Alternate Clonal Dominance in Richter Transformation Presenting as Extranodal Diffuse Large B-Cell Lymphoma and Synchronous Classic Hodgkin Lymphoma

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ABSTRACT

Objectives: Richter transformation (RT) represents the rare occurrence of a secondary aggressive lymphoma in the setting of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL).

Methods: Here we describe the peculiar case of a patient with trisomy 12+ and atypical (CD5+, CD23−) CLL/SLL who developed a two-step RT with complex morphologic and molecular features.

Results: Molecular analysis of a CLL/SLL population detected two different immunoglobulin rearrangement patterns corresponding to a main peak and a minor peak. Transformation took place both as gastric diffuse large B-cell lymphoma and as a synchronous bone marrow classic Hodgkin lymphoma with the same immunoglobulin rearrangement pattern corresponding to the minor peak detected in CLL/SLL at diagnosis. During chemotherapy, progression occurred as axillary nodal involvement by a CD5+ high-grade lymphoma with an immunoglobulin rearrangement pattern corresponding to the main CLL peak.

Conclusions: In this case, the elaborate clinical and molecular picture may be correlated to an alternate dominance of two distinct clonal populations probably influenced by therapeutic and environmental factors.

Richter transformation (RT) consists of a secondary aggressive lymphoma that develops in the setting of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). Commonly, a diffuse large B-cell lymphoma (DLBCL) is observed,1,2 while other entities, such as classic Hodgkin lymphoma (cHL), are infrequent.3–6 During the past years, it has been learned that the transformation may be clonally related to CLL/SLL or developed de novo, probably because of the immunosuppression determined by the primary disease or from the prior treatments.7 RT may be constituted by two different lymphomas at the same time, but there are just a few cases in the literature about this extremely rare presentation. Moreover, to our knowledge, no one has ever tried to study the clonal origin of these entities, and the few reports about this limit their analysis to a morphologic point of view.

In this article, we describe the case of a patient affected by atypical (CD5+, CD23−) CLL/SLL who developed a
two-step RT with complex morphologic and molecular features. In a first phase, transformation took place as separate and synchronous gastric DLBCL and bone marrow cHL. Despite their different morphology, phenotype, and Epstein-Barr virus (EBV) infection status, they shared the same immunoglobulin rearrangement pattern corresponding to the minor peak detected in CLL/SLL, thereby suggesting the same clonal origin.

During chemotherapy, after an initial complete response, progression occurred as axillary nodal involvement by a high-grade lymphoma with an immunoglobulin rearrangement pattern corresponding to the main CLL peak found at diagnosis. In light of the recent emerging hypothesis that clone heterogeneity in neoplasms may result in nonlinear progression, the elaborate clinical and pathologic picture we observed in this case may be correlated to an alternate dominance of two distinct clonal populations probably influenced by therapeutic and/or environmental (EBV infection) factors.

This case represents a very rare example of how a neoplasm can differentiate during its natural history into subclonal populations with different morphologic and biologic features, including a different response to the treatments, thereby potentially conditioning the clinical management of the patient.

Case Report

A 74-year-old man came to our institution with multiple lymphoadenomegaly (laterocervical, axillary, crural, and retrocephalopancreatic) and peripheral lymphocytosis (peripheral blood lymphocyte count, 7,860/mm3). Bone marrow and lymph node biopsies were performed, and blood samples were collected showing a population of small neoplastic B lymphocytes Image 1A characterized by CD20 and CD5 expression; bright expression of CD20, CD79b, and immunoglobulin λ light chain by flow cytometry;
presence of chromosome 12 trisomy; and absence of CD23 by both flow cytometry and immunohistochemistry, CD38 by flow cytometry, and cyclin D1 expression and t(11;14) translocation. FMC7 expression was positive with a moderate intensity. SOX-11 immunohistochemical staining was negative. Zap-70 and immunoglobulin gene mutational status were not investigated. Fluorescent in situ hybridization (FISH) performed on bone marrow aspirate using standard CLL FISH panel probes and on lymph node samples revealed the presence of elements positive for chromosome 12 trisomy. A diagnosis of atypical CLL/SLL, stage Rai B, was made. Subsequently, the patient underwent six cycles of rituximab, fludarabine, and cyclophosphamide chemotherapy and achieved a complete response.

Three years after the diagnosis, the patient had a severe episode of hematemesis. A peripheral blood test showed a WBC count of 4.430/mmc (lymphocytes, 700/mmc), hemoglobin concentration of 6.4 g/dL, and a platelet count of 164,000/mmc. A diagnostic esophagogastrroduodenoscopy was performed, revealing multiple gastric ulcers that were biopsied.

