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Reassessment of small lymphocytic lymphoma in the era of monoclonal B-cell lymphocytosis

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Short title: SLL in the era of MBL

Key words: chronic lymphocytic leukemia, small lymphocytic lymphoma, monoclonal B-cell lymphocytosis, MBL.

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ABSTRACT

Background. In the 2008 World Health Organization Classification, small lymphocytic lymphoma is defined as a neoplasm with tissue morphology and immunophenotype of chronic lymphocytic leukemia, but with absence of leukemia. Minimal criteria of tissue involvement to separate small lymphocytic lymphoma from monoclonal B-cell lymphocytosis have not been defined.

Design and Methods. We reviewed the clinicopathologic features of 36 patients with extramedullary tissue biopsies containing chronic lymphocytic leukemia-type cells and $<5x10^9$/L peripheral blood monoclonal B cells. Pathologic features (extent and patterns of involvement, architectural preservation, presence of proliferation centers) as well as cytogenetic and radiologic findings were examined in relation to clinical outcome.

Results. The biopsies were performed to evaluate lymphadenopathy in 20 patients and for other reasons (most frequently staging of a non-hematologic neoplasm) in 16 patients. At latest follow-up (median 23 months), 21 untreated patients had no or stable lymphadenopathy, 3 had regressed lymphadenopathy, and 12 developed progressive lymphadenopathy and/or received therapy for chronic lymphocytic leukemia/small lymphocytic lymphoma. Features associated with progression/treatment included lymph nodes ≥ 1.5 cm on imaging studies ($p=0.01$) and presence of proliferation centers in the biopsied tissue ($p=0.004$). Neither the size nor extent of involvement of the excised lymph node correlated with progression/treatment.

Conclusions. Our findings suggest that biopsies containing chronic lymphocytic leukemia-type cells, but lacking proliferation centers and with non-enlarged or only slightly enlarged lymph nodes on imaging, represent a very indolent disease that may best be considered a tissue equivalent of monoclonal B-cell lymphocytosis rather than overt small lymphocytic lymphoma.

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We propose that such cases be designated as *tissue involvement by chronic lymphocytic leukemia/small lymphocytic lymphoma-like cells of uncertain significance*.

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INTRODUCTION

The 2008 World Health Organization (WHO) Classification definition of chronic lymphocytic leukemia (CLL) requires $\geq 5x\ 10^9$/L peripheral blood (PB) monoclonal B-cells (MBC) with a CLL-phenotype.(1) The 2008 International Workshop on Chronic Lymphocytic Leukemia (IWCLL) allows the diagnosis also to be made with lower MBC counts if the patient has cytopenias or symptoms attributable to the CLL.(2) Patients who in the past would have been diagnosed with CLL but who no longer fulfill these new criteria are now classified as monoclonal B-cell lymphocytosis (MBL) with a CLL phenotype (CLL-type MBL). CLL-type MBL has been identified in up to 12% of adults with normal blood counts using highly sensitive flow cytometry techniques.(3-6) Patients in whom peripheral blood MBC are detected due to medical workup (so-called *clinical MBL*) tend to have higher MBC counts than MBL detected in population screening studies (7). The biologic association between clinical MBL and CLL is suggested by the similar proportion of deletion 13q14 and trisomy 12 detected in clinical CLL-type MBL compared to CLL and the finding that the vast majority of patients with CLL have B-cell clones detectable by flow cytometric or molecular genetic analyses up to 77 months before diagnosis.(4-9) Compared to CLL, patients with clinical MBL have superior treatment-free survival and longer lymphocyte doubling times.(10-12) While the vast majority of clinical MBL patients maintain stable counts over time, a small proportion progress to CLL at a rate that has been estimated at approximately 1-2% per year.(4-5,13) The most important predictor of outcome in clinical MBL appears to be the B-cell count at diagnosis, with several studies demonstrating that the B-cell count predicts progression to CLL, treatment-free survival, and overall survival as a continuous variable.(5, 9-11) Currently, $5x10^9$/L PB MBC is the WHO criterion that differentiates MBL from CLL; however there are differences in the literature

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regarding the optimal B-cell thresholds that best predict the risk of progression, treatment-free survival and overall survival, ranging from $1.2x10^9$/L to $11x10^9$/L.(8, 10, 12)

Although a distinction between clinical MBL and CLL has been defined in peripheral blood, criteria for the diagnosis based on involvement of bone marrow or extramedullary tissue have not been extensively investigated. The IWCLL report states that lymphocytes typically comprise more than 30% of nucleated cells in the bone marrow aspirate in patients with CLL. However, bone marrow examination is not recommended at the time of the diagnosis of CLL, and the guidelines do not specify a level of bone marrow involvement that would discriminate between MBL and CLL.(14) According to the 2008 WHO Classification, the diagnosis of small lymphocytic lymphoma (SLL) is used for non-leukemic cases with the tissue morphology and immunophenotype of CLL. Whereas the IWCLL requires a PB MBC count of $<5 \times 10^9$/L for a diagnosis of SLL, the WHO Classification does not provide a specific PB MBC count definition of *non-leukemic* (1-2). The histologic and immunophenotypic features of SLL are indistinguishable from lymph nodes involved by CLL, and therefore in the WHO classification, CLL and SLL are considered as a single disease entity (CLL/SLL) (1). Neither the WHO nor IWCLL provide guidelines defining the minimal level of tissue involvement that would qualify for a diagnosis of SLL. While palpable lymphadenopathy (LAD) and/or splenomegaly are considered exclusion criteria for MBL(7), these criteria are not part of the WHO or IWCLL definitions of SLL and the diagnosis of SLL can therefore be rendered independent of the extent of nodal involvement or presence of LAD.

