International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Immunohistochemical Characterization of Canine Lymphoma

Shailja Pandey[1], Yamini Verma[2]

[1]Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Jabalpur, Madhya Pradesh, 482001
PhD Scholar, Department of Veterinary Pathology, NDVSU Jabalpur

[2]Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Jabalpur, Madhya Pradesh, 482001
Head of the Department, Department of Veterinary Pathology, NDVSU Jabalpur

Abstract: ***Lymphomas occur by clonal expansion of lymphoid cells and have distinctive morphological and immunophenotypic features. Determination of canine lymphoma immunophenotype is useful for accurate prognosis and further therapy. The current review focus on the molecular characterization that is associated with the gene mutation that leads to protein expression causing neoplastic transformation of lymphoid cells. Further there is discussion regarding immunohistochemical characterization of lymphoma for the diagnostic point of view to identify the lympohocyte lineage for confirmatory diagnosis of lymphoma and its prognosis. In this review there is details regarding immunohistochemical markers B lymphocyte (CD79a, CD20, Pax5), and T lymphocytes (CD3).***

Keywords: Lymphoma, Immunohistochemical markers, B lymphocyte, T lymphocyte

1. Introduction

Lymphoma is among the most frequently diagnosed malignancies in the dog and represents the most commonly managed neoplasia in veterinary medical oncology. (Baba and Cătoi, 2007). Lymphoma accounts for approximately 7% up to 24% of all canine neoplasms and about 83% of all malignant haematopoietic tumors of this species (Valli et al., 2017; Vail et al., 2013). Although canine lymphoma is often viewed as a single disease, it actually comprises a number of clinically and morphologically distinct forms of lymphoid cell neoplasia. Nevertheless, cL represents the most common hematopoietic neoplasia in dogs with an estimated minimal annual incidence rate of 13 - 114 per 100, 000 dogs. (Dobson et al.2002) Although cL can affect any dog breed but middle - sized to larger dog breeds are overrepresented with the condition (Edwards et al.2003; Villamil et al.2009). This finding could not be related to growth hormone levels (Lantinga van Leeuwen et al.2000) and more likely reflects a genetic susceptibility in some of the larger dog breeds.

Molecular Biology of Canine Lymphoma

Comparative genomic hybridization has demonstrated genetic abnormalities in dog chromosomes number 13 and 31 (Thomas et al.2011). The proto - oncogene c - kit, a tyrosine protein kinase, is an important factor in the proliferation, survival, and differentiation of hematopoietic stem cell including mast cells. The expression of c - kit in cL is typically low, but found to be increased in some high - grade T cell lymphomas (Giantin et al.2013). Mutations in the N - ras oncogene are common in leukemia (Usher et al.2009), but rare in cL (Mayr et al.2002). Mutations in the tumor suppressor gene p53 are relatively rare in in cL (Tomiyasu et al.2010). Increased Rb (retinoblastoma) phosphorylation and subsequent activation of CDK4, is common in high - grade canine T - cell lymphoma and might result from deletion of p16 or loss of dog chromosome number 11 (Fosmire et al.2007), hypermethylation of the CpG island of the p16 gene (Fujiwara - Igarashi et al.2014), and from a deletion of the p15, p14 and p16 locus (Fujiwara - Igarashi et al.2013). Increased Rb phosphorylation in high - grade canine B - cell lymphoma appears to correlate with c - Myc overexpression and trisomy of dog chromosome number 13 (Fosmire et al.2007).

The Bcl - 2 family consists of approximately 25 proteins that regulate apoptosis through controlling the formation of the mitochondrial outer membrane permeabilization pore (MOMPP). The canine anti - apoptotic Bcl - 2 (B - cell lymphoma 2) (Chaganti et al.1992) is not upregulated in canine B - cell lymphoma cases (Tomiyasu et al.2010). Loss - of - function mutations and/or deletions in the tumor suppressor genes, PTEN and CDKN2A/B, have, respectively, not been studied in cL or not been found (Thomas et al.2011).

Bcl - 6 mRNA and protein expression are low or absent in canine high - grade B - cell lymphoma. The tumor suppressor gene tissue factor pathway inhibitor 2 (TFPI - 2) is associated with inhibition of tumor invasion and hypermethylation of the gene and subsequent downregulation of TFPI - 2 expression is identified in most canine high - grade B - cell lymphomas (Ferraresso et al.2014). Canonical activation of NF - kB, a regulator of genes that control cell proliferation and apoptosis, and increased NF - kB target gene expression has been demonstrated in a subset of dogs with B - cell lymphoma (Gaurnier - Hausser et al.2011; Mudaliar et al.2013; Richards et al.2013.

