cytogenetic analysis

Cytogenetic analysis using FISH to detect IgH/BCL2 fusion was carried out with the LSI IGH/BCL2 Dual Color, Dual Fusion Translocation Probe Set™ (Vysis, Downers Grove, IL), following the manufactures' instructions. In those cases whose differential diagnosis was mantle cell lymphoma, IgH/Cyclin D1 (CCND1) fusion was examined with the LSI IGH/CCND1 Dual Color, Dual Fusion Translocation Prove Set™ (Vysis).

treatment and outcome

As the primary site was limited to the duodenum, the direct measurement of size was more subjective than the standardized response criteria. The relative size was evaluated by comparing the inner diameter and height of folding of the duodenum between the current and previous endoscopic image. If disappearance was observed, biopsy was mandatory. If the biopsy failed to demonstrate lymphoma cells in the original region, the response was judged to be complete remission (CR). Random biopsy was carried out at the original site during EGD every time to confirm CR. Progressive disease (PD) was defined as enlargement of the tumor, an increase in the size of the lesion, lymph node enlargement, or the appearance of a new lesion. Enlargement of the intestinal region was defined as >20% increase compared with the previous measurement. PD also included cases who showed no change in endoscopic findings, and who had a previous biopsy that demonstrated a proven CR, but who subsequently suffered reappearance of the tumor cells on biopsy. Stable disease was defined as a treatment effect that was not included in the above definitions.

Progression-free survival (PFS) was measured from the date of diagnosis to the first date when PD was documented or death. Time to next treatment was measured from the last day of primary treatment (when patients were treated) or from the date of diagnosis (when patients were not treated upon diagnosis) to the first date when treatment began. PFS was calculated by the Kaplan–Meier method using SPSS software package (version 11.0.1 J; SPSS Inc., Chicago, IL).

Table 1. Definition of primary DFL

Stage I	Follicular lymphoma with only duodenal involvement
	Follicular lymphoma with duodenal and other gastrointestinal involvement, but this lesion must be smaller than or equal to the duodenal lesion
Stage II_1	Local lymph node involvement smaller than the duodenal lesion
Stage II_2	Mesenteric or infradiaphragmatic lymph node involvement smaller than the duodenal lesion
Stage IV	Any nodal disease smaller than the duodenal lesion excluding disease of the mesenteric and infradiaphragmatic lymph node[a]
	Any extranodal disease other than disease of the intestines smaller than the duodenal lesion[b]
	Bone marrow involvement detected by either aspiration or biopsy[c]

[a]Four patients with buccal mucosal, subcutaneous, bone, and pulmonary lesions.
[b]Thirteen patients including nine patients with bone marrow involvement.
[c]Four patients with larger mesenteric lesions, which were found by CT or abdominal ultrasonography by chance or due to abdominal symptoms and each lesion was much larger than the relevant duodenal lesion.
DFL, duodenal follicular lymphoma; CT, computed tomography.

results

patient characteristics, pathological, phenotypical, and cytogenetic analyses

From 1999 to 2007, 479 patients were diagnosed as FL at the National Cancer Center Hospital. We found that 49 FL patients had involvement of FL in the duodenum. Among them, 21 patients were found during the staging procedure of FL originating from other sites, usually nodal ones. Table 1 shows the excluded cases.

Excluding these patients, 28 patients satisfied the criteria of primary DFL according to the definition of primary intestinal lymphoma by Lewin et al. [4]. As one patient was referred to our institute for a second opinion, we could not follow-up the patient's clinical course. Excluding this case, 27 patients were subject to this retrospective study. The patient characteristics are shown in Table 2. Eight patients (30%) were symptomatic; three had abdominal pain arising from the whole abdomen, three patients had gastric discomfort after meal, and two had epigastralgia at the upper abdomen.

