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Screening for monoclonal B-lymphocyte expansion in a hospital-based Chinese population with lymphocytosis: an observational cohort study

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ABSTRACT

Objectives Screening of monoclonal B-cell lymphocytosis (MBL) has improved the early detection of B-cell lymphoproliferative disorders (B-LPDs). This study was designed to find the most cost-effective way to screen for asymptomatic B-LPD.

Design Observational study.

Setting A lymphocytosis screening project was conducted at a large-scale hospital among the Chinese population.

Participants For 10 consecutive working days in 2018, 22 809 adult patients who received a complete blood count (CBC) were reviewed. These patients were selected from the outpatient, inpatient and health examination departments of a National Medical Centre in China.

Results A total of 254 patients (1.1%, 254/22 809) were found to have lymphocytosis (absolute lymphocyte count (ALC) >3.5×10^9/L). Among them, a population of circulating monoclonal B-lymphocytes were detected in 14 patients, with 4 having chronic lymphocytic leukaemia (CLL) and 10 having MBL, indicating an overall prevalence of 5.5% for B-LPD (3.9% for MBL). The prevalence of CLL among the elderly patients with lymphocytosis (≥60 years) was determined to be 4.3% (4/92). In the patients over 60 years of age, the prevalence of MBL was found to be 8.7%. CD5 (–) non-CLL-like MBL was observed to be the most common subtype (8, 80%), followed by CLL-like phenotype (1, 10.0%) and atypical CLL phenotype (1, 10.0%). The receiver operating characteristic curve analysis for the CBC results revealed that the ALC of 4.7×10^9/L may serve as the optimal and cost-effective cut-off for screening for early-stage asymptomatic B-LPD.

Conclusion In Chinese patients with lymphocytosis, there was a relatively high proportion of patients with CLL among individuals over 60 years of age. MBL is an age-related disorder. Non-CLL-like MBL was the most common MBL subtype, almost all of whom displayed a pattern of 'marginal zone lymphoma (MZL)-like' MBL. Lymphocytosis screening among the elderly would be effective in the detection of B-LPD and MBL.

Strengths and limitations of this study

- In this study, we screened a hospital-based cohort of 22 809 Chinese patients.
- All patients with lymphocytosis underwent flow cytometer analysis to screen for early-stage asymptomatic B-cell lymphoproliferative disorder and get a preliminary understanding of the prevalence and spectrum of monoclonal B-cell lymphocytosis in China.
- The hospitalised patients with lymphocytosis participating in this study are not representative of the entire Chinese population, hence, the presented estimates may have bias.

INTRODUCTION

B-cell lymphoproliferative disorders (B-LPDs) generally refer to a group of heterogeneous malignant diseases derived from the monoclonal expansion of B cells, of which typical clinical manifestation is lymphocytosis. B-LPDs are the most frequently encountered haematological malignancies in adults, accounting for over 90% of non-Hodgkin's lymphoma (NHL) and more than half of leukaemia.[1] However, large geographical diversity exists with regard to the incidence and disease spectrum in China, Europe and USA, which may reflect differences in racial and hereditary disparities, environmental influence and diagnostic capacity. According to GLOBOCAN 2018,[2] the age-standardised annual incidence per 100 000 people for NHL and leukaemia in China was 9.4, lower than that of USA (10.9) and Europe (15.5).

Chronic lymphocytic leukaemia (CLL) is the most common adult leukaemia in the West, accounting for 45% of lymphocytosis cases.[3] CLL is a relatively rare haematological malignancy in China, with an annual incidence of only 0.05/100 000 according to a previous population-based study.[4 5] However, an increase in the frequency of CLL was recently observed in several large medical centres in China,[6] indicating that the actual incidence of CLL in China may have been underestimated due to high misdiagnosis rates as well as high diagnostic delay.

Monoclonal B-cell lymphocytosis (MBL), characterised by distinguishable monoclonal B cells in peripheral blood (PB) with an absolute B-cell count of less than 5×10^9 cells/L,[7] is a precursor to B-LPD with a reported prevalence ranging from 0.12% to 14.3%.[8] MBL may be detected in asymptomatic adults and may serve as a model in screening for early B-LPD. MBL is categorised into three types: the CLL-like phenotype, atypical CLL phenotype and non-CLL-like phenotype.[7 9] Though CLL-like MBL accounts for over 60% of MBL in USA and Europe, non-CLL-like phenotype is relatively rare in this population.[8 10 11] However, the spectrum of MBL in the Chinese population has yet to be accurately defined.

