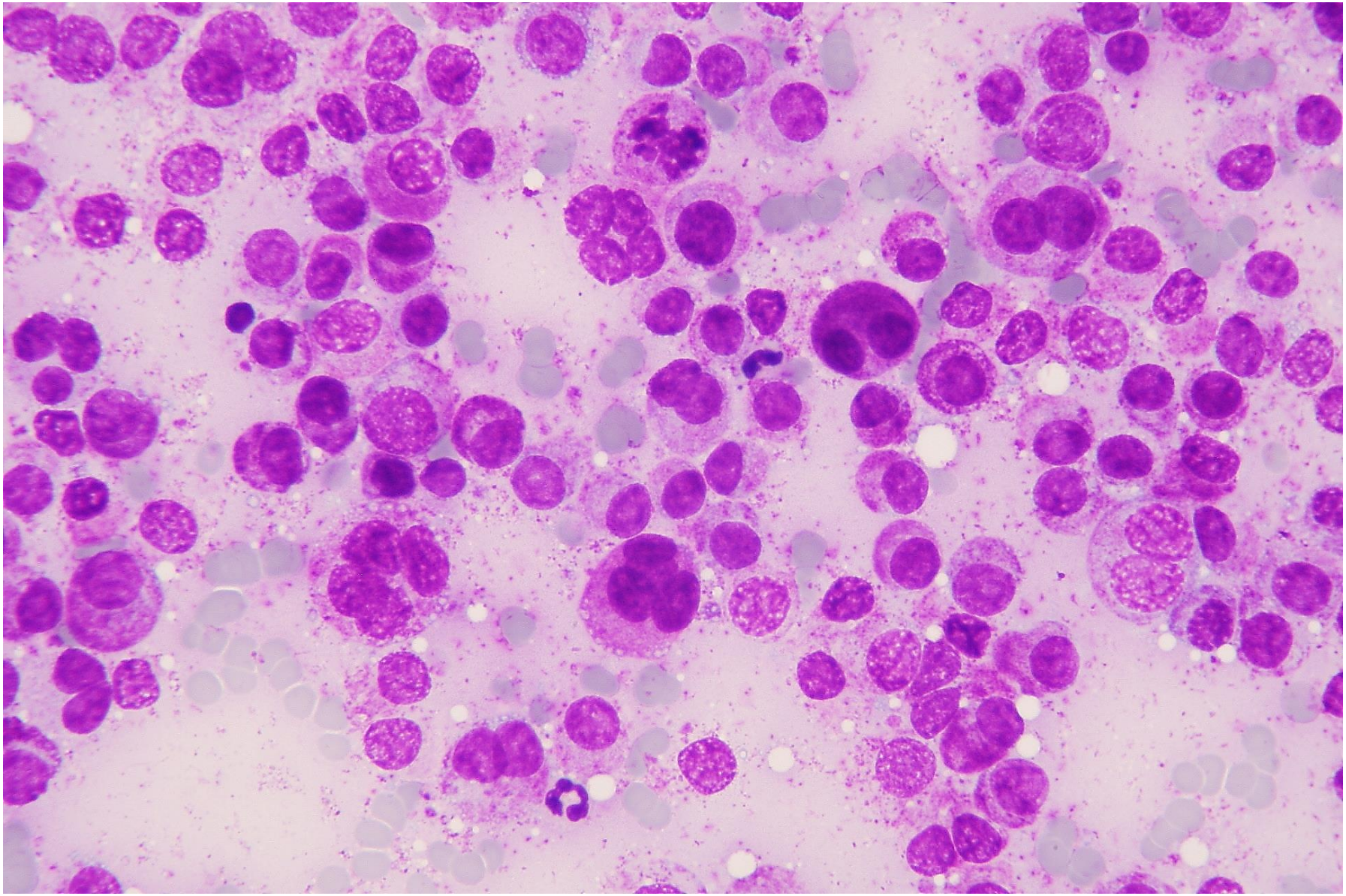
- Eosinophilic granulocytes
- Rare lymphocytes











Diagnosis

- Cytological diagnosis: poorly differentiated mast cell tumor

- Histological diagnosis: mast cell tumor, grade III (Patnaik, 1984), high grade (Kiupel, 2011)

Classification of mast cell tumor in dogs

Class. Patnaik, 1984

I grade	II grade	III grade
Subepidermical and periadnexal distribution	Deep dermal and subcutanoeus distribution	Deep dermal and subcutanoeus distribution
Rows and clusters of neoplastic cells infiltratin mature bundles of collagen	Hyalinization and ispissation of collagen; oedema and necrosis	Hyalinization and ispissation of collagen; oedema and necrosis
Granular cytoplasm	Variable amount of granules into the cytoplasm	Poorly granulated cytoplasm
Round, monomorphic cells	Round cells; rare giant cells	Many binucleated, multinucleated and giant cells
Round nucleus	Round to folded nucleus	Round to folded nucleus
No mitosis detectables	Mitosis from 0 to 2/HPF	Mitosis from 3 to 6/HPF

Class. Kiupel, 2011

Low grade	High grade
<7mitoses/10HPF	>7mitoses/10HPF
<3 multinucleated cells/10HPF	>3 multinucleated cells/10HPF
No bizarre nuclei	>3 bizarre nuclei/10HPF
No karyomegaly	Karyomegaly in more than 10% of the neoplastic cells

Cytological grading of canine cutaneous mast cell tumors

F.Scarpa, S.Sabattini, G.Bettini

Vet Comp Oncol. 2016 Sep;14(3):245-51

- Attempt to apply the grading of Kiupel to cytological samples
- The cytograding correctly predicts the histological grade in 94% of cases:
  - Sensitivity 84,6%
  - Specificity 97,3%
- Loss of diagnostic meaning of cytoplasmic granules in diagnosis of low-grade mast cells tumor

Low grade (74%)	High grade (26%)	
	>7 mitoses	76,9% sens: 10%
	>3 mutinucleated cells	53,8% sens: 84,6%
	>3 bizarre nuclei	69,2% sens: 11%
	Karyomegaly in >10% cell	53,8% sens: 85,7%

Use of a 2-tier histologic grading system for canine cutaneous mast cell tumors on cytology specimens

Hergt F.,Von Bomhard W., Kent M.S., Hirschberger J

Vet Clin Pathol. 2016 Sep;45(3):477-83

- Attempt to apply the grading of Kiupel to cytological samples
- The cytograding correctly predicts the histological grade in 94,3% of cases
  - Sensitivity 97,1
  - Specificity 94%

Low grade (74%)	High grade (26%)	
	>7 mitoses	30,7% sn 18,5% sp 81,8%
	>3 mutinucleated cells	34,3% sn 66,7% sp 75%
	>3 bizarre nuclei	37,5% sn 13,3% sp 82,6%
	Karyomegaly in >10% cell	82,9% sn 90,9% sp 62,5%

Poor prognosis in 17-56% of dogs with mast cell tumor grade II (Blackwood, 2012)

Discussion

- Use of cytological classification in diagnosis of mast cell tumor
  - Just "mast cell tumor" or use of cytological grade?

- Poor prognosis in 17-56% of dogs with mast cell tumor grade II (Blackwood, 2012)

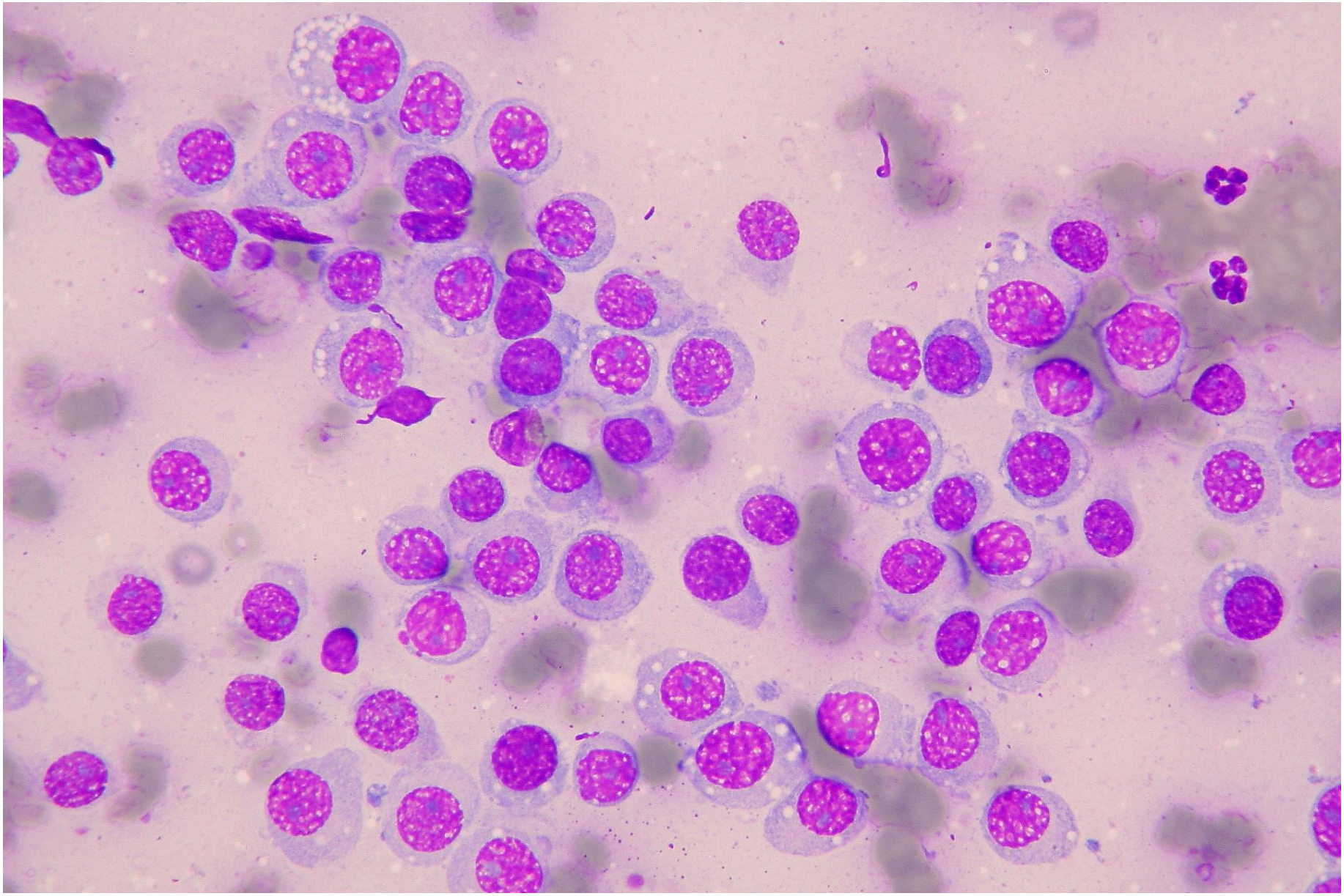
Case #4

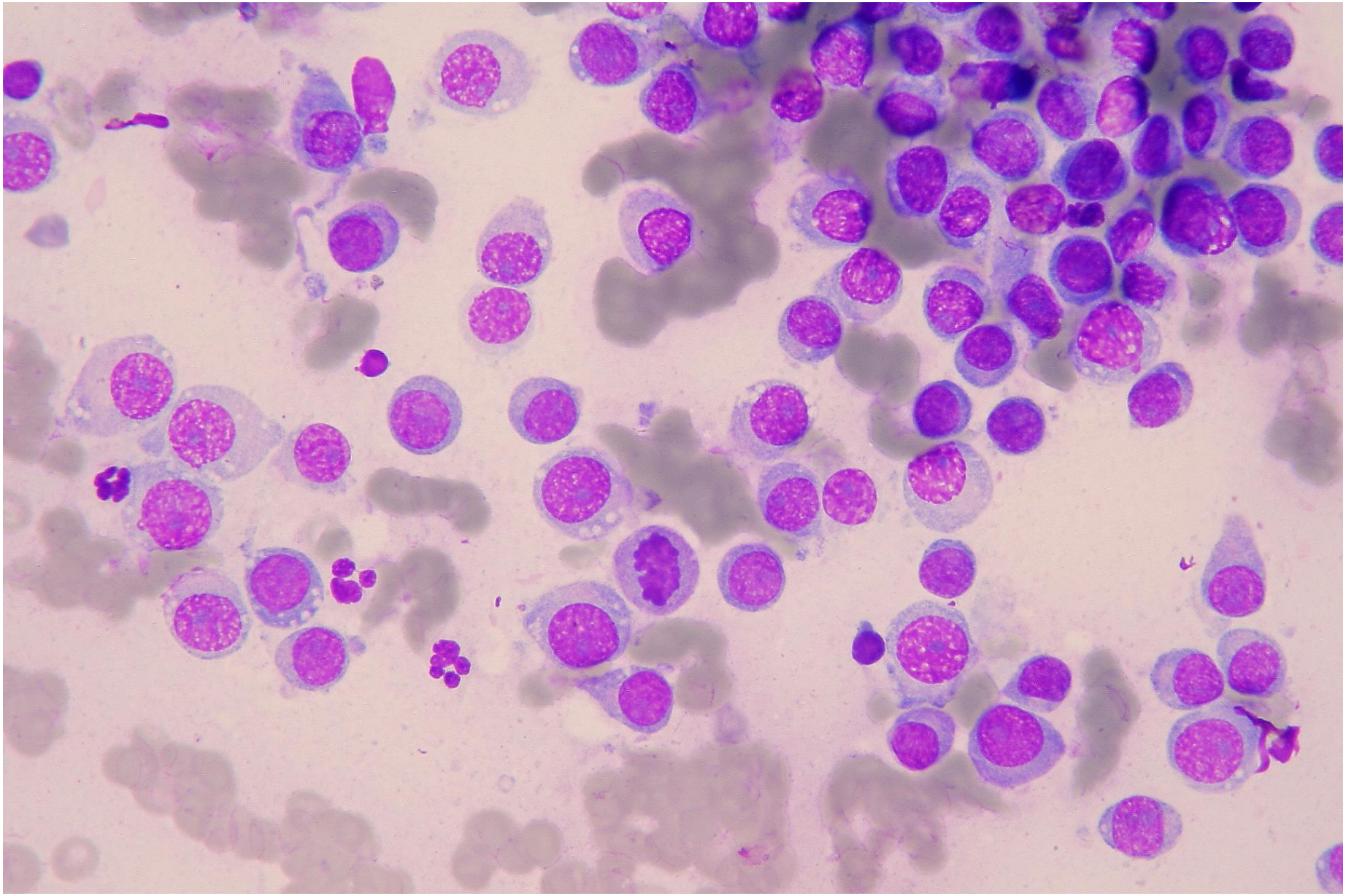
- Dog, mongrel, 6-year-old, female
- Vulvar mass

- Sample: FNCS
- Stain: MGG

Cytologic findings

- Round cells with slightly basophilic cytoplasm
  - Frequent presence of achromatic microglobules
- Round nucleus
  - Mild anisokaryosis
  - Granular to clumped chromatin
  - Rare nucleoli
  - Rare mitoses

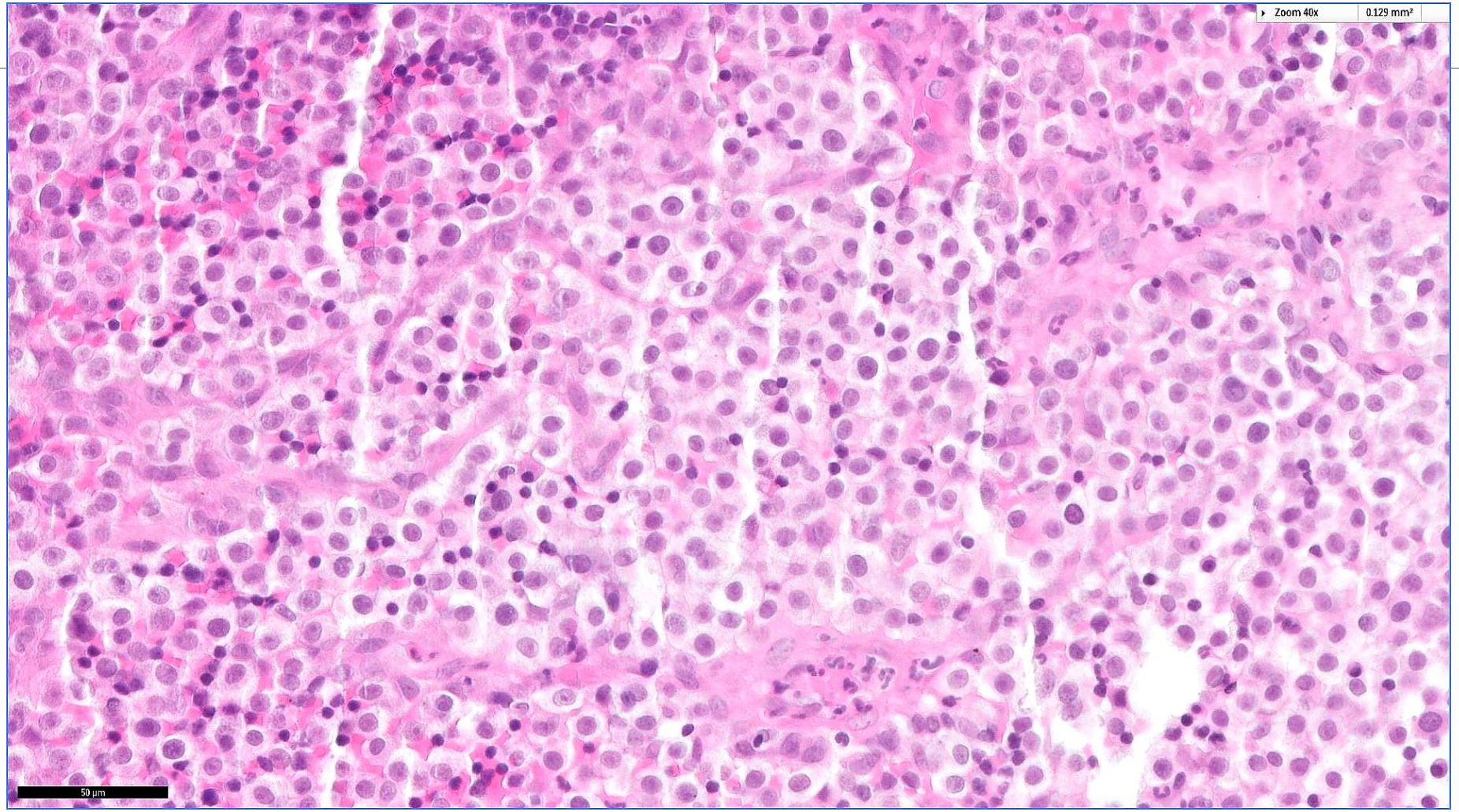




Diagnosis

- Cytological diagnosis: round cell tumor, morphologically indicative of Transmissible Venereal Tumor (TVT) or Sticker sarcoma

