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Diagnosis and Classification of Primary Nodal Lymphomas in Dogs: A Consensus of the Oncology-Pathology Working Group

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ABSTRACT

One of the primary objectives of the Oncology Pathology Working Group (OPWG) is for oncologists and pathologists to collaboratively generate consensus documents to standardise aspects of and provide guidelines for oncologic pathology in veterinary species. Consensus is established through critical review of the peer-reviewed literature relevant to a subgroup's particular focus. In this article, the authors provide a critical review of the current literature regarding methods for the diagnosis and classification of primary nodal lymphomas of dogs, including histopathology, cytopathology, immunophenotyping and assessment of molecular clonality. Knowledge gaps in the current literature and recommendations for future study are also reported. Major conclusions of this consensus include: (1) Histopathology with immunohistochemistry is required for complete diagnosis and classification of nodal lymphomas; (2) Immunohistochemistry and flow cytometry are the most reliable methods of immunophenotyping lymphomas, though neither is clearly superior to the other; (3) Molecular clonality testing should not be used in favour of immunophenotyping assays for classifying lymphomas; and (4) The use of emerging molecular tests for diagnosing lymphomas in the absence of histopathologic, cytopathologic, or immunophenotypic disease characterisation should be restricted to investigational settings until their diagnostic validity and the clinical benefit they confer to patients are more thoroughly characterised. This document represents the opinions of the OPWG and the authors; it does not constitute a formal endorsement by the American College of Veterinary Pathologists or the Veterinary Cancer Society.

1 | Introduction

Naturally-occurring lymphomas in domestic dogs represent a heterogeneous family of cancers with distinct pathological and clinical features. The clinical classification of lymphomas is conventionally based upon the organ site(s) at which they are most apparent, otherwise known as the cancer's *anatomic form*. The most common of these is the so-called "multicentric" form, in which the disease burden is most apparent in peripheral lymph nodes. These peripheral nodal lymphomas are the focus of this OPWG consensus.

The diagnosis and classification of nodal lymphomas is based upon histomorphologic, cytomorphologic, immunophenotypic and, increasingly, molecular biological criteria. This consensus manuscript offers a critical review of recent literature on the use of histopathology, cytopathology, immunophenotyping, and assessment of molecular clonality to diagnose and classify these cancers. The emerging applications of advanced molecular diagnostics such as next generation RNA and DNA sequencing to this process are also briefly discussed. Whilst some of these diagnostic tests also play increasingly important roles in staging lymphomas (including assessment of minimal residual disease (MRD) burden following chemotherapy), disease staging was not a focus of this consensus and will not be discussed in detail.

From this literature review, evidence-based recommendations for best practise in the diagnosis and classification of primary nodal lymphomas of dogs are made, knowledge gaps in the literature are identified, and directions for future study are recommended. It should be acknowledged that, in routine clinical scenarios, the "best practise" for diagnosing and classifying nodal lymphomas in dogs often must strike a balance between the ideal and the practical, as exhaustive diagnostic evaluation may not be feasible or required to inform treatment decisions in all cases. It should be further acknowledged that, though the focus of this review is not disease prognostication, diagnostic and prognostic assessment are inextricable from one another. Therefore, the significance of a specific disease diagnosis to patient prognosis (in terms of response to therapy or survival time) will be discussed when relevant to this review. Finally, the importance of *prior probability of disease* [1] to selecting and interpreting diagnostic tests for lymphomas in dogs is also discussed on several occasions in this manuscript. Prior probability reflects the expected prevalence of disease in a population of animals with the clinical characteristics of the individual animal undergoing diagnostic evaluation. These include not only clinical signs of disease but also genetic (i.e., breed-related) and environmental risk factors for the disease. Because it concerns disease prevalence, prior probability directly affects the positive predictive value of a diagnostic test. Since no diagnostic test for lymphoma has perfect sensitivity, specificity and predictive value, a *combination* of diagnostic tests is often preferred (and in some cases, necessary) to establish a diagnosis.

The consensus opinion offered here was developed by the Canine Lymphoma Subgroup of the Oncology Pathology Working Group (OPWG), an initiative jointly supported by the American College of Veterinary Pathologists (ACVP) and Veterinary Cancer Society (VCS). The information presented here reflects the expert opinion of the Canine Lymphoma Subgroup, with review, input, and approval by the OPWG membership at large; a formal endorsement of this consensus statement by the ACVP or VCS is not implied.

2 | Materials and Methods

In the summer of 2022, the Canine Lymphoma Subgroup conducted a thorough search of the scientific peer-reviewed literature on canine nodal lymphomas published during the preceding 20 years (2002–2022). The initial search was broad to capture any articles reporting on the value or limitations of current diagnostic tools, including cytopathology, histopathology, immunohistochemistry, flow cytometry, immunocytochemistry and clonality assays as they relate to the diagnosis and classification of nodal lymphomas of dogs. Peer-reviewed literature that discussed the role of emerging molecular diagnostics in this process was also captured. There were no restrictions to the search engines used to identify relevant articles, although PubMed, Scopus, Google Scholar and Cab Direct were used most commonly. The subgroup consisted of 4 veterinary medical oncologists board certified by the American College of Veterinary Internal Medicine (PB, MC, WK, AM-W), 1 veterinary medical oncologist board certified by the European College of Veterinary Internal Medicine (LaM), 1 Fellow of the Australian and New Zealand College of Veterinary Scientists in Oncology (LB), 6 veterinary pathologists board certified by the ACVP (EB-K, LuM, RD, KH, PR, SS), 1 PhD-trained veterinary clinical pathologist with expertise in lymphoid pathology (VM), 1 veterinary molecular geneticist (LA), and 1 veterinary immunologist (AA), all of whom are co-authors of this consensus.

All subgroup members participated in the initial literature search. Articles identified through the search were saved to a shared Google drive. The subgroup co-chairs (MC and LA) reviewed each article for scientific rigor and relevance to the purpose and scope of the consensus document. Following this initial review, 164 articles were retained and 53 were discarded due to being published in non-peer-reviewed journals or due to their irrelevance to the subject of this consensus statement (e.g., concerning staging, prognostication, or treatment of lymphoma rather than diagnosis and classification). The retained articles were then distributed to the 15 subgroup members for critical in-depth review, in accordance with the OPWG's standard review process. Each subgroup member was asked to review 10–11 articles and to summarise their findings using a standardised review template, as described in previous OPWG-drafted consensus statements [2]. In their reviews, the subgroup members recommended that nearly half of the 164 reviewed articles not be referenced in the consensus statement due to redundancy, irrelevance, or methodological concerns (e.g., limited statistical power, outdated/inappropriate analytical techniques). Review articles and single case reports were included amongst the materials used to generate the consensus statement if they contained unique clinical data or viewpoints not stated as clearly in other reports. All articles referenced in the final consensus statement were reviewed by at least 2 subgroup members, including one or both of the co-chairs. An initial consensus document was drafted by the co-chairs and was then sent to the entire subgroup for review and discussion. During the review process, additional references were added to support specific statements

in the final draft,[1] which, following approval by the OPWG Executive Committee for adherence to consensus document guidelines and procedures, was distributed electronically to the full OPWG membership in July 2024 for review and comment. A four-week open comment and review period ensued, after which the subgroup considered all input received from the OPWG membership and revised the document accordingly whilst providing responses to each member comment. The final consensus document was fully approved by the OPWG in December 2024.