The most common clinical presentation of cL is the multicentric form that affects the peripheral lymph nodes, but extra nodal forms exist and include mediastinal, abdominal (gastrointestinal (GI), hepatic, splenic, renal), cutaneous, ocular, central nervous system, and pulmonary lymphoma. The clinical presentation of cL can be further complicated by the presence of paraneoplastic syndromes.

Immunohistochemical Markers

Immunohistochemical examination is used to determine the type of tumoral cells, but this technique is also useful for the determination of the initial diagnosis. (Comazzi and Gelain,

2011). The immunophenotype of a lymphocyte is identified by determining the expression of specific molecules for B lymphocytes (e. g., CD79a, CD20, Pax5), and T lymphocytes (e. g., CD3) (Vail et al., 2013).

Many studies have reported that T - cell lymphomas are characterised by shorter survival times and disease - free intervals than lymphomas of B - cell origin (Ponce et al.2003; Valli et al.2013). Because of this dissimilar prognosis, histopathological classification with immunophenotyping is important for chemotherapeutic treatment in canine lymphomas (Rebhun et. al.2011).

In this paper there is description of histochemical markers for immunophenotyping of canine lymphoma. According to immunophenotype lymphomas are divided into three categories B, T and null lymphomas. The term null cell is generally used for lymphocytes that have not been immunoreactive for B or T cells. It may be a poorly differentiated cell of unknown origin, or it may be a natural killer cell. There are no antibodies documented to recognize NK - cell lymphomas in animals.

Immunohistochemical Markers for B Cell Lymphoma

CD 79 a

A transmembrane heterodimer in the immunoglobulin superfamily which consist of two glycoproteins, CD79a and CD79b. CD79 is noncovalently associated with surface immunoglobulins, constituting the B-cell receptor complex. Overall, approximately 97% of B - cell neoplasms are CD79a positive (Chu and Arber 2001). It is expressed in the cytoplasm of pre-pro-B cell to the plasma cell differentiation stage, CD79a has mostly a cytoplasmic membrane expression from the pro-B cell stage throughout the B cell differentiation. CD79 has been considered the marker of choice for B cell lymphomas in multiple animal species; including canine, however, it may express an aberrant nuclear labeling, which is considered nonspecific. (Meuten., 2020)

CD 20

CD20 is a tetraspanning transmembrane phospho - protein that is expressed predominantly in pre - B cells and in mature peripheral B cells. The function of CD 20 is associated with receptor - induced calcium signals. Engagement of CD20 using antibodies leads to increased intracellular calcium (Corina et. al., 2017). CD20 has a similar pattern of staining to that of CD79, and is considered an excellent alternative to the CD79 a. it has membranous activity. CD20 is among the first molecules that have been successful as immunotherapeutic targets. This molecule was an attractive target because its expression can be determined in both fresh and archival tissues and because it is stable in cell membranes (i. e., it is not readily internalized or shed upon antibody binding). Some anti - bodies decorate the cytoplasm of B cells, whereas others have a cytoplasmic membrane labeling. (Mutan., 2020. Corina et. al., 2017)

CD21

CD21, also known as complement receptor 2 (CR2), is a protein that is expressed on the surface of B cells, follicular dendritic cells, and some epithelial cells. It plays a role in the regulation of the complement system and is involved in the activation and differentiation of B cells. CD21 is often used as an immunohistochemical marker for the diagnosis of canine B cell lymphoma. (Zandvliet., 2016)

PAX 5

Pax5 is a member of the paired - box domain family of transcription factors that encodes the B - cell - specific activator protein. Its important roles are to control B - cell identity, development and differentiation. Pax5 protein is expressed in normal and neoplastic cells from the pro - B to mature B - cell stages (Horcher et al.2001). It serves as a pan pre B - cell marker and was shown to be more specific than CD79a (Willmann et al.2009). In studies, Pax5 expression was restricted to B - cell malignancies including those that lacked CD20 and CD79a expression (Jensen et al.2007). It has nuclear reactivity. (muten., 2020)

Immunohistochemical Marker for T Cell Lymphoma

CD3

CD3 antigen consists of at least four structurally different membrane glycoproteins comprising extracellular, transmembrane, and intracellular domains. It is noncovalently associated with the T-cell receptor (TCRα/β or TCRγ/δ) complex, and appears in the cytoplasm before its detection on the cell surface of thymocytes. (Sompuran et. al 2006) CD3 is one of the earliest T cell lineage antigens, expressed from the pro - thymocyte stage to the mature T cell stage.7 It is used in veterinary oncology, because of its high specificity, to confirm T cell differentiation of lymphomas. (Muten., 2020).

There are other markers for T cell such as CD4, CD8. CD4 is a protein expressed on the surface of T - helper lymphocytes, which play a crucial role in the immune response against foreign antigens, including cancer cells. In veterinary medicine, CD4 is often used as an immunohistochemical marker for the diagnosis and classification of canine lymphoma.

