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Review

Cyodiagnosics of canine lymphomas – possibilities and limitations

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Abstract

Malignant lymphomas are one of the most common malignant tumours occurring in dogs. The basic method of lymphoma diagnosis in human, as well as in canine oncology is histopathology supported by immunohistochemistry. It was suggested that in veterinary medicine excisional biopsy of lymph node and histopathology should be considered only where the cytologic diagnosis is equivocal or needs to be confirmed. There are at least three basic reasons for which cytological examination ought to be accepted as a sufficient and reliable diagnostic method for lymphoma in dogs. Firstly, most dog owners consider the fine-needle biopsy as an acceptable non-harmful method of sample collection. Secondly, an increasing number of studies recommend cytology as an accurate test for diagnosing and subtyping canine lymphoma. Finally, the vast majority of canine lymphoma subtypes belong to 4-5 categories characterized by a typical cytological picture. Immunocytochemical staining of cytological smears gives new diagnostic possibilities, such as detection of markers better characterizing given growth or a potential goal for target therapy in individual cases (for example inhibitors of platelet-derived growth factor).

Key words: cytopathology, dogs, fine-needle biopsy, Kiel classification, lymphoma

Introduction

Malignant lymphomas are one of the most common malignancies in dogs, it has been estimated that from 13 to 33 per 100 000 dogs may become affected each year (Edwards et al. 2003, Pastor et al. 2009, Sapieryński et al. 2010, Regan et al. 2012). Boxers, Scottish terriers, Airedale terriers, Basset hounds, German shepherds, Bulldogs and Bernese Mountain

dogs are most commonly affected. Some breeds appear to be predisposed to lymphomas of a certain immunophenotype, i.e. boxers and dog de Bordeaux to T-cell lymphoma, whereas German shepherds and Rottweilers are predisposed to B-cell lymphoma (Fournel-Fleury et al. 2002, Jagielski et al. 2002, Lurie et al. 2008, Pastor et al. 2009, Jankowska et al. 2015).

Classification of canine lymphomas

In recent decades many lymphoma classification systems have been used in dogs, including classifications based on the organ affected (multicentric, alimentary, mediastinal etc.), histological grade (low-grade, intermediate-grade, high-grade), cell immunophenotype (B-cell lymphomas, T-cell lymphomas, and non-B cell non-T-cell lymphomas) as well as cellular morphology, and tissue architecture (Kiel classification, WHO classification and others). Nowadays, it is clear that the above-mentioned classification systems are no longer sufficient in veterinary oncology (Comazzi et al. 2014). The previously widely accepted statement that better prognosis was associated with B-cell rather than T-cell lymphomas, has been recently refuted (Flood-Knapik et al. 2012, Valli et al. 2013). Nonetheless, identifying lymphoma cell immunophenotype and tumor cytological classification still remain important elements of the definitive diagnosis. As in human medicine the canine lymphoma is not one disease but a heterogeneous group of many conditions with different clinical presentations, requiring various therapeutic methods and characterized by different prognoses (Valli et al. 2006, Pastor et al. 2009, Ponce et al. 2010, Rebhun et al. 2010, Poggi et al. 2013, Valli et al. 2013, Jankowska et al. 2015). For example, the WHO classification lists more than 30 subtypes of canine lymphoma, with a few specific subtypes which occur only in dogs. Unfortunately, the recognition of specific disease entities based on a combination of morphologic and behavioral characteristics is not yet established in veterinary oncology (Comazzi et al. 2014).

The basic classification system of hematopoietic tumors in humans as well as in dogs is the WHO lymphoma classification, which defines histoclinical disease entities based on clinical appearance, tumor morphology and immunophenotype, and genetic alterations. However, it is important to underline that lymphoma subtyping according to the WHO classification requires histopathological examination of tissue samples (preferably the entire lymph node), immunophenotyping, and often also cytogenetics and molecular biology. In general, the WHO lymphoma classification divides lymphomas according to maturity of neoplastic cells into: precursor lymphoid lymphomas (which usually originate from primary lymphoid tissue – bone marrow or thymus) and peripheral or mature cell lymphomas (originating from secondary lymphoid tissues). The great advantage of the WHO lymphoma classification is that it allows the biologic behavior of neoplastic cells to be predicted, and thus the response to therapy and prognosis in treated animals (Aresu et al. 2013, Flood-Knapik et

al. 2013, Valli et al. 2013). The vast majority of lymphoma subtypes are common for humans and dogs and their biological behavior is also virtually identical (Valli et al. 2010).

