

**Case Reports****Morphologic Confounders and CD19 Negativity in a Case of Hairy Cell Leukemia**

Pulkit Rastogi, Sreejesh Sreedharanunni, Uday Yanamandra, Man Updesh Singh Sachdeva and Neelam Varma

Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh - 160012, India.

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Abstract. Objectives: We report a case of hairy cell leukemia (HCL) initially misdiagnosed as plasma cell dyscrasia due to various clinical, morphological and immunophenotypic confounders.

Methods and results: In a patient diagnosed of marrow plasmacytosis and serum monoclonal protein elsewhere and referred to our hospital, morphological evaluation of bone marrow aspirate smears and trephine biopsy, immunophenotyping, and molecular testing (*BRAFV600E* mutation) were done. Clinically, the patient was asymptomatic; bone marrow revealed plasmacytosis, mastocytosis, and lymphocytosis with a few "hairy" cells. Immunophenotyping showed features of HCL with aberrant CD10 expression and a large subclone of CD19^{neg} cells. A diagnosis of HCL with reactive plasmacytosis and mast cell hyperplasia was made and confirmed by immunophenotyping and molecular studies.

Conclusion: Hematopathologists must be aware of various confounding factors and should judiciously use flow cytometric and molecular studies for attaining a proper diagnosis of HCL. We also report a very rare immunophenotypic aberrancy (CD 19 negativity) in HCL.

Keywords: Hairy cell leukemia; Aberrancy; Immunophenotype; CD19 negativity.**Citation:** Rastogi P., Sreedharanunni S., Yanamandra U., Sachdeva M.U.S., Varma N. Morphologic confounders and CD19 negativity in a case of hairy cell leukemia. *Mediterr J Hematol Infect Dis* 2017, 9(1): e2017033, DOI: <http://dx.doi.org/10.4084/MJHID.2017.033>**Published:** May 1, 2017**Received:** January 23, 2017**Accepted:** April 1, 2017This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by-nc/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.Correspondence to: Dr. Sreejesh Sreedharanunni, MD, DM. Assistant Professor, Dept of Hematology, 5th Floor, Research Block A, Postgraduate Institute of Medical Education and Research, Chandigarh – 160012, India. Tel: +91-9478053220, Fax: +91-172-2747124. sreejesh.s@pgimer.edu.in

Introduction. Hairy cell leukemia (HCL) is an uncommon yet unique hematomorphoid neoplasm exhibiting a characteristic cytomorphology, immunophenotype, and well defined molecular features. It accounts for 2% of all lymphoid leukemias.¹ Typically, it presents with splenomegaly, pancytopenia, and monocytopenia; and shows a characteristic immunophenotype. The cells are universally CD19^{positive} with co-expression of CD25, CD11c, CD103 and CD123. Patients of HCL can present with a spectrum of different clinical and pathological characteristics often puzzling a clinician or a pathologist. However, the clinical and pathological findings

often complement each other to clinch the diagnosis of HCL. A correct diagnosis is mandatory as specific therapy in the form of purine analogues can provide long-term remissions in such patients.² We present a case of HCL with atypical clinical and laboratory features confounding the primary hematological abnormality.

Case Report. Clinical history: A 69-year-old male with no known co-morbidities presented with complaints of breathlessness on exertion for 15 days. It was not associated with fever, cough, or purulent expectoration. He had moderate pallor,

tachycardia, and tachypnoea. There was no lymphadenopathy or hepatosplenomegaly. Respiratory system evaluation revealed features suggestive of consolidation confirmed by chest roentgenogram. He improved with parenteral antibiotics. Meanwhile, he was detected to have pancytopenia [Hemoglobin (Hb) – 91g/L, total leukocyte count (TLC) – $2.3 \times 10^9/L$, and platelet count – $122 \times 10^9/L$] with the nadir absolute neutrophil count (ANC) of $252/\mu L$. He was subjected to bone marrow evaluation which revealed plasmacytosis (plasma cells – 10%). A serum protein electrophoresis (SPEP) performed subsequently, showed M-spike (0.3g/dL), though no immunofixation studies were done. He was referred to our center for further evaluation of suspected plasma cell dyscrasia.

On the assessment at our center, he was asymptomatic. He did not complain of bone pains. Physical examination was not contributory. His pancytopenia recovered (Hb-112 g/L, TLC- $3.2 \times 10^9/L$, ANC- $1.4 \times 10^9/L$, platelets- $522 \times 10^9/L$). A repeat bone marrow examination was performed to evaluate suspected plasma cell dyscrasia. Bone

marrow aspirate (BMA) revealed 5% plasma cells; however showed 36% larger lymphoid cells with clumped chromatin and a moderate amount of pale basophilic cytoplasm. A few cells had grooved/reniform nucleus or cytoplasmic projections (**Figure 1A**). An increase in mast cells was also noted. Trepine biopsy showed an interstitial infiltrate, typical of hairy cell leukemia (**Figure 1B, C**) along with an increase in mast cells confirmed by mast cell tryptase immunohistochemistry (**Figure 1D**). No significant clusters of mast cells were highlighted. Repeat SPEP and immunofixation study, done at our center, revealed polyclonal hypergammaglobulinemia.