With the frequent use of flow cytometry and immunohistochemistry to evaluate lymph nodes, it is possible to identify subtle populations of CLL-type cells in tissues. While a PB MBC count threshold is being used to distinguish MBL from CLL, it is uncertain if there should be a

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threshold of tissue involvement that would define SLL. Recently, *in situ* proliferations of follicular lymphoma cells and mantle cell lymphoma cells have been recognized that are not considered equivalent to overt lymphoma and have relatively indolent behavior compared to their bona fide lymphomatous counterparts(15-17); no analogous type of CLL/SLL with minimal tissue involvement is currently recognized. We studied the clinicopathologic features of 36 extramedullary tissue biopsies containing monoclonal B cells with a CLL phenotype, but with $<5x10^9$/L PB MBC, in order to better determine if a subset of these cases might represent a more indolent disorder than SLL (i.e. a tissue equivalent of MBL).

DESIGN AND METHODS

Case selection and clinical review

The study was approved by the institutional review boards of the University of Pittsburgh and Massachusetts General Hospital (MGH). The records of the pathology departments of the University of Pittsburgh Medical Center (2000-2009) and MGH (2004-2009) were searched for lymph node and other extramedullary tissue biopsy specimens on which a diagnosis of SLL had been made, or that contained monoclonal B cells with the immunophenotype of CLL, and in which the patients had $<5 \times 10^9$/L PB MBC. Thirty-six extramedullary tissue biopsies (34 lymph nodes and 2 extranodal sites) were identified. All available clinical data were reviewed. Patients were staged when sufficient clinical data were available using the Rai and Binet staging systems and the International Prognostic Index (IPI).(18-20) An additional cohort of 10 consecutive patients from MGH diagnosed with clinical MBL in 2009 on peripheral blood flow cytometry and who underwent radiologic staging studies was evaluated for comparison.

DOI: 10.3324/haematol.2011.042333

Morphologic and immunophenotypic review

All routine hematoxylin and eosin (H&E)-stained sections, existing immunohistochemical stains, and flow cytometric immunophenotypic studies performed on the extramedullary tissue biopsies were reviewed. The following pathologic features were examined in all lymph node specimens: lymph node size (greatest diameter), extent and patterns of lymph node involvement by cells with a CLL phenotype, preservation of normal lymph node architectural features (sinuses, mantles, and germinal centers), presence or absence of proliferation centers, number of germinal centers, the presence of any additional pathological processes, and percentage of CLL-type cells in the tissue as evaluated by flow cytometry. The aforementioned features were scored together by 3 observers (SEG, SHS, RPH) and separately by a fourth observer (NLH); any discrepancies were resolved by discussion and a consensus score determined for each parameter. All observers were blinded to the clinical outcome of the cases. In addition, one observer (NLH) blinded to clinical outcome and immunohistochemical/flow cytometry studies evaluated if an abnormal infiltrate could be detected on H&E-stained sections. The morphologic and immunophenotypic features of concurrent and/or subsequent peripheral blood and bone marrow specimens were also reviewed when available.

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Classical cytogenetic and fluorescence in situ hybridization studies

All classical cytogenetic studies and FISH studies performed for routine clinical purposes on peripheral blood, bone marrow, and/or extramedullary tissue biopsies were reviewed. Additional FISH studies were performed on formalin-fixed, paraffin-embedded 5 μm whole tissue sections from 13 extramedullary tissue biopsies without prior cytogenetic studies using the following probes: Vysis LSI p53 / LSI ATM multi-color, LDI D13S319 / CEP12 multicolor, LSI IGH dual color break apart, and LSI MYB (6q23) SpectrumAqua (Abbott Molecular, Des Plaines, IL). The signal patterns from a minimum of 200 cells were scored, and cutoffs to determine positive samples were established for each probe based on individual laboratory experience with clinical specimens.

Follow-up

Progression was defined as new or increased LAD detected on radiologic studies or physical examination after the time of diagnosis. Dates and types of treatments and clinical responses were recorded. Patients who progressed or were treated for CLL/SLL were compared to patients with at least one year of follow-up who showed no progression and were not treated. Overall and disease-specific survivals were determined for all patients.

Statistical analysis

Statistical analyses, including two-tailed t-tests, two-tailed Fisher's exact tests, Mann-Whitney test, and survival curves (log rank test) were calculated using the Prism software package, version 5 (GraphPad Software, Inc, La Jolla, CA, USA).

DOI: 10.3324/haematol.2011.042333

RESULTS

Clinical features

The 36 patients included 19 men and 17 women with a median age of 71 years (range 47 – 88 years)(Table 1). In 16 patients, the tissue was biopsied for reasons other than to evaluate palpable LAD. These tissues included 9 lymph nodes removed during staging for carcinoma or melanoma, 4 removed during surgery for benign diseases, and 1 removed when LAD was detected on radiologic studies performed to evaluate another process, as well as 2 extranodal tissue biopsies (one nasal biopsy in a patient with nasal obstruction without a mass lesion and one breast biopsy performed to evaluate breast calcifications without a mass lesion). Palpable LAD was subsequently discovered on physical examination after the tissue diagnosis in 8 of these 16 patients. Twenty patients underwent lymph node biopsies because of palpable LAD or splenomegaly. Palpable LAD was localized in 14 cases and more extensive in 6 cases. On radiologic staging studies (chest and abdominal CT scans), LAD (>1 cm in smallest dimension) was detected in 32/36 (90%) patients and involved 3 or more lymph node regions in 22/36 patients (61%). In comparison, only 2/10 (20%) clinical MBL patients diagnosed on peripheral blood flow cytometry had any LAD detected on chest and abdominal CT scans ($p<0.0001$ compared with the patients in the current study), and none of these MBL patients had LAD involving more than 2 lymph node regions ($p<0.0001$ compared with the patients in the current study). Combining physical exam and radiologic studies, 34/36 patients (94%) had detectable LAD. Three patients had splenomegaly, while hepatomegaly was not identified in any patients.

