bjh review

Diagnostic criteria for monoclonal B-cell lymphocytosis

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Summary

Very low levels of circulating monoclonal B-cell subpopulations can now be detected in apparently healthy individuals using flow cytometry. We propose the term 'monoclonal B-cell lymphocytosis' (MBL) to describe this finding. The aim of this document is to provide a working definition of MBL for future clinical, epidemiological and laboratory studies. We propose that the detection of a monoclonal B-cell population by light chain restriction is sufficient to define this condition in individuals not meeting the diagnostic criteria for other B-lymphoproliferative disorders. The majority of individuals with MBL will have cells that are indistinguishable from chronic lymphocytic leukaemia (CLL). However, this blood cell clonal expansion of $CD5^+$ or $CD5^-$ B-lymphocytes is age-dependent and immunophenotypic heterogeneity is common. Longitudinal studies are required to determine whether MBL is a precursor state to CLL or other B-lymphoproliferative disease in a situation analogous to a monoclonal gammopathy of undetermined significance and myeloma. Future studies of MBL should be directed towards determining its relationship to clinical disease, particularly in individuals from families with a genetic predisposition to developing CLL.

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The increasing technological ability to detect monoclonal B cells using three- and four-colour flow cytometry has led to the identification of very low levels ($<0{\cdot}005 \times 10^9$ cells/l) of circulating clones of B cells with surface features similar to chronic lymphocytic leukaemia (CLL) in apparently healthy individuals. Such clones are now being detected within the context of an absolute lymphocyte count of $5{\cdot}0 \times 10^9$/l or below the level required for a diagnosis of CLL. If these clonal expansions are implicated in the aetiology of CLL then their study has the potential to offer insight into the molecular development of CLL in general. A variety of terms have entered the literature to designate this finding. Followed by a meeting of the International Workshop on CLL at the National Cancer Institute (NCI) in Bethesda, MD, a subcommittee of the International Familial CLL consortium was formed to summarize the literature and to propose a unified nomenclature to describe the finding of a monoclonal expansion in healthy individuals. Here, we define monoclonal B-cell lymphocytosis (MBL) as the flow cytometric detection of a light chain restricted lymphocytosis. In addition to providing a working definition of MBL we suggest areas for further study.

History and population prevalence of MBL

Chronic lymphocytic leukaemia is defined by the presence of monoclonal B-lymphocytes co-expressing CD19, CD5 and CD23, with weak or no expression of CD20, CD79b, FMC7 and surface immunoglobulin. The monoclonal B cells must represent the majority of leucocytes with an absolute lymphocyte count $>5 \times 10^9$ cells/l, which has persisted for at least 1 month (Cheson *et al*, 1996). Several investigators have demonstrated that patients fitting within these criteria had disease that was stable for long periods of time, and have used a variety of different names to denote this situation. It has also

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been noted that patients with $CD5^-$ B-cell disorders may have stable disease. These reports are characterized in Table I. These studies have been critical to the understanding that there is a spectrum of disease in CLL and other B-cell lymphoproliferative disorders, both in terms of the tumour burden at presentation and the probability that the disease will progress to a stage that requires treatment. The application of basic diagnostic flow cytometry to the general population has led to the detection of a MBL in otherwise healthy individuals.

Monoclonal B cells in healthy individuals with normal peripheral blood counts were first noted in studies of unaffected siblings families with a genetic predisposition to CLL (Marti *et al*, 1992a,b; Marti, 1993). In 1995, a US Public Health Service (USPHS) Workshop reviewed the laboratory, clinical, and population data and recognized that both $CD5^+$ and $CD5^-$ MBL could be identified in healthy individuals and outpatients with no clinically apparent evidence of haematological disease (Cartwright, 1997; Marti *et al*, 1997; Sarasua *et al*, 1997; Vogt *et al*, 1997). In a large cross-sectional population study of 1491 individuals (Marti *et al*, 1997; Sarasua *et al*, 1997; Vogt *et al*, 1997), US investigators at the Agency for Toxic Substances and Disease Registry, Centres for Disease Control and the Food and Drug Administration found evidence of a MBL phenotype in 11 individuals (0·8%). Research from the Haematological Malignancy Diagnostic Service Laboratory using three-colour flow cytometry demonstrated a higher prevalence of approximately 1·7% in an outpatient clinic population (Jack *et al*, 1997). Following recommendations from the USPHS Workshop, Slade (1999) selected patients with an absolute B-cell lymphocytosis or relative increase in the proportion of $CD5^+$ B cells for monoclonality assessment, and demonstrated a prevalence of 0·6% (13/1985). More recently, Rachel *et al* (2002) examined MBL using a similar screening strategy in a study of mid-Western US blood donor population. Among donors aged 39–80 years, they found a prevalence of MBL of 0·14% (7/5138). These studies demonstrated that an MBL may be detected in the absence of an absolute lymphocytosis and in individuals with a normal proportion of $CD5^+$ B cells. However, the reported prevalence varied dependent on the technique used, and the development of CLL-specific assays suitable for minimal residual disease detection that are more sensitive than polymerase chain reaction for immunoglobulin heavy chain gene rearrangement (IgH-PCR) (Rawstron *et al*, 2001) potentially allows the detection of monoclonal B cells in individuals with an apparently normal kappa:lambda ratio.

