

Original Article

# Clinicopathological significance of CD79a expression in classic Hodgkin lymphoma

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Classic Hodgkin lymphoma (CHL) is a lymphoid neoplasia characterized by the presence of large tumor cells, referred to as Hodgkin and Reed-Sternberg (HRS) cells, originating from B-cells in an inflammatory background. As the clinical significance of B-cell markers has yet to be fully elucidated, this study aimed to clarify the clinicopathological significance of CD79a in 55 patients with CHL. They were immunohistochemically divided into two groups, comprising of 20 CD79a-positive and 35 CD79a-negative patients. There was no significant correlation between CD79a and CD20 expression ( $r_s = 0.125$ ,  $P = 0.362$ ). CD79a-positive patients were significantly older at onset ( $P = 0.011$ ). There was no significant correlation between CD79a-positivity and clinical stage ( $P = 0.203$ ), mediastinal involvement ( $P = 0.399$ ), extranodal involvement ( $P = 0.749$ ), or laboratory findings, including serum levels of lactate dehydrogenase ( $P = 1$ ) and soluble interleukin-2 receptor ( $P = 0.251$ ). There were significant differences in overall survival (OS) ( $P = 0.005$ ) and progression-free survival (PFS) ( $P = 0.007$ ) between CD79a-positive and CD79a-negative patients (5-year OS: 64.6% and 90.5%; 5-year PFS: 44.0% and 76.6%, respectively). Five patients in whom the majority (> 80%) of HRS cells expressed CD79a consisted of 4 males and 1 female aged between 52 and 81 years; 4 of them were in a limited clinical stage. We concluded that CD79a-positive CHL may have unique clinicopathological features.

**Keywords:** classic Hodgkin lymphoma, CD79a, prognosis, immunohistochemistry

## INTRODUCTION

Classic Hodgkin lymphoma (CHL) is a lymphoid neoplasia characterized by the presence of large pathognomonic cells, such as Hodgkin and Reed-Sternberg (HRS) cells, in an inflammatory background.<sup>1</sup> In Japan, CHL represents 5–10% of all lymphomas, with a bimodal age distribution, and peak incidences being between 15 and 34 years of age and between 55 and 84 years of age.<sup>2</sup> In contrast, peak incidences of CHL in Western countries, such as the United States<sup>1</sup> or Germany,<sup>3</sup> are predominantly in a younger cohort. The majority of patients with CHL have a good clinical course with chemotherapy, including doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) therapy, and/or radiation therapy. Currently, the 5-year survival rate for patients has been reported to be over 95% in a limited stage<sup>4,5</sup> and over 80% in an advanced stage.<sup>5,6</sup>


The origin of HRS cells had been unknown until recently because they frequently express markers of different hematopoietic lineages.<sup>7,8</sup> Recent studies revealed HRS cells to have clonally rearranged immunoglobulin genes with a high load of somatic mutations.<sup>9-11</sup> Most cases of CHL originate from germinal center B-cells.<sup>12</sup> However, immunohistochemical analysis often cannot detect B-cell markers, such as CD20 and CD79a, in HRS cells.<sup>9,13-18</sup> In contrast, the expression of PAX5 is usually conserved.<sup>19</sup> Expression of OCT-2 and BOB.1, essential transcription factors for immunoglobulin genes, can also be detected in some cases.<sup>14,15,20</sup> Previous studies reported varying results regarding the expression patterns of these B-cell markers,<sup>16,18</sup> although their clinical significance has yet to be fully elucidated. We therefore aimed to clarify the clinicopathological significance of CD79a by comparing CD79a-positive and CD79a-negative CHL.

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## MATERIALS AND METHODS

### Patients

Fifty-five patients, who were initially diagnosed with CHL, were examined at Hiroshima Red Cross Hospital & Atomic-bomb Survivors Hospital via excisional biopsy between 2002 and 2016. Original diagnoses of CHL were confirmed to meet the diagnostic criteria of the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, Revised 4th Edition.<sup>1</sup> Patients, who had been administered immunosuppressants, such as methotrexate, before the initial diagnosis of CHL were excluded. The use of patient specimens and medical records was approved by the Institutional Review Board of Hiroshima Red Cross Hospital & Atomic-bomb Survivors Hospital.

