De novo CD5 Positive Diffuse Large B-cell Lymphomas with Bone Marrow Involvement in Korean

In CD5 positive (CD5+) mature B-cell lymphomas, newly recognized CD5+ diffuse large B-cell lymphoma (DLBCL) has been characterized by aggressive features. We studied twenty-five cases with CD5+ lymphomas involving bone marrow. Eleven cases were diagnosed as chronic lymphocytic leukemia, six cases were diagnosed as mantle cell lymphoma (MCL), and three cases with morphologic characteristics of MCL and without both the cyclin D1 expression and IGH/CCND1 rearrangement were unclassifiable. The remaining five cases, showing large to medium-sized lymphoid cells with prominent nucleoli and a moderate amount of cytoplasm, were diagnosed as DLBCL. Five DLBCL cases were positive for CD5, CD20, surface immunoglobulin, but negative for CD23. Patients with CD5+ DLBCL showed a high age of onset (median, 68 yr) and two patients expired one month after the diagnosis. Since CD5+ DLBCL forms a distinct subgroup of DLBCL, a study of CD5 expression in DLBCL would be helpful to predict prognosis and to determine future therapeutic strategy. To the best of our knowledge, this is the first report on de novo CD5+ DLBCL in Koreans.

Key Words : *Antigens, CD5; Leukemia, Lymphocytic, Chronic; Lymphoma, Mantle-Cell; Lymphoma, Large-Cell, Diffuse*

Sun-Young Kong[*,‡]**, Eun Hae Cho**[*]**,**
Hee Yeon Woo[*]**, Qeuhn Park**[*]**,**
Young Hyeh Ko[†]**, Sun-Hee Kim**[*]

Departments of Laboratory Medicine[*] and Pathology[†], Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul; Department of Diagnostic Laboratory[‡], Center for Clinical Services, National Cancer Center, Goyang, Korea

Address for correspondence
Sun-Hee Kim, M.D.
Department of Laboratory Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, 50 Ilwon-dong, Gangnam-gu, Seoul 135-710, Korea
Tel : +82.2-3410-2704, Fax : +82.2-3410-2719
E-mail : sunnyhk@smc.samsung.co.kr

INTRODUCTION

Mature B-cell neoplasms, which are clonal proliferations of B cells at various stages of differentiation, comprise over 90% of lymphoid neoplasms in western countries and about 75% of non-Hodgkin's lymphomas (NHLs) in Korea (1-3). The distinctive morphology and immunophenotypes of mature B-cell neoplasms allow them to be readily classified by postulated cell of origin. Among many types of mature B-cell lymphoma, B-cell chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL) typically contain CD5 positive B cells, which can be observed within the period from naive B-cell to mantle cell. Recently, in addition to the two well-known lymphomas, another type of lymphoma with CD5 antigen expression has been reported in Japan (4, 5). This type lymphoma, so-called CD5 positive diffuse large B cell lymphoma (CD5+ DLBCL), was first described in western country, and has received much attention lately because it has been suggested to possess more aggressive clinical features and a poor outcome (6). Thus attempts have been made to categorize CD5+ DLBCL as new disease entity, distinct from CD5- DLBCL, CLL or MCL (7-9). The identification of a high-risk subgroup of DLBCL would be particularly important for determining therapeutic strategy, given that treatment is successful in only 35 to 40% of DLBCL patients (10). Therefore, we investigated DLBCL cases in all

CD5 positive lymphoid malignancies with bone marrow involvement, as proven by flow cytometry, and also examined the clinical data, the histo-morphology, and immunophenotyping and cytogenetic result in these cases.

