Effects of pre-analytical variables on flow cytometric diagnosis of canine lymphoma: A retrospective study (2009-2015)

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Abstract

An increasing number of veterinarians use flow cytometry (FC) for immunophenotyping and staging of canine lymphoma, since it is minimally invasive and cost effective. The aim of this retrospective study was to assess pre-analytical variables that might influence the diagnostic utility of FC of lymph node (LN) fine needle aspirate (FNA) specimens from dogs with lymphoproliferative diseases. The database of the Department of Veterinary Medicine, University of Milan, Italy, from 2009 to 2015 was interrogated. Cases were selected if a LN FNA sample was sent for immunophenotyping and if the final FC report was available. Cases were grouped into ‘diagnostic’ and ‘non-diagnostic’. The following pre-analytical factors were analysed by univariate and multivariate analysis: patient-related factors (breed, age, sex, size), operator-related factors including sampling and shipping (year, season, shipping method, submitting veterinarian), and sample-related factors (type of sample material, cellular concentration, cytological smears, artefacts).

A total of 987 cases fulfilled the inclusion criteria. Among all evaluated variables, the submitting veterinarian, sample material, sample cellularity and artefacts affected the likelihood of having a diagnostic sample. The availability of specimens from different sites and of cytological smears increased the odds of obtaining a diagnostic result. Major artefacts affecting diagnostic utility included poor cellularity and the presence of dead cells. Flow cytometry on LN FNA samples yielded conclusive results in more than 90% of cases with adequate sample quality and sampling conditions.

Keywords: Canine lymphoma; Diagnosis; Fine needle aspirate; Flow cytometry; Pre-analytical
**Introduction**

Lymphoma is the most common haematopoietic malignancy in dogs, with an incidence of about 84 cases/100,000 dogs per year (Dorn et al., 1970). Classification schemes, and diagnostic and therapeutic approaches tend to reproduce those applied in human medicine, with only a few exceptions, including a higher prevalence of multicentric lymphomas and diffuse versus nodular lymphoma (Marconato et al., 2013a). These features favour the use of minimally invasive diagnostic techniques such as fine needle aspirate (FNA) cytology and subsequent flow cytometry (FC) (Comazzi and Gelain, 2011).

Fine-needle aspiration and cytology is now considered to be the first diagnostic approach for canine lymphoma (Marconato et al., 2013a), since it is cost-effective, minimally invasive and well accepted by the owners. The information provided by cytology in association with other ancillary techniques sets the basis for therapeutic decisions in the majority of cases, as reported by a recent survey (Regan et al., 2013). Recently, the use of FC has significantly increased in veterinary medicine due to the increased number of canine-specific monoclonal antibodies available. Flow cytometry is routinely used for immunophenotyping of lymphoma and to refine the diagnosis of specific lymphoma subtypes (Seelig et al., 2014; Martini et al., 2015). It can also be used for lymphoma staging (Marconato et al., 2013b) and to evaluate minimal residual disease after treatment (Aresu et al., 2014). However, veterinary FC facilities are currently limited to a few reference laboratories, mainly due to economic reasons. Most of them are part of academic institutions and provide services for veterinary hospitals, veterinary laboratories and private practices. The specific requirements for sample preparation and shipping may partially limit the utility when FC is not readily available and could also bias results, even though specific studies are still lacking.
Since 2008, the FC facility of the authors’ institutions offer a reference service for canine lymphoma and leukaemia flow cytometry, receiving samples from veterinary oncologists working in the same institution and external veterinarians from Italy, Switzerland, Portugal, Spain, Slovenia and the UK. Given our experience, we hypothesised that different sampling techniques, shipping and storage conditions might bias the results and influence the diagnostic performance of FC. To the authors’ knowledge, only one study on the influence of pre-analytical variables on diagnostic performance of FC is available in cats (Martini et al., 2017), but a study evaluating the effects of such variables in a high number of dogs is still lacking.

The aim of this retrospective study was to assess whether and how pre-analytical variables might influence the diagnostic utility of lymph node (LN) FNA samples obtained from dogs with clinically suspected lymphoproliferative disease analysed by FC. The goal was to create recommendations for sampling techniques, sample storage and shipping in order to decrease pre-analytical errors and to increase the diagnostic utility of FC for the diagnosis of lymphoma and leukaemia in dogs.

