Concurrent CD5-negative small lymphocytic lymphoma (SLL) and CD5-positive metastatic carcinoma of unknown primary in a lymph node biopsy

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Abstract CD5 expression is considered a key marker for the diagnosis of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). We report an unusual case of CD5-negative (CD5[−]) SLL with a concurrent CD5-positive (CD5⁺) adenocarcinoma of unknown primary involving the same lymph node. An 83-year-old woman with a history of CD5[−] B cell lymphoma diagnosed on a core biopsy of a cervical lymph node presented with jaundice and was found to have a 2.5-cm pancreatic mass. A fine needle aspiration of the mass revealed a CD5[−] monotypic B cell population. A subsequent excisional biopsy of a cervical lymph node showed morphologic findings typical for SLL, including the presence of multiple proliferation centers. However, the neoplastic lymphocytes were negative for CD5 by both flow cytometric analysis and immunohistochemistry. Interestingly, multifocal metastatic adenocarcinoma was identified and was positive for CD5. Lack of CD5 expression often leads to the exclusion of CLL/SLL, particularly in small biopsy samples. However, rare cases of CD5[−] CLL/SLL have been reported. In addition, CD5 is not an exclusive marker for lymphoid cells; its expression has been observed in several non-hematopoietic neoplasms. This report briefly reviewed and discussed CD5[−] CLL/SLL and CD5⁺ carcinoma reported in the literature.

Keywords Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) · Immunophenotype · CD5 · Metastatic carcinoma

Introduction

Chronic lymphocytic leukemia (CLL) is the most common leukemia of adults in Western countries. Small lymphocytic lymphoma (SLL) is the tissue counterpart of the same disease. In peripheral blood and bone marrow, the CLL cells are small with condensed chromatin and scant cytoplasm. In tissue sections, SLL shows diffuse proliferation of neoplastic lymphocytes with an effacement of the normal lymph node architecture. The presence of “proliferation centers” in lymph node sections is virtually pathognomonic for SLL. The typical immunophenotype of CLL/SLL is dim CD20⁺, CD5⁺, CD19⁺, CD23⁺, CD79b[−], and FMC7[−] with dim surface immunoglobulin (Ig) staining [1]. CD5 expression is the most consistent immunophenotype and is considered a key marker for the diagnosis of CLL/SLL. However, cases with unusual morphologic or immunophenotypic features have been reported. Here, we reported a rare case of CD5[−] SLL with concurrent CD5⁺ metastatic adenocarcinoma of unknown primary in a lymph node biopsy.

Case report

The patient was an 83 year-old woman with a past medical history of essential thrombocythemia treated with anagrelide and hydroxyurea and with a recent diagnosis of CD5[−] small B cell lymphoma on a needle core biopsy of a cervical lymph node at an outside hospital. The B cell lymphoma could not be further subclassified in the core biopsy based on the morphologic findings and the immunostaining results (CD20⁺, CD5[−], CD10[−], CD23⁺, and cyclin D1[−]). Specifically, mantle cell lymphoma was unlikely because of the negative staining for both CD5 and cyclin D1. The patient presented to our hospital with jaundice and was found to

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have a 2.5-cm mass in the pancreatic head with biliary obstruction, dilated common bile duct, and significant abdominal lymphadenopathy. A fine needle aspiration of the pancreatic mass revealed numerous small lymphocytes with condensed chromatin and scant cytoplasm. Flow cytometric analysis demonstrated a predominantly CD5– and CD10– monotypic B cell population with similar immunophenotype to that reported in the referring hospital. Her CBC at the time of presentation was within normal range with WBC of $7.8 \times 10^3/\mu\text{L}$, hemoglobin of 11.9 g/dL, and platelets of $409 \times 10^3/\mu\text{L}$. A morphologic review of the peripheral smear was unremarkable with no absolute lymphocytosis.

