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Role of MYC in the tumor microenvironment in high grade non-Hodgkin B cell lymphomas

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Abstract. High-grade non-Hodgkin's B cells lymphomas (NHLs) are malignant tumours of the lymphoid system. MYC protein was known correlated with poor prognosis. In addition to promoting cell-cycle progression, MYC plays an important role activation of an alternative pathway of a tumour associated macrophage (TAM). We further determined if MYC protein expression correlated with the high density of TAM. This was a cross-sectional study of 47 specimens of high-grade non-Hodgkin B cell lymphomas. Their immunophenotype was re-evaluated, and stratified into DLBCL and others non-Hodgkin lymphomas. All samples were immunostained with MYC and CD163 antibodies. The expression of MYC and CD163 were scored according to the previous study. Statistical analysis was performed by using SPSS version 22. MYC positive expression was detected in 72,3% of patients. Its found mostly in male patient, age ≤60, and Ki67 >70%. Average density of TAMs was 7,29. An average density of TAM in MYC positive lymphomas was higher rather than MYC negative lymphomas. MYC may facilitate the high density of TAM and contribute to worst prognosis in younger male patient and Ki67 >70%, of high-grade B cell lymphomas.

1. Introduction

Non-Hodgkin lymphoma is the tenth most frequent cancer worldwide and diffuse large B-cell lymphoma (DLBCL) is the most common histologic subtype [1]. Diffuse large B-cell lymphoma (DLBCL) is an aggressive lymphoma with heterogeneous morphologic, clinical, and genetic features [2]. Alterations of *MYC* oncogenes can drive the pathogenesis of NHL [3]. *MYC*, a proto-oncogene, is a key regulator of cellular proliferation and apoptosis [4]. Deregulation of *MYC* as resulted of either chromosomal translocation or gene amplification, point mutations or epigenetic reprogramming, enhanced translation and increased protein stability resulting in overexpression of MYC protein [5]. To date, several prognostic biomarkers of lymphoma have been investigated, in which MYC is one of the most prominent factors [6].

MYC is an essential cellular protein that regulates nucleotide metabolism, ribosome and protein synthesis, RNA transcription and processing, and DNA replication [7]. Green et al [8] and Johnson et al [3] reported that DLBCL patients with MYC/BCL2 coexpression, with or without MYC or BCL2 gene rearrangements, have a poorer prognosis. The tumor microenvironment is one of an important factor in the progression of malignant tumors including lymphoma, and TAM is one of the tumor microenvironment components of B cell lymphoma [9]. TAM in non-Hodgkin lymphoma have been shown to be a prognostic indicator using CD163 as immunohistochemical markers [10]. The mechanisms by which TAM affect cancer progression are still unclear and are probably multifactorial,

one of the potential tumor-promoting functions of TAM is a proto-oncogene *MYC* [11]. Pello et al in his study revealed MYC role in the alternative pathway of macrophage activation [12]. Studies about MYC expression and the density of TAM is still limited according to our knowledge. In this study, we used IHC to assess the association of MYC and the density of TAM stained with anti-CD163.

2. Methods

A cross-sectional study was performed using archival materials in the Department of Pathology, Dr Mohammad Hoesin Palembang general hospital, Indonesia from January 1st 2016 to September 30th 2017. The age of the patients ranged from 4 to 88 years with a mean age of 46 years. All histological and immunophenotypic data of the 47 patients with high grade Non-Hodgkin B cell lymphoma were reviewed by expert pathologists in accordance with the 2008 World Health Organization (WHO) criteria. The samples were available as formalin-fixed, paraffin-embedded (FFPE) tissue blocks and immunohistochemical slide stained with CD20 and or CD79A and Ki67. We further graded the proliferative index value into aggressive (≥40-70%) and very aggressive (70%). The subtype stratified into DLBCL and others NHL. This study was approved by ethical committee of dr. Mohammad Hoesin General Hospital Palembang Indonesia.

2.1. Immunohistochemistry

The blocks were sectioned with a standard microtome (Thermo, shandon, finesse 325) at 4 μm thickness. Subsequently, the slides were dried in a lab heating and drying oven (Sharp, Japan) at power level 8 for 10 min. For immunohistochemical detection, the following antibodies were used: MYC (clone Y69 rabbit monoclonal, dilution 1:100, Abcam, Cambrigde, MA), and CD163 (clone 10D6, rabbit, monoclonal, dilution 1:100, Thermo Fisher, USA) Immunohistochemical staining were performed using manual system according to standardized immunohistochemical protocol. The slides were dehydrated in a graded ethanol serial and immediately covers lipped.

2.2. Assessment of MYC expression and cell counting of TAM

One hundred tumor cells per 5 high-power fields (500 tumor cells per case) were analyzed for quantitative assessment of MYC expression by pathologists. The high-power fields were randomly selected at highest expression of MYC. The MYC IHC scores, as reported by the Jhonson *et al* in their study were used for subsequent analyses, score of ≥40% nuclear staining considered as positive [3]. The density distribution of macrophage stained by CD163 was manually counted as follows: total positively stained cells counted using an ocular grid with a × 400 times microscope field using a BX51 microscope, (olympus, Japan). At least five areas were counted in each case. In this analysis, the most densely distributed areas were selected and the number of TAM were averaged.

