

# CD10, BCL6, and MUM1 Expression in Diffuse Large B-Cell Lymphoma on FNA Samples

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**BACKGROUND:** Gene expression profiling has divided diffuse large B-cell lymphoma (DLBCL) into 2 main subgroups: germinal center B (GCB) and non-GCB type. This classification is reproducible by immunohistochemistry using specific antibodies such as CD10, B-cell lymphoma 6 (BCL6), and multiple myeloma oncogene 1 (MUM1). Fine-needle aspiration (FNA) plays an important role in the diagnosis of non-Hodgkin lymphoma, and in some cases FNA may be the only available pathological specimen. The objectives of the current study were to evaluate CD10, BCL6, and MUM1 immunostaining on FNA samples by testing the CD10, BCL6, and MUM1 algorithm on both FNA cell blocks (CB) and conventional smears (CS), evaluating differences in CB and CS immunocytochemical (ICC) performance, and comparing results with histological data. **METHODS:** Thirty-eight consecutive DLBCL cases diagnosed by FNA were studied. Additional passes were used to prepare CB in 22 cases and CS in 16 cases; the corresponding sections and smears were immunostained using CD10, BCL6, and MUM1 in all cases. The data obtained were compared with histological immunostaining in 24 cases. **RESULTS:** ICC was successful in 33 cases (18 CB and 15 CS) and not evaluable in 5 cases (4 CB and 1 CS). The CD10-BCL6-MUM1 algorithm subclassified DLBCL as GCB (9 cases) and non-GCB (24 cases). ICC data were confirmed on histologic staining in 24 cases. **CONCLUSIONS:** CD10, BCL6, and MUM1 ICC staining can be performed on FNA samples. The results herein prove it is reliable both on CB and CS, and is equally effective and comparable to immunohistochemistry data. *Cancer (Cancer Cytopathol)* 2015;000:000-000. © 2015 American Cancer Society.

**KEY WORDS:** B-cell lymphoma 6 (BCL6); CD10; cell block; diffuse large B-cell lymphoma (DLBCL); fine-needle aspiration (FNA); non-Hodgkin lymphoma; multiple myeloma oncogene 1 (MUM1).

## INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma (NHL); it may arise de novo or from the transformation of a former NHL, and accounts for approximately 40% of NHL cases.<sup>1,2</sup> DLBCL is a heterogeneous disease with a variable clinical course, and patients currently are treated with a combination of immunotherapy and chemotherapy.<sup>3</sup> The clinical presentation and outcome are remarkably variable, reflecting biologic and pathogenetic heterogeneity.<sup>4</sup> On the basis of gene expression profiling (GEP), DLBCL can be divided into distinct subgroups that differ in terms of molecular features and reflect the origin from different stages of B-cell differentiation during germinal center maturation.<sup>5</sup> This classification based on the cell of origin divides DLBCL into at least 3 different groups: the germinal center B cell-like (GCB), the activated B cell-like (ABC), and the unclassifiable DLBCL. This subclassification appears to have prognostic and predictive

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value, because patients with ABC and unclassifiable DLBCL have worse overall survival compared with patients with GCB DLBCL and respond less effectively to the current therapeutic regimens; an overall cure rate of approximately 40% is reported.<sup>4,6</sup> GEP may not be used in routine clinical practice because of high costs and the need for specific technologies; therefore, different immunohistochemical (IHC) algorithms have been proposed within the last decade to classify DLBCL subgroups by means of specific phenotypic profiles.<sup>7-13</sup> These algorithms include various antibodies, with CD10, B-cell lymphoma 6 (BCL6), and multiple myeloma oncogene 1 (MUM1) being the most frequently used.<sup>7-10</sup> The combination of CD10, BCL6, and MUM1 may classify DLBCL into GCB DLBCL and non-GCB DLBCL (ABC and unclassified subgroup), with approximately 80% concordance with GEP.<sup>3,7</sup>

Fine-needle aspiration (FNA) plays an important role in the diagnosis and management of NHL and may represent the only source of diagnostic material in specific clinical contexts.<sup>14</sup> Therefore, an accurate FNA diagnosis with prognostic/predictive information is advisable, with or without subsequent histological examination. Immunocytochemical (ICC) assessment is commonly used in FNA on different cytological samples, such as conventional smears (CS), cytopsin preparations, ThinPrep, and cell blocks (CB), with different fixation of the samples and varying procedures.<sup>15-18</sup> Because the 2 institutions involved in the current study routinely use CB and CS, respectively, for ICC, this difference was examined to assess whether there might be differences in terms of ICC performance.

The objectives of the current study were the evaluation of CD10, BCL6, and MUM1 ICC on FNA samples; testing of the CD10-BCL6-MUM1 algorithm on FNA CB and CS preparations; and identification of differences in CS and CB ICC performance in comparison with histological data.