In order to find the most cost-effective way to screen for early-stage asymptomatic B-LPD, get a preliminary understanding of the prevalence and spectrum of MBL in China and determine the most appropriate time-point for only highly suspected patients to undergo more accurate tests, a hospital population-based lymphocytosis screening project was carried out at our institute. The institute is a large reference centre delivering medical services to over 4 000 000 patients nationwide annually. The obtained data demonstrated a substantial proportion of patients having lymphocytosis who presented with diagnostic findings consistent with MBL. Furthermore, a high frequency of CD5 (−) MBL was detected in Chinese patients with MBL, which contrasted with data taken from western populations.

MATERIALS AND METHODS

Study population and sample collection

Over 10 continuous working days (16–19, 23–26 and 30–31 July) in 2018, the authors of this study reviewed all complete blood count (CBC) results performed on all visiting patients from the outpatient, inpatient and health examination departments in Zhongshan Hospital, Fudan University. CBC are performed on more than 2000 patients daily and more than 98% of whom are patients visiting non-haematological department. Adolescents were excluded from the study. All adult patients demonstrating lymphocytosis with an absolute lymphocyte count (ALC) of over 3.5×10^9/L, which was the normal upper limit of ALC at our institutional laboratory, were enrolled in this study. All procedures performed involving human participants were in accordance with the ethical standards of the institutional and/or national research committee, adhering to the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All samples and clinical data were collected after informed consent was obtained from each individual. The clinical data of the patients were collected from an electronic record database at our hospital. A total of 2 mL/case of EDTA-anticoagulated PB samples of the enrolled patients were collected from the residual materials stored in the hospital clinical laboratory, which had a storage time of less than 24 hours. Patients with healthy problems all received oral and written information.

Immunophenotyping and diagnosis

First, resuspended blood cells were stained with four antibody mixtures: Kappa-FITC (Dako FR481); Lambda-PE (Dako FR481); CD19-PC5 (Beckman Coulter A07770); and CD45-PC7 (Beijing Tongsheng Shidai Biotech, Z6410009). On a FC500 flow cytometer (Beckman Coulter), at least 30 000 events were acquired and analysed by the FCS Express software, where B cells were identified by their characteristics of CD19 and light scatter. If necessary, the acquired number of events would be increased to 10 000 000. A positive marker was defined as having more than 20% positive cells. When the κ/λ ratio was >3:1 or <1:3, immunoglobulin light chain expression was considered to be restricted, that is, when a monoclonal B-cell clone was detected. When the CD19 B-cell population carry a balanced κ/λ light chain ratio (κ/λ ratio=1–0.3), the polyclonal B cell clones were detected. All cases carrying a CD19 B-cell population with an unbalanced κ/λ light chain ratio were further analysed. The following five antibody stains were used: CD5-FITC (BD Pharmingen 347303), CD23-PE (BD Pharmingen 341007), CD20-ECD (Beckman Coulter IM3607U), CD10-FITC (Beckman Coulter A07759), CD200-PE (BD Pharmingen 552475). The entire flow diagram is illustrated in figure 1.

The diagnosis of MBL was made according to the criteria put forward by Shanafelt *et al*[7] and was classified according to phenotype. CLL-like was defined as a phenotype with CD5 (+) and CD23 (+), while atypical CLL possessed CD5 (+) and CD23 (−) (excluding mantle cell lymphoma by t (11;14)) and non-CLL-like had CD5 (−).

Statistical methods

The Student t-test was performed to analyse continuous variables, while the χ^2 test (or Fisher's exact test) compared the categorical variables. Receiver operating characteristic (ROC) curves were used to evaluate the discriminatory ability of the evaluated screening procedure. Line charts and logarithm trendlines were drawn using Microsoft Office Excel (V.2016, Microsoft). Statistical analyses were performed using IBM SPSS Statistics V.23.0 and R V.3.5.2 based on actual needs. Two-sided p value ≤0.05 was considered to be statistically significant.

