Unexpected Cytological Findings in a Feline Neck Mass

Contributors

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Specimen

Fine needle aspiration of a subcutaneous mass located on the right side of the neck, close to the trachea, firm, approximately 1 cm diameter

Signalment

Alessia, Cat, Domestic shortair (DHS), female spayed, 5 years old,

History

Alessia is an indoor - outdoor cat (access to a private garden), living with another cat and regularly vaccinated. It was asymptomatic except for the presence of a small firm mass located on the right side of the neck, near the trachea.

Clinical findings

Fine needle aspirate (FNA) of the subcutaneous neck mass. Staining: May – Grünwald Giemsa.

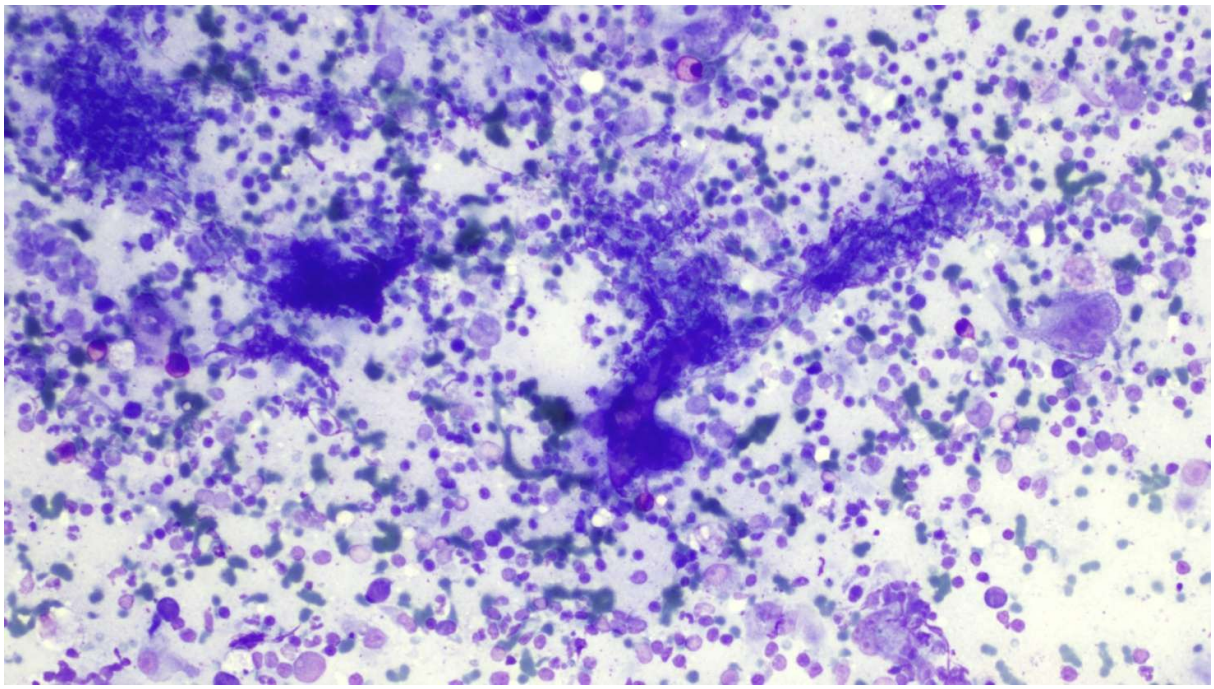

Figure 1. 20x Objective

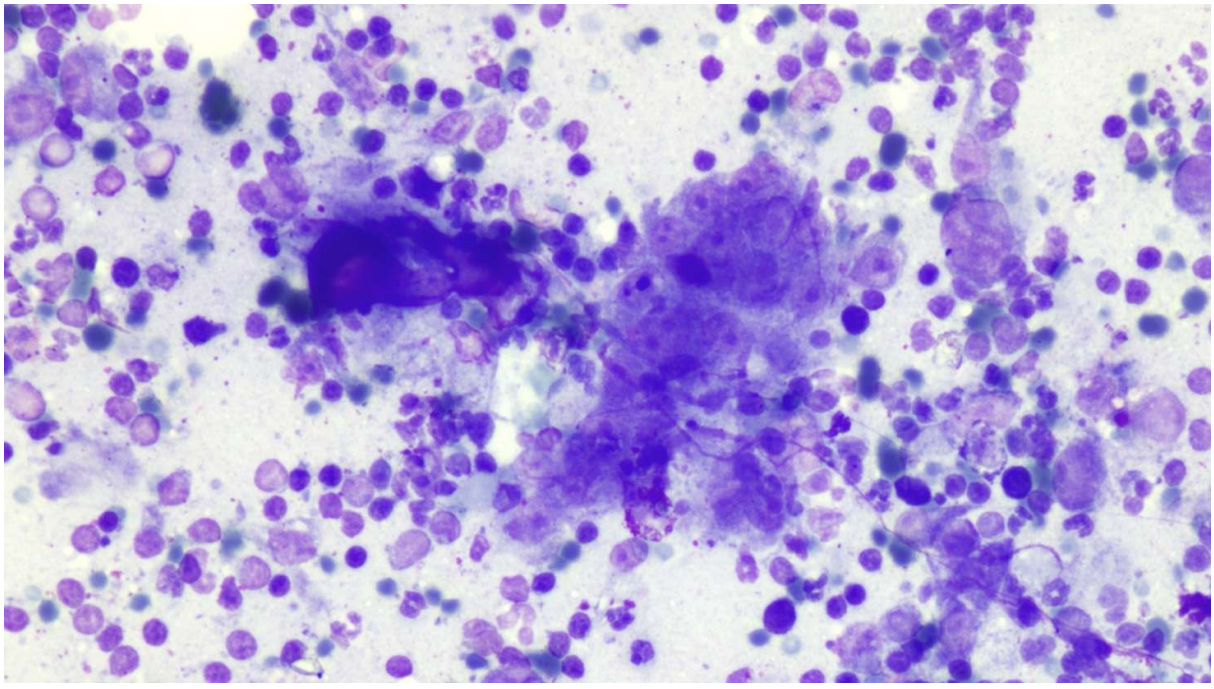