At histologic examination, gastric mucosa was infiltrated by large lymphoid cells with a prevalent centroblast/paraimmunoblast-like morphology Image 1B and the following immunophenotype: CD20+ Image 1D, CD79a+, CD45+, Pax-5+, CD3−, CD5−, CD10−, CD15−, CD23−, Bcl-6−, Bcl-1−, and Bcl-2 rare elements weakly positive; the Ki-67 proliferation index was 70%.

Image II (cont) D, Large CD20+ cells in gastric mucosa (CD20; ×20). E, Rare CD79a+ small cells in bone marrow; large cells were negative (CD79a; ×20). F, Bone marrow nodule with fibrosis and polymorphous infiltration composed of inflammatory cells and large neoplastic Hodgkin elements (H&E; ×20). G, Neoplastic cells were CD15+ in bone marrow infiltrate (CD15; ×20).
These findings indicated a gastric localization of DLBCL configuring an aggressive transformation of CLL/SLL (ie, RT). Consequently, staging bone marrow biopsy and a computed tomography (CT) scan were performed. The CT scan was negative for significant lymphadenopathies, while on histologic examination, bone marrow showed the following unexpected features: increased cellularity with fibrosis and multinodular polymorphous infiltrate, including small lymphoid cells (prevalently T CD3+, CD4+), histiocytes, eosinophils, plasma cells, and scattered large neoplastic mononuclear cells, frequently with macro-nucleoli.

These large elements were positive for CD30 Image 1C, CD15 Image 1G, Pax-5, and MUM1 and negative for CD45, CD3, CD5, CD23, and CD79a Image 1E. Rare cells were also weakly positive for CD20. These results were compatible with bone marrow localization of cHL.

In light of this new finding, a comparative evaluation of the original lymph node biopsy specimen with CLL/SLL and of the gastric and bone marrow biopsy specimens was made in terms of morphologic and immunohistochemical features, EBV, and cytogenetic and clonality status.

On immunohistochemistry, a discrete percentage of large cells in the gastric biopsy specimen were CD30+ but CD15−; the few small-sized CD5+ elements detected, both in gastric and bone marrow infiltrates, had a distribution similar to CD3+ T lymphocytes; almost all CD20+ and CD79a+ elements in the gastric mucosa were of large size; and no CD79a reaction was found among bone marrow large cell infiltrate. FISH analysis in the gastric biopsy specimen did not detect trisomy 12.

EBV evaluation was performed by immunohistochemical staining for latent membrane protein 1 (LMP-1), EBV-related protein (EBER), and the in situ hybridization technique. LMP-1 was positive in a few large Hodgkin-like cells in the bone marrow, as well as in very rare small/medium lymphoid cells in the gastric mucosa, and was negative in the original lymph node biopsy specimen; the in situ hybridization technique staining showed no positive elements in the original lymph node biopsy specimen, rare large elements positive on the gastric biopsy specimen, and a moderate number of elements in the bone marrow.

In conclusion, the immunomorphologic evaluation did not show any microscopical evidence of CLL/SLL residual infiltration and demonstrated that the gastric and bone marrow localizations had morphologic/phenotypical features and EBV infection patterns clearly distinct from each other.

Immunoglobulin gene rearrangement analysis by polymerase chain reaction revealed two different immunoglobulin gene rearrangement patterns in the original CLL/SLL specimen corresponding, respectively, to a major peak and a minor peak. In RT biopsy specimens, the latter grew to be dominant, while the former was feebly detectable Image 1F.

The evidence of an identical rearrangement pattern in both gastric and bone marrow biopsy specimens indicated a common molecular background for the two lymphomas, despite the clear morphologic and phenotypical differences. Consequently, the patient received an intensive support treatment with multiple blood transfusions and intravenous proton-pump inhibitors to stabilize the clinical picture; then he started a salvage treatment with doxorubicin and prednisone, achieving a partial reepithelization of the gastric mucosa, and finally an immunohemotherapy regiment, with six cycles of rituximab, cyclophosphamide, vincristine, liposomal doxorubicin, and prednisone, targeted toward the more aggressive lymphomatous component.

In September 2012, at the end of the therapy, despite negative gastric and bone marrow biopsy specimens, multiple adenopathies in the right axillary region were evident on the CT scan, indicating disease progression. A lymph node biopsy specimen showed a proliferation of large CD5+ lymphoid cells with an immunoglobulin gene rearrangement pattern corresponding to the original dominant CLL/SLL one and the presence of trisomy 12. Neoplastic cells were also positive for CD45, OCT2, Pax-5, and focally for CD79a and negative for CD20, CD23, CD10, CD15, Bcl-1, Bcl-2, Bcl-6, and CD3. A discrete number of elements were CD30+ and MUM1+ as well. In situ hybridization and immunohistochemical staining for EBV were negative.

