Mixed cutaneous round cells tumor in a cock (*Gallus domesticus*): A case report

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Abstract:

Cutaneous round cell tumors have been classified as mast cell tumor (MCT), histiocytoma (HCT), lymphosarcoma, undifferentiated round cell tumors and occasionally rhabdomyosarcoma in veterinary medicine. An adult cock (*Gallus domesticus*) showing a large solitary integument mass raised on dorso bilateral of cervical part, extending to intercapsular and cranial mid part of the back was referred to the birds clinic of the faculty of veterinary medicine at the university of Tehran. Microscopic examination revealed sheeted cells with large, round to oval nuclei with each one containing one or more prominent nucleoli with scant cytoplasm. The myofibrils of the neck were degenerated by aggressive tumor cells. The condition was differentiated from other round cell tumors by electron microscope, histochemical staining, as well as the application of a large panel of antibodies. Polymerase chain reaction failed to confirm the involvement of both Marek's disease virus and avian leukosis virus subgroup-J. It was concluded that the tumor cells were consistent with both B-cell lymphocytes and histiocytes that unusually covered the entire dermal layer of dorsal neck skin. This unusual cutaneous lymphoma was named as lymphoblastic histiocytoma.

Case history

Cutaneous round cell tumors have been classified as mast cell tumor (MCT), histiocytoma (HCT), lymphosarcoma, undifferentiated round cell tumors and occasionally rhabdomyosarcoma (Fernandez-Bellon et al., 2003; Fernandez et al., 2005) in veterinary medicine. Lymphosarcoma has been subdivided into two groups as T-cell lymphosarcoma (TCLA) and B-cell lymphosarcoma (BCLA). The plasmacytoma (PCT) is also known a subgroup of the BCLA. However, the definite diagnosis of similar histopathologic morphology without application of differential diagnostic tests like histochemical staining and immunohistochemistry, especially in poorly differentiated tumors, is almost impossible. Most of the antibodies now utilized in immunohistochemistry of animal samples have been originat-ed from human sources but have shown reliable and valuable results in differential diagnosis of animal round skin tumors (Copie-Bergman et al., 1998; Fernandez et al., 2005).

Clinical presentation

A 15-months-old cock (*Gallus domesticus*) with a widespread and raised surface mass on the dorsal part of neck and back was referred to our birds clinic in the faculty of veterinary medicine, university of Tehran. The cock was being kept with several other chickens in a free-range grazing environment and fed with a supplementary diet of pellets and millet. The bird's general condition was good, only an unfeathered, large solitary, firm, and raised (~ 3 cm) dermal mass was noticed. According to the owner, other birds were not suffering from such description. On cut surface, the tumor mass was intradermal or subcutaneous, well-encapsulated, and non-adherent to adjacent epithelial tissues. It was a firmed, slight rubbery, and glistening with gray/white colour covered by unfeathered thickened epidermis.

Diagnostic testing

Pathology. A biopsy of thick section was fixed in 10% neutral buffered formalin, routinely processed and embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin (HE), Toluidine blue (TB), and Giemsa. For immunohistochemical analysis, the following antibodies were applied in appropriate dilutions on the sample sections: mouse monoclonal antibodies (MoAb) to CD1a (Dako, Glostrup, Denmark), CD20 (Dako), CD45 (Leucocyte common antigen, Dako), CD68 (Dako), CD79a (Dako), IgG (clone A57H, Dako), IgM (clone R1/69, Dako), desmin (clone D33, Dako), myogenin (Dako), vimentin (clone V9, Dako) and rabbit polyclonal antibodies (PoAb) to alpha-1-antitrypsin (Dako), lysozyme (Dako) and S100 (Dako), using the labeled strptavidin-biotin method (LSABTM Kit, Dako). The slides were counterstained with Mayer's hematoxylin. The specificity of CD20 and CD79a antisera was ascertained in normal thymus and bursa of Fabricius of chicken. For transmission electron microscopy (TEM), small pieces of paraffin embedded tissues were post-fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) and 1% Osmium tetroxide in 0.1 M phosphate buffer (pH 7.3), embedded in epon. The thin sections were stained with uranyl acetate and lead citrate and examined with a Philips 208S electron microscope. All immunohistochemical and electron microscopic examinations were performed in the Cancer Institute of Tehran's Imam Khomeini Hospital.

