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*Original Research Article*

# Leucocyte Counts and Derivatives as Inflammatory Indicators in Active Tuberculosis

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ABSTRACT

Tuberculosis (TB) is a disease of public health concern in Nigeria as the country is listed among the 30 high TB burden countries in the world. It is caused by *Mycobacterium tuberculosis*, an airborne pathogen. Tuberculosis disease is a manifestation of an active state of the infection when host immune response has been overwhelmed. Leucocytes participate in immune response, and traditionally, the total and differential counts have been useful in offering general outlook on inflammation as well as immunodeficiency. Lately, leucocyte derivatives such as neutrophil-to-lymphocyte ratio (NLR) and lymphocyte-monocyte ratio (LMR) have been reported as better markers of inflammation in some disease conditions. This study assessed total and differential leucocyte counts together with the derivatives in active tuberculosis infection among 80 tuberculosis patients and equal number of matched controls. Total and differential leucocyte counts were determined using standard haematology autoanalyzer, while the ratios were mathematically derived. Data were analyzed and hypotheses tested using student's t-test on statistical package for social sciences (SPSS) version 21. A p-value of ≤0.05 was considered as statistically significant. Neutrophil-lymphocyte ratio was significantly higher in TB patients, while lymphocyte-monocyte ratio was significantly lower in TB patients than the control. Furthermore, neutrophil-lymphocyte ratio was significantly higher, while the lymphocyte-monocyte ratio was significantly lower in TB patients at diagnosis (n=26) in comparison with those on treatment (n=54). There is derangement in leucocyte derivatives in association with tuberculosis. These derivatives improved significantly as anti-TB treatment progressed. Therefore, these variables could serve as useful indicators in the monitoring of treatment for TB patients.

***Keywords***: Tuberculosis, leucocytes, inflammation, immune response

## Introduction

Tuberculosis is a disease of public health concern in Nigeria as the country is listed among the thirty high TB nations across the globe as determined by Sustainable Development Goals (SDGs) indicators. According to the Global TB Report 2020, thirteen percent of Nigerians are undernourished, with 7.9% of the male population being smokers and a prevalence of 6% for diabetes; these are risk factors for the acquisition of tuberculosis infection.[1] In 2015, the WHO proposed an End TB strategy with 2020 as the milestone. It was the target to reduce TB incidence rate by 20% between 2015 and 2020 but only 11% reduction was achieved. The number of TB deaths reduced by 9.2%, a far cry from the targeted 35%. Furthermore, the goal was to eliminate catastrophic costs but as at 2020, 47% of people with TB were still faced with high costs of treatment. Treatment targets also fell short of the mark; hence TB is still a major cause of ill health and death globally.[2]
Tuberculosis is caused by *Mycobacterium tuberculosis*, an airborne pathogen that is spread as droplet particles and released into the air when pulmonary tuberculosis patients cough or sneeze. The bacteria are first taken up by alveolar macrophages which after engulfment, activate other immune cells (neutrophils and dendritic cells) for clearance and presentation of mycobacterial antigens to different subsets of T cells; this results in cell activation and secretion of different cytokines which orchestrate an adaptive immune response.

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*Mycobacterium tuberculosis* becomes active when the immune system is overwhelmed by the invading organism leading to tuberculosis disease.[2,3] Total and differential leucocyte counts are vital in assessing systemic inflammation owing to the participation of these cells in immune response.[4-10] Additionally, derivatives of leucocyte counts are being recognized as better markers and reported in different conditions. Neutrophil-to-lymphocyte ratio (NLR) depicts a simple relationship between absolute neutrophil and lymphocyte counts as derived from differential white blood cell count (WBC) of complete blood cell (CBC) count.[11] The NLR has been recongnised as an inflammatory marker and its increase has been linked to poor prognosis across different disease conditions. This has been reported with regards to solid tumours in which high NLR associated with adverse overall survival.[12] Moreso, NLR has been observed to be of independent prognostic value in different clinical utilities for cancer patients.[9] For cardiovascular conditions, NLR has been found to correlate with adverse outcomes in patients with acute coronary syndrome as well as coronary heart disease, while it also aids the screening for the latter in asymptomatic general population cohort.[13] The utility of NLR has also been observed across other conditions such as kidney transplantation, bacterial pneumonia, Alzheimer's disease as well as schizophrenia.[14] The lymphocyte-monocyte ratio (LMR) is also a marker of systemic inflammation and has been used for its predictive prognosis in breast cancer and other solid tumours as well as in colo-rectal cancer. A low LMR indicates poor survival outcomes.[15,16] Tuberculosis infection induces a state of chronic systemic inflammation in the body. This study seeks to determine the leucocyte counts and derivatives (neutrophil- lymphocyte and lymphocyte-monocyte ratios) of TB patients in Calabar, Cross River State, Nigeria as this may aid in the monitoring and management of tuberculosis patients.

