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Hairy cell leukemia diagnostic criteria and differential diagnosis

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Abstract

Hairy cell leukemia (HCL) is a disease with distinctive clinical findings, as well as a unique morphology and immunophenotype. These features typically allow for a reliable and reproducible diagnosis in nearly all situations. However, certain morphological features of HCL, such as villous cytoplasmic projections or characteristic tissue specific infiltrative patterns, including red pulp expansion with pseudosinuses, may be seen in other B-cell lymphoproliferative disorders. A methodical and thorough approach evaluating the clinical, cytological, histological, architectural, and immunophenotypic features is described and will aid in rendering the appropriate diagnosis. This is paramount as current data indicate that hairy cell leukemia – variant and other splenic B-cell lymphomas must be distinguished from HCL, as the response to therapy differs in these disorders.

Keywords

Hairy cell leukemia; bone marrow; spleen; B-cells; immunophenotyping; lymphocytes; lymphocytosis

The World Health Organization (WHO) 2008 classification utilizes a disease-oriented approach for the classification of hematopoietic neoplasms [1]. By providing pathologists with comprehensive disease definitions, accurate diagnoses are rendered and in turn, appropriate treatments can be implemented by clinicians. These treatments are usually disease tailored and specific, and often achieve a significant therapeutic response. Hairy cell leukemia (HCL) is one such disease that when recognized and diagnosed accurately, is highly responsive to appropriate therapy.

HCL is a distinctive entity both clinically and pathologically. Clinically, HCL commonly presents with peripheral blood monocytopenia, significant splenomegaly, and bone marrow involvement. Although distinctive, the clinical presentation of splenomegaly and bone marrow disease is not specific for any single diagnostic entity. The differential diagnosis for small B-cell neoplasms that often present with splenomegaly and bone marrow disease (Table I) is broad and includes: chronic lymphocytic leukemia/small lymphocytic lymphoma

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(CLL/SLL), prolymphocytic lymphoma (PLL), splenic marginal zone lymphoma (SMZL), hairy cell leukemia (HCL), the category of splenic lymphoma/leukemia, unclassifiable, which includes splenic diffuse red pulp small B-cell lymphoma and hairy cell leukemia – variant (HCL-v), lymphoplasmacytic lymphoma (LPL), and mantle cell lymphoma (MCL). Fortunately, HCL is a morphologically and immunophenotypically distinctive disorder which allows for its accurate diagnosis in essentially all cases.

Cytologically the ‘hairy cell’ is approximately twice the size of a mature lymphocyte and contains a nucleus which occupies the majority of the cell’s area. The nuclei are more often than not oval, but can also range in shape from round to spindled to horseshoe-shaped. Uncommonly the cells have marked nuclear irregularities. In such cases accurate diagnosis rests on a characteristic immunophenotype. The chromatin is variably smooth and granular in appearance and nucleoli are commonly inconspicuous. The hairy cell’s cytoplasm is characteristic and its appearance is dependent upon the preparation. In Wright–Giemsa stained smears and touch preparations the cytoplasm stains a soft, blue-gray with a tissue paper-like texture that appears frayed at the border [Figure 1(A)]. The villous projections seen in peripheral blood smears are not evident in touch preparations from spleen or bone marrow [Figure 1(B)]. Ultrastructurally, the hairy cell possesses hair-like cytoplasmic projections, hence its name, and a distinctive organelle: the ribosome–lamella complex.

The hairy cell expresses isoenzyme 5 of acid phosphatase. This isoenzyme imparts a resistant to treatment with tartaric acid to the hairy cell and therefore, essentially all cases of HCL express tartrate resistant acid phosphatase (TRAP) cytochemically to some degree. Although expression of TRAP is not pathognomonic for HCL, bright cytochemical expression of TRAP is almost exclusive to HCL.

