Diagnosis of Chronic Lymphocytic Leukemia Using iwCLL 2018 Compared with NCI-WG96 Criteria in Cipto Mangunkusumo Hospital: A Practical Consideration in Resource Limited Setting

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ABSTRACT

Background*: The diagnosis of chronic lymphocytic leukemia (CLL) is mainly based on blood count, morphology, and immunophenotyping. In Indonesia, the diagnosis is more challenging as the availability of immunophenotyping tests is limited. The European Society of Medical Oncology (ESMO) stated flowcytometry as a prerequisite to establishing diagnosis of CLL, meanwhile in the original International Workshop on Chronic Lymphocytic Leukemia (iwCLL) 2018 criteria, which has been widely accepted by physicians caring for patients with CLL, the diagnosis of CLL can be made in patients with cytopenia using bone marrow biopsy where flowcytometry test is not available. The aim of the study was to compare the utility of iwCLL 2018 compared with National Cancer Institute Working Group 96 (NCI-WG96) criteria in the diagnosis of CLL in Indonesia, especially in limited resource settings.* ***Methods****: The data of newly diagnosed CLL patients, including baseline demographic, clinical, and laboratory characteristics was retrieved retrospectively from medical records in Cipto Mangunkusumo General Hospital from 2015 until 2021. Diagnosis of CLL using iwCLL 2018 diagnostic criteria were then compared with NCI-WG96 criteria.* ***Results****: Thirty-eight patients were enrolled to this study. The median age was 59.5 years and dominated by males. Most of them were classified in the late-stage disease (63.4% in Binet C and about 70% in Rai III-IV). Four cases were CD5-negative CLL. Based on NCI-WG96 guideline, only 24 patients (63.2%) fulfilled all four criteria for CLL. Similarly, using the iwCLL 2018 flowcytometric criteria without biopsy data, 26 patients (68%) were diagnosed as CLL. However, if bone marrow biopsy in patient with cytopenia was taken into account, all patients (100%) can be confirmed as CLL.* ***Conclusion****: The iwCLL 2018 criteria which included bone marrow biopsy in the presence of cytopenia was more applicable to establish the diagnosis of CLL in Indonesia where flowcytometry is not available.*

Keywords: *chronic lymphocytic leukemia, diagnosis, NCI-WG96 criteria, IWCLL 2018 criteria.*

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is defined as a clonal expansion of small, mature, CD5-positive neoplastic B-cell lymphocytes having a characteristic immunophenotype in the peripheral blood, spleen, bone marrow, and other lymphoid tissues.[1] This disease is the most prevalent leukemia among adults in Western countries, accounting for 37% of newly diagnosed leukemia in the United States, followed by acute

myeloid leukemia (AML).[2] However, CLL in Asia differs from Western countries due to its lower prevalence.[3,4] This might be explained by variation in genetic background between different regions.[5] Another reason for this lower incidence is due to the asymptomatic nature of most CLL cases, many are left undiagnosed, particularly in resource limited countries. Until now, there is no population-based study regarding epidemiology of CLL in Indonesia, especially regarding prevalence and diagnostic aspects of CLL. This lack of data can hinder the efforts to evaluate the incidence and trends of the disease among different populations.

Over the last few decades, the criteria to diagnose CLL has undergone substantial revisions. The first consensus criteria to standardize the diagnosis of CLL was available in 1988. At that time, automated blood counter and immunophenotyping test were not routinely available, thus absolute lymphocyte counts (ALC) above $5x10^9$/L was used as the threshold for diagnosis.[6,7] In the 2008 revisions to the CLL diagnostic criteria, B-cell count rather than ALC above $5x10^9$/L was adopted as the basis for diagnosis.[8] Nowadays, the diagnosis of CLL is mainly based on laboratory features, including blood count, morphology, and immunophenotyping, as described in the iwCLL 2018.[9] Society guidelines for CLL stressed on the importance of CLL diagnosis from peripheral blood using B-cell count and flowcytometry alone. In the National Comprehensive Cancer Network (NCCN) guidelines, bone marrow biopsy is recommended if the initial workup is non-diagnostic.[10] Moreover, the European Society of Medical oncology (ESMO) guidelines suggest doing bone marrow biopsy if flowcytometry result remain inconclusive.[11] This is considered convenient for patients since these recommendations prevent the procedural risk and unnecessary pain from bone marrow biopsy. However, flowcytometry is not widely available in developing countries. After thorough review of the iwCLL 2018 diagnostic criteria, it was stated that "the presence of a cytopenia caused by a typical marrow infiltrate establishes the diagnosis of CLL regardless of the number of peripheral blood B lymphocytes or of the lymph node involvement."[7] Therefore, in the absence of immunophenotyping facility, bone marrow biopsy can be used to establish CLL diagnosis.

