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Association of Neutrophil-Lymphocyte Ratio and Monocyte-Lymphocyte Ratio with Opportunistic Infections in Patients with HIV Infection

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Abstract: ***Opportunistic infection is a problem that is often found in patients with HIV infection which contributes to high rates of morbidity and mortality. The incidence of opportunistic infections will increase with advanced progression of HIV infection and further decline of the immune system. Several leucocyte subsets, including neutrophil-lymphocyte ratio (NLR) and monocyte-lymphocyte ratio (MLR) have been shown its potential of inflammatory marker in various diseases and infection. The objective of this study is to evaluate the relationship of NLR and MLR with opportunistic infections in patients with HIV infection. This is a single center, cross-sectional, and retrospective study conducted in Voluntary Counseling and Testing (VCT) clinics at Wangaya General Hospital. Patients aged 18 years old or older confirmed with HIV infection are included, except those with co-existing chronic hepatitis, malignancy, and pregnancy. From 110 subjects included, 45 (40.9%) had opportunistic infections with 25 (55.6%) and 20 (44.4%) of them are naïve and on HAART respectively. Oral-esophageal candidiasis was the most common opportunistic infection (26.4%), followed by pulmonary tuberculosis (11.8%), toxoplasmosis and pneumocystis pneumonia (7.3%), herpes zoster (6.4%), and extrapulmonary tuberculosis and CMV infection (0.9%). NLR, MLR, and CD4 count had significant relationship with the presence of opportunistic infections (p<0.001), with correlation coefficient of NLR (r = 0.47, p <0.001) MLR (r = 0.59, p <0.001) and CD4 count (r= -0.69, p <0.001). The result of this study suggests the role of NLR and MLR as a simple and widely available parameter available to raise suspicion of possible opportunistic infection in HIV infected patients in areas of limited resources.***

Keywords: opportunistic infections, HIV patients, neutrophil-lymphocyte ratio, monocyte-lymphocyte ratio

1. Introduction

There are approximately 37.6 million people lived with HIV infection globally by 2020, with 1.5 million people newly diagnosed with HIV infection in 2020. HIV infection has contributed to high rates of morbidity and mortality worldwide. In 2020, 690.000 people have died due to AIDS-related illness. This number added to a total of 34.7 million people have died due to AIDS-related illness since the beginning of the epidemic.[1]In 2019, HIV/AIDS has made it as the second most disability-adjusted life-years (DALY) lost in young adult (25-49 years). HIV/AIDS also showed a 58.5% increases of age-standardized DALY rates between 1990 and 2019.[2]

People with chronic HIV infection, especially those who are not on treatment or non-responsive to treatment with antiretroviral agents, will have low CD4+ counts. As the CD4+ counts progressively drop, those people are in vulnerable condition to various infection which are hardly occur in healthy, immunocompetent people. Therefore, this condition is referred to as an opportunistic infection. Study showed that people lived with HIV infection with CD4 counts of <200/µL were associated with six times higher chance to develop opportunistic infection compared to those with >350/µL of CD4 counts.[3]

Leucocyte has been known have major role in in infections and inflammatory processes. Several leucocyte subsets, including neutrophil-lymphocyte ratioand monocyte-lymphocyte ratio, are frequently used to assess the severity of inflammation in cancer, inflammatory arthritis, cardiovascular disease, and as a bacterial infection marker. Previous study evaluated neutrophil-lymphocyte ratio, monocyte-lymphocyte ratio for the purpose of predicting active TB infection and the risk of future TB infection in HIV patient.[4-6] Neutrophil-lymphocyte ratio also shown to have role in predicting bacterial infections in HIV patients[7].

Incorporating all the previous findings that have been described above, this study purpose is to find correlation between neutrophil-lymphocyte ratio, monocyte-lymphocyte ratio with incidence of opportunistic infections in HIV patients. As these parameters is readily available within peripheral blood examination, this study aims to provide a screening criterion to help diagnosis the presence of opportunistic infections in HIV patients.

2. Method

This is a single center, cross-sectional, and retrospective study conducted in Voluntary Counseling and Testing (VCT) clinics at Wangaya General Hospital. Patient diagnosed with HIV infection from 2010 to 2020 were enrolled. Patients aged 18 years old or older confirmed with HIV infection by positive result in all 3 combination of rapid HIV test are included during the study. Patients with existing chronic hepatitis, malignancy, and pregnancy is excluded. The total of 110 subjects were included in the study. All data were taken from medical record.

