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Clinicopathological Features of Malignant Lymphoma in Japan: The Miyagi Study

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The Miyagi Study is an epidemiological study of malignant lymphoma, including immunological and genetic analyses, constructed by a population-based registration system covering Miyagi prefecture, Japan. A total of 1,552 newly diagnosed cases in Miyagi between 2002 and 2008 were enrolled in this study; 75% were B-cell lymphomas, 19% were T-cell and natural killer-cell (T/NK-cell) lymphomas, and 5% were Hodgkin's lymphomas. The most frequent subtype of B-cell lymphoma is diffuse large B-cell lymphoma, followed by follicular lymphoma and extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (51%, 24% and 8%, respectively). Thus, follicular lymphoma accounts for 18.2% of newly diagnosed cases in Miyagi; unexpectedly, its frequency is similar to that reported in Western countries. The common subtypes of T/NK-cell lymphoma are peripheral T-cell lymphoma, angioimmunoblastic T-cell lymphoma, and adult T-cell leukemia/lymphoma (30%, 15% and 14%, respectively). Most of the data are similar to those reported in Asian countries, except for follicular lymphoma. We also analyzed the CD20 expression in B-cell lymphomas by flow cytometry for the cell membrane expression and by immunohistochemistry for the cytoplasmic expression. The cell membrane expression of CD20 protein may determine the susceptibility of B-cell lymphomas to anti-CD20 antibody therapy. The lack of CD20 expression was confirmed by both methods in 4 cases of 585 newly diagnosed cases (0.7%) and in 5 of 67 recurrent cases (7.5%). Furthermore, 23 cases (6.5%) showed the discrepancy of CD20 expression between both methods. The Miyagi Study has revealed the latest epidemiological features of malignant lymphoma in Japan.

Keywords: CD20; epidemiology; flow cytometry; malignant lymphoma; Miyagi Study
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Malignant lymphoma is a group of haematological malignancies with various clinical presentations, histological subtypes and biological behaviours. The subtypes of malignant lymphoma were defined in the World Health Organization (WHO) classification of tumours of haematopoietic and lymphoid tissues published in 2008 (Swerdlow et al. 2008). They can be divided into 2 major categories: Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL). NHL is also classified into 2 immunological categories, B-cell neoplasms and T-cell and natural killer-cell (T/NK-cell) neoplasms. Each can be further divided into small subtypes according to the stages of differentiation, clinical, histological, immunophenotypic and genetic findings. The relative incidences of the subtypes are known to differ according to geographic areas, which may be ascribed to genetic and environmental factors. Compared with Western countries, Asian countries are known to have higher rates of T/NK-cell lymphoma and lower rates of follicular lymphoma (FL), chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL/SLL), and HL (Ko et al. 1998; Sukpanichnant 2004; Lee et al. 2006; Morton et al. 2006).

Several epidemiological studies in Japan have been reported to date. However, the data were collected from selected representative institutions, such as university hospitals and cancer centres (Lymphoma Study Group of Japanese Pathologists 2000; Aoki 2008). Therefore, the results may not reflect the actual incidences and characteristics of malignant lymphoma in Japan. In addition, these studies did not include immunological and genetic information of tumours, which are essential for diagnosis according to the updated WHO classification. The Miyagi Study is a comprehensive epidemiological study of malignant lymphoma, including immunological and genetic information, constructed by a population-based registration system covering Miyagi prefecture, Japan, with a population of 2.35

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million. Miyagi is an area of Japan in which human T-cell leukaemia virus type 1 (HTLV-1) is non-endemic, and the population composition by age group and the population growth rate in Miyagi are similar to the respective national average figures. Therefore, we assumed that the clinicopathological features of malignant lymphomas in Miyagi represent those of Japan, excluding the Kyushu area where HTLV-1 is endemic.

The purpose of this study was to evaluate the relative incidences and features of subtypes of malignant lymphoma in Japan, and compare the results with those of other countries.

Materials and Methods

A total of 1,885 cases, including 231 recurrent cases, diagnosed at institutes in Miyagi prefecture between January 2002 and December 2008 were enrolled in this study. The 23 participating institutes included small hospitals in rural areas as well as large hospitals representative of each local area in Miyagi. This study was based on the findings from tissue biopsy materials and clinical information, including age and gender. Cases of acute lymphoblastic leukaemia and CLL were not included in this study, as they are usually diagnosed from bone marrow aspirates.

