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Mucocutaneous and cutaneous generalized candidiasis in a thymectomized dog

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ABSTRACT

Cutaneous candidiasis is usually related to immunosuppressive diseases and/or therapies as predisposing factors. In humans, chronic mucocutaneous candidiasis (CMC) is observed with thymoma and thymectomy. In this case report we describe the clinical and pathological findings, laboratory analysis, treatment, and follow-up of a thymectomized dog with cutaneous and mucocutaneous generalized candidiasis.

1. Introduction

Candida spp. are ubiquitous, yeast-like organisms. Among the species of the genus, *Candida albicans* is the one most commonly isolated in dogs and cats [1]. This commensal organism normally inhabits the gastrointestinal, upper respiratory, and genitourinary tracts, and may cause localized infections of the skin and mucous membranes, as well as life-threatening systemic infection with multisystem organ failure, mostly in patients having underlying immunosuppressive disorders [1,2].

The thymus is a primary lymphoid organ essential for the development of T lymphocytes which orchestrate adaptive immune responses. It provides surveillance and protection against several pathogens, tumors, antigens, and mediators of tissue damage [3]. In humans, thymic dysfunction caused by neoplasia or absence of thymus resulting from thymectomy is itself a precipitating factor for the development of opportunistic infections such as candidiasis [4,5].

To the best of our knowledge, this is the first report of cutaneous and mucocutaneous candidiasis in a thymectomized dog affected by thymoma. The diagnostic work-up and treatment outcome are described.

2. Case presentation

A 9-year-old spayed Labrador retriever was presented for a computed tomography (CT) scan because a mediastinal mass was suspected on the thoracic radiographs made by the referring veterinarian (day −60). Associated clinical signs included tremors, dyspnea, lethargy, regurgitation, inappetence, and temporal muscular atrophy. No significant alterations were detected during the pre-anesthetic blood assessment, and CT-scan confirmed a well-defined space-occupying mass within the cranio-ventral aspect of the mediastinum, with no vascular invasion. Thymectomy was performed by median sternotomy with no post-operative complications after surgery.

Histopathological findings confirmed a thymoma with tissue mainly consisting of a mixed cell proliferation of epithelial cells arranged in sheets and ranging from polygonal to elongated in form, with abundant amounts of clear-to-eosinophilic cytoplasm, rounded to ovoid nuclei with fine chromatin as well as mature or intermediate lymphocytes infiltrating the capsule (Fig. 1A). Thymic epithelial cells were immunoreactive to the epithelial marker cytokeratin, with $CD3^{+}$ lymphocytes in the stromal area of the sample, respectively (Fig. 1B and C).

Two months later, the dog was referred due to severe pustular and crusting dermatitis (day 0). On Day −12 before referral, the regular veterinarian performed a complete cell blood count (CBC) and

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biochemistry profile, tested the dog for Leishmaniosis by indirect fluorescent antibody test (IFAT), and made repeated thoracic radiographs together with abdominal ultrasound examination. All results were within normal limits except for serum biochemical, with hypoalbuminemia [albumin (Alb) 1.8 g/dL, range 2.8–4.3], hyperglobulinemia [globulin (Glob) 5.9 g/d; range 2.4–4.3], and C-reactive protein [18.7 mg/l; range 0–10.7] associated with skin inflammation.

On Day 0, physical examination revealed persistence of temporal muscular atrophy, and upon dermatological examination a diffuse alopecia with severe scaling and crusting dermatitis was detected in the face, limbs, and dorsally in the trunk; ventrally, pustules, crusts, and hyperpigmentation were also observed (Fig. 2A and B). Moreover, a whitish-gray exudate that left behind underlying erythematous plaques after removal by gentle scraping was present in the oral cavity (Fig. 2C).

Initially, because of the clinical signs together with mucocutaneous involvement, the differential diagnoses included immunologic diseases as systemic lupus erythematous, pemphigus vulgaris and bullous pemphigoid, erythema multiforme, and epitheliotropic lymphoma. As a further complication, a fungal infection, also including candidiasis, and superficial bacterial folliculitis, were also considered. Wood lamp and microscopic hair examination of plucked hairs were negative. Cytologic examination of direct smear impressions revealed suppurated inflammation together with scattered oval-yeasted organisms. Bacterial culture, fungal culture from both skin and oral cavity, and skin biopsy were therefore performed.

