

Association Between CD30 Expression and EBV-DNA Detection Status With ABVD Chemotherapy Response in Classical Hodgkin Lymphoma Patients

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ABSTRACT

CD30 is expressed in Hodgkin lymphoma (HL). To date, research efforts have focused on understanding its contribution to lymphomagenesis through anti-apoptotic mechanisms, and its effect on cell survival. It has been suggested that Epstein-Barr virus (EBV) plays a role in the pathogenesis of positive cases, as an early event in HL development. The presence of EBV is associated with prognosis. More than 40% of Hodgkin lymphoma (HL) patients are chemoresistant and show early relapse. The purpose of this study is to determine if there is an association between CD30 and EBV-DNA in HL with doxorubicin, bleomycin, vinblastine and dacarbazine (ABVD) regimen response therapies. This study is a cross-sectional study that was conducted at 3 hospitals in Bandung, Indonesia. We analyzed paraffin sections from 60 cases with histologically diagnosed with Classical Hodgkin lymphoma. ABVD chemotherapy response was evaluated by RECIL criteria. Detection of EBV-DNA by Real-Time PCR and CD30 expression using immunohistochemistry with semiquantitative evaluation was performed on all samples. All data were analyzed using a Chi-Square test with a p-value <0.05 for significant levels. From 60 cases, 28 cases (46.7%) showed a complete/partial response and 32 cases (53.3%) showed no response to the ABVD regimen. EBV-DNA were present in 33 cases (55%), 10 cases from the complete/partial response group and 23 cases from the no response group. Low CD30 expression distribution ($\leq 25\%$) dominated the complete response group (68%), whereas high CD30 distribution ($> 25\%$) dominated the nonresponse group (68.7%). The results showed a statistically significant EBV-DNA detection status ($p=0.005$) and CD30 expression ($p=0.009$) between the complete/partial response and nonresponse groups. In conclusion, the ABVD chemotherapy response of CHL is associated with CD30 expression and EBV-DNA infection.

Keywords: “CD30”, “EBV”, “ABVD”, “Classical Hodgkin Lymphoma”

1. INTRODUCTION

Hodgkin lymphomas (HL) are lymphoid malignancies that are composed of large mononuclear and multinucleated cells, with a cellular background rich in inflammatory cells¹. In Europe and the US, HL is present in 2-3 per 10,000 people. Age distribution is bimodal, occurring in early adulthood (20-30 years) and late adulthood (over 55 years).² In terms of histological structure, HL are divided into two major categories: nodular lymphocyte predominant HL (NLPHL) and classic HL (CHL). 90% of all HL cases are classic HL¹.

Previous studies have revealed that after receiving primary therapy with ABVD chemotherapy regimens (doxorubicin, bleomycin, vinblastine, and dacarbazine), over 80% of patients with advanced Hodgkin lymphoma (HL) enter complete remission; however, approximately 40% of patients relapse over time and require additional

therapy. Between 66% and 89% of patients respond to these regimens, meaning that the non-response rate for refractory and relapsed HL patients is 10%–30%.³ Salvage chemotherapy followed by high-dose therapy (HDT) and autologous stem cell transplantation (ASCT) is only able to cure 50% of patients⁴.

The Epstein-Barr virus (EBV) is a human herpes gamma-1 virus. EBV is involved in the pathogenesis of many emerging B-cell lymphomas, including Burkitt's lymphoma, Natural killer (NK)/T cell lymphoma, diffuse large B cell lymphoma (DLBCL) and Hodgkin's lymphoma⁵. Epidemiological studies indicate that CHL infects more than 90% of individuals who have previously been exposed to Epstein-Barr virus (EBV) who reach adulthood⁶. Latent EBV genomes express five EBV-encoded nuclear antigens (EBNA) and two latent membrane proteins (LMPs): EBV-encoded small RNA (EBER) and non-transcribed BART (*BamHI-A* region rightward transcript) RNAs⁷. The

development and survival of Hodgkin Reed Sternberg cells can also be encouraged by EBNA1⁸.