From each institution, the tissue specimens were sent to the registration-examination-analysis-description (READ) system, which is a comprehensive diagnostic laboratory for lymphoproliferative disorders (Koutou Biseibutsu Laboratories and Special Reference Laboratories, Tokyo, Japan), for diagnosis and classification by pathologists. Tissue was fixed in 10% formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin, and immunochemical staining was performed. A standard panel of flow-cytometry (FCM) was performed on each case including CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD13, CD19, CD20, CD22, CD30, CD45, CD56, TCR-$\alpha\beta$, TCR-$\gamma\delta$, kappa chain and lamda chain. All cases were examined by FCM to determine immunological subtypes. G-banding analysis was performed in most cases to identify chromosomal abnormalities. Southern blotting analysis was performed in most cases to examine tumour clonality, immunophenotype and detection of viral DNA, such as Epstein-Barr virus (EBV) and HTLV-1, when the amounts of biopsied materials were sufficient to carry out the analysis. Polymerase chain reaction (PCR) analyses, and fluorescence in situ hybridization (FISH) studies were performed as needed, and all cases were diagnosed according to the criteria of the WHO classification (Jaffe et al. 2001). The study was approved by the Ethics Committee of Tohoku University Graduate School of Medicine, and complied with the Helsinki Declaration.

The results regarding the incidences of subtypes were compared with those of a previous nationwide study reported in 2000, which analysed the diagnoses of 3,194 cases at 18 institutes between 1994 and 1996 (Lymphoma Study Group of Japanese Pathologists 2000).

For statistical analysis, the χ^2 test was used to compare differences between 2 groups.

This study was approved by the ethical committees of the Medical School of Tohoku University. Informed consent was obtained in accordance with the Declaration of Helsinki.

Results

Patient characteristics

Of the total of 1,885 cases, 1,552 cases were newly diagnosed, 231 cases were recurrent, and status was unknown for the remaining 102 cases. The patient population consisted of 1,028 men and 857 women with a median age of 66 years.

Relative frequency of each subtype

Of the 1,552 cases of newly diagnosed malignant lymphoma, 1,166 cases (75.1%) were B-cell lymphomas and 287 cases (18.5%) were T/NK-cell lymphomas, while only 81 cases (5.2%) were HL (Fig. 1a).

The largest subtypes in the 3 major groups were diffuse large B-cell lymphoma (DLBCL) (38.6%), followed by FL (18.1%), extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) (5.9%) and peripheral T-cell lymphoma-unspecified (PTCL-U) (5.5%) (Table 1).

The most frequent subtype of B-cell lymphoma was DLBCL, which accounted for 51.4% of all B-cell lymphoma cases, followed by FL and MALT lymphoma (24.1% and 7.9%, respectively) (Fig. 1b). It is noteworthy that the relative frequency of indolent B-cell lymphomas in women accounted for 39.6% of all B-cell lymphomas, which was higher than that in men (29.6%). There was no notable change in the proportion of FL in B-NHL over the 7 years included in the study, as it accounted for 20.2% in 2002, 28.9% in 2005 and 23.5% in 2008.

The frequent subtypes of T/NK-cell lymphoma were PTCL-U, angioimmunoblastic T-cell lymphoma (AILT) and adult T-cell leukaemia/lymphoma (ATLL), which accounted for 30%, 15% and 14% of cases of T/NK-cell lymphoma, respectively (Fig. 1c). Extranodal NK/T-cell lymphoma, and Mycosis fungoides or Sézary syndrome had incidence rates of 7% and 1%, respectively.

Of 81 cases of HL, the most common subtype was mixed cellularity classical Hodgkin's lymphoma (46%), followed by nodular sclerosis classical HL (43%) (Fig. 1d).

Incidences of disease according to age and gender

The median ages at onset were 67 years for B-cell lymphoma and T/NK-cell lymphoma and 44 years for HL (Table 1). With regard to histological subtypes, the median age was younger in precursor B-lymphoblastic lymphoma (18 years), nodular sclerosis classical Hodgkin's lymphoma (28 years), mediastinal large B-cell lymphoma (29 years) and precursor T-lymphoblastic lymphoma (32 years), and older in blastic NK-cell lymphoma (76.5 years), SLL (73 years), Burkitt's lymphoma (73 years), AILT (72.5 years) and PTCL-U (71 years).

The peak age of disease was in the 70s for all newly diagnosed cases (Fig. 2). The peak age of NHL was in the 70s and that of HL was biphasic in the 20s and in the 60s.

