

CASE REPORT

HAIRY-CELL LEUKEMIA: A RARE BLOOD DISORDER IN ASIA

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Abstract. We report a 68-year-old Indian man who was referred to the Hematology Unit for investigation for thrombocytopenia, an incidental finding during a pre-operative screening for prostatectomy. Physical examination was unremarkable. There was no splenomegaly, hepatomegaly or lymphadenopathy. Complete blood counts showed normal hemoglobin and total white cell count with moderate thrombocytopenia. Hairy-cell leukemia was diagnosed based on peripheral blood film, bone-marrow aspirate and trephine biopsy findings, supported by immunophenotyping results by flow cytometry. The purpose of this report is to create awareness of this uncommon presentation and to emphasize that a single-lineage cytopenia or absence of splenomegaly does not exclude the diagnosis of hairy-cell leukemia. Careful attention to morphological detail is important for early diagnosis, especially when low percentages of "hairy" cells are present in the peripheral blood and bone marrow. Early diagnosis is important to ensure that patients obtain maximum benefit from the newer therapeutic agents that have greatly improved the prognosis in this rare disorder.

INTRODUCTION

Chronic lymphoproliferative disorders are a heterogeneous group of clonal disorders with varying clinical features and prognoses. It is the most common hematological malignancy in the western world, but uncommon in Asia (Wells and Lau, 1960; Haenszel and Kurihara, 1968; Young *et al*, 1981; Brinker, 1982; Boggs *et al*, 1987; Kuperan *et al*, 1997). Hairy-cell leukemia (HCL) is an uncommon subtype of chronic B-cell lymphoproliferative disorder and accounts for around 2% of all forms of leukemias (Grey *et al*, 2000). It was first reported by Ewald in 1923 as leukemic reticuloendotheliosis (Ewald, 1923) but described as a distinct clinical entity by Bouroncle and colleagues in 1958. The disease occurs most frequently in middle-aged men and is traditionally characterized by splenomegaly, pancytopenia and the presence of abnormal mononuclear cells with irregular cytoplasmic projections in blood, bone marrow and other tissues, especially the spleen. Immunoglobulin gene rearrangement studies have confirmed that HCL is a clonal B-cell malignancy (Cheary *et al*, 1984). The disease runs an indolent course. However, many patients eventually develop life-threatening pancytopenia, symptomatic splenomegaly, or constitutional symptoms that necessitate treatment. Recently, HCL has attracted considerable attention because of the significant advances in its treatment;

the disease is unusually sensitive to therapy with 'unconventional' agents such as interferon-alpha and nucleosides (Pettitt *et al*, 1999).

The common clinical presentations of this disorder are abdominal discomfort, fatigue, weight loss, bruising, anemia, and infections. The usual laboratory findings are low hemoglobin levels, leukopenia with absolute neutropenia and monocytopenia, and the presence of a variable proportion of 'hairy cells' in the peripheral blood film, bone marrow aspirate and trephine biopsy. An isolated thrombocytopenia with absence of splenomegaly at presentation is rare. We report a 68-year-old Indian man who was referred to the Hematology Unit for investigation of thrombocytopenia, an incidental finding during a routine preoperative screening for prostatectomy.

CASE REPORT

A 68-year-old Indian man was referred to the University Hospital for investigation of thrombocytopenia, an incidental finding during a pre-operative screening for prostatectomy in a private medical center. There was no history of fever or any bleeding tendency. The patient denied all other constitutional symptoms, except for a weight loss of two kilograms in six months. At presentation, he appeared well. There was no pallor, hepatomegaly, splenomegaly, or lymphadenopathy. No other abnormal physical findings were noted. Laboratory investigations revealed a hemoglobin level of 133 g/l, white-blood-cell count of $7.0 \times 10^9/l$ and a platelet count of $54 \times 10^9/l$. Peripheral blood film showed normochromic red cells, absolute monocytopenia, atypical lymphoid cells with surface projections--'hairy cells' (Fig 1)--