Table 2. Primary duodenal follicular lymphoma patients

Number of patients	$N = 27$	%
Age (years)		
Median	58	
Range	42–83	
Sex		
Female	14	52
Male	13	48
Clinical symptoms		
Present	8	30
Absent	19	70
Primary site		
Second portion (anastomosis of the gastrojejunum)	23 (2)	85 (7)
Third portion	1	4
Second + third portion	3	11
Stage		
I	20	74
II_2	7	26
IV	0	0
IPI		
Low risk	25	93
Low–intermediate risk	2	7
FLIPI		
Low	25	93
Intermediate	2	7
Grading of histology		
Grade 1	20	74
Grade 2	6	22
Grade 3a	1	4
Immunostaining		
CD20+	27	100
CD10+	24	89
BCL-2+	27	100
IgH/Bcl2 fusion by FISH		
Positive cases/examined	20/24	83

IPI, International Prognostic Index; FLIPI, Follicular Lymphoma International Prognostic Index.

Reflecting the high frequency of early-stage disease, the International Prognostic Index (IPI) and Follicular Lymphoma International Prognostic Index (FLIPI) was low risk in the majority of these cases. Small lesions were limited to the duodenum, and the most common site of involvement was the second portion of the duodenum (26 cases, 96%) including those three patients with involvement of both the second and the third portions. These cases included two in which DFL had developed on the suture line after gastrectomy. Only one patient had a lymphomatous lesion at the third portion.

Among the 21 patients examined, only 1 (5% of the DFL) had lymphoma involvement in the large intestine (Table 3). In order to detect other small intestinal involvement, a capsule endoscope was used in two patients, but no other lesions were detected. Bone marrow involvement was negative according to the staging system by Cheson et al. [7].

A PET scan test was carried out in 10 cases, and in five cases, the primary site was positive. Among the positive cases, three were stage I, and two were stage II disease. A gallium scan test was carried out in 12 cases. Only two cases with stage II disease were positive, which were also positive on the PET scan.

The histology of FL was determined by morphology. Histological grade 1 was most common; 20 cases (74%) were grade 1. Analyses of surface marker showed that 24 of the 27 cases (89%) were also positive for CD10. All cases were clearly discernable from mantle cell lymphoma. CD5 and cyclin D1 were tested in 18 and 12 cases, respectively. CD5 was weakly positive in one case, however, cyclin D1 was negative. *H. pylori* was positive in 9 among 14 cases. No tumors showed plasma cell differentiation or monocytoid B cells characteristic of mucosa-associated lymphoid tissue (MALT) type lymphoma. IgH/BCL2 fusion was examined in 24 cases in which formalin-fixed paraffin-embedded tissues were available. Twenty (83%) cases showed IgH/BCL2 fusion, which is indicative of translocation of immunoglobulin and *bcl-2* genes.

treatment and outcome

The patients opted for watchful waiting or treatment with radiation or chemotherapy. The choice was determined according to the patient's preferences after the physician had provided appropriate information. As a certain fraction of patients with stage I nodal FL is expected to be cured by radiation alone, some patients preferred radiation.

All patients have survived with a median follow-up time of 47.9 months (range 7.7–118.6 months). A recent increase in the number of patients contributed to the relative short median time. Fourteen patients (52%) received treatment upon diagnosis (Table 4). The overall response rate was 86% (Figure 1A), and their estimated PFS rates after 1, 2, and 3 years were 93%, 70%, and 70%, respectively (Figure 1B). The treatment-free survival rates estimated from time to next treatment after 1, 2, and 3 years are 100%, 85%, and 77%, respectively. Table 5 shows three patients who achieved CR, but relapsed later, and two patients that did not achieve CR.

The other 13 patients did not initially receive therapy. Those who did not mind the frequent EGD tended to choose watchful waiting. In patients who wished to be free of the disease or symptoms, therapy was preferred (Table 4). However, the frequency of EGD was not significantly different between those patients who were observed and treated: the observed patients underwent EGD once every 3 months in the first year and once every 6 months thereafter, while the treated patients underwent EGD once every 4 months in the first year and once every 6 months thereafter.

These 13 patients' estimated PFS rates after 1, 2, and 3 years were 85%, 74%, and 74%, respectively in these patients (Figure 1B). The treatment-free rate estimated from time to next treatment after 1, 2, and 3 years were 75%, 67%, and 57%, respectively. In one patient, spontaneous regression occurred 57.0 months after diagnosis and became free of disease 93.0 months after the initial diagnosis. In three patients, progression was observed after chemotherapy had started but durable responses were achieved. In two patients, radiation therapy was started after the patient changed their mind and they also achieved CR.

