Utilization of a Scoring System for Diagnosis of Chronic Lymphocytic Leukemia in Thai Patients

Orathai Promsuwicha MSc*,
Wayuree Songmuang BSc*, Chirayu U Auewarakul MD, PhD*

** Division of Hematology, Department of Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand*

Background: *Chronic lymphocytic leukemia (CLL) is a rare B-lymphoid malignancy in Southeast Asia. We evaluated whether a scoring system based on the expression of CD5, CD23, FMC7, CD79b and surface immunoglobulin (SIg) could be utilized to distinguish CLL from other types of lymphoid neoplasms in the Thais.*

Material and Method: *One-hundred and forty-five samples with a clinical suspicion for CLL were analysed by flow cytometry. A score of one was assigned if the following marker was identified: $CD5^+$, $CD23^+$, $FMC7^-$, $CD79b^-$ and $SIg^{-/weak}$. A cut-off score of ≥3 was required for the definitive diagnosis of B-CLL.*

Results: *Only 50 cases (34.5%) were confirmed as B-CLL (scores ≥3). Cases with scores ≥ 3 had significantly higher leukocyte counts and marrow/blood lymphocytes than cases with scores ≤ 2. Dual CD5/CD23 expression was found in 87.5% of CLL cases. In 81 cases with scores ≤ 2, a variety of non-CLL disorders predominated, such as marginal zone lymphoma, splenic lymphoma with villous lymphocytes, mantle cell lymphoma, and prolymphocytic leukemia.*

Conclusion: *A score of ≥ 3 and dual CD5/CD23 expression are essential for the diagnosis of CLL while a score of ≤ 2 mostly indicative of non-CLL. The majority of clinical cases of CLL turned out to be non-CLL by flow cytometry. Increased utilization of this scoring system should increase the accuracy of diagnosis of this rare type of leukemia in the Thai population.*

Keywords: *Chronic lymphocytic leukemia, Flow cytometry, Immunophenotyping, Scoring system, Leukemia*

J Med Assoc Thai 2011; 94 (Suppl. 1): S232-S238
Full text. e-Journal: *http://www.mat.or.th/journal*

Chronic lymphocytic leukemia (CLL) as the most common type of leukemia in the Western countries is surprisingly rare in the East and Southeast Asian populations[1-3]. In a large retrospective series of Chinese leukemia patients diagnosed in 1952-1986, only 5% of the cases were CLL. In Thailand, small lymphocytic lymphoma (SLL) which is a pathologic counterpart of CLL was relatively uncommon, comprising < 10% of all cases of malignant lymphoma[4]. A retrospective study at Siriraj Hospital which is the largest hospital in Thailand revealed that CLL was a rare clinical entity with only 184 cases clinically and morphologically diagnosed between 1963 and 1988[5]. In clinical practice, the diagnosis of CLL is usually made through a careful blood cell enumeration and morphologic reviews of peripheral blood and bone marrow which requires the presence of at least 5 x 10^9/L (5,000/mm^3) of small, mature-looking lymphocytes in the peripheral blood and at least 30% of such lymphocytes in the bone marrow[6]. As CLL lymphocytes aberrantly express the T-cell antigen CD5 in addition to the normal B-cell surface antigens CD19, CD20, and CD23, immunophenotyping has become an important tool to establish and confirm the diagnosis of CLL[7,8]. A scoring system for CLL diagnosis was initially proposed by the Royal Marsden Hospital (RMH) group based on the expression of five membrane markers, *i.e.* CD5, CD23, FMC7, surface immunoglobulin (SIg) and CD22[9]. This system was subsequently refined by substitution of CD22 by CD79b based on the finding that the level of CD79b was characteristically low in neoplastic B-CLL cells when contrasted to that of normal B cells[10]. The accuracy of the RMH scoring system was over 96% when a cutoff value of 3 points or higher was used to distinguish B-CLL from other types of B-cell lymphoma.