Unfortunately, application of the WHO lymphoma classification in canine oncology is troublesome in some cases. As it requires the assessment of lymph node architecture (diffuse lymphoma vs. follicular lymphoma) the sample can only be collected through surgery in general anesthesia and this procedure may be unacceptable in some patients in a severe condition. As a consequence, only 10% of canine lymphoma cases enrolled in our own studies were recognized in histopathology and the remaining 90% were diagnosed cytologically (Sapieryński 2010, Sapieryński et al. 2012). Moreover, in the USA in routine veterinary practice, the definitive diagnosis of lymphoma is based on the results of histopathology only in 28% of cases. The vast majority of veterinary oncologists (88% of responders) tend to introduce anticancer chemotherapy on the basis of the cytological examination and immunophenotyping, and they consider this practice recommendable (Regan et al. 2012).

Furthermore, some data necessary for full characterisation (epidemiological, molecular, genetic and clinical) of specific lymphoma subtypes in the WHO classification have not been sufficiently included in routine examination of canine lymphomas (Foulner-Fleury et al. 2002, Ponce et al. 2010, Comazzi et al. 2014). Moreover, this system of classification needs some improvement because some subtypes of T cell lymphomas with different biological behavior (including indolent, low grade and high grade subtypes) are grouped together as mature T- and NK-cell neoplasms (Aresu et al. 2013). Finally, there are some subtypes of canine lymphomas, e.g. T zone lymphomas, plasmacytoid high-grade lymphomas not included in the WHO classification. It is also worth emphasizing, that despite the high diagnostic accuracy of histopathology false diagnoses can still be made unless immunohistochemistry is applied, especially in low grade lymphomas (Flood-Knapik et al. 2012). Such mistakes have also been observed in human non-Hodgkin lymphomas, most commonly in T zone lymphomas, which are quite common in canine patients (Uherowa et al. 2002, Jankowska et al. 2015).

To minimize the disadvantages mentioned above there are attempts to classify canine lymphoma according to the WHO classification on the basis of cytological examination of samples collected by fine-needle biopsy. Although in some subtypes it is quite simple (clear cell lymphoma can be treated as T zone lymphoma, macronucleolated medium-sized cell lymphoma as marginal zone lymphoma, centrob-

lastic pleomorphic lymphoma as diffuse large B cell lymphoma), it seems that such a method of classification is not reasonable because of lack of knowledge about histological architecture.

The updated Kiel classification (uKc) adopted for dogs is the second classification system commonly used for canine lymphoma subtyping (Fournel-Fleury et al. 1997a, Fournel-Fleury et al. 2002, Sozmen et al. 2005, Ponce et al. 2010, Jankowska et al. 2015, Fernandes et al. 2015). This system is based on neoplastic cell morphology and immunophenotype, with regard to similarity to normal lymphocytes in various stages of maturity. Both of these criteria can be established in microscopic examination of cellular samples. The microscopic criteria of classification include cell and nucleus size, volume, distribution and basophilia of the cytoplasm, nucleus shape, chromatin structure, presence and distribution of nucleoli, and mitotic activity. The prognostic usefulness of the updated Kiel classification was shown in a group of dogs with various subtypes of canine lymphomas (Ponce et al. 2004). Some authors have tried to adapt the canine lymphoma subtype determined on the basis of the uKc to the canine lymphoma subtype according to the WHO classification (Ponce et al. 2010).

The significant advantage of the updated Kiel classification is the strong relationship between the morphology of neoplastic cells and their immunophenotype, which allows diagnosis of lymphoma subtype to be established with 90% accuracy (Ponce et al. 2010, Sapierzyński et al. 2012). The only exceptions are lymphoblastic lymphomas, characterized by the same morphology regardless of the immunophenotype – in these cases immunocytochemistry is essential for definitive tumor identification (Fournel-Fleury et al. 2002, Sapierzyński et al. 2012).