To further characterize this B cell lymphoma, an excisional biopsy of the cervical lymph node was performed. The lymph node was approximately $3.5 \times 3.0 \times 1.6$ cm. Hematoxylin–eosin-stained tissue sections showed complete effacement of normal lymph node architecture with diffuse proliferation of a monotonous population of small lymphocytes with round nuclei, condensed chromatin, and scant cytoplasm. Multiple “proliferation centers” characteristic for CLL/SLL were seen (Fig. 1a, b). In addition, multiple foci of metastatic adenocarcinoma were identified and showed well-formed, irregularly-shaped glandular structure with background fibrosis. The glands were haphazardly arranged and composed of cuboidal cells with vesicular nuclei, prominent nucleoli, and moderate amount of amphophilic cytoplasm (Fig. 2).

Flow cytometric analysis of the lymph node biopsy revealed a monotypic B cell population with kappa-restricted surface immunoglobulin light chain expression. The monotypic B cells were CD19+, dim CD20+, predominantly CD5–, CD23+, CD10–, CD79b–, and dim FMC7+ (Fig. 3). The negative staining for CD5 was confirmed by repeated staining for CD5 with two additional independent

Fig. 2 The lymph node biopsy shows foci of metastatic adenocarcinoma (hematoxylin–eosin, original magnification $\times 400$)

anti-CD5 antibodies. Immunohistochemical staining demonstrated that the neoplastic lymphocytes were positive for CD20 but negative for CD5, CD10, BCL-6, and cyclin D1 (Fig. 4). The morphologic findings along with the negative staining for both CD5 and cyclin D1 essentially ruled out mantle cell lymphoma. Follicular lymphoma was also ruled out based on the morphologic findings and negative staining for both CD10 and BCL-6. Although the immunophenotype alone may lead to the possible diagnosis of a marginal zone lymphoma, the presence of proliferation centers makes this diagnosis unlikely. We also performed immunostaining for LEF-1 in this case since our recent findings indicate that nuclear overexpression of LEF-1 is highly associated with CLL/SLL among small B cell lymphomas [2]. The results

Fig. 1 **a** Low power view of the lymph node biopsy demonstrates multiple proliferation centers (arrows) (hematoxylin–eosin, original magnifications $\times 40$). **b** The proliferation center contains prolymphocytes and paraimmunoblasts (hematoxylin–eosin, original magnifications $\times 600$)

Fig. 3 Flow cytometric immunophenotyping reveals a CD19+ B cell population that is predominantly CD5–, CD10–, and CD23+, with kappa-restricted surface immunoglobulin light chain expression

showed that virtually all neoplastic lymphocytes were strongly positive for LEF-1 (Fig. 4c). To further confirm the diagnosis of CLL/SLL, interphase fluorescent in situ hybridization (FISH) analysis for CLL panel was performed on touch slides prepared from the lymph node biopsy and showed that 82 % of the cells analyzed have

a deletion of the ATM locus (11q22.3). No other abnormalities involving CEP6 and MYB (6q23), CEP12, P53 (17p13.1), D13S319, and LAMP1 (13q34) loci were present. Translocation or rearrangement involving the IGH and CCND1 was not identified using dual fusion and IGH break-apart probes.

Fig. 4 **a** Immunohistochemical stain for CD20 shows that the neoplastic lymphocytes are positive. (immunoperoxidase, original magnification $\times 400$). **b** Immunohistochemical stain for CD5 shows that the neoplastic lymphocytes are negative, but the adenocarcinoma cells are

positive (immunoperoxidase, original magnification $\times 400$). **c** Immunohistochemical stain demonstrates strong nuclear staining of LEF1 in neoplastic lymphocytes (immunoperoxidase, original magnification $\times 400$)

Thus, a diagnosis of CD5[−] CLL/SLL was established based on the characteristic morphologic findings in the lymph node, the remaining immunophenotype (dim CD20⁺, CD23⁺, CD10[−], CD79b[−], and dim FMC7⁺), and exclusion of other types of small B cell lymphoma such as follicular lymphoma and CD5[−] mantle cell lymphoma. The diagnosis of CLL/SLL was further supported by positive staining for LEF-1.