### Histology and immunohistochemistry

The tissue samples used consisted of 51 lymph nodal and 4 anterior mediastinal biopsy specimens. The spleen was also examined for one patient. Tissue samples were fixed in 10% formalin and embedded in paraffin. Four-micron-thick sections were stained with HE. Immunohistochemical studies were performed using standard manual methods with the Dako REAL™ EnVision™ Detection Systems or via the automated stainer BOND-III. The primary antibodies used were as follows: anti-CD30 (Ber-H2, 1:100, Agilent Technologies, Santa Clara, CA), anti-CD15 (BY87, 1:40, Leica Biosystems, Nussloch, Germany), anti-CD20 (L26, 1:200, Nichirei Biosciences, Tokyo, Japan), anti-CD79a (JCB117, 1:400, Agilent Technologies, Santa Clara, CA), anti-PAX5 (1EW, 1:100, Leica Biosystems, Nussloch, Germany), anti-CD3 (PS1, pre-dilute, Nichirei Biosciences, Tokyo, Japan), anti-BOB.1 (sc-955, 1:200, Santa Cruz Biotechnology, Dallas, TX), anti-OCT-2 (sc-233, 1:500, Santa Cruz Biotechnology, Dallas, TX), and PD-L1 (E1L3N, 1:200, Cell Signaling Technology, Danvers, MA). Based on previous studies, a sample was considered positive if  $\geq 10\%$  of the tumor cells were stained.<sup>14</sup> Furthermore, CD20 and CD79a expression was classified into 11 groups by every 10% cut-off value.

Dual immunohistochemistry for CD79a and CD30 was performed for two representative patients using the Leica ChromoPlex™ 1 Dual Detection for BOND and BOND-III stainer. The clones of primary antibodies, and their dilution, source, enzyme, and chromogen for primary and secondary stains were as follows: anti-CD79a (JCB117, 1:25, Agilent Technologies, Santa Clara, CA), horseradish peroxidase, DAB; anti-CD30 (Ber-H2, 1:20, Agilent Technologies, Santa Clara, CA), alkaline phosphatase, and Fast Red, respectively.

Detection of latent Epstein-Barr virus (EBV) infection was performed by means of *in-situ* hybridization for EBV-encoded small RNAs (EBERs) using PNA Probe/Fluorescein and anti-fluorescein isothiocyanate rabbit polyclonal antibody.

### Statistical analysis

Fisher's exact test, the chi-square test, and Mann-Whitney U test were used to examine the differences in characteristics between two groups, as appropriate. Correlation between the proportions of CD79a- and CD20-positive cells was examined by the Spearman's rank correlation coefficient. The Kaplan-Meier method was used for analyzing patient survival data. The log-rank test was applied to analyze the differences in survival; overall survival (OS) and progression-free survival (PFS) were considered for the evaluation of survival.

The results were considered significant if the *P*-value was less than 0.05. All data were analyzed using R version 3.4.2.

## RESULTS

### Clinicopathological findings

Clinical characteristics of the 55 patients with CHL at the time of biopsy are summarized in Table 1. The patients included 34 males and 21 females, with a median age of 51 years (range, 15–86 years). According to the Lugano Classification 2014,<sup>21</sup> 35 patients were in a limited clinical stage (CS) (stage I or II) and 20 were in an advanced CS (stage III or IV). Thirty-eight (69.1%) patients underwent biopsy from cervical lymph nodes, whereas 7 (12.7%) underwent biopsy from infra-diaphragmatic regions. Extranodal involvement included bone marrow, subcutis, lung, liver, pleural effusion and/or pleura, kidney, and stomach in 8, 4, 3, 2, 2, 1, and 1 patients, respectively. Histological subtypes consisted of 27 cases with mixed cellularity (MCCHL), 20 cases with nodular sclerosis (NSCHL), 6 cases with lymphocyte-rich (LRCHL), and 2 unspecified cases. Forty-eight patients (87.3%) were administered ABVD therapy, 12 of them also received radiotherapy. Five patients only received radiotherapy. The 5-year OS and PFS rates were 77.8% and 57.1%, respectively.