## MATERIALS AND METHODS

A prospective study of DLBCL FNA was performed at the cytopathology services of the university hospitals of the "Federico II" University of Naples and the University of Salerno, both in Italy. The study design was approved by the Campania Sud Ethics Committee (cometicocampaniasud@asl3sud.it). Forty-one consecutive DLBCL cases, diagnosed by FNA over a 2-year period (January 2013-December 2014), were retrieved from the files of the 2

institutions. Three of these cases had been histologically diagnosed as high-grade follicular lymphoma and were not considered in the current study, leaving 38 proven DLBCL cases.

### **FNA Procedures and Enrollment Criteria**

FNA and enrollment criteria were similar in the 2 institutions. FNA procedures and related risks were discussed with patients and informed consent was obtained. FNA generally was performed with a 23-gauge needle under ultrasound guidance, with the exception of 2 axillary lymph nodes that were aspirated under direct palpation and 1 para-aortic lumbar lymph node in which a 22-gauge, 20-cm long Chiba needle was used with a probe adaptor. In all the cases, rapid on-site evaluation (ROSE) of Diff-Quik-stained smears was performed to evaluate the adequacy of the sample and for a primary diagnosis.

### **Flow Cytometry Procedure**

A second pass was flushed in phosphate-buffered saline and used for flow cytometry (FC) analysis using the following fluoresceinated antibodies: CD3, CD5, CD19, CD23, FMC7, and CD10; kappa and lambda light chains; and a 3-color analysis technique on a Becton Dickinson FACS scan (BD Biosciences, San Jose, Calif) as previously described.<sup>19,20</sup> FC was considered not effective (NE) when fluoresceinated antibodies, including those targeting light chains, were not expressed or evaluable.<sup>19,21</sup> When DLBCL was suspected at the time of ROSE, additional passes were then flushed in 5 mL of buffered formalin to prepare paraffin-embedded CB using the Shandon Cytoblock CB preparation system (Thermo Fisher Scientific, Waltham, Mass), according to the manufacturer's instructions; alternatively,  $\geq 3$  additional CS were fixed in 95% alcohol for ICC analysis. CB were used to analyze 22 cases and CS were used in 16 cases.

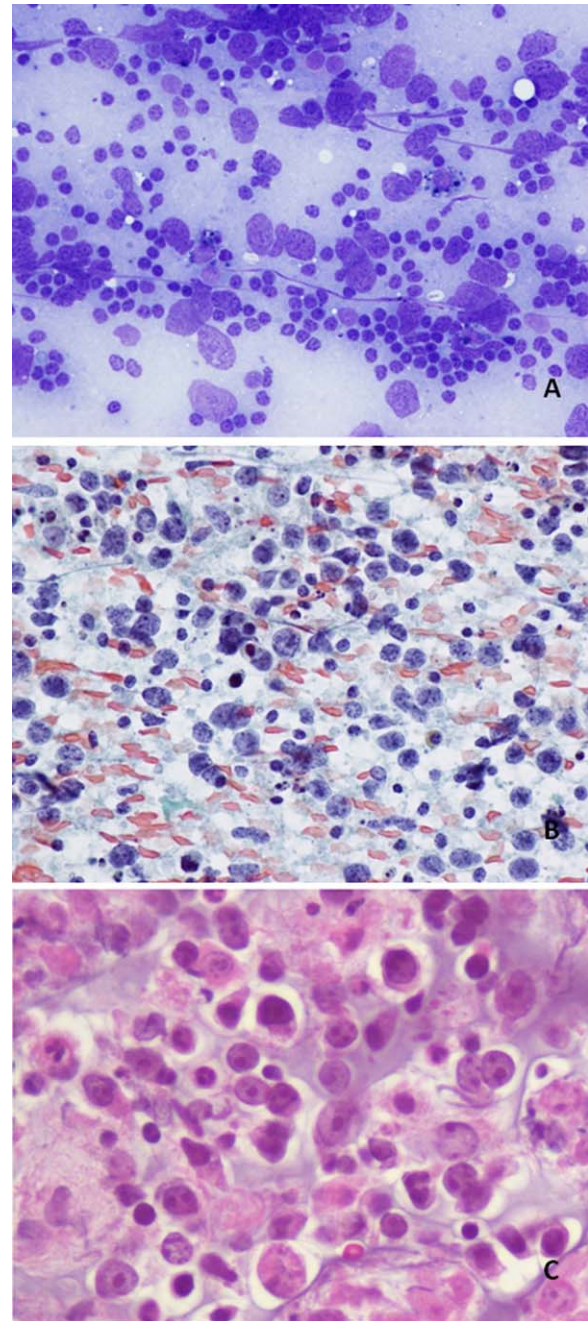
### **ICC Procedure**

An ICC study was performed on 4- $\mu$ m sections of dewaxed and dehydrated formalin-fixed, paraffin-embedded CB and on alcohol-fixed CS. After heat-induced antigen retrieval, slides were processed using the BenchMark Autostainer (Ventana Medical Systems Inc, Tucson, Ariz) using the iVIEW 3,3'-diaminobenzidine detection kit (Ventana Medical Systems Inc) according to the manufacturer's instructions. The following prediluted monoclonal antibodies were used in all cases: CD10 (clone SP67;

