Identification of peripheral blood involvement in dogs with large B-cell lymphoma: Comparison of different methods

IDENTIFICATION OF PERIPHERAL BLOOD INVOLVEMENT IN DOGS WITH LARGE B-CELL LYMPHOMA: COMPARISON OF DIFFERENT METHODS

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Abstract

Stage V lymphoma is defined as the presence of neoplastic cells in peripheral blood (PB), bone marrow, or any other non lymphoid tissue. Still, official guidelines do not specify which technique should be used to assess infiltration.

We assessed the agreement among flow cytometry (FC), blood smear evaluation, and ADVIA120 (LUC and BASO) to quantify PB infiltration in 100 dogs with large B-cell lymphoma (LBCL).

Significant errors were found for all methods compared to FC. A moderate agreement was present between FC and blood smear evaluation, whereas LUC and BASO had excellent specificity but unsatisfactory sensitivity in detecting FC infiltrated PB samples.

The different techniques should not be used alternatively. We support the use of LUC/BASO as a speedy preliminary test to detect infiltrated samples, and the joined use of blood smear evaluation and FC to quantify definitively the infiltration. Our results are valid only within canine LBCL staging workup, once the diagnosis has been confirmed.

Keywords: ADVIA 120; blood smear evaluation; flow cytometry; infiltration; lymphoma; staging

Introduction

Lymphoma, the most common hematopoietic neoplasm in dogs, comprises many different entities. Accordingly, different classification schemes have been developed: in particular, the World Health Organization (WHO) scheme is mainly based on the histopathological characteristics (Valli et al. 2002; Valli et al. 2011), whereas the updated Kiel scheme is mainly based on cyto-morphological characteristics (Fournel-Fleury et al., 1997; Ponce et al., 2010); phenotype assessment is also required by both classification schemes. Regardless of the classification scheme used, the most frequently diagnosed forms share the B phenotype and the medium to large size of the cells (Aresu et al., 2013; Ponce et al., 2010; Teske et al., 1994; Valli et al., 2011) and can be referred to as Large B cell Lymphomas (LBCL) (Rout and Avery 2017). Irrespective of the tumor subtype, however, all lymphoma-bearing dogs are routinely staged according to the TNM system provided by the WHO (Owen, 1980). This system provides five stages for classifying the spread of the tumor, ranging from involvement of a single node or lymphoid tissue in a single organ (stage I) to generalized lymph node involvement with infiltration of liver and/or spleen (stage IV). When neoplastic cells are detected in the peripheral blood (PB), bone marrow (BM) and/or other organs in addition to the primary solid tumor, dogs are classified as having stage V disease. To date, different techniques have been used to assess PB and/or BM infiltration: namely, blood smear evaluation (Abbo and Lucroy, 2007; Flory et al., 2007; Graff et al., 2014; Ponce et al., 2004), flow cytometry (FC) (Marconato et al., 2013; Martini et al., 2015; Riondato et al., 2016), or PCR for Antigen Receptor Rearrangement (PARR) (Lana et al., 2016). This lack of consistency may affect staging results (Flory et al., 2007) and prevents the comparison of the data obtained by different studies.

Our research group already demonstrated the excellent analytical and diagnostic performances of FC, in detecting and quantifying PB and BM infiltration by LBCL cells (Riondato et al., 2016). Although PARR is more sensitive than FC in detecting the presence of neoplastic cells (Aresu et al., 2014), it is unable to quantify the extent of infiltration. The combined used of this two techniques may improve the accuracy of clinical staging of lymphoma in dogs.

However, clinicians may occasionally prefer to use less expensive and time-consuming techniques, such as automated CBC or blood smear evaluation (Regan et al., 2013). Today, a number of different hematology analyzers with specific veterinary software are commercially available. In particular, the laser-based hematology analyzer ADVIA 120 (Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA) provides a differential leukocyte count in the PEROX-channel based on the complexity and peroxidase content of the cells, enumerating neutrophils, eosinophils, lymphocytes, monocytes and large unstained cells (LUC, consisting of activated lymphocytes and blasts) both as percentage and absolute number. In addition, in the BASO-channel, an acidic reagent and a surfactant disrupt platelets and erythrocytes and strip membranes from all other white blood cells except basophils. ADVIA basophil count is not accurate in dogs and other lyse-resistant cells may fall within the gate, including blasts (Lilliehöök and Tvedten, 2011). This phenomenon is called ‘pseudobasophilia’ and it has been described also in humans (Gibbs et al., 2009). In particular, the presence of blasts may be suspected when cells spread from the mononuclear or polymorph nuclear gate into the lyse-resistant cells area in the BASO-channel, or a “blast-nose” in the BASO cytogram is found, with cells creating a tip at the left bottom of the mononuclear area (Stirn et al., 2014). Thus, although

never reported in the veterinary literature, LUC and lyse-resistant cells in the BASO channel (BASO) may be used to assess PB infiltration in dogs with lymphoma.

The aim of the present study was to compare retrospectively different methods and parameters (blood smear evaluation, LUC, BASO and FC) for detecting PB infiltration in dogs with LBCL.

Other methods can be used to stage lymphomas and our intention was not to define which of the available techniques has to be considered the gold standard. Our ultimate goal was to determine whether the techniques and parameters evaluated in our study are interchangeable and equally informative for the clinician, or not.