Using this assay, Rawstron *et al* (2002a)) detected CLL-phenotype cells in 2·7% of adults aged over 18 years (0·3% in individuals younger than 40 and 5·2% in individuals older than 60). Incidental to CLL-specific assay, these studies identified $CD5^-$ non-CLL phenotype MBL in nine of 910 (1·0%) individuals over 40 (Rawstron *et al*, 2002a). Using a similar approach in a population-based study of 500 normal Italian subjects over the age of 65 years with a normal blood cell count, Ghia *et al* (2004) found 29 individuals with a clonal expansion of lymphocytes with either a CLL-phenotype ($n = 22/442$, prevalence 5·5%) or $CD5^-$ non-CLL-phenotype ($n = 7/500$, 1·4%) showing good consensus between the two independent studies. All cases, regardless of the phenotype, were CD10 negative and *BCL1* or *BCL2* rearrangements were not found. In both studies, a monoclonal IgH rearrangement was confirmed by IgH-PCR in the majority of cases. The absolute monoclonal B-cell count ranged from 0·002–$1{\cdot}5 \times 10^9$/l. There was a male predominance, and an increasing prevalence with age similar to that observed in clinical disease. It has also been demonstrated that the CLL-phenotype cells in otherwise haematologically normal outpatients have similar karyotypic abnormalities to clinical disease with respect to 13q14 deletions (O'Connor *et al*, 2003).

In summary, studies assessing light chain clonality only will identify MBL in approximately 0·5–1% of the adult population. Studies combining a disease-specific phenotype with clonality assessment detect MBL in approximately 2–3% of the population. The prevalence increases with age, rising to over 5% for adults aged 60 years or older.

Diagnostic criteria

The diagnosis of MBL is based on the identification of a clonal lymphocyte population by immunophenotypic characterization. Different laboratories have used diverse approaches to identify minimal B-cell monoclonal lymphocytosis, making comparisons across geographic, ethnic, and in different risk groups difficult. In order to standardize and facilitate future studies, we propose the following set of guidelines for the diagnostic characterization of a blood B-cell monoclonal lymphocytosis.

1 Detection of a monoclonal B-cell population in the peripheral blood with
   - i overall kappa:lambda ratio >3:1 or <0·3:1, or
   - ii greater than 25% of B cells lacking or expressing low level surface immunoglobulin or
   - iii a disease-specific immunophenotype.

2 Repeat assessment should demonstrate that the monoclonal B-cell population is stable over a 3-month period.

3 Exclusion criteria
   - i lymphadenopathy and organomegaly, or
   - ii associated autoimmune/infectious disease, or
   - iii B-lymphocyte count $>5 \times 10^9$/l, or
   - iv any other feature diagnostic of a B-lymphoproliferative disorder. However, a paraprotein may be present or associated with MBL and should be evaluated independently.

4 Subclassification:
   - i $CD5^+23^+$: this is the major subcategory and corresponds to a CLL immunophenotype (Cheson *et al*, 1996).
   - ii $CD5^+23^-$: correlate moderate level of CD20 and CD79b expression with atypical CLL.
   - iii $CD5^-$: corresponds to non-CLL lymphoproliferative disease (LPD).

Table I. Reported studies of subclinical chronic lymphocytic leukaemia (CLL) states and previous terminology to describe monoclonal B-cell lymphocytosis (MBL)*.

