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Diagnostic Value of Leukocyte Count Abnormalities in Newly Diagnosed Tuberculosis Patients

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Abstract

Background: The diagnosis of tuberculosis (TB) is frequently challenging given that the clinical and radiographic features of TB are often nonspecific. Altered leukocyte count ratios could serve as new tools of diagnostic orientation of tuberculosis. The aim of this study was to assess the diagnostic value of the leukocyte count ratios for the diagnosis of TB. **Methods:** This was a cross-sectional study including cases of newly diagnosed TB patients from registers of the TB treatment center of the Douala General Hospital. Control subjects were healthy volunteers, age and sex matched, recruited at the blood bank. Sociodemographic, clinical data and peripheral blood parameters were collected. The diagnostic value of leukocyte counts was determined using receiver operating characteristics curve analysis. **Results:** In total, 204 TB patients and 204 control subjects were included in the study. The gender of the participants was equitably distributed in the 2 study groups (male 61.8%; female 38.2%). The median age of TB patients was 33 years while that of control patients was 32 years. The monocyte-lymphocyte count ratio (MLR) and neutrophil-lymphocyte count ratio (NLR) were significantly higher in the TB patients group compared to control group. A NLR > 1.19 and MLR > 0.29 were identified as cut-off values for discriminating TB patients. The areas under the curves (AUC) were 0.77 and 0.84 for the MLR and NLR respectively. **Conclusion:** A raised NLR > 1.19 and MLR > 0.29 are predictive of tuberculosis. The NLR has greater diagnostic ability as evidenced by its higher AUC. Further research is needed to confirm or refute our findings.

Keywords

Leukocytes, Diagnosis, Tuberculosis, Africa

1. Introduction

Tuberculosis (TB) is highly prevalent chronic infectious disease caused by Mycobacterium tuberculosis (MTB), an aerobic intracellular bacterium. MTB is the second leading cause of death from a single infectious agent, after the human immunodeficiency virus (HIV). It ranks amongst the first worldwide pathogens latently infecting one third of the population [1]. This reservoir of infected individuals resulted in an estimated 10.4 million incident TB cases, with 1.4 million deaths reported worldwide in 2015 [1]. In 2014 the African Region had the most severe burden relative to population of the disease with 275 incident cases per 100,000 population on average, more than double the global average of 133 [2]. Cameroon records an estimated 25,000 new cases of TB annually since 2006 while the mortality rate in the general population is 57/100,000 inhabitants and 6% among notified TB case [1] [3].

For decades, myeloid-specific cells have been known to serve as host cells for MTB growth and lymphoid cells are thought to be the major effector cells in tuberculosis immunity. Given the central role of monocytes and lymphocytes in the induction of immune responses, their levels in peripheral blood might be expected to reflect the state of an individual's immunity to infection. Therefore, the relative abundance may reflect a balance between effector and target cells [4]. Recent clinical analysis of peripheral blood mononuclear cells supports the hypothesis that a significantly high or low monocytes/lymphocytes count ratio (MLR) does not only correlate with risk of developing tuberculosis but is also predictive of active TB [5] [6].

Evidence has emerged in recent years suggesting that neutrophils may also play a role in early innate immunity against MTB. Neutrophils are the most commonly infected phagocytic cell in sputum samples in the airways of patients with active tuberculosis (TB). They may play an important role as part of the innate host response to mycobacterium and contribute to the early control of MTB infection [5] [7]. Previous reports have shown that the neutrophils/lymphocytes count ratio (NLR) is significantly lower in tuberculosis compared to bacterial community acquired pneumonia and sarcoidosis with cut-off values of 7 and 2.5 respectively [8] [9].

Diagnosis of TB is frequently challenging, given that clinical and radiographic features of TB are not specific and acid-fast bacilli sputum smear-the most widely available in resource-limited settings is of a variable sensitivity from 74% to 93% and a specificity from 71% to 96% [10] [11] [12]. Delays in diagnosing pulmonary tuberculosis can have a negative effect on patient morbidity and mortality and increase disease transmission [9]. Studies on changes in leukocyte

subsets in newly diagnosed TB patients are discrepant. In addition, values of the NLR and MLR between TB patients and healthy subjects have not been established.