Among the newly diagnosed cases, there were 830

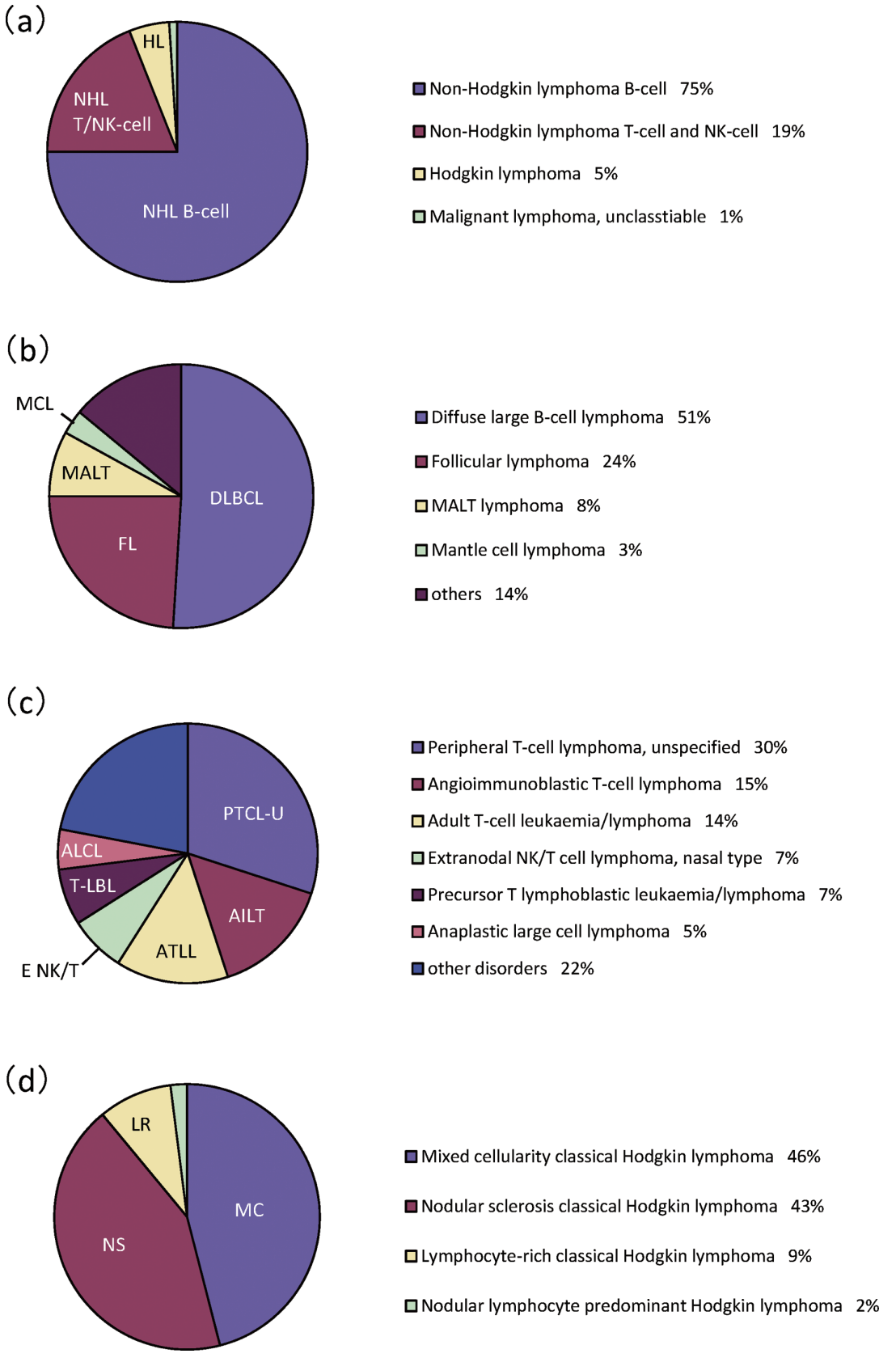

Fig. 1. Relative frequencies of lymphoid neoplasm subtypes.
Malignant lymphomas in Miyagi, 2002-2008. (a) Overall proportions of malignant lymphomas. (b) The proportions of different B-cell lymphoma entities. (c) The proportions of different T/NK-cell lymphoma entities. (d) The proportions of different Hodgkin's lymphoma entities.

Table 1. Data of malignant lymphoma newly diagnosed in Miyagi, 2002-2008.