Term	Subjects and study setting	Definition	Reference
Benign monoclonal B-cell lymphocytosis	20 of 500 CLL cases	Rai stage 0, no progression	Han *et al* (1984)
Benign monoclonal B-lymphocytosis	327 of 1777 CLL patients	Rai stage 0, no deep nodes	Mandelli *et al* (1987)
Idiopathic, persistent, chronic lymphocytosis	Six of 13 individuals with chronic lymphocytosis	Monoclonal ALC 4·4–13·4 × 10^9/l	Bassan *et al* (1988)
B-monoclonal lymphocytosis of undetermined significance (BMLUS)	25 patients in one study	Blood and bone marrow lymphocytosis only decreased CD23, CD25 and CD71 expression	Aguilar-Santelises *et al* (1989, 1992); Garcia *et al* (1989); Kimby *et al* (1989); Aman and Mellstedt (1991)
Smoldering CLL	127 of 261 CLL did not progress; 31% progression at 3 years	Hb ≤13 g/dl, ALC <30 × 10^9/l and LDT >12 months	Montserrat *et al*, 1988
Smoldering CLL (French Co-operative Group)	231 of 309 natural history Binet stage A CLL; two groups defined from two studies	A1′: Hb >12 g/dl, ALC <30 × 10^9/l; A1″: Hb,12, ALC >30 k	
		A2′: Hb >12, ALC <30 × 10^9/l, BM <80%,<2 areas involved A2″: Hb <12, ALC >30 × 10^9/l, BM >80% or two involved areas	French Cooperative Group on Chronic Lymphocytic Leukemia (1990)
B-cell monoclonal lymphocytosis	Two CLL kindreds	Downregulation of CD20	Marti *et al* (1992a); Marti (1993)
B-cell monoclonal lymphocytosis	Residents in areas near hazardous waste dumps; stratified random sample by age and sex; 10 USA sites, 1991–4; age >45 years; 11 of 1499 (0·7%);	B-cell-like phenotype defined as: lymphocytosis with B-lymphocytes >50 percentile, CD20 dim, abnormal CD5	Vogt *et al* (1997)
B-cell monoclonal lymphocytosis	Same base population as Vogt *et al* (1997); three of 11 subjects with increased B cells	Monoclonality demonstrated by kappa–lambda analysis	Marti *et al* (1997)
B-cell monoclonal lymphocytosis	Clonal B-population in subjects age >40 years estimated 1·5–2%; 1000 Yorkshire hospital outpatients	Three-colour flow cytometry with light chain expression	Jack *et al* (1997)
Detectable, sub-clinical BCLL B-cell monoclonal lymphocytosis	37 Samples showed light chain restriction with normal morphology	87% Confirmed by PCR analysis for Ig gene rearrangement	Maiese and Braylan (1997)
B-cell monoclonal lymphocytosis	13 of 1985 (0·6%)	B-cell follow-up study	Slade (1999)
CLL-like immunophenotype non-CLL-like immunophenotype	32 of 910 (3·5%) clinic outpatients	Four-colour flow cytometry Ig gene spectral typing	Rawstron *et al* (2002a)
CLL-like immunophenotype non-CLL-like immunophenotype	Eight of 59 (13·5%); apparent unaffected cases from 21 CLL kindreds	Four-colour flow cytometry	Rawstron *et al* (2002b)
B-cell monoclonal lymphocytosis	Seven of 5138 (0·14%); adult blood donors	Three-colour flow cytometry; PCR IgH rearrangement	Rachel *et al* (2002)
CD5 negative BMLUS	Seven patients stable chronic lymphocytosis	$CD5^-$, $CD23^-$ not viewed as precursor CLL	Wang *et al* (2002a,b)

Table I. Continued

Term	Subjects and study setting	Definition	Reference
Clonal lymphocytosis of unknown significance (CLUS)	CLUS	Blood Editorial	Byrd (2002)
B-cell monoclonal lymphocytosis	Six of 33 (18·5%); apparent unaffected cases from nine CLL kindreds	Three-colour flow cytometry colour PCR IgH rearrangement	Marti *et al* (2003)
Monoclonal B-lymphocyte expansion atypical CLL-like immunophenotype	500 Normal Italian subjects ≥65 years old	Four-colour flow cyotometry light chain restriction PCR IgH rearrangement	Ghia *et al* (2004)
Monoclonal B-cell lymphocytosis	Longitudinal study	Four-colour flow cytometry and FISH	Rawstron *et al* (2003)
Monoclonal gammopathy of unknown significance (MGUS); monoclonal gammopathy, unattributed/unassociated [MG(u)]	The relative risk of progression for multiple myeloma is 25 and macroglobulinaemia is 46	M-protein in serum <30 g/l; BM clonal plasma cells <10%; no evidence of other B-cell proliferative disorders. No related organ or tissue impairment (no end organ damage, including bone lesions)	The International Myeloma Working Group (2003)

*Definitions of smoldering CLL and monoclonal gammopathy of undetermined significance (MGUS) added for the sake of comparison with MBL.

