EVALUATION OF HEMATOLOGICAL PROFILES AND THEIR INFLUENCE ON TREATMENT OUTCOMES IN NEWLY DIAGNOSED PULMONARY TUBERCULOSIS PATIENTS IN WESTERN KENYA

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NOVEMBER 2024

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DEDICATION

I dedicate this thesis to Augustine Mwilitsa Bulemi, Sarah Ngasi Bulemi and more so, to my daughter Hailey Emille Mwilitsa.

ACKNOWLEDGEMENT

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LIST OF ABBREVIATIONS AND ACRONYMS

AECs	Airway Epithelial Cells
AFB	Acid Fast Bacilli
BMI	Body Mass Index
CCL5	Chemokine Ligand 5
CCL9	Chemokine Ligand 9
CLSI	Clinical and Laboratory Standards Institute
CRP	C Reactive Protein
DC	Dendritic Cell
ESR	Erythrocyte Sedimentation Rate
FDCs	Fixed Drug Combinations
HGB	Hemoglobin
HCT	Hematocrit
IDA	Iron Deficiency Anemia
IFN	Interferon gamma
IL	Interleukin
KCTRH	Kakamega County Teaching and Referral Hospital
KPHC	Kenya Population and Housing Census
KTTA	Kenya Tissue and Transplant Authority
LFTs	Liver Function Tests
LF-LAM	Lateral Flow-LipoArabinoMannan Assay
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MMUST	Masinde Muliro University of Science and Technology

M.TB	Mycobacterium tuberculosis
NACOSTI	National Commission for Science, Technology and Innovation
NK	Natural Killer cell
DNTLD-P	Division of National tuberculosis, Leprosy and Lung Disease Program
PBF	Peripheral Blood Film
PCT	Plateletcrit
PCV	Packed Cell Volume
PDW	Platelet Distribution Width
PLT	Platelets
PRRs	Pattern Recognition Receptors
PTB	Pulmonary Tuberculosis
RBCs	Red Blood Cells
RDW	Red blood cell Distribution Width
RH	Rifampicin, Isoniazid
RHZE	Rifampicin, Isoniazid, Pyrazinamide, Ethambutol
TB	Tuberculosis
WBCs	White Blood Cells
WHO	World Health Organization

OPERATIONAL DEFINITION OF TERMS

Anti-tubercular treatment- standard treatment for *Mycobacterium tuberculosis* consisting of RHZE for two months and RH for four months.

Hematological changes- denotes deviations in the values of hematological parameters compared to normal reference values for the parameters.

Hematological parameters- refers to counts for absolute and differential white blood cells, red blood cells and platelets with their respective indices.

Hematological profile- refers to measurable blood indices that can be used to identify and monitor disease states.

Morphological characteristics- refers to physical attributes of cells that can be observed microscopically such as poikilocytosis, anisocytosis, hyper-segmentation, toxic granulation and cellular inclusions.

Optimal treatment outcomes- desirable treatment outcomes including completion of treatment, treatment success and those who get cured

Poor clinical outcomes- undesirable treatment outcomes including treatment failure, lost to follow-up, death, and confirmed drug resistance, at month 6 of anti-TB treatment.

Sputum smear conversion- change in sputum smear results from positive to negative following anti-TB treatment

Treatment outcomes- categorization of patients into well-defined groups based on response to treatment and sputum smear results at month five and six of treatment. Patients are categorized as cured, treatment completed, treatment success, treatment failure, died, lost to follow-up, moved to category 4 (A patient confirmed to have drug resistant TB while on first line treatment), and not evaluated for outcomes.

ABSTRACT

Pulmonary tuberculosis (PTB) is among the leading infectious diseases globally in terms of morbidity and mortality with an estimated 1.3 million people having succumbed to the disease in 2022 despite the availability of effective anti-tubercular treatment. In Kakamega County, mortality is estimated to be 10.8%, which is double the national target of <5% for TB-related deaths. The potential factors contributing to this persistently high mortality rate are not fully understood. To date, there are no reliable prognostic markers for PTB monitoring. The objectives of this study were to evaluate the hematological profiles of newly diagnosed PTB patients, changes during the course of treatment and their influence on treatment outcomes. The study was conducted at Kakamega County Teaching and Referral Hospital in Kakamega County. The study adopted a prospective longitudinal study design and used convenience sampling to recruit 55 participants for both the PTB and control groups. The sample size was determined by G-power sample calculation software with a power of 0.80, alpha of 0.05 and a large effect size of 0.5. Venous blood was collected for hematological profile analysis in both the PTB and control group. However, for the PTB group, hematological profile analysis was done at diagnosis, at months 2 and 5 of treatment. Microscopy analysis of sputum smears at months 2 and 6 was used to monitor for sputum smear conversion and outcomes, respectively. Data were analyzed using Chi-square, Mann-Whitney U test, Kruskal-Wallis test with a post hoc of Dunn's multiple comparisons test and multivariate logistic regressions where applicable in SPSS version 29.0 and Graph Pad prism version 6.0. Males were 80% (n=44) in the PTB group and 81.8% (n=45) in the control group. At diagnosis, the PTB group had a significantly lower median RBC count compared to controls ($4.79x10^6$/µL vs $5.2x10^6$/µL, P=0.001). Similarly, relative to the control group the PTB group had lower HGB levels (12.8g/dL vs 14.3g/dL), HCT (37.9% vs 42.05%), mean platelet volume (8.9fL vs 10.5fL) and platelet distribution width (10.4fL vs 13.0fL) (P<0.001). Further, the PTB group had significantly higher median WBC counts ($6.82x10^3$/µL vs $4.60x10^3$/µL), RDW-CV% (12.8 vs 11.9), RDW-SD (42.3fL vs 40.4fL) and median platelet count ($314.0x10^3$/µL vs $237.0x10^3$/µL) (P<0.001) compared to the control group. Peripheral blood film analysis at diagnosis revealed normocytic normochromic anemia was predominant at 54.5% (n=30) followed by microcytic hypochromic anemia in 38.2% (n=21) of the PTB patients. Controls had normocytic normochromic anemia and microcytic hypochromic anemia at 90.9% (n=50) and 3.6% (n=2), respectively. Comparison of hematological profiles for PTB patients at diagnosis, month 2 and month 5 revealed a significant decrease in WBC counts, neutrophil counts and platelet counts. Low lymphocyte count, low HGB, high WBC count and high Platelet count were significantly associated with increased odds of having a positive sputum smear post-intensive phase of treatment (P<0.001). Normal counts for WBC, MCV, MCH, MCHC, RDW-CV% and platelet showed a statistically significant association with treatment outcomes among PTB patients at month 6 (P<0.05). Therefore, newly diagnosed PTB patients at KCTRH present with deranged hematological profiles, with abnormal peripheral features such as microcytic hypochromic anemia. The hematological changes following treatment have significant effects on sputum smear conversion suggesting the need for routine monitoring of their hematological profiles. The study recommends that the hematological profiles of

newly diagnosed PTB patients be monitored a diagnosis and during course of treatment so as to tailor patient specific treatment approaches for better clinical outcomes.

CHAPTER 1: INTRODUCTION

1.1 Background

Pulmonary tuberculosis (PTB) is a common disease in the developing nations and accounts for a large portion of the global heath burden. PTB has surpassed other infectious diseases to become a leading killer infectious disease with an estimated 1.3 million people having succumbed to the disease in the year 2022 (Global TB report, 2023). PTB ranks second as a leading infectious killer disease and 13th as a major cause of death globally according to the World Health Organization (WHO) global TB report, 2023. Of the 1.3 million deaths in 2022, 1.13 million were HIV negative patients whereas HIV-related TB accounted for approximately 167, 000 deaths. The WHO South-East Asia region, African region and the Western pacific contributed 46%, 23% and 18% of the cumulative global TB infections in 2022, respectively (Global TB report, 2023).

Several African countries are among the high TB burdened countries in the world (Global TB report, 2023). For the year 2022, Kenya recorded a total of 90, 560 drug-sensitive tuberculosis cases of TB (all forms) with a TB/HIV co-infection rate of 23% (MoH-annual TB report, 2022). The country had a death rate of 6% for the 2021 cohort that was evaluated for treatment outcomes among patients with drug-sensitive TB. Kakamega County notified 1, 899 cases of TB (all forms) with a TB/HIV co-infection rate of 30.8%. The County recorded a death rate of 10.8% above the national target of <5% for TB-related deaths (MoH-annual TB report, 2021). This high mortality rate remains despite the availability of effective anti-tubercular treatment. The factors that could be contributing to this persistently high mortality rate are not fully understood.

Acquisition of *M. tuberculosis* bacterium by healthy individuals is mainly through exposure to micro aerosols deposited in air from infected persons (Global TB report, 2023). Inhalation of the bacteria gets it into the lungs where release of several chemokines is induced. Resident macrophages in the lungs produce these cytokines which attract various inflammatory cells such as monocytes, natural killer cells, neutrophils and lymphocytes, particularly $CD4^+$ T helper cells. $CD4^+$ are known to effect key immune functions related to control of *M. tuberculosis* infection (Sia and Rengarajan, 2019). Consequently, interaction between CD4+ and *M. tuberculosis* or its related bacterial components initiates a series of events that lead to production of TNF-α and IFN-γ, which are inflammatory cytokines (Sia and Rengarajan, 2019). These inflammatory cytokines affect the hematopoietic system resulting in either over-activity or under-activity of the myeloid and lymphoid pathways of blood formation (Khan *et al*., 2020).

As such, pulmonary TB has been documented to cause several reversible peripheral blood abnormalities (Abaker and Mohammed, 2016). Of these abnormalities, anemia is the most common. Estimates shows that anemia is present among 50% to 90% of PTB patients in various countries (Shafee *et al.,* 2014; Erhabor *et al.,* 2020). The cause of this anemia has not been fully elucidated, but it is postulated to be multifactorial. For example, several inflammatory mediators induced by *M. tuberculosis* suppress erythropoiesis directly, consequently leading to reduction in red blood cell production. Moreover, TNF-α, interleukin (IL)-6, and IL-1 have been suggested as possible causes of hypoferremia in PTB (Abaker and Mohammed, 2016). Furthermore, the inflammatory response during the process of PTB infection induces hepcidin synthesis

by macrophages and liver cells (Minardi *et al*., 2021). Increased hepcidin levels lead to a reduction in iron utilization, malabsorption, and bone marrow impairment (Minardi *et al*., 2021). Apart from the *M. tuberculosis*, several region specific factors, including genetics, and diet could influence development and type of anemia among PTB. However, there is paucity of data on anemia patterns and their association with treatment outcomes among PTB patients in western Kenya.

Pulmonary TB is also associated with an elevated number of platelets (thrombocytosis) (Rathod *et al.,* 2017; Kahase *et al.,* 2020; Shah *et al.,* 2022). This PTB-associated thrombocytosis is thought to be due to liberation of interleukin (IL)-6, a cytokine that is required for control of *M. tuberculosis* (Kahase *et al*., 2020). Not only does platelet numbers increase, but there is also qualitative changes, including altered transcriptome, structure and function. For instance, pro-inflammatory mediators contained in alpha granules of platelets such as TNF-α and IL-1 are known to be markedly increased in PTB patients (Kirwan *et al*., 2021). There is growing clinical evidence that platelets are involved in the rapid immune responses in *M. tuberculosis* invasion (Kirwan *et al*., 2021).

Fox *et al* (2018) in UK investigated the regulatory role of thrombocytes in pulmonary inflammation and the resultant tissue destruction in newly diagnosed patients. Fox and colleagues documented that platelet associated mediators such as platelet factor 4 (PF4) were upregulated in the plasma of TB patients in comparison with controls. Furthermore, platelets were implicated in the upregulation of matrix metalloproteinase-1, a monocyte collagenase involved in collagen destruction at tissue

level, demonstrating the possible role played by thrombocytes in PTB pathology. Thrombocytosis has been suggested to mediate the inflammatory processes and tissue degradation that leads to cavitation in pulmonary tuberculosis patients (Fox *et al*., 2018; Cox and Keane, 2018). However, thrombocytosis is not a consistent indicator as some PTB patients present with thrombocytopenia during the acute phase or during the chronic stages of pulmonary TB (Rohini *et al.,* 2015; Batool *et al.,* 2022; Shah *et al.,* 2022). Therefore, hematopoietic changes might be key systemic factors in the pathogenesis and outcomes of PTB that are not often considered.

Treatment of drug susceptible pulmonary TB consists of an intensive phase of 2 months followed by a continuation phase of 4 months. During the intensive phase, patients are put on RHZE (MoH integrated guideline, 2021). In the continuation phase, patients take RH. If sputum smear conversion does not occur at the end of the intensive phase, treatment with the four drugs is continued until a negative sputum smear result is obtained (MoH integrated guideline, 2021). In Kenya, PTB patients undergoing treatment are monitored for toxic effects of drugs on peripheral nerves and the liver. Furthermore, for bacteriologically confirmed cases, sputum conversion from positive to negative is monitored for (MoH integrated guideline, 2021). However, little or no attention is paid to hematological changes of newly diagnosed PTB patients or those undergoing treatment.

Therefore, this study evaluated the hematological profiles of newly diagnosed PTB patients at diagnosis, month 2 and 5 of their treatment at KCTRH, Western Kenya. Moreover, the study also evaluated morphological characteristics of white blood cells,

red blood cells and platelets in newly diagnosed PTB patients. Furthermore, the influence of hematological changes on sputum smear conversion at month two and their associations with treatment outcomes at month six was determined. We herein report that PTB patients at KCTRH in Western Kenya exhibit various hematological and morphological derangements at diagnosis, month 2 and month 5 of anti-TB treatment in their hematological profiles. Furthermore, there is a significant influence and association between hematological changes with sputum smear conversion and treatment outcomes in PTB patients.

1.2 Problem statement

Pulmonary TB according to WHO is the 13th leading cause of morbidity and mortality worldwide. In 2022, 7.5 million people were newly diagnosed with TB worldwide. For the year 2022, 1.3 million people died due to TB including those people with HIV (all forms) (Global TB report, 2023). This high mortality rate remains despite availability of effective anti-TB drugs. The reasons for this persistently high mortality remains unknown. Also, there is still poor prognostication for the disease, which makes it difficult to tailor management approaches for patients (Harries and Kumar, 2018). In most healthcare systems, pharmaco-therapeutic management of PTB patients entirely depends on sputum monitoring and occasional investigation for hepatotoxicity (MoH integrated guideline, 2021).

Kakamega County bears one of the highest mortality rates due to TB, which was reported to be at 10.8% in a cohort of PTB patients that were evaluated for treatment outcomes in 2020. This is higher than the national target of < 5% for TB-related deaths.

In spite of this, the effects of the TB associated hematological changes on treatment outcomes is largely unexplored although there is growing evidence that PTB may be characterized by marked hematological derangements. For this reason, the present study evaluated the hematological profiles of pulmonary tuberculosis patients at diagnosis, the morphological characteristics of blood cells in pulmonary tuberculosis patients at diagnosis, and the hematological changes during anti-tubercular treatment and their influence on treatment outcomes in pulmonary TB patients.

1.3 Justification

Evaluating the hematological profiles of pulmonary tuberculosis (PTB) patients at diagnosis is crucial for understanding the systemic impact of the disease. Tuberculosis, a bacterial infection primarily affecting the lungs, can lead to significant alterations in blood cell counts and morphology. These changes may include anemia, leukocytosis, and thrombocytopenia, reflecting the body's inflammatory response and the infection's severity. By studying these hematological parameters at diagnosis, healthcare providers can understand the disease's progression and the patient's overall health status. This information is important for planning treatment plans and managing any potential complications, as well as enhancing patient care.

In addition, the examination of the morphological characteristics of blood cells and the hematological changes during anti-tubercular treatment provides a comprehensive view of the disease's impact and treatment efficacy. Morphological changes in blood cells, such as abnormal shapes or sizes, can indicate the presence of chronic inflammation or other underlying conditions that may complicate TB treatment.

Monitoring these changes over the course of treatment allows for the evaluation of treatment success. Moreover, understanding how hematological parameters evolve during therapy can also help in predicting treatment outcomes, guiding adjustments in treatment strategies, and improving patient prognosis. This study is therefore critical for improving the overall management of pulmonary TB patients in Kakamega and the country at large.

Furthermore, selection of the study area was influenced by the high death rate that was recorded in the county in 2020 at 10.8%, above the set national target of <5%. Kakamega County is among the counties with the highest transmission rates with 1,562 new TB cases, bacteriologically and clinically diagnosed, having been confirmed in 2021 (MoH-annual TB report, 2021). Additionally, selection of newly diagnosed HIV negative PTB patients was meant to rule out any occurrence of prior hematological abnormalities either due to relapse, treatment failure or prior treatment with anti-tubercular treatment.

1.4 Significance of the study

Evaluating the hematological profiles and morphological characteristics of blood cells in pulmonary tuberculosis (TB) patients has the potential to enhance diagnostic accuracy and treatment management. Hematological abnormalities are common in TB patients and can serve as indicators of disease severity and systemic involvement. By documenting these abnormalities at diagnosis, healthcare providers can identify patterns that may be predictive of disease outcomes or complications. It is important to generate this knowledge because it will be used for more precise patient stratification, ensuring that those at higher risk receive more intensive treatment and monitoring.

Evidence based data on the occurrence of hematological abnormalities in PTB patients such as anemia and thrombocytosis has been generated from this study. Findings on the occurrence of these hematological abnormalities in pulmonary tuberculosis patients can be further explored to tailor patient specific treatment approaches. Moreover, understanding the hematological landscape in TB patients could lead to the identification of novel diagnostic markers, thereby improving early detection and reducing the burden of TB in high-risk populations, such as in western Kenya. Findings from the study confirm to policy makers that PTB patients need to be screened for hematological abnormalities at diagnosis and when undergoing treatment.

Incorporation of this screening into the standard PTB management protocol will help avert hematological associated mortalities in PTB patients undergoing treatment. This will significantly reduce the high mortality rate in Kakamega County. Peer reviewed articles have been generated from this study. The study has expanded on the scientific knowledge on pulmonary tuberculosis and the involvement of the hematopoietic system. Therefore, more significant information has been made available to policy makers, health care personnel, researchers and other relevant stakeholders. Lastly, this work forms the basis for future research in development of novel treatment therapies for pulmonary tuberculosis patients.

1.5 Objectives

1.5.1 General objective

To evaluate the hematological profiles and their influence on treatment outcomes in newly diagnosed pulmonary tuberculosis patients in Western Kenya.

1.5.2 Specific objectives

i. To determine the hematological profiles of pulmonary tuberculosis patients at diagnosis.
ii. To identify the morphological characteristics of blood cells in pulmonary tuberculosis patients at diagnosis.
iii. To determine hematological changes during anti-tubercular treatment and influence on treatment outcomes in pulmonary tuberculosis patients.

1.6 Research questions

i. What are the hematological profiles of pulmonary tuberculosis patients at diagnosis?
ii. What are the morphological characteristics of blood cells in pulmonary tuberculosis patients at diagnosis?
iii. What are the hematological changes during anti-tubercular treatment and influence on treatment outcomes in pulmonary tuberculosis patients?

1.7 Scope of the study

The scope of the study was limited to recruiting 110 adult participants, 55 for the control group and 55 for the PTB group. The PTB group participants were limited to newly bacteriologically confirmed HIV negative PTB patients. The recruitment period lasted 8 months when all 110 participants had been recruited. The study was limited to hematological profile and morphological analysis of blood cells. Hematological profile analysis was quantitative while a qualitative morphological analysis was also performed.

1.8 Limitation

Participants who were recruited as controls were not screened for PTB either through chest X-rays or laboratory approaches (microscopy/GeneXpert). However, the study relied on a validated donor screening questionnaire developed by the Kenya Tissue and Transplant Authority for presumptively screening eligible donors. Additionally, this study was conducted at a single facility in Kakamega County with the assumption that it serves patients from different parts of the County. Findings from this study cannot therefore be entirely extrapolated to other regions of Kakamega County or Kenya due to genetic variations among people in different parts of the country. The effects of confounders such as BMI and nutritional status of PTB patients on results at baseline, at months 2 and 5 of anti-tubercular treatment were not determined in this study due to inconsistencies in available data on BMI and nutritional status for PTB patients.

1.9 Conceptual framework

The independent variables for this study were the type of PTB, that is, either drug sensitive or drug resistant PTB and the PTB regimen the patient was put on during the course of treatment. The dependent variables for this study were hematological parameters and blood cell morphological characteristics for WBC, RBC and platelets evaluated at diagnosis, month 2 and month 5 of treatment.

Participants recruited presented with drug sensitive PTB. The treatment regimen comprised of first line anti-TB drugs. Having either drug susceptible PTB or drug resistant PTB can have an effect on the hematological parameters and blood cell morphological characteristics of infected patients. The type of regimen (first line or second line) can also affect hematological parameters and blood cell morphological

characteristics of patients initiated on anti-tubercular treatment (Kassa *et al.*, 2016; Mirlohi *et al.*, 2016).

Similarly, factors such as age, sex, and staging of the disease may have an effect on parameters. Derangement in hematological parameters and morphological characteristics of blood cells can lead to poor clinical progression among patients undergoing treatment for PTB with resultant poor clinical treatment outcomes.

Independent Variables **Dependent Variables** **Outcomes**

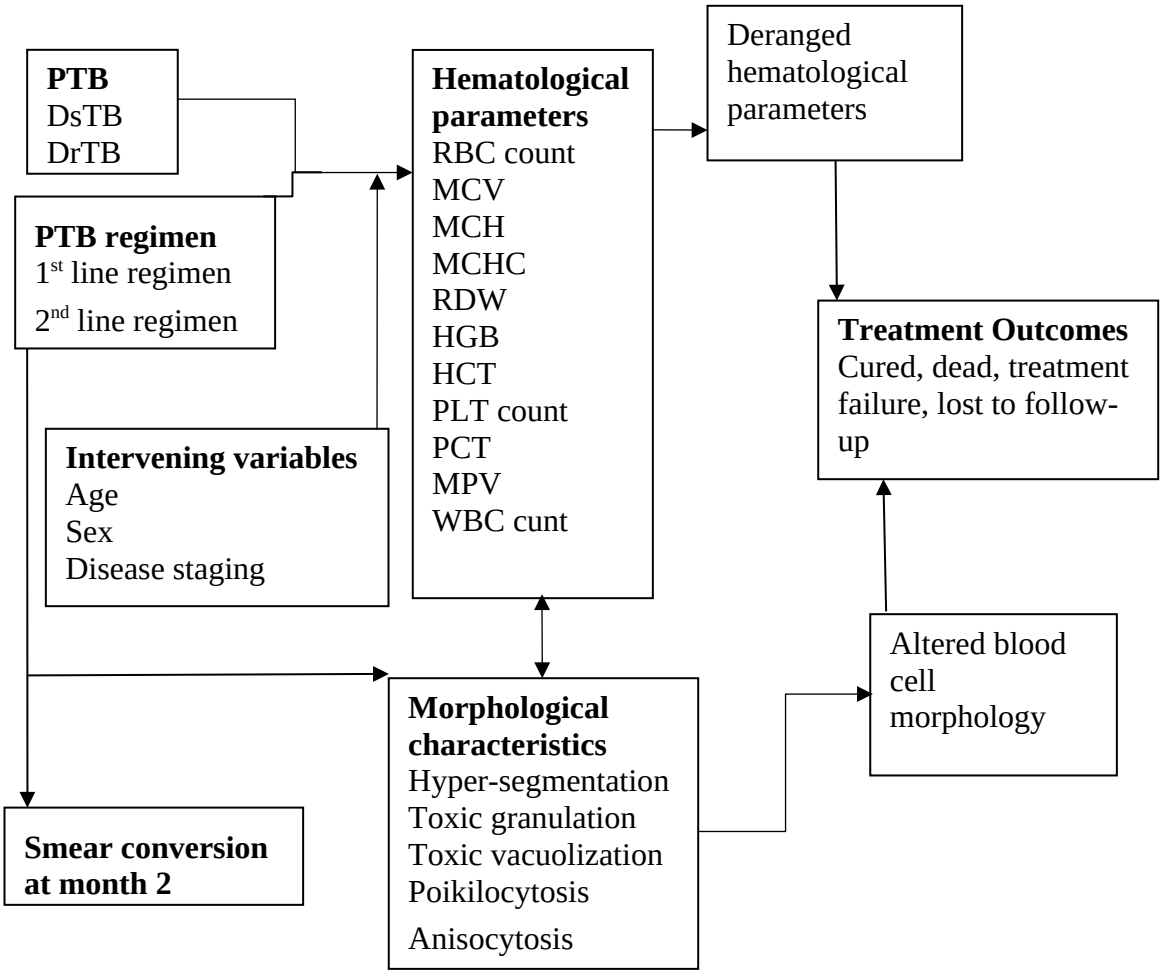

Figure INTRODUCTION.1: Conceptual framework showing the interrelationship between independent variables, intervening variables, dependent variables and the outcomes.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

The following is discussed under this chapter: descriptions of PTB, epidemiology, immune response to *M. tuberculosis*, immunopathogenesis of *M. tuberculosis*, hematopoiesis in human beings, effects of cytokines on hematopoiesis, PTB associated hematological abnormalities, diagnosis of PTB, treatment, treatment outcomes and monitoring response to treatment.

2.2 Pulmonary tuberculosis (PTB)

The microorganisms that cause PTB are termed as *Mycobacterium tuberculosis* bacilli. Mycobacterium infection occurs when a person harbors mycobacterial bacilli inside his/her body. Normally, Mycobacteria are kept in check by the host's defense system. In case of a breach in the immune system, individuals become susceptible to *Mycobacterium tuberculosis* infection (Global tuberculosis report, 2023). The transmission of *M. tuberculosis* mainly occurs when aerosols of infectious droplet nuclei are generated through the action of expectoration, sneezing, or even talking by a PTB patient. The amount of droplet nuclei deposited in the air and amount taken in by individuals during breathing determine whether an infection will occur or not (Global tuberculosis report, 2023). The common signs of PTB infection include unintentional weight loss, profuse night sweats, persistent cough, chest pain, fatigue, fever and loss of appetite. Some serious complications of PTB include secondary infection by fungi, expectoration of blood and permanent damage to the lungs (Global tuberculosis report, 2023).

2.3 Epidemiology of TB

TB cases that were newly diagnosed in 2022 were 7.5 million globally (Global tuberculosis report, 2023). A total of 10.6 million people are estimated to have fallen ill with TB in 2022 up from best estimates of 10.3 million in 2021 and 10.0 million in 2020. Globally, the estimated TB incidence rate per 100, 000 population per year was 133 in 2022. In the same year, TB caused an estimated 1.3 million deaths representing a decrease in estimates from 1.4 million both for 2020 and 2021 (Global tuberculosis report, 2023).

The estimated number of deaths in the South-East Asia and Western pacific regions caused by TB was relatively stable between 2021 and 2022. The number of deaths that occurred in the WHO African and South East Asia due to TB among HIV negative people was 81% of the global number of deaths. Of this deaths, 29% occurred in India (Global tuberculosis report, 2023). The 30 WHO TB high burden countries accounted for 87% of the world's TB cases reported in 2022. India, Indonesia, China, the Philippines, Pakistan, Nigeria, Bangladesh and the Democratic Republic of Congo contributed two-thirds of the global total of TB cases at 27%, 10%, 7.1%, 7.0%, 5.7%, 4.5%, 3.6% and 3.0%, respectively (Global tuberculosis report, 2023).

In Kenya, the incidence was estimated to be 133, 000 in 2022. Vihiga, Murang'a, Marsabit, Kajiado, Nakuru, Uasin Gishu, Kiambu, Nyeri and Garissa reported an increase in case notification rates. Case notification rate in Kenya, in 2022, stood at 168 per 100, 000 population; representing an increase from 2021, which was 147 per 100, 000 population. About 23% of the cases reported were from a TB/HIV co-infection in the year 2022 (MoH-annual TB report, 2022).

2.4 Immune response to pulmonary TB

The innate and adaptive arms of the immune system execute important processes that are essential in the fight against *M. tuberculosis*. Innate immunity is mediated by a diverse group of cells made up of macrophages, neutrophils, mast cells, dendritic cells (DCs), natural killer cells (NK) and the complement system (Jasenosky *et al*., 2015; de Martino *et al*., 2019). The airway epithelial cells (AECs) are thought to be key players in innate immune responses to *M. tuberculosis* infection. AECs are the first cells that contact the invading *M. tuberculosis*. Although termed as 'non-professional' cells, AECs can recognize *M. tuberculosis* via their cell surface pattern recognition receptors (PRRs) that are critical in detecting unusual antigens (Pai *et al*., 2016).

The activation of cell surface PRRs on airway epithelial cells culminates in the production of pro-inflammatory cytokines that consequently activate invariant T cells on the mucosal surfaces (Cliff *et al*., 2015; de Martino *et al*., 2019). This leads to release of cytokines such as TNF-α and IFN-γ. Macrophages are the major first responders against invasion by *M. tuberculosis*. Recognition of *M. tuberculosis* by macrophages allows for its ingestion by phagocytosis (Jasenosky *et al*., 2015). Within the properly matured phagosomes in the macrophage, *M. tuberculosis* is subjected to a toxic environment of zinc and copper. Macrophages have been demonstrated to block delivery of any essential nutrients to the *M. tuberculosis* including iron and manganese to diminish its survival (Lerner *et al*., 2015; de Martino *et al*., 2019).

In the innate response, DCs are termed as the most potent antigen presenters. However, the role of this group of cells in PTB immunity is controversial (Pai *et al*., 2016). It is

not known whether dendritic cells enhance activities of cellular immunity or whether their susceptibility to manipulation by *M. tuberculosis* diminishes the specific T-cell response. Manipulation of DCs through coupling of lipoarabinomannan mannose to CD209 of DCs by *M. tuberculosis* appears to be one of the winning strategies by the bacterium, which leads to disruption of dendritic cell activity (Pai *et al*., 2016; de Martino *et al*., 2019). Suppression of dendritic cell activity impairs potent antigen presentation cells thereby affecting crucial immune processes. Conversely, dendritic-cell specific intercellular adhesion molecule 3-grabbing non-integrin receptor (DC-SIGN) has been postulated to promote host protection by maintaining balanced inflammatory state thereby preventing tissue pathology (de Martino *et al*., 2019).