- Histologic diagnosis: Transmissible Venereal Tumor


Zoom 40x
0.129 mm²
50 µm

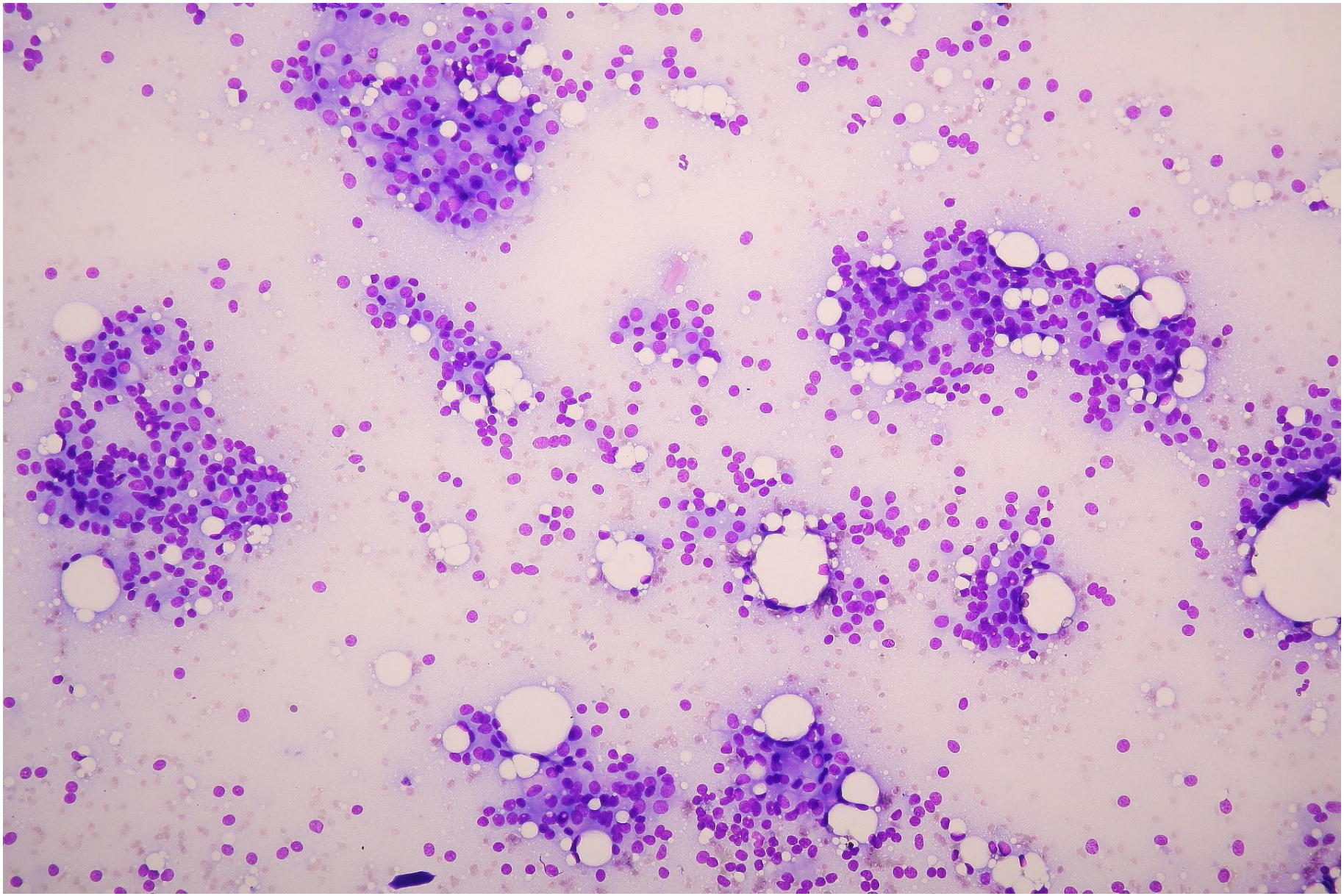
Transmissible Venereal Tumor (TVT)

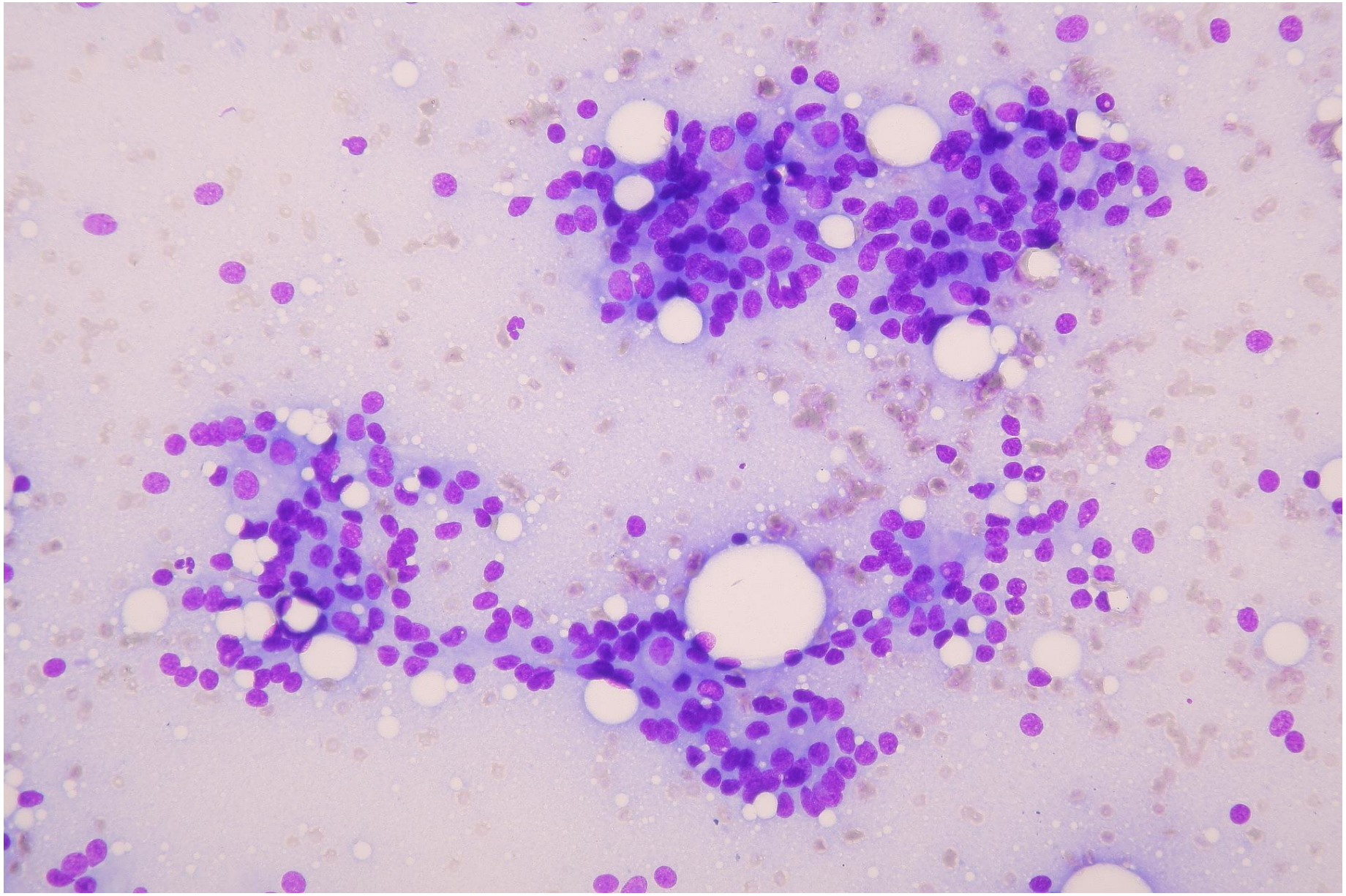
- Contagious, sexually transmitted tumor occuring in both gender
  - The first described transplantable neoplastic process
  - 58 or 59 chromosomes
    - Normal in dog: 78 chromosomes
- Strongly suspected to be of histiocytic origin
  - Macrophages-specific immunostain
  - Phagocytosis of Leishmania spp
- Neoplastic behaviour similar to the ulcerative facial dermatitis of Tasmanian devil

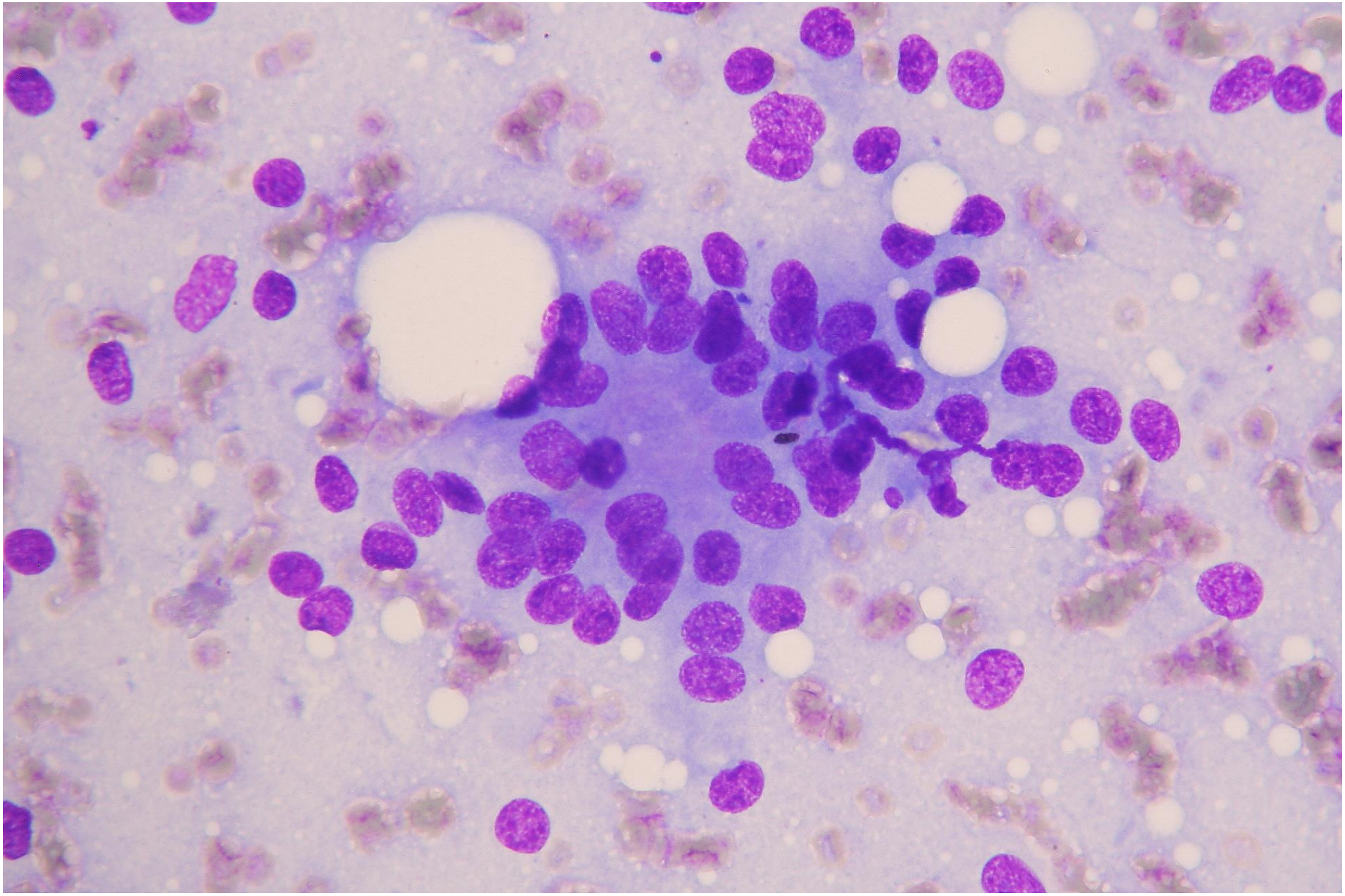
Case #5

- Dog, Pinscher, 13-year-old, female neutered
- Pancreatic nodule.

- Sample: US-guided FNCS
- Stain: MGG





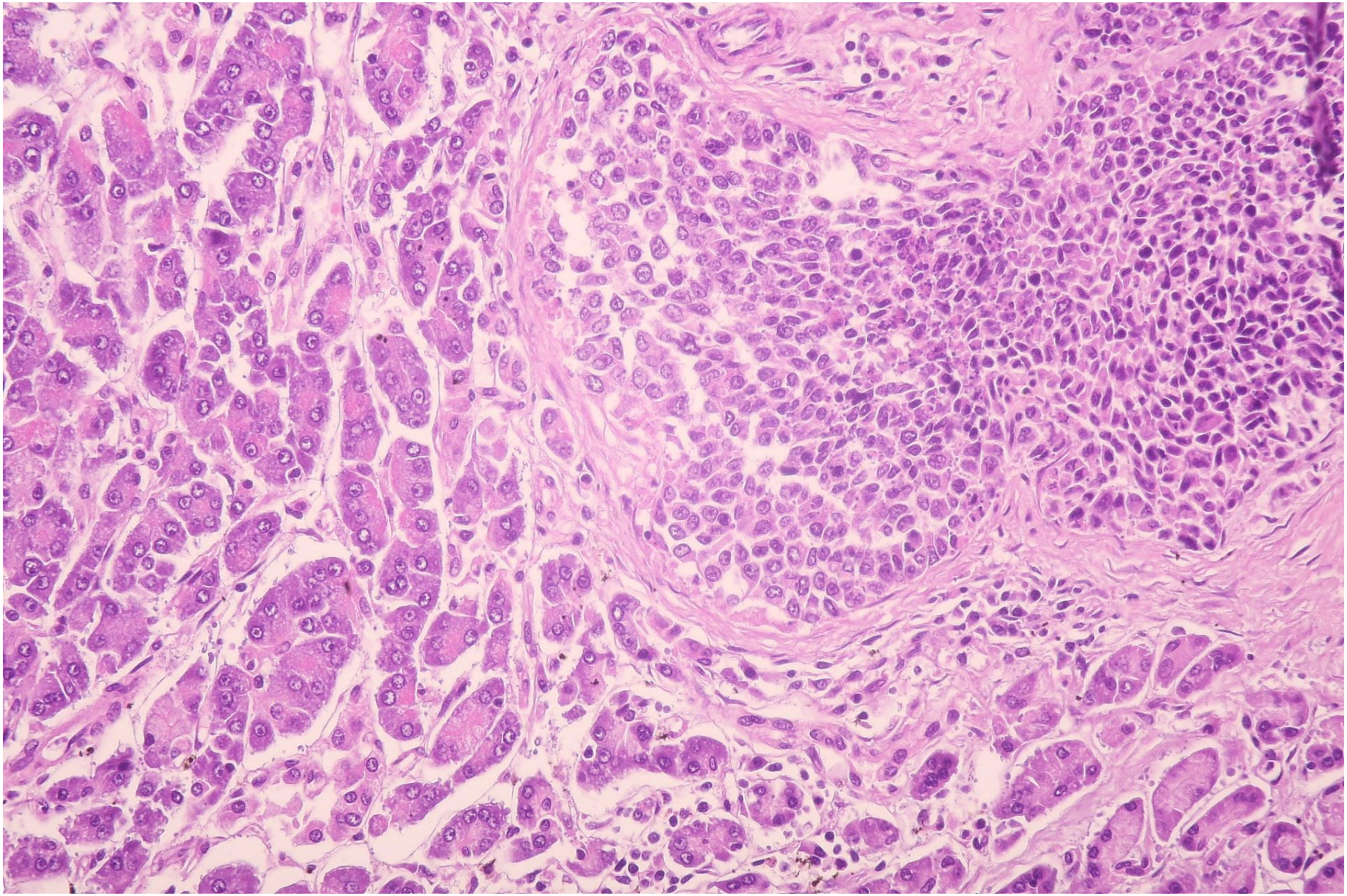


Cytologic findings

- Epithelial cells
  - Indisintict cytoplasm
  - Round ot ovoid nucleus
  - Finely granular chromatin
  - Rare nucleoli
- Discohesive aggregates
- Many naked nuclei

Diagnosis

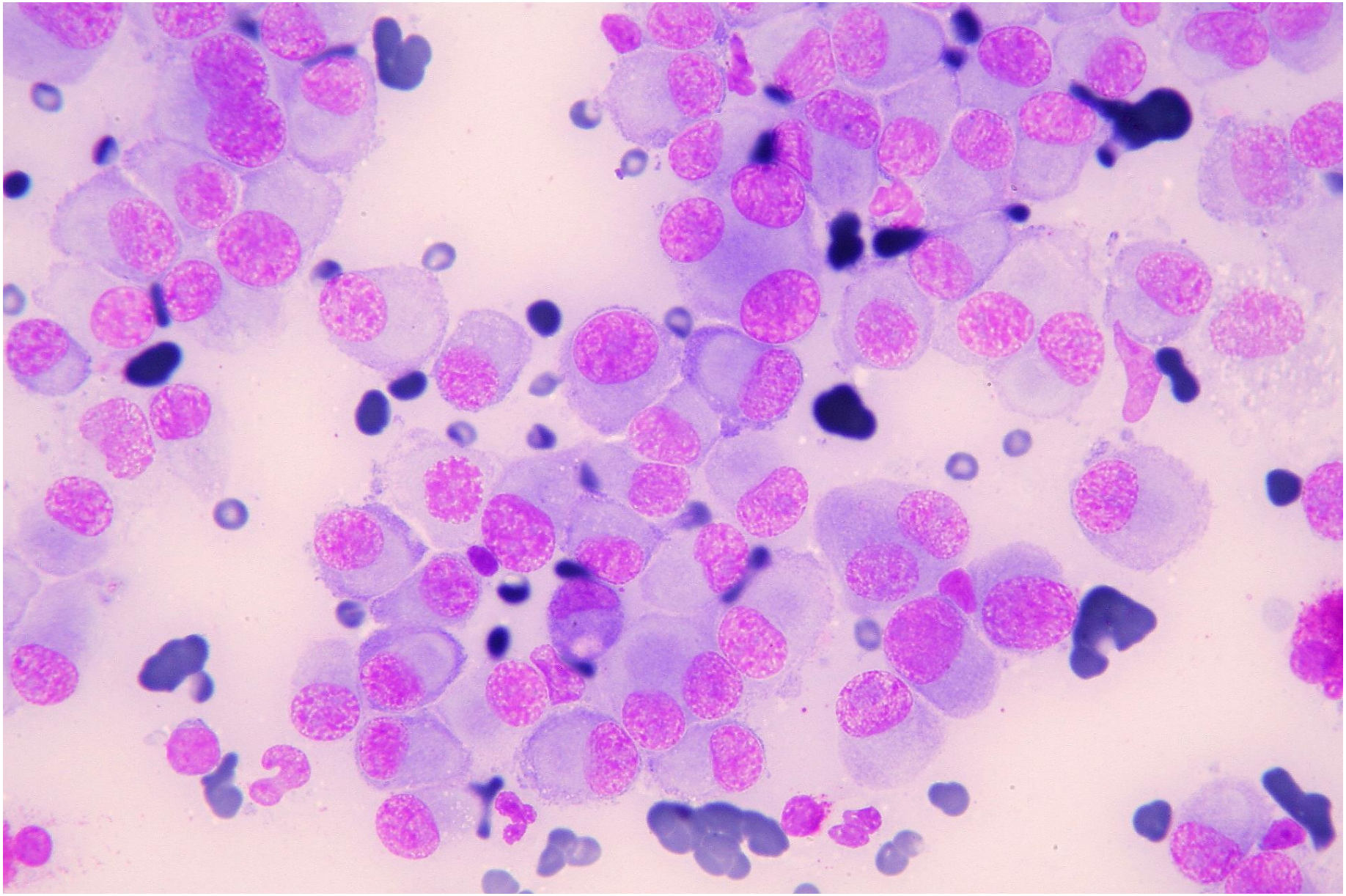
- Cytological diagnosis: epithelial neoplasm with neuroendocrine features
- Histological diagnosis: neuroendocrine carcinoma
- IHC:
  - Insulin ++

