CLONAL B-CELL LYMPHOCYTOSIS EXHIBITING IMMUNOPHENOTYPIC FEATURES CONSISTENT WITH A MARGINAL ZONE ORIGIN: IS THIS A DISTINCT ENTITY?

Running title: Clonal MZ B-Cell Lymphocytosis

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KEYPOINTS

1. Clonal B-cell lymphocytosis of potential marginal zone origin (CBL-MZ) rarely progresses to a well-recognized lymphoma.
2. CBL-MZ does not require treatment in the absence of progressive disease.
ABSTRACT

The biological and clinical significance of a clonal B-cell lymphocytosis with an immunophenotype consistent with marginal zone origin (CBL-MZ) is poorly understood. We retrospectively evaluated 102 such cases with no clinical evidence to suggest a concurrent MZ lymphoma. Immunophenotyping revealed a clonal B-cell population with Matutes score ≤2 in all cases; 19/102 were weakly CD5-positive and all 35 cases tested expressed CD49d. Bone marrow biopsy exhibited mostly mixed patterns of small B-lymphocytic infiltration. 48/66 (72.7%) cases had an abnormal karyotype. Immunogenetics revealed overusage of the IGHV4-34 gene and somatic hypermutation in 71/79 (89.8%) IGHV-IGHD-IGHJ gene rearrangements. With a median follow-up of 5 years, 85 cases remain stable (Group A), whereas 17 cases (Group B) progressed, of whom 15 developed splenomegaly. Neither the clonal B-cell count, degree of marrow infiltration, immunophenotypic nor immunogenetic findings at diagnosis distinguished between the 2 groups. However deletions of chromosome 7q were confined to Group A and complex karyotypes were more frequent in Group B. Although CBL-MZ may antedate SMZL/SLLU, most cases remain stable over time. These cases, not readily classifiable within the WHO classification, raise the possibility that CBL-MZ be considered as a new provisional entity within the spectrum of clonal marginal zone disorders.
INTRODUCTION

The 2008 WHO classification of hematological malignancies utilizes clinical, morphological, immunophenotypic and genetic data to define distinct and provisional entities based on cell lineage. The 2008 version of the classification and a subsequent perspective recognized the increasing detection of small B-cell clones both in the blood and tissues and the uncertainty surrounding their biological and clinical significance.

In 2005, the term monoclonal B cell lymphocytosis (MBL) was introduced to describe the presence of circulating small B-cell clones of less than $5 \times 10^9/\text{l}$ persisting for more than three months in healthy individuals who had no evidence of lymphadenopathy, organomegaly, an associated autoimmune disease or any other feature diagnostic of a B-cell lymphoproliferative disorder other than the presence of a paraprotein. MBL encompasses the very small clones detectable in individuals with a normal lymphocyte count (population MBL) as well as larger clones detectable in patients presenting with a slight lymphocytosis (clinical MBL).

MBL was subclassified into a CD5 +ve, CD23 +ve CLL-like category, a CD5 +ve, CD23 -ve, CD20<sup>bright</sup> atypical CLL category and a CD5 -ve category. Many subsequent studies have confirmed the biological similarities between clinical CLL-like MBL and early stage CLL and the low rate of progression to CLL requiring therapy. Although the diagnostic criteria for CLL-like clinical MBL have been used to refine the diagnosis of CLL, the cut-off of $5 \times 10^9/\text{l}$ clonal B lymphocytes for distinguishing clinical MBL from CLL is arbitrary, lacking clinical and/or biological justification.

