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Case Report / *Olgu Sunumu*

A case of thymic lymphoma in a rabbit

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Abstract: A five-year-old male rabbit was presented with the request of necropsy. Post mortem examination revealed a mass in the cranial mediastinum. Histopathologically, it was seen that the mass consisted of a limited mesenchymal tissue with medium-sized mature lymphocytes and a large proportion (approximately 90%) of lymphoblast-like round cell infiltration. While tumour cells were stained positively for CD3, they were negative for CD79a. Pan-Cytokeratin was localized in small number of epithelial cells in the background. Histopathological and immunohistochemical evaluation showed that the case was compatible with thymic lymphoma and it was the first case reported in rabbits in Turkey.
Keywords: Immunohistochemistry, rabbit, thymic lymphoma

Bir tavşanda timik lenfoma vakası

Özet: Beş yaşlı, erkek bir tavşan nekropsi isteği ile getirildi. Yapılan nekropside kranial mediastinum bölgesinde bir kitle saptandı. Histopatolojik olarak, kitlenin sınırlı bir mezenşimal doku ile birlikte orta büyüklükte olgun lenfositler ve büyük bir bölümünün (yaklaşık % 90'ı) lenfoblast benzeri yuvarlak hücre infiltrasyonundan oluştuğu görüldü. Tümör hücreleri CD3 ile pozitif boyanmalarına rağmen, CD79a için boyanma negatifti. Pan-Cytokeratin ile arka planda az sayıda epitel hücresi gösterildi. Histopatolojik ve immunohistokimyasal değerlendirmeler sonucunda vakanın timik lenfoma ile uyumlu olduğu ve Türkiye'de tavşanlarda bildirilen ilk vaka olduğu gösterilmiştir.
Anahtar sözcükler: İmmunohistokimya, tavşan, timik lenfoma

Although primary neoplasms of the thymus are rare, thymic epithelial tumours (thymomas and thymic carcinomas) and thymic lymphomas are the most common primary tumours of the thymus. Thymoma is a neoplasm that derived from the epithelial components of the thymus and is composed of lymphoid and reticuloepithelial cells, whereas thymic lymphoma denotes that the neoplasm is of T-lymphocytic origin (14). The 2004 WHO classification system identifies four major types of thymic tumours: epithelial cell tumours (thymomas and thymic carcinomas), neuroendocrine tumours, thymolipomas and other rare tumours (neuroblastoma, ganglioneuroblastoma, malign melanoma, thymic hemangioma and myoid tumors) (12, 13). This classification is based on the cell morphology of the neoplastic epithelial cells and the ratio of non-neoplastic lymphocytes (14). Thymic tumours in animals are classified based on the predominant cell population within the mass (lymphocytic, epithelial or lymphoepithelial) (1, 14). This classification is important because the treatment and prognosis may vary according to the cell type observed in cytology or histopathology (15). Immunohistochemistry is often necessary to confirm the presence of neoplastic thymic epithelial cells in lymphocyte rich-thymoma and to differentiate thymoma from the more common mediastinal lymphoma in most animals (8). The lymphoid tumours of the cranial mediastinum are most commonly precursor T lymphoblastic lymphomas seen in cats, cattle and less commonly dogs. Thymic lymphoma is seen in young beef breeds in cattle, and it is thought to be unassociated with bovine leukaemia virus infection (14). The purpose of this study is to describe a thymus lymphoma that spontaneously occurred in a five-year-old male rabbit using immunohistochemical techniques. To the best of the author's knowledge, this is the first described case of thymic lymphoma in a rabbit in Turkey.

A five-year-old male Angora rabbit was presented with the request of necropsy by the animal's owner. The

owner declared that the rabbit was generally weak and depressed before dying; it was unable to breath normally and died within two days. The systemic necropsy revealed a large mass in the cranial mediastinum extending from the second to the sixth ribs with numerous necrosis and petechial haemorrhages (Figure 1). The mass was yellowish-grey with irregular lobulated areas of infarction with hyperaemic borders. No lesion was found in other organs except a caudodorsal displacement of the lungs. There was no evidence of metastatic lesions in other organs.