Multiparametric flow cytometry (**Figure 2**) was performed on the BMA using four/six color antibody panels by lyse-wash-stain method (antibodies from BD Biosciences, San Jose, CA). One tube containing unstained leukocytes was used as negative control. A minimum of one lakh events was acquired on dual laser BD FACS Canto II and analyzed using BD FACS Diva software. Bright $CD19^{positive}$ low side scatter events (5.3% of

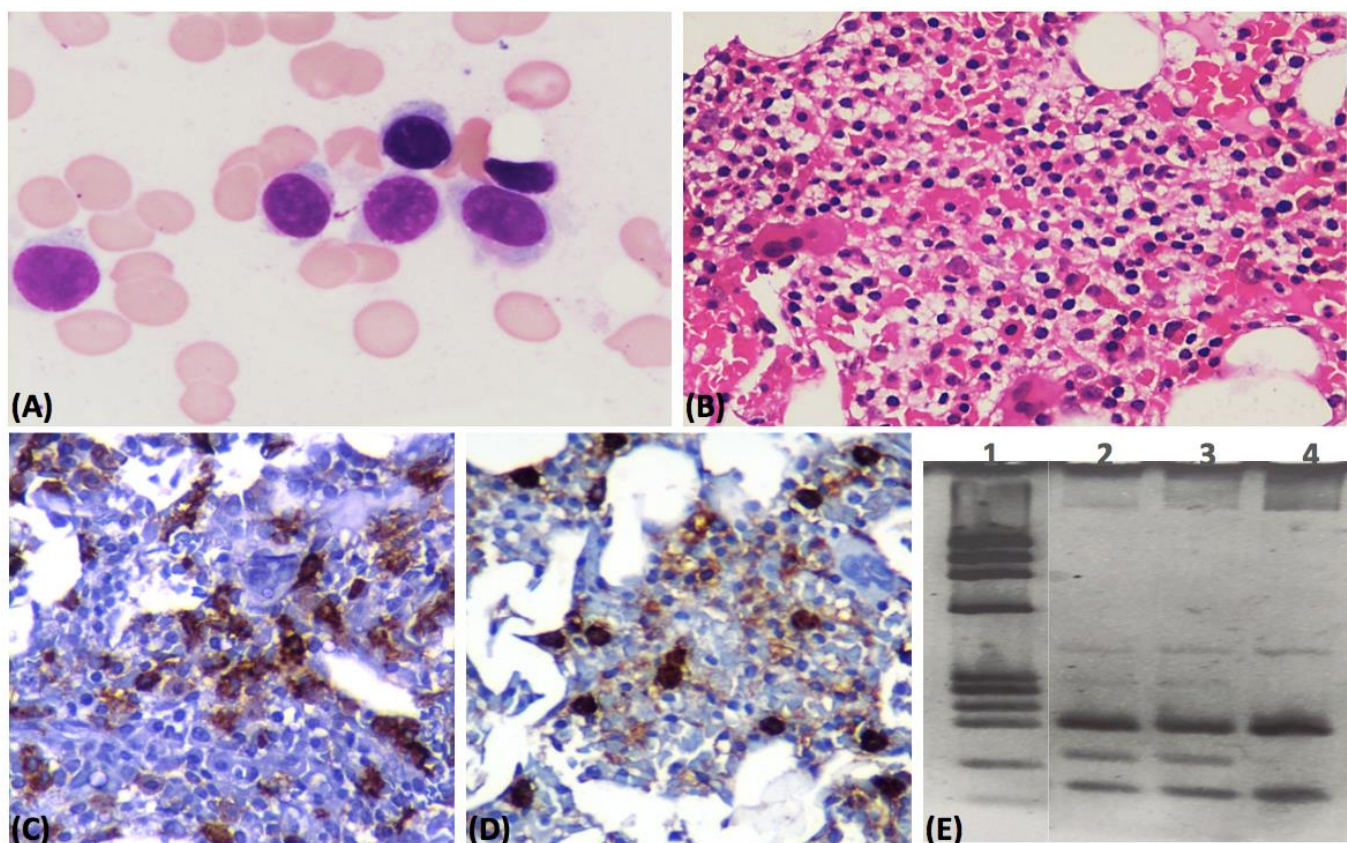


Figure 1. (A) Larger lymphoid cells/hairy cells in bone marrow aspirate smear (May-Grunwald Giemsa stain, x1000); (B) Trepine biopsy showing interstitial infiltrate of hairy cell having a typical “fried egg” appearance (Hematoxylin and Eosin stain, x600); (C) Immunohistochemistry for DBA.44 highlights hairy cells (Hematoxylin counterstain, x600); (D) Immunohistochemistry for mast cell tryptase highlights mast cells (Hematoxylin counterstain, x600). (E) ARMS-PCR and agarose gel electrophoresis showing positivity for *BRAF* V600E mutation (lane 3); 100bp ladder (lane 1), positive control (lane 2), and negative control (lane 4).