The present study aimed to evaluate the diagnosis of CLL using iwCLL 2018, which allowed CLL diagnosis from bone marrow biopsy where flowcytometry test is not available, compared with NCI-WG96 and to determine demographic and immunophenotypic profiles of CLL in Indonesia , . This is the first study to compare the diagnosis of CLL according to the NCI-WG96 and the iwCLL 2018 guidelines in such settings.

METHODS

Based on the NCI-WG96 guideline, the diagnosis criteria of CLL include: (1) Peripheral absolute lymphocyte count $>5x10^9$/L; (2) Lymphocytes were positive for CD5 and CD19/CD20/CD23; (3) Atypical cells (prolymphocytes) <55%; (4) Bone marrow lymphocytes ≥ 30%.[12] Meanwhile, in iwCLL 2018 criteria, CLL diagnosis can be made if there are: (1) Peripheral B-cell count $\geq 5x10^9$/L; AND (2) Lymphocytes were positive for CD5 and CD19/CD20/CD23. Alternatively, CLL can also be diagnosed by "the presence of cytopenia caused by a typical bone marrow infiltrate, regardless of the number of peripheral B-cell count or lymph node involvement".[9]

This was a cross-sectional study done at Department of Hematology and Medical Oncology, Cipto Mangunkusumo General Hospital, Jakarta from 2015 until 2021. Patients with newly-diagnosed CLL were recruited from the database of patients who visited the Hematology and Medical Oncology outpatient clinic. Sampling was done by total sampling obtained from the list of CLL patients in the registry. Inclusion criteria was patients diagnosed with CLL. Exclusion criteria was incomplete laboratory data.

Information regarding baseline demographic, clinical (including age, sex, Rai and Binet stage, lymphadenopathy, hepatosplenomegaly), and laboratory (including complete blood count, absolute lymphocyte count, erythrocyte sedimentation rate, LDH, serum protein electrophoresis, and immunophenotyping)

characteristics were obtained retrospectively from medical records. The sample of bone marrow aspirate and biopsy were collected to confirm the diagnosis by flow cytometry immunophenotyping and morphologic analysis were performed to examine the percent of bone marrow lymphocytes.

The study protocol was approved by the Local Ethics Committee of Faculty of Medicine Universitas Indonesia (No. KET-452/UN.2.F1/ETIK/PPM.00.02/2021). The data was expressed in frequency (percentage, %) for categorical variables and median (range) for continuous variables. All analysis was performed using SPSS version 24 for Mac.

RESULTS

There were thirty-eight CLL cases in Cipto Mangunkusumo General Hospital, Jakarta between 2015 and 2021, which were recorded in the registry. All subjects fulfilled the inclusion criteria and were recruited into this study. The characteristics of the subjects are summarized in **Table 1**. Their age at diagnosis ranged from 41-82 years (median 59.5 years) and was dominated by individuals aged ≤65 years (89.5%) and male (60.5%). Two of those patients (5.3%) was diagnosed as prolymphocytic leukemia (PLL), the aggressive form of CLL. Hypertension (28.9%), dyspepsia/GERD/gastritis (15.8%), and diabetes mellitus (13.2%) were the most common comorbidities in the patients. Two of the patients had history of malignancy: thyroid cancer (2.6%) and breast cancer (2.6%). Most of the patients with CLL at time at diagnosis were found in the late stage of the disease, 63.4% of the patients were in Binet stage C and around 70% of the patients were in Rai stage III-IV. Additionally, palpable splenomegaly was presented in 63.2% of the patients.

The median hemoglobin, leucocyte count and absolute lymphocyte count at diagnosis were 8.9 g/dL, 59.86 x 10^9/L, and 47.83 x 10^9/L, respectively. Serum LDH levels were available in 24 of 38 patients, and only 9 of 38 patients (23.7%) had an elevated LDH levels.

