ELSEVIER

European Journal of Cancer 40 (2004) 725–733

European Journal of Cancer

www.ejconline.com

Pathology review for paediatric non-Hodgkin's lymphoma patients in Japan: a report from the Japan association of childhood leukaemia study (JACLS)

A. Nakagawa[a,*], S. Nakamura[b], H. Nakamine[c], T. Yoshino[d], T. Takimoto[e], K. Horibe[e], K. Ueda[f]

[a]*Department of Pathology, Aichi Medical University, Nagakute, Aichi 480-1195, Japan*
[b]*Department of Pathology and Clinical Laboratories, Aichi Cancer Center Hospital, Nagoya, Japan*
[c]*Department of Clinical Laboratory Medicine, Wakayama Medical University, Wakayama, Japan*
[d]*Department of Pathology, Okayama University Graduate School of Medicine and Dentistry, Okayama, Japan*
[e]*Department of Pediatrics, Nagoya National Hospital, Nagoya, Japan*
[f]*Department of Pediatrics, Hiroshima University, Hiroshima, Japan*

Received 20 May 2003; received in revised form 19 August 2003; accepted 3 September 2003

Abstract

A central pathology review system with an immunophenotyping laboratory was established in Japan to support the clinical trial, the Japan Association of Childhood Leukaemia Study (JACLS) NHL-98, for patients with paediatric non-Hodgkin's lymphoma (NHL). Pathology samples from 155 clinically-suspected NHL cases were evaluated centrally initially using the Revised European-American Lymphoma (REAL) classification in a rapid review (within 2 weeks after surgery/biopsy) and then later at the consensus review (once a year). The samples were subsequently re-classified according to the new World Health Organisation (WHO) classification. After the pathology review, 96 (62%) patients were eligible for the study, and 58 of them (60%) had extra-nodal primaries. These NHL cases included B-cell lymphomas (precursor B-cell, 11; Burkitt, 18; diffuse large B-cell, 18; not otherwise specified, 3) and T/Natural Killer (NK)-cell lymphomas (precursor T-cell, 23; anaplastic large cell, 20; others, 3). There was excellent concordance in making the diagnoses (95/96, 99%) and typing (93/96, 97%) of NHL between the rapid and consensus reviews. Five cases, initially diagnosed as diffuse large B-cell lymphoma by the review, were re-classified as Burkitt lymphoma according to the immunocytochemical criteria by the WHO classification. A total of 59 (38%) cases were excluded from the study: they were Hodgkin lymphoma (7), leukaemias (11), reactive lymphoid hyperplasia (20), necrotizing lymphadenitis (7), no consensus diagnosis (1), insufficient materials (2), and others (11). This is the first report of the central pathology review from the paediatric NHL group study in Japan. Because various diseases, either neoplastic or reactive, mimicked NHL, clinically and histopathologically, the central pathology review system was critical and essential for patient enrollment and protocol assignment in our clinical trial. Through the two-step review system, highly reliable data were generated to support this study.

Keywords: Non-Hodgkin's lymphoma; Japanese children; Pathology; Central review; WHO classification; Group study

1. Introduction

Historically, different systems have been applied for classifying and typing non-Hodgkin's lymphoma (NHL) cases in different countries: the Working Formulation was the most popular classification in the United States (US) [1] and the Kiel/Updated Kiel classifications were commonly used in European countries [2,3], while most of the pathologists in Japan used the Lymphoma Study Group (LSG) classification [4] that was not well accepted worldwide. This often resulted in a serious problem for haematologists/oncologists when analysing and comparing clinical data with those from other countries. In 2001, the new World Health Organisation (WHO) classification was published for international use in classifying NHL cases [5].

In 1998, before the introduction of the new WHO classification, a central pathology review system was

* Correspondence author. Tel.: +81-52-264-4811; fax: +81-561-62-9318.
E-mail address: atsukon@aichi-med-u.ac.jp (A. Nakagawa).

0959-8049/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2003.09.039

established to support a nationwide group study, the Japan Association of Childhood Leukemia Study (JACLS) NHL-98, for Japanese paediatric NHL patients aged between 0 and 16 years old. 95 institutions/hospitals from 24 different prefectures participated in this study, covering approximately 40% of paediatric population in Japan. The pathology review system for this study initially used the Revised European-American Lymphoma (REAL) classification [6] that was the prototype of the new WHO classification [5]. Fortunately, there are no major differences between the REAL Classification and the new WHO classification in defining the most common categories of paediatric NHL cases, such as precursor B lymphoblastic lymphoma (B-LBL), Burkitt lymphoma (BL), diffuse large B-cell lymphoma (DLBCL), precursor T lymphoblastic lymphoma (T-LBL), and anaplastic large cell lymphoma (ALCL). However, in the new WHO classification, immunostaining was mandatory for the critical distinction between atypical Burkitt/Burkitt-like variant of BL (>99% cells positive for Ki-67) and DLBCL. Accordingly, some of the cases arbitrarily diagnosed as either DLBCL or BL by the REAL classification need to be re-classified by the new WHO classification after determination of the proportion of Ki-67-positive cells [5].