Patient and public involvement

Patients and the public were neither involved in the development of the research question and the outcome nor the design, conduct, recruitment or reporting of this research. No clinical intervention was made for patients. Dissemination of the general results (no personal data) will be made on demand.

RESULTS

Patients with lymphocytosis

During the 10-day study, the results for the 22 809 adult patients (18–90 years, with a male to female ratio of 1.2:1) who had CBCs carried out were reviewed, of which 254

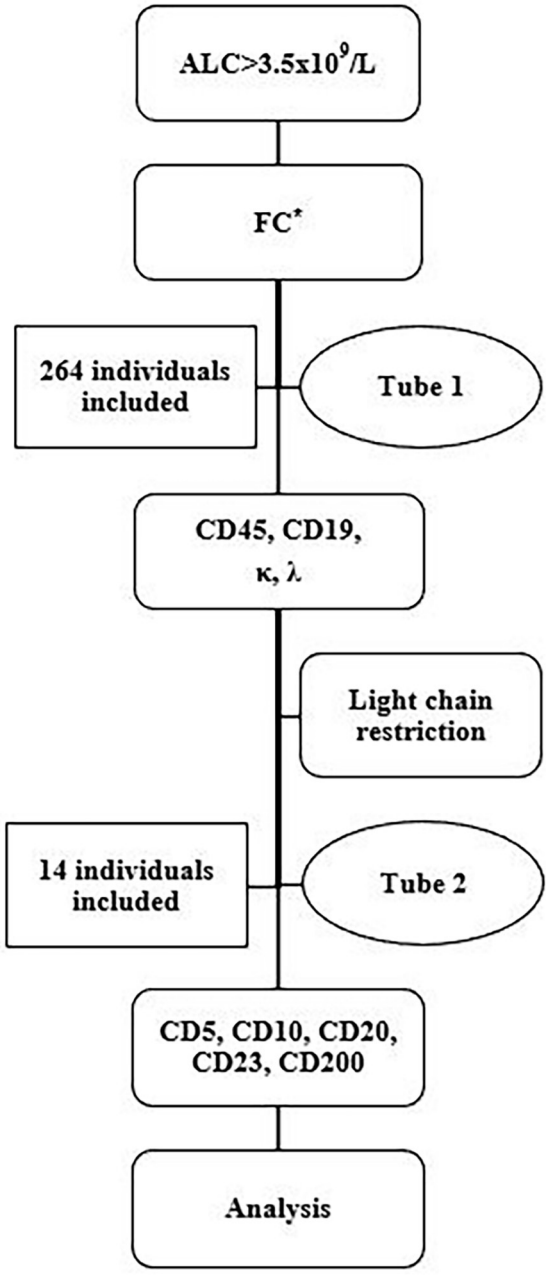

Figure 1 The flow process diagram of this screening project. ALC, absolute lymphocyte count; FC, flow cytometry.

patients (1.1%, 254/22 809) were found to have lymphocytosis with an ALC of more than 3.5×10^9/L. The median age of patients with lymphocytosis was 49 years. In the 254 patients, 36.2% (92/254) were older than 60 years and 62.2% (158/254) were male. The median cell count of absolute lymphocytes was 3.9×10^9/L ($3.6–33.8\times10^9$/L). Moreover, 51 of the 254 patients were apparently 'healthy' individuals undergoing a routine health examination.

Excluding 91 adult patients younger than 40 years (18–39 years), the median age of the remaining 167 patients with lymphocytosis was 60 years (40–90 years). Among these patients, 63.2% (103/163) were male, and 56.4% (92/163) patients were older than 60 years

