Cytometry Part B (Clinical Cytometry) 51B:41–44 (2003)

Case Report:

Guess What: Chronic 13q14.3$^+$/CD5$^-$/CD23$^+$ Lymphocytic Leukemia in Blood and t(11;14)(q13;q32)$^+$/CD5$^+$/CD23$^-$ Mantle Cell Lymphoma in Lymph Nodes!

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We report a case of a patient with two B-cell lymphoproliferative disorders: CD5$^-$/CD23$^+$ B-cell chronic lymphocytic leukemia and CD5$^+$/CD23$^-$ mantle cell lymphoma. These disorders were diagnosed simultaneously based on flow cytometry, immunohistochemistry, fluorescence in situ hybridization, and polymerase chain reaction–based molecular studies. The B-cell lymphocytic leukemia clone predominated in the blood and bone marrow, whereas the mantle cell clone predominated in lymph nodes. Cytometry Part B (Clin. Cytometry) 51B:41–44, 2003. © 2002 Wiley-Liss, Inc.

Key terms: leukemia; lymphoma; flow cytometry; fluorescence in situ hybridization; B cells; biclonal; mantle cell; chronic lymphocytic leukemia; t(11;14); 13q

Over recent years, the recognition that lymphoproliferative disorders (LPDs) comprise distinct clinicopathologic entities defined by a combination of morphologic, immunophenotypic, and genetic features and the availability of specific therapies for certain of these tumors make accurate diagnosis imperative. In most cases, detailed immunophenotypic analysis of the leukemia/lymphoma cells contributes to a specific classification. In fact, the pattern of reactivity for CD20, CD23, CD79b, FMC7, surface immunoglobulins (Igs), and adhesion molecules proved to be of great help in distinguish between small lymphocytic lymphoma/chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL) (1–5). Moreover, techniques based on polymerase chain reaction (PCR) and fluorescence in situ hybridization (FISH) to detect specific molecular and chromosomal abnormalities (6,7) and the use of antibodies against novel fusion proteins (8,9) greatly improved the ability to detect specific genetic abnormalities. For instance, the chromosomal translocation (11;14)(q13;q32) that fuses the IGH and CCND1 genes and leads to cyclin D1 overexpression has been identified as the genetic hallmark of MCL (10). Although an equivalent genetic marker has not been found for B-CLL, numerical or structural chromosomal abnormalities are detected by conventional cytogenetics or FISH analysis in 50% of B-CLL cases, the most common changes being the trisomy 12 and the structural abnormalities of 13q, 11q, 6q, 14q, and 17p (11,12). Karyotypic analysis and FISH- and PCR-based molecular studies may be helpful in clarifying the diagnosis especially when the B-cell LPD is difficult to classify based on clinical, morphologic, and phenotypic grounds such as in establishing the differential diagnosis between an MCL and a morphologically atypical B-CLL (13–15) and in identifying B-CLL forms with specific chromosomal abnormalities with prognostic significance (11,16)

We report a case of a patient in whom a CD5$^-$/CD23$^+$ B-CLL and CD5$^+$/CD23$^-$ MCL were diagnosed simultaneously based on flow cytometry and FISH- and PCR-based molecular studies. Aside from the rarity of a true CD5$^-$/CD23$^+$ B-CLL, to the best of our knowledge, this is the

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Received 8 May 2002; Accepted 29 August 2002

Published online in Wiley InterScience (www.interscience.wiley.com).