Since CLL is such a rare entity in Thailand, we

Correspondence to:
Auewarakul C U, Division of Hematology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, 2 Prannok Road, Bangkoknoi, Bangkok 10700, Thailand.
Phone: 0-2419-4449, Fax: 0-2411-2012
E-mail: sicaw@mahidol.ac.th

wish to determine if the scoring system could be of value in the differentiation of CLL from other more common types of leukemia and lymphoid neoplasms that can sometimes masquerade as CLL. Samples of blood and/or bone marrow from patients with a clinical diagnosis of CLL were received in the flow cytometry laboratory and immunophenotypic analysis was performed with a panel of monoclonal antibodies. CLL was then diagnosed based on 1) the scores of at least ≥3 and/or 2) dual expression of CD5 and CD23 antigens on neoplastic B-cells.

Material and Method

Leukemia samples

This study was a retrospective analysis approved by the Ethical Committee for Human Research, Faculty of Medicine Siriraj Hospital, Mahidol University. Blood and/or bone marrow samples from 145 Thai patients who underwent routine hematologic work-ups for lymphocytosis from January 2007 to December 2009 were received at the flow cytometry laboratory. All cases had a presumptive clinical diagnosis of CLL. Clinical and biological characteristics of patients were collected including age, sex, white blood cell (WBC) counts, differential counts, hemoglobin (Hb) level and platelet (Plt) counts.

Immunophenotypic analysis

Leukemic samples were labeled with the following panel of monoclonal antibodies (BD Biosciences, CA, USA): CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD11c, CD16, CD19, CD20, CD22, CD23, CD25, CD38, CD45, CD56, CD57, CD79b, CD103, FMC7, kappa and lambda. After 15-minute incubation at room temperature in the dark, cells were lysed in FACS lysing buffer and washed with phosphate-buffered saline (PBS). CD45 and side scatter (SSC) gates were used to select blast windows for multiparameter flow cytometric analysis (FACScalibur; Becton Dickinson, San Jose, CA, USA)[11]. A membrane marker was considered positive when more than 20% of the cells expressed it.

Scoring system and statistical analysis

The RMH scoring system modified by Moreau et al[10] was utilized in this study cohort based on the five standard markers. A score of one was assigned if one of the following markers was identified: $CD5^+$, $CD23^+$, $FMC7^-$, $CD79b^-$ and $SIg^{-/weak}$. A cut-off score of ≥ 3 was required for the definitive diagnosis of B-CLL by flow cytometry. A cut-off score of ≥ 4 was also used to achieve another set of scores for further comparison.

Differences in the distribution of variables between groups were determined using the Mann-Whitney test. A p-value of less than 0.05 was considered to be statistically significant. All statistical analyses were performed using the SPSS program for Windows (version 17.0).

Results

Scores and CD5 expression of cases with a clinical suspicion of CLL

The RMH scores and the percentage of CD5 positivity of 145 patients included in the study are summarized in Table 1. The scores were calculated and used for subclassification of cases into scores ≥ 4/5 (n = 43), ≤ 3/5 (n = 102), ≥ 3/5 (64) or ≤ 2/5 (81). All cases (100%) with scores ≥ 4/5 expressed CD5, whereas 20.6% of cases with scores ≤ 3/5 did. If categorized based on a cut-off score of ≥ 3/5, 86% of cases with scores ≥ 3/5 expressed CD5, whereas 11% of cases with scores ≤ 2/5 did.

Table 1. Subclassification of 145 clinical cases based on immunophenotypic scores (CD5, CD23, CD79b, FMC7, SIg) and CD5 positivity

	Score ≥ 4/5	Score ≤ 3/5	Score ≥ 3/5	Score ≤ 2/5
Total cases	43	102	64	81
CD5 positive (no. of cases/percentage)	43 (100%)[a]	21 (20.6%)	55 (86%)[b]	9 (11%)[c]
CD5 negative(no. of cases/percentage)	0 (0%)	81 (79.4%)	9 (14%)[d]	72 (89%)[e]

[a] All classified as CLL

[b] Include CLL (46), MCL (8), CLL s/p treatment (1)

[c] Include $CD5^+CD23^+$ atypical CLL (2), $CD5^+CD23^-$ MCL (7)

[d] Include atypical $CD5^-CD23^+CD79b^+SIg^-FMC7^-$CLL (1), CLL s/p treatment (2), MCL s/p treatment (1), B-PLL (1), MZL/SLVL (4)

[e] Include CLL s/p treatment (4)

Clinical and hematologic parameters of all cases based on scores ≤2/5 or scores ≥3/5

Most of the patients in this cohort belonged to the older age group (mean age of 64) regardless of their derived scores (Table 2). The majority of cases were males with mild anemia (mean Hb of 10 g/dL) and a normal Plt count. Cases with scores ≥3/5 had a higher WBC count and a higher percentage of marrow lymphocytes and blood lymphocytes than cases with scores ≤ 2/5 ($p < 0.001$).

