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CLINICAL REVIEW

Review of Systemic Aspergillosis in Dogs

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1. Introduction

Fungi of the genus *Aspergillus* are ubiquitous in the environment and may occasionally cause opportunistic infections in humans and animals. In dogs, aspergillosis most commonly occurs as a localized sinonasal infection. Disseminated infections (systemic aspergillosis) are less frequently observed and typically involve the intervertebral disks, bones, thoracic lymph nodes, lung and renal pelvis. [11] A bronchopulmonary form of aspergillosis also occurs rarely in dogs. Achieving a definitive diagnosis of any form of aspergillosis can be challenging and/or invasive. Detection of *Aspergillus* galactomannan antigen in canine urine or serum has recently been demonstrated as a noninvasive, sensitive and specific method for diagnosis of systemic aspergillosis.

2. Causative Agents

Aspergillus fumigatus, *Aspergillus flavus*, *Aspergillus terreus*, *Aspergillus niger* and *Apergillus deflectus* have all been shown to cause disease in dogs [2,8,9,12-14,18,21,22]. Sinonasal aspergillosis in dogs is primarily caused by *A. fumigatus* (and occasionally *A. flavus*); however, systemic aspergillosis is more frequently attributed to *A. terreus* and *A. deflectus* [19]. Recent reports describe isolated cases of systemic aspergillosis caused by *Aspergillus alabamensis* and *Aspergillus versicolor* [26].

3. Pathogenesis

Aspergillus species are ubiquitous in soil and decaying vegetation. Over 200 species of aspergilli are known, and these organisms produce small hydrophobic conidia (asexual spores) that disperse easily into the air [4]. Conidia are inhaled and deposited in the bronchioles and alveolar spaces. Due to the abundance of fungal conidia in the environment, the average human may inhale up to 200 *A. fumigatus* conidia per day [15]. In healthy individuals, conidia are cleared by mucociliary defenses or phagocytosed by alveolar macrophages. Any remaining germinating conidia are targeted by infiltrating neutrophils that can destroy fungal hyphae. Neutropenic and otherwise immunocompromised patients are at risk for pulmonary colonization leading to tissue injury, uncontrolled fungal growth and potential dissemination by angioinvasion [4].

4. Clinical Findings

German shepherd dogs (especially young-middle aged females) are markedly overrepresented in cases of systemic aspergillosis [6, 10], possibly due to a hereditary IgA deficiency or dysfunction leading to defective mucosal immunity [7-9]. One recent study of 30 dogs with systemic aspergillosis included 20 German shepherd dogs and reported a mean age of 4.5 years and a female: male ratio of 3:1 [19]. Clinical signs commonly include limb pain/lameness due to long bone involvement, vertebral pain with possible paraparesis/paraplegia, ataxia, obtundation/mental dullness, visual impairment and other ocular signs related to uveitis, coughing, chronic vomiting, and nonspecific signs (anorexia, weight loss and lethargy) [6,19] Fungal granulomas frequently develop in the spleen, lymph nodes and kidneys [6].

5. Clinicopathologic Abnormalities

Common abnormalities on the complete blood count include normocytic normochromic anemia, leukocytosis, left shift and neutrophil toxicity. Serum biochemical profile may reveal hyperglobulinemia, azotemia, hypercalcemia and/or hypoalbuminemia. Urinalysis commonly shows isosthenuria, hematuria and pyuria. Fungal hyphae may occasionally be observed in the urine sediment (as well as in aspirates from other affected areas such as lymph nodes, kidneys, pleural effusion, lung, bone, joint fluid, and transtracheal wash). Pyogranulomatous inflammation is also frequently observed in cytologic specimens. In dogs with neurologic signs, CSF often shows neutrophilic pleocytosis [19].

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Diagnostic Imaging

Radiographic abnormalities commonly include evidence of diskospondylitis, productive and destructive bony changes consistent with osteomyelitis and hilar lymphadenomegaly [11, 19]. Abdominal ultrasonographic changes are most often detected in the kidneys (e.g., pyelectasia, echogenic debris in a dilated renal pelvis, distinct nodules/masses), spleen, lymph nodes and liver. In dogs with neurologic signs, MRI features may be similar to other inflammatory brain diseases [19].

Fungal Culture

Definitive diagnosis of systemic aspergillosis has historically required positive identification of the organism through culture of normally sterile body fluids and tissues. Samples may be acquired from blood, urine, pleural effusion, lymph node aspirates, CSF, bone biopsies, intervertebral disc aspirates, synovial fluid, etc. Although many aspergilli grow rapidly in culture, identification of some species may be a challenging and lengthy process. In addition, sensitivity is low from sites such as urine or blood. One study showed only 52% sensitivity of urine culture in dogs with systemic aspergillosis [19]. Collection of culture specimens from other sites (i.e., bone, intervertebral disc) may be more challenging and invasive.

