Systemic Mycosis in a dog involving a *Penicllium sp*.

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Cytological interpretation of FNA from sublumbar lymph node.

The smears were moderately cellular and of moderate diagnostic quality. The background was palely basophilic with a moderate amount of cellular debris and denuded nuclei (necrosis). Nucleated cells consisted of degenerate/necrotic neutrophils with fewer epithelioid macrophages. These were large cells, with palely to deeply basophilic cytoplasm, eccentric nuclei with coarse chromatin pattern and 1-3 nucleoli. They were often bi- or multi-nucleated. Inflammatory cells were often closely associated with each other and, in these areas, narrow diameter, tubular, non-staining structures were visible. They measured approximately 3µm wide and varied in length. Branching was evident. The diagnosis was necrotising pyogranulomatous lymphadenitis with intralesional fungi.

Cytological interpretation of pleural fluid.

The smears were moderately cellular and of good quality. The background was clear with rare red blood cells and finely granular, eosinophilic, proteinaceous material. Nucleated cells were predominantly non-degenerate neutrophils, with activated mesothelial cells and macrophages. Closely associated with some groups of cells there were narrow, variable diameter, tubular, non-staining structures similar to those observed in the lymph node.

The high protein content, elevated cellular count, with predominance of non-degenerate neutrophils, activated mesothelial cells and macrophages, and the negatively stained tubular structures correlated with a diagnosis of an exudate with intralesional fungi.

Special stains were utilised on both specimens to identify the fungal hyphae and, as shown in Figures 1 and 2, they demonstrated large numbers of fungal hyphae, in which segmentation and terminal swellings are evident.

Figure 1. Sublumbar lymph node. PAS stained, x600

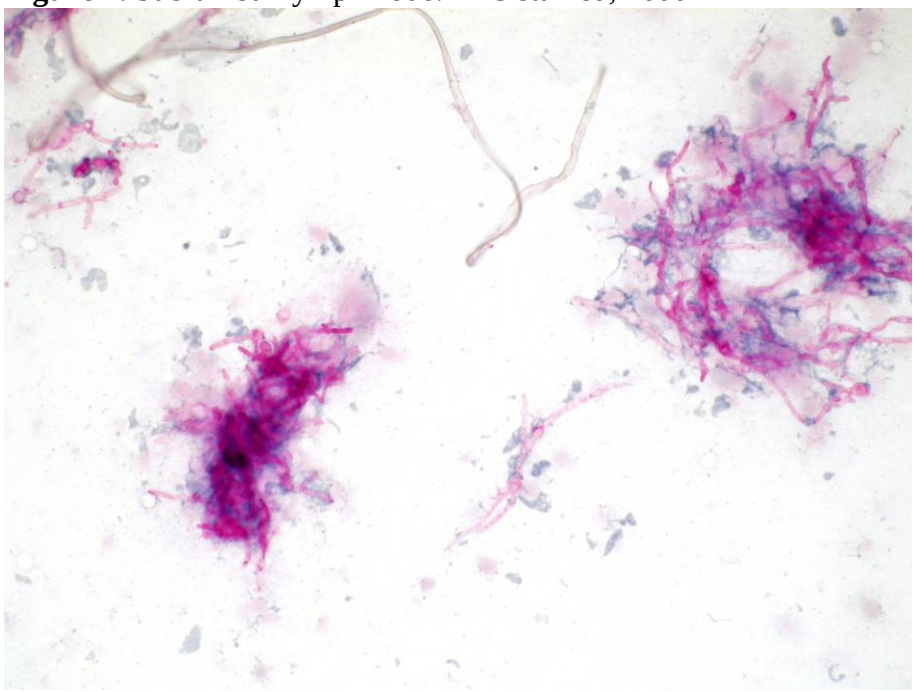
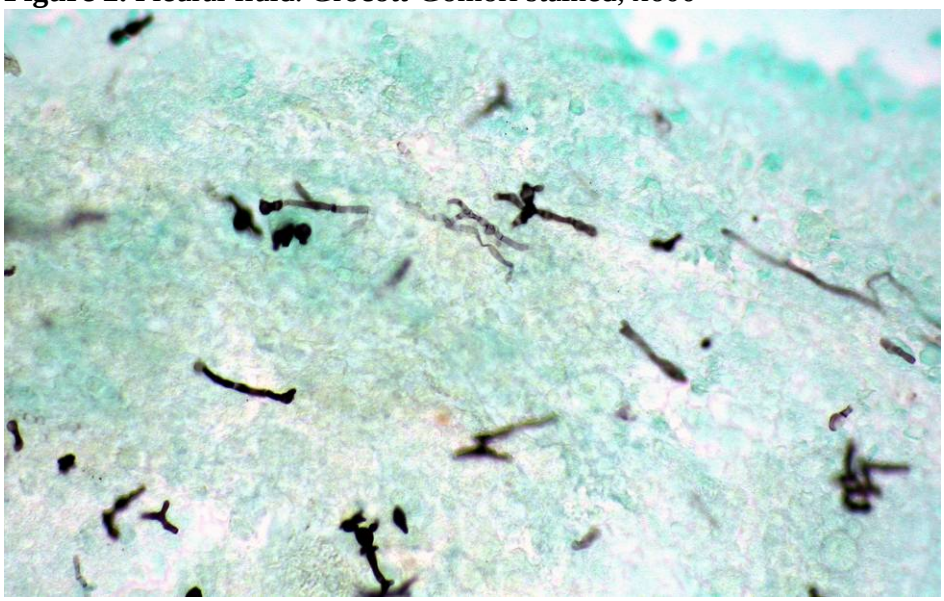