PCR for Marek's disease virus (MDV) and avian leukosis virus-J (ALV-J): for detection of MDV, vaccine strains of CVI988 (serotype enseignement-MDV1) and non-pathogenic turkeys HVT FC 126 (serotype 3- MDV3) were obtained from commercial vaccine vials. These viruses were used for the extraction of total cellular DNA. A piece of tissue sample was also used for DNA extraction. Total extracted DNAs were used for PCR to amplify a part of glycoprotein A gene using primers described previously (Zhu et al., 1992). PCR reaction mixture (50 µl) included 5 µl of 10 x PCR buffer containing 1.5 mM MgCl2 (Roche, Germany), 250 ng of each primer (TIB, Germany), 2mM of dNTPs (Roche), 0.5 U of *Taq* DNA polymerase (Roche), and sterile dinonized water. Template DNA was added to the reaction mixture at a concentration of 0.5-2 µg. The amplification process was performed in a thermocycler (Eppendorf, Germany) and consisted of one cycle at 94°C for 3 min, 94°C for 30 s and 72°C for 30 s, repeated for 30 cycles, and followed by a cycle at 72°C for 10 min. The Amplified products were electrophoresed, stained with ethidium bromide, visualized by UV transluminator, and photographed (Sambrook et al., 2001). For the detection of ALV-J, the procedures described by Rajabi et al., 2009 was followed.

Assessments

In histopathological examination, the tumor was found as diffuse infiltrate of closely packed, large pleomorphic cells, most of which had a thin rim of cytoplasm with well-defined margin and strikingly pale round or ovoid vesicular nuclei. The chromatin was more concentrated at the nuclear membrane and most of the nuclei contained a single large prominent central but ocassionally eccentric nucleolus (Figures 1,2). Mitotic figures were numerous. The disintegrated dense collagen bundles dispersed throuought the lesion. Most of the thin-walled blood vessels were remarkabley dilated but without any evidence of the presence of tumoric cells. The skeletal muscles of the neck were splitted by invasive tumor cells. In the deep portion of dermal layer (Figure 2), there were

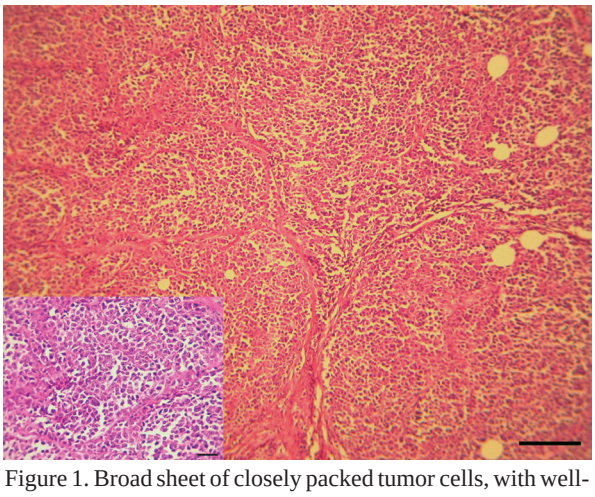

Figure 1. Broad sheet of closely packed tumor cells, with well-defined cytoplasmic borders (HE Bar = 200 µm). Inset: The same micrograph under higher magnification. The tissue consists of variable shaped of tumor cells with well-defined boundaries. Mitotic figures are also present. (HE Bar = 30 µm).