CD 30

It is expressed by large neoplastic cells which are also known as Anaplastic large - cell lymphoma or null - cell lymphoma. Null - cell lymphoma occurs when when tumor cell neither expresses CD 79 or CD 3 but the cells labelled for CD 30. There is membranous reactivity of this marker. (Pittaway et. al 2018)

2. Conclusion

In this paper there is description of various markers for B cell such as CD 79 a, CD 20, CD21, PAX 5 but the results are best obtained with CD 79 a when we talk about diagnosis of canine B cell lymphoma. Likewise when we talk about T cell lymphoma the most commonly used histochemical marker is CD 3 with which the results are best obtained. The reports for CD4 and CD 8 as immunohistochemical markers are less although there are evidences of flowcytometry using these markers**.**

References

[1] Baba AI, Cătoi C (2007). Comparative oncology. The Publishing House of Romanian Academy, 573 - 625

[2] Chaganti SR, Mitra J, LoBue J.1992. Detection of canine homologs of human MYC, BCL2, IGH, and TCRB genes by southern blot analysis. Cancer Genet Cytogenet.62: 9 - 14.
[3] Chu PG, Arber DA. CD79: a review. Applied Immunohistochemistry & Molecular Morphology: AIMM.2001 Jun; 9 (2): 97 - 106.
[4] Comazzi S, Gelain ME (2011). Use of flow cytometric immunophenotyping to refine the cytological diagnosis of canine lymphoma. Vet J, 188 (2): 149 - 155.
[5] Corina, T. O. M. A. and CĂTOI, C., 2017. Immunohistochemical characterization of canine lymphomas. *Bulletin UASVM Veterinary Medicine*, *74*, p.2.
[6] Dobson JM, Samuel S, Milstein H, Rogers K, Wood JL.2002. Canine neoplasia in the UK: estimates of incidence rates from a population of insured dogs. J Small Anim Pract.43: 240 - 246.
[7] Edwards DS, Henley WE, Harding EF, Dobson JM, Wood JL.2003. Breed incidence of lymphoma in a UK population of insured dogs. Vet Comp Oncol.1: 200 - 206.
[8] Ferraresso S, Bresolin S, Arico A, Comazzi S, Gelain ME, Riondato F, Bargelloni L, Marconato L, Kronnie G, Aresu L.2014. Epigenetic silencing of TFPI - 2 in canine diffuse large B - cell lymphoma. PLoS One.9: e92707.
[9] Fosmire SP, Thomas R, Jubala CM, Wojcieszyn JW, Valli VE, Getzy DM, Smith TL, Gardner LA, Ritt MG, Bell JS, et al.2007. Inactivation of the p16 cyclin - dependent kinase inhibitor in high - grade canine non - Hodgkin's T - cell lymphoma. Vet Pathol.44: 467 - 478.
[10] Fosmire SP, Thomas R, Jubala CM, Wojcieszyn JW, Valli VE, Getzy DM, Smith TL, Gardner LA, Ritt MG, Bell JS, et al.2007. Inactivation of the p16 cyclin - dependent kinase inhibitor in high - grade canine non - Hodgkin's T - cell lymphoma. Vet Pathol.44: 467 - 478.
[11] Fujiwara - Igarashi A, Goto - Koshino Y, Sato M, Maeda S, Igarashi H, Takahashi M, Fujino Y, Ohno K, Tsujimoto H.2014. Prognostic significance of the expression levels of the p16, p15, and p14 genes in dogs with high - grade lymphoma. Vet J.199: 236 - 244.
[12] Gaurnier - Hausser A, Patel R, Baldwin AS, May MJ, Mason NJ.2011. NEMO - binding domain peptide inhibits constitutive NF - kappaB activity and reduces tumor burden in a canine model of relapsed, refractory diffuse large B - cell lymphoma. Clin Cancer Res.17: 4661 - 4671.
[13] Giantin M, Aresu L, Arico A, Gelain ME, Riondato F, Comazzi S, Dacasto M.2013. Evaluation of tyrosine - kinase receptor ckit mutations, mRNA and protein expression in canine lymphoma: might c - kit represent a therapeutic target? Vet Immunol Immunopathol.154: 153 - 159.
[14] Horcher M, Souabni A, Busslinger M (2001): Pax5/bsap maintains the identity of B cells in late B lymphopoiesis. Immunity 14, 779–790.
[15] Jensen KC, Higgins JP, Montgomery K, Kaygusuz G, Van De Rijn M, Natkunam Y (2007): The utility of pax5 immunohistochemistry in the diagnosis of undifferentiated malignant neoplasms. Modern Pathology 20, 871–877.
