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Proliferation centers in bone marrows involved by chronic lymphocytic leukemia/small lymphocytic lymphoma: a clinicopathologic analysis☆

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ABSTRACT

Objectives: Proliferation centers (PCs) are a characteristic finding in chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) lymph nodes, and their presence and extent in this site are not currently felt to be related to clinical course. In contrast, detailed clinicopathologic analyses of bone marrow (BM) PCs have not been previously reported.
Methods: The PCs in 88 CLL/SLL BMs from 45 patients (pts) were graded (0-4) and were correlated with other morphologic, immunophenotypic, cytogenetic, and laboratory features.
Results: Proliferation centers were present in 69 BMs (78%) from 32 pts (71%) and were distinct/prominent (grades 2-4) in 21 pts (47%), with the latter more commonly found in follow-up BMs (1/7 diagnostic BMs vs 49/81 follow-up BMs; $P = .04$). When present, PCs were most commonly graded as distinct nodules easily visible on ×10. No relationships were identified between PCs and any complete blood count parameter, serum lactate dehydrogenase or IgG levels, degree or pattern of BM involvement, blood morphology, CD38 and FMC7 expression by flow cytometry, or fluorescence in situ hybridization results, when the first encountered BM was considered for each patient.
Conclusions: This represents the first detailed analysis of PCs in CLL/SLL BMs. In our tertiary center, PCs were seen frequently, in approximately three-fourths of cases. There were no statistical associations identified between PCs and cytogenetic, immunophenotypic, or other laboratory and morphologic findings.

1. Introduction

Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) is a clonal lymphoproliferative disorder composed of small, mature B cells in the peripheral blood (PB), bone marrow (BM), and lymph nodes. It is the most common leukemia in Western countries, representing approximately 30% of all leukemias [1].

Lymph nodes involved by CLL/SLL typically show effacement of architecture, with a diffuse or vaguely nodular pattern, and with pale, irregular nodules distributed in a darker cellular background [2]. The pale areas represent proliferation centers (PCs), which are concentrations of prolymphocytes and paraimmunoblasts, a spectrum of larger cells with more dispersed chromatin, conspicuous nucleoli, and more abundant cytoplasm [2]. Proliferation centers are thought to represent the site of T-cell–dependent and stromal cell–dependent immune responses, resulting in the selection and proliferation of clonal B cells [3]. Not surprisingly, PCs often demonstrate genetic and immunophenotypic differences compared with the surrounding small lymphocytes, including increased expression of Ki-67, CD71, MUM1/IRF4, HLA-DC, HLA-DR, CD20, and CD23, and down-regulation of IgD and CD9 [4-8]. Although some studies have not found a correlation between clinical features and the extent of PCs in lymph nodes [9], other studies suggest that the presence of confluent PCs in CLL/SLL or SLL lymph nodes was associated with a worse clinical course and/or specific cytogenetic abnormalities [2,10-11].

Bone marrow involvement by CLL/SLL may show nodular, interstitial, diffuse, or mixed patterns of infiltration [12-14]. The pattern of involvement in CLL/SLL was once regarded as an important prognostic factor, but more recent literature has described other, more powerful predictive markers, such as cytogenetics and molecular abnormalities. Although the presence of PCs, in the setting of the appropriate immunophenotype, is essentially diagnostic of CLL/SLL in the BM, it is generally considered to be uncommon in BM biopsy specimens. Despite the robust literature devoted to CLL/SLL infiltration patterns in BM and their relationship to prognosis, there are few data on the frequency of PCs in the BM; therefore, we sought to systematically study this morphologic finding in a large cohort of CLL/SLL BM biopsies. To the best of our knowledge, no previous studies have examined the presence

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and extent of BM PCs in correlation with cytogenetic abnormalities, immunophenotypic features, and other laboratory findings.

2. Materials and methods

2.1. Patients

All patients with CLL/SLL diagnosed according to the World Health Organization 2008 criteria [15,16], who had a BM biopsy performed at Froedtert Hospital/Medical College of Wisconsin from 2006 to 2010, were reviewed. Cases with less than 5% BM involvement by CLL/SLL were excluded. Patient demographics, the complete blood count, serum lactate dehydrogenase (LDH), and IgG immunoglobulin were recorded. This study was approved by the institutional review board at the Medical College of Wisconsin.

2.2. Morphologic studies

The hematoxylin and eosin–stained histologic sections of the BM biopsy and clot section were reviewed. The BM PCs were graded together by 2 of the authors (JCC and SHK) as follows: 0, absent; 1, present, but small and ill-defined, not visible on ×10 objective; 2, distinct, easily recognized on ×10 objective; 3, extensive; and 4, diffuse increase in prolymphocytes without discrete PC formation. Absent/ill-defined PCs equated to grades 0 to 1, and distinct/prominent PCs equated to grades 2 to 4. In addition, the patterns of BM infiltrate were classified into nodular, diffuse, interstitial, and mixed by previously described definitions [12-14]. In cases where more than one pattern existed, the primary and secondary patterns were noted. The degree of BM involvement by the CLL/SLL infiltrate was semiquantitatively classified into 4 categories: less than 25%, 26% to 50%, 51% to 75%, and more than 75%. If present, the location of the PCs was noted (ie, perisinusoidal, perivascular).