Tissue samples were collected from the rabbit during systemic necropsy. Then samples were fixed in 10% neutral formalin at room temperature. Afterwards, they were dehydrated with graded series of ethanol, embedded in paraffin and cut using microtome (4-5 μm). Tissue samples were stained with Haematoxylin-Eosin. To further characterize the mass, the sections were also stained immunohistochemically by the avidin-biotin-peroxidase complex technique. Briefly, after deparaffinising with xylene and rehydrating through a graded ethanol series, sections were immersed in 10 mM citrate buffer pH6.0 and heated in microwave oven at 560W for 15 min to retrieve antigens. Endogenous peroxidase activity was quenched by treating slides with 0.3% hydrogen peroxide in methanol. Then the sections were coated with 10% goat serum (50062Z, Life technologies, USA) for reduce nonspecific binding, and incubated with primary antibodies. The following primary antibodies were used: mouse monoclonal anti-CD3 (Biorbyt-orb323391; dilution 1:100), mouse monoclonal anti-CD79a (Thermo Fisher Scientific-13212; dilution 1:400) and mouse monoclonal anti-Pan-Cytokeratin PCK26 (Merck-C1801; dilution 1:300). Following incubation of slides with biotin-labelled secondary antibody (biotinylated goat anti-mouse IgG BA-9200, Vector Laboratories, Burlingame, CA, USA) at 1:100 dilution, a streptavidin-peroxidase Vectastain ABC-peroxidase kit was applied according to the manufacturer's instructions (Vector Laboratories, Burlingame, CA, USA). Immune reactions were visualized with the Liquid DAB+substrate kit (K3467, Dako, North America) and the slides were counterstained with Gill's Hematoxylin.

Histopathological examination confirmed dense infiltration of medium-sized lymphocytes and 90% lymphoblast-like round cells with dispersed chromatin and indistinct nucleoli (Figure 2a). Large areas of necrosis with extensive haemorrhage were also observed in the mass (Figure 2b). While tumour cells were stained positively for CD3 (Figure 2c), they were negative for CD79a. Pan-Cytokeratin highlights small number of epithelial cells in the background (Figure 2d). CD3 staining was localized in the cytoplasmic membrane, signals for Pan-Cytokeratin were observed in the cytoplasm. In this case, neoplastic cell population was identified as T-lymphocytes by using immuno-histochemistry. In the view of the immunohistochemistry and histopathological examination, the present case was diagnosed as a thymic lymphoma.

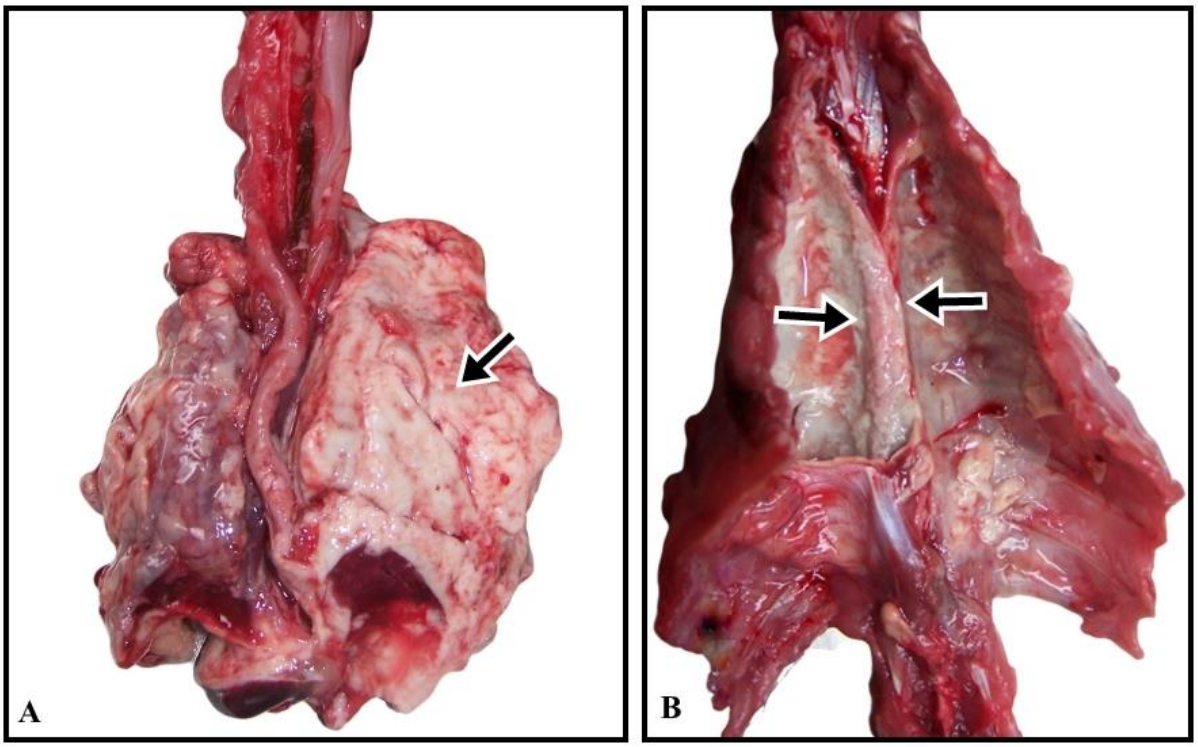

Figure 1. Systemic necropsy revealed a large mass in the cranial mediastinum. The overview of numerous petechial haemorrhages (arrow) (**A**), and necrosis in the thoracic cavity (arrows) (**B**).