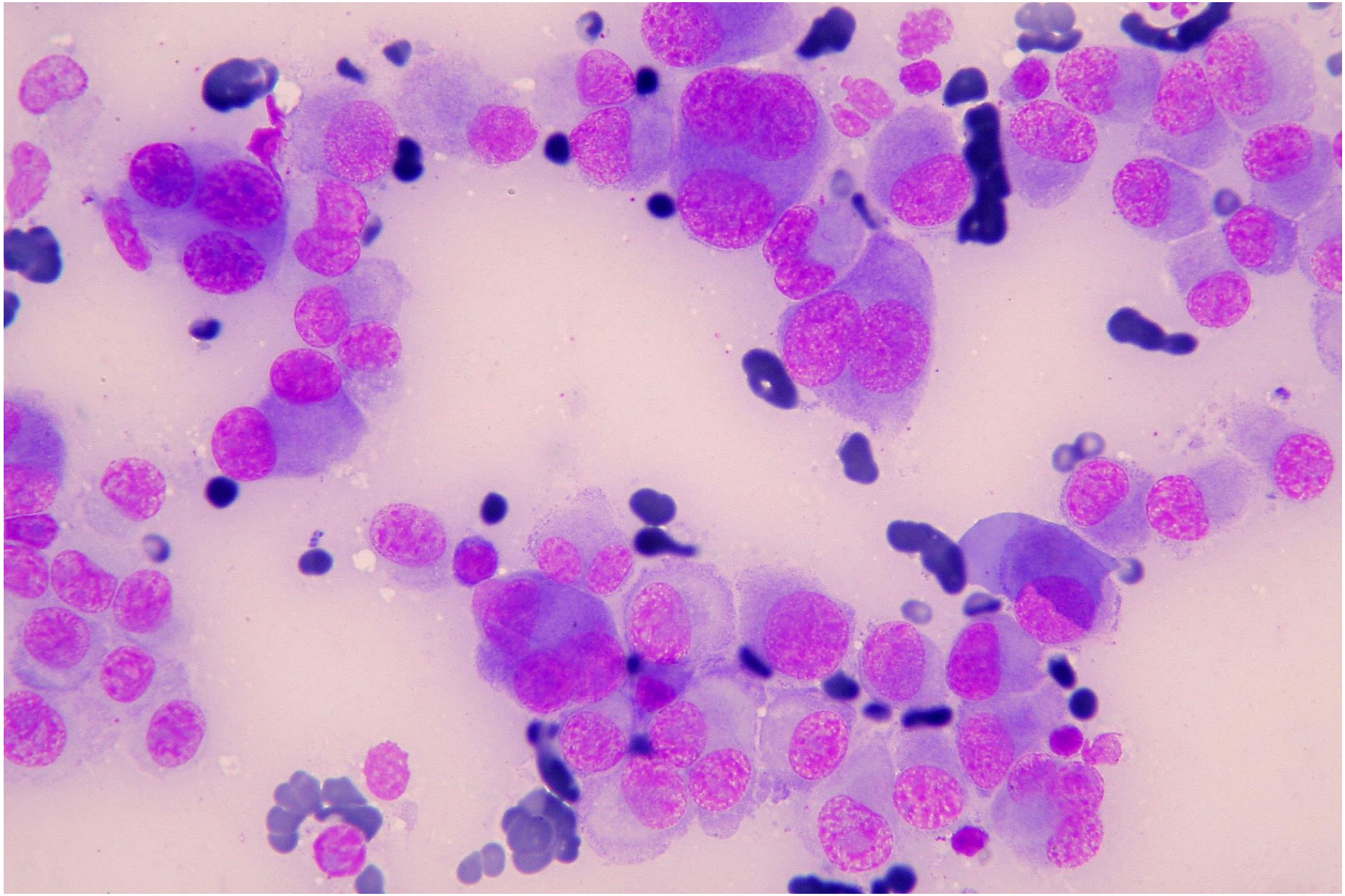


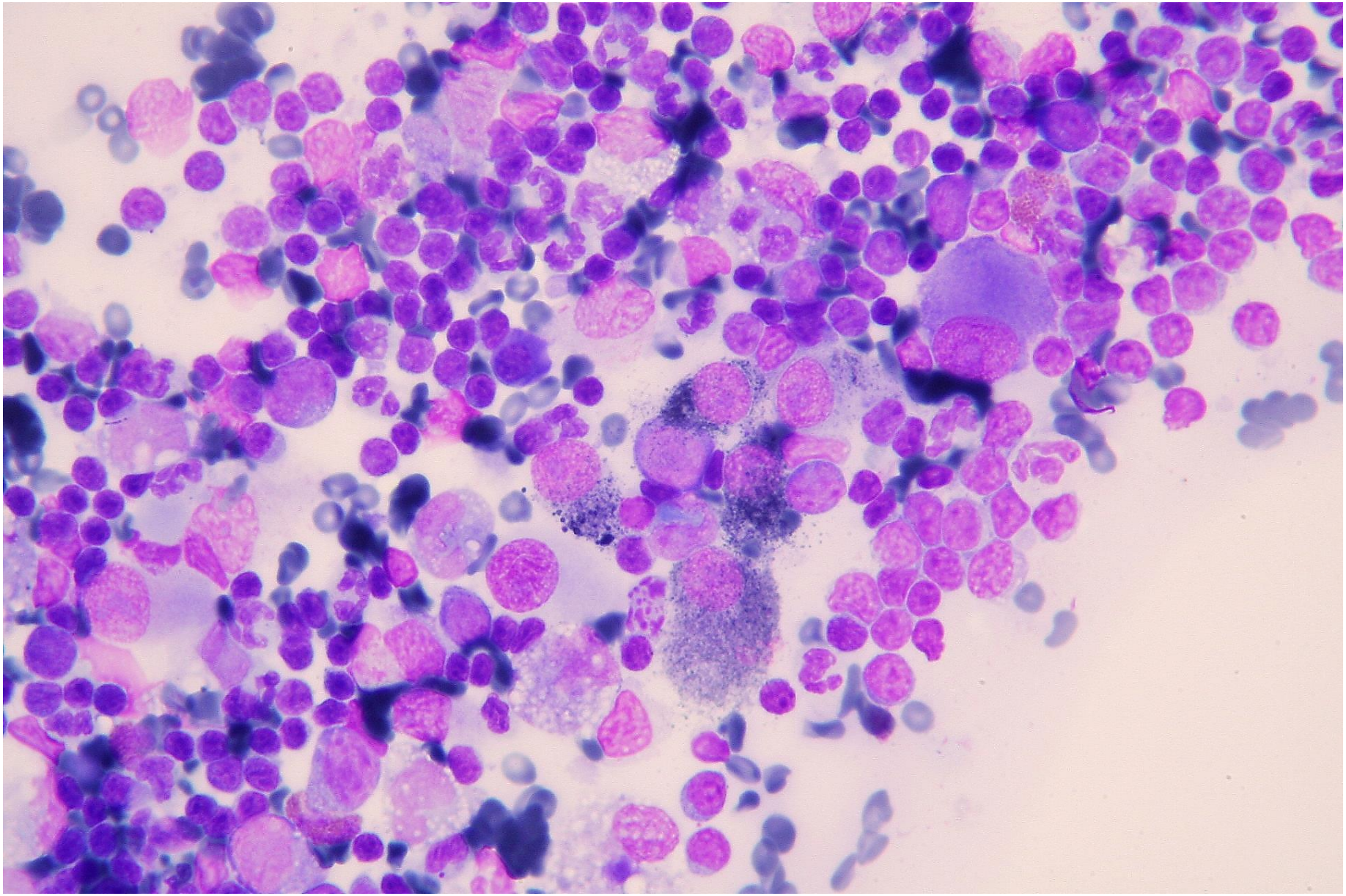
Case #6

- Cat, DSH, 12-year-old, female, neutered
- Cutaneous nodule on the foreleg
- Enlarged prescapular lymph node

- Sample: FNCS
- Stain: MGG







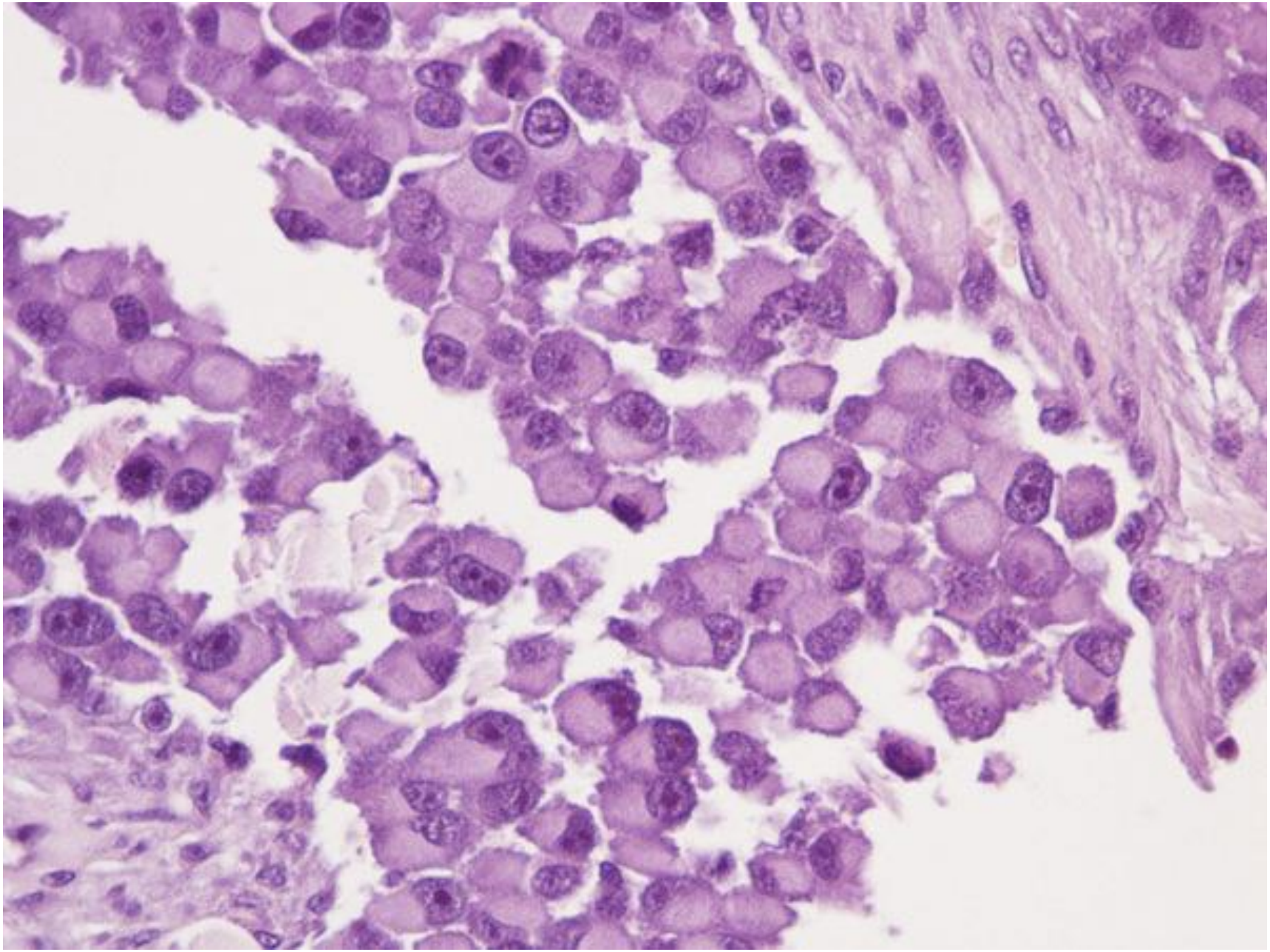
Cytologic findings

- Discrete, round to ovoid cells
  - Some short tails
  - Slightly blue cytoplasm
  - Achromatic, perinuclear area
  - Presence of melanin (slide from lymph node)
- Eccentric nucleus
  - Anisokaryosis
  - Multiple nuclei
  - Clumped chromatin
  - Mitoses

Diagnosis

- Cytological diagnosis: signet-ring cells melanoma; lymph node metastasis of melanoma

- Histological diagnosis: signet-ring cells melanoma
- IHC:
  - Vimentin:+
  - Melan A:+/-
  - PNL2:+/-



Discussion

Cutaneous Malignant Melanomas in 57 Cats: Identification of (Amelanotic) Signet-ring and Balloon Cell Types and Verification of Their Origin by Immunohistochemistry, Electron Microscopy, and In Situ Hybridization

J. S. VAN DER LINDE-SIPMAN, M. M. L. DE WIT, E. VAN GARDEREN, R. F. MOLENBEEK, D. VAN DER VELDE-ZIMMERMANN, AND R. A. DE WEGER

Vet Pathol 34:31–38 (1997)

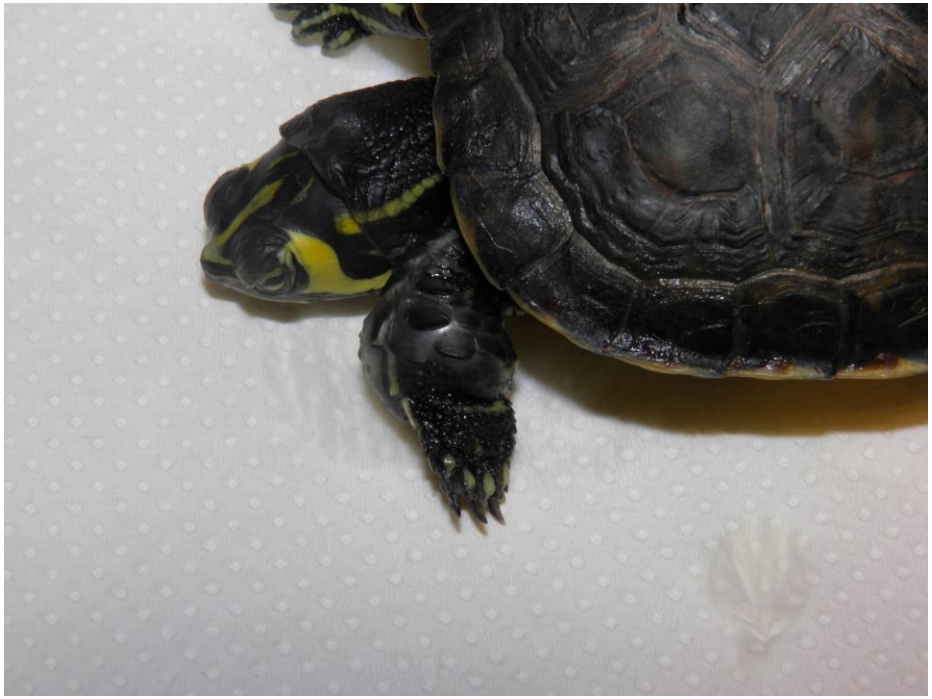
- Signet ring cells melanoma
  - Very rare
  - Only described in cat, dog, human and hamster
- Electron microscopy
  - Abundant amount of cytoplasm in which extensive paranuclear accumulation of intermediate filaments were present.
  - Remaining organelles were concentrated at the edge of the cell or around the nucleus

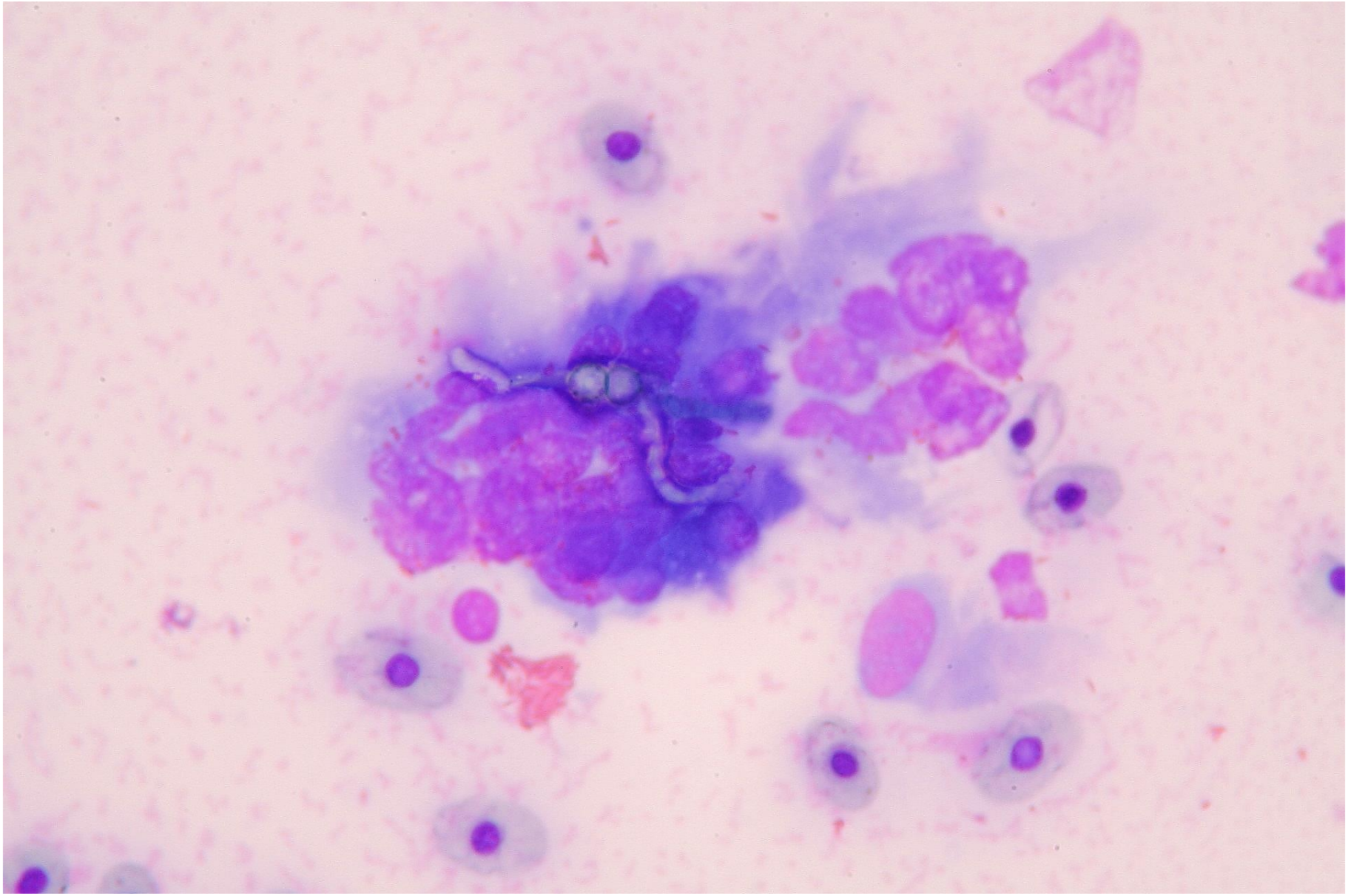
Case #7

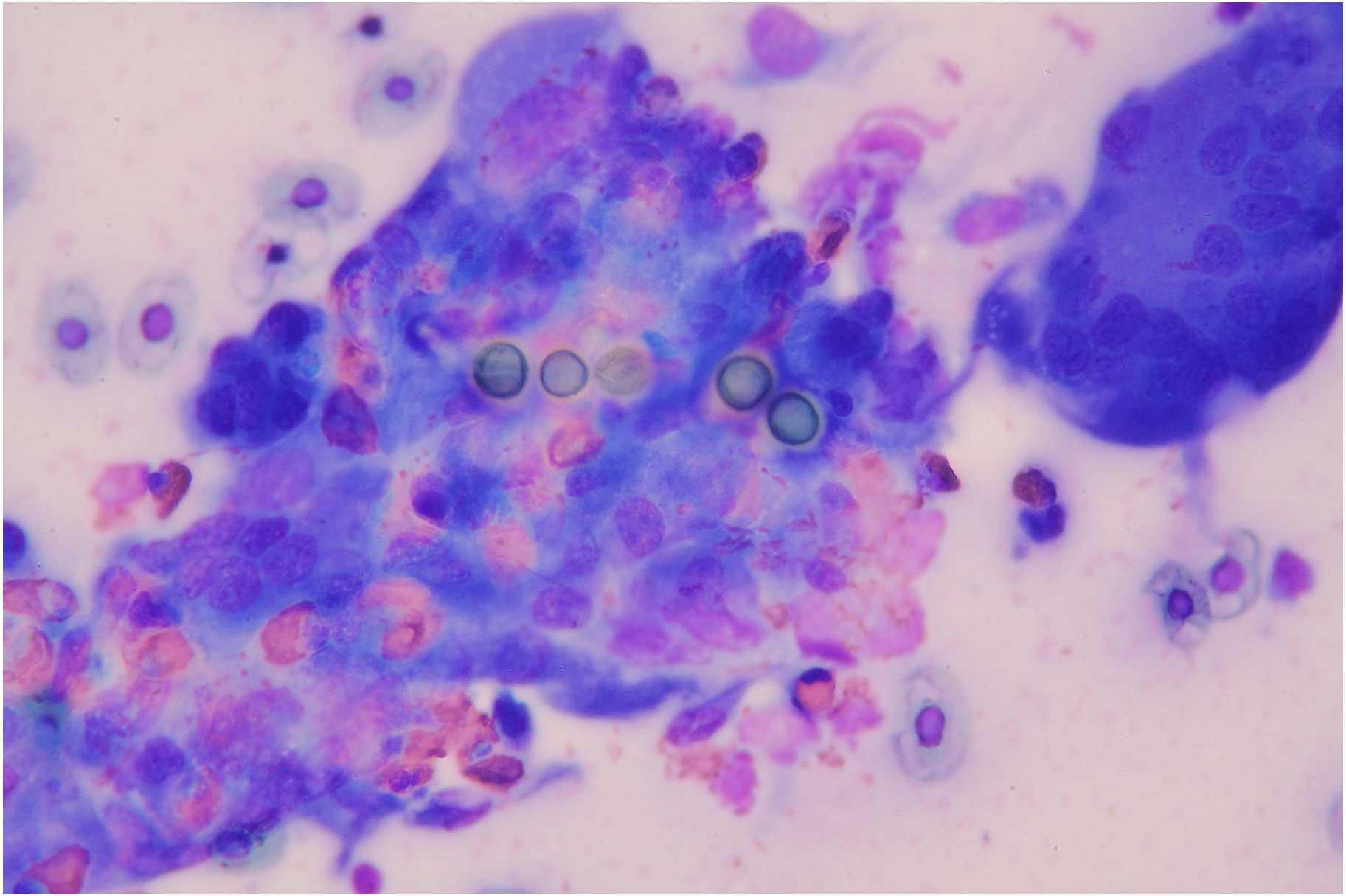
- Turtle, (Trachemys scripta), 10-year-old, female
- Swelling and crusting lesions on the legs