Materials and methods

The FC database of the laboratory of the Department of Veterinary Sciences (University of Turin, Turin, Italy) was interrogated and consecutive cases from January 2012 to February 2014 fulfilling the following inclusion criteria were selected: 1) a final diagnosis of LBCL based on cytology, FC and possibly histopathology of an enlarged peripheral lymph node (LN); 2) availability of FC data of lymph node (LN) and PB; 3) availability of a CBC performed via ADVIA120 and/or of a good quality PB smear stained with May Grünwald-Giemsa for a cytological review. All cases were classified according to the updated Kiel classification. Acute lymphoid leukemia was excluded based on bone marrow examination and/or number and degree of hematological alterations, clinical history and signs and follow up data. Cases in which acute leukemia could not be definitively ruled out were not included in the study. No case had been previously treated with corticosteroids or chemotherapy.

All PB samples were collected in EDTA tubes and processed within 24 hours from collection. When not provided along with the EDTA tube, PB smears were prepared at time of sample delivery to the laboratory.

Our FC service receives samples from both the internal oncology service and many different private veterinarians outside the Institution itself making uneven the collection of clinical and histopathological data. These data are not useful for the specific purposes of the present study and are not reported.

All dogs were privately owned and sampled for diagnostic purposes with a written informed consent of the owners. Thus, a formal approval of the Institution Committee for Animal Care of the University of Turin was not necessary.

Both a skilled hematologist (Operator 1) and a recently graduated student (Operator 2) reviewed all PB smears. Both operators were aware of the confirmed LBCL diagnosis, but blinded to the FC results and to the results obtained by the other operator. A 200-cells manual differential count was done and the percentage of immature lymphoid cells was recorded: these cells had to be medium to large in size (nucleus diameter ≥2 RBCs), with scant to moderate basophilic cytoplasm and a round nucleus with dispersed or finely granular chromatin and possibly one or more nucleoli. The presence of other atypical lymphocytes was also recorded when cells with a combination of the following features were detected: nucleus diameter <2 RBCs, moderate basophilic cytoplasm, round nucleus with clumped chromatin, no nucleoli. These cells were considered reactive and excluded from the calculation of infiltrating cells. According to Flory et al. (2007), samples were considered infiltrated if any immature lymphoid cell was present (0.5%).

LUC and BASO values were recorded for each case. Samples were considered infiltrated if LUC or BASO exceeded the upper reference limit calculated using data from 49 healthy dogs (2.4% and 1.6%, respectively).

Sample processing for FC was performed as previously described (Riondato et al., 2016). Samples were acquired with a BD Accuri C6 (Becton Dickinson, San Josè, CA, USA) and data were analyzed with the specific software CFlow Plus (Becton Dickinson). PB infiltration was defined as the percentage of large CD21 positive cells out of total CD45-positive cells and samples were considered infiltrated if the percentage was ≥0.56% (Riondato et al., 2016).

All methods was compared to FC, since diagnostic performances of this latter technique have already been described in our laboratory (Riondato et al., 2016).

A Shapiro-Wilk test was performed to assess whether the data obtained by Operator 1 and 2 through blood smear evaluation were normally distributed. A non-parametric test (Spearman test) was then performed to assess the possible correlation between the infiltration percentages reported by the two operators.

Passing-Bablok regression analysis and Bland-Altman plots (Analyze-it, Analyze-it Software Ltd, Leeds, UK) were used to assess agreement between percentages of infiltration obtained by FC and blood smear evaluation from either operator, LUC and BASO, respectively.

Contingency tables were prepared to compare results from the different techniques and to estimate the diagnostic accuracy of each test, including concordance (κ), sensitivity (Se) and specificity (Sp), positive and negative likelihood ratio (LR+ and LR-, respectively), diagnostic odds (DO), positive and negative predictive value (PPV and NPV, respectively). The website http://www.quantitativeskills.com/sisa was used for these calculations.

159 Finally, since different cutoffs have been used in the literature (Flory et al., 2007;
160 Graff et al., 2014) a Receiver Operating Characteristic (ROC) curve was drawn, to
161 identify the cytological percentage of immature lymphoid cells best discriminating
162 between FC infiltrated and not infiltrated PB samples. Also, ROC curves coordinates
163 were used to assess the sensitivity and specificity of the 10% cutoff described in the
164 literature for cytological assessment of PB infiltration in dogs with large cell
165 lymphoma (Graff et al., 2014). Only results obtained by the most experienced
166 operator (Operator 1) were used to this aim.
167 The statistical software SPSS v19.0 (SPSS Inc, Chicago, IL, USA) was used for
168 Shapiro-Wilk and Spearman tests and to draw ROC curves. Significance was set at
169 $p \leq 0.05$.

170 **Results**

171 Overall, samples from 100 dogs with LBCL were included in the study. The diagnosis
172 of LBCL was made based on cytology and FC of an enlarged LN in all cases; in 31
173 cases, this diagnosis was also confirmed via histopathology. FC was available for all
174 PB samples. In addition, 87 (87%) cases had both blood smear evaluation performed
175 and ADVIA data available, 11 (11%) had blood smear evaluation performed but
176 lacked ADVIA data, and 2 (2%) cases had ADVIA data but the blood smear was not
177 available for review.
178 Based on FC results, overall mean large B-cells percentage was 7.23±10.03%
179 (median, 2.75%; min-max 0-52%). In particular, 27 samples (27%) were not
180 infiltrated. For the remaining 73 samples (73%), mean PB infiltration was
181 9.84±10.62% (median, 7%; min-max, 0.6-52%).
182 PB smears were available for 98 cases. According to Operator 1, overall mean
183 immature lymphoid cells percentage was 4.7±9.5% (median, 0%; min-max 0-60%):

48 samples (49%) were infiltrated, with a mean immature lymphoid cells percentage of 9.5±11.7% (median, 6%; min-max, 1-60%). According to Operator 2, overall mean immature lymphoid cells percentage was 8.1±10.0% (median, 3%; min-max 0-55%): 87 samples (88.8%) were infiltrated, with a mean immature lymphoid cells percentage of 8.8±10.3% (median, 5%; min-max 1-55%). A significant correlation between operators was found (p=0.000, r=0.740).

