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Characteristics Based on Molecular Subtypes in Diffuse Large B-Cell Lymphoma Cases

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Abstract

Diffuse B-cell origin large cell lymphoma (DLBCL) is a type of non-Hodgkin's malignant lymphoma most commonly found in adults. Based on the gene or molecular expression profile, DLBCL can be divided into GCB (Germinal Center B-Cell-Like) and ABC / Non-GCB (Activated B-Cell-Like). However, identification of this grouping is still rarely done in Indonesia, especially Makassar City. This study aims to determine the characteristics of DLBCL cases based on molecular examination. This study used a sample of 44 cases of paraffin block for DLBCL patients from 2017 to 2019, then categorized using the Hans algorithm. Of the 44 cases, the number of GCB was 8 cases (18.2%) and ABC / Non-GCB 36 cases (81.8%). The incidence of female sex was slightly lower (47.7%) than in men (52.3%) with the most frequent location being nodal with 36 cases (81.8%) and extranodal as many as 8 cases (18.2%) . Meanwhile, age was found more frequently in patients with age <60 years (32 cases, 72.7%) than those aged> 60 years (12 cases, 27.3%) with a mean age of 52.57 years. The results showed that the ABC / Non-GCB type DLBCL was more common than the GCB type in patients in Makassar city.

Keywords: GCB; ABC; Non-GCB; DLBCL; Makassar.

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1. Introduction

Lymphoma is the seventh most common malignancy and has continued to increase over the past four decades [1]. In America there was an increase of about 3-4%, and in Korea accounted for 3.69% of cases of all types of malignancy in 2008. Data from the Ministry of Health of the Republic of Indonesia in 2015 estimated that 14,905 people had malignant lymphoma. Data in Makassar, there were 222 cases from 2015 to 2017 with the number of deaths as many as 26 cases (3.5%) [2]. DLBCL most frequently affects elderly adult patients, especially in patients in the seventh decade. Diffuse large B-cell lymphoma or DLBCL is the most common and aggressive type of lymphoma from B-cell origin and accounts for 30-35% of total cases of non-Hodgkin malignant lymphoma [3,4]. DLBCL has heterogeneous clinical features, histologic features, immunophenotypic, cytogenetic, and molecular features, which can provide different disease entities [5]. Based on its Gene Expression Profile (GEP), DLBCL is divided into two main subtypes, namely GCB (Germinal Center B-Cell-Like) and ABC / Non-GCB (Activated B-Cell-Like). Examination of the gene expression profile is very rarely performed in the daily diagnosis of DLBCL cases. The common algorithm and most often used in classifying this DLBCL is Hans's algorithm, using the CD10, BCL6, and MUM1 immunohistochemical examination panels. In the western population it was found that the incidence of GCB was more common than ABC / Non-GCB. However, it is different from the incidence rate in China, where ABC / Non-GCB incidence is more common [5]. Research and data regarding the division of DLBCL subtypes based on molecular subtypes have not been carried out.

2. Materials and Methods

2.1. Collections of Samples

In this study, we collected 44 paraffin block samples of patients with DLBCL diagnosis from a total of 187 cases diagnosed with large cell non-Hodgkin malignant lymphoma during the period 2017 to 2019 from 3 places, namely the Anatomical Pathology Laboratory of Hasanuddin University Hospital, Wahidin Sudirohusodo Hospital, and Sentra Diagnostik Patologia Laboratory in Makassar. Samples were divided into 2 subtypes based on Gene Expression Profile (GEP), GCB and ABC / Non-GCB using the Hans algorithm through the immunohistochemical panel examination CD10, BCL6, and MUM1.

2.2. Immunohistochemistry Staining (Hans Algorithm)

After collecting the tissue blocks, the tissue was cooled back in the refrigerator, then cut with a 4 µm thick microtome. Furthermore, the tissue cut from the microtome is put into a water bath. The cut in the water bath was taken using a poly-L-lysine slide, then deparaffinized. Immunohistochemical staining using the standard method Avidin-Biotin-Peroxidase Complex (ABC). The unstained slides were incubated with peroxidase-1 for 5 minutes at room temperature and followed by the ABC procedure. Immunohistochemical staining using CD10 concentrated monoclonal antibody (dilution: 1/50) with positive control on tonsil tissue. BCL6 is ready to use monoclonal antibody, with positive control on tonsil tissue. MUM1 is ready to use monoclonal antibody, with positive control on the tonsil tissue. CD10 was colored on the cytoplasmic membrane of tumor cells, while

BCL6 and MUM1 were colored on the tumor cell nucleus. The results of immunohistochemical staining were assessed by two pathologists. The cut-off values of interpreted CD10, BCL6, and MUM1 were positive, stained in >30% of tumor cells. (Coutinho, 2013). Based on the Hans algorithm, CD10+ is categorized as GCB. If CD10-, then staining using BCL6. If the result is CD10- / BCL6- then it is categorized as ABC / Non-GCB. If CD10- / BCL6+, staining can be continued using the MUM1 antibody. Furthermore, staining with CD10- / BCL6+ / MUM1+ results was categorized as ABC / Non-GCB, while CD10- / BCL6+ / MUM1- was categorized as GCB [3].

