Accurate Flow Cytometric Gating of the Large Lymphocyte Region Is a Powerful Screening Method for Detecting Hairy Cell Leukemia Presenting with a Low Tumor Burden

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Abstract

Hairy cell leukemia typically presents with pancytopenia and often mimics aplastic anemia. Making an accurate diagnosis is crucial, as treatment with the purine analogues cladribine and pentostatin brings about durable complete remission in the majority of patients. Surface kappa and lambda flow cytometric analyses of peripheral blood or bone marrow are a powerful screening tool, although routine gating of the entire lymphocyte region may fail to show light chain restriction due to a low tumor burden. We herein demonstrate that accurate subgating of the large lymphocyte region is essential and recommend the application of this method in all cases of pancytopenia of unknown etiology.

Key words: hairy cell leukemia, aplastic anemia, pancytopenia, flow cytometry, large lymphocyte gate

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Introduction

Hairy cell leukemia (HCL) is a disease that is diagnosable only when suspected, as many patients present with a limited number of circulating neoplastic cells and the predominant manifestation is usually pancytopenia (1). We herein show that flow cytometric gating of the large lymphocyte region is especially important when screening for HCL and strongly recommend that all patients with pancytopenia of unknown etiology be tested.

Case Report

A 62-year-old man was referred to our hospital due to pancytopenia found on a routine health check. The patient’s white blood cell (WBC) count, hemoglobin level and platelet count were 1.8×10⁹/L, 11.9 g/dL and 61×10⁹/L, respectively. The WBC differential showed profound neutropenia, with 5.0% bands, 11.0% neutrophils, 82.5% lymphocytes, 0.5% monocytes and 1.0% eosinophils. Peripheral blood (PB) flow cytometry (FCM) revealed no light chain restriction when analyzed on the bulk of the lymphocyte gate (Figure A); however, subgating of the large lymphocyte region constituting a mere 1.9% of the entire sample disclosed clear lambda restriction (Figure B). A further FCM analysis revealed these cells to be positive for CD11c (Figure B), CD19, CD20, CD22, CD25 and CD103 (Figure B) and negative for CD3, CD4, CD5, CD8, CD10, CD23, CD38 and CD56. A PB morphological analysis of an air-dried smear specimen demonstrated a low number of abnormal lymphocytes with villous cytoplasmic projections (Figure C). Aspiration and a biopsy showed hypocellular bone marrow (BM) resembling that of aplastic anemia (Figure D), and immunohistochemistry of the BM biopsy specimen revealed invasion of CD20-positive tumor cells (Figure E, F). Meanwhile, BM FCM showed approximately 9% tumor cells, and a BM Southern blot analysis revealed IgH-JH rearrangement, with the BRAFV600E mutation confirmed on an Ion AmpliSeq™ Cancer Panel (Life Technologies, Carlsbad, USA) analysis. HCL was thus diagnosed. Treatment with cladribine was administered at a dose of 0.1 mg/
kg/day as continuous intravenous infusion for seven days. The patient currently remains well with nearly resolved pancytopenia on day 130 of cladribine treatment, a WBC count of $4.3 \times 10^9/L$, hemoglobin level of 15.0 g/dL, platelet count of $134 \times 10^9/L$ and normalized WBC differential. A subsequent BM analysis confirmed a normalized kappa/lambda ratio, and a Southern blot analysis no longer showed IgH-JH rearrangement. In addition, the soluble interleukin-2 receptor level decreased from 5,520 to 223 U/mL.

**Discussion**

HCL is an indolent B-cell lymphoproliferative disorder manifesting with pancytopenia, splenomegaly, BM infiltration and circulating neoplastic cells with hair-like cytoplasmic projections. HCL often presents with a low tumor burden, and, without conducting an exhaustive investigation, microscopic techniques alone can result in failure to detect
the disease (1). Surface kappa and lambda light chain FCM analyses are a powerful supplementary screening tool, as light chain restriction is indicative of B-cell malignancy. However, in cases of HCL with a low tumor burden, routine FCM gating of the entire lymphocyte region may fail to show light chain restriction due to dilution with normal polyclonal B lymphocytes, as observed in the present case. Robbins et al. demonstrated that, in their study, 148 of 161 cases (92%) of HCL were diagnosable on PB FCM when accompanied by subgating of the large lymphocyte region (2). The large lymphocyte gate is usually abundant in monocytes, although monocytopenia often occurs in cases of HCL, with tumor cells tending to dominate this gate. FCM should ideally be performed using BM as well because this tissue is usually more heavily infiltrated than PB (3). In patients with HCL, the BM may be hypocellular or inaspirable due to myelofibrosis and thus often mimics the characteristics of aplastic anemia or idiopathic myelofibrosis (1, 4). Without conducting a FCM analysis of the large lymphocyte gate, our patient may have been misdiagnosed with aplastic anemia, and inappropriate and possibly harmful therapies, such as cyclosporine and anti-thymocyte globulin, may have been administered, as described in a previous case report (4). Making an accurate diagnosis, including the exclusion of other B-cell malignancies, is crucial in such cases because treatment with the purine analogues cladribine and pentostatin brings about durable complete remissions in 79% to 95% of patients with HCL (3). Distinguishing HCL from other B-cell malignancies is traditionally performed based on the detection of a constellation of clinical, morphological and immunophenotypical findings; however, the BRAFV600E mutation has recently been shown to be a helpful discriminator (5). Wells et al. reported that four of 56 patients (7.1%) with unexplained pancytopenia and four of 65 patients (6.2%) with a presumptive diagnosis of myelodysplastic syndrome were found to have HCL (6). In a retrospective study by Devitt et al., seven of 132 patients (5.3%) initially presenting with pancytopenia were diagnosed with HCL (7). Physicians confronted with cases of pancytopenia should therefore be aware that HCL is not as rare as often perceived, and kappa/lambda FCM analyses with subgating of the large lymphocyte region should be considered as part of routine screening in such patients.

The authors state that they have no Conflict of Interest (COI).

References


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