

Short Communication

# Flow Cytometric Analysis of Kappa and Lambda Light Chain Expression in Endoscopic Biopsy Specimens before the Diagnosis of B-Cell Lymphoma

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Forty-eight patients with gastrointestinal (GI) tract B-cell lymphoma (BCL) were analyzed retrospectively. The diagnosis was based on the histological examination of specimens obtained by endoscopic biopsy. Before the diagnosis was made, single-color flow cytometry was performed to analyze the expression of light chains and B-cell antigens including CD10 in the specimens. Restricted light chain (RLC) expression, a marker of B-cell clonality, was defined as  $\kappa$  and  $\lambda$  ratios of either more than 3.0 or less than 0.5. The specimens from 30 patients (62.5%) showed RLC expression. No RLC expression or RLC expression not examined was divided into two groups: those showing CD10 positivity in more than 20% of cells (4 patients, 8.3%) and those showing no positivity (14 patients, 29.2%). The cell number analyzed in the latter group was significantly smaller than that in the other two groups. Abnormal karyotypes were found in the specimens from 8 patients (16.7%). These results indicate that the flow cytometric analysis of endoscopic biopsy specimens is useful when BCL is suspected if an adequate number of cells are obtained. [*J Clin Exp Hematopathol* 52(2) : 127-131, 2012]

**Keywords:** flow cytometry, B-cell lymphoma, gastrointestinal tract, endoscopy

## INTRODUCTION

Flow cytometry (FCM) is widely used for immunophenotyping of leukemia, lymphoma, myeloma, and myelodysplastic syndrome. FCM can determine B-cell clonality by analyzing  $\kappa$  and  $\lambda$  ratios in a B-cell population.<sup>1,2</sup> Usually, the samples for flow cytometric analysis are from peripheral blood and bone marrow; however, fluid samples and fine needle aspiration biopsy samples can also be analyzed using FCM.<sup>3</sup> B-cell lymphoma (BCL) is diagnosed on the basis of histological examination including immunostaining in samples collected from nodal or extranodal lesions. The gastroin-

testinal (GI) tract is the most common site for extranodal BCL, which includes marginal zone B-cell lymphoma, diffuse large B-cell lymphoma (DLBCL), and other subsets of BCL.<sup>4,5</sup> If GI tract lesions are suspected, gastrointestinal endoscopy with biopsy is performed as routine practice in Japan.<sup>6</sup> Previously, we reported that FCM was useful for evaluating B-cell clonality in endoscopic biopsy specimens from 10 patients with GI tract BCL.<sup>7</sup> We extended our work to confirm these results in a large number of patients with GI tract BCL.

## PATIENTS AND METHODS

### Patients

Patients admitted to Jichi Medical University Hospital from January 1992 to August 2010 were retrospectively surveyed. Ten patients previously reported were included in this study.<sup>7</sup> Gastrointestinal endoscopy with biopsy was performed on patients complaining of GI tract symptoms at initial presentation. Biopsy specimens collected from each patient were used for both histological examination including immunostaining and FCM.<sup>7</sup> In cases where sufficient num-

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bers of cells from the biopsy specimens could be obtained, chromosomal analysis was performed. Histological subtypes were defined according to the World Health Organization's Classification.<sup>8</sup>

### Flow cytometry

Single-color FCM was performed as reported previously.<sup>7</sup> Specimens were dissected and suspended in phosphate-buffered saline containing 1% bovine serum albumin to obtain single-cell suspensions. Then, cells were stained with a panel of fluorescein isothiocyanate- or phycoerythrin-conjugated monoclonal antibodies: CD10, CD19, CD20, CD2, CD3, CD4, CD5, CD7, CD8, CD25, TCR $\alpha\beta$ , TCR $\gamma\delta$ , CD33, CD34, CD117, CD56, HLA-DR,  $\kappa$  light chain, and  $\lambda$  light chain. For the negative controls, cells were stained with isotype-matched control antibodies. Stained cells were analyzed using a flow cytometer (FACSCalibur, Becton Dickinson Biosciences, San Jose, CA). Flow cytometric data were evaluated by two independent FCM specialists before the diagnosis of BCL was made. B-cell clonality was determined by the quantification of the  $\kappa$  and  $\lambda$  light chain expression in a gated cell population. B-cell clonality was defined as  $\kappa$  and  $\lambda$  ratios of either more than 3.0 or less than 0.5.<sup>1,2</sup>

