NOTE ***Pathology***

Thymoma and Multiple Thymic Cysts in a Dog with Acquired Myasthenia Gravis

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(Received 11 January 2002/Accepted 20 March 2002)

ABSTRACT. An anterior mediastinal cystic lesion in an 11-year-old mongrel dog was examined. The dog showed dysbasia and vomiting due to megaoesophagus, and anterior mediastinal round mass lesion, approximately 35 mm in diameter, was found by X ray. Based on clinical examinations, the dog was diagnosed as acquired myasthenia gravis and was successfully controlled by anticholinesterase treatment for approximately 4 months. The dog died of thermic stroke and was necropsied. Grossly, fatty tissues with cysts containing yellowish fluid and white nodules were found in the anterior mediastinal area. Histopathologically, multiple cysts, neoplastic tissues, and atrophic thymus were found within the examined tissues. The cysts were lined by thin wall consisting of ciliated long cuboidal and non-ciliated round cells and were filled with eosinophilic colloidal fluid. Some extended cysts contained neoplastic foci within their lumen and walls. The neoplastic tissues consisted of mixed population of large epithelial cells with abundant clear cytoplasm and large oval nuclei, and lymphocytes. Immunohistochemically, proliferating epithelial cells were intensely positive for keratin and cytokeratin, and more than half number of infiltrating lymphocytes were intensely positive for CD3 suggesting T cells. All these findings indicate the neoplastic lesion is thymoma and multiple cysts are considered as thymic or brachial cleft cysts.

KEY WORDS: canine, thymic cyst, thymoma.

J. Vet. Med. Sci. 64(7): 637–640, 2002

Thymomas are rather common tumors in the anterior mediastinal area of dogs [2, 3, 5, 7, 16], and some are associated with acquired myasthenia gravis and autoimmune paraneoplastic syndrome [6, 8, 10, 12, 17, 18, 22]. The clinical and pathological features of thymomas in domestic animals are well documented. Histopathologically, thymomas are characterized by mixed population of thymic epithelial cells and lymphocytes, and can be classified into epithelial dominant, lymphocyte dominant, and mixed type. On the other hand, several cystic changes including brachial cleft, thymic, bronchial, and esophageal cysts, may appear in the anterior mediastinal area and most of them are originated from the endoderm of the 3rd and 4th pharyngeal pouches. These cystic lesions are broadly classified according to their morphological features of the cyst walls and location. Interestingly, there are a few case reports of human thymomas arising in the wall of these thymic cysts [4, 13, 19], which are distinguished from cystic changes of thymomas.

The present paper describes the morphological features of multiple cysts containing neoplastic foci mimicking mixed type thymoma in the anterior mediastinal area of a dog suffering from acquired myasthenia gravis. The relationship between thymoma and multiple cysts is discussed.

A 11-year-old mongrel male dog started to show dysbasia, vomiting, and bad appetite on March 12, 2001, and was presented to a private animal hospital. Since the dog had typical clinical features of acquired myasthenia gravis, anticholinesterase drug, endrophonium chloride, was injected to confirm the diagnosis. By endrophonium chloride injection, apparent amelioration of dysbasia was observed. In addition, X-ray examination revealed the presence of mass lesion in the anterior mediastinal area and megaesophagus. Needle biopsy of the anterior mediastinal mass revealed a large number of neutrophils, macrophages, and a few mesothelial cells and mast cells, but no neoplastic cells were detected. Diagnosis of the anterior mediastinal mass was not made at this time. Based on these findings the dog was clinically designed as acquired myasthenia gravis associated with some thymic lesions. The dog was successfully controlled for 134 days by anti-cholinesterase drug, pyridostigmine bromide, and predonisolone. The dog suddenly died of thermic stroke on July 25, 2001. Immediate necropsy at the hospital revealed diffuse pulmonary hemorrhage and fatty tissues in the anterior mediastinal area measured by 9.0 $\times$ 2.0 $\times$ 0.8 cm. Whole the anterior mediastinal tissue was fixed by 10% formalin for further pathological examinations.

