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THE CONTRIBUTION OF FLOW CYTOMETRY TO THE DIAGNOSIS OF AGGRESSIVE B CELL LYMPHOMA: ANALYSYS OF 97 LYMPH NODE BIOPSIES. A SINGLE CENTER EXPERIENCE

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We describe our flow-cytometric (FC) diagnostic approach to lymph node biopsies in patients (pts) with histological diagnosis of aggressive B cell lymphomas, in particular diffuse large B cell lymphoma (DLBCL) to evaluate the correlation between FC and immunohistochemistry (IHC).
Cell suspensions were prepared by mechanical disaggregation of 97 solid tissues (65 surgical resections, 19 radiologically guided biopsies and 13 endoscopic core biopsies) using the Medimachine. The cell suspensions were incubated with 8 surface markers including the main diagnostic antigens of B-cell lymphomas (KappaV450/CD45V500/CD20FITC/CD79bPE/ CD5PerCpCy5.5/CD19PECy7/CD10APC/LambdaAPCH7). Data were acquired with BDFACSCantoII (BD) and DXFlex (Beckman Coulter) cytometers.
Our FC algorithm to detect an aberrant CD19+ B cell population suggestive for DLBCL consisted in the identification of a surface immunoglobulin light chain clonality or the absence of light chains expression in combination with increased FSC-A and SSC-A physical parameters. We observed a phenotypically aberrant mature B cell cluster in 77/97 cases. A clonal B cell population was observed in 71 cases. In 6 cases we noted the absence of surface light chain on pathological B cells and four of these had an histological diagnosis of primary mediastinal large B cell lymphoma.
WBC count of the cell suspension was an important factor for the diagnostic sensitivity. The median leukocyte count was 68000/µl in diagnostic cases and 1350/µl in non-diagnostic cases respectively ($p=0.04$).
FSC-A and SSC-A median values were evaluated on pathological CD19+ B cells and CD5+ T cells. We defined FSC-ratio and SSC-ratio as the ratio between the median value of FSC-A and SSC-A in these two populations respectively. By calculating the ROC curve, the best cut-off value to differentiate DLBCL from 34 cases of different B-cell NHL (28 FL, 4 MCL, 2 MZL) were FSC-ratio >1.28 and SSC-ratio >1.44, with an AUC=0.90. This cut-off value provided a sensitivity of 76% and a specificity of 94%. The positive predictive value was 82%.
Moreover, we compared expression of markers assessed in FC with IHC results; there was a high concordance for CD20, CD10 and CD5 positivity (Fisher test $p<0.0001$).
CD5 expression, that defines a subgroup of DLBCL with an inferior prognosis, was identified in 18% of cases. Moreover, we observed that CD20 MFI value was significantly lower in CD5+ DLBCL (Mann-Whitney p 0.02).
No correlation was found between CD79b expression in FC (positive in 55/77 cases) and CD79a expression in IHC (positive in 75/77 cases).

We conclude that FC is a useful and rapid tool to identify with a high sensitivity and specificity aggressive B cell lymphomas and can support the IHC work-up in the diagnosis of DLBCL. Therapeutic targets, such as CD20 and CD79b can be detected in a quantitative approach with the perspective to identify markers not only for prediction of the pts's prognosis but eventually to guide therapeutic choices.