A CBC performed with ADVIA 120 was available for 89 samples. Overall mean LUC percentage was 2.43±3.44% (median, 1.5%; min-max 0-26.9%): 29 samples (32.6%) were LUC-positive, with a mean percentage of 5.31±4.87% (median, 3.6%; min-max, 2.5-26.9%). Overall mean BASO percentage was 0.67±0.63% (median 0.5%; min-max 0-3.1%): 8 samples (9%) were BASO-positive, with a mean percentage of 2.3%±0.49% (median, 2.2%; min-max, 1.7-3.1%). Five FC infiltrated cases (median, 5%; min-max, 1-14%) were negative at blood smear evaluation by both operators and positive at LUC (median, 3.4%; min-max, 3.1-3.6%); 1 out of these 5 (FC = 1%) was positive both at LUC (3.1%) and BASO (1.7%).

When comparing blood smear evaluation and FC, significant proportional error and bias were found for Operator 1 but not for Operator 2 (Figure 1 and 2). Operator 1 performance was as follow: accuracy = 0.724, κ = 0.454, Se = 0.648, Sp = 0.926, LR+ = 8.746, LR- = 0.38, DO = 23, PPV = 0.958, NPV = 0.5 (Table 1). Operator 2 performance was as follow: accuracy = 0.786, κ = 0.252, Se = 0.934, Sp = 0.273, LR+ = 1.285, LR- = 0.24, DO = 5.3, PPV = 0.816, NPV = 0.5 (Table 2). The ROC curve (drawn based on Operator 1 results) identified 0.5% of immature lymphoid cells as the best cutoff for blood smear evaluation to discriminate between FC infiltrated and not infiltrated PB samples, with a 67.6% sensitivity and a 92.6% specificity

(AUC=0.819). Finally, sensitivity and specificity for the 10% cutoff described in the literature (Graff et al., 2014) were 18.3% and 100%, respectively.

When comparing LUC and FC, significant bias with constant and proportional errors were found (Figure 3). LUC performance was as follow: accuracy = 0.573, κ = 0.274, Se = 0.433, Sp = 1, LR- = 0.567, PPV = 1, NPV = 0.367 (Table 3).

When comparing BASO and FC, significant bias with constant and proportional errors were found (Figure 4). BASO performance was as follow: accuracy = 0.337, κ = 0.063, Se = 0.119, Sp = 1, LR- = 0.881, PPV = 1, NPV = 0.272 (Table 4).

LR+ and DO for LUC and BASO could not be calculated because no false positive result was obtained.

The combined use of blood smear evaluation (Operator 1), LUC and BASO (infiltration detected if at least one out of three was positive) reported the following performance: accuracy = 0.816, κ = 0.588, Se = 0.785, Sp = 0.909, LR+ = 8.631, LR- = 0.237, PPV = 0.962, NPV = 0.588 (Table 5).

Discussion

An official staging system for canine lymphoma has been created many years ago (Owen, 1980). In spite of this, staging procedures vary largely among veterinarians (Regan et al., 2013) and among published studies (Abbo and Lucroy, 2007; Flory et al., 2007; Graff et al., 2014; Lana et al., 2006; Marconato et al., 2013; Martini et al., 2015; Ponce et al., 2004; Riondato et al., 2016). Far from defining the best staging system, or the “gold standard”, we retrospectively analyzed PB samples from 100 dogs with LBCL, and discovered only a moderate agreement between blood smear evaluation and FC in detecting and quantifying infiltration. Furthermore, LUC and BASO results provided by ADVIA 120 are reliable in detecting PB infiltration in dogs

with large B-cell lymphoma only in case of LUC and/or BASO positive results. We conclude that these techniques provide contrasting results and are not interchangeable.

Discriminating reactive and neoplastic lymphoid cells via cytological evaluation alone may be challenging, as they can present with similar morphological features, including increased size, more abundant basophilic cytoplasm and cleaved or bilobed nuclei (Stockham and Scott, 2008). Misclassification of reactive lymphocytes would bring to an erroneous overestimation of PB infiltration by neoplastic cells, possibly driving the clinicians toward a worse prognosis (Jagielski et al., 2002). Therefore, skilled hematologist may be more prone to classify cells of ambiguous nature (not completely corresponding to the description provided above) as reactive lymphocytes than as neoplastic cells, in order to avoid false positive results. This hypothesis is also supported by the different results obtained by the two operators involved in the present study: the unskilled operator provided data with a slightly higher sensitivity, but an 8-fold higher percentage of false-positive results. Still, we strongly agree that cytological evaluation should always be done in conjunction with FC, in order to support and confirm FC results, and to assess possible abnormalities of the other cellular populations.

Blood smear evaluation has been used previously to assess PB infiltration in dogs with lymphoma, and different cutoffs have been proposed to define positive samples. On one hand, Flory and colleagues considered as positive PB samples when any lymphoblast was present (Flory et al., 2007). On the other hand, in a more recent study by Graff et al. (2014) a threshold of 10% large (neoplastic) lymphocytes was adopted, claiming that any clinical pathologist would reproducibly identify neoplastic cells present in such a high percentage. In the present study, we applied the same

cutoff used by Flory et al, and found only a moderate agreement with FC, mainly due to the low sensitivity of blood smear evaluation. Raising the cutoff value toward 10% improved specificity, but unfortunately sensitivity fell down to unacceptable values. These results support our assumption that lower cutoffs work better. Since the cutoff with the best sensitivity and specificity in the ROC curve corresponds with detection limit of the 200-cells differential count (0.5% = 1 blast out of 200 leukocytes), it remains to be determined if counting more leukocytes would increase the sensitivity providing a lower detection limit.