After 4 years, the JACLS NHL-98 study was successfully closed in March 2002. Towards the end of the study, we had an opportunity to re-evaluate and re-classify all the NHL cases according to the new WHO classification. This report illustrates our experience of the first central pathology review system established for the paediatric NHL patients in Japan.

2. Patients and methods

Pathology material from 155 clinically-suspected NHL cases were examined through the central review system for the JACLS NHL-98 study (1 April 1998–31 March 2002). Those materials, including haematoxylin-eosin (H&E)-stained sections (172), unstained sections (1520), and snap-frozen tissues, were submitted to the Pathology Center at the Department of Pathology, Aichi Medical University, Aichi, Japan, from the participating institutions/hospitals (see Appendix). The review system was composed of two steps: i.e., rapid review and consensus review. The rapid review accepted pathology material from those cases with clinically-suspected NHL without waiting for the final pathological diagnosis from the contributing institutions, and was completed by the responsible pathologist (AN) within 2 weeks after surgery/biopsy. The rapid review diagnosis was required for patient eligibility, stratification, and protocol assignment in the study. Immunophenotyping of the proliferating cells in each case was performed at the central laboratory of the Pathology Center to support the rapid review diagnosis by using the unstained sections provided by the contributing institutions/hospitals (see the panel of primary antibodies and typical staining pattern listed in Table 1). Snap-frozen tissues were filed and kept at the Pathology Center for future investigations. The consensus review took place once every year and involved four haematopathologists (AN, SN, HN, TY) in order to ensure the reproducibility of the rapid review diagnosis and determine the final eligibility of the cases for the analysis of the study results. In selected cases, additional tests, such as detection of clonal gene rearrangements and/or chromosomal translocations, were performed to support the consensus diagnosis. Appropriate informed consent procedures were followed, and consent was obtained from patients or guardians.

All those clinically-suspected cases were evaluated histologically and placed into two major groups: i.e., NHL and other diagnoses. The tumours in the NHL group, originally evaluated by the REAL classification through this review system, were re-classified using the new WHO classification. In order to complete the process of re-classifying those NHL cases according to the criteria of the new WHO classification, additional immunostaining for Ki-67 was performed for all of the cases initially diagnosed as DLBCL and BL.

3. Results

Of the 155 cases, 95 patients were diagnosed as having NHL at the time of both the rapid and consensus reviews. One case was diagnosed as a DLBCL at the time of the rapid review, but a panel of four haematopathologists did not reach a consensus diagnosis (two voted for DLBCL and two voted for atypical lymphoid proliferation) at the time of the consensus review: a clonal B cell population was not detected by the polymerase chain reaction (PCR) method using paraffin-embedded material from this case. 57 cases were classified into the group of other diagnoses at the rapid review. 56 of them were excluded from the study after the consensus review. However, one case was diagnosed as a "fulminant T-cell proliferation in Epstein–Barr Virus (EBV) infection" at the rapid review, but was eventually included in the study with a diagnosis of "peripheral T-cell lymphoma, unspecified" after the consensus review because of an aggressive growth pattern with soft tissue infiltration and a clonal rearrangement of T-cell receptor beta chain gene by Southern hybridisation. There were an additional two cases that were also excluded from the study due to insufficient pathology material for evaluation at the time of both reviews.

In summary, 96 (62%) of 155 cases evaluated by the central pathology review system were eligible for the

Table 1a
Panel of antibodies for immunophenotyping

Antibody	Clone	Company	Antibody	Clone	Company
Anti-CD3ϵ	Polyclonal	DAKO	Anti-CD4	1F6	Novocastra
Anti-CD43	DF-T1	DAKO	Anti-CD8	C8/114B	DAKO
Anti-CD20	L26	DAKO	Anti-CD56	1B6	Novocastra
Anti-CD79a	JCB117	DAKO	Anti-CD45RO	UCHL-1	DAKO
Anti-TdT	Polyclonal	Supertechs	Anti-CD10	56C6	Novocastra
Anti-Ki-67	MIB-1	MBL	Anti-Bcl-2	124	DAKO
Anti-CD30	Ber-H2	DAKO	Anti-Granzyme B	GrB-7	MONOSAN
Anti-ALK-1	ALK1	DAKO	Anti-CD34	BI-3C5	DAKO
Anti-EMA	E29	DAKO	Anti-CD68	KP-1	DAKO
Anti-CD15	C3D1	DAKO	Anti-CD99 (MIC2)	12E7	DAKO

Antibodies to be tested were selected after reviewing the haematoxylin and eosin (H&E) sections from individual tumours.

