

Correlation of CD4 Count and Neutrophil- Lymphocyte Ratio in Human Immunodeficiency Virus-Acquired Immunodeficiency Syndrome (HIV- AIDS) Patients

by A Makbul Aman

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Correlation of CD4 Count and Neutrophil-Lymphocyte Ratio in Human Immunodeficiency Virus-Acquired Immunodeficiency Syndrome (HIV-AIDS) Patients

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Abstract: Background Human Immunodeficiency Virus (HIV) is an RNA virus that causes a decrease in the immune system and destroys specific white blood cells called T-helper lymphocytes or Cluster of Differentiation 4 (CD4); CD4 is a parameter in HIV-AIDS patients while NLR is a simple parameters that were used to assess inflammatory status.

Purpose: This study aims to determine the relationship between CD4 and NLR levels in HIV-AIDS patients

Methods: This is an observational study with a cross-sectional design conducted in September 2019-September 2020. The study populations were all HIV patients that hospitalized and outpatient at Dr. Wahidin Sudirohusodo Hospital and his network hospital. Data were analyzed descriptively to found out the association between CD4 and NLR levels in HIV-AIDS patients using the Spearman trial.

Results: A total of 150 subjects, consist of 62 (41.3%) subjects at age 30-39 years, 122 (81.3%) male subjects, 108 (72%) subjects who had PEM nutritional status, and 102 subjects (68%) subject with CD4 count <50. Based on the Spearman test, there is negative correlation of CD4 and NLR in HIV-AIDS patient.

Conclusion: There are significant negative correlation between CD4 and NLR in HIV-AIDS patients at Dr. Wahidin Sudirohusodo Makassar, which higher NLR correlates with the lower value of CD4..

Keywords: human immunodeficiency virus, acquired immunodeficiency virus, cluster differentiation 4, neutrophil-lymphocyte ratio

1. INTRODUCTION

Human Immunodeficiency Virus (HIV) is an RNA virus belonging to the Retroviridae family, the sub-family Lentivirinae. This virus causes Acquired Immunodeficiency Syndrome (AIDS), causing a complete decrease in the immune system, destroying specific white blood cells called T-helper lymphocytes or Cluster of Differentiation 4 (CD4) such as macrophages, dendritic cells, and lymphnodes.¹

Human Immunodeficiency Virus has been known since the early 1980s and has infected millions of people worldwide. All HIV-infected patients are at risk for illness and death due to complications such as opportunistic infections, neoplastic infections, and other complications.²

It is reported that the total number of HIV patients worldwide is around 60 million people. In 2009 33.3 million people were living with HIV worldwide, and AIDS cause about 17 million deaths.¹ In Indonesia, since 1999, there has been an increase in the number of people with HIV AIDS (PLWHA) in groups of people who have a high risk of contracting HIV, namely commercial sex workers and drug abusers in several provinces such as Riau, Bali, West Java and East Java. It is estimated that there are 186,000 people with HIV in Indonesia in 2009.³

The cluster of differentiation 4 (CD4) is a type of white blood cell or lymphocyte that plays a role in the immune system. The cluster of differentiation 4 is also called T cells because the production site is in the bone marrow, but maturation is in the thymus. These cells have the main function of binding to antigens through TCR, besides having general functions in inflammation, activation of macrophage phagocytosis, activation of B cell proliferation in the antibody process, and recognition of the destruction of infected cells.^{4,5} Until now, CD4 cell count is still an important parameter in HIV AIDS patients. Low CD4 counts in HIV patients who experience opportunistic infections where there is a decrease in CD4 counts <200 / μ L.^{4,5}

The neutrophil-lymphocyte ratio (NLR) is a simple parameter used to assess inflammatory status. The neutrophil-lymphocyte ratio is the result of dividing the total neutrophils by the total lymphocytes.^{7,8} Neutrophils are myeloid precursors that act as the first line in response to a pathogen and innate immune response responses. Neutrophils inhibit microorganisms, especially bacteria or fungi; they phagocyte and kill pathogens by requiring NADPH oxygenase or producing an antibacterial protein in the phagosome. While lymphocytes are lymphoid precursors that function as adaptive immune cells and have a significant role in the

5 immune process because lymphocytes respond specifically to micro-organism infections.⁴The neutrophil-lymphocyte ratio is often used to determine a degree of inflammation in malignant disease, cardiovascular disease and is also used as a marker of bacteria. There is no ideal marker or marker that can be done quickly to see viral infection and bacterial infection. Currently, CRP, leukocyte, and neutrophil count are the parameters most often used in diagnosing bacterial infections.^{7,8}

12 This aim of the study is to analyse the relationship between CD4 cell count and NLR in HIV-AIDS patients in the <50 / μ L, <100 / μ L, and <200 / μ L groups.