Ventana), BCL6 (clone GI191/A8; Cell Marque Corporation, Rocklin, Calif), and MUM1 (clone MRQ-43; Cell Marque Corporation). CD3, CD15, CD30, and *ALK-1* were also used in 4 cases, in which a differential diagnosis of T-cell lymphoma, Hodgkin lymphoma (HL), and anaplastic (*ALK-1* positive or negative) lymphoma was considered.

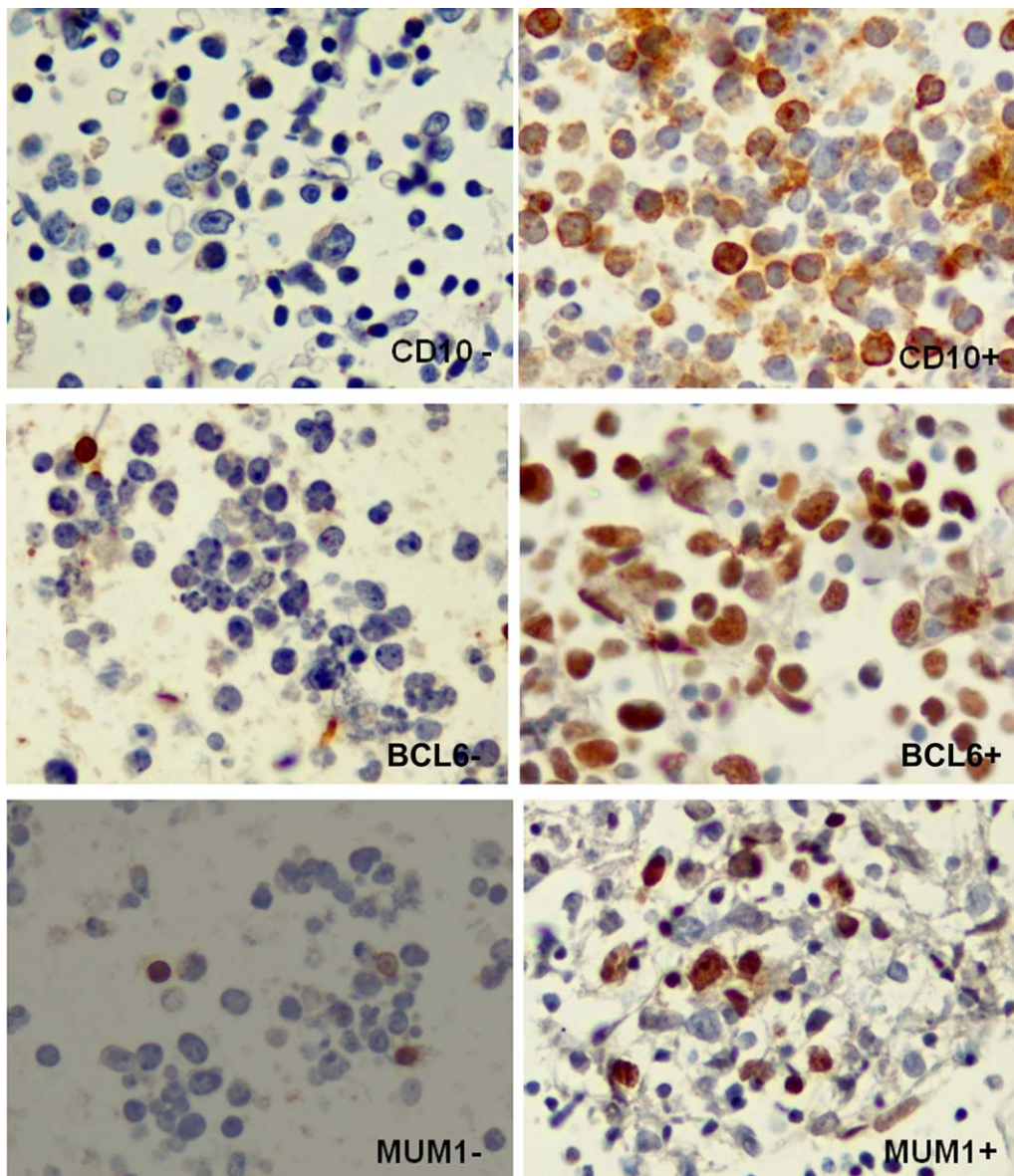
### Evaluation Criteria for ICC

To determine CD10, BCL6, and MUM1 expression, ICC evaluation was performed through the identification of nuclear (BCL6 and MUM1) or cytoplasmic (CD10) membrane positivity. According to the literature,<sup>7,11,13,22</sup> the corresponding antibodies were considered positive when expressed in at least 30% of the diagnostic cells. Smaller or indeterminate DLBCL cells were not considered in cell counting; only large and definitely atypical DLBCL cells were incorporated into the count. ICC was considered NE when insufficient cells were present on the CB or none of the cells on the CS was immunostained, and negative when DLBCL cells were not immunostained, apart from background positivity (namely granulocytes, medium-sized follicular center cells, occasional stromal cells, and plasma cells). The intensity of the signal was not considered.<sup>7,11,13,22</sup> For ICC evaluation on CB sections, routine histological or hematopathological samples were used as positive controls and tested with the same antibodies during the same IHC run. Negative controls were obtained by omitting the primary antibody. With regard to CS, negative and positive cells in the background of the corresponding smears were used as internal controls. Quantification was then performed by counting the number of positive cells in 5 to 10 fields at  $\times 430$  high-power fields. Cases were then classified as