CD30, CD15, CD50, and PAX5 Expressions as Diagnostic Markers for Hodgkin Lymphoma (HL) and Systemic Anaplastic Large Cell Lymphoma (sALCL)

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ABSTRAK

Latar belakang: *ekspresi dari CD30, CD15, CD50, dan PAX5, digunakan untuk membantu mengkonfirmasi diagnosis HL dan sALCL; namun belum ada data mengenai proporsi ekspresi dari penanda-penanda ini. Penelitian ini bertujuan mengetahui proporsi ekspresi positif dari CD30, CD15, CD50, dan PAX5 serta karakteristik pasien HL dan sALCL di RS Pusat Kanker Dharmais pada periode tahun 2005-2015.* ***Metode:*** *studi retrospektif observasional ini dilakukan dengan menggunakan data rekam medis dan hasil patologi pada pasien dewasa HL dan sALCL yang mendapatkan terapi di rumah sakit antara tahun 2005 dan 2015. Pemeriksaan imunohistokimia dilakukan dan data proporsi ekspresi positif dari CD30, CD15, CD50, dan PAX5 dianalisis secara deskriptif.* ***Hasil:*** *sebanyak 45 pasien direkrut dalam studi ini dengan mayoritas (42 pasien, 93,3%) adalah pasien HL dan hanya 3 (6,7%) yang merupakan pasien sALCL. Nilai median usia pasien HL lebih rendah dibandingkan pasien sALCL, yaitu 35 (18-72) tahun versus 54 (49-61) tahun. Dari hasil pemeriksaan imunohistokimia pada pasien HL, ditemukan ekspresi positif dari CD15, CD30, CD50, PAX5 berturut-turut sebesar 37,5%; 88,9%; 31,2%; dan 31,2%. Dengan keterbatasan jumlah pasien sALCL, ditemukan ekspresi positif dari CD30, CD15, CD50, dan PAX5 berturut-turut sebesar 100%; 66,7%; 50%; dan 50%. Pada keseluruhan pasien HL dan sALCL proporsi ekspresi positif dari CD15, CD50, dan PAX5 sebesar 39.5%, 32.4%, dan 32.4%, sedangkan ekspresi positif dari CD30 sebesar 89.5%.* ***Kesimpulan:*** *studi ini menunjukkan bahwa hampir 90% pasien memberikan ekspresi positif CD30, sedangkan yang mengekspresikan positif CD15, CD50, dan PAX5 terdapat pada kurang dari 40% pasien. Hasil studi ini mengindikasikan bahwa pemeriksaan CD30 merupakan penanda diagnosis yang penting untuk HL dan sALCL dan mungkin dapat digunakan untuk memperbaiki strategi pengobatan.*

Kata kunci: *ekspresi C30, CD15, CD50, PAX5, limfoma hodgkin, systemic anaplastic large cell lymphoma (sALCL), limfoma, imunohistokimia.*

ABSTRACT

Background: *the expression of CD30, CD15, CD50, and PAX5 are used to help in confirming diagnosis of HL and sALCL; however data on the proportion of these markers have not been available. The study was aimed to identify the proportion of CD30, CD15, CD50 and PAX5 expressions and characteristics of patients with HL*

and sALCL at Dharmais National Cancer Center Hospital between 2005 and 2015. ***Methods:*** *a retrospective observational study was conducted using data from medical records and histopathological results of HL and sALCL adult patients who sought treatment at the hospital between 2005 and 2015. Immunohistochemistry (IHC) examinations were performed and data on the proportion of positive CD30, CD15, CD50, and PAX5 expressions were analyzed descriptively.* ***Results:*** *a total of 45 patients were recruited in this study, with the majority (42 patients, 93.3%) were HL patients and only 6.7% were sALCL patients. The median age of HL patients was younger than sALCL patients; 35 (18-72 years old) versus 54 (49-61 years old). Moreover, the immunohistochemistry examination demonstrated that the positive CD15, CD30, CD50, and PAX5 expressions were found respectively in 37.5%, 88.9%, 31.2%, and 31.2% patients with HL; while in patients with sALCL, in spite of their small sample size, positive CD30, CD15, CD50 and PAX5 expressions were found in 100%; 66,7%; 50%; and 50%, respectively. Overall, CD15, CD50, and PAX5 positive expressions were found in 39.5%, 32.4%, and 32.4% patients who had HL and sALCL; while positive expression of CD30 was found in 89.5% of them.* ***Conclusion:*** *present study shows that almost 90% patients have positive CD30 expression; while the positive expressions of CD15, CD50, and PAX5 are found in less than 40% patients. It indicates that CD30 is an important diagnostic marker for HL and sALCL and it may improve treatment strategy.*