	No. of patients	M/F	age (years)		Miyagi (%)
			median	range	
Total	1,552	1.1	66	0-98	100
B-cell neoplasms	1,166	1.1	67	0-97	75.13
T/NK-cell neoplasms	287	1.6	67	1-98	18.49
Hodgkin Lymphoma	81	1.3	44	4-88	5.22
Lymphoid neoplasmas-not otherwise specified	17	0.5	66.5	0-79	1.10
B-cell neoplasms					
Precursor B-lymphoblastic leukemia/lymphoma	5	1.5	18	5-68	0.32
B-cell small lymphocytic lymphoma	15	1.5	73	52-91	1.00
Lymphoplasmacytic lymphoma	5	4	69	54-77	0.32
Mantle cell lymphoma	35	71	71	53-87	2.26
Follicular lymphoma	281	1.2	58	24-90	18.11
Nodal marginal zone B-cell lymphoma	14	0.8	69.5	47-84	0.0
Extranodal marginal zone B-cell lymphoma	92	0.6	69.5	24-91	5.93
Plasma cell neoplasmas	17	3.2	57	32-84	1.45
Diffuse large B-cell lymphoma	599	1.1	69.5	0-94	38.60
Mediastinal large B-cell lymphoma	7	1.3	29	17-80	0.45
Intravascular large B-cell lymphoma	5	4	67	55-81	0.32
Primary effusion lymphoma	1	F only	79	79	0.06
Burkitt lymphoma	7	2.5	73	13-97	0.45
B-cell neoplasmas-not otherwise specified	83	1	70	0-89	5.34
T /NK-cell neoplasms					
Precursor T-lymphoblastic leukemia/lymphoma	19	1.7	32	1-74	1.22
Blastic NK cell lymphoma	10	4	76.5	18-89	0.64
T-cell prolymphocytic leukemia	1	M only	39	39	0.06
Aggressive NK cell leukemia	2	1	35.5	23-48	0.13
Adult T-cell leukemia/lymphoma	41	1.7	65	36-80	2.64
Extranodal NK/T-cell lymphoma, nasal type	20	3	59.5	21-91	1.29
Mycosis fungoides/Sezary syndrome	3	M only	60	13-83	0.19
Primary cutaneous CD30+ T-cell LPD	2	M only	55	52-58	0.13
Peripheral T-cell lymphoma, unspecified	85	1.3	71	12-90	5.48
Angioimmunoblastic T-cell lymphoma	42	2.5	72.5	41-98	2.71
Anaplastic large cell lymphoma	15	0.9	68	16-79	0.97
T-cell neoplasmas-not otherwise specified	41	1	68	37-89	2.64
T/NK-cell neoplasmas-not otherwise specified	6	5	58.5	38-78	0.39
Hodgkin's lymphoma					
Nodular lymphocyte predominant Hodgkin's lymphoma	2	M only	37	21-53	0.13
Classical Hodgkin's lymphoma					
Nodular sclerosis	35	0.9	28	9-85	2.26
Mixed cellularity	37	1.8	64	4-88	2.38
Lymphocyte rich	7	0.4	57	34-87	0.45

men and 722 women, showing a slight male predominance (M/F ratio: 1.1) (Table 1). The male/female ratios were 1.1 in B-cell lymphoma, 1.6 in T/NK-cell lymphoma and 1.3 in HL. Notable male predominance was seen in plasmacytoma (M/F ration: 10), mantle cell lymphoma (M/F ratio: 4.8), lymphoplasmacytic lymphoma (M/F ratio: 4.0) and blastic NK-cell lymphoma (M/F ratio: 4.0).

CD20 expression of each B-cell lymphoma subtype

We evaluated CD20 expression of newly diagnosed B-cell lymphomas by FCM and immunohistochemistry (IHC). Plasma cell neoplasms were excluded, because they