Subsequently, a staging bone marrow biopsy showed extensive interstitial and nodular non-paratrabeular lymphoid infiltrates with rare proliferation centers. The infiltrate is composed of small lymphocytes with condensed chromatin. The immunophenotype of the lymphocytes was similar to that previously identified in the lymph node. These findings are consistent with CLL/SLL with an extensive bone marrow involvement.

Interestingly, a metastatic adenocarcinoma was incidentally found in the lymph node biopsy (Fig. 2), and CD5 staining for workup of the lymphoma highlighted the metastatic adenocarcinoma in a background of CD5[−] SLL (Fig. 4b). Immunostaining to evaluate the metastatic carcinoma showed that the carcinoma cells were positive for CK7 and negative for CK20, CDX2, ER, PR, mammaglobin, and BRST-2. Negative staining for mammaglobin and BRST-2 essentially excludes breast primary, and negative staining for ER and PR does not support ovarian or endometrial origin. The positive staining for CK7 along with the negative staining for CK20 and CDX2 makes colorectal adenocarcinoma unlikely but is suggestive of pancreatic and biliary primary. However, biopsy to confirm the pancreatic primary of the carcinoma was not performed due to the patient's overall health condition. Extensive radiology workup did not reveal any other mass lesions. She was treated with rituximab for lymphoma and a nucleoside analog gemcitabine for carcinoma and was later discharged after a biliary drainage in place to relieve the obstructive symptoms. She was later followed up with her primary oncologist at a local hospital for additional chemotherapy.

Discussion

CLL/SLL is a B cell leukemia/lymphoma composed of small mature lymphocytes involving the peripheral blood, bone marrow, lymph node, and spleen. In the peripheral blood, the neoplastic lymphocytes are usually small with condensed chromatin and scant cytoplasm; smudge cells are often present. In tissue section, CLL/SLL shows effacement of normal architecture and may demonstrate proliferation centers that contain variable numbers of prolymphocytes. The presence of proliferation centers is virtually diagnostic for SLL.

The typical immunophenotype of CLL/SLL is as follows: CD19⁺, dimCD20⁺, CD22⁺, CD5⁺, CD10[−], CD23⁺, dim surface immunoglobulin IgM/IgD⁺, and negative or weakly positive for FMC7 and CD79b [1]. Variations in immunophenotype in CLL/SLL are recognized [1]. Among the immunophenotypic markers, the expression of CD5 appears the most consistent finding in CLL/SLL and thus becomes a key marker in differential diagnosis of CLL/SLL from other small B cell lymphoma/leukemia. However, CD5[−] CLL cases have been reported [3–6]. Since the classification of mature B cell lymphoma/leukemia has evolved rapidly in recent years, many of these cases reported in the early literature may represent other types of lymphoma/leukemia based on the current classification. Nonetheless, the literature and our current case indicate that CD5[−] CLLs are rare but do exist. Thus, lack of CD5 expression should not completely exclude the possibility of CLL/SLL. Correlation with morphologic findings such as the presence of proliferation centers is important when immunophenotype is not typical for CLL/SLL.

Our case also illustrates the importance of excisional biopsy in evaluating low-grade B cell lymphoma. The excisional lymph node biopsy of our patient showed the presence of multiple proliferation centers, but this diagnostic feature was not appreciable on the initial needle core biopsy. In addition, ancillary studies such as FISH CLL panel for evaluation of the abnormalities commonly observed in CLL/SLL may be helpful in the differential diagnosis. In our case, FISH analysis on the touch slides of the lymph node revealed a deletion of the ATM locus on chromosome 11q22.3 in 82 % of cells analyzed. Although not specific for CLL/SLL, a deletion of ATM is a recurring abnormality, detected by FISH in about 15–20 % of CLL, and is observed in patients with advanced disease. The diagnosis of CLL/SLL in this case was also confirmed by a new immunohistochemical marker we previously reported, LEF-1, that is highly associated with CLL/SLL [2]. The strong nuclei staining of LEF1 in this case also support the diagnosis of CLL/SLL.