The aim of this study was to find out the association of various leukocyte count ratios, monocytes/lymphocytes, neutrophils/lymphocytes ratios and tuberculosis and to evaluate their diagnostic abilities.

2. Methods

2.1. Study Design and Setting

A cross-sectional study was conducted in the TB treatment center, and in the blood bank and hematology unit of the laboratory of the DGH from January 2016 to May 2016. This hospital with a capacity of 300 beds is a tertiary care and teaching hospital. In the TB treatment center, all patients diagnosed with TB are registered in the TB register before starting the treatment. The diagnosis and the treatment are based on guidelines of the national tuberculosis control program described below. The blood bank is part of the diagnostic laboratory of the DGH. The hematology unit is endowed with an automated hematology analyzer and is headed by a clinical hematologist. Socioeconomic and clinical data of donors are collected before blood donation. They also benefit from free HIV and hepatitis B and C screening. Donors are composed mainly of young adults from the Douala sub region as well as family members of hospitalized patients. They are usually healthy, without any clinical signs or symptoms.

2.2. Participants

Our study population was made of 2 groups: 1) patients aged 18 years and above, diagnosed TB and registered in the TB treatment center. These included smear positive and negative pulmonary TB, as well as pleural and lymph node TB. Smear negative pulmonary TB was defined according to the guidelines of the National Tuberculosis Control Programme (13) 2) healthy controls were patients recruited at the blood bank of the DGH. They were age and sex-matched to TB patients.

Subjects with known chronic conditions such as HIV infection, viral hepatitis, hematological disorders, diabetes mellitus, pregnancy, steroid therapy within 3 months before the study were excluded from both groups. Participants with clinical suspicion of infectious diseases other than tuberculosis were also excluded, as well as patients who did not perform differential white blood cell count.

A consecutive sampling was used for the 2 groups of subjects. TB patients were recruited from TB register while control subjects were consecutively included among blood donors at the blood bank of the DGH.

2.3. Data Collection

All the donors provided written informed consent and the study was approved by the ethics committee of the Faculty of Medicine and Pharmaceutical Sciences

of the University of Douala while administrative clearance was obtained from the Douala General Hospital authorities. Data were collected on sociodemographiccharacteristics such as age, sex and residence; Clinical forms of tuberculosis as well as peripheral blood parameters such as hemoglobin concentration, neutrophils, lymphocytes, monocytes, and platelet counts. About 3 - 4 ml of peripheral venous blood was drawn aseptically in the morning, with sterile needles and collected into ethylene-diamine tetra-acetic acid containing (EDTA) tubes. Quantitative analysis of blood cells was performed by the automated Cell Dyn Ruby Hematology analyzer (ABBOT model) at the clinical diagnostic laboratory of the Douala General Hospital. The NLR and MLR were evaluated as follows: NLR = absolute neutrophil count/absolute lymphocyte count, MLR = absolute monocyte count/absolute lymphocyte count

The diagnosis of TB was based on the guidelines of the National Tuberculosis Control Programme [13]. Smear-positive pulmonary TB was diagnosed on the basis of clinical symptoms, and the presence of at least one acid fast bacillus (AFB+) in at least one sputum sample from two samples submitted on 2 consecutive days for microscopic examination after staining by Ziehl–Neelsen's technique. The diagnosis of smear-negative TB was based on no improvement of symptoms after a 10-day course of nonspecific antibiotic therapy, persistent negative results of a new series of two sputum smear examinations, and chest X-ray abnormalities consistent with active pulmonary TB. The diagnosis of extrapulmonary TB was based on suggestive clinical signs and evidence of a predominantly lymphocytic exudate as in the case of pleural or peritoneal TB with ascites or granulomatous lesions on histopathological examination of lymph nodes or other tissue biopsy. Patients with pulmonary TB associated or not with extra pulmonary sites were considered pulmonary cases and recorded as such [13].