In the adaptive arm, T lymphocytes once activated undergo clonal expansion of specific cells sensitive to the different populations of *M. tuberculosis* antigens in the lymph nodes. $CD4^+$ T lymphocytes produce IFN-γ that mediates the process of phagosome maturation of macrophages (Jasenosky *et al*., 2015). Optimal production of IFN-γ and IL-17 largely depends on the cooperation between $CD4^+$ T lymphocytes and DCs. IFN- γ, in synergy with other cytokines such as IL-1, IL-6, and tumor necrosis factor alpha, work to ensure that the infection is controlled (Jasenosky *et al*., 2015; de Martino *et al*., 2019). Chemotactic cytokines such as chemokine ligand 5 (CCL5) and chemokine ligand 9 (CCL9) augment the effects of the immune response process by attracting other cell lines at the site of invading bacteria (de Martino *et al*., 2019).

The T cytotoxic $CD8^+$ cells employ the use of major histocompatibility class I molecules and are known to recognize *M. tuberculosis* antigens through major histocompatibility complex (MHC) class I molecules (Pai *et al*., 2016). $CD8^+$ T lymphocytes are also known to produce cytokines such as TNF-α, IL-2 and IFN-γ. Reviews by Donovan *et al.*, 2017, de Martino *et al.*, 2019 and Hunter, 2020 have demonstrated that these cytokines play an important role in controlling TB infection. Cytotoxic action against *M. tuberculosis* is initiated by cytotoxic $CD8^+$ lymphocytes with the release of perforin and granulysin mediated mechanisms (de Martino *et al*., 2019).

2.5 Immunopathogenesis of pulmonary TB

M. tuberculosis is known to enter the alveolar of humans through inhalation of aerosol droplet nuclei in the air (Lerner *et al*., 2015). Inhaled droplet nuclei are phagocytized by macrophages, which form part of the mononuclear phagocytic system. The process of engulfing *M. tuberculosis* is initiated by bacterial contact with macrophage mannose receptors and/or complement receptors (Lerner *et al*., 2015). Upon successful entry into the host macrophage, *M. tuberculosis* initially reside in endocytic vacuoles known as the phagosome (Sia and Rengarajan, 2019). Within the phagosome, the bacteria are exposed to an extremely hostile environment that is made up of an acidic pH, toxic peptides, lysosomal enzymes and reactive oxygen intermediates. This hostility is partly contributed by the normal phagosomal maturation that entails the fusion of a phagosome and a lysosome (Lerner *et al*., 2015; Sia and Rengarajan, 2019).

The bacteria that survive in the macrophages induce the production of chemokines that attract other cell lines such as neutrophils to the site of the invading bacteria. Monocytes, lymphocytes, and neutrophils, even though respond to the site of infection, they do not kill the bacteria efficiently (Sia and Rengarajan, 2019). Consequently, macrophage derived giant cells and lymphocytes form focal lesions that are granulomatous, common with TB infection (Hunter, 2020).

Progression of cellular immunity results in killing of macrophages loaded with bacilli. This process results in the formation of a caseous center of the granuloma (Sia and Rengarajan, 2019). In immunocompetent individuals, the *M. tuberculosis* is inhibited and the granulomas commence to heal, forming small lesions that are fibrous and calcified in nature. However, in immunocompromised individuals, the granuloma centers liquefy by a process that is not well understood.

The liquefied granulomas provide a rich medium that allows for survival and proliferation of *M. tuberculosis* in an uncontrolled manner (Hunter, 2020). *M. tuberculosis* replicates within these granulomas with viable ones escaping and spreading within the lungs, culminating into PTB. *M. tuberculosis* can also spread via the lymphatic drainage or blood to other tissues resulting in milliary or extra-pulmonary tuberculosis (Hunter, 2020).

2.6 Hematopoiesis and normal blood profiles

Hematopoiesis is a tightly controlled process of blood cell production. The process involves cell proliferation, differentiation, maturation and renewal. During early life, hematopoiesis largely takes place in the yolk sac, aorta-gonad mesonephros region and

fetal liver. In adults, hematopoietic tissue is largely located in the red bone marrow of long bones. However, the lymph nodes, spleen, liver and thymus retain hematopoietic capability (Keohane *et al.,* 2016). Erythropoiesis, a complex process of producing sufficient numbers of erythrocytes in peripheral blood, occurs in the bone marrow. The earliest identifiable colony of RBCs known as burst forming unit-erythroid (BFU-E) is derived from colony forming unit-granulocyte, erythrocyte, monocyte and megakaryocyte (CFU-GEMM).

Cytokines such as IL-3, granulocyte-macrophage colony stimulating factor (GM-CSF), thrombopoietin (TPO) and KIT ligand progressively influence the development of the colony forming unit-erythroid (CFU-E). The influence of EPO mediates the differentiation of CFU-Es into pronormoblasts, the earliest visually recognizable red blood cell precursor (Keohane *et al.,* 2016). Formation of WBCs is termed leukopoiesis. The process is divided into two: myelopoiesis and lymphopoiesis. Myelopoiesis involves differentiation of the CFU-GEMM into monocytes, neutrophils, eosinophils and basophils and is mediated by granulocyte-macrophage colony stimulating factor, granulocyte colony stimulating factor, macrophage colony stimulating factor, interleukin-3, IL-5, interleukin-11 and KIT ligand (Keohane *et al.,* 2016).

Lymphoid differentiation is largely mediated by IL-2, IL-7, IL-12, and IL-15 growth factors (Keohane *et al.,* 2016). The process by which platelets are formed and replenished is called megakaryopoiesis. Growth factors know to influence megakaryopoiesis include granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-3, interleukin-6, interleukin-11, KIT ligand and thrombopoietin.

Thrombopoietin is a growth factor required for platelet production and is mainly produced by the liver. Figure 2.1 below shows a schematic presentation of hematopoietic processes from a single pluripotent hematopoietic stem cell (Keohane *et al.*, 2016).

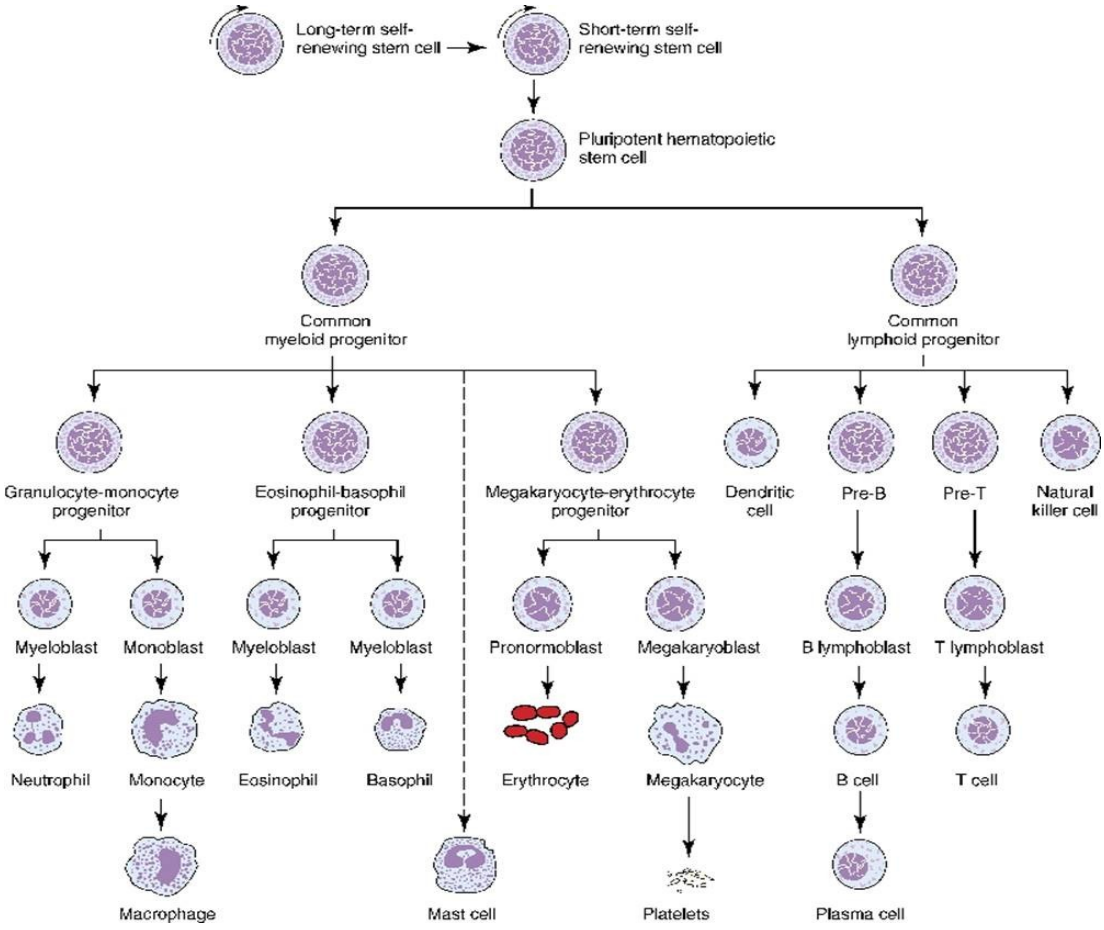

Figure LITERATURE REVIEW.2: Diagram of hematopoiesis showing derivation of cells from the pluripotent hematopoietic stem cell.
Source: *Rodak's Hematology, Fifth Edition: Clinical Principles and applications, Chapter 7, page 103*

The reference intervals for the various hematological parameters vary from one population to another depending on geographical location, diet, race and

environmental conditions. Table 2.1 shows the generally acceptable reference ranges for the common hematological parameters globally.

Table LITERATURE REVIEW.1: Reference intervals for hematological parameters

Assay	Reference interval
Red blood cell, M	4.2-6.0, X 10^6/µL
Red blood cell, F	3.8-5.2, X 10^6/µL
Hemoglobin, M	13.5-18.0, g/dL
Hemoglobin, F	12.0-15.0, g/dL
Hematocrit, M	40-54, %
Hematocrit, F	35-49, %
Mean cell volume	80-100, fL
Mean cell hemoglobin	26-34, Pg
Mean cell hemoglobin concentration	32-36, g/dL
Red blood cell distribution width	11.5-14.5, %
White blood cell	3.6-10.6, X 10^3/µL
Neutrophil	50-70, %
Neutrophil	1.7-7.5, X 10^3/µL
Lymphocyte	18-42, %
Lymphocyte	1.0-3.2, X 10^3/µL
Monocyte	2-11, %
Monocyte	0.1-1.3, X 10^3/µL
Eosinophil	1-3, %
Eosinophil	0-0.3, X 10^3/µL
Basophil	0-2, %
Basophil	0-0.2, X 10^3/µL
Platelet	150-450, X 10^3/µL
Mean platelet volume	7.0-12.0, L

Acceptable ranges for various hematological parameters. Source: *Rodak's Hematology, Fifth Edition: Clinical Principles and applications, page 2*. M-male F-female.

2.7 Effects of inflammatory cytokines on hematopoiesis

Immunity against *M. tuberculosis* largely depends on a number of cytokines usually secreted by inflammatory cells of the immune system. T helper 1 cells through their activities largely produce IFN-γ, IL-12 and TNF-α (Tania *et al.*, 2015). Interleukin 1β has been demonstrated to directly kill *M. tuberculosis* in murine and human macrophages. Interleukin 6 is required for resistance against *M. tuberculosis* by the

host. IFN-γ, IL-12, IL-7 and TNF-α are involved in formation of Mycobacterium-induced granulomas (Tania *et al.,* 2015).

However, as much as these cytokines are critical in combating *M. tuberculosis,* they have been demonstrated to have significant effects on erythropoiesis, leukopoiesis and megakaryopoiesis. Interleukin 6 is known to stimulate the production of hepcidin from the liver which is known for its inhibitory role in absorption of iron from the gut. (Koorts *et al.,* 2011). Further, interleukin-6 has been implicated in stimulation of megakaryopoiesis, with consequential increase in number of circulating platelets thought to be drivers of inflammatory processes in PTB patients.

Interferon gamma has been demonstrated to increase the expression of divalent metal transporter 1 on macrophages with resultant stimulation of ferrous iron (Fe^{2+}) uptake by macrophages (Koorts *et al.,* 2011; Domingo *et al.,* 2016). Interferon gamma is known to down-regulate ferroportin 1, an iron transporter on macrophages, leading to inhibition of iron export from macrophages. Interleukin-10, an anti-inflammatory cytokine, increases the expression of transferrin receptor on monocytes. This leads to increased uptake of transferrin bound iron into monocytes via transferrin receptors.

Tumor necrosis factor-alpha cytokine is involved in damaging of erythrocyte membranes thereby stimulating the process of phagocytosis by which red blood cells are removed from circulation (Domingo *et al.,* 2016). Moreover, TNF-α, IL-1, IL-6 and IL-10 have been shown to induce ferritin expression, storage and retention of iron within macrophages. Production of erythropoietin by the kidneys is inhibited by TNF-alpha and IFN-γ (Koorts *et al.,* 2011; Domingo *et al.,* 2016).

2.8 Pulmonary TB associated hematological abnormalities

2.8.1 Red blood cell associated anomalies

Pulmonary tuberculosis disease presents with several hematologic derangements in infected individuals. Anemia is the most common finding and abnormality found in people infected with PTB (Nagu *et al*., 2014; Chu *et al*., 2019). Ramadan and Shareef, 2019, conducted a study to determine abnormalities in hematological parameters for newly diagnosed patients. The predominant findings in their study, with relation to hematologic parameters, found that HGB, HCT, and RBC mass were significantly reduced in patients who had PTB (Ramadan and Shareef, 2019).

Furthermore, the predominant type of anemia among PTB individuals was normocytic anemia. In another study by de Mendonça *et al*., 2021, findings from their study corroborate the previously mentioned findings by Ramadan and Shareef, 2019. The general finding was that 61.2% of the PTB infected individuals had anemia. The predominant form of anemia among the patients was normochromic normocytic anemia followed by hypochromic microcytic anemia. Anemia is the commonest finding at TB diagnosis, with estimated prevalence of 9.5% to 96% (de Mendonça *et al*., 2021). Concrete evidence reveal that anemia is a predominant manifestation in individuals infected with *M. tuberculosis*.

Hemoglobin (HB) levels have also been shown to reduce as smear positivity increases (Nagu *et al*., 2014; de Mendonça *et al*, 2021). Anemia has been shown to worsen treatment outcomes and severity of TB in PTB individuals. Prevalence of this severity

is seen more in TB/HIV co-infected patients than in TB-HIV negative individuals, giving an indication that anemia is associated with more severe cases of TB (de Mendonça *et al*, 2021). Some studies document microcytic hypochromic anemia as the most predominant due to associated iron deficiency anemia (IDA). Sulochana *et al* (2018) in their study, showed that 77.3% of patients had anemia (normocytic normochromic and microcytic hypochromic types). Inflammatory responses affect critical processes of blood cell production, such as erythropoiesis.

Therefore, patients with PTB present with distinct types of anemia with altered biochemical profiles. Previous studies revealed that patients with PTB exhibited distinct biochemical profiles of gamma-glutamyl transferase, CRP, ESR, ferritin and uric acid. Increased levels of CRP, ESR and uric acid exhibited a positive correlation with active cases of PTB (Gil-Santana *et al*., 2019). Furthermore, the study demonstrated that significant differences in the levels of albumin and ferritin occur in PTB infected individuals (Gil-Santana *et al*., 2019), suggesting that anemia associated with TB infection is caused by chronic inflammation mediated by *Mycobacterium tuberculosis* pathogenesis.

Several reversible abnormalities in peripheral blood are commonly associated with PTB infection (Shafee *et al*., 2014). The hematological changes induced by PTB can be used as markers for prognosis, as well as monitoring for therapeutic response. Clinical TB causes derangements in the normal physiology of an individual especially through modulation of key physiological processes (Okafor *et al*., 2014). PTB results in

significant anomalies in both the marrow and peripheral blood thereby interfering with normal production of several blood cells (Reece *et al*., 2018).

In PTB patients, several biochemical derangements and hematological abnormalities occur which are crucial in monitoring disease progression and response to treatment (Shafee *et al*., 2014). Shafee and colleagues evaluated the hematological changes that occur in PTB patients and associated risk factors for the transmission of PTB. The findings suggest that ESR is elevated in most of the PTB patients. Additionally, hematocrit was significantly low in most of the patients with majority predominantly exhibiting signs of anemia due to critically low hemoglobin values (Shafee *et al*., 2014). Alteration of the bone marrow hematological elements in PTB infected individuals indicates dysfunctional hematopoietic processes that culminate into reduced synthesis of blood components and altered immunity.

Javed *et al*., 2018 examined the hematological profiles of PTB patients and associated co-morbidities. Evaluation of hematological parameters among the PTB group revealed significant variation in hematological parameters. In addition, Javed *et al*., 2018 demonstrated a significant reduction in PCV and red blood cell count with consequential reduction in hemoglobin levels. Kutiyal *et al*., 2017 also reported similar findings where 75.78% of patients were anemic with 98.43% having raised ESR values. A closely related study was conducted by Thatoi and Khadanga (2013). The investigation of PTB with its hematologic correlates reported that most patients were anemic, with normocytic anemia being predominant, followed by microcytic anemia

with hypochromia. Rohini and colleagues also reported a significant decrease in HGB and red blood cell (RBC) count among the patients they studied.

Several such studies have been conducted within the African region to understand the pathological changes that occur in both biochemical and hematological parameters of PTB patients. Mulenga *et al*., 2017 investigated hematological attributes of newly diagnosed PTB patients. The assessment of serum transferrin revealed two types of anemia; anemia associated with low serum transferrin levels and that associated with high levels of serum transferrin. Hypo-albuminemia was positively associated as a contributory risk factor for anemia development among PTB patients (Mulenga *et al*., 2017). Abay and colleagues assessed hematological changes in PTB patients and reported significant hematological findings. The predominant finding was that almost all the patients presented with some form of anemia, ranging from mild, moderate to severe anemia (Abay *et al*., 2018).

Kahase *et al*., 2020 determined that, there was significant variability in the different hematological parameters they assessed in PTB patients. Hemoglobin levels, hematocrit and MCHC were found to be reduced in the PTB group. From the reviewed articles, no study assessed the predictive effect of anemia on conversion of sputum at month 2 of treatment and treatment outcomes at month 6.

2.8.2 White Blood cell associated anomalies

White blood cell changes have been reported in PTB patients in several studies around the world with a lot of variability. A study by Ramadan and Shareef, 2019 in Iraq

reported non-significant changes in total WBC counts, neutrophils, monocytes and lymphocyte counts compared to a healthy control group. Rohini *et al.,* 2015 in India observed a 1.1 fold increase in WBC counts among PTB patients compared to controls. This increase in WBC counts in PTB patients was attributed to an increase in macrophages and granulocyte leukocytes as part of the body's immune defense mechanism to combating invasion by *M. tuberculosis.*

In yet another study by Sulochana *et al.,* 2018 in India, leukocytosis was reported in 45.3% of PTB patients with neutrophilia being reported in 90 patients out of the 150 that were recruited. Additionally, eosinophilia was observed in 15 patients whereas lymphocytopenia was seen in 70 patients. Shafee *et al.,* 2014 in Pakistan examined the hematological profiles of 600 PTB patients and reported lymphocytopenia in 59% and 43% of male and female patients, respectively. The study also observed neutrophilia among 64% of males and 60% of females. Neutropenia was reported in 2.1% of males and 2.2% of females. Furthermore, leukopenia was observed in 8% and 6% of male and female patients, respectively.

Obeagu *et al.,* 2019 in Nigeria examined the hematological parameters of PTB patients at baseline, month 2, month 4, and at month 6 of treatment. Variable results were reported in total WBC and differential WBC counts for the patients at the various time points. The study reported a significant increase in total WBC count at baseline, at month 4 and at month 6 compared to month 2 of treatment. On differential WBC count, neutrophils significantly decreased at month 6 of treatment compared to values at baseline, month 2 and month 4 of treatment. Similarly, lymphocytes were observed to

be significantly lower at baseline and month 2 of treatment compared to month 4 and month 6 of treatment.

The Nigerian study recommended that decrease in WBC counts in patients can be linked to improvement in their health status with increase in lymphocyte counts being used as a crude measure of disease progression (Obeagu *et al.,* 2019). Of the forty pulmonary tuberculosis patients that were studied by Kahase *et al.*, 2020 in Ethiopia, 27.5% had leukocytosis while 60% presented with lymphopenia. In general, the study demonstrated a significantly higher total WBC count along with neutrophilia in PTB patients compared to controls. According to Kahase and colleagues, the increase in total WBC count, especially polymorphonuclear leukocytes and macrophages, is due cell-mediated immunity meant to combat the invading *M. tuberculosis*

In Kenya, a study by Ongwae *et al.,* 2023 reported that 20.5%, 63.6% and 15.9% of PTB patients had leukopenia, normal leukocyte counts and leukocytosis, respectively. Absolute neutrophil counts among the patients revealed that 13.6% had neutropenia while neutrophilia was observed in 18.2%. Additionally, lymphocytopenia and lymphocytosis was noted in 13.6% and 6.8%, respectively. Ongwae *et al.,* 2023 remarked that assessing total WBC count can provide indication of pulmonary tuberculosis infection. The studies reviewed above reported significant findings on total WBC and deferential WBC counts in PTB patients. However, the effect and association of these changes with sputum smear conversion and treatment outcomes was not determined.

2.8.3 Platelet associated anomalies

A study by Ramadan and Shareef, 2019 reported thrombocytosis as a predominant finding among PTB patients. The study demonstrated that majority of the PTB patients presented with an elevated number of platelets relative to a healthy control group. Additionally, it was noted that majority of the patients who presented with thrombocytosis were males. Ramadan and Shareef (2019) attributed the occurrence of thrombocytosis among the study subjects to production of platelet antibodies that led to reactive myeloid hyperplasia.

Kahase *et al*., 20202 reported occurrence of thrombocytosis in 65% of the patients that were studied. The proportion of PTB patients having thrombocytosis was significantly higher compared to healthy controls. The study attributed the increase in platelet counts to interlukin-6, a cytokine released during the acute phase of the infection, which is known to promote megakaryopoiesis. A study in India by Sulochana *et al.,* 2018 reported thrombocytosis in 33.3% of patients with the abnormality occurring more among men than females. Additionally, Sulochana and colleagues observed thrombocytopenia in 5 cases only, from the 150 patients that were sampled. Increase in platelet numbers in this study was attributed to increase in inflammatory cells, cytokines and other mediators that are involved in formation of granulomatous lesions in PTB.

In a closely related study by Shafee *et al.,* 2014 in Pakistan, the major finding on platelets was thrombocytopenia in which 15% and 13% of male and female patients, respectively, presented with a lower than normal number of platelets. On the other hand, thrombocytosis was reported in 12% and 10% of male and female patients,

respectively. The study by Shafee *et al.,* 2014 suggested that occurrence of thrombocytosis among PTB patients can be assumed to be due to increase in thrombopoietic factors occasioned by the inflammatory response. Drug immune mechanisms, hypersplenism and bone marrow fibrosis were implicated as possible causal factors for thrombocytopenia in their study. Investigations by Obeagu *et al.,* 2019 in a prospective study in Nigeria, determined the values of platelet counts at baseline, month 2, month 4 and month 6 of treatment.

The study reported significant increase in platelet counts at month 4 and month 6 of treatment compared to baseline and month 2 of treatment. The study attributed the decrease in platelet counts at month 2 to the suppressive effect of anti-tubercular treatment. A study conducted in Kenya by Ongwae *et al.,* 2023 reported thrombocytopenia in 6.8% of PTB patients whereas 25% presented with thrombocytosis. Occurrence of thrombocytosis was attributed to interleukin-6, a promoter of megakaryopoiesis, known to be elevated in the acute phase of PTB infection. From the reviewed articles, thrombocytopenia and thrombocytosis appear to be PTB associated hematological derangements. Therefore, determination of their prognostic effect on conversion of sputum and clinical outcomes in PTB patients would be critical in monitoring disease progression.

2.9 Management of PTB

2.9.1 Diagnosis of PTB

In the Kenyan healthcare system, GeneXpert MTB/Rif is the first choice for diagnosis of PTB in places where the technology is available (MOH integrated-guideline, 2021).

The test is also capable of detecting rifampicin resistance in new cases. However, when GeneXpert MTB/Rif technology is not available, 2 sputum samples are usually collected. One sample is used for smear microscopy and the other is referred to a nearby GeneXpert MTB/Rif testing laboratory (MOH integrated-guidelines, 2021). GeneXpert MTB/Rif is an automated *in vitro* diagnostic test that utilizes nested polymerase chain reaction for qualitative detection of *M. tuberculosis* and rifampicin resistance. The 81 base pair core region of the *rpoB* gene is amplified by primers incorporated in the GeneXpert MTB/Rif cartridge. A sample processing control in the cartridge checks for adequate processing of the target bacteria while a probe check control verifies probe integrity, PCR tube filling and dye stability in the cartridge. A rapid diagnostic test, LF-LAM, is available but only considered for use in people living with HIV (PLHIV) as per the Kenyan TB diagnostic algorithm.

2.9.2 Pharmaco-treatment for PTB

Upon positive diagnosis of active drug susceptible PTB; the patient is immediately enrolled on first line anti-TB treatment. This anti-TB treatment regimen consists of a 2 months intensive phase and a four months continuation phase. During the intensive phase, patients are put on RHZE after which the patient transitions to a 4 months continuation phase of rifampicin (R) and isoniazid (H) following sputum conversion at month 2 of sputum follow-up (MOH integrated-guideline, 2021).

2.10 Pulmonary TB anti-tubercular treatment associated hematological anomalies

The common adverse effects associated with anti-tubercular treatment include hepatotoxicity, peripheral neuropathy and optic neuritis (MOH integrated-guidelines, 2021). Pyrazinamide, rifampicin and isoniazid have been strongly associated with acute hepatotoxicity in patients taking such medication. Likewise, peripheral neuropathy and optic neuritis has been associated with isoniazid and ethambutol medications, respectively (MOH integrated-guideline, 2021). Similarly, anti-tubercular treatment has been demonstrated to induce hematological changes in patients undergoing treatment.

Previous investigations reported a significant increase in hemoglobin for patients following anti-tubercular treatment (Mirlohi *et al*., 2016). The study further reported a significant increase in hematocrit percentages for patients on treatment. In addition, the study reported variable results in relation to other hematological parameters such as platelet counts, RBC indices and the differential WBC counts (Mirlohi *et al*., 2016). Studies by Kassa *et al*., 2016 found out that hematological profiles of PTB patients demonstrated a statistically significant difference in hematocrit, hemoglobin and platelet values before and after completion of the intensive phase of anti-tubercular treatment.

Furthermore, the study reported lower RDW values for TB patients who were yet to commence treatment compared to RDW values after 2 months on anti-tubercular therapy (Kassa *et al*., 2016). Investigations by Lin *et al*., 2015 also reported significant

findings on the impact of anti-tubercular treatment on circulating levels of peripheral leukocytes.

2.10.1 Treatment outcomes

At the end of the regimen, the patients are classified into well-defined treatment outcomes namely; cured, treatment completed, treatment success, treatment failure, loss to follow-up, dead and moved to Cat (MT4) (MOH integrated-guideline, 2021). A cured patient is one who had bacteriologically confirmed PTB at the beginning of treatment with smear or culture negative results in the last month of treatment with at least one other previous occasion. Treatment completed category involves patients who finish treatment without any evidence of treatment failure and have no record to show sputum smear or culture negative results in the last one month and at least on one previous occasion.

Treatment success category involves the sum total of patients cured plus those who complete treatment. A PTB patient with a positive sputum smear or culture at month 5 or later in the course of treatment is categorized as treatment failure. Lost to follow-up patients for some reason fail to start treatment after diagnosis or get their treatment interrupted for more than two months. Moved to Cat patients are those who are confirmed to develop drug resistant TB while on first line regimen. Lastly, patients who die for any reason before start of anti-tubercular treatment or during course of treatment are classified as dead.

2.10.2 Monitoring of PTB patients on treatment

During the course of treatment, the patient is monitored for response to anti-tubercular treatment through sputum conversion monitoring at the 2nd, 5th and 6th months. Besides monitoring response to treatment, patients are also monitored for common adverse effects associated with first line medications including acute hepatotoxicity, peripheral neuropathy and optic neuritis. Liver function tests (LFTs), as per the Kenya integrated guidelines for TB management, are recommended in patients undergoing PTB treatment due to drug associated hepatotoxicity (MOH integrated-guideline, 2021). However, no attention is paid to the potential hematological changes that occur in such patients either due to *M. tuberculosis* infection or anti-tubercular treatment.

Anti-tubercular treatment has been demonstrated to cause alterations in the hematological profiles of several patients undergoing treatment. Minardi *et al* (2021) reported the different anomalies associated with drugs used in the management of PTB. Most of the drugs have been shown to have a suppressive effect on key hematopoietic processes thereby affecting normal blood cell levels (Minardi *et al*., 2021). Further, limited information is available on how such changes affect treatment outcomes in PTB patients on anti-tubercular treatment. During treatment of pulmonary tuberculosis patients, no attention is paid to the potential hematological impairments that occur. To avert further mortalities among PTB patients, especially those that die from hematological-associated impairments, an integrated approach to the monitoring of these patients should considered.

To improve on desirable treatment outcomes in HIV negative PTB patients, attention need to be paid to potential hematological changes that can occur either due to *M. tuberculosis* infection or anti-tubercular treatment. The evaluation of hematological profiles at baseline and during treatment in infected persons is critical for tailoring further supportive care that can enhance improved treatment outcomes. This study evaluated hematological profiles in newly diagnosed HIV negative PTB patients and their possible influence on treatment outcomes in PTB patients. The study also compared hematological changes with sputum smear conversion during follow- up of PTB patients at month 2 and 5.

CHAPTER 3: MATERIALS AND METHODS

3.1 Introduction

This chapter presents a detailed description of study area, study design, study population, sample size determination, laboratory sample analyses, statistical analyses and ethical considerations.

3.2 Study area

The research was conducted in Kakamega County located in the Western part of Kenya. The County borders Vihiga and Siaya Counties to the South and West, respectively. The county also borders Bungoma and Trans Nzoia Counties to the North and Nandi and Uasin Gishu County to the East. The County covers an approximate area of 3, 033.8KM^2. According to the KNBS, the County contributes to a greater percentage of the population in the country as it is ranked the second most populous. The county has a total population of 1, 867, 579 people (KPHC, 2019). The county lies at an altitude of between 1,240 meters and 2,000 meters above sea level.

