

# A rare case of hairy cell leukemia with co-expression of CD5 and cyclin D1: A diagnostic pitfall

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**Abstract.** Hairy cell leukemia (HCL) is an uncommon chronic B-cell lymphoproliferative disease with an indolent course. It mainly occurs in elderly men, although abdominal lymphadenopathy is rare. HCL cells are mostly found in the bone marrow, peripheral blood, and spleen and typically express CD11c, CD20, CD25 and CD103. We present a case of HCL with a novel immunophenotype. A 48-year-old woman presented with pancytopenia and splenomegaly. The diagnosis was HCL with lymph node infiltration. Unlike previously described HCL cases, the current case showed strong expression of CD5 and cyclin D1 in the lymph nodes. The patient underwent cladribine chemotherapy, and the leukocyte count increased during and after treatment. The 8-month follow-up revealed that she had recovered well. This case highlights the distinctive immunophenotype of HCL infiltrating the lymph nodes and the potential misdiagnosis of HCL as mantle cell lymphoma. It also adds to our limited understanding of HCL.

## Introduction

Hairy cell leukemia (HCL) is a very rare, indolent disease that typically occurs in middle-aged adults; the disease was first recognized by the World Health Organization in 2008 (1). Its most common symptoms include fatigue and left upper quadrant pain, with most patients presenting with splenomegaly. The diagnosis is typically based on the presence of hairy cells with a 'fried-egg' appearance

in bone marrow biopsy samples. Hairy cells are small- to medium-sized mature B lymphoid cells with oval nuclei and pale blue cytoplasm (2).

The HCL immunophenotypic profile is characterized by the expression of CD19, CD20, CD22, and CD200. Cyclin D1 is expressed in approximately 50-70% of cases, whereas CD5 is weakly expressed in only approximately 0-2% (1,3). The BRAF V600E gene mutation is found in >97% of HCL cases (4), and is associated with poor prognosis (2). As its clinical symptoms are not obvious, HCL must be differentiated from splenic diffuse red pulp lymphoma, mantle cell lymphoma (MCL), and other B-cell lymphomas (2). A combination of clinical, morphological, immunohistochemical, and molecular features are required for the diagnosis of HCL.

In the present study, we report a rare case of HCL with lymphadenopathy in multiple nodes and a distinctive immunophenotype characterized by CD5 and cyclin D1 expression in the lymph nodes.

## Case report

*Patient and clinical data.* A 48-year-old woman was admitted to our hospital with a 2-year history of pancytopenia and splenomegaly. She had worked in an oil refinery and was often exposed to ammonia, hydrogen sulfide, and other gases for many years. Peripheral blood analysis showed low neutrophil counts ( $2.0 \times 10^9/l$ ), hemoglobin levels (98 g/l), and platelet counts ( $59 \times 10^9/l$ ) (Table I). Positron emission tomography-computed tomography (CT) revealed splenomegaly, high local infarction metabolism, active bone marrow hyperplasia, and multiple metabolically active lymph nodes around the mediastinum and aorta (Fig. 1A). B-scan ultrasonography revealed splenomegaly and mediastinal, neck, and upper clavicle multiple lymphadenopathy (Fig. 1B). Abdominal CT showed marked enlargement of the spleen. Thus, lymphoma with splenic and bone marrow infiltration was considered (Fig. 1C).

On physical examination, the patient appeared to be well and was not anemic or pale. No skin xanthochromia, petechiae, or ecchymosis was noted. Other findings included arrhythmia without a pathological murmur, clear pulmonary respiration, a soft abdomen without tenderness or rebound pain, an intact liver and a spleen subcostal region of 10 cm. There was no edema in the lower extremities or significant abnormalities in kidney and

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*Abbreviations:* HCL, hairy cell leukemia; CT, computed tomography; MCL, mantle cell lymphoma; CLL, chronic lymphocytic leukemia/small lymphocytic lymphoma

*Key words:* CD5, cyclin D, hairy cell leukemia, immunophenotype, mantle cell lymphoma, immunophenotype

Table I. Laboratory data.