### CD79a expression

Out of the 55 patients with CHL, 20 were positive for CD79a (Fig. 1); 3 patients with 10–20%, 3 with 20–30%, 5 with 30–40%, 4 with 40–50%, none with 50–60%, 60–70%, or 70–80%, 1 with 80–90%, and 4 with 90–100% (Fig. 2). Most of the CD79a-positive cells (19 patients) exhibited weaker staining than normal B-cells and plasmacytes. Each HRS cell demonstrated homogenous CD79a staining intensity inside the cytoplasm.

Dual immunohistochemistry in a selected case demonstrated some HRS cells to be both CD30- and CD79a-positive, whereas others were CD30-positive but CD79a-negative.

### CD20 expression and its correlation with CD79a

Out of the 55 patients, 25 were positive for CD20; 15 patients with 10–20%, 4 with 20–30%, 1 with 30–40%, 2 with 40–50%, 2 with 50–60%, none with 60–70%, 1 with

**Table 1.** Patient characteristics, and histological subtypes of CD79a-positive and CD79a-negative CHL

Variables	All CHL cases	CD79a-positive CHL	CD79a-negative CHL	<i>P</i>
Number of patients	55	20	35	
Age, median (range)	51 (15–86)	69 (15–82)	37 (17–86)	0.011 †
Sex, male	34 (61.8)	14 (70.0)	20 (57.1)	0.399
LDH > normal *	15 (27.8)	5 (26.3) *	10 (28.6)	1
sIL-2R > normal *	45 (81.8)	14 (73.7) *	31 (88.6)	0.251
Clinical stage				0.203 ‡
I	9 (16.4)	6 (30.0)	3 ( 8.6)	
II	26 (47.3)	7 (35.0)	19 (54.3)	
III	9 (16.4)	3 (15.0)	6 (17.1)	
IV	11 (20.0)	4 (20.0)	7 (20.0)	
Mediastinal involvement	30 (54.5)	9 (45.0)	21 (60.0)	0.399
Extranodal involvement **	13 (23.6)	4 (20.0)	9 (24.3)	0.749
Bone marrow	8 (14.5)	3 (15.0)	5 (14.3)	1
Others	9 (16.4)	2 (10.0)	7 (25.0)	0.462
Histological subtype, specified				0.108 ‡
Mixed cellularity	27 (49.1)	6 (30.0)	21 (60.0)	
Nodular sclerosis	20 (36.4)	10 (50.0)	10 (28.6)	
Lymphocyte-rich	6 (10.9)	3 (15.0)	3 ( 8.6)	
Histological subtype, unspecified	2 ( 3.6)	1 ( 5.0)	1 ( 2.9)	
Initial treatment				N/A
Chemotherapy only	38 (69.1)	13 (65.0)	25 (71.4)	
ABVD	36 (65.5)	12 (60.0)	24 (68.6)	
Other regimens	2 ( 3.6)	1 ( 5.0)	1 ( 2.9)	
Radiotherapy only	5 ( 9.1)	4 (20.0)	1 ( 2.9)	
Combined therapy	12 (21.8)	3 (15.0)	9 (25.7)	
ABVD + radiotherapy	12 (21.8)	3 (15.0)	9 (25.7)	
Overall survival, months				0.005 §
Median	Not reached	141.0	Not reached	
Range	3–196	4–181	3–196	
Five-year survival rate (%)	80.2	64.6	90.5	
Progression-free survival, months				0.007 §
Median	Not reached	28.0	Not reached	
Range	0–183	2–176	0–183	
Five-year survival rate (%)	63.7	44.0	76.6	

\* Laboratory data were not obtained for one patient

\*\* Mediastinal and splenic lesions are regarded as nodal involvement.