The PB lymphocyte morphology was assessed by reviewing the corresponding Wright-stained PB smears. Cases were considered to have increased prolymphocytes if they comprised more than 10% of the lymphocytes. Cases were categorized as atypical morphology if more than 10% of lymphocytes show cleaved or irregular nuclear contours. Correlations with concomitant lymph node biopsies could not be performed as too few cases had such concomitant biopsies.

2.3. Flow cytometric analysis

Flow cytometric studies were performed using previously described methods [17]. Briefly, specimens were analyzed by 4-color flow cytometry on a FACSCanto flow cytometer with FACSDiva software (Becton Dickinson, San Jose, CA). Antibodies relevant to the current analysis included CD38 and FMC7 (Becton Dickinson, Franklin Lakes, NJ). Isotype controls were performed in all cases. Cluster analysis was performed with Paint-A-Gate software (Verity House, Topsham, ME). CD38 and FMC7 expression was assessed by the degree of overlap between the neoplastic cluster and the same population in the isotype control tube, using a 20% cutoff at a 2% isotypic control threshold.

2.4. Fluorescence in situ hybridization

A CLL/SLL fluorescence in situ hybridization (FISH) panel, consisting of a commercially available set of 5 probes (Vysis, Des Plaines, IL) to evaluate for trisomy 12, IgH rearrangements, and deletions 11q22, 13q14, and 17p13, was performed according to manufacturer's instructions. Two hundred interphase nuclei were analyzed for each probe.

2.5. Statistical analysis

The histopathologic characteristics of the BM PCs were correlated with other morphologic, immunophenotypic, FISH, and laboratory findings. The associations between 2 categorical variables were examined using the Fisher exact test. Comparisons between the means of continuous variables were performed using the *t* test. When examining for trends across unique patients, findings from the first BM available for review were considered in the analysis. Statistical significance was set at $P < .05$. Statistical analyses were performed using SPSS for Macintosh version 11 (SPSS, Chicago, IL) software.

3. Results

3.1. Cases

The study population consisted of 88 BM examinations from 45 patients. The patients included 37 men and 8 women ranging from 48 to 81 years (median age, 63 years). Seven cases were diagnostic BMs, whereas the remaining 81 were follow-up assessments.

3.2. Morphologic features

Histologically, PCs were present in 69 (78%) of 88 cases and 32 (71%) of 45 patients in the first BMs from each available for review. The PCs in BMs were histologically identical to their counterparts in lymph nodes, appearing as pale staining, vaguely nodular collections of prolymphocytes, paraimmunoblasts, and small lymphocytes that were noticeable against a background of dark staining typical small, round CLL/SLL lymphocytes. Nineteen (22%) cases had grade 1 PCs (Fig. 1A and B). Distinct/prominent PCs (grades 2-4) were relatively common and were observed in 50 (57%) of 88 cases and 21 (47%) of 45 patients at first BM review. Grade 2 PCs were the most common pattern, observed in 38 (43%) of 88 cases, whereas grade 3 and grade 4 PCs were present in 7 (8%) and 5 (6%) of 88 cases, respectively (Fig. 1C-F). The PCs were observed adjacent to marrow sinusoids in 13 (15%) of 88 cases and in a perivascular distribution in 14 (16%) of 88 cases (Fig. 2). (See Fig. 2.)

Distinct/prominent PCs were seen in 1 (14%) of 7 diagnostic BMs and 49 (60%) of 81 follow-up BMs ($P = .04$). However, PCs were not a temporally consistent feature in patients. Many patients with multiple follow-up biopsies had some biopsies showing distinct/prominent PCs and others with complete absence of PCs (data not shown).

The most common pattern of BM involvement was the mixed pattern, seen in 58 (66%) of 88 cases, followed by interstitial (23/88; 26%), diffuse (5/88; 6%), and nodular patterns (2/88; 2%). Among cases with a mixed pattern, the most common primary pattern was the interstitial pattern (40/58 cases; 69%) and the most common secondary pattern was the nodular pattern (35/58 cases; 69%). Overall, interstitial, nodular, and diffuse patterns were observed in 81 (92%) of 88, 49 (56%) of 88, and 16 (18%) of 88 cases, respectively, either as the sole pattern or as the primary or secondary pattern in a mixed pattern. Nodular and diffuse patterns did not coexist in any given case; whenever there was a mixed pattern with either the nodular or diffuse pattern, the other component was always the interstitial pattern. Distinct/prominent PCs were seen in 33 (66%) of 50 cases containing a nodular pattern compared with those cases with absent/ill-defined PCs 16 (42%) of 38 ($P = .03$; Table 1), but this association disappeared when first available BMs were analyzed for each patient ($P = .377$). In contrast, a diffuse or interstitial pattern of infiltrate was not associated with the presence of distinct/prominent PCs in total cases (both $P = 1.00$; Table 1) or first available BMs ($P = .729, .421$).

When the degree of BM involvement by CLL/SLL was semiquantitatively assessed, 11 (13%), 13 (15%), 28 (32%), and 36 (41%) of 88 of cases showed less than 25%, 26% to 50%, 51% to 75%, and more than 75% involvement, respectively. However, the degree of involvement did not correlate with the presence of distinct/prominent PCs ($P = .62$).