Histology

Histologic evaluation of samples frequently reveals infiltration with fungal hyphae and pyogranulomatous inflammation. Involvement has been observed in a wide variety of tissues, including kidney, spleen, lymph nodes, bone, vertebral endplates, intervertebral disk, liver, heart, pancreas, brain, lungs, eyes, bone marrow, meninges, pleura, small intestine, skin, spinal cord, thyroid, prostate, and trachea/larynx [19]. Fungal hyphae appear as 3-8µm wide, septate, hyaline hyphae with parallel walls and acute angle branching [3,16]

Antibody Detection

In dogs with nasal aspergillosis, detection of serum anti-*Aspergillus* antibodies (by either agar gel immunodiffusion or enzyme-linked immunoassay [EIA]) has moderate sensitivity and high specificity.(1) In contrast, current antibody tests appear to have little clinical utility in the diagnosis of systemic aspergillosis. Sensitivity was only 20% in a small sample of 5 dogs [19].

Galactomannan Antigen Detection

The Platelia™ *Aspergillus* antigen assay (Bio-Rad Laboratories; Redman, WA) is an EIA for the detection of *Aspergillus* galactomannan antigen in body fluids. In humans, the assay is useful in the diagnosis of systemic aspergillosis which most often occurs in immunosuppressed patients (e.g., neutropenia, solid organ transplant, bone marrow transplant). Sensitivity and specificity vary based on underlying condition and cutoff for positivity (galactomannan index; GMI). A meta-analysis of human studies showed an overall sensitivity of 71% and specificity of 89% [17]. A GMI ≥ 0.5 is considered to be positive for galactomannan antigen, as recommended by the assay manufacturer.

In a 2012 study of dogs with systemic aspergillosis (using cutoff GMI ≥ 0.5), the sensitivity was 92% in serum and 88% in urine, while the specificity was 84% in serum and 92% in urine [11] Any false negatives in this study were cases of localized pulmonary aspergillosis; therefore, the assay sensitivity was 100% (in either serum or urine) for disseminated disease. False positives could be decreased slightly by using a higher cutoff value (GMI ≥ 1.5) without affecting the sensitivity in urine. False positives may be attributed to other fungal infections with cross-reactive galactomannan (i.e., disseminated penicilliosis, paecilomycosis, geotrichosis, etc.) or administration of *Penicillium*-derived antibiotics (amoxicillin-clavulanate and other beta lactam antibiotics) or Plasma-Lyte® (Baxter, Deerfield, IL) or other gluconate-containing fluids [11,25] In humans, false positivity has occasionally been reported in cases of cryptococcosis [5] and blastomycosis [20], as

well as approximately half of cases of histoplasmosis [24]. In contrast with the pattern described in human patients, all dogs with systemic aspergillosis in this study had profound increases in GMI in both serum (>5.0) and urine (>4.0). Additional studies are necessary to determine the optimum specimen type for the assay; however, at this time, both serum and urine appear to be acceptable for diagnosis [11]. *Aspergillus* galactomannan may also be detected in bronchoalveolar lavage fluid and CSF [23].

6. Treatment

Prognosis is often poor in cases of systemic aspergillosis, and many dogs are euthanized due to severe CNS signs, pain or respiratory distress. Antifungal therapy agents such as itraconazole, amphotericin B and/or terbinafine should be considered in dogs with less severe signs. In one study, survival times of treated dogs had a wide range with one dog surviving 25 months after diagnosis [19]. This dog had received lipid complex amphotericin B, newer azole derivatives and echinocandins. Another report described survival >3 years in a dog treated only with itraconazole [14]. Antifungal resistance by *Aspergillus* has emerged as a problem, and therefore newer azole drugs such as voriconazole, posaconazole, ravuconazole and isavuconazole have been utilized to treat invasive aspergillosis in humans. The newer azoles have had limited use in veterinary medicine due to high cost; however, voriconazole now is available in generic form. One recent case report described successful treatment of aspergillosis in a bottlenose dolphin with high dose posaconazole [27].

7. Conclusion

Systemic aspergillosis is an uncommon condition observed primarily in young-middle aged female German shepherd dogs (and occasionally other breeds). Fungal colonization initially occurs in the lungs, followed by dissemination to numerous other body sites. Common clinical signs are attributed to disease in the musculoskeletal, neurologic, respiratory and GI systems. Diagnosis by identification of organisms through histology and/or culture can be challenging or invasive. The galactomannan antigen test is a noninvasive, sensitive and specific assay to aid in the diagnosis of disseminated *Aspergillus* infection in dogs. Although prognosis is poor, treatment should be considered in dogs with less severe clinical signs.

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