CHL: classic Hodgkin lymphoma; LDH: lactate dehydrogenase; sIL-2R: soluble interleukin-2 receptor.

Fisher's exact test, two-sided

† Mann-Whitney U test.

‡ Chi-square test.

§ Log-rank test.

N/A, Not applicable.

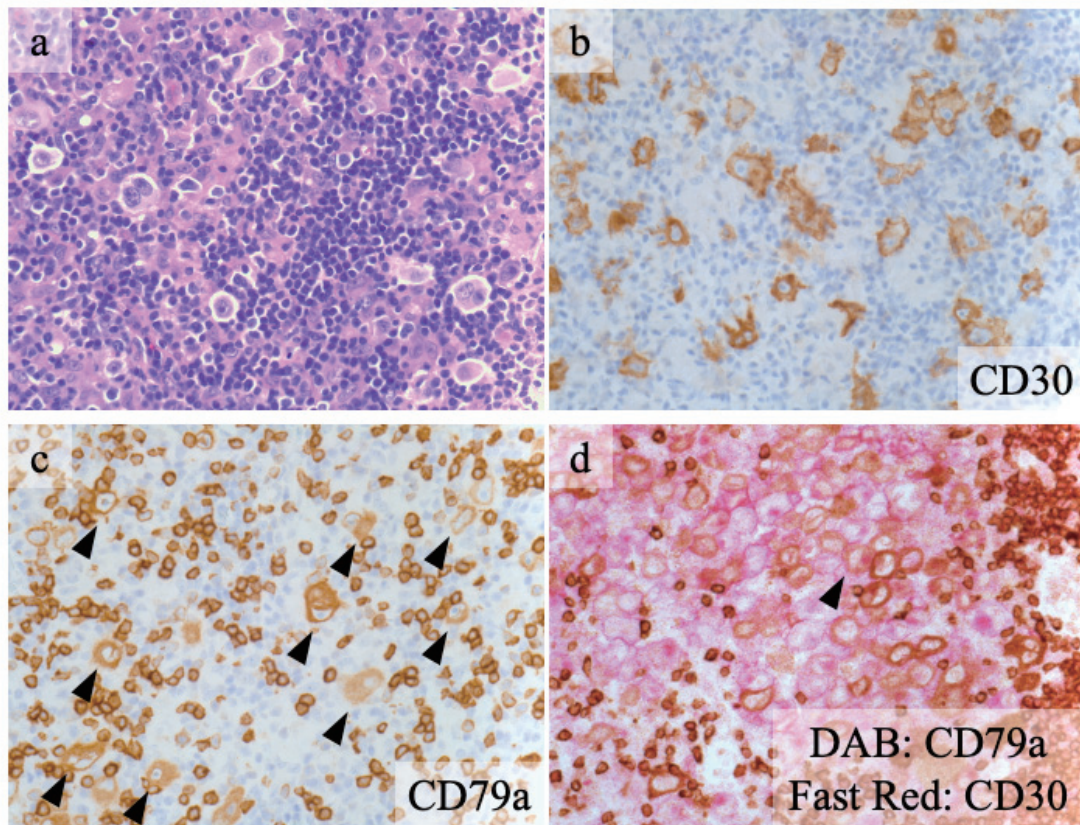
70–80%, and none with 80–90% or 90–100% (Fig. 2). Irrespective of the strength of the staining intensity, it was generally confined to the plasma membrane. There was no significant correlation between CD79a and CD20 expression ( $r_s = 0.125$ ,  $P = 0.362$ ).

### PAX5 expression

PAX5 was immunohistochemically examined in 15 CD79a-positive and 20 CD20-positive patients. Ten were positive for PAX5 in the CD79a-positive group, in contrast to 8 in the CD20-positive group ( $P = 0.141$ ).