3 | Results

3.1 | Histopathology

The World Health Organisation (WHO) classification system [3] recognises over 30 histopathologic subtypes of lymphoma in the dog (Table 1), defined on the basis of morphologic and immunophenotypic criteria. The five most common subtypes—diffuse large B-cell lymphoma (DLBCL), marginal zone lymphoma (MZL), peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS), T-zone lymphoma (TZL), and T-lymphoblastic lymphoma (T-LBL)[2]—account for nearly 80% of all cases [3]. The rate at which pathologists reach a consensus diagnosis using the WHO system—approximately 80%–90%—underscores its clinical reproducibility [3]. The purpose of the WHO system is to segregate lymphomas into distinct disease entities with characteristic clinical features, including rates of progression and responsiveness to therapy. Under the WHO system, lymphomas are classified most broadly by the maturation stage of their constituent cells. *Precursor* neoplasms derive from immature lymphocytes that have not yet migrated from the bone marrow (in the case of B- or T-cells) or thymus (in the case of T-cells), whereas *mature* neoplasms derive from lymphocytes that have migrated from the bone marrow and thymus to complete their maturation in secondary lymphoid tissues. Except for T-LBL, the most common lymphomas of dogs are derived from mature lymphocytes. Additional pathological classification of lymphomas is based on cellular morphology, lesional topography and immunophenotype [3]. In humans, gene expression, cell of origin, and cytogenetic abnormalities also play important roles in the WHO classification of lymphomas [5]. It is likely that these features will also be reflected in updated classification schemes in dogs as new data on the molecular pathogenesis of canine lymphomas are reported.

Under the WHO system, nodal lymphomas are also classified broadly as either *aggressive* or *indolent* cancers. Aggressive nodal lymphomas recognised in the dog include DLBCL, PTCL-NOS, T-LBL and Burkitt-like lymphoma (B-LL). They are associated with rapid clinical progression and high mortality, particularly in the absence of treatment [6]. The indolent nodal lymphomas include small lymphocytic lymphoma (SLL) and TZL. These cancers are generally characterised by slow, insidious clinical progression, prolonged survival and low disease-specific mortality [6–8]. However, these generalisations cannot be considered absolute, as aggressive clinical phenotypes and short survival times have been documented in some dogs with "indolent" histopathologic subtypes. Most notable amongst these is nodal MZL, which is classified as indolent by some sources [4],

TABLE 1 | Histological classification of lymphomas of dogs according to World Health Organization criteria.

B-cell neoplasms	T-cell and natural killer (NK) cell neoplasms
Precursor B-cell neoplasms	Precursor T-cell Neoplasms
• B-lymphoblastic lymphoma/leukaemia	• T-lymphoblastic lymphoma/leukaemia
Mature B-cell neoplasms	Mature T-cell neoplasms
• B-cell chronic lymphocytic leukaemia (CLL)/small lymphocytic lymphoma (SLL) • B-cell prolymphocytic leukaemia • Lymphoplasmacytic lymphoma • Marginal zone lymphomas ◦ Splenic marginal zone lymphoma ◦ Extranodal marginal zone lymphoma of MALT ◦ Nodal marginal zone lymphoma • Plasma cell neoplasms ◦ Plasmacytoma ◦ Multiple myeloma • Follicular lymphoma • Mantle cell lymphoma • Diffuse large B-cell lymphoma ◦ T-cell rich large B-cell lymphoma/lymphomatoid granulomatosis • Mediastinal large B-cell lymphoma ◦ Large cell immunoblastic lymphoma ◦ Intravascular large B-cell lymphoma • Burkitt-like lymphoma • Primary effusion lymphoma	• T-cell chronic lymphocytic leukaemia • Peripheral nodal T-zone lymphoma • T-cell prolymphocytic leukaemia • Large granular lymphocytic leukaemia/lymphoma • Aggressive NK cell leukaemia/Blastic NK cell lymphoma • Mature nodal and extranodal T-cell lymphomas ◦ Angioimmunoblastic T-cell lymphoma ◦ Hepatosplenic T-cell lymphoma ◦ Intestinal T-cell lymphoma (+/− enteropathy associated) ◦ Subcutaneous panniculitis-like T-cell lymphoma • Mycosis fungoides/Sézary syndrome • Anaplastic large cell lymphoma • Adult T-cell lymphoma/leukaemia • Intravascular large T-cell lymphoma • Peripheral T-cell lymphoma, not otherwise specified

yet has an aggressive clinical behaviour [9, 10]. It is likely that, as new information regarding clinical outcomes in dogs with specific lymphoma subtypes (particularly uncommon subtypes) continues to emerge, the classification of these subtypes as aggressive vs. indolent will evolve as well.

In addition to WHO subtype, histopathologic evaluation of nodal lymphomas typically assigns a tumour grade. Under the WHO system, grade is assigned entirely according to mitotic count, with tumours demonstrating 0–5, 6–10, and >10 mitotic figures per high-power field (0.237 mm^2) classified as low, intermediate and high grade, respectively [3, 6]. The histopathologic grade of nodal lymphomas has not been shown to correlate completely with clinical phenotype; thus, aggressive cancers such as DLBCL can be assigned a low histopathologic grade based upon the mitotic count [6]. This can be a point of confusion amongst veterinary oncologists and cytopathologists accustomed to conceptualising nodal lymphomas according to a *clinical* grade based upon cell size, cytomorphological features and clinical course [11].

The determination of WHO subtype and grade requires evaluation of high-quality biopsy samples, although the ideal method of nodal biopsy has not been fully established. Surgical biopsy (via nodal extirpation or incisional wedge biopsy) of an affected lymph node has the advantage of providing a greater volume of tissue for histopathologic evaluation and is the only method that allows assessment of some morphologic features associated with clinical behaviour, such as tumour invasion through the lymph node capsule into perinodal adipose tissue. In the case of some morphologically ambiguous and/or indolent lymphomas, large samples collected by surgical biopsy may be needed to fully assess the lymph node architecture and pattern of infiltration.