While the 'atypical -CLL' variant of MBL is recognised to include cases of indolent mantle cell lymphoma, the nature of CD5-ve MBL remains unclear. Furthermore, although very small B-cell clones carrying the t(14;18) translocation detectable by PCR can frequently be found in the blood of healthy individuals and follicular lymphoma in situ is a well recognized entity, it is extremely rare for CD5 -ve clinical MBL to show the immunophenotypic or genetic features of a germinal centre-derived clonal B-cell disorder. In contrast, many cases of CD5 -ve clinical MBL have immunophenotypic and morphological features typically associated with...
marginal zone lymphomas involving the spleen. In fact, early studies on splenic lymphoma with villous lymphocytes, now regarded as a leukemic manifestation of a marginal zone splenic lymphoma, included cases with circulating villous lymphocytes in the absence of splenomegaly that either pursued an indolent course or subsequently developed splenic enlargement.

Uncertainty about the biological significance of CD5-ve clinical MBL also extends to its clinical management. Whereas the clinical relationship between CLL-like MBL and early CLL is well understood and neither bone marrow examination nor imaging studies are recommended in the former at diagnosis, it is still not clear whether those investigations are mandatory in clinical CD5-ve MBL diagnosis.

In view of this clinical and biological uncertainty, we have reviewed the morphological, clinical, cytogenetic and immunogenetic features of cases presenting predominantly with a lymphocytosis, or more rarely with a paraprotein and normal lymphocyte count, whose immunophenotype was consistent with a marginal zone lymphoma. We provide data to suggest that at least a proportion of these cases are not readily classifiable according to the current WHO or MBL criteria and discuss whether there is sufficient evidence to warrant the introduction of a new term such as clonal B cell lymphocytosis with marginal zone features (CBL-MZ) as a provisional entity.
PATIENTS AND METHODS

Patients
This retrospective study included 102 patients from three centers selected on the basis of the demonstration of a clonal B-cell population with immunophenotypic features consistent with MZ derivation yet without lymphadenopathy, signs of chronic and/or active inflammation, autoimmunity, organomegaly or cytopenias. Clonal B-cell populations were identified during the investigation of a persistent (>6 months) lymphocytosis of >3.0x10^9/l or paraproteinememia. Information regarding patient demographics and clinical presentation are given in the Results section.

The study was approved by the Ethics Review Committee of each participating institution. Informed consent was obtained in accordance with the Declaration of Helsinki.

Clinical evaluation
A detailed medical history and physical examination were available for all patients. The majority were screened by serum immunoelectrophoresis for the presence of hypogammaglobulinaemia and a paraprotein, for the hepatitis C virus, and also underwent CT scanning of the chest and abdomen and/or ultrasonography of abdominal organs (including the spleen). Gastroscopy, with concurrent mucosal biopsies and bone marrow examination were performed on selected patients at the discretion of each center.

Blood and bone marrow smear morphology
Blood and bone marrow smears were morphologically evaluated for the presence of clonal lymphocytes after staining with the May-Grunwald/Giemsa technique.

Immunophenotyping of lymphomatous cells in the blood and bone marrow aspirate
Clonal lymphocytes in the blood and/or bone marrow were immunophenotypically studied by flow cytometry using standard techniques. In the panel of this immunophenotyping study the following monoclonal
antibodies were used: κ and λ clonality, CD19, CD20, CD5, CD10, CD23, CD79b, FMC7, CD38, CD49d.

Histopathological and immunohistochemical studies
Bone marrow biopsy (BMB) samples were obtained at diagnosis based on the policy of the participating center from 35 cases and reviewed by experienced hematopathologists (TP, PK). Tissue processing and immunohistochemistry were performed as previously described.

Cytogenetic Studies
Cytogenetic studies were performed at diagnosis on peripheral blood mononuclear cells, as previously described. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature (ISCN, 2005). Cut-offs used for chromosome gain, loss or rearrangement were 5%, 10% and 5%, respectively. A karyotype was defined as complex when ≥3 chromosomal aberrations were observed (structural and/or numerical). Individual patient cases were analyzed in the Mitelman Database (http://cgap.nci.nih.gov). Ideograms of translocation breakpoints, gains and losses of chromosomal material in the studied cohort were prepared with the use of the Cydas software (freely available at http://www.cydas.org).