positive, negative, and NE for the corresponding antibodies. The cases from each institution were blindly and reciprocally reviewed by 2 of the authors (I.C. and P.Z), who confirmed the original diagnoses, ICC evaluation, and quantification. The data obtained were compared with the histological data in 24 of 38 cases. To evaluate ICC performance on FNA DLBCL, a linear regression analysis was performed between ICC and IHC data on the 24 cases by plotting the 6 possible combinations generated by the expression of CD10, BCL6, and MUM1 in a scatter plot graph. The six categories are the following: 1) CD10-, BCL6-,



**Figure 1.** (A) Cytological features of diffuse large B-cell lymphoma (DLBCL) showing large isolated cells with an irregular nuclear shape; small lymphocytes are present in the background. Note the expression of nuclear fragility indicated by the nuclear strips (Diff-Quik stain,  $\times 430$ ). (B) DLBCL smear showing large irregular nuclei with coarse, granular, dispersed chromatin and  $\geq 1$  nucleoli; erythrocytes and small lymphocytes are present in the background (Papanicolaou stain,  $\times 430$ ). (C) Cell block appearance of DLBCL showing large, isolated, irregular cells with dispersed chromatin and large nucleoli (Papanicolaou stain,  $\times 430$ ).





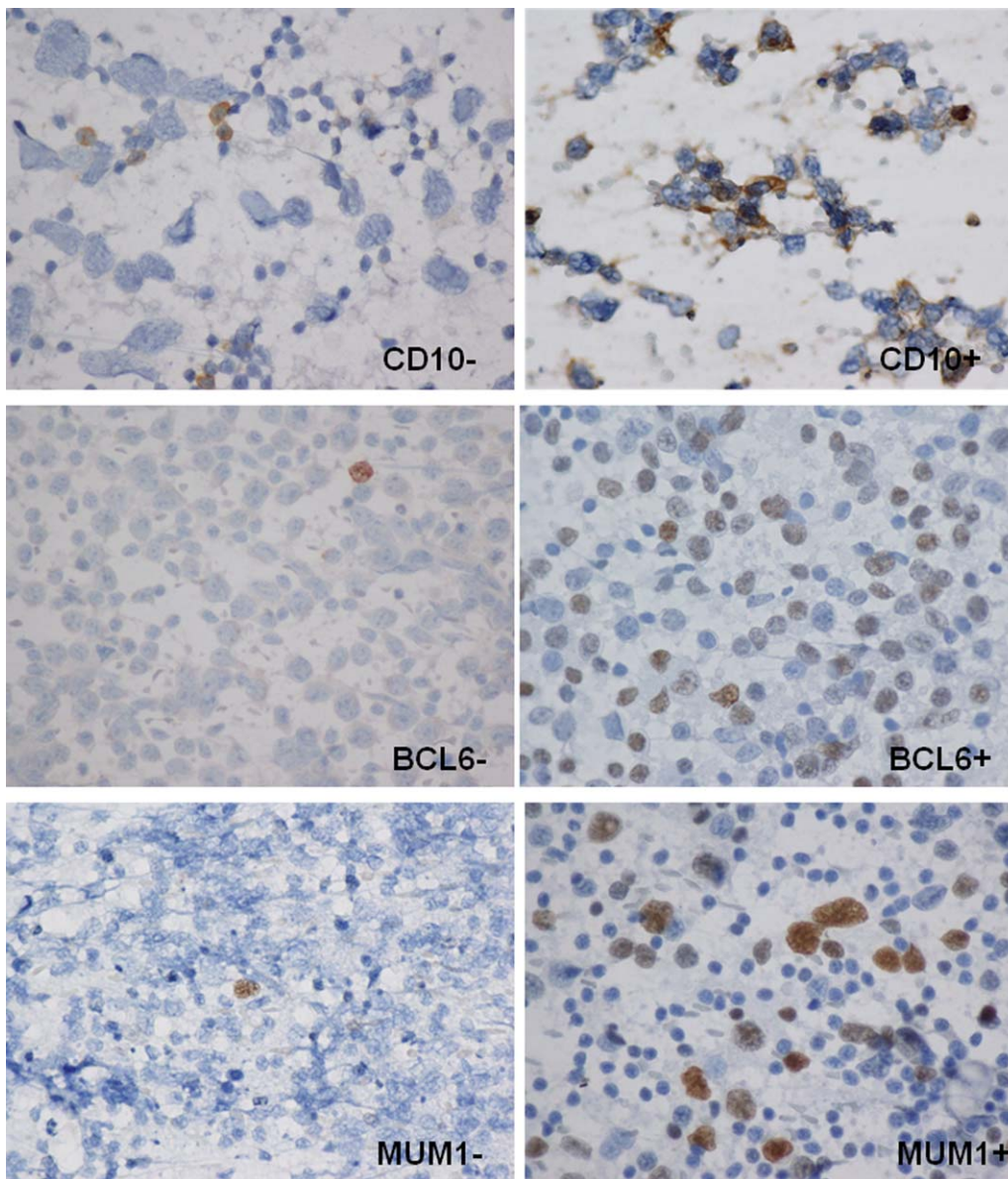
**Figure 2.** Immunostaining with CD10, B-cell lymphoma 6 (BCL6), and multiple myeloma oncogene 1 (MUM1) in diffuse large B-cell lymphoma cell blocks. In positive cases, >30% of the diagnostic cells show nuclear (BCL6 and MUM1) and cytoplasmic (CD10) positivity. In negative cases, occasional diagnostic cells or small cells in the background represent positive internal controls (CD10-BCL6-MUM1 immunostain,  $\times 430$ ). - indicates negative; +, positive.