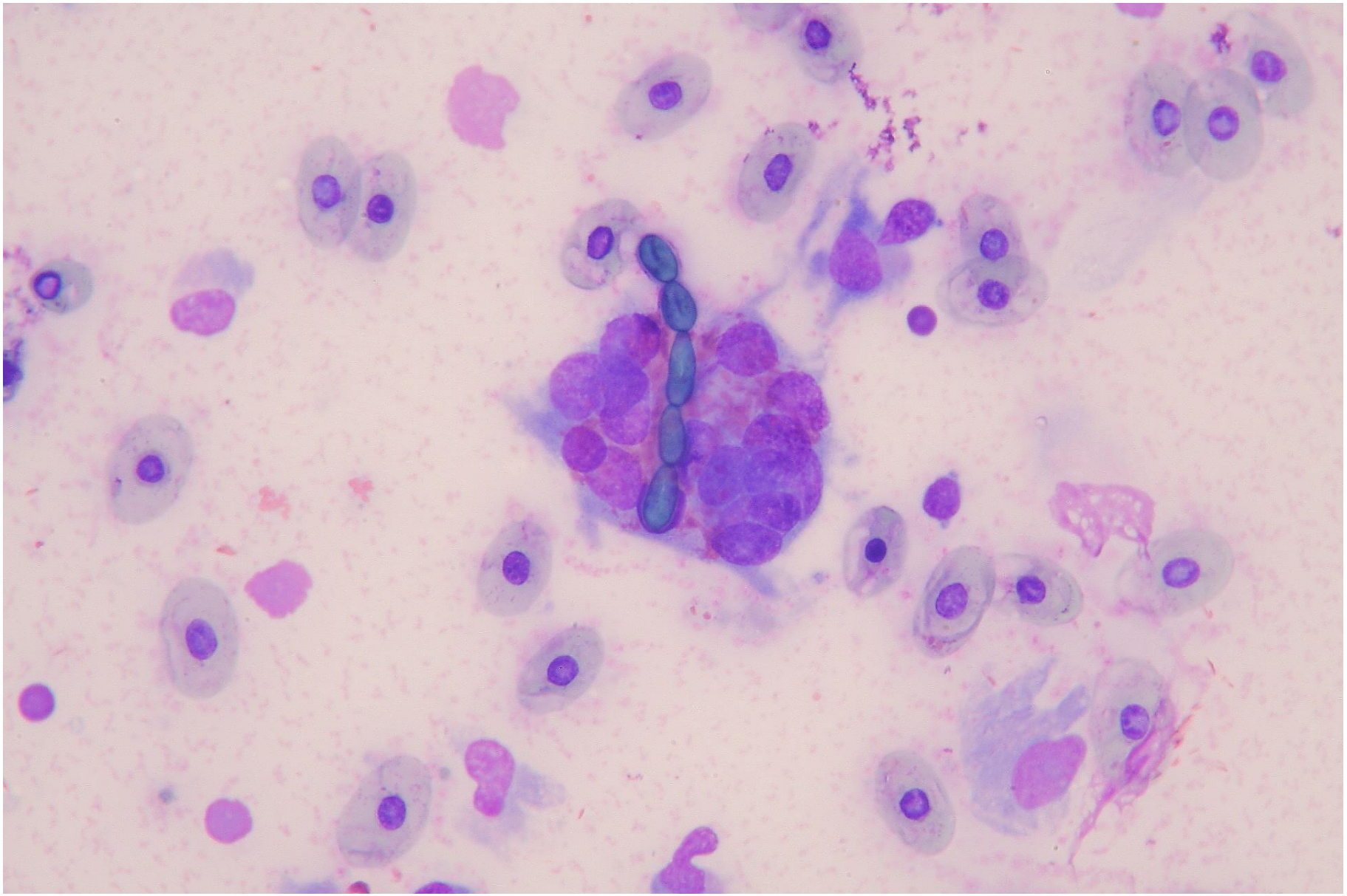
- Sample: FNCS
- Stain: MGG

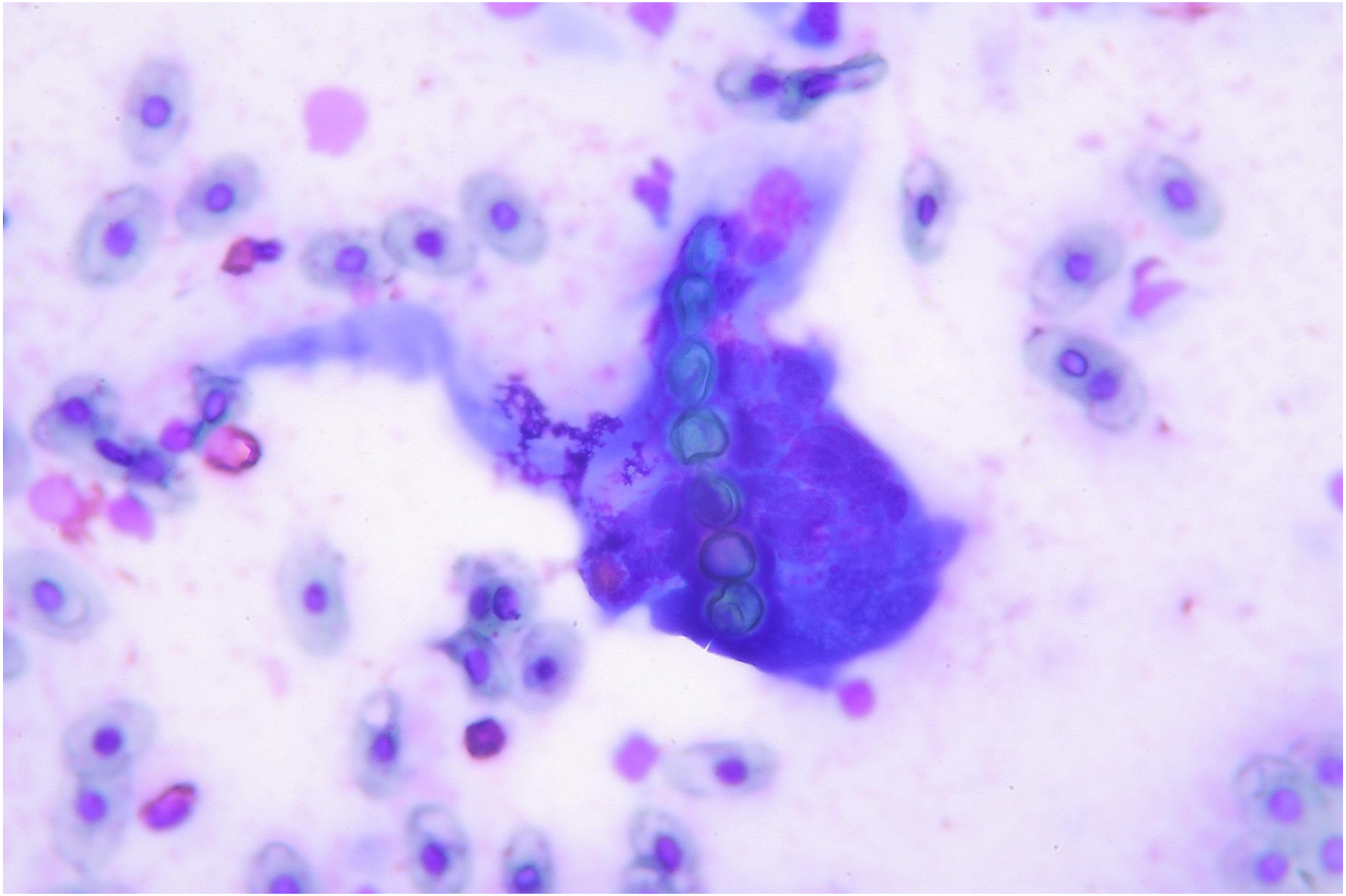


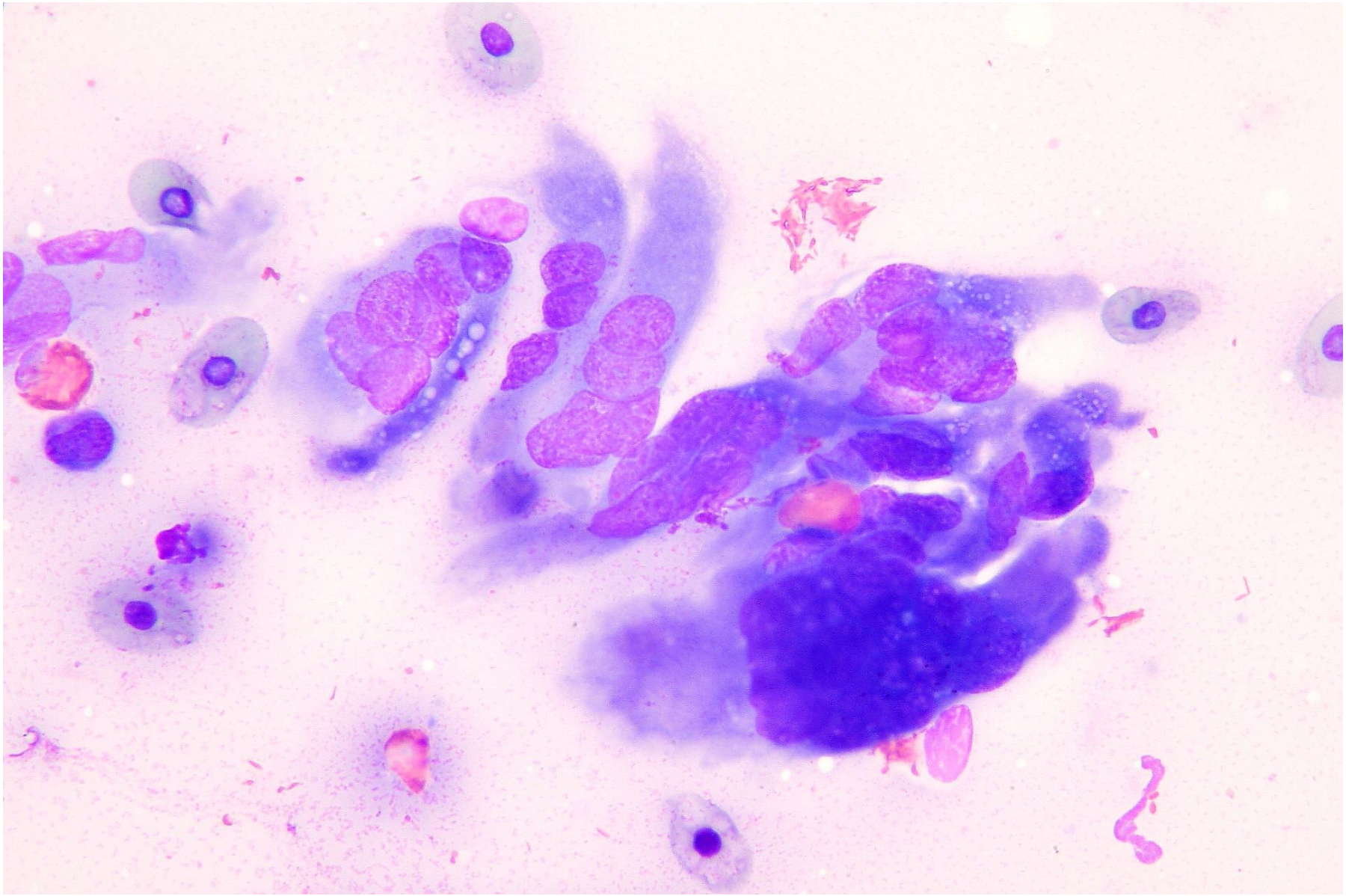


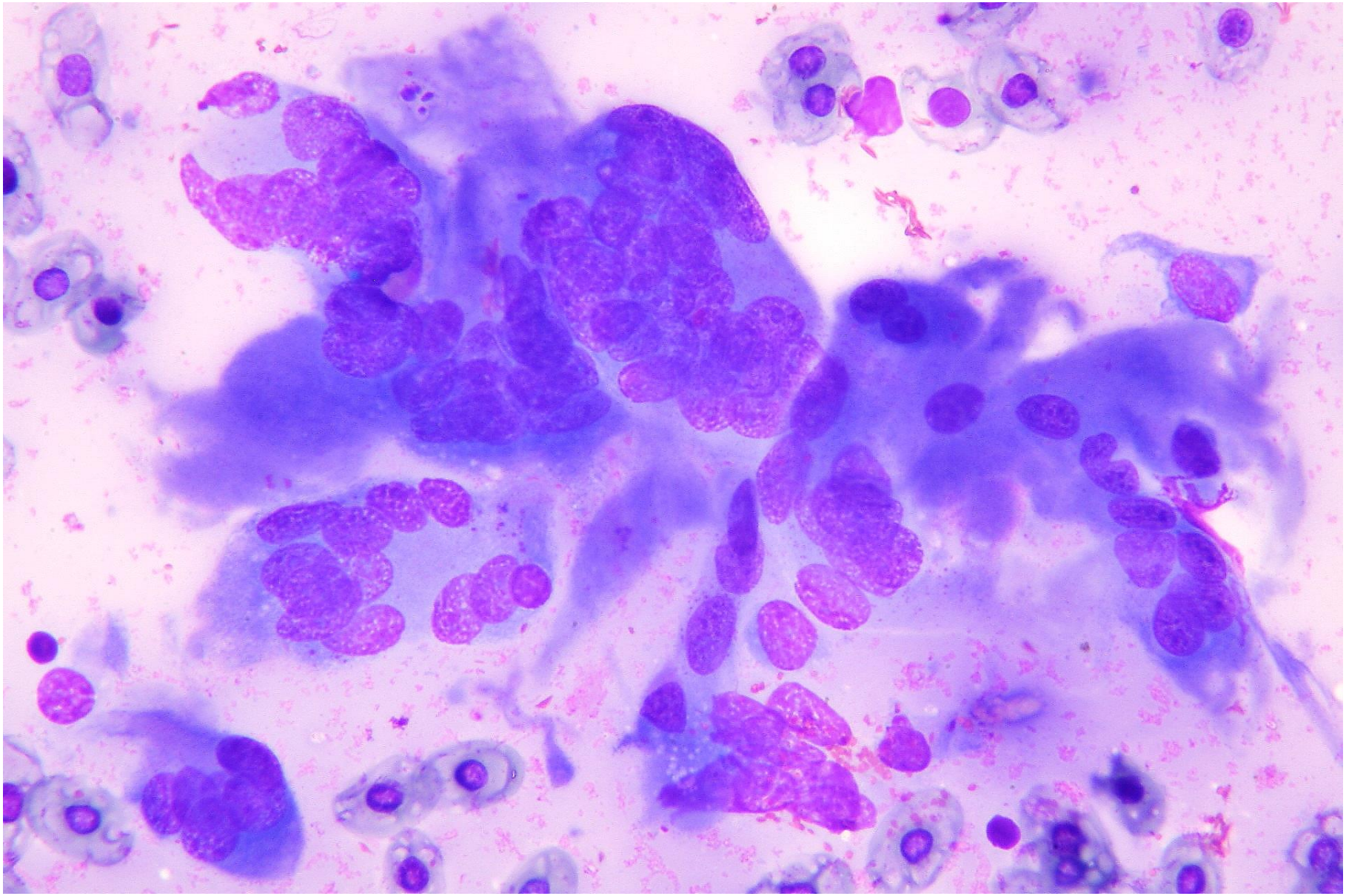













Cytological findings

- Epithelioid aggregates of macrophages
  - Multinucleated giant cells
  - Spindle/stellate shape of the cytoplasm
- Heterophils
- Concentration of inflammatory cells around a fungal hyphae (pseudohyphae?)