This notwithstanding, the superiority of surgical biopsy to less invasive approaches (such as needle core biopsy; NCB) for diagnosing and classifying lymphomas has not been established in an evidence-based fashion. One small study comparing the diagnostic utility of NCB with lymphadenectomy found that NCB accurately confirmed the presence of lymphoma in 12/14 (86%) cases [12]. However, the study made no attempt to use NCB samples to determine the WHO subtype, and all tumours included in the study were characterised by diffuse effacement of nodal architecture, making assessment of the presence of lymphoma relatively straightforward. Two large studies [3, 6] reporting WHO subtype in 300 and 992 dogs with nodal lymphomas, respectively, report a significant proportion of specimens in which WHO subtype was assigned to samples acquired by NCB; however, a formal comparison of the rate at which pathologists accurately assign a WHO subtype from NCB vs. lymphadenectomy specimens was not performed in these studies. Given that NCB has the advantages of technical simplicity and decreased surgical morbidity when compared to lymphadenectomy, a formal comparison of the accuracy of these two methods for assigning WHO subtype seems warranted.

3.2 | Cytopathology

Although lymphadenectomy may represent an ideal means for procuring samples to diagnose and classify nodal lymphomas, it is invasive, time-consuming and impractical in many clinical settings. Cytopathologic evaluation of lymph node samples collected by fine needle aspirate (FNA) is comparatively less technically demanding, less costly, and has a shorter turnaround time to yield diagnostic results than surgical lymph node biopsy. Fine needle aspirate samples can also be submitted for rapid immunophenotyping via flow cytometry or immunocytochemical staining. These factors likely contribute to the far greater popularity of FNA when compared to surgical lymph node biopsy amongst veterinarians in clinical practise [13].

Although cytopathology is commonly accepted as an accurate means for diagnosing the presence of nodal lymphomas, particularly aggressive subtypes in which nodal architecture is effaced by monomorphic large lymphocytes [14], the extent to which its diagnostic performance has been critically evaluated is quite limited. In one retrospective study [15] evaluating the diagnostic accuracy of lymph node cytopathology in dogs and cats with a variety of nodal pathologies, an accurate diagnosis of lymphoma was made in 24/26 (92.3%) dogs for which histopathology and immunophenotyping results from the same lymph node were available as a reference standard. However, no attempt was made in this study to correlate cytopathology results with specific histopathologic subtypes of lymphoma. Such an attempt was made in an older study [16], in which the correlation between cytomorphologic diagnosis and histologic diagnosis was relatively strong (Cramér's V 0.65–0.70) [17]. However, this report predates the publication of the WHO system, so translating these results to contemporary methods of lymphoma classification is problematic.

The subgroup identified only one report [18] in which an attempt was made to correlate the results of cytopathology with histopathologic subtype defined by WHO criteria. In this report, six evaluators reviewed FNA samples from 161 lymph nodes for which histopathologic sections of lymphadenectomy specimens were also available for review. Of these lymph nodes, 146 were affected by lymphoma, whilst 15 were affected by other pathologies (reactive hyperplasia or metastatic non-lymphoid cancer). The proportion of lymph nodes in which the presence of lymphoma was correctly identified (i.e., classification accuracy) amongst the 6 evaluators ranged from 82.9% to 93.2%. The overall sensitivity of FNA cytology for diagnosing lymphoma was estimated at 92.6%. The classification accuracy of these evaluators when predicting clinical phenotype (high/low grade) and immunophenotype (B/T-cell) from cytopathology results was generally lower and highly variable. The accuracy with which the evaluators identified high-grade B-cell tumours, low-grade B-cell tumours and high-grade T-cell tumours ranged from 23.8%–77.4%, 2.4%–40.5%, and 33.3%–75%, respectively. The clinical phenotype that the evaluators accurately identified most consistently was low-grade T-cell lymphomas, which 5/6 evaluators identified with ≥75% accuracy. The classification accuracy when predicting WHO subtype was best for TZL (≥75% for 5/6 evaluators) and DLBCL (≥65% for 5/6 evaluators), but poor for other common subtypes such as PTCL-NOS (≤40% for all evaluators). Importantly, the proportion of cases in which lymphoma was present, yet a diagnosis of non-lymphoma was reported by at least one of the evaluators (i.e., rate of false negative diagnosis), ranged from 27.7% to 60.9%. These results indicate

that, whilst many nodal lymphomas can be accurately diagnosed using cytopathology alone, complete disease classification requires additional testing. Furthermore, they suggest that FNA results indicating absence of lymphoma should be viewed with some degree of scepticism in dogs with a high prior probability of disease (i.e., cytopathology has a low negative predictive value when disease prevalence is high).

3.3 | Immunophenotyping

Immunophenotyping involves the identification of proteins specific to a given lymphocyte lineage using antibody-based detection methods (Table 2). It is an essential adjunct to histopathology and/or cytopathology to allow complete diagnostic classification of nodal lymphomas of dogs, since neither cytologic nor histologic features reliably predict a tumour's immunophenotype [19, 20]. In its earliest incarnations, immunophenotyping was used primarily to classify lymphomas as either T- or B-cell tumours, which are considered one of the strongest predictors of clinical outcome in dogs with aggressive histopathologic subtypes [20–22]. However, as immunophenotyping techniques in veterinary medicine have evolved, they have been used to classify tumours derived from more restricted lymphocyte subsets or stages of maturation [23, 24], as well as to characterise other tumour traits such as the growth fraction [25, 26].

The most commonly used methods for immunophenotyping nodal lymphomas of dogs are immunohistochemistry (IHC) and flow cytometry (FC), although immunocytochemistry (ICC) and multiple immunofluorescence (IF) have also been described. There is no "ideal" method for immunophenotyping lymphomas; all methods have relative advantages and disadvantages, which are described in the sections that follow. The preferred immunophenotyping method for a given clinical situation depends highly on the type of tissue sample available (e.g., FNA vs. larger tissue biopsy), the diagnostic information desired, and whether the immunophenotyping lab has validated assays for detecting the immunophenotypic marker(s) of interest. As some immunophenotypic markers can be detected by many methods, the antibody clones used by individual labs for detecting these markers are critically important. Some clones perform well across multiple detection methods, whilst others are only validated for use in a single method. Clinicians are highly encouraged to consult with a pathologist to guide the selection of the most appropriate immunophenotyping method in each clinical scenario.

It is noteworthy that all methods currently used for immunophenotyping nodal lymphomas of dogs suffer from a common limitation, which is a lack of standardisation in reagents, instrumentation and techniques used amongst different laboratories [27–29]. Whilst this limitation makes comparing immunophenotyping results across different laboratories challenging, it should not be construed to invalidate the results produced by an individual laboratory for an individual dog. Rather, it speaks to a need for greater harmonisation across laboratories so that new knowledge disseminated about specific disease entities can be interpreted consistently by all stakeholders involved in the diagnosis and treatment of dogs with nodal lymphomas.