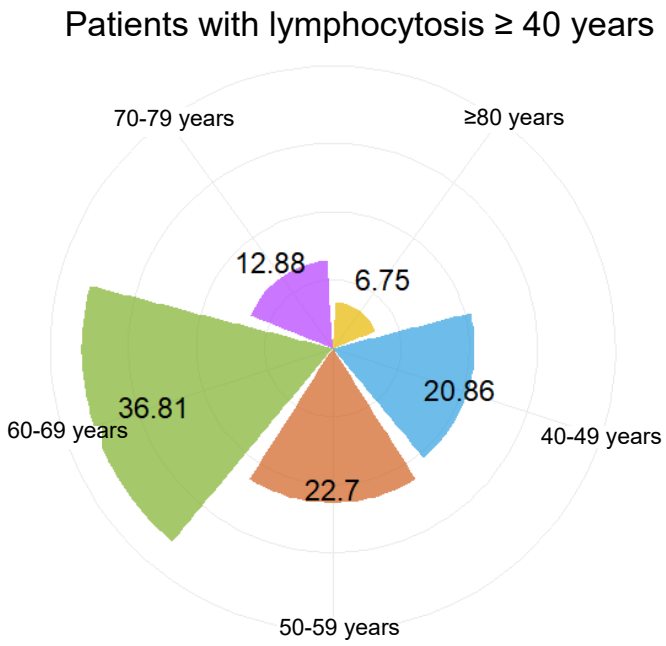

Figure 2 The age distribution of patients older than 40 years with lymphocytosis (40–49 years: 20.86%, 50–59 years: 22.70%, 60–69 years: 36.81%, 70–79 years: 12.88%, ≥80 years: 6.75%).

(figure 2, 60–69 years: 36.8%, 70–79 years: 12.9%, ≥80 years: 6.8%).

Patients with circulating monoclonal B-lymphocytes in PB

The population of circulating monoclonal B-lymphocytes in PB was detected in 14 of the 254 adult patients with lymphocytosis via flow cytometry (FCM) immunophenotyping, indicating a prevalence of 5.5% (14/254) in the adult cohort. In patients with lymphocytosis over 40 years, the percentage was 8.6% (14/163). The median age of the corresponding patients was 68.5 years (43–85 years) of age, with prevalence of 6.3% (10/158) in male and 4.2% (4/96) in female (p=0.58).

The diagnosis and clinical features of the analysed patients are depicted in table 1. Accordingly, among the 14 patients, 4 were diagnosed with CLL, while 10 were found to have MBL. The prevalence of CLL among the elderly patients with lymphocytosis (≥60 years) was determined to be 4.3% (4/92). In the cohort that comprised of 4233 healthy patients who underwent health examinations, 51 adults were found to have lymphocytosis while 3 had monoclonal B-lymphocytes in PB (5.9%). In the corresponding elderly population of 624 individuals older than 60 years, 14 had lymphocytosis while 2 had monoclonal B-lymphocytes in PB (14.3%).

Prevalence of MBL and MBL subtype

The data mentioned above showed that the percentage of MBL in adult population was 3.9%, which increased with age. In patients over 40 years, the percentage was 6.1%. In elderly over 60 years, the percentage was 8.7%. Compared

Table 1 Disease spectrum and clinical features of 14 patients with circulating monoclonal B-lymphocytes

Diagnosis	CLL (n=4, 28.6%)					MBL (n=10, 71.4%)								
Patient No	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Age (years)	67	86	81	67	82	64	43	70	81	71	64	52	61	81
Gender	M	M	F	M	F	M	M	M	F	M	M	F	M	M
Combined diagnosis	HCC, BT, CRC, LC	CRC	GU	None	BC	PMDN	MI	HT	CRC	None	None	BI	None	PC
CBC during screening														
LY	18.6	28.6	33.8	9.1	3.7	5.1	3.8	3.7	4.0	4.0	4.7	3.6	4.2	5.8
Lymphocytosis recorded in our hospital for the first time														
Time	1/31/12	3/31/09	5/13/09	12/1/10	8/25/15	IV	2/18/16	7/8/14	IV	7/19/14	3/22/17	IV	IV	IV
LY	4.7	20.4	66.7	19	3.3	5.1	5.4	4.9	4.0	3.3	4.9	3.6	4.2	5.8

BC, breast cancer; BI, basilar invagination; BT, bladder tumour; CBC, complete blood count; CLL, chronic lymphocytic leukaemia; CRC, colorectal cancer; F, female; GU, gastric ulcer; HCC, hepatocellular carcinoma; HT, hyperthyreosis; IV, initial visit; LC, lung cancer; LY, lymphocyte; M, male; MBL, monoclonal B-cell lymphocytosis; MI, myocardial infarction; PC, prostate cancer; PMDN, primary malignant duodenal neoplasms; UK, unknown.