Table 1b
Typical patterns of immunophenotyping for paediatric non-Hodgkin's lymphoma cases

Histological type	CD45	TdT	CD3ϵ	CD43	CD45RO	CD20	CD79a	CD99	CD15	CD30	EMA	ALK-1
B-LBL	+/−	+	−	−/+	−	−	+	+	−	−	−	−
BL	+	−	−	−	−	+	+	−	−	−	−	−
DLBCL	+	−	−	−	−	+	+	−	−	−/+[a]	−/+	−
T-LBL	+/−	+	+	+	+	−	−/+	+	−	−	−	−
ALCL	−/+	−	+/−	+/−	+/−	−	−	−	−	+	+	+

B-LBL, precursor B lymphoblastic lymphoma; BL, Burkitt lymphoma; DLBCL, diffuse large B-cell lymphoma; T-LBL, precursor T lymphoblastic lymphoma; ALCL, anaplastic large cell lymphoma; +, positive; −, negative; +/−, positive in most cases; −/+, negative in most cases.
[a] Expression, extensive in anaplastic variant and variable in mediastinal primary tumour.

JACLS NHL-98 study. Among these, only two (2%) cases had discrepancies in determining their types between the rapid and consensus reviews. In those two cases (final typing from B-NHL to DLBCL and from BL to B-NHL, one case each), the changes were made after reassessment of quality/quantity of the pathology sample at the consensus review. There were no differences, except for five cases, in typing of the NHL tumours between the original review by using REAL classification and the subsequent re-classification using the new WHO classification. The five cases, all initially diagnosed as DLBCL in the review (5/23, 22%), were re-classified as BL (atypical Burkitt/Burkitt-like variant of BL) after evaluation and determination of a Ki-67 fraction of close to 100% in their tumour tissues. Those cases, as shown in Fig. 1, had ambiguous morphological features: all were positive CD10 and 4/5 were negative for Bcl-2 immunohistochemically. Two of them were reported to show a normal karyotype. During the same 4-year period, 23 cases were enrolled on the JACLS NHL-98 study without central pathology review: those cases were excluded from further analysis in the present study.

Table 2 shows the final diagnosis (typing) according to the new WHO classification and clinical information (age, gender, and primary site) for all the NHL cases in this study. These cases included B-LBL (11 cases, 11%), BL (18 cases, 19%), DLBCL (18 cases, 19%), B-cell non-Hodgkin's lymphoma, not otherwise specified (B-NHL, NOS: 3 cases, 3%, B-cell phenotype determined by either immunostaining or flow cytometry, but further subclassification not feasible due to limited amount/quality of the samples), T-LBL (23 cases, 24%), ALCL (20 cases, 21%; including 13 cases with a T-cell phenotype and 7 cases with a null-cell phenotype), and peripheral T-cell/natural killer (NK) cell lymphoma (pT/NK-NHL: 3 cases, 3%, including hepatosplenic T-cell lymphoma, extranodal NK/T cell lymphoma, nasal type, and peripheral T-cell lymphoma, unspecified).

There were 60 males and 36 females with ages ranging between 11 months and 16 years old (median 9 years old) at diagnosis. Of these cases, 38 (40%) had primary nodal lymphoma, whereas 58 (60%) had primary extranodal lymphoma. Nineteen (20%) patients had bone marrow involvement (less than 25%), while no children had disease in the central nervous system (CNS) at the time of diagnosis. Those patients who had extramedullary masses showing histology that was indistinguishable from B-LBL, T-LBL, or BL with 25% or more blasts in their bone marrow were diagnosed as having acute lymphoblastic leukaemia (ALL) and placed in the other diagnoses group (see below). As shown in Table 2, most (39/50, 78%) of the tumours with a B-cell phenotype were diagnosed in the cervical lymph node, extra-nodal head and neck region or gastrointestinal tract. Children with BL were predominantly male (Male (M):Female (F) = 16:2), while those with B-LBL were more frequently female (M:F = 4:7). Children with T-LBL were predominantly male (M:F = 17:6), and

almost exclusively diagnosed either in the mediastinal region (15/23, 65%) or cervical lymph node (7/23, 30%). Children with ALCL were more frequently female (M:F = 8:12) and often had nodal primaries (14/20, 70%).

A total of 59 (38%) cases were excluded from the study due to other diagnoses (56 cases, Table 3), no consensus diagnosis (one case), and insufficient material (2 cases). Those cases with other diagnoses are listed in Table 3. The cases in the other diagnosis group were summarized as follows: (1) Hodgkin lymphoma was diagnosed by its characteristic histology and the presence of CD30-positive and, less frequently, CD15-positive, Reed-Sternberg cells [5,8]. However, the hallmark cells of ALCL and activated lymphocytes in reactive lymphadenopathy were also positive for CD30 [8]. (2) Like Burkitt lymphoma and B-ALL, T/B-LBL and precursor T/B-lymphoblastic leukaemias were indistinguishable cytologically [5]. All of the ALL cases in this series had more than 25% of bone marrow involvement at the time of diagnosis. In contrast, other leukaemia cases presented with an extramedullary mass, and no or few leukaemic blasts were found in the bone marrow at the time of diagnosis. (3) More than 1/3 of the cases in the other diagnoses group were classified into a category of reactive lymphoid hyperplasia (benign polyclonal lymphoproliferation). It seemed to be a unique situation in paediatric age group to have such a large number of cases of reactive lymphoid hyperplasia as one of the differential diagnoses from NHL. (4) A case of Ewing's/peripheral Primitive Neuroectodermal Tumour (pPNET) presented us with a diagnostic difficulty, since the tumour cells, like those in many of the LBL cases, showed positive staining for CD99 (MIC2 gene product). Detection on *EWS* gene translocation and expression of neural markers confirmed the diagnosis of this particular case [9].