Variable	On admission	Reference range, adults
Hemoglobin (g/l)	98	113-151
White-cell count ( $\times 10^9/l$ )	2.0	3.69-9.16
Differential count (%)		
Neutrophils	88.6	50-70
Lymphocytes	7.8	20-40
Monocytes	0.6	3-10
Eosinophils	2.5	0.5-5
Basophils	0.5	<1.0
Red-cell count ( $\times 10^{12}/l$ )	2.09	3.68-5.13
Platelet count ( $\times 10^9/l$ )	59	101-320
Albumin (g/l)	30	35-55
Total bilirubin ( $\mu\text{mol}/l$ )	12.2	4.7-24
Direct bilirubin ( $\mu\text{mol}/l$ )	2.5	0-6.8
D-dimer (mg/l)	0.61	<0.55
Fibrinogen (g/l)	1.5	1.8-3.5
Epstein-Barr virus viral capsid antigen IgG antibody	Negative	Negative
Epstein-Barr virus viral nuclear antigen IgG antibody	Negative	Negative

Reference range values are affected by the patient population and the laboratory methods used. They may therefore not be appropriate for all patients.

liver function tests. Flow cytometric immunophenotypic analysis of bone marrow aspirates showed expression of CD5 and CD19 (Fig. 2A). The typical morphological features of circulating hairy cells in the bone marrow can be seen in peripheral blood smear photomicrographs (Fig. 2B). The morphological features identified following the bone marrow biopsy are shown in Fig. 2C and D. Histological analysis of upper clavicle lymph node biopsy samples showed a proliferation of small- to medium-sized lymphoid cells with a vaguely nodular growth pattern (Fig. 2E). The lymphoid cells had slightly irregular nuclear contours, and many small vessels were evident (Fig. 2F). The pathological diagnosis was challenging.

Cladribine chemotherapy was then administered to the patient. The patient experienced bone marrow depression and fever accompanied by agranulocytosis. Anti-inflammatory drugs including cephalosporin, were administered. The patient's body temperature returned to normal. The 8-month follow-up revealed that the patient had recovered well.

This study was approved by the Ethics Committee of Ruijin Hospital, Shanghai Jiaotong University School of Medicine (Shanghai, China). Written informed consent was obtained from the patient for publication of this case report and accompanying images with preservation of the patient's anonymity.

**Pathological findings.** The patient's bone marrow had morphologic features typical of HCL: Circulating small- to medium-sized hairy cells with abundant clear cytoplasm and oval or indented nuclei. Bone marrow biopsy showed a diffuse infiltration of lymphoid cells with oval nuclei, abundant cytoplasm, and prominent borders. Mitotic figures were absent. The B-cell infiltrate was positive for CD5, CD20, CD79, cyclin D1,

Bcl-2, CD25, CD103, CD11C, whereas IgD, CD3, CD10, CD23, and Mum-1 were not expressed (Fig. 3). Immunohistochemistry of the hairy cell leukemia cells in the bone marrow biopsy shows strong and diffuse expression of CD20, CD5, CD103 and partially positive expression of Annexin A1 (Fig. 4).

Immunohistochemical staining was performed using the Envision 2-step method with 3,3'-diaminobenzidine as the substrate. The slides were counterstained with hematoxylin, and CD5, CD11C, CD20, CD25, CD79, CD103, cyclin D1, Bcl-2, CD25, CD103, CD11C, IgD, CD3, CD23 and Mum-1 were all prediluted from DAKO. CD10 (DAKO) dilution of 1:80 was used and appropriate positive controls was used for all assays.

