T and B immunophenotype determination using specific markers (CD3, CD79A, λ and κ light chains) in canine biopsies with suspicion of malignant lymphoma


SUMMARY

Thirty four canine biopsy specimens (lymph node, spleen, intestine and skin) with suspicion of malignant lymphoma were investigated in this study. Histopathological classification and B/T immunophenotypes were determined on the routinely processed formalin fixed, paraffin embedded tissue sections using rabbit anti-human CD3, mouse anti-human CD79a and rabbit anti-human kappa- and lambda-light chain as primary antibodies and streptavidin-biotin peroxidase complex procedure for immunolabelling. The T cell lymphomas (CD3 positive cells) constituted 61.5% (16/26) and B cell lymphomas (CD79a positive cells) 38.5% (10/26) of all immunostained specimens, whereas 8 cutaneous tumours with lymphoid-like lesions did not react with any marker of differentiation and could not be diagnosed as lymphosarcomas. Nodal and cutaneous lymphosarcomas were in majority T lymphomas (64.3 % and 62.5 % respectively) which 2 subtypes were identified based on histological criteria: non epitheliotrophic cutaneous T lymphoma in skin and T cell lymphoblastic lymphoma in lymph nodes and in spleen. Among the ten B lymphomas, 9 were positive for lambda and 1 for kappa light chain and 3 subtypes not associated with any specific tissue localisation were evidenced: B lymphocytic lymphomas (4/10), B lymphoplasmacytic lymphomas (4/10) and diffuse large B cell lymphomas (2/10). When considering the different subtypes, the 26 immunopositive neoplastic tissues consisted in 15.3% lymphocytic, 15.3% lymphoplasmacytic and 7.6% large cell B lymphomas whereas 19.2% and 42.3% comprised non epitheliotrophic and lymphoblastic T subtypes respectively. These results emphasize the interest of B and T immuno-phenotyping for the diagnosis of malignant lymphoma in dog.

Keywords: Malignant lymphoma, dog, immunophenotype, lymphocyte, CD3, CD79a, lymph node, skin.

RéSUMÉ

Détention des immunophénotypes B et T par utilisation de marqueurs spécifiques (CD3, CD79A, chaînes légères λ et κ) sur des biopsies présentant une suspicion de lymphome malin chez le chien

Trente quatre biopsies (de noeuds lymphatiques, de rate, d’intestins ou de peau) de chien présentant une suspicion de lymphome malin ont été analysées, après fixation au formol et inclusion en paraffine, en histologie et en immunohistochimie en utilisant un anticorps de lapin anti-CD3 humain, un anticorps de souris anti-CD79a humain et des anticorps de lambda et kappa comme anticorps primaires et révélation des complexes immuns formés par le complexe péroxydase-streptavidine. Les lymphomes à cellules T (lymphocytes exprimant le CD3) ont constitué 61.5% (16/26) et ceux à cellules B (lymphocytes exprimant le CD79a) 38.5% (10/26) des lymphomes malins alors que le diagnostic de lymphosarcome a été écarté sur 8 tumeurs cutanées présentant également des infiltrats lymphocytaires mais ne réagissant avec aucun des marqueurs de différenciation utilisés. Les lymphosarcomes des noeuds lymphatiques et de la peau ont été de type T en majorité (64.3 % et 62.5 % respectivement) et 2 sous-types ont été identifiés grâce aux critères histologiques : lymphome cutané non épithéliodermique spécifique de la peau et lymphome T lymphoblastique dans les noeuds lymphatiques et la rate. Parmi les 10 lymphosarcomes de type B, 9 ont produit des chaînes lambda et 1 seul des chaînes kappa ; 3 sous-types, n’admettant pas de localisation tissulaire privilégiée, ont été mis en évidence : lymphome B lymphoblastique (4/10), lymphome B lymphoplasmoctytaire (4/10) et lymphome diffuse à larges cellules B (2/10). Au total, les fréquences respectives des différents sous-types identifiés à partir des 26 tumeurs positives ont été les suivantes : 15.3% de lymphomes B lymphoblastiques, 15.3% de lymphomes B lymphoplasmoctytaires, 7.6% de lymphomes B à large cellules, 19.2% de lymphomes T non épithétopsiques et 42.3% de lymphomes T lymphoblastiques. Ces résultats soulignent l’intérêt de l’immunophénotypage B ou T dans le diagnostic de lymphosarcome chez le chien.

