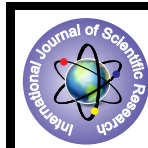


Buffy Coat : A Clue to The Diagnosis of Hairy Cell Leukemia



Medical Science

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ABSTRACT

Hairy cell leukemia (HCL) is a rare disease, comprising 2% of lymphoid leukemias. HCL is B-cell lymphoproliferative disorder affecting middle-aged to elderly adults. HCL is an indolent neoplasm of small mature B lymphoid cells with oval nuclei and abundant cytoplasm with hairy projections involving peripheral blood and diffusely infiltrating the bone marrow and splenic red pulp. Occasional cases may have nuclei that are monocyteoid, folded or even bilobed in shape. Buffy coat preparation in pancytopenia cases has proved to be an important modality in clinching the diagnosis of HCL which can be further confirmed with positive markers like DBA.44(CD72) and Annexin A1 on immunohistochemistry. An accurate diagnosis is of critical importance because of the unique approach to the management of HCL patients.

INTRODUCTION

Hairy cell leukemia is a rare neoplasm of mature B lymphoid cells. Tumour cells are found predominantly in the Bone Marrow and spleen. The median age of presentation is 50 years. The male to female ratio is 5:1. The most common presenting symptoms are weakness, fatigue, fever and bleeding tendencies.¹ Most patients present with splenomegaly and pancytopenia with few circulating neoplastic cells. Hairy cells are small to medium sized cells with an oval or indented (bean shaped) nuclei with homogeneous, spongy, ground glass chromatin that is slightly clumped than that of normal lymphocyte. Nucleoli are typically absent or inconspicuous. The cytoplasm is abundant and pale blue with circumferential hairy projections on smears. The edges of the cytoplasm are indistinct. Many hairy cells have a cytoplasmic border best described as 'frayed', although the most typical cells have a visible circumferential halo of long slender villous cytoplasmic projections that produce the classic 'hairy' appearance. Monocytopenia is characteristic. Hairy cells have a characteristic appearance under the electron microscope. In the past, prior to the wide availability of reliable immunophenotyping, electron microscopy has been useful for diagnosis. The presence of ribosome-lamellar structures in the cytoplasm is the characteristic ultrastructural feature of HCL. In practical terms, with the advent of widely available immunostaining and flow cytometry, electron microscopy is rarely useful in the diagnosis of HCL.² HCL has good prognosis by the fact that the overall 10 year survival rate is 90%.¹

CASE REPORT

A 45 years old female was admitted with complaints of fever, weakness, giddiness, easy fatigueability and excessive bleeding per vaginum. On examination severe pallor was noted. Abdominal examination revealed enlarged, firm, non-tender splenomegaly 5 cms below the costal margin. On ultrasonography, the spleen measured 8x6cm with normal echotexture. Hematological examination revealed severe pancytopenia with hemoglobin (Hb) concentration of 3.6gm/dl, a total leukocyte count (TLC) of $2.1 \times 10^9/L$, red blood cell (RBC) count $1.31 \times 10^9/L$, platelet count (Plt) of $37 \times 10^9/L$, hematocrit (Hct) 12.1%, red cell distribution width (RDW) of 22.2%, MCV 92.0 fl, Mean Platelet volume (MPV) of 10.8 fl. PS revealed normocytic normochromic red cells with few atypical lymphoid cells. Platelets were markedly reduced in number. Since the TLC was too low and peripheral smear showed few atypical cells, buffy coat preparation was made and morphology was studied on Field and Leishman's stained smears. DLC from buffy coat smears revealed 21% polymorphs, 65% lymphocytes, 0% eosinophils, 4% monocytes and 10% atypical lymphoid cells. The atypical cells

had pale cytoplasm containing numerous hairy projections on the outer surface. The nucleus was oval and indented (kidney shaped) with coarse chromatin (Fig. 1). The buffy coat findings were suggestive of hairy cell leukemia, thus bone marrow aspiration and trephine biopsy was advised. Bone marrow aspiration smears were diluted and revealed suppression of erythroid and myeloid series, occasional megakaryocytes and predominantly atypical lymphoid cells. Bone marrow biopsy revealed total replacement of the marrow by sheets of malignant lymphoid cells showing typical "fried egg" appearance with abundant pale cytoplasm and round to indented nuclei with coarse chromatin. Intervening areas show residual hemopoietic cell precursors and few adipocytes (Fig 2). IHC panel was done. Positive markers were LCA, CD20, CD10, DBA.44, Annexin A1. CD5 was negative.

