Best Practice & Research Clinical Haematology 32 (2019) 229–238

Contents lists available at ScienceDirect

Best Practice & Research Clinical Haematology

journal homepage: www.elsevier.com/locate/issn/15216926

Monoclonal B-cell lymphocytosis

Elsa Maitre, Xavier Troussard*

Laboratoire d'hématologie biologique, CHU de Caen Normandie, Caen, 14033, CEDEX 9, France

ARTICLE INFO

Keywords:
Monoclonal B-Cell lymphocytosis
Chronic lymphocytic leukemia
Flow cytometry
Progression

ABSTRACT

Flow cytometry diagnostic practices can detect very low levels of clonal B cells in the peripheral blood. In the absence of clinical symptoms, cytopenia or organomegaly, the small clones may correspond to monoclonal B-cell leukemia (MBL) diagnosis. Most MBLs harbor a chronic lymphocytic leukemia (CLL) phenotype (e.g., $CD5^+$, $CD23^+$) and are referred to as CLL-type MBL. The two other types are atypical CLL-type MBL and non-CLL-type MBL. In addition to the phenotypical classification, the clonal B count is a major issue because of the impact on the prognosis and the risk of progression in CLL. It allows for the discrimination of two distinct types: high-count (HC) MBL and low-count (LC)-MBL based on a cutoff value of 0.5×10^9/L clonal B cells. LC MBL appears to be very stable over time and is probably related to immunosenescence. Conversely, HC MBL could be a premalignant state before the occurrence of CLL.

Introduction

Monoclonal B-cell lymphocytosis (MBL) is characterized by the presence of less than 5×10^9 clonal B cells in the peripheral blood in the absence of lymphadenopathy, splenomegaly and hepatomegaly, any other features of B-cell chronic lymphoproliferative disorder (B-CLPD) or disease-related symptoms (unintentional weight loss, significant fatigue with Eastern Cooperative Oncology Group (ECOG) performance scale 2 or worse, fever for 2 or more weeks without evidence of infection, night sweats without evidence of infection). In contrast, chronic lymphocytic leukemia (CLL), the most prevalent lymphoid malignancy, is defined by the identification of more than 5×10^9 clonal B cells in the peripheral blood. CLL is a heterogeneous disease, with patients who survive for many years or decades and other patients who have a rapidly fatal disease despite treatment. Based on the immunophenotypic profiles of abnormal peripheral lymphoid cells, MBL is classified into three types: CLL-type, atypical CLL-type and non-CLL-type. Because of the improved detection and identification of clonal B cells by increasingly sensitive flow cytometric techniques, a very low level of clonal B cells can also be detected in the peripheral blood, especially in the healthy elderly population. Similar to CLL, MBL appears to be heterogeneous with different profiles. Low-count (LC) MBL, defined by the presence of less than 0.5×10^9/L clonal B cells, can be a very stable condition over time. Conversely, high-count (HC) CLL-type MBL ($> 0.5 \times 10^9$/L clonal B cells) can progress from a premalignant disease to a true CLL. This review aims to describe the epidemiologic, clinical and biological features of the three different types of MBL.

Abbreviations: B-CLPD, B-cell chronic lymphoproliferative disorder; HC MBL, High-count monoclonal B cell lymphocytosis; LC MBL, low-count monoclonal B cell lymphocytosis

* Corresponding author. Laboratoire d'hématologie biologique, CHU de Caen Normandie, Avenue de la Côte de Nacre, 14033, Caen Cedex 9, France.

E-mail addresses: maitre-e@chu-caen.fr (E. Maitre), troussard-x@chu-caen.fr (X. Troussard).

https://doi.org/10.1016/j.beha.2019.06.002
Received 2 April 2019; Accepted 4 June 2019

How to define MBL?

In 1975, the original Rai staging system was introduced to divide CLL patients into five different prognostic subgroups. Patients with stage 0, a low-risk subgroup, presented with lymphocytosis > 15×10^9/L, bone marrow infiltration with more 40% abnormal lymphoid cells and no enlargement of the lymph nodes, spleen or liver. Furthermore, red blood cell and platelet counts were normal [1]. The original Rai classification was subsequently modified to reduce the number of prognostic groups from 5 to 3. The criteria required for CLL also changed over time with a decrease in the absolute lymphocyte count (ALC) to ≥5×10^9/L in 1996. Consensus guidelines from the World Health Organization (WHO) classification and International Workshop on Chronic Leukemia (iwCLL) now require the presence of ≥5×10^9/L clonal B cells for the diagnosis of CLL [2–5]. MBL is defined by a monoclonal B-cell count < 5×10^9 cells/L (< 5,000 B lymphocytes/μL) in the peripheral blood of people who have no associated clinical lymph node enlargement or organomegaly, no disease-related cytopenias, any other feature of B-cell chronic lymphoproliferative disorder (B-CLPD) or disease-related symptoms [2,3]. The concept of nodal MBL was introduced because a gray zone can exist between MBL and small lymphocytic lymphoma (SLL). The term SLL is used for cases with a circulating CLL cell count < 5×10^9/L and documented nodal, splenic or extramedullary involvement. Patients with nodal MBL can have a subtle and/or focal abnormal lymphoid population with no detectable lymphadenopathy or a spontaneous regression of lymph nodes that were enlarged at initial diagnosis [4]. An ALC ≥5×10^9/L needs clinical and biological investigation to clarify the etiology of lymphocytosis [5,6]. An interrogation, physical examination and a careful review of peripheral blood smears are the first steps of the clinical investigation. Immunophenotypic analysis must be performed if absolute lymphocytosis is sustained for at least three months [7]. The most common causes of lymphocytosis must be eliminated: reactive lymphocytosis (viral infections, autoimmune conditions, stress or hypersensitivity reactions), smoking, splenectomy or persistent polyclonal B-cell lymphocytosis with binucleated lymphocytes (PPBL) [8]. Morphologic assessment of peripheral lymphoid cells eventually suggests monomorphic infiltration. In CLL and MBL, mature lymphocytes with a narrow border of cytoplasm and a dense nucleus lacking discernable nucleoli and partially aggregated chromatin are present. Gumprecht nuclear shadows, or smudge cells, found as cellular debris, are additional common morphologic features. In the case of low-count (LC MBL) (< 0.5×10^9/L), morphologic examination is not sufficiently sensitive. Flow cytometry assessment of peripheral lymphoid cells remains necessary in all cases. A screening panel based on surface protein markers, such as Lymphoid Screening Tube Euroflow (CD45, CD19, CD20, lambda, kappa, CD38, DC3, CD4, CD8, CD56, TCR$\gamma\delta$ and CD5), could be useful to eliminate polyclonal lymphocytosis [9]. In the case of a monotypic abnormal B-cell population, other markers had to be added to achieve a better classification of B-CLPD. The European Research Initiative on CLL (ERIC) and the European Society for Clinical Cell Analysis (ECCA) recommended analyzing CD23, CD43, CD79b, CD81, CD200, CD10 and ROR1 [10]. To calculate the Royal Marsden Hospital (RMH) score, FMC7 expression is also needed [11,12]. Most MBL cases are CLL-type (> 75%), characterized by a CLL phenotype including $CD5^+$, $CD23^+$, weak expression of SmIg (often IgM with or without IgD), absence of FMC7 and weak or no expression of CD79b or CD22. The MBL CLL-type immunophenotypic signature is indistinguishable from that observed in CLL. In the other 25% of cases of MBL, B-cell clones have a different immunophenotypic profile and are classified as atypical CLL MBL ($CD5^+$, $CD20^{bright}$, variable expression of CD23) and non-CLL-type (CD5- without evidence of other typical markers of lymphoproliferative disorders such as CD10 in follicular lymphoma) [13]. To distinguish CLL-type from atypical CLL MBL, CD200 expression could be useful [14] but doesn't exclude the need for t(11; 14) cytogenetic analysis based on some previously described non-nodal CD200 + mantle cell lymphoma (MCL) studies [15]. A typical example of CLL-type MBL is shown in Fig. 1, with morphologic and phenotypic features.