We also investigated the potential value of ADVIA 120 LUC and BASO in quantifying PB infiltration. The cutoff we adopted to define positive samples is the upper reference limit obtained using samples from healthy dogs analyzed with our instrument in our laboratory. These values (both LUC and BASO) are higher than the reference limits previously reported (Moritz et al., 2004). This discrepancy once again highlights the importance of in-house made reference ranges for any laboratory test. However, adopting lower cutoff values could be beneficial in case of diagnosed LBCL and it has to be further investigated.

Overall accuracy of both ADVIA 120 parameters was low, but these unsatisfying values were mostly due to the low sensitivity, whereas specificity was optimal, as no false positive result was obtained when analyzing PB samples from dogs with LBCL. It means that a positive LUC/BASO result is conclusive for the presence of PB infiltration in dogs with previously diagnosed LBCL. Unfortunately, a large number of false negative LUC/BASO results occur. LUC/BASO might therefore be used only as a preliminary test and LUC/BASO negative samples should be further tested to rule out minor infiltration. Combining blood smear evaluation, LUC and BASO data slightly improves the results, increasing the number of cases correctly identified as

infiltrated. Anyway, both a constant and a proportional error were found when comparing LUC/BASO and FC. Thus, it would be better to analyze by FC even LUC/BASO-positive samples, in order to quantify the infiltration with higher accuracy. A possible decisional algorithm is suggested in Fig.5

In the recent years, many upgrades have been made in the diagnostic procedures and classification systems for canine lymphoma, and most recent studies are focusing on specific lymphoma subtypes. Following on from this background, only dogs with a confirmed diagnosis of LBCL, which is the highly prevalent subtype in dogs (Aresu et al., 2013; Ponce et al., 2010; Teske et al., 1994; Valli et al., 2011), were included in the present study. Therefore, our results may be not applicable to other lymphoma subtypes, such as small cell or T-cell lymphomas. Further studies are needed to assess whether the techniques investigated here have similar performances when applied to different lymphoma entities. In addition, all our results and conclusions are valid only within a staging workup, once the LBCL diagnosis has been confirmed: the occasional detection of positive PB samples by any technique should never be considered conclusive for LBCL if a primary lesion has not been detected or investigated.

The main pitfall of the present study is the lack of PARR data: this analysis was not performed on the included samples, due to the retrospective nature of this study. Although FC has great sensitivity and specificity in identifying infiltrated blood samples detected by PARR (Riondato et al., 2016) discordance between these two techniques may occur in few cases (Aresu et al., 2014): further studies are warranted to assess whether the concomitant use of other techniques might be of aid in this subset of cases.

Finally, we did not investigate the clinical relevance of PB infiltration. A recent prospective study highlighted a prognostic role for BM infiltration quantified by FC in dogs with LBCL, but failed to recognize such a role for PB infiltration (Marconato et al., 2013). Prospective studies are needed to assess if PB infiltration quantified by other techniques (including PARR, blood smear evaluation, ADVIA's LUC or BASO) may have any clinical or prognostic role.

In conclusion, the present study demonstrated that FC, blood smear evaluation and ADVIA 120 LUC and BASO have only a moderate agreement in the quantification of PB involvement in dogs with LBCL. Thus, results from these different methods and parameters are not comparable and they should not be used alternatively. We suggest the use of LUC/BASO only as a fast preliminary test having optimal specificity but unsatisfactory sensitivity, and the joined use of blood smear evaluation and FC to quantify definitively the degree of PB infiltration. Also, PARR analysis may be of benefit in many cases. Further studies are needed to assess the prognostic role of PB infiltration evaluated by these techniques.

Conflict of interest

There is no conflict of interest of any authors in relation to the submission.

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407

Table 1: contingency table showing the distribution of 98 dogs diagnosed with large B-cell lymphoma, according to the peripheral blood infiltration status, assessed via flow cytometry (reference method) and blood smear evaluation by a skilled hematologist (PB smear Op.1).

	Flow cytometry		
	negative	**positive**	**Total**
PB smear Op.1 negative	25	25	50
positive	2	46	48
Total	27	71	98

Table 2: contingency table showing the distribution of 98 dogs diagnosed with large B-cell lymphoma, according to the peripheral blood infiltration status, assessed via flow cytometry (reference method) and blood smear evaluation by an unskilled hematologist (PB smear Op.2).

	Flow cytometry		
	negative	**positive**	**Total**
PB smear Op.2 negative	6	5	11
positive	16	71	87
Total	22	76	98

Table 3: contingency table showing the distribution of 89 dogs diagnosed with large B-cell lymphoma, according to peripheral blood infiltration status, assessed via flow cytometry (reference method) and large unstained cells (LUC) detected with ADVIA 120 hematology analyzer.

	Flow cytometry		
	negative	**positive**	**Total**
LUC negative	22	38	60
positive	0	29	29
Total	22	67	89

Table 4: contingency table showing the distribution of 89 dogs diagnosed with large B-cell lymphoma, according to peripheral blood infiltration status, assessed via flow cytometry (reference method) and lyse-resistant cells (BASO) detected with ADVIA 120 hematology analyzer.

	Flow cytometry		
	negative	**positive**	**Total**
BASO negative	22	59	81
positive	0	8	8
Total	22	67	89

Table 5: contingency table showing the distribution of 87 dogs diagnosed with large B-cell lymphoma, according to peripheral blood infiltration status, assessed via flow cytometry (reference method) and the concomitant evaluation of a blood smear and the LUC and BASO percentages detected with ADVIA 120 hematology analyzer (smear/LUC/BASO).