At diagnosis, the patients had a median absolute lymphocyte count (ALC) of $2.5x10^9$/L (range $0.3–7.5x10^9$/L). Flow cytometric immunophenotypic studies performed on peripheral blood (PB) specimens in 18 patients demonstrated CLL-type MBC in all patients, with a median absolute

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MBC of $1.2x10^9$/L and a range of 0.02–$4.4x10^9$/L. All 3 cases with ALC >$5x10^9$/L had PB flow cytometry performed and had an absolute MBC of 3.0, 3.5, and $4.4x10^9$/L, while all 18 cases in which flow cytometry was not performed had ALC of <$5x10^9$/L. Bone marrow involvement was identified in 10/10 patients who underwent a bone marrow biopsy. The extent of bone marrow involvement varied from less than 5% to over 50% (median 30%) of the biopsy cellularity. Twenty-eight of 36 (78%) patients had palpable LAD or splenomegaly. Among the remaining 8 (22%) patients, all 4 examined by PB flow cytometry would have been classified as clinical MBL according to IWCLL (because LAD or splenomegaly were absent or only detectable on imaging studies), while flow cytometry was not performed in the other 4 patients. Among the 28 patients with palpable LAD, 23 were Rai Stage I, 2 were Stage II, and 3 were Stage III (although 5 patients were anemic with hemoglobin <11 gm/dL, in two cases the anemia was attributable to causes other than CLL/SLL). Twenty-five patients were Binet stage A and 3 patients Binet stage B. Rai and Binet staging was not applicable in the 8 patients lacking palpable LAD.

DOI: 10.3324/haematol.2011.042333

Gross and microscopic features of the extramedullary tissue biopsies

The specimens included 31 excisional lymph node biopsies, 3 lymph node core biopsies, 1 nasopharyngeal tissue biopsy, and 1 breast core biopsy (Table 2). Excisional lymph node biopsies performed in 31 patients had a median greatest dimension of 2.0 cm (range 0.9–5.2 cm). In 4 cases, the lymph node appeared enlarged as a result of other pathology, including metastatic tumor, paracortical (T-zone) hyperplasia, granulomas, and/or fatty replacement. Lymphoma was evident on routine H&E sections in 25/32 (78%) evaluable cases (excluding 3 core biopsies of lymph node and one core biopsy of breast, in which tissue architecture could not be reliably ascertained). These cases exhibited morphologic features typical for CLL/SLL, manifesting as a diffuse infiltrate of small lymphoid cells with round nuclear contours and clumped chromatin (Figure 1). In these 25 cases, as well as in an additional 3 cases after using immunohistochemistry to highlight CD20+/CD5+ cells, the neoplastic B cells were found to involve >50% of the lymph node area. Most cases exhibited either a predominantly diffuse pattern, in which sinuses were obliterated, or an interfollicular and intersinus pattern, in which follicles and/or sinuses were preserved (Figure 1). Three cases had a predominantly follicular pattern of growth, where the neoplastic B cells infiltrated existing germinal centers, while in one case the neoplastic cells exhibited a predominantly perifollicular growth pattern (Figure 2). Seven cases with mainly diffuse or interfollicular/intersinus growth patterns had follicular (2 cases) or perifollicular (5 cases) patterns as prominent secondary growth patterns. Proliferation centers were identified in 25/36 (69%) cases; when present, the proliferation centers were usually scattered, but were perifollicular in distribution in the case with a predominantly perifollicular growth pattern. Interobserver reproducibility was high for assessing extent of nodal involvement

DOI: 10.3324/haematol.2011.042333

(94% agreement) and presence or absence of proliferation centers (92% agreement), but was lower for assessing the primary pattern of nodal involvement (79% agreement).

Many normal architectural features were often present in the lymph node biopsies. Residual follicles with germinal centers were identified in 23/32 (72%) evaluable lymph node biopsies, and in 17 cases at least 3 residual germinal centers were noted. Mantle zones were thinned (<3 cells wide) in 5 cases and absent in 11 of the 17 cases with sufficient germinal centers for evaluation. In the 11 cases with absent mantle zones, the neoplastic B cells surrounded the normal residual germinal centers. Lymph node sinuses were intact in 16/30 (53%) evaluable cases, while sinuses were entirely absent or rare in only 4/30 cases (13%).

Immunophenotypic and cytogenetic features

All 36 cases showed expression of CD20 (which was dim to intermediate in intensity in the 15 cases studied with flow cytometry) and were CD5 positive. Thirty of 32 cases (94%) were positive for CD23, while all evaluated cases were negative for FMC7 (0/10 cases) and cyclin D1 (0/33 cases). There was dim surface immunoglobulin light chain expression in all 15 cases evaluated by flow cytometry. CD38 was expressed in 10/22 cases (45%), and ZAP-70 was positive in 4/15 cases (27%). Cytogenetic abnormalities were assessed by conventional cytogenetics and/or FISH in 27 cases. The most common abnormality identified was deletion 13q14.3 (9/26 cases, isolated in 8 cases and together with trisomy 12 in 1 case), followed by trisomy 12 (5/24 cases), deletion 11q22.3 (2/26 cases), and deletion 17p13.1 (2/27 cases). No cases with deletion 6q23 were identified (0/27 cases).

DOI: 10.3324/haematol.2011.042333

Follow-up

Five patients were treated for CLL/SLL. These included 2 patients treated within one month of diagnosis due to symptomatic disease (progressive myopathy attributed to CLL cell infiltration in one patient and bulky LAD in one patient) and 3 patients treated for disease progression at 5, 19, and 19 months following diagnosis. Treatment included rituximab monotherapy, rituximab with bendamustine, cyclophosphamide or fludarabine, or a combination of cyclophosphamide and prednisone. Two achieved a complete response, 1 a partial response, 1 had persistently progressive LAD, and 1 died of other causes within 1 month of therapy. After a median follow-up time of 26 months, an additional 7 patients developed progression (new or increasing LAD on physical exam and/or radiologic studies) but were not treated during the follow-up period. The median time to lymph node progression in these 7 patients and the 3 patients treated for progression was 22 months (range 4 – 53 months). (Figure 3A). One untreated patient with progression died of other causes 13 months after diagnosis. At last follow-up, 24 patients had no evidence of progressive LAD and remained untreated. These included 17 patients who were alive (median follow-up 27 months, including 3 patients without any detectable LAD [2 post surgical excision of a single enlarged lymph node], 3 patients with regression of LAD without therapy, and 11 patients with stable LAD) and 7 patients who died of other causes 0.5 – 21 months after diagnosis. No patients died due to CLL/SLL. Among the 24 patients with no progression or treatment, 18 had follow-up of at least 1 year and constituted the *no progression/treatment group* used for comparison with the group of patients who progressed or required therapy.