Keywords: *CD30, CD15, CD50, PAX5 expression, hodgkin lymphoma, systemic anaplastic large cell lymphoma (sALCL), lymphoma, immunohistochemistry.*

INTRODUCTION

The incidence of Hodgkin Lymphoma (HL) in Asian countries is relatively lower than Western countries. The annual incidence rate in the US and the UK is 2.8 and 2.4 per 100,000 population, respectively. These numbers are particularly higher than most Asian countries.[1-3] The 5-year survival rate has improved from less than 10% in the 1960s to over 80% in 2012. It is considered one of the most curable cancers.[4,5] The improved survival rate might be due to improved physicians' skill in classifying disease and determining the most effective treatment based on different pattern of gene expression including providing targeted therapy or monoclonal antibody.[6] Despite the high success rate of the initial treatment with chemotherapy and radiotherapy for HL and sALCL, the relapse rate is 20-30%.[7]

Approximately 9% of lymphoma patients or about 10 patients per year at Dharmais Hospital National Cancer Center are diagnosed with Hodgkin Diseases based on our unpublished internal report. Unfortunately, 41% of all subjects are classified as HL not otherwise specified (NOS), due to financial inability to conduct immunohistochemistry (IHC) testing for all subjects.[8]

IHC is an essential part of diagnostic tools for lymphoma malignancies; however, it has not been considered a routine test in daily practice. Various types of lymphoma have different and specific expressions of IHC markers. WHO classifies HL based on the immunophenotype of Reed-Stemberg (RS) cells into two subtypes: nodular lymphocyte predominant HL (NLPHL) with immunohistochemical characteristics of CD45RB+, CD20+, Epithelial Membrane Antigen (EMA)+/-, EBER- and Classical HL (CHL) with CD45RB-, CD30+, CD15+, EBV-encoded RNA(EBER)+/-.[9] The gene experession of CD30+ has been reported in almost patients with CHL, while the experession of CD15+ has been found in 59-93% of CHL cases.[10,11]

Results from IHC in patients with HL and sALCL can help in diagnosis as well as treatment decision. Antibody-based immunotherapy has emerged as the novel treatment for patients with HL and sALCL. These new therapies could target malignant cells which express certain antibody. For instance, brentuximab vedotin is an anti-CD30 antibody-drug conjugate (ADC), while CD30 is an important therapeutic target for the treatment of patients with HL and sALCL who have positive CD30 expression.

Currently, little is known about the IHC profile of lymphoma patients in Indonesia; while the use of IHC testing has become increasingly

significant for the prognosis and treatment. The expressions of CD30, CD15, CD50 and PAX5 are used to provide help in establishing diagnosis of HL and sALCL. CD30 is highly expressed in patients with HL and sALCL.[12,13] Furthermore, CD30 may also contribute to prognosis and treatment decision.[14,15] The aim of our study was to identify the proportion of positive CD30, CD15, CD50, and PAX5 expressions and characteristics of patients with HL and sALCL at Dharmais Hospital, National Cancer Center between 2005 and 2015.

METHODS

The study was a retrospective observational study using secondary data from Dharmais National Cancer Center Hospital between January 2005 and October 2015. Data were obtained from medical records and results of IHC analyses, which were performed using paraffin blocks with specimens obtained from patients with HL and/or sALCL.

The IHC testing was performed based on routine standard operational procedure at the hospital using labeled Streptavidin-Biotin (LSAB) staining method. Paraffin blocks of the study sample were cut into 4 micron pieces and then attached to a SuperfrossPluss (Ventana) glass object, and then heated over a warmer slide at 60°C for 30 minutes.

The evaluation of CD30, CD15, CD50 and PAX5 expressions were carried out using the Benchmark XT, an automated IHC slide staining instrument, according to the standard IHC protocol. All data were evaluated descriptively and were presented in narrative texts and tables.

Ethical Approval

The study was conducted in compliance with the Good Clinical Practice (GCP) principle as has been defined by the International Conference on Harmonization (ICH) and was also performed in accordance with the Indonesian National Agency for Drug and Food Control Guideline. Ethical approval was obtained from the Health Research Ethics Committee, Dharmais National Cancer Center Hospital on December 15th, 2015, with a reference number: KEPK/046/XII/2015.