Table 1. Clinical and laboratory characteristics of the subjects

		CLL/PLL (n=38)
Age at diagnosis (years), median (range)		59.5 (41-82)
Age group (years), n (%)	≤65	34 (89.5)
	>65	4 (10.5)
Sex, n (%)	Male	23 (60.5)
	Female	15 (39.5)
Diagnosis, n (%)	CLL	36 (94.7)
	PLL	2 (5.3)
Comorbidities, n (%)	Hypertension	11 (28.9)
	Dyspepsia/ GERD/ gastritis	6 (15.8)
	Diabetes mellitus	5 (13.2)
	Hyperuricemia	4 (10.5)
	Chronic kidney disease	2 (5.3)
	Congestive heart failure	2 (5.3)
	Stroke	1 (2.6)
	AIHA	1 (2.6)
	Beta thalassemia trait	1 (2.6)
	Thyroid cancer	1 (2.6)
	Breast cancer	1 (2.6)
	Hepatitis C	1 (2.6)
	Asthma/ allergy	2 (5.2)

Binet, n (%)	A	11 (28.9)
	B	3 (7.9)
	C	24 (63.2)
RAI, n (%)	0	1 (2.6)
	I	2 (5.3)
	II	7 (18.4)
	III	20 (52.6)
	IV	8 (21.1)
Lymphadenopathy >1 cm, n (%)	Yes	16 (42.1)
	No	22 (57.9)
Palpable hepatomegaly, n (%)	Yes	9 (23.7)
	No	29 (76.3)
Palpable splenomegaly, n (%)	Yes	24 (63.2)
	No	14 (36.8)
Hemoglobin (g/dL), median (range)		8.9 (2.8-16.1)
Hematocrit (%), median (range)		28.25 (12.6-47.1)
Leukocyte count (x10^9/L), median (range)		59.86 (4.04-558.35)
Absolute lymphocyte count (x10^9/L), median (range)		47.83 (2.00-530.43)
Platelet count (x10^9/L), median (range)		163.5 (41-468)
ESR (mm/h), median (range)		57.5 (2-153)
LDH, n (%)	Above the limit (>350 IU/L)	9 (23.7)
	Normal levels	15 (39.5)
	N/A	14 (36.8)
Serum protein electrophoresis, n (%)	Monoclonal	5 (84.2)
	Polyclonal	1 (2.6)
	N/A	32 (84.2)

ESR, erythrocyte sedimentation rate; LDH, lactate dehydrogenase; N/A, not available

Immunophenotyping results from thirty-eight subjects were shown in **Table 2**. Cells from all cases were positive with CD19, and the majority were CD5, CD20, CD23, and kappa positive. Lambda were positive in a minority of cases. Four cases were CD5-negative.

Table 2. Membrane markers of CLL/PLL cases

Marker	Number of cases		% Positive
	Tested	Positive	
CD5	31	27	87.1%
CD19	33	33	100%
CD20	36	34	94.4%
CD23	21	17	81%
Kappa	15	11	73.3%
Lambda	9	3	3.3%

For NCI-WG 1996, 37 patients fulfilled criteria of absolute lymphocytosis > 5x10^9/L in the peripheral blood, 27 patients fulfilled immunophenotyping criteria (lymphocytes were positive for CD5 and > 1 B-cell marker), 36 patients fulfilled atypical cells (prolymphocytes) < 55%, and 35 patients fulfilled bone marrow lymphocytes > 30% (**Figure 1**). Using the immunophenotyping criteria, a total of eleven patients that did not fulfill the criteria. However, it should be noted that 7 out of 11 patients did not have complete immunophenotyping examinations.