Characterization of disease entities with scores ≤ 2

Table 3 delineates the diseases that were diagnosed among 81 cases with a score of 0, 1, or 2. The majority of cases with a score of 2 were marginal zone B-cell lymphoma (MZL)/splenic lymphoma with villous lymphocytes (SLVL) (n = 11), mantle cell lymphoma (MCL) (n = 7) and B-prolymphocytic leukemia (PLL) (n = 5). Three cases of B-PLL, 5 cases of MZL/SLVL and 1 case of Hairy cell leukemia (HCL) had a score of 1. Four cases of B-CLL previously treated with chemotherapy were found in a group with a score of zero and 2 cases of untreated CLL were found in a group with a score of 2 based on the dual expression of CD5/CD23 antigens on neoplastic B-cells. Other diseases within a zero-score group included HCL (n = 2), MCL s/p treatment (n = 2), T/NK-large granular lymphocyte leukemia (T/NK-LGLL) (n = 6), acute leukemia (n = 2), myelodysplastic syndrome (MDS) (n = 5), MZL/SLVL (n = 7), T-PLL (n = 5) and normal specimens (n = 10).

Scores and immunophenotypic profiles of cases with definitive diagnosis of CLL

Table 4 summarizes all 56 cases with a definitive diagnosis of CLL by flow cytometry. Thirty-one cases had a score of 5/5, 12 cases had a score of 4, 7 cases had a score of 3, 2 cases had a score of 2, and 4 cases had a zero score. Forty-nine cases were $CD5^+/CD23^+$ (87.5%), 39 were $CD5^+/CD23^+/CD79b^-$, 45 cases were $CD5^+/CD23^+/FMC7^-$, and 34 cases were $CD5^+/CD23^+/SIg^{-/weak}$. Representative flow cytometric profiles and morphological smears of a case of CLL with a high score of 5 and another case of marginal zone B-cell lymphoma with a low score of 2 are shown in Fig. 1.

Table 2. Clinical and laboratory parameters of cases with scores ≤ 2/5 and scores ≥ 3/5

	Scores ≤ 2 (n = 81)	Scores ≥ 3 (n = 64)	p-value
Age			
mean (SD)	64 (15.23)	64.31 (12.52)	ND
median (min: max)	67 (24:89)	68 (21:84)	
Sex (M/F)	44/37	40/24	ND
WBC count ($x10^9$/L)			
mean (SD)	33.59 (62.29)	54.32 (57.73)	$p < 0.001$
median (min: max)	9.68 (1.46:372)	31.65 (2.3:224.9)	
Hb (g/dL)			
mean (SD)	10.05 (2.68)	10.80 (2.36)	$p = 0.058$
median (min: max)	9.8 (4.3:16.4)	5.7 (5.7:16.0)	
Platelet count ($x10^9$/L)			
mean (SD)	172.34 (134.65)	169.2 (119.58)	$p = 0.97$
median (min: max)	157 (5.6:964)	164 (7:839)	
Absolute blood lymphocytes			
mean (SD)	21.01 (41.88)	44.68 (53.46)	$p < 0.001$
median (min: max)	4.39 (0.13:257.94)	23.69 (0.49:205.99)	
Marrow lymphocytes by flow cytometry (%)			
mean (SD)	47.22 (27.80)	70.68 (21.86)	$p < 0.001$
median (min: max)	40.93 (1.79:94.3)	76.61 (8.56:96.83)	
Blood lymphocytes by flow cytometry (%)			
mean (SD)	52.56 (24.13)	79.71 (14.77)	$p < 0.001$
median (min: max)	51.23 (11.66:89.24)	83.2(35.38:96.60)	
Lymphadenopathy (%)	22.2	29.7	ND
Hepatomegaly (%)	34.6	28.1	ND
Splenomegaly (%)	44.4	39.1	ND