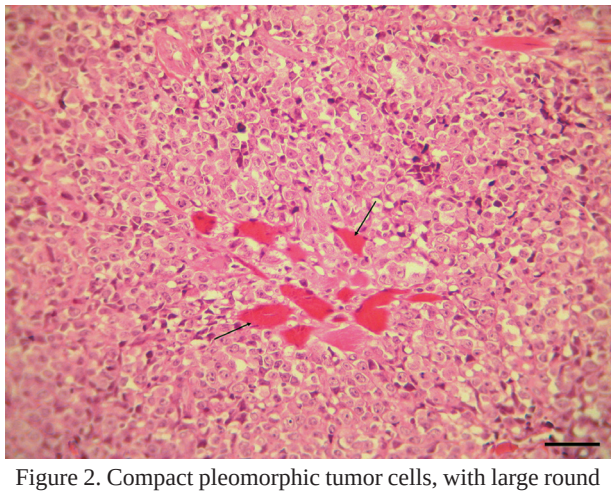

Figure 2. Compact pleomorphic tumor cells, with large round or oval vesicular nuclei each containing one or more basophilic nucleoli. The amount of pale eosinophilic cytoplasm is small and well-defined. They infiltrated into dermal layer of skin and splitted the muscle fibers (arrows). (HE Bar = 30 µm).

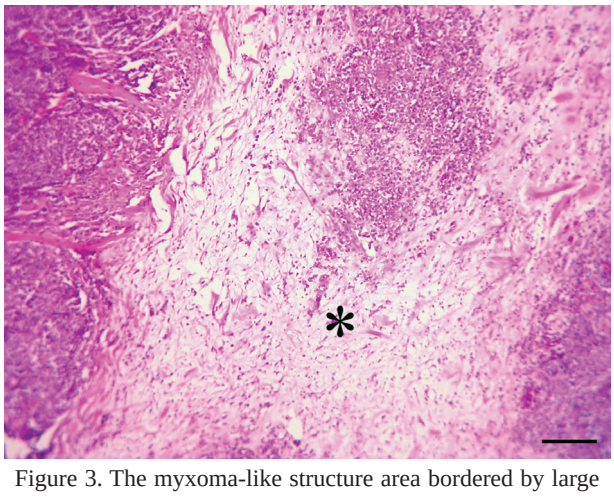

Figure 3. The myxoma-like structure area bordered by large population of neoplastic cells (astrisk).(HE Bar = 200 µm).

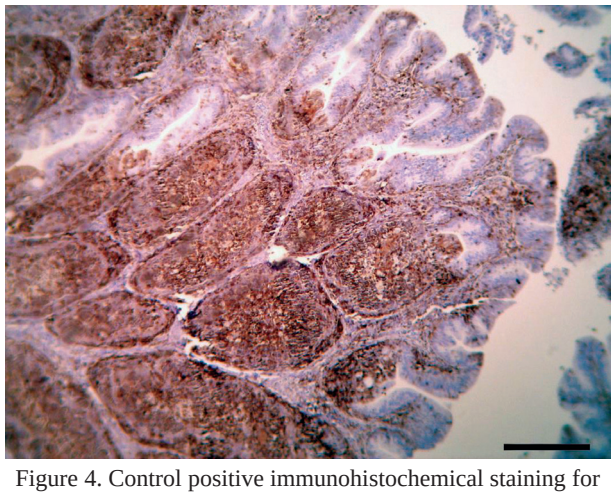

Figure 4. Control positive immunohistochemical staining for CD20 in normal bursa of Fabricius. SAB immunolabelling, Mayer's hematoxylin counter stain. (Bar = 200 µm).

myxoma-like structures, rimmed by tumor cells (Figure 3). The tumor cells of this pattern were scattered in transparent mucinous stroma.

Histochemical results showed that both Toluidine blue and Giemsa stains were negative for the presence of metachromatic cytoplasmic granules. Immunohistochemical staining of normal thymus and bursa of Fabricius revealed that thymic cells were negative for both anti CD79a and CD20. Lymphoid cells in the bursa stained strongly with CD20 (Figure4), but were negative for CD79a. Most of neoplastic cells intensively reacted with anti-CD20 antibody (Figure 5). A low population of neoplastic cells, dispersed all over the tissue, also immunolabelled against anti lysozyme (Figure 6). No immunoreaction was found in the third population of cells for any markers. The splitted muscles were noticed in histopathological examination labeled for both desmin and myogenin as internal control (Figure 7).