PCR amplification, sequence analysis and sequence interpretation of IGHV-IGHD-IGHJ rearrangements
Reverse transcriptase-polymerase chain reaction (RT-PCR) or genomic DNA-PCR amplification of IGHV-IGHD-IGHJ rearrangements were performed as previously described. Purified PCR amplicons were directly sequenced on both strands. Sequence data were analyzed using the international IMGT® database and the IMGT/V-QUEST tool (http://www.imgt.org).

Statistical analysis and definitions
Descriptive statistics were used for the presentation of data in terms of frequency distributions (discrete variables) and mean, median values
RESULTS

Overview of the patient cohort

The present series included 102 cases, 49 males and 53 females, retrospectively selected for the presence of a clonal B-cell population with immunophenotypic features consistent with MZ derivation. In all cases, physical examination was negative for lymphadenopathy and/or organomegaly and this was further confirmed by radiological assessment. No concurrent cytopenias were observed in any of the studied cases. None had any clinical or laboratory features to indicate a diagnosis of mantle cell lymphoma, hairy cell leukemia, follicular lymphoma or diffuse large B-cell lymphoma. The median age at presentation was 71 years (range, 38-91).

Eighty of 102 (78.4%) cases presented with a lymphocytosis of >4.0 x10^9/l [median lymphocyte count: 6.63 x 10^9/l; (range: 4.0-37.1) x 10^9/l] and thirteen cases (12.7%) with a lymphocyte count between 3.0-3.97 x 10^9/l (median 3.7 x 10^9/l) detected incidentally on a routine blood test. In 9 cases (8.8%), all with normal CBC, a clonal lymphocytic population was identified during the evaluation of paraproteinemia.

Of 81 cases with available information, 27 were positive for serum paraprotein (median level: 0.7 g/dl, range: 0.3-3.8 g/dl). Data concerning the type of paraprotein were available in 25 cases, as follows: IgGκ, n=8; IgGλ, n=4; IgMκ, n=9; IgMλ, n=4. None of the studied cases with available data (87/102) had positive serology for the hepatitis C virus.

Blood smear lymphocyte morphology

Examination of May-Grunwald/Giemsa-stained peripheral blood (PB) smears revealed a heterogeneous lymphocytic population consisting of small, medium and large cells (Figure 1A). Cytoplasm was variable in amount. Nuclei were round-to-oval, centrally or eccentrically placed with dense or sparse chromatin. Variable numbers of villous lymphocytes and lymphocytes with plasmacytoid differentiation were identified in some cases. Overall, although
morphologic heterogeneity of the lymphocytic population was evident in all cases, the cytologic features of different cases were generally similar.

**Immunophenotypic findings by flow cytometry**

In all cases, PB immunophenotyping by flow cytometry revealed the presence of a clonal B-cell population with a Matutes score 0-2. Cells from all cases expressed B-cell antigens (CD19, CD20 strong), whereas they were consistently negative for the expression of CD10. Other individual markers were expressed as follows: CD5: 19/102 cases (18.6%); CD23: 16/102 cases (15.6%); CD79b: 77/85 cases (90.5%), FMC7: 74/93 cases (79.5%), CD38: 6/53 cases (11.3%). Intriguingly, all 35 analysed cases expressed CD49d. Only two cases exhibited co-expression of CD5 and CD23, both with a Matutes score of 2.

**Histopathological and immunohistochemical findings**

In all 35 BMB specimens studied at presentation a lymphocytic infiltration was found, ranging from 7% (single case) to greater than 70%; 17/35 cases (48.5%) exhibited ≥ 30% BM lymphocytic infiltration. No clear association was observed between the extent of BM lymphocytic infiltration and the peripheral blood lymphocyte count.

Most cases exhibited mixed patterns of predominantly interstitial and, to a lesser extent, nodular or intrasinusoidal infiltration (Figure 1B). In 27/35 cases the lymphoid infiltrate consisted of small cells, with round-to-oval nucleus and clumped chromatin. In five cases, a proportion of the small B-lymphocytic infiltration exhibited plasmacytoid differentiation.