Table 3. Categorization of 81 cases with a score ≤ 2

Score	Number of cases														
	Atypical CLL	CLL s/p treatment	B-PLL	T-PLL	MZL/ SLVL	SMZL/ SLVL s/p treatment	MCL	MCL s/p treatment	HCL	T/NK-LGL	Reactive marrow	Acute leukemia	MDS	CML	Normal marrow
2	2	0	5	0	11	0	7	0	0	0	0	0	0	0	0
1	0	0	3	0	5	0	0	0	1	0	0	0	0	0	0
0	0	4	0	5	7	1	0	2	2	6	2	2	5	1	10
Total	2	4	8	5	23	1	7	2	3	6	2	2	5	1	10

Table 4. Summary of cases diagnosed as CLL

	Total diagnosed cases of CLL	
	n = 56	%
$CD5^+$	49	87.5
$CD5^+CD23^+$	49	87.5
$CD5^+CD23^+CD79b^-$	39	69.6
$CD5^+CD23^+CD79b^+$	10	17.9
$CD5^+CD23^+FMC7^-$	45	80.4
$CD5^+CD23^+FMC7^+$	4	7.1
$CD5^+CD23^+SIg^-$	34	60.7
$CD5^+CD23^+SIg^+$	15	26.8
Score 5	31	55.4
Score 4	12	21.4
Score 3	7	12.5
Score 2	2	3.6
Score 1	0	0
Score 0	4	7.1

Discussion

CLL is characterized by extensive proliferation and accumulation in the blood, bone marrow and lymphoid organs of the characteristically small, mature lymphocytes with scant cytoplasm and dense nuclei with indistinct nucleoli[12]. The recent World Health Organization (WHO) classification of tumours of haematopoietic and lymphoid tissues categorizes CLL as a leukemic counterpart of SLL with the distinction between CLL and SLL being an absolute number of circulating lymphocytes not exceeding 5 x 10^9/L in SLL[13]. CLL/SLL represents a continuum of indolent small B-cell lymphoid neoplasm that needs to be distinguished from other small to medium-sized B-cell lymphomas and leukemias such as mantle cell lymphoma (MCL), marginal zone lymphoma (MZL), splenic lymphoma with circulating villous lymphocytes (SLVL), follicular lymphoma (FL), prolymphocytic leukemia (PLL) and hairy cell leukemia (HCL)[14-16]. Moreover, it is essential to exclude other types of T/NK-cell lymphoid neoplasms including a previously described entity, T-CLL, which is now called T-PLL, and T/NK-large granular lymphocyte leukemia (T/NK-LGLL)[17].

The RMH scoring system was initially developed for CLL patients in the Western population[8-10] although its application to the Southeast Asian populations has never been reported. We set out to determine if such scoring system could be of value for the differential diagnosis of various types of small lymphoid neoplasms in the Thais. We found that only