### Statistical analysis

A Kruskal-Wallis test was used to investigate the mean differences between three dependent populations. Values of  $P < 0.05$  were considered significant. Calculations were performed with the JMP version 5 program Stat View 512 (Berkley Software; Berkley, CA).

## RESULTS AND DISCUSSION

Forty-eight patients were diagnosed as having GI tract BCL. Twenty-seven were males and 21 were females, and the median age was 65.7 (range, 33-88). Endoscopic biopsy specimens were obtained from the stomach (18 patients), ileum (19), and colon (11). The histological subtypes were as follows: 31 patients, DLBCL; 6, follicular lymphoma (FL); 4, lymphoma involving mucosa-associated lymphoid tissue; 1, mantle cell lymphoma; 1, Burkitt lymphoma; and 5, unclassified BCL. Patients were divided into three groups according to FCM (Table 1): patients with  $\kappa$  and  $\lambda$  ratios outside the normal range in endoscopic biopsy specimens were designated as FCM-BCL. When  $\kappa$  and  $\lambda$  ratios were within the normal range or not examined, but CD10 positivity was more than 20% in the specimens, patients were designated as FCM-probable BCL (FCM-pBCL). Patients having other flow cytometric patterns including insufficient analysis were designated as FCM-ND. Table 1 shows the characteristics of the patients with GI tract BCL. There were no differences in

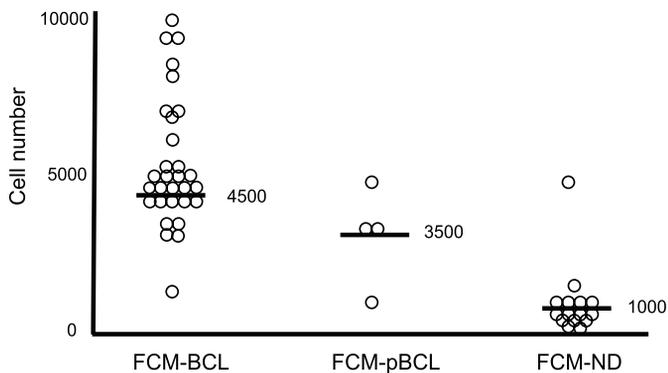
age, sex, specimen site, histological diagnosis, and chromosomal analysis among the three groups. The most common BCL was DLBCL in the three groups. Analyzed cell number was significantly different among the groups; the FCM-ND group had the lowest cell number (Table 1, Fig. 1). In the FCM-BCL group, 19 patients showed  $\kappa$  light chain restriction, while 11 showed  $\lambda$  light chain restriction (Table 1, Fig. 2). Both FCM-BCL and FCM-pBCL groups showed high positivity for CD19, CD10, and CD20, while the FCM-ND group showed low positivity for the antigens. Specimens from one patient in the FCM-pBCL group and 6 patients in the FCM-ND group did not show RLC expression. In the FCM-pBCL group, CD10 positivity was confirmed in specimens from two patients by immunostaining: one sample was CD10<sup>+</sup>, CD20<sup>+</sup>, BCL-2<sup>+</sup>, BCL-6<sup>+</sup>, and CD5<sup>-</sup> and the other was CD10<sup>+</sup>, CD20<sup>+</sup>, CD23<sup>-</sup>, BCL-2<sup>+</sup>, and CD5<sup>-</sup>. These patients were diagnosed as having FL. The remaining specimens showed CD10 negativity by immunostaining: one was CD10<sup>-</sup>, CD20<sup>+</sup>, CD23<sup>-</sup>, and CD5<sup>-</sup> and the other was CD10<sup>-</sup>, CD20<sup>+</sup>, CD23<sup>-</sup>, and CD5<sup>-</sup>. These patients were diagnosed as having DLBCL. The evaluation of light chain expression by immunostaining was not performed in all three groups. The highest cell number in the FCM-ND group (5,000) was from a specimen from a patient with DLBCL. In this specimen, CD19 positivity, CD20 positivity, and  $\kappa/\lambda$  ratio were 32%, 39%, and 2.1, respectively. Chromosomal analysis could be performed in the specimens from 21 patients. Of these 21 patients, only specimens from 8 patients designated as having FCM-BCL showed abnormal karyotypes.