	Flow cytometry		
	negative	**positive**	**Total**
Smear/LUC/BASO negative	20	14	34
positive	2	51	53
Total	22	65	87

Figure 1. Passing–Bablok regression analyses (left) and Bland–Altman difference plots (right) for flow cytometric and smear evaluation assessment of blood infiltration in dogs with large B cell lymphoma (*n* = 98). % FC = % of large B cells detected on flow cytometric analysis; % PB smear Op.1 = % of lymphoblasts in a 200-cells manual differential count on a peripheral blood smear detected by a skilled hematologist

Figure 2. Passing–Bablok regression analyses (left) and Bland–Altman difference plots (right) for flow cytometric and smear evaluation assessment of blood infiltration in dogs with large B cell lymphoma (*n* = 98). % FC = % of large B cells detected on flow cytometric analysis; % PB smear Op.2 = % of lymphoblasts in a 200-cells manual differential count on a peripheral blood smear detected by an unskilled hematologist

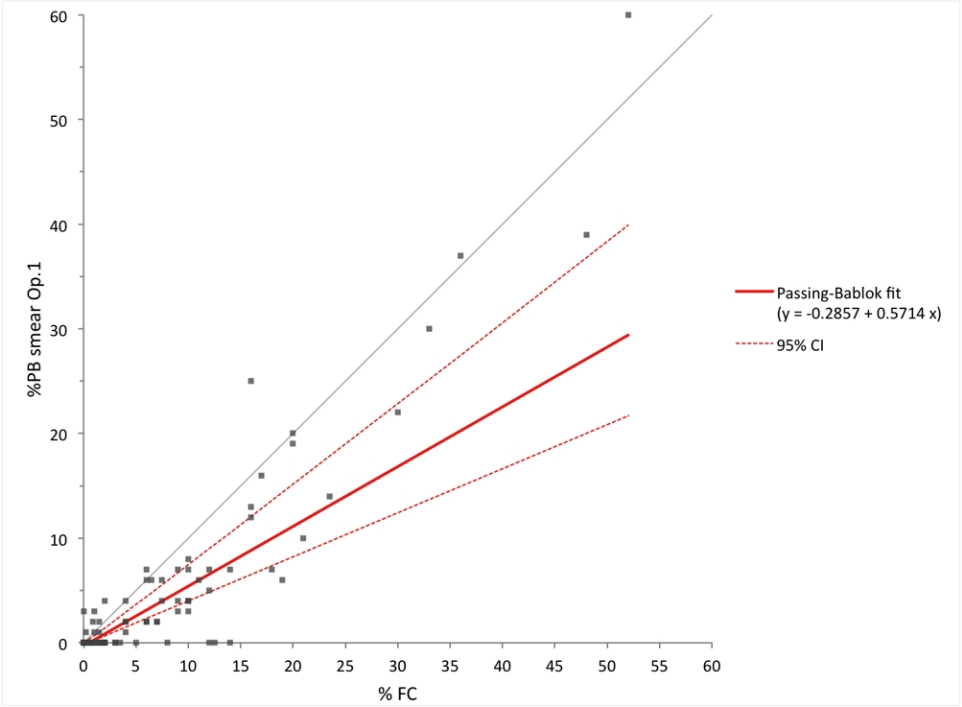
Figure 3. Passing–Bablok regression analyses (left) and Bland–Altman difference plots (right) for flow cytometric and ADVIA120 LUC assessment of blood infiltration in dogs with large B cell lymphoma (*n* = 89). % FC = % of large B cells detected on flow cytometric analysis; % LUC = % of leukocytes in the LUC region (PEROX channel) of the ADVIA120 differential count

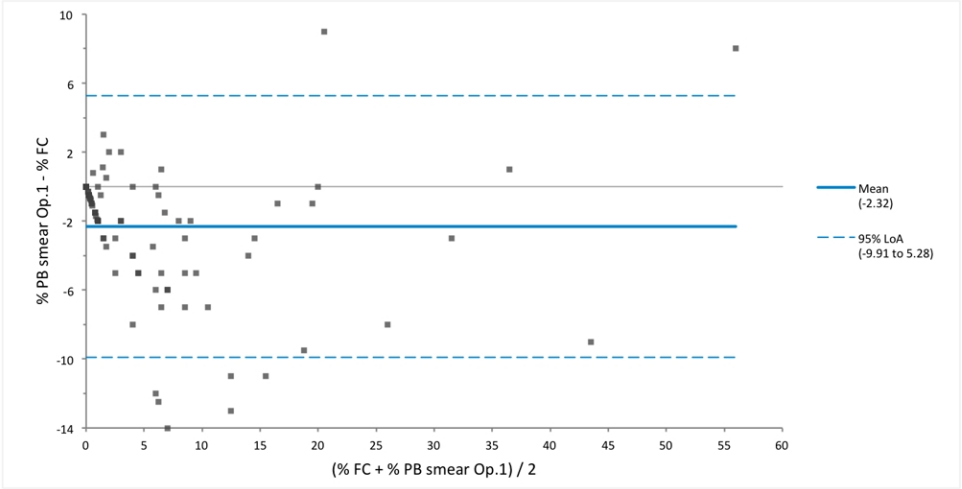
Figure 4. Passing–Bablok regression analyses (left) and Bland–Altman difference plots (right) for flow cytometric and ADVIA120 BASO assessment of blood infiltration in dogs with large B cell lymphoma (*n* = 89). % FC = % of large B cells detected on

462 flow cytometric analysis; % BASO = % of leukocytes in the basophils region (BASO
463 channel) of the ADVIA120 differential count