### Comparison of the characteristics of patients with CHL based on CD79a expression

Patient characteristics were compared between CD79a-positive and -negative CHL patients (Table 1). CD79a-positive patients were significantly older in age at onset ( $P = 0.011$ ). There was no significant correlation between CD79a expression and laboratory disease characteristics, including serum levels of LDH ( $P = 1$ ) and sIL-2R ( $P = 0.251$ ), CS ( $P = 0.203$ ), mediastinal involvement ( $P = 0.399$ ), extranodal involvement ( $P = 0.749$ ), or the histological subtype ( $P = 0.108$ ). However, the OS and PFS of CD79a-positive patients were



**Fig. 1.** (a–c) Classic Hodgkin lymphoma with CD79a expression (> 90%). (a) HE-stained Hodgkin and Reed-Sternberg (HRS) cells distributed among non-neoplastic small lymphocytes and histiocytes. (b) Neoplastic cells identified based on CD30 staining. (c) Most HRS cells were positive for CD79a and showed variable staining intensity (arrowhead). (d) Dual immunohistochemistry of CD30 (Fast Red) and CD79a (DAB) in another case of classic Hodgkin lymphoma with CD79a expression (30–40%) in which CD79a-positive HRS cells expressed CD30, as indicated by an arrowhead.

significantly inferior to those of CD79a-negative patients ( $P = 0.005$  for OS and  $P = 0.007$  for PFS) (Fig. 3). The 5-year OS rates for CD79a-positive and negative patients were 64.6% and 90.5%, respectively; the PFS rates were 44.0% and 76.6%, respectively. Among 7 CD79a-positive patients with advanced-stage disease (CS III or IV), 3 exhibited disease progression and 2 died despite receiving therapy. In contrast, among 13 CD79a-negative patients with advanced-stage disease, 2 exhibited disease progression and 1 died.

Subsequently, the proportion of CD79a expression in HRS cells was assessed between younger (< 50 years of age) and older ( $\geq 50$  years) age groups (Fig. 4). The older age group exhibited a significantly higher proportion of CD79a than the younger age group ( $P = 0.001$ ). Of note, all patients with CD79a expression higher than 80% were in the older age group. In addition, the proportion of CD79a expression was compared between limited (CS I or II) and advanced stage groups (Fig. 4); both groups had a similar CD79a distribution ( $P = 0.884$ ).