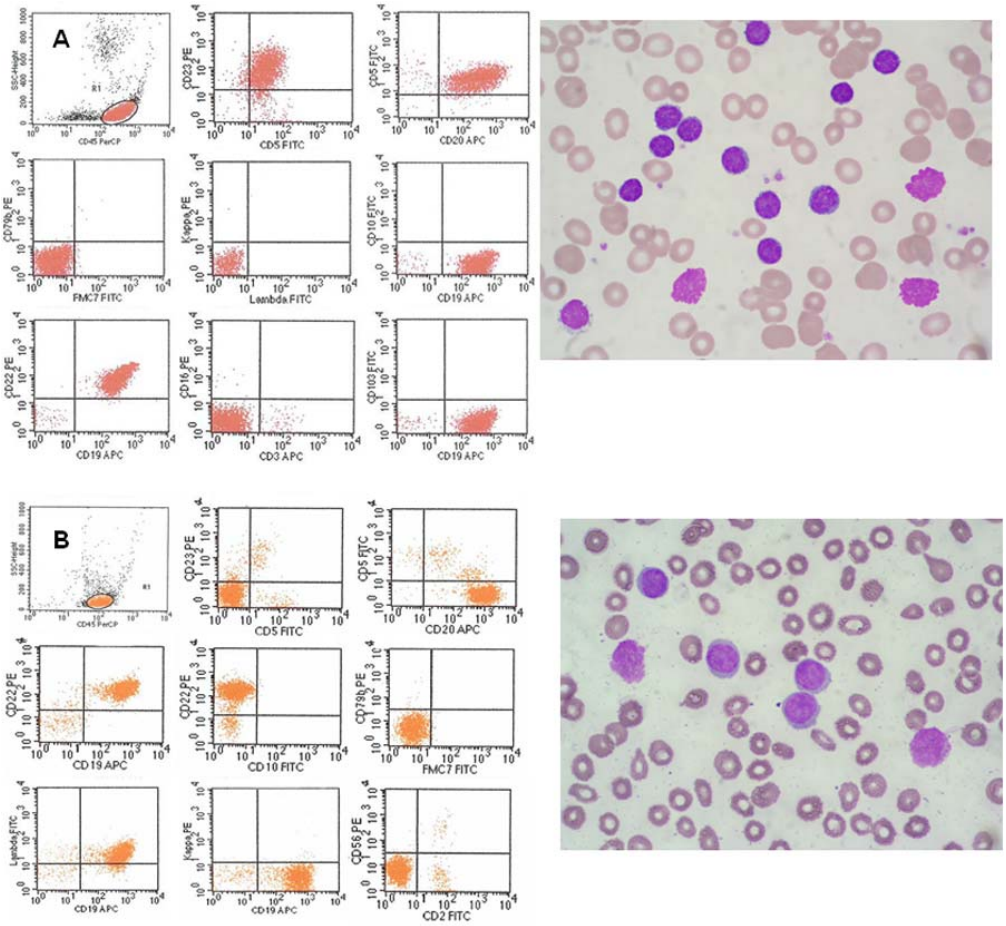

Fig. 1 A representative immunophenotypic profile and morphologic of a case of CLL with a score of 5 (A); and a case of CD5-negative/CD23-negative marginal zone B-cell lymphoma with a score of 2 (B)

50 cases out of 145 cases (34.5%) could be confirmed as B-CLL based on the scores of $\geq$ 3 and only 49 cases (33.7%) had a dual expression of CD5 and CD23. However, when the results of CD5/CD23 expression and prior clinical information were considered, 56 cases of CLL could be diagnosed regardless of the patients' scores. Among these, only 31 cases had a perfect score of 5/5 while 12 cases had a score of 4, 7 cases had a score of 3, 2 cases had a score of 2 and 4 cases had a zero score. Two atypical cases with scores $\leq$ 2 were allowed as CLL due to the dual expression of CD5 and CD23 on neoplastic B-cells which fits the diagnostic criteria according to the WHO 2008[13]. All 4 CLL patients with a zero score had previously received chemotherapy treatment and appeared to have lost their CD5 expression as well as other typical features of CLL resulting in lower scores in treated cases than in newly diagnosed cases. Therefore, clinical history is very important for the accurate diagnosis of CLL cases after treatment.

With respect to each scoring antigen, it was of interest to find that lack of FMC7 expression seemed to occur more frequently (45/56) than lack of CD79b (39/56) and weak/negative SIg (34/56) expression in Thai CLL. FMC7 may represent a more sensitive marker to diagnose CLL in the Thais and should always be included in the diagnostic panel. A clinical correlation was also found between the WBC counts and % lymphocytes in the bone marrow and peripheral blood and the patients' score. Cases with scores $\geq$ 3 (*i.e.* mostly CLL) had higher WBC counts and % marrow lymphocytes and % blood lymphocytes than cases with scores $\leq$ 2 (*i.e.* mostly non-CLL), reflecting the dominant leukemic nature of CLL in contrast to lymphomas with a more subtle leukemic phase.

As CLL is an uncommon disease compared to

other types of lymphoid neoplasms in the Thai population[4,5], it was not unexpected to find that the majority of cases in this cohort turned out to be non-CLL disorders, particularly lymphomas with a leukemic phase such as MCL[18] and MZL[19]. MCL can be differentiated from CLL based on the expression of CD5, FMC-7, CD43 and cyclin D1 with moderate SIg while lacking CD23 expression. Other types of lymphomas normally do not express CD5 and therefore can be distinguished from CLL and MCL based on the lack of expression of CD5. MZL usually express pan-B-cell markers such as CD19, CD20, and CD22 but lack CD5, CD10, CD23 and CD43 expression. In this series, other types of leukemias were also discovered in the group with scores ≤ 2, including PLL and HCL. B-PLL cases expressed CD19, CD20, CD22, CD79b and FMC7, but the majority did not express CD5. Three of our HCL cases did not express CD5 but expressed CD11c, CD25 and/or CD103; therefore they could easily be distinguished from CLL. We were also able to exclude other types of T-cell disorders based on the expression of T-cell markers as well as to exclude other types of marrow disorders based on the CD45/SSC profiles.