In all cases, immunohistochemistry showed that the lymphocytic infiltrate was immunoreactive for CD20 and CD79a, while it was consistently negative for CD10, CD3, Bcl-6 and cyclin D1. One and four cases expressed CD5 and CD23, respectively; while no case exhibited co-expression of CD5 and CD23. DBA44 was positive in 7/21 (33.3%) cases.

**Cytogenetic findings**

Among 67 cases analyzed by G-banding cytogenetics, 48 (71.6%) displayed an aberrant karyotype (Figure 2). Within this subgroup, 11 (22.9%) cases carried three or more cytogenetic abnormalities and were considered to exhibit a
complex karyotype. The chromosomes most frequently involved were: 3, 12, 17 and 7. Aberrations with involvement of chromosome 7 included del(7q) \( [n=7 (14.5\%)] \) as well as translocations involving 7q \( [n=6 (12.5\%), \) of which 3 concerned a t(2;7)(p11;q22)]. Isochromosome 17q was identified in 8 (16.6%) cases; in 3/8 cases, this was the sole aberration.

**Immunogenetics**

A total of 79 productive IGHV-IGHD-IGHJ rearrangements were obtained from 77 analyzed cases as 1 case each carried two and three productive rearrangements, respectively. In the latter case, exhibiting a uniform immunophenotype, the most plausible explanation is biclonality i.e. the presence of two MZ-like populations. Overall, 28 different IGHV genes were identified. The IGHV4-34 gene predominated \( (18/79 \text{ rearrangements, } 22.8\% \) ), followed by the IGHV3-23 \( (5/79, 6.3\% \) ), IGHV1-2 \( (5/79, 6.3\% \) ) and IGHV4-59 genes \( (4/79, 5\% \) ) (Figure 3). Concerning somatic hypermutation (SHM), 8/79 rearrangements \( (10.1\% \) ) carried IGHV genes with no SHM \( (100\% \GI) \) and were assigned to a “truly unmutated” subgroup. The remainder \( (71/79, 89.8\% \) ) showed some impact of SHM activity ranging from minimal-to-pronounced. For statistical comparisons, sequences with 97-99.9% gene identity were classified as “borderline/minimally mutated” \( (n=11/79, 13.9\% \) ), whereas those with less than 97% gene identity as “significant mutated” \( (n=60/79, 75.9\% \) )

**Clinical outcomes**

With a median follow-up of 5 years \( \text{range 0.4-20.2 years} \) ), 85 cases exhibited isolated MZ-like lymphocytosis without organ involvement \( \text{(Group A)} \). The remaining 17 cases were classified separately \( \text{(Group B)} \) as they evolved clinical signs and/or had laboratory/imaging findings consistent with/suggestive of a well-recognized lymphoma entity. Signs of progression were noted at a median of 24 months from diagnosis \( \text{range, 8-79} \) ). In particular: (i) fifteen cases developed progressive enlargement of the spleen, assessed both clinically and radiologically, with no cytopenias or lymphadenopathy; in such cases, a diagnosis of SMZL or SLLU is a definite possibility, however this could not be formally established as no case
underwent splenectomy; (ii) one case was eventually diagnosed with gastric MALT lymphoma (see below); (iii) one case developed diffuse large B cell lymphoma (DLBCL) of the skin; the clonal relationship of the DLBCL with the pre-existing MZ-like lymphocytosis could not be investigated due to the inability to obtain by PCR the clonotypic IGHV-IGHD-IGHJ gene rearrangement of the DLBCL, likely because of technical reasons (formalin-fixed, paraffin-embedded material yielding low-quality DNA).