RLC expression in B-cells has been used as a traditional marker of BCL evaluated by FCM.<sup>1-3</sup> In our study, specimens from 7 patients did not show RLC expression. Several reasons for the negative results can be considered. The first is the single-color FCM used in this study. It is difficult to isolate neoplastic B-cells from residual T-cells and monocytes by single-color FCM. The second is the distribution of neoplastic B-cells in the specimens. If samples do not contain abundant neoplastic B-cells, FCM cannot detect RLC expression. This is the case of the highest cell number in the FCM-ND group. No RLC expression in this specimen may have been due to an insufficient number of DLBCL cells in it. The third is the sensitivity of the antibodies that reacted to the light chains used in this study. Recently, Horna *et al.* reported that it is important to evaluate  $\kappa$  and  $\lambda$  light chain expression with both monoclonal and polyclonal antibodies.<sup>9</sup> Patients with BCL sometimes show a lack of surface immunoglobulin light chain expression.<sup>10,11</sup> In our study, immunostaining to light chains in specimens was not performed. Therefore, it cannot be ruled out that BCL with a lack of surface immunoglobulin light chain expression was present in the FCM-ND group. In addition, RLC expression can be found in the germinal center B-cells in reactive follicular hyperplasia of lymph nodes.<sup>12</sup> We do not know whether RLC

**Table 1.** Characteristics of the patients with gastrointestinal tract B-cell lymphoma

	FCM-BCL	FCM-pBCL	FCM-ND	<i>p</i>
Patients (no.)	30	4	14	
Age (years) <sup>#</sup>	65 (33-88)	63 (49-77)	65 (50-83)	0.458
Sex (no. ; male/female)	17/13	1/4	9/5	0.987
Specimen				0.991
Stomach	13	1	4	
Ileum	12	1	6	
Colon	5	2	4	
Histological diagnosis (no.)				0.744
Diffuse large B-cell lymphoma	20	2	9	
Follicular lymphoma	4	2	0	
MALT lymphoma	1	0	3	
Mantle cell lymphoma	1	0	0	
Burkitt lymphoma	1	0	0	
Unclassified	3	0	2	
Analyzed cell numbers <sup>#</sup>	4,500 (1,500-10,000)	3,500 (1,000-5,000)	1,000 (200-5,000)	< 0.001
Phenotypes of the cells (%) <sup>#</sup>				
CD19 <sup>+</sup>	77.9 (90.7 ± 4.6)	88.5 (90.1 ± 9.3)	23.4 (25.1 ± 8.3)	< 0.01
CD10 <sup>+</sup>	85.6 (94.0 ± 4.4)	47.0 (53.3 ± 15.5)	11.2 (4.2 ± 6.4)	< 0.05
CD20 <sup>+</sup>	74.2 (83.3 ± 4.3)	73.4 (74.8 ± 14.8)	14.7 (13.9 ± 2.9)	< 0.05
$\kappa/\lambda$ ratios <sup>#</sup> (no.)				
$\kappa/\lambda > 3.0$	13.0 (23.4 ± 5.7)(19)	N/A	N/A	
$0.5 \leq \kappa/\lambda \leq 3.0$	N/A	1.3 (1)	1.3 (1.3 ± 0.1)(6)	
$\kappa/\lambda < 0.5$	0.03 (0.07 ± 0.02)(11)	N/A	N/A	
Chromosomal analysis (no.)				0.462
Normal karyotype	6	2	5	
Abnormal karyotype	8	0	0	
No metaphase	3	1	2	
Not examined	13	1	7	

no., number ; #, median ; N/A, not applicable ; MALT lymphoma, lymphoma involving mucosa-associated lymphoid tissue.