Histologically, tumor cells are commonly encountered within the bone marrow and spleen, and less commonly within the liver and lymph nodes. Bone marrow involvement is variable and displays a broad spectrum of histological appearances, ranging from hypocellular, aplastic-appearing infiltrates to diffuse sheets of monotonous cells replacing marrow elements. The most common pattern observed within the bone marrow is an interstitial infiltrate, which tends to infiltrate throughout the marrow cavity preserving the marrow adipose tissue, rather than a nodular growth pattern [Figure 2(A)]. At low power the infiltrate appears paucicellular and may often be overlooked. The hairy cell’s abundant cytoplasm imparts a well-spaced appearance to the lymphoid cells, as compared to other lymphoid neoplasms. Well-preserved cytoplasmic borders result in a ‘fried egg’ appearance. Hairy cells may disrupt the microvasculature of the bone marrow leading to extravasation of red blood cells that may impart a pseudosinus appearance. Similar pseudosinuses and blood lakes are invariably seen in the spleen. Additionally, hairy cells produce and excrete fibronectin which results in pericellular deposition of fibronectin that may also contribute to the widely spaced appearance of the lymphoid cells [2]. It is this pericellular fibronectin that is the most probable cause for the increase in marrow reticulin fibers seen in essentially all HCL cases and is often the culprit of the characteristic ‘dry tap’ observed in this disease.

Individual hairy cells can histologically mimic many common bone marrow elements (monocytes, myelocytes, mast cells, etc.) and, as mentioned previously, when HCL

infiltrates interstitially it can be quite inconspicuous. Immunohistochemical analysis is invaluable in highlighting subtle involvement, particularly early in the disease [Figure 2(B)]. CD20 is strongly expressed. Other markers useful in differential diagnosis of the bone marrow core include positive staining for DBA.44, annexin A1, CD25, weak staining for cyclin D1, and typically negative staining for CD5, CD23, and CD10. Caution is advised to always compare annexin A1 staining to that for a B-cell antigen, as annexin A1 is also present in myeloid cells and a proportion of T-cells. Annexin A1 is highly specific for HCL and can be used to differentiate HCL from SMZL and HCL-v, as annexin A1 is not expressed in any other B-cell lymphoma other than HCL [3]. Cyclin D1 expression is not secondary to translocations involving the *CCND1* gene. Interestingly, as in MCL, Sox11 may be expressed as well [4]. Enzyme cytochemistry for TRAP activity is infrequently done for diagnosis currently. Immunohistochemical detection of TRAP is feasible, but less specific; false negatives due to limited expression of the antigen are also a problem.

HCL by flow cytometry commonly gates within the monocyte region when analyzed by CD45 versus side scatter due to the characteristic surface irregularities of hairy cells. Additionally, this region is often devoid of actual monocytes. The classic phenotype of HCL is bright surface immunoglobulin, with bright co-expression of CD20, CD22, and CD11c. HCL also expresses CD103 and CD25, although these markers are not HCL specific. It is nearly always negative for CD5.

Spleens are enlarged macroscopically with expansion of red pulp areas and zones of hemorrhage, which are often referred to as red blood cell lakes. The integrity of the cord-sinus architecture is disrupted, leading to pseudosinus formation in which sinusoidal-like spaces are lined by hairy cells with a loss of normal sinusoidal lining cells [5]. The HCL infiltrate is histologically similar to that described in the bone marrow and composed of small-to-medium sized lymphocytes with ample cytoplasm. White pulp areas are atrophic.

Lymph nodes, when involved, are largely confined to retroperitoneal and abdominal nodes, with rare involvement of peripheral nodes. The infiltrate displays an interfollicular or paracortical distribution within the node, and may extend into surrounding adipose tissue. Sinuses are typically intact and follicular structures are spared. The cells have a fried-egg appearance, due to the abundant cytoplasm (Figure 3).

The differential diagnosis of HCL in the bone marrow includes CLL, SMZL, and other forms of splenic lymphoma, including splenic diffuse red pulp small B-cell lymphoma and HCL-v. These latter two disorders are included in the 2008 WHO classification under splenic B-cell lymphoma/leukemia, unclassifiable (SBCL/L,u). The process known as splenic lymphoma with villous lymphocytes (SLVL) is probably heterogeneous and includes cases of SMZL and the disorders included under SBCL/L,u.

HCL-v cases share some features with classic HCL but show differences in hematologic, cytologic, and immunophenotypic features, and lack responsiveness to classic HCL therapies [6]. Some hematologic variances include leukocytosis and the presence of monocytes. Cytologic variances include the presence of prominent nucleoli, resembling prolymphocytes. As mentioned earlier, HCL-v, even with all its similarities to HCL, should

be thought of as a completely separate disease and treated as such. Within the spleen, HCL-v maintains the pure red pulp distribution of HCL and can similarly form blood lakes. Bone marrow involvement by HCL-v is also similar to HCL; however, unlike HCL the bone marrow is aspirable and without significant reticulin fibrosis. Additionally, the degree of peripheral blood involvement is usually greater. HCL-v cells variably express TRAP; CD25, cyclin D1, and annexin A1 are characteristically absent, although CD103 may be expressed.