The study was conducted at KCTRH, situated within Kakamega town. KCTRH is located 402 Km west of Nairobi, 51 KM north of Kisumu, along the Kisumu-Webuye road. The facility is situated in Lurambi sub-county which has a population of about 188, 212 people (KPHC, 2019). The hospital serves as the major referral facility within Kakamega County for people who reside within the County and outside the County. Therefore, the hospital was a perfect catchment area for most of the TB cases occurring within the County and neighboring counties. The hospital has a well-functioning TB clinic with integrated services for TB and /or TB with other co-morbidities.

3.3 Study design

A prospective longitudinal study design was used for this research. The study period lasted from April 2023 to April 2024. The research assessed the hematological profiles (RBCs, MCV, MCH, MCHC, HCT%, WBC, WBC differential count, PLT, PCT%, and RDW) of newly diagnosed HIV negative PTB patients. These parameters were evaluated at enrollment, end of month 2 and at month 5 of anti-tubercular treatment.

Anti-tubercular treatment was for six months and included an intensive and a continuation phase of treatment. During the intensive phase, patients took RHZE while in the continuation phase, RH was administered. Therefore, patients were followed up for the entire treatment period.

3.4 Study population

The participants who were recruited comprised of adult patients who were newly diagnosed with pulmonary tuberculosis through GeneXpert MTB/RIF (Cepheid Inc Sunnyvale, CA, U.S.A). The study population comprised of two study groups; HIV negative patients newly diagnosed with PTB and a control group. The control group served as a comparator group for the PTB patients to establish whether any hematological abnormalities occur at diagnosis for PTB patients. They were recruited from among persons who met the minimum set criteria for donors as per the KTTA donor questionnaire. These individuals were presumed healthy and therefore eligible for recruitment as normal controls.

3.4.1 Inclusion criteria

HIV negative adult patients newly diagnosed with pulmonary tuberculosis were included. Persons who met the minimum set criteria for donors as per the KTTA donor questionnaire and were free from syphilis, HIV, hepatitis B and hepatitis C were included as controls.

3.4.2 Exclusion criteria

Patients suffering cancer, heart disease, renal diseases, diabetes, obstructive and pulmonary disease were not recruited into the study. Furthermore, patients suffering from pneumonia, meningitis and infectious diseases such as HIV were not recruited. In addition, patients with a history of suffering from sickle cell disease, thalassemia, hemophilia, and Von Willebrand disease were not recruited into the study.

Furthermore, patients presenting with a relapse of tuberculosis and those who did not consent in writing were not recruited into the study. The patients were screened using GeneXpert MTB/RIF as per the TB diagnostic algorithm in Kenya. Blood donors who were pregnant, lactating, had tattoos or tested positive for HIV, Hepatitis C, hepatitis B and syphilis were also excluded from the study.

3.5 Sampling design and recruitment

Convenience sampling, a non-probability technique, was used in recruiting study participants. Newly diagnosed PTB patients who met the inclusion criteria and were willing to give consent were recruited into the study with no predetermined order until the target sample size was attained. Control participants who met the requirements of the KTTA questionnaire and were free from TTIs were also recruited into the study at the blood donation center. Participants were recruited after a thorough explanation of the study objectives to them. The participation was voluntary and those who participated provided written informed consented.

3.6 Sample size determination

G power computer application, a sample size determination software, was used to compute a sample size for this study (Kang, 2021). A power of 0.80, an alpha of 0.05 and a large effect size of 0.50 was used to compute the sample size which gave a sample size of 51 participants per group. The statistical test was set at differences between two independent means computed by the T tests family. Instead of 51, 55 participants per group were recruited by the researcher, with the extra number of participants meant to cover for any attrition among the PTB group.

3.7 Laboratory analyses

3.7.1 Sputum collection for PTB diagnosis

In line with the MOH integrated guideline (2021), sputum samples were collected according to the following instructions that were issued to patients. The patient was directed to a designated sputum collection area. The patient used water to wash their mouth to get rid of any food particles. The patient inhaled deeply 2-3 times with strong breathing at each instance. On the last inhalation, the patient coughed deeply from their chest to produce sputum which was directed into an open sputum container. The reason for deep inhalation was to enhance production of sputum via expectoration rather than saliva.

The container was closed tightly and labeled appropriately with the date of collection and the patient's serial number. Collected sputa from the presumptive cases of pulmonary tuberculosis at the chest clinic were transported in a dedicated cooler box to the laboratory. The samples were analyzed using GeneXpert MTB/Rif technique according to the Ministry of Health screening and diagnostic algorithm.

3.7.2 Follow-up sputum at month 2 and month 5

Sputa were collected from the pulmonary tuberculosis patient group after 60 days post intensive treatment phase and 150 days into the anti-tubercular maintenance treatment phase (MOH integrated-guideline, 2021). Sputum smears were then prepared and examined for conversion.

3.7.3 Sputum smear preparation

Sputum smear preparation for the follow-up samples was carried out in a biosafety cabinet to prevent formation and exposure to droplet nuclei micro aerosols. The biosafety cabinet used was Class II type A2. Smears were prepared using the following procedure:

Glass slides were labelled with the date of preparation and patients' serial number for each sample. Direct sputum smear preparation was performed by selecting small portions of purulent/mucopurulent material with an applicator stick. The selected material was transferred to an appropriately labelled glass slide. The material was spread carefully in repeated circular motions over an area equal to about 2-3 cm x 1-2 cm, until an even lawn and satisfactory thickness was achieved. The prepared smears were air dried at room temperature inside the biosafety cabinet and fixed with absolute methanol for 10 minutes.

Sputum samples that remained after GeneXpert/smear preparation were disposed of according to laboratory standard procedures. The samples were treated with 1% hypochlorite inside the biosafety cabinet and then segregated off in the highly

infectious red bin in the laboratory. The waste was then collected by trained waste handlers at the facility for incineration.

3.7.4 Staining of air-dried sputum smears and Microscopic examination

Staining of fixed sputum smears for the follow-up samples was performed using the Auramine O staining technique (Appendix 4: Sputum smear microscopy guideline). The smear was flooded with 0.1% Auramine O solution, a fluorescent dye, and allowed to stand for 20 minutes. After 20 minutes, the smear was gently rinsed using tap water. Thereafter, 0.5% acid-alcohol was used to decolorize the smear followed by a gentle rinse in running tap water.

Finally, 0.3% methylene blue solution was added to the smear and allowed to stand for 2 minutes. This was followed by a gentle rinse in running tap water with subsequent blotting of excess water from the glass slides for purposes of air-drying the smears. The air-dried smears were examined using 40X objective lens to assess for material distribution and *M. tuberculosis* bacilli. The Auramine O staining procedure was used due to its high sensitivity and specificity especially in determining paucibacillary cases.

The reporting of results after examination of smears by fluorescent microscopy was done using semi-quantitative grading (Appendix 4: Sputum smear microscopy guideline). Examination of sputum smears at 2 months and 5 months during the course of patient treatment was critical in monitoring sputum smear conversion. Furthermore, evaluation of follow-up sputum smears acted as a general form of evaluating patients'

treatment outcomes according to the Kenya integrated guideline for tuberculosis management (2021).

3.8 Venous blood collection

At enrollment, month 2 and 5 during subsequent clinic visits, venous blood (5mL) samples were collected from study participants into purple evacuated vacutainer tubes that contained ethylene diamine tetra- acetic acid. The venous blood samples were used to evaluate hematological profiles of study participants for determination of any hematological abnormalities. The intervals for sample collection were in line with MOH integrated-guideline (2021) which provides for the intervals at which PTB patients on treatment should be monitored.

3.8.1 Procedure for venous blood collection

The following procedure was adopted for venous blood collection as recommended by CLSI (GP41ED7, 2017). The median cubital vein was selected for venipunctures. A tourniquet was applied and an ideal puncture site selected on the vein. The venipuncture site was cleansed using 70% isopropyl alcohol in concentric circles. Blood was drawn using the evacuated system of blood collection tubes. Immediately after blood flow was established, the tourniquet was released.

After drawing sufficient volumes of blood, the needle was withdrawn from the vein with subsequent application of slight pressure at the puncture site with sterile gauze. The EDTA vacutainer tube was gently inverted 4-8 times to allow for anticoagulation of collected blood samples. The puncture equipment and other biohazard waste were

disposed-off appropriately. The vacutainer tubes were labelled with appropriate accompanying information; the participant's serial number, the date and time of collection, and the required tests. Labelled samples were transported to the hematology laboratory in a dedicated cooler box for analysis. Hematological profile analysis and peripheral blood film preparation was done immediately the samples were received in the hematology laboratory.

3.8.2 Automated blood cell counts

Automated blood cell analysis was used to generate data on hematological parameters. Whole blood samples collected in K_2EDTA were used in determination of cell counts. Full hemogram analysis was performed using the HumaCount 5D automated hematology analyzer (Human Gesellschaft für Biochemica und Diagnostica GmbH, Germany). The instrument provided information on a wide array of parameters including RBCs, MCV, MCH, MCHC, HCT%, absolute WBC count, WBC differential count, PLT, PCT%, RDW.

Daily internal quality control checks were performed on the analyzer using quality controls (low, medium and high) as part of the internal quality control scheme for laboratory analysis of specimen. Additionally, manual generation of Levey-Jennings charts was periodically done for corroboration with automated charts that were provided by the analyzer as part of monitoring quality (Appendices IX-XIX). Corrective action was taken where equipment errors occurred with appropriate documentation in hematology occurrence book. The parameters that were assessed

provided key information on the occurrence of any hematological abnormalities in the study participants.

3.8.3 Hematological abnormalities

A white blood cell count below $4.0x10^3$/uL was considered to be leukopenia whereas a white blood cell count above $10.0x10^3$/uL was termed as leukocytosis. Neutropenia was defined as a count below $2.0x10^3$/uL while neutrophilia as a count above $7.0x10^3$/uL. Lymphocytopenia, monocytopenia and Eosinopenia were defined as counts below $0.80x10^3$/uL, $0.12x10^3$/uL, and $0.02x10^3$/uL respectively (Keohane *et al.,* 2016). Lymphocytosis, monocytosis and Eosinophilia were defined as counts above $4.0x10^3$/uL, $1.20x10^3$/uL and $0.50x10^3$/uL, respectively.

Erythropenia was defined as a count below $3.50x10^6$/uL and erythrocytosis as a count above $5.50x10^6$/uL. Anemia was assessed based on measured hemoglobin concentrations. Mild anemia had a cut-off of 11.0-12.9g/dl while moderate anemia ranged from 8.0-10.9g/dl (Keohane *et al.,* 2016). Severe anemia was classified as hemoglobin values below 8.0g/dl. Thrombocytopenia and thrombocytosis were defined as counts below and above $150x10^3$/uL and $450x10^3$/uL, respectively (Keohane *et al.,* 2016).

3.8.4 Preparation of peripheral blood films

PBFs were prepared in triplicate. An aliquot of the collected anti-coagulated venous blood was used to prepare peripheral thin films and analyzed to assess morphological characteristics and distribution of blood cells in PTB patients and controls. The smears

were prepared using the normal standard technique of thin film preparation (Keohane *et al*., 2019). Briefly, two clean grease-free slides were selected with no chipped edges. The EDTA tube sample was inverted 4-8 times to remix the blood cellular components. A small amount of blood (2mm in diameter) was placed at the center of the base slide, 1/4 inch from the opaque edge of the slide. A spreader slide held at an angle of 30º was used to quickly spread the drop of blood along the base slide with even pressure, avoiding jerky movements. The prepared smears covered 2/3 of the total slide area. The smears were labelled with dates of preparation and patients' serial numbers. Thereafter, they were air-dried and assessed for quality.

3.8.5 Staining of peripheral blood smears

Fixation of the peripheral blood smears was done after the smears had completely dried up. Absolute methanol was used to fix the thin films. The smears were placed on a staining rack and 1-2 drops of methyl alcohol added. The alcohol was allowed to completely dry on the film to ensure complete fixation (Keohane *et al*., 2019). The smears were stained using the Leishman's staining technique. The air-dried prefixed thin smears were covered with undiluted Leishman stain. The stain was allowed to stand for 2 minutes. Buffered water (pH 6.8) double in volume was added to the undiluted stain on the smear. After proper mixing, the stain was left undisturbed for 10 minutes. After 10 minutes, the stain was gently washed off using running tap water, slides were wiped clean at the back and placed in a drying rack. Dried films were examined for set quality standards before examination microscopically.

To further enhance quality of results that were obtained from peripheral film reporting, a three tier reporting system was adopted. Three morphologists were involved in

reading and reporting the films, two in Kakamega and one in Eldoret, Dr. Lotodo Cherop. Peripheral blood films for referral were packed in designated slide holders. Silica gel was added inside the slide holder boxes for moisture removal so as to preserve the quality of the dried stained blood smears. Additionally, the slides were padded with sufficient amounts of cotton wool to absorb shock during transit to avoid slide breakages. The slide holders were packed in envelopes and addressed to Dr. Lotodo and shipped via Wells Fargo Kakamega to Eldoret. Qualitatively, there was 100% agreement on the findings that were reported by the morphologists.

3.8.6 Microscopic examination of peripheral blood films

Air dried smears were examined using a compound microscope; Olympus CX23 model. The 40X objective lens was used to assess the quality of the smears and check for material distribution. Thereafter, 100X oil immersion objective lens was used to assess the films for cellular morphology. Red blood cells were examined morphologically with respect to poikilocytosis, anisocytosis, distribution and red blood cell inclusions. For poikilocytosis, red blood cells were examined for any abnormal shapes including target cells, spherocytes, ovalocytes, stomatocytes, achantocyte and tear drops.

Anisocytosis involved examination of red blood cells based on size to check for microcytes and macrocytes. The cellular inclusions that were targeted included Heinz bodies, siderotic granules, basophilic stippling, Howell-Jolly bodies and cabot rings. White blood cells were assessed for distribution, hyper-segmentation, toxic granulation, toxic vacuolization, and presence of Dohle bodies. Shift to the left

phenomena was also examined for in white blood cells. Platelets were examined for size, granularity, adequacy and abnormal phenomena such as platelet satellitism.

3.9 Statistical analyses

Data was cleaned, entered into Microsoft excel and extracted into SPSS (Inc, Chicago, USA) version 29.0 and Graph pad prism version 6.0 (California, USA) for analysis. A normality test to check for normal distribution of data for objective one and two was done using Kolmogorov-Smirnov test which showed that data was skewed.

Descriptive statistics were used to analyze participant characteristics (weight, age and gender). For the first objective, Mann-Whitney U test was used to compare hematological profiles of controls and PTB patients for statistically significant differences. Data was presented as median values. Chi-square test (X^2) was used to compare proportions of hematological derangements in WBCs, RBCs and platelets for the two groups for any statistically significant differences. For objective two, morphological data was presented as percentage values for study participants. X^2 test was used to compare cellular morphological data between study groups for any statistically significant differences.

For objective three, Shapiro-Wilk normality test was performed to check for normal distribution of data, results showed that data was skewed. Therefore, Kruskal-Wallis test was used to compare hematological profiles of patients at diagnosis, month 2 and month 5 of anti-tubercular treatment for statistically significant differences. Dunn’s

multiple comparisons post hoc test was performed to check for statistically significant mean rank differences at diagnosis, month 2 and month 5 of anti-TB treatment.

McNemar's test was used to compare proportions of RBC, WBC and platelet abnormalities at month 2 and 5 for PTB patients. Chi-square and multivariate linear regression tests were used to check for association between hematological profiles of PTB patients with sputum smear conversion and treatment outcomes. For outcomes at month 6, it was not possible to do multivariate linear regression because of the small number of participants in the outcome subcategories of death and treatment failure. A *P* value of <0.05 was considered statistically significant.

3.10 Ethical approval

Ethical approval to conduct the study was obtained from Masinde Muliro University institutional ethical review committee, approval number **MMUST/IERC/120/2023**. A permit to undertake the research was obtained from the national Commission for Science, Technology and Innovation (NACOSTI), license number **NACOSTI/P/23/23989**. Ethical approval was also obtained from the ethical review committee of Kakamega County Research Directorate, approval number **ERC/190-03/2023**. The researcher in general ensured that the declarations of Helsinki were upheld throughout the study (Halonen *et al*., 2020). Written informed consent was obtained from study participants. Participants who were below eighteen years but above sixteen years of age assented to the study verbally with written proxy informed consent from their parents/legal guardian. Patients who did not consent to the study

were not coerced into the study. Patients were informed about their voluntary inherent right to either participate in the study or not.

3.11 Ethical considerations

3.11.1 Confidentiality

The researcher ensured confidentiality of participants' information and data by use of lockable cabinets to prevent unauthorized access to patient particulars. Participants' data was serialized and there was no use of real personal identifier information throughout the research. The data was password protected to prevent non-authorized access of participants' information.

3.11.2 Minimization of risk

Risks were minimized during the course of research to avoid harm to the patients and researcher. The researcher utilized validated methods of sample collection that were in line with the goals of patient safety. Standard operating procedures for sample collection were used at all times to ensure that sample collection was done procedurally without any risk or harm to the participants.

3.11.3 Beneficence

The study was designed to improve on the quality of care for patients undergoing PTB treatment. The study purposed to enrich healthcare policies and practices to ensure that patients receive the highest attainable standards of care while undergoing treatment for PTB. Therefore, the design of the study was meant to benefit participants and not the contrary. Furthermore, participants directly benefited from the evaluation of their hematological profiles at diagnosis, months 2 and 5 of treatment. Clinicians' at the

chest clinic used this information to advice and augment the treatment needs of the patients

3.11.4 Maleficence

Study participants were protected from any form of psychological, physical, social or legal harm throughout the entire period of research process. The researcher conducted the study in a manner that promoted the physical and psychological well-being of those who agreed to participate in the study.

CHAPTER 4: RESULTS

4.1 Introduction

Participant characteristics, results of hematological profile analysis and blood cell morphological analysis at diagnosis, end of month 2 and at month 5 of treatment are presented in this chapter. Furthermore, respective statistical analyses are presented in this chapter.

4.2 Participant characteristics at enrollment

Participant characteristics are as presented in table 4.1. Males comprised of 80.0% (n=44) and 81.8% (n=45) for the PTB and control groups, respectively. The number of

females in the PTB and control groups were 11 (20.0%) and 10 (18.2%), respectively. The two groups were evenly matched on gender and age with no statistically significant difference between them (P=0.059 and P=0.121 Chi-square test). However, relative to the control group, the PTB group had a significantly lower mean weight (57.8±12.1 vs 69.9±12.1, P=0.046). This finding corroborates the occurrence of unintentional weight loss in most PTB patients at diagnosis.

Table RESULTS.2: **Characteristics of study participants at baseline**

Parameter	Control group (n=55)	PTB group (n=55)	*P*-value
Gender			
Male (n (%))	45 (81.8)	44 (80.0)	0.059
Female (n (%))	10 (18.2)	11 (20.0)	
Weight (mean±SD)	69.9 ±12.1	57.8 ±12.1	**0.046***
Age (mean±SD)	32.8 ±10.5	38.0 ±13.1	0.121
16-30 (n (%)	25 (45.5)	14 (25.5)	
31-44 (n (%)	22 (40.0)	24 (43.6)	
45-58 (n (%)	7 (12.7)	12 (21.8)	
59-71 (n (%)	1 (1.8)	4 (7.3)	
>71 (n (%)	0 (0.0)	1 (1.8)	

Data presented as n (%). Statistical significance determined by X^2 test was used to test for differences between the groups, with statistical significance set at $P<0.05$. *Statistical significance

All the fifty five PTB patients recruited had drug susceptible pulmonary tuberculosis at the time of enrollment. Drug susceptible pulmonary TB patients were treated with RHZE for a 2-month intensive phase followed by RH for a 4-month continuation phase, lasting for a total period of 6 months. Additionally, patients were also supplemented with vitamin A and pyridoxine as a preventive measure against peripheral neuropathy. Where there was delayed sputum conversion at month 2, treatment with RHZE was extended for one more month until sputum conversion was achieved.

4.3 Newly diagnosed pulmonary tuberculosis patients exhibit deranged hematological profiles

The hematological parameters of pulmonary TB and blood donor controls are presented in table 4.2. The results presented here reveal that percentage of neutrophils ($P<0.001$), monocytes ($P=0.044$), RDW-CV ($P<0.001$) and PCT ($P=0.002$), were

elevated in the PTB group relative to the control group. However, the percentage of lymphocytes (P<0.001), eosinophils (P=0.001), HCT (P<0.001) and P-LCR (P<0.001) were reduced in the PTB group compared to the control group. The percentage of basophils (P=0.878) was comparable between the groups.

Additional analyses demonstrated that absolute counts for WBCs (P<0.001), neutrophils (P<0.001) monocytes (P<0.001), basophils (P=0.002), and PLT (P<0.001) were higher in the PTB group relative to the control group. Conversely, absolute counts for eosinophils (P=0.040) and RBCs (P=0.001) were significantly lower in the PTB group compared to the control group. However, absolute lymphocyte counts (P=0.193), were comparable between the groups. Whereas, the RDW-SD (P=0.001) was higher in the PTB group than the control group, the converse is true for HGB (P<0.001), MCHC (P=0.010), MPV (P<0.001) and PDW (P<0.001). In addition, the MCV (P=0.152), MCH (P=0.067) and P-LCC (P=0.479) were comparable between the groups.

Table RESULTS.3: Comparison of hematological profiles between study groups at baseline

Parameter	Control group Median (min-max)	PTB group Median (min-max)	*P* value
Neutrophil%	51.1 (31.0-78.2)	64.2 (42.17-92.6)	<**0.001***
Lymphocyte%	41.8 (19.2-59.6)	26.2 (5.3-49.6)	<**0.001***
Monocyte%	4.5 (1.2-12.6)	5.6 (1.0 – 16.2)	**0.044***
Eosinophil%	2.2 (0.3-11.3)	1.2 (0.0-9.2)	**0.001***
Basophil%	0.7 (0.1-1.5)	0.7 (0.1-1.8)	0.878
Hematocrit%	42.05 (31.3-54.8)	37.9 (22.2-53.1)	<**0.001***
Red blood cell distribution width CV%	11.9 (10.8-13.7)	12.8 (11.2-18.7)	<**0.001***
Plateletcrit %	0.25 (0.14-0.38)	0.27 (0.16-0.64)	**0.002***
P-LCR %	37.6 (21.9-53.4)	25.8 (9.9-47.0)	<**0.001***
White blood cell (10^3/uL)	4.6 (2.84-8.22)	6.82 (3.22-13.12)	<**0.001***
Neutrophil	2.41 (1.18-5.01)	4.31 (1.78-11.61)	<**0.001***
Lymphocyte	1.91 (1.14-4.27)	1.74 (0.61-4.77)	0.193
Monocyte	0.23 (0.05-7.0)	0.38 (0.08-1.89)	<**0.001***
Eosinophil	0.12 (0.01-7.0)	0.08 (0.0-1.07)	**0.040***
Basophil	0.03 (0.01-7.0)	0.05 (0.01-0.15)	**0.002***
Platelet count (10^3/uL)	237 (123.0-367.0)	314 (185.0-868.0)	<**0.001***
Red blood cell count (10^6/uL)	5.2 (3.78-6.50)	4.79 (2.9-6.33)	**0.001***
Hemoglobin (g/dL)	14.3 (10.4-19.10)	12.8 (7.30-18.3)	<**0.001***
Mean cell hemoglobin (fL)	81.9 (68.10-96.0)	80.2 (55.70-93.7)	0.152
Mean cell hemoglobin (pg)	27.9 (22.8-33.50)	27 (17.60-32.4)	0.067
Mean cell hemoglobin concentration (g/dL)	34.1 (32.9-35.10)	33.8 (30.60-35.7)	**0.010***
Red blood cell distribution width SD (fL)	40.4 (36.5-44.8)	42.3 (36.3-70.7)	**0.001***
Mean platelet volume (fL)	10.5 (8.50-12.6)	8.9 (6.60-11.7)	<**0.001***
Platelet distribution width (fL)	13 (9.50-19.5)	10.4 (7.10-18.0)	<**0.001***
P-LCC (10^9/L)	86 (51.0-146.0)	88 (49.0-189.0)	0.479

Mann-Whitney U test was used to compare medians between the two study groups, with statistical significance set at *P* value of 0.05. *Statistical significance. **Abbreviations: WBC**-white blood cell count, **RBC**-red blood cell count, **HGB**-hemoglobin concentration, **MCV**-mean corpuscular volume, **MCH**-mean corpuscular hemoglobin, **MCHC**-mean corpuscular hemoglobin concentration, **RDW-CV**-red blood cell distribution width-coefficient of variation,

RDW-SD-red blood cell distribution width-standard deviation, **HCT**-hematocrit, **PLT**-platelet count, **MPV**-mean platelet volume, **PDW**-platelet distribution width, **PCT**-plateletcrit, **P-LCR**-platelet-large cell ratio, **P-LCC**-platelet-large cell count

The WBC counts of patients were further examined as presented in table 4.3 below. The PTB group had a higher proportion of leukocytosis and a lower proportion of leukopenia relative to the control group that had a higher proportion of leukopenia and no case of leukocytosis (P<0.001). The PTB group had a higher proportion of neutrophilia and a low proportion of neutropenia (P<0.001). However, the proportions for WBC differential counts were comparable between the groups.

Table RESULTS.4: Proportions of WBC and WBC differential counts among study participants at baseline

Parameter	Control group, n (%)	PTB group n (%)	*P* Value
WBC (10^3/uL)			
Leukopenia	10 (17.9)[a]	2 (3.6)[b]	**0.001**[*]
Normal	46 (82.1)[a]	45 (81.8)[a]	
Leukocytosis	0 (0.0)[a]	8 (14.5)[b]	
Neutrophil			
Low	19 (34.5)[a]	3 (5.5)[b]	
Normal	36 (65.5)[a]	42 (76.4)[a]	< **0.001**[*]
High	0 (0.0)[a]	10 (18.2)[b]	
Lymphocytes			
Low	0 (0.0)[a]	2 (3.6)[a]	
Normal	54 (98.2)[a]	52 (94.5)[a]	0.361
High	1 (1.8)[a]	1 (1.8)[a]	
Monocytes			
Low	7 (12.7)[a]	2 (3.6)[a]	
Normal	46 (83.6)[a]	51 (92.7)[a]	0.219
High	2 (3.6)[a]	2 (3.6)[a]	
Eosinophils			
Low	1 (1.8)[a]	5 (9.1)[a]	
Normal	50 (90.9)[a]	48 (87.3)[a]	0.185
High	4 (7.3)[a]	2 (3.6)[a]	
Basophils			
Low	0 (0.0)[a]	0 (0.0)[a]	
Normal	52 (94.5)[a]	51 (92.7)[a]	
High	3 (5.5)[a]	4 (7.3)[a]	0.696

X^2 test was used to test differences between the two study groups with statistical significance set at ***P*** =0.05. *Statistical significance. Similar superscripts denote column proportions that are similar.

The classification of anemias was based on hemoglobin concentration measurement as shown in figure 4.1 below. Relative to the control group, the PTB group had significantly higher proportions of mild and moderate anemias (*P*<0.001, respectively). Since there were only two participants with severe anemia in the PTB group and none in the control group, this class of anemia was not subjected to statistical analysis.

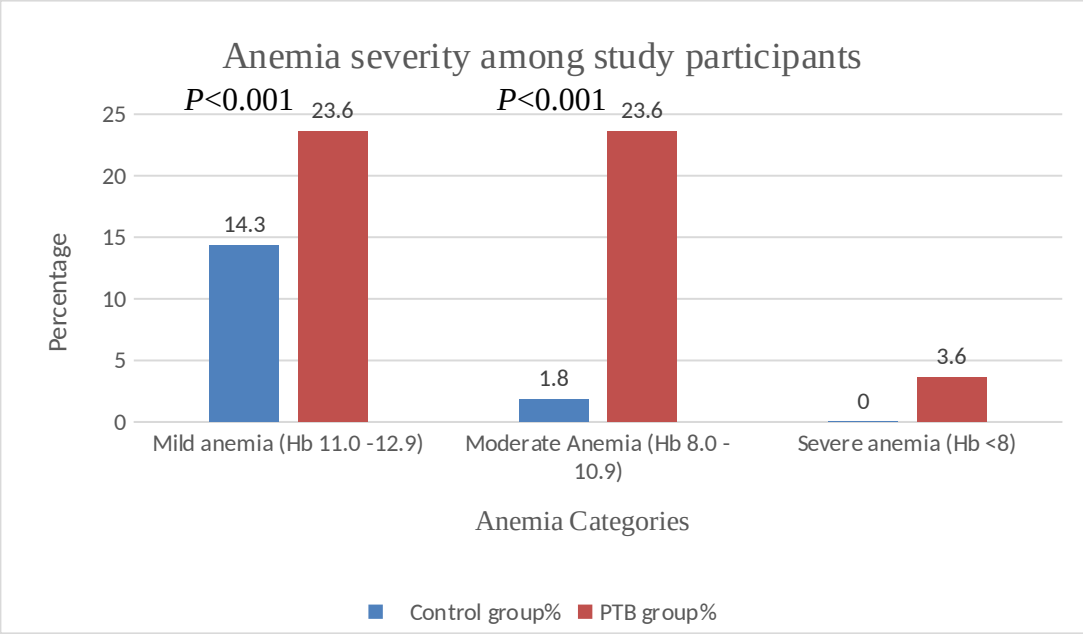

Figure RESULTS.3: Anemia severity among study participants.
Comparison between the two groups was done using Chi-square test, *P*<0.05

The proportion of abnormalities in the red blood cell indices was also assessed as shown in table 4.4. The results obtained indicate that a higher proportion of the PTB patients had low HCT%, while a lower proportion of the PTB patients had normal HCT % (*P*=0.001), relative to the control group. In addition, the PTB group had a higher

proportion of individuals with low MCH and lower proportion with normal MCH (*P*=0.032). Additional analysis showed that the proportions of erythrocytopenia and erythrocytosis (*P*=0.078) were comparable between the groups. Similarly, the proportions of low, normal and high MCV (*P*=0.249) and MCHC (*P*=0.079), were comparable between the groups.