MUM1+; 2) CD10+, BCL6-, MUM1-; 3) CD10+, BCL6+, MUM1-; 4) CD10-, BCL6-, MUM1-; 5) CD10-, BCL6+, MUM1-; 6) CD10-, BCL6+, MUM1+.

## RESULTS

FNA samples from 38 DLBCL cases were used. Lymph nodes were lateral cervical in 18 cases, inguinal in 8 cases,

submandibular in 5 cases, supraclavicular in 4 cases, axillary in 2 cases, and para-aortic lumbar in 1 case. The male-to-female ratio was 20:18, and the median age of the patients was 62.4 years (range, 36-91 years). DLBCL smears were generally highly cellular. The predominant cell population was represented by large isolated cells with irregular nuclear membranes and coarse, granular chromatin with an overall pale appearance when compared with small lymphoid cells (Figs. 1A-1C). One or 2 large,

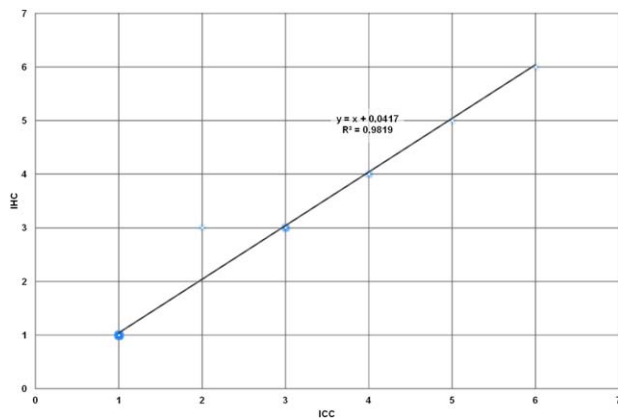


**Figure 3.** Immunostaining with CD10, B-cell lymphoma 6 (BCL6), and multiple myeloma oncogene 1 (MUM1) in diffuse large B-cell lymphoma conventional smears. In positive cases, >30% of the diagnostic cells show nuclear (BCL6 and MUM1) and cytoplasmic (CD10) positivity. In negative cases, occasional diagnostic cells or small cells in the background represent positive internal controls (CD10-BCL6-MUM1 immunostain,  $\times 430$ ). - indicates negative; +, positive.

irregular, eccentric nucleoli were often present (Figs. 1B and 1C). Cytoplasm was thin, often ill-preserved, and occasionally completely absent, giving the cells the appearance of naked large nuclei. Nuclear fragility was often present, with nuclear disruption or strips observed when compared with cell integrity in the background (Figs. 1A and 1B) of small and medium-sized lymphocytes, granulocytes, occasional eosinophils, and macrophages. In 2 cases

with scant diagnostic cells and relatively numerous eosinophils, the differential diagnosis of HL was considered. In such cases (cases 12 and 35), ICC for CD15 and CD30 on the CB and CS was negative, thus excluding the diagnosis of HL. In another 2 cases with more marked nuclear atypia (cases 25 and 22), the differential diagnosis with anaplastic lymphoma or T-cell NHL was considered and excluded by FC and by CD30 and ALK ICC negativity,