A comparison of Group A versus Group B showed no differences with regards to age; gender; lymphocyte count; incidence of paraproteinemia; immunophenotype; frequency of infection by *H. pylori* (HP), IGHV gene repertoire and mutational status. In contrast, the cytogenetic profiles of the two groups were distinct. In particular: (i) del7q was identified exclusively among Group A cases; (ii) i(17q) as single aberration and/or co-existing with other aberrations predominated in Group A (7/50 versus 1/17 Group B cases, respectively); (iii) a complex karyotype was less frequent in Group A compared to Group B (6/50 versus 5/17 cases, respectively); (iv) translocation t(2;7)(p11;q22) was only identified in Group B cases. Interestingly, of 5 group B cases with karyotypic complexity, 4 belonged to the “possible SMZL/SLLU” category. The differences between Groups A versus B regarding i(17q) and karyotype complexity did not reach statistical significance (p values of 0.3 and 0.09, respectively), likely due to small numbers.

In very rare cases, MZ-like lymphocytosis can be the presenting feature of occult gastric MALT lymphoma

We have previously reported that MZ-like lymphocytosis can be the presenting feature of occult gastric MALT lymphoma. To exclude this possibility in the current series, a subset of 34 cases of the present cohort were subjected to upper gastrointestinal tract (GI) endoscopy. All cases but one had no endoscopic or histopathological evidence of lymphoma; 21/33 cases were found with gastritis and amongst them 10 were *HP*-positive; 5 cases received *HP* eradication treatment and were *HP*-negative with normal GI endoscopy on re-evaluation. Lymphocytosis persisted in all 5 cases after *HP* eradication, indicating that the detection of HP may be a coincidental finding of no direct aetiopathogenic significance.
The remaining case with GI endoscopic evaluation concerned an asymptomatic 74-year-old female with MZ-like lymphocytosis who was diagnosed with gastric MALT lymphoma, t(11;18)-negative, *H. pylori (HP)*-positive, 8 months after the initial presentation. The patient received HP eradication therapy, followed by chlorambucil (12 cycles) and attained a complete remission, however the monoclonal lymphocytosis has persisted (46 months from the initial presentation). Molecular immunogenetic analysis of PB and gastric biopsy samples documented clonal identity of the circulating cells to the gastric MALT lymphoma (identical IGHV-IGHD-IGHJ rearrangements).
DISCUSSION

We describe the presenting clinical and laboratory features and subsequent natural history of 93 patients presenting with a CD5 -ve lymphocytosis and a further 9 patients investigated for presence of a paraprotein. All had a circulating clonal B-cell population which was either CD5 negative or weakly CD5 positive. This was a retrospective collaborative study incorporating data from 3 centres. The diagnostic tests performed other than the core evaluation, namely clinical, morphological and immunophenotypic, varied upon each center’s policy. Even though this heterogeneity may be regarded as a weakness, suggesting that different centers may have studied different kinds of cases, the uniformity of the core evaluation findings leaves no doubt about the homogeneity of the study group.

All patients had a normal blood count apart from a lymphocytosis and had no clinical or radiological evidence of lymphadenopathy or organomegaly. All had a circulating clonal B-cell population with an immunophenotype typically associated with MZ B cells. Interestingly, all evaluated cases expressed CD49d in the majority of cells together with a low frequency of CD38 expression. CD49d, the a4 integrin subunit, is critically implicated in microenvironmental interactions through the binding to fibronectin and VCAM-1. In CLL, the CD49d/CD29 integrin complex is physically associated with CD38, being part of a macromolecular complex that impacts on migration and adhesion capacity, cell proliferation and survival. In this context, the dissociation of CD49d from CD38 expression in the present series (at least for the great majority of cases) is noteworthy, however, it is unclear whether the observed phenotype reflects the cell of origin or the neoplastic process.

