

# Anti Rituximab antibody titer and therapeutic response in Non-Hodgkin Lymphoma Patient Receiving R-Chop Treatment

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## ANTI RITUXIMAB ANTIBODY TITER AND THERAPEUTIC RESPONSE IN NON-HODGKIN LYMPHOMA PATIENT RECEIVING R-CHOP TREATMENT

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### Abstract

**Background:** Rituximab as a combination of chemotherapy (R-CHOP) is more effective in the treatment of Lymphoma Non-Hodgkini (NHL). Production of humoral immune response frequently decreases the efficacy of antibody therapy due to premature clearance of antibodies, thus limiting the effectiveness of anti-tumor responses.

We determined the correlation between anti-rituximab antibody levels and the response of R-CHOP-treated NHL patients who receiving.

**Methods:** This study was an observational analytic cross-sectional study on NHL patients in the Division of Hematology of Medical Oncology at Dr. Soetomo General Hospital Surabaya. Anti rituximab antibody titer was measured by ELISA from serum samples and the treatment response was evaluated according to category of response criteria for clinical trial (complete response, partial response, stable disease or progressive disease).

**Results:** This study was enrolled 54 subjects that consisted of 64.8% of male, mean age of patients was  $49.167 \pm 12,075$  years old, stage III was highest (61.6%). Median anti-rituximab antibody titer of 21.55 (9.24 - 197.17) pg/mL. The most progressive disease therapeutic response group was 46.3%. Comparative analysis of anti-rituximab antibody titer in each treatment response group was not significant ( $p$ -value = 0.08). The results of the correlation test are significant ( $p$ -value = 0.04;  $r$  Spearman = -0.27).

**Conclusion:** There was weak negative correlation between anti-rituximab antibody titer and therapeutic response in R-CHOP-treated NHL patients.

**KEYWORDS:** non-hodgkin's lymphoma, anti rituximab antibody, therapeutic response

### INTRODUCTION

Non-Hodgkin's lymphoma (NHL) is a group of heterogeneous malignancies derived from a lymphoid system that generally express B cells and/or T cells and it indicates a disruption in the development of normal precursor stage (1). The addition of rituximab (R) to CHOP regimens has now been widely accepted as a standard regimen for NHL treatment. Another study showed that 6-cycle R-CHOP resulted in CR treatment response of 56% and 58%, respectively (2-4).

In Indonesia, Hodgkin's disease and leukemia,

NHL ranks the sixth most common malignancy. The disease has caused a high rate of morbidity and mortality (5). Based on data obtained in the US between 2009 – 2013, there were 19.5 new cases of NHL found every 100.000 people per year, with mortality of 6 per 100.000 people each year (6). NHL can affect the quality of life of its patients, depending on the degree of symptoms severity, support of social environment and other needs (7). The addition of rituximab to standard CHOP regimen will increase the cost of treatment. Therefore, study about the correlation between anti rituximab antibody titer and therapeutic response in NHL patients with R-CHOP treatment needs to be conducted.

The CHOP regimen consisting of cyclophosphamide, anthracycline, vincristine and prednisone (9) has been used as the gold standard for aggressive lymphoma therapy for more than 25

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years. Patients with aggressive type of NHL are very likely to be cured and CHOP is considered to successfully cure 50% of cases in that population (8). The CHOP regimen. Lymphomas have typical histologic appearances such as immune phenotype with or without CD20 as the surface marker. With the discovery of CD20, the standard of treatment nowadays changes along with the addition of rituximab. Rituximab is one of the examples of the most successful anti-CD20 that has been widely approved to be used as combination of chemotherapy (10). Rituximab is a chimeric monoclonal antibody that is recognized by host's body as a foreign protein, thus stimulating the immune response. As a result, humoral immune response is subsequently generated and encountered Rituximab. (1, 11). Murine-derived rituximab fragments were reported to induce the formation of human anti-chimeric antibody (HACA) in patients receiving rituximab therapy, as in pemphigus patients and other autoimmune diseases such as Systemic lupus erythematosus (12, 13). HACA was also formed in NHL patients receiving rituximab therapy, enabling it to bind to rituximab, disrupt the therapeutic mechanism and reduce the efficacy (14) (10).

According to Association of Indonesia Hematology-Medical Oncology of Internal Medicine guidelines, the therapeutic response to NHL can be evaluated after 4 cycles of chemotherapy, therefore antibody rituximab antibody titer screening will also be performed after the 4th cycle of R-CHOP (5). Based on these considerations, the researchers wanted to analyze the correlation between anti-rituximab antibody titer and therapeutic response in NHL patients who received R-CHOP therapy in Dr. Soetomo General Hospital, Surabaya.

