

Others

Flow cytometric evaluation of endoscopic biopsy specimens from patients with gastrointestinal tract B-cell lymphoma: a preliminary report

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Abstract

Based on histological examinations of endoscopic biopsy specimens, gastrointestinal (GI) tract B-cell lymphoma was diagnosed in 18 patients who had GI tract symptoms as their initial presentation. The affected sites were the stomach, small intestine and large intestine in nine, seven, and two patients, respectively. The histological classifications were as follows: thirteen patients had diffuse large B-cell lymphoma, one had mucosa-associated with lymphoid tissue (MALT) lymphoma, one had mantle cell lymphoma, while three patients had other types. The flow cytometric analysis of biopsy specimens showed restricted light chain expression in the specimens from seven out of nine patients. The specimens from three out of the another seven patients showed a high expression of CD19 or CD20, however, no light chain expression was determined in any of the seven patients because of insufficient cell numbers. Abnormal karyotypes were observed in the specimens from two of five patients. The analyses using histological examinations combined with flow cytometry (FCM) for ordinary endoscopy and biopsy specimens were thus found to have a significant value for the diagnosis of GI tract B-cell lymphoma.

(Keywords: Flow cytometry, B-cell lymphoma, gastrointestinal tract, endoscopy, biopsy)

Introduction

Primary GI tract lymphoma is most frequently shown in the stomach, followed by the small intestine and colon and the majority of gastric lymphoma arises from MALT^[1, 2]. MALT lymphoma is defined as an extra-nodal marginal zone B-cell lymphoma of the MALT type in peripheral B-cell lymphoma and is associated with *Helicobacter pylori* infections^[3]. MALT lymphoma cells express B-cell antigens CD20 and CD 79a, but not CD5, CD10 or CD23^[3]. The evaluation procedures for GI tract lymphoma includes endoscopy, endoscopic ultrasound, conventional abdominal ultrasound, and abdominal computed tomography scans. Usually, all types of B-cell lymphoma show a restricted light chain expression^[4]. Therefore,

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to diagnose B-cell lymphoma, the most essential finding is restricted light chain expression in biopsy specimens. In Japan, ordinal examinations for symptoms and signs arising from the GI tracts are normally performed by gastrointestinal endoscopy. We evaluated the role of FCM for biopsy specimens obtained by ordinal gastrointestinal endoscopy.

Patients and Methods

Patients

Patients admitted to Jichi Medical University Hospital from January 1992 to August 2006 were retrospectively surveyed. An original endoscopic examination was performed on patients complaining of GI tract symptoms as their initial presentation. Biopsy specimens from each patient were collected and used for both histological examination and FCM. The number of biopsy specimens taken by endoscopic procedures was determined by the discretion of the gastroenterologists and the number of cells containing samples was not counted. In cases where sufficient numbers of cells from the biopsy specimens could be obtained, either a chromosomal analysis, a Southern blot analysis or both were performed.

Histological examination

The biopsy specimens were fixed in formalin and stained with a solution containing Hematoxylin-Eosin and Wright-Giemsa. Immunostaining was performed using a panel of monoclonal antibodies including CD20 (L26; DakoCytomation, Glodtrup, Denmark), CD10 (Dako), CD3 (Dako) and cyclin D1 (Dako). The histological subtypes were defined according to the World Health Organization Classification^[5].