Table 2 Age-adjusted prevalence of MBL among 254 adult patients with lymphocytosis

Age (years)	N*	Prevalence estimate† (95% CI)
≥18	10/254	3.9 (0.02 to 0.06)
40–49	1/34	2.9 (−0.03 to 0.09)
50–59	1/37	2.7 (0.0 to 8.2)
≥60	8/92	8.7 (2.80 to 14.60)

*Number of patients with MBL/number of all patients in the category.
†Prevalence estimate per 100 persons.
MBL, monoclonal B-cell lymphocytosis.

with adult female (3.1%), a higher prevalence of MBL in male was observed (4.4%), but no significant differences were confirmed. Age-adjusted prevalence of MBL among 254 adult patients with lymphocytosis is shown in table 2.

Among the 10 confirmed MBL cases, non-CLL-like phenotype was the most prevalent subtype (8, 80%), followed by the CLL-like phenotype (1, 10.0%) and atypical CLL phenotype (1, 10.0%). Table 3 depicts the clinical features and phenotypes of these patients. Among the eight non-CLL MBL cases, the ALC ranging from 3600 to 5800/µL, the absolute clonal B-cell count was observed to range from 500 to 2400/µL (median 1050/µL) and all cases were observed as high count (HC) MBL (≥500/µL). The clonal population constituted from 9.1% to 57.5% of lymphocytes. The mean age was 65.4 years. All of them displayed CD10 (−), CD200 (−) and CD20 (+). The frequency of non-CLL-like MBL cases increased with age ranging from 2.9% among 71 patients with lymphocytosis aged 40–59 years to 7.55% among 81 individuals aged 60–79 years and even to 18.18% among 11 individuals over 80 years of age (figure 3A). For male patients, logarithm trendlines suggested that as the age increased, the prevalence of non-CLL MBL (R^2=0.84) would be the most frequent, followed by CLL (R^2=0.69), atypical CLL MBL (R^2=0.11) and finally CLL-like MBL (R^2=not available) (figure 3B). Moreover, the prevalence of atypical-CLL MBL and CLL-like MBL was always at a very low level. For female patients, logarithm trendlines suggested that as the age increased, the prevalence of CLL (R^2=0.43) would be the highest, followed by non-CLL-like MBL (R^2=0.55), CLL-like MBL (R^2=0.43) and finally atypical-CLL MBL (R^2=not available) (figure 3C). Meanwhile, the prevalence of non-CLL-like MBL and CLL-like MBL remains very closely.

Determination of optimal screening cut-off

In order to explore the most appropriate time-point for a patient with lymphocytosis to receive the FCM analyse, the diagnostic performance of ALC in the detection of B-LPD and MBL was evaluated in elderly patients over 60 years of age. The ROC curve analysis revealed an area under the ROC curve (AUC) of 0.76 in the discrimination of B-LPD (figure 4A) and an AUC of 0.61 in the discrimination of MBL (figure 4B). The ALC of 4.7×10^9/L served

Table 3 Clinical features and phenotypes of 10 MBL cases

	CLL-like	Atypical-CLL	Non-CLL							
Percentage	10.0	10.0	80.0							
Age (years)	82	64	43	70	81	71	64	52	61	81
LY ($\times 10^9$/L)	3.7	5.1	3.8	3.7	4.0	4.0	4.7	3.6	4.2	5.8
κ/λ restriction	λ	κ	κ	κ	κ	κ	κ	κ	λ	κ
Clonal B cells/LY (%)	54.2	21.3	32.4	17.3	27.2	48.3	20.4	55.7	57.5	9.1
Absolute clonal B cells ($\times 10^9$/L)	2.0	1.1	1.2	0.6	1.1	1.9	1.0	2.0	2.4	0.5
Level	HC	HC	HC	HC	HC	HC	HC	HC	HC	HC
CD10	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)
CD20	(+)	(+)	Partial (+)	(+)	(+)	(+)	Partial (+)	(+)	(+)	(+)
CD200	(+)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)

Patients' number: CLL-like: No 5; atypical-CLL: No 6; non-CLL: No 7–14.
CLL, chronic lymphocytic leukaemia; HC, high count, clonal B-cell counts above 0.5×109/L; LY, lymphocyte; MBL, monoclonal B-cell lymphocytosis.

as the optimal and cost-effective cut-off for the maximum Youden Index, and the sensitivity and specificity in detecting B-LPD and MBL were 0.80 and 0.60, respectively. Therefore, although a threshold of 3.5×10^9/L was selected in this study, the final result confirms that the usual threshold (4.0×10^9/L) could be taken into account in routine practice.