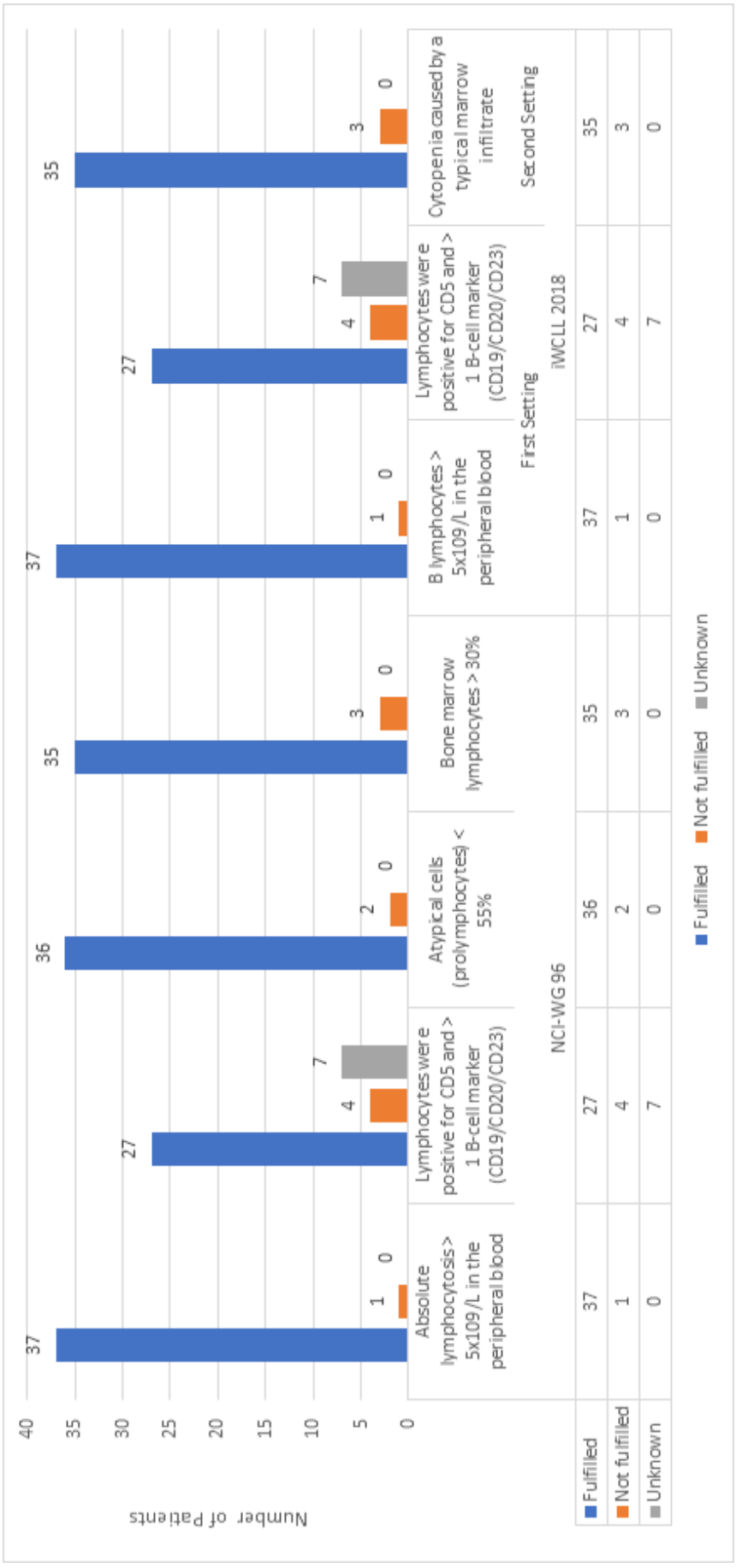

Figure 1. Number of Patients that Fulfilled NCI-WG 96 Guideline and iwCLL 2018 Diagnostic Criteria.