FCM

Biopsy specimens were dissected and suspended in phosphate-buffered saline containing 1% bovine serum albumin to obtain single cell suspensions. Single-color FCM was performed as reported previously^[6]. Briefly, the cells were stained with a panel of fluorescein isothiocyanate- or phycoerythrin-conjugated monoclonal antibodies as follows: CD19 (B4; Coulter Immunology, Hialeah, FL), CD10 (Dako), CD20 (B1; Coulter), CD5 (Leu1; Becton Dickinson Immunocytometry Systems, San Jose, CA), CD7 (Leu 9; Becton), CD2 (OKT11; Ortho Diagnostic Systems, Beersse, Belgium), CD1 (Dako), CD3 (OKT6; Ortho), CD4 (OKT4; Ortho), CD8 (OKT8; Ortho), CD14 (Mo2; Coulter), CD33 (Leu-M9; Becton), CD56 (Leu-19; Becton), CD25 (anti-IL-2 receptor; Becton), HLA-DR (OKDR; Ortho), immunoglobulin (Biosource International, Camarillo, CA or Dako), kappa light chain (Biosource or Dako) and lambda light chain (Biosource or Dako). For the negative controls, the cells were stained with isotype-matched control antibodies. The stained cells were analyzed using a flow cytometer (Cytron, Ortho Diagnostic Systems, Raritan, NJ or FACSCalibur, Becton Dickinson Biosciences, San Jose, CA). The B-cell clonality was determined by the quantification of the kappa and lambda light chain expression. Because normal kappa/lambda ratios are within the range of 0.5 to 3.0^[6], any values outside this range were regarded as indicating the existence of clonal B-cells.

Results

Based on the histological examination of endoscopic biopsy specimens, 18 patients were diagnosed as having GI tract B-cell lymphoma as shown in Table 1. Ten were males and eight were females, and the

Table 1 Characteristics of the patients

Case	Age	Sex	Specimen	Analyzed cell number	Antigen expression (%)										Kappa/Lambda ratio	FCM diagnosis	Histologic diagnosis	IgH	Chromosomal analysis
					CD19	CD20	CD10	CD5	HLA-DR	Smlg	Kappa	Lambda							
1	50	M	Ileum	500	87.8	ND	ND	5.6	98.6	ND	ND	ND	ND	ND	s/o B-cell lymphoma	DLBCL	G	46,XY (20/20 cells)	
2	56	M	Stomach	1,000	14.1	ND	0.2	4.2	5.2	ND	ND	ND	ND	ND	Undefined	DLBCL	ND	ND	
3	77	F	Ascending colon	5,000	97.7	44.4	47.6	4.8	99.8	76.3	8.6	79.9	0.1	0.1	B-cell lymphoma	DLBCL	ND	46,XX (20/20 cells)	
4	77	F	Stomach	1,000	5.7	4.9	4.6	6.0	9.6	ND	ND	ND	ND	ND	Undefined	DLBCL	ND	ND	
5	61	M	Stomach	5,000	90.8	91.6	3.0	96.9	95.4	90.7	5.1	90.2	0.06	0.06	B-cell lymphoma	MCL	ND	ND	
6	65	F	Ileum	2,000	2.6	11.3	ND	3.1	ND	ND	ND	ND	ND	ND	Undefined	DLBCL	ND	ND	
7	64	F	Ileum	1,000	96.6	98.2	46.4	6.0	95.1	ND	ND	ND	ND	ND	s/o B-cell lymphoma	DLBCL	ND	ND	
8	74	M	Ileum	1,000	93.1	98.9	2.0	9.4	100.0	82.8	29.7	26.1	1.1	1.1	s/o B cell lymphoma	DLBCL	ND	ND	
9	81	M	Ileum	5,000	93.4	97.4	1.3	5.0	97.5	90.9	94.9	22.0	8.6	8.6	B-cell lymphoma	DLBCL	ND	ND	
10	61	F	Ascending colon	10,000	90.7	93.9	3.9	9.8	90.6	83.9	2.6	82.1	0.03	0.03	B-cell lymphoma	B-cell lymphoma	G	ND	
11	65	F	Stomach	10,000	73.6	76.2	15.8	34.2	77.2	76.9	69.8	0.0	>3.0	>3.0	B-cell lymphoma	B-cell lymphoma	ND	46,XX	
12	33	F	Stomach	4,000	80.7	89.6	4.7	18.5	96.7	38.5	9.8	34.4	0.29	0.29	B-cell lymphoma	DLBCL	ND	46,XX,-3,+der(3)t(3;2)(q26?)-13,-15,+21,+mar1 (15/20 cells) 46,XX (5/20 cells)	
13	47	M	Stomach	10,000	66.8	70.9	0.7	30.2	73.7	63.9	56.0	13.2	4.2	4.2	B-cell lymphoma	MALT	ND	48,XY,+3,+18 (10/10 cells)	
14	64	M	Stomech	5,000	60.6	51.5	22.7	1.2	79.3	ND	ND	ND	ND	ND	Undefined	DLBCL	ND	ND	
15	79	M	Stomach	5,000	65.8	90.5	0.6	25.4	77.4	11.5	9.6	8.0	1.2	1.2	s/o B cell lymphoma	DLBCL	ND	ND	
16	54	F	Ileum	1,000	3.5	ND	1.2	9.7	15.8	ND	ND	ND	ND	ND	Undefined	DLBCL	ND	ND	
17	47	M	Stomach	1,000	99.1	ND	ND	0.4	99.3	ND	99.2	2.1	47.0	47.0	B-cell lymphoma	DLBCL	ND	ND	
18	52	M	Ileum	1,000	84.2	78.8	17.9	19.6	76.6	77.7	2.0	77.2	0.03	0.03	B-cell lymphoma	FL	ND	ND	