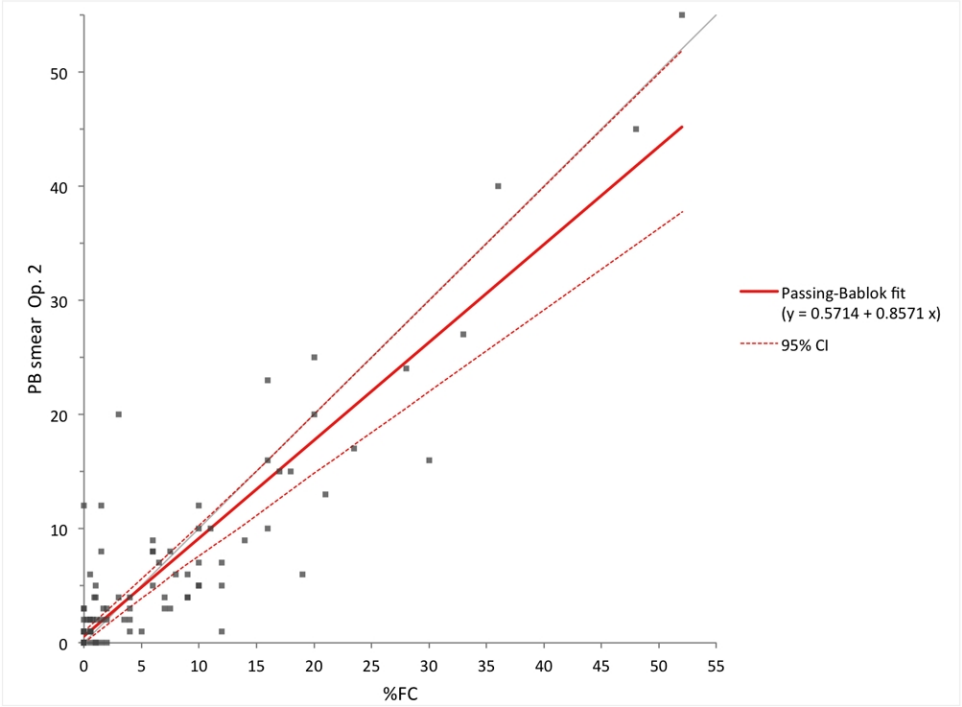
464

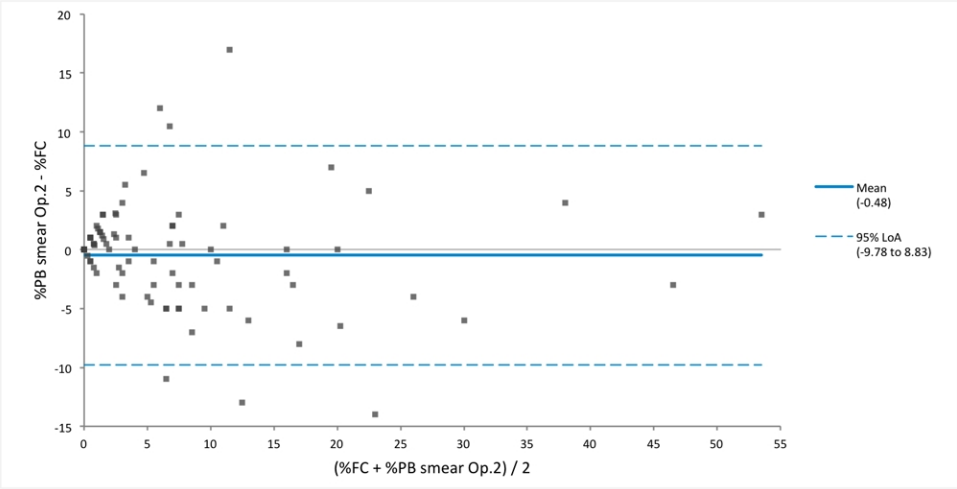
465 **Figure 5**. Decisional algorithm integrating ADVIA120, peripheral blood (PB) smear
466 evaluation and flow cytometric (FC) analysis of peripheral blood infiltration in dogs
467 with large B-cell lymphoma (LBCL).