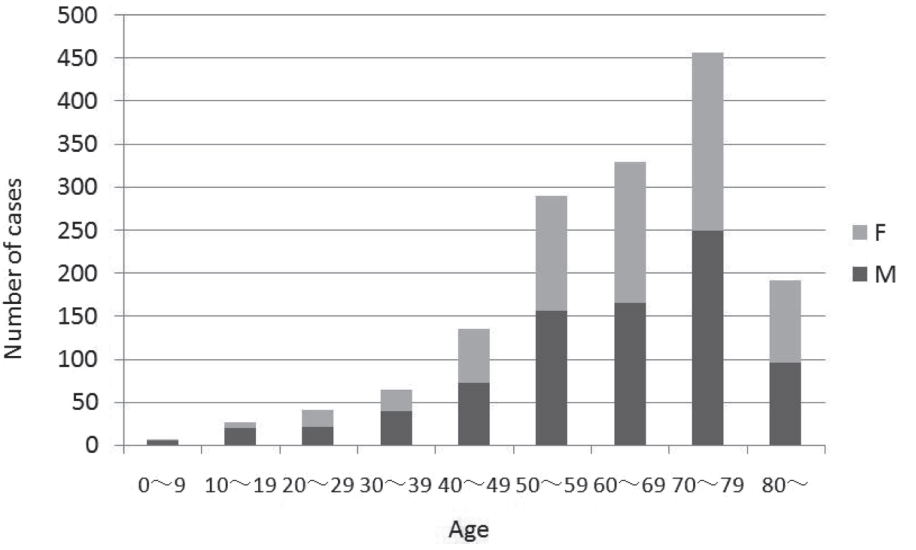

Fig. 2. Case number of malignant lymphoma by age.
Frequency of malignant lymphomas by age. n = 1,540. Six cases for which age was unknown were excluded.

are usually negative for CD20. CD20 was detected in 94%-100% of the B-cell lymphomas. The positive rates of CD20 as determined by FCM were 94.9% in DLBCL, 99.2% in FL, 98.4% in MALT lymphoma and 100% in Mantle cell lymphoma (MCL). The positive rates of CD20 determined by IHC were 96.5% in DLBCL, 100% in FL, 94.0% in MALT lymphoma and 100% in MCL.

CD20 expression in newly diagnosed and recurrent cases

We assessed the frequency of B-cell lymphoma with CD20 expression ($CD20^+$) in the newly diagnosed group (870 cases) and the recurrent group (132 cases) by FCM or IHC. FCM analysis revealed that the proportion of $CD20^+$ B-cell lymphoma was 97.1% (845 out of 870 newly diagnosed cases), while 123 of 132 recurrent cases were positive for CD20 (93.2%). Subsequently, 956 cases of B-cell lymphoma (854 newly diagnosed cases and 102 recurrent cases) were examined by IHC on CD20 expression. The proportion of $CD20^+$ cases among the newly diagnosed cases was 96.6% (825 out of 854). In contrast, 86 of 102 cases were positive for CD20 (84.3%) in the recurrent cases. Thus, there were significant differences in the frequency of CD20 expression between newly diagnosed and recurrent cases (FCM: 845/870 vs. 123/132, $p = 0.02$; IHC:l 825/854 vs. 86/102, $p < 0.01$).

Discrepancy in CD20 expression between FCM and IHC

There appears significant difference in the frequency of CD20 expression in B-cell lymphoma, depending on the methods employed. Therefore, we analyzed the discrepancy rate of CD20 expression in 670 cases of B-cell lymphoma (585 newly diagnosed cases, 67 recurrent cases, and 18 cases with unknown status) by FCM and IHC. Among 670 cases, 619 cases were positive for CD20 and 9 cases were negative for CD20, as judged by both methods. The remaining 42 cases (6.3%) showed a discrepancy in CD20 expression between the two methods. Twenty-four cases (3.6%) were negative for CD20 by FCM but positive by IHC, while 18 cases (2.7%) were positive for CD20 by FCM but negative by IHC.

Table 2. Discordant CD20 expression by FCM and IHC.

CD20 expression	Newly diagnosed cases	Recurrent cases
FCM + • IHC +	547 (93.5%)	54 (80.6%)
FCM − • IHC −	4 (0.7%)	5 (7.5%)
FCM + • IHC −	14 (2.4%)	4 (6.0%)
FCM − • IHC +	20 (3.4%)	4 (6.0%)
	585	67

FCM, flow cytometry; IHC, immunohistochemistry.

We then assessed the CD20 expression patterns in newly diagnosed and recurrent cases (Table 2). Among the 585 newly diagnosed cases, 34 cases (5.8%) showed a discrepancy in CD20 expression, while 8 of 67 recurrent cases (12%) showed a discrepancy. Thus, recurrent cases were likely to show a discrepancy between the results of FCM and IHC regarding CD20 expression. Rituximab, which is a therapeutic antibody against CD20, is effective when CD20 is expressed on the cell surface. The discrepancies between the results of FCM and ICH regarding CD20 expression suggest that ICH analysis may not be sufficient to determine whether rituximab should be used.