3.3.1 | Immunohistochemistry

The major advantage to immunohistochemical evaluation is that it allows concurrent evaluation of both histomorphology and immunophenotype. This allows precise determination of which cells within a tumour section express a given antigen (i.e., neoplastic cells, resident stromal cells, etc.), as well as at the subcellular localization of antigen expression (i.e., membranous, cytoplasmic, nuclear). Immunohistochemical analysis is therefore well suited to the initial characterisation of an antigen's expression pattern within a tumour [30, 31], particularly if the antigen is expressed in the nucleus, cytoplasm, or internal surface of the cell membrane, locations that are all less easily evaluated using FC. Immunohistochemistry also can be used to distinguish reactive pathologies from neoplasia when this distinction cannot be made reliably based upon histomorphology alone [32]. Finally, IHC can be used to assess the tumour growth fraction through detection of proliferation-associated proteins like Ki-67. Whilst Ki-67 expression has been more thoroughly researched as a prognostic biomarker, it bears some relevance to lymphoma diagnosis and classification because of its association with tumour grade. One study found Ki-67 expression to be significantly lower in low grade tumours when compared to intermediate or high-grade tumours [26]. Interestingly, this study reported 2 aggressive lymphomas (one each of DLBCL and PTCL) with a low mitotic count yet high percentage of Ki-67-expressing cells, suggesting that Ki-67 expression may be more reliable than mitotic count for assessing tumour grade. However, further study is needed to support such a conclusion.

The major disadvantage to IHC is that it is typically performed on formalin-fixed, paraffin-embedded tissues in which the three-dimensional conformation of the antigens of interest has been altered during the fixation process. This renders the epitopes recognised by some diagnostic antibodies physically inaccessible, thereby preventing antibody recognition [27]. As a result, the palette of antigens detectable using IHC in many diagnostic laboratories is quite limited (most frequently to CD3, CD79a, CD20 and/or Pax-5 expression [30, 31, 33–35]), although selected laboratories report the use of IHC to detect additional antigens of diagnostic interest (e.g., CD5, CD45, CD21, CD30, CD45, FOXP3, granzyme B) [32, 36, 37]. Another disadvantage to IHC is that it requires samples obtained by relatively invasive methods of surgical or needle core biopsy, which are not suitable for all patients.

3.3.2 | Flow Cytometry

Perhaps the greatest advantage to FC is that it can be performed on samples collected by FNA, allowing immunophenotyping of samples collected in a minimally invasive fashion. Given FNA's popularity in the diagnosis of lymphomas, FC is likely the most frequently used method for immunophenotyping these cancers. When compared to IHC analysis as a gold standard, FC correctly identified the immunophenotype of a lymphoma (in terms of B-cell or T-cell derivation) in 94% of cases [38]. In contrast to IHC analysis, however, FC is typically performed on cells that have been immunolabelled whilst still alive. Consequently, antigenic degradation due to tissue fixation, which limits the range of antigens detectable

TABLE 2 | Immunophenotypic markers used to classify lymphomas of dogs.

Marker	Specificity	Subcellular localization	Method(s) of detection	Biological function
CD3	T-lymphocytes	Cell membrane, cytoplasm	IHC, FC, ICC, IF	T-cell activation following antigen recognition
CD4	Helper T-lymphocytes, neutrophils, dendritic cells, macrophages	Cell membrane	FC, ICC	Co-receptor for MHCII; promotes T-cell activation
CD5	T-lymphocytes	Cell membrane	FC, IHC	Negative regulator of T-cell receptor signalling; promotes immune tolerance
CD8	Cytotoxic T-lymphocytes	Cell membrane	FC, ICC	Co-receptor for MHCI; promotes T-cell activation
CD18	Pan-leukocyte	Cell membrane	IHC, FC	Cell-ECM adhesion
CD20	B-lymphocytes	Cell membrane	IHC, IF, FC	Uncertain
CD21	B-lymphocytes	Cell membrane	FC	Complement receptor; promotes B-cell activation after antigen binding
CD22	B-lymphocytes	Cell membrane	FC	Negative regulator of B-cell receptor signalling
CD34	Haematopoietic stem cells; precursor lymphoid neoplasms	Cell membrane	FC	Cell–cell and cell-ECM adhesion
CD45	Pan-leukocyte; absent in most TZL	Cell membrane	FC, IHC	Protein tyrosine phosphatase; regulates several intracellular signalling pathways
CD79a	B-lymphocytes	Cell membrane, cytoplasm	IHC, ICC, IF, FC	Signal transduction through B-cell receptor
MHCII	In health: macrophages, dendritic cells, B- and T-lymphocytes Expressed variably by lymphoid neoplasms	Cell membrane	FC	Antigen presentation
Pax5	B-lymphocytes	Nucleus	IHC	Transcription factor critical to B-cell development

Note: Reported methods for detecting these markers are listed in the 4th column. For each marker, the detection method most frequently reported in the literature is listed first, with other reported methods of detection listed subsequently. Information reported in this column is not comprehensive, as individual labs may have developed and validated antibody clones used in assays not reflected here.
Abbreviations: CD = Cluster of differentiation; ECM = extracellular matrix; FC = flow cytometry; ICC = immunocytochemistry; IF = immunofluorescence; IHC = immunohistochemistry; MHC = Major histocompatibility complex; TZL = T-zone lymphoma.

using IHC analysis, is less impactful on FC. Thus, whilst routine immunophenotyping using IHC analysis typically classifies lymphomas solely on the basis of B-cell or T-cell origin, FC can characterise a diverse range of immunophenotypes based on the expression of a far greater array of antigens. Flow cytometric quantification of Ki-67 expression may also offer some clues as to a lymphoma's clinical aggressiveness [26]. In some cases, the versatility of FC has important diagnostic implications.

One way that FC may contribute to a lymphoma diagnosis is through detection of aberrant antigen expression by lymphocytes, such as loss of CD45 or CD18 expression, or coexpression of antigens normally restricted to a single lineage (e.g., $CD4^+/CD8^+$, $CD3^+/CD21^+$). In doing so, FC can support a diagnosis of lymphoid neoplasia in morphologically ambiguous cases [39–42]. Additionally, some immunophenotypes readily identified by FC are correlated with specific morphologic subtypes of lymphoma. Most notable amongst these subtypes is TZL, which typically expresses the T-cell antigens CD3 and CD5, one, both, or neither of the T-cell subset antigens CD4 and CD8, and high levels of major histocompatibility complex (MHC) II. Interestingly, TZL also expresses the complement receptor CD21, an antigen whose expression usually is restricted to B-cells, and typically does not express the common leukocyte antigen CD45 [24, 43]. This unique immunophenotype, particularly the absence of CD45 expression, allows TZL to be reliably diagnosed by a combination of cytopathology and FC. However, CD45 expression can be decreased or aberrant in subpopulations of cells within more aggressive nodal T-cell lymphomas [40]. Furthermore, CD45 expression was recently documented using IHC in 2/27 cases of histopathologically confirmed TZL [36]. Thus, the absence of CD45 expression in a homogeneous population of T-cells evaluated by FC cannot be used as a sole criterion by which to diagnose TZL.