DOI: 10.1002/cyto.b.10005

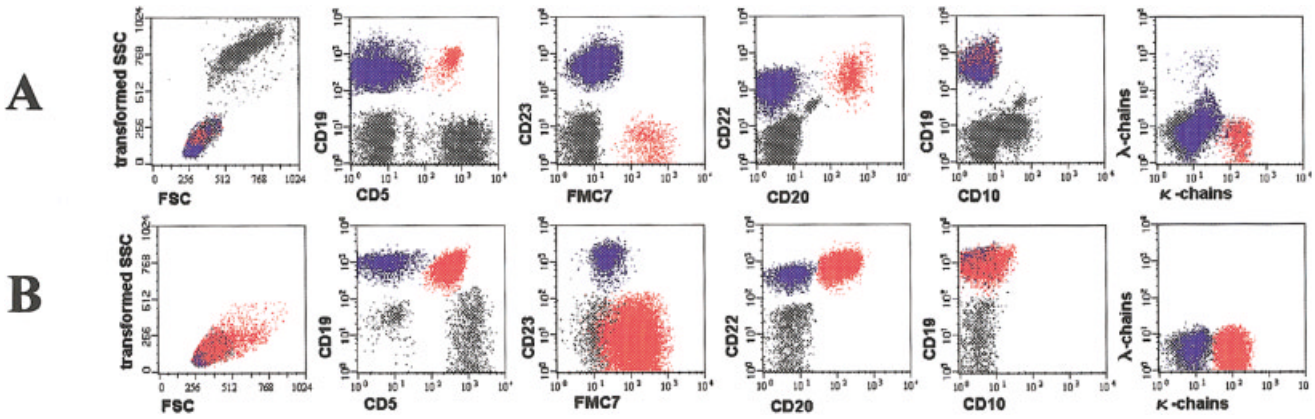

FIG. 1. Dot plots showing the main immunophenotyping characteristics of peripheral blood (**A**) and lymph node (**B**) B cells. $CD19^+/CD5^-$ B lymphocytes are represented as blue dots and $CD19^+/CD5^+$ B lymphocytes are represented as red dots. Gray dots represent $CD19^-$ white blood cells. Flow cytometric studies were performed by using a method of staining and washing lyses (FACS lysing solution, Becton Dickinson, San Jose, CA) and four-color staining that combined allophycocyanin-conjugated anti-CD19 and phycoerythrin–cyanine 5 conjugated anti-CD5 with a large panel of other phycoerythrin and fluorescein isothiocyanate conjugated monoclonal antibodies directed against other markers combined as follows: FMC7/CD23, CD20/CD22, CD10/CD38, CD103/CD25, CD21/CD79b, CD11a/HLA-DR, κ/λ chains. Other specific combinations were used whenever necessary to better discriminate the B-cell populations. Data acquisition was carried out in a FACScalibur flow cytometer equipped with a 488-nm argon laser and a 635-nm red-diode laser (Becton Dickinson). For data analysis, Paint-a-Gate PRO software (Becton Dickinson) was used. FSC, forward scatter; SSC, side scatter.

first report documenting the simultaneous diagnosis of a B-CLL and an MCL.

CASE REPORT

An 86-year-old man was evaluated at the Hematology Department of the Hospital Geral de Santo António (Porto, Portugal) for persistent, absolute lymphocytosis and adrenomegalies. White blood cell count was 16.4×10^9/liter (neutrophils, 22%; lymphocytes, 74%), hemoglobin was 9.1 g/dl, and platelet count was 201×10^9/liter. The physical examination revealed multiple cervical, axillary, and inguinal adrenomegalies and hepatomegaly.

The peripheral blood (PB) smear showed a marked predominance of small lymphocytes with clumped chromatin and scarce cytoplasm and a small population (<15%) of large lymphoid cells with less condensed chromatin, frequently with a visible nucleoli and a more abundant, sometimes basophilic, cytoplasm. Gumprecht's shadows also were present. Flow cytometry–based immunophenotyping of PB lymphocytes revealed two populations of phenotypically abnormal B cells (Fig. 1A). The first one, comprising 90% of B cells, consisted of $CD19^+$, $CD22^{+low}$, $CD23^{+high}$, $CD25^{+low}$, $FMC7^-$, $CD5^-$, $CD10^-$, $CD11a^-$, $CD11c^-$, $CD20^-$, $CD38^-$, $CD79b^-$, $CD103^-$, and surface and cytoplasmic Ig^-. The second population, comprising only 10% of B cells, consisted of $CD19^+$, $CD20^{+high}$, $CD22^{+high}$, $CD25^{+low}$, $CD38^{+low}$, $CD79b^+$, $FMC7^+$, $CD5^+$, $CD10^-$, $CD11a^+$, $CD11c^-$, $CD23^-$, $CD103^-$, and surface Ig^{+high} (IgM, κ). A few (<1%) residual polyclonal B cells were also present. FISH analysis showed the (11;14) translocation in only 9% of PB lymphocytes (Fig. 2A) and the 13q14.3 deletion in another 21% (Fig. 2B); trisomy 12 and the (14;18) translocation were not detected (data not shown).

On bone marrow smears, lymphocytes comprised 60% of the total cells counted, and immunophenotyping studies revealed that most (59%) were B cells consisting of $CD19^+/CD5^-/CD23^+$/surface Ig^- (73%) and $CD19^+/CD5^+/CD23^-/\kappa$ chains$^+$ (27%), mature B lymphocytes, and a few residual $CD19^+/CD20^-/CD38^{+high}/CD10^+$/surface Ig^- B-cell precursors (<1%). The bone marrow trephine biopsy showed a mixed nodular and interstitial infiltration pattern consisting mainly of $CD20^-/CD5^-/CD23^+$ small lymphocytes.