2. METHODS.

Research Design

6 This is a cross-sectional study that conducted at Dr. Wahidin Sudirohusodo Hospital in Makassar, South Sulawesi, Indonesia.

Research Subjects

HIV-infected patients which hospitalized and at outpatient clinic at Dr. Wahidin Sudirohusodo and his network hospital from September 2019 to September 2020.

Research Data Collection

The inclusion criteria in this study were patients that diagnosed with HIV with CD4 counts <50 / μ L, 51-100 / μ L, and 101-200 / μ L, and willing to participate in the study, and signed informed consent.

Research Data Analysis

4 Data analysis was performed using SPSS version 22. Statistical analysis performed using descriptive statistical calculations and frequency distribution and chi-square statistical tests, independent t-test, ANOVA test, and Spearman correlation test. The test results are significant if the p-value <0.05.

Ethical Clearance

This study protocol was approved by the Health Research Ethics Commission of Hasanuddin University, Medical Faculty, with approval letter number 500/UN4.6.4.5.31/PP36/2020.

3. RESULTS

Subject Characteristics

Overall, 150 HIV subjects were included in this study from September 2019 to September 2020. it was found that 46 subjects (30.7%) were <30 years old, 62 subjects (41.3%) were 30-39 years old, and 42 subjects (28%) were \geq 40 years old (Table 1). This table also shows that out of 150 subjects, 122 subjects (81.3%) were male, and 28 (18.7%) female subjects. This table also shows that 108 subjects (72%) had PEM nutritional status, and 42 subjects (28%) had PEM nutritional status. CD4 count was divided into 3 groups. CD4 count <50 counted 102 subjects (68%), CD4 count 51-100 as many as 32 subjects (21.3%), and CD4 count 101-200 as many as 16 subjects (10.7%). (Table 1)

Mean WBC (white blood cell) level was $6,856.7 \pm 3,710.6$ / ul. The mean haemoglobin was 10.8 ± 2.8 g / dl. The platelet mean was 290.0 ± 126.8 / dl. The mean of

neutrophils was 74.3 ± 14.4 . The mean lymphocyte was 14.0 ± 9.2 . The mean absolute number of neutrophils (ANC) was $5,288.7 \pm 3,370.7$. The mean total leukocyte count (TLC) was 842.0 ± 540.1 . The mean ratio of lymphocyte to neutrophil (NLR) was 9.5 ± 9.5 . The mean CD4 count was 43.0 ± 41.5 . (Table 2)

Analysis of correlation CD4 count with NLR

Based on figure 1 shows that there is negative correlation of CD4 and NLR, where the higher CD4, the lower NLR and vice versa. The decrease of CD4 was followed by increase of NLR value (Figure 1). Based on the Spearman test results, there is a significant negative correlation between CD4 and NLR, where the higher the NLR value, the lower the CD4 value ($R = -0.321$ $p < 0.001$). (figure 2)

Analysis of correlation CD4 and NLR with nutritional status

Analysis correlation of CD4 and NLR with nutritional status shows that mean CD4 was lower on severe PEM (29.8) compared with moderate PEM (76.8). There was significant correlation of CD4 and nutritional status ($p < 0.001$). The mean NLR was higher in severe PEM (10.4) than in moderate PEM (7.1). There was a significant correlation between NLR and nutritional status ($p < 0.05$) (Table 3)

4. DISCUSSION

This study included 150 study subjects aged 18-69 years with a mean of 35.2 ± 10.2 years. In this study, it was found that the age group of 30 - 39 years was the largest group of people with HIV AIDS, namely 62 subjects (41.3%), then the <30 years age group was 46 subjects (30.7%), and the age group ≥ 40 years was 42 subjects (28%). This study's results are in line with previous research conducted by Elisda Yusra et al. in 2018 in Padang, which found that the research subjects had a maximum age of 30-39 years, namely 48.6%.⁸ In fact, research conducted by Yuneti Octavianus et al. in 2010 - 2016 in East Sumba found that 51.4% or as many as 57 research subjects were in the 25-34 year age group.⁹ Research conducted by Myeongso Yoo in 2010-2015 in Korea found that the incidence of HIV AIDS was increased in young adults aged 20-29 years. HIV AIDS testing conducted on young adults has increased to 200,000 cases over the past 6 years and has doubled the HIV-AIDS prevalence.⁹ The incidence of HIV AIDS in the age group of 20 - 39 years (late adolescence and adulthood) increases because this age group is still active and sexual unhealthy behavior such as changing partners and not using protection during sexual intercourse, thus allowing transmission through body fluids such as semen and vaginal fluids.¹⁰