SMZL is characterized by loosely organized nodular, non-paratrabecular, lymphoid infiltrates in the bone marrow, rather than the interstitial pattern of HCL. The lymphoid cells have a moderate amount of pale cytoplasm, with indistinct cytoplasmic borders. Some cases may show an intrasinusoidal distribution of tumor cells. As in HCL, there may be marked splenomegaly without peripheral lymphadenopathy. The pattern of involvement in the spleen is starkly different from that of HCL, with predominantly white pulp, rather than mainly red pulp involvement.

Splenic diffuse red pulp small B-cell lymphoma is included as a provisional entity in the 2008 WHO classification. It appears to correspond to many cases previously diagnosed as SLVL [7,8]. A typical feature is an intrasinusoidal localization pattern in the bone marrow (Figure 4). Peripheral blood involvement is generally more prominent than seen in HCL, especially early in the course of the disease. The cells have abundant pale cytoplasm, often with villous projections mimicking hairy cells. As the name implies, in the spleen the neoplastic cells diffusely infiltrate the red pulp, involving both cords and sinuses. Blood lakes are usually absent. Immunophenotypic studies are useful in the distinction with HCL, as the cells express B-lineage markers, but annexin A1 is consistently negative; the cells usually lack immunoglobulin D (IgD), CD5, CD25, CD103, and CD11c.

HCL is a characteristic entity that demonstrates a significant response to disease appropriate therapy. An appropriate diagnosis can be rendered in nearly all situations due to the reliable and reproducible cytomorphologic and immunophenotypic features of this disease. Morphologically similar lymphoproliferative disorders differ in their response to HCL therapy, and therefore careful diagnostic evaluation is essential.

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Figure 1. Hairy cell leukemia, cytological features. (A) Peripheral blood smear showing characteristic 'hairy' cell with cytoplasmic projections and frayed cytoplasmic borders ($\times 1000$; Wright–Giemsa). (B) Touch preparation from spleen demonstrating representative hairy cells. Note loss of villous cytoplasmic projections ($\times 1000$; Wright–Giemsa).

Figure 2.
Hairy cell leukemia, bone marrow biopsy. (A) Bone marrow with relatively inconspicuous interstitial infiltrate of medium-sized lymphoid cells with ample cytoplasm ($\times 200$; H&E). (B) CD20 immunohistochemical stain aids in identification of neoplastic cells ($\times 200$; IHC).

Figure 3. Hairy cell leukemia involving lymph node. Replacement of lymph node paracortex and interfollicular areas by a relatively monotonous infiltrate of medium-sized lymphoid cells with ample cytoplasm. Sinuses are widely patent ($\times 40$; H&E). Inset: high-power image of atypical infiltrate. The lymphoid cells have abundant cytoplasm and distinct cytoplasmic borders ($\times 400$; H&E).

Figure 4. Differential diagnosis of hairy cell leukemia. (A) Peripheral blood with features of splenic lymphoma with villous lymphocytes (SLVL). This pattern is most commonly associated with diffuse red pulp small B-cell lymphoma. The cells have ample cytoplasm and villous cytoplasmic projections morphologically consistent with 'hairy' cells ($\times 400$; Wright–Giemsa). (B) CD20 immunohistochemical stain highlights the typical intrasinusoidal localization of the neoplastic cells ($\times 40$; IHC).

Table 1.

Immunohistochemical profiles of small B-cell lymphomas which present with splenomegaly and bone marrow disease.

	CD5	Cyclin D1	Sox11	CD23	CD25	IgD	TRAP	Annexin A1	CD103	CD11c
Hairy cell	-	+	+/-	-	+	-	+	+	+	+
Splenic red pulp	-	-	ND	-	-	-	-	-	-	-/+
HCL – variant	-	-	ND	-	-	-	+/-	-	+	+
CLL/SLL	+	-	-	+	-	+	-	-	-	-/+
SMZL	-	-	-	-	-	+	-	-	-	-
MCL	+	+	+	-	-	+	-	-	-	-

HCL, hairy cell leukemia; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; SMZL, splenic marginal zone lymphoma; MCL, mantle cell lymphoma; IgD, immunoglobulin D; TRAP, tartrate resistant acid phosphatase; ND, not determined.