Detection of t(14;18) in follicular lymphoma

Chromosomal translocation t(14;18)(q32;q21) involving the immunoglobulin heavy chain gene (*IGH*) and the *BCL2* gene is a frequent chromosomal abnormality in FL, which results in a reduction in expression of the bcl-2 pro-

Table 3. Immunophenotypic features of FL with and without t(14;18).

	t(14;18) positive	t(14;18) negative	χ^2 test
No. of the cases	183	71	–
Grade 1	73 (39.9%)	13 (18.3%)	$p < 0.01$
Grade 2	75 (41.0%)	28 (39.4%)	NS
Grade 3a	35 (19.1%)	30 (42.2%)	$p < 0.01$
M/F ratio	83/100 (0.8)	38/33 (1.2)	NS
Median age	57	59	NS
(range)	(32-90)	(27-84)	
CD10 (FCM)	89.7%	73.4%	$p < 0.01$
CD10 (IHC)	90.7%	76.3%	$p < 0.05$
BCL2	97.6%	81.6%	$p < 0.01$
BCL6	90.2%	81.5%	NS
MUM1	7.5%	16.7%	NS
Ki67 (> 30%)	20.7%	41.4%	$p < 0.05$

*NS: not significant
FCM, flow cytometry; IHC, immunohistochemistry.

Table 4. Comparison of immunophenotypic features between De novo $CD5^+$ DLBCL and $CD5^-$ DLBCL.

	$CD5^+$ DLBCL	$CD5^-$ DLBCL	χ^2 test
No. of the cases	64	232	–
Median age (range)	71 (40-92)	71 (19-94)	NS
≥ 60 years	50 (78.1%)	172 (74.1%)	NS
M/F ratio	28/36 (0.8)	117/115 (1.0)	NS
CD10 (FCM)	15.6%	32.5%	$p < 0.01$
CD19 (FCM)	98.4%	98.7%	NS
CD20 (FCM)	92.2%	94.4%	NS
CD10 (IHC)	9.8%	26.1%	$p < 0.01$
CD20 (IHC)	98.4%	96.1%	NS
BCL6	60.5%	73.9%	NS
MUM1	88.6%	69.4%	$p < 0.05$
Ki67 (≥ 70%)	89.6%	78.8%	$p < 0.01$
GCB type	12.5%	32.6%	$p < 0.01$

*NS: not significant
GCB type, germinal centre B-cell like type; FCM, flow cytometry; IHC, immunohistochemistry.

tein. In the present study, t(14;18)/*BCL-2* rearrangement was detected by several methods including G-banding, FISH and PCR analyses. In PCR analysis, the *BCL-2* gene rearrangements were detected at both the major breakpoint region (MBR) and the minor cluster region (mcr). The t(14;18) was present in 133 out of 229 cases (58.1%) by G-banding analysis, whereas *BCL2/IgH* rearrangement was present in 58 out of 80 cases (72.5%) by FISH and in 47 out of 65 cases (72.3%) by PCR analysis.

Then we divided FL into two groups, FL with and without t(14;18)/*BCL-2* rearrangement, to clarify the differences in phenotype. Among 201 t(14;18) positive cases, the numbers of cases of Grade 1, Grade 2, Grade 3a and Grade 3b were 73, 75, 35 and 16, respectively. The Grade of 2 cases was unknown. The t(14;18)-negative group consisted of 94 cases, including 13 cases of Grade 1, 28 of Grade 2, 30 of Grade 3a, 22 of Grade 3b and 1 of Grade Unknown. Based on previous reports that FL Grade 3b differs molecularly from FL of Grades 1-3a (Ott et al. 2002; Katzenberger et al. 2004), we excluded Grade 3b and Grade Unknown cases from both groups. As shown in Table 3, the t(14;18)-negative group had lower positive rates in CD10 by both FCM (73.4% *vs*. 89.7%, $p < 0.01$) and IHC (76.3% *vs*. 90.7%, $p < 0.05$), and BCL2 by IHC (81.6% *vs*. 97.6%, $p < 0.05$). In contrast, the proportion of cases that showed Ki-67 labelling in more than 30% of the tumour cells was higher in the t(14;18)-negative group (41.4% *vs*. 20.7%, $p < 0.05$). The proportion of Grade 3a cases in the

t(14;18)-negative group was higher than that of the t(14;18)-positive cases (19.1% vs. 42.2%, $p < 0.01$).