Application of cytology in canine lymphomas

The basic diagnostic method for lymphoma diagnosis in human and canine oncology is histopathology together with immunohistochemistry (Uherowa et al. 2002, Flood-Knapik et al. 2013). However, it has been suggested that in veterinary medicine excisional biopsy of the lymph node and histopathology should only be considered when cytological examination gives an inconclusive result (Messick 2008, Comazzi et al. 2014). Cytology is a cheap, easy to perform, safe and reliable method of lymphoma diagnosis with high accuracy compared to histopathological examination (Amores-Fuster et al. 2015). In human oncology, cytological examination allows non-Hodgkin lymphoma to be recognized in 80-95% of cases; additionally, subtyping is possible in 67-86% of lymphomas

(Das 1999, Alam et al. 2010). In canine oncology it is especially valuable for diagnosing the most common subtypes of lymphomas (Fournel-Fleury et al. 1997a, Fournel-Fleury et al. 2002, Sapierzyński et al. 2010, Comazzi et al. 2014). Recently, many authors have recommended using cytology as a sufficient diagnostic method for canine lymphomas, especially if supported by immunocytochemistry and/or flow cytometry (Messic 2008, Regan et al. 2012, Avery et al. 2014, Martini et al. 2013, Comazzi et al. 2014, Curran and Thamm 2015, Fernandes et al. 2015, Jankowska et al. 2015, Martini et al. 2015). The great advantage of this method is the possibility of performing immunocytochemical staining. The technical aspects of this diagnostic procedure and possible application of the immunocytochemistry (ICC) were described previously (Sapierzyński and Przędziecki 2012). The antibodies most commonly used in ICC procedures in dogs with lymphoma suspicion include: anti-cluster differentiation (CD) antigens (especially CD3, CD4, CD8, CD21 and CD79 α), and anti-Ki67 antigen. Nowadays, these antibodies are used in correct cytological classification of lymphomas in dogs, and other antibodies could be applied in the future for better characterization of individual cases (e.g. anti-survivin antibodies, anti-P-glycoprotein antibodies or anti-PDGF antibodies).

In dogs with lymphoma cytological examination may be performed for the following indications:

- to establish the final diagnosis, including specific subtype,
- to choose the most representative lymph node for the excisional biopsy and histopathological examination,
- to establish clinical extent of the disease (staging),
- to monitor disease course, especially the response to therapy and posttreatment recurrence,
- and prospectively to detect potential targets for individual targeted therapy.

There are at least three basic reasons for which cytological examination ought to be accepted as a sufficient and reliable diagnostic method for lymphoma in dogs. Firstly, most dog owners consider the fine-needle biopsy as an acceptable non-harmful method of sample collection. Secondly, an increasing number of studies recommend cytology as an accurate test for diagnosing and subtyping canine lymphoma. Finally, the vast majority of canine lymphoma subtypes belong to 4-5 categories characterized by a typical cytological picture; if necessary additional immunocytochemistry with anti-CD3 and anti-CD79 α antibodies allows to any doubts to be dispelled. This especially applies to the high grade diffuse B cell lymphomas (centroblastic type), T zone/clear cell lym-

phoma, marginal zone lymphoma/medium-sized macronucleolated lymphoma, high grade peripheral T cell lymphomas, and lymphoblastic lymphomas (Sapiernyński et al. 2010, Regan et al. 2012, Comazzi et al. 2014).

General cytological criteria. Cytological diagnosis of lymphoma is based on the percentage of cells considered to be neoplastic in the collected smears (usually young immature blastic cells arrested at a certain stage of differentiation). The morphological classification criteria used for diagnosing and subtyping lymphomas are based on the cell size („medium”, „small” or „large”, i.e., nucleus equal to, smaller than, or larger than the diameter of two red blood cells), the shape of the nucleus, the density and structure of the chromatin, the number, size and distribution of the nucleoli, the extension and basophilia of the cytoplasm, and the mitotic index. These criteria are thoroughly described by many authors (Fournel-Fleury et al. 1994, Fournel-Fleury et al. 1997a, Fournel-Fleury et al. 2002, Ponce et al. 2010), and their detailed analysis is beyond the scope of this review. Generally speaking, more than 50% of blastic cells on smears strongly suggest lymphoma; however, usually the neoplastic population comprises more than 70-80% of the cells (Fournel-Fleury et al. 1994, Messick 2008, Sapiernyński et al. 2010, Poggi et al. 2013). Depending on the proportion of small cells in the total population of neoplastic cells lymphomas are classified as large-cell (<50% of small cells), mixed-cell (50-70% of small cells) or small-cell (>70% of small cells) lymphomas (Fournel-Fleury et al. 1994, Sapiernyński et al. 2010). In the study of Ponce et al. (2010) subtyping was determined by the size of cells (majority of small-sized cells or majority of medium- and large-sized cells) and by mitotic index (MI). Cases showing a majority of small-sized cells and a low or medium MI were classified as low-grade lymphomas, whereas those showing a majority of medium- and large-sized cells and a moderate to high MI were classified as high-grade lymphomas.