RESULTS

As many as 42 HL patients and 3 sALCL patients were enrolled in the study. In general, patients who were diagnosed with HL were younger than those with sALCL (median age: 35 vs. 54 years) as can be seen in **Table 1**. Based on tumor staging, 12 out of 22 (54.5%) HL patients were found in advanced stage (stage 3 and 4). There were limited clinical data about out study subjects. In the group with HL, we found four patients who had relapsed after receiving initial treatment and five patients with partial responses. Moreover, regarding the laboratory results, one patients in the sALCL group had a high erythrocyte sediment rate (ESR) [**Table 2**].

Table 1. Subject characteristics

Characteristics	HL (n=42)	sALCL (n=3)
Age, Median (Min-Max)	35 (18-72)	54 (49-61)
Sex, n (%)		
- Male	20 (47.6)	3 (100.0)
- Female	22 (52.4)	0 (0.0)
Weight (Kg), Median (Min-Max)	54.5 (37-100)	62.5 (57-68)
Height (cm), Median (Min-Max)	161 (142-180)	166 (164-168)
ECOG Performance status		
- 0	0 (0.0)	1 (100.0)
- 1	4 (100.0)	0 (0.0)
B-symptoms		
- Yes	13 (76.5)	0 (0.0)
- No	4 (23.5)	1 (100.0)
Comorbidities		
- Yes	13 (40.6)	0 (0.0)
- No	19 (59.4)	3 (100.0)
Stage of disease		
- 1	1 (4.2)	0 (0.0)
- 2	9 (37.4)	1 (100.0)
- 3	6 (25.0)	0 (0.0)
- 4	6 (25.0)	0 (0.0)
Progressive	1 (4.2)	0 (0.0)
Refracter	1 (4.2)	0 (0.0)
Bulky tumor		
- Yes	8 (29.6)	0 (0.0)
- No	19 (70.4)	1 (100.0)
Extranodal		
- Yes	7 (28.0)	0 (0.0)
- No	18 (72.0)	1 (100.0)

Table 2. Laboratory results of the study subjects

Variables	Median (min-max) (n)	
	HL	sALCL
Hb (g/dL)	11.2 (7.6-17.2) (36)	10.5 (9.4-11.7) (2)
WBC (cells/µL)	9,930 (4,070-35,410) (35)	11,200 (11,200-11,200) (1)
Platelets (103 cells/µL)	341.5 (153.0-666.0) (36)	388.50 (325.0-452.0) (2)
Lymphocyte (%)	13.0 (1.0-42.0) (23)	17.5 (17.0-18.0) (2)
Erythrocyte sediment rate (ESR)	31.0 (5.5-53.0) (9)	115.0 (115.0-115.0) (1)
Ureum (mg/dL)	18.0 (6.0-82.0) (35)	32.5 (23.0-42.0) (2)
Creatinine (mg/dL)	0.79 (0.30-2.20) (34)	1.30 (0.91-1.70) (2)
AST (unit/L)	16.5 (6.0-238.0) (32)	20.5 (15.0-26.0) (2)
ALT (unit/L)	18.0 (5.0-292.0) (33)	17.5 (16.0-19.0) (2)
Albumin (g/dL)	3.25 (2.10-26.00) (16)	- (0)
Glucose (mg/dL)	90 (69-180) (24)	100(100-100) (1)
LDH (unit/L)	524.5 (222.0-6327.0) (28)	331.5 (307.0-356.0) (2)
HBsAg, n (%)		
- Positive	1 (6.2)	0 (0.0)
- Negative	15 (93.8)	1 (100.0)
AntiHCV, n (%)		
- Reactive	0 (0.00)	0 (0.0)
- Non-reactive	14 (100.00)	1 (100.0)

Histopathology and Expressions of CD30, CD15, CD50, and PAX5

Histopathological data was obtained from 34 out of 42 HL patients. In **Table 3**, we can see that most HL subjects have histopathology types of mixed cellularity (10 out of 34) and nodular sclerosis (9 out of 34). We also assessed the IHC markers of the subjects. In HL patients, CD30 were positively expressed in almost all patients (32 out of 36), while CD15, CD50 and PAX5 were positively expressed in 37.5%, 31.2%, and 31.2% patients respectively. Despite the small sample size of of sALCL patients, positive expressions of CD30 and CD15 were found in 100% and 66.7% subjects, respectively. [**Table 4**].