4. Discussion

This is the first report illustrating the central pathology review system of paediatric NHL and the experience of the JACLS NHL-98 study. Paediatric NHL cases are different from adult NHL cases: more than 90% of them are high-grade, approximately 75% present at advanced stages at diagnosis, and they often show early dissemination or leukaemic manifestation [7,10]. Tumours in paediatric NHL are frequently found in extra-nodal locations, and are difficult to diagnose, clinically as well as histopathologically [7,11,12]. Furthermore, because of striking differences in proliferative kinetics, treatment protocols for such patients should be appropriately determined based on a precise diagnosis

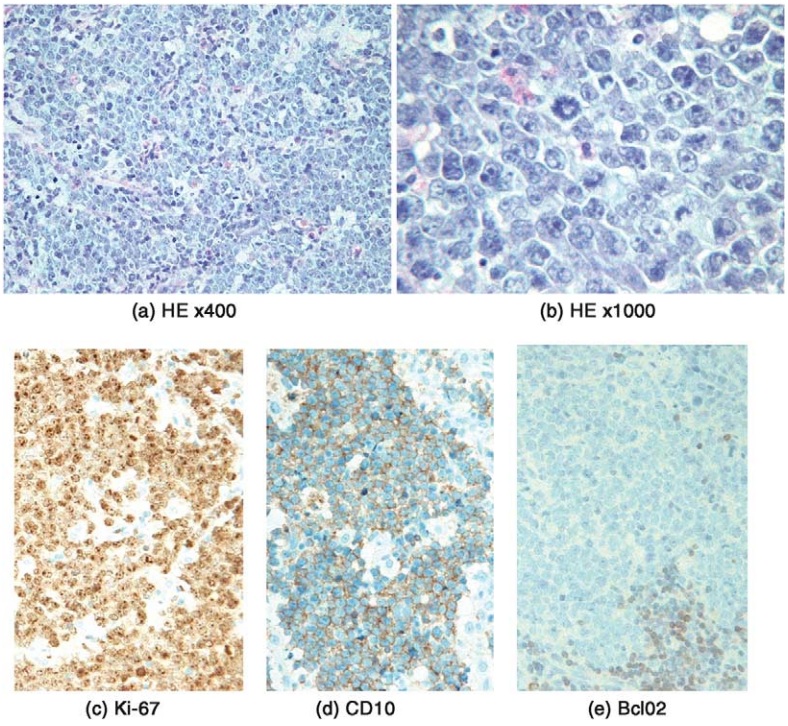

Fig. 1. Atypical Burkitt/Burkitt-like variant of Burkitt lymphoma. (a) Diffuse proliferation of medium-sized to large lymphocytes. There are some macrophages, but atypical "starry-sky" pattern is not observed. Haematoxylin and eosin (H&E) stain. (b) Tumour cells show greater pleomorphism in nuclear size and shape than those commonly seen in classical Burkitt lymphoma. There are numerous mitotic figures. Nucleoli are more prominent. A few eosinophils are recognised. H&E stain. (c) Nearly 100% of the tumour cells are positive for Ki-67. Immunostain for Ki67. (d) Tumour cells are positive for CD10. Immunostain for CD10. (e) Tumour cells are negative for Bcl-2. Residual lymphocytes are positive for Bcl-2 (lower right). Immunostain for Bcl-2.

Table 2
Paediatric non-Hodgkin's lymphoma cases from the JACLS NHL-98

Histological type	Age (median)	Gender	Nodal					Extra-nodal						
			Cervical LN	Axillary LN	Inguinal LN	Mesenteric LN	Other LN	Head and neck	Mediastinum	Abdomen	Gastrointestinal	Bone	Skin	Other
B-LBL ($N=11$)	11 m–12 y (3 y)	M4:F7	4							1	3	1	1	1 Testis
BL ($N=18$)	1 y–13 y (9 y)	M16:F2	6					5		1	5			1 Kidney
DLBCL ($N=18$)	5 y–16 y (9.5 y)	M10:F8	3					8	2		3	1		1 Testis
B-NHL, NOS ($N=3$)	3 y, 12 y, 14 y	M3:F0	2							1				
T-LBL ($N=23$)	2 y–14 y (10 y)	M17:F6	7				1 Elbow		15					
ALCL ($N=20$; T 13, Null 7)	1 y–13 y (10 y)	M8:F12	7	2	2	2	1 Lung hilar		2				2	2 Soft tissue
pT/NK-NHL ($N=3$)	5 y, 6 y, 9 y	M2:F1	1					1		1				

Primary site (Nodal and Extra-Nodal), determined based on the clinical information. B-LBL, precursor B lymphoblastic lymphoma; BL, Burkitt lymphoma; DLBCL, diffuse large B-cell lymphoma; B-NHL, NOS: B-cell non-Hodgkin's lymphoma, not otherwise specified; T-LBL, precursor T lymphoblastic lymphoma; ALCL, anaplastic large cell lymphoma; pT/NK-NHL, peripheral T-cell/natural killer-cell lymphoma; m, months; y, years; M, male; F, female; LN, lymph node; JACLS, Japan Association of Childhood Leukaemia Study.