Figure 2. 40x Objective

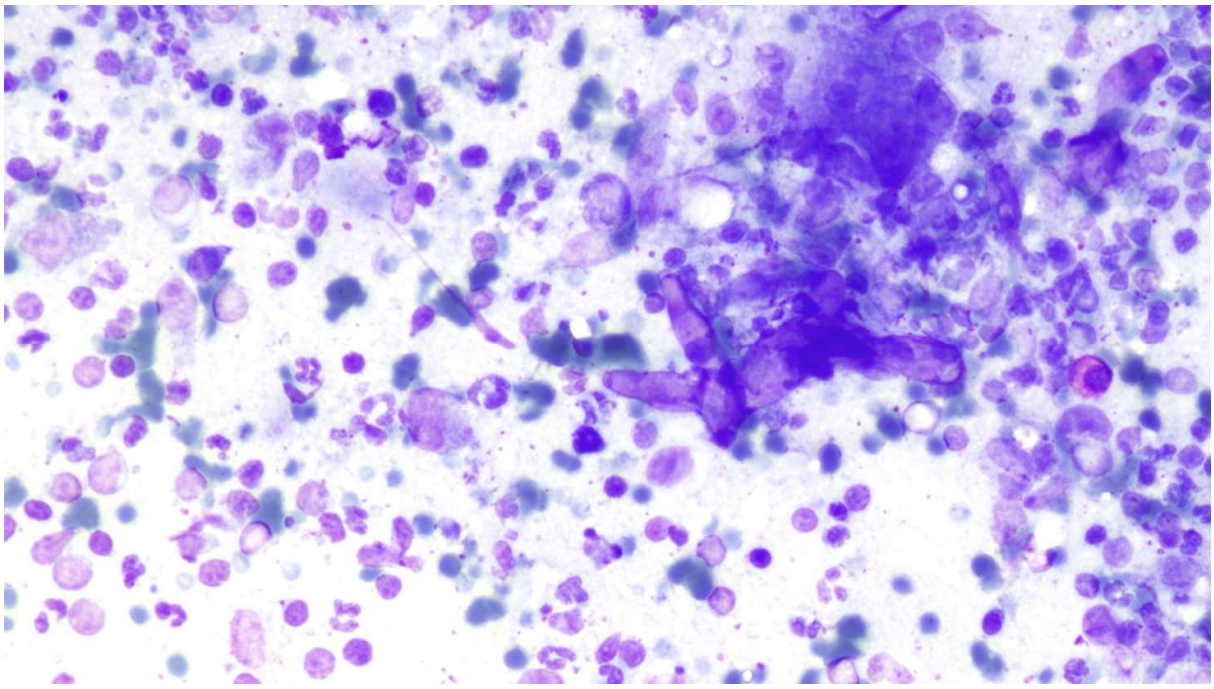

Figure 3. 40x Objective

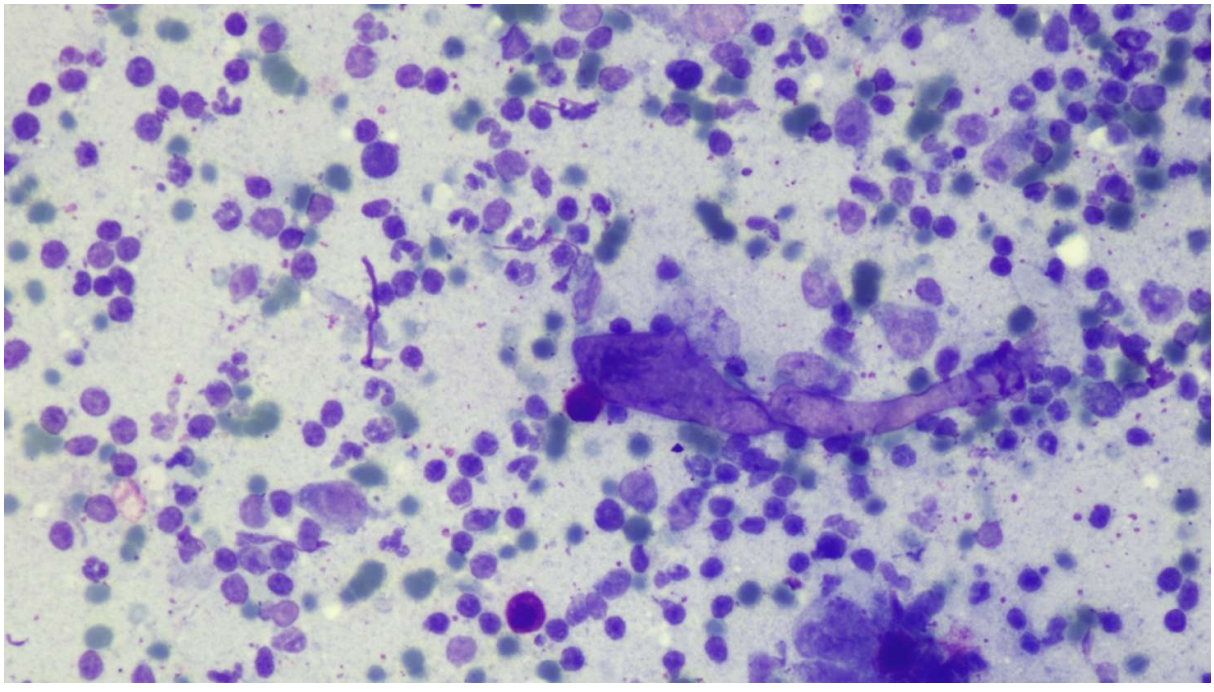

Figure 4. 40x Objective

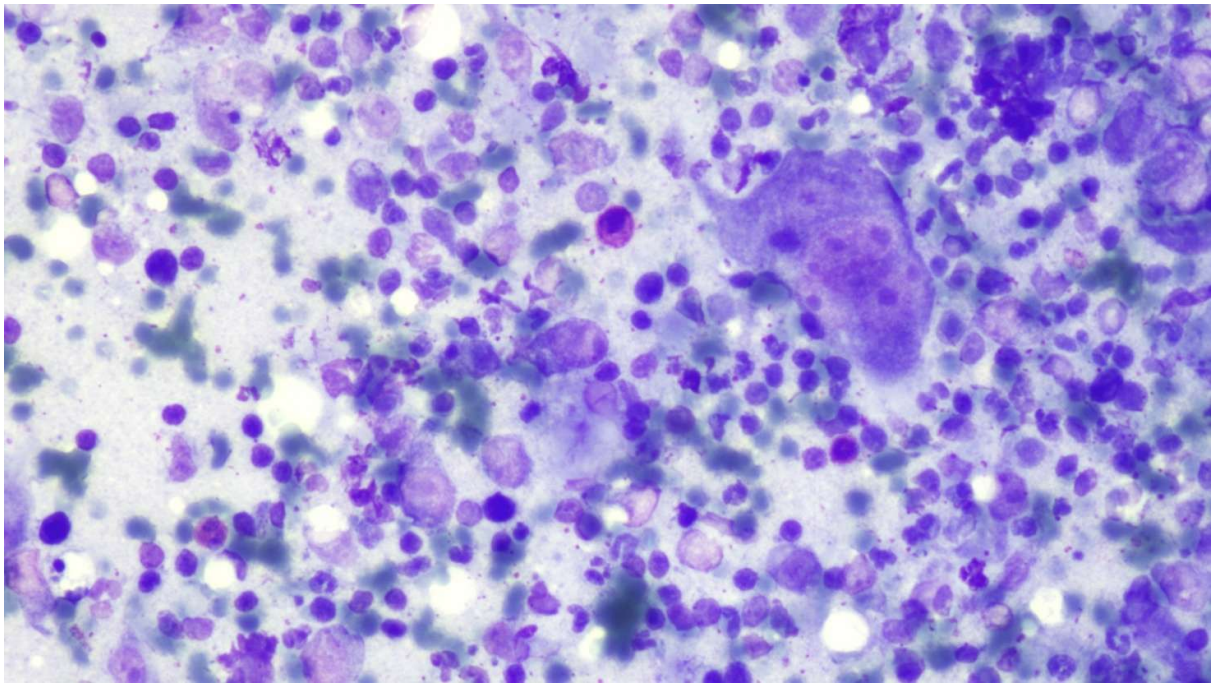

Figure 5. 40x Objective

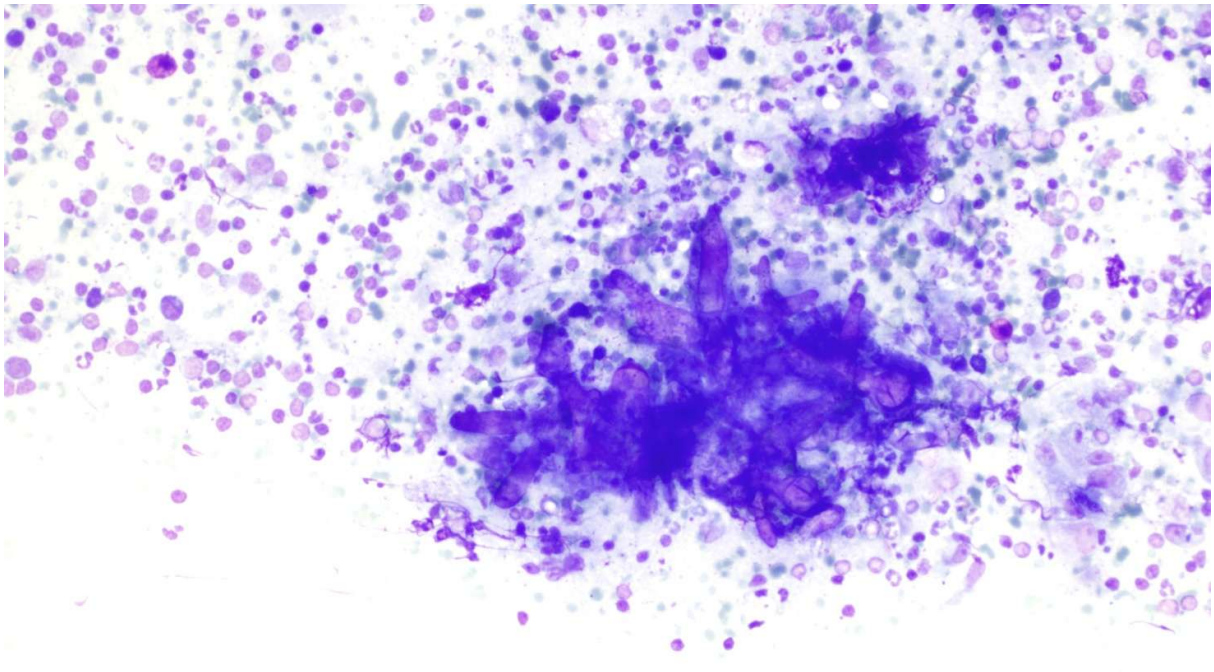

Figure 6. 40x Objective

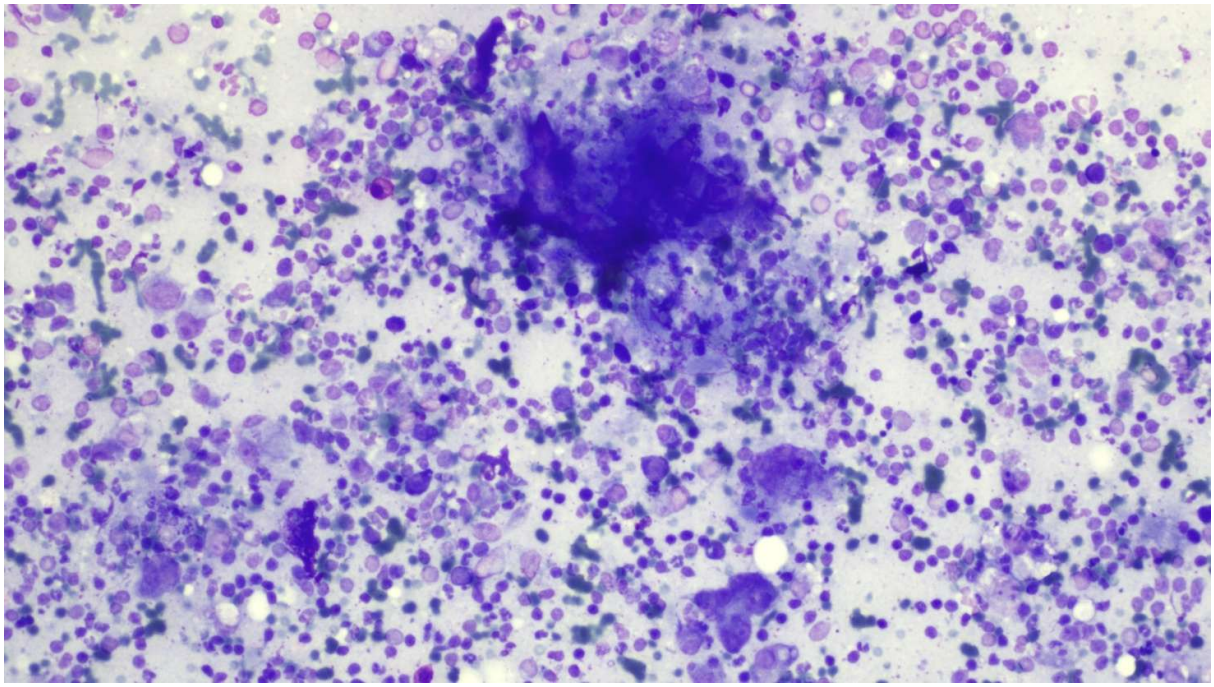

Figure 7. 20x Objective

Questions

What is your cyological interpretation and diagnosis?

Which further analysis would you recommend?

Interpretation/Diagnosis

The cytological interpretation of the case was the following:

Description: The sample had high overall cellularity and good preservation. Moderately bloody background. Presence of a mixed cell population composed mainly by small to medium – sized lymphocytes, followed by a significant proportion of non degenerated neutrophils and macrophages