One of several tumor necrosis factor receptors (TNFR) is Cluster of Differentiation 30 (CD30). Malignancies have recorded the highest rate of elevated CD30 expression in diseased tissue. Hodgkin lymphoma and ALCL are two of the foremost malignancies with elevated CD30 expression⁹. The neoplastic cells in Hodgkin's lymphoma are often CD30-positive, and CD30 expression on the surface of Reed–Sternberg cells associated with the size and invasiveness of the tumor¹⁰. Due to the variable expression of CD30, the focus of previous research has been on examining the pathogenesis of CD30 upregulation, its contribution to lymphomagenesis through anti-apoptotic mechanisms, and its effect on cell survival¹¹. Due to the fact that its expression is often limited to particular disease states, CD30 has become an important molecule in the field of targeted therapy⁹.

The synergistical implications of EBV infection on the function of Cluster of Differentiation (CD30) is not known. However, Hu et al.'s research revealed considerably worse OS and PFS in patients with CD30⁺EBV⁺ DLBCL compared to those with CD30⁺EBER⁻ DLBCL. There is still limited data on Classical Hodgkin Lymphoma cases¹².

Therefore, we have attempted to evaluate the frequency of CD30 expression and EBV-DNA status in Hodgkin lymphoma patients, and its relationship with ABVD chemotherapy.

2. MATERIAL AND METHODS

2.1. Study design

The study is a cross-sectional following a retrospective design and involving 60 cases of classical Hodgkin lymphoma diagnosed between 2014 and 2019. The data for all cases data were re-evaluated and tumor biopsies re-examined focusing on histology. Immunohistochemistry staining of CD30 was performed on all samples. EBV-DNA presence was identified. Tumor stage and ABVD chemotherapy response were evaluated by an haematologist (according to RECIL criteria) and the relevant information taken from patient's medical records.

2.2. Patients and ethical approval

Data were retrieved from 60 consecutive patients whose tissue biopsies were examined by the Anatomical Pathology Departments at Hasan Sadikan General Hospital, Santosa Hospital and Advent Hospital in Bandung, Indonesia, between 2014–2019, and treated with ABVD chemotherapy. From these cases, the original slide and formalin-fixated paraffin-embedded (FFPE) primary biopsy material from the cancer lesions were retrieved from the archives. Patient records were reviewed for age, sex, cancer stage and ABVD chemotherapy response. Stage were assessed according to Ann-Arbor staging and chemotherapy

response was evaluated using RECIL criteria. Ethical approval was granted by the research ethics committee at Padjadjaran University Bandung (2019/1012). In accordance with the ethical approval, informed consent was not required due to this being an examination of historical biopsy material.

2.3. Histopathologic review

All histological slides were reviewed and classified according to the World Health Organization (WHO) Classification of tumors of Haematopoietic and Lymphoid Tissues (2017 edition) which categorizes CHL into four histological subtypes, i.e. nodular sclerosis (NS), mixed cellularity (MC), lymphocyte rich (LR) and lymphocyte depletion (LD). The pathologists (B.S and H.A.U) were unaware of the outcome of EBV-DNA analyses, while performing the classification.

2.4. Immunohistochemistry of CD30

Paraffin sections were stained using the labeled streptavidin biotin immunoperoxide complex method, using the Starr Trek Universal HRP Detection system (Biocare Medical, California, USA). The primary antibody used was CD30 using a mouse monoclonal antibody from Biocare Medical (California, USA). As the positive control, we used tonsil tissue and for the negative control we used the same FFPE sample but we eliminate the antibody reaction. The CD30 immunohistochemistry results were scored by 2 pathologists (BTS and HAU) on a 3-tiered scale (1+: 1-25% positive HRS cells, 2+ 26-75% positive HRS cells and 3+ 76-100% positive HRS cells), then divided into low (+1) and high (+2 and +3) categories.

2.5. EBV-DNA detection

Sections of FFPE blocks were prepared. They were cut into four 5 µm thick section. These dissected tumor tissues were then put into 1.5 ml microcentrifuge tubes, and DNA extracted and purified using Quick-DNA™ FFPE Kits (Zymo Research, California, USA). After purification, DNA template concentration was counted for every sample. The data was used to equalize the concentration (15 ng DNA/µl).

From each sample, 10µl was analyzed for the quantity of EBV DNA (single copy gene encoding the Epstein-Barr virus Nuclear Antigen 1 (EBNA1)) by the use of a commercial kit (EBV/ISIN/100, GeneProof, Brno, CZ) for real time PCR (Rotor-gene, Qiagen). 10µl sample and 30µl MasterMix were added into the PCR tubes. During PCR preparation, all components were kept at +2°C to +8°C. There are three major steps that make up each cycle in a qPCR reaction. Reactions are run for 40 cycles. qPCR steps are as follows: denaturation 95°C for 5s, annealing 55°C for 40s and extension 72° for 20s.