CCND1 t(11;14)(q13;q32) gene fusion and BRAF V600E mutation were assessed in bone marrow samples via *in situ* hybridization and amplification refractory mutation system polymerase chain reaction (ARMS-PCR), respectively. The BRAF V600E mutation was detected positively (Fig. 5), whereas the CCND1 t(11;14)(q13;q32) gene fusion was not. Finally, the overall findings were diagnostic of HCL with lymph node, peripheral blood, and bone marrow infiltration.

## Discussion

This report describes a rare case of HCL involving the lymph nodes and bone marrow and the immunohistochemical expression of CD5 and cyclin D1. Given these characteristics, HCL was initially misdiagnosed as MCL. However, additional analyses revealed features characteristic of HCL but not MCL, namely, the absence of the CCND1 gene fusion, the presence of the BRAF V600E mutation, and the strong expression of CD11C, CD25, and CD103.

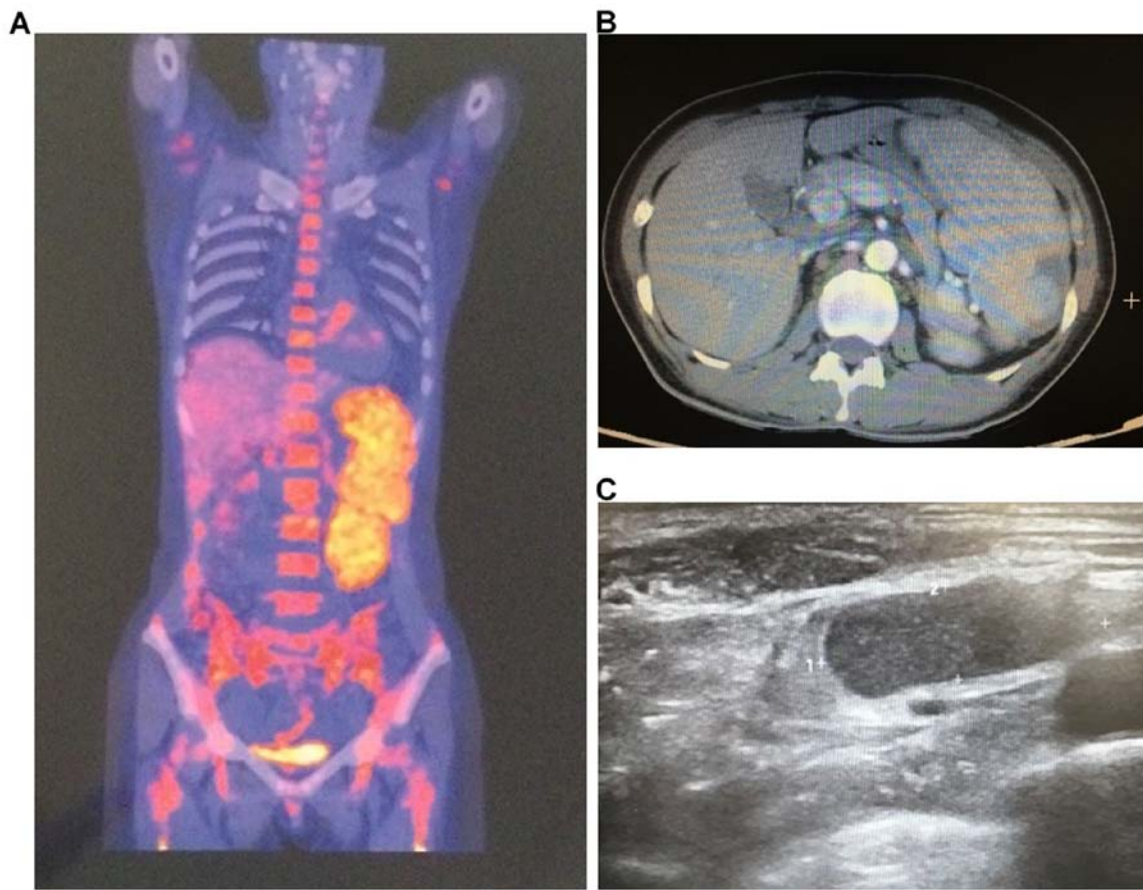


Figure 1. (A) Positron emission tomography-computed tomography (CT). (B) Abdominal CT shows marked enlargement of the spleen. (C) Ultrasound shows enlarged lymph nodes in the clavicle.