DISCUSSION

In the present study, a hospital-based cohort comprising of 22 809 patients were examined for the presence of circulating monoclonal B-lymphocytes. Patients having an ALC over 3.5×10^9/L (defined as lymphocytosis in this study) underwent further FCM analysis. The flow diagram provided an economical and cost-effective screening method for clinicians and patients.

From the present cohort, the CLL prevalence was observed to be 4.3% among individuals with lymphocytosis over 60 years of age. Another recent retrospective study conducted in China showed that CLL accounted for 55.9% of all 653 cases of B-LPD (median age: 61 years, IQR: 54–70 years), which was treated at a single haematological centre.[12] Similarly, Yang *et al*[6] reported an increased frequency of CLL at their centre in Beijing, China.These data seem to overthrow the view that CLL is much less common in Asians compared with persons of European descent. It is suggested that as the huge population base in China, many cases with CLL may have been underestimated and misdiagnosed, especially in the elderly population.

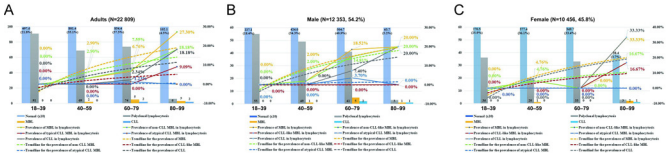

Figure 3 (A) Prevalence of MBL, MBL subtypes and CLL in a hospital cohort of 22 809 individuals; (B) prevalence of MBL, MBL subtypes and CLL in a male hospital cohort of 12 353 male individuals; (C) prevalence of MBL, MBL subtypes and CLL in a hospital cohort of 10 456 female individuals. CLL, chronic lymphocytic leukaemia; MBL, monoclonal B-cell lymphocytosis.

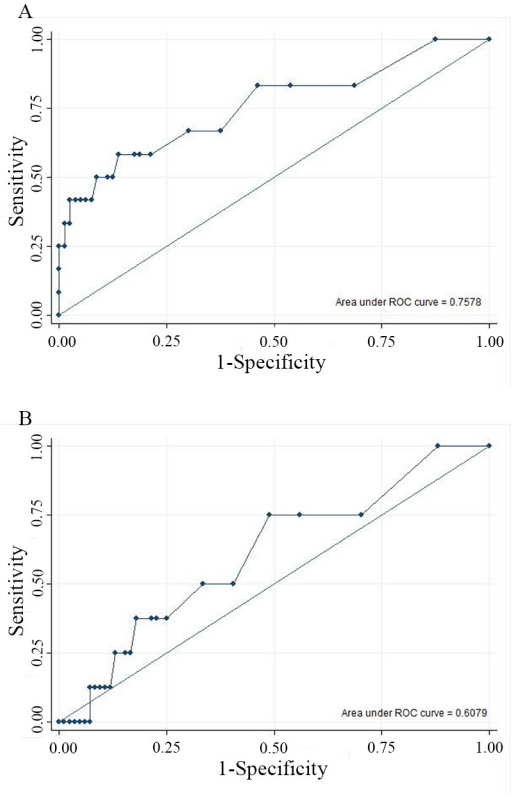

Figure 4 Receiver operating characteristic (ROC) curves of sensitivity vs specificity for absolute lymphocyte counts in detecting B-cell lymphoproliferative disorder (A) and monoclonal B-cell lymphocytosis (B).