For quantification, we made standard curve (included in the kit) from 10x10¹ to 10x10⁴ copies/µl. Quantification was extrapolated from a linear regression standard curve.

2.6. Statistics

A Chi-squared test was performed to explore the association between CD30 expression and ABVD chemotherapy response, and EBV-DNA status and ABVD chemotherapy response. All statistical analyses were performed using SPSS version 24.0 for Windows, and *p* values less than 0.05 were considered significant.

3. RESULTS

The clinicopathological features of the 60 cases are listed in Table 1. The median age at diagnosis was 39.5 years (ranging from 5–76 years). The male to female (MF) ratio was 1.14:1. Mixed Cellularity Classical Hodgkin Lymphoma was the most common subtype (30% of all cases), slightly higher than Lymphocyte Rich followed by Nodular Sclerosis and Lymphocyte Depleted subtypes (28.3%, 25% and 16.7% of all cases, respectively). Most patients were classified as stage III (41.6%) according to Ann Arbor criteria.

Table 1. Sample Characteristics

Variable	N=60
Age (years)	
Median	39.50
Range (min-max)	5-76
Sex	
Male	32 (53.3%)
Female	28 (46.7%)
Histological subtype	
MCCHL	18 (30%)
NSCHL	15 (25%)
LRCHL	17 (28.3%)
LDCHL	10 (16.7%)
Stage	
I	4 (6.7%)
II	16 (26.7%)
III	25 (41.6%)
IV	15 (25%)

A comparison between patient characteristics and CD30 expression can be seen in Table 2. Statistical tests showed that there was no significant association between age, gender, histological subtype and tumor stage and CD30 expression (*p* value > 0.05). In the table, it can be seen that cases in the High CD30 category had a mean age of 39.26 years, and were more common in the male gender, the Nodular Sclerosis histological subtype and stage III tumors.

Table 2. Distribution of age, sex, histological subtypes and stage by CD30 expression

Variable	CD30 (low) (N = 29)	CD30 (high) (N = 31)	p value
Age (years)			
Mean	40.66	39.26	0.762
(95% CI)	(33.66–47.65)	(32.98–45.54)	
Sex			
Male	14 (43.8%)	18 (56.2%)	0.605
Female	15 (53.6%)	13 (46.4%)	
Histological subtype			
MCCHL	9 (50%)	9 (50%)	0.353
NSCHL	5 (33.3%)	10 (66.7%)	
LRCHL	8 (47.1%)	9 (52.9%)	
LDCHL	7 (70%)	3 (30%)	
Stage			
I	2 (50%)	2 (50%)	0.402
II	10 (62.5%)	6 (37.5%)	
III	10 (40%)	15 (60%)	
IV	7 (46.7%)	8 (53.3%)	

A comparison between patient characteristics and EBV-DNA status can be seen in Table 3. Statistical tests showed that there was no significant association between age, gender, histological subtype and tumor stage and EBV-DNA status (*p* value > 0.05). In the table, it can be seen that cases that were EBV-DNA positive had a median age of 42 years, and were more common in male gender, the Mixed Cellularity histological subtype and stage III tumor.

The association between CD30 expression and ABVD chemotherapy response can be seen in Table 4. Statistical tests showed that there is a significant association between CD30 expression and ABVD chemotherapy response (*p* value = 0.009) which means that CD30 expression and ABVD chemotherapy response are associated with each other. In the response group, 67,9% were cases with low-expression of CD30 and in non-response group, 68,7% were cases with high-expression of CD30.