Table RESULTS.5: Proportions of Red Blood Cell counts among study participants at baseline

Parameter	Control group, n (%)	PTB group n (%)	*P* Value
RBC (10^6/uL)			
Erythrocytopenia	0 (0.0)[a]	3 (5.5)[a]	0.078
Normal	43 (78.2)[a]	46 (93.6)[a]	
Erythrocytosis	12 (21.8)[a]	6 (10.9)[a]	
HCT%			
Low	7 (12.5)[a]	25 (45.5)[b]	
Normal	47 (85.5)[a]	30 (54.5)[b]	0.001*
High	1 (1.8)[a]	0 (0.0)[a]	
MCV (fL)			
Low	21 (38.2)[a]	27 (49.1)[a]	0.249
Normal	34 (61.8)[a]	28 (50.9)[a]	
High	0 (0.0)[a]	0 (0.0)[a]	
MCH (pg)			
Low	16 (29.1)[a]	27 (49.1)[b]	0.032*
Normal	39 (70.9)[a]	28 (50.9)[b]	
High	0 (0.0)[a]	0 (0.0)[a]	
MCHC (g/dL)			
Low	0 (0.0)[a]	3 (5.5)[a]	0.079
Normal	0 (0.0)[a]	0 (0.0)[a]	
High	55 (100.0)[a]	52 (94.5)[a]	
RDW-CV%			
Low	2 (3.6)[a]	0 (0.0)[a]	
Normal	53 (96.4)[a]	50 (90.9)[a]	0.029*
High	0 (0.0)[a]	5 (9.1)[b]	
RDW-SD (fL)			
Low	0 (0.0)[a]	0 (0.0)[a]	
Normal	55 (100.0)[a]	53 (96.4)[a]	0.154
High	0 (0.0)[a]	2 (3.6)[a]	

X^2 test was used to compare differences between the two study groups with statistical significance set at ***P***=0.05. *Statistical significance. Similar superscripts denote column proportions that are similar.

Further analysis focused on the platelet abnormalities between the groups as indicated by table 4.5. These analyses reveal that a high proportion of PTB group had thrombocytosis (*P*<0.001) and higher PCT% (*P*=0.030). However, the proportions of low, normal and high MPV (*P*=0.154), PDW (*P*=0.069), P-LCR% (0.153) and P-LCC (P=0.702), were comparable between the groups.

Table RESULTS.6: Proportions of Platelet counts among study participants at baseline

Parameter	Control group, n (%)	PTB group n (%)	*P* Value
PLT (10^3/uL)			
Thrombocytopenia	0 (0.0)[a]	0 (0.0)[a]	
Normal	49 (89.1)[a]	27 (49.1)[b]	**< 0.001***
Thrombocytosis	6 (10.9)[a]	28 (50.9)[b]	
MPV (fL)			
Low	0 (0.0)[a]	0 (0.0)[a]	
Normal	53 (96.4)[a]	55 100.0)[a]	0.154
High	2 (3.6)[a]	0 (0.0)[a]	
PDW (fL)			
Low	0 (0.0)[a]	5 (9.1)[a]	0.069
Normal	52 (94.5)[a]	48 (87.3)[a]	
High	3 (5.5)[a]	2 (3.6)[a]	
PCT %			
Low	0 (0.0)[a]	0 (0.0)[a]	
Normal	40 (72.7)[a]	29 (52.7)[b]	**0.030***
High	15 (27.3)[a]	26 (47.3)[b]	
P-LCR %			
Low	0 (0.0)[a]	1 (1.8)[a]	
Normal	50 (90.9)[a]	53 (96.4)[a]	0.153
High	5 (9.1)[a]	1 (1.8)[a]	
P-LCC (10^9/L)			
Low	0 (0.0)[a]	0 (0.0)[a]	
Normal	31 (56.4)[a]	29 (52.7)[a]	0.702
High	25 (43.6)[a]	26 (47.3)[a]	

X^2 test was used to test for differences between the two study groups with statistical significance set at ***P***=0.05. *Statistical significance. Similar superscripts denote column proportions that are similar.

4.4 Morphological characteristics of blood cells of newly diagnosed PTB patients

To further elucidate the hematological changes that occur in newly diagnosed PTB patients, peripheral blood films were prepared and examined. The various morphological characteristics and distribution of RBCs, WBCs and platelets were as reported below.

4.4.1 Red blood cell morphologies at baseline, end of month 2 and month 5 of treatment

Red blood cell morphological characteristics at baseline are as shown in table 4.6 and figures 4.2, 4.3, 4.4. A significantly lower proportion of normocytic normochromic anemia was observed in the PTB group at 54.5% (n=30) compared to the control group who had 90.9% (50) (P=0.005). Microcytic hypochromic anemia was significantly higher in the PTB group at 38.2% (n=21) compared to 3.6% (n=2) for the control group (P=0.005). Erythropenia, polycythemia and poikilocytosis were reported in 1.8% (n=1), 1.8% (n=1) and 3.6% (n=2) of the PTB patients compared to 0% (n=0), 3.6% (n=2) and 1.8% (n=1), respectively, for the control group. However, no statistically significant difference was established on these findings between the two groups. From the data presented, PTB patients had more red blood cell morphological anomalies than controls.

Table RESULTS.7: Red blood cell morphologies among study participants at baseline

Red blood cell morphologies among study participants			
	Control, n (%)	**PTB, n (%)**	***P*-value**
Normocytic normochromic RBCs	50 (90.90)[a]	30 (54.50)[b]	
Microcytic hypochromic RBCs	2 (3.60)[a]	21 (38.20)[b]	
Erythrocytopenia, normocytic normochromic	0 (0.0)[a]	1 (1.8)[a]	
Polycythemia, Normocytic normochromic	2 (3.6)[a]	1 (1.8)[a]	**0.005**[*]
Poikilocytosis (Elliptocytes and target cells)	1 (1.8)[a]	2 (3.6)[a]	

X^2 test was used to test for differences between the two study groups with statistical significance set at ***P***=0.05. [*]Statistical significance. Similar superscripts denote proportions between columns that are similar.

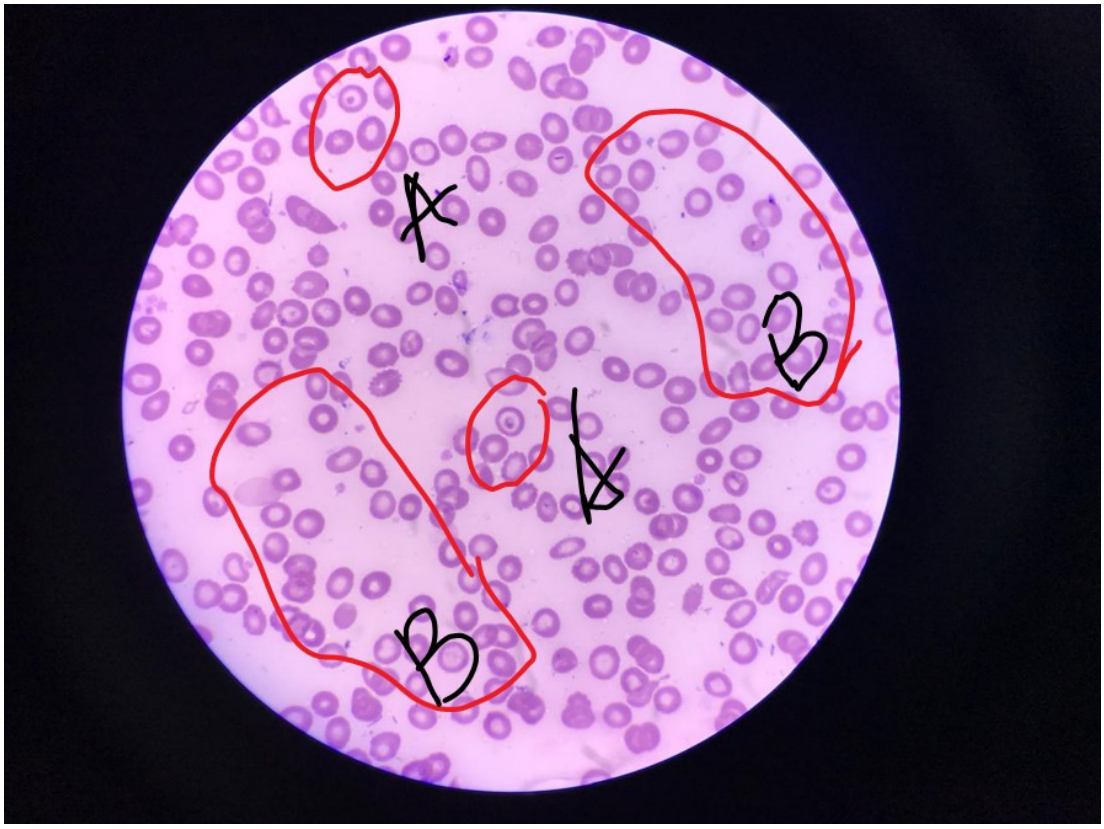

Figure RESULTS.4: Red blood cell morphologies for a PTB patient indicating different morphological anomalies.

A represents target cells, indicating poikilocytosis. B represents microcytic hypochromic red bloods cells, consistent with microcytic hypochromic anemia.

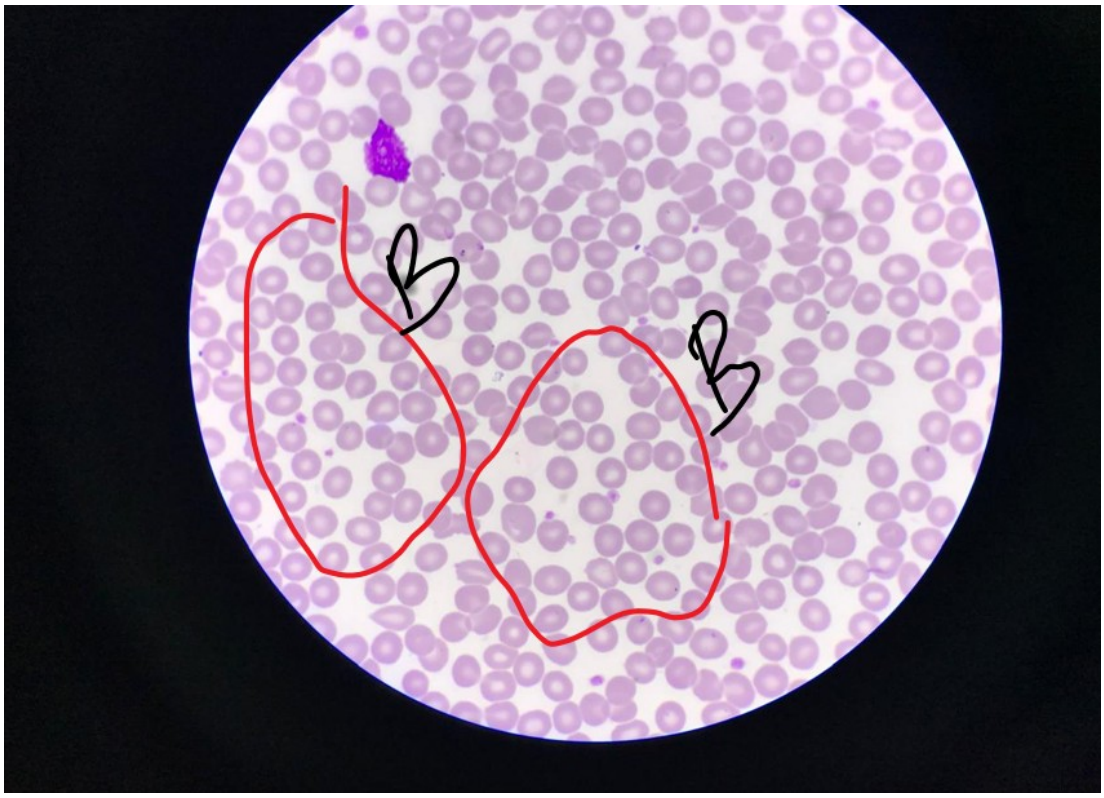

Figure RESULTS.5: Red blood cell morphologies for a PTB patient indicating a normal peripheral blood film with normocytic normochromic cells labelled as B.

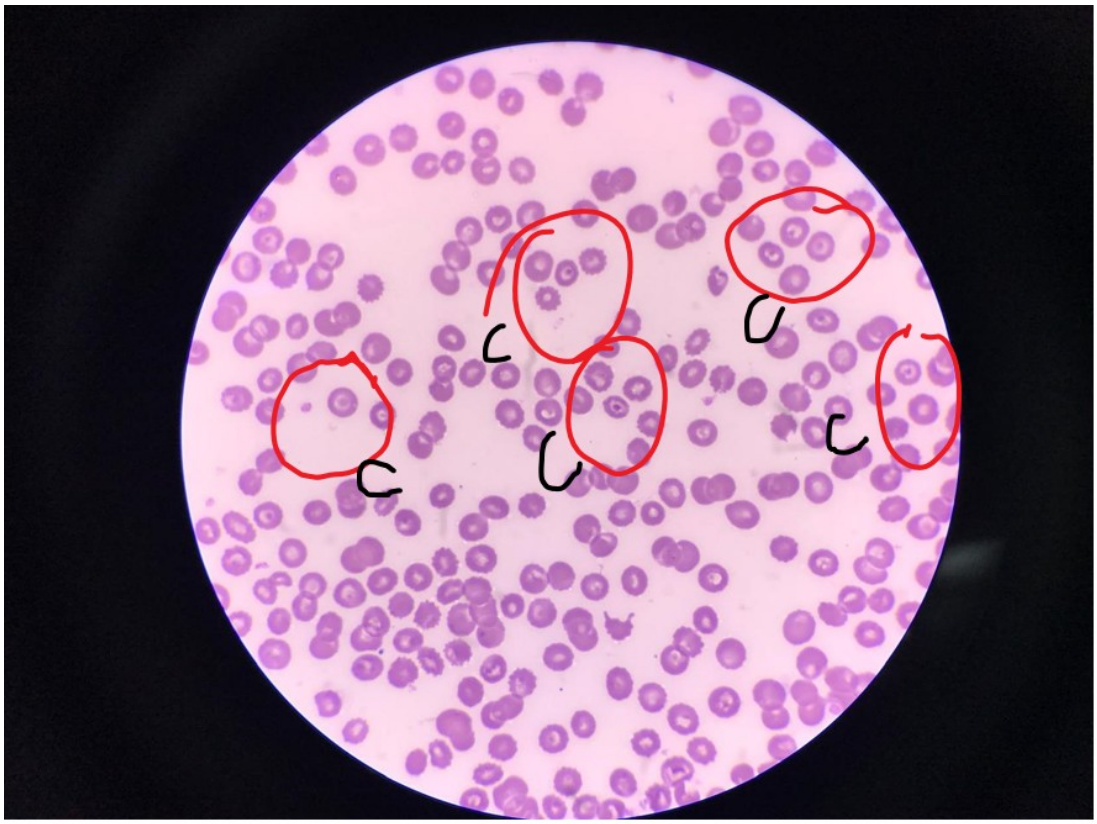

Figure RESULTS.6: Red blood cell morphologies for a PTB patient indicating different morphological anomalies.
Anisopoikilocytosis is present with majority of cells being target cells as labelled by C.

The red blood cell morphologies of PTB patients post intensive treatment phase and at month 5 are as presented in table 4.7 and figures 4.5, 4.6, 4.7, 4.8. The proportion of patients with normocytic normochromic anemia at month 5 was 53.8% (n=28), an increase from 38.2% (n=21) at month 2 of treatment. The proportion of patients who presented with microcytic hypochromic RBCs at month 2 and month 5 of treatment were 43.6% (n=24) and 34.6% (n=18), respectively. Variation in red blood cell shape (poikilocytosis) was seen in 9.1% (n=5) of the patients at month 2 of treatment compared to 5.8% (n=3) of the patients at month 5 of treatment. Generally, RBC morphological abnormalities increased at month two compared to diagnosis and slightly decreased at month 5 compared to month 2.

Table RESULTS.8 Red blood cell morphologies at month 2 and 5 of anti-tubercular treatment for PTB patients

Red blood cell morphologies/distribution among study participants		
	Month 2, n (%)	**Month 5, n (%)**
Normocytic normochromic	21 (38.2)	28 (53.8)
Microcytic hypochromic RBCs.	24 (43.6)	18 (34.6)
Erythrocytopenia, microcytic hypochromic RBCs	5 (9.1)	3 (5.8)
Microcytic hypochromic RBCs with poikilocytosis (Target cells, dacrocytes, bite cells)	5 (9.1)	3 (5.8)

Data is presented as number (percentage) values.

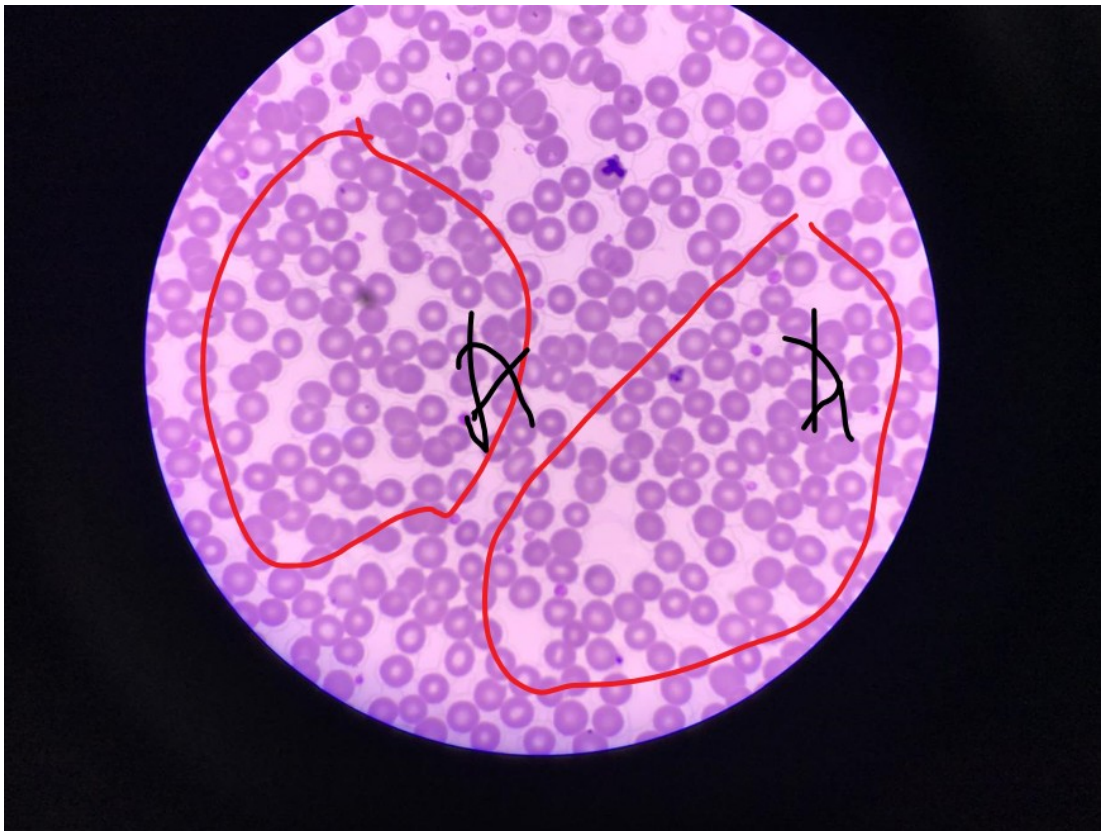

Figure RESULTS.7: Red blood cell morphologies for a PTB patient at month 2 of treatment indicating a normal peripheral blood film.

A represents normocytic normochromic red blood cells with an even area of central pallor.

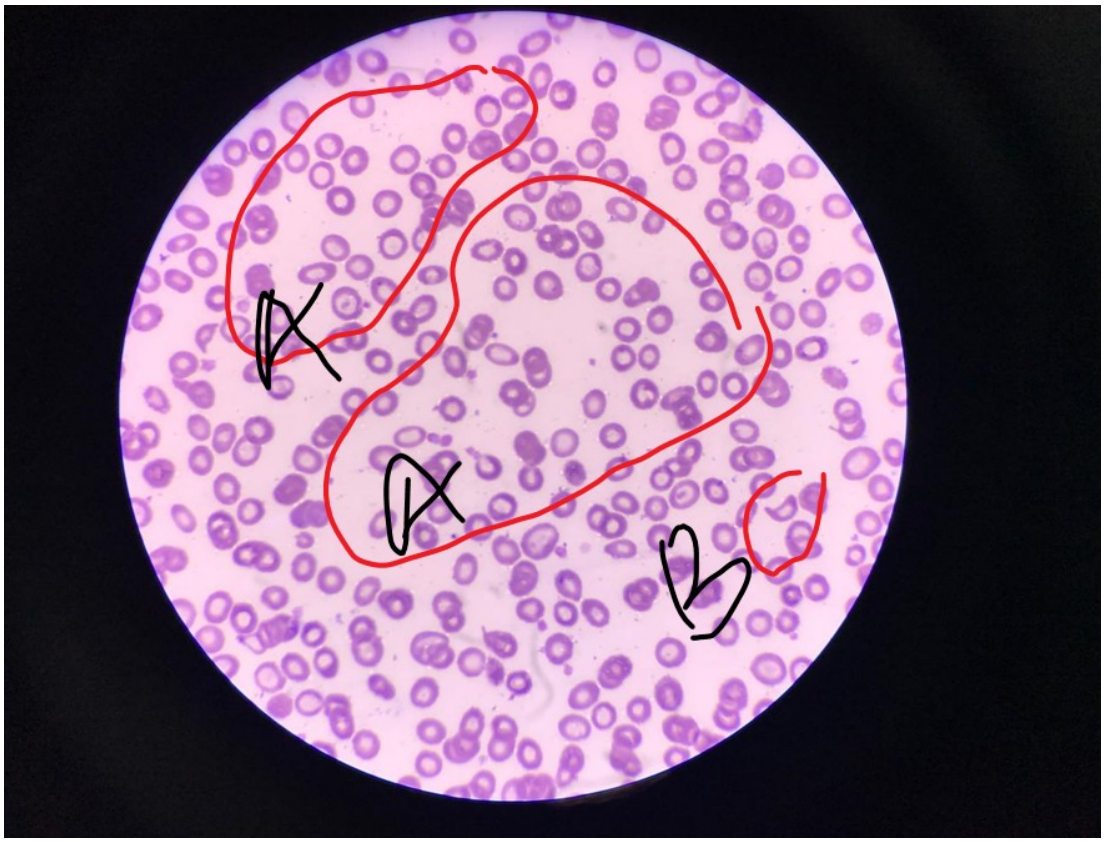

Figure RESULTS.8: Red blood cell morphologies for a PTB patient at month 2 of treatment indicating abnormal peripheral blood film.

A represents microcytic hypochromic red blood cells with a large area of central pallor. B is a helmet cell indicative of anisopoikilocytosis.

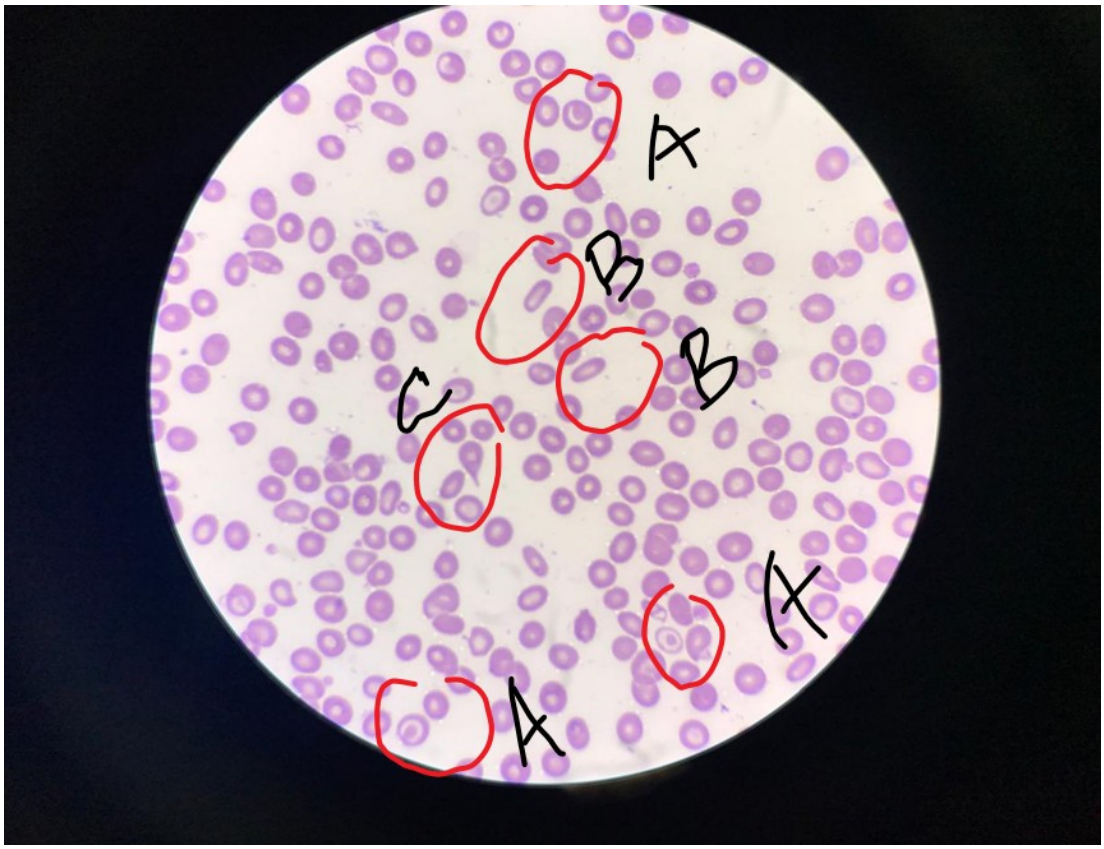

Figure RESULTS.9: Red blood cell morphologies for a PTB patient at month 2 of treatment indicating abnormal peripheral blood film.

A-Target cells, B-Ovalocytes, C-Dacrocyte. A, B, and C indicate variation of red blood cell shapes in the film.

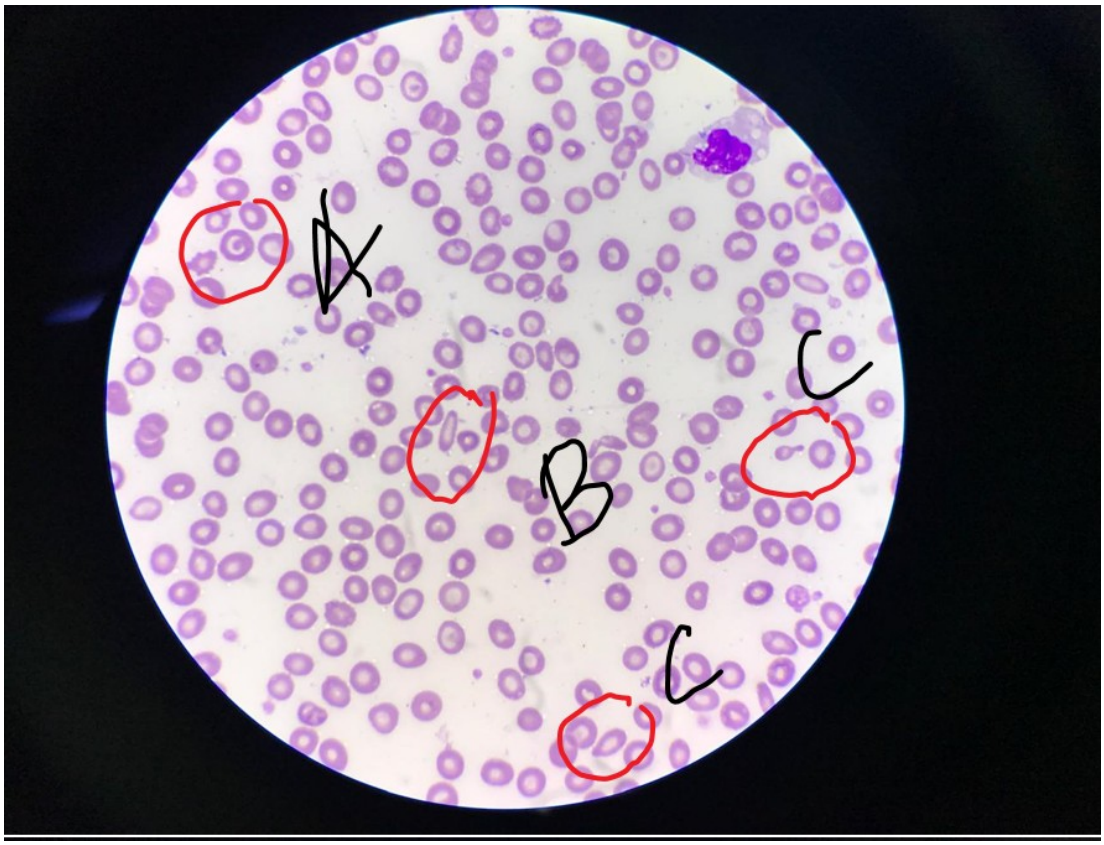

Figure RESULTS.10: Red blood cell morphologies for a PTB patient at month 5 of treatment indicating abnormal peripheral blood film.

A –Codocyte, B-Ovalocyte, C-Dacrocytes. The other red blood cells are microcytic hypochromic indicating presence of microcytic hypochromic anemia.

4.4.2 White blood cell morphologies at baseline, end of month 2 and month 5 of treatment

As shown in table 4.8 at baseline, a significantly higher proportion of neutrophilic leukocytosis with lymphopenia was recorded in the PTB group at 12.8% (n=9) compared to 0.0% (n=0) for the control group (P=0.015). Similarly, PTB patients exhibited a higher proportion of neutrophilia with a left shift at 10.9% (n=6) compared to 0.0% (n=0) for the control group. Varying proportions for leukopenia, toxic granulation, eosinophilic leukocytosis and lymphocytosis were reported for the two groups. However, no statistically significant difference was established between

groups for leukopenia, toxic granulation, eosinophilic leukocytosis and lymphocytosis. Generally, a high proportion of PTB patients presented with WBC derangements compared to controls.

Table RESULTS.9: White blood cell morphologies of study participants at baseline

White blood cell morphologies among study participants			
	Control, n (%)	**PTB, n (%)**	***P*-value**
Normal	54 (98.20)[a]	36 (65.50)[b]	
Leukocytosis-neutrophilia and lymphopenia	0 (0.00)[a]	9 (12.80)[b]	
Leukopenia	1 (1.80)[a]	0 (0.00)[a]	
Band neutrophils.	0 (0.00)[a]	6 (10.90)[b]	**0.015**[*]
Neutrophils with-toxic granulation	0 (0.00)[a]	3 (5.40)[a]	
Slight eosinophilic leukocytosis with monocytosis.	0 (0.00)[a]	1 (1.80)[a]	
Lymphocytosis	0 (0.00)[a]	2 (3.60)[a]	

X^2 test was used to test for differences between the two study groups with statistical significance set at P=0.05. [*]Statistical significance. Similar superscripts denote proportions between columns that are similar.