Incidence rate of de novo CD5$^+$ DLBCL

The prognosis of *de novo* CD5$^+$ DLBCL is poor (Yamaguchi et al. 2002). CD5 expression was examined by both FCM and by IHC in 296 cases of newly diagnosed DLBCL. CD5 was shown to be expressed in 64 cases (21.6%) of DLBCL by either method. Particularly, 28 cases that were CD5- by IHC were CD5$^+$ by FCM. In contrast, only 1 case was CD5$^+$ by IHC but CD5$^-$ by FCM.

We compared immunophenotypic features with regard to CD5 status in DLBCL cases (Table 4). These cases were classified into a germinal centre B-cell-like (GCB) group and non-GCB group according to the previous reports (Hans et al. 2004). The positive rates of CD10 in CD5$^+$ DLBCL were lower than those of CD5$^-$ DLBCL by FCM (15.6% *vs*. 32.5%, respectively; $p < 0.01$) and IHC (9.8% *vs*. 26.1%, respectively; $p < 0.01$), whereas the positive rate of MUM1 was higher in CD5$^+$ DLBCLs (88.6% *vs*. 69.4%, $p < 0.05$). Consequently, GCB cases comprised 32.5% of the CD5$^-$ DLBCLs but only 12.5% of the CD5$^+$ DLBCLs ($p < 0.01$).

Discussion

Considering the number of cases included in the present study, we believe that our data represent the status of the relative incidence rates of malignant lymphoma subtypes in Japan except the Kyushu region.

In the present study, B-cell lymphomas, T/NK-cell lymphomas and HL accounted for 75.1%, 18.5% and 5.2% of all lymphoma cases, respectively. The low frequency of HL, which accounted for less than 10% of malignant lymphomas, was similar to that of Korea (5.3%), Taiwan (7.0%) and Thailand (7.9%) (Ko et al. 1998; Sukpanichnant 2004; Lee et al. 2006). This is a common finding in Asian countries in comparison with the high frequency (40%-45%) reported in Western countries (Morton et al. 2006).

We showed that DLBCL comprised the largest group, followed by FL and MALT lymphoma. SLL accounted for only 1.3% of B-cell lymphomas, which is one of the major differences between Asian and Western countries. FL is the most frequent B-cell lymphoma in Western countries, especially in North America (Anderson et al. 1998). On the contrary, the lower rate of FL has been reported in Asian countries (Lymphoma Study Group of Japanese Pathologists 2000; Chuang et al. 2000). However, in the present study, FL accounted for 18.2% of newly diagnosed cases, which was similar to the relative frequency in Western countries. High relative frequency of FL has been also reported in Taiwan and in Osaka, Japan (Miyazato et al. 2002; Lee et al. 2006; Aoki et al. 2008). An increase in relative frequency was also noted in Korea, although the rate was still low (5.88%) (Ko et al. 1998). Therefore, it appears that the incidence of FL is increasing in East Asia, although the factors responsible are still unclear. Some reports suggested that westernisation of lifestyle may be one factor involved in these changes (Miyazato et al. 2002). The increase in relative frequency of FL suggests that the pattern of malignant lymphoma occurrence in Japan may be gradually changing.

HTLV-1-associated ATLL is endemic in several areas around the world, including southwestern Japan, especially Kyushu. As Miyagi is a non-endemic area of HTLV-1, T/NK-cell lymphomas comprised 18.5% of cases, which was similar to the rate for Japan excluding the Kyushu area reported previously (Lymphoma Study Group of Japanese Pathologists 2000). Extranodal NK/T-cell lymphoma, nasal type, has a higher prevalence rate in Asian countries and is closely linked to EBV infection (Jaffe et al. 2001). In comparison with the incidence in previous studies performed in Japan and other Asian countries, the present study showed a relatively low incidence rate of this type of lymphoma (1.3%) (Ko et al. 1998; Lee et al. 2006; Lymphoma Study Group of Japanese Pathologists 2000). However, the recent report from Japan also showed a lower late of extranodal NK/T-cell lymphoma, nasal type (1.4%) (Aoki et al. 2008). The difference of incidence among reports may arise from the difference of geographic area or period the survey was performed.

Rituximab is a chimeric monoclonal antibody that recognises the CD20 protein, which has played an important role in treating B-cell lymphomas (Coiffier et al. 2002; Feugier et al. 2005; Fisher et al. 2005). Recent reports indicate that loss of CD20 expression occurs in some patients with B-cell lymphomas during rituximab therapy, but the incidence has not been clarified (Hiraga et al. 2009; Johnson et al. 2009). We showed significantly higher rate for loss of CD20 expression by IHC in the recurrent cases (15.7%, 16/102 cases) than in newly diagnosed cases (3.4%, 29/854 cases) in this largest scale of analysis among the previous reports. However, biopsy was not performed in most patients at relapse; therefore, the accurate incidence of recurrent cases negative for CD20 expression is unknown.