The presence of villious lymphocytes, lymphocytes with plasmacytoid differentiation, an intrasinusoidal pattern of bone marrow infiltration and cytogenetic abnormalities of chromosome 7q, observed in many cases are all seen in splenic MZ lymphomas and lend support to a MZ origin. Although none of the above features are pathognomonic of MZ lymphomas, our cohort did not have features to suggest alternative diagnoses. In particular, there were no morphological, histological or cytogenetic data to indicate a germinal center origin or hairy cell leukemia and 0/45 cases evaluated had the MYD88...
L265P mutation (unpublished data), closely associated with lymphoplasmacytic lymphoma/Waldenstrom’s macroglobulinemia. Most cases pursued an indolent clinical course but 15 developed splenomegaly detected clinically or radiologically, one additional case was subsequently diagnosed with a clonally-related gastric MALT lymphoma and one developed a cutaneous diffuse large B-cell lymphoma whose clonal relationship to the circulating clonal lymphocytes could not be established.

There are a number of possible, and in some cases, closely related explanations for the presence of a small circulating B-cell clone with a marginal zone phenotype in apparently healthy individuals: 1) it could be the leukemic manifestation of an pre-existing undiagnosed lymphoma; 2) it could be the early stage of a lymphoma with a high chance of clonal expansion and clinical progression given sufficient time; 3) it could be the precursor of a lymphoma, requiring additional transforming events for disease progression; 4) it could represent a genomically stable clonal disorder with little of no risk of evolution to lymphoma; and, 5) it could represent a heterogeneous group of patients including two or more of the above possibilities.

The 2008 WHO Classification recognized three distinct types of MZ lymphomas: (i) nodal MZ lymphomas; (ii) extranodal MZ lymphomas of MALT type; and (iii) splenic marginal-zone lymphoma (SMZL) as well as a broad category of variably well-defined provisional entities, involving primarily the spleen, that do not fall into any of the other distinct types of splenic B-cell neoplasms. The best characterized provisional entities in this category of “splenic B-cell lymphoma/leukaemia, unclassifiable” (SLLU) are hairy cell leukemia variant (HCL-v) and splenic diffuse red pulp lymphoma (SDRL).

By definition, our cohort excluded cases with lymphadenopathy or splenomegaly but could have included cases with disease at extranodal sites such as the GI tract and bone marrow. Gastroscopy, performed in 34 asymptomatic patients revealed gastritis in 22 and a single gastric MALT lymphoma, detected 8 months after presenting with a lymphocytosis, consistent with the rarity of leukemic involvement in extranodal MZ lymphoma. In contrast, all cases showed clonal B-lymphocytic infiltration of the bone marrow which was >30% in 17/35 patients. This raises the question as to whether these cases should be considered to have a MZ
lymphoma of the bone marrow\textsuperscript{38}. However none presented with or developed cytopenias or have subsequently required treatment and use of the term lymphoma to describe these cases would seem clinically inappropriate. The observation that 15 patients developed splenomegaly strongly supports options 2 or 3, at least in a subset of cases. The absence of splenic histology limits the conclusions that can be drawn, although both cytogenetic and immunogenetic data provide some insights into the biology of MZ-like lymphocytosis. Among the whole cohort, the incidence of chromosomal abnormalities was 71.6\%, identical to that seen in our recent large-scale multi-institutional study of 330 cases of SMZL/SLLU\textsuperscript{34}. The pattern of cytogenetic abnormalities was also similar (Figure 4) with a high incidence of aberrations involving chromosome 7, including 3 cases with the t(2;7)(p11-12;q21-22) translocation leading to dysregulation of CDK6 expression. This translocation has been reported previously in rare cases of SMZL\textsuperscript{39} and a single case designated as CD5-negative MBL\textsuperscript{40}. Compared to SMZL/SLLU, MBL-MZ exhibited a significantly lower frequency of complex karyotypic abnormalities (3 or more aberrations) and gain of chromosome 3/3q, but interestingly 4/5 cases with complex karyotypes developed splenomegaly suggesting a link between clonal evolution and progressive disease. Eight patients, of whom 7 had stable disease, had an isochromosome of 17p resulting in TP53 loss. Several also had p53 dysfunction and a TP53 mutation (unpublished data), analogous to the findings in CD5+ve MBL and early CLL, in which TP53 abnormalities may be associated with indolent disease especially in patients with mutated IGHV genes\textsuperscript{9,41}.