Both the patients who had been followed up without therapy and those who relapsed after treatment responded well to subsequent chemotherapy. All patients are alive.

Table 3. Intestinal involvement other than in the duodenum

Stage	Intestinal involvement	Nodal involvement
I[a]	Jejunum, sigmoid colon, and transverse colon	None
I[a]	Ileum	None
II_2[b]	Ileum	Mesenteric lymph node

[a]According to the criteria described in Table 1, which were modified from those described by modified from the International Workshop for Gastrointestinal Lymphoma [5], lymphomas restricted to the gastrointestinal tract were defined as stage I, even if the lesions were skipped.

[b]Ileal lesions were found in two patients by colonoscopy, which can observe the ileum at least 30 cm beyond Bauhin's valve. As one of the two also demonstrated involvement of the regional lymph node, the case was staged as II_2. As regional local lymph nodes of the duodenum were not detected in any cases, no cases belong to stage II_1.

discussion

We found that 49 among 479 cases with FL had involvement of the duodenum. We had previously analyzed 26 such cases in 2005 [8], and those cases were included in this study with a longer follow-up period.

Twenty-one cases among these 49 cases (43%) were found to have lymphoma involvement in the duodenum during the staging procedures of systemic FL. The lesions are small and easily missed by CT or even PET scans [9]. If these 21 patients had not undergone screening with EGD, the duodenal involvement might not have been detected. As we routinely carry out EGD as a staging procedure for lymphoma patients at our institution, it is likely that we had the chance to evaluate the relative frequency of the duodenal involvement of FL at the initial manifestation.

Of these 21 cases, 4 cases with mesenteric FL need mentioning. The larger or largest lesion in these cases was the mesenteric tumor; therefore, these cases were not included as

Table 4. Initial management

Therapy	Symptoms		Stage I, $n = 20$ (%)	Stage II_2, $n = 7$ (%)	Total, $N = 27$ (%)
	Yes	No			
Watchful waiting	4	9	10 (77)	3 (23)	13 (48)
Rituximab only	1	5	6 (100)	0 (0)	6 (22)
Chemotherapy[a] +/− rituximab	3	3	2 (33)	4 (66)	6 (22)
Radiotherapy	0	2	2 (100)	0 (0)	2 (7)

[a]Two cases treated with R-CHOP, two with COP, one with C-MOPP, and one with CHOP.
R-CHOP, a combination chemotherapy with cyclophosphamide, doxorubicin, vincristine and prednisone plus rituximab; COP, a combination chemotherapy with cyclophosphamide, vincristine, prednisone; C-MOPP, a combination chemotherapy with cyclophosphamide, vincristine, prednisone, procarbazine; CHOP, a combination chemotherapy with cyclophosphamide, doxorubicin, vincristine and prednisone.

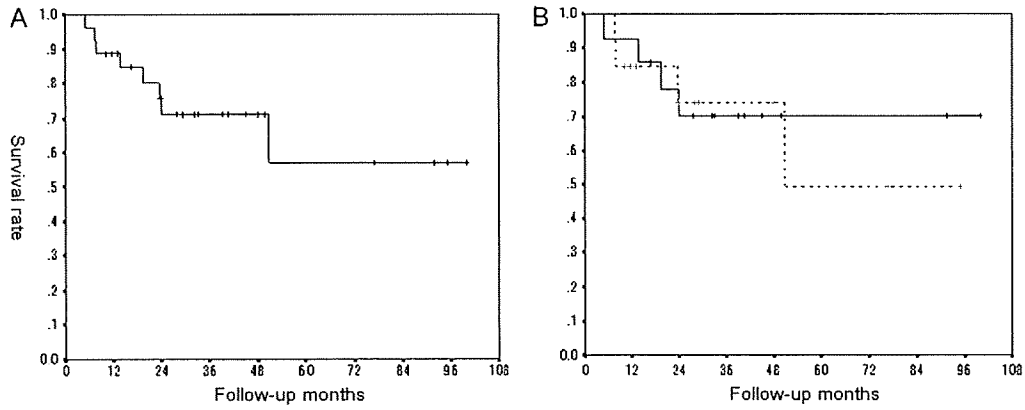

Figure 1. Progression-free survival (PFS). (A) PFS of all patients. The median follow-up period was 28.0 months (range 5.0–100.0 months). (B) PFS according to the initial treatment. The solid line shows that of treated patients, and the dotted line shows that of untreated patients.