Over the follow-up period, the peripheral blood ALC remained $<5.0x10^9$/L in 25/36 patients (69%), all of whom had CBC results available at the latest followup. Eight of the remaining 11 patients at least doubled their ALC at a median of 27 months after diagnosis (range 3-104

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months). Although flow cytometric immunophenotypic studies were not performed to determine if the absolute MBL count qualified for a diagnosis of CLL, the ALC in these 8 patients ranged from 8.6-38.2x10^9/L and represented 228% - 1091% of the original ALC at diagnosis. These 8 patients included 2 patients who did not develop progressive LAD and 6 patients who had been treated and/or developed progressive LAD.

Correlation of pathological and clinical features

The following parameters showed no association with progression/treatment: age; gender; Rai or Binet stage; number of involved lymph node sites; palpable LAD on physical exam; splenomegaly; or IPI score. The 3 patients with spontaneously regressed LAD all had more than one site of LAD at diagnosis. There was no association between any hematologic parameters, including ALC and absolute PB MBC count at diagnosis (see Table 1), and progression/treatment. In 4/12 patients (33%) who progressed or required therapy, the CLL infiltrate was discovered incidentally in a biopsy performed for reasons other than LAD, and palpable LAD as reason for the biopsy was not associated with progression/treatment. There were no statistically significant differences in the size of the biopsied lymph node between patients with and without progression/treatment, nor were maximal lymph node diameters of greater than 1 cm, 1.5 cm, or 3 cm associated with progression or treatment. However, detection of any lymph node $\geq$ 1.5 cm in diameter on radiologic staging studies was associated with progression/treatment ($p=0.02$, log rank test for time to progression/treatment) and the largest lymph node size measured on CT at diagnosis was greater in patients who subsequently experienced progression or were treated ($p=0.01$, Mann-Whitney test) (Figure 3B). Of note, the

DOI: 10.3324/haematol.2011.042333

largest lymph node size detected by CT on staging studies was not correlated with the diameter of the biopsied lymph node.

The degree of histologic or flow cytometric involvement of the biopsied tissue by CLL/SLL was not associated with progression/treatment. An abnormal infiltrate characteristic of CLL/SLL could be identified on routine histology in all 9 patients with evaluable biopsy tissue who progressed or required therapy, versus in 11/17 (65%) patients with evaluable biopsy material and followup of at least 12 months who did not progress or require therapy (p=0.06, Fisher's exact test). The only histologic parameter that was associated with progression/treatment was the presence of proliferation centers, which were found in all 12 patients who progressed or required therapy versus in 9/18 (50%) of patients who did not progress (p=0.004, Fisher's exact test and p=0.03, log rank test for time to progression/therapy) (Figure 3C). There was no significant difference in CD38 or ZAP-70 expression between those who did or did not progress or require therapy. Deletion 13q14.3 was identified in only 1/8 (13%) patients who progressed or required therapy, versus in 7/13 (54%) of patients with adequate followup who did not progress (p=0.08, Fisher's exact test and p=0.13 log rank test for time to progression/treatment)(Figure 3D). No other cytogenetic/FISH abnormalities correlated with progression/therapy.

DOI: 10.3324/haematol.2011.042333

DISCUSSION

The concept of MBL is relatively new, having evolved from the recognition that small MBC populations with a CLL-type phenotype can be identified in healthy adults with normal peripheral blood counts. (3-6) Although a recent study has suggested that CLL is almost always preceded by a period of MBL, it has been estimated that persons with clinical CLL-type MBL only progress to CLL at a rate of 1-2% per year. (4-5, 8,13) Several studies have demonstrated that the B-cell count predicts progression to CLL/SLL, treatment-free survival, and overall survival as a continuous variable.(5, 9-11) While the likelihood of treatment remains lower in clinical MBL as compared to Rai stage 0 CLL, it remains uncertain if there is a clinically significant difference in survival between patients with clinical MBL and those with Rai stage 0 CLL with a relatively low MBC. (9)

Criteria for the diagnosis of MBL are based solely on investigation of MBC in PB specimens, with few existing studies of bone marrow involvement (10, 21) and no studies to date on the appearance of extramedullary tissues. Although the IWCLL report states that lymphoid cells usually comprise over 30% of the nucleated cells in the bone marrow aspirate in patients with CLL, this cutoff has not been validated in subsequent studies.(2) Indeed, one recent study found a subset of clinical MBL patients to have more extensive bone marrow involvement in spite of clinically stable disease, thus implying that, unlike the PB MBC count, the degree of bone marrow involvement may not affect the prognosis of clinical MBL.(21) According to the 2008 WHO criteria, any extramedullary tissue involvement by CLL-type MBC not fulfilling the PB criteria for CLL is defined as SLL.(1) However, the IWCLL definition of SLL requires palpable LAD and/or splenomegaly.(2) The current recommendations are that patients diagnosed with

DOI: 10.3324/haematol.2011.042333

MBL and lacking clinically evident LAD do not require radiologic staging, implying that non-palpable LAD would not exclude a diagnosis of MBL, or that it would only rarely be found.(7)

In this study, we investigated the clinicopathologic features of 36 extramedullary tissue biopsies fulfilling the current WHO criteria for SLL, but with $<5 \times 10^9$/L PB MBC, to better characterize SLL in the MBL era and to determine whether or not there may be a tissue equivalent of MBL that should not be diagnosed as overt SLL. Although 16 of our cases were found incidentally, the clinicopathologic features and outcome of these cases did not differ significantly from cases biopsied for LAD. We found in a small cohort of clinical MBL patients who underwent CT staging at diagnosis that 2/10 patients had radiologically detectable LAD. The influence of radiologically detectable LAD on progression of clinical MBL patients is unknown; however, one recent study found that CT-detected abdominal LAD in Rai Stage 0 CLL predicted progression and earlier treatment requirement. (22) Even though up to 15% of patients with clinical MBL are reported to develop LAD or eventually progress to SLL, the proportion of patients that develop LAD with stable PB MBC counts is unknown.(5, 9) A subset of the patients in the study by Rawstron *et al*. had LAD during follow-up, but this feature was reported only in patients with progressive lymphocytosis.(5) In contrast, Rossi and colleagues identified 19 patients (15%) with clinical MBL that progressed to SLL after a median of 42.7 months.(10) Although the outcome of this group of patients was not specified, the authors did speculate that some of the cases could have been SLL with very low disease burden at diagnosis.(10)