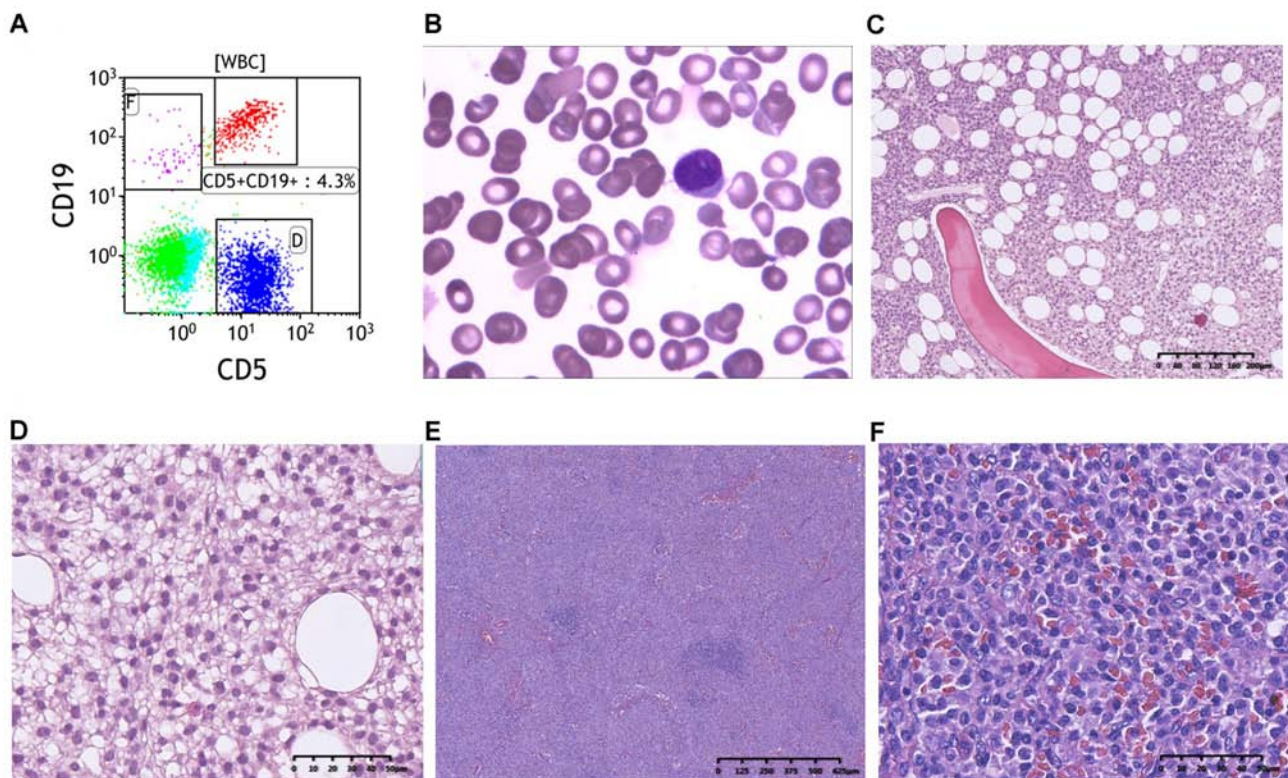


Figure 2. (A) Flow cytometric immunophenotypic analysis of bone marrow aspirates showed expression of CD5. (B) The typical morphological features of circulating hairy cells in the bone marrow. (C and D) The morphological features in the bone marrow biopsy (x100, x400, respectively). (E and F) Morphologic characteristics of upper clavicle lymph node biopsy (x40, x400, respectively).