How common is MBL?

CLL is the most common leukemia in the Western world and in the United States, with an incidence of 4.2:100 000/year [16,17]. The MBL frequency is estimated to be hundred times more common than CLL. CLL-type MBL is a common finding in the peripheral blood of healthy subjects regardless of their geographic origin or context. The prevalence of MBL varies considerably by function of the population studied (general population with normal blood count, hospital outpatient, age, sex) and sensitivity of the flow cytometry analysis. In the initial reports, two colors were used until 10 colors were used in the more recent studies. The number of acquired events is also a crucial element to improve the sensitivity (between 80000 and 5000000 cells). Thus, frequencies vary between 0.08% and 20.9% for CLL-type MBL, near 1% for atypical CLL-type MBL and 0.04–6% for non-CLL-type MBL (Table 1). CLL-type MBL varies with age: MBL is very rare under 40 years, and the prevalence increases with age, reaching 75% in patients older than 90 years (Table 1). Most MBL patients have a normal absolute B-cell count, ranging from 0.170 to 0.300 G/L, the clonal B cells accounting for a minimal percent of total B cells (less than 10%) (Table 1) [18–22]. In contrast to CLL, male predominance is not so clear, except for the elderly patient group or MBL occurring in CLL families [18–20,23,24].

Risk factors for developing MBL

In addition to age, which is closely associated with MBL [25,32], a familial history of hematological or solid malignancies among first-degree members of the family has been reported [32]. Among 505 first-degree relatives with no personal history of lymphoproliferative disease from 140 families having at least two cases of CLL, 17% of relatives had MBL. Age was the most important determinant where the probability for developing MBL by age 90 years was 61%. As is the case with CLL, males had a significantly higher risk for MBL than did females. In first-degree relative patients with a family history of CLL, the relative risk of developing MBL is four times higher than that observed in the general population, reaching 17-fold in adults < 40 years [33]. The MBL frequency has been reported up to 13–18% [21,24,34]. Furthermore, the probability for a relative increases with male gender and age [24]. Another

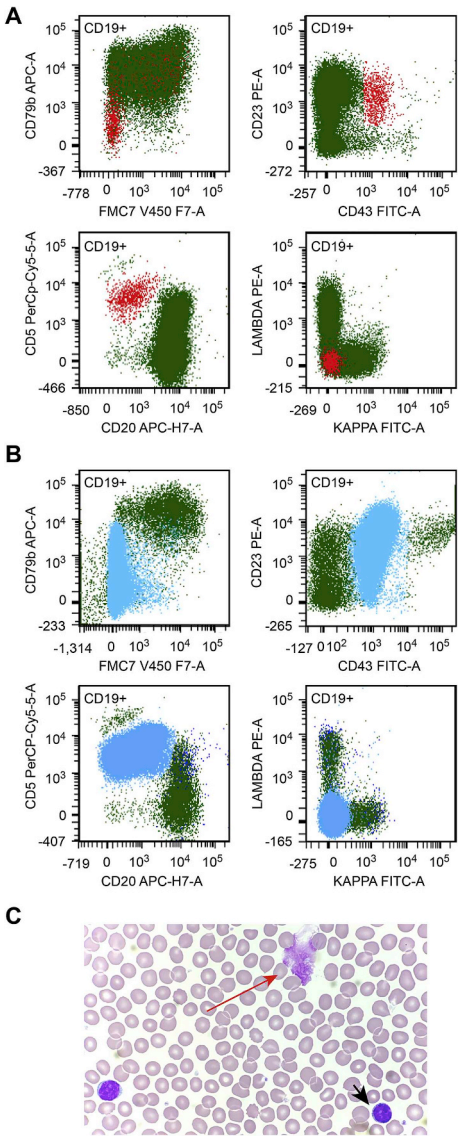

Fig. 1. Chronic lymphocytic leukemia (CLL)-type monoclonal B-cell lymphocytosis (MBL). Flow cytometry. A: Example of low-count CLL-type MBL in blood samples with CD19/CD5/CDCD23/CD24/CD79b/FMC7/kappa/lambda combination. Normal B cells are in green, and CLL-type MBL cells are shown in red. CLL-type MBL represents 0.4% of total lymphocytes (absolute abnormal B lymphocyte count = 0.006×10^9/L). B: Example of high-count CLL-type MBL in blood samples with CD19/CD5/CDCD23/CD24/CD79b/FMC7/kappa/lambda combination. Normal B cells are in green, and CLL-type MBL cells are shown in blue. CLL-type MBL represents 81% of the total lymphocyte (absolute abnormal B lymphocyte count: 4.4×10^9/L). Peripheral blood smears. C: Cytologic features. Mature lymphoid cells similar to those observed in chronic lymphocytic leukemia. Patient with mature lymphoid cells CLL-type with mature clumped chromatin (black arrow) and Gumprecht shadow (red arrow).

risk factor for developing MBL is exposure to infections and immunodeficiency. A high frequency of MBL is observed in patients with hepatitis C virus infection (HCV) [35]. Using a cross-sectional study design, MBL was significantly less common among individuals vaccinated against pneumococcal infection or influenza. MBL patients were also more likely to report infectious diseases among their children, respiratory disease among their siblings and personal history of pneumonia and meningitis [32]. Interestingly, kidney transplantation does not seem to be a risk factor for developing MBL. Alfano et al. [36] described five cases of MBL in a series of 157 patients: 4 patients (2.6%) with non-CLL-type MBL and only 1 patient with CLL-type MBL (0.6%)) at a median age of 65 years which is similar to the general population [36].