In TEM, it was found that the tumor was composed of two distinct but inter mingled cell populations. The first group that included almost majority of the cells, were characterized by lack of intercellular junctions, basal lamina, and tonofilament. Cytoplasm showed few organelles and their nuclei contained mainly disaggregated chromatin with a minimal heterochromatin mostly associated with the inner nuclear membrane with one prominent nucleolus. The second group of cells were also non-cohesive and conatined more abundant cytoplasm

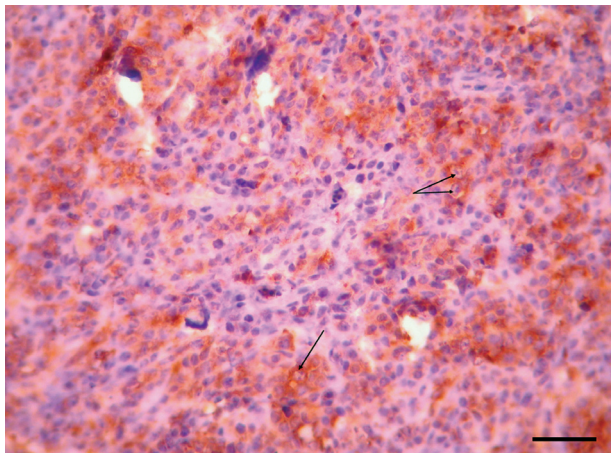

Figure 5. The large number of cells strongly immunostained with CD20 (arrows). The other cells in this field with the same morphological structure are not reacted to this marker. SAB immunolabelling, Mayer's hematoxylin counter stain. (Bar = 30 µm).

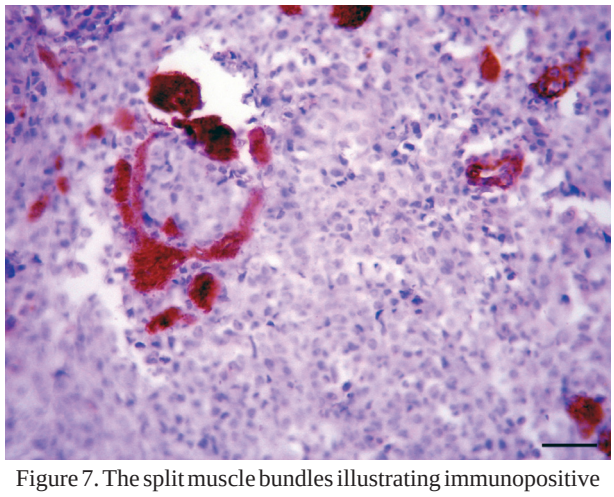

Figure 7. The split muscle bundles illustrating immunopositive reaction for desmin. SAB immunolabelling, Mayer's hematoxylin counter stain. (Bar = 30 µm).

than the former cells. The second group of cells showed smooth plasma membranes and large irregular nuclei with often markedly convulted nuclear membranes and finely clumped marginated heterochromatin. Nucleoli which were usually large and frequently multiple.

Amplification of the MDV-1 gA gene sequence of non-pathogenic serotype MDV-enseignement vaccine virus (CVI988-Rispens vaccine) gave a positive result of DNA amplification with the expected size of 200bp, using MDV-gA1 primers. No amplified product was observed when DNA template was from tissue specimen, HVT strain, or negative control (uninoculated chicken embryo fibroblast cell culture). When HTV-gA-3 primers were used in PCR, a 388 bp