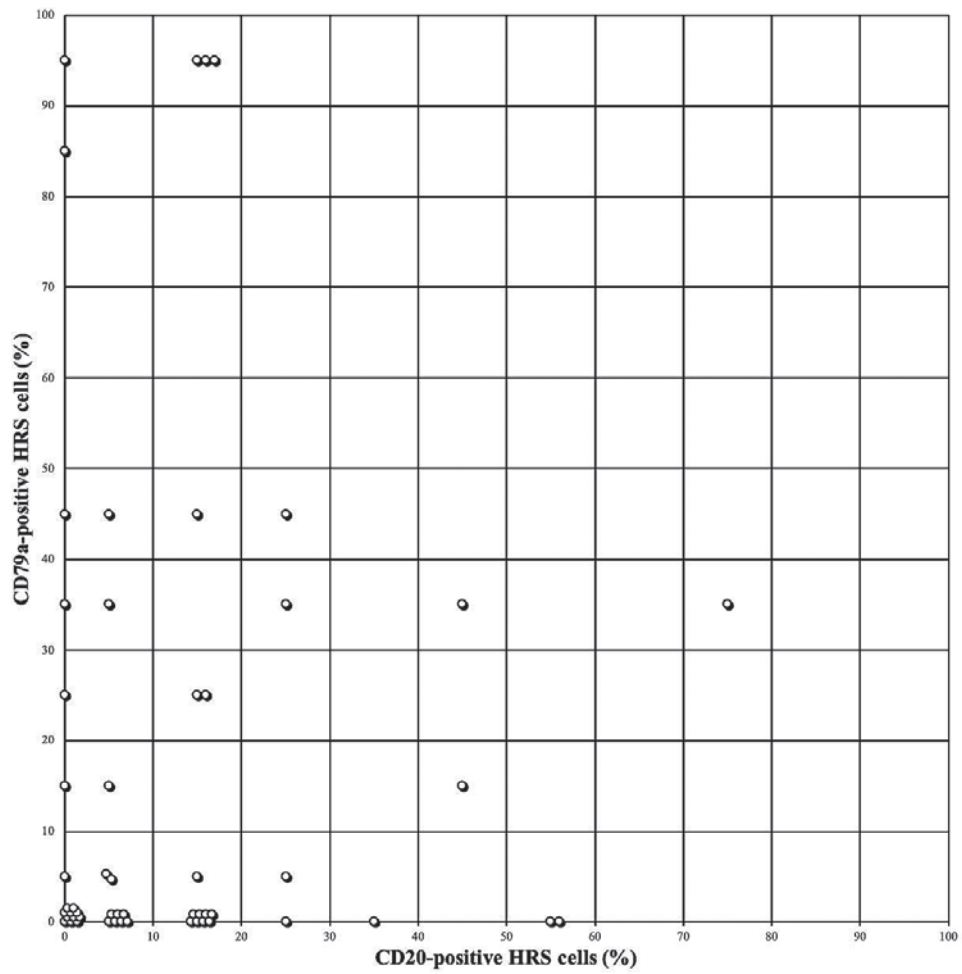
#### Clinicopathological features of 5 patients with a high proportion of CD79a-positive HRS cells

Clinicopathological findings in 5 patients in whom the majority (> 80%) of HRS cells expressed CD79a are described

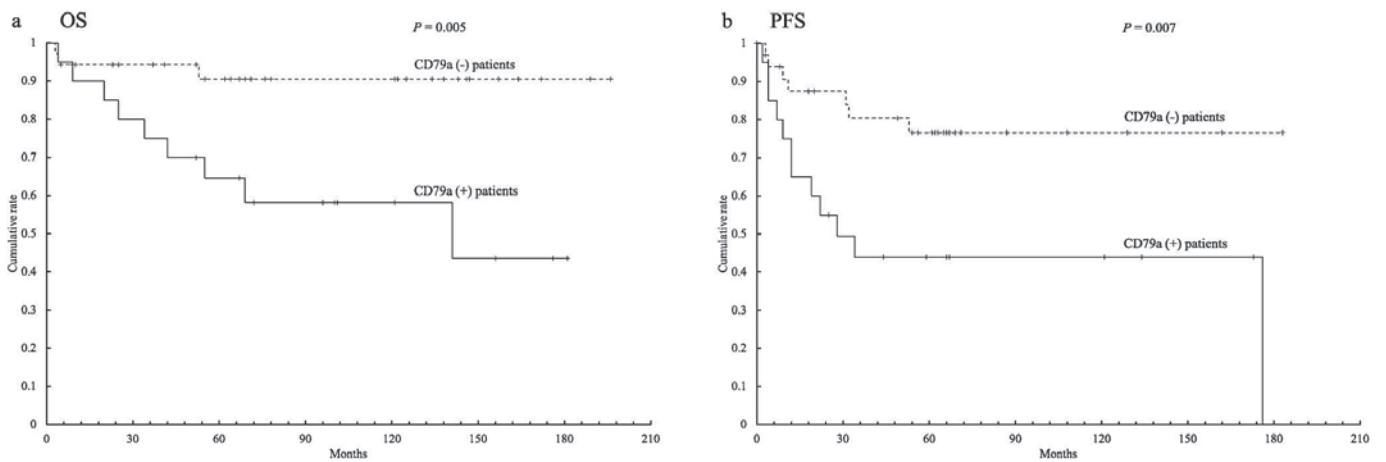
in Table 2. The patients consisted of 4 males and 1 female, aged between 52 and 81 years. At the time of biopsy, patients 1–4 were in a limited stage, whereas patient 5 was in CS III. Patient 5 was in CS II at initial presentation without mediastinal involvement. The four patients in a limited stage were only administered radiotherapy; 3 of them achieved complete response (CR), whereas one had progressive disease (PD). In contrast, patient 5 was treated using ABVD therapy, resulting in CR. Patient 2 relapsed after 26 months and died of the disease after 55 months. Patient 3 also died of the disease after 141 months. All other patients survived during the observation period between 52 and 176 months. The histological subtypes consisted of 3 MCCHL and 2 NSCHL. The intensities of CD79a were weaker than in normal B-cells in all patients, except in the splenic lesion of patient 5. Percentages of CD20-positive HRS cells were small (< 20%) in all 5 lymph node specimens, in contrast to that in the splenic lesion of patient 5 (70–80%). EBV was not detected in HRS cells of any patient.