## Materials and Methods

Cross-sectional study design was used in this study. The study site is University of Calabar Teaching Hospital (UCTH), Calabar, Nigeria. Information from patients' records indicate that diagnosis of TB was by sputum smear microscopy and Gene Xpert techniques. A total of 80 tuberculosis patients comprising 26 newly diagnosed patients and 54 patients on treatment were enrolled. Another 80 apparently healthy individuals who were mantoux test negative within the preceding six months were selected to serve as control. The study was carried out in 2022. Ethical approval and informed consent were sought and obtained from the Health Research Ethics Committee of UCTH (UCTH/HREC/33/Vol.III/036) and the study subjects respectively. Subjects with TB-HIV co-infection and healthy individuals who did not give their consent were excluded.

Two (2) ml of venous blood was collected aseptically with minimal stasis from a prominent vein of each subject and was transferred into di-potassium ethylene diamine tetra acetic acid to a final concentration of 2 mg/ml. Blood samples were analyzed within four hours of collection. Total white cell counts were determined by automation using Mindray BC-5000 Haematology Analyzer (Mindray Medical International Limited, China). This equipment was controlled and calibrated according to manufacturer's instructions to ensure its fitness for use. Leucocyte count derivatives were mathematically calculated in the form of ratios.

*Statistical analysis*

Data were analyzed and hypotheses tested using student's t-test on statistical package for social sciences (SPSS) version 21. Values are presented as Frequencies and Mean ± SD (standard deviation). A p-value of ≤0.05 was considered as statistically significant.

## Results and Discussion

Leucocyte counts as well as count ratios of eighty (80) tuberculosis patients and controls were assessed in this study. Table 1 shows the demographic parameters of the tuberculosis patients and their controls. Forty eight percent of the TB patients were within the age bracket of 15-30 years. Sixty nine percent of the TB patients were males with 53% being married and eighty one percent having had only secondary level of education.

Table 2 shows total and differential white cell counts, neutrophil-lymphocyte and lymphocyte-monocyte ratios of tuberculosis patients and their controls. The total white cell count of TB patients was observed to be significantly higher ($p<0.05$) than that of the controls while the neutrophil count was observed to be higher though not significant ($p=0.056$), in TB as compared to the control. Absolute lymphocyte count was significantly lower ($p<0.05$) while the eosinophil count was comparable ($p>0.05$) between TB patients and their controls. Conversely, the monocyte count was significantly higher ($p<0.05$) for TB patients than the controls. The neutrophil-lymphocyte ratio was significantly higher ($p<0.05$), while the lymphocyte-monocyte ratio was significantly lower in TB patients than the controls.

The total and differential white cell counts, neutrophil-lymphocyte and lymphocyte-monocyte ratios of untreated tuberculosis patients and those on anti-TB treatment is presented in Table 3. The total white cell count and absolute neutrophil count were observed to be significantly higher ($p<0.05$) for TB patients at diagnosis when compared to their counterparts on anti-TB treatment. On the other hand, the lymphocyte count was observed to be significantly lower ($p<0.05$) while the monocyte and eosinophil counts were comparable ($p>0.05$) between untreated TB patients and those on anti-TB treatment. The neutrophil-lymphocyte ratio was significantly higher ($p<0.05$) while the lymphocyte-monocyte ratio was significantly lower for TB patients at diagnosis in comparison with those on treatment.