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and thrombocytopenia. Bone marrow aspiration was difficult; however, the smears so obtained, stained by May Grunwald-Giemsa stain (Lewis *et al*, 2002) showed depressed normal hemopoiesis and infiltration of the marrow by large abnormal lymphoid cells (Fig 2). These cells had moderate to ample amounts of pale blue cytoplasm, with irregular hair-like surface projections, round to oval nuclei with smooth nuclear chromatin and inconspicuous nucleoli. Acid phosphatase cytochemical staining (John and Lewis, 1991) of these cells revealed strong tartrate-resistant acid phosphatase activity (Fig 3). Trephine biopsy stained by haematoxylin & eosin (Dacie and Lewis, 1995) showed the marrow to be diffusely infiltrated by loosely scattered hairy cells with plenty of spaces between the cells. In focal areas, clear zones were noted around individual cells (Fig 4). Normal hemopoietic cells were significantly decreased. The marrow also showed a diffuse increase in reticulin fibers (Fig 5) demonstrated by Gordon and Sweets' technique (Brancroft *et al*, 1995). Immunophenotyping of these abnormal cells by flow cytometry revealed strong expression of CD19, CD22, CD11c, CD25, CD103 and surface membrane immunoglobulin IgG with kappa light chain restriction. The cells were negative for CD5, CD23, CD34, CD10 and myeloid markers. A diagnosis of hairy-cell leukemia was made. The patient has since been started on the purine analogue, cladribine.

DISCUSSION

Hairy-cell leukemia is a clonal, B-cell lymphoproliferative disorder with distinct clinical and laboratory features. Traditionally, this disease entity has been characterized by splenomegaly, pancytopenia and the presence of abnormal mononuclear cells with irregular cytoplasmic projections in peripheral blood, bone marrow and other tissues, especially the spleen (Catovsky *et al*, 1974). Hence, the common clinical presentations of this disorder are abdominal fullness

or discomfort associated with splenomegaly, fatigue, weight loss, weakness, bruising associated with thrombocytopenia, and bacterial and opportunistic infections associated with severe neutropenia and monocytopenia. Splenomegaly is frequently massive and this may be the most prominent physical finding in many patients. However, small number of patients may present without the usual clinical findings with mild unexplained cytopenias (Tytherleigh *et al*, 2001); early diagnosis in these cases is important, to ensure that patients obtain maximum benefit from the newer therapeutic agents currently available. According to the guidelines (British Society for Haematology, 2000) for the diagnosis and therapy of hairy-cell leukemia, the incidence of splenomegaly at diagnosis has decreased to 60 - 70 % when compared with earlier studies, the reason being better and early recognition of the disease. Enlargement of the lymph nodes is rare, and when present, is minimal and localized. Mild hepatomegaly occurs occasionally.

The common laboratory findings in this disorder are cytopenias, usually affecting two or three lineages; anemia results from reduced bone marrow production and splenic pooling. Leukopenia is most common, and is characterized by both absolute neutropenia and monocytopenia. The white cell count is usually $< 5 \times 10^9/l$ and very rarely $> 10 \times 10^9/l$; monocytopenia is a consistent feature of this disease and the peripheral blood film and bone marrow usually show the presence of a variable proportion of 'hairy cells.' Hairy cells (HCs) are about twice the size of a small lymphocyte with moderate to abundant amounts of pale blue cytoplasm with irregular 'hair-like' surface projections. The nuclei, positioned in a central or eccentric location are round, oval or slightly indented in shape, with reticular or 'sieve-like' chromatin pattern and indistinct or absent nucleoli (Bruce, 2001).

A review of the biology of HCs has shown these cells to be clonal late B cells with specific activation

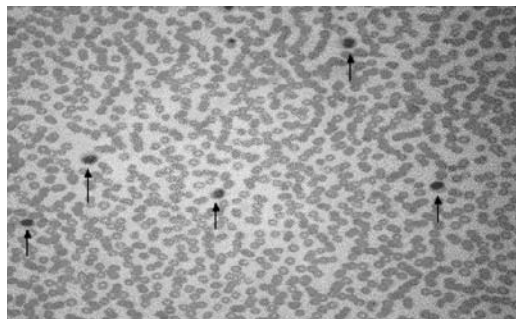


Fig 1- Peripheral blood film shows hairy cells.