FCM, flow cytometry; IgH, Southern blot analysis for immunoglobulin heavy chain genes; Smlg, surface immunoglobulin; G, germ line; DL-BCL, diffuse large B-cell lymphoma; MCL, mantle cell lymphoma; MALT, MALT lymphoma; FL, follicular lymphoma; s/o, suspicion of; ND, not done.

median age was 62.5 years (range: 33-81 years). The endoscopic biopsy sites were in the stomach, in the ileum and in the ascending colon in nine, seven, and two patients, respectively. The histological subtypes were as follows: thirteen patients with diffuse large B-cell lymphoma, one with MALT lymphoma, one with mantle cell lymphoma, one with follicular lymphoma and two with unclassified B-cell lymphoma. For FCM, 500 to 10,000 cells were analyzed using each antibody. Over 60% of B-cell antigen expression including CD19, CD20 and CD10 were observed in the specimens from 14 of the 18 patients. The light chain expression was observed in the specimens from 11 of the 18 patients using FCM; the specimens from nine of the 11 patients were assessed to include clonal B-cells, according to the previously published criteria^[8]. Of the nine patients, the specimens from four patients were dominant for kappa light chain expression and those from five patients were dominant for lambda light chain expression (Figure 1). The specimens from two other patients, case 8 and case 15, showed no restricted light chain expression, although these specimens showed more than 90% in CD20 expression. The light chain expression was not analyzed in the specimens from another set of seven patients because of insufficient cell numbers. However, the specimens from two patients among these, case 1 and case 7, included cells expressing CD19 or CD20 by more than 85%. No rearrangement of immunoglobulin heavy chain genes was detected in the specimens from the two patients, although the restricted light chain expression was shown in one of the two specimens. Abnormal karyotypes were observed in the specimens from two of five patients and in cases where there were chromosomal abnormalities, restricted light chain expression was observed.

Discussion

Neoplastic B-cells usually express either kappa light chain or lambda light chain on their surface but not both^[4]. Therefore, restricted light chain expression is the most important diagnostic marker for B-cell lymphoma. FCM is a powerful tool for identifying B-cell lymphoma infiltrating into the lymph nodes, peripheral blood and bone marrow^[6-8]. Using FCM, it is possible to obtain not only restricted light chain expression but specific antigen expression associated with B-cell lymphoma, such as the dual positivity of CD5 and CD20^[9,10]. An endoscopic examination is essential for the diagnosis of GI tract B-cell lymphoma and the potential advantage of the method resides in patients whose condition may be too unstable for undergoing general anesthesia and open surgical biopsy. There have been only a few reports on the flow cytometric analysis of biopsy specimens obtained from original endoscopic examination^[11, 12]. In this study, we performed flow cytometric evaluation on endoscopic biopsy specimens obtained from the stomach, small intestine and large intestine. In cases where sufficient numbers of cells have been obtained from the endoscopic biopsy specimens, over 80% of the patients (nine of 11 patients) had biopsy specimens showing restricted light chain expression, while two patients did not. The reason for the lack of restricted light chain expression in the latter cases is not certain. The neoplastic B-cells in these specimens may have dually expressed both light chains or demonstrated an absence of light chain expression^[13, 14]. The cell numbers in the biopsy specimens obtained by GI tract endoscopy are crucial for a flow cytometric analysis. In our study, the specimens from seven patients contained insufficient cell numbers to perform a flow cytometric analysis and as a result, the light chain expression was not determined in these patients. However, among these patients, the specimens from two patients showed a high expression of CD19 or CD20, thus suggesting the presence of clonal B-cells. Monoclonal immunoglobulin heavy chain gene rearrangement was not detected in the two patients by a Southern blot analysis and abnormal