2.4. Data Analysis

The data were collected and analyzed with the program the IBM Statistical Package for the Social Sciences, version 20.0 (IBM Corp, Armonk, NY, USA). Quantitative data was summarized using the median and interquartile range (IQR). Categorical data were summarized using number and percentage. Comparison of continuous data was done using Mann-whitney test. Chi 2 test was used for the comparison of categorical data. The diagnostic value of leukocyte count was estimated using receiver operating characteristics curve (ROC) analysis for the MLR and NLR. Sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) were also calculated.

The cut-off values of these ratios correspond to the plots on the respective curves that yield the best sensitivity and specificity. The areas under the curves (AUC), which quantifies the ability of the test to differentiate between two outcomes were determined and graded as follow: perfect test (AUC of 1); excellent test (AUC 0.9 - 0.99), good test (AUC 0.8 - 0.89), fair test (AUC 0.7 - 0.79), non useful test (AUC < 0.70). A P-value less than 0.05 was considered significant.

3. Results

From the 438 files of TB patients selected, we excluded 234 for various reasons as illustrated in Figure 1.

3.1. General Characteristics of the Study Population

A total of 204 consecutive diagnosed TB patients' files and 204 blood donors were included in the study. They were 126 (61.8%) males for the two groups giving a male: female sex ratio of 1.61. The median age was 33 (IQR 26 - 42) years and 32 (IQR 25 - 40) years respectively for TB patients and control subjects. As shown in Table 1, pulmonary TB was observed in 95 (46.6%). There were statistically significant differences concerning total white blood cells and differential counts in the two groups.

Table 1. Baseline characteristics of the participants.

Characteristics	TB patients (N = 204)	Healthy controls (N = 204)	P-value
Sex			
Male	126 (61.8%)	126 (61.8%)	0.9
Female	78 (38.2%)	78 (38.2%)	
Age			
Median age (IQR)	33.00 (26 - 42)	32.00 (25 - 40)	0.9
<25	41 (20.1%)	51 (25%)	
25 - 34	72 (35.3%)	68 (33.3%)	
35 - 44	3 (26.0%)	51 (25%)	
45 - 54	29 (14.2%)	30 (14.7%)	
≥55	9 (4.4%)	4 (2.0%)	
Level of education			
Primary school	53 (26%)	71 (34.8%)	
Secondary school	91 (44.6%)	79 (38.7%)	0.18
University	60 (29.4%)	54 (26.5%)	
Tuberculosis			
Smear positive pulmonary TB	95 (46.6%)		
Smear negative pulmonary TB	38 (18.6%)		
Extrapulmonary TB	71 (34.8%)		
Cell count (Giga/l) (median, IQR)			
Total white blood cells	6.5 (4.80 - 8.47)	4.25 (3.68 - 5.05)	<0.001
Neutrophils	4.25 (2.83 - 5.75)	1.87 (1.51 - 2.39)	<0.001
Lymphocytes	1.54 (1.18 - 1.96)	1.67 (1.42 - 2.23)	0.002
Monocytes	0.60 (0.38 - 0.77)	0.32 (0.24 - 0.41)	<0.001

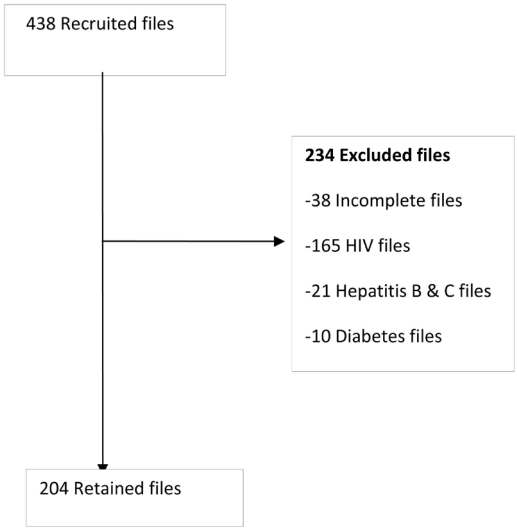

Figure 1. Flow chart.