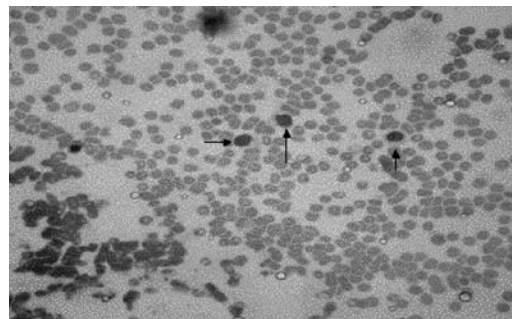


Fig 2- Bone marrow smear shows hairy cells.

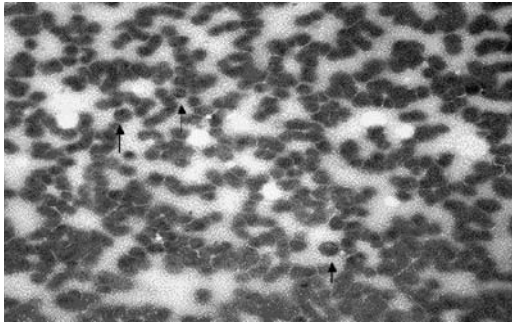


Fig 3- Bone marrow smear shows positivity in hairy cells with tartrate-resistant acid phosphatase (TRAP) stain and no staining in the late normoblast.

features. The cell surface structures associated with normal B-cell activation, such as CD22, CD25, CD72 and CD40 ligand, are strongly expressed by HCs, and the markers normally lost after B-cell activation, such as CD21 and CD24, are expressed only at low levels. The most obvious feature of HC activation is the unique morphology; the distinctive surface pattern of microvilli and ruffles that characterize the HCs reflect ongoing cytoskeleton and signalling activity. These cells have a low proliferative rate and their accumulation is therefore primarily the result of abnormally prolonged cell survival. Although the nature of the underlying oncogenic events is unknown, it results in the distinctive activated phenotype of HCs (Pettitt *et al*, 1999).

Bone marrow aspirates are usually unsuccessful in this disease due to diffuse reticulin fibrosis, the result of fibronectin synthesis by HCs through the interaction of their surface CD44 with hyaluronan, abundantly present in the bone marrow (Aziz *et al*, 2001) When aspirable, hairy cells are often observed in the smear; the demonstration of positive tartrate-resistant acid phosphatase (TRAP) activity, which corresponds to a unique isoenzyme 5 (Hoffbrand, Lewis *et al*, 1999) found in these cells helps confirm a diagnosis of HCL. Although positive TRAP is not specific for HCL, and is therefore not a prerequisite for diagnosis, a positive TRAP stain, together with a morphologically characteristic trephine biopsy, is essentially diagnostic of HCL (Bruce, 2001). The TRAP reaction can be performed on peripheral blood films, marrow aspirate smears, or touch preparations of bone marrow. Examination of the bone marrow trephine biopsy is essential in establishing a diagnosis of HCL (Burke *et al*, 1978; Bartl, 1983; Burke and Rappaport, 1984). The marrow biopsy nearly always contains an infiltrate of mononuclear cells, with round

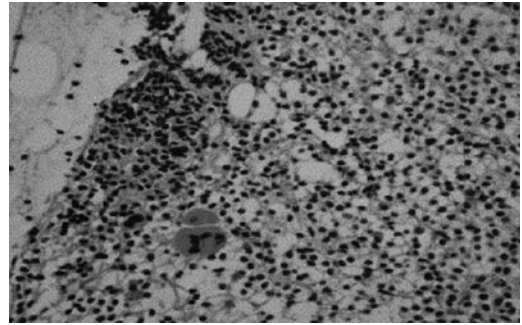


Fig 4- Bone marrow biopsy shows shows extensive replacement of normal hemopoietic tissue by hairy cells. The nuclei are typically surrounded by a clear zone of cytoplasm.