2.3. Data Processing

The data in this study were processed using descriptive statistical techniques that present measures of central tendency such as mean, median, and mode. Descriptive statistical calculations were performed using SPSS 20 for Windows software. The distribution of data in this study was classified based on gender, age, tumor location, and molecular subtypes.

3. Results

3.1. Patients Characteristics

Of the 44 samples of DLBCL cases taken in Makassar, the distribution of cases based on male gender was 23 cases (52.3%) and women were 21 cases (47.7%). The frequency of cases at ≤60 years was 32 cases (72.7%), and >60 years were 12 cases (27.3%), with a mean age of 52.57 years (range, 18-81 years). Based on location, 36 cases (81.8%) were nodal disease and 8 cases (18.2%) were extra-nodal disease at initial diagnosis (Table 1).

3.2. Molecular Subtypes

There are two molecular subtypes that are differentiated based on the gene expression profile described by the Hans algorithm, which were obtained: a) GCB, 8 of 44 cases (18.2%) (3 cases of CD10+, 5 cases of BCL6+ / MUM1-) and b) ABC / Non-GCB, 36 of 44 cases (81.8%) (Table 2), of which all cases showed CD10- features with some variation from other antibody stains (CD10- / BCL- and CD10- / BCL6+ / MUM1+) (Table 3)

Table 1: Clinical Features of Diffuse Large B-Cell Lymphoma Patients in Makassar (Total n=44)

Clinical Features	Distribution	
N		%
Gender		
Male	23	52.3%
Female	21	47.7%
Age		
≤60	32	72.7%
>60	12	27.3%
Site		
Nodal	36	81.8%
Extra-nodal	8	18.2%

Table 2: Molecular subtype (Total n=44)

Molecular subtype	Distribution	
	N	%
GCB	8	18.2%
ABC/Non-GCB	36	81.8%

GCB,Germinal Centre B-Cell Like; ABC, Activated B-Cell Like

Table 3: Molecular subtype based on CD10 BCL6/MUM1 (Total n=44)

	CD10			BCL6				MUM1			
	(+) (n)	(-) (n)	∑ (n)	(+) (n)	(-) (n)	N/A (n)	∑ (n)	(+) (n)	(-) (n)	N/A (n)	∑ (n)
GCB	3	5	8	5	0	3	8	0	5	3	8
ABC/Non-GCB	0	36	36	13	23	0	36	13	0	23	36

GCB,Germinal Centre B-Cell Like; ABC, Activated B-Cell Like

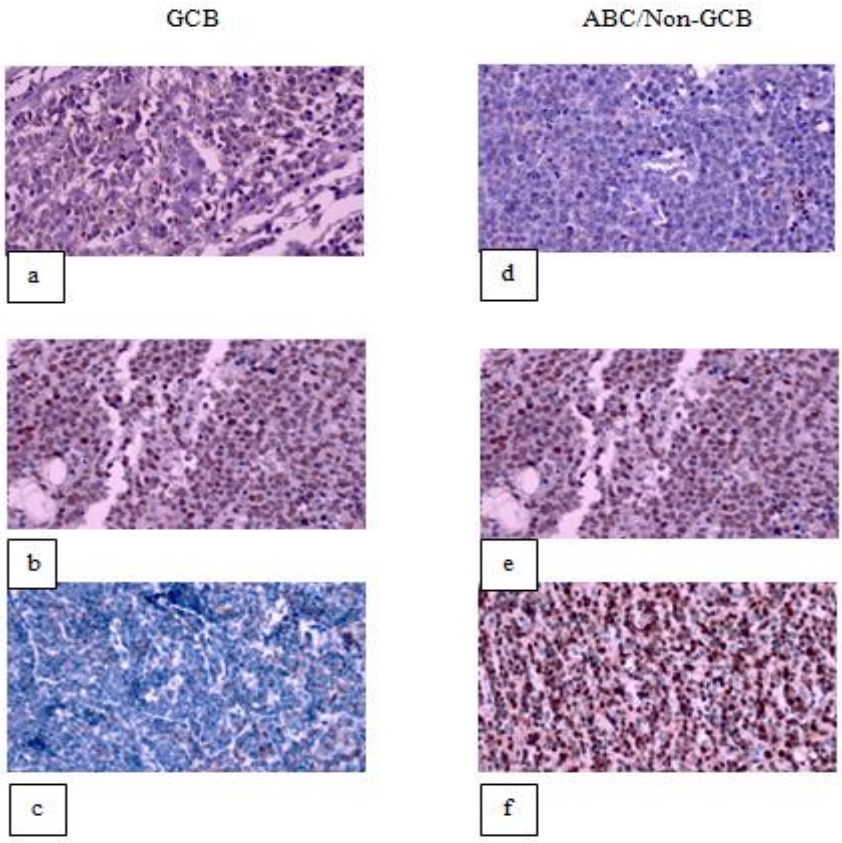

Figure 1: Expression of GCB Subtype by immunohistochemistry with (a) CD10+, (b) BCL6+, (c) MUM1- and Non-GCB Subtype with (d) CD10-, (e) BCL6+, (f)MUM1+ (x400)