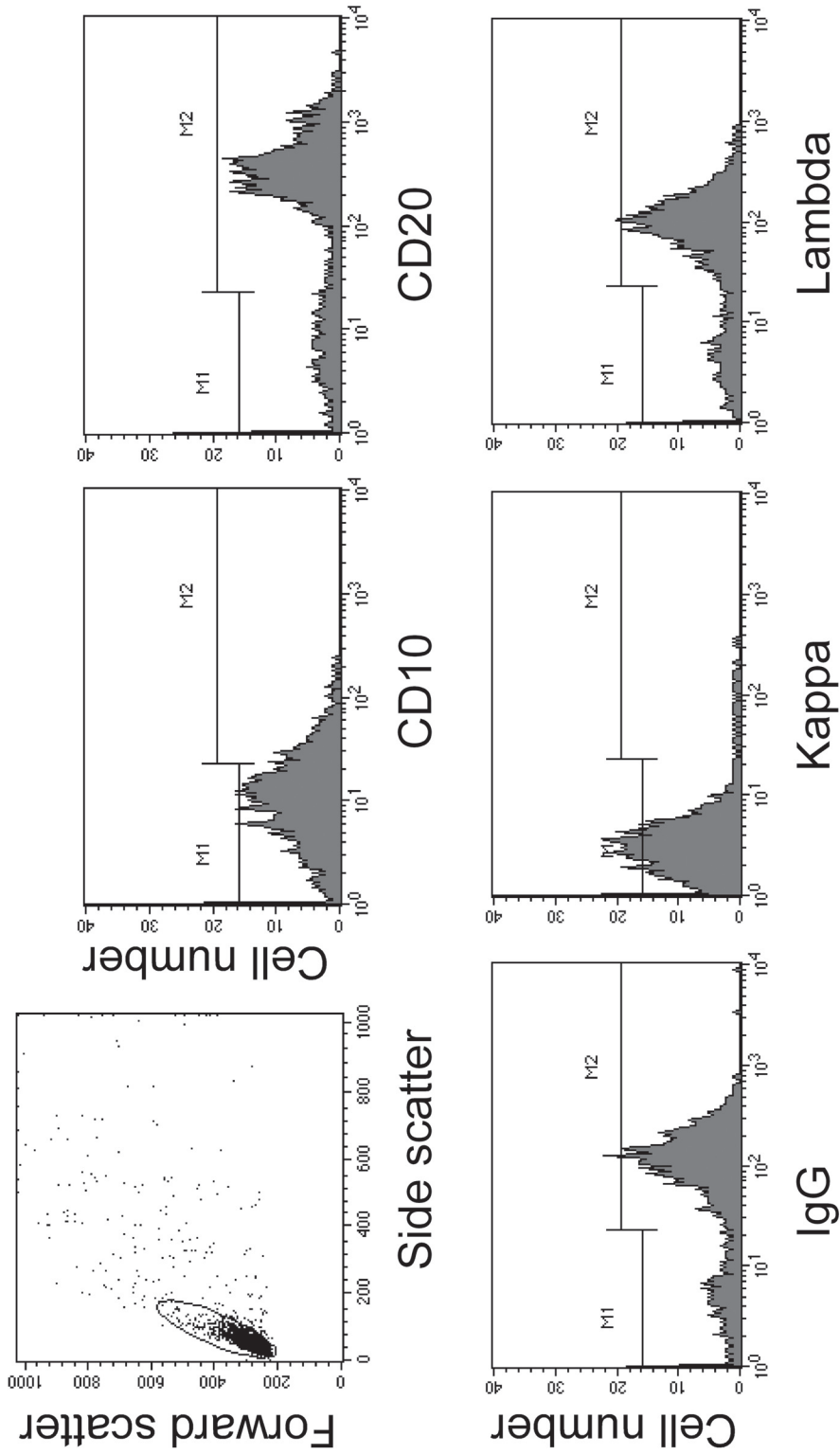


Figure 1 Flow cytometric analysis

The cells in the endoscopic biopsy specimens obtained from case 18 were CD20+, IgG+ and lambda light chain+, and a subset of the cells showed a weak CD10-positivity.

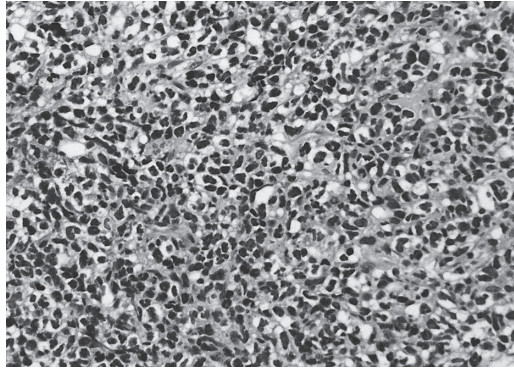


Figure 2 The histological finding of the endoscopic biopsy specimens from case 18. Infiltration of intermediate-sized atypical lymphoid cells with nuclear membrane irregularity is shown (Hematoxylin-Eosin staining, $\times 400$).

karyotypes were detected in the specimens from only two of five patients. These results indicate that the cell numbers are essential for FCM, a Southern blot analysis and a karyotypic analysis of endoscopic biopsy specimens.

In the classification of GI tract B-cell lymphoma, most of our patients had diffuse large B-cell lymphoma, while only one patient had MALT lymphoma. Since the transformation from low-grade to high-grade MALT lymphoma and diffuse large B-cell lymphoma has been shown^[15], diffuse large B-cell lymphoma in our patients may have progressed from low-grade