with abundant, slightly basophilic, finely vacuolated cytoplasm. Numerous multinucleated giant cells (up to 30 nuclei) were present, displaying a large amounts of cytoplasm and oval nuclei irregularly arranged. Less numerous were eosinophils, moderately granular mast cells and occasional spindle cells with intensely basophilic cytoplasm and nuclei showing prominent nucleoli. In the sample there were numerous hyphae-like structures, with irregular diameters (5-30 µm), occasionally septate or terminating in globose end. These structures sppeared slightly basophilic to amphophilic, sometimes surrounded by a faint capsule that did not take up the stain. They frequently formed large aggregates and were occasionally observed within the cytoplasm of multinucleated cells.

Cytological diagnosis: Findings are most consistent with sampling of a lymph node showing pyogranulomatous lymphadenitis, likely secondary to the presence of the aforementioned structures, which are consistent with fungal organisms.
Given the lesion's location, a primary fungal granuloma with concurrent sampling of an adjacent lymph node should be considered as a differential diagnosis.

Comment: Further histopathological and mycological investigations, along with complete hematological and biochemical profiles, are recommended to assess the lesion's biological behavior and confirm the etiological agent.

Answers to questions:

Cytological findings are consistent with pyogranulomatous lymphadenitis associated with fungal elements. Specifically, histopathology of the neck mass along with mycological examination are suggested to better characterize the etiological agent.