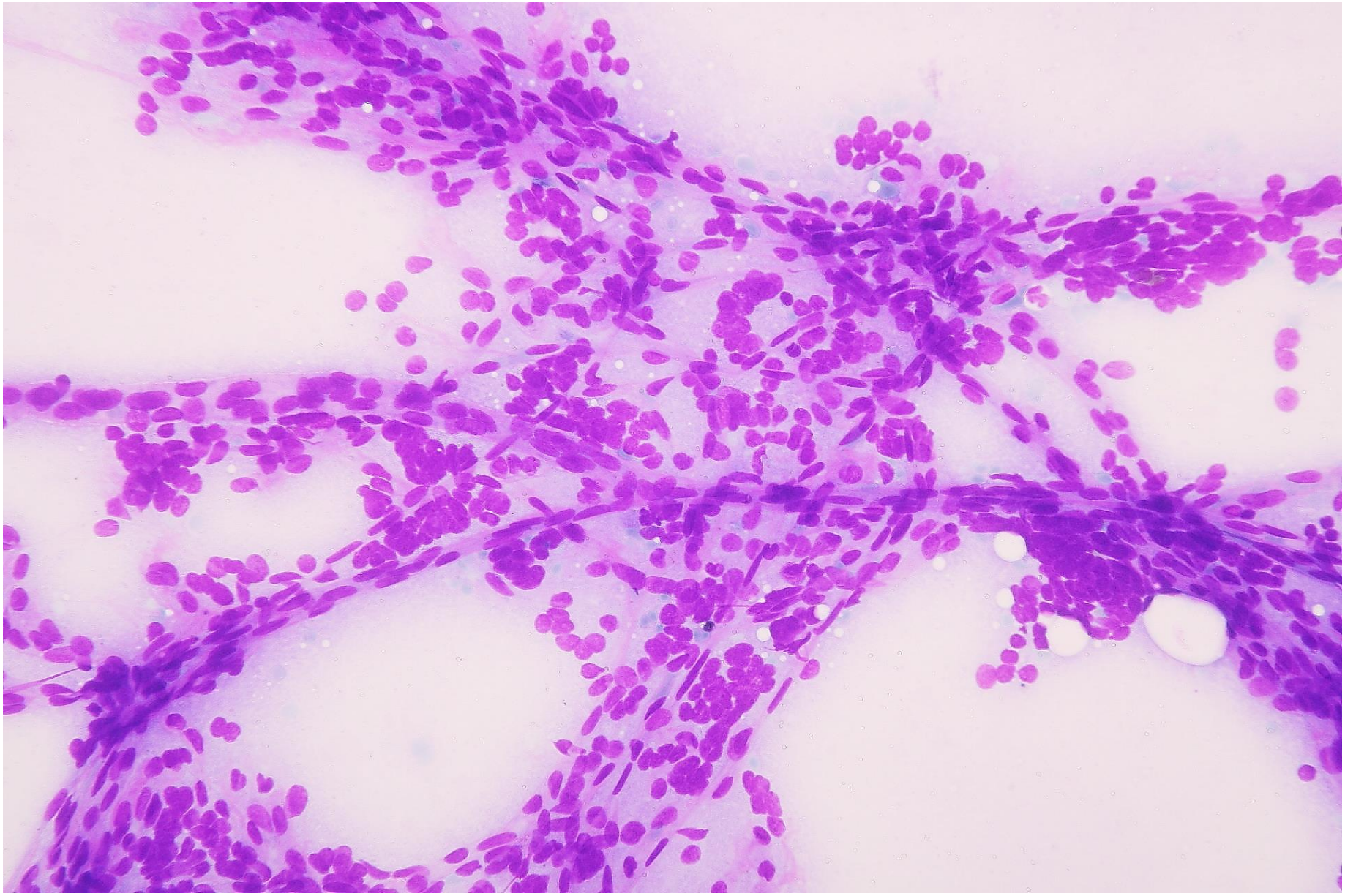
Diagnosis

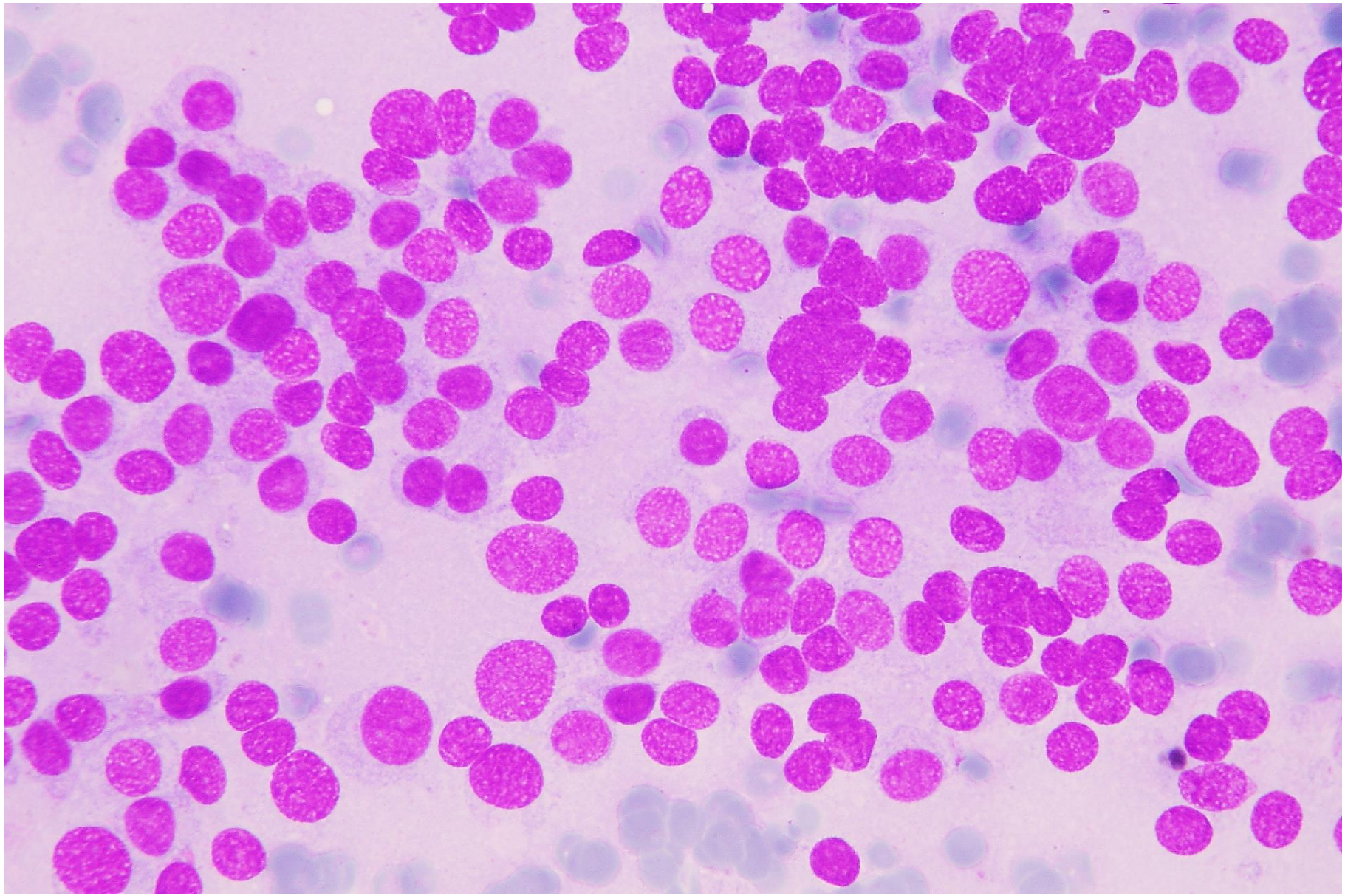
- Cytological diagnosis: fungal granulomatous inflammation
- Microbiology: no growth

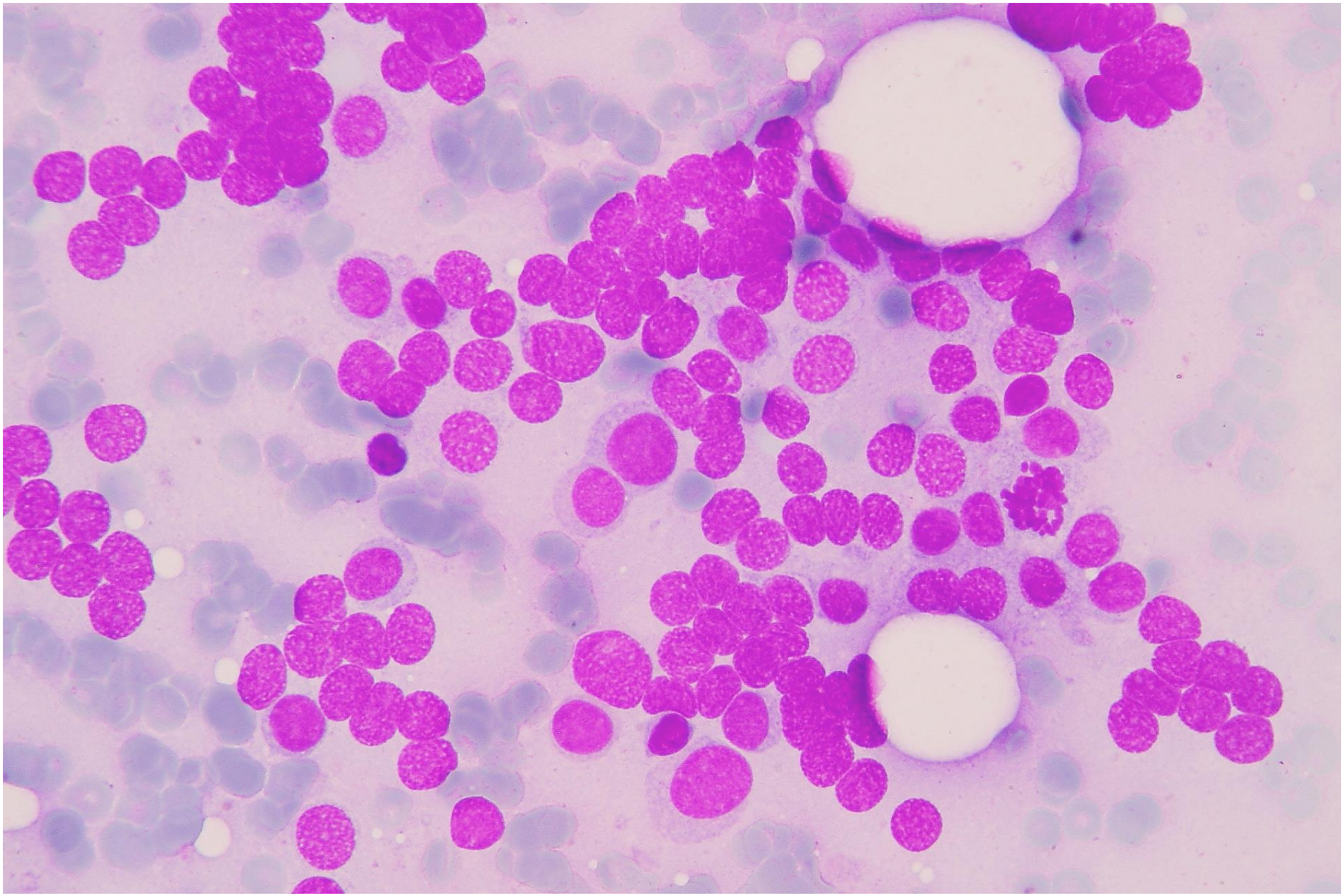
Case #8

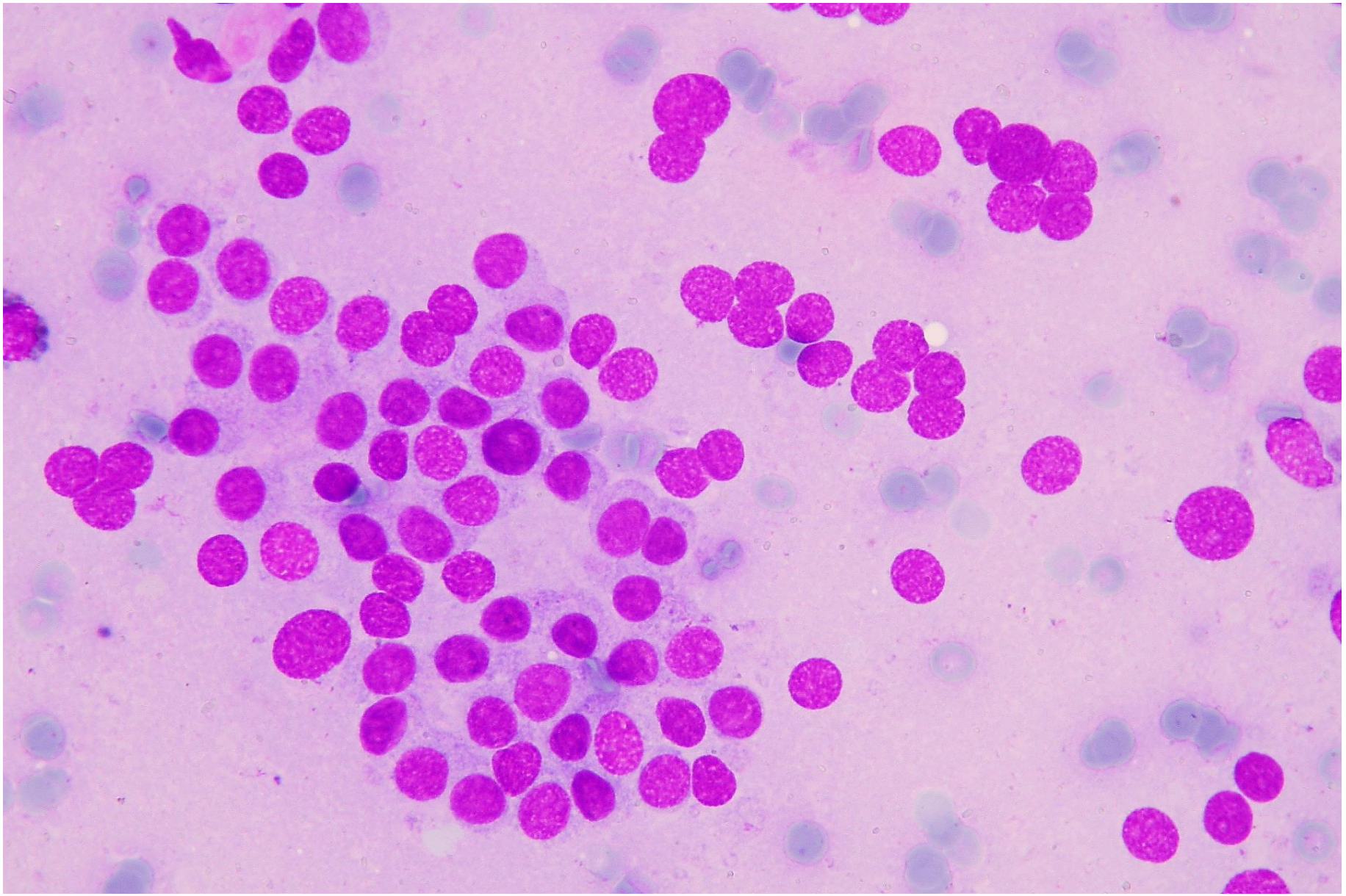
- Dog, Shih-tzu, 10-year-old, female, neutered
- Adrenal mass.

- Sample: US-guided FNCS
- Stain: MGG









Cytologic findings

- Epithelial cells
  - Round, frequently indistinct cytoplasm
  - Round nucleus,
  - Finely granular or compact chromatin
- Small aggregates or dispersed singularly
  - Perivascular arrangements
- Many naked nuclei scattered on the background

Diagnosis

- Cytological diagnosis: epithelial neoplasm with neuroendocrine features, morphologically indicative of pheocromocytoma

- Histological diagnosis: pheocromocytoma

ORIGINAL RESEARCH

Accuracy of cytology in distinguishing adrenocortical tumors from pheochromocytoma in companion animals

Walter Bertazzolo[1], Martine Didier[1], Maria Elena Gelain[2], Silvia Rossi[3], Luca Crippa[4], Giancarlo Avallone[5], Paola Roccabianca[5], Ugo Bonfanti[6], Luca Giori[5], Federico Fracassi[7]

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Table 1. Cytologic key features of adrenocortical tumors and pheochromocytoma in dogs and cats (after ref. 9).

	Adrenocortical Tumors	Pheochromocytomas
General architecture	Many intact cells, singly or in cohesive clusters, with distinct cellular borders (Figure 1)	Many uniform naked nuclei, often on a finely granular and basophilic background, often in rows or rosette (Figure 2) Rare intact round and plasmacytoid cells
	Perivascular pattern possible (Figure 3)	Perivascular pattern possible (Figure 4)
Intact cells	Common (Figure 5A, B)	Rare (Figure 6)
Nuclear to cytoplasmic ratio	Low (Figure 5A, B)	High (Figure 6)
Cytoplasm	Basophilic and markedly vacuolated (small to medium lipid vacuoles) (Figure 5A, B)	Pale blue, finely granular (Figure 6)
Nucleus	Round to oval, central to peripheral, with coarse/condensed chromatin (Figure 5A, B)	Round to oval, with fine chromatin (Figure 6)
Nucleoli	Indistinct to prominent (Figure 5A, B)	Indistinct (Figure 6)
Other features	Hematopoietic precursors cells (extramedullary hematopoiesis) (Figure 7)	

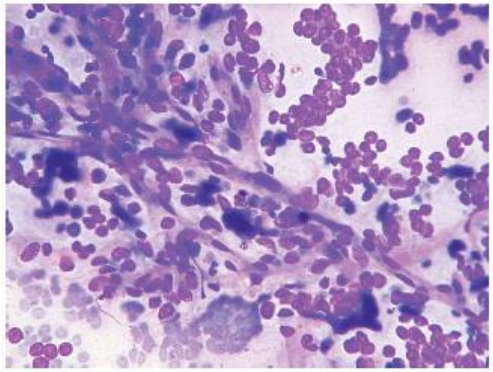
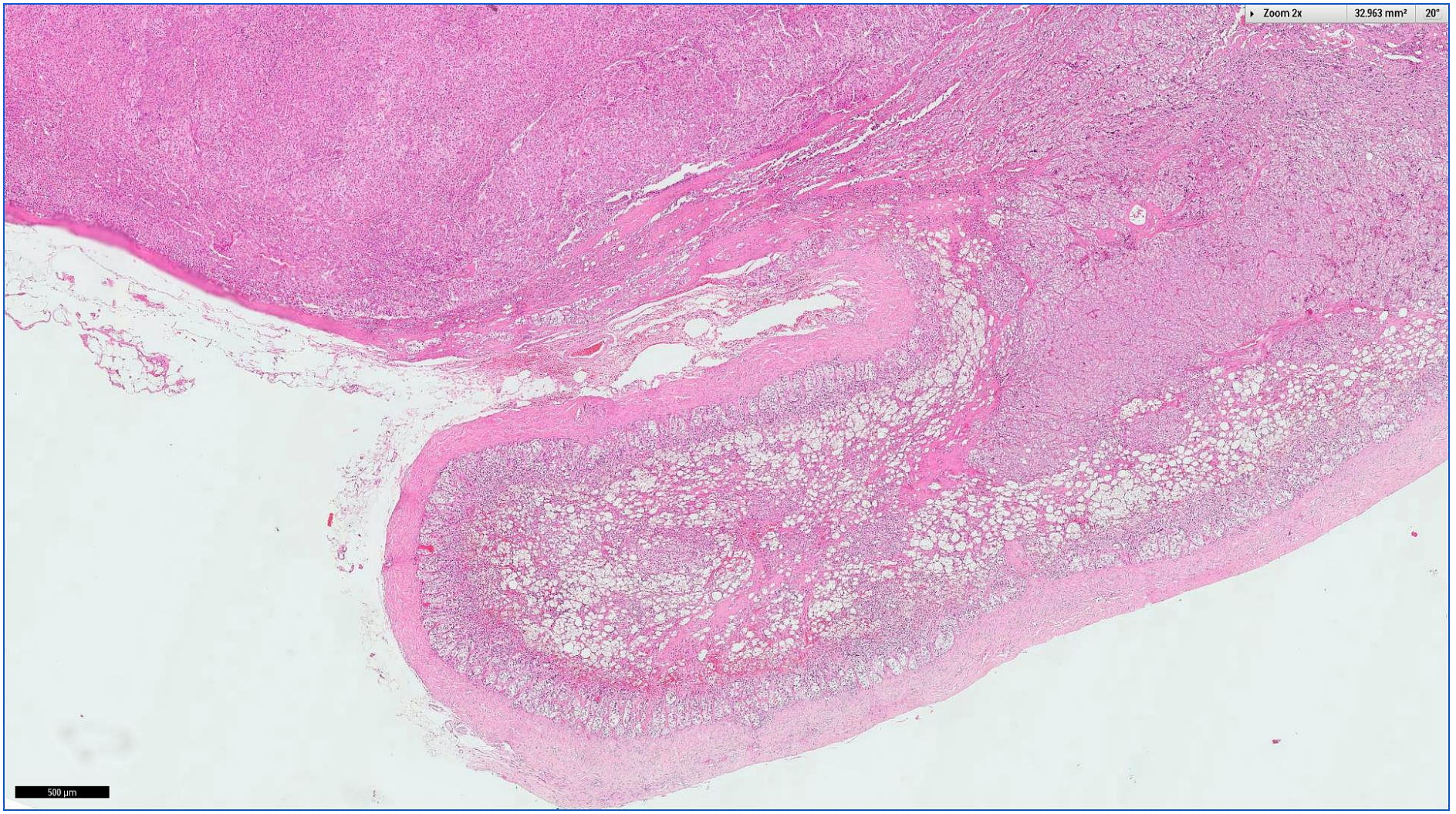