Moreover, the obtained data demonstrated that MBL may be detected in a substantial proportion of the hospital population having lymphocytosis, with a relatively high prevalence of 8.7% in elderly patients over 60 years of age. The previous reported prevalence of MBL varied according to population and study, which ranged from 0.14% to 28.5%.[13 14] The prevalence of MBL in the Chinese population has yet to be elucidated. This study's results were compared with data taken from other prevalence studies performed in various populations and countries (table 4).[3 10 11 15–18] In cohorts with normal blood counts in European countries and the USA,[10 11 16] the enrolled patients were always older than 45 years. In order to be comparative with the other studies so far published, we additionally add the information of our patients over 40 years in table 4. In contrast with references 3 and 18, which also studied the patients with ALC, it clearly indicates that the percentage of MBL, especially for the CLL-like MBL, in the Chinese population is much lower. In contrast with the remaining five references,[10 11 15–17] although the percentage of this study seems not significantly lower, the reason may be attributed to that all of these investigations studied patients with normal blood count or healthy blood donors. Therefore, the percentage of MBL in the whole Chinese population is still supposed to be lower than the other countries. The notable difference in prevalence of CLL-like MBL may be partially attributed to geographical and hereditary diversity among the Western and Asian populations.

It is worth noting that in our cohort, despite a low prevalence of MBL in the Chinese population, non-CLL-like MBL cases (80.0%) were observed at a much greater percentage of patients than CLL-like and atypical-CLL subtype. The frequency of non-CLL-like MBL cases among individuals over 80 years of age even increased to 18.18%. The present cohort shared a similar percentage of CD5 (−) MBL with the African cohort. In the recent cross-sectional epidemiological study comparing MBL in the UK and Uganda, the percentage of patients with non-CLL-like MBL was surprisingly much higher in the Ugandan cohort than in the UK cohort (Uganda 97.6% vs UK 24.0%), where further genotype analysis reflected fundamental differences in the pathogenesis of MBL subtypes in different races.[16] This finding is evidently contrary to the results attained in previous studies regarding Western populations, where the CLL-like phenotype was generally the most prevalent subtype.[3 7 10 16]

Moreover, all of our CD5 (−) MBL patients displayed CD10 (−) and CD20 (+), which is suggested to be the 'marginal zone lymphoma (MZL)-like' MBL. In 2014, Kalpadakis *et al*[19] have disclosed that CD5 (−) MBL have similar features with splenic MZL (SMZL) with regard to morphology of lymphomatous cells, bone marrow infiltration pattern and immunophenotypic findings. Recent several studies also have highlighted CD5 (−) non-CLL-like MBL resemblance to the marginal zone lymphoproliferative disorders.[20–22] In respect to Chinese literatures, Chinese patients appear to have higher prevalence of MZL than the Western population. In 2014, the calculated age-standardised incidence rate for malignant lymphomas in Chinese was 4.18/100 000.[23] According to a Chinese study analysing 10 002 lymphoma cases in 2012, SMZL, extranodal MZL and mucosa-associated lymphoid tissue lymphoma accounted for 0.41%, 0.99% and 6.85% of all lymphomas, respectively. Therefore, these data suggested that the overall annual age-adjusted incidence of MZL in China would be 0.34 per 100 000 persons per year. While according to the 8 years of data (2001–2008) from the Surveillance, Epidemiology and End Results (SEER), the overall annual age-adjusted incidence of MZL in USA was only 0.13 per 100 000 persons per year.[24] The higher incidence of MZL and higher incidence of CD5 (−) non-CLL-like MBL that displays as 'MZL-like MBL' could confirm each other.

Therefore, although a large-scale population-based screening for B-LPD and MBL is not currently recommended, screening for B-LPD and MBL in high-risk populations, like the geriatric hospitalised population having lymphocytosis, may be rational and serve as a cost-effective approach in detecting early-stage B-LPD and MBL.

As this is an observational cohort study based on hospital population, its limitations should be illustrated in the interpretation of its results. First, the hospital population may confer a concurrent transient immune response. Several studies have demonstrated an association between MBL and infectious diseases.[13 25 26] Moreover, the hospitalised population in this study could not be representative of the entire Chinese population. Second, as the CLL-like MBL with low counts (<500/μL) may appear without the presence of lymphocytosis, as well as part of the HCs, therefore, the true and accurate prevalence needs further studies and validation. Third, patients without lymphocytosis were not analysed by FCM in this study, and the accurate frequency of MBL in the whole hospital population was not reported. Finally, as only 2 mL/case of PB samples of the enrolled patients was collected, further gene analyses such as the information about oligoclonality in B-cell malignancies could not be reported, temporarily. Consequently, the corresponding results may be biased, and larger population-based studies are required for further study.