The white blood cell morphologies for pulmonary TB patients at month 2 and month 5 of treatment are as presented in table 4.9. After the intensive phase of treatment, 63.6% (n=35) of patients had normal white blood cell morphology and distribution compared to 69.2% (n=36) at month five of treatment. Neutrophilia with a left shift was only present in 1.8% (n=1) of the patients at post intensive phase of treatment. There was a significant reduction in the number of patients who presented with neutrophilic leukocytosis at month five of treatment at 3.8% (n=2) compared to 14.5% (n=8) at month two of anti-tubercular treatment. Quite notably, lymphocytes increased gradually at month two, 12.7% (n=7) and month five, 15.4% (n=8) of treatment compared to values that were recorded at baseline.

Table RESULTS.10: White blood cell morphologies at month 2 and 5 of anti-tubercular treatment for PTB patients

White blood cell morphologies/distribution among study participants		
	Month 2, n (%)	**Month 5, n (%)**
Normal morphology& distribution.	35 (63.6)	36 (69.2)
Neutrophilia with left shift.	1 (1.8)	0 (0.0)
Neutrophilic leukocytosis.	8 (14.5)	2 (3.8)
Mild leukopenia, normal morphology.	0 (0.0)	4 (7.7)
Relative neutropenia.	2 (3.6)	0 (0.0)
Relative monocytosis.	2 (3.6)	2 (3.8)
Relative lymphocytosis.	7 (12.7)	8 (15.4)

Data is presented as number (percentage) values.

4.4.3 Platelet morphologies at baseline, end of month 2 and month 5 of anti-tubercular treatment

On peripheral platelet examination, no morphological abnormalities were detected in the two groups at baseline. However, the PTB group had a significantly higher proportion of thrombocytosis at 20.0% (n=11) as shown in table 4.10 and figure 4.9.

Table RESULTS.11: Peripheral platelet presentation for study participants a baseline

Platelet distribution among study participants			
	Control, n (%)	**Pulmonary TB, n (%)**	***P*-value**
Normal platelets count	55 (100.00)[a]	44 (80.00)[b]	**0.007***
High platelet count	0 (0.00)[a]	11 (20.00)[b]	

X^2 test was used to test for differences between the two study groups with statistical significance set at ***P***=0.05. *Statistical significance. Similar superscripts denote proportions between columns that are similar.

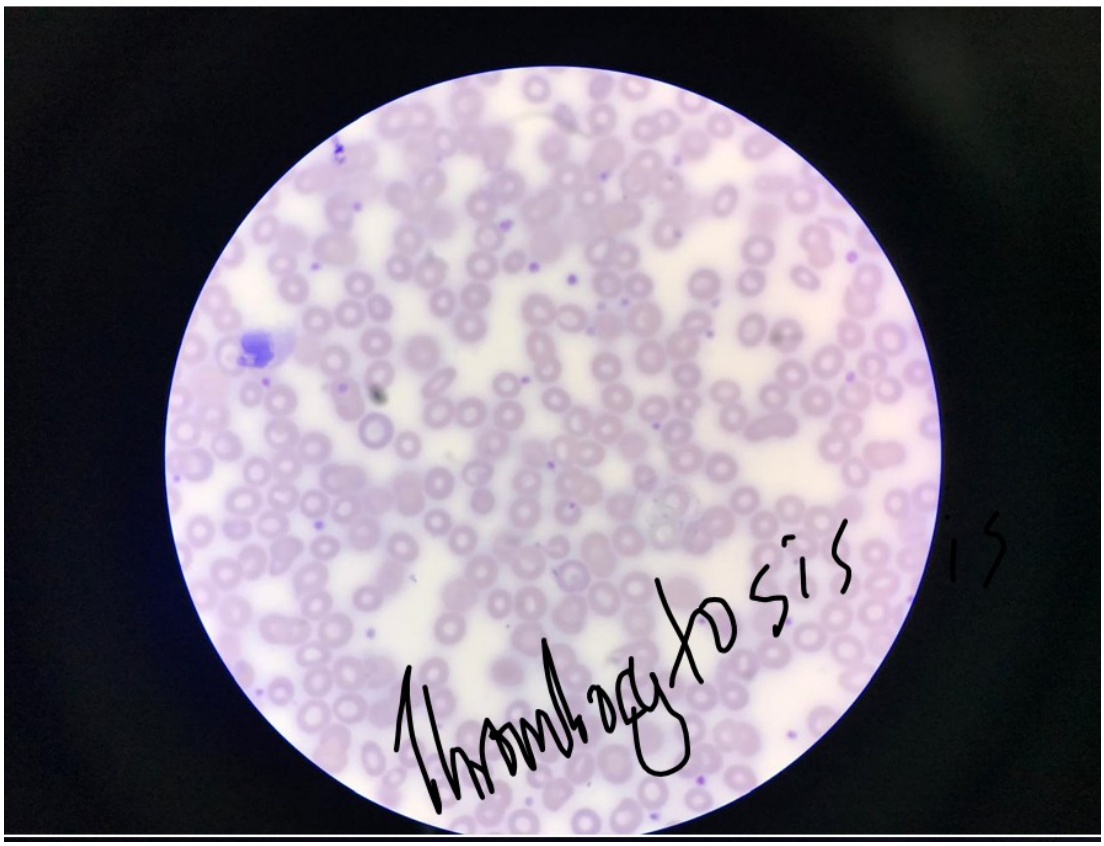

Figure RESULTS.11: Peripheral blood film of a PTB patient at diagnosis showing an elevated number of platelets (purple dots) (Thrombocytosis).
Background red blood cells are microcytic hypochromic indicative of microcytic hypochromic anemia.

Distribution of platelets in peripheral blood films at month 2 and 5 of anti-tubercular treatment for PTB patients is presented in table 4.11. The number of patients who had adequate numbers of platelets on peripheral film examination increased at month five of treatment (84.6% (n=44)) compared to month two of treatment (74.6% (n=41)). There was a slight increase in PTB patients who presented with thrombocytosis at month two of treatment, 25.4% (n=14) compared to values that were recorded at baseline, 20.0% (n=11). At month five of treatment, the number slightly decreased to 13.5% (n=7) for patients who presented with thrombocytosis on peripheral blood film examination. Interestingly, there was no case of thrombocytopenia at baseline and at

month two of treatment among patients. However, at month five of treatment, one patient presented with thrombocytopenia.

Table RESULTS.12: Platelet distribution at month 2 and 5 of anti-tubercular treatment for PTB patients

Platelet distribution among study participants		
	Month 2, n (%)	**Month 5, n (%)**
Adequate number of platelets	41 (74.6)	44 (84.6)
Thrombocytosis	14 (25.4)	7 (13.5)
Slight thrombocytopenia, no platelet clumps	0 (0.0)	1 (1.9)

Data is presented as number (percentage) values.

4.5 Hematological changes during anti-tubercular treatment and influence on treatment outcomes in pulmonary tuberculosis patients

4.5.1 Dynamic changes in white blood cell counts

As shown in table 4.12, there was a significant decrease in absolute WBC counts from the time of diagnosis of PTB to month 2 and 5 of anti-tubercular treatment ($6.82x10^3$/uL vs $6.58x10^3$/uL vs $5.87x10^3$/uL, P=0.035). Contributing to this significant overall decrease in absolute WBC counts in PTB patients was the significant reduction in neutrophil counts at month 2 and 5 of TB treatment ($3.28x10^3$/uL vs $2.97x10^3$/uL, P=0.002). The lymphocyte population steadily increased at month 2 ($2.11x10^3$/uL) and 5 ($1.94x10^3$/uL) when compared to the value that was recorded at diagnosis for PTB patients ($1.74x10^3$/uL, P=0.039). There was no statistically significant difference in monocyte, eosinophil and basophil counts at diagnosis, month 2 and month 5 of anti-tubercular treatment for PTB patients (P=0.180, P=0.127 and P=0.221, respectively).

Table RESULTS.13: Comparison of absolute WBC counts and differential WBC counts at diagnosis, month 2 and month 5 for PTB patients

Parameter	Diagnosis Median (min-max)	Month 2 Median (min-max)	Month 5 Median (min-max)	*P*= Value
WBC count (10^3/uL)	6.82 (3.22-13.12)[a]	6.58 (3.26-21.14)	5.87 (2.72-10.3)[a]	**0.035***
Neutrophil count (10^3/uL)	4.31 (1.78-11.61)[a]	3.28 (0.73-18.9)	2.97 (1.21-7.78)[a]	**0.002***
Lymphocyte count (10^3/uL)	1.74 (0.61-4.77)	2.11 (0.69-5.25)	1.94 (0.76-4.67)	**0.039***
Monocyte count (10^3/uL)	0.38 (0.08-1.89)	0.42 (0.08-1.65)	0.34 (0.10-0.97)	0.180
Eosinophil count (10^3/uL)	0.08 (0.0-1.07)	0.12 (0.0-0.9)	0.095 (0.010-0.95)	0.127
Basophil count (10^3/uL)	0.05 (0.01-0.15)	0.04 (0.01-0.17)	0.04 (0.02-0.12)	0.221

Data was analyzed using Kruskal-Wallis test and presented as median (min-max) with a *P* value of <0.05. *Statistical significance. Similar superscripts and subscripts denote column proportions that are significant.

Dunn's multiple comparisons post hoc test was performed to check for statistically significant differences in mean rank differences for parameters at diagnosis, month 2 and month 5 of anti-tubercular treatment as shown in figure 4.2. For WBC and neutrophil counts, there was no significant difference in mean rank differences at diagnosis vs 2 months and 2 months vs 5 months. However, a statistically significant mean rank difference was demonstrated between diagnosis vs 5 months of anti-tubercular treatment. There was no statistically significant mean rank difference for lymphocytes, monocytes, eosinophils and basophils at diagnosis, month 2 and month 5 of anti-tubercular treatment.

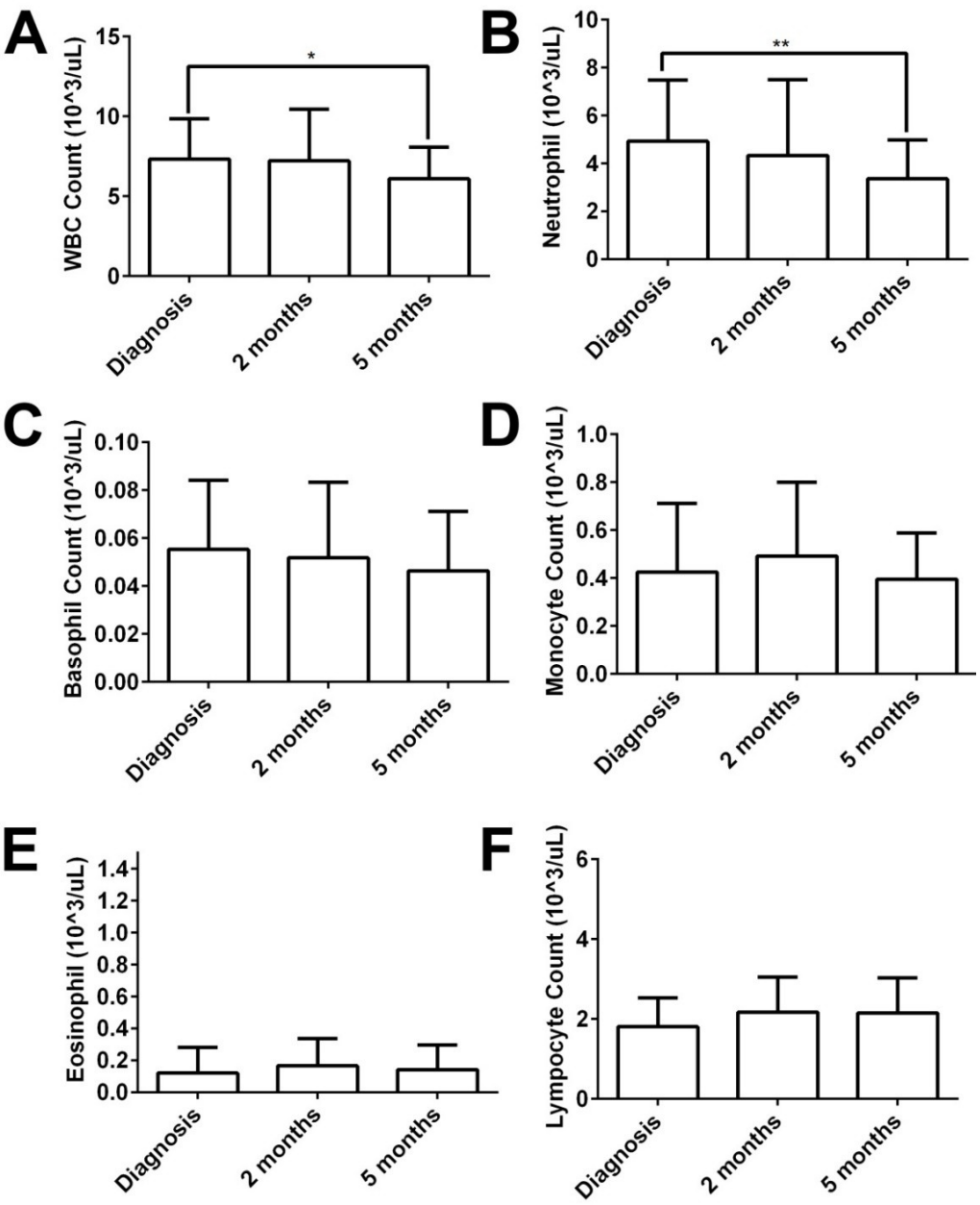

Figure RESULTS.12: Time dependent changes in white blood cell parameters from diagnosis to the fifth month of treatment.
A-Denotes a statistically significant difference in WBC counts between mean rank differences at diagnosis versus month 5. B-Denotes a strong statistically significant difference in neutrophil counts between mean rank differences at diagnosis versus month 5. C-Denotes no statistically significant difference in basophil counts between mean rank differences at diagnosis, month 2 and month 5. D-Denotes no statistically significant difference in monocyte counts between mean rank differences at diagnosis, month 2 and month 5. E-Denotes no statistically significant difference in eosinophil counts between mean rank differences at diagnosis month 2 and month 5. F-Denotes no statistically significant difference in lymphocyte counts between mean rank differences at diagnosis, month 2 and month 5. Data was analyzed using Dunn's

Multiple Comparisons tests and presented as means with a P value of <0.05. (*Continuation of figure 4.2*)

4.5.1.1 Proportion of absolute WBC and differential WBC counts at month 2 and 5 of anti-tubercular treatment among study participants

At month 2 of treatment, 7.7% (n=4) of PTB patients presented with leukopenia compared to 5.8% (n=3) at month 5 as shown in table 4.13. Notably, there was a significant reduction in the number of cases who had leukocytosis at diagnosis compared to month 2 and 5. At month 2 and 5, 11.5% (n=6) and 1.9% (n=1) of PTB patients presented with leukocytosis compared to 14.5% that presented with leukocytosis at diagnosis. On white blood cell differential counts, the percentage with low neutrophil counts at month 2 and month 5 among PTB patients was 17.3% (n=9) and 40.4% (n=21). The percentages represent a significant increase in the percentage of subjects with low neutrophil counts among PTB patients compared to the value recorded at diagnosis. Similarly, the percentage of high neutrophil counts at month 2 and 5 of treatment was 11.5% (n=6) for both. These values represent a significant decline in the number of patients with high percentage neutrophil counts at month 2 and 5 compared to the value that was recorded at diagnosis before the commencement of anti-tubercular treatment. Variable white blood cell abnormalities were recorded in the other differential types of WBCs at month 2 and 5. However, no statistically significant difference was demonstrated from their statistical comparison.

Table RESULTS.14: Proportions of WBC and differential WBC counts among study participants at month 2 and month 5 of anti-tubercular treatment

Parameter	Month 2, n (%)	Month 5, n (%)	*P* Value
WBC (10^3/uL)			
Leukopenia	4(7.7)[a]	3(5.8)[a]	
Normal	45(80.8)[a]	48(92.3)[a]	0.156
Leukocytosis	6(11.5)[a]	1(1.9)[a]	
Neu%			
Low	9(17.3)[a]	21(40.4)[a]	
Normal	40(71.2)[a]	25(48.1)[b]	**0.019***
High	6(11.5)[a]	6(11.5)[a]	
Lym%			
Low	9(17.3)[a]	6(11.5)[a]	
Normal	30(51.9)[a]	27(51.9)[a]	0.825
High	16(30.8)[a]	19(36.5)[a]	
Mon%			
Low	1(1.9)[a]	5(9.6)[a]	
Normal	49(88.5)[a]	43(82.7)[a]	0.245
High	5(9.6)[a]	4(7.7)[a]	
Eos%			
Low	4(7.7)[a]	3(5.8)[a]	
Normal	42(75.0)[a]	44(84.6)[a]	0.478
High	9(17.3)[a]	5(9.6)[a]	
Bas%			
Normal	45(80.8)[a]	38(73.1)[a]	0.503
High	10(19.2)[a]	14(26.9)[a]	
Neu#			0.325
Neutropenia	10(18.2)	6(10.9)	
Normal	39(70.9)	44(80)	
Neutrophilia	6(10.9)	2(3.6)	
Lym#			
Lymphopenia	2(3.8)	1(1.9)	
Normal	53(96.2)	49(94.2)	0.120
Lymphocytosis	0(0.0)	2(3.8)	
Mon#			
Monocytopenia	1(1.9)	1(1.9)	0.400
Normal	53(96.2)	51(98.1)	
Monocytosis	1(1.9)	0(0.0)	
Eos#			
Eosinopenia	1(1.9)[a]	1(1.9)[a]	
Normal	52(94.2)[a]	49(94.2)[a]	1.000
Eosinophilia	2(3.8)[a]	2(3.8)[a]	
Bas#			
Normal	50(90.4)[a]	48(92.3)[a]	1.000
Basophilia	5(9.6)[a]	4(7.7)[a]	

Data is presented as number (percentage) values. McNemar's test was used to compare differences between month 2 and month 5, a ***P*** value of 0.05. *Statistical significance

4.5.2 Dynamic changes in red blood cells counts

Table 4.14 shows the time trend analysis for red blood cell parameters and indices at diagnosis, month 2 and month 5 of anti-tubercular treatment. Generally, the RBC counts, HGB concentration and HCT% were not statistically significantly different when compared between the three time points (P=0.093, P=0.070 and P=0.093, respectively). Notably, the RBC indices improved at month 5 (MCV (fL) 83.75, MCH (pg) 28.40, MCHC (g/dL) 33.95) when compared to values recorded at diagnosis (MCV (fL) 80.20, MCH (pg) 27.0, MCHC (g/dL) 33.80) and month 2 (MCV (fL) 78.60, MCH (pg) 26.50, MCHC (g/dL) 33.50) of anti-tubercular treatment (P<0.001, P<0.001 and P=0.007, respectively). Furthermore, at diagnosis, month 2 and month 5 of TB treatment, the RDW-CV% and RDW-SD did not record statistically significant differences (P=0.149 and P=0.081, respectively).

Table RESULTS.15: Comparison of RBC parameters and indices at diagnosis, month 2 and month 5 for PTB patients

Parameter	Baseline Median (min-max)	Month 2 Median (min-max)	Month 5 Median (min-max)	*P*= Value
Red blood cell count (10^6/uL)	4.79 (2.88-6.33)	4.58 (2.42-5.69)	4.51 (2.79-5.68)	0.093
Hemoglobin (g/dL)	12.8 (7.3-18.3)	11.6 (6.6-16.7)	13 (9.0-15.9)	0.070
Hematocrit%	37.9 (22.2-53.1)	34.7 (19.9-49.8)	37.8 (26.6-47.1)	0.093
Mean cell volume (fL)	80.2 (55.7-93.8) [a]	78.6 (56.7-92.6)[a]	83.75 (57.3-95.3) [a]	<**0.001***
Mean cell hemoglobin (pg)	27 (17.6-32.4) [a]	26.5 (18.7-31.4) [a]	28.4 (18.4-32.3) [a][a]	<**0.001***
Mean cell hemoglobin concentration (g/dL)	33.8 (30.6-35.7) [a]	33.5 (30.5-35.4) [a]	33.95 (31.3-35.1) [a][a]	**0.007***
Red cell distribution width-CV%	12.8 (11.2-18.7)	13 (11.2-18.9)	12.6 (11-17.8)	0.149
Red cell distribution width-SD	42.3 (36.3-70.7)	41.9 (33.9-57.9)	43.65 (36.8-54)	0.081

Data was analyzed using Kruskal-Wallis test presented as median (min-max) with a *P* value of <0.05. *Statistical significance. Similar superscripts and subscripts denote column proportions that are significant.

Dunn's multiple comparisons post hoc test to check for statistically significant differences in mean rank differences for parameters at diagnosis, month 2 and month 5 of PTB treatment as shown in figure 4.3. For the red blood cell count, hemoglobin, hematocrit%, red blood cell distribution width-coefficient of variation and red blood cell distribution width-standard deviation, there was no statistically significant difference in mean rank differences at diagnosis, month 2 and month 5 of anti-tubercular treatment. The RBC indices: mean cell volume, mean cell hemoglobin and mean cell hemoglobin concentration, showed no statistically significant differences in mean ranks at diagnosis vs 2 months. However, statistically significant differences in mean ranks for mean cell volume, mean cell hemoglobin and mean cell hemoglobin

concentration were shown at diagnosis vs 5 months and 2 months vs 5 months of anti-tubercular treatment.

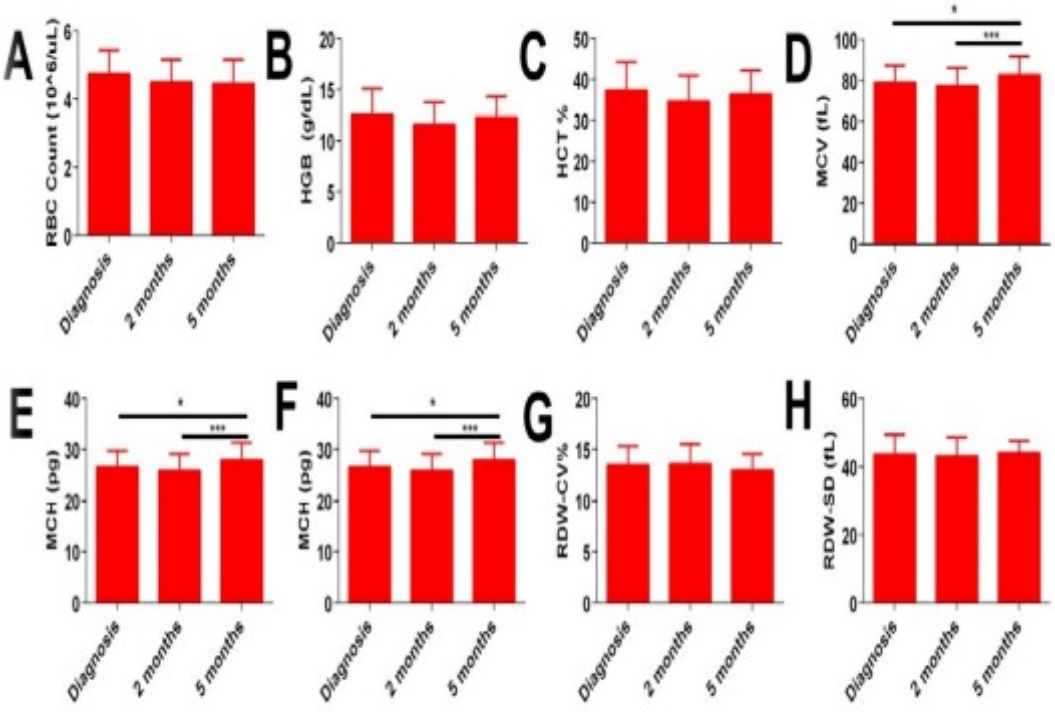

Figure RESULTS.13: Time dependent changes in red blood cell parameters from diagnosis to the fifth month of treatment.
A-Denotes no statistically significant difference in RBC counts between mean rank differences at diagnosis, month 2 and month 5. B-Denotes no statistically significant difference in HGB between mean rank differences at diagnosis, month 2 and month 5. C-Denotes no statistically significant difference in HCT% between mean rank differences at diagnosis, month 2 and month 5. D-Denotes a statistically significant difference in MCV between mean rank differences at diagnosis vs 5 months and month 2 vs month 5. E-Denotes a statistically significant difference in MCH between mean rank differences at diagnosis vs month 5 and month 2 vs month 5. F-Denotes a statistically significant difference in MCHC between mean rank differences at diagnosis vs month 5 and month 2 vs month 5. G- Denotes no statistically significant difference in RDW-CV% between mean rank differences at diagnosis, month 2 and month 5. H- Denotes no statistically significant difference in RDW-SD between mean rank differences at diagnosis, month 2 and month 5. Data was analyzed using Dunn's Multiple Comparisons tests and presented as means with a *P* value of <0.05.

4.5.2.1 Proportion of RBC counts among study participants at month 2 and 5 of anti-tubercular treatment

As shown in table 4.15, there was a steady increase in the number of patients who had a low number of RBC counts at month 2 (5.8% (n=3) and month 5 (9.6% (n=5) compared to the number at diagnosis. Interestingly, there was a steady decrease in the number of patients who had a high number RBC counts at month 2 (5.8% (n=3) and month 5 (3.8% (n=2). At month 2, 34.6% (n=18) of PTB patients presented with low hemoglobin concentration compared to 40.4% (n=21) at month 5. Statistically significant differences were noted in HCT%, MCV and MCH values at month 2 compared to month 5 (P=0.041 and P<0.001, respectively). There was a general improvement in the values for HCT%, MCV and MCH at month 5 compared to month 2. No statistically significant difference was noted in the MCHC and RDW-CV% values of patients at month 2 and month 5 (P=0.289 and P=1.000, respectively).

Table RESULTS.16: Proportions of RBC counts at month 2 and month 5 of anti-tubercular treatment among study participants

Parameter	Month 2, n (%)	Month 5, n (%)	*P* Value
RBC (10^6/uL)			
Erythrocytopenia	3(5.8) [a]	5(9.6) [a]	
Normal	49(88.5) [a]	45(86.5) [a]	0.513
Erythrocytosis	3(5.8) [a]	2(3.8) [a]	
HGB (g/dL)			
Low	18(34.6) [a]	21(40.4) [a]	0.105
Normal	35(61.5) [a]	31(56.9) [a]	
High	2(3.8) [a]	0(0.0) [a]	
HCT%			
Low	33(63.5) [a]	23(44.2) [b]	0.041*
Normal	22(36.5) [a]	29(55.8) [b]	
MCV (fL)			
Microcytic	27(51.9) [a]	9(33.3) [b]	<**0.001***
Normocytic	28(48.1) [a]	43(82.7) [b]	
MCH (pg)			
Low	29(55.8) [a]	11(21.2) [b]	<**0.001***
Normal	26(44.2) [a]	41(78.8) [b]	
MCHC (g/dL)			
Low	7(11.5) [a]	2(3.8) [a]	0.289
High	48(88.5) [a]	50(96.2) [a]	
RDW-CV%			
Normal	50(90.4) [a]	48(92.3) [a]	1.000
High	5(9.6) [a]	4(7.7) [a]	

Data is presented as number (percentage) values. McNemar's test was used to compare differences between month 2 and month 5, a ***P*** value of 0.05 was used.*Statistical significance.

4.5.3 Dynamic changes in platelet counts

Comparison of platelet counts at diagnosis, month 2 and month 5 of anti-TB treatment showed a significant reduction in platelet counts among PTB patients as shown in table 4.16 (314.0x10^3/uL vs 312.0x10^3/uL vs 232.0x10^3/uL, P<0.001). There was significant improvement in MPV (fL) and PDW (fL) values at month 5 (10.15 and 12.5, respectively) when compared to month 2 (9.2 and 10.7, respectively) of anti-tubercular treatment and at diagnosis (8.90 and 10.40), P<0.001 and P=0.002,

respectively. Additionally, the PCT% for the patients was significantly lower at month 5 when compared to values that were recorded at diagnosis and month 2 of anti-tubercular treatment (P=0.003).

Table RESULTS.17: Comparison of platelet parameters at diagnosis, month 2 and month 5 for PTB patients

Parameter	Baseline Median (min-max)	Month 2 Median (min-max)	Month 5 Median (min-max)	*P*= Value
Platelet count (10^3/uL)	314.0 (185.0-868.0)[a]	312.0 (129.0-730.0)[a]	232.0 (71.0-683.0)[a][a]	**<0.001**
MPV (fL)	8.90 (6.60-11.70)[a]	9.2 (6.6-11.1)[a]	10.15 (8.0-11.7)[a][a]	**<0.001**
PDW (fL)	10.40 (7.10-18.0)[a]	10.7 (7.0-16.0)[a]	12.5 (6.5-16.3)[a][a]	**0.002**
PCT%	0.273 (0.161-0.643)[a]	0.269 (0.143-0.649)[a]	0.235 (0.061-0.677)[a][a]	**0.003**

Data was analyzed using Kruskal-Wallis test and presented as median (min-max) with a P value of <0.05. Significant values are bolded. Similar superscripts and subscripts denote column proportions that are significant.

Dunn's multiple comparisons post hoc test to determine statistically significant differences in mean rank differences for platelet parameters at diagnosis, month 2 and month 5 of anti-tubercular treatment. The post-hoc analysis revealed a statistically significant mean rank difference for platelet count, MPV, PDW and PCT% at diagnosis vs 5 months and 2 months vs 5 months of anti-TB treatment. However, no statistically significant difference was shown for the parameters at diagnosis vs 2 months as shown in figure 4.4.