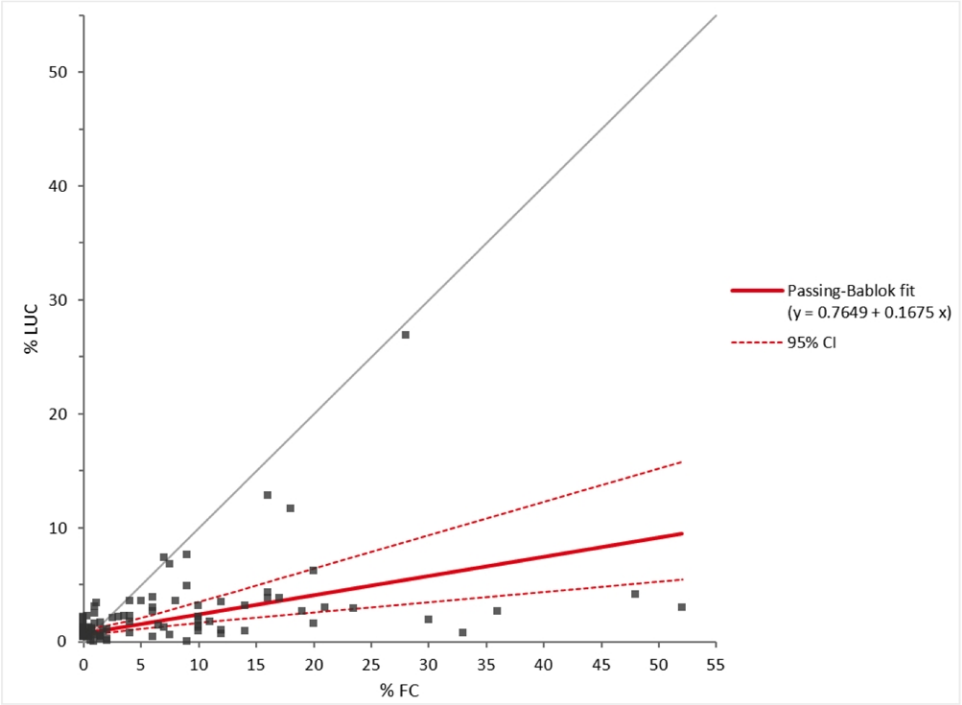


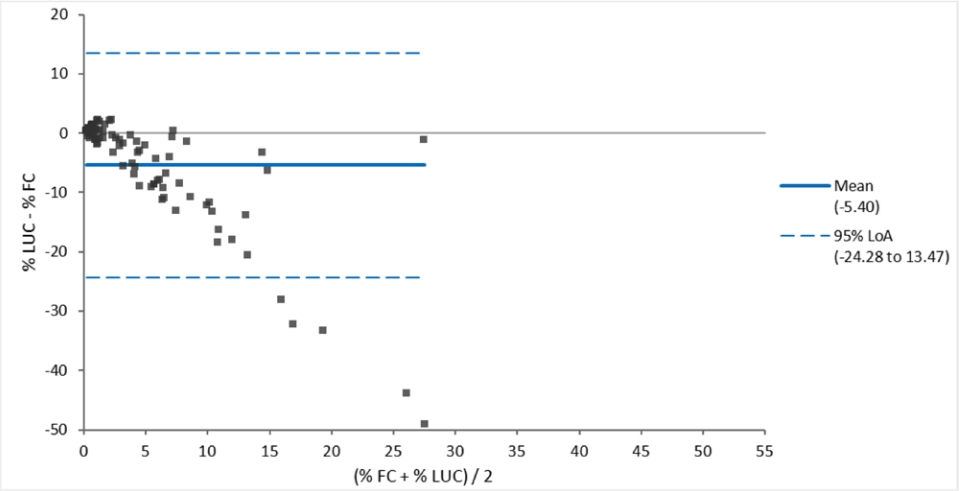
	Passing-Bablok		*Bland-Altman*	
	Slope (95% CI)	*Intercept (95% CI)*	*Bias mean (95% CI)*	*Bias SD*
FC vs PB smear Op.1	0.57 (0.42-0.77)	-0.29 (-0.38-0)	-2.32 (-3.10 - -1.54)	3.87



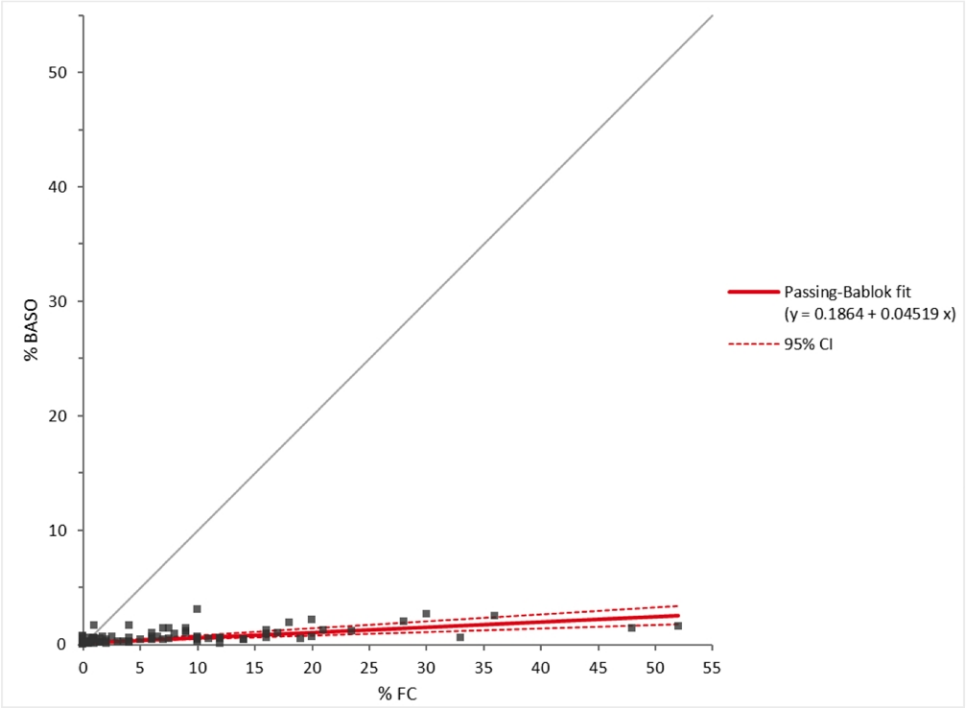


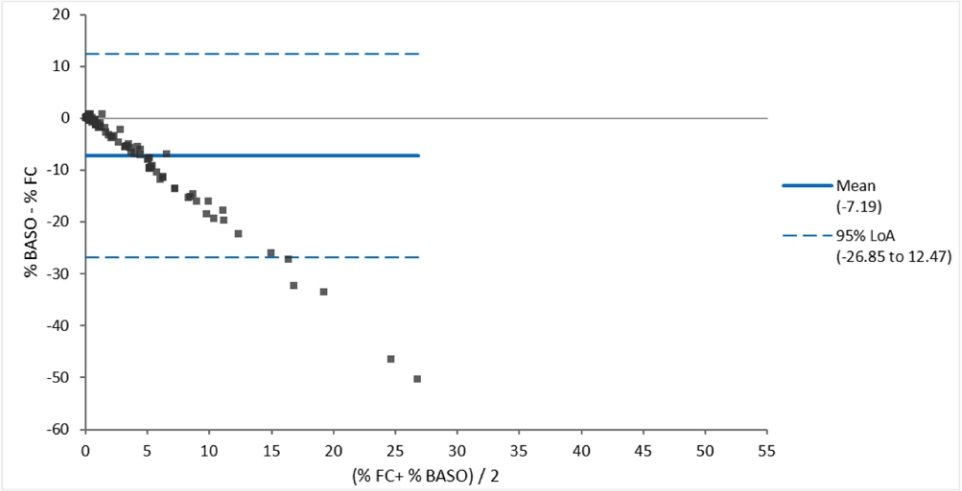
	Passing-Bablok		*Bland-Altman*	
	Slope (95% CI)	*Intercept (95% CI)*	*Bias mean (95% CI)*	*Bias SD*
FC vs PB smear Op.2	0.86 (0.71-0.99)	0.57 (0-1)	-0.48 (-1.53 – 0.57)	4.75