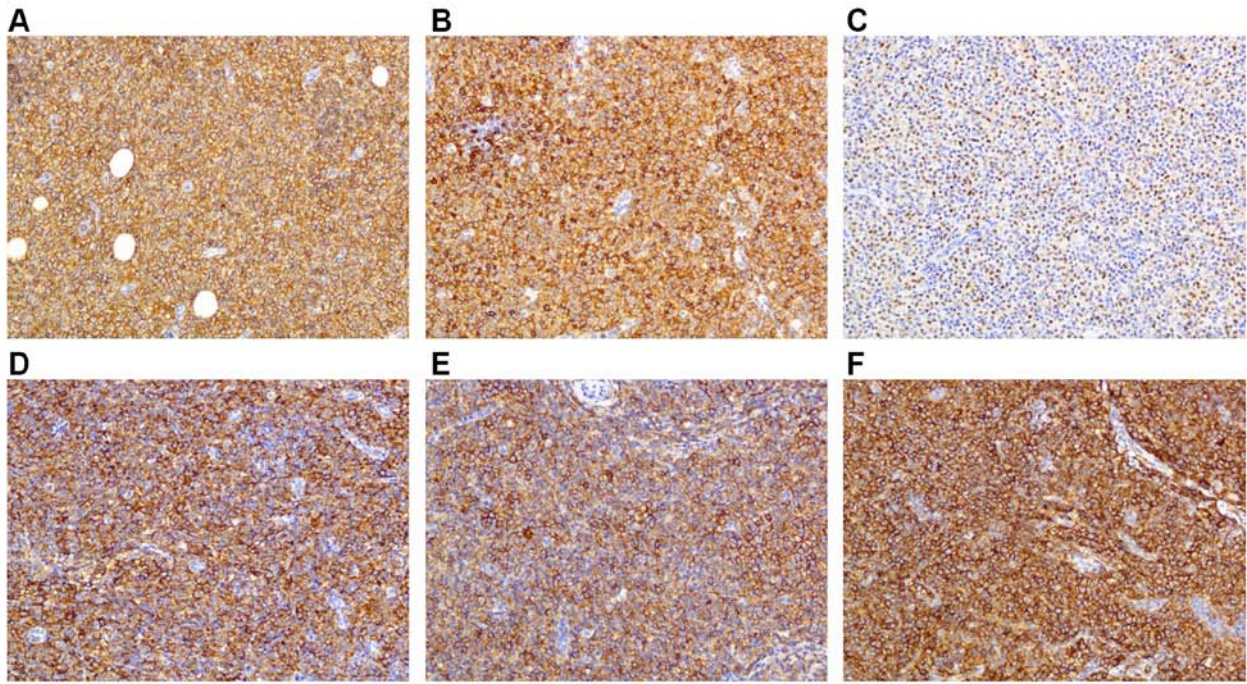


Figure 3. Immunohistochemistry of the hairy cell leukemia cells in the lymph nodes shows strong and diffuse expression of (A) CD20, (B) CD5, (C) cyclin D1, (D) CD25, (E) CD11c, and (F) CD103. Original magnification x400.