**Material and methods**

**Inclusion criteria**

The FC database of the Department of Veterinary Medicine, University of Milan, Italy, was searched retrospectively and canine cases were identified over a period of six years (2009-2015). Inclusion criteria were: (1) LN FNA sent for flow cytometric immunophenotyping; and (2) presence of a final FC report in the database. If other sample types, such as peripheral blood (PB), bone marrow (BM) aspirates, body cavity effusions, FNA from mass lesions, spleen, liver or other tissues, were submitted, these cases were included in the present caseload only if a LN FNA from the same patient was analysed, regardless of the diagnostic pathway used and what tissue (e.g. PB or BM for leukaemias) was considered first in the diagnostic pathway. Exclusion criteria included:
(1) cases composed of tissues other than LN aspirates; and (2) cases sent for minimal residual disease analysis.

Sample collection

Samples were collected from one or more enlarged LNs by multiple aspirations, with or without suction, using a fine needle (21-22 ga). The collected material was suspended in 1 mL of a transport medium (Roswell Park Memorial Institute, RPMI 1640, recommended; phosphate buffered saline; saline solution, not recommended), refrigerated at 4-6 °C and shipped to the laboratory within 24 h from sampling with a cold pack in the envelope to maintain sample cooling.

At admission, samples were analysed visually and cellularity was evaluated using an automated analyser (Sysmex XT-2000iV). Cellularity was generally considered to be suitable if the cell concentration in 1 mL was > 5 x 10^9 cells/L (corresponding to > 5 x 10^6 cells in total), whereas samples with < 1 x 10^9 cells/L (corresponding to < 1 x 10^6 cells in total) were generally excluded from processing, although slight variability may have occurred due to the preferences of the operator dealing with the sample.

Flow cytometry

Processing for FC was performed as described previously (Gelain et al., 2008). The erythrocyte lysis step was generally not considered necessary for LN FNA samples unless gross haemodilution was detected at visual inspection. For PB and BM samples, red blood cells were lysed by adding a lysis solution containing 8% ammonium chloride.

The panel of antibodies applied to LN samples was adapted over the years due to the increased availability of conjugated antibodies against canine leukocytes. Samples processed before 2011 were analysed using mainly a two-colour approach. Starting from 2011, a multicolour
approach was applied with the addition of CD45 as tracking label in all tubes. The diagnostic algorithm varied throughout the years, but a basic panel included antibodies against CD5, CD21, CD34, and CD45. On the basis of the staining results obtained with this panel, expression of other antigens was evaluated, including CD3, CD4, CD8, CD11b, CD14, CD18, CD20, CD25, CD44, CD79a, CD117 and MHCII. The antibody panel used to label PB and BM samples varied based on the phenotype of neoplastic cells identified in the LN sample. If the LN sample was not adequate for FC, the basic antibody panel was applied to PB and BM, with the addition of CD4 and CD8. Specificity, sources and clones of antibodies are listed in Novacco et al. (2015). Samples were acquired using a flow cytometer (FACScalibur, Becton Dickinson) and analysed using Cell Quest software (Becton Dickinson). All FC interpretations and reports were made by one board-certified clinical pathologist (SC).

The final diagnosis was based on a comprehensive evaluation of all the data provided by the referring veterinarian, including different combinations of history, presenting complaint, clinical signs, LN cytological smear, haematology data and LN/PB/BM FC analysis. Cases included in the present study were classified based only on the FC report, without any re-evaluation of the raw FC data.