2.3. Statistical analysis

The fisher exact test was used to determine MYC expression and its correlation with age and subtype variables and chi square test was used to determine the correlation between MYC expression with gender, tumor location and Ki67 proliferative index category. The Mann Whitney U test was used to determine the average value of TAM and its correlation with MYC expression and others variable. All statistical analyses were performed using IBM SPSS statistics software version 22. Differences were considered significant when p-values were < 0.05.

3. Results

A total of 47 cases were collected from files from the Department of Pathology Dr. Mohammad Hoesin general hospital. Of forty-seven cases of high grade non-Hodgkin B cell lymphoma, the reviewed diagnoses comprised of DLBCL non-GC in 29 cases, lymphoma with morphology of DLBCL but with limited marker in three cases, BCLU in two cases, Burkitt lymphoma (BL) in one cases, lymphoblastic lymphoma in one cases, blastoid mantle cell lymphoma in one cases, T cell rich LBCL in one cases, and seven cases showed DLBCL morphology but with limited marker, others high grade non-Hodgkin lymphoma with limited immunohistochemistry panel in 2 cases. DLBCL non-GC subtype were mostly found at age ≤ 60. Clinical characteristics regarding age, site location, sex distribution and Ki67 category are summarized in table 1.

Table 1. Patient characteristic.

Clinicopathologic characteristic	n	(%)
Gender		
Male	26	55,3
Female	21	44,7
Age		
≤ 60	35	74,5
>60	12	25,5
Ki67 proliferation index		
≥40-70 (agressive)	21	44,7
>70 (very aggressive)	26	55,3
Subtype		
DLBCL	40	85
Others Non-Hodgkin lymphomas	7	14,9
Tumor location		
Nodal	25	53,2
Extra nodal	22	46,8

3.1. MYC immunohistochemistry and its association with the density of TAMs

Overall, of 47 cases showed MYC nuclear positivity in 34 (72,3%) cases and negative expression were found 13 cases. Most of positive MYC expression were found in DLBCL subtype. The highest mean score of the density of TAM were also found in DLBCL subtype. CD 163 expression observation in the same spot of the highest MYC expression was found variable, case number 33 with MYC expression was 80%, the mean score of TAM is 27.06, case number 18 which is MYC expression 90%, the TAM density mean score is 13.06 (figure 1).

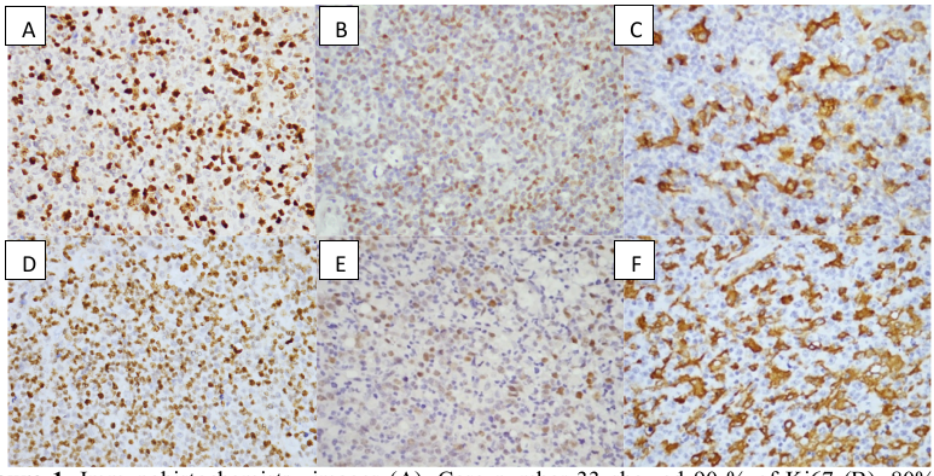

Figure 1. Immunohistochemistry images (A). Case number 33 showed 90 % of Ki67 (B). 80% of MYC expression and (C). Density of TAM (mean score 27.06) (D). Case number 18 showed 90% of Ki67 (E). 90% of MYC expression (F). Density of TAM (mean score 13.06).

In bivariate analysis, MYC protein expression were significantly correlated with the density of TAM (P <0.05). The average of TAM density in MYC positive was 9,15 and MYC negative was only 5,12. MYC positive expression and higher density of TAM were found mostly in a male patient, higher in age ≤ 60, more in nodal location for the densest of TAM and showed Ki67 > 70%. MYC expression and all clinicopathologic variables was found not correlated with p value > 0,05.

Observation of the two categories of Ki67 with MYC expression and the density of TAM, in this study, were found not correlated with all variables, however we found significant correlation between Ki67 with tumour location (p-value=0,05 and odd ratio>4). MYC expression, TAM density and its correlation with all variables were summarized in table 2.

Table 2. MYC expression and the density of TAM and their correlation with clinicopathologic variables.