**Figure 4.** Immunocytochemistry (ICC)/immunohistochemistry (IHC) scatter plot graph. The graph shows a scatter plot of 6 ICC immunophenotypes plotted versus the corresponding IHC immunophenotypes among 24 patients analyzed. The correlation coefficient value of 0.9819 revealed a strong concordance between ICC and IHC data.

respectively. FC was effective in 26 cases, demonstrating the following phenotypes: CD19+/CD5-/CD10-/CD23-/FMC7- in 10 cases; CD19+/CD5-/CD10+/CD23-/FMC7- in 11 cases; CD19+/CD5-/CD10+/CD23-/FMC7+ in 2 cases; and CD19+/CD5-/CD10-/CD23-/FMC7+ in 3 cases. Kappa and lambda light chain restriction was observed in 18 and 8 cases, respectively. In 12 cases in which FC was NE, the FNA diagnosis of DLBCL was basically morphological and confirmed by the clinical history in cases of DLBCL recurrence; by using CD3, CD15, CD30, and ALK in selected cases; by the CD10-BCL6-MUM1 algorithm; and by the follow-up histological controls when available. Therefore, the diagnosis of DLBCL was performed on the basis of morphological, cytological, FC, and ICC data. Histological controls were available in 24 of 38 cases, and confirmed the FNA diagnosis of DLBCL in 24 cases. Eight cases were DLBCL recurrences, which had been previously diagnosed histologically. At the time of recurrence, the FNA diagnosis was consistent with the clinical presentation, and histology was not required by clinicians. In case 20 (para-aortic lymph node) and cases 10 and 29 (patients aged 85 years and 91 years, respectively), lymph nodes were not removed for the histological evaluation for clinical reasons and because the FNA diagnosis matched with the clinical data. Finally, cases 2, 21, and 32 were histologically confirmed at other institutions, but IHC data were not available for the algorithm evaluation. ICC for CD10, BCL6, and MUM1 was successfully performed on 18 CB and 15 CS, and was NE in

5 cases (Figs. 2 and 3). ICC combinations were CD10-/BCL6-/MUM1+ (20 cases), CD10+/BCL6-/MUM1- (1 case), CD10+/BCL6+/MUM1- (8 cases), CD10-/BCL6-/MUM1- (2 cases), CD10-/BCL6+/MUM1- (1 case), and CD10-/BCL6+/MUM1+ (1 case) (Figs. 2 and 3). The CD10-BCL6-MUM1 algorithm classified DLBCL as GCB in 9 cases (27%; 2 CB and 7 CS) and non-GCB in 24 cases (73%; 16 CB and 8 CS). The histological controls confirmed the ICC data in 23 of the 24 available IHC cases, being discordant in only 1 case (case 6) that was found to be negative for BCL6 on CB and positive on IHC lymph node section. NE ICC results were reported in 4 CB cases and 1 CS case (Table 1). No significant differences were detected in terms of ICC quality when comparing ICC performance on CS and CB; the higher number of NE findings among CB in comparison with CS (4 cases vs 1 case) were caused by the scant cellularity of CB sections in the corresponding cases.

The linear regression analysis obtained by grouping the 6 combinations of CD10, BCL6, and MUM1 expression and plotting the ICC against the corresponding IHC revealed a correlation coefficient value equal to 0.9819, thus demonstrating a strong concordance between ICC versus IHC data (Fig. 4).

## DISCUSSION

Using the CD10-BCL6-MUM1 ICC algorithm applied to CS and CB, we were able to subclassify DCBLC into GCB and non-GCB in a series of 38 lymph node FNA cases. The original algorithm proposed by Hans et al<sup>7</sup> was based on the expression of CD10, BCL6, and MUM1 and was effective in the classification of DLBCL with a GEP concordance in approximately 80% of the cases. Additional studies have proposed other antibodies (such as BCL2, Ki-67, and human leukocyte antigen [HLA])<sup>8-12</sup> or new antibodies (such as Forkhead box-P [FoxP], GCET1 (centerin),<sup>22</sup> and LMO2<sup>10,13</sup>) to be used in addition to the 3 original ones (CD10, BCL6, and MUM1) or by replacing BCL6.<sup>23,24</sup> The algorithm by Choi et al<sup>22</sup> introduced the germinal center B cell-expressed transcript 1 (GECT1) as the first discriminator of DLBCL cells, and FOXP1 as a discriminator of CD10-/BCL6+ cases achieving a 93% concordance with GEP. In other recently proposed algorithms, BCL6 was replaced by BCL2<sup>24</sup> or included CD10, GCET, FOXP1, and MUM1 antibodies, which appear to be the most specific for GCB and non-GCB, respectively.<sup>25</sup>