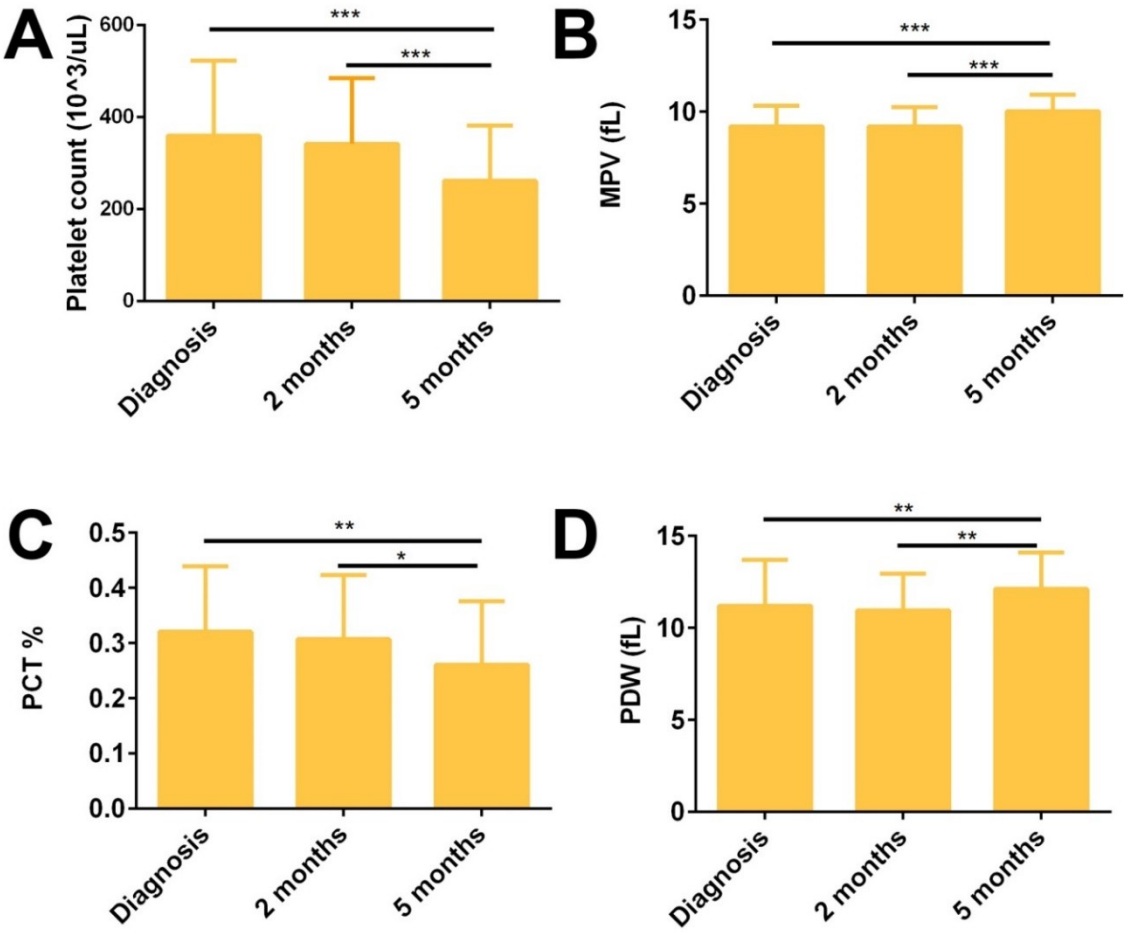

Figure RESULTS.14: Time dependent changes in red blood cell parameters from diagnosis to the fifth month of treatment.
A)-Denotes a statistically significant difference in platelet counts between mean rank differences at diagnosis vs month 5 and month 2 vs month 5. **B)-** Denotes a statistically significant difference in MPV between mean rank differences at diagnosis vs month 5 and month 2 vs month 5. **C)-** Denotes a statistically significant difference in PCT% between mean rank differences at diagnosis vs month 5 and month 2 vs month 5. **D)-** Denotes a statistically significant difference in PDW between mean rank differences at diagnosis vs month 5 and month 2 vs month 5. Data was analyzed using Dunn's Multiple Comparisons tests and presented as means with a *P* value of <0.05.

4.5.3.1 Proportion of platelet counts among study participants at month 2 and 5 anti-tubercular treatment

Thrombocytosis was noted in 48.1% (25) of patients at month 2 and 23.1% (n=12) at month 5 of treatment as shown in table 4.17. This represent a significant decrease in the number of cases that had thrombocytosis at month 2 and 5 compared to values recorded at diagnosis. Interestingly, 3.8% of patients at month 5 presented with thrombocytopenia, which did not occur at diagnosis and at month 2. There was also a significant decrease in the number of patients who had a high PCT% at month 2 (44.2% (n=23)) and month 5 (23.1% (n=12)) compared to the value recorded at diagnosis (47.3% (n=26)).

Table RESULTS.18: Proportions of platelet counts among study participants at month 2 and Month 5 of anti-tubercular treatment

Parameter	Month 2, n (%)	Month 5, n (%)	*P* Value
PLT (10^3/uL)			
Thrombocytopenia	0(0.0)	2(3.8)	0.090
Normal	30(51.9)	38(73.1)	
Thrombocytosis	25(48.1)	12(23.1)	
PDW (fL)			
Low	8(15.4) [a]	2 (3.8) [a]	0.109
Normal	47(84.6) [a]	50 (96.2) [a]	
PCT %			
Low	0(0.0)	2(3.8)	0.123
Normal	32(55.8)	38(73.1)	
High	23(44.2)	12(23.1)	
P-LCR %			
Low	1(1.9)	0(0.0)	0.401
Normal	54(98.1)	48(92.2)	
High	0(0.0)	4(7.7)	

Data is presented as number (percentage) values. McNemar's test was used to compare differences between month 2 and month 5, a ***P*** value of 0.05 was used. *Statistical significance.

4.5.4 Sputum smear conversion and treatment outcomes

4.5.5 Smear conversion post-intensive phase of anti-TB treatment

At the end of month 2, 13% of the pulmonary TB patients had smear positive results whereas 87% had smear negative results as shown in figure 4.5.

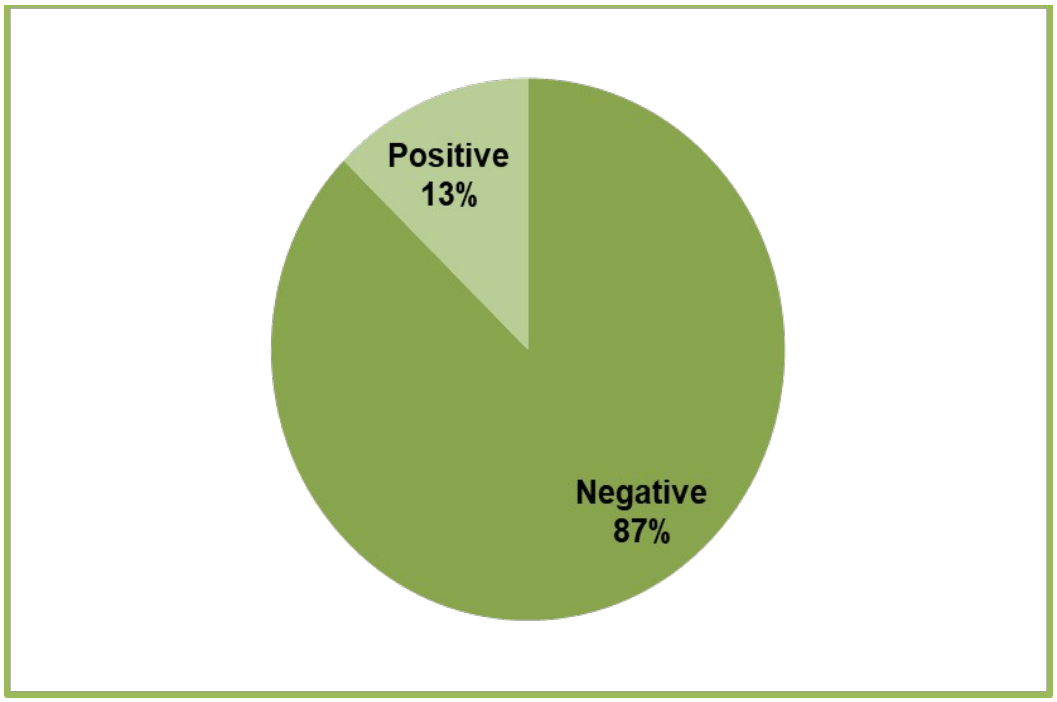

Figure RESULTS.15: Smear conversion results at month two of anti-tubercular treatment.

4.5.6 Association of hematological profile and sputum conversion at month 2

A high WBC count at month two was associated with a 2.4 times higher likelihood of a positive sputum smear post intensive phase of anti-TB treatment for pulmonary TB patients. [OR=2.42, 95% CI= 2.07-2.82, *P*<0.001] in comparison to a normal range, whereas patients with a high platelet count at month two, and lymphocyte count were found to be 1.5 and 1.19 times as likely as those with normal levels to have a positive sputum smear post-intensive phase. [OR= 1.5, 95% CI= 1.01-2.22, *P*= 0.043], and [OR=1.19, 95% CI= 1.08-1.31, *P*= 0.001], respectively. On the other hand, high levels

of neutrophil count [OR=0.44, 95% CI= 0.40-0.48, *P*<0.001], monocyte [OR=0.41, 95% CI= 0.36-0.48, *P*<0 .001], eosinophil, basophil, RBC and HGB were associated with lower likelihood of having a positive sputum smear at the completion of intensive phase of treatment in comparison to normal ranges at month two. A positive sputum smear post-intensive phase was equally likely between patients with low lymphocyte count and those in the normal range, [OR= 1.00, 95% CI= 1.00-1.00), *P*< 0.001], while a low HGB level was found to increase the odds of having a positive sputum smear at month two of anti-tubercular treatment [OR= 2.58, 95% CI= 1.68-3.95, *P*<0.001], as did low PDW [OR= 1.62; 95% CI= 1.16-2.27; *P*= 0.004]. At month two, low WBC, monocyte, RBC, MCV (fl), MCH (pg), RDW-SD (fL), and PDW (fl) were associated with lower odds of having a positive sputum smear post-intensive phase of treatment as presented in table 4.18.

Table RESULTS.19: The predictive influence of hematological changes at month 2 on sputum smear conversion post-intensive phase

Parameter	Level	OR (95% CI)	*P* value
WBC (10^3/uL)	High	2.42(2.07-2.82)	**0.001***
	Low	0.58(0.38-0.88)	**0.011***
Neutrophil count	High	0.44(0.4-0.48)	**0.001***
	Low	0.69(0.44-1.09)	0.112*
Lymphocyte count	High	1.19(1.08-1.31)	**0.001***
	Low	1(1-1)	**0.001***
Monocyte count	High	0.41(0.36-0.48)	**0.001***
	Low	0.44(0.4-0.48)	**0.000***
Eosinophil count	High	0.53(0.37-0.75)	**0.000***
Basophil count	High	0.44(0.4-0.48)	**0.000***
RBC (10^6/uL)	High	0.68(0.5-0.91)	**0.009***
	Low	0.65(0.44-0.97)	**0.033***
HGB (g/dL)	High	0.62(0.44-0.88)	**0.008***
	Low	2.58(1.68-3.95)	**0.001***
HCT%	Low	1(0.67-1.49)	0.999
Mean cell volume (fL)	Low	0.63(0.45-0.88)	**0.006***
Mean cell hemoglobin (pg)	Low	0.65(0.47-0.91)	**0.013***
Mean cell hemoglobin concentration (g/dL)	Low	0.99(0.81-1.22)	0.929
RDW-CV%	High	0.93(0.73-1.17)	0.528
RDW-SD (fL)	Low	0.59(0.41-0.84)	**0.004***
PLT (10^3/uL)	High	1.5(1.01-2.22)	**0.043***
PDW (fL)	Low	1.62(1.16-2.27)	**0.004***
PCT %	High	1.09(0.84-1.42)	0.515

Data is presented as odds ratio (95% confidence interval) values. Multivariate logistic regression was used to determine the predictive effect of hematological parameters at month two of treatment on sputum smear conversion post-intensive phase of treatment. A *P* value of 0.05 was used. *Statistical significance.

4.5.7 Treatment outcomes at the end of month sixth of anti-TB treatment

Patients were classified into well-defined treatment outcome categories following response to treatment as evidenced by sputum smear results at month five and six of treatment. In this study, 93% of PTB patients were classified as cured while 2% were lost to follow-up. Additionally, 2% were categorized as treatment failure while 3% died as shown in figure 4.6. Combining death, treatment failure and loss to follow-up, the unsuccessful treatment outcome rate was 7%.

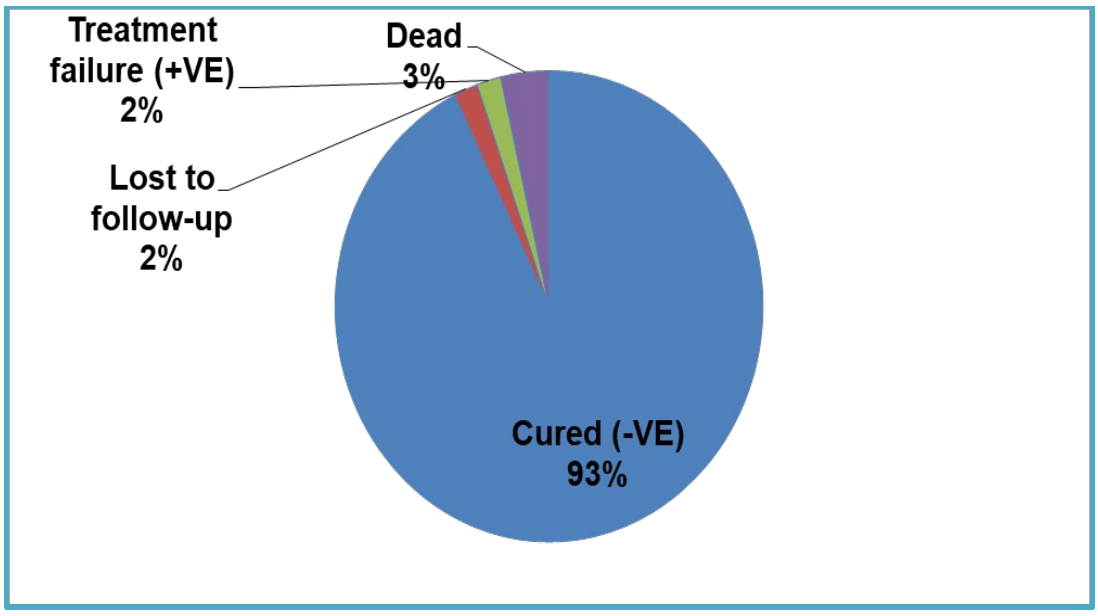

Figure RESULTS.16 Treatment outcomes at month six post treatment.
93% of patients had successful treatment outcomes whereas 7% had unsuccessful treatment outcomes (death, treatment failure, loss to follow-up)

4.5.8 Association of hematological profiles and treatment outcomes at month 6

A statistically significant association was demonstrated between normal counts for WBC, neutrophil, lymphocyte and monocyte counts with treatment outcomes (P=0.001). On the contrary, no association was shown between eosinophil and basophil counts with treatment outcomes (P=0.0606 and P=0.9482, respectively). Similarly, there was no association between RBC counts, HGB and HCT% with treatment outcomes for PTB patients (P=0.9950, P=0.1529 and P=0.1948, respectively). Interestingly, a significant association between treatment outcomes with MCV, MCH, MCHC and RDW-CV% was demonstrated (P=0.0016, P=0.0073 and P=0.0001, respectively). Normal counts for platelets showed a statistically significant association with treatment outcomes (P=0.0001) as opposed to PDW and PCT% (P=0.9818 and P=0.3590, respectively).

CHAPTER 5: DISCUSSION

5.1 Introduction

In this section, the participant's characteristics are first discussed briefly followed by in-depth discussion of hematological profiles at diagnosis, followed by morphological findings and finally the association of hematological changes and anti-TB treatment outcomes.

5.2 Participant characteristics

Several factors besides PTB influence the hematological profiles in humans. These include age, gender, nutrition, pregnancy, drugs, lifestyle and altitude among other factors. Therefore, it is important to control for most of these factors in studies of hematological profiles. In this study, the characteristics of recruited participants in the PTB and control groups were similar except for weight, which was significantly lower in the PTB group (Table 4.1).

Weight loss is a consistent clinical manifestation of PTB in newly diagnosed patients in different countries (Loddenkemper *et al.,* 2016) This unintentional weight loss has been linked to several factors including massive production of inflammatory mediators and up regulation of anorexigenic leptin upon *M. tuberculosis* infection (Zheng *et al*., 2013). However, the role of this weight loss in the PTB-associated hematological changes observed in this study and other previous studies remains unclear and should be considered in future studies. In the PTB group, there was male predominance at 80%. This is consistent with studies by Molay *et al.,* 2015 in India, Alamlih *et al.,* 2020 in Qatar, Batool *et al.,* 2022 in Pakistan and Shah *et al.,* 2022 in India.

However, a study by Abdelkareem *et al* (2019), reported more cases of PTB among women than men at 58% in Egypt. One potential reason of male predominance among PTB participants could be biased recruitment of males. However, this is unlikely to be the case in this study, because for the year 2023, 74.8% (N=232) of all the cases of diagnosed PTB at KCTRH were males (KCTRH chest clinic records). Therefore, male predominance in our study is a reflection of higher incidence of PTB among males visiting the hospital compared to females. Importantly, the controls were gender-matched to avoid confounding the results at diagnosis. One potential explanation is that the risk factors for acquisition of *M. tuberculosis* such as smoking and alcoholism are more associated with males than females.

Moreover, it could be linked to differences in health seeking behaviors between male and females living within the catchment areas of KCTRH. However, this needs further investigation in future studies. Additionally, majority of the participants were aged between 31 and 44. This was also confirmed by the laboratory data on diagnosed cases at KCTRH in 2023 where majority of the cases (121 (39.0%)) fell in the age group of

31-44 (KCTRH chest clinic record). Furthermore, this trend is in line with national epidemiological surveys and reports on PTB distribution based on age stratification. This study and previous documented reports/surveys confirm that PTB affects adults in their productive age.

5.3 Hematological profiles of PTB patients at diagnosis

The median absolute white blood cell count in the current study for PTB group was significantly higher than that of the control group (Table 4.2). This finding is similar to reports by Ongwae *et al.,* 2023, which documented leukocytosis in 15.9% of the PTB patients. The proportion of PTB patients with leukocytosis in the current study, however, is much lower when compared to other studies including, Kulkarni and Jaju, 2017 in India, Kahase *et al.,* 2020 in Ethiopia, Shah *et al.,* 2022 in India and Batool *et al.,* 2022 in Pakistan which reported leukocytosis in 38.4%, 27.5%, 53% and 46.2% of PTB patients, respectively.

Previous reports in Nigeria and India by Erhabor *et al.,* 2020 and Shah *et al.,* 2022 documented leukopenia in 3.75% and 4% of cases studied, respectively. Their findings agree with our finding of leukopenia in 3.6% of PTB patients in the current study (Table 4.3). However, the low prevalence of leukopenia at diagnosis in this study is contrary to the studies by Abbas *et al.,* 2020 and Batool *et al.,* 2022, which reported higher proportions of leukopenia among PTB patients in their study subjects at 27.94% and 20.4%, respectively. These variations in WBC counts with this study could be due to differences in the time at which patients sought care, socio demographic differences such as age, and differences in sample size.

Physiologically, the human body mounts concerted immune responses against bacteria via cells of the immune system. This always occurs through detection of bacteria by

their pattern recognition receptors followed by expansion of the different leukocyte populations. Release of cytokines from activated macrophages and T lymphocytes results in recruitment of more cells to combat the invading bacteria. Furthermore, expansion of the different white blood cell populations especially neutrophils contributes overwhelmingly to the increase in total white blood cell counts. This explains the occurrence of leukocytosis in PTB patients in the current study (Lerner *et al.,* 2015; Sia and Rengarajan, 2019).

Of note is there was no significant increase in the absolute lymphocyte population in PTB patients (Table 4.2). This could have been due to the possible early staging of PTB in our patients where phagocytic and polymorphonuclear responses predominate in the acute phase of the infection. Lymphocytes form part of the adaptive immunity which develops following robust responses from innate immunity. As a result, these adaptive lymphocyte responses in tuberculosis begin later on when *M. tuberculosis* spreads to the lymphatics (de Martino *et al.,* 2019).

The observed leukopenia in a small number of PTB patients could have occurred as a result of immunosuppression. This is because during the acute phase of the infection several cytokines including tumor necrosis factor alpha and interferon gamma are released into circulation in large amounts by activated monocytes and tissue macrophages. Upon interaction with their receptors in bone marrow, these cytokines inhibit leukopoiesis leading to decreased production of white blood cells (Lin *et al.,* 2015).

Differential white blood cells analysis revealed the presence of neutrophilia in 18.2% of PTB patients at diagnosis (Table 4.3). This proportion was lower when compared to a previous report by Shah *et al* (2022) who documented neutrophilia in 56% of PTB

patients in India. The higher proportion of neutrophilia among PTB patients can be explained by the fact that neutrophil activation and expansion is among the very first immune defense mechanism against *M. tuberculosis* (de Martino *et al.,* 2019). The recruitment of neutrophils to the site of infection usually occurs via secretion of antimicrobial molecules and inflammatory mediators (Sia and Rengarajan, 2019).

Surprisingly, a higher proportion of controls were neutropenic compared to newly diagnosed PTB patients in the current study (Table 4.3). Neutrophil numbers can decrease due to an increase in neutrophil marginalization and T-lymphocyte activities that hinder granulopoiesis. Neutropenia can also be due to viral infections, bacterial infections such as brucellosis, splenomegaly, bone marrow failure and drugs such as anti-cancer drugs (Boxer, 2012). Possibly, therefore, the occurrence of neutropenia among our controls could have been due to factors that were not captured by the Kenya Tissue and Transplant Authority donor screening questionnaire. Furthermore, blood donor controls were only screened in the laboratory for transfusion transmissible infections, and thus infections like seasonal influenza cannot be ruled out.

Besides derangement in white blood cells counts, this study also revealed significantly deranged RBC parameters among PTB patients at diagnosis (Figure 4.1 and Table 4.4). Key among this was anemia, which was recorded in a proportion of 50.8% (Figure 4.1) of PTB patients, a finding comparable to that documented by Abay *et al* (2018) in Ethiopia. Several previous studies have reported a much higher prevalence of anemia in newly diagnosed PTB patients. Shah *et al* (2022) reported anemia in all the seventy cases of PTB that were studied with the predominant type being mild anemia at 50% of the cases.

Similarly, studies by Kulkarni and Jaju (2017) in India reported anemia among 69.2% of PTB patients whereas Mulenga *et al* (2017) in Democratic republic of Congo documented anemia prevalence of 69% among PTB patients. Mukherjee *et al* (2019) in India in their study, documented anemia at 72.7% while Erhabor *et al* (2020) in Nigeria recorded 88.7% of PTB cases being anemic. Furthermore, a study by Batool *et al* (2022) in Pakistan reported a higher proportion of anemia among PTB patients at 82.6%. The predominant type of anemia that was reported in these studies was mild anemia.

In another Ethiopian study by Kahase *et al* (2020) anemia was documented in 25% of PTB cases studied, a proportion lower than that reported in the current study. In addition to low HGB levels, PTB patients also exhibited lower HCT%, MCV and MCH at diagnosis (Table 4.2). This confirms the findings of several previous studies in Democratic republic of Congo, Ethiopia and Pakistan (Mulenga *et al.,* 2017; Kahase *et al.,* 2020; Batool *et al.,* 2022).

The differences in red blood cell parameters and indices reported in our study compared to other studies could be attributed to differences in patient genetics, geographical location, nutritional status and time at which healthcare was sought by different patients. Occurrence of anemia in PTB patients has been correlated with cytokine release during the acute phase of the infection. Inflammatory mediators such as interleukin-6, tumor necrosis factor alpha and interferon gamma have been demonstrated in prior studies as inhibitors of erythropoietin formation in PTB patients (Shah *et al.,* 2022). Consequently, a decrease in erythropoietin production leads to inhibition of erythropoiesis and bone marrow suppression. This results in low red

blood cell production reducing the number of circulating red blood cells (Shah *et al.,* 2022).

Additionally, other studies have demonstrated that one of the survival mechanisms for *M. tuberculosis* is the utilization of heme as an iron source. This has been suggested to lead to decrease in hemoglobin levels in infected persons hence development of anemia among PTB patients (Kahase *et al.,* 2020). Furthermore, hepcidin synthesis induction by activated macrophages and liver cells during the acute phase of PTB has also been demonstrated by prior studies (Minardi *et al.,* 2021). Hepcidin is a key regulator of iron absorption from the gastrointestinal tract and therefore its increased production in PTB decreases levels of available serum iron.

Consequently, decreased levels of serum iron lead to decreased iron utilization and bone marrow impairment leading to anemia among persons infected with *M. tuberculosis* (Minardi *et al.,* 2021). The high prevalence of anemia among PTB patients in this study and other several studies call for screening of anemia among PTB cases at diagnosis to tailor patient-specific treatment approaches.

The current study documented thrombocytosis in 50.9% of the PTB patients with no case of thrombocytopenia at diagnosis (Table 4.2). In comparison, a study in India by Shah *et al* (2022), reported thrombocytosis in 70% of the cases while 5% presented with thrombocytopenia. Similarly, Rathod *et al* (2017) in India and Kahase *et al* (2020) in Ethiopia reported higher proportions of thrombocytosis among cases at 75% and 65%, respectively. However, a study by Batool *et al* (2022) in Pakistan reported a lower proportion of thrombocytosis in 26.2% of the PTB patients while 10.6% presented with thrombocytopenia. These findings altogether, point out to

thrombocytosis as the more consistent platelet abnormality finding in PTB patients at diagnosis.

However, Rohini *et al* (2015) in a study they conducted in India reported thrombocytopenia as the predominant finding among cases studied as opposed to thrombocytosis which was the predominant finding in most of the previous reports and the current study. Differences in platelet counts could be due to differences in patient populations and staging of the disease at diagnosis. Increase in platelet counts in pulmonary tuberculosis patients has been postulated to be due to interleukin-6 which is released during the acute phase of the infection by prior studies (Abbas *et al.,* 2020; Kahase *et al.,* 2020).

Interleukin-6 is reportedly involved in granuloma formation and is known to promote megakaryopoiesis consequently leading to elevated numbers of platelets (Abbas *et al.,* 2020; Kahase *et al.,* 2020). More recent studies have implicated platelets as contributors to the inflammatory and immune responses in pulmonary tuberculosis infection. A study by Kirwan *et al* (2021) demonstrated upregulation of specific platelet gene transcripts in PTB patients. Changes in platelet structure and function have also been described in most patients with active pulmonary tuberculosis infection. Furthermore, mean platelet volume has been suggested as an important inflammatory marker in some chronic inflammatory conditions like tuberculosis by previous studies. Lee *et al* (2016) hypothesized that mean platelet volume can be used as a marker to determine course of pulmonary tuberculosis disease in infected patients.

5.4 Blood morphological characteristics of newly diagnosed pulmonary tuberculosis patients

The results of this study show altered morphological characteristics in a substantial number of PTB patients at diagnosis (Table 4.6). This corroborates previous observations among PTB patients in other countries. For example, studies by Abdelkareem *et al* (2019) in Egypt and Shah *et al* (2022) in India reported microcytic hypochromic anemia in 55% and 52%, respectively, which is higher than 38.2% reported in this study.

Lower proportions of microcytic hypochromic anemia in PTB patients were reported by Molay *et al* (2015) in India and Ongwae *et al* (2023) in Kenya at 29.67% and 20%, respectively. The variation in the number of patients presenting with microcytic hypochromic anemia may be due to differences in patient populations, staging of the disease and patient nutritional status.

Further, the current study documented normocytic normochromic anemia in 54.5% of the studied PTB subjects. This finding is comparable to that reported by Ongwae *et al* (2023) at 55% of cases that had normocytic normochromic anemia in their study. On the contrary, Abdelkareem *et al* (2019) and Shah *et al* (2022) reported lower proportions for normocytic normochromic anemia than that documented in this study at 37% and 40%, respectively.

Additionally, 3.6% of PTB subjects in the current study had poikilocytosis with the predominant finding being target cells. These marked variations could be due to differences in patient populations, staging of the disease and patient nutritional status. Elucidation of the exact mechanism leading to the observed abnormal red cell morphologic changes was beyond the scope of the current study. Nevertheless,

infection with *Mycobacterium tuberculosis* leads to release of several inflammatory mediators that can negatively impact on erythropoiesis (Shah *et al.*, 2022).

Furthermore, infection with *Mycobacterium tuberculosis* interferes with iron absorption and metabolism (Minardi *et al.*, 2021). Consequently these might ultimately lead to altered red cell morphology including microcytes and hypochromia. Occurrence of target cells in PTB is thought to be an accompanying effect of iron deficiency in infected subjects (Adewoyin and Nwogoh, 2014).

The study also revealed presence of bands forms of neutrophils, toxic granulation and neutrophilic leukocytosis among PTB patients at diagnosis (Table 4.8). This extends the findings of several previous studies indicating that PTB is associated with white blood cell morphological abnormalities. Ongwae *et al* (2023), reported band forms of neutrophils in 25% of PTB patients, which is higher than the findings of this study. Moreover, Ongwae *et al* (2023) reported a much higher proportion of 65% for toxic granulation among study participants than that documented by this study.

Majority of the patients in this study had neutrophilia as the predominant white blood cell anomaly morphologically. This is consistent with the results of the automated full WBC counts. Notably increase in neutrophils in PTB patients is associated with the inflammatory response and bacillary load (Muefong and Sutherland, 2020). Invasion by *M. tuberculosis* triggers release of several potent inflammatory mediators (TNF-α; IL-18) that activate rapid expansion of the neutrophils (Domingo-Gonzalez *et al.*, 2016). This explains the high proportion of PTB patients presenting with neutrophilia on peripheral blood film.

The platelet morphology was relatively less affected as demonstrated by the finding that 80.0% of PTB patients had normal estimates for platelets (Table 4.10).

Nevertheless, 20% of the PTB participants had thrombocytosis. Thrombocytosis in PTB have been postulated to be due to the inflammatory cytokines particularly interleukin-6 (Abbas *et al.,* 2020). Interleukin-6 is a known promoter of megakaryopoiesis which leads to increase in platelet counts.

Importantly, platelets act as active drivers of immune response, as demonstrated by upregulation of gene transcripts of immune mediators in PTB patients (Kirwan *et al.,* 2021).

5.5 Hematological changes during anti-tubercular treatment and influence on treatment outcomes in pulmonary tuberculosis patients

Prospective follow-up of PTB patients on treatment revealed significant time-dependent changes in hematological profiles. Statistically significant decreases were noted in WBC counts, neutrophil counts and platelet counts, with significant improvements in RBC indices (Tables 4.12, 4.14, 4.16 and figures 4.2, 4.3 and 4.4). Similar findings have been reported by other studies in various countries.