The disease burden in MBL is presumably reflected by the PB MBC count, which has been shown to directly correlate with progression to CLL/SLL and/or requiring therapy in multiple

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studies. (5, 9-11) Disease burden is more difficult to assess in lymph node-based disease. In our series of patients with tissue-based disease, the ALC was not associated with progressive LAD or treatment. However, the presence of any enlarged lymph node at least 1.5 cm in diameter on CT staging at diagnosis correlated with progression/treatment. In contrast, the size of the biopsied lymph node correlated with neither progression/treatment nor the size of the largest lymph node detected on radiologic staging, suggesting that a single lymph node sampled for biopsy may not be representative of overall lymph node enlargement. There was also no correlation between palpable LAD and progression/treatment, a finding that challenges the notion that SLL should be defined by palpable LAD and suggests that CT staging of patients with CLL/SLL cells identified in tissue biopsies may help in identifying patients more likely to progress.

The extent of lymph node involvement by CLL/SLL cells did not correlate with progression/treatment and nearly half of the cases in this series demonstrated a predominant interfollicular/intersinus growth pattern. An interfollicular growth pattern, with preservation of reactive follicles and open sinuses, has been described in 8-17% of CLL/SLL involving lymph nodes.(23-24) Similar to the cases in our study, the majority of interfollicular CLL/SLL cases have been reported to have low PB absolute lymphocyte counts at diagnosis.(25-27) However, this interfollicular/intersinus pattern is likely not helpful in predicting clinical behavior, as it has been described in both leukemic (CLL) and non-leukemic (SLL) cases and in cases with both focal and extensive LAD (25-28) and was not correlated with progression in our series. In contrast, although perifollicular proliferation centers can occur in interfollicular CLL/SLL(25), perifollicular and follicular growth patterns are unusual in CLL/SLL and are more often seen in marginal zone and mantle cell lymphomas. These latter possibilities were excluded in our cases

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by the characteristic CLL phenotype (CD20 dim, surface immunoglobulin dim, CD5 and CD23 positive) and lack of cyclinD1 expression. Perifollicular and follicular growth patterns were relatively common in this series, manifesting as the predominant or secondary infiltration patterns in 33% of assessable cases. These findings suggest that perifollicular and follicular infiltration patterns may identify a unique subset of CLL/SLL cases that often lack a significant leukemic involvement (<5 x 10^9/L circulating MBC). The only pathologic feature that correlated with progression or treatment in this series was the presence of proliferation centers. The clinical significance of this latter finding is uncertain, as the majority of previous studies have not shown a correlation between the extent of proliferation centers and clinical behavior in CLL/SLL.(23, 29-31) However, one recent study did suggest that expanded and confluent proliferation centers predict a worse outcome in patients with CLL/SLL.(32)

While the proportion of cases with deletion 13q14.3 was relatively lower in our study than what has been reported in the literature for CLL/SLL and clinical MBL, the proportions of trisomy 12 and deletions of 11q22.3, 17p13.1 and 6q23 were similar.(5, 11, 33-34) Cytogenetic abnormalities are important predictors of disease progression and survival in CLL and have been shown to predict treatment-free survival in clinical MBL(11, 34) There was a trend for more frequent identification of deletion 13q14.3 in patients who did not progress or require therapy in our series, but 1/8 patients with deletion 13q14.3 did progress and this finding did not reach statistical significance. Among the 4 cases with the poor prognostic markers deletion 17p13.1 or deletion 11q22.3, only two developed progressive LAD or required therapy. The clinical variability of these *high-risk* cytogenetic abnormalities has also recently been suggested in a

DOI: 10.3324/haematol.2011.042333

study of 99 CLL patients with deletion 17p13.1, in which approximately half of the patients maintained a relatively stable disease for up to 70 months of follow-up.(35)

An unusual finding in our study was the observation that LAD regressed in 3 patients without treatment. Spontaneous clinical regression of CLL has been previously described in the literature, although a recent study suggested that a residual MBC population remains detectable by flow cytometry or molecular analysis in such patients.(36-37) While the 3 patients in our study with regression had no evidence of an absolute lymphocytosis, LAD, or splenomegaly at last follow-up, PB specimens were not available to evaluate for the presence of a persistent clonal B-cell population by flow cytometry. Continued follow-up is necessary to determine if these patients will remain in clinical remission.

In summary, we identified 36 extramedullary tissue biopsies fulfilling the WHO criteria for SLL, nearly half of which were diagnosed in lymph nodes removed for incidental reasons other than evaluation of unexplained palpable LAD. Most patients had disseminated disease and low-level peripheral blood involvement was present in all patients tested. The majority of cases, including those diagnosed incidentally, were associated with variably enlarged lymph nodes that were usually extensively involved by lymphoma, resembling overt SLL. In a significant subset of cases, however, the abnormal lymphoid population was more subtle and/or focal, and could not be recognized without the use of immunohistochemistry and/or flow cytometry. Most patients had stable LAD and did not develop lymphocytosis over the followup period and a minority of patients either never manifested LAD or showed spontaneous regression of lymph nodes that were enlarged at initial diagnosis. Although the incidence of progression or treatment appears to be greater in the cases reported here compared with many cases of clinical MBL as currently defined, only 5 of the 36 patients in our series required treatment. Our data suggest that some

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CLL/SLL-like tissue infiltrates may represent an indolent or even potentially regressing disease and may be better considered as a tissue equivalent to clinical MBL rather than full-fledged lymphoma (SLL). An absence of lymph nodes ≥1.5 cm on radiologic staging studies and an absence of proliferation centers were correlated with freedom from progression or treatment in our series. Cases with these features may be more appropriately diagnosed as "involvement by CLL/SLL-like cells of uncertain significance" rather than SLL, analogous to terminology proposed for the "in situ" versions of follicular lymphoma and mantle cell lymphoma and as suggested at the recent European Association of Hematopathologists/Society for Hematopathology 2010 Workshop (Table 3). (38) A deletion 13q14.3 abnormality was more common in cases that did not progress, but this parameter did not attain statistical significance. Achieving an appropriate definition for "involvement by CLL/SLL-like cells of uncertain significance" will require larger validation studies with longer follow-up. The results of our study suggest that cases lacking recognizable proliferation centers in patients who do not have lymphadenopathy ≥1.5 cm on CT scans are the most likely candidates for such a diagnosis.