CONCLUSION

This study reported on preliminary data regarding the prevalence of B-LPD and MBL in patients with lymphocytosis from the hospital-visiting population in China. By screening lymphocytosis in this large cohort, a relatively high proportion of patients with CLL (4.3%) was observed among individuals with lymphocytosis over 60 years of age. The prevalence of MBL was 3.9% and increased with age. Despite a low prevalence of MBL in the Chinese population, the CD5 (−) non-CLL-like MBL was the most common subtype, which displayed a pattern of 'MZL-like' MBL. Finally, the ROC curve analysis was

Table 4 Comparison of the studies that reported the prevalence of MBL in different population and countries

Reference	Country	Population	Size	MBL criteria	Overall	CLL-like	Atypical-CLL	Non-CLL-like
This study	China	Inpatients and outpatients ► Age: ≥18 years ► ALC >3500/μL	254 from 22 809	B-cell count $<5\times10^9$/L AND κ-to-λ ratio >3:1 or <0.3:1	3.9% (10/254)	0.4% (1/254)	0.4% (1/254)	3.1% (8/254)
		Inpatients and outpatients ► Age: ≥40 years ► ALC >3500/μL	167 from 22 809		6.0% (10/167)	0.6% (1/167)	0.6% (1/167)	4.8% (8/167)
3	UK	Subjects ► Age: 62–80 years ► No history of cancer ► Normal blood count	1520	B-cell count $<5\times10^9$/L AND κ-to-λ ratio <1:1 or >2.1:1	6.9% (105/1520)	5.1% (78/1520)	None	1.8% (27/1520)
		Patients ► Age: 39–99 years ► Current or previous lymphocytosis (ALC >4000/μL)	2228		Not reported	13.9% (309/2228)	Not reported	Not reported
18	USA	Emergency, in-clinics and out-clinics ► Age ≥50 years ► ALC ≥4000/μL	178	B-cell count $<5\times10^9$/L AND light chain restriction	19.1% (34/178)	11.2% (20/178)	2.2% (4/178)	1.7% (3/178)
15	Turkey	Volunteers blood donor ► Age: 18–78 years	999	B-cell count $<5\times10^9$/L AND κ-to-λ ratio >3:1 or <0.3:1	1.8% (18/999)	1.6% (16/999)	None	0.2% (2/999)
16	UK	Outpatients ► Age >45 years ► No history of Cancer ► Normal blood count	302	κ-to-λ ratio >3:1 or <0.3:1 OR more than 25% of B cells without surface Ig or expressing low levels of surface Ig	8.3% (25/302)	7.0% (21/302)	None	2.0% (6/302)
16	Uganda	Volunteers in rural community ► Age >45 years ► Seronegative for HIV-1	302	Same as above	13.9% (42/302)	1.0% (3/302)	None	13.6% (41/302)
17	Germany	Inpatients ► Age >50 years ► Non-haemato/oncological patients ► Normal blood count	1657	κ-to-λ ratio >3:1 or <0.3:1	3.8% (63/1657)	0.8% (14/1657)	0.8% (13/1657)	2.2% (36/1657)
10	USA	Healthy blood donor ► Age >45 years ► No evidence of HBV, HCV and HIV	2098	B-cell count $<5\times10^9$/L AND κ-to-λ ratio >3:1 or <0.3:1	7.1% (149/2098)	4.7% (99/2098)	1.1% (22/2098)	0.9% (19/2098)
11	Italy	Outpatients ► Age >65 years ► No history or suspicion of cancer ► Normal blood count	500	κ-to-λ ratio >3:1 or <0.3:1	6.4% (32/500)	4.4% (22/500)	0.6% (3/500)	1.4% (7/500)

ALC, absolute lymphocyte count; CLL, chronic lymphocytic leukaemia; HBV, hepatitis B virus; HCV, hepatitis C virus; MBL, monoclonal B-cell lymphocytosis.

suggestive, in that screening for lymphocytosis (ALC $\geq 4.7\times10^9$/L) by FCM in the elderly may be beneficial in the detection of B-LPD and MBL.

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