The research subjects consisted of 122 women (81.3%) and 28 men (18.7%). This is in line with previous research. Research conducted by Emily et al. in 2013 in Africa found that the number of HIV AIDS sufferers in men is more than in women.¹¹ Also, Sang Bagus et al research in 2018 in Bali found that out of 95 samples, 69 (72.6%) were male, and 26 (27.4%) were female. Men are more at risk of contracting HIV AIDS because they tend to have unhealthy sexual behavior, homosexual, and often change partners. However, women remain a serious concern because they are susceptible to infection from men and can infect the baby later if pregnant.⁹

In this study, 108 subjects (72%) had PEM nutritional status, and 42 subjects (28%) had PEM nutritional status. This is following previous research. Research conducted by Shinyoung et al. in 2017 in the United States shows that HIV AIDS can directly cause malnutrition in HIV AIDS patients due to reduced appetite, difficulty due to infection in the mouth, poor absorption of nutrients due to diarrhea, or drug interactions, which is consumed.¹⁴

CD4 counts in HIV patients <50 were 102 subjects (68%), CD4 counts 51-100 were 32 subjects (21.3%), and CD4 counts 101-200 were 16 subjects (10.7%). This is in line with the previous research. Research conducted by Mirna et al. in 2013 in Jayapura that 40% of subjects had CD4 <200 cells / mm³. Also, a study conducted by Rini et al in 2013 in Riau found 64 subjects with CD4 <200 cells / mm³.¹⁶ In people with HIV AIDS, there is a decrease in CD4 T cells due to CD4 death. After acute infection, there is an asymptomatic period in which the decrease in CD4 cell counts gets sharper at an advanced stage. This is because at CD4 count <200 cells / mm³, the risk of opportunistic infections will increase.¹⁵

HIV AIDS patients had a mean WBC (white blood cell) level of $6,856.7 \pm 3,710.6$ / ul. The mean hemoglobin was 10.8 ± 2.8 g / dl. The platelet mean was 290.0 ± 126.8 / dl. The mean of neutrophils was 74.3 ± 14.4 . The lymphocyte mean was 14.0 ± 9.2 . The mean ANC (absolute number of neutrophils) was $5,288.7 \pm 3,370.7$. The mean TLC (total leukocyte count) was 842.0 ± 540.1 . The mean NLR (ratio of lymphocyte to neutrophil) was 9.5 ± 9.5 . The mean CD4 count was 43.0 ± 41.5 . Research conducted by Subhas B et al. in 2015-2018 in India found that there are hematological disorders at all stages in HIV AIDS patients; the disorders can be anemia, leukopenia, lymphocytopenia, and thrombocytopenia.¹³ Anemia is the most common hematological disorder in people with HIV AIDS. Research conducted by Sang Bagus et al. in 2018 in Bali found a significant positive TV obtained between Hb levels and the number of CD4 + T lymphocytes. Anemia can be used as a marker for a decrease in the number of CD4 + T lymphocytes so that a simple complete blood count can be used as a support to determine the decrease in CD4 +.¹²

Based on the Spearman trial results, there are significant negative correlation between CD4 and NLR, where the higher the NLR value, the lower the CD4 value ($R = -0.321$ $p < 0.001$). Research conducted by I Nyoman et al. in 2017 in Bali found that there are weak positive correlation between CD4 and NLR in people with HIV AIDS. CD4 is a type of white blood or lymphocyte produced in the bone marrow and plays a role in the immune system. CD4 becomes the target cell for HIV infection. A decreased CD4 count in HIV infection makes the patient susceptible to infection. He causes a cytopathic effect on CD4 through immune system activation resulting in PMN cell apoptosis, stem cell damage, and lymphoid tissue, including one thymus. No new cells are formed. This disrupts the function and number of neutrophils. Neutrophil dysfunction makes HIV patients prone to bacterial infections.¹

Research conducted by Emokpae et al. in 2016 in Africa found that the NLR in HIV patients was higher than that in the control group of healthy patients ($p < 0.001$). while the CD4 count in HIV patients was lower than that in the control group of healthy patients ($p < 0.001$). NLR is a marker of inflammation for diagnosing HIV and warning of HIV severity. The inflammatory process causes neutrophil apoptosis, which disrupts the function and number of neutrophils and makes HIV patients susceptible to bacterial infection.¹⁷

The mean CD4 count was lower in severe PEM (29.8) than in moderate PEM (76.8). There was a statistically significant association between CD4 cell count and nutritional status (p