Mots-clés : Lymphosarcome, chien, immunophénotype, lymphocyte, CD3, CD79a, nœud lymphatique, peau.

Introduction

Malignant Non – Hodgkin’s lymphoma (NHL) is the most common haematological malignancy in dogs [31] and it is the third type of tumours diagnosed in dogs behind mammary and skin tumours [8, 20]. The incidence was reported to be 13 - 24 dogs per 100,000 in some studies [14, 31].

Formerly canine malignant lymphomas (CML) had been classified especially on the basis of their histological features. Meanwhile histological criteria alone were found to be unreliable and only immunophenotyping (and determination of cellular proliferation markers) was shown to be associated with the survival time and the clinical outcome of patients [31]. The identification of the cell type of lymphomas (B or T lymphocyte) is of great prognostic importance [8, 20] because dogs with T cell lymphomas have a lower complete response to chemotherapy as well as shorter remission and
survival times than dogs with B cell tumours [10, 15, 28]. Consequently, the current classification system, based on immunophenotype and genetic features of the tumour as well as on its cellular morphology, has included criteria of differentiation of B and T lymphomas and identification of their subgroups [18]. This classification system has been adopted from that of humans [8, 17, 18] and was proposed by the World Health Organisation (WHO) [19].

Antibodies used to identify the type of the lymphocyte in dogs are directed against antigenic receptors found on the surface of the normal canine lymphocytes. To date most immunophenotyping has been performed by means of fluorescent immunocytochemistry on frozen sections and fine-needle aspiration biopsy [4, 26], by using a panel of canine monoclonal antibodies directed against various antigens including CD45RA, Thy-1, CD49d, CD3, CD4, CD8, CD21 and IgM, IgG and IgA. The identification of this panel of cell markers is not practical for routine histopathology on formalin-fixed paraffin wax-embedded tissue sections for 2 reasons. Firstly, there are technical constraints, since many positive reactions to monoclonal antibodies are inconclusive. Secondly, application of such a wide panel of cell markers is cost effective in veterinary medicine. However, human polyclonal CD3 antiserum [DAKO A 452-rabbit anti-human DAKO A/S, Produktionsvej 42, DK-2600 Glostrup, Denmark] has been documented as effective on canine formalin-fixed paraffin wax-embedded tissue sections and would appear to be adequate for routine histopathology as a pan-T cell marker in CML [7, 12, 24, 32]. As CD3 in T cells, the CD79a, specific for B cells, is involved in transmembrane signal transduction [22, 34]. Antibody to CD79a has been successfully applied to formalin-fixed paraffin wax-embedded tissue sections in cases of NHL and Hodgkin’s lymphoma in man [21, 22]. MILNER et al. [23] reported the successful application of CD3 and CD79a cell marker systems in the immunophenotypic identification of T and B-cell CML in formalin-fixed paraffin wax-embedded tissues.

No true correlation has been evidenced between the cytomorphological and immunophenotypical features of the cells in lymphomas as previously reported [14, 33]. For the diagnosis of lymphomas, it is necessary to provide to the clinician both morphological and immunohistochemical determination of B or T cell type. Lymphoblastic lymphomas are frequently of T cell type [18]. Characterization of tumour beyond routine histology is important since non-morphological parameters have been shown to be significantly associated with response to therapy and survival times. Significant morphologic-immunophenotypic correlations included shorter remission and survival times for T-cell tumours than B-cell tumours. However, the phenotype could not be predicted by the morphologic characteristics alone.

The objective of this study was to classify canine malignant lymphoma (CML) according to their immunophenotype features by means of four cellular markers (CD3, CD79a, lambda - and kappa - light chain) on paraffin sections from canine biopsies. In the current study, selected dogs showed clinical signs which included lymphadenopathy, splenomegaly and nodular swellings on the skin. The biopsies consisted of lymph nodes, intestinal, splenic and cutaneous tissues.
with a secondary biotinylated antibody including both polyclonal and monoclonal antibodies (goat anti-rabbit and goat antimouse serum) and then treated one hour with peroxidase conjugated with streptavidin according to the manufacturer’s instructions (Dako LSAB2 System peroxidase). The slides were rinsed in TBS (Tris Buffer Solution) between each step during the whole staining procedure. Finally, the sections were incubated 10 minutes with diaminobenzidine tetrahydrochloride (Dako DAB, Chromogen), treated in 0.02 % H₂O₂ as a substrate. After the slides were washed in tap water, the sections were counterstained with Mayer’s haematoxylin for 1 minute, rehydrated in alcohol at different concentrations and mounted with Entellan (Merck 1.07960.0500). Normal lymph node and tonsil sections were used as positive controls, while negative control was developed by replacement of the primary antibody with TBS and the rest of the procedure was followed as described.