Clinical characteristic	n /%	MYC expression			The density of TAMs	
		Positive n/%	Negative n%	*p-value*	Mean±SD	*p-value*
Gender				0,840		0,373
Male	26/55.3	19/70,4	8/30,8		7,80 ± 5,56	
Female	21/44,7	16/76,2	5/23,8		8,33 ± 5,60	
Age				1,000		0,756
≤ 60	35/74,5	25/71,4	10/28,6		8,46 ± 6,76	
>60	12/25,5	9/75.0	3/25,0		6,80 ± 6,48	
Ki67 proliferation index				1,000		0,697
≥40-70%	21/44,7	15/71,4	6/28,6		8,52 ± 7,53	
>70%	26/55,3	19/73,1	7/25,9		7,46 ± 5,99	
Subtype				1,000		0,965
DLBCL	40/85	29/72,5	11/27,5		8,08 ± 6,82	
Others non-Hodgkin lymphomas	7/14,9	5/71,4	2/28,6		7,77 ± 6,10	
Tumor location				0,702		0,394
Nodal	25/46,8	17/68	8/32,0		7,10 ± 6,27	
Extra nodal	22/53,2	17/77,3	5/22,7		8,63 ± 6,96	

4. Discussions

High grade non-Hodgkin B cell lymphoma encompasses a broad group of B-cell neoplasms that are histologically characterized by proliferation of intermediate to large B-cell [13,14]. Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin's lymphoma. It has heterogeneous clinicopathological, immunophenotypic, and genetic features [2,15]. Of 47 cases in this study,85% are DLBCL. DLBCL can be stratified into several subtype comprised of germinal centre B-cell (GCB)–like or activated B-cell (ABC)–like subtypes, and the ABC subtype of DLBCL have an inferior prognosis [16]. MYC rearrangements can be found in aggressive B-cell non-Hodgkin lymphomas, including most B-cell lymphomas, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and BL (BCLU), and in 10% to 15% of diffuse large B-cell lymphomas (DLBCL) [7].

MYC protein expression in our study was found positive in 72,3% cases. MYC overexpression was confirmed as a negative prognostic parameter in multiple studies [2,3,8,15,17]. MYC overexpression was also correlated with high proliferation index [18]. Broyde et al, in their study about Ki67 and lymphoma grade reported the mean score of Ki-67 PI increased from 26.6% for indolent lymphomas to 67.2% for aggressive lymphomas to 97.6% for very aggressive lymphomas [19]. The cut of value of Ki67 for lymphoma grade was not establish yet, then our study used ≥40-70% for aggressive and > 70% for very aggressive and we found there is no significant correlation with others variable (p>0.005).

Non-Hodgkin lymphoma pathogenesis is not only the result of autonomous cell growth but also relies on survival and proliferation signals from the tumor microenvironment [20]. MYC is interesting in that it mediates the tumorigenic mechanisms of both cancer cells and macrophages and MYC is found overexpressed in human TAM [21]. TAM is M2-like macrophage which stained specifically by CD163 [22]. To our knowledge, this present study reports for the first time the MYC expression and

its association with TAM in high-grade non-Hodgkin lymphoma. We initiated this immunohistochemical study to evaluate the possibility of positive MYC expression will be followed by the high density of TAM, as its reported by Pello et al [12] in their study of MYC and its role in alternative activation of macrophage. Our study found MYC expression and the density of TAM tend to higher in male, more in nodal location for the densest of TAM, higher in younger subject age ≤ 60 and showed more Ki67 > 70%.

The high percentage of MYC expression was almost always followed by high average score of TAM. TAM may activate by another mechanism, referring to other literature that mention, TAM was induced by antibody immune complex, IL-1, IL-6, IL-10, glucocorticoid and transforming growth factor (TGFβ) [23]. TAM may contribute to the worst prognosis of lymphoma because TAM can produce growth factors, proteases and cytokines that initiate tumorigenesis, enhance tumor progression, and promote angiogenesis and metastasis [10]. Our finding in this study, is similar with Pello et al [12]. This study showed MYC may has role in alternative pathway of macrophages activation. In summary, our review of MYC IHC data collected during routine clinical practice has revealed several findings, high percentage MYC expression was almost always followed with high density of TAM. Secondly for DLBCL non-GC subtype in our study mostly ≤ 60 in contrast with other literature which reported age predilection for this subtype is over 60 [13]. Several factors might cause this differentiation, it may cause by life style, diet habit, occupation and others unknown factor.

We also found association between Ki67 expression and tumor location. Taken together, our findings suggest that both of MYC IHC and CD 163 are important for a comprehensive understanding of high-grade non-Hodgkin B cell lymphomas, since these tests provide complementary diagnostic information that is useful for clinicians triaging patients who may require more aggressive therapies. The limitation of our study is the distinction between positively stained in macrophages and tumor cell by MYC antibody is difficult to observe in our study, therefore, future multi-color immunohistochemistry will be required for more detailed.

5. Conclusions

Our study showed MYC positive expression and high density of TAM, almost always found in male, younger patient, nodal location for the densest of TAM and showed more Ki67 > 70%. Based on our finding we conclude MYC may facilitate the high density of TAM and contribute to worst prognosis in younger male patient of high grade non-Hodgkin B cell lymphomas.

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