Bacterial culture of skin lesion followed by antibiotic sensitivity testing identified a coagulase positive staphylococci bacterium that was sensitive to multiple antibiotics. For fungal culture, hair and scraped scales were collected and incubated onto Dermasel agar plates (Oxoid, UK) at 25 ± 1 °C for about two weeks and then observed daily, and at the same time a PCR protocol was set up. The primers were DMTF18SF1 (5′-CCAGGGAGGTTGGAAACGACCG-3′), DMTF28SR1 (5′-CTACAAATTACAACTCGGACCC-3′) and DMTFITS1R (5′-CCGGAACCAAGAGATCCGTTGTTG-3′), designed for the region ITS+ of nuclear ribosomal DNA. PCR positive reactions were purified using QIAquick PCR Purification Kit (Qiagen®). Sequencing was performed in both directions, with primers DMTF18SF1 and DMTFITS1R, using BigDye Teminator v1.1 Cycle Sequencing Kit, and reactions were separated through an ABI PRISM 310 Genetic Analyzer (Applied Biosystems®). Consensus sequences were created with BioEdit Sequence Alignment Editor software v 7.0.9.0 and then aligned on the Genbank database. Samples from oral cavity and skin were taken with sterile swab friction and inoculated onto Sabouraud Dextrose agar (Oxoid, UK), and then incubated at 36 ± 1 °C for 48–72 hours. Yeast colonies grown were subjected to PCR amplification and sequencing analysis. DNA was extracted using the QIAamp DNA mini kit (Qiagen®) and then amplified by PCR of D1/D2 region with NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL2 (5′-GGTCCGTGTTTCAAGACGG-3′) primers. Sequencing was performed in both directions with BigDye Teminator v1.1 Cycle Sequencing Kit and reactions were separated through an ABI PRISM 310 Genetic Analyzer (Applied Biosystems®). Consensus sequences were created with BioEdit Sequence Alignment Editor software v 7.0.9.0 and then aligned on the Genbank database.

Yeast sensitivity to Amphotericin B, Anidulafungin, Caspofungin, Micafungin, 5-fluorocytosine, Fluconazole, Itraconazole, Posaconazole, and Voriconazole was tested through a Thermo Scientific™ Sensititre™ YeastOne™ kit, with both broth micro-dilution and minimum inhibitory concentration (MIC).

Fungal culture and molecular test from skin were both negative for dermatophytes. Oral and skin swabs showed, after 24–48 hours, a pure growth of roundish, ivory-white, creamy colonies, macroscopically referable to yeasts. The molecular typing of these colonies showed significant identity (99.18%, e-value 0.0) with the sequences recorded in NCBI GeneBank for *Candida albicans*. The strain was resistant to all tested antifungals.

Biopsy specimens were collected under local anesthesia using an 8 mm skin biopsy punch, and histopathological findings on routine examination with hematoxylin and eosin (HE) consisted of serocellular crusts with a prevalence of degenerated neutrophils associated with hyperkeratosis, moderate epidermal hyperplasia, dermal edema, and perivascular to interstitial lymphoplasmacytic dermatitis (Fig. 3A). Periodic acid Schiff (PAS) staining highlighted the presence of tangled pseudohyphae in the stratum corneum (Fig. 3B).

Based on these results, a mucocutaneous and cutaneous generalized *Candida albicans* infection was confirmed. Finally, to identify overall immune dysfunction, the $CD4^+/CD8^+$ ratio was also tested, but no alteration was detected.

Terbinafine hydrochloride at 30 mg/kg per os daily was initiated (day +18) together with econazole nitrate shampoo 10 mg/ml two-three times weekly and clinical signs improved significantly after 4 weeks without reporting of adverse effects. Therapy was continued for 10 days beyond clinical cure and a blood test was submitted which showed no significant alteration. Over the course of a year, the dog had two relapses of dermatological clinical signs at intervals of 3–4 months, and despite normal recheck x-rays and blood test that only revealed alterations attributable to chronic inflammation, fungal cultures confirmed a multidrug resistant *Candida* spp. infection that, however, responded to terbinafine.