#### DISCUSSION

In this study, 36.4% of the CHL patients were positive for CD79a. This proportion was higher than that reported in



**Fig. 2.** Scatterplot of the proportion of CD20-positive (x-axis) and CD79a-positive (y-axis) HRS cells. No significant correlation was found between these B-cell specific antigens ( $r_s = 0.125$ ,  $P = 0.362$ ).



**Fig. 3.** Survival curves of CHL patients with or without CD79a expression. **(a)** Overall survival (OS). **(b)** Progression-free survival (PFS). There were significant differences between the OS ( $P = 0.005$ ) and PFS ( $P = 0.007$ ) of the two groups.



**Table 2.** Clinicopathological features of patients in whom the majority ( $\geq 80\%$ ) of HRS cells were positive for CD79a

Patient no.	1	2	3	4	5
Tissue site	LN	LN	LN	LN	LN
Initial symptoms/Reason for consultation	follow-up PET-CT for B-cell lymphoma	CT for detailed examination of rheumatoid arthritis	LN swelling	LN swelling	Abdominal pain and fever of unknown origin
Clinical findings at biopsy					
Sex/Age	M/74	M/81	M/66	F/52	M/65
Site of involvement	Bilateral cervical LN and mediastinum	Left axillary LN	Left cervical LN	Right cervical LN	Para-aortic, mesenteric, and bilateral axillary LN, mediastinum, and spleen
Bulky tumor, $\geq 10$ cm	-	-	-	-	-
Clinical stage	II	I	I	I	III
LDH (U/L)	128	193	141	152	313
sIL-2R (U/mL)	432	1,110	391	647	3,690
Initial treatment	Radiotherapy	Radiotherapy	Radiotherapy	Radiotherapy	ABVD
Initial response	CR	CR	PD	CR	CR
Outcome after biopsy	Alive without relapse of either lymphoma, 52 months	Relapsed at 26 months, died of disease, 55 months	Progression at 4 months, died of disease, 141 months	Alive without relapse, 67 months	Development of B-cell lymphoma at 140 months, CR, alive without relapse of CHL, 176 months
Pathological findings					
Histological subtype	Mixed cellularity	Nodular sclerosis	Mixed cellularity	Mixed cellularity	Nodular sclerosis
Size and number of neoplastic cells	Medial to large, medial	Small to medial, medial	Medial to large, numerous	Small to medial, few	Small to large, numerous
CD79a (percentage and intensity)	+ , 90–100, moderate	+ , 90–100, weak	+ , 80–90, moderate	+ , 90–100, moderate	+ , 90–100, strong
CD20 (percentage)	-	+ , 10–20	-	+ , 10–20	+ , 70–80
PAX5 (intensity)	+ , weak	+ , moderate	+ , moderate	+ , weak	+ , moderate
CD30	+	+	+	+	+
CD15	+	+	-	-	-
OCT-2	+	+	±	±	+
BOB.1	-	-	-	-	-
PD-L1 (percentage and intensity)	+ , 50–60, moderate	+ , 10–20, weak	+ , 20–30, moderate	+ , 70–80, moderate	+ , 90–100, strong
EBER	-	-	-	-	-

HRS cell: Hodgkin and Reed-Sternberg cell; LN: lymph node; CHL: classic Hodgkin lymphoma; LDH: lactate dehydrogenase; sIL-2R: soluble interleukin-2 receptor. CR: complete response; PD: progressive disease; ABVD: adriamycin, bleomycin, vinblastine, and dacarbazine.