**Results**

**IMMUNOHISTOCHEMICAL FINDINGS**

Eight of the 34 biopsies did not react either with T or B cell markers and could not be classified as CML.

Within the other 26 biopsies, 10 (38.5%) were immunostained with anti-CD79a, showing B cell proliferation (figure 1) and 16 (61.5%) with anti-CD3, showing T cell proliferation (figure 2). Immunostaining was mostly located in the cell membranes and, to a lesser extent, within the cytoplasm (figures 1 and 2). On the basis of these findings, the ratio of T lymphomas to B lymphomas was estimated to be 1.6 (16/10), in favour of T cell lymphoma. Among B lymphomas (CD79a positive biopsies, n = 10), 9 (90%) positively react with anti-λ light chains and 1 (10%) with anti-κ light chains.

**HISTOPATHOLOGICAL FINDINGS**

**In tumours positively reacting with anti-CD79a antibody (B lymphomas)**

Four tumours (2 arising from lymph nodes, 1 from spleen and 1 from skin) were found to be constituted by round to ovoid cells with small cleaved vesicular nuclei (figure 3). The mitotic index was found to be less than ten per ten high power fields (HPF). The present case was classified as B cell lymphocytic lymphoma.

Four cases (2 arising from lymph nodes, 1 from skin and 1 from intestine) revealed a similar morphology than lymphocytic lymphoma, but the cells were found to have larger cytoplasm and some of them presented an eccentric nucleus. Consequently, these cases were classified as lymphoplasmacytic B lymphoma (figure 4). The mitotic index was found to be less than ten per ten HPF.

Two cases (arising from skin and lymph node respectively) revealed diffuse architecture consisting of large cells with round to oval nuclei, branched chromatin, multiple nucleoli and with fine basophilic cytoplasm (figure 5). The mitotic index was found to be five to six per ten HPF. The present case was classified as diffuse large B cell lymphoma.

**In tumours positively reacting with anti-CD3 antibody (T lymphomas)**

Four specimens (originating from skin) showed diffuse architecture with scattered pale lymphoid foci infiltrating the deep dermis. The mass was composed of lymphoid cells with slightly cleaved hyperchromatic nuclei and markedly clear cytoplasm (figure 6). These cases were diagnosed as non-epitheliotropic cutaneous T lymphoma on the basis of the cellular morphology. The mitotic index was found to be less than ten per ten HPF.

Two cases (arising from spleen) and nine specimens (from lymph nodes) revealed morphologic features consistent with T-cell lymphoblastic lymphomas. The tumours showed diffuse architecture, consisting of medium-sized cells with slightly cleaved large nuclei with finely dispersed chromatin (figure 7). Mitoses were frequently observed in each ten HPF. Starry-sky appearance was visible in some specimens.

**In tumours negatively reacting with anti-CD79a and with anti-CD3 antibodies**

In eight cases (originating from skin) round to oval cells with large nuclei and single prominent nuclei were observed beneath the epidermis. The mitotic index was found to be nine per ten HPF. Lymphocytes and plasmocytes with normal morphology and fibrous proliferation were also present. Severe anaplastic changes in the neoplastic cells were detected in some areas in some cases. These cells had prominent irregular nucleoli, with chromatin located in periphery and relatively abundant cytoplasm.