In conclusion, the scoring system is very useful for the definitive diagnosis of CLL. The majority of clinical cases of CLL turned out to be non-CLL by the scoring criteria. Over-diagnosis of CLL by clinicians may be in part due to the under-recognition of the leukemic phases of other more common types of lymphomas, PLL and T/NK-LGLL. Increased utilization of the scoring system should improve the accuracy of diagnosis of this rare type of leukemia in the Southeast Asian populations.

Potential conflicts of interest

None.

References

1. Boggs DR, Chen SC, Zhang ZN, Zhang A. Chronic lymphocytic leukemia in China. Am J Hematol 1987; 25: 349-54.
2. Chan LC, Lam CK, Yeung TC, Chu RW, Ng M, Chow EY, et al. The spectrum of chronic lymphoproliferative disorders in Hong Kong. A prospective study. Leukemia 1997; 11: 1964-72.
3. Tamura K, Sawada H, Izumi Y, Fukuda T, Utsunomiya A, Ikeda S, et al. Chronic lymphocytic leukemia (CLL) is rare, but the proportion of T-CLL is high in Japan. Eur J Haematol 2001; 67: 152-7.
4. Sukpanichnant S, Sonakul D, Piankijagum A, Wanachiwanawin W, Veerakul G, Mahasandana C, et al. Malignant lymphoma in Thailand: changes in the frequency of malignant lymphoma determined from a histopathologic and immunophenotypic analysis of 425 cases at Siriraj Hospital. Cancer 1998; 83: 1197-204.
5. Sriphatphiriyakun T, Auewarakul CU. Clinical presentation and outcome of Thai patients with chronic lymphocytic leukemia: retrospective analysis of 184 cases. Asian Pac J Allergy Immunol 2005; 23: 197-203.
6. Cheson BD, Bennett JM, Grever M, Kay N, Keating MJ, O'Brien S, et al. National Cancer Institute-sponsored Working Group guidelines for chronic lymphocytic leukemia: revised guidelines for diagnosis and treatment. Blood 1996; 87: 4990-7.
7. Matutes E, Attygalle A, Wotherspoon A, Catovsky D. Diagnostic issues in chronic lymphocytic leukaemia (CLL). Best Pract Res Clin Haematol 2010; 23: 3-20.
8. 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: 2088-95.
9. Matutes E, Owusu-Ankomah K, Morilla R, Garcia MJ, 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.
10. 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: 378-82.
11. Craig FE, Foon KA. Flow cytometric immunophenotyping for hematologic neoplasms. Blood 2008; 111: 3941-67.
12. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposals for the classification of chronic (mature) B and T lymphoid leukaemias. French-American-British (FAB) Cooperative Group. J Clin Pathol 1989; 42: 567-84.
13. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al., editors. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon: IARC; 2008.
14. Karoopongse E, Khuhapinant A, Auewarakul CU. A rare occurrence of hairy cell leukemia in the Thai population: a case report. J Med Assoc Thai 2010; 93 (Suppl 1): S196-202.
15. Dungarwalla M, Matutes E, Dearden CE. Prolymphocytic leukaemia of B- and T-cell sub-

type: a state-of-the-art paper. Eur J Haematol 2008; 80: 469-76.
16. Kurtin PJ. Indolent lymphomas of mature B lymphocytes. Hematol Oncol Clin North Am 2009; 23: 769-90.
17. 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: 5446-56.
18. Wong KF, Chan JK, So JC, Yu PH. Mantle cell lymphoma in leukemic phase: characterization of its broad cytologic spectrum with emphasis on the importance of distinction from other chronic lymphoproliferative disorders. Cancer 1999; 86: 850-7.
19. Zucca E, Bertoni F, Stathis A, Cavalli F. Marginal zone lymphomas. Hematol Oncol Clin North Am 2008; 22: 883-901, viii.