Table 5. Secondary treatment after PD or SD

Clinical stage	Initial management (treatment)	Response	Duration to PD	Site of progression	Secondary or third management (treatment)	Response	Time from PD (months)
I	R	CR then PD[a]	23.8	Duodenum	R	CR	21.4
I	COP × 10	CR then PD[a]	5.0	Duodenum	RT (40 Gy)	CR	99.4
II_2	R-CHOP × 6	CR then PD[a]	19.4	Duodenum	WW	SD	28.4
II_2	CHOP × 4	SD[b]	13.6	Duodenum	R-C-MOPP	CR	73.0
I	R	SD[b]	27.7[c]				
I	WW		23.5	Duodenum	R	CR	81.9
II_2	WW		50.8	Duodenum	R	SD	36.6
II_2	WW		7.5	Paraaortic lymph node	R-CHOP × 6	CR	54.7
I	WW		13.3[d]		RT (36 Gy)	CR	35.7
I	WW		11.6[d,e]		RT (36 Gy); CHOP × 3 + RT (40 Gy)	CR then PD; CR	4.7
I	WW		10.0[d]		RT (36 Gy)	CR	20.2

[a]Patients who relapsed. Two were treated differently from their initial treatment and achieved CR. The other patient was observed without progression of >20% compared with the tumor at the initial diagnosis.
[b]Patients who did not achieve CR. One patient who did not respond to CHOP and stayed in SD underwent C-MOPP and achieved CR.
[c]The other patient who did not respond to rituximab stayed in SD.
[d]Patients who changed their minds and received radiation.
[e]One patient achieved CR of 4.7 months; however, histological transformation to diffuse large B-cell lymphoma developed with a subsequent relapse at the cervical lymph nodes. The patient received three cycles of R-CHOP and involved-field radiotherapy and has remained in CR for 6 years.
PD, progressive disease; SD, stable disease; R, rituximab; CR, complete remission; RT, radiation and dose in Gy; R-CHOP, a combination chemotherapy with cyclophosphamide, doxorubicin, vincristine and prednisone plus rituximab; WW, watchful waiting; CHOP, a combination chemotherapy with cyclophosphamide, doxorubicin, vincristine and prednisone.

primary DFL in this study; but the tumor was located only at mesenteric lymph nodes and duodenum. This definition was rather a practical one, as we had not searched for jejunal or ileal lesions extensively, so the possibility that these four cases had primary jejunal or ileal lesions, including the duodenum that had extended into the local mesenteric lymph node cannot be ruled out. Alternatively, retrograde progression of the duodenum from the development of mesenteric lymph nodes might have occurred.

We analyzed 27 cases of primary DFL. The median age was middle aged, and female cases were more common than male cases, which were in agreement with a previous report by Sato et al. [10]. We found that 70% of our cases had no symptoms, in which the disease was found by chance, mainly by screening for gastric cancer. Yoshino et al. [1] and others also reported that none of their cases had symptoms [11]. In Japan, routine screening for gastric cancer is recommended by the government, and a significant number of patients are screened through EGD, including duodenum. A number of case reports from Japan indicate that high prevalence rate of the disease in this country might be derived from its screening system.

We found that in 23 cases (85%), the primary site was in the vicinity of Vater's ampulla in the second portion of the duodenum, and among them, 2 cases had received gastrectomy. This is the first report to reveal that patients who undergo gastrectomy might be associated with the subsequent development of DFL. It is relatively high incidence in the present study that might be partly attributed to the frequent routine examination by EGD after gastrectomy.