Recapitulative immunohistochemical and histological findings were given in the table I. Among canine biopsies with suspicion of lymphosarcoma, T lymphomas (characterized by positive CD3 cells) were the preponderant type (47.1%). The B lymphomas (characterized by CD79a positive cells) were observed in 29.4% of cases and lymphoma-like tumours (no B or T cells) exclusively of a cutaneous origin in 23.5% of cases. The respective proportions of each subtype of B and T lymphomas were: 42.3% for T lymphoblastic lymphomas, 19.2% for non epitheliotrophic T lymphomas, 15.3% for B lymphocytic lymphomas, 15.3% for B lymphoplasmacytic lymphomas and 7.6% for diffuse large B cell lymphomas. The preferential localisation of B and T lymphomas were firstly the lymph nodes and secondly the skin and again, T lymphomas were in majority in these 2 sites (64.3% and 62.5% respectively). The T-cell lymphoblastic lymphoma was the more frequent type of T lymphomas (68.7%) identified in this retrospective study and it was evidenced in lymph nodes and in spleen, whereas the non epitheliotrophic cutaneous subtype was less often diagnosed (31.3%). Among B lymphomas, 2 preponderant subtypes (lymphocytic and lymphoplasmacytic lymphomas) were identified with a frequency of 40% while the third subtype (diffuse large B cell lymphoma) was more rarely observed (20%). Nevertheless, no preferential tissue localisation was associated with a particular B lymphoma subtype.

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FIGURE 1: B cell lymphoma positively reacted with anti-CD79a antibody in a lymph node.

FIGURE 2: T cell lymphoma positively reacted with anti-CD3 antibody in the skin.

FIGURE 3: Lymphocytic B lymphoma in a lymph node.

FIGURE 4: Lymphoplasmacytic B lymphoma in a lymph node.

FIGURE 5: Diffuse large B cell lymphoma in a lymph node.

FIGURE 6: Non epitheliotrophic cutaneous T lymphoma in skin.

FIGURE 7: Lymphoblastic T lymphoma in a lymph node.

B lymphomas: LBL: Lymphocytic B Lymphoma, LPBL: Lympho-Plasmacytic B Lymphoma, DLBL: Diffuse Large B cell Lymphoma.

and the diagnosis of canine transmissible venereal tumour diagnosed as NHL on the basis of immunohistochemical features (23.5% of all the cases). These 8 skin tumours could not be the proportion of skin tumours of unknown origin was elevated the preponderant type (61.5% of the attested cases). In parallel, was lowered (38.5% of the identified lymphomas and 29.4% of all suspected cases) and the T lymphoma was surprisingly was the most common subtype, with 33 cases (40.2%). Of these, 21 (63.6%) were of the B subtype and seven

Table 1: Recapitulative immunohistochemical and histological findings found in the canine biopsy specimens with suspicion of malignant lymphoma (n = 34).

<table>
<thead>
<tr>
<th>Origin and subtypes</th>
<th>Number and frequency in %</th>
</tr>
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<tbody>
<tr>
<td></td>
<td><strong>B lymphomas</strong> (positive CD79a cells)</td>
</tr>
<tr>
<td>Lymph node (n = 14)</td>
<td>10 (29.4%)</td>
</tr>
<tr>
<td>Intestine (n = 1)</td>
<td>5 (2 LBL, 2 LPBL, 1 DLBL)</td>
</tr>
<tr>
<td>Spleen (n = 3)</td>
<td>1 (LPBL)</td>
</tr>
<tr>
<td>Skin (n = 16)</td>
<td>3 (1 LBL, 1 LPBL, 1 DLBL)</td>
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Discussion

Various classification systems have been used in the past for canine Non – Hodgkin’s lymphomas (NHL), most of which have been adopted from human medicine. BREUER et al. reported the compatibility of human classification systems at various points [2]. In veterinary medicine, all of these systems were applied as they were developed, and their application demonstrated a surprising level of variation in subtypes of animal leukaemia and lymphomas. Major differences between human and animal lymphomas include a higher proportion of high-grade lymphomas in animals and a lower proportion of follicular neoplasms. Also, Hodgkin-like neoplasms are rarely identified in animals. The haematopoietic tumours in animals tend to mimic their counterparts in humans, including their biological behaviour and their response to therapy [19].

The classification of haematopoietic tumours of domestic animals proposed by WHO is the current classification system, which takes into account morphology as well as immunophenotype features [19]. This classification is based on the biological behaviour of neoplasms in order to assess the appropriate therapeutic approach [9]. The necessity of establishing immunophenotype criteria in canine lymphomas have been indicated in many studies [20]. Thus it was reported that a T - cell phenotype is associated with a high rate of relapse and significantly shorter survival times [27, 28]. In the present study, for the first time in Turkey, the criteria proposed by WHO, have been applied to evaluate tumour tissues. Immunohistochemical studies have been carried out on 34 canine suspected malignant lymphomas in terms of distinction of the predominating cell type [5, 6, 11, 29].