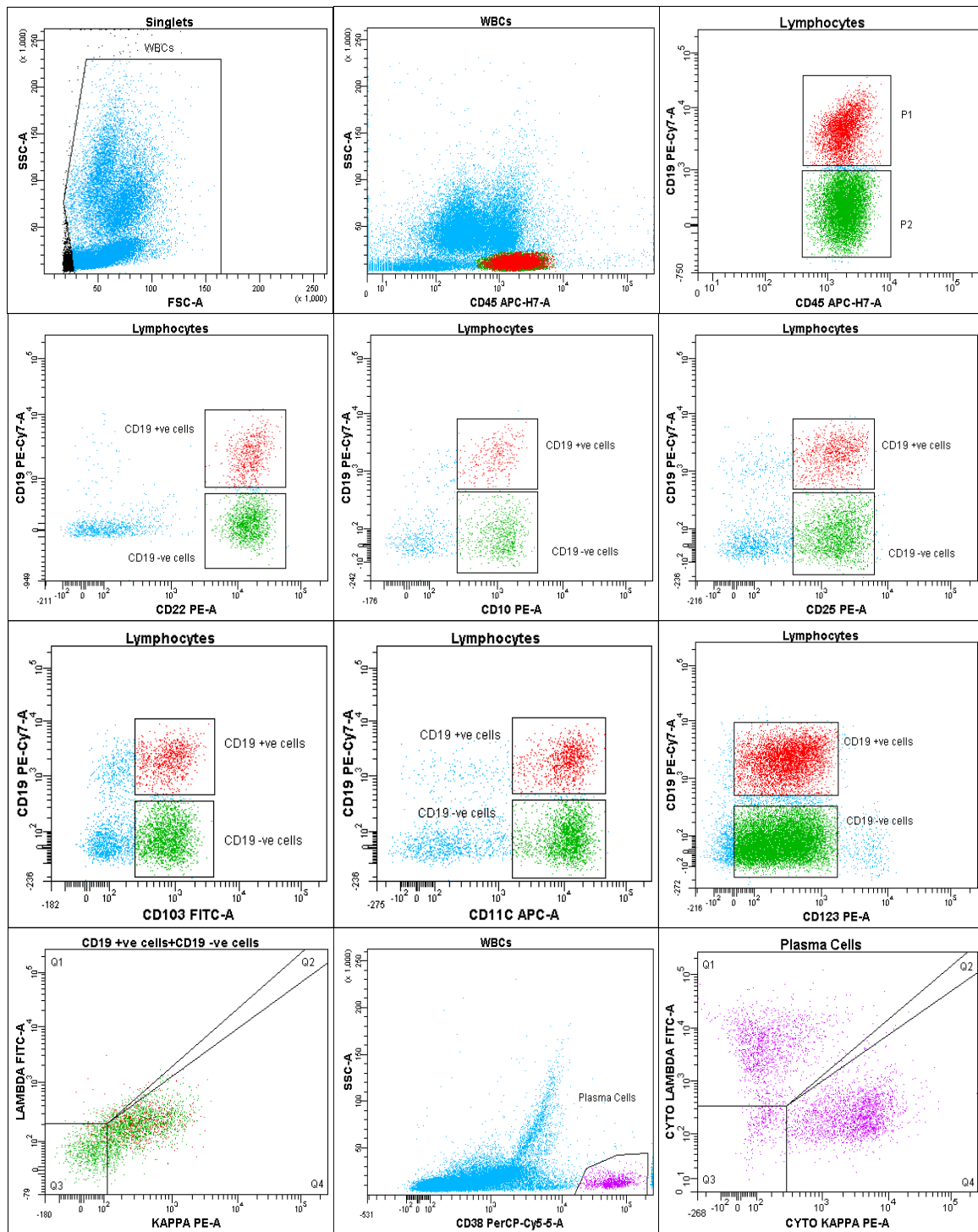


Figure 2. Multiparametric flow cytometry shows two distinct clones (CD19^{POS} and CD19^{NEG}) of cells both of which are positive for CD45, CD22, CD10, CD25, CD103, CD11c, CD123 and surface Igk in similar intensities. The plasma cells do not show clonal restriction.