MATERIALS AND METHODS

We searched for CD5 positive cases of B-cell lymphoid malignancy involving the bone marrow among patients admitted to the Samsung Medical Center between 1997 and 2003. CD5 expression was confirmed by immunophenotyping using flow cytometry, which was performed using the following panel of lymphoid cell associated monoclonal antibodies: anti-CD2, CD3, CD5, CD7, CD10, CD19, CD20, CD23, FMC7, and surface immunoglobulin (Ig) (Becton Dickinson, CA, U.S.A.). The analyzed cells were gated on FSC/SSC and revealed lymphoid light scatter and moderate CD45 expression. Twenty-five cases of B-cell lymphoid malignancies showed CD5 expression; corresponding medical records were reviewed. Bone marrow histomorphologies of the 25 cases were re-evaluated using Giemsa and hematoxylin-eosin stains by three different pathologists. Diagnosis was based on the criteria documented in the REAL/WHO classification (11). In brief, CLL was defined as a case showing monomorphic small lymphocytes with CD5 and CD23 expression, and mantle cell

Table 1. The key features of CD5 positive mature B-cell lymphomas in the present study

	No. of cases	Morphology	CD5/CD23 in flow cytometry	Cyclin D1/ IGH/CCND1 rearrangement*
B-CLL	11	Small lymphocytes with clumped chromatin and scant cytoplasm	+/+	−/−
MCL	6	Small to medium sized cells with irregular nuclear contour and slightly dispersed chromatin	−/+	+/±
CD5+ DLBCL	5	Medium to large sized cells with distinct nucleoli and a moderate amount cytoplasm	+/−	−/−
Unclassified	3	Small to medium sized cells with irregular nuclear contour and slightly dispersed chromatin	+/−	−/−

B-CLL, B-cell chronic lymphocytic leukemia; MCL, mantle cell lymphoma; DLBCL, diffuse large B-cell lymphoma.
*Cyclin D1 expression was studied by immunohistochemistry and the IGH/CCND1 rearrangment was determined by dual color FISH with IGH/CCND1 probes (Vysis Inc., IL, USA).

Table 2. Clinical and laboratory findings of patients with de novo CD5+ DLBCL

Case	Age (yr)/ Sex	Initial site of involvement	WBC (×10⁹/L)	Tumor cells (PB/BM%)	CD5/ 20 (%)	Chromosome	Survival Months
1	53/M	BM, Liver, Spleen, LN	97.9	76/36	96/95	47,XY,del(4)(p15.2),-7,add(7)(p22),add(9)(p24), del(9)(p13),+18,+mar[14]/46,XY[6]	1
2	65/M	BM, Liver, Spleen	4.4	0/31	33/33	47-48,X,-Y,dup(1)(q21q25),t(1;4)(q42;q31), t(1;5)(p13;q13),del(7)(p13),t(8;9)(q13;q22), +12,add(14)(q32),+2mar[cp16]/46,XY[3]	1
3	65/M	BM, LN	47.5	75/76	50/85	No mitotic cells	1*
4	70/F	BM, LN	11.4	70/52	73/91	47,XX,+6,t(9;16)(p22;q24),inv(11)(p15q23), add(14)(q32),add(18)(q23)[2]/47,XX,idem, t(8;12)(q24.3;q24.1)[10]/46,XX[8]	60*
5	79/F	BM, Liver, Spleen, LN, Pleural effusion	12.9	20/49	89/89	46~48,der(1)add(1)(p13)dup(1)(q12q32)add(1)(q32), der(1)add(1)(p36.3)dup(1)add(1)(q32),+der(3)r(3;?)(p21;?), +der(3)t(3;?)(p21;?),-8,add(13)(q32),15,add(17)(p13), -del(17)(p11.2),+18,add(21)(p11.2)[cp15]/46,XX[5]	3*

*, Alive. CD5+ DLBCL, CD5 positive diffuse large B-cell lymphoma; BM, bone marrow; LN, lymph node.

lymphoma was restricted to cases with cyclin D1 expression by immunohistochemistry (Novocastra, CA, U.S.A.) in either a lymph node or bone marrow. Cytogenetic analysis was performed as follows: unstimulated bone marrow cells were cultured for 24 hr and G-banded, and twenty metaphase cells were analyzed and karyotyped, according to the guidelines of the International System for Cytogenetic Nomenclature (ISCN) 1995 (12). Dual color FISH with IGH/CCND1 probes was undertaken according to the manufacturer's instructions (Vysis Inc., Downers Grove, IL, U.S.A.), and 200 nuclei from interphase cells were analyzed. To determine the reference range of IGH/CCND1 FISH, 200 nuclei from 20 normal bone marrow specimens were observed; no true positive fusion signals (1G2O1F) were observed.