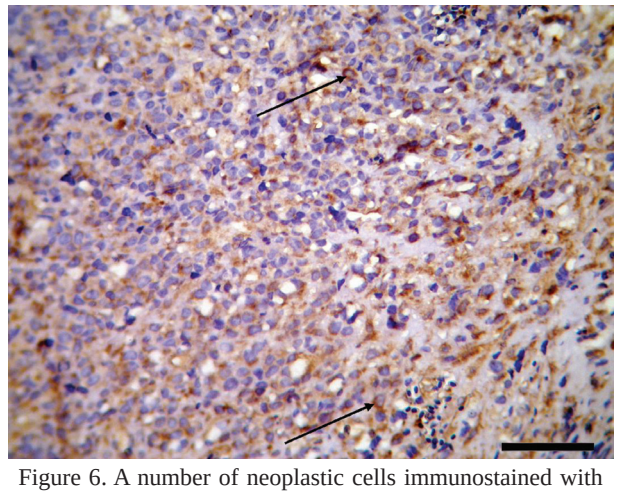

Figure 6. A number of neoplastic cells immunostained with lysozyme (arrows). SAB immunolabelling, Mayer's hematoxylin counter stain. (Bar = 30 µm).

PCR product was detected with DNA template from HVT strain but not with DNA template from tissue specimen and uninoculated chicken embryo fibroblast cell culture. Attempt to detect the ALV-J target gene, also, failed to show the avian leukosis virus subgroup-J.

Based on the above findings and our differential diagnosis methodoloy, we concluded that these types of tumor cells were consistent with B-cell lymphocytes that have unusually covered the entire dermal layer of dorsal neck skin. (Fernandez et al., 2005), previously, classified cutaneous round cell tumors in dogs as mast cell tumor, histiocytoma, T cell or B cell lymphosarcoma, plasmacytoma, and unidentified round cell tumors with utilization of immunohistochemical markers. Mastocytoma can be differentiated by histochemical staining for metachromatic granules and by TEM observation for the presence of secretory granules found in mast cells (Swayne and Weisbrode, 1990; Hafner and Latimer, 1997). In addition to the neoplastic histiocytes in histiocytoma that express MHC class II and CD18, both B and T lymphocytes are also immunopositive to these markers (Fernandez et al., 2005). Therefore, with anti-CD20 positive staining for tumor cells, in this case with extreme caution, histiocytoma or even cutaneous histiocytic lymphoma, and malignant histiocytosis that must all express the mono/histiocyte markers and show no reaction to lymphoid markers, were excluded (Fernandez et al., 2005). At this point in time, none of these tumors have been reported in poultry related literature.

Diffuse immunolabeling of lysozyme, which was demonstrable in many cells, revealed that many cells characteristically were consistent with histiocytes. But it was impossible to determine wether the cells that stained for lysozyme were infiltrating histiocytes or neoplastic cells. Lysozyme and alpha-1-anti-trypsin are both well characterized immune-markers of mononuclear phagocyte differentiotion in human beings. Moore and Rossin (1986), in a retrospective case series of malignant histiocytosis of dogs, demonstrated the reaction of lysozyme with all neoplastic histiocytes whereas only half of the tumor cells were identified for alpha-1-antitrypsin. This means that lysozyme is more reliable for evaluation of normal or tumor histiocytes than alpha-1-antitrypsin.

Although we used anti-CD1a, the most applicable marker for identifying of histiocytic cells in formalin-fixed, paraffin-embedded (FFPE) tissue, this marker is exclusively valuable in human samples and was not able to detect any canine cells expected to be CD1a positive in animals (Fernandez et al., 2005).

Lymphoproliferative lesions in Marek's disease may also be misdiagnosed with the type of lesion in this report. However, our PCR results were negative for MDV and on the other hand, in MD, mainly, the feather follicles are involved and contain intranuclear inclusions in the epithelium of feather follicles (Silva, 1992; Zhu et al., 1992; Schat and Venugopal, 2008).

Other tumors that are histologically very similar to the neoplastic round cells and should be excluded, are rhabdomyosarcoma that might be seen as subcutaneous mass (Fernandez-Bellon et al., 2003) and plasmacytoma (Fernandez et al., 2005). However, the tumor cells in the former are negative for both myogenin and desmin, and in the later, most of the cells have been shown to be CD79a positive in dogs (Fernandez et al., 2005). Furthermore, the ultrastructural features such as eccentric nuclei and prominent rough endoplasmic reticulum (rER) which are found in normal plasma cells, and also PCT, were not obsereved in our diagnostic electron microscopy (Dardick et al., 1996)

The tumor cells that were negative to all stainings may indicate an undifferentiated or poorly stage of development. Three weeks after skin biopsy, the cock died and it was not submitted to our clinic for necropsy procedures to examine the possible metastasis to other organs.