3.2. Leukocyte Count Ratios

The monocyte-lymphocyte count ratio was higher in the patient group (median 0.38; IQR 0.24 - 0.56) as compared to the control group (0.19; IQR 0.14 - 0.25) ($p < 0.001$). The neutrophil-lymphocyte count ratio was also significantly increased in the patient group, 2.71 (IQR: 1.62 - 4.29) in comparison to the control group, 1.03 (IQR: 0.78 - 1.48) ($p < 0.001$).

3.3. Neutrophils-Lymphocytes Ratio (NLR) and Monocytes-Lymphocytes Ratio (MLR)

As illustrated in Figure 2, the area under the curve for NLR (AUC: 0.84) was significantly greater than that for MLR (AUC: 0.76) ($P < 0.001$). A MLR > 0.29 was identified as the optimal cut-off value for discriminating patients with TB from healthy subjects. Using this cut-off value, the MLR showed 67.2% sensitivity, 83.3% specificity, a positive predictive value (PPV) of 80.12% and a negative predictive value of (NPV) of 71.73%. A NLR > 1.79 was identified as the optimal cut-off value for discriminating patients with TB from healthy subjects, yielding 70.6% sensitivity, 87.3% specificity, a PPV of 84.7% and NPV of 74.79%.

4. Discussion

The aim of this study was to determine the relationship between various leukocyte count ratios and TB and to evaluate their diagnostic abilities. The NLR and MLR were significantly raised in TB patients compared to the control group. A MLR > 0.29 and a NLR > 1.79 were optimal cut-off values for differentiating TB patients from healthy subjects. The NLR had greater diagnostic ability as evidenced by its high AUC.

The neutrophil lymphocyte count ratio is an expedient marker of inflammation for foreseeing bacterial infection [9] [14]. The physiological immune responses of circulating leukocytes to various stressful events are characterized by

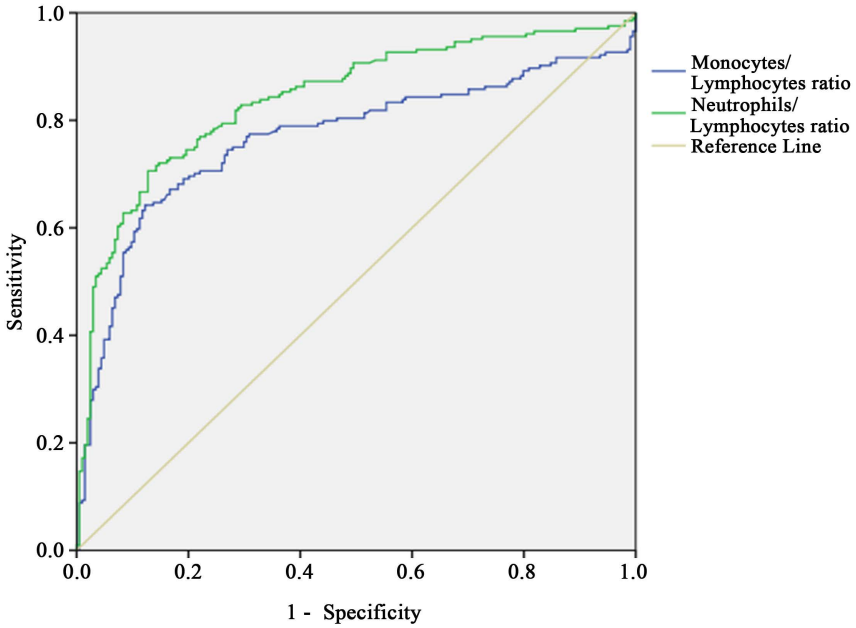

Figure 2. Receiver Operating characteristics curves of MLR and NLR for discriminating TB patients from healthy subjects. 1-specificity (X-axis); Sensitivity (Y axis).