Opportunistic infections were diagnosed clinically, pathologically, and/or immunologically, as infection that develops in conditions of decreased immune function due to chronic HIV infection in pre-treatment or non-responsive to treatment. Peripheral blood examination is performed within visit in outpatient care or inpatient care in patients who presented with suspected opportunistic infection. The number of CD4 counts in the plasma is measured in cells per milliliter (cells/ml) using flow-cytometry methods. CD4 counts were examined on the presence of opportunistic infections. Collected data then analyzed by Statistical Package for the Social Sciences (SPSS) version 25. Multivariate analysis and correlation coefficient analysis were performed, with significant statistical test is determined by p value <0.05.

3. Result

The study included 63 males (57.3%) and 47 (42.7%) subjects with mean age of 38.1 and 38.7 years old respectively. Among 80.9% subjects are married, and most subjects reported have heterosexual (95.5%) as risk factor of HIV infection. As much as 58.3% of the subjects already received highly active antiretroviral therapy (HAART), with 90% of them were in good adherence to treatment. The number of subjects who had opportunistic infections was 40.9%, and 42.2% among them had 2 or more concurring opportunistic infection. The most common reported opportunistic infection is oral-esophageal candidiasis and followed by pulmonary tuberculosis. From the laboratory parameters, the mean leucocyte counts of the subjects was 5.82 x 10^3/µL, followed by a mean neutrophil counts of 3.35 x 10^3/µL, lymphocyte counts of 1.62 x 10^3/µL, monocyte counts of 0.54 x 10^3/µL, and CD4 counts of 246.74 cell/µL (table 1.).The incidence of opportunistic infection in pre-HAART subjects is higher than in subjects already received HAART (55.6% vs 44.4%). The distribution of opportunistic infection among treatment group is presented in table 2.

Table 1: Characteristics of the Subjects

Sex	N	%
Male	63	57.3
Female	47	42.7
Marital Status		
Single	17	15.5
Married	89	80.9
Divorced	4	3.6
Risk Factor		
Homosexual	2	1.8
Heterosexual	105	95.5
Bisexual	1	0.9
IVDU	1	0.9
Treatment Status		
Pre-HAART	46	41.8
On-HAART	64	58.2
Adherence		
>95%	99	90
95-80%	1	0.9
<80%	3	2.7
Lost to Follow Up	7	6.4
Opportunistic Infection		
Positive	45	40.9
Negative	65	59.1
Number of Opportunistic Infection		
1	26	23.6
2	15	13.6
3	4	3.6
Opportunistic Infection		
Pulmonary tuberculosis	13	11.8
Extrapulmonary Tuberculosis	1	0.9
Toxoplasmosis	8	7.3
Pneumocystis Pneumonia (PCP)	8	7.3
Herpes zoster	7	6.4
Oral-Esophageal Candidiasis	29	26.4
CMV infection	1	0.9
Laboratory Parameter		
Total Leucocytes	5.82 (1.33-17.73)	
Neutrophil	3.35 (0.60-16.61)	
Lymphocyte	1.62 (0.08-4.60)	
Monocyte	0.54 (0.2-1.34)	
CD4 count	246.74 (1.0-1177.0)	

Table 2: Incidence of Opportunistic Infection Among Treatment Status

Treatment Status	Opportunistic Infection	
	Positive	Negative
Pre-HAART	25 (55.6%)	21 (32.3%)
On-HAART	20 (44.4%)	44 (67.7%)
Total	45 (40.9%)	65 (59.1%)

The data distribution of NLR, MLR, and CD4 count was evaluated using the Kolmogorov-Smirnoff normality test. NLR, MLR, and CD4 count data distribution resulted in non-normally distributed data with value of p<0.001 among all of them. Incidence of opportunistic infections were further classified into (1) no opportunistic infection, (2) 1 opportunistic infection, and (3) ≥2 opportunistic infections. Kruskal-Wallis test then performed to analyze NLR, MLR, and CD4 count within incidence of opportunistic infections occurred in the subjects. The analysis showed that there was a statistically significant difference between the NLR, MLR, and CD4 count values in subjects with no opportunistic infections and subjects who had opportunistic infections. However, further analysis showed that NLR, MLR, and CD4 count weren't different between subjects with 1 opportunistic infection and ≥2 opportunistic infection (p = 0.99, p = 0.68, p = 0.60). The result of Kruskal-Wallis test of NLR, MLR, and CD4 count with the incidence of opportunistic infections is further shown in table 3 and figure 1.