RESULTS

Among the 25 cases of B-cell lymphoid malignancies showing CD5 expression, 11 cases showed small sized lymphoid cells with clumped chromatin and scant cytoplasm express-

ing CD5 and CD23. These cases were diagnosed as B-CLL (Table 1). Another 9 cases showed small to medium sized cells with the characteristics of mantle cells; these were CD5+ and CD23-. Among these 9 cases, six expressed cyclin D1 by immunohistochemistry, but only two cases revealed the IGH/CCND1 rearrangement by FISH, and the remaining three cases showed neither cyclin D1 expression nor the IGH/ CCND1 rearrangement. We finally diagnosed six cases showing cyclin D1 expression as MCL; the other three cases were unclassifiable. Finally, the remaining five cases showed medium to large-sized lymphoid cells with prominent nucleoli and a moderate amount of cytoplasm. Immunophenotypically they were positive for CD5, CD19, CD20 and surface Ig κ or λ, and negative for CD23 and CD10 (Fig. 1 and 2). All five cases were confirmed to be cyclin D1 negative, and a FISH study identified no case with the IGH/CCND rearrangement. After considering morphology, immunophenotype, immunohistochemistry, and FISH results, we ruled out CLL and MCL, and diagnosed the five cases as centroblastic type DLBCL with CD5+.

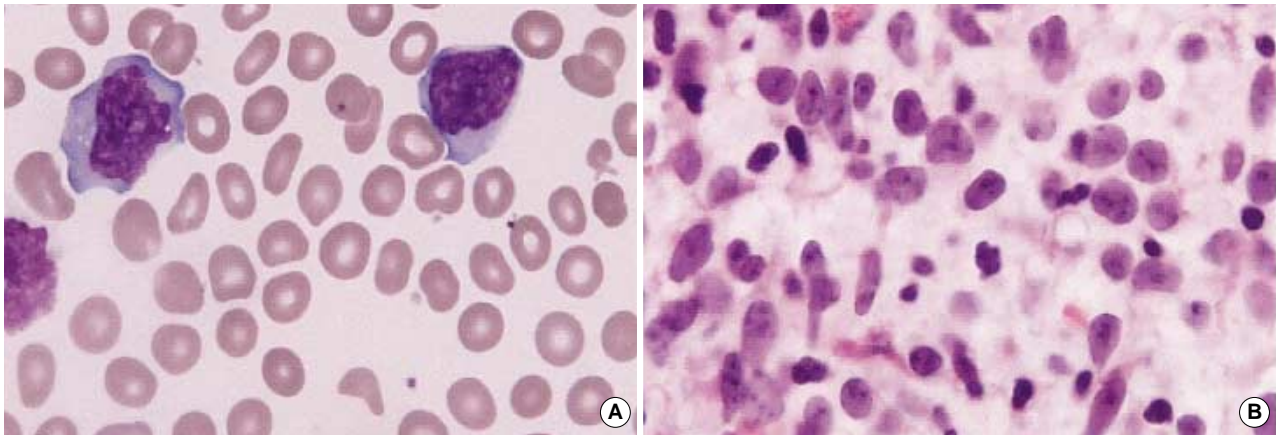The characteristics of CD5+ DLBCL cases at presentation

Fig. 1. The morphologic findings of a case with CD5+ diffuse large B-cell lymphomas. Medium sized cells with moderately condensed cleaved nuclei with distinct nucleoli, and a moderate amount of cytoplasm are observed. (A) Bone marrow aspirate, ×1,000, Wright-Giemsa stain, (B) Bone marrow biopsy, ×400 Hematoxylin-Eosin stain.
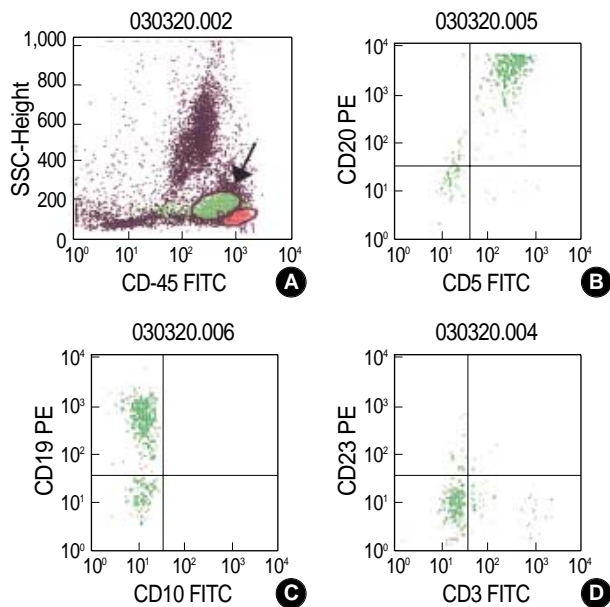

