Received: 3 August 2020 | Revised: 18 November 2020 | Accepted: 1 December 2020

DOI: 10.1002/cyto.b.21980

LETTER TO THE EDITOR-CASE REPORT

CLINICAL CYTOMETRY WILEY

A rare biclonal Hairy Cell Leukemia disclosed by an integrated diagnostic approach: A case report

Hairy Cell Leukemia (HCL) is a rare indolent disease affecting mature B lymphocytes. While general symptoms are rather nonspecific (splenomegaly, fatigue, pancytopenia), HCL cells show a characteristic morphologic, immunophenotypic, and mutational profile that features prominently in the diagnostic process. Malignant cells have oval nuclei, abundant cytoplasm, and typical cytoplasmatic projections. Flow cytometric immunophenotyping includes key antigens such as CD11c, CD25, CD103, and CD200, which are strongly expressed, in addition to the ordinary panel used for the detection of B cell lymphoproliferative diseases (LPD). Moreover, a recurrent V600E BRAF mutation has been recently uncovered in HCL but not in its mimickers and is now considered the molecular hallmark of the disease (Cross & Dearden, 2020).

Usually, LPD present one single clonal population, but bi- or multiclonal cases, where two or more distinct aberrant clones coexist in a single anatomic site, are well known and described. Multicolor flow cytometry (MFC) plays a key role in the detection of these cases for being both highly sensitive and specific (Mahdi et al., 2018). Although the association of HCL with other non-Hodgkin's lymphoma is a known phenomenon, among the so-called composite lymphoma, pure biclonal HCL is to be considered an exceptional finding.

We report a case of biclonal HCL found by MFC in a 80 y.o. male patient referred to our clinic because of new onset of peripheral blood leucopenia and thrombocytopenia. A full blood count showed WBC 2310/μl, with 800 neutrophils/μl. Hb was 13.8, and platelets 73,000/μl. Past medical history showed an ischemic stroke and a metastatic squamous cell carcinoma (SCC) of unknown primary origin, for which he underwent several surgical treatments but no systemic therapy. A routine CT scan of the thorax and a neck, and abdomen US performed for SCC follow-up showed multiple lung lesions and enlarged laterocervical and mediastinal lymph nodes, all interpreted as metastatic SCC sites, as well as a 15.5 cm spleen. The cytopenias and splenomegaly were interpreted as possible signs of myelophthisis or myelodysplastic syndrome. Therefore, a peripheral blood smear was performed, and a second sample was sent to the Flow Cytometry Laboratory. This sample was marked with a two-tube eight-color panel initially addressing monocyte and neutrophil maturation and myeloid blast count, in addition to a limited lymphocyte subpopulation analysis for total T, B, and NK subsets. The immunophenotypic evaluation was done using FACSDiva software on an eight-color FACSCanto II Flow Cytometer (BD Biosciences). The analysis confirmed neutropenia (40%) and an inversion of the leukocyte formula. A subset of CD20+ B lymphocytes with high Side Scatter (SSC), accounting for 15% of all lymphocytes, was noted. These cells overlapped monocytes in the CD45/SSC dot-plot suggesting an increased cellular complexity, as typically observed in HCL (Figure 1). To investigate this hypothesis, B lymphocytes were further analyzed using an eight-color panel including kappa FITC, lambda PE, CD5 PerCP-Cy5.5, CD19 PE-Cy7, CD11c APC, CD38 APC-H7, CD20 V450, CD45 V500 monoclonal antibodies, directed to identify a possible CD11c+ clonal subpopulation, coupled with a second tube including antibodies against the HCL-associated CD25 and CD103 markers labeled with FITC and PE respectively. Then CD10, CD22, CD200 and FMC7 were also tested (all purchased from BD Biosciences).

CD19+/SSC high-gated B lymphocytes turned strongly positive for CD19 and CD20 as well as for CD11c, CD25, CD103, CD22, CD200, and FMC7, while CD5, CD10, and CD38 were not detected. These results, consistent with HCL, were also supported by the blood smear analysis that identified a subset of lymphocytes with atypical protrusions. Unexpectedly, the ratio between the Ig Kappa and Lambda light chains was within the normal range (1.8) (Figure 1 panel (a)). However, due to the immunologic profile and the peculiar SSC values, a possible biclonal HCL with one kappa- and one lambda-restricted subclones was strongly suspected.

In order to provide molecular evidence supporting our unusual MFC finding, DNA was extracted from peripheral blood leukocytes of the patient and subjected to multiplex polymerase chain reaction (PCR) for size discrimination of immunoglobulin heavy chain (IgH) and IgK light chain gene rearrangement with the IdentiClone™ IgH + IgK B-cell Clonality Assay-ABI Fluorescence Detection kit (InVivoScribe Technologies) according to the Biomed-2 assay protocol. The results were evaluated with the aid of GeneMapper™ Software (Applied Biosystems™). Two prominent peaks were generated by the rearrangement of both IgH (FR3-JH) and IgK (V-Kde and JC intron-Kde) genes, confirming the clonal origin of the "hairy" population (Figure 1 panels (b,c)). Moreover, the BRAF V600E gene mutation was demonstrated by Allele Specific PCR (AS-PCR) and confirmed by direct sequencing (Figure 1 panels (d,e)) and a diagnosis of HCL was finally rendered. Subsequently, an extended bone marrow localization was proved by histologic evaluation.