**TABLE 1.** Clinical and Phenotypic Classification of 38 Cases of DLBCL Diagnosed by FNA

| Case No. | Localization               | Age, Years | Sex | CB/CS | CD10 | BCL6 | MUM1 | DLBCL Subtype | Histological Concordance |
|----------|----------------------------|------------|-----|-------|------|------|------|---------------|--------------------------|
| 1        | Left lateral cervical      | 43         | F   | CB    | -    | -    | +    | Non-GCB       | Yes                      |
| 2        | Bilateral lateral cervical | 66         | F   | CB    | -    | -    | +    | Non-GCB       | Yes <sup>a</sup>         |
| 3        | Right submandibular        | 47         | F   | CB    | -    | -    | +    | Non-GCB       | Yes                      |
| 4        | Right lateral cervical     | 49         | M   | CB    | NE   | NE   | NE   | NP            | NA                       |
| 5        | Left supraclavicular       | 52         | F   | CB    | -    | -    | +    | Non-GCB       | Yes                      |
| 6        | Left lateral cervical      | 46         | F   | CB    | +    | -    | -    | GCB           | No BCL6+                 |
| 7        | Left lateral cervical      | 80         | F   | CB    | -    | -    | +    | Non-GCB       | NA                       |
| 8        | Left supraclavicular       | 55         | F   | CB    | -    | -    | +    | Non-GCB       | NA                       |
| 9        | Left supraclavicular       | 68         | F   | CB    | -    | -    | -    | Non-GCB       | Yes                      |
| 10       | Left lateral cervical      | 85         | M   | CS    | NE   | NE   | NE   | NP            | NA                       |
| 11       | Left inguinal              | 72         | M   | CB    | +    | +    | -    | Non-GCB       | NA                       |
| 12       | Right lateral cervical     | 77         | M   | CB    | -    | -    | +    | Non-GCB       | Yes                      |
| 13       | Right lateral cervical     | 55         | M   | CS    | -    | -    | +    | Non-GCB       | Yes                      |
| 14       | Right lateral cervical     | 43         | M   | CB    | NE   | NE   | NE   | NP            | NA                       |
| 15       | Right lateral cervical     | 65         | F   | CS    | +    | +    | -    | GCB           | NA                       |
| 16       | Right submandibular        | 76         | M   | CS    | +    | +    | -    | GCB           | Yes                      |
| 17       | Left inguinal              | 81         | M   | CS    | -    | -    | +    | Non-GCB       | Yes                      |
| 18       | Right inguinal             | 58         | F   | CS    | +    | +    | -    | GCB           | Yes                      |
| 19       | Right lateral cervical     | 49         | M   | CS    | -    | -    | +    | Non-GCB       | Yes                      |
| 20       | Para-aortic lumbar         | 72         | M   | CB    | NE   | NE   | NE   | NP            | NA                       |
| 21       | Right inguinal             | 51         | F   | CS    | +    | +    | -    | GCB           | Yes <sup>a</sup>         |
| 22       | Left supraclavicular       | 66         | F   | CB    | -    | -    | +    | Non-GCB       | Yes                      |
| 23       | Left lateral cervical      | 69         | M   | CB    | -    | +    | -    | GCB           | Yes                      |
| 24       | Left axillary              | 69         | M   | CB    | -    | +    | +    | Non-GCB       | Yes                      |
| 25       | Left submandibular         | 39         | F   | CB    | -    | -    | +    | Non-GCB       | Yes                      |
| 26       | Left lateral cervical      | 60         | M   | CB    | -    | -    | +    | Non-GCB       | Yes                      |
| 27       | Left axillary              | 69         | M   | CB    | -    | -    | -    | Non-GCB       | Yes                      |
| 28       | Left inguinal              | 54         | F   | CB    | -    | -    | +    | Non-GCB       | Yes                      |
| 29       | Left lateral cervical      | 91         | M   | CB    | -    | -    | +    | Non-GCB       | NA                       |
| 30       | Left lateral cervical      | 65         | M   | CS    | -    | -    | +    | Non-GCB       | NA                       |
| 31       | Right submandibular        | 76         | M   | CS    | +    | +    | -    | GCB           | Yes                      |
| 32       | Left lateral cervical      | 72         | F   | CS    | +    | +    | -    | GCB           | Yes <sup>a</sup>         |
| 33       | Left lateral cervical      | 53         | M   | CS    | -    | -    | +    | Non-GCB       | Yes                      |
| 34       | Left submandibular         | 62         | M   | CB    | NE   | NE   | NE   | NP            | NA                       |
| 35       | Left inguinal              | 36         | M   | CS    | -    | -    | +    | Non-GCB       | Yes                      |
| 36       | Left lateral cervical      | 53         | F   | CS    | -    | -    | +    | Non-GCB       | Yes                      |
| 37       | Right inguinal             | 65         | F   | CS    | +    | +    | -    | GCB           | Yes                      |
| 38       | Right inguinal             | 81         | F   | CS    | -    | -    | +    | Non-GCB       | Yes                      |

Abbreviations: -, negative; +, positive; BCL6, B-cell lymphoma 6; CB, cell block; CS, conventional smear; DLBCL, diffuse large B-cell lymphoma; F, woman; FNA, fine-needle aspiration; GCB, germinal center B; M, man; MUM1, multiple myeloma oncogene 1; NA, not available; NE, not effective; NP, not performed.

