Abstract

Background: Diffuse large B-cell lymphoma (DLBCL) is one of the most common lymphomas identified in tissue biopsies during routine practice. Various references have propagated the perception that DLBCLs are difficult to assess by flow cytometry (FC) given their large cell size, fragility, and frequent association with necrosis/debris, sclerosis, and increased mitotic activity. Notably, however, there is essentially no data to substantiate this. Failure to identify an abnormal large B-cell population by FC may increase turnaround time and possibly misdirect subsequent immunohistochemical evaluation. Anecdotally, we have observed high rates of DLBCL detection by FC in tissue specimens and therefore chose to systematically study this observation.

Design: We retrospectively analyzed in a blinded fashion 4-color FC data from tissue specimens containing DLBCL. The following antibodies were assessed routinely: CD5, CD10, CD45, CD20, CD3, CD8, PD1, CD27, and FCappa and/or FClambda. Reactive, polyclonal lymphoid tissue specimens served as (-) controls. Bone marrow and fluid specimens were excluded. Lymphoma populations were identified using cluster analysis and defined as FC(+) based on immunophenotypic aberrancy and/or light chain restriction. FC cytospin morphology was reviewed in select cases.

Results: We collected 68 DLBCLs (35 females and 33 males), 22-92 y/o (median 62) and 11 (-) controls (7 females and 4 males), 18-88 y/o (median 54). The DLBCL specimens included 22 soft tissue (ST), 19 lymph nodes (LN), 6 bone, 4 each of mediastinal, lung, and sinonasal, 3 retroperitoneal, 5 brain, and 1 each of skin, bowel, and spleen; the (−) controls consisted of 8 LNs and 2 tonsils. FC(+) was observed in 53/68 (78%) of DLBCLs with (±) FC results, with tumor cells accounting for 0.03-75% (median 9%) of events [Figure 1A-E].

Conclusions: Abnormal B-cell populations were identified in 78% of DLBCL tissue specimens by FC, highlighting its use as an important diagnostic study in aggressive B-cell lymphomas. There was no statistically significant differences between detection rates in different tissue sites, although sample size in individual tissue sites was small. Interestingly, the majority of FC(−) DLBCL cases had morphologic evidence of lymphoma in the processed FC specimens; the cause of the (−) analyses in these cases is unclear.

Most Diffuse Large B-cell Lymphomas are Identified by Flow Cytometry

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Introduction

Diffuse large B-cell lymphoma (DLBCL) accounts for 25-30% of non-Hodgkin lymphomas and is therefore one of the most common lymphoma diagnoses rendered on patients in Western countries. Flow cytometry (FC) is an ancillary tool in DLBCL diagnosis and also serves to categorize BCL-based lymphomas into subgroups such as CD5(+) DLBCL and germinal center-like DLBCL.

Several references cite difficulties in flow cytometric analysis of DLBCLs, however, noting negative findings in up to 25% of analyzed cases. Though several theories for this are proposed, including the large size and/or fragility of DLBCL cells, factors responsible for abnormal light scatter properties, frequent associations with necrosis and/or sclerosis, and the high mitotic rate, there is virtually no data in the literature supporting these theories. Additionally, several references identify a subset of DLBCLs which do not show light chain restriction, further complicating flow cytometric analysis of this tumor type. As we have anecdotally observed high rates of lymphoma detection in tissue sections with FC, we chose to systematically study this observation.

Materials and Methods

- Study specimens were identified using the following inclusion criteria: DLBCL diagnosis according to 2008 WHO criteria, available FC data, and tissue biopsy sites. Bone marrow and peripheral blood analyses were excluded. H&E-stained tissue sections were reviewed in all controls. Controls were defined as reactive lymphoid tissue based on FC and morphologic assessment.

- 4-color FC was performed, assessing the following antigens: CD5, CD20, CD10, CD45, CD22, CD8, PD1, and FCappa and/or FClambda.

- Monoclonal and polyclonal anti-kappa and lambda antibodies were used in the majority of cases.

- FC data was analyzed in a blinded fashion.

- B cell populations were identified using cluster analysis. Clonal (lymphoma) populations were identified as aberrant and/or light chain restricted populations, while reactive populations were identified with polytypic light chain expression.

- Wright Giemsa-stained FC cytospin preparations were reviewed in select cases when available.