High-count MBL: a first step before the occurrence of CLL

An elegant study analyzed stored blood samples from healthy individuals enrolled in a prospective cohort study: 45 individuals developed CLL. In the peripheral blood obtained up to 6.4 years before CLL diagnosis, a typical B-cell clone was identified in 44/45

Table 1
MBL frequencies from different series.

Study	Study group			FCM		CLL-type MBL					Atypical CLL-type MBL	Non-CLL-type MBL
	Population-based	Median of age	N	Numbers of color	Events	< 40 years frequency (%)	≥60 years frequency (%)	≥90 years frequency (%)	Total frequency (%)	Absolute count clonal B cells (x10^9/L)	Frequency (%)	Frequency (%)
[18]	Italy general population	55.2 [18–102]	1725	5	500,000	0.2	8.96	ND	5.2	0.34 [ND]	1.1	1.2
[33]	US general population	ND	1242	4	500,000	0.3	5.2	ND	2.7	ND	ND	ND
[25]	UK general female population ≥64 years	ND [64–94]	597	11	ND	NA	20.9[b]	21.1	20.9	ND	0.8	4.2
[19]	Italy outpatients ≥65 years	[d] 74 [65–98]	500	4	200,000	NA	ND	ND	5.5	ND	0.6	1.4
[26]	Australia	ND	10,000	4	ND	0	,	ND	3.2[a]	[d] 2.36 [0.01–5.21]	ND	0.9[a]
[20]	Spain outpatients	62 [40–97]	608	8	5,000,000	NA	> 17.5[a]	75.0	12.0	ND	ND	ND
[27]	US blood donors	45 [18–79]	5141	2	ND	ND	0.37[c]	ND	0.08	ND	ND	0.04
[21]	UK hospital outpatients ≥40 years	57 [40–90]	910	4	200 000	NA	5.0	NA	3.5	0.013 [0.003–1.458]	ND	ND
[22]	UK hospital outpatients ALC < 4G/L	74 [62–80]	1520	4	500,000	NA	ND	ND	5.1	ND	ND	1.8
	UK hospital outpatients ALC≥4G/L	71 [39–99]	2228	4	500,000	ND	ND	ND	13.9	ND	ND	ND
[28]	UK hospital outpatients ≥45 years	ND > 45 years old	302	ND	ND	NA	ND	ND	7	0.005 [0.001–1.773]	ND	2
	UgaNDa general population ≥45 years	ND > 45 years old	302	ND	ND	NA	ND	ND	1	< 0.001	ND	14
[29]	US general population ≥40 years	ND [40–76]	1926	2	ND	NA	ND	ND	0.6	ND	ND	ND
[30]	US blood donors ≥45 years	57 [45–91]	2098	6	500 000	NA	ND	ND	4.8	0.005 [ND]	1.1	1.0
[31]	Australia general population ≥90 years	93 [90–101]	50	10	80 000	NA	NA	4	4	ND	ND	6

[a] Estimated from data.
[b] ≥64 years.
[c] ≥65 years.
[d] Mean and not median; ALC: absolute lymphocyte count; ND: data not done.

Table 2
CLL-type MBL characteristics.

Study	Population	FCM			FISH				IGHV	IGHV1		IGHV3			IGHV4			Mutations	
		CD38 (%)	CD49d (%)	Zap70 (%)	Del17p (%)	Del13q (%)	Del11q (%)	+12 (%)	UM[a]	Total (%)	1-69 (%)	Total (%)	3-7 (%)	3-23 (%)	Total (%)	4-59/61 (%)	4-34 (%)	TP53 (%)	NOTCH1 (%)
[20]	Spain outpatients	ND	ND	ND	ND	27.0	ND	5.4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
[22]	UK hospital outpatients	ND	ND	ND	2.3	47.9	3.7	21.8	12.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
[30]	US general population	ND	ND	ND	ND	ND	ND	ND	19.4	0	0	59.4	15.6	3.1	40.1	15.6	9.3	ND	ND
[46]	Selected high-count MBL	21[c]	ND	19.3[b]	4	44.6	2	18.6	22.8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
[43]	Selected low and high-count MBL	ND	ND	ND	0	29.4	2.7	15.4	29.5	14.1	5.1	62.8	5.1	21.8	16.7	1.3	10.3	ND	0
[41]	Selected high-count MBL	18.5[c]	ND	30.0[b]	0.7	34.6	2.3	11.5	19.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
[44]	CLL families	12.3[c]	19.0[d]	25.9[c]	3.0	73.8	0	6.6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
[53]	Selected high-count MBL	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	11.0
[45]	Selected MBL	17.0[ND]	ND	13.3[ND]	1.6	46.0	3.2	0	15.3	ND	ND	ND	ND	ND	ND	ND	ND	ND	3.2
[18]	Italy general population	ND	ND	ND	ND	ND	ND	ND	19.5	0	0	62.7	7.8	7.8	37.3	23.5	3.9	ND	ND
[42]	Selected MBL	22.7[c]	38.1[c]	40.2[c]	3.8	35.2	0	18.1	20.0	ND	8.6	ND	ND	11.4	ND	ND	16.9	3.0	ND
[50]	Selected low and high-count MBL	ND	ND	ND	ND	ND	ND	ND	24.8	16.1	6.8	49.3	8.5	8.7	24.5	6.8	11.8	ND	ND
[42]	Rai 0 CLL	26.0[c]	29.2[c]	36.3.1[b]	9.0	46.9	6.2	14.5	28.1	ND	8.7	ND	ND	7.2	ND	ND	15.2	11.5	ND
[46]	Rai 0 CLL	19.9[c]	ND	24.1[b]	0	56.8	4.9	8.0	18.8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND: data not done; UM unmutated.
[a] Homology≥98%.
[b] Threshold 20%.
[c] Threshold 30%.
[d] Threshold 45%.

patients [37]. This study demonstrates that MBL could be an early step in the process of CLL development. Xenogeneic transplantation of hematopoietic stem cells of CLL patients (HSC-CLL) in immunocompromised mice generates secondary B-cell monoclonal or oligoclonal clones simulating MBL phases, suggesting that MBL is a preleukemic phase and that new oncogenic events are necessary to transform MBL into CLL [38]. The results were recently confirmed *in vivo* in a large whole-genome analysis of MBL patients. Clonal hematopoiesis was demonstrated in most MBL patients, as in myelodysplastic syndromes or acute myeloid leukemia [39]. The annual rate to progression to CLL is 1–2% [13,22,40]. In a study including 227 patients with HC MBL, 34.5% of the patients progressed after a median follow-up of 76 months, regardless of the MBL type [41]. In one another study, 15.5% of MBL patients needed treatment compared to 30.5% of Rai 0 CLL cases after a median follow-up of 42.7 months [42].