Table 3
Cases in the other diagnoses group

	Gender	Age (median)	Nodal				Extra-Nodal				
			Cervical LN	Axillary LN	Inguinal LN	Mesenteric LN	Head and neck	Mediastinum	Abdomen	Skin/soft tissue	Other
Classical Hodgkin lymphoma ($N=7$)											
Mixed cellularity ($N=3$)	M1:F2	3 y, 5 y, 7 y	3								
Nodular sclerosis ($N=2$)	M1:F1	11 y, 13 y	2								
Lymphocyte-rich ($N=2$)	M1:F1	8 y, 9 y		1	1						
Leukaemia ($N=11$)											
ALL (precursor B) ($N=3$)	M1:F2	6 y, 7 y, 10 y	1						1	1	
ALL (precursor T) ($N=4$)	M3:F1	6 y–11 y (7 y)	3		1						
ALL (B) ($N=1$)	M1:F0	13 y							1		
AML (acute monoblastic leukaemia, M5a) ($N=1$)	M1:F0	1 y									1 (testis)
Precursor myeloid/NK cell leukaemia ($N=1$)	M1:F0	1 y								1	
Mixed lineage leukaemia (Ph1-positive) ($N=1$)	M0:F1	1 y					1				
Reactive lymphoid hyperplasia ($N=20$)	M16:F4	4 m–16 y (8.5 y)	11		2	3	1		2	1	
Histiocytic necrotising lymphadenitis ($N=7$)	M5:F2	7 y–15 y (13 y)	6	1							
EBV-associated diseases ($N=4$)											
Infectious mononucleosis ($N=2$)	M1:F1	4 y, 6 y	2								
EBV-associated LPD (T-cell) ($N=1$)	M1:F0	10 y							1		
PTLD (DLB) ($N=1$)	M1:F0	12 y									1 (systemic)
HIV-related lymphadenopathy ($N=1$)	M0:F1	6 m				1					
Miscellaneous ($N=3$)											
Extramedullary plasmacytoma ($N=1$)	M1:F0	10 y	1								
Castleman's disease ($N=2$)	M1:F1	4 y, 13 y	2								
Others ($N=3$)											
Ewing's sarcoma/pPNET ($N=1$)	M0:F1	5 y								1	
Panniculitis ($N=1$)	M0:F1	15 y								1	
Hypertrophic thymus ($N=1$)	M0:F1	4 y						1			

Primary site (Nodal and Extra-Nodal), determined based on the clinical information. EBV, Epstein–Barr Virus; ALL, acute lymphoblastic leukaemia; pPNET, peripheral Primitive Neuroectodermal Tumour; PTLD, post-transplant lymphoproliferative disorder; m, months; y, years; M, male; F, female; LN, lymph node.

and classification [11,13]. Therefore, the central pathology review system, consisting of the rapid and consensus review supported by immunophenotyping results, was essential and critical to carry out our clinical trial. As described in this report, our system successfully generated the pathological data with excellent agreement rates in terms of the diagnosis and typing of the NHL cases between the rapid and consensus reviews.

All cases were initially reviewed using the REAL classification, and, subsequently, were re-classified according to the new WHO classification. There were 5 patients whose diagnoses changed from DLBCL to BL during this process of re-classification. Fortunately, we had the same treatment approaches for patients with NHL of the mature B-cell phenotype. Accordingly, the treatment protocols for these patients did not change in our clinical trial. In the classification, there still seems to be some difficulty/confusion in distinguishing between BL (atypical Burkitt/Burkitt like variant of BL) and DLBCL. For the diagnosis of BL (atypical Burkitt/Burkitt like variant of BL), a growth fraction of nearly 100% in the tumour tissue is critical according to the WHO guideline. In addition, CD10 and Bcl-2 immunostainings are reported as useful for the distinction between BL and DLBCL [14]: in our series CD10 was positive in 15/16 BL cases and 7/12 DLBCL cases, whereas Bcl-2 was positive in 1/6 BL cases and 4/8 DLBCL cases. Cytogenetic analysis for the detection of MYC translocation was largely unsuccessful in our study.