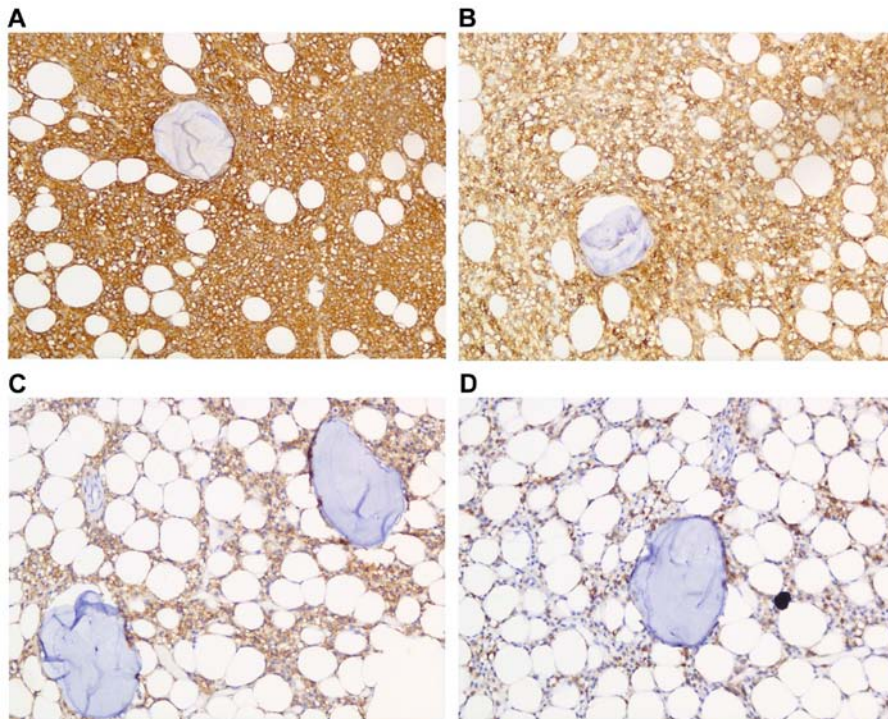


Figure 4. Immunohistochemistry of the hairy cell leukemia cells in the bone marrow biopsy shows strong and diffuse expression of (A) CD20, (B) CD5, (C) CD103 and (D) partially positive expression of Annexin A1. Original magnification x200.

HCL is a chronic malignant hematological malignancy characterized by dysplastic pallidum-like cells. Bouroncle *et al* first described it in 1958 (5), and Schrek and Donnelly coined the term 'HCL' in 1966 in a report of two patients with leukemia who had cells identified with numerous hairy processes on the edges of the peripheral blood cells (6). HCL is very rare, accounting for only 2% of all lymphoid leukemias. It mainly affects the elderly,

but is not uncommon in young and middle-aged adults (7). Most patients present with pancytopenia and splenomegaly, and anemia and infection have also been reported (8). All the patients with HCL have different degrees of splenomegaly, and diffuse expansion of the red pulp can be seen macroscopically. HCL infiltrates the lymph nodes in a marginal zone or interfollicular pattern, and the nodal sinuses are often preserved (8). In the