[16] Lantinga van Leeuwen IS, Teske E, van Garderen E, Mol JA.2000. Growth hormone gene expression in normal lymph nodes and lymphomas of the dog. Anticancer Res.20: 2371 - 2376
[17] Mayr B, Winkler G, Schaffner G, Reifinger M, Brem G.2002. N - ras mutation in a feline lymphoma. low frequency of N - ras mutations in a series of feline, canine and bovine lymphomas. Vet J.163: 326 - 328.
[18] Meuten, D. J. ed., 2020. *Tumors in domestic animals*. John Wiley & Sons.
[19] Mudaliar MA, Haggart RD, Miele G, Sellar G, Tan KA, Goodlad JR, Milne E, Vail DM, Kurzman I, Crowther D, et al.2013. Comparative gene expression profiling identifies common molecular signatures of NF - kappaB activation in canine and human diffuse large B cell lymphoma (DLBCL). PLoS One.8: e72591.
[20] Pittaway, R., Wu, Y., Szladovits, B., Suárez - Bonnet, A., Scurrell, E. J., Garden, O. A., Polton, G. and Priestnall, S. L., 2018. Diagnosis of anaplastic large - cell lymphoma in a dog using CD30 immunohistochemistry. *Journal of Veterinary Diagnostic Investigation*, *30* (3), pp.455 - 458.
[21] Ponce F, Magnol JP, Marchal T, Chabanne L, Ledieu D, Bonnefont C, Felman P, Fournel - Fleury C (2003). High - grade canine T - cell lymphoma/leukemia with plasmacytoid morphology: a clinical pathological study of nine cases. J Vet Diagn Invest, 15 (4): 330 - 337.
[22] Rebhun RB, Kent MS, Borrofka SA, Frazier S, Skorupski K, Rodriguez CO (2011): CHOP chemotherapy for the treatment of canine multicentric T - cell lymphoma. Veterinary and Comparative Oncology 9, 38–44.
[23] Richards KL, Motsinger - Reif AA, Chen HW, Fedoriw Y, Fan C, Nielsen DM, Small GW, Thomas R, Smith C, Dave SS, et al.2013. Gene profiling of canine B - cell lymphoma reveals germinal center and postgerminal center subtypes with different survival times, modeling human DLBCL. Cancer Res.73: 5029 - 5039.
[24] Sompuran, S. R., Vani, K., and Bogen, S. A. (2006) A molecular model of antigen retrieval using a peptide array. Am J Clin Pathol 125: 91–98
[25] Thomas R, Seiser EL, Motsinger - Reif A, Borst L, Valli VE, Kelley K, Suter SE, Argyle D, Burgess K, Bell J, et al.2011. Refining tumor - associated aneuploidy through 'genomic recoding' of recurrent DNA copy number aberrations in 150 canine non - hodgkin lymphomas. Leuk Lymphoma.52: 1321 - 1335.
[26] Tomiyasu H, Goto - Koshino Y, Takahashi M, Fujino Y, Ohno K, Tsujimoto H.2010. Quantitative analysis of mRNA for 10 different drug resistance factors in dogs with lymphoma. J Vet Med Sci.72: 1165 - 1172.
[27] Usher SG, Radford AD, Villiers EJ, Blackwood L.2009. RAS, FLT3, and C - KIT mutations in immunophenotyped canine leukemias. Exp Hematol.37: 65 - 77.
[28] Vail DM, ME Pinkerton, KM Young (2013). Hematopoietic Tumors, Chapter 32, p.608 - 678. In Withrow SJ, Page R, Vail DM (Eds.). Withrow and MacEwen's small animal clinical oncology.5th Edition, Elsevier Health Sciences.
[29] Valli VEO, Bienzle D, Meuten DJ (2017). Tumors of the Hemolymphatic System, Chapter 7, p.203 - 210. In Meuten DJ (Ed.). Tumors in Domestic Animals, 5th

Edition, John Wiley & Sons, Inc., Wiley Blackwell, Iowa, USA.

[30] Villamil JA, Henry CJ, Hahn AW, Bryan JN, Tyler JW, Caldwell CW.2009. Hormonal and sex impact on the epidemiology of canine lymphoma. J Cancer Epidemiol.

[31] Willmann M, Mullauer L, de Arespacochaga AG, Reifinger M, Mosberger I, Thalhammer JG (2009): Pax5 immunostaining in paraffin - embedded sections of canine nonHodgkin lymphoma: A novel canine pan pre - B - and B - cell marker. Veterinary Immunology and Immunopathology 128, 359–365.

[32] Zandvliet, M., 2016. Canine lymphoma: a review. *Veterinary Quarterly*, *36* (2), pp.76 - 104.