The fixed tissue contained multiple cysts ranging from 2 to 10 mm in diameter. Some cysts were filled by yellowish pink fluid and some contained yellowish white solid nodules (Fig. 1). Paraffin sections of 2 to 4 μm thick were made and stained with hematoxylin and eosin (HE) and periodic acid Schiff (PAS) for routine histopathological examination. Immunohistochemical analysis was performed using Envision polymer reagent (Dako-Japan, Kyoto, Japan). As primary antibodies, rabbit antisera against human CD3 (1:40, Dako-Japan), keratin (wide range, prediluted, Dako-Japan), and mouse monoclonal antibody against cytokeratin (prediluted, Nichirei, Tokyo, Japan) were used. By routine histological examination, multiple cysts (Fig. 2), neoplastic tissues within cyst, and atrophic thymus were observed in submitted anterior mediastinal tissue. The cysts were lined by thin wall consisting of single to double layer of ciliated cuboidal and non-ciliated round epithelial cells (Fig. 3) and

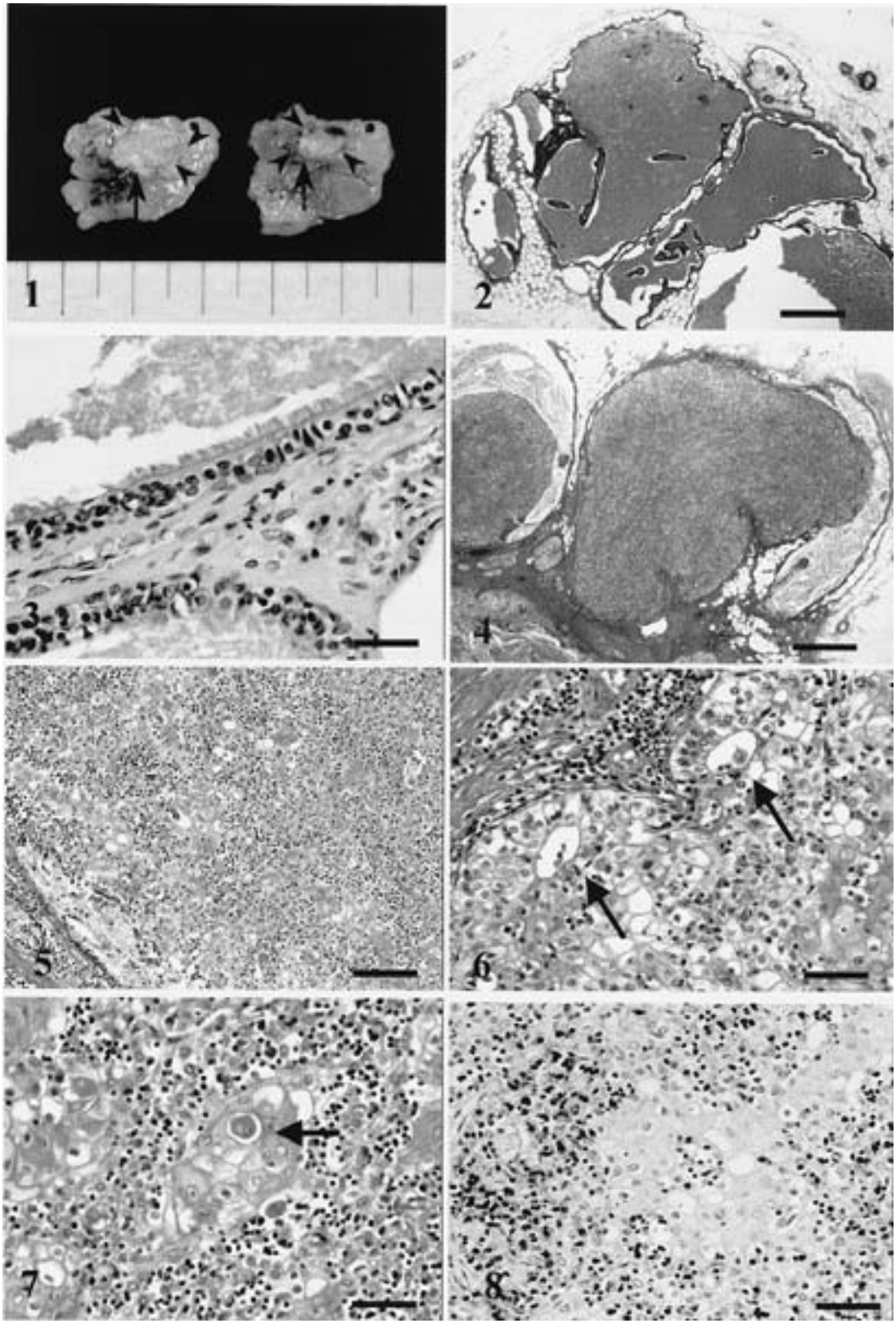
the lumen was filled with eosinophilic colloidal fluid. Immunohistochemically, the ciliated epithelial cells were negative for keratin and cytokeratin, although non-ciliated epithelial cells were intensely positive for both antigens. Some largely extended cysts contained neoplastic tissue within the lumen and their cyst walls (Fig. 4). The neoplastic tissues consisted of mixed proliferation of large epithelial cells with abundant clear cytoplasm and large oval nuclei and lymphocytes (Fig. 5). The large epithelial cells sometimes formed glandular structures (Fig. 6) and sometimes showed single cell keratinization representing as eosinophilic spheroids (Fig. 7). By immunohistochemistry, proliferating epithelial cells were intensely positive for keratin and cytokeratin. In addition, infiltration of lymphocytes was abundant within the neoplastic foci, and more than half number of infiltrating lymphocytes was intensely positive for CD3 suggesting T cells (Fig. 8). Within the lumen of large cysts or around the neoplastic foci, there were abundant granulomatous areas consisting of accumulation of macrophages with yellowish brown pigments and cholesterin deposits.