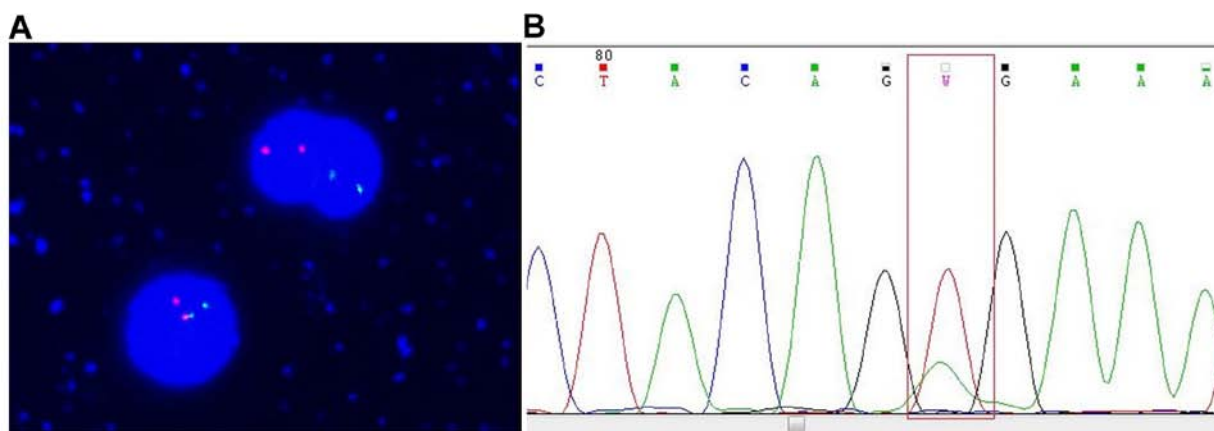


Figure 5. CCND1 t(11;14)(q13;q32) fusion was not detected via fluorescent *in situ* hybridization, we used CCND1 t(11;14)(q13;q32) gene fusion probe. (A) The red and green signals are separated, and no fusion is evident in the figure: The red signals represent probe signals (cyclinD1[CCND1];11q13) and the blue signals represent immunoglobulin heavy chain [IgH];14q32). (B) The BRAF V600E mutation was detected in the bone marrow via amplification refractory mutation system polymerase chain reaction (ARMS-PCR).

present case, multiple lymph nodes were enlarged and CD5 and cyclin D1 were strongly co-expressed in the infiltrating lymph nodes and bone marrow. However, co-expression of CD5 and cyclin D1 in the lymph nodes of patients with HCL has never been previously reported.

Cortazar *et al* reported that the immunophenotype of nodal/extranodal diseases overlapped with that of other small B-cell lymphomas; proteins expressed by both sets of diseases included CD5 and cyclin D1 (3). MCL is a mature B-cell neoplasm that accounts for 3-10% of malignant lymphomas. It has an atypical 'hairy cell-like' morphology that can easily be confused with that of other malignant lymphomas with hairy cytoplasmic projections (9). Its hallmark is CD5 and cyclin D1 positivity (10,11). As the HCL in our case strongly expressed CD5 and cyclin D1, it was difficult to differentiate it from MCL based on immunophenotype alone.

HCL should be differentiated from the HCL-variant, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL), and other indolent B-cell lymphomas. The HCL-variant is a rare disease that was first identified by Cawley *et al* (12). Immunophenotypically, HCL cells usually express the mature B-cell markers CD19 and CD20, as well as CD11c, CD25, CD103, and CD123. By contrast, the HCL-variant cells often express CD11c and CD103 but not CD25 or CD123. Moreover, the *BRAF* V600E mutation is present in essentially all HCL cases, but is not present in HCL-variant cases (13-15). CLL is characterized by mature B lymphocyte clonal proliferative tumors characterized by lymphocyte aggregation in the peripheral blood, bone marrow, and spleen. The diagnostic requirements for CLL are a peripheral blood B lymphocyte count  $>5 \times 10^9/l$  and CD5 and CD23 expression in B cells. The lymph node in our case was CD23-negative, which distinguishes our case from CLL.

Immunohistochemical and molecular analyses are essential for distinguishing HCL from other small B-cell lymphomas. HCLs co-express CD25, CD103, and CD123. Although common to both HCL and MCL, cyclin D1 is weakly expressed in the former and strongly in the latter. CCND1-IGH translocations are present in MCLs but absent in

nearly all HCLs (16,17). Conversely, the BRAF V600E mutation is present in nearly all HCLs but is not present in other B-cell lymphomas (18).

In conclusion, diagnosis of HCL requires collective consideration of cytological, histological, immunohistochemical data and cytogenetic abnormalities.

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#### Availability of data and materials

All data generated or analyzed during this study are included in this published article.

#### Authors' contributions

LZ performed the histological examination of this disease, and was a major contributor in writing the manuscript. HX performed immunohistochemical staining and analysis. JZ and BO collected the clinical data. CW participated in the design of the study and assisted in writing the manuscript. All authors read and approved the final manuscript.

#### Ethics approval and consent to participate

This study was approved by the Ethics Committee of Ruijin Hospital, Shanghai Jiaotong University School of Medicine (Shanghai).

#### Patient consent for participation

Written informed consent was obtained from the patient for publication of this case report and accompanying images with preservation of the patient's anonymity.

## Competing interests

The authors declare that they have no competing interests.

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