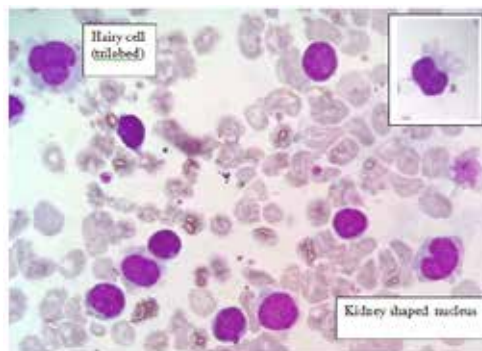


Figure 1 A buffy coat blood smear demonstrating atypical cells with light basophilic cytoplasm containing numerous hairy projections [inset] on the outer surface. (Leishman's stain; x 1000)

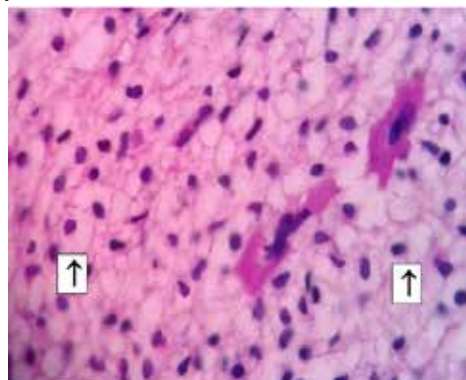


Figure 2. A bone marrow biopsy demonstrating the typical fried egg appearance of sheets of hairy cells (arrows) with abundant pale cytoplasm and round to indented nuclei with coarse chromatin.

megakaryocytes (Hematoxylin and Eosin; x 1000)**DISCUSSION**

Hairy cell leukemia is a rare disorder, accounting for 2% of all leukemias. HCL was first recognized by Ewald in 1923, who described the condition as leu-kemische reticuloendotheliose. The disease is more common in Caucasians and particularly frequent in Ashkenazi Jewish males, with an overall male to female ratio of approximately 5:1. The median age of onset is 50 years. HCL has been classified into three types: HCL-classic, variant HCL (HCL-V, type II HCL) and Japanese variant HCL (HCL-J). Morphological evaluation of a buffy coat smear is an extremely valuable tool in screening for HCL particularly in cases of severe pancytopenias as the disease may go undetected when very low levels of hairy cells are present in the peripheral smears and even in bone marrow aspiration smears due to marrow fibrosis. Differential diagnosis of HCL include B-Chronic Lymphocytic Leukemia (CLL), Prolymphocytic leukemia and T-cell lymphoproliferative disorders such as Hepatosplenic T-cell lymphoma and Splenic B-cell lymphoma including splenic lymphomas with villous lymphocytes (SLVL). The cells of CLL differ from hairy cell leukemia as they have more coarsely clumped

chromatin and round or ovoid nuclei. Hairy cells are intermediate-sized lymphocytes that possess round to indented nuclei and an abundant light blue agranular cytoplasm with characteristic "hairy" projections. They strongly express CD103, CD22 and CD 11C. These cells typically infiltrate the bone marrow, the spleen and to a lesser extent the liver, lymph nodes and skin. Recently, immunohistochemical demonstration of **Annexin A1** has been reported to be a **100% specific** marker for HCL. Careful attention to morphological details by making smears from buffy coat is important for early diagnosis, particularly when low percentages of atypical cells are present in peripheral smears.

CONCLUSION

In conclusion it should be noted that even if the clinical presentation and the haematological profile of the patient indicates a lymphoproliferative disorder especially in severe pancytopenia cases, **buffy coat smear studies** (in addition to further confirmation by IHC markers /Flow Cytometry) **should be done** so that an erroneous diagnosis of other lymphoreticular disorders is avoided.

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REFERENCE

- [1]. Swerdlow Steven H et al 2008;4th edition .WHO classification of tumors of haematopoietic and Lymphoid Tissues 188-190. | [2]. Brunning RD & Parkin J. Ribosome-lamella complexes in neoplastic hairy cells. American Journal of Pathology 1975; 79: 565-578. | [3]. Burke JS. Surgical pathology of the spleen: an approach to the differential diagnosis of splenic lymphomas and leu-kemias. Part I. Diseases of the white pulp. Am J Surg Pathol. 1981;5(6):551-563. | [4]. Zafrani ES, Degos F, Guigui B et al. The hepatic sinusoid in hairy cell leukemia: an ultrastructural study of 12 cases. Human Pathology 1987; 18: 801-807. | [5]. Mercieca J, Puga M, Matutes E et al. Incidence and significance of abdominal lymphadenopathy in hairy cell leukaemia. Leukemia & Lymphoma 1994; 14 (supplement 1): 79-83. | [6]. Mercieca J, Matutes E, Moskovic E et al. Massive abdominal lymphadenopathy in hairy cell leukemia: a report of 12 cases. British Journal of Haematology 1992; 82: 547-554. | [7]. Bilsland D, Shahriari S, Douglas WS et al. Transient leukaemia cutis in hairy-cell leukemia. Clinical and Experimental Dermatology 1991; 16: 207-209. | [8]. Salomon-Nguyen F, Valensi F, Troussard X & Flandin G. The value of the monoclonal antibody DBA44 in the diagnosis of B-lymphoid disorders. Leukemia Research 1996; 20: 909-913. |