Fig. 2. Immunophenotyping of malignant lymphoid cells of CD5 positive diffuse large B-cell lymphomas shows CD5+CD10-CD 19+CD20+CD23- phenotype. (A) CD45/SSC (B) CD5/20 (C) CD 10/19 (D) CD3/23, Note the gated areas, black head arrowed.

are summarized in Table 2. They showed a high age of onset (median, 68 yr), and no case had a history of low-grade lymphoma. Thus, they were categorized as de novo CD5+ DLBCL. They all received chemotherapy with the CHOP regimen (cyclophosphamide, doxorubicin, vincristine, and prednisone) and two patients (cases 1 and 2) expired one month after initial diagnosis.

DISCUSSION

The undefined three cases showing mantle cell morphology without cyclin D1expression and IGH/CCND1 rearrangement, which required further study including DNA microarray analysis, to define a diagnostic category. The presence of these cases implies that the REAL/WHO classification is not sufficient to classify diverse mature B-cell lymphoid malignancies. Recently, clusters of cases between typical CLL and MCL were reported based on flow cytometry, and they were found to be correlated with prognosis (13). Thus, an additional study should contribute to our understanding of mature B-cell lymphoid malignancies.

CD5+ cases account for approximately 5-10% of DLBCL, and usually the frequency of infiltration of bone marrow, the most frequent site of extranodal involvement, is reported to be around 25% (4, 5). In the present study, the frequency of CD5+ DLBCL among the 25 cases of CD5 positive B-cell lymphoid malignancies involving the bone marrow was 24%, which is not an insignificant number. In view of the fact that reports of CD5+ DLBCL are rare in Korea, a serious underestimation of CD5+ DLBCL is suspected.

De novo CD5+ DLBCL has been recognized to have distinct features; a high age at onset, a female preponderance, a frequent association with poor prognostic components, and an aggressive clinical course (5). Our cases also showed a higher average age of onset than general DLBCL in Korea (2) and frequent extranodal infiltration of both liver and spleen.

To explain the correlation between CD5 expression and an unfavorable prognosis, genetic aberrations of CD5+ DLBCL have been evaluated in a number of ways. Initially, an investigation targeting D13S25, p16, and p53 was performed, and the deletion at the D13S25 locus and loss of p16 were revealed, which implies an early transforming event resulting in aggressive DLBCL (8). In addition to the above mentioned genetic aberrations, the expressions of several other markers such as BCL2, cyclin D3, cyclin B, or survivin have been proposed to be associated with poor outcome (10). Following a DNA microarray analysis, it was reported that the outcome

of patients with DLBCL might be influenced by gene expression profiles other than histologic profiles (14), and that the gene signature is associated with the control of cell kinetics and host immune response (15). Thus, study of the genetic aberrations of CD5+ DLBCL would help elucidate previously indistinct diagnostic categories.

The aggressive behavior of CD5+ DLBCL has also been attributed to a tendency to spread widely within tissue, and this phenomenon was associated with the CD5 molecule interacting as a ligand with heavy-chain variable framework regions of surface Ig (16). The latest study on this topic revealed that the aggressiveness of CD5+ DLBCL might be due to the overexpression of integrin $\beta 1$ and/or CD36 adhesion molecules (17). Integrin $\beta 1$, an adhesion molecule, plays an important role in B-cell lymphoma adhesion and chemotaxis on fibronectin (18). The protein expression of integrin $\beta 1$ has already been reported to be associated with extranodal involvement in NHL (19). The CD36 antigen is transcriptionally regulated by Oct-2, which is a regulator of B-cell differentiation, and its expression is reported to be an indicator of tumor metastasis (20, 21). However, CD36 in CD5+ DLBCL tissues is expressed in the vascular endothelial cells rather than in malignant cells. This finding implies the pathophysiologic role of the vascular endothelium as an integral part of the cancer, and would explain the high frequency of intravascular or intrasinusoidal infiltration observed in de novo CD5+ DLBCL (5).