Notes:

1 More than one set of kappa/lambda light chain reagents may be used; the detection of any B-cell monoclonal population by light chain restriction is sufficient. Confirmation with IgH-PCR may be helpful but is not essential.

2 The monoclonal B-cell population may represent a minority of total B cells when identified by a disease-specific immunophenotype. These may be demonstrable even if the overall kappa:lambda ratio is normal, although clonality must be demonstrated within the cellular population identified by the disease-specific phenotype.

3 MBL lacking surface immunoglobulin is associated with $CD5^{+}CD23^{+}$ MBL.

4 A minimum of three colours (CD19 or CD20, anti-kappa and anti-lambda) should be used to confirm clonality, although four or more colours are preferable.

5 The fluorescence intensity of surface immunoglobulin, CD20 and CD79b expression if moderately increased should be noted.

6 The number of cells analysed should allow the formation of a cluster containing at least 50 events.

7 Repeat flow cytometric analysis is not necessary for research applications if monoclonality is confirmed by other approaches, e.g. fluorescence *in situ* hybridization or PCR, but may be useful for monitoring.

8 A disease-specific phenotype exists for hairy cell leukaemia ($CD5^{-}CD103^{+}CD11c^{+}CD25^{\pm}$) but it is probable that a full diagnosis of hairy cell leukaemia will be made in the presence of any level of circulating disease.

9 Other subclassifications may be included if sufficiently specific tests with evidence of a clinical association can be confirmed.

Natural history of the disorder

As MBL has a greater prevalence than CLL, even if it were to be a precursor of CLL, a significant proportion of cases must either regress or remain static. This is in keeping with the finding that a large proportion of patients with early stage CLL show stable disease and, occasionally, decreasing CLL cell counts (Table I). Faguet *et al* (1992) reported a series of 39 patients with a slight increase in their absolute lymphocyte count; 24 had an abnormal B-cell clone and eight of the 24 patients progressed to clinical B-cell CLL over 5 years. In the limited NCI follow-up of familial MBL, progression to CLL has not yet been observed. Rawstron *et al* (2003) have begun to assess outcome in $CD5^{+}23^{+}$ MBL in a clinical population of patients who do not meet the diagnostic criteria for CLL. These are patients with an incidental finding of MBL meeting the criteria outlined above. Initial data (5-year median follow-up) suggests that approximately 60% have stable or regressive lymphocytosis; 35% show increasing lymphocyte cell counts, and 5% have required treatment. Of note, six of 47 patients showed bi-phasic progression kinetics, with initially stable disease followed by a more pronounced increase in lymphocyte count at a median 3·5 years (range 0·5–7·0) from presentation, suggestive of a secondary event.

These data reinforce the concept that CLL presents with a very broad spectrum of tumour burden, and that risk of progression is independent of the absolute level at presentation. There is currently no evidence that early diagnosis of CLL is of any benefit to the patient. However, identifying CLL at its earliest stages is likely to be of significant value in identifying and defining the biological mechanisms responsible for disease aetiology and progression.

Populations to consider

One group (Kyle & Rajkumar, 1999; Kyle *et al*, 2002) established a significant relationship between multiple myeloma (MM) and monoclonal gammopathy of undetermined significance (MGUS). Over a 30-year period, there is a 25-fold relative risk of MGUS progressing to MM and a 40-fold relative risk of developing macroglobulinaemia in subjects with IgM MGUS. At present, the notion that MBL is a precursor of CLL and other non-Hodgkin lymphomas (NHLs) is presumptive and a major goal of future studies should be to define this relationship. However, given the high population prevalence, screening of the general population would be prohibitively expensive and clinically uninformative and is not recommended outside of research applications. It is likely that, as with MGUS, MBL will predominantly be identified as an incidental finding in individuals being found medically for other reasons.