#### METHODS

This research was an observational research with the cross-sectional study design. The population of the study was NHL patients treated in Dr. Soetomo Surabaya from July 2015 to February 2016. The research samples were patients who meet the inclusion criteria but not the exclusion criteria. NHL patients with either nodal or extranodal who have received 4 cycles of chemotherapy R-CHOP regimen and have never received other

chemotherapy regimen or radiotherapy before, willing to participate in research and signed informed consent were categorized as inclusion criteria. While patients with age above 80 years were excluded. The sampling was done by consecutive sampling and interview in IRJ POSA-HOM Outpatient Installation and Medical Inpatient Installation of Dr. Soetomo General Hospital, Surabaya.

Anti-rituximab antibody titer was a parameter that describes the antibody concentration of rituximab in rituximab-treated NHL patients, that was measured using quantitative ELISA from serum samples after 4 cycles of chemotherapy RCHOP regimen, and expressed in pg/mL units. The R-CHOP regimen was a therapeutic regimen in NHL patients, a combination of immunotherapy and chemotherapy consisting of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone, administered every 21 days cycle (5, 15).

The instruments used during this research were the ELISA Kit (Sincere Biotech Co., Ltd., Beijing, China) to detect and identify the human anti-rituximab ELISA quantitatively and Ruler or meter with a scale of cm with precision up to 0.1 cm or 1 mm to measure the target lesion, measured at the two longest/bidimensional diameters.

NHL patients, both nodal and extra nodal in IRJ POSA-HOM unit Dr. Soetomo General Hospital that performed anamnesis symptoms related to the disease; examination of the size of the tumor physically and other investigations that meet the inclusion and exclusion criteria which has been given 4 cycles of R-CHOP will be taken serum samples for antititer antibody examination and evaluated the response of therapy based on the measurement of the target lesion physically and the presence or absence of disease-related symptoms, compared with data before chemotherapy, categorized by definition of response criteria for clinical trials including Complete Response, Partial Response, Stable Disease or Progressive Disease (5, 16). The data obtained then processed statistically.

Data analysis were analyzed using R statistic program, a system for calculation and graph (17). The difference analysis on anti rituximab antibody titer were determined using analysis of variance (ANOVA) test if data were normally distributed. Kruskal-Wallis would be used if the data were not normally distributed. Spearman correlation test was

used to determine the relationship between titer antibody anti rituximab with therapeutic response .

**RESULTS**

There were 54 of NHL patients who fulfilled the inclusion criteria, with the proportion of male 35 patients (64.8%) and female by 19 patients (35.2%). The mean age of the patient was  $49.167 \pm 12.075$  years, with the youngest was 16 years old and the oldest was 75 years old. The highest stage was III as much as 33 (61,1%), mostly found severity degree was intermediate grade as much as 33 (61,1%), appearances were diffuse mixed as much as 23 patients (42,6%) (Table 1). We found that the lowest concentration of anti-rituximab antibody titer in 54 LNH patients receiving R-CHOP was 9.24 pg/mL and highest concentration was 197.17 pg/mL, with median antibody concentration of rituximab antibody as much as 21.55 pg/mL.

The responses of therapy were grouped into the progressive disease, stable disease, partial response and complete response, with the mostly found response was progressive disease while least found was stable disease. In this study, the highest distribution of treatment groups was a progressive disease (46.3%), followed by a partial response (31.5%), then the complete response (14.8%) and at least stable disease (7.4%). The subject distribution according to the therapeutic response can be seen in Figure 1.

The statistical test was performed to see the comparison of antibody titer of anti rituximab in each treatment response group by using Kruskal-Wallis test because of abnormal data distribution. The Kruskal-Wallis test of anti rituximab antibody titer in each treatment response group can be seen in

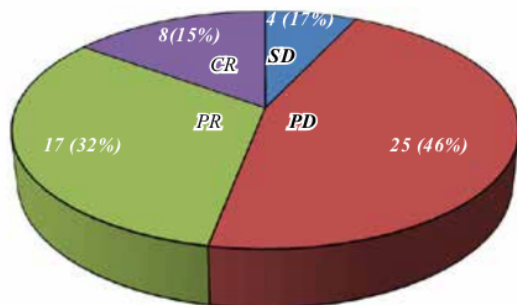


FIGURE 1. Distribution of subjects according to therapeutic response

NOTES: PD – Progressive Disease, PR – Partial Response, SD – Stable Disease, CR Complete Response

TABLE 1

Characteristics of research subjects		
Characteristics	Amount (n = 54)	Percentage (%)
<b>Sex</b>		
Male	35	64.8
Female	19	35.2
<b>Age</b>		
≤ 60 year	44	81.5
> 60 year	10	18.5
<b>Stage</b>		
I	4	7.4
II	16	29.6
III	33	61.1
IV	1	1.9
<b>Clinical and histopathologic degree</b>		
<b>Low grade</b>		
Small lymphocytic	5	9.2
Follicular mixed cell	6	11.1
<b>Intermediate grade</b>		
Diffuse mixed	23	42.6
Follicular large cell	1	1.9
Difuse large cell	9	16.7
<b>High grade</b>		
Large cell, immune blastic	5	9.2
Lymphoblastic	3	5.6
Small non cleaved cell	2	3.7