Spearman correlation was performed to evaluate the relationship between NLR, MLR, and CD4 count with the incidence of opportunistic infection. Significant positive relationship was found in NLR (r = 0.47, p <0.001) and MLR (r = 0.59, p <0.001) with the incidence of opportunistic infection. Significant inverse relationship was found between CD4 count with the incidence of opportunistic infection (r= -0.69, p <0.001).

Table 3: Kruskal-Wallis Analysis of NLR, MLR, and CD4 count with Incidence of Opportunistic Infection

Number of Opportunistic Infection	NLR	MLR	CD4 count
No Opportunistic Infection	1.58 (0.46-6.55)	0.24 (0.01-1.38)	332 (14-1177)
1 Opportunistic Infection	2.88 (0.73-16.04)	0.64 (0.27-1.51)	37 (4-468)
≥2 Opportunistic Infections	2.63 (0.99-20.82)	0.43 (0.20-1.74)	60 (1-433)
p value	<0.001	<0.001	<0.001

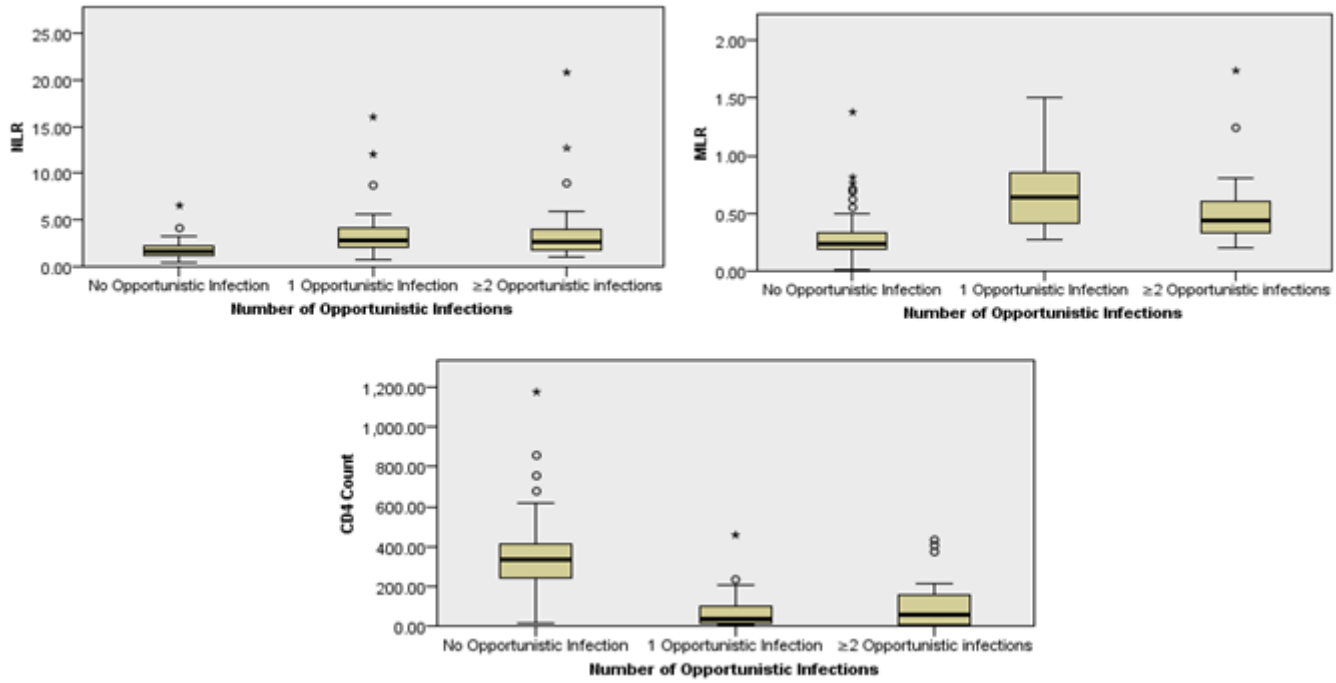

Figure 1: NLR, MLR, and CD4 count based on number of opportunistic infections of HIV patients

4. Discussion

Opportunistic infections have become a major burden in patients with HIV infection. Even after initiation of HAART, incidence of opportunistic infection in HIV infected patients was 46.7/1,000 person-years with 45% of mortality is contributed by AIDS-related diseases.[8]The incidence of opportunistic infections is also an economic burden. Based on a cost-per-event calculation, CMV infection was the most costly ($1100), followed by Cryptococcosis ($913), PCP ($708), Candidiasis ($587), Tuberculosis ($489), and Varicella zoster virus infection ($127).[9]