Table 3. Distribution of age, sex, histological subtypes and stage by EBV-DNA status

Variable	EBV-DNA Positive (N = 33)	EBV-DNA Negative (N = 27)	p value
Age (years)			
Median	42	33	0.135
Range (min-max)	(17–75)	(5–76)	

Variable	EBV-DNA Positive (N = 33)	EBV-DNA Negative (N = 27)	p value
Sex			
Male	20 (62.5%)	12 (37.5%)	0.212
Female	13 (46.4%)	15 (53.6%)	
Histological subtype			
MCCHL	11 (61.1%)	7 (38.9%)	0.730
NSCHL	8 (53.3%)	7 (46.7%)	
LRCHL	10 (58.8%)	7 (41.2%)	
LDCHL	4 (40%)	6 (60%)	
Stage			
I	2 (50%)	2 (50%)	0.916
II	10 (62.5%)	6 (37.5%)	
III	13 (52%)	12 (48%)	
IV	8 (53.3%)	7 (46.7%)	

Table 4. Association between CD30 expression and ABVD chemotherapy response

Variable	ABVD Chemotherapy		p value
	Response (N=28)	Non-response (N=32)	
CD 30 expression			
High (>25%) (N=31)	9 (29%)	22 (71%)	0.009
Low (≤ 25%) (N=29)	19 (65.5%)	10 (34.5%)	

The association between EBV-DNA status and ABVD chemotherapy response can be seen in Table 5. Statistical tests showed that there is a significant association between EBV-DNA status and ABVD chemotherapy response (p value = 0.005) which means that EBV-DNA status and ABVD chemotherapy response are associated with each other. In the response group, 66,7% of cases were EBV-DNA negative and in the non-response group, 69,7% of cases were EBV-DNA positive.

Table 5. Association between EBV-DNA and ABVD chemotherapy response

Variable	ABVD Chemotherapy		p value
	Response (N=28)	Non-response (N=32)	
EBV DNA			
Positive (N=33)	10 (30.3%)	23 (69.7%)	0.005
Negative (N=27)	18 (66.7%)	9 (33.3%)	

After multivariate testing, the variable that most influenced the ABVD chemotherapy response was the CD30 expression variable, displaying because it has the lowest p value and an OR value that is greater than the EBV-DNA variable. Therefore, it can be concluded that, simultaneously, CD30 expression is more dominant than EBV-DNA status in affecting ABVD chemotherapy response.

Table 6. Multivariate analysis of ABVD chemotherapy response

Variable	B	df	Nilai P	OR	CI 95%	
					Lower	Upper
CD30 Histoscore	1.539	1	0.010	4.661	1.446	15.020
EBV-dna	1.530	1	0.011	4.616	1.427	14.928

4. DISCUSSION

There is a striking variance in the rate of EBV infection in patients with CHL. According to previous studies, the range of EBV incidences is between 31.8% to up to 74.2% in different countries.^{13,14} The EBV frequency in this study was 55%. Likely causes of the high variability in EBV prevalence include ethnic background and genetic variation. In accordance with previous findings, male participants and the MCCHL subtype in the present study showed higher but not significant incidences of EBV-positive cells.^{6,15,16} The majority of cases in the current study were advanced (III and IV stage), according to Ann Arbor classification with Cotswold modification.¹⁷

The first-line chemotherapy regimen for treating HL is ABVD.¹⁸ The response rate to ABVD chemotherapy in our study was low (46.67%) compared to studies in Serbia (83.8%), Australia (96%) and Italy (79%). EBV negative cases had a slightly higher response rate (69.7%) than EBV positive cases (66.7%). A similar finding was also made in Murray et al.'s study, in which EBV negative cases were more responsive to chemotherapy (94.17%) than EBV positive cases (90.24%).¹⁹ The pathogenesis of Hodgkin's lymphoma may be a factor in the variance in chemotherapy responses.

The pathogenesis of Classical Hodgkin Lymphoma (CHL) is still unclear, partly due to the rarity and difficulty in analyzing Hodgkin and Reed-Stenberg (HRS) cells. The current study reveals that the emergence of different pathogenetic pathways is triggered by different rates of viral infections, genetic alterations, and interactions with microenvironmental components. HRS cells are imperative in regulating the microenvironment milieu associated with HL. The findings indicate complexities in communication between the tumor cells and the reactive infiltrate of the microenvironment and the occurrence of multiple mechanisms, meaning that CHL is extremely heterogeneous.²⁰