Genetic aberrations in CLL-type MBL

Most biological studies have focused on CLL-type MBL. Cytogenetic abnormalities are very close to those found in low-risk cytogenetic CLL [42]. 13q14 deletion and +12 are the most frequent chromosomal aberrations involving nearly 50% and 10–20% of MBL cases [22,41-46] (Table 2). The chromosomal abnormalities detected in high-risk cytogenetic CLL are uncommon in MBL: deletions 17p13 and 11q22-q23 are present in less than 5% of MBL cases compared to 7% and 18% in CLL [47]. Gain of 2p [48] and overexpression of miR-155 [49] or LEF-1, a crucial pro-B survival transcription factor, are rarely observed in MBL patients. CLL patients are classified as mutated or unmutated based on the degree of somatic mutations in the gene of the heavy variable chain of immunoglobulin (*IGHV*). The *IGHV* mutational status is clinically relevant, with an aggressive disease in unmutated *IGHV* CLL patients and a more indolent course in mutated CLL patients. A mutated profile is observed in 70–80% of cases in MBL patients, a frequency much higher than the rate observed in CLL patients but similar to Rai 0 CLL patients [42,43,46,50]. The *IGHV* gene repertoire is different in MBL and CLL: the use of *IGHV3-23* and I*GHV4-59/61* is overexpressed in MBL, and IGHV1-69 is underexpressed (Table 2) [18,43]. *IGHV1-69*, the most frequently rearranged V(H) gene, is associated with an unmutated profile and unfavorable biologic characteristics in CLL, which is in line with T-independent B cell receptor (BCR) maturation [43,51-52]. In contrast, *IGHV 3–23* and *IGHV 3–7* are associated with a high rate of somatic mutations [51]. No significant differences in the mutational landscape were observed when using whole-genome analysis in LC CLL-type MBL, HC CLL-type MBL and stable CLL patients for a very long time [39]. CLL is characterized by an adverse genetic landscape with driver gene mutations in pathways considered central to disease pathogenesis, e.g., NOTCH and NF-κB signaling. The frequency of most driver gene mutations in CLL tends to increase in aggressive/refractory cases, supporting their involvement mainly in disease progression. Few data concern the genetic lesions in MBL: mutations in *NOTCH1*, known to confer a worse prognosis in CLL patients, are found in 10% of CLL-type MBL cases with a lower variant allelic frequency compared to Rai 0 CLL (e.g., 20% in CLL vs. 4% in MBL cases for the same tumor burden) [43,45,53]. Using transcriptomic analyses, significant differences were identified between normal memory B cells, CLL-type MBL and CLL. CLL-type MBL upregulated crucial oncogenic genes, such as CTLA4, LEF1, ROR1, and TCL1A, but the differences were less pronounced between CLL-like MBL and CLL. An enrichment of genes regulating the cell cycle is found in CLL compared to MBL [44]. As in CLL, CD38, CD49d and ZAP70 positive expression were also suggested as poor prognostic factors [40]. Interestingly, the frequency of CD38-positive cases is identical in MBL and CLL (approximately 20% each) [42,46]. Because of new criteria of MBL [2], some Rai 0 LLC were reclassified in MBL. In that case, bone marrow analysis showed a lesser involvement with a median of 20% of lymphocytes and a lower prevalence of diffuse pattern compared to Rai 0 CLL [42].

High-count (HC) MBL versus low-count (LC) MBL: is it important to distinguish both types?

HC MBL, also named clinical MBL, is characterized by the presence of clonal B cells in the range of 0.5–5.0 $\times$ 10^9/L. LC is defined by an absolute count of clonal B cells $< 0.5 \times 10^9$/L and is more likely to be "accidentally" found in the general population in screening studies. Oligoclonal or biclonal features are more common in LC MBL [50]. Genetic abnormalities are less frequent in LC MBL than in HC MBL [43]. The somatic *IGHV* mutated profile is identical in LC and HC MBL [43]. Cytogenetic aberrations between LC and HC MBL are similar, except for a lesser frequency or trisomy 12 [43]. Compared to HC CLL-type MBL, LC MBL have overrepresented IGHV-4 (notably *IGHV4-59/61*) and underrepresented *IGHV1,* but the frequency of unmutated cases is comparable [18,50]. The overrepresentation of IGHV4-59/61 could be related to age, with a relationship being reported between IGHV-4-59/61 and age [18,50].

The decrease in immunocompetence in the elderly population is well described, with compromised B-cell compartments and shrunken T cell repertoires that contribute to increased sensitivity to developing cancer [54-55]. In a study exploring the MBL frequency in an HCV cohort, only LC MBL (monoclonal BALC [0.006–0.27 $\times$ 10^9/L]) was reported [35]. These data support that LC MBL could be preferentially related to an immunosenescence process or a chronic antigenic stimulation (Fig. 2). LC MBL seems to be very stable over time. In a study with a follow-up of 7-years, the clone size increased in 69% of LC MBL patients, 1/54 patients progressed to HC MBL, and no patient progressed to CLL [56].

Are there biomarkers predictive of evolution?

The outcome of MBL patients seems to be closely related to the absolute B-cell count regardless of the MBL type [22,40-42,57]. Generally, the median size of the MBL clone increased in HC MBL but also decreased and even disappeared in the case of LC MBL [56,58]. Studies defined a threshold of approximately 3.7–4 x10^9 G/L of monoclonal CLL lymphocytes as a risk factor for predicting progression to CLL/SLL [22,41,42]. MLB harboring driver mutations (SF3B1, BIRC3, DDX3X, CHD2, NOTCH1) had shorter time-to-

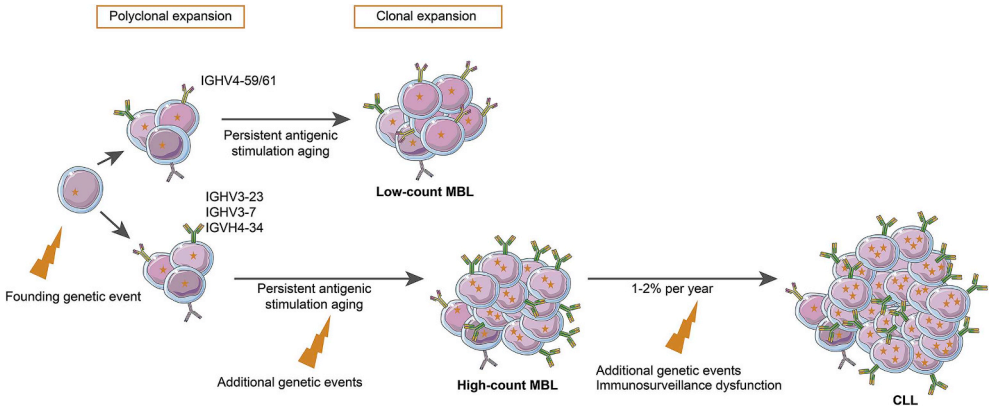

Fig. 2. Model illustrating MBL progression. Designed according to Agathangelidis et al. [39], Kikushige et al. [38]. The figure was produced using Servier Medical Art: www.servier.com.