Additional information

Histopatological examination

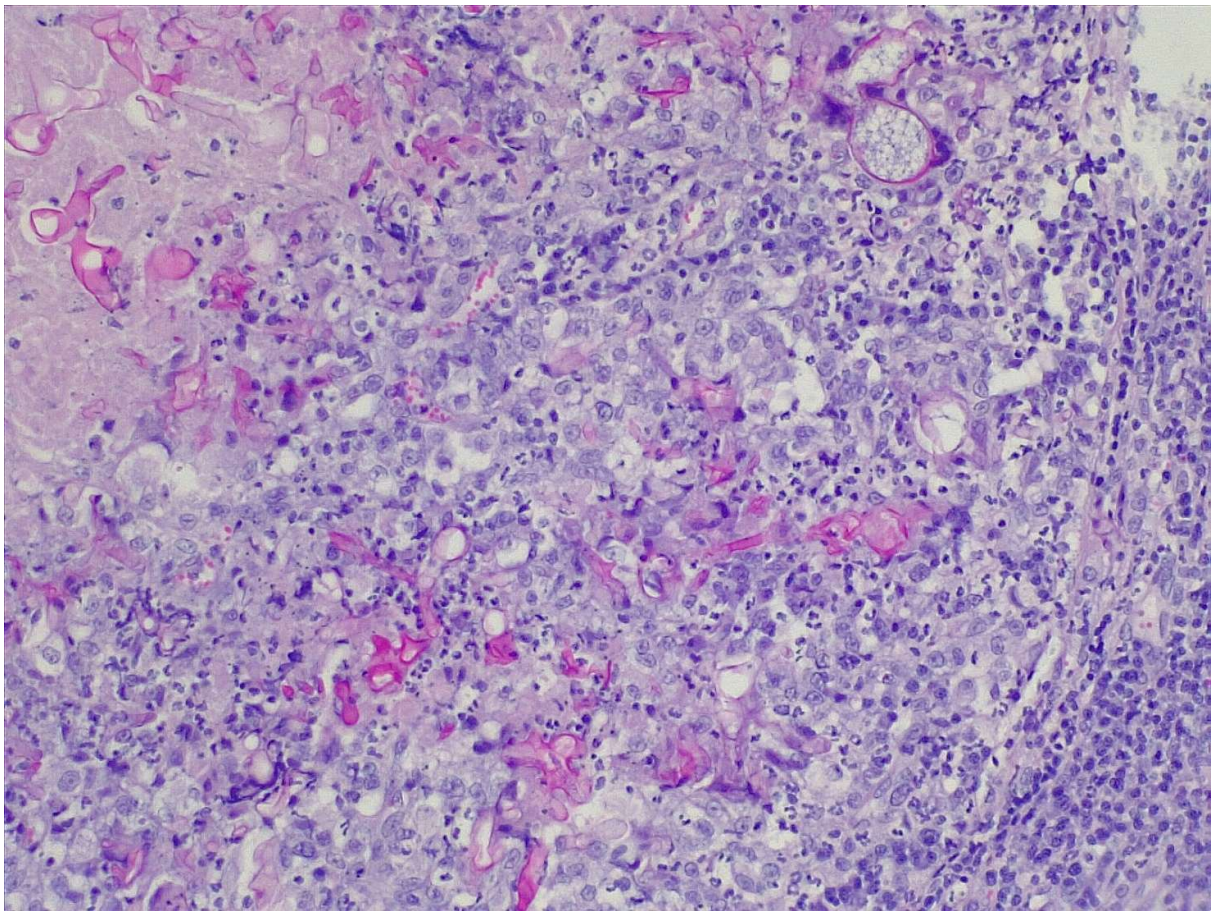

Figure 11. HE staining, 20x Objective.