Our findings are consistent with those of previous reports that the second portion is the most common site [12–15]; however, the third portion is not examined routinely using endoscopy, and our cases also did not include routine capsule endoscopy or double-balloon endoscopy. In our series, 21 patients (78%) underwent colonoscopy, and ileal involvement was found in 2 among 21 cases (10%) and sigmoid colon involvement in 1 (5%) (Table 3). As lower intestinal endoscopy is not mandatory in the routine staging procedures for FL, the exact incidence of large intestinal involvement is unclear.

In order to rule out MALT type and mantle cell lymphoma, we examined whether these cells contained t(14;18). In the present study, 83% of these cases had a translocated *bcl-2* gene, as detected by FISH analysis. This frequency was higher compared with one obtained in a previous study of Japanese patients with nodal FL in our institute, where 28 (60%) of 48 patients demonstrated IgH/BCL2 fusion [16]. The test is sensitive and is free of the false-negative results, which are sometimes found in standard karyotypic analysis. Sato et al. [10] reported that the incidence of t(14;18) was only 26%; however, the methodology they used was to detect only major IgH/BCL2 fusion at major breakpoint cluster redion (MBR) and not minor breakpoint cluster region.

Immunoglobulin and *bcl-2* gene rearrangement at the MBR locus was present in 11 of 14 patients in another report [17]. It is likely that the frequency shown in this study represents the true one, which is higher than our previous frequency of ~60%, found in nodal FL using the same method. The frequent occurrence of the translocation is consistent with the findings of nodal disease in Europe and the United States. Kodama et al. [18] also reported that 7 of 10 cases of GI FL including DFL demonstrated fusion, using the same method as ours.

Since this study was retrospective, we cannot determine the most appropriate strategy for primary DFL. The high prevalence of stage I disease should be taken into consideration, and if we apply the general treatment policy of FL to this subset of the diseases, involved-field radiation might be the first choice, which is recommended as a standard therapy because more than half of the patients are cured. However, currently we do not know the exact frequency of lymphoma involvement of the small intestine, and the lack of this information makes this modality less relevant. Also, the duodenum is located close to the kidney, which is a radiation-sensitive organ.

To detect small intestinal involvement, we tried capsule endoscopy in two cases; however, no other lesions were detected. Colonoscopy was carried out in 20 cases, and 2 cases were positive for ileocecal lesion. Without a double-balloon endoscope or capsular endoscope, observation of the jejunum and most of the ileum are impossible. Until we confirm that FL is limited to the duodenum, we still need to examine the incidence of lymphoma involvement in the GI tract other than the duodenum.

It is impossible to compare the efficacy of systemic treatment because the number in each treatment group was too small as shown in Table 4. It is notable that six cases were treated with rituximab alone, and one case did not achieve CR after eight cycles of rituximab monotherapy, and a second round was necessary.

We found that the prognosis for patients with primary DFL was excellent. Shia et al. [19] have also reported that among 25 cases, 14 were disease free and 11 were alive with disease at a mean follow-up of 43 months. In our study, regardless of the therapy, both transformation and emergence of new lesions were rare.

In this retrospective study, we found that the patients who were not treated at the initial presentation did well, and treatment after confirmation of progression was effective as well. This indicates that a watchful waiting policy is an acceptable approach for a significant fraction of cases of DFL. Damaj et al. [17] reported that seven patients did not receive any treatment, and four of them had progressed after a median follow-up of 37.5 months, but despite this, overall survival was excellent. As the initial management was determined by patient's preference in this series, even though the PFS is the same as that of patients who were treated upon diagnosis, we cannot conclude that watchful waiting is as good as early intervention as there might be a difference between these two groups, and a comparative study is required. It is also unclear whether these results would apply to symptomatic patients diagnosed using other methods. However, it indicates that a longer watchful waiting policy be applied to a certain subset of cases of this disease.

summary

The majority of primary DFL is positive for t(14;18), localized, of low-grade histology, and with low risk according to IPI and FLIPI. Due to the indolent course of primary DFL, a watch and wait policy may be an acceptable initial management strategy.

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