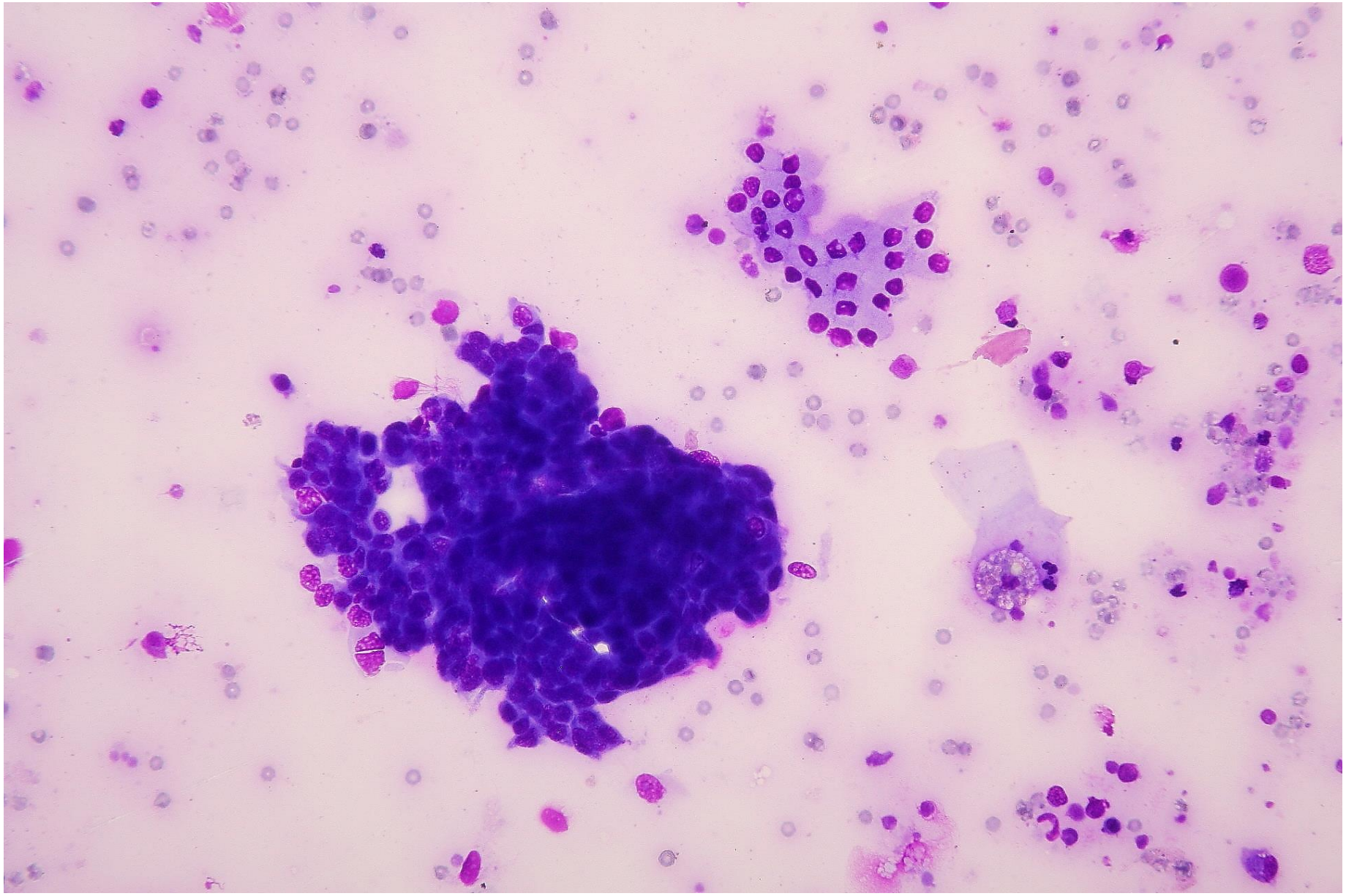
Figure 4. A group of medullary cells from a canine pheochromocytoma showing a perivascular arrangement. May–Grünwald–Giemsa. ×40 objective.

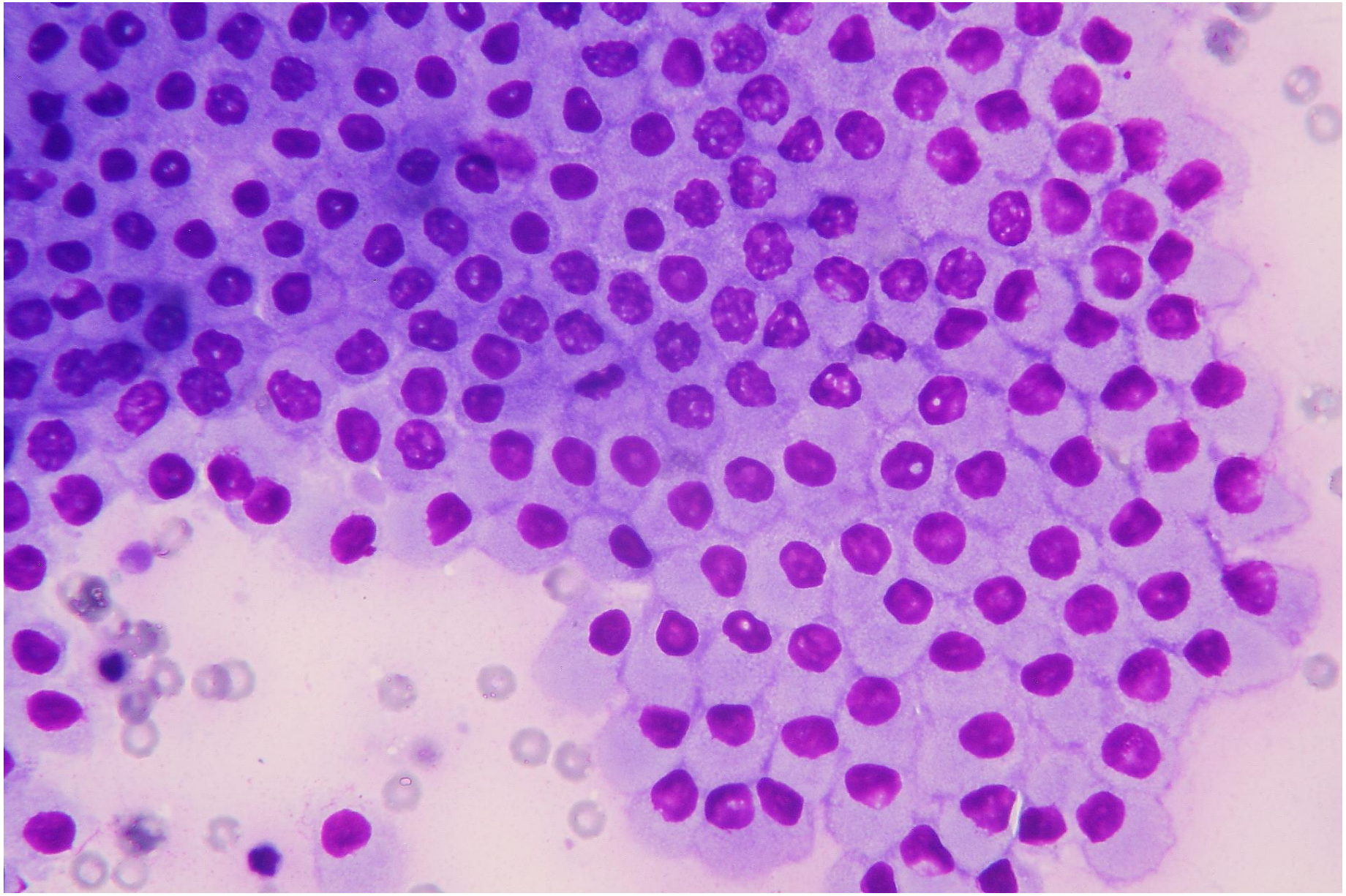

Zoom 2x
32.963 mm²
20°
500 µm

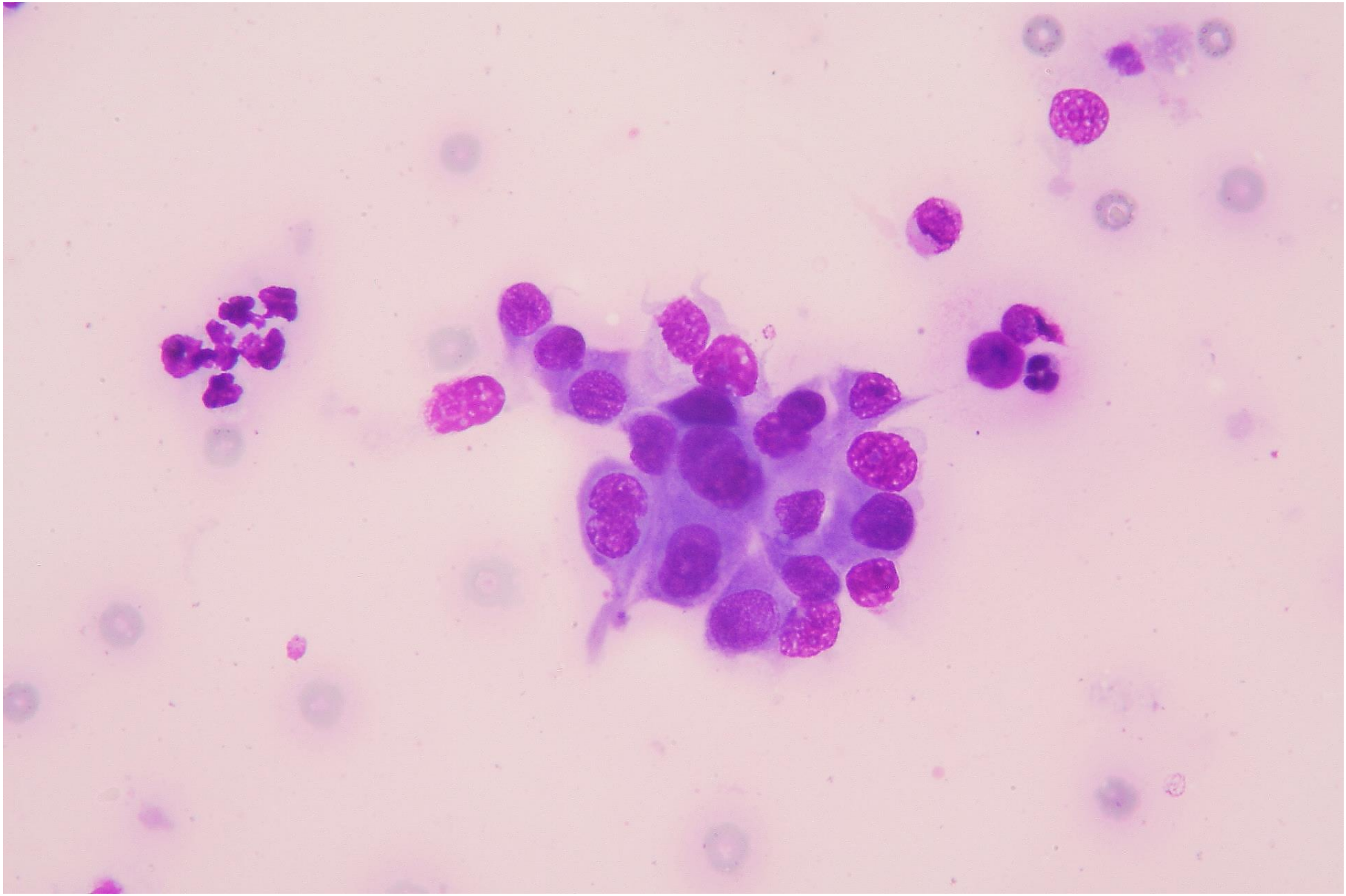
Case #9

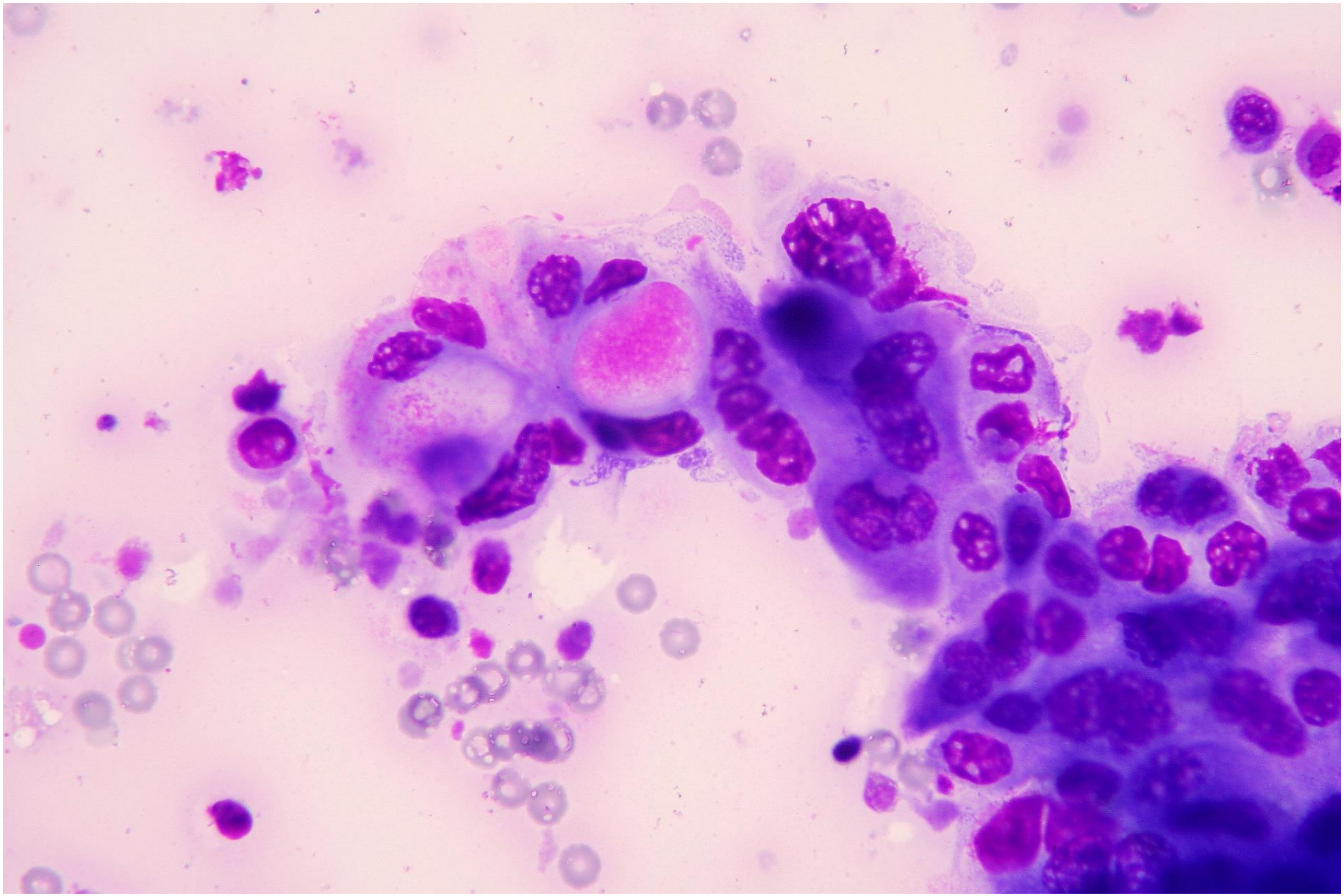
- Dog, mongrel, 7-year-old, male
- Hematuria
- Prostatic enlargement