In Ethiopia, for example, Kassa *et al* (2016) determined the hematological profiles of PTB patients at diagnosis and after the 2 months intensive phase of treatment. Their study reported a significant reduction in WBC counts post intensive phase compared to baseline. Similarly, total WBC reductions were reported between baseline and week 8 of anti-TB treatment in Nigeria (Bonsome (2017) and India (Yadav *et al* (2022).

The decrease in total WBC observed the current and previous similar studies can be attributed to reduction in bacillary load occasioned by response to anti-tubercular treatment as evidenced by sputum smear conversion at month 2 and month 5. Reduction in bacillary load possibly reduces the amount of inflammatory cytokines

liberated by the cells of the immune system thereby reducing the number of immune cells released into circulation.

Additionally, granulocytes are largely responsible for the increase in WBC counts in PTB patients. Therefore, response to treatment with consequent decrease in granulocyte populations ultimately translated to the observed lower WBC counts at month 2 and 5 of anti-TB treatment. In particular, there was a significant reduction in neutrophil counts at month 2 and month 5 of anti-tubercular treatment compared to diagnosis in the current study (Table 4.12 and figure 4.2). This finding agrees with a report by Obeagu *et al* (2019) and Yadav *et al* (2022) who documented a significant decrease in neutrophil counts at the different time points in their studies.

However, the finding is inconsistent with that documented by Kassa *et al* (2016) who reported an increase in neutrophil counts post-intensive phase. This disagreement on neutrophil dynamics could be due to differences in bacillary loads in study patients. Obeagu *et al* (2019) further reported a significant decrease in monocyte and basophil counts at month 6 compared to baseline values with no statistically significant difference reported in eosinophil counts.

These findings contradict this study findings, showing non-significant changes in monocyte, eosinophil and basophil counts at diagnosis, month 2 and month 5 of anti-TB treatment. Analysis of red blood cells parameters revealed significant time-dependent changes in MCV, MCH and MCHC values, but not RBC counts, HGB and HCT %. Specifically, the MCV, MCH and MCHC values were higher at month 5 when compared to values recorded at month 2 and at diagnosis (Table 4.14 and Figure 4.3). The gradual increase in MCV, MCH and MCHC can be used as potential markers reflecting response to treatment (Shafee *et al.,* 2014)

A study in Nigeria by Obeagu *et al* (2019) showed significant increase in MCHC alone through the different time points with no statistically significant difference in MCV and MCH values at baseline, month 2, month 4 and month 6 of treatment. Contrary to our finding of improvement in MCV, MCH and MCHC among patients, Bonsome (2017) reported reductions in MCV, MCH and MCHC values for patients at the various time points of treatment. Similarly, Kassa *et al* (2016) in Ethiopia reported reductions in MCH and MCHC with no significant changes in MCV at baseline and post-intensive phase. Improvement in these RBC indices can be due to anti-TB - mediated clearance of *M. tuberculosis* bacillary load as evidenced by negative sputum smear results at month 2 and month 5 among majority of the patients.

Clearance of tubercle bacilli by anti-tubercular treatment possibly leads to reduction in inflammatory cytokines produced during the acute phase of the disease. This would translate to less inhibition of erythropoietin with improved erythropoiesis in patients undergoing treatment. However, it is important to note that anemia persisted in 34.6% and 40.4% of the patients at month 2 and 5 of treatment, respectively. These findings are comparable to those that were reported by Bonsome, 2017 in Nigeria, Yadav *et al.,* 2022 in India and Reta *et al.,* 2023 in Ethiopia

The cause of this persistent anemia despite sputum smear conversion should be investigated, since this can inform adjunctive therapeutic strategies. In some studies, anti-TB drugs has been associated with hematological syndromes such as hemolytic anemia, megaloblastic anemia, methemoglobinemia, sideroblastic anemia and red blood cell aplasia (Kassa *et al* (2016). Therefore, a putative role of anti-TB drugs in persistence of anemia in a substantive number of patients observed in this study cannot be ruled out.

There was a significant decrease in platelet counts and consequently the proportion of patients with thrombocytosis at month 5 compared to values recorded at month 2 and at diagnosis (Table 4.17). This was accompanied by significant improvement in MPV and PDW values. This is consistent with a finding reported in Ethiopia by Kassa *et al* (2016), which documented a decrease in platelet values post-intensive phase compared to baseline values. The finding is also in agreement with findings that were documented in studies conducted in Nigeria, India and Ethiopia by Bonsome (2017), Yadav *et al* (2022) and Reta *et al* (2023), respectively.

Significant reduction in proportions of patients who had thrombocytosis can be attributed clearance of *M. tuberculosis* by anti-tubercular drugs. This might have led to reduction in the production of interleukin-6, which has been implicated by prior studies to promote megakaryopoiesis (Kahase *et al.,* 2020; Kirwan *et al.,* 2021). Additionally, reduction in platelet counts among PTB patients has also been attributed to anti-TB drugs mediated immune destruction of platelets (Visentin and Liu, 2007; Aster and Bougie, 2007; Yakar *et al.,* 2013).

Rifampicin for example has been shown to non-covalently bind to membrane glycoproteins on platelets producing compound epitopes that induce conformational changes where antibodies can bind. Platelets with bound antibodies are then made susceptible to destruction by macrophages (Yakar *et al.,* 2013). Altogether the dynamic changes of various hematological parameters observed in this study and by others, warrant consideration of monitoring hematological profiles of PTB patients at diagnosis and during the course of treatment.

5.5.1 Association of hematological changes with anti-TB treatment outcomes

In our study, high absolute white blood cell counts at the end of the second month of treatment were significantly associated with having a positive sputum smear post-intensive phase. An increase in white blood cells in pulmonary TB patients usually denotes a physiological response to invading *M. tuberculosis.* Therefore, the persistently high numbers of white blood cells even after two months of treatment could be a key indicator of residual bacilli.

Similar findings were observed by Ndlovu *et al.,* 2020 in South Africa who documented the prognostic significance of neutrophils and CD15 expression. Ndlovu and colleagues found that CD15 expression level was predictive of culture positivity at the second month of treatment for PTB patients, suggesting its potential value in triaging of subjects into those likely to respond well to treatment. Similarly, our finding on high absolute WBC counts at the end of month 2 of treatment for PTB patients can be used as a monitor for response to treatment.

Patients with a low lymphocyte count at month two of treatment were also more likely to have a positive sputum smear post-intensive phase. This finding in our study is comparable to that reported by Nagu *et al* (2014) in Tanzania. According to the Tanzanian study, low lymphocyte count was demonstrated to occur in patients with severe forms of TB, which partly explains delays in conversion. Additionally, lymphocytes are known to execute effector immune functions, therefore, a decrease in their numbers would potentially sustain high bacterial loads leading to delays in sputum conversion at the end of second month of treatment.

Our study also demonstrated a significant association between low hemoglobin values (anemia) at the end of month two of treatment with sputum smear conversion.

Pulmonary TB patients with anemia have a 2 times likelihood of sputum non-conversion post-intensive phase of treatment. This finding agrees to that documented by Nagu and colleagues in Tanzania. However, actual mechanisms explaining how anemia is associated with delayed sputum conversion have not yet been fully understood.

Nagu *et al* postulated the involvement of iron deficiency in pulmonary TB patients as one of the mechanisms that would explain delayed sputum conversion. Iron deficiency among PTB patients impairs host T-cell immunity through interference with effector cell activity (Weiss, 2002; Weiss, 2009). Additionally, PTB patients with low ferritin levels have been predicted to have treatment failure at the end of month one of anti-TB treatment (Isanaka *et al.,* 2012).

Physiologically, *M. tuberculosis* induces a massive 'cytokine storm' that affects several hematological processes. Persistent anemia in patients can be due to this inflammatory response that impairs erythropoiesis. The association of anemia with delayed sputum conversion in the current and previous studies suggest need for managing anemia in PTB patients.

Having high platelet counts at month two of treatment was demonstrated to increase the odds of having a positive sputum smear post-intensive phase by 1.5 times. Platelets have been demonstrated to significantly upregulate production of monocyte matrix metalloproteinase-1(MMP-1), a tissue collagenase, known to cause lung cavitation in PTB patients (Fox *et al.,* 2018). Extensive cavitation has been linked to continuous and enhanced survivability of *M. tuberculosis* in infected individuals.

A study by Fox *et al* (2018) demonstrated that platelets alter the pro-inflammatory response in PTB patients consequently leading to increased tissue destruction with

development of an interleukin (IL)-1β, interleukin (IL)-10 high and interleukin (IL)-12 low milieu associated with increased pathogen growth. Therefore, high platelets counts favor *M. tuberculosis* survivability and proliferation, which accounts for the observed negative association of thrombocytosis with anti-TB treatment outcomes.

Our evaluation of hematological changes with treatment outcomes revealed a significant association between treatment outcomes with normal counts for WBC, neutrophil, lymphocyte and monocyte counts. A reduction in the numbers of circulating WBCs especially neutrophils, translates to a decrease in the amounts of inflammatory mediators secreted. Consequently, this decreases the associated damages caused by inflammatory mediators improving on outcomes.

Similarly, normal values for MCV, MCH, MCHC and RDW-CV% were strongly associated with desirable treatment outcomes in PTB patients. Improvement in these RBC indices could have potentially lessened severity of anemia occurring among PTB cases. Less severe forms of anemia have been postulated to improve outcomes in PTB patients (Nagu *et al.,* 2014).

Lastly, a significant association between platelet counts with treatment outcomes was observed. Normal counts of platelets have been shown as good predictors of successful outcomes as opposed to sustained high platelet counts (thrombocytosis). High counts enhance the inflammatory state and further worsen lung physiology (Fox *et al.,* 2018) with associated unsuccessful clinical outcomes (treatment failure/death).

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

i. Newly diagnosed pulmonary tuberculosis patients at KCTRH present with various hematological derangements in their profiles like anemia, thrombocytosis, leukocytosis, neutrophilia and low HCT% at diagnosis.

ii. Newly diagnosed pulmonary tuberculosis patients at KCTRH present with various blood cell morphological abnormalities at diagnosis like neutrophilic leukocytosis, left shift in neutrophils, toxic granulation, anisocytosis, poikilocytosis and microcytic hypochromic anemia at diagnosis.

iii. Hematological parameters in the profiles of PTB patients undergo dynamic changes during treatment including improvements in RBC indices, decrease in WBC and platelet counts. These changes have a significant influence on sputum smear conversion at month two and significant

association with treatment outcomes at month six in newly diagnosed PTB patients undergoing anti-tubercular treatment.

6.2 Recommendations

6.2.1 Recommendations for action

i. Hematological profiles of newly diagnosed PTB should be screened at diagnosis before commencement of anti-tubercular treatment. Screening for hematological disorders in their profiles will be critical in ensuring that patients with hematological aberrations are given tailored treatment both for PTB and hematological disorders.

ii. A simple peripheral blood film examination should be made mandatory as part of the panel tests that are offered to newly diagnosed pulmonary tuberculosis patients at diagnosis. Examination of such films will be invaluable in making early detection of abnormalities in patient blood pictures for tailored management.

iii. During treatment, PTB patients should be monitored with respect to hematological changes in their profiles. This would help detect abnormal hematological changes for proper management of the patient. Additionally, detecting such changes would enable the clinicians adjust patient treatment approaches accordingly.

6.2.2 Recommendations for future research

Future studies need to explore transcriptome expression in PTB patients. Similarly, the role of platelets in PTB pathogenesis and inflammation needs to be investigated further. The mechanisms by which anemia is associated with a likelihood of sputum non conversion post-intensive phase of treatment needs to be explored further.

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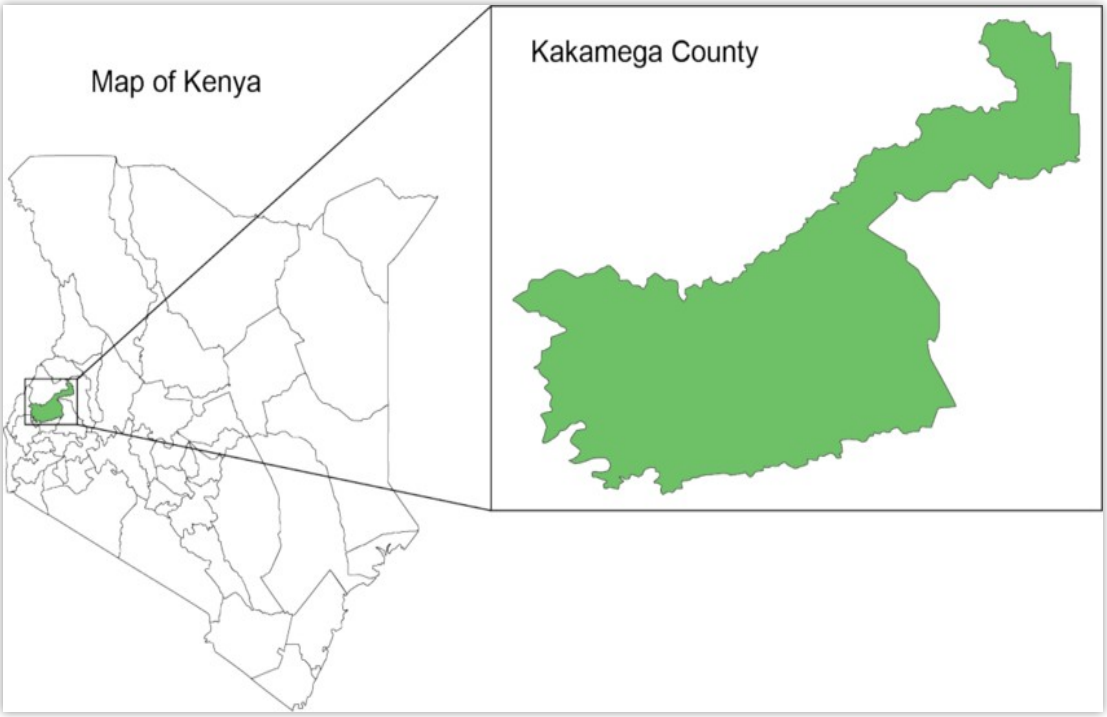
APPENDICES

APPENDIX I : Study Area



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APPENDIX II: Informed consent form (PTB patients)

EVALUATION OF HEMATOLOGICAL CHANGES AND THEIR EFFECT ON TREATMENT OUTCOMES IN NEWLY DIAGNOSED PULMONARY TUBERCULOSIS PATIENTS

Dear participant,

My name is …… ……..and this is a consent form, which provides information that you need to understand this study. You are free to ask questions and to freely accept or decline to participate in this study.

Jina langu ni……..na hii ni fomu ya kukubali, inayokupa habari unayohitaji kuhusu utafiti nitakaofanya. Kama una maswali, uko na haki ya kuuliza na pia haki ya kukataa kushiriki utafiti huu.

What you need to know?

Pulmonary tuberculosis is a disease caused by the bacterium called *Mycobacterium tuberculosis*. The disease can be spread from one person to another through infectious droplet nuclei generated through sneezing, coughing or talking. The bacterium causes significant alterations in normal body processes.

Kifua kikuu ni ugonjwa unaosababishwa na bakteria inayoitwa Mycobacterium tuberculosis. Ugonjwa huu unaweza kuambukizwa kutoka mtu mmoja hadi mwingine kupitia kupiga chafya, kukohoa au kuongea. Bakteria hii huadhiri utendakazi wa viungo kwa mwili.

Study purpose

The purpose of this study is to document any hematological changes associated with Mycobacterium tuberculosis infection and how treatment of the same can be used to augment anti-tubercular treatment for patients.

Lengo la utafiti huu ni kuchunguza mabadiliko ya damu ambayo yanahusiana na maambukizi ya Mycobacterium tuberculosis na jinsi ambavyo mabadiliko haya yanaweza kushughulikiwa ili kuboresha matibabu ya wagonjwa.

What I will do

To get this information, I will be collecting blood samples at respective intervals of treatment during the six months course period.

Kupata ujumbe huu, nitatoa damu muda baada ya muda wakati wa matibabu katika muda wa miezi sita.

What are the Potential Benefits?

The direct benefit from this study is that patients on care will be able to receive optimized and individually tailored treatment interventions as informed by results of hematological analysis and sputum smear conversion.

Utafiti huu utasaidia wagonjwa kupata matibabu yaliyo boreshwa kama vile tutakavyo arifiwa na matokea ya uchunguzi wa damu na kikohozi.

What are the risks? There are No foreseen risks in this study.

Utafiti huu hauna madhara yeyote

Privacy, confidentiality and right to participate/Decline

I will protect your privacy, no name or any other personal identifier will be included anywhere during the data collection period, on reports or publication. Participant data will be serialized throughout the study

Majina au habari nyingine yeyote kuhusu mgonjwa haitatumika mahali popote. Ila, nambari maalum kuwatambulisha wahusika katika utafiti itatumika. Uko na haki ya kubali au kukataa kushiriki.

<table>
<tr><td rowspan="2">The above has been explained to me and I agree to take part in the study. I understand that I am free to participate in this study and that declining will have No consequences for me.
Nimeelezwa masuala ya utafiti huu na nakubali kushirika. Niko huru kushiriki au kutoshiriki bila matatizo yeyote.</td><td colspan="2">If you agree to participate, tick “YES,” if you do Not agree, tick ‘NO’.
Kama unakubali kushiriki, weka alama kwa “NDIO”. Kama unakataa, weka alama
kwa “HAPANA”.</td></tr>
<tr><td>YES:
NDIO:</td><td>NO:
HAPANA:</td></tr>
</table>

APPENDIX III : **Informed consent form (Donors)**

EVALUATION OF HEMATOLOGICAL CHANGES AND THEIR EFFECT ON TREATMENT OUTCOMES IN NEWLY DIAGNOSED PULMONARY TUBERCULOSIS PATIENTS

Dear participant,

My name is …… ……..and this is a consent form, which provides information that you need to understand this study. You are free to ask questions and to freely accept or decline to participate in this study

Jina langu ni……..na hii ni fomu ya kukubali, inayokupa habari unayohitaji kuhusu utafiti nitakaofanya. Kama una maswali, uko na haki ya kuuliza na pia haki ya kukataa kushiriki utafiti huu.

Study purpose

The purpose of this study is to establish whether Mycobacterium tuberculosis modulates the process of hematopoiesis. The researcher will be comparing the hematological parameters of people who qualify as blood donors with those of newly diagnosed HIV negative pulmonary tuberculosis positive patients. For purposes of this study, blood donors are included to serve as a comparator group for the HIV negative pulmonary tuberculosis group.

Lengo la utafiti huu ni kuchunguza kama bakteria inayosababisha kifua kikuu ina madhara kuhusu utengenezaji wa damu kwa mwili. Matokeo ya uchunguzi yatalinganishwa kati ya kundi la kutoa damu na kundi litakalo kuwa na kifua kikuu.

What I will do

I will collect blood and run the routine hematology tests, viz: complete blood count and preparation of a peripheral blood film. The results will be sorted, organized and

analyzed for comparison with results that will be obtained from the HIV negative pulmonary tuberculosis positive group.

Tutachukua damu kufanya vipimo vya kuangalia hali ya damu. Matokeo ya uchunguzi huu yatalinganishwa na matokea ya wale walio na kifua kikuu

What are the Potential Benefits?

Participants will get the benefit of having complete blood counts and peripheral films done. This will inform them about their overall blood picture and any anomalies therein.

Wahusika watapata kufanyiwa vipimo ambavyo vitapeana ujumbe kuhusu hali ya damu yao na kama kuna shida yeyote.

What are the risks? There are No foreseen risks in this study.

Utafiti huu hauna madhara yeyote

Privacy, confidentiality and right to participate/Decline

I will protect your privacy, no name or any other personal identifier will be included anywhere during the data collection period, on reports or publication. Participant data will be serialized throughout the study. You have the right to either accept to decline to participate in the study.

Majina au habari nyingine yeyote kuhusu mgonjwa haitatumika mahali popote. Ila, nambari maalum kuwatambulisha wahusika katika utafiti itatumika. Uko na haki ya kubali au kukataa kushiriki.

The above has been explained to me and I agree to take part in the study. I understand that I am free to participate in this study and that declining will have	If you agree to participate, tick “YES,” if you do Not agree, tick ‘NO’.

<table>
<tr><td rowspan="2">No consequences for me.
Nimeelezwa masuala ya utafiti huu na nakubali kushirika. Niko huru kushiriki au kutoshiriki bila matatizo yeyote.</td><td colspan="2">Kama unakubali kushiriki, weka alama kwa “NDIO”. Kama unakataa, weka alama kwa “HAPANA”.</td></tr>
<tr><td>YES:
NDIO:</td><td>NO:
HAPANA:</td></tr>
</table>

APPENDIX IV : **Proposal approval**

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY (MMUST)

Tel: 056-30870
Fax: 056-30153
E-mail: directordps@mmust.ac.ke
Website: www.mmust.ac.ke

P.O Box 190
Kakamega – 50100
Kenya

Directorate of Postgraduate Studies

Ref: MMU/COR: 509099

13th January 2023

Edwin Mwilitsa
HML/G/01-70108/2021,
P.O. Box 190-50100,
KAKAMEGA.

Dear Mr. Mwilitsa,

RE: APPROVAL OF PROPOSAL

I am pleased to inform you that the Directorate of Postgraduate Studies has considered and approved your Masters Proposal entitled: "***Evaluation of Hematological Changes and Their Effects on Treatement Outcomes in Newly Diagnosed Pulmonary Tuberculosis Patients)***" and appointed the following as supervisors:

1. Dr. Raballah Evans - MMUST
2. Dr. Sammy Kimoloi - MMUST

You are required to submit through your supervisor(s) progress reports every three months to the Director Postgraduate Studies. Such reports should be copied to the following: Chairman, School of Public Health, Biomedical Sciences and Technology Graduate Studies Committee and Chairman, Medical Laboratory Sciences Department. Kindly adhere to research ethics consideration in conducting research

It is the policy and regulations of the University that you observe a deadline of three years from the date of registration to complete your Master's thesis. Do not hesitate to consult this office in case of any problem encountered in the course of your work.

We wish you the best in your research and hope the study will make original contribution to knowledge.

Yours Sincerely,

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY
DIRECTORATE OF POSTGRADUATE STUDIES
P.O. BOX 190 - 50100, KAKAMEGA

Prof. Stephen O. Odebero, PhD, FIEEP
DIRECTOR, DIRECTORATE OF POSTGRADUATE STUDIES

APPENDIX V : Masinde Muliro university ethical approval

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY

Tel: 056-31375
Fax: 056-30153
E-mail: ierc@mmust.ac.ke
Website: www.mmust.ac.ke

P. O. Box 190,
50100.
Kakamega,
KENYA

Institutional Scientific and Ethics Review Committee (ISERC)

REF: MMU/COR: 403012 Vol 6 (01)

Date: February 17th, 2023

To: Edwin Mwilitsa

Dear Mr.,

RE: EVALUATION OF HEMATOLOGICAL CHANGES AND THEIR EFFECT ON TREATMENT OUTCOMES IN NEWLY DIAGNOSED PULMONARY TUBERCULOSIS PATIENTS

This is to inform you that the ***Masinde Muliro University of Science and Technology Institutional Scientific and Ethics Review Committee (MMUST-ISERC)*** has reviewed and approved your above research proposal. Your application approval number is **MMUST/IERC/120/2023.** The approval covers for the period ***February 17th, 2023 to February 17th, 2024.***

This approval is subject to compliance with the following requirements;

i. Only approved documents including informed consents, study instruments, MTA will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***MMUST-ISERC.***
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***MMUST-ISERC*** within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to ***MMUST-ISERC*** within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to ***MMUST-ISERC.***

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed

Yours Sincerely,

Prof. Gordon Nguka (PhD)
Chairperson, Institutional Scientific and Ethics Review Committee

Copy to:
- The Secretary, National Bio-Ethics Committee
- Vice Chancellor
- DVC (PR&I)

APPENDIX VI : Nacosti research permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **462488**

Date of Issue: **16/March/2023**

RESEARCH LICENSE

This is to Certify that Mr.. EDWIN MWILITSA of Masinde Muliro University of Science and Technology, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Kakamega on the topic: Evaluation of hematological changes and their effect on treatment outcomes in newly diagnosed pulmonary tuberculosis patients for the period ending : 16/March/2024.

License No: **NACOSTI/P/23/23989**

462488

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

See overleaf for conditions

APPENDIX VII: Kakamega County ethical approval

COUNTY GOVERNMENT OF KAKAMEGA

E-mail: wpgh15@yahoo.com
Telephone: Kakamega 0702930346
When replying, please quote:

REF: CGH/KAK/ERC/VOL.I/198

COUNTY GENERAL HOSPITAL
P.O. Box 15-G.P.O-50100
KAKAMEGA

DATE: 24th March 2023

MINISTRY OF HEALTH SERVICES

EDWIN MWILITSA,
REF: NACOSTI/P/23/23989

RE: RESEARCH AUTHORIZATION FOR DATA COLLECTION – NO. ERC/190-03/2023

This is to inform you that Kakamega County General Hospital Ethics Review Committee (KCGH ERC) acting on behalf of the Kakamega County Department of Health, has reviewed and authorized your data collection for the protocol titled: ***"Evaluation of Haematological Changes and their Effects on Treatment Outcomes in Newly Diagnosed Pulmonary Tuberculosis Patients."*** The approval period shall apply until 16th March 2024.

This authorization is subject to compliance with the following requirements:

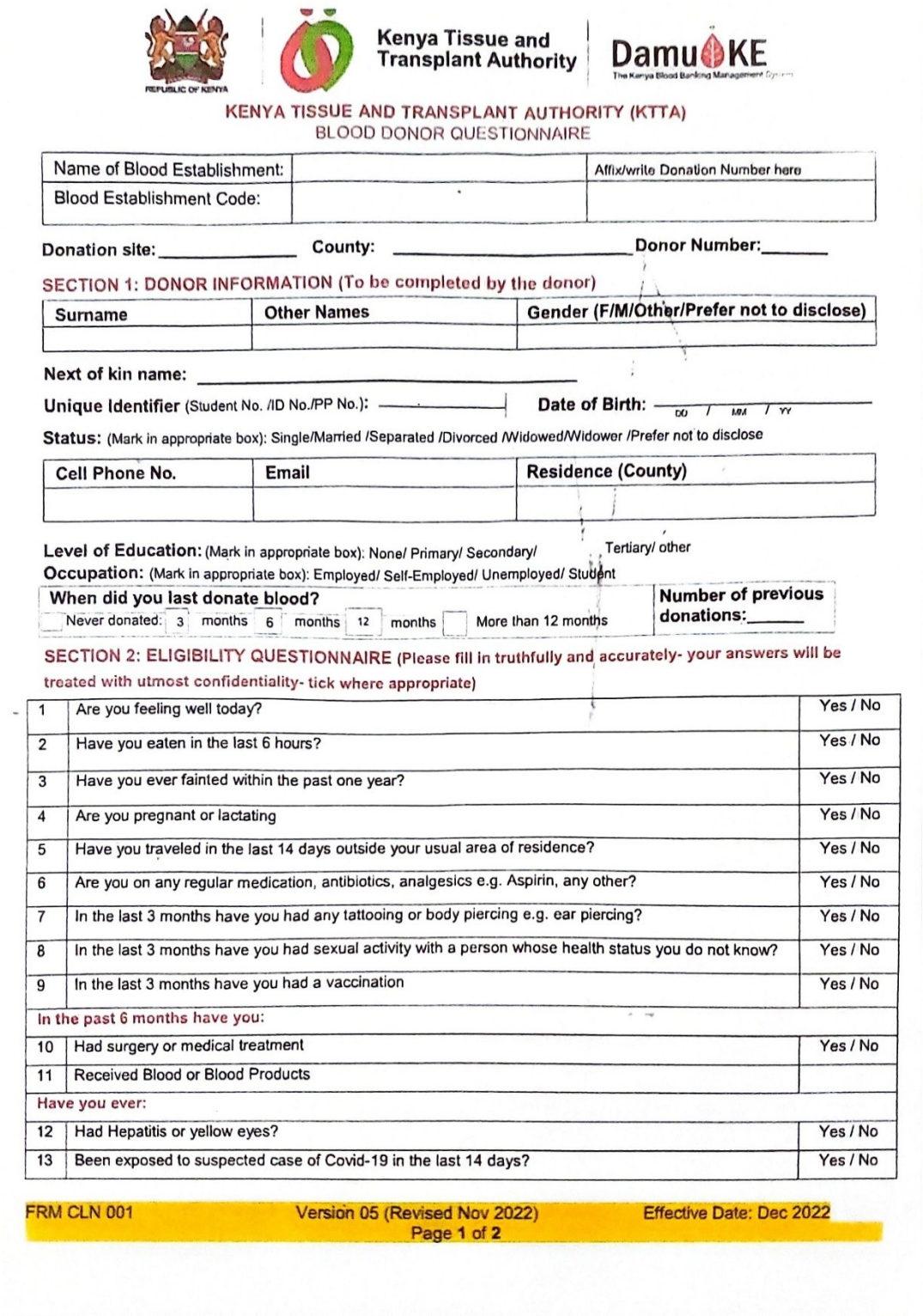
i. Only approved documents including informed consent, study instruments, will be used.
ii. All changes including amendments, deviations and violations are submitted for review and approval by the **KCGH ERC.**
iii. Death and life-threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to **KCGH ERC** within 24 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety of welfare of the study participants and others or affect the integrity of the research must be reported to **KCGH ERC** within 24 hours.
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to **KCGH ERC.**
viii. Submission of quarterly progress report to the **KCGH ERC** and dissemination of preliminary findings at the end of the study is expected from the researcher
ix. This authorization for data collection is for Kakamega County General Hospital only.

This authorization should be attached to your research license from National Commission for Science, Technology and Innovation (NACOSTI) and also other necessary clearances. Preliminary dissemination of your study findings to **KCGH ERC** is mandatory prior to publications.