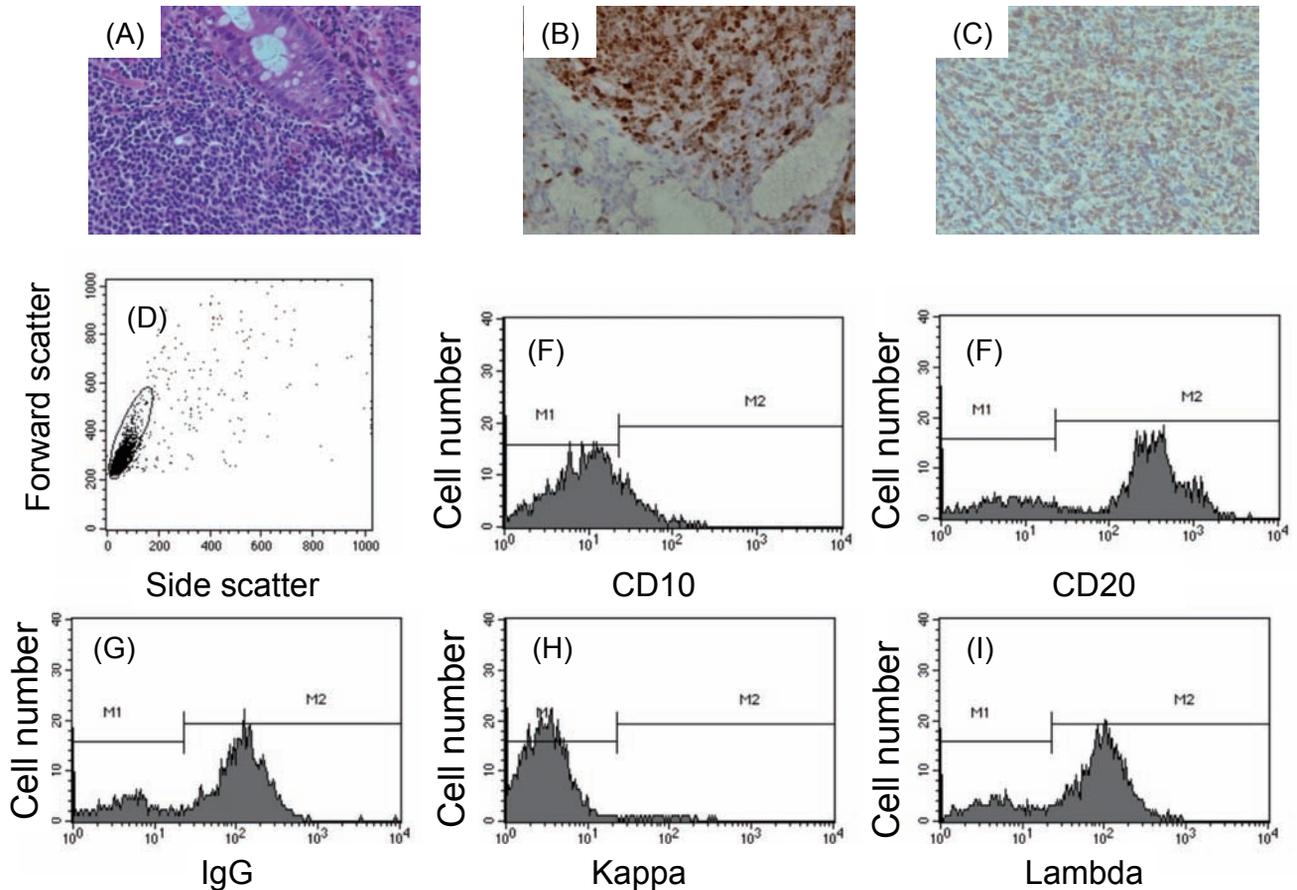
**Fig. 1.** Dot plots of analyzed cell number in each group. The bars indicate median cell number.

expression is found in B-cells in the reactive GI tract lesions.