The present study enrolled 80 tuberculosis patients accessing medical care at the study site. Persons within reproductive age with fair literacy level were more in number. While marital status was almost evenly distributed, more males were observed among the study participants. Tuberculosis has remained a public health challenge particularly in resource-poor settings where factors responsible for its spread are yet to be addressed. Such militating factors against the effective control of infectious diseases within the study locality extend from poor living conditions to inadequate health care coverage[17-20]. Concerning the later, financial capability remains a driving force in the health-seeking behaviors of our populace.[19] The demographic finding of the present study, therefore, may not necessarily apply to the actual population of persons living with tuberculosis. Rather, these findings reflect the characteristics of those who are able to seek medical care. Moreso, considering the contagious nature of tuberculosis, community-based studies are highly recommended for better appreciation of tuberculosis demographics within our setting.

Full blood count is an integral part of the laboratory testing protocol for the diagnosis, prognosis and/or monitoring of various medical conditions. Beyond the direct assessments of anaemia and coagulation information derived from the erythrocyte and thrombocyte parameters respectively, leucocyte parameters are currently attracting research attention particularly for their prognostic values.[21,22] Although the mean value was still within the reference range, this study recorded significantly higher total white cell count in the infected group compared to control. This is obviously due to the presence of infection with *Mycobacterium tuberculosis* which triggers an inflammatory response.[23] Furthermore, differential white cell count ratios have been found to be quite informative with regards to inflammation compared to the individual counts.[24,25] The neutrophil count was observed to be slightly higher for TB patients versus controls. In TB infection, neutrophils have been reported to be the first line of defense as they are recruited and mobilized to the pulmonary vasculature as an acute inflammatory response to the infective agent *M. tuberculosis*. Indeed, neutrophil numbers have been found to be higher with the establishment of infection but drops to near normal levels within six weeks.[26] The findings in the present study shows neutrophil counts of TB patients slightly different from the uninfected controls probably because most (67.5%) of the TB patients enrolled in this study were already on anti-TB treatment hence the reduction in neutrophil response as pathogen clearance progressed. Again, being a chronic infection, TB does not sustain the increase in neutrophil numbers expressed in acute bacterial infections. Although increment in absolute neutrophil count was not significantly different from the control value, the neutrophil-lymphocyte ratio was significantly higher for TB patients than the control group thus underscoring the importance of ratios. Depletion of lymphocytes with or without increased neutrophil count could give rise to higher values of neutrophil-lymphocyte ratio.

**Table 1:** Demographic data of Tuberculosis Patients and Controls

| Parameter | | TB Patients 80 (100%) | Control 80 (100%) |
|---|---|---|---|
| Age (Years) | 15-30 | 38 (48%) | 48 (60%) |
| | 31-45 | 23 (28%) | 21 (26%) |
| | >46 | 19 (24%) | 11 (14%) |
| Gender | Males | 55 (69%) | 56 (70%) |
| | Females | 25 (31%) | 24 (30%) |
| Marital Status | Married | 42 (53%) | 35 (44%) |
| | Single | 37 (46%) | 45 (56%) |
| | Divorced | 1 (1%) | 0 (0%) |
| | Widowed | 0 (0%) | 0 (0%) |
| Education | No Formal | 0 (0%) | 0 (0%) |
| | Primary | 14 (18%) | 8 (10%) |
| | Secondary | 65 (81%) | 24 (30%) |
| | Tertiary | 1 (1%) | 48 (60%) |