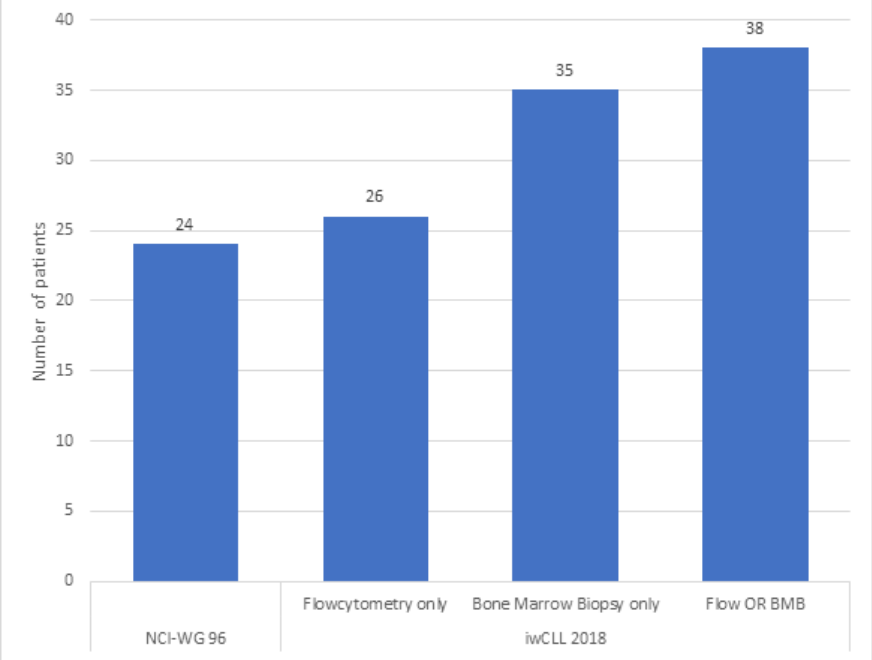

Figure 2. Number of patients categorized as CLL based on NCI-WG 96 guideline, iWCLL 2018 guideline flowcytometric criteria, bone marrow biopsy criteria, and flowcytometry OR bone marrow biopsy. Flow: flowcytometry; BMB: bone marrow biopsy.

Based on NCI-WG96 guideline, only twenty-four patients (63.2%) fulfilled all four criteria and thus were diagnosed as CLL (**Figure 2**). Similarly, using the iwCLL 2018 criteria without bone marrow biopsy, twenty-six patients (68%) were diagnosed as CLL. Using the iwCLL 2018 bone marrow biopsy part of the criteria only, there were thirty-five cases (92%) of CLL (**Figure 2**). Overall, using the bone marrow biopsy criteria of iwCLL 2018 yielded more CLL diagnosis than using all four NCI-WG96 criteria for CLL diagnosis or iwCLL flowcytometric criteria. If bone marrow biopsy in patient with cytopenia was taken into account in the patients who did not fulfill the flowcytometric criteria of iwCLL 2018, all patients (100%) can be confirmed as CLL.

DISCUSSION

In the present study, the median age of study population at diagnosis was 59.5 years and CLL cases were predominantly among males. This results is in accordance to prior studies.[13,14] Chronic illnesses, such as hypertension, gastrointestinal problem (dyspepsia or gastritis), and diabetes mellitus were the top three comorbid conditions in our patients. Although CLL incidence may be secondary to aging, chronic illnesses may be a contributing factors despite the mechanisms remaining unclear.[15–17]

According to CLL international data, CLL incidence rate (IRs) is highest in Western countries (e.g. North America, Europe), and the lowest in Asian countries.[18–20] Indeed, CLL incidence was varied by race as noted in a multicenter study by Dores et al that the IRs of CLL among whites, blacks, and Asian/pacific islander were 4.18, 3.01, and 0.84 cases per 100,000 people, respectively.[20] The variability of CLL incidence cases between different regions might be influenced by the variations in genetic background.[5] The sustained low IRs of CLL in Asians who have migrated to the United States support that genetic influence is greater than environmental factors.[21,22] This low IRs in Asians might explain why we had limited sample size. In addition, underdiagnosis and shortened life expectancy may contribute to the low incident cases. Since patients with early stage CLL are mostly asymptomatic, the diagnosis is often overlooked. Moreover, in Indonesia there is lack of infrastructure to diagnose CLL properly. Flowcytometry, which is essential to determine B-cell clonality, is only available in few large cities.

Call et al demonstrated that the majority of CLL incident cases diagnosed using the NCI-WG96 criteria were observed in the early stages: 60.9%, 33.9%, and 5.2% for Rai stage 0, I/II, and III/IV, respectively as it was mostly diagnosed in primary care settings.[23] Molica et al, Villavicencio et al, and Strati et al also provided similar results.[16,24,25] This is truly contrast to our data where most of CLL cases was diagnosed in the advanced-stage disease, either Binet C or Rai III-IV, because patients in Indonesia usually sought medical help when they developed symptoms (B-symptoms, lymphadenopathy, marrow failure); and only a minority of the patients were diagnosed through incidental finding of an ALC above the defined threshold. Apparently, most of the cases were diagnosed in tertiary care setting because of the limited availability of flow cytometry immunophenotyping.