The clinical features including megaesophagus and myasthenia gravis, were quite typical for the patients with thymoma [6, 9, 10, 12, 14, 17, 18, 22]. The present dog was clinically diagnosed as acquired myasthenia gravis before pathological examinations and was successfully controlled by anti-cholinesterase treatment. This fact indirectly suggests the present dog might be involved in autoimmune condition mediated by autoantibodies such as for acetylcholine, titin, or ryanodine receptors associated to thymic lesions [9, 18]. Since serological analysis to detect autoantibody was not performed in this dog, the real pathogenesis of myasthenia gravis remained unknown. However, these clinical signs were very informative for pathological diagnosis of thymomas or related thymic changes including thymic cysts.

The neoplastic lesions are considered as mixed type thymoma consisting of solid proliferation of thymic epithelial cells and lymphocytes. Glandular differentiation of large clear epithelial cells is unique morphological character of the present case, although similar changes have been also reported previously in canine thymomas [1, 15, 21]. Occasional keratinization of the epithelial cells might imitate Hassall's bodies that are specific feature of thymus and its tumors. Moreover, most of the infiltrating lymphocytes showed intense reactivity for CD3, suggesting T cells. Thus, all these morphological features are consistent with those of canine thymomas [2, 3, 5, 7, 16]. Interestingly all neoplastic foci were formed within the lumen and wall of largely extended cysts. Cystic changes of thymomas were sometimes recognized in animals and humans and some were reported as cystic thymomas [8, 11]. Recently, Sugio *et al.* [19] reported a case of thymoma arising in the wall of the thymic cyst in a 77-year-old woman. In addition, several malignant thymic lesions arising from the thymic cysts have been reported in humans [4, 13]. In the present dog, there were multiple cysts varied in size in the anterior mediastinal area, together with atrophic thymus and some large cysts contained neoplastic lesions. It would be very difficult to decide whether present thymoma was arisen from the thymus with non-neoplastic cyst formation, or originated from the cyst walls. Since almost all neoplastic foci were recognized within the lumen and wall of large cysts, the present thymoma would be considered to arise in congenitally formed thymic cysts that related to brachial cleft tissues. The epithelial cells of these cysts may differentiate to thymic epithelial cells when they start to proliferate forming thymic tumors. In addition, some neoplastic epithelial cells may retain enough functional ability to mediate T cell-differentiation of lymphocytes, because most infiltrating lymphocytes in the neoplastic foci were immunopositive for CD3. On the other hand, human acquired thymic cysts are described as multiocular and consisted of variously thickened walls with severe inflammation [20]. Present thymic cysts represented as multiple, although inflammatory reactions were limited within the neoplastic foci. In addition, the cysts without neoplastic lesions were lined by thin wall consisting of single to double layer of ciliated and non-ciliated epithelial cells. Thus, except for the multiple formations, the present cysts contradict to multiocular thymic cysts in humans.

In conclusion, the present paper describes a unique morphology of canine thymoma probably arising from multiple thymic or brachial cleft cysts. The present case indicates that careful consideration should be needed for the diagnosis of such cystic changes in the mediastinal area, especially in dogs with acquired myasthenia gravis or other clinical signs associated to thymomas.

Fig. 1. The cut surface of large cysts (arrow heads), approximately 10 mm in diameter, are filled by yellowish fluid and contain white solid neoplastic tissues (arrows).
Fig. 2. Multiple cysts varied in size are filled by eosinophilic homogeneous fluid. HE. Bar = 1 mm.
Fig. 3. Ciliated cuboidal and non-ciliated round epithelial cells lining the cyst. HE. Bar=20 μm
Fig. 4. Two large cysts containing neoplastic foci. HE. Bar=1 mm.
Fig. 5. Mixed population of large epithelial cells and lymphocytes within the neoplastic foci. HE. Bar=200 μm.
Fig. 6. Glandular formation of proliferating large clear epithelial cells. HE. Bar = 20 μm.
Fig. 7. Keratinization of proliferating large clear epithelial cell imitating Hassall's body. HE. Bar=20 μm.
Fig. 8. Infiltration of CD-3 positive lymphocytes within the neoplastic tissue consisting of large epithelial cells. CD-3-immunostaining. Bar=20 μm.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