การใช้ระบบการให้คะแนนเพื่อการวินิจฉัยมะเร็งเม็ดเลือดขาวเรื้อรังชนิดลิมฟอยด์ในคนไทย

อรทัย พรหมสุวิชา, วยุรี สองเมือง, จิรายุ เอื้อวรากุล

***ภูมิหลัง**: มะเร็งเม็ดเลือดขาวเรื้อรังชนิดลิมฟอยด์ หรือ ซีแอลแอล เป็นมะเร็งของเซลล์บีลิมฟอยด์ที่พบได้น้อยในแถบเอเชียตะวันออกเฉียงใต้*

***วัตถุประสงค์**: เพื่อศึกษาว่าการใช้ระบบการให้คะแนนตามการแสดงออกของแอนติเจนชนิดต่าง ๆ ได้แก่ CD5, CD23, FMC7, CD79b และ surface immunoglobulin (SIg) มีประโยชน์ในการแยกมะเร็งชนิดซีแอลแอลออกจากมะเร็งลิมฟอยด์ชนิดอื่น ๆ ในคนไทยหรือไม่*

***วัสดุและวิธีการ**: ทำการตรวจวิเคราะห์เซลล์มะเร็งด้วยวิธีโฟลซัยโตเมทรี โดยใช้ผู้ป่วยจำนวน 145 ราย ซึ่งได้รับการวินิจฉัยเบื้องต้นจากแพทย์ว่าเป็นซีแอลแอล โดยกำหนดการให้คะแนน 1 คะแนน สำหรับการแสดงออกแต่ละอย่างดังต่อไปนี้ CD5 เป็นบวก CD23 เป็นบวก FMC7 เป็นลบ CD79b เป็นลบและ SIg เป็นลบหรือแสดงออกอย่างอ่อน การวินิจฉัยว่าเป็นซีแอลแอล ผู้ป่วยจะต้องได้คะแนนเท่ากับหรือมากกว่า 3*

***ผลการศึกษา**: มีผู้ป่วยเพียง 50 ราย หรือร้อยละ 34.5 ที่ได้รับการยืนยันว่าเป็น ซีแอลแอล (ได้คะแนนเท่ากับหรือมากกว่า 3) ผู้ป่วยที่มีคะแนน ≥ 3 จะมีจำนวนเม็ดเลือดขาวและลิมโฟซัยท์ทั้งในไขกระดูกหรือเลือดสูงกว่าผู้ป่วยที่มีคะแนน ≤ 2 อย่างมีนัยสำคัญ การแสดงออกร่วมกันของ CD5 และ CD23 พบในร้อยละ 87.5 ของผู้ป่วยซีแอลแอลในผู้ป่วย 81 ราย ที่มีคะแนน ≤ 2 พบว่าเป็นโรคชนิดอื่นที่ไม่ใช่ซีแอลแอลเป็นส่วนใหญ่ เช่น มะเร็งต่อมน้ำเหลืองชนิดต่างๆ หรือมะเร็งเม็ดเลือดขาวชนิดโปรลิมโฟซัยท์*

***สรุป**: คะแนน ≥ 3 และการแสดงออกร่วมกันของ CD5/CD23 มีความจำเป็นสำหรับการวินิจฉัยซีแอลแอล ในขณะที่คะแนน ≤ 2 ส่วนใหญ่จะไม่ใช่ซีแอลแอล จากการศึกษานี้พบว่า ผู้ป่วยที่ได้รับการวินิจฉัยจากทางคลินิกว่าเป็นซีแอลแอล ส่วนใหญ่จะกลายเป็นโรคอื่นที่ไม่ใช่ซีแอลแอลเมื่อได้รับการตรวจโดยวิธีโฟลซัยโตเมทรี การนำระบบการให้คะแนนของแอนติเจนมาใช้จึงมีประโยชน์อย่างยิ่ง โดยจะช่วยเพิ่มความถูกต้องแม่นยำในการวินิจฉัยมะเร็งเม็ดเลือดขาวชนิดนี้ซึ่งพบได้ไม่บ่อยในคนไทย*