The immunogenetic signature of cases with MZ-like lymphocytosis reported in our study is strongly indicative of antigen selection, reflected in significantly mutated IG genes in the great majority (~76\%) of cases and predominance of the IGHV4-34 gene (22.8\% of all cases). Immunogenetic comparisons revealed interesting similarities to SDRL, distinct from SMZL (Figure 5). In particular, most sequences in both entities were classified as significantly mutated, in contrast to SMZL (p=0.004), where a large proportion of cases carry borderline/minimally mutated IGHV genes\textsuperscript{42}. A further significant distinction from SMZL concerned the usage of certain IGHV genes, in particular IGHV1-2*04\textsuperscript{42}. This gene, predominating by far in the SMZL
repertoire (32%), was used much less frequently in either the present series (6.3%; p<0.0001) or SDRL (5.8%)\textsuperscript{42}. In contrast to the cytogenetic findings, there was no correlation between splenomegaly and immunogenetic data.

In summary, our cohort comprises cases with a clonal lymphocytosis, likely to have arisen from a MZ B cell. We have no evidence for an underlying causative agent or for a unique cytogenetic or immunogenetic profile. All cases present with bone marrow involvement without splenomegaly suggesting preferential homing and/or origin in the bone marrow. However expression of DBA.44 (normally associated with primary splenic lymphomas) in many cases and the subsequent splenic enlargement in some cases means that we cannot exclude a splenic origin. Currently it is not possible to reliably distinguish the majority of cases of MZ-CBL that will remain clinically stable despite prolonged follow up, from those destined to progress. Distinguishing between options 2, 3 and 4 therefore requires additional information. Recent next generation sequencing studies have identified recurring genomic abnormalities in SMZL. It will be of interest to determine whether similar abnormalities are found within our cohort at presentation or are acquired during the course of their disease. These studies are in progress.

This data raises two questions of practical importance, firstly, mindful of increasing concerns about the implications of over-diagnosis, how should asymptomatic individuals found to have a lymphocytosis with marginal zone features be managed and, secondly, what name should be given to their condition?

The policy of all 3 centers contributing to this study has been to perform either CT scanning or ultrasonography to exclude nodal and especially splenic enlargement. Based on our findings in this study we would not recommend routine screening for extranodal lymphomas (e.g. gastroscopy) in the absence of specific symptoms. While bone marrow examination is mandatory in patients presenting with or developing cytopenias and provided additional diagnostic information in our cohort, we recognize that many of our patients were elderly and frail and marrow examination would not alter their management. The observation that disease progression can occur, often many years after presentation indicates the need for long-term follow up.
Studies on CLL-like MBL suggest an increased incidence of bacterial infections even in the absence of progressive disease\textsuperscript{43,44}. Our cohort was too small to comment definitively but we did not find an increased risk of significant infections despite the presence of mild hypogammaglobulinaemia in some cases. 

Regarding nomenclature, many cases could be classified as CD5 -ve MBL but the cohort also included cases with weak CD5 positivity as seen in MZ lymphomas. It has been suggested that MBL could be more simply subclassified into CLL-like and non Hodgkin-lymphoma-like categories but our data would suggest that it is possible to define a distinct subgroup with MZ features. Our cohort also included cases with a clonal B-cell count exceeding $5 \times 10^9$/l. These cases did not differ in any other respect from cases with clonal B-cell counts of $<5 \times 10^9$/l and, as with the distinction between clinical MBL and early CLL, raises questions about the clinical and biological validity of a numerical cut off.