The possible HCL origin of the multiple adenopathies was ruled out by molecular analysis of biopic material of an axillary lymph node, which was negative for BRAF V600E and consistent with SCC involvement. In reason of the comorbidity described above and because cytopenias were not severe, we elected the patient for a watch and wait approach.

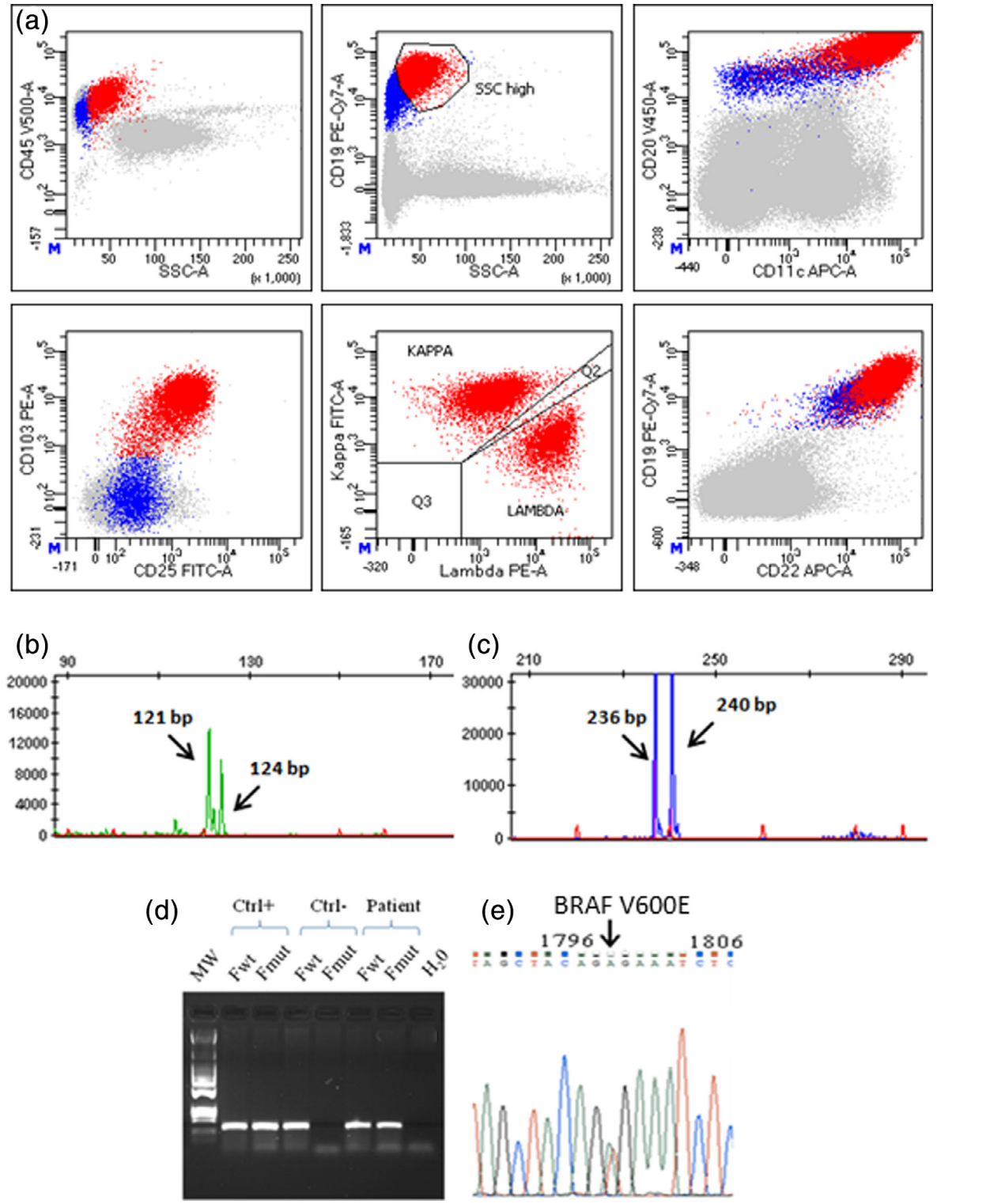

FIGURE 1 Flow cytometric Immunophenotyping, B-cell clonality analysis and BRAF V600E mutation detection. (a) Highly scattered lymphoid cells (colored red) are strongly stained with the B-lineage CD19, CD20, CD22 antibodies. Unlike normal B lymphocytes (colored blue), they also show bright expression of CD11c and are positive for CD103 and CD25. The kappa/lambda ratio is within the normal range (1.8). (b,c): IgH (FR3-JH) gene rearrangement (HEX dye) and IgK (IgKV/intron-Kde) gene rearrangement (6FAM dye), respectively. Two peaks, indicated by arrows, are detected with each set of primers. (d) AS-PCR results (agarose gel). Lane 1: Molecular weight marker. Lanes 2 and 3: Heterozygous V600E positive control (JVE-109 cell line) displaying amplification of both mutated (Fmut) and wild type (Fwt) alleles. Lanes 4 and 5: Negative control (HNT 34 cell line) showing amplification only with the wt primer. Lanes 6 and 7: Patient sample; the amplification of both fragments indicates the presence of the V600E substitution. Lane 8: No template PCR control. (e) The graph shows the direct sequencing result of BRAF exon 15 T to A transversion at position bp 1799 (arrowheads) present in the patient's cells [Color figure can be viewed at wileyonlinelibrary.com]

Our case highlights the fundamental role of MFC and molecular biology in discovering a rare biclonal HCL. MFC is the most available method for detecting bi- or multiclonal LPD (Mahdi et al., 2018), and an atypical immunologic profile, even in the presence of a normal kappa/lambda ratio, is highly suggestive of malignant proliferation and should drive further investigations.

Biclonality is a rare event among B-LPD; it presents either in the form of composite lymphoma or as an expansion of two or multiple concurrent subclones in a process of intraclonal evolution within the same disease, as frequently reported in B-CLL (Mahdi et al., 2018). Overall, HCL has been seldom detected in the setting of composite lymphoma and, to our knowledge, pure HCL with two simultaneous subclones is an extremely rare finding. The only report about a biclonal HCL, dated 1986, was based on Ig gene rearrangement studies. In this short article, Raghavachar et al. described two coexisting kappa and lambda clones, one of which was resistant to IFN-α therapy. In our cohort of 1090 newly diagnosed B-LPD detected in a 10-year period, including 26 HCL (2.4%), bi- or multiple clonality was found in 4.7% of all cases. Only one HCL turned out biclonal.