Figure 2. Pleural fluid. Grocott-Gomori stained, x600



Treatment was started with oral itraconazole (Sporanox®, Janssen-Cilag Ltd.) at 100mg once daily. A prophylactic broad-spectrum antibiotic (amoxicillin and clavulanic acid, [Synulox®, Pfizer Ltd.] 250g twice daily per os) was also initiated as an underlying immunosuppressive disease was suspected. The pitting oedema resolved within 24 hours of starting therapy and the patient showed a significant clinical improvement. However, within 48 hours, the patient had again deteriorated. Inappetence continued and the patient lost 4 kg during hospitalization, due to fluid loss and cachexia. In an attempt to mitigate the pyogranulomatous response, trial therapy with dexamethasone was commenced. Although contraindicated in immunosuppressed individuals, this treatment achieved an improvement in the patient's demeanour. He was sent home with a grave prognosis and was euthanized a few days later. A post mortem examination was not performed and any underlying disease processes remain unknown, as well as the extent of the mycosis.

A *Penicillium* sp. was isolated on routine fungal culture. Because of the high numbers of saprophytic fungi, reference laboratories usually identify only human pathogenic species. A sample of the fungal culture was sent to a national reference laboratory, but they were unable to identify the specific *Penicillium* sp. involved, because it was not a recognised human pathogen. The original fungal culture was not retained, therefore further attempts to identify the species using PCR were not possible.

Discussion

The presence of intralesional fungal hyphae in the lymph node aspirate supported the diagnosis of systemic mycosis. This was confirmed by the special stains and then by culture of the pleural fluid. Cytologically, fungal hyphae with branching septae and rounded terminal swellings were present. Considering the history and the presence of extensive necrosis in the cytological specimens, there was a high suspicion that an underlying immunocompromising disease was present.

The peripheral oedema localised to the hind limbs was likely due to the compression of the caudal vena cava caused by the enlarged sublumbar lymph nodes, associated with an overwhelmed lymphatic system.

The haematological data indicated a non-regenerative, normocytic, normochromic anaemia. The pathogenesis of anaemia in this case was most likely due to chronic disease.
The leukogram showed a moderate neutrophilia, with initially a left shift and a mild to severe lymphopaenia. On the day of discharge a marked monocytosis was also present. These findings were consistent with the inflammatory response to the fungal invasion followed by massive necrosis/inflammation consequential to antifungal therapy.

The major features of the biochemistry were mild hypoalbuminaemia, moderately increased ALP, a mild increase in bile acids, urea, creatinine and inorganic phosphate. The hypoalbuminaemia may have occurred because albumin is a negative acute phase protein. The possibility that drainage of the effusion contributed to hypoalbuminaemia cannot be discounted although it was present before drainage was initiated. The bile acids may have increased because of functional cholestasis. A hepatopathy was not identified on abdominal ultrasonography. The initial increase in urea may have been pre-renal in origin due to mild dehydration or anorexia with increased protein catabolism. As treatment was initiated, fluid drainage could also have contributed to dehydration. The elevated creatinine (although very mild) and inorganic phosphate may reflect mildly decreased glomerular filtration. Urinalysis was not performed. The mild hypocalcaemia was considered to be a result of hypoalbuminaemia.

Systemic fungal diseases are not very common in Europe and are more frequently seen in patients with compromised immunity. The ability of fungi to cause disease is proportional to the number of organisms and their pathogenicity and is indirectly proportional to the host resistance. Systemic fungal disease may be caused by primary pathogenic fungi or by opportunistic fungi. Primary pathogenic fungi are often dimorphic (e.g. Histoplasma, Blastomyces, Coccidiomyces, Paracoccidiodomyces and Sporothrix), i.e. they are present as a yeast form in the host tissue (at 37^{o}C) and as mould (filamentous-form) in the environment (at ~25^{o}C). In contrast, opportunistic fungi are of low pathogenicity and can infect immunocompromised hosts. More common examples include aspergillosis, systemic candidosis and cryptococcosis, and only exceptionally other saprophytic fungi such as *Penicillium* and *Trichosporum* spp.

The only *Penicillium* sp. that has been identified as pathogenic in humans is *Penicillium marneffei.* This is an unusual dimorphic fungus (all other *Penicillium* are not dimorphic) which has a restricted geographical distribution being localised only to southeast Asia. Systemic fungal disease caused by this *Penicillium* sp. has increased dramatically in the human population with the increase in susceptible subjects (i.e. patients with AIDS, on immunosuppressive therapy for transplantation, or receiving chemotherapy). Other systemic human penicillioses are very rare, and only 40 cases have been described in the medical literature in the past century.

References

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