<sup>a</sup>Not available for immunohistochemistry algorithm.

The most recently proposed algorithm by Visco et al<sup>10</sup> based on the expression of CD10, FOXP1, GCET-1, MUM1, and BCL6<sup>10</sup> gained a 92.6% concordance with GEP. All the proposed algorithms have advantages and limitations, and none appears to dramatically improve either the DLBCL subclassification or the concordance with GEP. Moreover, in terms of predictive implications, the retrospective evaluation of patients treated with different protocols might determine different biologic behaviors and survival rates in individuals affected by the same DLBCL subtype (GCB or non-GCB).<sup>11,25</sup> The CD10-BCL6-MUM1 algorithm, as originally proposed,<sup>7</sup> may classify DLBCL into GCB and non-GCB (ABC and unclassifiable), with approximately 80% concordance with GEP.<sup>7</sup> The new algorithms suggested in the literature,<sup>10,22,25</sup> such

as the algorithm by Visco et al,<sup>10</sup> might improve the concordance with GEP significantly, but such algorithms require the use of antibodies that are not used routinely.

The use of cytological material, which is quantitatively limited by definition, necessitates a more limited number of antibodies. We chose CD10, BCL6, and MUM1, which are available and routinely used in our laboratories. Moreover, CD10, BCL6, and MUM1 were routinely tested on the corresponding histological data with the same procedure. FNA is generally used as the first diagnostic step or in the follow-up of lymph node and extra lymphoproliferative processes, and it is followed by histological examination in the majority of cases. Therefore, an accurate cytological subclassification of DLBCL might be considered to an objective that goes beyond the limits of

FNA. Nonetheless, in specific clinical situations, FNA may be the only diagnostic procedure, and the diagnosis of DLBCL with an accurate subclassification may be useful, providing additional clinical information. The main difficulties in DLBCL subclassification on FNA samples include the management of the diagnostic material, the choice of an effective and limited panel of antibodies, and the interpretation of cytological results and their reproducibility when compared with histological data.

In our experience, as well as the experience of others,<sup>15,26</sup> the management of diagnostic material is determined by ROSE. In fact, after the routine preparation of CS and cell suspension for FC, when DLBCL was considered in the differential diagnosis, additional passes and residual material were used to prepare CB and additional CS for ICC assessment. DLBCL diagnostic cells are often intermingled with other inflammatory, nonlymphomatous cells; as a result, antigen expression has to be evaluated in relation to the cytological features. Therefore, ICC, performed either on CS or CB, was appropriate for comparing the obtained results with the histological data and was the ancillary technique used for the current study. The panel of CD10, BCL6, and MUM1 was chosen because it was specific and available in both institutions, as well as in all the IHC controls, and left extra material for further diagnostic tests. In this respect, CD3, CD15, CD30, and ALK were initially tested in 4 cases to exclude T-cell NHL; HL; and anaplastic, ALK+ lymphoma, respectively, and diagnostic material was still available to include corresponding cases in the current study. When the final FNA diagnosis of DLBCL was achieved, the corresponding CB and CS were tested by the CD10-BCL6-MUM1 algorithm. As reported earlier, ICC was successful in 33 of the 38 cases and classified as positive or negative for the corresponding antibodies. ICC was NE in 5 of the 38 cases (in 4 CB due to the lack of cells and in 1 CS because of defective fixation). The same criteria were used to evaluate histological data, and a general concordance was achieved between FNA data and the 24 cases of corresponding histological data. Moreover, in the current series, CD10 and MUM1 expression were mutually exclusive, helping to distinguish between the GCB and non-GCB samples.

The results of the current study suggest that the CD10-BCL6-MUM1 ICC algorithm may be hampered mainly by insufficient cells on CB but, when performed, is effective and reproducible when compared with histological data. With regard to CB and CS efficacy as a technical

support for ICC, CS appears to be more effective than CB because of the possible scant cellularity of the latter, being ICC quality equally effective on CB and CS. The clinical value of the DLBCL classification is not accepted unanimously, and the current series was too small to have any clinical and predictive value. However, we believe it is large enough to assess the reproducibility of the algorithm when compared with the corresponding histological data.

The CD10-BCL6-MUM1 ICC algorithm is reliable for FNA specimens and equally effective on CB and CS, with cell adequacy being the main technical limitation. Therefore, ICC data are comparable to the histological data. FNA subclassification of DLBCL may provide additional prognostic information that might be useful when histological data are not available.

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#### CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

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