CD10 is a proteolytic enzyme expressed on the surface of germinal center B-cells and lymphomas derived from these cells, that is, FL.<sup>13,14</sup> High-intensity CD10 expression is associated with FL and DLBCL can also express the antigen.<sup>13,14</sup> We used 20% as the cut-off value of CD10 positivity for identifying BCL because mature B-cells do not express CD10 and reactivity is generally defined as positive when more than 20% of cells are stained with a monoclonal antibody.<sup>15</sup> This

cut-off value was adapted to only 4 patients designated as having FCM-pBCL. Of these patients, two specimens showed CD10 positivity by immunostaining, while the other two did not. The reason for the discrepancy in CD10 positivity between FCM and immunostaining is not yet understood. It may be due to the difference of sensitivity to detect antigen expression between the two methods. It is necessary to verify whether this cut-off value is appropriate to identify BCL.

Recently, endoscopic ultrasound-guided fine needle aspiration biopsy has been introduced and this technique combined with FCM has been used to diagnose BCL.<sup>16,17</sup> This approach is particularly important to diagnose deep-seated lymphoma, the lesions of which occur in the mediastinum, para-aorta, pancreas, spleen, and so on.<sup>18</sup> In Japan, GI tract lesions are usually examined by GI endoscopy with biopsy as routine practice.<sup>6</sup> To our knowledge, there are only a few reports of the flow cytometric analysis of endoscopic biopsy specimens for the diagnosis of BCL.<sup>7,19,20</sup> FCM is accurate and quantitative. Its data can be reanalyzed easily. FCM analysis provides significant information on B-cell clonality associated with BCL before a morphological assessment is completed.



**Fig. 2.** Flow cytometric analysis of the biopsy specimen obtained from colon fiberoscopy. (2A) Diffuse large B-cell lymphoma (DLBCL) in the colon mucosa (H&E stain,  $\times 200$ ). (2B) Positivity of the leukocyte common antigen staining in the DLBCL cells ( $\times 200$ ). (2C) Positivity of CD20 staining in the DLBCL cells ( $\times 200$ ). (2D) The gated region is indicated by a circle; from 2E to 2I, DLBCL cells express CD20, IgG, and  $\lambda$  light chain, while the cells express CD10 at a low level.