The second rearrangement pattern was still present but reverted to the former condition of being the minor component. Therefore, the patient started a salvage treatment with gemcitabine and oxaliplatin. At the time of writing this report, the patient was still alive and continued the planned therapy.

Discussion

Richter syndrome or transformation identifies the infrequent phenomenon occurring in CLL/SLL when a secondary aggressive lymphoma takes rise. Usually the transformation results in DLBCL, but a small number of cases instead have developed cHL or other entities.

The pathobiologic substratum of CLL/SLL progression remains largely unknown. Some studies have demonstrated that RT cells may share the same clonal background with the original population, supporting the hypothesis of their derivation from “transformed” CLL/SLL cells. Secondary lymphomas in RT may be “de novo” notwithstanding. The peculiarity of our case lies mainly in the clinicopathologic presentation of this syndrome with two neoplastic entities, clearly distinct in terms of morphology and phenotype, arising synchronously in two separate
In the literature, but regardless of the evidence of co-localization, the concomitant presence of non-Hodgkin lymphoma and cHL as Richter syndrome remains an extremely rare occurrence. The case also exhibited evidence of EBV infection and previous fludarabine treatment, which according to the literature may be both involved in the development of RT. In particular, EBV infection, frequently associated with Reed-Sternberg cell morphology and phenotype, was observed between chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) and Richter transformation (RT): the CLL clone gave rise to a 247–base pair (bp) peak with FR2-JH primers, a 146-bp peak with Vk-J primers, and 280- and 283-bp peaks with Vk-de primers. In the RT, a new clone developed, visible as 260-bp (FR2-JH), 149-bp (Vk-J), and 239-bp (Vk-de) signals. No amplification was achieved with FR2-JH primers in the bone marrow biopsy specimen, due to low DNA quality, but IgK analysis demonstrated the same rearrangement pattern in diffuse large B-cell lymphoma (DLBCL) and classic Hodgkin lymphoma (cHL). As shown by FR2-JH and Vk-J results, minute traces of the CLL clone were still detectable in the RT samples (247-bp and 283-bp signals, respectively, both in the DLBCL and cHL). On the other hand, IgK/Vk-J analysis showed that the more aggressive clone leading to RT was already detectable in the original lesion (149-bp peak). All the results were confirmed on PCR duplicates.
observed accordingly in the bone marrow biopsy specimen where chL features prevailed. The molecular analysis showed that the two distinct RT components were characterized by the same immunoglobulin gene rearrangement pattern, indicating a reciprocal biologic relation. Furthermore, this pattern was observed in a CLL/SLL lymph node biopsy specimen as a weak signal besides the dominant one, indicating a possible molecular link between CLL/SLL and the two transformed components. Eventually, the complexity of our case further increased: despite regression of gastric lesions and a negative bone marrow biopsy specimen, progression occurred with axillary lymph node involvement by a high-grade lymphoma showing immunophenotypical and molecular features (CD5 expression, an immunoglobulin gene rearrangement pattern identical to the original dominant CLL/SLL one, and the presence of trisomy 12), indicating a close connection with previous CLL/SLL.

The case described presents a high degree of complexity, which probably gravitates toward the modulation of morphologic, phenotypical, and molecular features of a unique disease. In fact, our data suggest the hypothesis that the tumor was heterogeneous “ab initio,” with the two immunoglobulin gene rearrangement patterns probably indicating the existence of two different neoplastic populations that may have given rise, in turn, to a two-phase transformation: a first population was trisomy 12+ and histologically and clinically evident at the time of CLL/SLL diagnosis, while the other was trisomy 12− and immunomorphologically and clinically undetectable at that time. The latter may have grown to give rise to the initial phase of the transformation presenting with two different morphologies and subsequently decreasing, while the original main CLL/SLL population reappeared in axillary lymph nodes with a high-grade morphology during the second phase of RT.

This process could be seen as an extraordinary example of the recent emerging concept that tumor progression may follow a nonlinear course, and this may be correlated to substantial clone diversity, as shown in the latest massive parallel sequencing studies in myeloma.15-17 The composite picture and the clonal mosaic we observed seem to suggest that a “Darwinian” evolutionary selection might have taken place with alternating dominance of two distinct clonal populations over time, probably influenced by therapeutic or environmental factors.

References


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