Table 3. Histopathological subtypes of HL patients (n=42)

Histopathological subtypes	n (%)
Mixed cellularity	10 (23.8)
Nodular sclerosis	9 (21.4)
Lymphocyte predominance	8 (19.1)
Lymphocyte depletion	4 (9.5)
Lymphocyte rich	3 (7.1)
Not available	8 (19.1)

Table 4. IHC marker expressions of the study subjects

Variables	HL	sALCL	HL + sALCL
CD15, n (%)			
- Positive	15 (37.5)	2 (66.7)	17 (39.5)
- Negative	25 (62.5)	1 (33.3)	26 (60.5)
CD30, n (%)			
- Positive	32 (88.9)	2 (100.0)	34 (89.5)
- Negative	4 (11.1)	0 (0.0)	4 (10.5)
CD50, n (%)			
- Positive	10 (31.2)	1 (50.0)	11 (32.4)
- Negative	22 (68.8)	1 (50.0)	23 (67.6)
Pax 50, n (%)			
- Positive	10 (31.2)	1 (50.0)	11 (32.4)
- Negative	22 (68.8)	1 (50.0)	23 (67.6)

DISCUSSION

In our study, we reported the proportion of patients with positive CD30, CD15, CD50, and PAX5 gene expression within a 10-year period. The analysis was conducted retrospectively on paraffin blocks of HL and sALCL patients who previously may or may not have IHC examination. We observed that less than 40% of the patients had positive CD15, CD50 and PAX5 gene expressions; while almost 90% of the patients had positive CD30 expression.

More than 80% of our patients had common type HL histopathological subtypes,[16] i.e. mixed cellularity and nodular sclerosis. The age distribution in our HL population (median age of 35 years old) was similar to HL population in Korea (median age of 39 years old);[17] however,

our patients were much older than patients in Taiwan (median age of 26 years old).[18] Our study has also demonstrated typical features of HL and sALCL cases with rare B symptoms, extra nodal involvement and bulky tumor.

Data about treatment outcomes using first-line treatment, i.e. conventional chemotherapy and radiotherapy were only obtained from HL patients. After first-line treatment, our follow-up data showed that 5 out of 9 patients had partial response, while others had progressive course of illness. Four HL patients had relapses after having first remission. Wu et al[18] in Taiwan observed a cumulative relapse rate of 23% at 10 years among their 115 HL patients.

HL and sALCL are difficult to diagnose. Confirmed diagnosis is important since 80% cases of sALCL can be cured with modern regimens of chemotherapy and radiotherapy.[19,20] HL diagnosis is usually confirmed by IHC examination. The findings of positive CD15 gene expression can be an indicator for the presence of sALCL.[16,21] However, in our study, 12 HL patients who were found with positive CD30 expression also showed positive CD15 expression, whereas only one sALCL patient had positive expression of both CD15 and CD30 since there was only a small sample size for this group. Further studies with larger size are recommended in order to confirm these findings.

In addition to positive CD15 expression, 9 out of 29 HL patients also had positive PAX5 expression; while 1 sALCL patient also had positive PAX5 expression. PAX5 is a B-cell-specific activator protein, which has a major role in B-cell differentiation and can be used to confirm the diagnosis.[22] It indicates that IHC examination alone is not sufficient as a diagnostic tool. Further phenotypic testing is necessary to confirm the diagnosis. CD30, which is a cell surface receptor, has been identified in 1982 as an important therapeutic target for treatment of lymphomas. Several monoclonal antibodies that bind CD30 (and another receptor) may attract immune cells that generate anti-tumor response.[21] Due to a high response rate of HL and sALCL patients with positive CD30 expression to some targeted therapy, the CD30 testing is considered as an important tool to determine the therapeutic options.[21,23]

There are some limitations in our study including the absence link between paraffin block and medical record data, which might affect the number of recruited subject. Nevertheless, our study is the first study in our center, as well as the first in Indonesia, which demonstrate the profile of CD30, CD15, CD50 and PAX5 in patients with HL and sALCL. It is expected that our study can provide the basic information on clinical, histological and immunohistochemical characteristics of patients with lymphoma in Indonesia.

CONCLUSION

89.5% of the patients have positive CD30 expression, while the proportion of patient with positive CD15, CD50 and PAX5 gene expression are less than 40%. Since HL and sALCL are not commonly found in Asia, we hope that our study could provide essential information about HL and sALCL patients in Asia, which eventually can provide information for better therapeutic options.

CONFLICT OF INTEREST

The authors declare that there was no conflict of interest in this study.

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