3. Discussion

To the best of our knowledge, dermatological clinical signs of

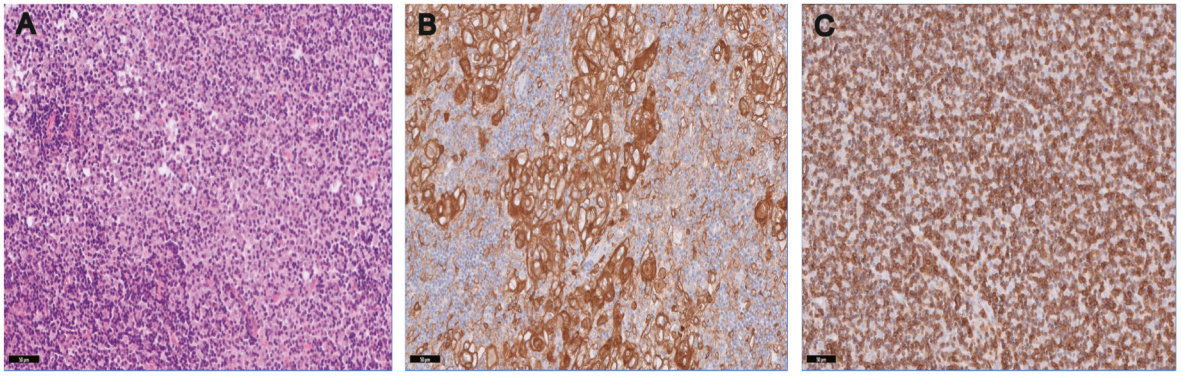

Fig. 1. Hematoxylin-eosin histology Photograph. Thymoma. Two cell populations composed of small lymphocytes admixed with larger epithelioid neoplastic cells having paler stain affinity. Hematoxylin and eosin (H&E). 20X magnification (A). Cytokeratin Photograph. Thymoma. Larger neoplastic cells clustered or scattered among small lymphocytes are diffusely positive for pancytokeratin confirming epithelial origin. Immunolabeling with anti-pan-cytokeratin, hematoxylin counterstain. 20X magnification (B). CD3 Photograph. Thymoma. Small lymphocytes are diffusely positive for CD3 indicating T-cell origin. Immunolabeling with anti-CD3, hematoxylin counterstain. 20X magnification (C).

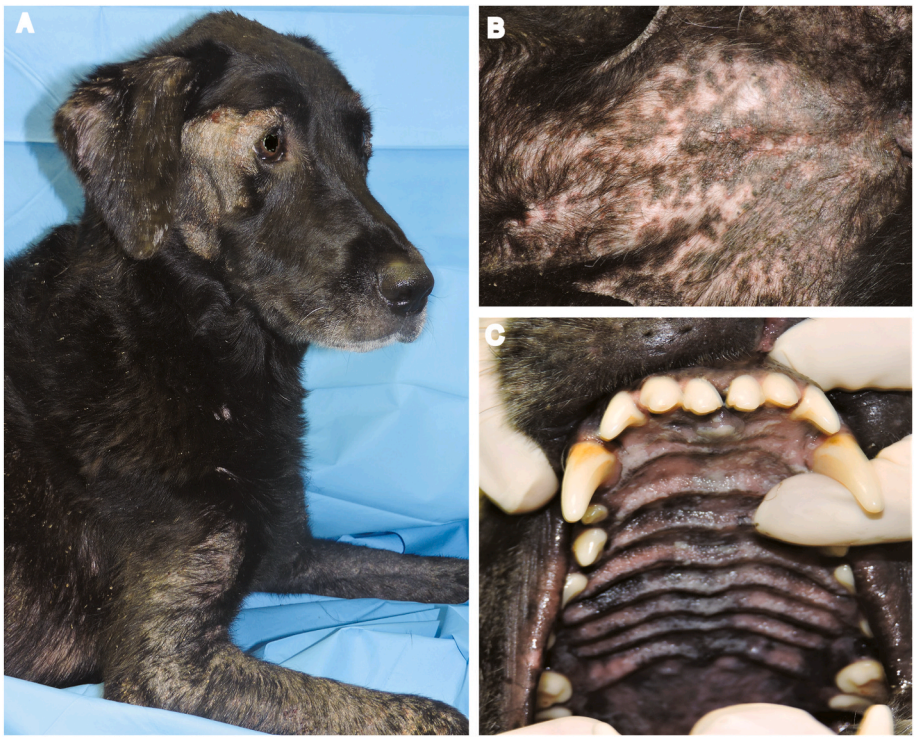

Fig. 2. Macroscopic lesions: pustular and crusting dermatitis in a Labrador retriever. Alopecia and crusts affect the face, ears, and legs (A); pustules and mottled hyperpigmentation are observed in the abdomen (B), and a whitish-gray exudate in the oral cavity (C).