In conclusion, the present study found that de novo CD5+ DLBCL, with its distinct clinicopathologic features, is present in Korea. Moreover, the characteristics of our CD5+ DLBCL cases share many similarities with those previously reported. Since CD5+ DLBCL may form a distinct subgroup of DLBCLs, there is a need to determine the presence or absence of CD5 antigen expression in all DLBCL cases. It is thus essential to study CD5 expression in DLBCL by immunohistochemistry or flow cytometry, as such findings would be helpful for to predict prognosis and determine future therapeutic strategy. In addition, cytogenetic studies and molecular analyses for proto-oncogenes and tumor suppressor genes would provide insights into the pathogenesis of CD5+ DLBCL, and provide information relevant to the establishment of innovative therapeutic strategies. Furthermore, regarding the difficulty in making a diagnosis of heterogeneous mature B-cell lymphomas, comprehensive flow cytometry, cytogenetic and molecular genetic study would aid in the diagnosis of all mature B cell lymphoid malignancies.

REFERENCES

1. Harris NL, Jaffe ES, Diebold J, Flandrin G, Muller-Hermelink HK, Vardiman J, Lister TA, Bloomfield CD. *The World Health Organization classification of neoplasms of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting--Airlie House, Virginia, November, 1997. Hematol J 2000; 1: 53-66.*

2. Ko YH, Kim CW, Park CS, Jang HK, Lee SS, Kim SH, Ree HJ, Lee JD, Kim SW, Huh JR. *REAL classification of malignant lymphomas in the Republic of Korea: incidence of recently recognized entities and changes in clinicopathologic features. Hematolymphoreticular Study Group of the Korean Society of Pathologists. Revised European-American lymphoma. Cancer 1998; 83: 806-12.*

3. Armitage JO, Weisenburger DD. *New approach to classifying non-Hodgkin's lymphomas: clinical features of the major histologic subtypes. Non-Hodgkin's Lymphoma Classification Project. J Clin Oncol 1998; 16: 2780-95.*

4. Yamaguchi M, Ohno T, Oka K, Taniguchi M, Ito M, Kita K, Shiku H. *De novo CD5-positive diffuse large B-cell lymphoma: clinical characteristics and therapeutic outcome. Br J Haematol 1999; 105: 1133-9.*

5. Yamaguchi M, Seto M, Okamoto M, Ichinohasama R, Nakamura N, Yoshino T, Suzumiya J, Murase T, Miura I, Akasaka T, Tamaru J, Suzuki R, Kagami Y, Hirano M, Morishima Y, Ueda R, Shiku H, Nakamura S. *De novo CD5+ diffuse large B-cell lymphoma: a clinicopathologic study of 109 patients. Blood 2002; 99: 815-21.*

6. Matolcsy A, Chadburn A, Knowles DM. *De novo CD5-positive and Richter's syndrome-associated diffuse large B cell lymphomas are genotypically distinct. Am J Pathol 1995; 147: 207-16.*

7. Pileri SA, Dirnhofer S, Went P, Ascani S, Sabattini E, Marafioti T, Tzankov A, Leoncini L, Falini B, Zinzani PL. *Diffuse large B-cell lymphoma: one or more entities? Present controversies and possible tools for its subclassification. Histopathology 2002; 41: 482-509.*

8. Katzenberger T, Lohr A, Schwarz S, Dreyling M, Schoof J, Nickenig C, Stilgenbauer S, Kalla J, Ott MM, Muller-Hermelink HK, Ott G. *Genetic analysis of de novo CD5+ diffuse large B-cell lymphomas suggests an origin from a somatically mutated CD5+ progenitor B cell. Blood 2003; 101: 699-702.*