AUTHORSHIP AND DISCLOSURES

SEG collected and analyzed data and wrote the manuscript; SHS and NLH collected and analyzed data and assisted in writing the manuscript; JAF collected data; US and PDC analyzed data; RPH collected and analyzed data and assisted in writing the manuscript.

SEG, SHS, JAF, US, NLH, PDC, and RPH have no disclosures related to this work to report.

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Table 1. Clinical and laboratory features of tissue infiltrates of CLL/SLL cells.

	All cases (n=36)	No progression or therapy* (n=18)	Progression or therapy (n=12)	Comparison
Median age in years (range)	71 (47-88)	66 (47-80)	73 (57-88)	NS
Gender	19M/17F	8M/10F	7M/5F	NS
Reason for biopsy				
Incidental	16	9	4	NS
Palpable LAD	20	9	8	
Extent of LAD on staging studies (including physical exam)				
None	2	2	0	NS
1-2 LN regions involved	12	5	5	
≥ 3 LN regions involved	22	11	7	
Largest LN on CT, median diameter in cm (range)	2.1 (<1.0-7.1)	2.0 (<1.0-3.3)	2.7 (1.5-7.1)	p=0.01
% of cases with bone marrow involvement	100% (10/10)	100% (6/6)	100% (2/2)	NS
Median CBC data (range)				
ANC at diagnosis ($x10^9$/L)	4.5 (1.6-11.4)	4.2 (1.8-6.7)	4.4 (1.6-11.4)	NS
Hemoglobin at diagnosis (g/dL)	12.9 (9.9-15.9)	13.2 (10.0-15.9)	13.4 (10.9-15.0)	NS
Platelets at diagnosis ($x10^9$/L)	242 (146-577)	242 (166-420)	233 (146-577)	NS
ALC at diagnosis ($x10^9$/L)	2.5 (0.3-7.5)	3.2 (0.8-7.5)	2.3 (0.8-6.8)	NS
Maximum ALC ($x10^9$/L)	3.5 (0.3-38.2)	3.5 (1.4-38.2)	6.5 (1.7-15.5)	NS
Absolute PB MBC at diagnosis ($x10^9$/L)	1.2 (0.02-4.4)	1.1 (0.02-3.5)	1.6 (1.1-4.4)	NS
ALC increase of >=100% from diagnosis	22% (8/36)	11% (2/18)	50% (6/12)	p=0.03
Splenomegaly	8% (3/36)	6% (1/18)	8% (1/12)	NS
Rai stage at diagnosis				
NA	8	4	2	
0	0	0	0	NS
I	23	11	10	
II	2	1	0	
III-IV	3	2	0	
IPI score at diagnosis				
Low	10	7	3	
Low/Intermediate	13	6	5	NS
High/Intermediate	6	1	3	
High	0	0	0	
Not available	7	4	1	
Median follow-up time	23 months	27 months	30 months	NS
Status at last follow-up				
DOC	9	1	2	
AWSD	11	11	0	
AWProgD	7	0	7	NA
AWRegD	3	3	0	
PR or CR, post-chemo- or immunotherapy	3	0	3	
ANED, post-surgery	2	2	0	
ANED	1	1	0	

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LAD: lymphadenopathy; LN, lymph node; CBC, complete blood count; ANC, absolute neutrophil count; ALC, absolute lymphocyte count; PB, peripheral blood; MBC, monoclonal B-cells; IPI, international prognostic index; NA, not applicable; DOC, dead of other causes; AWSD, alive with stable disease; AWProgD, alive with progressive disease; AWRegD, alive with regressed disease; ANED, alive with no evidence of disease; PR, partial remission; CR, complete remission; M, male; F, female; CLL, chronic lymphocytic leukemia; NS, not significant.
*Only patients with >12 months of follow-up are included in this group.

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Table 2. Gross and microscopic features of tissue infiltrates of CLL/SLL cells.

	All cases (n=36)	Noprogression therapy* (n=18)	Progression therapy (n=12)	Comparison
Biopsy site				
Lymph node	34	16	12	NS
Extranodal tissue	2	2	0	
Median LN greatest dimension, cm (range)	2.0 (0.9-5.2)	1.6 (0.9-5.2)	1.6 (1.0-4.0)	NS
Median MBC by flow cytometry (range)	67% (12-89%)	52% (14-82%)	73% (50-87%)	NS
Lymphoma evident on routine histology†	78% (25/32)	65% (11/17)	100% (9/9)	p=0.06
Extent of histologic involvement by lymphoma†				
<10% involved	1	1	0	NS
10-50% involved	3	1	1	
>50-90% involved	12	6	3	
>90% involved	16	9	5	
Primary pattern of involvement‡				
Follicular-appearing	3	3	0	NS
Perifollicular	1	1	0	
Interfollicular/intersinus	15	5	6	
Diffuse	15	7	6	
Proliferation centers present	69% (25/36)	50% (9/18)	100% (12/12)	p=0.004
Median number of germinal centers/2.5x field (range)†	3 (0-74)	5 (0-51)	1 (0-20)	NS
Preservation of sinuses§				
None or rare	4	3	0	NS
Few or focal	10	6	4	
Numerous	17	7	5	
Other pathology				
Metastatic non-hematologic neoplasm	4	2	1	
Granulomas	2	0	0	NS
T-zone hyperplasia	3	2	1	
Fatty replacement	1	1	0	
Dermatopathic change	1	1	0	

LN: lymph node; MBC, monoclonal B-cells; LAD, lymphadenopathy; HPF, 40x microscopic field; NS, not significant. *Only patients with >12 months of follow-up are included in this group. †Not assessed in 3 core LN biopsies and 1 breast core biopsy. ‡Not assessed in 2 biopsies of extranodal tissue (breast and nasopharynx) §Not assessed in 3 core LN biopsies or 2 biopsies of extranodal tissue

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Table 3. Proposed classification scheme for CLL/SLL cells in blood and tissues.