In addition to its important role in identifying some WHO-defined subtypes of lymphoma, FC may also identify immunophenotypically distinct subsets of tumours *within* these subtypes. As an example, PTCL-NOS, the most common aggressive T-cell lymphoma of dogs, typically has an immunophenotype of $CD3^+/CD4^+/MHC\ II^-$ [23]. A minority of cases are $CD4^-/CD8^+$, $CD4^+/CD8^+$, or $CD4^-/CD8^-$, or may express high levels of MHC II. Importantly, tumours bearing these rarer immunophenotypes are histomorphologically indistinguishable from those with the typical immunophenotype. Although one report [44] suggested superior survival times for dogs with some of these rarer immunophenotypes, this was not corroborated by another report [24]. Similarly, one of these reports [24] noted improved survival time for PTCL-NOS characterised by small cell size, whilst the other did not [44]. Therefore, although the greater breadth of immunophenotyping afforded by FC adds to the diagnostic assessment of some lymphoma subtypes, its impact on prognostic assessment has not been established conclusively. Furthermore, whilst FC can identify immunophenotypically distinct subsets of some T-cell lymphomas, the same is not true of B-cell tumours [45]. The most common B-cell lymphomas, including both aggressive and indolent subtypes, all express a similar repertoire of cell surface antigens, and thus require histopathology and IHC for complete diagnostic classification.

Whilst FC is a versatile technique, it does have some disadvantages, the most significant of which is its requirement for relatively fresh, living cells. This may limit its utility for analysing samples collected outside of normal clinical working hours or other situations in which transit to a FC laboratory may take longer than is ideal [28, 46]. Determining the immunophenotype of samples from lymph nodes characterised by extensive necrosis also may be difficult using FC. Relative to IHC, FC provides less information about cell morphology, although forward light scatter measured by FC does provide a reliable, objective measurement of cell size, which is of diagnostic (and possibly prognostic [24, 47]) importance.

3.3.3 | Immunocytochemistry and Immunofluorescence

Compared to IHC and FC, ICC and IF are used less commonly to immunophenotype nodal lymphomas of dogs [48–52]. As with FC, these techniques can conveniently be performed on samples collected by minimally invasive FNA. An additional convenience of ICC is that it can be performed more rapidly than IHC due to differences in techniques for sample fixation and preparation. ICC also allows more direct visualisation of individual cellular morphology than FC. However, IF and ICC have important disadvantages, including: (1) they provide less quantitative immunophenotypic information, particularly when compared to FC; (2) they are offered at fewer laboratories (whereas FC and IHC are offered at numerous commercial and institutional diagnostic laboratories); (3) their sample quality cannot be assessed prior to staining, which may necessitate collecting multiple FNA samples for complete morphologic and immunophenotypic assessment; and (4) they have undergone little to no validation against a reference standard (such as IHC) [29].

3.4 | Assessment of Molecular Clonality

Monoclonality—the derivation of a cell population from a single ancestral cell—is a hallmark feature of malignancy. Although the monoclonal nature of cancer has been demonstrated by multiple methods, the technique most commonly used in lymphoid tumours is PCR-based evaluation of the genes encoding lymphocyte antigen receptor proteins: the immunoglobulin (Ig) receptor protein in B-lymphocytes and the T-cell receptor (TCR) protein in T-lymphocytes. Specifically, these assays target the DNA sequences encoding the 3rd complementarity determining region (CDR3) of these proteins, which confers exquisite specificity for their cognate antigens [53]. Because the genes encoding the CDR3s possess a unique base sequence and length in each mature lymphocyte in the body, PCR-based amplification of this region of DNA yields a uniquely sized amplicon from each lymphocyte (and its clonal progeny) within a sampled population. Once the PCR reaction is complete, the amplicons are separated by size to allow visualisation of their size distribution. This separation is typically carried out by capillary electrophoresis in contemporary clonality assays, with some laboratories also employing sequencing instruments with fluorescent primers (e.g., GeneScanning) [54], which improves assay efficiency and specificity. If the sampled lymphocyte population is monoclonal, the amplicons produced in the PCR reaction all will be of

identical size. If, on the other hand, the population is polyclonal, the amplicons will be of varying sizes, usually arranged in a Gaussian distribution. Clonality assays used in diagnostic veterinary medicine are typically multiplexed, using primer sets that span several gene loci involved in Ig and TCR sequence determination. This endows a single assay with great sensitivity to detect monoclonal populations attributable to either T- or B-lymphocyte lineages, depending upon the primer(s) resulting in amplicon formation.

Clonality assays are of several potential uses in the diagnostic evaluation of nodal lymphomas of dogs. First, they can confirm the presence of cancer in morphologically or immunophenotypically ambiguous lesions, particularly when the diagnostic distinction must be made between a neoplastic vs. a reactive or benign condition [7]. The identification of a monoclonal population of lymphocytes within a diagnostic sample is supportive of (though not specific to) a diagnosis of lymphoma, whereas a polyclonal population is inconsistent with lymphoma [53]. Second, they can specify the lineage assignment (B-lymphocyte or T-lymphocyte) of a lymphoid cell population in cases where immunophenotyping is not practical (such as when additional or more invasive sample collection is required), not possible (such as when sample quality precludes immunophenotypic assessment), or yields atypical results (e.g., MUM1+/CD3+) [55]. Finally, because of their high sensitivity, clonality assays can be used to identify clonally rearranged Ig and TCR genes in lymph node aspirates [56], bone marrow [57], or peripheral blood [57–60] of dogs whose lymphoma is in a complete clinical remission following chemotherapy (i.e., in the minimal residual disease (MRD) setting). The detection of MRD using clonality assays in dogs that have completed a course of chemotherapy is of demonstrable prognostic value [58, 59]. Notably, all dogs in these studies had histologically or cytologically confirmed lymphoma; the validity of clonality assays for confirming a diagnosis of lymphoma *in the absence of cytopathology or histopathology* has not been established.