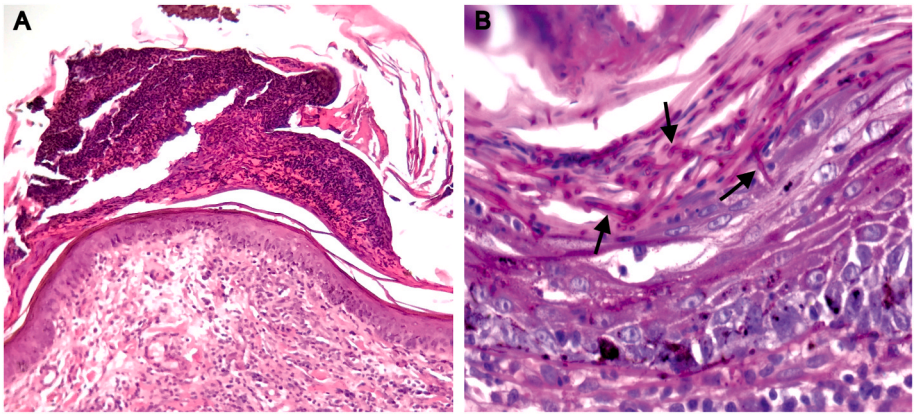

Fig. 3. Dermatopathological findings. Serocellular crusts with a prevalence of degenerated neutrophils associated with hyperkeratosis, moderate epidermal hyperplasia, dermal edema, and perivascular to interstitial lymphoplasmacytic dermatitis (A). Hematoxylin-eosin (10x). Elevated numbers of PAS-positive pseudohyphae (black arrows) in the stratum corneum (B). 400x

cutaneous and mucocutaneous generalized candidiasis after extended thymectomy have never been described in dogs.

C. albicans is the main pathogen involved in cutaneous and systemic candidiasis although other *Candida* species have also been reported [6–8]. Even if fungi of the genus Candida are normally considered commensal organisms of both human and animals' microbiota, the immune status of the host and the opportunistic fungal pathogenicity might have an impact on disease onset [9]. However, disseminated *Candida* spp. infection in immunocompetent dogs has also been described [10,11].

In humans, chronic mucocutaneous candidiasis (CMC) has been reported both in a comorbid state with thymoma and post-thymectomy, with a defective immune regulation that can increase susceptibility to disseminated disease [4,5]. Moreover, there is evidence of an adult-onset acquired immunodeficiency characterized by thymoma and hypogammaglobulinemia whose clinical constellations are highly heterogeneous, ranging from various infections to concurrent autoimmune disorders, and with a diagnosis that remains challenging [12,13]. $CD4^+$ T cells have been thought to play a role in both the resolution and worsening of superficial and invasive fungal disease, with Th17 cells, a CD4 subtype, essential for preventing mucosal fungal infection [14,15].

In veterinary medicine, thymomas represent the second most common mediastinal mass in middle-aged and older dogs with Labrador retrievers, as in this clinical case, over-represented [2,16]. In our patient, a generalized yeast disease due to a dysregulation in the immune system after thymectomy, thus mimicking the disease in humans, was hypothesized. However, no blood alterations such as hypogammaglobulinemia were detected when the dog had thymoma or after thymectomy, and T-cell response by $CD4^+/CD8^+$ ratio also was within normal limits. However, it is interesting to note that age-, breed-, and perhaps

also gender-related differences in lymphocyte subset distribution in the peripheral blood of dogs have been documented [17]. Accordingly, our patient ratio results may be considered poor support by itself to assess the immune response of the patient.

Myasthenia gravis (MG) is often a presentation of thymomas [18]. Even though it was suspected in this case because of the initial presentation, no ancillary diagnostic investigation such as acetylcholine receptor (AChR) antibody test was performed. However, after thymectomy, a progressive resolution of all related clinical signs occurred, strongly suggesting the possibility of acquired MG in our patient.

Terbinafine is an allylamine antifungal drug approved to treat fungal infections in humans. Moreover, several studies have demonstrated its efficacy against non-dermatophytic infections including azole-resistant candidiasis [19]. It is not registered for systemic use in dogs but there is evidence of its usefulness in several fungal conditions such as in Malassezia dermatitis [20].

In conclusion, our patient developed a cutaneous and mucocutaneous candidiasis that was suspected of being related to thymectomy because of a thymoma. Systemic anti-fungal treatment and topical therapy were useful in controlling the yeast infection. However, clinical relapses occurred, confirming a *Candida* spp infection. Although it was not possible to detect any immunologic dysfunction, close surveillance of the patient was incumbent.

Declaration of competing interest

There are none.

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