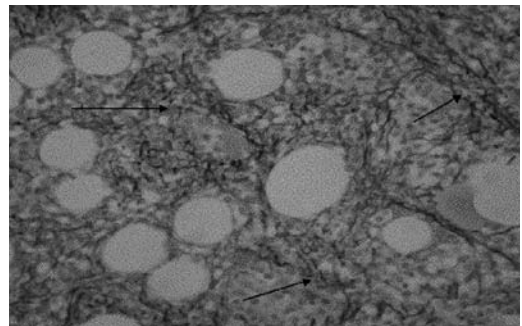


Fig 5- Bone marrow trephine biopsy shows the characteristic increased reticulin deposition as indicated by arrows.

or indented nuclei surrounded by a halo of clear or pale cytoplasm. This characteristic appearance is the result of the abundant cytoplasm and some shrinkage that occurs during fixation of the biopsy. Mitosis and nuclear pleomorphism are uncommon in trephine biopsy.

Immunophenotyping of the malignant cells by flow cytometry is now common and has become the standard method for confirming diagnosis (Bruce, 2001). Malignant cells exhibit a mature B-cell phenotype and express strong monoclonal surface immunoglobulin with either kappa or lambda light chains and pan-B-cell antigens CD19, CD20, and CD22, but lack CD5, CD10 and CD23 (Robbins *et al*, 1993). The cells also strongly express CD103 and CD25 and bright CD11c in contrast to dim co-expression in chronic lymphocytic leukemia (CLL), and the leukemic cells of many lymphomas.

The differential diagnosis of HCL includes other B-cell lymphoproliferative disorders associated with splenomegaly, such as CLL, B- cell prolymphocytic leukemia (PLL), splenic lymphoma with villous lymphocytes (SLVL), and hairy-cell variant. CLL and PLL can be distinguished from HCL by marked

lymphocytosis, different cell morphology, and differences in immunophenotyping profile; CLL is positive for CD5 and negative for CD103, while PLL shows negativity for CD11c and CD103. SLVL shares some clinical and morphological features with HCL; however, the cells usually do not exhibit strong TRAP positivity, the bone marrow infiltrate are sharply demarcated, unlike HCL, and the immunophenotyping profile is usually negative for CD103 and CD25. HCL variant exhibits morphological features intermediate between hairy cells and prolymphocytes, with prominent leukocytosis and lack of monocytopenia; they are CD25-negative (Bruce, 2001).

The clinical management and outcome of HCL patients has greatly improved in the last decade with new therapeutic agents that have enabled effective treatment. Splenectomy was first-line therapy until alpha-interferon was reported to provide more effective and systemic treatment (Quesada *et al*, 1984). More recently, excellent complete remission rates have been reported with the purine nucleoside analogues, pentostatin (2 deoxycoformycin) (Spiers *et al*, 1987) and cladribine (2-chlorodeoxyadenosine; 2-CdA) (Piro *et al*, 1990), although increasingly recognized adverse reactions have also been reported, which include severe myelotoxicity (Betticher *et al*, 1993; Cheson, 1995) and cutaneous reactions (Meunier *et al*, 1996). Over 90% of cases respond to treatment with purine analogues and complete remission is induced in at least 75% of cases (Pettitt *et al*, 1999). Interferon is less effective in all aspects, but may retain a place in the initial treatment of very cytopenic patients and in patients refractory to cladribine treatment. (Seymour *et al*, 1995). Rituximab (anti- CD20 monoclonal antibody), is another exciting new agent suggested to have a place in treating patients who have relapsed, or are refractory to cladribine treatment (Hoffman *et al*, 2000).

Our patient had an uncommon presentation; he was fairly well, with only isolated thrombocytopenia. There was no splenomegaly or other abnormal physical finding, normal haemoglobin level and white cell count. The patient's bone marrow aspirate was difficult; however, the smears obtained showed the presence of 'hairy cells' which revealed strong positivity for tartrate-resistant acid-phosphatase activity. Trephine biopsy showed the morphologically characteristic appearance of HCL and immunophenotyping of the malignant cells by flow cytometry confirmed the diagnosis. This report emphasizes the need to pay careful attention to morphological details when examining peripheral blood films and bone marrow smears containing low percentages of cells, especially

when there is no clinical suspicion of this disorder. It is also important to be aware that patients may present without the classical, clinical and laboratory findings of HCL and hence a single lineage cytopenia or absence of splenomegaly does not exclude a diagnosis of HCL. Early diagnosis is important to ensure that patients obtain maximum benefit from newer therapeutic agents that have enabled effective treatment and have greatly improved prognosis in this rare disorder.

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