It is usually admitted that B and T cell lymphomas represent 55% and 42% of all canine NHL respectively and 3 % did not react with any of the B-or T-cell lineage markers [31]. In the present study, the observed frequency of B lymphomas was lowered (38.5% of the identified lymphomas and 29.4% of all suspected cases) and the T lymphoma was surprisingly the preponderant type (61.5% of the attested cases). In parallel, the proportion of skin tumours of unknown origin was elevated (23.5% of all the cases). These 8 skin tumours could not be diagnosed as NHL on the basis of immunohistochemical features and the diagnosis of canine transmissible venereal tumour was proposed. The tumours were composed of loose sheets, rows and cords of relatively uniform round to ovoid cells. Cell margins were generally indistinct. Nuclei were large, round, with a single centrally placed nucleolus surrounded by margined chromatin. The cells had moderate amount of light pink to clear cytoplasm. Variable numbers of lymphocytes, plasma cells and macrophages were seen infiltrating the tumour tissue and numerous mitotic figures were found. Areas of necrosis and fibrosis were seen in the regression parts. It was reported that this neoplasm which showed histological similarities with NHL did not react with anti-CD3 or with anti-λ light chains antibodies [18].

Lymphosarcomas located in lymph nodes were reported to be B lymphomas in majority (B cell phenotype: 74% and T cell phenotype: 26%) [13] and proliferating cells preferentially produced λ light chains (82.2%) than the κ type (14.8%) [1, 3, 13]. By contrast, in our study, 64.3% of nodal malignant lymphomas are T lymphomas, especially lymphoblastic lymphoma vs. 35.7% B lymphomas (3 identified subtypes), but this discrepancy could be related to the relative low number of biopsy samples of lymph nodes. Moreover, the proportions of cancerous B cells expressing λ or κ chains (90% and 10% respectively) were in agreement with previous studies [1, 3, 13]. For cutaneous lymphosarcomas, the T lymphoma (non epitheliotrophic cutaneous subtype) was again the preponderant type (62.5%), but B lymphomas were also relatively commonly observed (37.5%).

A predominance of T subtype (11%) was also shown in a study [16] whereas 1.2% comprised B cell lymphomas. In the same study, the lymphoblastic lymphoma group represented 8.5% of cases, three being of the B-cell subtypes, three of the T-cell subtypes and one being unclassifiable due to lack of immunolabelling. In a similar study [30], the proportions for lymphocytic and lymphoplasmacytic B subtypes were reported to be 1.8% and 10.9% respectively. The T cell lymphoblastic lymphomas comprised 17.5 % of the whole cases in another study by PONCE et al. [25]. We did not define any case of lymphoblastic T cell lymphoma in the current study and the majority (42.3%) of the T cell lymphomas consisted of lymphoblastic subtype. Lymphoblastic T cell lymphomas comprised 23.9% of the cases in another study [13].

In the study of SUEIRO et al. [30], the large cell lymphoma was the most common subtype, with 33 cases (40.2%). Of these, 21 (63.6%) were of the B subtype and seven
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(21.2%) the T subtype [30], which conflicted with our findings that revealed the predominance of lymphoblastic T lymphomas and that diffuse large cell lymphoma was immunophenotyped as of B cell origin. These findings revealed that data obtained with respect to the prevalences of the CML subtypes have not been in accordance with each other, and consequently further studies are needed to clarify the lacking points.

Non epitheliotrophic lymphosarcoma was reported to be mostly of T-cell origin in the dog, although it could be of B-cell origin [7]. We found an equal distribution of T-cell and B-cell lymphosarcomas within the non epitheliotrophic group in the present study. It was also reported that T lymphosarcomas were more often encountered in the gastrointestinal tract (in 75% of cases) than B lymphosarcomas evidenced by a positive anti CD20 reaction in dogs [6].

In conclusion, B or T immunophenotype determination of malignant lymphomas with specific T (CD3) and B (CD79a, λ, and κ light chains) markers efficiently completes the classical histological analysis by offering a positive diagnosis gain (elimination of lymphoid-like tumours which do not react with any markers of lymphocyte differentiation) and probably a positive prognostic gain. Indeed, the determination of the phenotype of the neoplasm is of great prognostic and therapeutic importance since the recurrence rate was found to be higher with a shorter survival time in T-type lymphomas.

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References