treatment [59]; CD38, ZAP-70 and CD49d expression has also been associated with risk of progression, but del17p/+12 are the sole independent predictor factors of treatment in multivariate analysis [42]. Furthermore, some studies have shown that subclonal expansion was predictive of treatment or CLL progression [40,43,59]. In a large prospective series of HC MBL, acquisition of cytogenetic abnormalities (mostly acquired del(13q) or +12) was found during follow-up [41]. Taken together, these findings support the hypothesis that progressive accumulation of genetic defects is necessary for the progression of MBL to CLL (Fig. 2). When performing immunogenetic profiling in 333 CLL-like MBL cases (60 cases with LC MBL, 273 cases with HC MBL), the *IGHV* profile was unmutated in 24.5% of cases: a frequency identical in LC MBL and HC MBL. An overrepresentation of *IGHV4-59/61* and an underrepresentation of *IGHV1-69* genes were used in LC MBL. Furthermore, a progressive increase in the frequency of B-cell receptor (BcR) stereotype was found: 5.5% in LC MBL and 21.9% in HC MBL, reaching 30.4% in all stages of CLL. HC MBL exhibits clear immunogenetic similarities with CLL-0 patients, suggesting that LC MBL may not represent a true preleukemic condition, thus differing from HC MBL/CLL-0 patients. As in CLL, the immune system is impaired in CLL-type MBL, and the risk of infections and/or secondary malignancies is increased [60,61]. Normal residual circulating B cells were significantly reduced in HC MBL and CLL 0 patients compared to healthy controls and LC MBL. Notably, normal B cell were decreased at the expense of pre-germinal center B cells (immature and naïve B cells) while no significant differences were observed in MBL regarding total peripheral blood memory B cells [62]. During follow-up, expansion of T and natural killer (NK) cells is described without a clear link to protective adverse effects [56], and a clonal T cell expansion was described in 75% of CLL-type MBL patients [58]. These findings suggest a progressive deterioration of the immune cell compartment in MBL progression (Fig. 2). Overall survival of CLL-type MBL is not significantly different compared to an age-sex matched population, which is in contrast to newly diagnosed Rai 0 CLL patients [46]. These crucial data allow for the interpretation of CLL-type MBL, especially with HC and CLL as a continuum to CLL, and the distinction between MBL and Rai 0 CLL should preferentially be made on progressive or nonprogressive status [63]. Interestingly, CLL-type MBL LC patients seem to have a higher frequency of death compared to healthy adults due to infections and cancer [56]. As a specialized

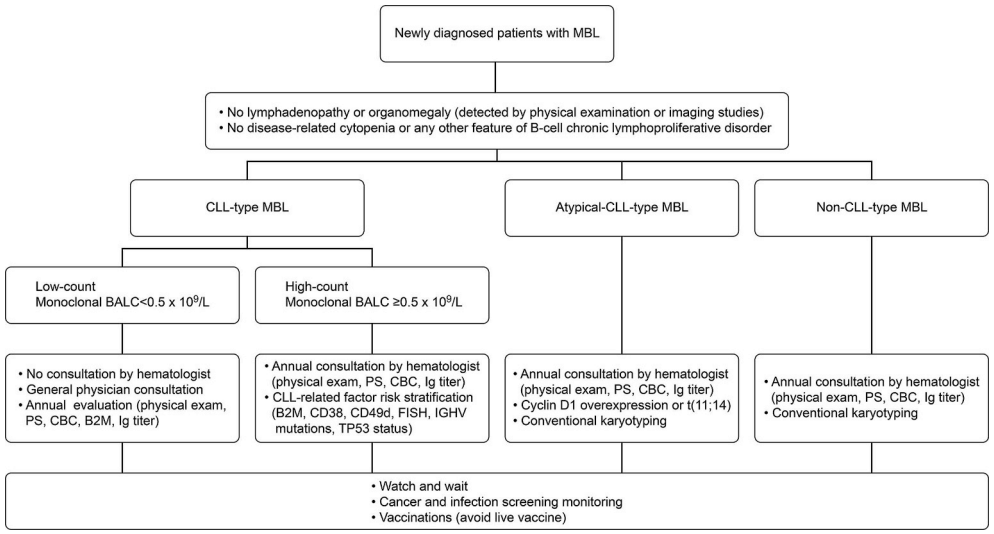

Fig. 3. General approach of MBL management. B2M: β-2-microglobulin; CBC: complete blood count; Ig: immunoglobulin; PS: performance status.

hematologic follow-up of LCMBL is not required, these data suggest particular attention by the general physician to prevent infection/neoplasms (Fig. 3).

Focus on non-CLL MBL

Although the majority of published data concerns CLL phenotype MBL, other categories of MBL are recognized in the general population or in the laboratory setting. Atypical CLL-type MBL and non-CLL-type MBL account for less than 25% of all diagnosed MBLs. Non-CLL MBLs are found in HCV-infected patients [35]. Cytogenetic abnormalities in atypical CLL-type MBL or non-CLL-type MBL showed distinct cytogenetic patterns [41,56]. t(11; 14)(q13; q32) was found in $CD5^+$ cases, while del7q and +3 were preferentially detected in CD5- MBL cases [49,64]. Some non-CLL-type MBLs display morphologic (villous lymphocytes) and/or phenotypic features of marginal zone lymphoma (MZL). In a series of 7 non-CLL-like MBLs, two patients had mutations in genes commonly involved in MZL (e.g., *NOTCH2*, *TNFAIP3* and *MYD88*); two patients progressed (one with a nodal form and one with diffuse B-cell lymphoma). In contrast, some bias in the *IGHV* repertoire is found with a predominance of *IGHV4-34* and a lower frequency of IGHV1-2 in non-CLL-like MBL compared to MZL [64]. In contrast to CLL-type MBL, non-CLL-type MBL seems to have a better prognosis compared to atypical CLL-type MBL: the median time to progression is 84 months and 45 months, respectively [49,72]. A progression to atypical CLL is possible [65]. Similar to CLL-type MBL, higher ALC is associated with a high risk of progression [41].

Conclusion

MBL is an entity composed of various diseases. A better comprehension of the mechanisms of progression is crucial for the best management of MBL patients. Current data support that HC CLL-type MBL is a premalignant state of CLL with biological features undistinguishable from those in Rai 0 CLL patients.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declarations of interest

None.

Practice points

- Improvement of flow cytometry procedures increases the detection of very low B-cell clones in peripheral blood samples
- MBL is defined by the presence of less than 5×10^9 clonal B cells in the peripheral blood and no symptoms or signs of lymphoproliferative disorders
- Three categories of MBL are recognized: CLL MBL, atypical CLL MBL and non-CLL MBL
- Low-count CLL MBL is stable over time, requiring a watch and wait therapeutic attitude, and is different from CLL in biological and clinical features and could be likely due to immunosenescence or chronic infection
- Conversely, high-count CLL-type MBL could progress to CLL
- Optimal understanding of the pathobiology of these pathologies is necessary for better management of MBL patients

Acknowledgments

No.