Hodgkin and Reed-Stenberg (HRS) cells and recruited immune and stromal cells produce a complex and dense network of signals mediated by direct cell contact and soluble mediator synthesis. Most of these signals have final

effect in the delivery of a prosurvival feedback to HRS cells. These include the engagement of surface CD30 molecules expressed on HRS cells by CD30L expressed on B lymphocytes, granulocytes, macrophages, and mast cells.²¹ The current study found high CD30 expression in non-response patients (71%), and low CD30 expression in response patient (65.5%). In another study, a high density of CD30 was also found to be present in patients with relapsed or refractory Hodgkin's lymphoma.^{22,23} According to Flangea et al., CD30 expression increases simultaneously with the progression of the disease.¹⁰ Su et al. concluded that Hodgkin Reed-Stenberg cells are able to inhibit the proliferation and activation of T cells through CD30-related interactions. CD30-related interactions result in an ineffective antitumor immunity, which clearly favors the growth and survival of the tumor cells.²⁴ Van der Weyden et al. also found that the effects of CD30 are mediated using several diverse signaling pathways, which put together benefit the survival of the cells on which CD30 is upregulated. Trimerization and signal mediation through tumor necrosis factor receptor associated proteins (TRAF) is caused by stimulation of the CD30 molecule, specifically TRAF2 to stimulate the nuclear factor-kappa B (NFkB) pathway. Furthermore, CD30 ligation is also signaled by mitogen-activated protein kinase (MAPK) pathways, including ERK1 and ERK2, which have diverse anti-apoptotic and pro-survival benefits in the neoplastic cell.¹¹

The inflammatory cell that infiltrates the tumor microenvironment displays prominent interactions with HRS cells and stimulates the production of molecules that support their growth and survival, including programmed death 1 (PD-1).^{25,26} EBV stimulates the production of many cytokines and chemokines by HRS cells, contributing to the peculiar microenvironment. HRS cells also produce immunosuppressive factors including PD-L1, which may inhibit cytotoxic T-cells from responding to EBV-infected HRS Cells. This latter finding indicates that in order to survive and grow, HRS cells evade immunosurveillance and interact with immune cells within the tumor microenvironment.²³ Higher PD-L1 expression levels were found in EBV-positive cases, suggesting that viral infections further upregulate PD-L1.²⁸

Wu et al. suggest that, by reducing their proapoptotic potential, PD-L1 overexpression renders tumor cells resistant to certain chemotherapy drugs. The study also suggests that chemoresistance as a result of PD-L1 is reliant on and requires an ERK/ MAPK pathway through the association of PD-L1 with DNA-PKcs. DNA-PKcs can activate MAPK/ERK pro survival signaling pathways. Increased activation of ERK in tumor cells was caused by overexpression of PD-L1, and p38 MAPK activation reduced by PD-L1 deficiency. Decreased activation of p38 MAPK consequently led to reduced expression of Bcl-2, a pro survival molecule.²⁹

In addition to playing a critical role in apoptosis, PD-L1, BCL (B Cell Lymphoma) proteins, as family member mutations, interfere with normal programmed cell death. Almost 38% of cases reported Bcl-2 protein expression in Hodgkin Lymphoma, and Reed Sternberg cells. Another

study revealed that Reed Sternberg cells were bcl-2 positive in 62% of Hodgkin Lymphoma cases. According to Chetty et al.'s study, most Reed Sternberg-like cells encountered in post-transplant lymphoproliferative disorders are strongly positive for both EBV and bcl-2 protein.³⁰ EBV-positive expression was found to result in the induction of Bcl-2, and Bcl-2 expression to confer a survival function upon the cells.³¹ [Angelica Gamboa-Cedeño](#) et al.'s study revealed that BCL2 expression rate correlated with a lack of response to conventional therapy and both early and late disease progression in CHL.^{32,33} Further research is required to determine the effect of PD-L1 and BCL2 in responses to ABVD and other chemotherapy regimens.

5. CONCLUSION

Low CD30 expression distribution dominated the complete response group, whereas high CD30 distribution dominated the nonresponse group. The results showed a statistically significant EBV-DNA detection status ($p=0.005$) and CD30 expression ($p=0.009$) between the complete/partial response and nonresponse groups. In conclusion, In conclusion, CHL responses to ABVD chemotherapy are found to be linked to CD30 expression and EBV-DNA status.

AUTHORS' CONTRIBUTIONS

BSH and HAU conceptualized and designed of the study, revised the manuscript critically for important intellectual content, and approved manuscript critically for important intellectual content.

ZN conception and designed the study, Acquired data, analyzed and interpreted of data, draft the manuscript, revised the manuscript critically for important intellectual content, and approved of the version of the manuscript critically for important intellectual content.

EP and A acquired data, analyzed and interpreted the data, draft the manuscript, revised themanuscript critically for important intellectual content

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