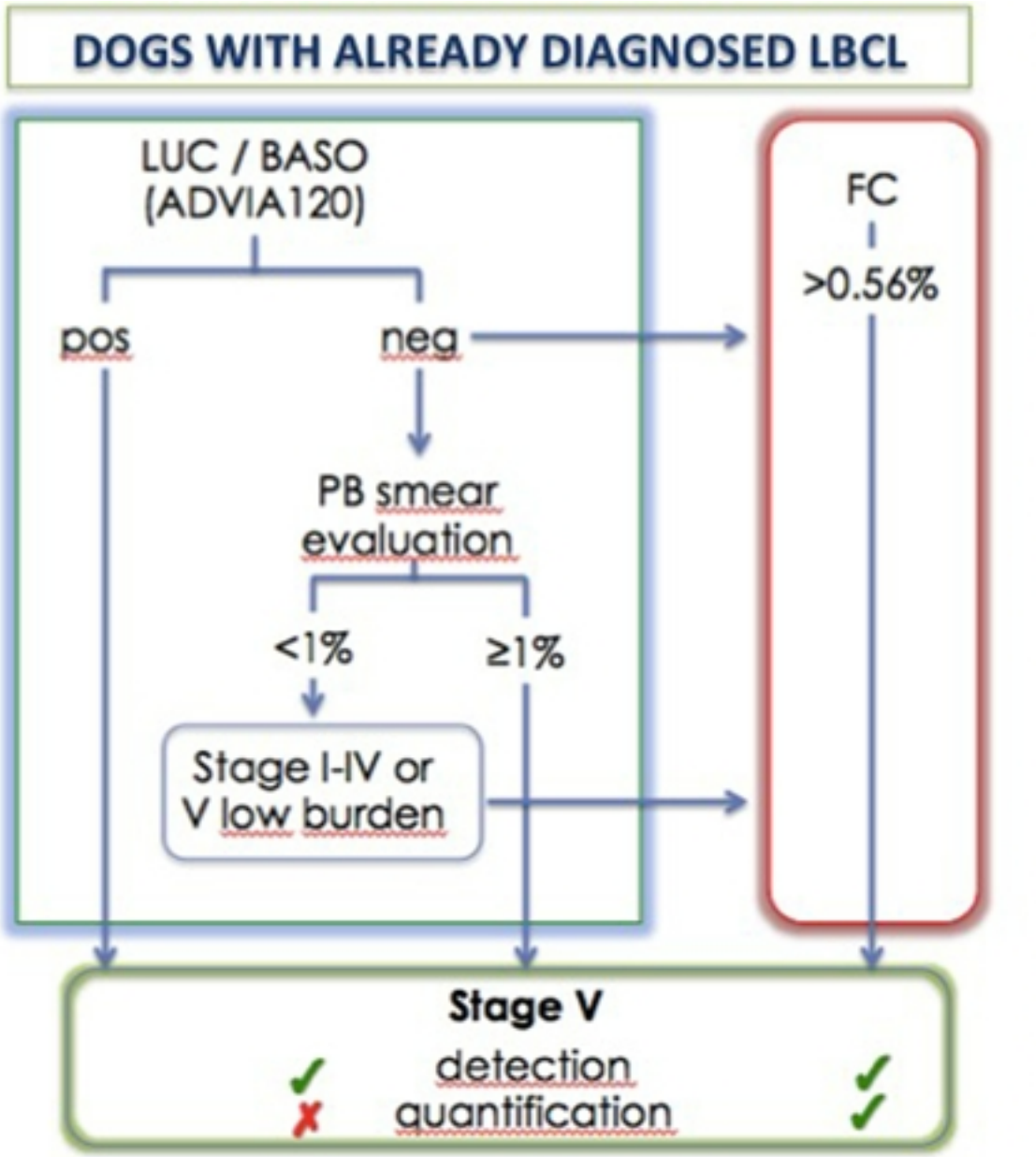


	Passing-Bablok		*Bland-Altman*	
	Slope (95% CI)	*Intercept (95% CI)*	*Bias mean (95% CI)*	*Bias SD*
FC vs LUC	0.17 (0.09-0.29)	0.76 (0.48-1.09)	-5.40 (-7.43 - -3.38)	9.63





	Passing-Bablok		*Bland-Altman*	
	Slope (95% CI)	*Intercept (95% CI)*	*Bias mean (95% CI)*	*Bias SD*
FC vs BASO	0.05 (0.03-0.06)	0.19 (0.14-0.28)	-7.19 (-9.30 - -5.08)	10.03


DOGS WITH ALREADY DIAGNOSED LBCL
LUC / BASO
(ADVIA120)
pos
neg
PB smear
evaluation
<1%
≥1%
Stage I-IV or
V low burden
FC
>0.56%
Stage V
detection
quantification