Although clonality assays are useful adjunctive tests for diagnosing lymphomas, they must be used thoughtfully, with appreciation of their limitations, to avoid misinterpretation of their results. Clonality assays should not be considered a substitute for immunophenotyping, as one study showed less than 70% agreement of lineage assignment determined by clonality assay with immunophenotype determined either by FC or IHC [38]. Causes of false positive and false negative results must be considered when interpreting the results of clonality assays. False positive results occur in the setting of benign clonal lymphocyte expansion, which has been documented in some dogs with infectious diseases, such as ehrlichiosis [61] and leishmaniasis [62], both of which present with clinical signs similar to those seen in dogs with peripheral nodal lymphomas. False negative results usually occur when the primers for the assay fail to hybridise with sample DNA [53]. This can occur due to mutations at the primer binding sites or inadequate primer coverage of all chromosomal loci used in Ig or TCR gene sequence determination. False negative results also may occur when the input DNA is of poor quality or insufficient quantity [53]. *Cross-lineage rearrangements*, in which clonal rearrangements of both Ig and TCR genes are present within the same tumour, can be another source of confusion when interpreting clonality assay results. Cross-lineage rearrangements are a feature of haematopoietic precursor neoplasms in humans, occurring in approximately 20% and 60%–90% of T- and B-acute lymphoblastic leukaemias, respectively [63–65]. True cross-lineage rearrangements have also been documented on rare occasions in precursor lymphoid tumours of dogs [66, 67]. Dogs with tumours bearing clonal rearrangements of both Ig and TCR genes must be carefully evaluated to determine whether a true cross-lineage rearrangement is present or whether the dog has two (or more) distinct lymphoid neoplasms [68, 69]. Thorough cancer staging with complete morphologic and immunophenotypic assessment of pathological specimens is necessary to resolve the confusion in such circumstances.

Another challenge to interpreting clonality assay results is that, reminiscent of the situation with immunophenotyping assays, there are no consensus standards regarding the methodology, sample quality control procedures, or primers used to perform them [70]. Thus, the sensitivity and specificity of a given assay are unique to the lab in which it was performed. Pre-analytical variables such as the type of sample used (e.g., fresh air-dried vs. formalin-fixed) can affect assay sensitivity and specificity and must also be considered when interpreting results.

3.5 | Advanced Diagnostic Testing for Molecular Subtyping and Early Disease Detection

A considerable body of literature has been published in the past decade providing detailed characterisation of canine lymphomas at the genomic, transcriptomic and epigenomic level. Two predominant themes from these reports are relevant to the diagnosis and classification of these cancers. First amongst these is that nodal lymphomas of dogs can be classified based upon molecular signatures that are distinct even amongst morphologically and immunophenotypically similar tumours. Second, these reports suggest methods by which lymphomas could be diagnosed using blood-based biomarkers in dogs with or without clinical signs of disease.

It has long been recognised in both animals and humans that patients with clinically and histologically similar cancers experience disparate outcomes following treatment. The molecular underpinnings of this disparity have been extensively described in human DLBCL over the past 20 years. Comprehensive transcriptomic and genomic profiling of human DLBCL identifies two major molecular subtypes—germinal center B-cell (GCB) and activated B-cell (ABC) – with characteristic gene expression and mutational profiles [71, 72]. Although additional molecular subtypes continue to be identified [73], the prognostic significance of GCB vs. ABC classification is clear: patients with the ABC subtype experience significantly lower cure rates and shorter survival times. In 2013, Richards et al. published the first report of GCB- and ABC-like subtypes of canine DLBCL, which exhibited similar signalling pathway dysregulation, but different gene expression patterns when compared to the analogous human subtypes [74]. Since the publication of this report, several groups have provided additional characterisation of the molecular pathogenesis of DLBCL. Two important generalisations can be taken from these reports. First, canine DLBCL typically shows molecular lesions more often recognised in the ABC subtype of human DLBCL than the GCB subtype, including

dysregulation of NF-κB signalling [74–79], upregulation of B-cell receptor signalling [74, 78], and overexpression of MYC and BCL2 [78, 80]. This may be one explanation for the inferior cure rate of canine DLBCL when compared to the human cancer. Second, canine DLBCL can be separated into molecularly defined subtypes associated with significantly different survival outcomes [74, 78, 81–84]. However, the genetic and epigenetic lesions defining these subtypes differ from one report to the next, so it is still unclear how these results can be applied to the diagnosis and classification of nodal lymphomas in routine clinical practise. Comprehensive molecular profiling of histopathologic subtypes other than DLBCL has been performed [81, 85], but to a much lesser extent, so the relevance of these data to clinical practise is even less clear.

Recently the genomic landscape of canine lymphoma was also described [82, 83]. Striking parallels were identified in the signalling pathways and cellular processes between canine lymphoma and its human counterpart. However, disparities in the mutation frequencies of key genes in B-cell lymphoma, such as TRAF3, SETD2, POT1, TP53, MYC, FBXW7, DDX3X and TBL1XR1, were also observed [86, 87]. Significantly, TP53 mutations were associated with a notable reduction in survival amongst dogs with DLBCL. Additionally, a prognostic model resembling the International Prognostic Index (IPI) for human DLBCL was developed. This model integrated exonic variants and clinical features to predict outcomes in dogs with DLBCL [87]. These findings present a comprehensive perspective of the common genomic lesions of canine DLBCL and hold potential for uncovering innovative therapeutic approaches.

In addition to their use in disease subtyping, numerous molecular species, including cell-free DNA [60, 88, 89], nucleosomes [90], antibodies [91] and microRNAs [92] have been advanced as possible blood-based biomarkers suitable for early disease detection or as an adjunctive tool for the diagnosis of canine lymphomas. Although many of these approaches show early promise for distinguishing lymphoma-bearing dogs from healthy dogs, the extent to which they have been evaluated in dogs with non-neoplastic diseases—particularly those with clinical signs mimicking lymphoma or other cancers—is limited. Additionally, these biomarkers have not been validated in independent laboratories using randomised patient samples, a critical step in the validation of human cancer biomarkers [93]. Therefore, their role in the diagnosis and classification of nodal lymphomas of dogs is currently uncertain.

4 | Consensus Recommendations for Lymphoma Diagnosis and Classification

A summary of the subgroup's recommended diagnostic approach to dogs with suspected nodal lymphomas based upon review of the current literature is presented in Figure 1. Histopathology paired with immunohistochemical staining is required for complete classification of nodal lymphomas in dogs. This is currently the only method by which most nodal lymphoma subtypes, as defined by the WHO system, can be definitively diagnosed. The ideal method for obtaining biopsy specimens for histopathologic evaluation and WHO subtyping has not been conclusively established. However, in the absence of a well-designed study comparing the accuracy of WHO subtype assignment to samples obtained by NCB vs. surgical lymphadenectomy as a gold standard, it is the consensus of this subgroup that lymphadenectomy specimens should be preferred when complete classification of nodal lymphomas is required, as they provide the greatest amount of morphologic information upon which to establish a diagnosis.