The cellular population collected from lymphoma-affected tissues can be monomorphic (cells with similar morphology) or pleomorphic (cells with various morphology), with the mitotic activity ranging from low to high. In the majority of cases, neoplastic lymphocytes are indistinguishable from normal lymphocytes in various stages of maturation, thus application of cytological criteria of cellular atypia is pointless in canine lymphoma (Messick 2008). Such features of marked cellular atypia are observed uncommonly in canine lymphomas, and are obvious only in anaplastic lymphomas. The presence of binucleated or multinucleated neoplastic cells and irregular nucleus shape appear to be the most apparent microscopic hallmarks

of malignancy in canine lymphomas, and have been linked to shorter remission and survival time (Munashinge et al. 2015). On the other hand, an increased number of nucleoli proved to be a positive prognostic factor, associated with longer total survival time (Munashinge et al. 2015).

It may be challenging to distinguish between low-grade lymphomas and some non-neoplastic growths of lymphoid tissue (Foulner- Fleury et al. 1994). Absolute domination of small mature lymphocytes is typical for low-grade tumors as well as for normal non-stimulated lymphoid tissue. However, normal lymphoid tissue should not be subjected to cytological examination, since it is not associated with lymphadenomegaly. Additionally, some cases of low-grade lymphomas can be difficult to distinguish from lymphoid hyperplasia; however, in the latter form medium-sized and large blastic cells as well as plasma cells are usually observed, and quite numerous mitotic figures can be seen. Some problems can be encountered in differentiating between clear cell/T zone lymphoma and T-cell hyperplasia. However, some authors suggest that the presence of numerous interdigitating cells and some number of larger blastic cells in association with some clinical information (e.g. chronic dermatitis) may favour diagnosis of T-cell hyperplasia (Foulner- Fleury et al. 1994). Other possible difficulties can be found in immunoblastic lymphomas which can be mistaken for massive immunoblastic hyperplasia, characterised by predominance of large immunoblasts in collected aspirates. Both pathologies are uncommon in veterinary practice and the latter is related mainly to some viral diseases, at least in human patients. This seems to be an indication for an excisional lymph node biopsy; however, in animals immunoblastic hyperplasia is usually transient whereas immunoblastic lymphoma will not resolve. Thus cytological re-examination after a 2-week observation seems to be reasonable, especially if systemic clinical signs are absent.

Presence of lymphoglandular bodies and tangible body macrophages. Lymphoglandular bodies (LGBs) are small round or irregular fragments of cytoplasm of lymphoid cells, especially larger ones (mainly lymphoblasts, immunoblasts and centroblasts) scattered on the smear between intact lymphocytes (Bavle 2014). They are basophilic, with smooth borders, their diameter varies between 2 and 7 μm , and are produced in response to the injury of lymphoid cells. They are useful in differentiating lymphoma from other malignant tumors, especially non-lymphoid round cell tumors (Bavle 2014). LGBs are observed in both non-malignant and malignant lymphocytic proliferations; however, they seem to be more numerous in lymphomas (Messick 2008). More-

over, it has been suggested that LGBs are more abundant in B-cell than T-cell lymphomas or myeloid leukemias (Rauh and Good 2014).

Histopathological examination of many high-grade lymphomas reveals a „starry sky” pattern (Foulner-Fleury et al. 1997a, Ponce et al. 2010, Valli et al. 2010). This microscopic picture is due to the presence of numerous tangible body macrophages (TBMs) randomly scattered among neoplastic lymphocytes. Tangible body macrophages are macrophages containing death apoptotic neoplastic cells or their fragments (apoptotic bodies). The presence of TBMs is a consequence of an increased rate of proliferation and apoptosis typically observed in most subtypes of high grade lymphomas, especially those of B of lymphocyte origin. The „starry sky” appearance is moderately to highly pronounced in the high-grade lymphomas, including large B-cell lymphomas (especially centroblastic subtype), Burkitt-type or lymphoblastic lymphomas, but is rarely observed in plasmacytoid B cell lymphoma and aggressive large granular lymphoma (Ponce et al 2010). This cytological parameter is never present in low grade lymphomas and very rarely in T cell lymphomas (regardless of proliferative activity) and hence it appears useful in lymphoma subtyping (Foulner-Fleury et al. 1997a, Valli et al. 2010, Ponce et al. 2010). Fortunately, TGMs are easily detectable in cytological smears.