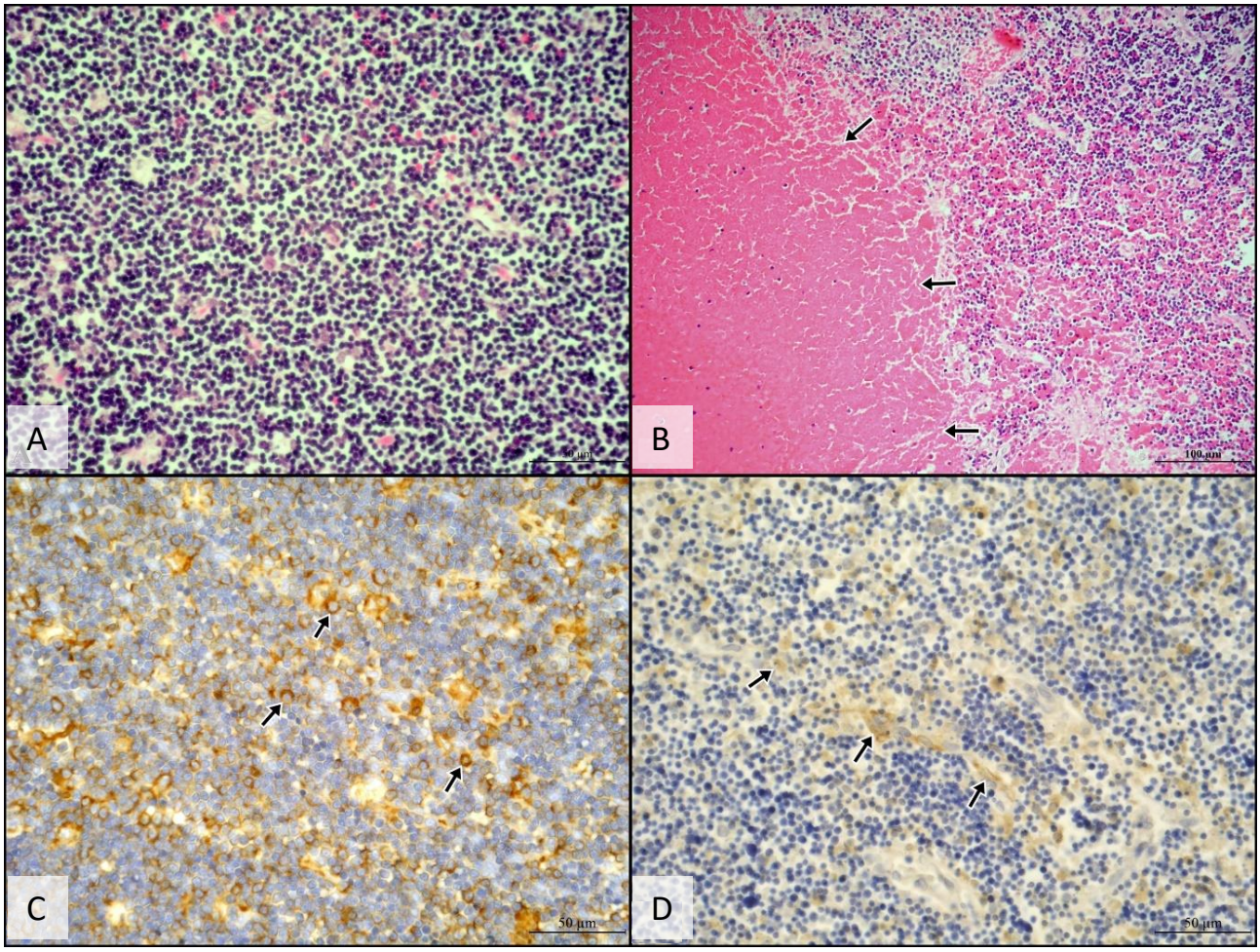

Figure 2. A. Dense infiltration of medium-sized lymphocytes and 90% lymphoblast-like round cells with dispersed chromatin, H&E, Bar: 50µm, **B.** Large areas of necrosis with extensive haemorrhage (arrows), H&E, Bar: 100µm, **C.** Lymphocytes demonstrate ubiquitous immunoreactivity with CD3 (arrows), CD3, Bar: 50µm, **D.** Immunohistochemistry for Pan-Cytokeratin highlights small number of epithelial cells in the background (arrows), Pan-Cytokeratin, Bar: 50µm.