Table 2. Kruskal-Wallis test is showed result of  $p = 0,08$ , suggesting that there was no significant difference of antibody anti rituximab titer in each group of therapeutic response. The median anti-rituximab antibody titer in the progressive disease group was 22.1 pg/mL; stable disease was 24.735 pg/mL, with the lowest concentration was 22.88 pg/mL and the highest concentration was 52.75 pg/mL. Stable disease was the least found therapeutic response group, which was obtained from 4 of 54 subjects (7.40%). The median anti-rituximab antibody titer in the partial response group was 18.95 pg/mL and at complete response was 16.095 pg/mL. In general, the median reduction of anti rituximab antibodies was accompanied by the increased thera-

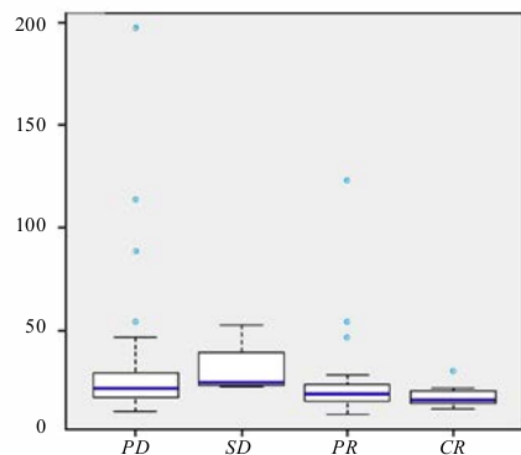
TABLE 2

Comparison of anti rituximab (pg/mL) antibody titer according to treatment response group

Disease	Medium	Min - Max
Progressive Disease (n=25)	22*	10.22-197.17
Stable Disease (n=4)	24.735*	22.88-52.75
Partial Response (n=17)	18.95*	9.24-123.2
Complete Response (n=8)	16.095*	11.95-30.17

Note: \* -  $p = 0.08$

peutic response in NHL patients receiving R-CHOP, although there was a slight increase in titer in the stable disease group. The difference between anti-rituximab antibody titers in each of these treatment response groups can be seen in Figure 2.



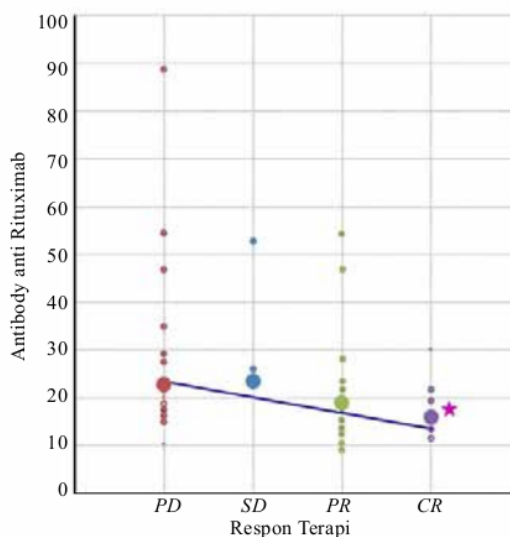
**FIGURE 2.** Differences between anti-rituximab antibody titers in each treatment response group

**NOTES:** PD – Progressive Disease, PR – Partial Response, SD – Stabile Disease, CR Complete Response

Statistically with the unbalanced number and distribution of samples present in each group of therapeutic responses, there was no significant difference in antibody titer of anti rituximab in each treatment response group, but the median trend line antibody titer of rituximab antibody on each - a therapeutic response group showed a antibody titer that decreased along with increasing therapeutic response (Figure. 4).

The result of analysis by using Spearman Rank showed a correlation between anti rituximab antibody titer and therapeutic response in with r value of -0.27 (-27%) and p-value of 0,04. Spearman's negative r value showed the inverse correlation between anti rituximab antibodies with therapeutic response with r value of 0.27 that was interpreted as a weak correlation strength.

This study showed that there was negative correlation between anti-rituximab antibody titer and the therapeutic response. The therapeutic responses would decrease along with the increase of anti-rituximab antibody titer, vice versa. This correlation can be seen through trend line median antibody titer anti rituximab on the therapeutic response (Figure 3).