**Criteria for diagnosis**

The final diagnosis was derived from the flow cytometry report and was based on cytology findings, if available, combined with a comprehensive interpretation of flow cytometric results obtained from LN aspirates and other sample material. Samples were considered to be ‘non-diagnostic’ if one or more of the following criteria were present: (1) acellularity (<1x10^6 cells in total); (2) sample composed of primarily dead cells; and (3) if flow cytometric results strongly differed from cytological evaluation, for example, when neoplastic cells disrupted during processing and only a small residual non-neoplastic population was labelled). Dead cells were
identified by gross evaluation of the sample (based on colour, odour or the presence of tissue debris) and/or with a viability stain (propidium iodide), which was included in the last 2 years of the study period. Samples were considered ‘negative for lymphoid tumour’ if: (1) a mixed population of predominantly small cells was present with a cytology supporting a reactive/hyperplastic lymph node; or (2) other causes of lymphadenomegaly were identified by immunophenotyping and cytology, for example, LN metastasis from solid tumours, histiocytic tumours and plasma cell tumours. Samples were considered as ‘likely lymphoid tumour’ if: (1) flow cytometry from a lymph node showed a highly prevalent population (> 65%) of lymphoid cells with a single phenotype, but only a reduced panel of antibodies was allowed and/or the lack of a good quality cytological smear precluded a definitive diagnosis; (2) flow cytometry from a lymph node was poorly cellular (< 1 x 10^6 cells in total) or provided equivocal results, but LN cytology was highly suggestive of lymphoma and immunophenotyping of PB and/or BM was suggestive of a lymphoid tumour. In these cases, a diagnosis of ‘likely lymphoid tumour’ was made. Cases were classified as ‘lymphoid tumour’ if a definitive diagnosis of lymphoma or leukaemia was made based on the results of immunphenotyping of LN, PB, or BM. In many cases, extended subtyping of lymphoid neoplasia was possible based on immunophenotyping of different tissues and cytological evaluation: (1) B cell lymphoma (irrespectively of the grade); (2) high grade T cell lymphoma (based on immunoreactivity to T cell markers and cytological aspects including high numbers of mitotic figures); (3) low grade T cell lymphoma (based on typical T zone pattern staining on FC and/or distinctive cytological features); (4) acute leukaemia (starting with PB or BM immunophenotyping of precursor cells confirmed by LN infiltration); and (5) chronic lymphocytic leukaemia (starting with PB or BM immunophenotyping confirmed by LN infiltration).

Statistical analysis

Statistical analysis was applied to identify pre-analytical variables possibly affecting the likelihood to reach a diagnosis; for this aim, samples were grouped into ‘diagnostic’ (if any
diagnosis had been made, regardless of the level of confidence) and ‘non-diagnostic’ (including samples not adequate for processing and samples processed but not diagnostic). Pre-analytical variables investigated were related to: (1) the patient: breed (pure or mixed), age (years), sex (female, spayed female, male, neutered male), size (toy/small, medium, large/giant); (2) sampling and shipping: year (from 2009 to 2015), season (cool to cold from October to March, warm to hot from April to September), referring veterinarian (veterinarians who sent > 10 cases over the study period were considered individually, while veterinarians sending less than 10 cases in total were grouped in a single category), origin and shipping (‘within institution’, ‘out of institution hand-delivered’, ‘out of institution delivered by express courier’); (3) sample: type of sample (LN alone, LN plus PB, LN plus PB and BM, LN plus BM), LN cytological smear (present or not), cellular concentration of the LN sample (x $10^9$ cells/L), presence of gross artefacts (haemodilution, dead cells, none). Univariate and multivariate binomial logistic regressions were performed using SPSS v20.0 for Windows (IBM). Multivariate analysis was performed with a backward step selection, including only variables with $P \leq 0.3$ at univariate analysis. Significance was set at $P \leq 0.05$.

Results

Out of 1273 samples, 264 cases were excluded due to lack of information regarding the sample ($n = 88$; 6.9%) or due to sampling of tissues other than LN ($n = 176$; 13.8%). Among the latter, 59 (33.5%) cases were represented by PB and BM, 49 (27.8%) cases by PB alone, 28 (15.9%) cases by extranodal masses (other than cutaneous), 18 (10.2%) cases by effusions, nine (5.1%) cases by spleen, seven (4.0%) cases by cutaneous masses, two (1.1%) cases by liver, and four (2.3%) cases by other tissues. Twenty-two cases (1.73%) were excluded because the final report was not available. Finally, 987 cases fulfilled the inclusion criteria and were included in the statistical analysis.
Seventy-two (7.3%) samples were non-diagnostic. Among the 915 diagnostic FNAs, 839 (91.7%) were 'conclusive for lymphoid tumour', 61 (6.7%) were 'negative for lymphoid tumour', and 15 (1.6%) were 'likely lymphoid tumour'. A specific diagnosis was available for 812 cases; 596 (73.4%) cases were B cell lymphomas, 143 (17.6%) cases were high grade T cell lymphomas, 60 (7.4%) cases were low grade T cell lymphomas, 8 (1.0%) cases were acute leukaemias, and five (0.6%) cases were chronic lymphocytic leukaemias. The results of univariate and multivariate analyses and the odd ratios for diagnostic samples are summarised in Table 1.