Immunophenotyping. Some cytological features strongly suggest the cell’s immunophenotype during microscopic examination of Giemsa-stained smears. For instance, morphological features indicating T cell immunophenotype include irregular nucleus shape, voluminous, pale cytoplasm with azurophilic fine granules, and the presence of plasma cells in the background of the smear (Fournel- Fleury et al. 2002). In some cases the presence of hyperplasia of postcapillary venules, which is typical for T zone lymphomas, can be observed in cytological samples. Proliferative activity of neoplastic cells measured by mitotic index or Ki67 immunocytochemistry varies among the lymphoma subtype; however, is usually higher in high-grade T cell lymphomas (Poggi et al. 2013). Valli et al. (2013) have suggested that the cytologic appearance of clear cell/T zone lymphomas is highly specific and the definitive diagnosis can be based solely on cytological examination.

As was previously mentioned, cytologic smears are excellent material for immunocytochemical staining. Even though microscopic examination of Giemsa-stained smears allow the immunophenotype of neoplastic cells to be predicted with 90% certainty in many lymphoma subtypes (Sapierzyński et al. 2012), unequivocal determination of immunophenotype requires immunocytochemical staining with at

least two antibodies: anti-CD3 (T lymphocyte marker) and anti-CD79 α (B lymphocyte marker). Immunocytochemistry is easy to perform, and the need to collect a few more smears of good quality and increased cost are the only shortcomings of this diagnostic method. Since the immunophenotype of neoplastic cells influences both treatment protocol and prognosis, immunophenotyping is recommended as an obligatory diagnostic method during characterization of canine patients with lymphoma by veterinary oncologists in the USA (Regan et al. 2012). ICC using other antibodies (CD4, CD8, CD21, CD45 and others) allows for precise subtyping of lymphoma cells and thus confirmation of less common or unusual types of tumors (Dzimira 2007, Martini et al. 2013, Avery et al. 2014, Pawlak et al. 2014, Seelig et al. 2014, Fernandes et al. 2015).

Proliferative activity. Proliferative activity of the neoplastic cells is one of the parameters determining the biologic behavior of the tumor and thus its clinical presentation and prognosis (Zuccari et al. 2004, Neuman and Kaup 2005, Dolka et al. 2015). In the majority of canine lymphomas high proliferative activity corresponds to high biological malignancy. Several methods of measuring proliferative activity exist, and mitotic index (MI) and Ki67 immunolabeling are the most commonly used in veterinary oncology. Counting of mitotic figures in cytological samples is a part of the routine cytological examination in canine lymphomas. It is widely accepted that low-grade lymphomas are characterized by a low mitotic index, whereas high-grade lymphomas are characterized a moderate to high number of mitotic figures (Fournel-Fleury et al. 1994, 1997, 2002, Ponce et al. 2010, Sapierzyński 2010). Determination of MI is easy to perform in histological slides, but some difficulties may be encountered during examination of this parameter in cytological smears. For example, the neoplastic cells are often unevenly distributed on cytological smears, and thus MIs determined may lack representativeness. The methods applied to MI determination vary between researchers, but the French method is used in most available studies. According to this method MI, is estimated in cytological smears by scanning five fields at 500 x magnification and counting mitotic figures: 0 to 1- low MI; 2 to 4 – medium MI; and >5 – high MI (Fournel- Fleury et al. 1997a, Fournel- Fleury et al. 2002, Sozmen et al. 2005).

Immunocytochemical measurement of cellular proliferative activity based on Ki67 immunostaining was shown to be useful in differentiation between malignant and non-malignant canine mammary tumors, and also in discrimination between non-neoplastic and neoplastic diseases of the canine liver (Zuccari et al. 2004, Neumann and Kaup 2005, Dolka et al. 2015). Ki67 ICC has also proved to be an excellent method

of differentiation between non-neoplastic and neoplastic proliferation of lymphatic tissue (Bauer et al. 2007, Fernandes et al. 2015). Moreover, Fournel-Fleury et al. (1997b) have confirmed that immunocytochemistry using Ki67 antibodies is a useful method of determination of proliferative activity of lymphoma cells, and have revealed high concordance between the results of Ki67 immunocytochemistry and immunohistochemistry performed on histological samples of lymphoma tissues. Additionally, in this study a significant difference in the percentage of Ki67 positive cells was found between low-grade and high-grade lymphomas (less and more than 21%, respectively) (Fournel-Fleury et al. 1997b). The percentage of Ki67 positive cells may vary between various subtypes of lymphomas, e.g. it was 5% in small lymphocytic B-cell lymphoma and clear cell lymphoma, 7% in medium-sized macronucleolated cell lymphoma, 40% in centroblastic pleomorphic large cell lymphoma, and as high as 80% in Burkitt-type lymphomas and aggressive large granular cell lymphoma (Phillips et al. 2000, Ponce et al. 2010, Poggi et al. 2013).