References

[1] Rai KR, Sawitsky A, Cronkite EP, Chanana AD, Levy RN, Pasternack BS. Clinical staging of chronic lymphocytic leukemia. Blood 1975;46:219–34.
[2] Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, Dohner H, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the international Workshop on chronic lymphocytic leukemia updating the national cancer institute-working group 1996 guidelines. Blood 2008;111:5446–56https://doi.org/10.1182/blood-2007-06-093906.
[3] Marti GE, Rawstron AC, Ghia P, Hillmen P, Houlston RS, Kay N, et al. Diagnostic criteria for monoclonal B-cell lymphocytosis. Br J Haematol 2005;130:325–32https://doi.org/10.1111/j.1365-2141.2005.05550.x.
[4] Ghia P. Another piece of the puzzle: is there a "nodal" monoclonal B-cell lymphocytosis? Haematologica 2011;96:1089–91https://doi.org/10.3324/haematol.2011.047712.
[5] Andrews JM, Cruser DL, Myers JB, Fernelius CA, Holm MT, Waldner DL. Using peripheral smear review, age and absolute lymphocyte count as predictors of abnormal peripheral blood lymphocytoses diagnosed by flow cytometry. Leuk Lymphoma 2008;49:1731–7https://doi.org/10.1080/10428190802251787.
[6] Trimoreau F, Galoisy AC, Genevieve F, Bardet V, Cornet E, Hurst JP, et al. Harmonisation of full blood count reports, recommendations of the French-speaking cellular haematology group (GFHC). J Clin Pathol 2017;70:395–402https://doi.org/10.1136/jclinpath-2016-204001.
[7] Strati P, Shanafelt TD. Monoclonal B-cell lymphocytosis and early-stage chronic lymphocytic leukemia: diagnosis, natural history, and risk stratification. Blood 2015;126:454–62https://doi.org/10.1182/blood-2015-02-585059.
[8] Troussard X, Cornet E, Lesesve J-F, Kourel C, Mossafa H. Polyclonal B-cell lymphocytosis with binucleated lymphocytes (PPBL). OncoTargets Ther 2008;1:59–66https://doi.org/10.2147/OTT.S4182.