The typical immunophenotypic feature of B-cell clone in CLL is the co-expression of CD5, CD19, CD20, and CD23. However, some cases of CLL have CD5-negative B-cell clone. Its incidence varies from 7% to 20%.[26,27] In our study, the incidence of CD5-negative CLL was 10.5% (four in 38 cases) and all of them had splenomegaly. Similarly, several studies reported a higher incidence of splenomegaly in CD5-negative CLL compared to CD5-positive CLL cases. Furthermore, some studies pointed out that CD5-negative CLL patients had a more advanced stage of disease and shorter survival.[26,28,29] In the present study, all those four patients was in Binet C and Rai III.

One patient in the present study had the ALC of $2x10^9$/L and no organomegaly, but presented with B-symptoms, anemia, typical bone marrow infiltrate, and immunophenotyping of CLL. In other cases, the diagnosis of CLL in CD5-negative cases was made according to clinical and morphological features. Accordingly, these patients met the iwCLL 2018 bone marrow criteria for CLL which only requires the presence of cytopenia caused by typical bone marrow infiltrate, regardless of B-cell count or nodal involvement.[9] The disadvantage of the immunophenotyping test is the cost and it is not widely available in Indonesia. In addition, very few pathologists have completed the flowcytometry interpretation training. The expensive testing cost pose another problem since it is not covered by the National Health Insurance. Hence, the use of immunophenotyping as stated in the flowcytometric criteria of iwCLL 2018 is not applicable in Indonesia. Based on our data, more than 90% of the patients can be diagnosed as CLL by bone marrow biopsy alone. Meanwhile, only 63.2%% of the patients fulfilled all the NCI-WG96 criteria and 68% of patients met the iwCLL flowcytometry without bone marrow biopsy) criteria. These findings showed that in our CLL patients, bone marrow biopsy alone is more sensitive to diagnose CLL, even without flowcytometry data.

This study depicted another insight into the iwCLL 2018 CLL diagnostic criteria. In the absence of flowcytometric immunophenotyping, bone marrow trephine biopsy showing marrow infiltrate can be utilized to diagnose CLL. In contrast to flowcytometric immunophenotyping, trephine biopsy is reimbursable by the national health insurance. The procedure can be performed in remote areas with limited resources, with the pathologic review done in large centers using the preserved samples sent in 10% formalin preservatives. In our study, only three patients (8%) did not fulfill the iwCLL 2018 bone marrow criteria for CLL. However, all three patients were confirmed for CLL by flowcytometry. It can be proposed that bone marrow biopsy is mandatory in diagnosis of CLL, especially in limited settings where flowcytometry is not available.

Our study is subject to several limitations. This study was a single center study which only had small number of subjects. Race or ethnicity was not gathered in our study as it might influence the incidence of CLL. Additionally, immunophenotypic analysis was not available for all patients. Immunophenotyping test is still limited and only available in several urban areas across Indonesia. Moreover, as CLL is usually diagnosed incidentally and patients present with minimal signs and symptoms, thus financial barriers to accessing primary health care providers or specialists or laboratory tests may hamper the detection rate of CLL. Further studies are needed to confirm the findings of this study,

especially with bigger sample size and emphasis on diagnostic agreement.

Another drawback of the bone marrow trephine biopsy is that this procedure is not routinely performed in diagnosis of CLL in all centers in Indonesia. This study showed that bone marrow biopsy in addition to bone marrow aspirate is the best available modality to diagnose CLL in Indonesia when flowcytometry is not available.

CONCLUSION

The iwCLL 2018 criteria involving bone marrow biopsy in the presence of cytopenia were more applicable to establishing CLL diagnosis in Indonesia. Also, it may be applied to other countries with limited access to immunophenotyping tests.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPLEMENTARY DATA

The 1996 National Cancer Institute-sponsored Working Group guidelines (NCI-WG 96) Guideline

No	Criteria
1	Absolute lymphocytosis > $5x10^9$/L in the peripheral blood
2	Lymphocytes were positive for CD5 and ≥ 1 B-cell marker (CD19/CD20/CD23)
3	Atypical cells (prolymphocytes) < 55%
4	Bone marrow lymphocytes ≥ 30%.

The 2018 International Workshop on chronic lymphocytic leukemia guidelines (IWCLL 2018) Guideline

B lymphocytes > $5x10^9$/L in the peripheral blood	AND	CLL cells were positive for CD5 and B-cell antigens (CD19/CD20/CD23)
OR		
Presence of cytopenia caused by a typical marrow infiltrate regardless of the number of peripheral blood B lymphocytes or of the lymph node involvement		