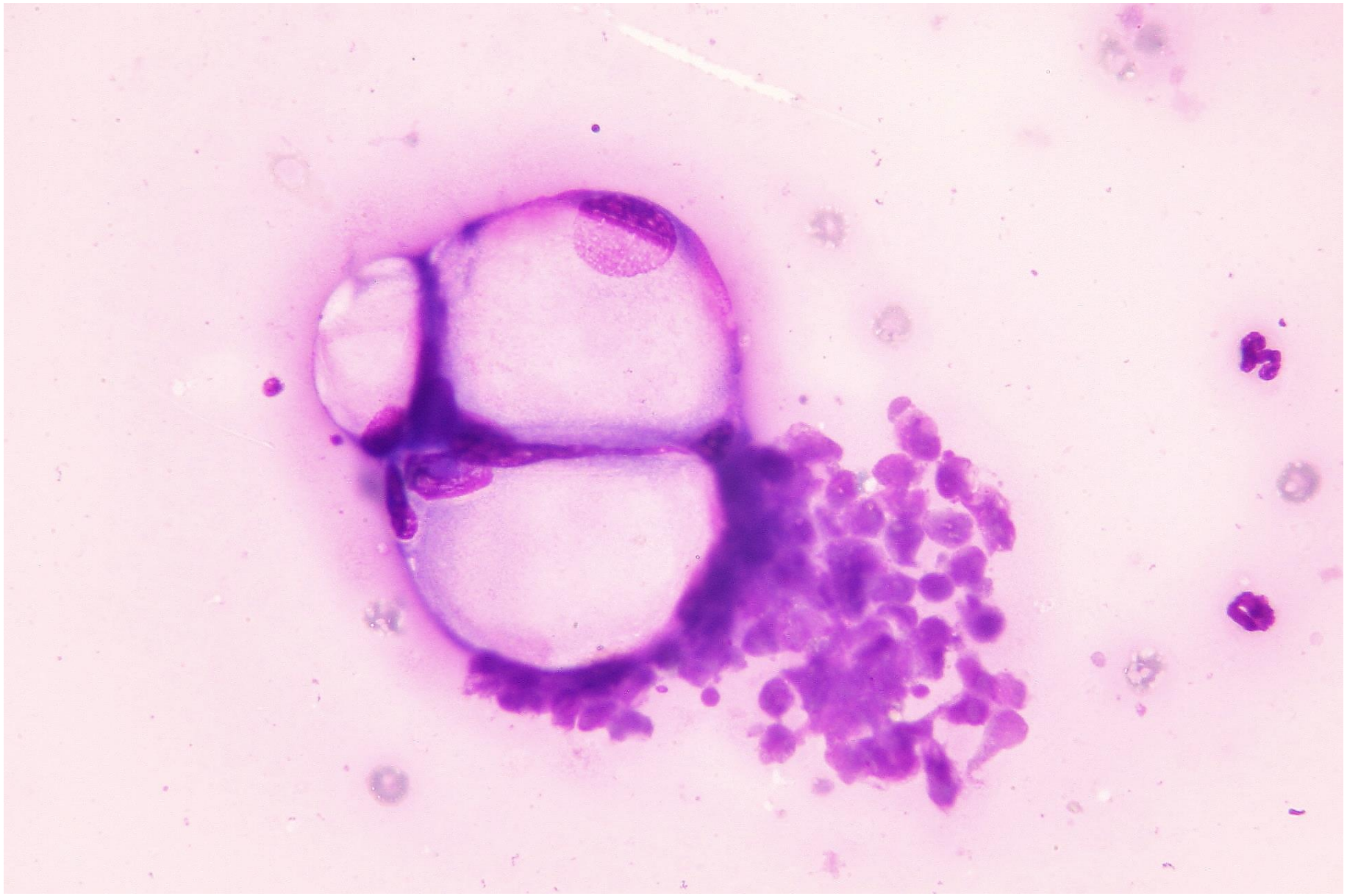
- Sample: US-guided FNCS
- Stain: MGG

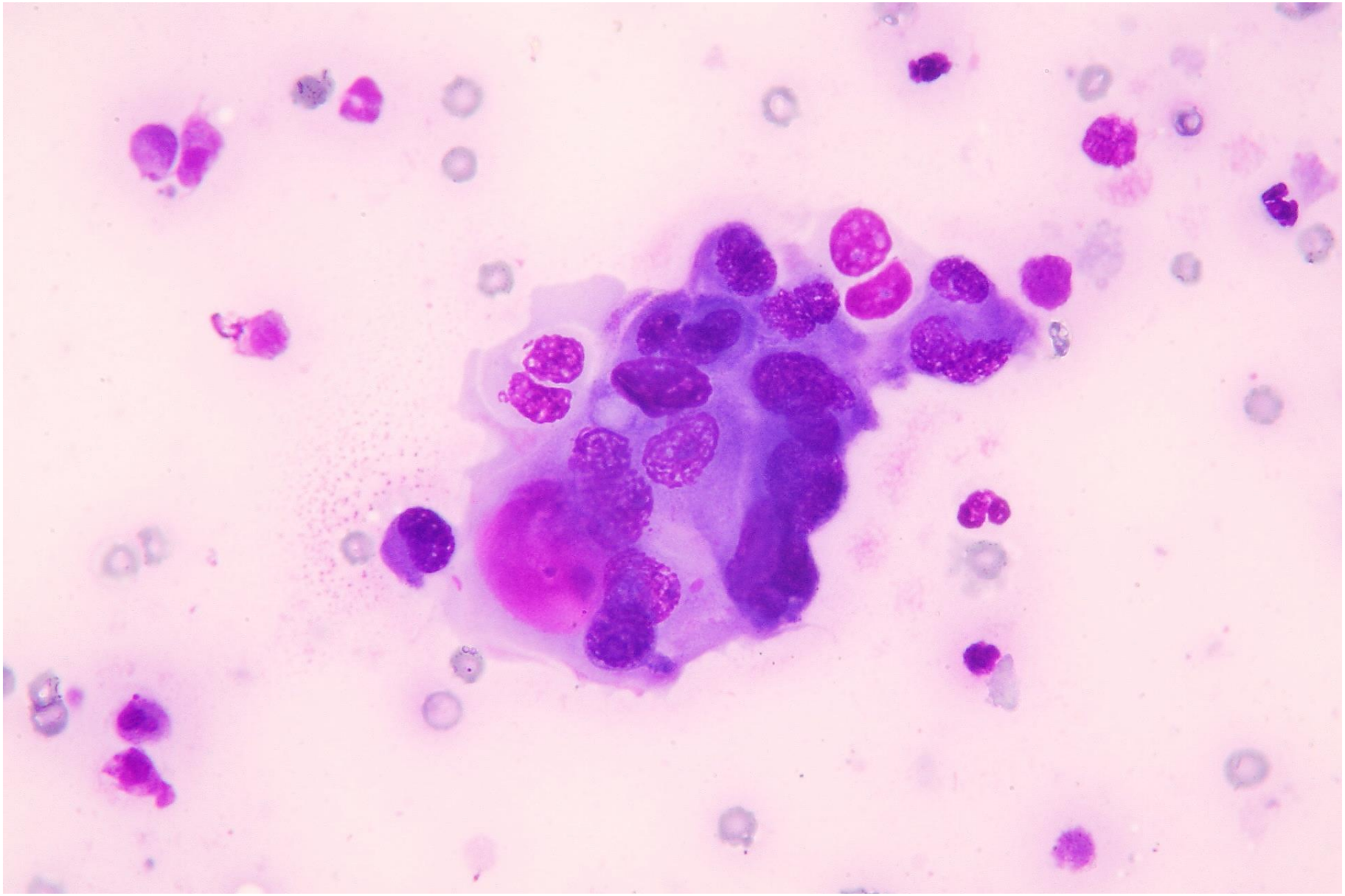










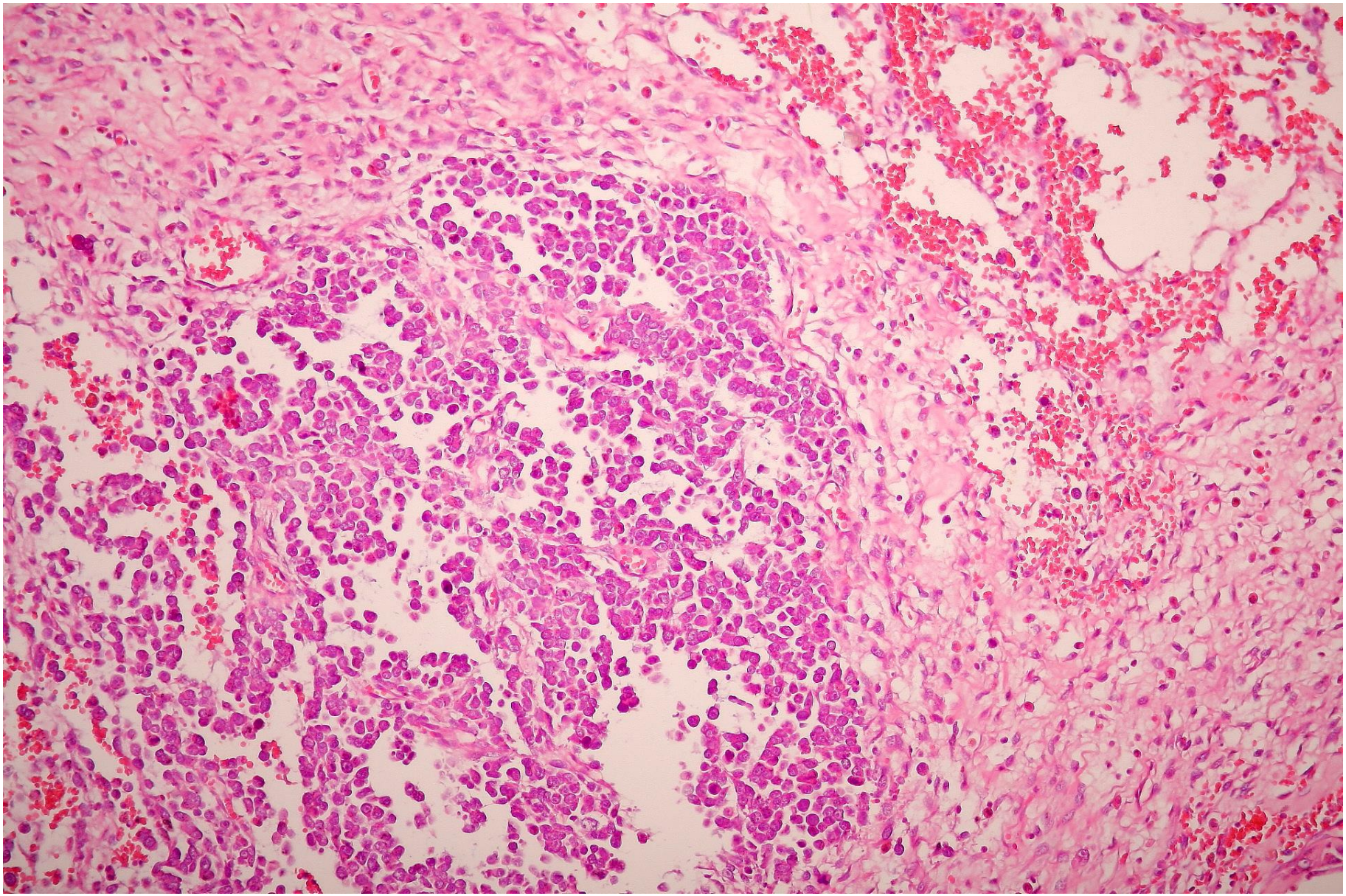


Cytologic findings

- Two distinct populations
  - Prostatic cells
  - Honeycomb aggregate
- Transitional cells
  - Round shape
  - Small amount of bluish cytoplasm
  - Round nucleus
  - Anisokaryosis
- Scattered inflammatory cells

Diagnosis

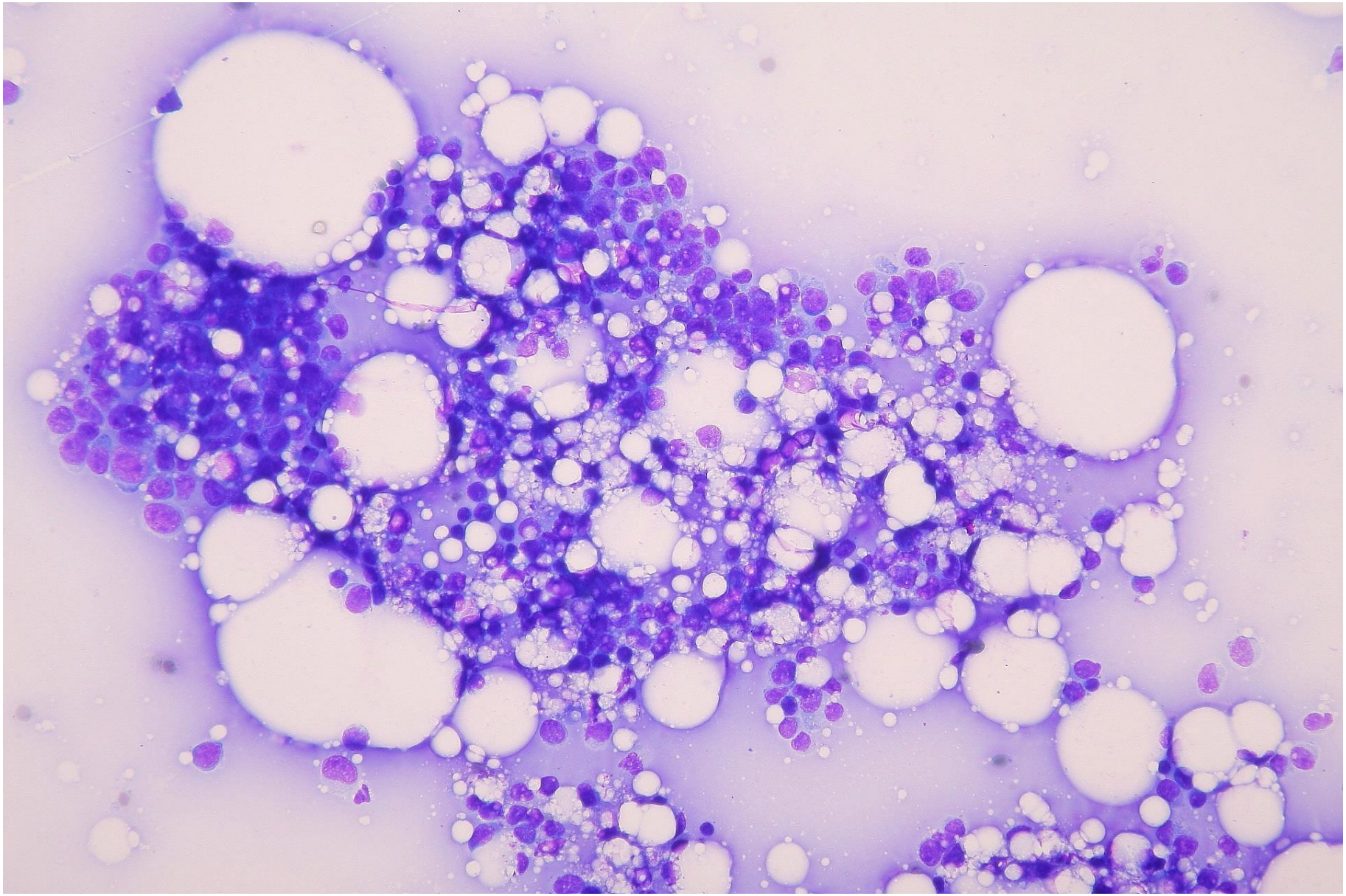
- Cytological diagnosis: transitional carcinoma of prostate
- Histological diagnosis: transitional carcinoma