Thymoma and T lymphoblastic lymphoma are both seen in the cranial mediastinum, and it is sometimes difficult to distinguish between these two lesions because of the similarity of thymoma-associated immature lymphocytes to T lymphoblastic lymphoma cells (7, 9). The presence of neoplastic epithelial cells in thymoma is the most important clue for differentiating thymoma from T lymphocytic lymphoma. The pathogenesis of T lymphoblastic lymphoma is still unknown, but there are two theories: (1) that the lymphocytes in thymoma may have transformed into T lymphocytic lymphoma (4, 10) and (2) that the incidental T lymphocytic lymphoma occurred and infiltrated into the thymoma (10). In the presented case, although the possibility of T lymphocytosis may be considered, the presence of cells with larger, more irregular nuclei and scattered keratin is suggestive of T lymphocytic lymphoma.

Allan et al. (1) diagnosed a T lymphocyte-rich thymoma based on the histological appearance and immunoreactivity components of a mass in the cranial thoracic region of a 10-year-old Siberian tiger. Although CD3 positive T lymphocytes constituted a large part of the tissue, they reported that the tumour was of thymic origin because of the neoplastic epithelial cells. In the present study, the case is considered to be a thymic lymphoma because the cells showed no staining for CD79, weak positivity for Pan-Cytokeratin and stained strongly for CD3. This is also in accordance with the study made in rabbit in which strong CD3 staining and rare positive staining for CD79a was observed in the neoplastic lymphocytes in thymic lymphoma (11).

The classification of thymomas is based on two main histological thymoma phenotypes showing benign cellular characteristics. The type A thymoma consists of spindle-shaped cells, while the type B thymoma consists of epithelioid cells. Tumours consisting of spindle-shaped cells and epithelioid cells are called AB-type thymoma. Type B thymomas are also subdivided into B1, B2 or B3 subtypes. Type B1 thymomas are very difficult to diagnose because they are very similar to normal thymus

and have a density lymphocyte population that can be misdiagnosed as lymphoid neoplasm (7, 14). Among the most characteristic features of type B1 thymomas are randomly distributed pale-stained medullary differentiation foci that can be confused with pale staining centres seen on low magnification in chronic B cell lymphocytic leukaemia. However, the epithelial cells with large ovoid nuclei and small nucleoli are easily recognizable in the background on high magnification (14). In the present case, the presence of small number of Pan-Cytokeratin-positive epithelial cells and the appearance of scattered keratinous and lymphoblast-like cells did not suggest type B1 thymoma.

Thymus tumours, as well as other diseases seen in the thymus, increase the likelihood of one or more autoimmune disorders, especially myasthenia gravis (1, 14). In the presented case, no evaluation could be made on this subject because the rabbit was dead. Thymoma-induced paraneoplastic syndromes in rabbits and cats are exfoliative dermatitis lesions affecting the face, neck and dorsum (2). Florizoone et al. (5) have reported the presence of thymoma-associated paraneoplastic dermal lesions in the superficial dermis in rabbits characterised by lymphocytic infiltration, lymphocytic folliculitis, and the absence of sebaceous glands. In the present case, contrary to previous research findings, paraneoplastic lesions associated with tumours were not found in the rabbit. According to Gupta (6), the lack of natural lymphoma reports in rabbits may be due to insufficient research interest because most animals are usually euthanized before these tumours develop, or there could be a reduced natural sensitivity in rabbits.

Although it was not possible to use them in the present case, there are some studies reporting that multimodal diagnostic approaches involving histological, flow cytometry immunophenotypic and molecular approaches are required for definitive differential diagnosis between thymoma and T lymphoblastic lymphoma in human medicine (3, 7).

To the best of the author's knowledge, this was the first case reporting thymic lymphoma in rabbits in Turkey, and further research is needed to characterise these tumours.

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Ethical Statement

This study does not present any ethical concerns.

Conflict of Interest

The authors declared that there is no conflict of interest.

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