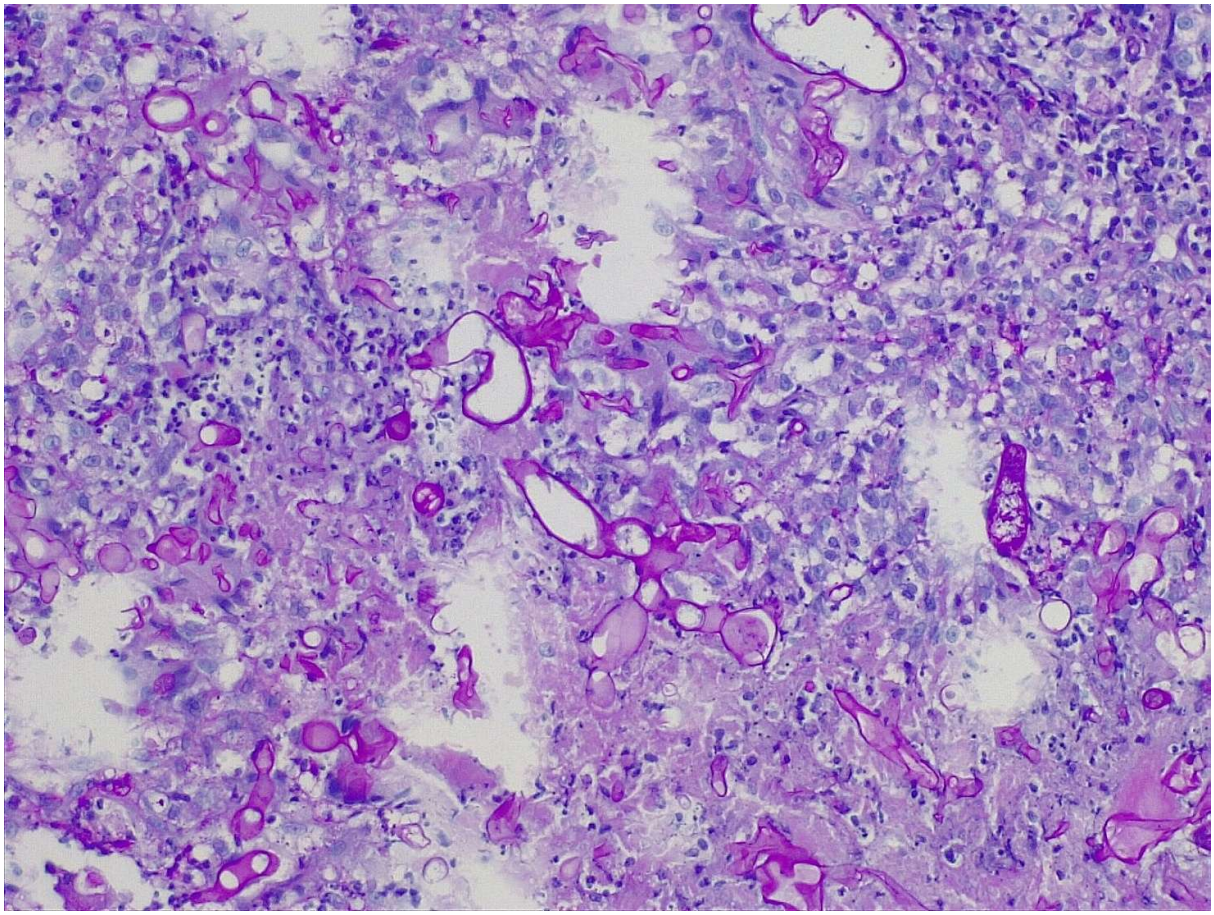

Figure 12. PAS staining, 20x Objective.

Sample: Biopsies of the subcutaneous mass

Macroscopic description: Non-orientable whitish tissue sample, up to 1 cm in diameter.

Microscopic description: Multiple sections of the specimen revealed exclusively coalescing leukocyte aggregates, with central areas composed of necrotic debris admixed with viable and degenerate neutrophils. Numerous fungal structures were observed, consistent with irregularly septate hyphae, characterized by non-parallel walls and non-dichotomous branching, measuring 6–30 µm in thickness and extending over 200 µm in length. Some of them formed "H-shaped" hyphal networks (features consistent with Zygomycetes). The necrosuppurative foci were surrounded by a variably thick rim of epithelioid macrophages, and occasional multinucleated giant histiocytes (pyogranulomas). Peripheral infiltration by small lymphocytes and scattered plasma cells was also noted. PAS staining revealed widespread and variably intense positivity of the fungal hyphae.

Morphological diagnoses: Subcutaneous tissue (based on reported site). Chronic-active pyogranulomatous and lymphoplasmacytic cellulitis with intralesional fungal hyphae consistent with Zygomycetes.

In light of the accompanying mycological findings, the histopathological features are consistent with Mucormycosis (Mauldin, 2016).

Mycological analysis

Sample: Skin biopsy

Result: *Rhizopus* spp.

Comment: *Rhizopus* spp. is a known etiological agents of subcutaneous or nasal mucormycosis. Members of the order *Mucorales* (including *Rhizopus* spp.) are responsible for acute, rapidly progressive infections, particularly in immunocompromised individuals or following traumatic injuries in otherwise healthy individuals (Grooters, 2022).

Fungal colonies grown on culture media were sent to an external laboratory for further fungal typing by sequence analysis. The identified pathogens was *Rhizopus microsporus*.

Follow up and clinical outcome