In the study of Munashinge et al. (2015) the counting of Ki67 positive cells was performed in 500 cells, however, it is worth emphasizing that this analysis does not have to be so labor-consuming because counting as few as 100 cells is sufficient to obtain reliable results of Ki67 immunostaining (Bauer et al. 2007). Counts can be performed in randomly chosen adjacent microscopic fields; however, unless positive cells are evenly distributed the analysis can be made in regions with the most intensive staining (Bauer et al. 2007, Munashinge et al. 2015).

Conclusion

Much information important from both the diagnostic and prognostic standpoint can be drawn from an examination of cellular samples collected from neoplastic lesions by low invasive methods. This is especially applicable to canine lymphomas, where accuracy of basic cytological techniques is high and can be further increased by immunocytochemical staining with the use of specific antibodies. The latter allows detection of cellular markers better characterizing the tumor as well as identification of potential targets for targeted therapy in individual patients, which appears to be the future of anticancer chemotherapy.

References

Alam K, Jain A, Maheshwari V, Siddiqui FA, Haider N, Khan AH (2010) Fine-needle aspiration cytology diag-

- nosis of non-Hodgkins lymphoma in a resource-challenged environment. *Diagn Cytopathol* 39: 461-467.
- Amores-Fuster I, Cripps P, Graham P, Marrington AM, Blackwood L (2015) The diagnostic utility of lymph node cytology samples in dogs and cats. *J Small Anim Pract* 56: 125-129.
- Aresu L, Martini V, Rossi F, Vignoli M, Sampaolo M, Arico A, Laganga P, Pierini A, Frayssinet P, Mantovani R, Marconato L (2013) Canine indolent and aggressive lymphoma: clinical spectrum with histologic correlation. *Vet Comp Oncol* 13: 348-362.
- Avery PR, Burton J, Bromberek JL, Seelig DM, Elmslie R, Correa S, Ehrhart EJ, Morley PS, Avery AC (2014) Flow cytometric characterization and clinical outcome of CD4+ T-cell lymphoma in dogs: 67 cases. *J Vet Intern Med* 28: 538-546.
- Bauer NB, Zervos D, Moritz A (2007) Argyrophilic nucleolar organizing regions and Ki67 equally reflect proliferation in fine needle aspirates of normal, hyperplastic, inflamed, and neoplastic canine lymph nodes (n=101). *J Vet Intern Med* 21: 928-935.
- Bavle RM (2014) Lymphoglandular bodies. *J Oral Maxillofac Pathol* 18, 334-335.
- Comazzi S, Guscetti F, Marconato L (2014) First meeting of the European canine lymphoma group. Workshop: state of the art and comparative aspects in canine lymphoma. CH-Lugano, 22 June 2013. *Hematol Oncol* 32: 68-71.
- Curran K, Thamm DH (2015) Retrospective analysis for treatment of naive canine multicentric lymphoma with 15-week, maintenance-free CHOP protocol. *Vet Comp Oncol* doi: 10.1111/vco.12163.
- Das DK (1999) Value and limitations of fine-needle aspiration cytology in diagnosis and classification of lymphomas. A review. *Diagn Cytopathol* 21: 240-249.
- Dolka I, Gruk-Jurka A, Wojtkowska A, Miśkiewicz A, Jurka P, Sapiernyński R (2015) Evaluation of canine mammary tumors using cytologic features, computerized cytometry and immunocytochemistry. The 33 Annual Meeting of ESVP 2-5 September, Helsinki, Finland.
- Dzimira S (2007) Immunocytochemical and cytometric diagnostics of malignant lymphomas in dogs. *Bull Vet Inst Pulawy* 51: 71-78.
- 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.
- Fernandes NC, Guerra JM, Ressio RA, Wasques DG, Etlinger-Colonelli D, Lorente S, Nogueira E, Dagli ML (2015) Liquid-based cytology and cell block immunocytochemistry in veterinary medicine: comparison with standard cytology for the evaluation of canine lymphoid samples. *Vet Comp Oncol* doi: 10.1111/vco.12137.
- Flood-Knapik KE, Durham AC, Gregor TP, Sanchez MD, Durney ME, Sorenmo KU (2012) Clinical, histopathological and immunohistochemical characterization of canine indolent lymphoma. *Vet Comp Oncol* 11: 272-286.
- Fournel-Fleury C, Magnol JP, Guelfi J-F. (1994) The lymph node. In: Fournel-Fleury C, Magnol JP, Guelfi J-F (eds) *Color atlas of cancer cytology of the dog and cat*. CNVSPA, Paris, pp 243-321.
- Fournel-Fleury C, Magnol JP, Bricaire P, Marchal T, Chabanne L, Delverdier A, Bryon PA, Felman P (1997a) Cytological and immunological classification of ca-