	Peripheral blood monoclonal B-cell count	Biopsy-proven tissue infiltrate of cells with CLL phenotype	Proliferation centers	Lymphadenopathy
Chronic lymphocytic leukemia (CLL)*	$\geq 5 \times 10^9$/L	Present or Absent	Present or Absent	Present or Absent
Monoclonal B-cell lymphocytosis (MBL)*	$<5 \times 10^9$/L	Absent	NA	No palpable lymphadenopathy or splenomegaly
Small lymphocytic lymphoma (SLL)	$<5 \times 10^9$/L	Present	Present or Absent	Enlarged lymph nodes ($\geq$1.5 cm) on CT staging
Tissue involvement by CLL/SLL-like cells of uncertain significance	$<5 \times 10^9$/L	Present	Absent	No lymph nodes $\geq$1.5 cm on CT staging

NA: not applicable. *Definitions according to the WHO 2008 Classification[1]

DOI: 10.3324/haematol.2011.042333

Figure 1. Examples of lymph nodes involved by CLL/SLL in patients with <5 x 10^9/L peripheral blood monoclonal B-cells. (A) Enlarged axillary lymph node diffusely involved by SLL with a residual germinal center (arrowhead; Hematoxylin-eosin [H&E], original magnificationx20). (B) Small cervical lymph node diffusely involved by SLL with numerous scattered proliferation centers (H&E, original magnificationx20). (C) Enlarged axillary lymph node with focal interfollicular/intersinus involvement by CLL/SLL cells (arrowhead; H&E, original magnificationx100). (D) Occasional proliferation centers are present (H&E, original magnificationx400). Immunohistochemical stains for CD20 (E), CD3 (F), and CD5 (G) highlight the infiltrate of CD20+, CD5dim+ SLL cells (original magnification x 400).

Figure 2. Examples of lymph nodes with subtle follicular and perifollicular involvement by CLL/SLL cells in patients with <5x10^9/L peripheral blood monoclonal B-cells. (A) Enlarged lymph node taken at the time of lung cancer staging, with preserved architecture and numerous follicular structures resembling primary follicles (follicular pattern)(H&E, original magnificationx100), highlighted by an immunohistochemical stain for CD20 (B, original magnificationx40). CD3 (C) and CD5 (D) reveal that the CD20+ CLL/SLL cells in the follicles weakly express CD5 (Original magnification x 200). (E) This non-enlarged inguinal lymph node shows preserved architecture (H&E, original magnificationx40). (F) Focal perifollicular proliferation centers are noted (arrowhead; H&E, original magnificationx400). Immunohistochemical stains for CD20 (G), CD3 (H), and CD5 (I) highlight the subtle

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perifollicular distribution of neoplastic CD20+, CD5+ CLL/SLL cells, surrounding the non-neoplastic CD20+, CD5- germinal center (Original magnification x 100).

Figure 3. Outcome of patients with CLL/SLL tissue infiltrates and <5 x 10^9/L peripheral blood monoclonal B-cells. (A) The median follow-up time for all 36 patients was 23 months (range 0.5–106 months) and median time to progression of lymphadenopathy or treatment was 52 months. (B) Patients with at least one lymph node $\geq$ 1.5 cm in diameter identified on CT staging studies were more likely to progress or be treated (median time 35 months) than patients lacking lymphadenopathy or with smaller lymph nodes on staging studies (median time not reached, p=0.02). (C) Patients with proliferation centers in the biopsied involved tissue were more likely to progress or be treated (median time 43 months) than patients lacking identifiable proliferation centers (median time not reached, p=0.03). (D) Patients lacking a del(13q) cytogenetic abnormality had a median time to progression or treatment of 35 months as compared to patients with a del(13q) cytogenetic abnormality (median not reached), but this did not reach statistical significance (p=0.13)(Figures 3B-D exclude patients with <1 year of progression/therapy-free follow-up).