Two days after cytologic sampling, there was a sudden worsening of the clinical condition. The cat developed purulent nasal discharge, anorexia and an increase in the size of the neck mass. Hematological analysis was performed in house by the referring veterinarian using Idexx ProCyte Dx® (no blood smear examination). Erythrocyte parameter were within reference interval. Concerning the leukocyte, even though the total count was within the reference interval, there was marked neutropenia (1.0x10 ^{3}/µL [2.30 – 10.29]), lymphocytosis (12.33x10 ^{3}/µL [0.92 – 0.67]) and mild monocytosis (0.99x10 ^{3}/µL [0.05 – 0.67]). The analyzer highlighted the possible presence of band neutrophils or immature myeloid forms which required blood smear microscopic confirmation. Mild instrumental thrombocytopenia (106x10 ^{3}/µL [151 – 600]) was also detected. Blood chemistry revealed a moderate hyperproteinemia and hyperglobulinemia (10.5 g/dL [5.7 – 8.9] and 7.7 g/dL [2.8 – 5.1] respectively) without any other abnormalities. FIV and FeLV tests were negative. Radiographs of the neck and thorax showed no significant findings.

One week later, the cat was found comatose and was humanly euthanazed by the referring veterinarian.

Discussion

Mucormycosis is an extremely severe fungal disease caused by members of the order Mucorales. The most common genera are *Rhizopus spp.* and *Mucor spp* (Skiada et al., 2017; Czech et Cuellar-Rodriguez, 2025). Among those, *Rhizopus* spp. was the most frequently isolated pathogen and it is mostly associated with an acute and rapidly progressive infection. These are saprophytic mold, ubiquitous in soil or food. In people, mucormycosis tipically occurs in immunocompromised patients (e.g. uncontrolled diabetes, hematologic malignancies, transplant patients), though it can also affect immunocompetent individuals following traumatic inoculation (Czech et Cuellar-Rodriguez, 2025). Infection often results from inhalation of sporangiospores that may remain dormant inside macrophages for a long period (Skiada et al., 2017). Common disease manifestations include rhino-orbito-cerebral, pulmonary and cutaneous forms (Czech et Cuellar-Rodriguez, 2025). Cutaneous mucormicosis can be primary (direct skin inoculation) or secondary (dissemination from other locations) (Castrejón-Pérez et al., 2017). Primary lesions may be gradual on onset or fulminant, typically presenting as indurated erythematous to purple plaques that progress to necrosis with