- nine malignant lymphomas: comparison with human non-Hodgkin's lymphomas. *J Comp Pathol* 117: 35-59.
- Fournel-Fleury C, Magnol JP, Chabanne L, Ghernati I, Marchal T, Bonnefond C, Bryon PA, Felman P (1997b) Growth fractions in canine non-Hodgkin's lymphomas as determined in situ by the expression of the Ki-67 antigen. *J Comp Pathol* 117: 61-72.
- Fournel-Fleury C, Ponce F, Felman P, Blavier A, Bonnefond C, Chabanne L, Marchal T, Cadore JL, Goy-Thollot I, Ledieu D, Ghernati I, Magnol JP (2002) Canine T-cell lymphomas: a morphological, immunological, and clinical study of 46 new cases. *Vet Pathol* 39: 92-109.
- Jagielski D, Lechowski R, Hoffman-Jagielska M, Winiarczyk S (2002) A retrospective study of the incidence and prognostic factors of multicentric lymphoma in dogs (1998-2000). *J Vet Med A* 49: 419-424.
- Jankowska U, Jagielski D, Czopowicz M, Sapieryński R (2015) The animal-dependent risk factors in canine T-cell lymphomas. *Vet Comp Oncol* doi: 10.1111/vco.12164.
- Lurie DM, Milner RJ, Suter SE, Vernau W (2008) Immunophenotypic and cytomorphologic subclassification of T-cell lymphoma in the boxer breed. *Vet Immunol Immunopathol* 125: 102-110.
- Martini V, Poggi A, Riondato F, Gelain ME, Aresu L, Comazzi S (2015) Flow-cytometric detection of phenotypic aberrancies in canine small clear cell lymphoma. *Vet Comp Oncol* 13: 281-287.
- Martini V, Marconato L, Poggi A, Riondato F, Aresu L, Cozzi M, Comazzi S (2015) Canine small clear cell/T-zone lymphoma: clinical presentation and outcome in a retrospective case series. *Vet Comp Oncol* doi: 10.1111/vco.12155.
- Messic JB (2008) The lymph nodes. In: Cowell RL, Meinkoth JH, Tyler RT, DeNicola DB (eds.) *Diagnostic Cytology and Hematology of the Dog and Cat*, Mosby Elsevier, St. Louis, pp 179-192.
- Munasinghe LI, Kidney BA, MacDonald-Dickinson V, Larson VS, Jackson ML, Fernandez NJ (2015) Evaluation of lymph node aspirates at diagnosis and relapse in dogs with high-grade multicentric lymphoma and comparison with survival time. *Vet Clin Pathol* 44: 310-319.
- Neumann S, Kaup FJ (2005) Usefulness of Ki-67 proliferation marker in the cytologic identification of liver tumors in dogs. *Vet Clin Pathol* 34: 132-136.
- Pastor M, Chalvet-Monfray K, Marchal T, Keck G, Magnol JP, Fournel-Fleury C, Ponce F (2009) Genetic and environmental risk indicators in canine non-Hodgkin's lymphomas: breed associations and geographic distribution of 608 cases diagnosed throughout France over 1 year. *J Vet Intern Med* 23: 301-310.
- Pawlak A, Rapak A, Drynda A, Poradowski A, Zbyryt I, Dzimira S, Suchański J, Obmińska-Mrukowicz B (2014) Immunophenotypic characterization of canine malignant lymphoma: a retrospective study of cases diagnosed in Poland Lower Silesia, over the period 2011-2013. *Vet Comp Oncol* doi: 10.1111/vco.12112.
- Phillips BS, Kass PH, Naydan DK, Winthrop MD, Griffey SM, Madewell BR (2000) Apoptotic and proliferation indexes in canine lymphoma. *J Vet Diagn Invest* 12: 111-117.
- Ponce F, Magnol JP, Ledieu D, Marchal T, Turinelli V, Chalvet-Monfray K, Fournel-Fleury C (2004) Prognostic significance of morphological subtypes in canine malignant lymphomas during chemotherapy. *Vet J* 167: 158-166.