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This study recorded significantly higher monocyte count alongside lower lymphocyte count for TB patients compared to controls. Considering that lymphocytes are involved in both humoral and cytotoxic immunological responses, derangement in its derivatives in addition to the direct assessment alludes to the magnitude of ongoing inflammation.[16] This is further reflected in the significantly lower lymphocyte-monocyte ratio recorded for TB patients in comparison to the controls. The mobilization of monocytes as tissue macrophages and their participation in driving inflammatory processes at sites of immunologic invasion is thought to signal increased peripheral presence resulting in higher count and lower ratio as seen in the present study.[16,23] The lymphocyte-monocyte ratio has been identified as an indicator of systemic inflammation where low ratios predict poor prognosis and signals unfavorable treatment outcomes. Generally, individual immunity and timely detection influence both the speed and degree to which most medical conditions are resolved.[27,28] It is interesting to note that antimicrobial therapy for tuberculosis which is aimed at eliminating the bacilli and reversing the morbidity occasioned by the invasive infectious agent, impacts positively on the measured morbidity indicators. Apart from the absolute monocyte count that was seen to be comparable between the newly diagnosed and those on treatment, other deranged parameters were observed to be showing significant improvement towards control values. The nature of TB as tissue-ravaging disease and the participation of monocytes in tissue inflammation could be the reason for comparable monocyte values between the newly diagnosed and those on treatment.

## Conclusion

This study has shown that total white cell count, absolute monocyte count as well as neutrophil-lymphocyte ratio were significantly higher while absolute lymphocyte count and lymphocyte-monocyte ratio were significantly lower in TB patients when compared to the control subjects. However, these values were observed to be within their reference ranges. Also, the total white cell count, neutrophil count, lymphocyte count, neutrophil-lymphocyte ratio and lymphocyte-monocyte ratio improved significantly as anti-TB treatment progressed. Therefore, these variables could serve as useful indicators in the monitoring of treatment for TB patients.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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**Table 2:** Total and differential white cell counts, neutrophil-lymphocyte and lymphocyte-monocyte ratios of Tuberculosis Patients and Control subjects

| | **TB Patients N=80** | **Control N=80** | **p- Value** |
|---|---|---|---|
| TWBC×$10^9$/L | 6.50 ± 3.11 | 4.84 ± 0.43 | 0.001 |
| NEUT×$10^9$/L | 4.71 ± 0.52 | 3.40 ± 0.40 | 0.056 |
| LYM ×$10^9$/L | 0.92 ± 0.50 | 1.45 ± 0.03 | 0.001 |
| EOSIN X $10^9$/L | 0.11 ± 0.08 | 0.09 ± 0.04 | 0.167 |
| MONO X $10^9$/L | 0.57 ± 0.09 | 0.34 ± 0.06 | 0.001 |
| NLR | 4.97 ± 0.88 | 2.30 ± 0.43 | 0.001 |
| LMR | 1.82 ± 0.46 | 4.08 ± 0.52 | 0.001 |

Values are presented as Mean ± SD
TWBC: Total White Blood Cell Count, NEUT: Neutrophil, LYM: Lymphocyte, EOSIN: Eosinophil, MONO: Monocyte, NLR: Neutrophil-to-Lymphocyte Ratio, LMR: Lymphocyte-to-Monocyte Ratio

**Table 3:** Total and differential white cell counts, neutrophil-lymphocyte and lymphocyte-monocyte ratios of Tuberculosis Patients at diagnosis and during anti-TB treatment

| | **TB Patients at Diagnosis 26 (32.5%)** | **Patients on Anti-TB Treatment 54 (67.5%)** | **p-Value** |
|---|---|---|---|
| TWBC×$10^9$/L | 8.58 ± 3.78 | 5.46 ± 2.11 | 0.001 |
| NEUT×$10^9$/L | 5.70 ± 0.62 | 2.86 ± 0.32 | 0.001 |
| LYM ×$10^9$/L | 0.86 ± 0.58 | 1.09 ± 0.03 | 0.001 |
| MONO x $10^9$/L | 0.67 ± 0.13 | 0.50 ± 0.06 | 0.107 |
| EOSIN x $10^9$/L | 0.13 ± 0.11 | 0.10 ± 0.05 | 0.633 |
| NLR | 2.72 ± 1.06 | 1.43 ± 1.00 | 0.013 |
| LMR | 1.26 ± 0.39 | 2.21 ± 0.46 | 0.050 |

Values are presented as Mean ± SD
TWBC: Total White Blood Cell Count, NEUT: Neutrophil, LYM: Lymphocyte, EOSIN: Eosinophil, MONO: Monocyte, NLR: Neutrophil-to-Lymphocyte Ratio, LMR: Lymphocyte-to-Monocyte Ratio

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