<0.001). Research conducted by Rashmi et al. in 2019 in America found that there was no significant show between the nutritional risk index and CD4 count in HIV AIDS patients.²⁰ Calculation of the nutritional risk index from the calculation $(1,519 \times \text{serum albumin (g / L)} + 41.7 \times (\text{current weight / usual body weight}))$, $\text{NRI} > 100$ means no risk of malnutrition, $\text{NRI} < 100$ means there is a risk of malnutrition, Riskoline $\text{NRI} 97.5-100$, low risk if $\text{NRI} 83.4 - 97.5$, and severe risk if $\text{NRI} < 83.5$. Research conducted by Muhammad Amin in 2017 in Pontianak also found that CD4 count did not affect the nutritional status of HIV AIDS patients.²¹ Mean NLR higher in severe PEM (10.4) than in moderate PEM (7.1), statistically significant association was found between NLR and nutritional status ($p < 0.05$). Research conducted by Shalini et al. 2011 in India found that malnutrition affects neutrophils and lymphocytes in HIV AIDS patients.¹⁹

Strengths and Weaknesses of Research

This study gives information on on the relationship between CD4 counts and levels of neutrophil-lymphocyte ratio (NLR) in HIV AIDS patients at Dr. Wahidin Sudirohusodo Makassar, which can be used as the basis for further research. However, this study could not see a comparison between the mean CD4 and NLR according to the type of opportunistic infection because the infection did not stand alone (coincided with other infections).

5. CONCLUSION

There is significant negative correlation of CD4 cell count and NLR in HIV AIDS patients at Dr. Wahidin Sudirohusodo Makassar.

Recognition.

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Table 1. Categories of research subjects (n = 150)

Variabel	N	%
Umur, tahun		
<30	46	30,7
30-39	62	41,3
≥ 40	42	28
Jenis Kelamin		
Laki-Laki	122	81,3
Perempuan	28	18,7
Status Nutrisi		
Severe PEM	108	72
Moderate PEM	42	28
CD4		
<50 u/L	102	68
51-100 u/L	32	21,3
101-200 u/L	16	10,7
PEM = Protein Energy Malnutrition, CMV = Cytomegalovirus, PCP = Pneumonia Pneumocystis, TB = Tuberkulosis, CD4 = Cluster of Differentiation 4		

Table 2. Mean of Research Subjects Laboratory Examination (n = 150)

Variabel	Minimum	Maximum	Mean	Std. Deviation
WBC	1.200	22.100	6.856,7	3.710,6
HB	3,08	27,00	10,8	2,8
PLT	20	605	290,9	126,8
Neutrofil	33,9	96,1	74,3	14,4
Limfosit	1,8	44,5	14,0	9,2
ANC	508,5	19.890,0	5.288,7	3.370,7
TLC	59,4	2.784,0	842,0	540,1
NLR	0,9	53,4	9,5	9,5
CD4	1	182	43,0	41,5

Table 3. Mean CD4 and NLR according to Nutritional Status

Variabel	Status Nutrisi	N	Mean	SD	P
CD4	Severe PEM	108	29,8	34,4	<0,001
	Moderate PEM	42	76,8	39,1	
NLR	Severe PEM	108	10,4	10,5	0,015
	Moderate PEM	42	7,1	5,8	

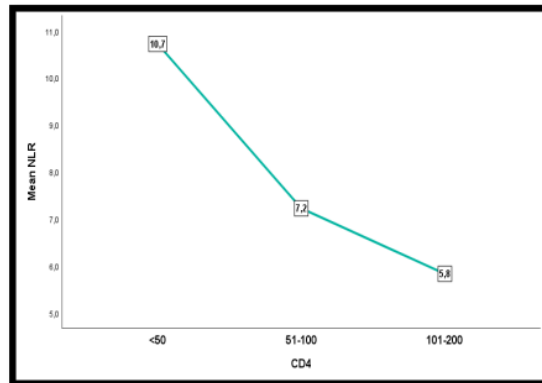


Figure 1 CD4 according with mean NLR

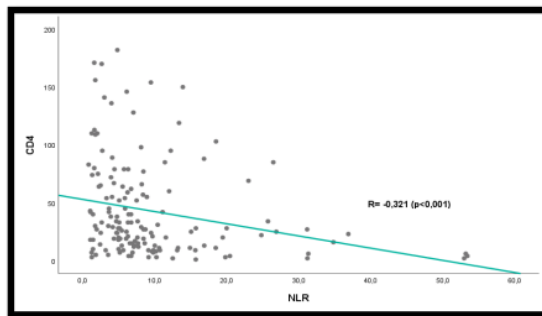


Figure 2. Figure CD4 correlation with NLR

Correlation of CD4 Count and Neutrophil- Lymphocyte Ratio in Human Immunodeficiency Virus-Acquired Immunodeficiency Syndrome (HIV- AIDS) Patients

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