Flow cytometry of lymph node (LN) lymphocytes showed that most (90%) had a $CD5^+/CD23^-/\kappa^+$ immunophenotype, whereas $CD5^-/CD23^{+high}/Ig^-$ B cells comprised only 10% of LN lymphocytes (Fig. 1B). FISH analysis showed the t(11;14)(q13;q32) in 94% of LN cells (Fig. 2C). The LN histology was consistent with MCL, and immunohistochemical studies confirmed that most B-lymphoma cells expressed CD20 and CD5 and were negative for CD23; a few $CD23^+$ B lymphocytes also were observed. PCR-based IgH rearrangement molecular studies confirmed that the B-cell clone found in the LN was different from the one that predominated in blood (Fig. 3).

DISCUSSION

An increasing number of cases of biclonal B-cell LPD have been reported in the past few years, likely a consequence of a more accurate laboratorian approach to diagnosis (17–20); in most of these cases, two different cell populations were detected by flow cytometry, and, unless they express different Ig light chains, additional studies are usually necessary to distinguish a true biclonal disease from phenotypic heterogeneity due to intraclonal diversity. Only a few cases in which two distinct B-cell popu-

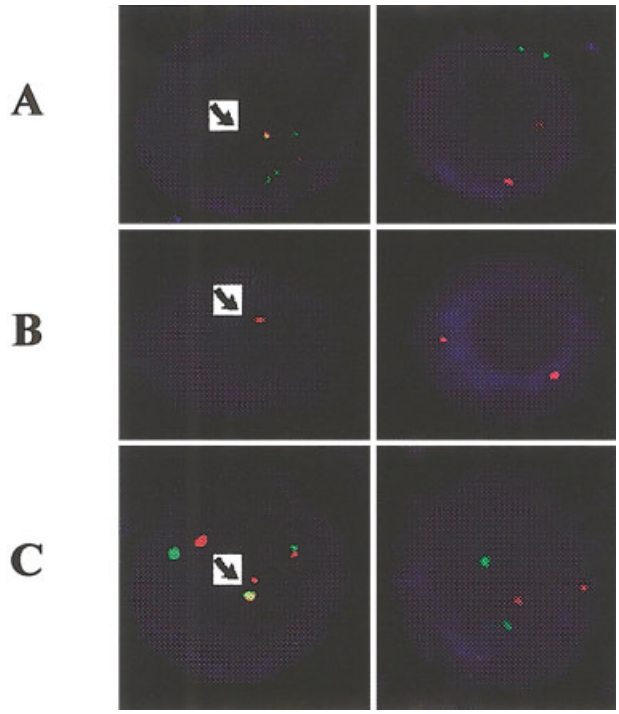

FIG. 2. Fluorescence in situ hybridization (FISH) images showing the t(11;14) translocation (**A**) and the 3q14.3 deletion (**B**) that were found in 9% and 24% of blood cells, respectively. **C** shows the t(11;14) translocation in most (94%) lymph node cells. The t(11;14) translocation is detected by the presence of a yellow FISH spot in the cells at the left parts of A and C, in addition to the normal red and green FISH spots observed in other cells from the same sample (right parts of A and C). The 3q14.3 deletion is demonstrated by the presence of one red FISH spot in the cell at the left part of B, in opposition to the two normal red FISH spots in other cells from the same sample (right part of B). Fluorescence hybridization studies were performed with the following DNA probes: IGH-specific BAC (covering the JH and first constant regions) and CCND1-specific probes to detect the IGH-CCND1 fusion gene associated with the t(11;14)(11q13;14q32) translocation (LSI IGH/CCND1); a D13S319 locus-specific probe to detect del(13q14.3) structural abnormalities (LSI D13S319).