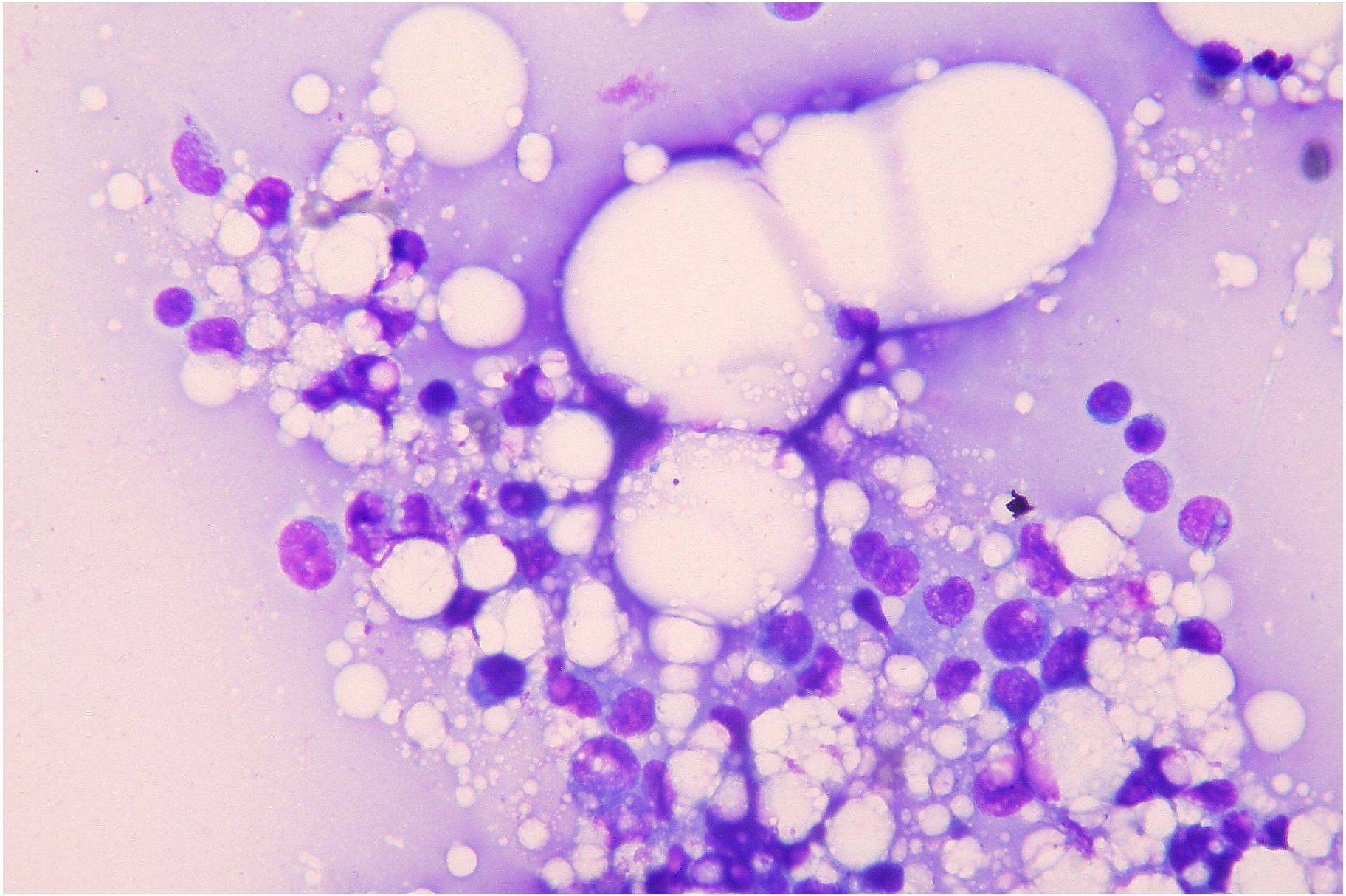


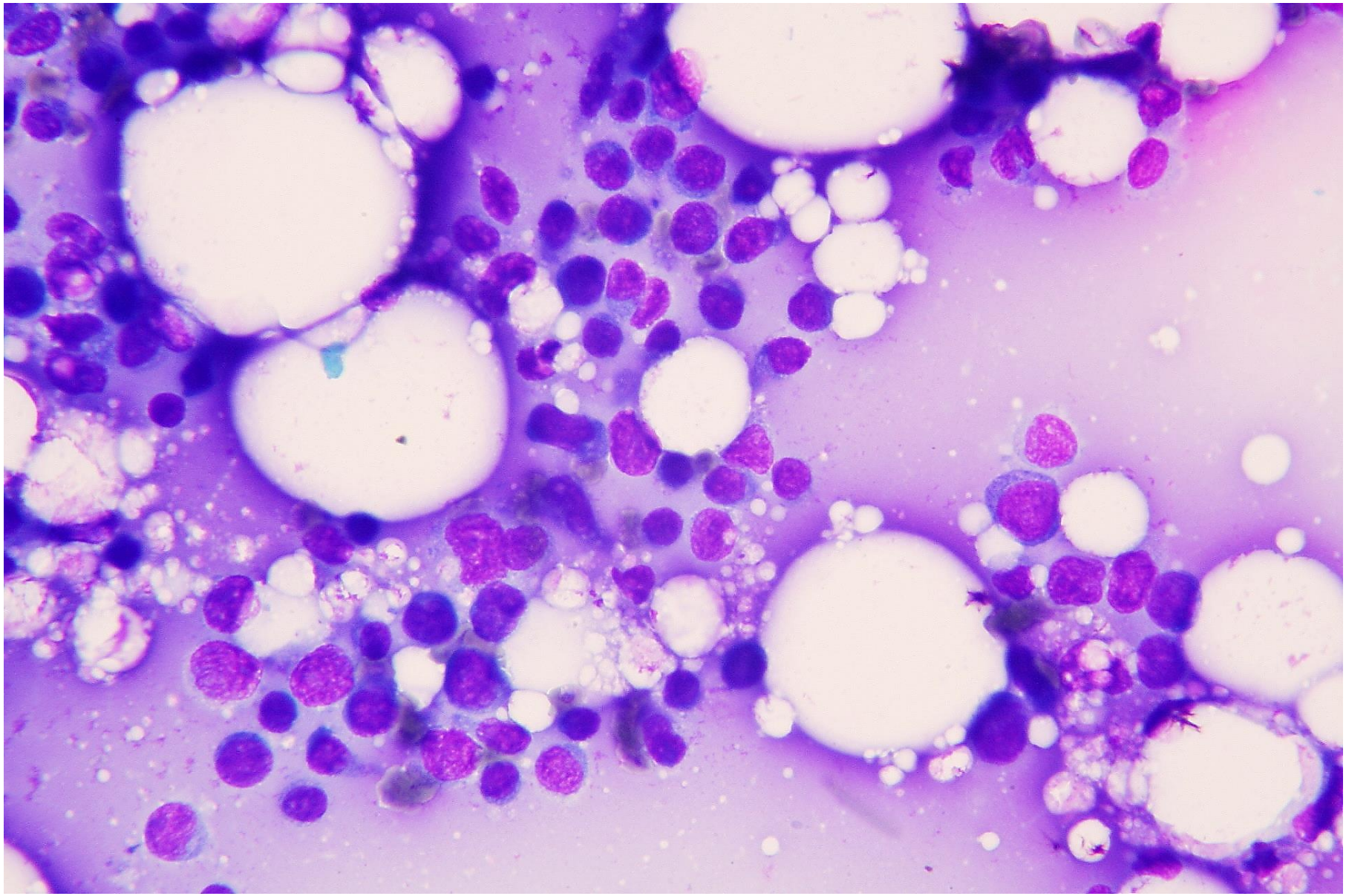
Case #10

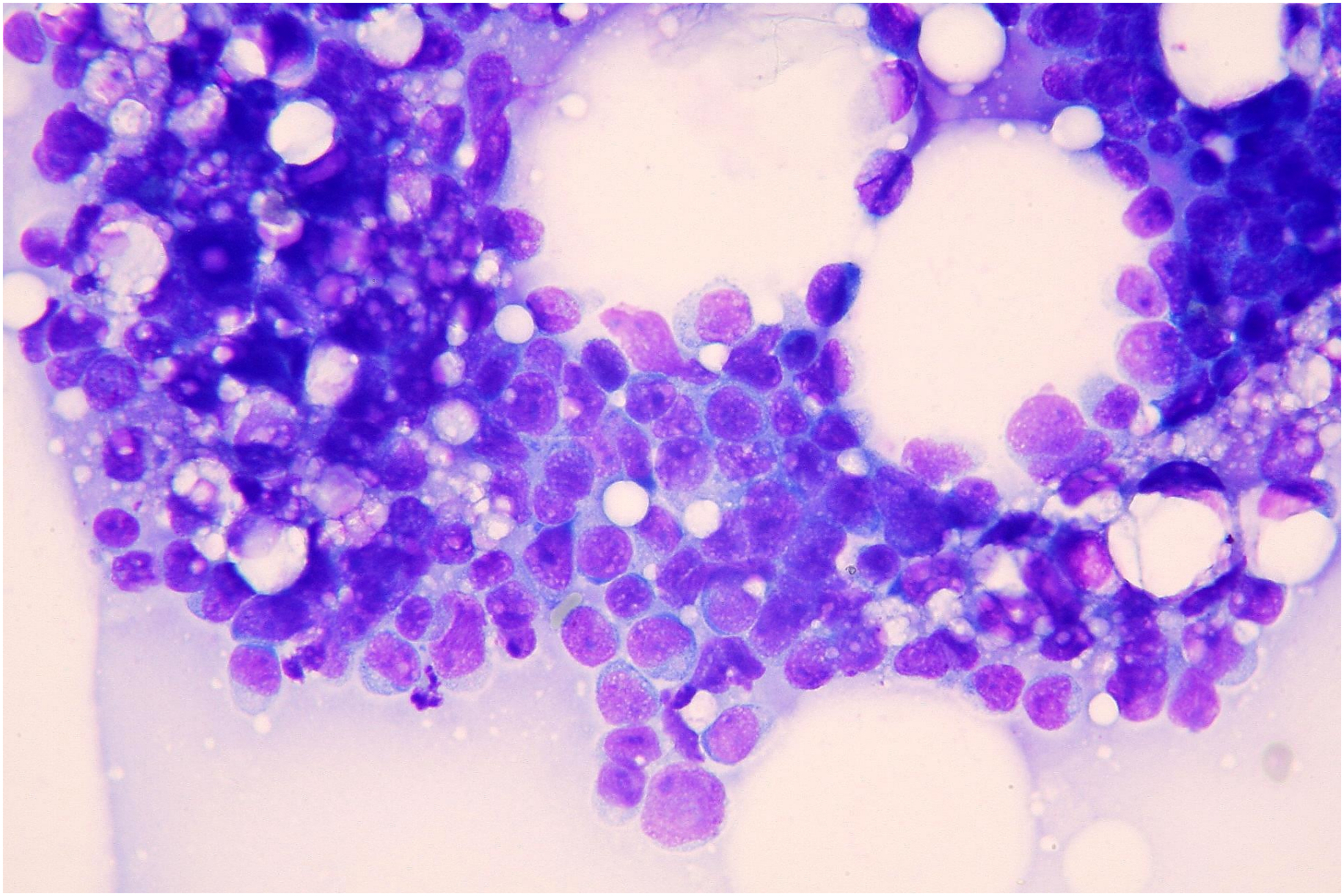
- Dog, mongrel, 12-year-old, male
- Subcutaneous mass on the shoulder

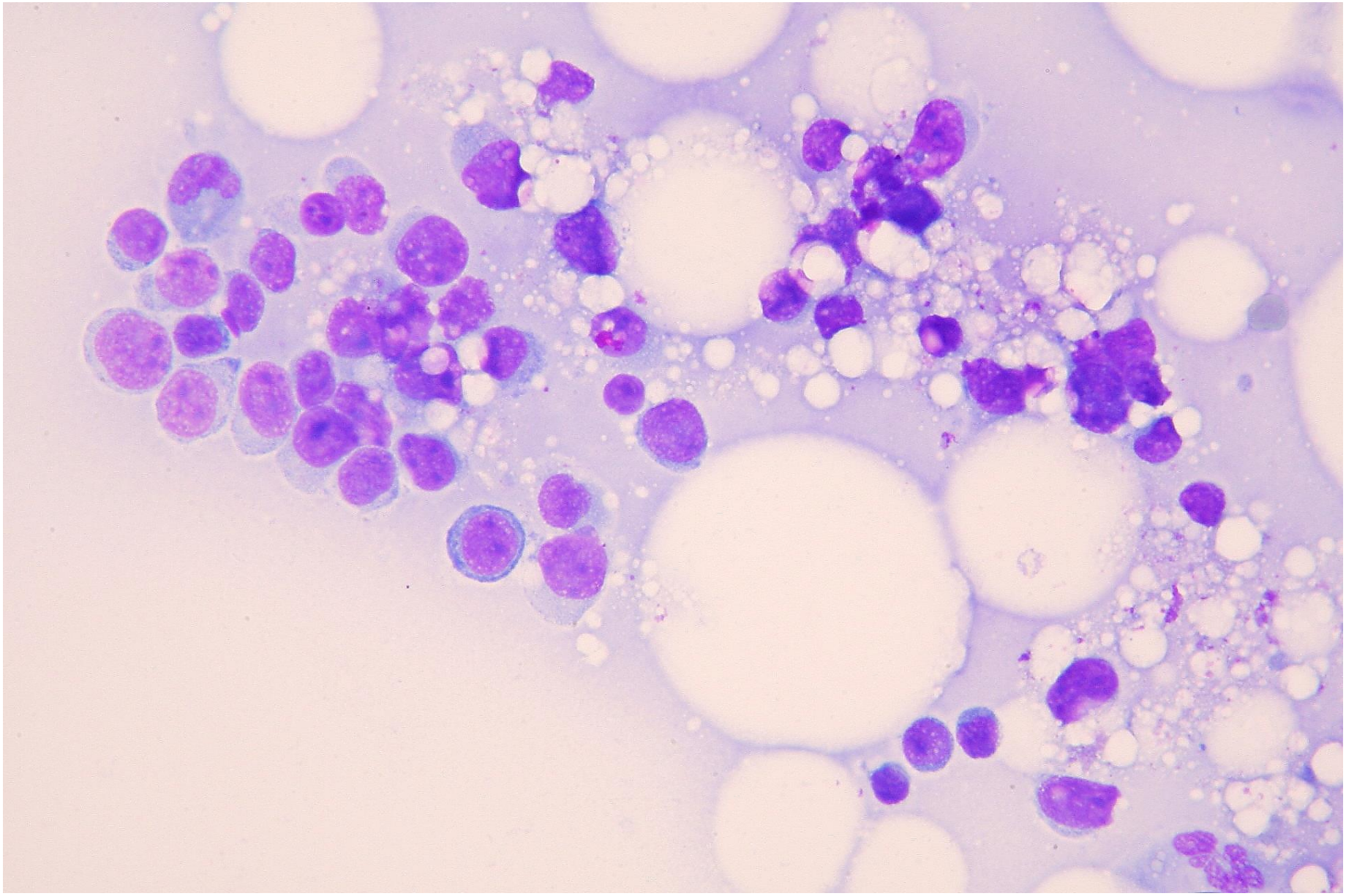
- Sample: FNCS
- Stain: MGG









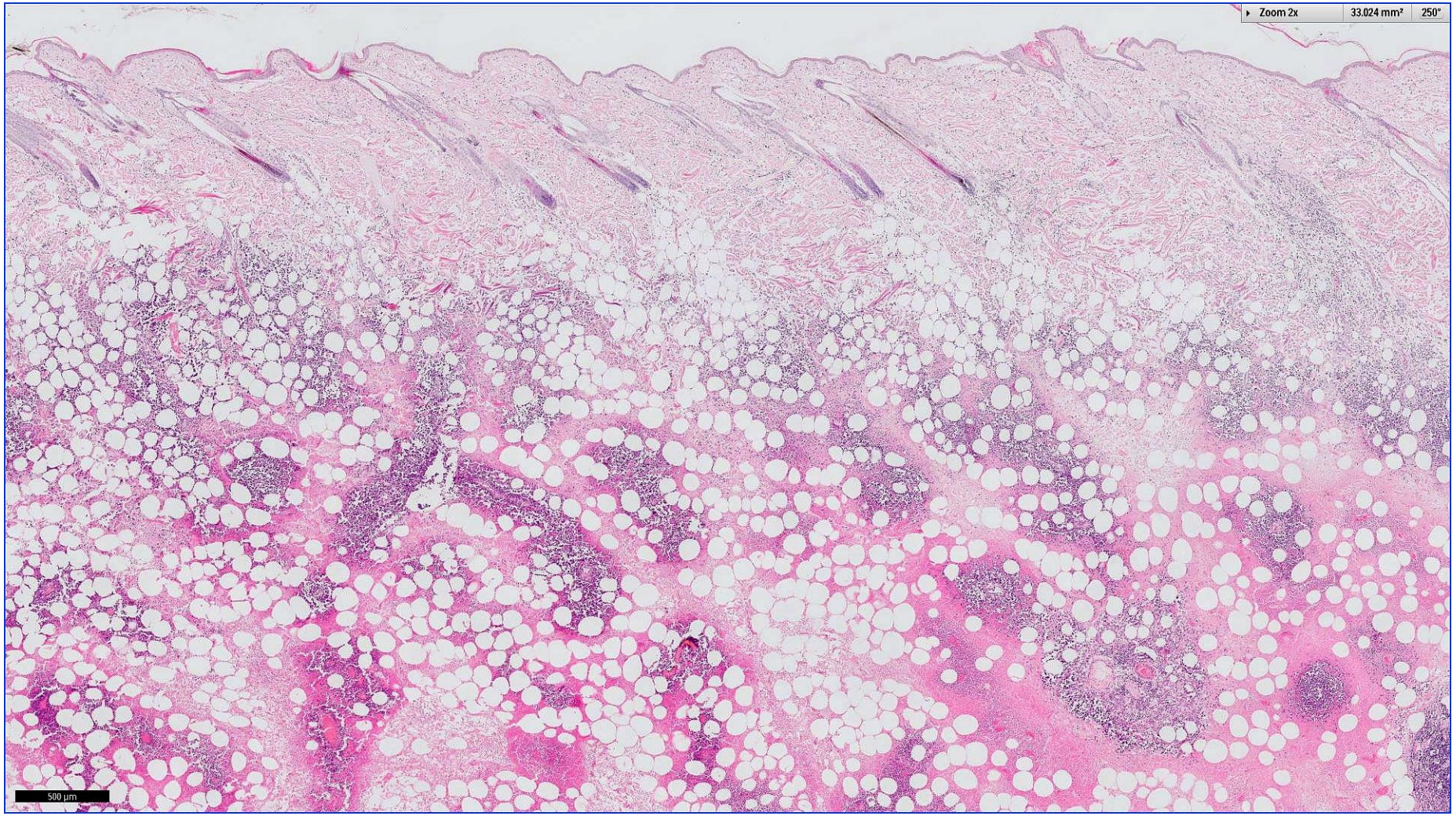


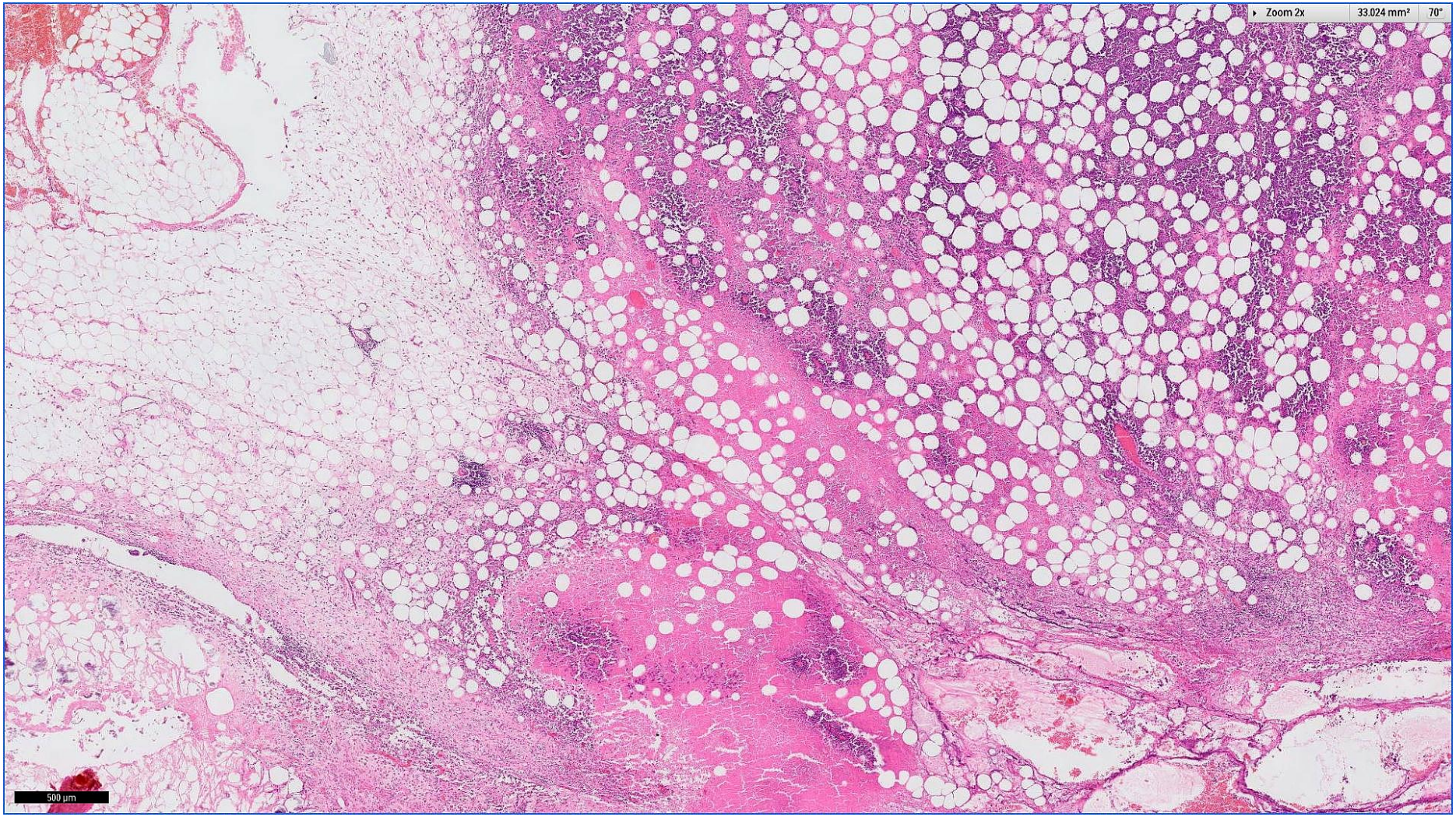
Cytologic findings

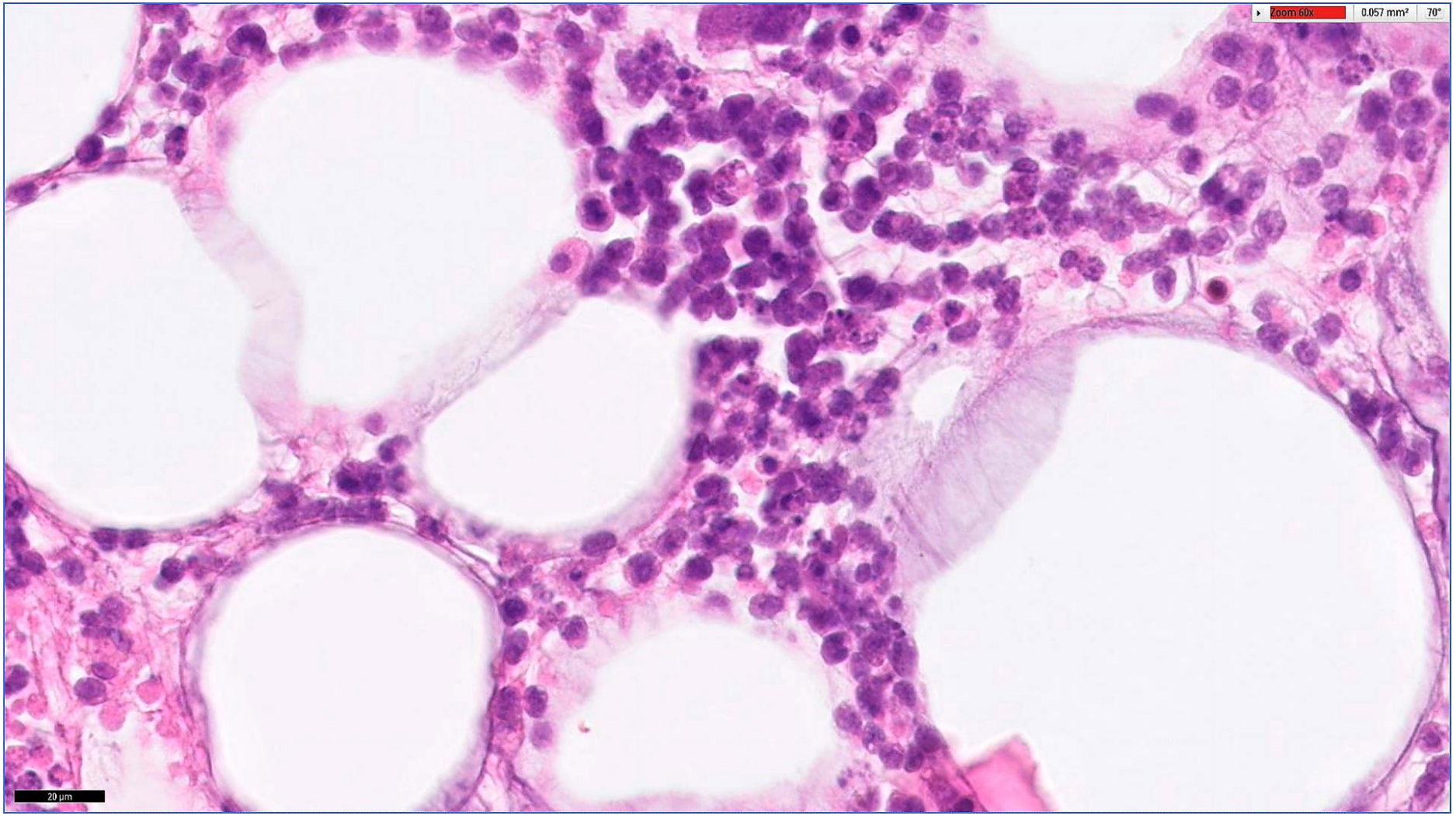
- Large, monomorphic, lymphoid cells
  - Medium-large size
  - Bluish cytoplasm
  - Clear halo
  - Round nucleus
  - Clumped chromatin
  - Nucleolus
- Presence of large amount of lipidic material, scatterde on the background
- Presence of mature adipocytes

Diagnosis

- Cytological diagnosis: large cells lymphoma, with involvement of panniculus
- Histological diagnosis: panniculitis-like T-cell lymphoma
- IHC:
  - CD20 -
  - CD3 ++


Zoom 2x
33.024 mm²
250°
500 µm


Zoom 2x
33.024 mm²
70°
500 µm


Zoom 60x
0.057 mm²
70°
20 µm

Discussion

- Subcutaneous panniculitis-like T-cell Lymphoma (SPTCL)
  - Subgroup of nonepitheliotropic cutaneous T-cell lymphoma (NECTCL) Noland, 2018
  - Subtypes of Peripheral T-cell Lymphoma Not Otherwise Specified (PTCL – NOS) Valli, 2017
- Distribution of neoplastic cells among adipocytes
- Sometimes heavy infiltrate of histiocytes with prominent phagocytosis
- Biological behavior in dogs remains to be determined