## REFERENCES

- Geary WA, Frierson HF, Innes DJ, Normansell DE: Quantitative criteria for clonality in the diagnosis of B-cell non-Hodgkin's lymphoma by flow cytometry. *Mod Pathol* 6:155-161, 1993
- Kawano-Yamamoto C, Muroi K, Izumi T, Saito K, Ozawa K: Two-color flow cytometry with a CD19 gate for the evaluation of bone marrow involvement of B-cell lymphoma. *Leuk Lymphoma* 43:2133-2137, 2002
- Kaleem Z: Flow cytometric analysis of lymphomas : current status and usefulness. *Arch Pathol Lab Med* 130:1850-1858, 2006
- Papaxoinis G, Papageorgiou S, Rontogianni D, Kaloutsi V, Fountzilas G, *et al*: Primary gastrointestinal non-Hodgkin's lymphoma : a clinicopathologic study of 128 cases in Greece. A Hellenic Cooperative Oncology Group study (HeCOG). *Leuk Lymphoma* 47:2140-2146, 2006
- Burke JS: Lymphoproliferative disorders of the gastrointestinal tract : a review and pragmatic guide to diagnosis. *Arch Pathol Lab Med* 135:1283-1297, 2011
- Yamamoto S, Nakase H, Yamashita K, Matsuura M, Takada M, *et al*: Gastrointestinal follicular lymphoma : review of the literature. *J Gastroenterol* 45:370-388, 2010
- Oka S, Muroi K, Sato K, Kawano-Yamamoto C, Ueda M, *et al*: Flow cytometric evaluation of endoscopic biopsy specimens from patients with gastrointestinal tract B-cell lymphoma : a preliminary report. *Jichi Med Univ J* 30:173-179, 2007
- Yin CC, Medeiros LJ, Bueso-Ramos CE: Recent advances in the diagnosis and classification of myeloid neoplasms -comments on the 2008 WHO classification. *Int J Lab Hematol* 32:461-476, 2010
- Horna P, Olteanu H, Kroft SH, Harrington AM: Flow cytometric analysis of surface light chain expression patterns in B-cell lymphomas using monoclonal and polyclonal antibodies. *Am J Clin Pathol* 136:954-959, 2011
- Kaleem Z, Zehnbauser B, White G, Zutter MM: Lack of expression of surface immunoglobulin light chains in B-cell non-Hodgkin lymphomas. *Am J Clin Pathol* 113:399-405, 2000
- Li S, Eshleman JR, Borowitz MJ: Lack of surface immunoglobulin light chain expression by flow cytometric immunophenotyping

- can help diagnose peripheral B-cell lymphoma. *Am J Clin Pathol* 118:229-234, 2002
- 12 Kussick S, Kalnoski M, Braziel R, Wood BL: Prominent clonal B-cell populations identified by flow cytometry in histologically reactive lymphoid proliferations. *Am J Clin Pathol* 121:464-472, 2004
  - 13 Almasri NM, Iturraspe JA, Braylan RC: CD10 expression in follicular lymphoma and large cell lymphoma is different from that of reactive lymph node follicles. *Arch Pathol Lab Med* 122:539-544, 1998
  - 14 Barcus ME, Karageorge LS, Veloso YL, Kornstein MJ: CD10 expression in follicular lymphoma versus reactive follicular hyperplasia: evaluation in paraffin-embedded tissue. *Appl Immunohistochem Mol Morphol* 8:263-266, 2000
  - 15 Muroi K, Suda T, Kawano C, Amemiya Y, Eguchi M, *et al*: Analysis of C-KIT, TIE and HTK expression on leukemic cells using flow cytometry: a preliminary report. *Leuk Res* 22:827-830, 1998
  - 16 Al-Haddad M, Savabi MS, Sherman S, McHenry L, Leblanc J, *et al*: Role of endoscopic ultrasound-guided fine-needle aspiration with flow cytometry to diagnose lymphoma: a single center experience. *J Gastroenterol Hepatol* 24:1826-1833, 2009
  - 17 Yasuda I, Goto N, Tsurumi H, Nakashima M, Doi S, *et al*: Endoscopic ultrasound-guided fine needle aspiration biopsy for diagnosis of lymphoproliferative disorders: feasibility of immunohistological, flow cytometric, and cytogenetic assessments. *Am J Gastroenterol* 107:397-404, 2012
  - 18 Stacchini A, Carucci P, Pacchioni D, Accinelli G, Demurtas A, *et al*: Diagnosis of deep-seated lymphomas by endoscopic ultrasound-guided fine needle aspiration combined with flow cytometry. *Cytopathology* 23:50-56, 2012
  - 19 Villar HV, Wong R, Paz B, Bull D, Neumayer L, *et al*: Immunophenotyping in the management of gastric lymphoma. *Am J Surg* 161:171-175, 1991
  - 20 Almasri NM, Zaer FS, Iturraspe JA, Braylan RC: Contribution of flow cytometry to the diagnosis of gastric lymphomas in endoscopic biopsy specimens. *Mod Pathol* 10:650-656, 1997