**Patient-related variables**

Breed was reported in 911 cases; there were 239 (26.2%) mixed breed dogs. Among the remaining 672 dogs, the most prevalent breeds included German shepherd (n = 59; 8.8%), Boxer (n = 56; 8.3%), Golden retriever (n = 46; 6.8%), Labrador retriever (n = 44; 6.5%), Rottweiler (n = 38; 5.7%) Doberman pinscher (n = 37; 5.5%), Beagle (n = 29; 4.3%), Bernese mountain dog (n = 23; 3.4%), English bulldog (n = 21; 3.1%) and Yorkshire terrier (n = 21; 3.1%). Many other breeds were represented, with less than 20 cases each. In 76 cases, the breed was not recorded. Since the size of mixed breed dogs was generally not available, this variable was analysed only within the pure-breed dog group. Among the latter, the majority of cases were of large/giant size (n = 415; 61.8%); 142 (21.1%) dogs were of toy/small size, and 115 (17.1%) were medium-sized. Sex was reported in 901 cases: there were 213 (23.6%) females, 210 (23.3%) spayed females, 397 (44.1%) males and 81 (9.0%) neutered males. The male/female ratio was 1.15:1. Age was known for 899 dogs. Median age was 8 years (mean 8.4 ± 3.2 years, range 1-17 years). None of the patient-related variables significantly influenced the likelihood of having a diagnostic sample.

**Variables related to sampling and shipping**

The number of cases per year steadily increased during the study period, ranging from 63 cases fulfilling the inclusion criteria in 2009 to 200 cases in 2015. The highest percentage of
diagnostic cases was reached in 2012 (124/128 cases, 96.9%) and the lowest in 2015 (174/200 cases, 87.0%). The likelihood of having a diagnostic sample significantly varied by years both with univariate and multivariate analysis ($P = 0.031$ and $P = 0.016$, respectively).

The percentage of samples collected in the cold and hot seasons was almost similar (51.2% and 48.8%, respectively) without any significant difference ($P=0.154$).

Thirteen veterinarians sent >10 samples, ranging from 13 to 207 samples; veterinarians sending < 10 samples were grouped all together, to a final count of 363 (36.8%) cases. Most of the samples for which this information was available had been sent to the laboratory by express courier (559/816, 68.5%), 159 (19.5%) were sampled outside the institution and hand-delivered, and 98 (9.9%) were sampled within the institution. The likelihood of a having a diagnostic sample was not influenced by these variables with univariate analysis. However, the proportion of diagnostic samples significantly varied among veterinarians based on multivariate analysis ($P = 0.004$).

Variables related to sample material

In the majority of cases (572; 58.0%), samples from all three sites (LN, PB and BM) of the same dog were available; LN and PB were sent in 225 (22.8%) cases, LN alone was submitted in 163 (16.5%) cases, and LN and BM were sent to the laboratory in 27 (2.7%) cases. Sample material significantly influenced the likelihood of obtaining a diagnosis ($P = 0.025$). In particular, cases for which both LN and PB or all three sites (LN, PB and BM) were obtained had a higher likelihood of being diagnostic, compared to samples from LN alone ($P = 0.011$ and $P = 0.006$, respectively). The type of sample material also significantly influenced the likelihood of having diagnostic samples at multivariate analysis ($P = 0.031$).

A LN cytological smear was provided in 634/987 (64.2%) cases, but the quality of these smears was not assessed in the present study. The presence of a cytological smear significantly
improved the likelihood of having a diagnostic sample \( (P = 0.009) \) only at univariate analysis.

Lymph node sample cellularity was highly variable among cases, ranging from 0.02 to 436.19 \( \times 10^9 \) cells/L (median 12.44 \( \times 10^9 \) cells/L, mean 29.21\( \pm \)48.13 \( \times 10^9 \) cells/L). Lymph node sample cellularity did not affect the likelihood of having diagnostic samples based on univariate analysis \( (P=0.104) \), but was statistically significant at multivariate analysis \( (P=0.013) \), with cellularity being higher in diagnostic than in non-diagnostic samples (median 13.1 \( \times 10^9 \) cells/L and 2.09 \( \times 10^9 \) cells/L, respectively). In the group of non-diagnostic cases, the cellularity was lower than the minimum adequate concentration \( (5.0 \times 10^9/L) \) in 52.8\% of cases, and lower than the lower cut-off value generally considered to be suitable for analysis \( (1.0 \times 10^9/L) \) in 37.5\% of cases. These percentages were much higher than those found in diagnostic samples \( (21.0\% \text{ and } 3.4\%) \), respectively).