Determination of the origin of the tumor cells is essential for the diagnosis of histiocytoma and lymphoma. In the literature, several descriptions for the classification of monocyte-macrophage lineages or lymphoma, are presented as follows: histiocytic lymphoma (HL) is recognized as lymphocytic lymphoma, lymphoblastic lymphoma and non Hodgkin's lymphoma which is not a tumor of the macrophage system. In this tumor, large lymphoid cells have cytoplasmic vacuoles and are similar to macrophage tumors (Wilson and Armitage, 2008). True histiocytic lymphoma (THL) is a type of neoplasm that reacts with monocyte/histiocyte associated markers like CD68, lysozyme, alpha-1-antitrypsin and CD45, and is negative for CD1a, epithelial, and B- and T-cell lineage-specific markers (Copie-Bergman et al., 1998). Hemophagocytic lymphohistiocytosis (HLH) is a reactive process resulting from prolonged and excessive activation of antigen-presenting cells (macrophages, histiocytes) and CD8+ cells (Filipovich, 2009). In our case, the tumor consisted of two distinct cell populations of lymphoblasts and histiocytes. Therefore, to name this unusual cutaneous lymphoma, we prefer to apply the combined term, lymphoblastic histiocytoma, which indicate the presence of two different precursor cells (lymphoblast and histiocyte) in one lesion. Using other definitions, as mentioned above, is a misnomer and causes diversion of the original meaning.

In conclusion, the population of neoplastic cells revealed immunopositive staining only with CD20, which serves as a diagnostic marker for lymphoma, and with lysozyme, which serves as one of the well-known definitive markers for histiocytoma. Considering the above findings, it may be better to call the present unusual cutaneous lymphoma as lymphoblastic histiocytoma.

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تومور جلدی مختلط ناشی از سلول‌های گرد در یک خروس (*Gallus domesticus*)

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چکیده

یک قطعه خروس بالغ نژاد لاری به علت تورم زیر جلدی سراسری از ابتدای ناحیه پس سر واقع در پشت گردن تا ناحیه جدوگاه به کلینیک پرندگان دانشکده دامپزشکی دانشگاه تهران ارجاع گردید. پس از انجام بیوپسی، حضور صفحات سلولی گرد با هسته‌های کروی یا بیضوی بزرگ به همراه یک یا چند هسته برجسته درون هسته‌ها و مقادیر کم سیتوپلاسم در بررسی میکروسکوپیک جلب نظر می‌نمود. از دیگر مشاهدات ریزبینی از بین رفتن رشته های ماهیچه‌ای مستقر در ناحیه عضلات گردن توسط تهاجم سلول‌های توموری بود. با استفاده از تکنیک میکروسکوپ الکترونی، رنگ‌آمیزی هیستوشیمی و کاربرد وسیع مارکرهای ایمونوهیستوشیمی، چهره افتراقی بین انواع تومور سلول‌های گرد برقرار شد. تکنیک واکنش زنجیره‌ای پلی مراز ابتلا به بیماری مارک و لوکوز پرندگان از زیر گروه J را تأیید نکرد. در نهایت تکنیک‌های فوق نشان داد شاکله توده مزبور با منشأ دو گروه متفاوت سلولی شامل لنفوسیت‌های B و هیستیوسایت می‌باشد. با شناسایی این دو تیپ از جمعیت سلول‌های توموری این ضایعه به نام لنفوبلاستیک هیستیوسایتوما نام گذاری شد.

واژه‌های کلیدی: لنفوسیت‌های B، هیستوسایتوما، تومور جلدی، خروس، تومور سلول‌های گرد.

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