Table 4 summarises paediatric NHL studies in the literature [15–19]. As shown in this table, paediatric NHL cases are mainly composed of only three or four different types according to the various classifications. Since these investigators used different classifications [1,3,19], it is difficult to make any direct comparisons for the incidence of NHL types between the previous studies and our current series using the new WHO classification [5] that distinguishes four major types: i.e., LBL, BL, DLBCL and ALCL. The small sample size of our study also means any conclusive statements must be cautiously made at this time. However, some or all of these types in the new WHO classification were included in the previous classifications and were given similar definitions. Recent advances in the field of haematopathology research, such as the introduction of a panel of systematic immunophenotyping with an increased number of good quality antibodies available for use on paraffin sections (Table 1), determination of a proportion of Ki-67- positive cells for the diagnosis of BL [5], and the detection of the ALK translocation for the diagnosis of ALCL [20], now enable us to recognise and distinguish these NHL types more precisely. After taking these into account, however, our study still seems to include less BL cases and more DLBCL and ALCL cases than previous reports from Western countries and Taiwan. Other investigators have reported that the distribution of adult NHL types in Japan was also different to that observed in Western countries [21]. These differ-

Table 4
Paediatric Non-Hodgkin's lymphoma studies in the literature

Author [Ref.] (country)	Study period (classification)	Number of cases[a]	Age (median)	Major histological type[b]
Wilson, JF [13] (U.S.)	1977–1980 (Rappaport)	213	⩽18 years	50.2% Undifferentiated (Burkitt's and non-Burkitt's) 34.3% LBL 13.6% Large cell/histiocytic lymphoma
Murphy, SB [15] (U.S.)	1962–1986 (Working Formulation)	338	7 months–21 years (10 years)	38.3% diffuse small non-cleaved cell 28.1% LBL 26.3% DLCL
Reighter, A [16] (Germany)	1986–1990 (Updated Kiel)	261	0.6 years–17.8 years	42.5% BL 28.0% LBL 7.7% DLBCL 6.9% ALCL
Wright, D [17] (U.K.)	1990–? (Updated Kiel)	293		44.4% BL 28.7% LBL 7.5% DLBCL 15.7% ALCL
Yang, C-P [18] (Taiwan)	1992–1998 (Working Formulation)	181	2.4 months–18.3 years	42.5% BL 29.8% LBL 27.6% DLCL (including ALCL)
Present study (Japan)	1998–2002 (WHO)	96	11 months–16 years (9 years)	18.8% BL 35.4% LBL 18.8% DLBCL 20.8% ALCL

Undifferentiated: undifferentiated lymphoma; LBL: lymphoblastic lymphoma; DLCL: diffuse large cell lymphoma; BL, Burkitt's lymphoma; DLBCL, diffuse large B-cell lymphoma; ALCL, anaplastic large cell lymphoma.

[a] Non-Hodgkin's lymphoma cases only (B-ALL cases, excluded).

[b] Only major types and their percentages are listed.

ences, in both paediatric and adult NHL types, between Japanese patients and patients from other countries could be due to different ethnic as well as environment/geographical backgrounds [21,22].

The classification of LBL according to the new WHO guidelines deserves a brief comment. LBL cases were once classified into three subsets due to the limited number of antibodies available for immunophenotyping: approximately 2/3 of the cases were classified in the T-cell subset and the rest of the cases were classified as either B-cell or indeterminate [16,17]. By using a panel of antibodies at the central laboratory, all LBL cases included in our study were classified into either T-cell or B-cell subsets, and no cases were classified as indeterminate. Among the various immunophenotyping markers used in our series, TdT+ and $CD3_{\epsilon}$+were useful for classifying a LBL in the T-cell lineage (23/34, 67.6%), and TdT+, CD20 +/−, and CD79a+were useful to define those belonging to the B-cell lineage (11/34, 32.4%). In our study 7/11 (64%) of the B-LBL cases were positive for both CD20 and CD79a, and 4/11 (36%) showed positive staining for CD79a only. In our series, there was only one case whose tumour was TdT+, CD3ϵ + and CD79a+. Since the T-cell receptor beta chain gene was clonally rearranged, but the immunoglobulin heavy chain gene showed a germline configuration, this particular case was classified as being in the T-LBL subset with an aberrant CD79a expression [23]. Both T-LBL and B-LBL tumours were diagnosed in nodal (cervical lymph node) and extra-nodal locations. In our series, the extra-nodal T-LBL tumours developed exclusively in the mediastinum, while the extra-nodal B-LBL tumours (as has been reported by other investigators) were found in unusual locations, such as the bone, skin, and testis, in addition to the gastrointestinal tract [24–26]. Positive TdT staining was often helpful, and even critical, for distinguishing B-LBL cases from BL cases, especially when the tumours developed in the gastrointestinal tract. This was particularly important since the patients with B-LBL were assigned to different treatment protocols from those with BL [12,26].

The central pathology review system of our study accepted pathology materials from those cases with clinically-suspected paediatric NHL immediately after surgery/biopsy, without waiting for the final pathological diagnosis from the contributing institutions. We decided to choose this system to review a large number of suspicious cases mainly because there are considerable numbers of neoplastic and reactive lesions that mimick NHL among paediatric patients. In addition, not all of the participating institutions were fully equipped with standardised immunohistochemical and molecular techniques to perform differential diagnoses and to precisely type the NHL cases. With this system, we successfully avoided a potential failure of enrolling on the study in a timely manner some of the NHL cases presenting diagnostic dilemma/difficulty. For example, some of ALCL cases with a feature, focally or diffusely, of either small-cell or lymphohistiocytic variant [5,27], comprising 35% (7/20) of all the ALCL cases in this series, could well have been missed because of difficulties in identifying the diagnostic hallmark cells in a background of intense inflammatory infiltrates [5,27]. Nevertheless, 59 (38%) cases were rejected from our study after the review.