Epidemiological studies have shown that the risk of CLL in relatives of patients with CLL is increased and it is likely that a subset of the disease is caused by a genetic susceptibility (Fraumeni *et al*, 1969; Neuland *et al*, 1983; Blattner *et al*, 1987; Caporaso *et al*, 1991, 1997; Goldin *et al*, 1999; Yuille *et al*, 2000; Ishibe *et al*, 2001, 2002a,b). As $CD5^{+}23^{+}$ MBL may be a precursor of CLL, it has been postulated that MBL might be overrepresented in apparently healthy relatives of familial CLL cases. Such a proposition has recently been examined by two research groups. Rawstron *et al* (2002b) found that 14% of relatives of familial cases had MBL compared with 3% of outpatient controls. Marti *et al* (2003) also reported overrepresentation of MBL in relatives of familial cases (18% compared with 0·7% in controls). These studies provide strong support for the notion that MBL with a CLL-like phenotype may be a surrogate marker for carrier status. Therefore, there is a powerful rationale for research into the risk of progression for individuals with MBL in CLL families. However, it is still far from clear whether the detection of MBL predicts a higher probability of eventually developing CLL within CLL family members. Therefore, as with the general population, screening apparently healthy members from CLL families is currently not clinically informative.

It is recommended that relatives who are potential donors for allogeneic transplant be screened for MBL. This is primarily because the transplanted MBL is likely to impact on residual disease monitoring in the donor, although the potential for transplanted MBL to expand and or transform in an immunocompromised host is also of concern. Genetic counselling for a familial disorder could be considered for high-risk individuals in certain settings (Hampel *et al*, 2004).

Recommendations for follow-up

There are two key problems that arise in the area of asymptomatic or subclinical disorders: the first is what to inform the patient or individual, and the second is how closely the patient should be investigated and monitored.

The risk of progression to clinical disease is not known, and even if an association with clinical disease is convincingly demonstrated, it will be sometime before any risk of progression can be calculated. In the absence of precise clinical information about outcome, the adverse consequences of inducing anxiety by informing an individual that they might have a preclinical blood cancer or precursor condition; the lack of any effective early intervention; and the potential for adverse individual health insurance concerns, it may be unethical to inform patients of their disorder when it would result in little or no clinical benefit whilst incurring the risk of significant adverse effects. It is therefore acceptable in a research setting, where extremely low levels of monoclonal B cells may be identified, to not provide the results of investigations to patients. If MBL is diagnosed after specific investigation, it is important to stress that the risk of progression is not known but is likely to be very low and it may be helpful to offer periodic monitoring in these cases.

How closely patients are investigated and monitored is a significant problem. Although population screening is not clinically indicated, a substantial and increasing number of individuals are demonstrated to have MBL as an incidental finding during routine medical assessment. As this report is aimed at providing diagnostic criteria, it is not possible to provide evidence-based guidelines for monitoring patients with MBL. However, some suggestions can be provided based on initial studies.

The close relationship between $CD5^{+}23^{+}$ MBL and CLL suggests that the levels in the peripheral blood are likely to be indicative of bone marrow involvement. Therefore bone marrow investigation is unlikely to be of any value for these patients. B-lymphoproliferative disorders with a non-CLL phenotype may show much greater tissue or bone marrow involvement than the peripheral levels suggest, and therefore marrow investigation may be of value in patients with $CD5^{+}23^{-}$ or $CD5^{-}$ MBL. The presence of peripheral lymphadenopathy or organomegaly will have been excluded as part of the initial physical examination. However, in selected cases, a baseline chest X-ray, abdominal ultrasound and or a computed tomography scan may be indicated. And a rapid rise in the absolute lymphocyte count might warrant a diagnostic/prognostic bone marrow aspirate and biopsy.

For MBL patients identified during investigation of a mild lymphocytosis, it may be helpful to confirm whether the monoclonal B-cell counts are regressive, stable or progressive over a 3–6-month period. Long-term prospective studies will be required to determine whether periodic monitoring (i.e. as in MGUS, approximately yearly assessment of the absolute lymphocyte count and/or flow cytometry) will be of value in identifying the small proportion of MBL patients who will show progression to clinical disease.

Monoclonal B cells that are indistinguishable from early CLL/NHL may be detected in up to 4% of the general population. We propose the term MBL to describe this phenotype. MBL is detectable at a higher frequency in apparently unaffected members of CLL kindreds and is more prevalent with advancing age. Appropriate studies are needed to establish the natural history of MBL in both the general population and high-risk settings. The relationship of MBL to other non-CLL LPD, MGUS and reactive lymph node hyperplasia (Kussick *et al*, 2004) remains to be defined. Continued investigation of the human B-cell repertoire in a defined MBL population may reveal further abnormalities relevant to the aetiology and pathogenesis of CLL.

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