Brief Communication

The Utility of Multiparametric Seven-Color Flow Cytometry in the Detection of Double Hit Lymphoma in Ascitic Fluid Samples

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Double-hit lymphoma (DHL) is a rare type of lymphoma with concurrent chromosomal translocations of C-MYC with BCL2 or BCL6, associated with unfavorable prognosis. We describe a case of DHL in a 79-year-old female patient previously diagnosed with diffuse large B-cell lymphoma (DLBCL) with an early relapse in the ascitic fluid. A seven-color multiparametric flow cytometry immunophenotyping study of the ascitic fluid was carried out, and revealed 99.78% of large in size and high cellular complexity B-cells positive for CD19, CD10 (64.27%), CD45 dim, CD22 dim, CD25 (60%), CD43 bright, CD38 bright, and IgM (18.53%); and negative for CD20, CD5, CD23, CD79b, CD103, CD200, CD11c, and FMC7, and 78.99% without light chain expression and 21% with Lambda chain restriction. Due to the expression of CD19 and CD10 with overexpression of BCL-2 protein and due to CD43 and CD38 positivity detected, those cells showed features between DLBCL and Burkitt lymphoma. Fluorescence in situ hybridization (FISH) confirmed both c-MYC/IGH and BCL2/IGH rearrangement. Our findings may help to identify cases requiring additional cytogenetic analysis.

Key terms: double-hit; lymphoma; flow cytometry

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INTRODUCTION

Double-hit lymphoma (DHL) is a rare type of lymphoma with concurrent chromosomal translocations of C-MYC with BCL2 or BCL6. DHL has been associated with unfavorable prognosis (1). We describe a case of DHL in a patient previously diagnosed with diffuse large B-cell lymphoma (DLBCL). The patient, a 79-year-old female, was initially treated with R-CHOP chemoimmunotherapy (rituximab, cyclophosphamide, doxorubicin, prednisone), achieving a partial response after the third and sixth course assessments. A new lymph-node biopsy was performed, and 1 week later she presented to the hospital because of abdominal pain and ascites. An ultrasound-guided paracentesis was performed to obtain an ascitic fluid sample for diagnosis.

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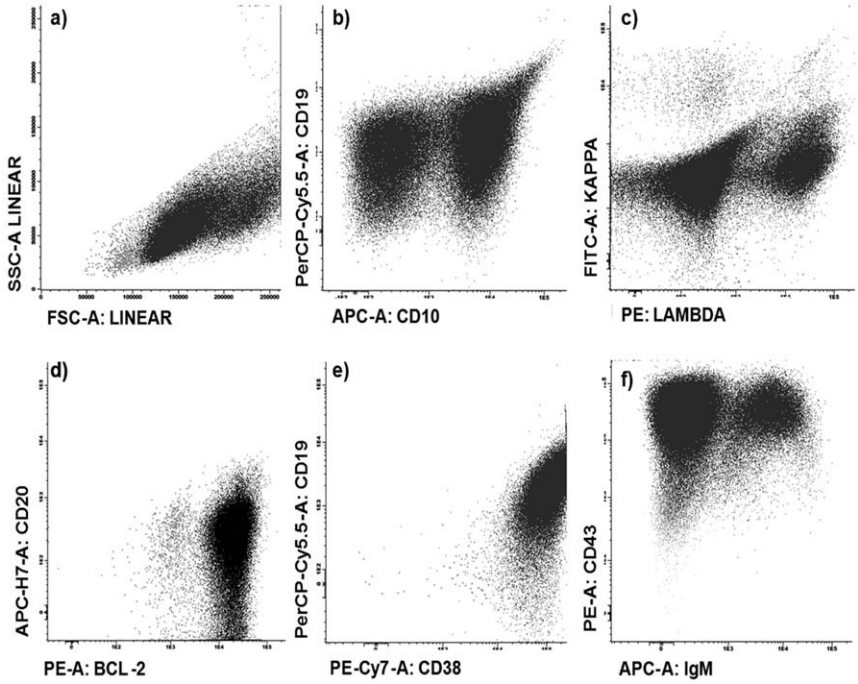

FIG. 1. Flow cytometric immunophenotype of neoplastic cells from ascitic fluid (99.78% of infiltration). The pathological cells show **a)** large size and cellular complexity, **b)** CD19 positive with partial expression of CD10, **c)** negative light chain expression with a 21% of Lambda chain restriction with **d)** overexpression of BCL-2 intracellular protein, **e)** high expression of CD38, and **f)** CD43 positivity with partial expression of IgM.

METHODS

A seven-color flow cytometric immunophenotyping study of the ascitic fluid was carried out, including Kappa/Lambda light chains in the 7-color combination. We did not study the phenotype of lymph node B cells at the time the ascitic fluid specimen was analyzed due to the difficulty in accessing the retroperitoneal lymph nodes. The cells were run on a 3-laser FACSCanto II analyzer (BD Biosciences, San Jose, CA) with FACSDiva software (BD Biosciences, San Jose, CA). >100,000 events were acquired. The cells were analyzed using Infinicyt software (Cytognos SL, Salamanca, Spain).

RESULTS

The immunophenotyping of the ascitic fluid revealed that 99.78% of B-cells were positive for CD19, CD10 (64.27%), CD45 dim, CD22 dim, CD25 (60%), CD43 bright, CD38 bright, and IgM (18.53%); and negative for CD20, CD5, CD23, CD79b, CD103, CD200, CD11c, and FMC7. The intracellular protein BCL-2 was clearly overexpressed. The study of Kappa and Lambda light chains was also evaluated in the B population, showing 78.99% without light chain expression and 21% with Lambda chain restriction (Fig. 1). The cells were large in size and with high cellular complexity. Due to the expression of CD19 and CD10 with overexpression of BCL-2 protein and due to CD43 and CD38 positivity detected, those cells showed features between DLBCL and Burkitt lymphoma. These results suggested the possibility of DHL with both C-MYC and BCL-2 translocation. Fluorescence in situ hybridization (FISH) confirmed both c-MYC/IGH and BCL2/IGH rearrangement.

DISCUSSION

In cases of suspected high-grade lymphoma, multicolor flow cytometry could be useful in the detection of DHL in patients exhibiting an immunophenotype with features intermediate between follicular lymphoma, diffuse large B-cell lymphoma, and Burkitt lymphoma, suggesting that further cytogenetic analysis of C-MYC and BCL-2 or BLC-6 translocations would be beneficial. DHL and triple-hit lymphomas could arise from follicular and DLBCL following the acquisition of MYC rearrangement in the molecular pathologic evolution of the disease (2). Seven-color flow cytometric immunophenotyping could be more accurate than four-color studies for DHL diagnosis (3). Controversy has arisen regarding the utility of flow cytometry for the diagnosis of DHL. Previous reports suggest that decreased CD20 and/or CD19 expression by flow cytometry is relatively common in DHL and may help to identify cases requiring additional cytogenetic analysis. However, only CD19 dim and CD20 coexpression are present in a minority of cases of DHL (3). Our findings support recent data suggesting that mature B-cell lymphomas with c-*MYC* translocations may have a common immunophenotype, meaning there might be a common immunophenotype also of DHL (4,5). Our data are novel because of the accuracy of the multiparametric immunophenotyping obtained with a seven-color screening panel; our findings may help to identify cases requiring additional cytogenetic analysis. In conclusion, flow cytometry could be

useful in the integrated analysis of histopathology and genetics to find the final diagnosis of DHL because it might identify these cases for cytogenetic analysis as this testing might not otherwise be routinely performed.

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