**Table 3.** Previous reports on immunohistochemical positivity of CD20 and CD79a in CHL

Reference	CD20-positive cases, n/N (%)	CD79a-positive cases, n/N (%)	Clone of CD79a	Cut-off value (%)
Present study	25/55 (45.5)	20/55 (36.4)	JCB117	10
Korkolopoulou <i>et al.</i> , <sup>22</sup> 1994*	20/67 (29.9)	19/94 (20.2)	JCB117	10
Watanabe <i>et al.</i> , <sup>18</sup> 2000	18/51 (35.3)	13/50 (26.0)	NS	10
Browne <i>et al.</i> , <sup>14</sup> 2003	17/57 (29.8)	3/53 ( 5.7)	HM57	10
Tzankov <i>et al.</i> , <sup>16</sup> 2003	84/253 (33.2)	26/253 (10.3)	NS	10**
García-Cosío <i>et al.</i> , <sup>15</sup> 2004	55/305 (18.0)	46/258 (17.8)	JCB117	NS
Valsami <i>et al.</i> , <sup>23</sup> 2007	NS	6/104 ( 5.8)	JCB117	10
Hoeller <i>et al.</i> , <sup>17</sup> 2010	76/269 (28.3)	24/244 ( 9.8)	JCB117	10
Di Napoli <i>et al.</i> , <sup>24</sup> 2013	13/51 (25.5)	17/51 (33.3)	NS	>0
Elsayed <i>et al.</i> , <sup>25</sup> 2017	45/173 (26.0)	9/25 (36.0)	NS	10

\* “Lymphocyte predominance” was excluded from CHL cases.

\*\* In case the tissue microarray core contains  $\geq 10$  HRS cells.

CHL: classic Hodgkin lymphoma; HRS cell: Hodgkin and Reed-Sternberg cell; NS, not stated.

downregulation of BOB.1 and/or OCT-2 can be useful. The unfavorable clinical outcome of patients with CD79a-positive CHL may represent the aggressive characteristics in common with DLBCL. Although patients with DLBCL are often classified in a higher CS than those with CHL,<sup>30-33</sup> the patients with CD79a-positive CHL in our study presented in both limited and advanced CS.

CHL highly expressing B-cell markers like CD20 and CD79a is controversial regarding its distinction from gray zone lymphoma (GZL) or primary mediastinal large B-cell lymphoma (PMLBCL).<sup>25,34-36</sup> However, mediastinal GZL or PMLBCL, whose differential diagnosis from CHL has been discussed in many studies, usually develops in younger adults. Therefore, the 5 CHL cases with CD79a-positivity higher than 80% had fundamentally different age distributions and initial tumor localization from mediastinal GZL or PMLBCL. Non-mediastinal GZL should also be taken into consideration when diagnosing CHL with high expression of B-cell markers; however, it is difficult to discuss due to its poorly established diagnostic criteria.<sup>37</sup>

In conclusion, we found CD79a-positivity in CHL to be associated with older age. In addition, CD79a-positive CHL patients had a poorer survival rate than CD79a-negative CHL patients. No positive correlation was observed between CD79a and CD20 expression. Our study suggests that CD79a-positive CHL involves unique clinicopathological features compared with CD79a-negative CHL. Further studies are needed to clarify the characteristics of CD79a-positive CHL, especially in Japan, where many patients are older at onset.

## CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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