an increased neutrophils count and decreased lymphocyte count. An increase in total WBC and neutrophils is an inflammatory reaction, particularly when caused by a bacterial infection [9]. Therefore, the NLR is thought to have stronger discriminative power for predicting bacteremia compared to discrimination based on neutrophilia or lymphocytopenia alone. Goodman *et al.* [15], Zahorec *et al.* [16] and recently, de Jager *et al.* [17] all demonstrated that the NLR was a better predictor of bacteremia than routine parameters such as CRP level, WBC count, and neutrophil count. Currently, the NLR has been garnering interest as a survival predictor in various clinical situations, ranging from oncological to cardiovascular diseases [18] [19]. Sumaria *et al.* reported increased NLR (3.12) in newly diagnosed TB patients compared to healthy controls (1.92) [14]. These observations were similar to our findings of 2.73 for the TB group and 1.03 for controls.

Monocytes are an essential component of the innate immune response that acts as a link to the adaptive immune system through antigen presentation to lymphocytes. Thus any factor that perturbs the function or relative numbers of either cell type could potentially affect an individual's response to infection [4]. The normal ML ratio is disrupted by MTB infection. It has been reported recently that MTB infection may alter subsets of hematopoietic stem cells [20] or directly infect bone marrow mesenchymal stem cells [21]. Studies in mice and humans have shown that subsets of hematopoietic stem cells have distinct biases in the ratio of myeloid to lymphoid cells they give rise to [22] [23] [24] [25]. The different proportion of myeloid biased or lymphoid-biased hematopoietic stem cells may underlie the peripheral difference of ML ratio. Therefore, it is reasonable that MTB infection may alter hematopoietic stem cells such that the ML ratio

is altered. The change of monocytes and lymphocytes, reflected in their ratio, may affect patients' ability to respond to mycobacterial infection. As demonstrated by our study, patients with active tuberculosis had a higher ML ratio compared to healthy controls, coinciding with the results of Jun Wang *et al.* 2015 [4].

Yoon 2013 identified a NLR < 7 as an optimal cut-off for differentiating pulmonary TB from bacterial community-acquired pneumonia [9]. Iliaz 2014 found a NLR < 2.5 as cut-off for differentiating TB from sarcoidosis [8]. We found a NLR < 1.19 and a MLR < 0.29 as cut-off for discriminating healthy subjects from TB patients. The NLR (AUC: 0.84) was a more powerful discriminative marker than the MLR (AUC: 0.77) as evidenced by its higher AUC. The NLR is thus a good while the MLR is a fair diagnostic test.

Although the present study is among the first studies to demonstrate and compare the diagnostic abilities of the neutrophil-lmphocyte and monocyte-lymphocyte count ratios in discriminating TB patients from healthy subjects, it faces some limitations. Firstly, it was a single center study, limitating the generalisability of our results. Also, the control group was not screened biologically for TB, raising the possibility of inclusion of subjects with subclinical disease among the controls. Furthermore, diagnosis of some forms of TB in this study was considered on the basis of suggestive clinical signs and response to treatment. This may be another source of bias in this study.

5. Conclusion

The neutrophil and monocyte-lymphocyte count ratios are raised in active TB. A NLR >1.19 and MLR >0.29 are optimal cut-offs to differentiate healthy subjects from TB patients. The NLR has greater diagnostic ability as evidenced by its higher AUC. Further research is needed to confirm or refute our findings.

Authors' Contributions

Conception and Design: MNBH, ATE; Data collection: ATE; Data analysis and Interpretation: MNBH; Drafting of the manuscript: ATE. Reviewing the manuscript: MNBH, NDE, ATE, MNER, KLF, CVL, NLH. All authors approved the final draft for publication.

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Conflicts of Interest

The authors declare no conflicts of interest.

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