4. Discussion

DLBCL is a type of non-Hodgkin's malignant lymphoma that most commonly affects adults [1]. DLBCL has a wide variety of clinical, histological, and immunophenotypic features [3]. It is important to differentiate the values for this type of DLBCL because each subtype has a different prognosis. So far, there are still many laboratory facilities in Indonesia that do not yet support the division of this DLBCL subtype. One of the many algorithms used for the division of this DLBCL subtype, the Hans algorithm is the most frequently used [6]. In this study, DLBCL cases were more often found in men with a percentage of 52.3% or 23 of 44 cases, in women slightly lower with a percentage of 47.7% or as much as 21 of 44 cases. This is in line with a study conducted by Sun and his colleagues which found that DLBCL cases were more common in men than women [7]. In terms of age, this study showed more DLBCL cases were under 60 years old, namely 72.7% compared to older people or more than 60 years which only had a percentage of 27.3%, where the median age was 52.57 years, this study is the same as several studies others that have already been carried out in various Asian countries such as Korea [8], China [7], and Malaysia [9]. A different thing happened in the western population where the average age of DLBCL patients was 68 years [10]. Based on the location, it was found that nodal disease was most frequently found in this study population, amounting to 81.8% or as many as 36 of 44 cases, while extra-nodal disease was 18.2% or as many as 8 of 44 cases. This is in line with a study conducted by Yuankai and his colleagues where in their study, 62.6% of cases of nodal disease were found in DLBCL and 37.4% of extra-nodal disease with a total sample population of 1,085 patients, the mean age of patients with nodal and extra-nodal disease was 50 and 56 years, respectively [11]. In this study, it was found that the ABC / Non-GCB type DLBCL had a higher frequency of 81.8% or as many as 36 of 44 cases, while the GCB type DLBCL was rarer, namely 18.2% or as many as 8 of 44 cases. This is in line with several studies that used samples of Asian patients such as those in China [5], Japan [6], and also researchers from Indonesia with a sample of Yogyakarta patients [3], showing that GCB type DLBCL was more common than ABC / Non-GCB type DLBCL. The results of a study conducted by Shiozawa and his colleagues where they combined and compared several studies from Asian countries and four studies from Western countries. In Asian countries, there were 102 out of 330 DLBCL patients who had GCB subtype as much as 31%, while in Western countries, 206 out of 416 patients had ABC / Non-GCB subtype or in a percentage of 50%, which showed that there was a significant difference between populations of Asia and the West ($p < 0.001$), where indeed GCB type DLBCL is less common in Asia than in Western countries [6]. DLBCL patients with elderly have a worse prognosis than young patients, the most logical thing that can explain this is that elderly patients involve comorbidities associated with elderly conditions, and this condition also affects the patient's chemotherapy response [4]. In this case age can be used as a reference for clinicians to predict the likely outcome of patient therapy, as in the study conducted by Coiffier and his colleagues adding rituximab to CHOP in elderly patients as many as eight cycles could significantly improve complete response, reducing the failure rate of treatment and reduced recurrence, and increased event-free survival and overall survival compared to providing standard CHOP therapy alone [12]. Based on the location, nodal disease is most commonly found in the lymph node route and for extranodal disease the most common is the gastrointestinal tract, whereas DLBCL patients with nodal disease have a better treatment outcome than patients with extranodal disease [11]. GEP is still not routinely used in daily clinical practice. There are several immunohistochemicals that can be used in several algorithm models among the two subtypes of DLBCL, namely CD10, BCL6, MUM1,

GCET1, and FOXP1 [12,13] . In Indonesia, Hans algorithm is generally used to distinguish these two DLBCL subtypes using CD10, BCL6, and MUM1, or Hans modification that only uses CD10 and MUM1 [15]. Kwak stated that in his study, it is important to distinguish the two subtypes in this DLBCL because it successfully predicted progression-free survival (PFS) and overall survival (OS) [13]. Another study by Liu and Barta stated that the ABC / Non-GCB subtype gave worse results with chemotherapy containing rituximab than the GCB subtype [16]. The limitations of this study are the limited number of samples and also the availability of one required immunohistochemical stain, MUM1, which is not available in our education center so we have to send samples out of town and this takes a long time.

5. Conclusions

In this study, we conclude that ABC / Non-GCB type DLBCL is more common than GCB type DLBCL. This division is important as a predictive factor as well as a prognostic.

6. Suggestions

Further research was carried out using this data and linking it to other molecular pathways that occur in DLBCL pathogenesis, such as Ki-67 or BCL2, with more samples.

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