MALT lymphoma. We believe that original endoscopic histological examination combined with FCM still has significance for the diagnosis of GI tract B-cell lymphoma, as a screening tool for the patients complaining of GI tract symptoms. The flow cytometric analysis of endoscopic biopsy specimens can provide clinically useful information in the diagnosis of GI tract B-cell lymphoma.

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消化管B細胞性リンパ腫患者から得られた内視鏡生検検体のフローサイトメトリーを用いた評価

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松山	智洋 ^{*1,2}	外島	正樹 ^{*1}	大嶺	謙 ^{*1}
尾崎	勝俊 ^{*1}	高德	正昭 ^{*1}	森	政樹 ^{*1,2}
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要 約

内視鏡生検検体の組織学的検査に基づき、初診時に消化管の症状を有した18人の患者がB細胞性悪性リンパ腫と診断された。病変部位は、胃が9人、小腸が7人、大腸が2人であった。組織学的診断は、びまん性大細胞型リンパ腫13人、MALTリンパ腫1人、マンツル細胞リンパ腫1人、他の病型が3人であった。内視鏡生検検体のフローサイトメトリーを用いた解析で、9人中7人の内視鏡生検検体にB細胞性悪性リ

ンパ腫に特異的な軽鎖の限定的な発現がみられた。残り7人のうち3人の内視鏡生検検体では、検体不良のため軽鎖の発現は検討されなかったが、CD19またはCD20の高発現を認めた。5人中2人の内視鏡生検検体で染色体異常を認めた。内視鏡生検検体に対して、組織学的検査とフローサイトメトリーを組み合わせることによって、消化管B細胞性リンパ腫の診断の価値が高まると思われた。

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