In summary, we established a central pathology review system with immunophenotyping facilities for paediatric NHL cases. Pathology materials from all clinically-suspected cases were reviewed, and NHL cases were classified according to the new WHO guidelines. With this system, highly reliable pathology data were provided to support the nationwide Japanese clinical trial, the JACLS NHL-98.

Acknowledgements

This study was supported (in part) by a Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (no. 13670194). The authors thank Mr. Yoshifumi Kaneko for his excellent laboratory assistance. They also thank Ms. Mika Ito for her dedication in developing and maintaining the database system for this study.

Appendix

Members of lymphoma committee:

T. Takimoto (Nagoya National Hospital), K. Horibe (Nagoya National Hospital), H. Misu (Kobe Children's Hospital), R. Kobayashi (Hokkaido University), M. Yazaki (Nagoya City University), M. Inoue (Osaka Medical Center and Research Institute for Maternal and Child Health), A. Akazai (Okayama Saiseikai General Hospital), N. Fujita (Hiroshima Red Cross Hospital & Atomic-Bomb Survivors Hospital), A. Nakagawa (Aichi Medical University)

Principal Investigators of JACLS NHL-98:

J. Miura (Asahikawa Red Cross Hospital), T. Kudo (Sapporo Medical University), R. Kobayashi (Hokkaido University), H.Naito (Sapporo City General Hospital), A. Iguchi (Kitami Red Cross Hospital), M. Yoshida (Asahikawa Medical College), T. Oda (Hokkaido Children's Hospital and Medical Center), N. Adachi (Kushiro City General Hospital), Y. Sakai (Municipal Muroran General Hospital), T. Matubayashi (Seirei Hamamatsu Hospital), T. Ito (Toyohashi Municipal Hospital), M. Yazaki (Nagoya City University), S. Kojima (Nagoya University), T. Matuyama (Nagoya Red Cross Hospital), K. Horibe (Nagoya

National Hospital), T. Ono (Komaki City Hospital), K. Isogai (Gifu University), Y. Komada (Mie University), A. Yoshioka (Nara Medical University), H. Kawasaki (Kansai Medical University), J. Hara (Osaka University), M. Sako (Osaka City General Hospital), H. Tanaka (Osaka Red Cross Hospital), A. Tawa (Osaka National Hospital), M. Yoshikawa (Osaka Prefectural General Hospital), K. Kawa (Osaka Medical Center and Research Institute for Maternal and Child Health), H. Miyata (Kinki University), N. Aoyagi (Wakayama Medical University), M. Yamamoto (Hyogo College of Medicine), Y. Kosaka (Kobe University), O. Mabuchi (Kobe Children's Hospital), Y. Sawamoto (Hoshigaoka Koseinenkin Hospital), M. Oda (Okayama University), K. Ueda (Hiroshima University), K. Hamamoto (Hiroshima Red Cross Hospital & Atomic-Bomb Survivors Hospital), T. Imai (Kagawa Medical University), Y. Ishida (Ehime University), N. Kodani (Matsuyama Red Cross Hospital), Y. Otoh (Ehime Prefectural Central Hospital), R. Kanai (Shimane Medical University), S. Suenobu (Oita Medical University), E. Ishii (Saga Medical School), H. Ayukawa (Yamaguchi University), T. Nakahata (Kyoto University), Y. Wakazono (Kyoto-Katsura Hospital), I. Usami (Kobe City General Hospital), K. Matsubara (Nishi-Kobe Medical Center), T. Momoi (Japanese Red Cross Society Wakayama Medical Center), T. Ishioka (Matsue Red Cross Hospital), M. Mayumi (Fukui Medical University), S. Seto (Kishiwada City Hospital).