9. Colomo L, Lopez-Guillermo A, Perales M, Rives S, Martinez A, Bosch F, Colomer D, Falini B, Montserrat E, Campo E. *Clinical impact of the differentiation profile assessed by immunophenotyping in patients with diffuse large B-cell lymphoma. Blood 2003; 101: 78-84.*

10. Coiffier B. *Diffuse large cell lymphoma. Curr Opin Oncol 2001; 13: 325-34.*

11. Harris NL, Jaffe ES, Diebold J, Flandrin G, Muller-Hermelink HK, Vardiman J, Lister TA, Bloomfield CD. *World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting-Airlie House, Virginia, November 1997. J Clin Oncol 1999; 17: 3835-49.*

12. Mitelman F. *ISCN 1995: An International System for Human Cytogenetic Nomenclature. S. Karger, Basel, Switzerland.*

13. Cro L, Guffanti A, Colombi M, Cesana B, Grimoldi MG, Patriarca C, Goldaniga M, Neri A, Intini D, Cortelezzi A, Maiolo AT, Baldini L. *Diagnostic role and prognostic significance of a simplified immunophenotypic classification of mature B cell chronic lymphoid leukemias. Leukemia 2003; 17: 125-32.*

14. Shipp MA, Ross KN, Tamayo P, Weng AP, Kutok JL, Aguiar RC, Gaasenbeek M, Angelo M, Reich M, Pinkus GS, Ray TS, Koval MA, Last KW, Norton A, Lister TA, Mesirov J, Neuberg DS, Lander ES, Aster JC, Golub TR. *Diffuse large B-cell lymphoma outcome predic-*

tion by gene-expression profiling and supervised machine learning. Nat Med 2002; 8: 68-74.

15. Rosenwald A, Wright G, Chan WC, Connors JM, Campo E, Fisher RI, Gascoyne RD, Muller-Hermelink HK, Smeland EB, Giltnane JM, Hurt EM, Zhao H, Averett L, Yang L, Wilson WH, Jaffe ES, Simon R, Klausner RD, Powell J, Duffey PL, Longo DL, Greiner TC, Weisenburger DD, Sanger WG, Dave BJ, Lynch JC, Vose J, Armitage JO, Montserrat E, Lopez-Guillermo A, Grogan TM, Miller TP, LeBlanc M, Ott G, Kvaloy S, Delabie J, Holte H, Krajci P, Stokke T, Staudt LM. *The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N Engl J Med 2002; 346: 1937-47.*

16. Pospisil R, Fitts MG, Mage RG. *CD5 is a potential selecting ligand for B cell surface immunoglobulin framework region sequences. J Exp Med 1996; 184: 1279-84.*

17. Kobayashi T, Yamaguchi M, Kim S, Morikawa J, Ogawa S, Ueno S, Suh E, Dougherty E, Shmulevich I, Shiku H, Zhang W. *Microarray reveals differences in both tumors and vascular specific gene expression in de novo CD5+ and CD5- diffuse large B-cell lymphomas. Cancer Res 2003; 63: 60-6.*

18. Yin Z, Giacomello E, Gabriele E, Zardi L, Aota S, Yamada KM, Skerlavaji B, Doliana R, Colombatti A, Perris R. *Cooperative activity of alpha4beta1 and alpha4beta7 integrins in mediating human B-cell lymphoma adhesion and chemotaxis on fibronectin through recognition of multiple synergizing binding sites within the central cell-binding domain. Blood 1999; 93: 1221-30.*

19. Terol MJ, Lopez-Guillermo A, Bosch F, Villamor N, Cid MC, Campo E, Montserrat E. *Expression of beta-integrin adhesion molecules in non-Hodgkin's lymphoma: correlation with clinical and evolutive features. J Clin Oncol 1999; 17: 1869-75.*

20. Corcoran LM, Karvelas M, Nossal GJ, Ye ZS, Jacks T, Baltimore D. *Oct-2, although not required for early B-cell development, is critical for later B-cell maturation and for postnatal survival. Genes Dev 1993; 7: 570-82.*

21. Rutella S, Rumi C, Di Mario A, Leone G. *Expression of thrombospondin receptor (CD36) in chronic B-cell lymphoproliferative disorders: a role in tumor metastasis? Eur J Histochem 1997; 41 (Suppl 2): 53-4.*