[9] van Dongen JJ, Lhermitte L, Bottcher S, Almeida J, van der Velden VH, Flores-Montero J, et al. EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. Leukemia 2012;26:1908–75https://doi.org/10.1038/leu.2012.120.
[10] Rawstron AC, Kreuzer KA, Soosapilla A, Spacek M, Stehlikova O, Gambell P, et al. Reproducible diagnosis of chronic lymphocytic leukemia by flow cytometry: an European research initiative on CLL (ERIC) & European society for clinical cell analysis (ESCCA) harmonisation project. Cytom B Clin Cytom 2018;94:121–8https://doi.org/10.1002/cyto.b.21595.
[11] Matutes E, Owusu-Ankomah K, Morilla R, Marco JG, Houlihan A, Que TH, et al. The immunological profile of B-cell disorders and proposal of a scoring system for the diagnosis of CLL. Leukemia 1994;8:1640–5.
[12] Moreau EJ, Matutes E, A'Hern RP, Morilla AM, Morilla RM, Owusu-Ankomah KA, et al. Improvement of the chronic lymphocytic leukemia scoring system with the monoclonal antibody SN8 (CD79b). Am J Clin Pathol 1997;108:378–82https://doi.org/10.1093/ajcp/108.4.378.
[13] Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al. WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon: WHO; 2017.
[14] Sorigue M, Junca J, Granada I. CD200 in high-grade lymphoma, chronic lymphocytic leukemia, and chronic lymphocytic leukemia-phenotype monoclonal B-cell lymphocytosis. Am J Clin Pathol 2015;144:677–9https://doi.org/10.1309/ajcpo2kkfhoexnzf.
[15] Hu Z, Sun Y, Schlette EJ, Tang G, Li S, Xu J, et al. CD200 expression in mantle cell lymphoma identifies a unique subgroup of patients with frequent IGHV mutations, absence of SOX11 expression, and an indolent clinical course. Mod Pathol 2017;31:327–36https://doi.org/10.1038/modpathol.2017.135.
[16] Dores GM, Anderson WF, Curtis RE, Landgren O, Ostroumova E, Bluhm EC, et al. Chronic lymphocytic leukaemia and small lymphocytic lymphoma: overview of the descriptive epidemiology. Br J Haematol 2007;139:809–19https://doi.org/10.1111/j.1365-2141.2007.06856.x.
[17] Le Guyader-Peyrou S, Belot A, Maynadie M, Binder-Foucard F, Remontet L, Troussard X, et al. Cancer incidence in France over the 1980-2012 period: hematological malignancies. Rev Epidemiol Sante Publique 2016;64:103–12https://doi.org/10.1016/j.respe.2015.12.017.
[18] Dagklis A, Fazi C, Sala C, Cantarelli V, Scielzo C, Massacane R, et al. The immunoglobulin gene repertoire of low-count chronic lymphocytic leukemia (CLL)-like monoclonal B lymphocytosis is different from CLL: diagnostic implications for clinical monitoring. Blood 2009;114:26–32https://doi.org/10.1182/blood-2008-09-176933.
[19] Ghia P, Prato G, Scielzo C, Stella S, Geuna M, Guida G, et al. Monoclonal CD5+ and CD5- B-lymphocyte expansions are frequent in the peripheral blood of the elderly. Blood 2004;103:2337–42https://doi.org/10.1182/blood-2003-09-3277.
[20] Nieto WG, Almeida J, Romero A, Teodosio C, Lopez A, Henriques AF, et al. Increased frequency (12%) of circulating chronic lymphocytic leukemia-like B-cell clones in healthy subjects using a highly sensitive multicolor flow cytometry approach. Blood 2009;114:33–7https://doi.org/10.1182/blood-2009-01-197368.
[21] Rawstron AC, Yuille MR, Fuller J, Cullen M, Kennedy B, Richards SJ, et al. Inherited predisposition to CLL is detectable as subclinical monoclonal B-lymphocyte expansion. Blood 2002;100:2289–90https://doi.org/10.1182/blood-2002-03-0892.
[22] Rawstron AC, Bennett FL, O'Connor SJ, Kwok M, Fenton JA, Plummer M, et al. Monoclonal B-cell lymphocytosis and chronic lymphocytic leukemia. N Engl J Med 2008;359:575–83https://doi.org/10.1056/NEJMoa075290.
[23] Call TG, Norman AD, Hanson CA, Achenbach SJ, Kay NE, Zent CS, et al. Incidence of chronic lymphocytic leukemia and high-count monoclonal B-cell lymphocytosis using the 2008 guidelines: CLL/MBL incidence-IWCLL 2008 guidelines. Cancer 2014;120. 2000–5 https://doi.org/10.1002/cncr.28690.
[24] Goldin LR, Lanasa MC, Slager SL, Cerhan JR, Vachon CM, Strom SS, et al. Common occurrence of monoclonal B-cell lymphocytosis among members of high-risk CLL families. Br J Haematol 2010;151:152–8https://doi.org/10.1111/j.1365-2141.2010.08339.x.
[25] Edlefsen KL, Cherian S, De Roos AJ, Getaneh A, Lessin L, Li W, et al. Detection of non-CLL-like monoclonal B cell lymphocytosis increases dramatically in the very elderly, while detection of CLL-like populations varies by race: findings in a multiethnic population-based cohort of elderly women. Ann Hematol 2016;95:1695–704https://doi.org/10.1007/s00277-016-2760-5.
[26] Mulligan CS, Thomas ME, Mulligan SP. Monoclonal B-lymphocytosis: demographics, nature and subclassification in 414 community patients. Leuk Lymphoma 2011;52:2293–8https://doi.org/10.3109/10428194.2011.598250.
[27] Rachel JM, Zucker ML, Fox CM, Plapp FV, Menitove JE, Abbasi F, et al. Monoclonal B-cell lymphocytosis in blood donors. Br J Haematol 2007;139:832–6https://doi.org/10.1111/j.1365-2141.2007.06870.x.
[28] Rawstron AC, Ssemaganda A, de Tute R, Doughty C, Newton D, Vardi A, et al. Monoclonal B-cell lymphocytosis in a hospital-based UK population and a rural Ugandan population: a cross-sectional study. Lancet Haematol 2017;4:e334–40https://doi.org/10.1016/s2352-3026(16)30192-2.
[29] Shim YK, Vogt RF, Middleton D, Abbasi F, Slade B, Lee KY, et al. Prevalence and natural history of monoclonal and polyclonal B-cell lymphocytosis in a residential adult population. Cytom B Clin Cytom 2007;72:344–53https://doi.org/10.1002/cyto.b.20174.
[30] Shim YK, Rachel JM, Ghia P, Boren J, Abbasi F, Dagklis A, et al. Monoclonal B-cell lymphocytosis in healthy blood donors: an unexpectedly common finding. Blood 2014;123:1319–26https://doi.org/10.1182/blood-2013-08-523704.
[31] Soosapilla A, Pepperell D, Best G, Brizzi C, Mulligan CS, van Bilsen N, et al. Monoclonal B-lymphocytosis in patients aged over 90 years is common but not inevitable, and has a prevalence comparable to that in individuals aged 65-90 years. Leuk Lymphoma 2015;56:2182–4https://doi.org/10.3109/10428194.2014.976822.
[32] Casabonne D, Almeida J, Nieto WG, Romero A, Fernandez-Navarro P, Rodriguez-Caballero A, et al. Common infectious agents and monoclonal B-cell lymphocytosis: a cross-sectional epidemiological study among healthy adults. PLoS One 2012;7:e52808https://doi.org/10.1371/journal.pone.0052808.
[33] de Tute R, Yuille M, Catovsky D, Houlston RS, Hillmen P, Rawstron AC. Monoclonal B-cell lymphocytosis (MBL) in CLL families: substantial increase in relative risk for young adults. Leukemia 2006;20:728–9https://doi.org/10.1038/sj.leu.2404116.
[34] Marti GE, Carter P, Abbasi F, Washington GC, Jain N, Zenger VE, et al. B-cell monoclonal lymphocytosis and B-cell abnormalities in the setting of familial B-cell chronic lymphocytic leukemia. Cytom B Clin Cytom 2003;52:1–12https://doi.org/10.1002/cyto.b.10013.
[35] Fazi C, Dagklis A, Cottini F, Scarfo L, Bertilaccio MT, Finazzi R, et al. Monoclonal B cell lymphocytosis in hepatitis C virus infected individuals. Cytom B Clin Cytom 2010;78(Suppl 1). S61–8 https://doi.org/10.1002/cyto.b.20545.
[36] Alfano G, Fontana F, Colaci E, Franceschini E, Ligabue G, Messerotti A, et al. Immunophenotypic profile and clinical outcome of monoclonal B-cell lymphocytosis in kidney transplantation. Clin Transplant 2018;32:e13338https://doi.org/10.1111/ctr.13338.
[37] Landgren O, Albitar M, Ma W, Abbasi F, Hayes RB, Ghia P, et al. B-cell clones as early markers for chronic lymphocytic leukemia. N Engl J Med 2009;360:659–67https://doi.org/10.1056/NEJMoa0806122.
[38] Kikushige Y, Ishikawa F, Miyamoto T, Shima T, Urata S, Yoshimoto G, et al. Self-renewing hematopoietic stem cell is the primary target in pathogenesis of human chronic lymphocytic leukemia. Cancer Cell 2011;20:246–59https://doi.org/10.1016/j.ccr.2011.06.029.
[39] Agathangelidis A, Ljungstrom V, Scarfo L, Fazi C, Gounari M, Pandzic T, et al. Highly similar genomic landscapes in monoclonal B-cell lymphocytosis and ultra-stable chronic lymphocytic leukemia with low frequency of driver mutations. Haematologica 2018;103:865–73https://doi.org/10.3324/haematol.2017.177212.
[40] Shanafelt TD, Kay NE, Rabe KG, Call TG, Zent CS, Maddocks K, et al. Brief report: natural history of individuals with clinically recognized monoclonal B-cell lymphocytosis compared with patients with Rai 0 chronic lymphocytic leukemia. J Clin Oncol 2009;27:3959–63https://doi.org/10.1200/jco.2008.21.2704.
[41] Kostopoulos IV, Paterakis G, Pavlidis D, Kastritis E, Terpos E, Tsitsilonis OE, et al. Clonal evolution is a prognostic factor for the clinical progression of monoclonal B-cell lymphocytosis. Blood Canc J 2017;7:e597https://doi.org/10.1038/bcj.2017.77.
[42] Rossi D, Sozzi E, Puma A, De Paoli L, Rasi S, Spina V, et al. The prognosis of clinical monoclonal B cell lymphocytosis differs from prognosis of Rai 0 chronic lymphocytic leukaemia and is recapitulated by biological risk factors. Br J Haematol 2009;146:64–75https://doi.org/10.1111/j.1365-2141.2009.07711.x.
[43] Henriques A, Rodriguez-Caballero A, Nieto WG, Langerak AW, Criado I, Lecrevisse Q, et al. Combined patterns of IGHV repertoire and cytogenetic/molecular alterations in monoclonal B lymphocytosis versus chronic lymphocytic leukemia. PLoS One 2013;8:e67751https://doi.org/10.1371/journal.pone.0067751.
[44] Lanasa MC, Allgood SD, Slager SL, Dave SS, Love C, Marti GE, et al. Immunophenotypic and gene expression analysis of monoclonal B-cell lymphocytosis shows biologic characteristics associated with good prognosis CLL. Leukemia 2011;25:1459–66https://doi.org/10.1038/leu.2011.117.
[45] Rasi S, Monti S, Spina V, Foà R, Gaidano G, Rossi D. Analysis of NOTCH1 mutations in monoclonal B-cell lymphocytosis. Haematologica 2012;97:153–4https://doi.org/10.3324/haematol.2011.053090.
[46] Shanafelt TD, Kay NE, Rabe KG, Call TG, Zent CS, Schwager SM, et al. Survival of patients with clinically identified monoclonal B-cell lymphocytosis (MBL) relative to the age- and sex-matched general population. Leukemia 2012;26:373–6https://doi.org/10.1038/leu.2011.211.