References

1. Non-Hodgkin's Lymphoma Pathologic Classification Project. National Cancer Institute sponsored study of classification of non-Hodgkin's lymphomas: Summary and description of a Working Formulation for clinical usage. *Cancer* 1982, **49**, 2112–2135.
2. Gerard-Marchant R, Hamlin I, Lennert K, *et al*. Classification of non-Hodgkin's lymphomas. *Lancet* 1974, **2**, 406–408.
3. Lennert K, Feller A. *Histopathology of Non-Hodgkin's lymphomas (Based on the Updated Kiel Classification)*. Berlin, Springer-Verlag, 1992.
4. Suchi T, Tajima K, Nanba K, *et al*. Some problems on histopathological diagnosis of non-Hodgkin's lymphoma—a proposal of a new type. *Acta Pathol Jpn* 1979, **29**, 755–776.
5. Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *Pathology and genetics of tumours of haematopoietic and lymphoid tissues*. Lyon, IARC Press, 2001.
6. Harris NL, Jaffe ES, Stein H, *et al*. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood* 1994, **84**, 1361–1392.
7. Murphy SB. Classification, staging, and end results of treatment of childhood non-Hodgkin's lymphomas: dissimilarities from lymphomas in adults. *Semin Oncol* 1980, **7**, 332–339.
8. Stein H, Mason DY, Gerdes J, *et al*. The expression of the Hodgkin's disease associated antigen Ki-1 in reactive and neoplastic lymphoid tissue: evidence that Reed-Sternberg cells and histiocytic malignancies are derived from activated lymphoid cells. *Blood* 1985, **66**, 848–858.
9. Ozdemirli M, Fanburg-Smith JC, Hartmann D-P, *et al*. Precursor B-lymphoblastic lymphoma presenting as a solitary bone tumor and mimicking Ewing's sarcoma. *Am J Surg Pathol* 1998, **22**, 795–804.
10. Sandlund JT, Downing JR, Crist WM. Non-Hodgkin's lymphoma in childhood. *N Engl J Med* 1996, **334**, 1238–1248.
11. Perkins SL. Work-up and diagnosis of pediatric non-Hodgkin's lymphomas. *Pediatric and Developmental Pathology* 2000, **3**, 374–390.
12. Pinkerton CR. The continuing challenge of treatment for non-Hodgkin's lymphoma in children. *Br J Haematol* 1999, **107**, 220–234.
13. Wilson JF, Jenkin RDT, Anderson JR, *et al*. Studies on the pathology of non-Hodgkin's lymphoma of childhood. *Cancer* 1984, **53**, 1695–1704.
14. Nakamura N, Nakamine H, Tamaru J, *et al*. The distinction between Burkitt lymphoma and diffuse large B-cell lymphoma with c-myc rearrangement. *Mod Pathol* 2002, **15**, 771–776.
15. Murphy SB, Fairclough DL, Hutchison RE, *et al*. Non-Hodgkin's lymphomas of childhood: an analysis of the histology, staging, and response to treatment of 338 cases at a single institution. *J Clin Oncol* 1989, **7**, 186–193.
16. Reiter A, Schrappe M, Parwaresch R, *et al*. Non-Hodgkin's lymphomas of childhood and adolescence: results of a treatment stratified for biologic subtypes and stage—a report of the Berlin-Frankfult-Munster group. *J Clin Oncol* 1995, **13**, 359–372.
17. Wright D, McKeever P, Carter R. Childhood non-Hodgkin lymphomas in the United Kingdom: findings from the UK Children's Cancer Study Group. *J Clin Pathol* 1997, **50**, 128–134.
18. Yang C-P, Hung I-J, Jaing T-H, *et al*. Treatment results of the TPOG-NHL92 protocols for childhood Non-Hodgkin's lymphomas in Taiwan: a report from the Taiwan Pediatric Oncology Group (TPOG). *Acta Pediatr Tw* 2000, **41**, 193–204.
19. Rappaport H. *Tumors of the hematopoietic system. Atlas of Tumor Pathology, Section III*. Washington, DC, Fascicle, AFIP, 1966 pp. 97–161.
20. Pulford K, Lamant L, Morris SW, *et al*. Detection of anaplastic lymphoma kinase (ALK) and nucleolar protein nucleophosmin (NPM)-ALK proteins in normal and neoplastic cells with the monoclonal antibody ALK1. *Blood* 1997, **89**, 1394–1404.
21. Lymphoma Study Group of Japanese Pathologists. The World Health Organization classification of malignant lymphoma in Japan: incidence of recently recognaized entities. *Pathol Int* 2000, **50**, 696–702.
22. Alessandri AJ, Pritchard SL, Schultz KR, *et al*. A population-based study of pediatric anaplastic large cell lymphoma. *Cancer* 2002, **94**, 1830–1835.
23. Pilozzi E, Müller-Hermelink H-K, Falini B, *et al*. Gene rearrangements in T-cell lymphoblastic lymphoma. *J Pathol* 1999, **188**, 267–270.
24. Ozdemirli M, Fanburg-Smith JC, Hartmann D-P, *et al*. Precursor B-lymphoblastic lymphoma presenting as a solitary bone tumor and mimicking Ewing's sarcoma: a report of four cases and review of the literature. *Am J Surg Pathol* 1998, **22**, 795–804.
25. Soslow RA, Baergen RN, Warnke RA. B-lineage lymphoblastic lymphoma is a clinicopathologic entity distinct from other histologically similar aggressive lymphomas with blastic morphology. *Cancer* 1999, **85**, 2648–2654.
26. Neth O, Seidermann K, Jansen P, *et al*. Precursor B-cell lymphoblastic lymphoma in childhood and adolescence: clinical features, treatment, and results in trials NHL-BFM 86 and 90. *Med Pediatr Oncol* 2000, **35**, 20–27.
27. Benharroch D, Meguerian-Bedoyan Z, Lamant L, *et al*. ALK-positive lymphoma: a single disease with a broad spectrum of morphology. *Blood* 1998, **91**, 2076–2084.