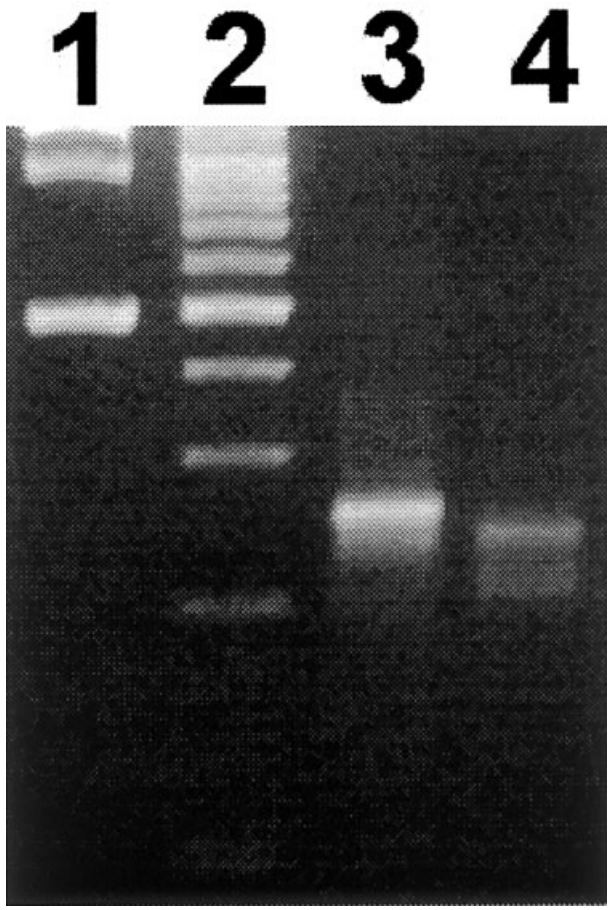

FIG. 3. Molecular identification of immunoglobulin H rearrangement clonality in samples of peripheral blood (**lane 3**) and lymph node (**lane 4**). **Lanes 1 and 2** show molecular weight markers (500-bp ladder in lane 1 and 100-bp ladder in lane 2). The lymph node was found to harbor a B-cell clone with a molecular weight different from that predominating in the peripheral blood. Molecular studies were performed with the B-Lymphoma Molecular Diagnostic Kit from MD-Master Diagnostica (Granada, Spain) as recommended by the manufacturer. Peripheral blood DNA was extracted by using a semi-automated procedure with the Nuclisens Extractor (Organon Technika Corporation, Durham, North Carolina); lymph node DNA was extracted with the Extracell Kit (Amplimedica spa-Bioline, Torino, Italy).

lations have been identified fulfill the criteria for diagnosis of two different entities (21).

The rare biclonal B-cell LPD described here requires further discussion concerning two points: the criteria for diagnosis of a $CD5^-$ B-CLL and its association with a $CD5^+$ MCL.

Although expression of the CD5 antigen by neoplastic cells is considered an important criterion for diagnosis of B-CLL, published series have frequently included a number of "$CD5^-$ B-CLL" cases. A careful review of cases that had been classified as "$CD5^-$ B-CLL" showed that most had other unusual immunophenotypic features, including bright-surface Ig expression, bright CD20 expression, and absence of CD23 expression, and displayed an adhesion molecule profile resembling Non Hodgkin Lymphoma (NHL), suggesting that the majority of them represented NHL in the leukemic phase rather than $CD5^-$ B-CLL (22,23). Nonetheless, it is usually accepted that $CD5^-$ B-CLL, although rare, does exist. We are convinced that the present case is one of the very rare cases of $CD5^-$ B-CLL because it met all the immunophenotypic criteria for the diagnosis of B-CLL except the expression of CD5 and the arbor 13q14.3 deletion, a structural abnormality commonly observed in B-CLL; moreover, the lymphocyte morphology was that usually observed in typical $CD5^+$ B-CLL and the possibility of a $CD10^-$ follicular cell lymphoma was ruled out by immunophenotypic and FISH studies.

Concerning the characterization of the $CD5^+$ B-cell population that accounted for only a minority of PB lymphocytes and predominated in LN, its characteristic $CD5^+$, $CD23^-$, $FMC7^+$, $CD20^{+high}$, $CD22^{+high}$, surface Ig^{+high} immunophenotype strongly pointed to the diagnosis of an MCL and made improbable the diagnosis of a B-CLL, a $CD5^+$ B-cell LPD that typically displays a $CD23^+$, $FMC7^-$, $CD20^{-/+low}$, $CD22^{-/+low}$, and surface $Ig^{-/+low}$

phenotype (4,24–26). FISH studies confirmed our suspicion by revealing the presence of the typical chromosomal abnormality t(11;14)(q13;q32).

To the best of our knowledge, this is the first case in which a rare form of 13q14.3^{+}/CD5^{-} B-CLL was associated with a typical t(11;14)(q13;q32)$^{+}$/CD5^{+} MCL, with each LPD predominating in different compartments (blood/bone marrow and lymph nodes).

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