As part of an integrated diagnostic pathway, molecular confirmation through size discrimination or nucleotide sequencing of IgH and IgK gene rearrangements is generally recommended to support MFC finding of bi- or multiple clonal B-NHL, especially when Ig light chain restriction is not directly demonstrated by MFC. In our patient, the IgH gene rearrangement analysis detected two predominant peaks, most likely consistent with biclonality. Similarly, IgK amplification generated two signals (specifically IgK V/Intron-Kde). Since this recombination occurs following a non-functional rearrangement of an IgK allele, we could not univocally demonstrate whether the two detected IgK peaks belonged to different subclones (one expressing kappa and the other one lambda light chains) or we were just catching the two nonfunctional IgK alleles of the lambda subclone. Nonetheless, the whole Ig gene molecular findings together with the flow cytometric immunophenotypic profile strongly supported the biclonal nature of the "hairy" B-cell population in spite of an apparent normal kappa/lambda ratio.

The finding of BRAF mutation served as the last piece of such an intriguing diagnostic puzzle. Notably, BRAF inhibitors, used in V600E positive metastatic melanoma, are a new potential approach for HCL patients who experienced refractory or relapsed disease (Cross & Dearden, 2020) and may become a potential therapy option if the conditions of our HCL patient would not be suitable for standard chemotherapy regimens. From a biological point of view, our findings also raise the question as to whether the BRAF mutation arose before the VDJ specification in a pro-B lymphocyte, or two separate clones acquired the same mutation in an example of convergent evolution; further studies are needed to address this issue.

In conclusion, we highlight the value of MFC in detecting an asymptomatic HCL in a clinical setting complicated by comorbidities and in dissecting two different subclones in spite of a balanced kappa/lambda ratio. An equally relevant contribution to the final diagnosis was provided by the molecular demonstration of biclonality and the finding of the specific HCL genetic signature. These data reinforce the notion that an integrated approach is an added value to the diagnostic pathway of B-LPD, allowing a thoroughly accurate response to the diagnostic framing.

Laura Vittoria[1]
Fabio Bozzi[1]
Iolanda Capone[1]
Cristiana Carniti[2]
Daniele Lorenzini[1]
Morena Gobbo[1]
Niccolò Bolli[2,3]
Antonella Aiello[1]

[1]*Department of Pathology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy*
[2]*Department of Medical Oncology and Hematology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy*
[3]*Department of Oncology and Onco-Hematology, University of Milan, Milan, Italy*

Correspondence
Laura Vittoria,
Email: laura.vittoria@istitutotumori.mi.it

ORCID

Laura Vittoria https://orcid.org/0000-0002-6220-7379

REFERENCES

Cross, M., & Dearden, C. (2020). Hairy cell leukaemia. *Current Oncology Reports*, *22*, 1–14.

Mahdi, T., Rajab, A., Padmore, R., & Porwit, A. (2018). Characteristics of lymphoproliferative disorders with more than one aberrant cell population as detected by 10-color flow cytometry. *Cytometry. Part B, Clinical Cytometry*, *94*(2), 230–238.