[47] Dohner H, Stilgenbauer S, Benner A, Leupolt E, Krober A, Bullinger L, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med 2000;343:1910–6https://doi.org/10.1056/nejm200012283432602.
[48] Fabris S, Mosca L, Cutrona G, Lionetti M, Agnelli L, Ciceri G, et al. Chromosome 2p gain in monoclonal B-cell lymphocytosis and in early stage chronic lymphocytic leukemia. Am J Hematol 2013;88:24–31https://doi.org/10.1002/ajh.23340.
[49] Ferrajoli A, Shanafelt TD, Ivan C, Shimizu M, Rabe KG, Nouraee N, et al. Prognostic value of miR-155 in individuals with monoclonal B-cell lymphocytosis and patients with B chronic lymphocytic leukemia. Blood 2013;122. 1891–9 https://doi.org/10.1182/blood-2013-01-478222.
[50] Vardi A, Dagklis A, Scarfo L, Jelinek D, Newton D, Bennett F, et al. Immunogenetics shows that not all MBL are equal: the larger the clone, the more similar to CLL. Blood 2013;121:4521–8https://doi.org/10.1182/blood-2012-12-471698.
[51] Murray F, Darzentas N, Hadzidimitriou A, Tobin G, Boudjogra M, Scielzo C, et al. Stereotyped patterns of somatic hypermutation in subsets of patients with chronic lymphocytic leukemia: implications for the role of antigen selection in leukemogenesis. Blood 2008;111:1524–33https://doi.org/10.1182/blood-2007-07-099564.
[52] Rossi D, Gaidano G. Biological and clinical significance of stereotyped B-cell receptors in chronic lymphocytic leukemia. Haematologica 2010;95. 1992–5 https://doi.org/10.3324/haematol.2010.033241.
[53] Lionetti M, Fabris S, Cutrona G, Agnelli L, Ciardullo C, Matis S, et al. High-throughput sequencing for the identification of NOTCH1 mutations in early stage chronic lymphocytic leukaemia: biological and clinical implications. Br J Haematol 2014;165:629–39https://doi.org/10.1111/bjh.12800.
[54] Aw D, Silva AB, Palmer DB. Immunosenescence: emerging challenges for an ageing population. Immunology 2007;120:435–46https://doi.org/10.1111/j.1365-2567.2007.02555.x.
[55] Fulop T, Kotb R, Fortin CF, Pawelec G, de Angelis F, Larbi A. Potential role of immunosenescence in cancer development: immunosenescence and cancer. Ann N Y Acad Sci 2010;1197:158–65https://doi.org/10.1111/j.1749-6632.2009.05370.x.
[56] Criado I, Rodríguez-Caballero A, Gutiérrez ML, Pedreira CE, Alcoceba M, Nieto W, et al. Low-count monoclonal B-cell lymphocytosis persists after seven years of follow up and is associated with a poorer outcome. Haematologica 2018;103:1198–208https://doi.org/10.3324/haematol.2017.183954.
[57] Shanafelt TD, Kay NE, Jenkins G, Call TG, Zent CS, Jelinek DF, et al. B-cell count and survival: differentiating chronic lymphocytic leukemia from monoclonal B-cell lymphocytosis based on clinical outcome. Blood 2009;113:4188–96https://doi.org/10.1182/blood-2008-09-176149.
[58] Fazi C, Scarfo L, Pecciarini L, Cottini F, Dagklis A, Janus A, et al. General population low-count CLL-like MBL persists over time without clinical progression, although carrying the same cytogenetic abnormalities of CLL. Blood 2011;118:6618–25https://doi.org/10.1182/blood-2011-05-357251.
[59] Barrio S, Shanafelt TD, Ojha J, Chaffee KG, Secreto C, Kortüm KM, et al. Genomic characterization of high-count MBL cases indicates that early detection of driver mutations and subclonal expansion are predictors of adverse clinical outc ome. Leukemia 2017;31:170–6https://doi.org/10.1038/leu.2016.172.
[60] Moreira J, Rabe KG, Cerhan JR, Kay NE, Wilson JW, Call TG, et al. Infectious complications among individuals with clinical monoclonal B-cell lymphocytosis (MBL): a cohort study of newly diagnosed cases compared to controls. Leukemia 2013;27:136–41https://doi.org/10.1038/leu.2012.187.
[61] Solomon BM, Chaffee KG, Moreira J, Schwager SM, Cerhan JR, Call TG, et al. Risk of non-hematologic cancer in individuals with high-count monoclonal B-cell lymphocytosis. Leukemia 2016;30:331–6https://doi.org/10.1038/leu.2015.235.
[62] Criado I, Blanco E, Rodriguez-Caballero A, Alcoceba M, Contreras T, Gutierrez ML, et al. Residual normal B-cell profiles in monoclonal B-cell lymphocytosis versus chronic lymphocytic leukemia. EuroFlow PID group and the primary health care group of salamanca for the study of MBL. Leukemia 2018;32:2701–5https://doi.org/10.1038/s41375-018-0164-3.
[63] Scarfo L, Ghia P. What does it mean I have a monoclonal B-cell lymphocytosis?: recent insights and new challenges. Semin Oncol 2016;43:201–8https://doi.org/10.1053/j.seminoncol.2016.02.013.
[64] Xochelli A, Oscier D, Stamatopoulos K. Clonal B-cell lymphocytosis of marginal zone origin. Best Pract Res Clin Haematol 2017;30:77–83https://doi.org/10.1016/j.beha.2016.08.028.
[65] Ugo V, Leporrier N, Salaun V, Letestu R, Radford-Weiss I, Ramond S, et al. Deciphering leukemic B-cell chronic lymphoproliferative disorders. Leuk Lymphoma 2006;47:2088–95https://doi.org/10.1080/10428190600727939.