viable gated leucocytes) were gated which were positive for CD10, CD20, CD22, CD79b, surface Igk, CD25, CD11c, CD103, and CD123. Serendipitously, we found a large subclone of cells (15% of viable gated leucocytes) expressing exactly the same immunophenotype markers except for CD19, indicating its loss of expression from hairy cells. The fluorochrome related

technical issues were ruled out as the cells showed similar profile using both anti-CD19PECy7 and anti-CD19APC-H7 (clone SJ25C1, BD Biosciences). The CD19 negative cells had an immune profile exactly similar to CD19+ve cells and revealed expression of hairy cell markers along with CD20, CD22, CD79b, CD45, and CD10. The plasma cells

(CD38^{pos}/CD138^{pos}/CD19^{pos}/CD81^{pos}/CD56^{neg} and no light chain restriction – **Figure 2**) and mast cells (CD117^{pos}/CD33^{pos}/CD2^{neg}/CD25^{neg}) showed normal immunophenotype indicating reactive plasmacytosis and mast cell hyperplasia. A diagnosis of hairy cell leukemia with atypical features (CD19 negative subclone, CD10 positivity, reactive plasmacytosis and mast cell hyperplasia) was made which was subsequently confirmed by amplification-refractory mutation system polymerase-chain-reaction (ARMS-PCR) for *BRAF* V600E mutation (**Figure 1E**).

The patient remained asymptomatic, and his laboratory parameters remained normal; not warranting purine analogue therapy. He has been keeping under close medical observation.

Discussion. HCL is a unique B-cell non-Hodgkin lymphoma (NHL) characterized by splenomegaly, cytopenias affecting two or more lineages and morphologically by typical hairy cells. Though the majority of cases have this typical presentation, there are scenarios in which the clinical, morphological or immunophenotypic features are atypical, leading to diagnostic confusion. The case presented here exemplifies this intriguing situation where the patient on evaluation for a lower respiratory tract infection was found to have cytopenias, had no palpable splenomegaly and the bone marrow showed only a few "hairy cells" along with confounders in the form of plasmacytosis and mastocytosis. All these together with a minor quantity of serum "M" protein led to initial misdiagnosis.

Splenomegaly is an important feature seen in up to 90% of patients with HCL. However, its absence should not exclude a diagnosis of HCL.³ And more importantly, a changing trend has been observed in the symptomatology of HCL over the past 30 years. Number of cases are being diagnosed at an early stage with a less marked splenomegaly.⁴

A co-existence of plasma cell myeloma with HCL as well as the development of myeloma in patients with HCL has been reported in the literature.⁵ At times, plasma cell myeloma/leukemia may mimic HCL also.⁶⁻⁷ Clonal plasma cells were excluded by flow cytometry and SPEP studies. The initial report of small monoclonal band in SPEP from outside our institute might represent a transient monoclonal gammopathy, as has been reported previously with

several infections.⁸⁻⁹ However, a wrong interpretation could not be conclusively resolved in the absence of immunofixation studies. The association of mast cell hyperplasia with HCL has been well characterized by Macon et al.¹⁰ This has been attributed to the angiogenesis and further progression of the disease, confirmed by a latter study.¹¹ There has also been a case report of systemic mastocytosis associated with a clonal hematopoietic non-mastcell lineage disease (SM-AHNMD) where the coexisting neoplasms were of both lymphoid and myeloid origin.¹² Our case shows a striking mast cell hyperplasia, however, a systemic mastocytosis has been ruled out based on immunophenotype studies.

Immunophenotype aberrancies have been well described in HCL, like negativity for CD103 or CD25; and positivity for CD10 or CD23.¹³ In our case, the cells showed positivity for CD10, and there was a subclone with absence of CD19 expression. While CD10 expression is relatively common (5-26% of cases) and explained by alternate origin of leukemic cells from germinal center,¹³ the absence of CD19 expression in HCL has not been previously reported in the literature. CD19 plays an important role in B-cell growth and differentiation and its expression increases as a B-cell matures. This characteristic is often the basis of using it in flow cytometry as a gating marker for the diagnosis and for minimal residual disease (MRD) testing in various B-cell malignancies. In fact, of all the B-NHLs, HCL cases show the maximum level of expression of CD19.¹⁴ The abnormal immunophenotypic pattern should be borne in mind while performing the MRD analysis during follow-up. An alternate marker (CD20) should also be considered for gating leukemic cells in these patients.¹⁵

Conclusions. We report a case of HCL with unique clinical, morphological and immunophenotypic features. A hematopathologist must be aware of these confounding factors and must deal such cases with a high index of suspicion and a supportive armamentarium of flow cytometry and molecular studies.

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