When we examined the incidence of discordant CD20 expression, 6.3% of B-cell lymphomas showed discordant CD20 expression between FCM and IHC. Interestingly, the relative frequency of the cases that showed discordant CD20 expression was also higher in the recurrent group. Loss of CD20 expression at relapse was reported in patients, who received Rituximab contained chemotherapy, genetic and epigenetic mechanisms have been suggested (Hiraga et al. 2009; Johnson et al. 2009, Terui et al. 2009). Discrepancy of CD20 expression between FCM and IHC was occurred more frequently in de novo DLBCL than in recurrent cases (Johnson et al. 2009). One of the reasons for the discrepancy is that the expression of CD20 on the cell surface was lost due to C-terminal deletion of CD20 (Terui et al. 2009). Theoretically, rituximab may not be effective when cell-surface expression of CD20 is reduced. Therefore, FCM should be performed on all biopsied lymphomas to determine the status of surface CD20, especially

in recurrent cases.

FL is characterised by the chromosomal translocation t(14;18)(q32;q21). It has been reported that there are variations in the detection rates of t(14;18) in FL between Asian and Western countries (Biagi and Seymour 2002). t(14;18) is found in 70%-95% of FL in Western countries and 60%-80% of FL in Japan, but the proportions depend on the technique used to identify the translocation (Hashimoto et al. 1995; Matsumoto et al. 2004; D'Haese et al. 2005; Guo et al. 2005). As it has been reported that FISH analysis is superior to other methods in detecting t(14;18)/ *BCL-2* gene rearrangement (Einerson et al. 2005; Belaud-Rotureau et al. 2007), FISH analysis showed the highest detection rate and in the present study. In the previous studies from Western countries, t(14;18) and/or *BCL-2* gene rearrangement were detected in 54.5%-89% by cytogenetic analysis, 89%-100% by FISH analysis and 41.2%-79% by PCR analysis, respectively (Yunis et al. 1982; Pezzella et al. 1990; Horsman et al. 1995; Godon et al. 2003; Einerson et al. 2005; Belaud-Rotureau et al. 2007). In comparison with those rates from Western countries, our results showed a relatively low rate by each method. There appears discordance in the frequency of t(14;18)/*BCL-2* gene rearrangement in FL between Japan and Western countries. This should be confirmed in larger epidemiologic studies excluding methodological differences.

Comparison of phenotypes between t(14;18)-positive and negative FL in this study indicated that the negative subgroup tended to have lower positive rates of CD10 and bcl-2 expression, and a higher rate of Ki-67 labelling and grade 1 and 2. These results support those of previous studies that indicated that t(14;18)-negative FL have different molecular characteristics (Karube et al. 2007, 2008; Leich et al. 2009). Then, detailed analysis of clinical features and prognosis would be needed to reveal the clinical differences of t(14;18) status in FL cases.

The prognosis of *de novo* $CD5^+$ DLBCL is poor in cases treated with CHOP therapy and is suggested to remain poor even after treatment with rituximab (Yamaguchi et al. 2002; Ennishi et al. 2008). CD5 antigen is expressed in the majority of SLLs and MCLs and in 9.8% of DLBCLs (Taniguchi et al. 1998). However, $CD5^+$ DLBCL detected both by FCM and by IHC accounted for 21.6% of all newly diagnosed DLBCLs in the present study. Among $CD5^+$ DLBCLs, 44% of the cases were $CD5^-$ by IHC. Usually, $CD5^+$ neoplastic B-cells express less CD5 than normal T cells and IHC for CD5 using paraffin sections is known to be less sensitive than FCM (Dorfman and Shahsafaei 1997). Our results indicated that FCM should be performed in combination with IHC to detect $CD5^+$ DLBCL.

In conclusion, Miyagi Study is a first comprehensive study of malignant lymphomas in Japan, including the relative frequencies of each subtype and the immunological and genetic information. We are colleting clinical information including clinical features, treatment and prognosis and will continue long follow-up of registered cases in this study to clarify the clinico pathological features of malignant lymphoma in more detail.

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Conflict of Interest

The authors report no conflict of interest.

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