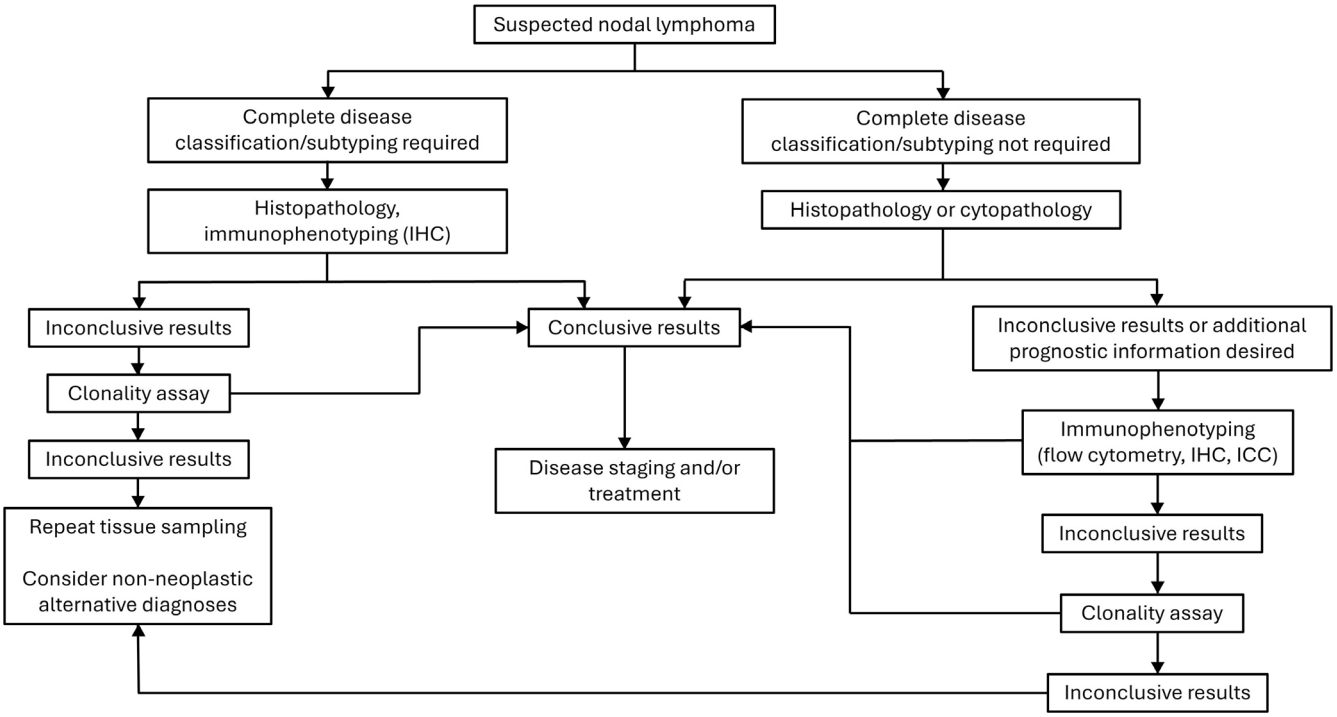

FIGURE 1 | Suggested algorithm for diagnostic evaluation of dogs with suspected nodal lymphomas. IHC = immunohistochemistry; ICC = immunocytochemistry.

Cytopathologic evaluation of lymph node specimens collected by FNA is a popular means for diagnosing nodal lymphomas in dogs. The accuracy and sensitivity of cytopathology for diagnosing many lymphomas appear good-to-excellent (> 80%–90%). It is plausible that the diagnostic accuracy of FNA would improve if cytopathologic and immunophenotypic data (such as from FC) were analysed concurrently. Agreement of cytopathological findings with immunophenotyping results has been reported [49, 50], although a critical evaluation of this subject, which should include determining the extent of agreement with histopathology and IHC results, is currently lacking for most lymphoma subtypes. It is therefore the consensus of the subgroup that cytopathology can be considered an acceptable means of diagnosing nodal lymphomas, particularly when cytopathology results are consistent with lymphoma in dogs with high prior probability of disease, and when an exacting level of disease subtyping is not necessary to inform treatment decisions. This scenario likely reflects many—if not most—routine clinical practise settings. Histopathology, on the other hand, should be considered imperative in cases where comprehensive diagnostic assessment of a nodal lymphoma is desired. These situations may include clinical trials where entry is restricted to dogs with a specific lymphoma subtype, or routine practise settings when other attempts at diagnosis have failed to clearly identify the disease present. The latter may be especially true when cytopathology results indicate the absence of lymphoma in dogs with high prior probability of disease. Histopathology also should be considered essential for characterising rare [94, 95] or novel [96] lymphoma subtypes.

Immunophenotyping is essential to the complete diagnosis and classification of nodal lymphomas of dogs, as neither histomorphology nor cytomorphology alone accurately distinguishes many lymphoma subtypes from one another. Thus, immunophenotyping should be performed in conjunction with histopathology in all situations where determination of WHO subtype is required. The methods most used for immunophenotyping nodal lymphomas—IHC and FC—have relative advantages and disadvantages. Neither affords a level of *prognostically significant* disease classification that is not afforded by the other. This may change as new disease subtypes are fully characterised. For now, however, the consensus of this subgroup is that IHC and FC should be regarded as equally acceptable methods of immunophenotyping a lymphoma. In cases where IHC and FC are not available, ICC and/or IF can be considered substitutes, although the latter two methods require additional study before being considered equivalent to IHC and FC in terms of the reliability of the diagnostic information they provide.

It should be noted that, at present, tumour immunophenotype may not affect treatment decisions, as the choice of therapy is often dictated more by the anticipated clinical course (i.e., indolent or aggressive) of the cancer, or simply by the desires of the dog owner. However, the extent to which immunophenotype should determine the choice of treatment for dogs with nodal lymphomas is currently a subject of much debate in the veterinary oncology community. Some reports [97, 98] suggest that dogs with aggressive T-cell lymphomas may benefit more from chemotherapy protocols enriched with alkylating agents than standard protocols incorporating cyclophosphamide, doxorubicin, vincristine and prednisone. However, randomised trials directly comparing clinical outcomes in dogs with aggressive T-cell lymphomas receiving these two alternative regimens have not been performed. Results that support the use of different treatment regimens for dogs with aggressive B- and T-cell tumours would further solidify the essential role of immunophenotyping in the diagnostic evaluation of dogs with these cancers.

Clonality assays should be used thoughtfully when diagnosing and/or classifying nodal lymphomas in dogs. Because the extent to which they agree with FC and IHC is only moderate, it is the consensus of the subgroup that, whenever possible, clonality assays should not be used as a sole diagnostic test for assigning a lymphoma to a given lymphocyte lineage. Immunophenotyping assays are the preferred diagnostic tests for this purpose. Ideally, clonality assays should be reserved for cases where both morphologic *and* immunophenotypic characterisation of a lymphoid cell population is inconclusive. In cases where immunophenotyping is impractical (e.g., due to an inability to perform it without acquiring additional diagnostic samples), but morphologic findings strongly support the presence of lymphoid neoplasia, it is reasonable to use clonality assays to determine lineage assignment. It is much less desirable to use clonality assays in the absence of immunophenotyping when morphologic findings are ambiguous or are derived from poor-quality samples. In these situations, the prior probability of lymphoma, as determined by clinical and pathological data, must be considered if ordering a clonality assay, as it significantly affects the assay's positive predictive value [1, 53]. Because the clinical consequences of misdiagnosing lymphoma can be significant, potential causes of false positive and false negative results from clonality assays should also be considered carefully. In cases where the prior probability of disease is only moderate (e.g., other diagnostic or clinical findings suggest an autoimmune or infectious cause for lymph node pathology), and lymph node cytopathology or histopathology results are ambiguous, repeat sampling of a lymph node (ideally combined with immunophenotyping), rather than a clonality assay, is the better means to achieve diagnostic clarity. This is particularly true in cases where histopathology may provide a more representative sample than was afforded by initial cytopathologic sampling.