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Figure 1

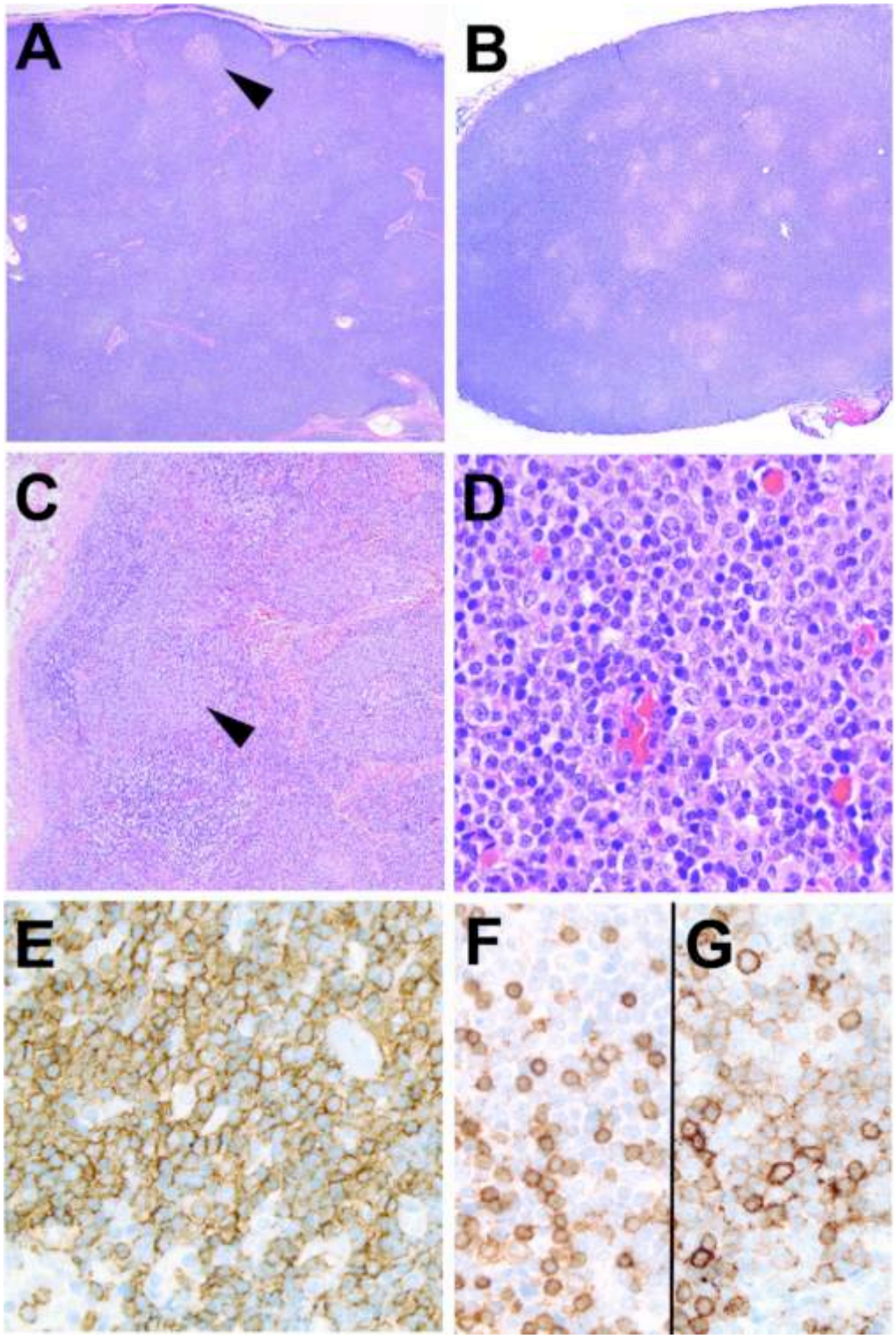

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Figure 2

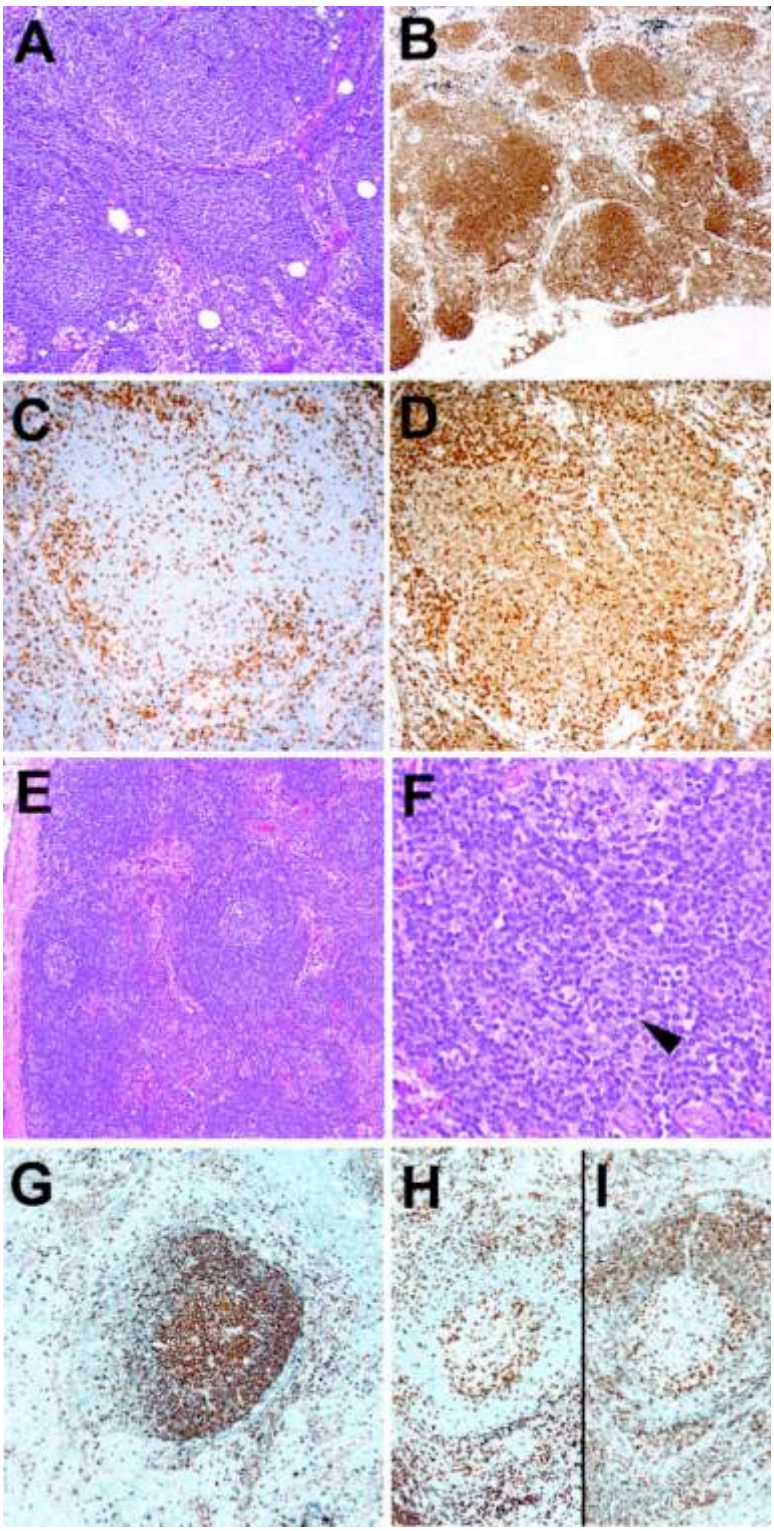

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Figure 3