Negative staining for CD5 may not always indicate negative expression of CD5 in the neoplastic lymphocytes; it could be caused by epitope deletion or masking. Current commercially available monoclonal antibodies are produced targeting specific epitope of the antigen. Deletion of even a single amino acid from the targeted epitope may result in reduced or complete loss of antigen–antibody reactivity. In addition, the three-dimensional structure of certain epitopes may limit the access of the antibody. Thus, in our laboratory, negative staining of CD5 as well as other “key” markers that may impact the diagnosis in a newly diagnosed B cell lymphoma is always confirmed by repeat staining with two additional monoclonal antibodies against different epitopes of the same molecules.

CD5 is not an exclusive marker for lymphoid cells. In normal skin, strong CD5 staining can be seen on apocrine glands and deep dermal eccrine glands, and weak CD5 staining is observed on the smooth muscle [7]. Expression of CD5 has been reported in several non-hematopoietic epithelioid neoplasms. Almost all benign and malignant apocrine lesions and 70–80 % of the eccrine tumor demonstrate at least focal CD5 expression [7]. CD5 expression was also reported in almost all cases of thymic carcinoma, 50 % of atypical thymoma, and all carcinoma showing thymus-like differentiation [8, 9]. Although the data on the expression of CD5 in pancreatic or biliary carcinoma are limited in the literature, Chu et al. reported a high CD5 expression in gastrointestinal tumors, including 6/13 pancreatic ductal carcinomas and 12/14 cholangiocarcinomas in their series [10]. Thus, CD5 expression may have a diagnostic value in evaluating carcinoma with unknown primary.

It is worth pointing out that different clones of commercially available antibodies or different laboratory protocols may impact the staining pattern. Walsh et al. reported that using one commercial CD5 monoclonal antibody, positive CD5 immunohistochemical staining was observed in 78 % of 60 breast infiltrating ductal carcinomas and there was a significant correlation between CD5 positivity and higher tumor grade. However, no significant CD5 staining was observed with two other CD5 clones [11]. Nonetheless, the published data indicate that CD5 expression can be detected in a variety of non-lymphoid tumors by immunohistochemistry. It is important to be aware of this and use a thorough immunohistochemical panel to differentiate CD5-positive hematopoietic neoplasm from poorly differentiated carcinoma. In our case, the immunostaining results of the CD5+ adenocarcinoma, the clinical findings (jaundice and pancreatic mass), and the lack of other detectable mass lesion on radiology all suggest pancreatic/biliary origin of the carcinoma. It is very unusual for a B cell lymphoma to first present as pancreatic mass and jaundice, although this remains as a possibility. But due to the patient's poor clinical condition, this could not be confirmed by biopsy or surgery.

Both carcinoma and CLL/SLL are more prevalent in elderly individuals. In addition, patients with CLL/SLL may have increased risk for developing a secondary malignancy [12]. Although identifying CLL/SLL and concurrent metastatic carcinoma in a lymph node biopsy has been reported before [13–16], the incidental finding of a metastatic carcinoma of unknown primary in a lymph node biopsy for lymphoma workup is relative uncommon.

Here, we report a unique case of incidental finding of a metastatic CD5+ adenocarcinoma of unknown primary in a lymph node with CD5– CLL/SLL. This rare case illustrates two important points. First, cases of CD5– CLL/SLL do exist, and lack of CD5 expression should not completely exclude the possibility of CLL/SLL. Second, CD5 is not an

exclusive marker for a hematopoietic neoplasm and may be expressed in epithelial tumors. Thus, it is important to use an appropriate immunohistochemical panel to differentiate CD5-positive hematopoietic neoplasms from poorly differentiated carcinoma.

Conflict of interest The authors declare that they have no conflict of interest.

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