**FIGURE 3.** Trend line median antibody titer of anti rituximab in each treatment

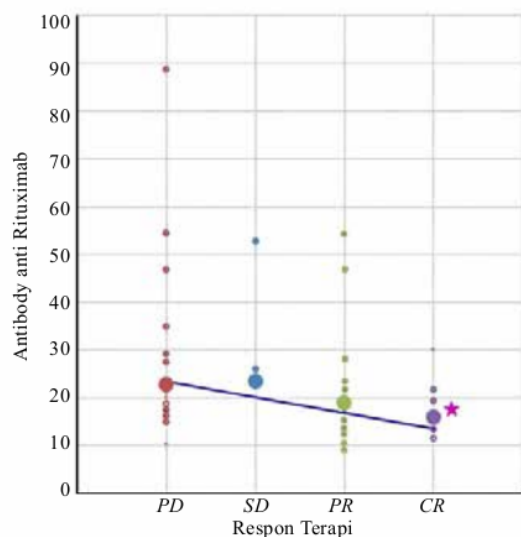
**NOTES:** PD – Progressive Disease, PR – Partial Response, SD – Stabile Disease, CR Complete Response, (star) - Median respon terapi, (stroke) - Linear median respon terapi

#### DISCUSSION

In this study, from 54 subjects that consisted of 35 male (64%) and 19 female (35.2%), the obtained ratio was 1.85: 1. This was in accordance with research conducted at Dr. Soetomo General Hospital Surabaya, with ratio of 1.5: 1 (21). The risk of NHL appears to increase with the aging process, which mostly occurs in the 60s or so (9). Median age was obtained in almost all NHL subtypes > 50 years, except for high-grade lymphoblastic and small non-cleaved lymphoma, more common in children and young adults. In 37% of patients with low-grade lymphoma were obtained at 35-64 years and only 16% at <35 years of age, but rarely found in children (20).

The subjects of this study had an average age of  $49.17 \pm 12.075$  years, with the youngest age range 16 years and the oldest 68 years, where age group with the highest proportion was age  $\leq 60$  years as many as 44 out of 54 patients (81.5%), previous research, got age  $\leq 60$  years counted 29 from 38 patient (76.32%) and got age  $\leq 60$  years counted 53 from 86 patient (61.63%) (3, 14).

The mostly found initial clinical stages in this study were stage III by 31 patients (57.40%), stage II by 17 patient (31.50%), stage I by 4 patient



**FIGURE 4.** Trend line median antibody titer of anti rituximab in each treatment

**NOTES:** PD – Progressive Disease, PR – Partial Response, SD – Stable Disease, CR Complete Response. (star) - Median respon terapi, (stroke) - Linear median respon terapi

(7.4%) and stage IV by 2 patient (3.7%). In contrast to the results of the study comparing two groups of elderly NHL patients who received the R-CHOP and CHOP regimens, in the two groups the highest stage was stage IV (49% vs 49%), stage III (26% vs 24%), stage II (19% vs. 20%) and at least stage I (6% vs. 7%) (22). This difference was most likely influenced by the use of multimodality that supported a systemic approach in staging.

The severity degree in this study was intermediate grade by 33 patients (61.1%), low grade 11 patients (20.4%) and high grade by 10 patients (18.5%). This was also in line with the data obtained the mostly found severity degree in the US were intermediate grade (41%), low grade (39%) and high grade (20%) (23).

In this study, the most histologic types were

diffuse mixed, intermediate grade of 23 patients (42.6%); diffuse large cell, intermediate grade of 9 patients (16.7%); follicular mixed cell, low grade by 6 patients (11.1%); small lymphocytic, low grade of 5 patients (9.2%); large cell, immunoblastic, high grade by 5 patients (9.2%); lymphoblastic, high grade of 3 patients (5.6%); small non cleaved cell, high grade of 2 patients (3.7%) and follicular large cell, intermediate grade of 1 patient (1.9%).

In this study, it was obtained that there was no difference in antibody titer of rituximab antibodies in each treatment response group, although it was generally found the median reduction of anti rituximab antibody titer in line with the improvement of therapeutic response in LNH patients who had received 4 RCHOP cycles. Only in the stable disease response group showed a slight increase in median antibody titer of anti rituximab. This was likely because the number of samples in each of the treatment response groups is unbalanced.

The correlation between anti rituximab antibody with LNH patient therapeutic response which got R-CHOP in this study obtained the result of r value equal to - 0.27 (- 27%) with p-value equal to 0.04 less than 0.05 ( $\alpha = 5\%$ ), meaning correlation between anti-rituximab antibody titers with therapeutic response in LNH patients receiving R-CHOP in Dr. Soetomo General Hospital, Surabaya was weak. Negative or opposite direction of correlation means the decrease of the therapeutic response, the antibody titer of anti rituximab will be higher.

#### CONCLUSION

There was weak negative correlation between anti-rituximab antibody titer and the therapeutic response. The therapeutic responses would decrease along with the increase of anti-rituximab antibody titer, vice versa.

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