Artefacts were reported in nine non-diagnostic samples \( (12.5\%) \), including one haemodiluted sample and eight necrotic samples, and in 18 diagnostic samples \( (2.0\%) \), including three haemodiluted and 15 necrotic samples. Haemodilution did not affect the likelihood of having a diagnostic sample. On the contrary, necrotic samples were significantly less likely to be diagnostic in both univariate and multivariate analysis \( (P < 0.001 \text{ for both analyses}) \).

Discussion

The results of the present study indicate that FC is suitable to refine a tentative diagnosis of canine lymphoma because it provided diagnostic results in the majority of cases \( (about 90\%) \). Among the pre-analytical variables investigated, no association was identified between signalment and the likelihood to obtain a diagnostic result. In particular, neither dog size nor breed \( (often related to breed specific temperament) \) influenced the odds of obtaining a diagnosis. Fine needle aspiration of a lymph node is usually carried out without sedation, and, in theory, obtaining a good quality fine needle aspirate is more difficult in small-sized or aggressive dogs. On the other hand,
the sampling of superficial lymph nodes may be more difficult in breeds in which the subcutaneous
tissue is abundant. However, the results of the present study indicate that FNA provides good
quality samples in the great majority of cases regardless of the animal’s signalment.

In contrast, the likelihood of obtaining a diagnosis varied among submitting
veterinarians/institutions, possibly reflecting different technical skills of the operator (veterinarian)
who performs the sampling. These data support the necessity of an adequate technical training in
order to increase the likelihood of having a diagnostic result.

Delivery method did not influence the diagnostic utility. No differences were found among
samples submitted from the internal oncology service of the University of Milan, which were
delivered within few minutes following sampling, those delivered in person within a few hours
following sample collection, and those shipped using an express courier.

Likewise, no differences were found between samples shipped during the cold and hot
seasons. Of notice, the standard requirements of our laboratory include a maximum shipping time of
24 h and shipment on ice packs for FC samples. Therefore, our results are only valid under these
conditions. In human medicine, refrigeration of FC samples generally is not recommended, since
some antigens may be internalised and bias the final results (Ekong et al., 1993). Studies on this
aspect in veterinary medicine are currently lacking, but it seems reasonable to refrigerate the
samples during shipment to prevent cell disruption due to high temperature, mainly in hot seasons
and in hot countries. It is currently unknown whether canine antigens vary their expression
following refrigeration.

Sample type and sample characteristics had the greatest influence on the diagnostic power of
flow cytometry. The major factor leading to a non-diagnostic result was poor cellularity, with more
than a half of non-diagnostic samples having a cell concentration lower than recommended.

Artefacts such as haemodilution and dead cells showed a minor effect on non-diagnostic samples. Haemodilution was rarely related to a non-diagnostic sample because a rapid lysis step was added to the procedure when gross haemodilution was evident. The presence of many non-viable cells is uncommon in LN FNAs, at least in untreated dogs. When present, the necrotic debris may be easily identified by gross evaluation (based on colour, odour or the presence of tissue debris) or using a viability stain, such as propidium iodide. The latter procedure was included in the last 2 years of the study period but it is not possible to determine if this inclusion could have influenced the percentage of diagnostic results.

Having sample material from different sites (LN, PB, BM) and a concurrent LN cytological smear available improved the likelihood of establishing a diagnosis of lymphoid neoplasia. Therefore, complete cases, including FNA from a LN, PB, BM and cytology smears, are desirable for a more comprehensive evaluation by a clinical pathologist. Specifically, cytological evaluation of a smear may help to differentiate reactive/hyperplastic lymph nodes from lymphoid tumours in which the neoplastic population, which tends to be more fragile, has been disrupted. In the first case, samples would be classified as ‘diagnostic, non-neoplastic’, while in the second case they would be classified as ‘non-diagnostic’. When a fresh cytological smear was not included, a cytospin or a cytology of the sediment from the cell suspension submitted for FC was generally prepared to better interpret FC results. However, these smears were often of poor quality for an adequate cytological evaluation and they were only used to confirm the FC results.