an erythematous halo and eventually eschar formation (Castrejón-Pérez et al., 2017). Secondary forms are more common than the primary ones and generally have a more acute onset with high mortality. The disease generally starts as a sinusitis associated with the presence of necrotic eschar (Castrejón-Pérez et al., 2017). Diagnosis is based on direct microscopy showing broad, nonseptate or pauci – septate, ribbon – like, hyaline hyphae (6 to 25 µm) (Skiada et al., 2017). These fungi tend to be fragile, thus the best mycologic results are obtained by culturing biopsy specimens on regular fungal media incubated at 30 – 37°C (Czech et Cuellar-Rodriguez, 2025). Histopathologic specimens stained with hematoxylin – eosin, periodic acid – Shiff or Grocott – Gomori methenamine silver allow for the identification of hyphae, which are generally associated with necrosis. Molecular identification using MALDI-TOF MS of PCR can confirm the species. (Czech et Cuellar-Rodriguez, 2025)

Direct microscopy is a rapid and cost-effective diagnostic tool that can support early suspicion of fungal infections, particularly in cases with acute clinical progression, demonstrating the presence of fungal elements togheter with an inflammatory reaction. In this case, cytological examination revealed the presence of fungal elements with features highly suggestive of Mucorales spp. However, direct microscopy may present some limitations. Morphological features may overlap between Mucorales spp. and other filamentous fungi reducing the diagnostic specificity. Moreover, cytologic and histopatological samples may be compromised by poor sample quality, artefacts, or the fragility of the fungal elements themselves (Knoll et al., 2023; Sangoi et al., 2009). Furthermore, they do not allow species-level identification, which may be relevant for therapy (Sangoi et al., 2009). Direct microscopy seems to have an overall accuracy of 79% in the fungal identification (Sangoi et al., 2009). In this case, cytology was essential in triggering further diagnostic efforts, and the definitive identification relied on both histopathology and mycological confirmation, highlighting the importance of a multimodal diagnostic approach.

Cases of mucromycosis are rarely reported in veterinary medicine (Seyedmousavi et al., 2017). In cats, only a few cases are documented. Most feline cases are associated with gastrointestinal involvement (Cunha et al., 2011; Martineau et al., 2023; Mavilio et Bottero, 2025) with two of them linked specifically to *Rizhopus microscoporus*: a gastric case in a young Ragdoll (Mavilio et Bottero, 2025) and a duodenal mass in a 5 year old domestic shorthair cat, the latter diagnosed as feline gastrointestinal eosinophilic sclerosing fibroplasia (FGESF) associated with fungal filaments (Martineau et al., 2023). Another report descibed *Rhizopus nigricans* in a Ragdoll cat with neurologic signs (Marinelly et al., 2025). All the feline cases showed aggressive clinical courses and high mortality rate, primarily caused by the fungus vasotropic effect, leading to angioinvasion, thrombosis and subsequent tissue necrosis. Only one case of subcutaneous *Mucor* spp. infection was succesfully treated with posaconazole for 5 months (Wray et al., 2008). Interestingly, in all the reported feline cases, FIV and FeLV status was negative. This supports the hypothesis that mucormycosis can occur in immunocompetent animals, possibly following unnoticed local trauma, bite wounds, or disruption of cutaneous barriers. The laboratory abnormalities observed in this case included marked neutropenia, lymphocytosis and mild monocytosis, that should be confirmed through blood smear microscopic examination. Neutropenia may be expected in cases of severe acute

inflammatory bacterial or fungal infections. This finding may result from either myeloid exhaustion or peripheral consumption. The suspected presence of immature myeloid forms also suggests a possible left shift, although confirmation via blood smear was not performed. Lymphocytosis may indicate a subacute or chronic inflammatory process in response chronic antigen stimulation. However, this finding also require confirmation on the blood smear, as the automated cell count may have been biased by the presence of immature neutrophils. The presence of increase total protein togehter with hyperglobulinemia likely reflects a polyclonal immune response to antigenic stimulation. To confirm this hypotesis a serum protein electrophoresis should have been performed. Although these abnormalities are non-specific, they are compatible with the rapidly progressive clinical deterioration observed in this case and are consistent with the immunologic dysregulation frequently associated with invasive mucormycosis. Despite the negative test result for FIV and FeLV, the possibility of transient immunosuppression due to unidentified factors or local trauma cannot be excluded.

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