Advanced diagnostic tests for molecular subtyping or early diagnosis of cancer do not have a clear role in the diagnosis or classification of nodal lymphomas in dogs at this time. It is the consensus of this subgroup that their use should be restricted to investigational settings until clear clinical indications for their use have been established. For molecular subtyping tests, a reasonable benchmark of clinical readiness would be the consistency of a prognostically significant molecular disease classifier across independent patient subsets, as has been demonstrated numerous times for GCB vs. ABC DLBCL in humans [71, 72]. For tests aimed at early cancer diagnosis, where the consequences of false positive and false negative results are significant, a more rigorous standard of validation should be the goal. An example of such a validation framework might resemble that proposed by Feng and Pepe [93], in which a test must not only show consistency in disease classification (i.e., cancer-bearing or cancer-free) across multiple independent patient cohorts but also demonstrate a clinically meaningful benefit—such as reduced cancer-related mortality—at the population level.

5 | Future Directions

The indispensability of histopathology and immunophenotypic analysis for the definitive diagnosis and classification of nodal lymphomas in dogs under the WHO system is clearly established, as is the correlation between WHO subtype and a lymphoma's typical clinical course [3, 6–9]. However, histopathology is recommended 3–4 times less frequently than cytopathology by veterinarians seeking to establish a lymphoma diagnosis [13]. This reality impels consideration of a crucial question: How much diagnostic information is "enough" to meaningfully inform treatment decisions for dogs with nodal lymphomas? The most important initial information a pathologist can provide to a clinician making these decisions is arguably whether the tumour has an aggressive or indolent phenotype. The only report thoroughly evaluating whether cytopathology conveys this information showed that it correctly predicted disease phenotype in only 20%–70% of cases [18]. This figure suggests that many lymphomas would be over- or under-treated if diagnosed by cytopathology alone.

With this in mind, we must also ask whether combining other diagnostic tests with cytopathology would provide the requisite information necessary to guide treatment. For example, it has already been shown that FC combined with cytopathology can predict histopathologic subtype and outcome in the two most common forms of nodal T-cell lymphoma, TZL and PTCL-NOS, neoplasms with vastly different clinical phenotypes [23, 99–101]. In human lymphomas, the diagnostic criteria for individual WHO subtypes vary—some have hallmark morphologic, architectural, molecular, or genetic features making diagnosis highly dependent on specific tests, whereas others must be diagnosed by synthesising results from multiple tests [5]. As the clinical phenotypes of currently recognised lymphoma subtypes in dogs are clarified [9, 10], new subtypes are identified and characterised [97], and new reagents and diagnostic testing methods become available, the selection of available diagnostic tests will likely depend upon the most relevant microscopic, molecular, and genetic features needed to specify a given subtype. Given the predominance of cytopathology as an initial diagnostic test for dogs with nodal lymphomas, an essential goal of future research will be to determine which diagnostic test(s) must be combined with it to accurately identify lymphoma subtypes and guide treatment decisions.

An additional element of the diagnostic process for lymphomas that requires reimagination is the terminology used to impute a clinical phenotype to a given tumour. The term historically used for this purpose, as it is for many other cancers, is *grade*. The use of this term to describe lymphomas, however, is problematic because it is used differently by anatomic pathologists, clinical pathologists and oncologists, robbing it of any consistent meaning. Moreover, the diagnostic features used to determine grade—mitotic count in histopathology and cell size and morphology in cytopathology—are inconsistently associated with the clinical course of a given tumour [6, 11, 96, 102]. To remedy the confusion surrounding the term grade, we propose adopting the terms *indolent* or *aggressive* in its place when attempting to ascribe a clinical phenotype to a given lymphoma. The term grade should be reserved for describing histopathologic samples for which a mitotic count can be determined, consistent with WHO guidelines. Immediate implementation of this proposal will likely be challenging, if not impossible, given the preeminence of cytopathology in the diagnosis of nodal lymphomas and the requirement for complete histopathologic subtyping to assign lymphomas to indolent or aggressive categories at present. Collaborative efforts between oncologists and pathologists are needed to better correlate the cytomorphologic features of specific lymphomas subtypes with their clinical disease course. Defining the situations in which additional diagnostic testing (such as immunophenotyping or assessment of cell proliferation markers like Ki67 [102, 103]) should be recommended when the distinction between indolent vs. aggressive disease is not clear from cytopathology alone will be an important goal of such collaborations.

Finally, a problem common to many assays used for diagnosing and classifying nodal lymphomas of dogs is a lack of standardisation in methodology and reagents across laboratories. Standardised methods of molecular testing for human lymphomas have resulted in improved assay sensitivity in some instances [104], and periodic re-assessment of consensus recommendations for diagnostic testing to account for new findings is commonplace [105]. The lack of standardisation in veterinary lymphoma diagnostics, in contrast, may have serious implications on the consistency with which these cancers are described in the literature. This in turn may impede any collective efforts to impose a greater sense of order on this diverse family of cancers through more refined phenotypic and genotypic characterisation. Whilst complete harmonisation of methods and reagents used across all laboratories is perhaps an unreasonable expectation, formal evaluation of the consistency of diagnostic results between laboratories would be an important and achievable goal. Developing and maintaining consensus standards for reporting the results of such tests is also an important goal which is already being pursued [106]. Collaborative efforts of veterinary oncologists, pathologists and molecular diagnosticians in the coming years will be vital to meeting these goals.

Author Contributions

M.O.C. and L.A. wrote the first draft of the manuscript. All authors participated in the review and revision of the first and subsequent drafts. All authors approved the final version of the manuscript.

Ethics Statement

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

No original data were generated for this manuscript.

Endnotes

[1] An asterisk (*) has been placed after the citation for each of these additional references in the list at the end of this document so that they may be easily identified by readers.

[2]The term "lymphoblastic lymphoma," though often used in clinical parlance to describe any lymphoma comprised of large or intermediate-sized cells, here refers only to a specific entity in the WHO system, which is a precursor lymphoid neoplasm comprised of cells morphologically similar to those of acute lymphoblastic leukaemia [4].

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