In this study, the prevalence of opportunistic infections was 40.9%. The most common opportunistic infections found in HIV infected patients was oral-esophageal candidiasis, followed by pulmonary tuberculosis, Toxoplasmosis, PCP, and herpes zoster. This finding is comparatively similar with previous study.[10]

The initial line of defense against invading pathogens, notably bacterial and fungal infections, is neutrophils. It also had role in containing and eliminating viral infections.[11]Neutrophils move to inflamed and infected areas, where they detect and phagocytize invading pathogens in order to kill them using various cytotoxic methods.[12]This inflammatory process will stimulate further needs of neutrophil through increasing bone marrow proliferation and release of the storage pool into the peripheral blood. HIV does not directly infect neutrophils, but is capable of disrupting the response of neutrophils to infection. HIV infection can interfere with phagocytic function, hyperactivation of neutrophils, the imbalance between apoptosis and neutrophil necrosis that also induce chronic inflammatory response. These conditions will contribute in the neutrophil count in response to infection in HIV infected patients.[13]

Monocyte activity is also impaired such as neutrophils in patients with HIV infection. Patients with HIV infection had a higher total monocyte count and inflammatory monocyte subset compared with healthy individual. Monocytes can be activated during HIV infection by replicating HIVvirus, microbial translocation, and coinfection with infective pathogen.[14]

Lymphocyte, especially $CD4^+$ T-lymphocyte, is the main target cell of HIV infection. Lower total lymphocyte count. Total lymphocyte counts have been found to be associated with the risk of disease progression in HIV infection. Total lymphocyte count also directly correlates with the CD4 count in peripheral blood.[15,16] Thus, lower lymphocyte count is associated with higher incidence of opportunistic infections. Patients with a total lymphocyte count of 1000–1500 cells/mm3 were estimated to have a 40% higher chance of contracting an opportunistic infection.[17]

All those findings could provide basic explanation to our findings. In this study, we found positive correlation between NLR and MLR value with the presence of opportunistic infections. This finding is in accordance with the findings in previous studies. Several studies have

assessed the relationship between NLR and MLR with the presence of opportunistic infections in HIV patients. In general, unspecified bacterial infections in HIV patients resulted in higher NLR compared to those HIV patients without bacterial infection (12.80 vs. 2.40; p<0.001).[7]The most studied opportunistic infection, especially in cases of active tuberculosis in HIV patients. Neutrophil count and neutrophil/lymphocyte ratio (NLR) were significantly higher in tuberculosis cases, irrespective of HIV co-infection at diagnosis.[18] This is also happened with MLR which previous studies showed active tuberculosis is associated with increased of total monocyte count compared with latent tuberculosis in HIV infected patients (5.1% vs. 3.7%; p = 0.013).[19]Increased monocyte count also resulted to higher MLR in HIV infected patient with active tuberculosis (0.407 vs. 0.207; p<0.01).[20]Incidence of herpes zoster is also correlated with NLR value. Although there were no studies examining herpes zoster in patients with HIV, studies in immunocompromised patients with rheumatic diseases showed that patients who experienced herpes zoster infection had higher neutrophil-to-lymphocyte ratio (4.9±6.2 vs 2.8±2.6; p<0.001).[21] Similar conditions also found in patients who underwent liver transplantation.[22] This findings supported the role of NLR and MLR in predicting the presence opportunistic infections in HIV patients. This could be a simple and widely available parameter to raise suspicion of possible opportunistic infection in areas of limited resources. The drawback is that this study did not show differences in NLR and MLR values associated with the number of opportunistic infections that were occurring in the subjects.

This study has several limitations. First, this study didn't evaluate separately incidence of opportunistic infections based on treatment status. Second, this study was not able to distinguish the effect of each type of opportunistic infection on the NLR and MLR values because the incidence of each opportunistic infection was relatively small. Other limitation that in some cases of infection, due to limited resources, the diagnosis is made based solely on clinical findings and response to therapy.

5. Conclusion

This study shows that 40.9% of subjects with HIV infection has opportunistic infection. NLR and MLR have been showed to have direct correlation, and CD4 count has inverse correlation with the presence of opportunistic infection in HIV infected patients. Further studies need to be done to evaluate NLR and MLR value in response to each type of opportunistic infections in HIV infected patients.

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