- Ponce F, Marchal T, Magnol JP, Turinelli V, Ledieu D, Bonnefond C, Pastor M, Delignette ML, Fournel-Fleury C (2010) A morphological study of 608 cases of canine malignant lymphoma in France with a focus on comparative similarities between canine and human lymphoma morphology. *Vet Pathol* 47: 414-433.
- Poggi A, Miniscalco B, Morello E, Comazzi S, Gelain ME, Aresu L, Riondato F (2015) Flow cytometric evaluation of ki67 for the determination of malignancy grade in canine lymphoma. *Vet Comp Oncol* 13: 475-480.
- Rauh MJ, Good DJ (2014) Bodies of evidence? Lymphoglandular bodies in aspirate smears of bone marrow involved by aggressive large B-cell lymphoma. *Blood* 123: 3695.
- Rebhun RB, Kent MS, Borroffka SA, Frazier S, Skorupski K, Rodriguez CO (2011) CHOP chemotherapy for the treatment of canine multicentric T-cell lymphoma. *Vet Comp Oncol* 9: 38-44.
- Regan RC, Kaplan MS, Bailey DB (2012) Diagnostic evaluation and treatment recommendations for dogs with substage-a high-grade multicentric lymphoma: results of a survey of veterinarians. *Vet Comp Oncol* 11: 287-295.
- Sapieryński R, Micuń J, Jagielski D, Jurka P (2010) Cytopathology of canine lymphomas (100 cases). *Pol J Vet Sci* 13: 653-659.
- Sapieryński R (2010) Practical aspects of immunocytochemistry in canine lymphomas. *Pol J Vet Sci* 13: 661-668.
- Sapieryński R, Dolka I, Fabisiak M (2012) High agreement of routine cytopathology and immunocytochemistry in canine lymphomas. *Pol J Vet Sci* 15: 247-252.
- Sapieryński R, Przeździecki R (2012) Application and possible application of immunocytochemistry in veterinary oncology. *Życie Wet* 87: 566-571.
- Seelig DM, Avery P, Webb T, Yoshimoto J, Bromberek J, Ehrhart EJ, Avery A (2014) Canine T-zone lymphoma: unique immunophenotypic features, outcome, and population characteristics. *J Vet Intern Med* 28: 878-886.
- Sozmen M, Tasca S, Carli E, de Lorenzi D, Furlanello T, Caldin M (2005) Use of fine needle aspirates and flow cytometry for the diagnosis, classification, and immunophenotyping of canine lymphomas. *J Vet Diagn Invest* 17: 323-330.
- Uherova P, Ross CW, Finn WG, Singleton TP, Kansal R, Schnitzer B (2002) Peripheral T-cell lymphoma mimicking marginal zone B-cell lymphoma. *Mod Pathol* 15: 420-425.
- Valli VE, Vernau W, de Lorimier LP, Graham PS, Moore PF (2006) Canine indolent nodular lymphoma. *Vet Pathol* 43: 241-256.
- Valli VE, San Myint M, Barthel A, Bienzle D, Caswell J, Colbatzky F, Durham A, Ehrhart EJ, Johnson Y, Jones C, Kiupel M, Labelle P, Lester S, Miller M, Moore P, Moroff S, Roccabianca P, Ramos-Vara J, Ross A, Scase T, Tvedten H, Vernau W (2010) Classification of canine malignant lymphomas according to the World Health Organization criteria. *Vet Pathol* 48: 198-211.
- Valli VE, Kass PH, San Myint M, Scott F (2013) Canine lymphomas: association of classification type, disease stage, tumor subtype, mitotic rate, and treatment with survival. *Vet Pathol* 50: 738-748.
- Zuccari DA, Santana AE, Cury PM, Cordeiro JA (2004) Immunocytochemical study of Ki-67 as a prognostic marker in canine mammary neoplasia. *Vet Clin Pathol* 33: 23-28.