None of our cases are readily classifiable within the current WHO criteria. We suggest that a case could be made for a provisional entity that would encompass the cases we describe. It would be clinically important for the name not to imply a neoplastic, malignant or lymphomatous process. Monoclonal B-cell lymphocytosis with marginal zone features would be attractive but is constrained by the current diagnostic criteria for MBL. Clonal B-cell lymphocytosis with marginal zone features (CBL-MZ) is a possible alternative.
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AUTHORSHIP
Aliki Xochelli, Christina Kalpadakis, Anne Gardiner and Panagiotis Baliakas performed research, analyzed data and wrote the paper. Theodoros Vassilakopoulos, Maria K. Angelopoulou, Sotirios Sachanas, Achilles Anagnostopoulos, and Helen A. Papadaki provided samples and associated clinical data and supervised research. George Kanellis and Penelope Korkolopoulou were responsible for histopathologic analysis. Sarah Mould, Zadie Davis, Evangelia Stalika, Neil McIver-Brown, Rachel Ibbotson and Anastasia Athanasiadou performed research. Theodora Papadaki, Kostas Stamatopoulos, Gerassimos A. Pangalis and David Oscier designed the study, supervised research and wrote the paper.

CONFLICT-OF-INTEREST DISCLOSURE
The authors have no relevant conflicts of interest to disclose.
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### TABLES

**Table 1.** Demographics and basic laboratory tests of the patients included in the present cohort. Δm: median value.

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<table>
<thead>
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<td>Number of cases</td>
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<tr>
<td>Males/Females</td>
<td>49/53</td>
</tr>
<tr>
<td>Age</td>
<td>Δm: 71 years (range: 38-91 years)</td>
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<tr>
<td>Lymphocyte count</td>
<td>Δm: 6.63 x 10^9/l (range: 2.2-37.1 x 10^9/l)</td>
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<td>Hemoglobin count</td>
<td>Δm: 136.5 g/L (range: 116-177 g/L)</td>
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<td>PLT count</td>
<td>Δm: 239 x 10^9/l (range: 145-524 x 10^9/l)</td>
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<tr>
<td>Paraproteinemia</td>
<td>27/81 cases (33%)</td>
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FIGURE LEGENDS

Figure 1. Peripheral blood cytology and bone marrow pathology. (A) Peripheral blood smear Lymphocyte cytology. Morphologic heterogeneity of the lymphocytic population was apparent in most CBL-MZ cases. (B) Patterns of bone marrow lymphocytic infiltration by predominately small B-lymphocytes: A) interstitial with minor extent of intrasinusoidal pattern of infiltration B) interstitial pattern of infiltration C) paratrabecular and interstitial pattern of infiltration (in all images B cells are depicted with the CD20 antibody).

Figure 2. Graphic representation of the cytogenetic findings. The ideograms were prepared with the CYDAS software package, freely available at www.cydas.org. A. Additions (green) and deletions (red). B. Chromosomal translocation breakpoints.

Figure 3. Immunogenetics of CBL-MZ. IGHV gene repertoire.

Figure 4. Comparison of main cytogenetic findings between CBL-MZ and primary splenic small B-cell lymphomas. CBL-MZ exhibits a heterogeneous cytogenetic profile that, apart from a similar incidence of abnormal karyotypes, is significantly different from both splenic marginal-zone lymphoma (SMZL) and splenic leukemia/lymphoma unclassifiable.

Figure 5. Comparison of immunogenetic features between CBL-MZ and primary splenic small B-cell lymphomas. (A) IGHV gene repertoire. The IGHV gene repertoire of CBL-MZ is significantly different from splenic marginal-zone lymphoma (SMZL) and resembles splenic diffuse red pulp lymphoma (SDRL). (B) Somatic hypermutation. Most CBL-MZ cases carry a significant mutational load as evidenced by the germline identity of the clonotypic IG genes. Comparisons with SMZL and SDRL.
Figure 4
Figure 5A
Figure 5B
Clonal B-cell lymphocytosis exhibiting immunophenotypic features consistent with a marginal zone origin: is this a distinct entity?

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