The presence of neoplastic cells detected via flow cytometry in PB and/or BM, together with a LN cytology compatible with lymphoma, may also permit a putative diagnosis of ‘likely lymphoma’, even if LN sample cellularity is too low for FC. This could be achieved when neoplastic cells can be differentiated from non-neoplastic cells, for example when the cells showed
an aberrant phenotype (such as a T zone pattern) or when a high percentage of large CD21 positive
cells were detected. Although it cannot be presumed that the cells in peripheral blood represented
the same neoplastic population than the cells in lymph node, we considered it very likely that they
were of similar origin when a cytological smear of lymph node was suggestive of lymphoma. In
such cases, we used a comprehensive approach and considered all available clinical and laboratory
information to make a diagnosis of ‘likely lymphoma’. Although this assumption should be taken
with care, we think that the stepwise approach of including FC of PB and/or BM when the LN
sample is not suitable could be of some clinical benefit in managing dogs with lymphoma. The
evaluation of PB and/or BM would also be crucial to differentiate acute and chronic lymphoid
leukaemias with secondary lymph node infiltration from nodal lymphoma with a haematogenous
phase. The results of our study suggest that, when LN cellularity was too low to perform FC, flow
cytometry of PB was most likely to be diagnostic in case it was infiltrated. This suggests that
submitting peripheral blood together with a LN aspirate will facilitate interpretation of FC results
and it will also assist with staging of lymphoma. In the present study, PB and BM samples were
frequently submitted to our laboratory in addition to a LN sample, mainly for staging purposes. Our
results indicate that having a BM aspirate available for FC did not increase the likelihood of having
a diagnostic sample if LN and PB were also submitted.

The purpose of this study was not to assess the diagnostic accuracy of FC in identifying
different lymphoma subtypes. However, the frequency of lymphoma subtypes reported here is
similar to frequencies reported in the literature (Ponce et al., 2010; Valli et al., 2011). This finding
suggests that FC may be useful to identify different subtypes in dogs with lymphoma. This may be
attributable to the high prevalence of diffuse lymphomas in dogs (Fournel-Fleury et al., 1994,
Comazzi and Gelain, 2011).
The limits of this study are inherent to its retrospective nature. Some possible sources of biases were not analysed, including the localisation and size of the LN sampled, the technique used for sampling (free hand vs. ultra-sound guided, suction vs. capillary technique), the use of different transport media (RPMI vs. phosphate buffer saline vs. saline solution), and the quality of the cytological smears. Another limitation is the lack of a gold standard for the final diagnosis, which precluded us from defining the diagnostic performances of both cytology and FC. This should be addressed in a future, prospective study.

Conclusions

Flow cytometry performed from canine LN FNA samples is a suitable diagnostic tool to confirm lymphoma in the majority of cases, regardless of patient characteristics, provided that the sample has sufficiently high cellularity, is submitted within 24 h following sampling and is shipped on ice packs. Packaging and shipping should be standardised to assure fast delivery within 24 h of sampling. Haemodilution has minimal effect on FC analysis, but veterinarians should make any possible effort to obtain a highly cellular sample. The centres of large, neoplastic nodes should be avoided if possible, they may provide more necrotic/dead cells. The addition of a PB sample and a LN cytological smear can be recommended to improve the likelihood of receiving a conclusive result.

Conflict of interest statement

None of the authors has any other financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Table 1
Results of univariate and multivariate analysis for different pre-analytical variables examined for their effect on flow cytometric analysis of samples from dogs with clinically suspected lymphoma.

<table>
<thead>
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<th>Pre-analytical variable</th>
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<th>Multivariate analysis</th>
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<td></td>
<td></td>
</tr>
<tr>
<td>Sample material</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LN alone</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>LN and PB</td>
<td>2.877</td>
<td>1.346-6.150</td>
</tr>
<tr>
<td>LN, PB and BM</td>
<td>2.078</td>
<td>1.182-3.653</td>
</tr>
<tr>
<td>LN and BM</td>
<td>1.849</td>
<td>0.408-8.380</td>
</tr>
<tr>
<td>LN cytological smear</td>
<td>No</td>
<td>Ref</td>
</tr>
<tr>
<td>Yes</td>
<td>1.904</td>
<td>1.176-3.083</td>
</tr>
<tr>
<td>Dead cells</td>
<td>No</td>
<td>Ref</td>
</tr>
<tr>
<td>Yes</td>
<td>0.133</td>
<td>0.054-0.326</td>
</tr>
<tr>
<td>Haemodilution</td>
<td>No</td>
<td>Ref</td>
</tr>
<tr>
<td>Yes</td>
<td>0.234</td>
<td>0.024-2.274</td>
</tr>
<tr>
<td>LN sample cellularity</td>
<td>1.908</td>
<td>0.998-1.017</td>
</tr>
</tbody>
</table>

LN, lymph node; PB, peripheral blood; BM, bone marrow; Ref, reference; ND, not detectable.

Odds ratio for the probability to provide a diagnostic result are also given.

* P ≤ 0.05