COUNTY DIRECTOR HEALTH SERVICES
27 MAR 2023
P.O. Box 359-50100, KAKAMEGA COUNTY

DR. AJEVI AUSTINE
CHAIRMAN; ETHICS AND RESEARCH COMMITTEE
CGH – KAKAMEGA
Copy to: Director, Health Services

APPENDIX VIII: Kenya Tissue and Transplant Authority donor screening questionnaire



Kenya Tissue and Transplant Authority

DamuKE

KENYA TISSUE AND TRANSPLANT AUTHORITY (KTTA)
BLOOD DONOR QUESTIONNAIRE

Name of Blood Establishment:		Affix/write Donation Number here
Blood Establishment Code:		

Donation site: _____ **County:** _____ **Donor Number:**_____

SECTION 1: DONOR INFORMATION (To be completed by the donor)

Surname	Other Names	Gender (F/M/Other/Prefer not to disclose)

Next of kin name: _____

Unique Identifier (Student No. /ID No./PP No.): _____ **Date of Birth:** _____ DD / MM / YY

Status: (Mark in appropriate box): Single/Married /Separated /Divorced /Widowed/Widower /Prefer not to disclose

Cell Phone No.	Email	Residence (County)

Level of Education: (Mark in appropriate box): None/ Primary/ Secondary/ Tertiary/ other

Occupation: (Mark in appropriate box): Employed/ Self-Employed/ Unemployed/ Student

When did you last donate blood?	Number of previous donations:_____
☐ Never donated: 3 months 6 months 12 months ☐ More than 12 months	

SECTION 2: ELIGIBILITY QUESTIONNAIRE (Please fill in truthfully and accurately- your answers will be treated with utmost confidentiality- tick where appropriate)

1	Are you feeling well today?	Yes / No
2	Have you eaten in the last 6 hours?	Yes / No
3	Have you ever fainted within the past one year?	Yes / No
4	Are you pregnant or lactating	Yes / No
5	Have you traveled in the last 14 days outside your usual area of residence?	Yes / No
6	Are you on any regular medication, antibiotics, analgesics e.g. Aspirin, any other?	Yes / No
7	In the last 3 months have you had any tattooing or body piercing e.g. ear piercing?	Yes / No
8	In the last 3 months have you had sexual activity with a person whose health status you do not know?	Yes / No
9	In the last 3 months have you had a vaccination	Yes / No
In the past 6 months have you:		
10	Had surgery or medical treatment	Yes / No
11	Received Blood or Blood Products	
Have you ever:		
12	Had Hepatitis or yellow eyes?	Yes / No
13	Been exposed to suspected case of Covid-19 in the last 14 days?	Yes / No

FRM CLN 001 Version 05 (Revised Nov 2022) Effective Date: Dec 2022
Page 1 of 2

SECTION 3: DECLARATION & CONSENT (Please read this before you sign the form)

i.	I declare that I have filled in all the information required in this form truthfully and accurately.
ii.	I declare that I will also answer truthfully and accurately to any other questions that may be asked of me, for purposes of verifying my eligibility to donate blood.
iii.	I consent to give blood; I understand that if found to be safe, it may be used for transfusion for the benefit of others.
iv.	The undersigned hereby releases the Kenya Tissue and Transplant Authority, its agents or employees, as well as any other users and exhibitors of said pictures, from any and all claims, demands, accountings and causes for which the aforesaid videotape, testimonial, motion picture, digital images, or photograph likeness may be used pursuant to this consent and general release. It is also my understanding that i will receive no compensation for my likeness and testimonial.
v.	I understand that whatever blood I donate will be screened for HIV, Hepatitis B & C, and Syphilis and the results of my tests may be obtained from the Kenya Tissue And Transplant Authority by myself.
vi.	I understand that should any of the screening tests give a reactive result, I will be contacted by use of any communication medium(s), to send **me important information and or to offer me counselling to make an informed decision** about further confirmatory testing and management. Such medium(s) shall include but are not limited to e-mail, post office, mobile telephone and/or fixed telephone.
vii.	I hereby give consent to KTTA to use the contact details provided in this form to communicate to me as the need may be.
viii.	I understand the blood may be used for scientific research, main objective being to improve the safety of the blood supply to patients. I further understand that this will be done in an anonymized manner, so as to not to reveal Personal Identifiable Information (PII), that may be linked directly to me No☐ Yes ☐

Donor Signature: _____ **Date:** _____

SECTION 4: FOR OFFICIAL USE

a. Donor Screening Report

Weight (kg)	Hb >12.5g/dl	BP	Pulse	Temp (°C)		Donor is Eligible	
						Yes	No
Donor Deferred (Y/N)	**Brief reason for deferral**						
Type of donor (Replacement/ Voluntary/ Autologous)			**Type of Donation (Normal / Apheresis)**				

Name of Nurse / Counselor:_____ **Date:**_____ **Sig** _____

b. Donation outcome & post donation adverse events: (Yes or no)

Underweight Unit (Y/N)	Underweight Unit (Y/N)	> 1Venipuncture (Y/N)	Hematoma (Y/N)	Fainting (Y/N)	Nausea (Y/N)
Vomiting (Y/N)	**Headache (Y/N)**	**Convulsion (Y/N)**	**Incontinence of urine/stool (Y/N)**		
Time Needle In		**Time Needle Out**	**Volume donated (ml)**	**Bag Type**	

SECTION 5: NOTIFICATION

Reported by:_____ **Date:**_____ **Sign:**_____

APPENDIX IX: Letter of agreement from the third tier level of quality assurance on peripheral blood films reporting

Dr. Teresa Cherop Lotodo

Lecturer and Pathologist

School of Medicine

Moi University

5th April, 2023

To: Edwin Mwilitsa

Masinde Muliro Science and Technology University

Kakamega

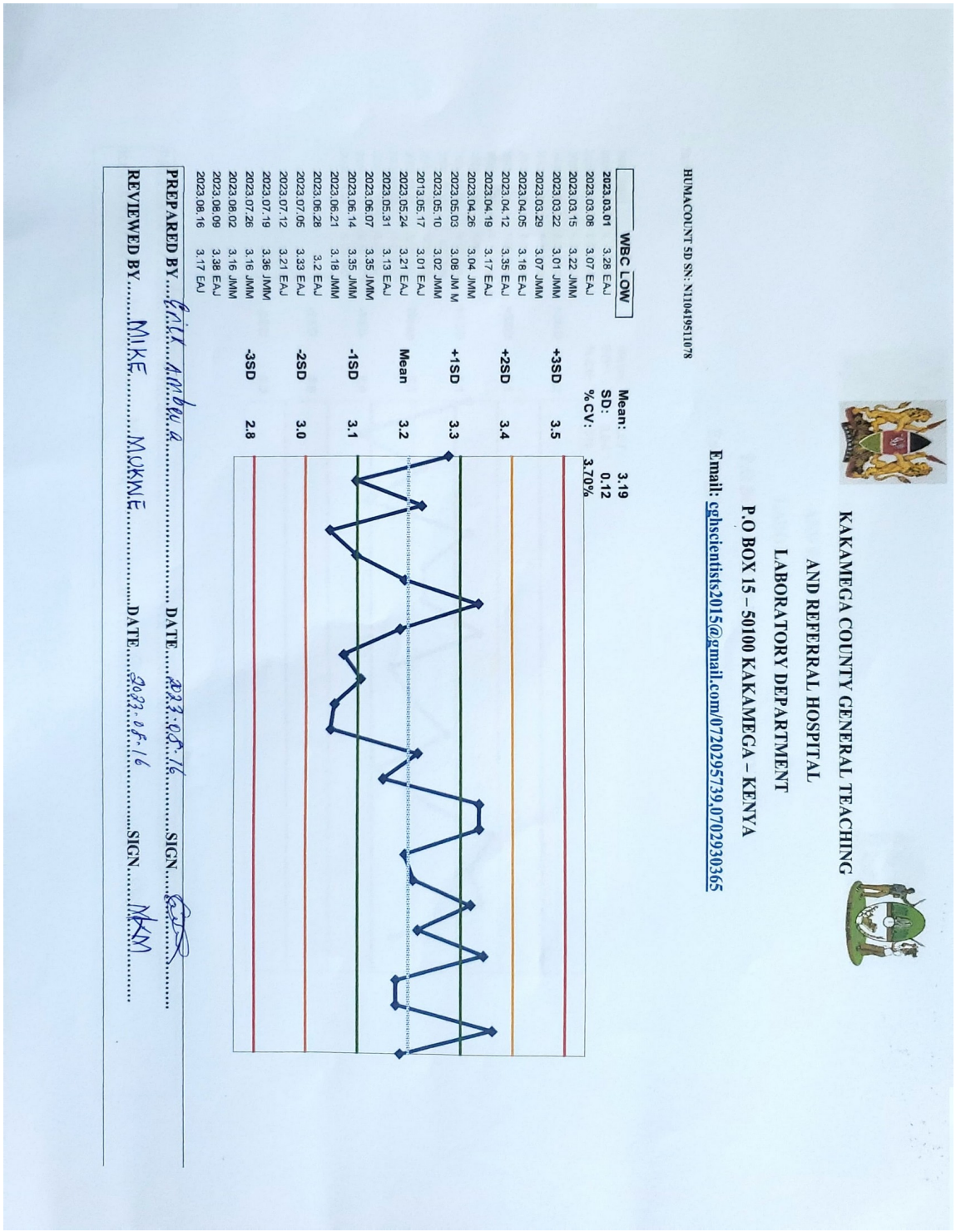
REF: REPORTING OF PERIPHERAL BLOOD SLIDES

Following your request on reporting of PBF slides in your Research titled: Evaluation of Hematological changes and their effect on treatment outcomes of Newly diagnosed pulmonary Tuberculosis patients, I would like to confirm that I agree to assist you.

Yours sincerely

Dr Teresa Cherop Lotodo

APPENDIX X: Levey-Jennings control chart- White Blood Cell Low



KAKAMEGA COUNTY GENERAL TEACHING
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LABORATORY DEPARTMENT
P.O BOX 15 – 50100 KAKAMEGA – KENYA
Email: cghscientists2015@gmail.com/0720295739,0702930365

HUMACOUNT 5D SN: N10419511078

	WBC LOW
2023.03.01	3.28 EAJ
2023.03.08	3.07 EAJ
2023.03.15	3.22 JNM
2023.03.22	3.01 JNM
2023.03.29	3.07 JNM
2023.04.05	3.18 EAJ
2023.04.12	3.35 EAJ
2023.04.19	3.17 EAJ
2023.04.26	3.04 JNM
2023.05.03	3.08 JN M
2023.05.10	3.02 JNM
2013.05.17	3.01 EAJ
2023.05.24	3.21 EAJ
2023.05.31	3.13 EAJ
2023.06.07	3.35 JNM
2023.06.14	3.35 JNM
2023.06.21	3.18 JNM
2023.06.28	3.2 EAJ
2023.07.05	3.33 EAJ
2023.07.12	3.21 EAJ
2023.07.19	3.36 JNM
2023.07.26	3.16 JNM
2023.08.02	3.16 JNM
2023.08.09	3.38 EAJ
2023.08.16	3.17 EAJ

Mean:	3.19
SD:	0.12
%CV:	3.70%
+3SD	3.5
+2SD	3.4
+1SD	3.3
Mean	3.2
-1SD	3.1
-2SD	3.0
-3SD	2.8

PREPARED BY...Erick Amoka.....DATE...23.08.16.....SIGN.....

REVIEWED BY.....MUKE.....MUKWE.....DATE...29.09.16.....SIGN.....MKM.....

APPENDIX XI: Levey-Jennings Control Chart- White Blood Cell Normal

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Email: cghscientists2015@gmail.com/0720295739,0702930365

HUMACOUNT 5D SN: N11041951078

NORMAL WBC			
2023.03.01	8.9 EAJ	Mean:	8.80
2023.03.08	8.5 EAJ	SD:	0.24
2023.03.15	8.7 EAJ	%CV:	2.73%
2023.03.22	8.4 EAJ	+3SD	9.5
2023.03.29	9 EAJ		
2023.04.05	9.1 CM		
2023.04.12	9 WN	+2SD	9.3
2023.04.19	9.1 WN		
2023.04.26	9 SN		
2023.05.03	9 EAJ	+1SD	9.0
2023.05.10	8.8 EAJ		
2013.05.17	9 CM		
2023.05.24	9 CM	Mean	8.8
2023.05.31	9 BA		
2023.06.07	9.1 BA		
2023.06.14	9.1 EAJ	-1SD	8.6
2023.06.21	8.9 EAJ		
2023.06.28	8.8 BM		
2023.07.05	8.8 BM	-2SD	8.3
2023.07.12	8.8 WN		
2023.07.19	8.7 JMM		
2023.07.26	8.6 JMM	-3SD	8.1
2023.08.02	8.5 EAJ		
2023.08.09	8.3 EAJ		
2023.08.16	8.5 EAJ		
2023.08.23	8.6 SN		

PREPARED BY...Erick Amboya.....DATE.....23.08.23.....SIGN.....

REVIEWED BY.....MIKE.....MUKWE.....DATE.....23-08-23.....SIGN.....MKM.....

APPENDIX XII: Levey-Jennings Control Chart- White Blood Cell High

KAKAMEGA COUNTY GENERAL TEACHING AND REFERRAL HOSPITAL

LABORATORY DEPARTMENT

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Email: ghscientists2015@gmail.com/0720295739,0702930365

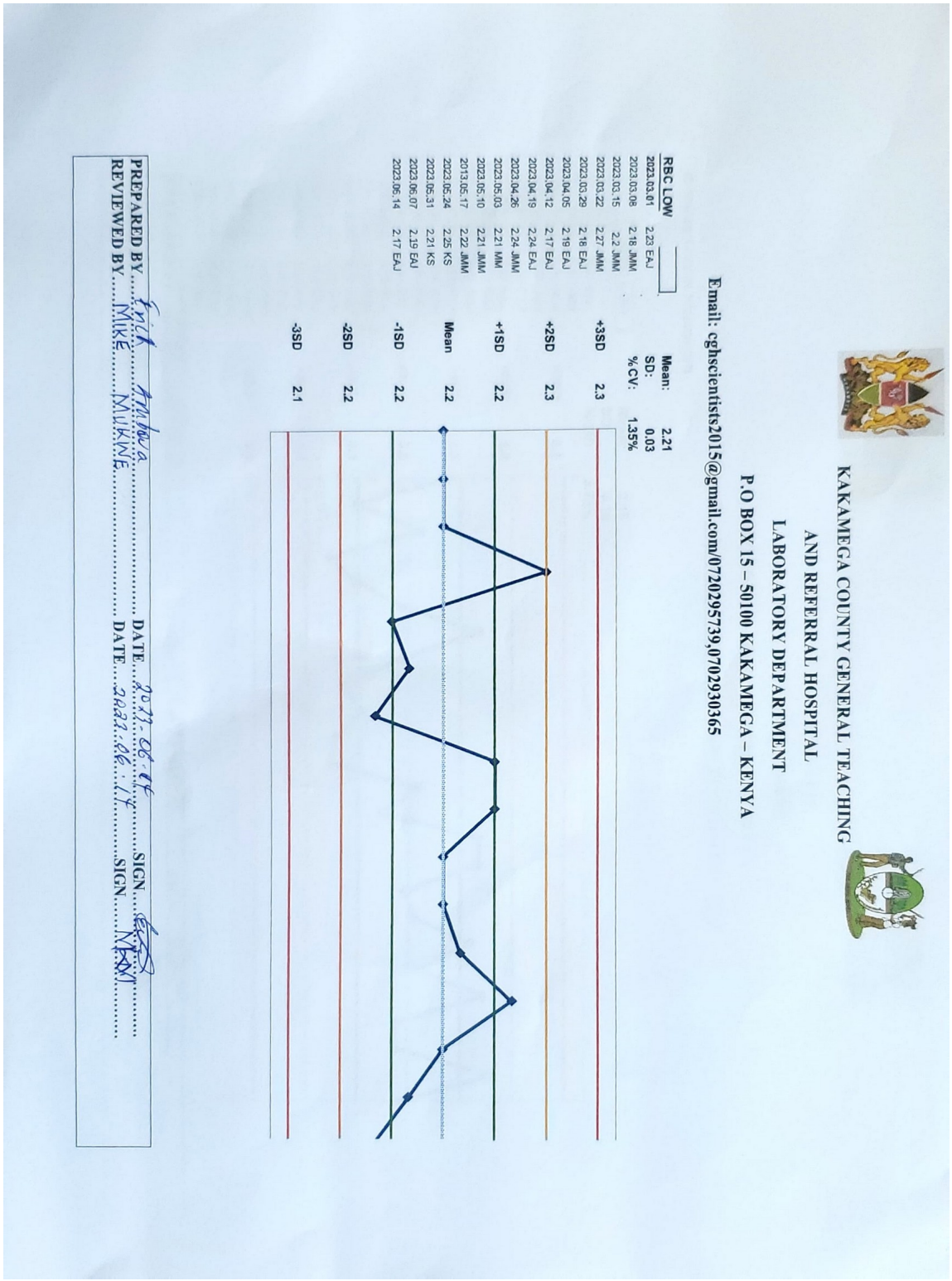
HUMACOUNT 5D SN: N11049511078

HIGH WBC		Mean:	21.28
2023.03.01	21 EAJ	SD:	0.36
2023.03.08	21.5 EAJ	% CV:	1.70%
2023.03.15	21.5 EAJ		
2023.03.22	21.5 JMM	+3SD	22.4
2023.03.29	21.9 JMM		
2023.04.05	21.4 JMM		
2023.04.12	21.3 JMM	+2SD	22.0
2023.04.19	21.6 EAJ		
2023.04.26	21.3 EAJ		
2023.05.03	21.4 EAJ	+1SD	21.6
2023.05.10	21.2 EAJ		
2013.05.17	21.8 JMM		
2023.05.24	21.2 JMM	Mean	21.3
2023.05.31	21.1 JMM		
2023.06.07	20.6 EAJ		
2023.06.14	20.7 EAJ	-1SD	20.9
2023.06.21	20.8 EAJ		
		-2SD	20.6
		-3SD	20.2

PREPARED BY.....DATE.....SIGN.....

REVIEWED BY.....DATE.....SIGN.....

APPENDIX XIII: Levey-Jennings Control Chart- Red Blood Cell Low



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Email: cghscientists2015@gmail.com/0720295739,0702930365

RBC LOW

2023.03.01	2.23 EAJ	Mean:	2.21
2023.03.08	2.18 JMM	SD:	0.03
2023.03.15	2.2 JMM	%CV:	1.35%
2023.03.22	2.27 JMM	+3SD	2.3
2023.03.29	2.18 EAJ		
2023.04.05	2.19 EAJ		
2023.04.12	2.17 EAJ	+2SD	2.3
2023.04.19	2.24 EAJ		
2023.04.26	2.24 JMM		
2023.05.03	2.21 MM	+1SD	2.2
2023.05.10	2.21 JMM		
2013.05.17	2.22 JMM		
2023.05.24	2.25 KS	Mean	2.2
2023.05.31	2.21 KS		
2023.06.07	2.19 EAJ		
2023.06.14	2.17 EAJ	-1SD	2.2
		-2SD	2.2
		-3SD	2.1

PREPARED BY.....Erick Amukoa.....DATE.....2023.06.14.....SIGN.....

REVIEWED BY.....MIKE.....MUKWE.....DATE.....2023.06.14.....SIGN.....

APPENDIX XIV: Levey-Jennings Control Chart- Red Blood Cell Normal

KAKAMEGA COUNTY GENERAL TEACHING

AND REFERRAL HOSPITAL

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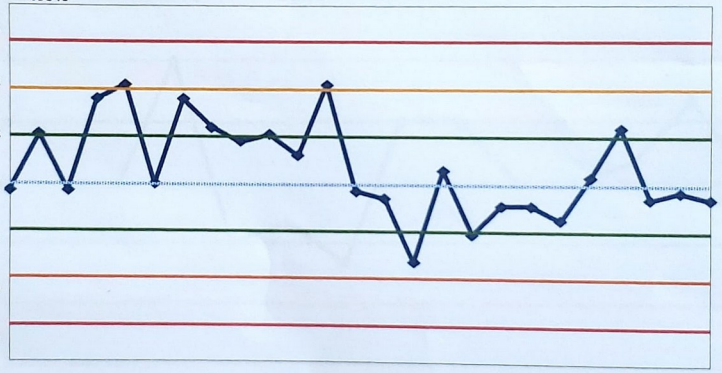
Email: cghscientists2015@gmail.com/0720295739,0702930365

HUMACOUNT 5D SN: N110419511078

	RBC NORMAL		
2023.03.01	4.08 EAJ		
2023.03.08	4.16 EAJ		
2023.03.15	4.08 EAJ		
2023.03.22	4.21 JMM	+3SD	4.3
2023.03.29	4.23 JMM		
2023.04.05	4.09 JMM		
2023.04.12	4.21 EAJ	+2SD	4.2
2023.04.19	4.17 EAJ		
2023.04.26	4.15 EAJ		
2023.05.03	4.16 JMM	+1SD	4.2
2023.05.10	4.13 JMM		
2013.05.17	4.23 JMM		
2023.05.24	4.08 EAJ	Mean	4.1
2023.05.31	4.07 EAJ		
2023.06.07	3.98 EAJ		
2023.06.14	4.11 EAJ	-1SD	4.0
2023.06.21	4.02 EAJ		
2023.06.28	4.06 BM		
2023.07.05	4.06 BM	-2SD	4.0
2023.07.12	4.04 WN		
2023.07.19	4.1 JMM		
2023.07.26	4.17 JMM	-3SD	3.9
2023.08.02	4.07 EAJ		
2023.08.09	4.08 EAJ		
2023.08.16	4.07 EAJ		
2023.08.23	4.09 SN		

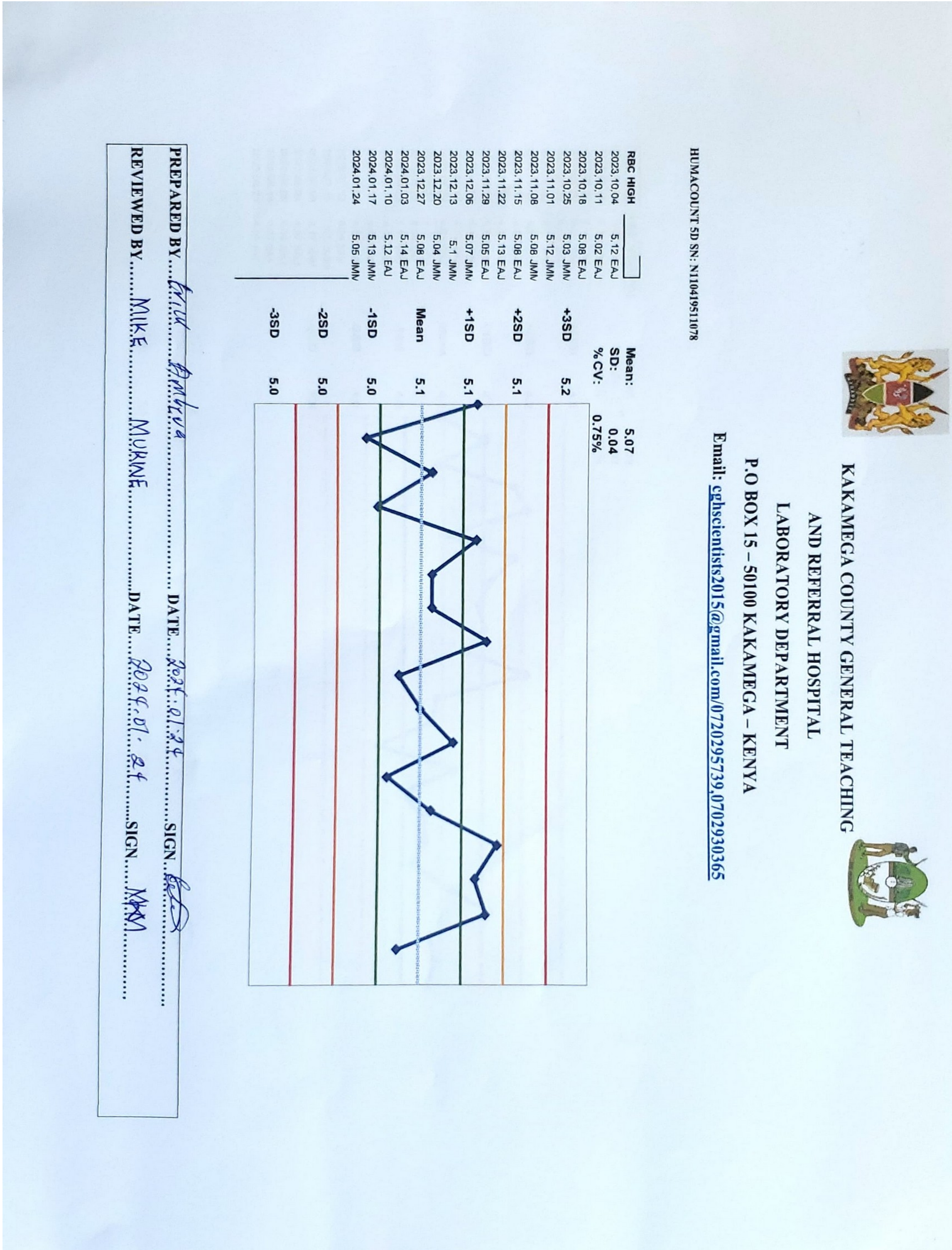
Mean: 4.09
SD: 0.07
%CV: 1.63%



PREPARED BY....Erick Ambeva..... **DATE**....2023.08.23.....**SIGN**.....

REVIEWED BY.....MIKE MUKINE.....**DATE**....2023.08.23.....**SIGN**.....

APPENDIX XV: Levey-Jennings Control Chart-Red Blood Cell High


KAKAMEGA COUNTY GENERAL TEACHING
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P.O BOX 15 – 50100 KAKAMEGA – KENYA
Email: cghscientists2015@gmail.com/0720295739,0702930365

HUMACOUNT 5D SN: N110419511078

RBC HIGH		
2023.10.04	5.12	EAJ
2023.10.11	5.02	EAJ
2023.10.18	5.08	EAJ
2023.10.25	5.03	JMM
2023.11.01	5.12	JMM
2023.11.08	5.08	JMM
2023.11.15	5.08	EAJ
2023.11.22	5.13	EAJ
2023.11.29	5.05	EAJ
2023.12.06	5.07	JMM
2023.12.13	5.1	JMM
2023.12.20	5.04	JMM
2023.12.27	5.08	EAJ
2024.01.03	5.14	EAJ
2024.01.10	5.12	EAJ
2024.01.17	5.13	JMM
2024.01.24	5.05	JMM

Mean: 5.07
SD: 0.04
%CV: 0.75%

PREPARED BY....Erick Ambuva.... DATE....2024.01.24.... SIGN....

REVIEWED BY....MIKE MURWE.... DATE....2024.01.24.... SIGN....

APPENDIX XVI: Levey-Jennings Control Chart-Hemoglobin Low

KAKAMEGA COUNTY GENERAL TEACHING
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Email: cghscientists2015@gmail.com/0720295739,0702930365

HUMACOUNT 5D SN: N11049511078

HB LOW			
2023.03.01	5.8 EAJ	Mean:	5.75
2023.03.08	5.7 EAJ	SD:	0.10
2023.03.15	5.7 EAJ	% CV:	1.75%
2023.03.22	5.6 EAJ	+3SD	6.1
2023.03.29	5.8 JMM		
2023.04.05	5.8 JMM		
2023.04.12	5.6 JMM	+2SD	6.0
2023.04.19	5.8 LL		
2023.04.26	5.6 LL		
2023.05.03	5.8 LL	+1SD	5.9
2023.05.10	5.9 JMM		
2013.05.17	5.8 EAJ		
2023.05.24	5.6 EAJ	Mean	5.8
2023.05.31	5.8 EAJ		
		-1SD	5.6
		-2SD	5.5
		-3SD	5.4

PREPARED BY.....DATE.....SIGN.....

REVIEWED BY.....DATE.....SIGN.....

APPENDIX XVII: Levey-Jennings Control Chart-Hemoglobin Normal

KAKAMEGA COUNTY GENERAL TEACHING
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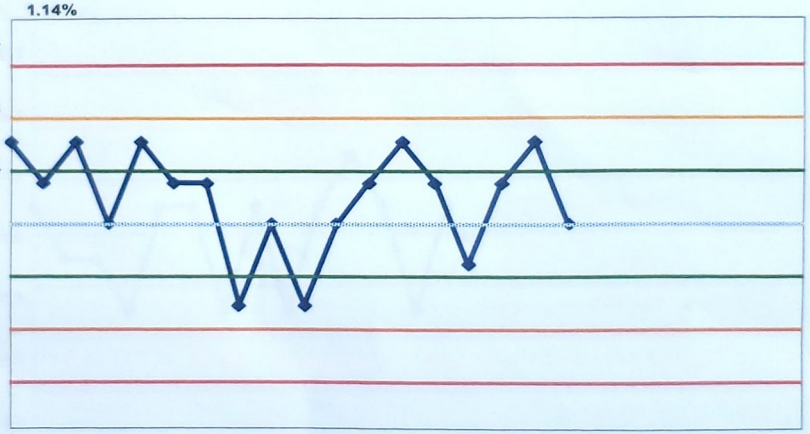
LABORATORY DEPARTMENT
P.O BOX 15 – 50100 KAKAMEGA – KENYA
Email: cghscientists2015@gmail.com/0720295739,0702930365

HUMACOUNT 5D SN: N110419511078

	NORMAL	HB
2023.03.01	11.5	JMM
2023.03.08	11.4	JMM
2023.03.15	11.5	JMM
2023.03.22	11.3	EAJ
2023.03.29	11.5	EAJ
2023.04.05	11.4	EAJ
2023.04.12	11.4	JMM
2023.04.19	11.1	JMM
2023.04.26	11.3	MM
2023.05.03	11.1	MM
2023.05.10	11.3	AM
2013.05.17	11.4	AM
2023.05.24	11.5	AM
2023.05.31	11.4	JMM
2023.06.07	11.2	AM
2023.06.14	11.4	AM
2023.06.21	11.5	AM
2023.06.28	11.3	EAJ

Mean:		11.30
SD:		0.13
%CV:		1.14%
+3SD	11.7	
+2SD	11.6	
+1SD	11.4	
Mean	11.3	
-1SD	11.2	
-2SD	11.0	
-3SD	10.9	



PREPARED BY Erick Amkeva DATE 2023.06.28 SIGN

REVIEWED BY MIKE MUKWE DATE 2023.06.28 SIGN MKM

APPENDIX XVIII: Levey-Jennings Control Chart-Hemoglobin High

KAKAMEGA COUNTY GENERAL TEACHING
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LABORATORY DEPARTMENT
P.O BOX 15 – 50100 KAKAMEGA – KENYA
Email: cghscientists2015@gmail.com/0720295739,0702930365

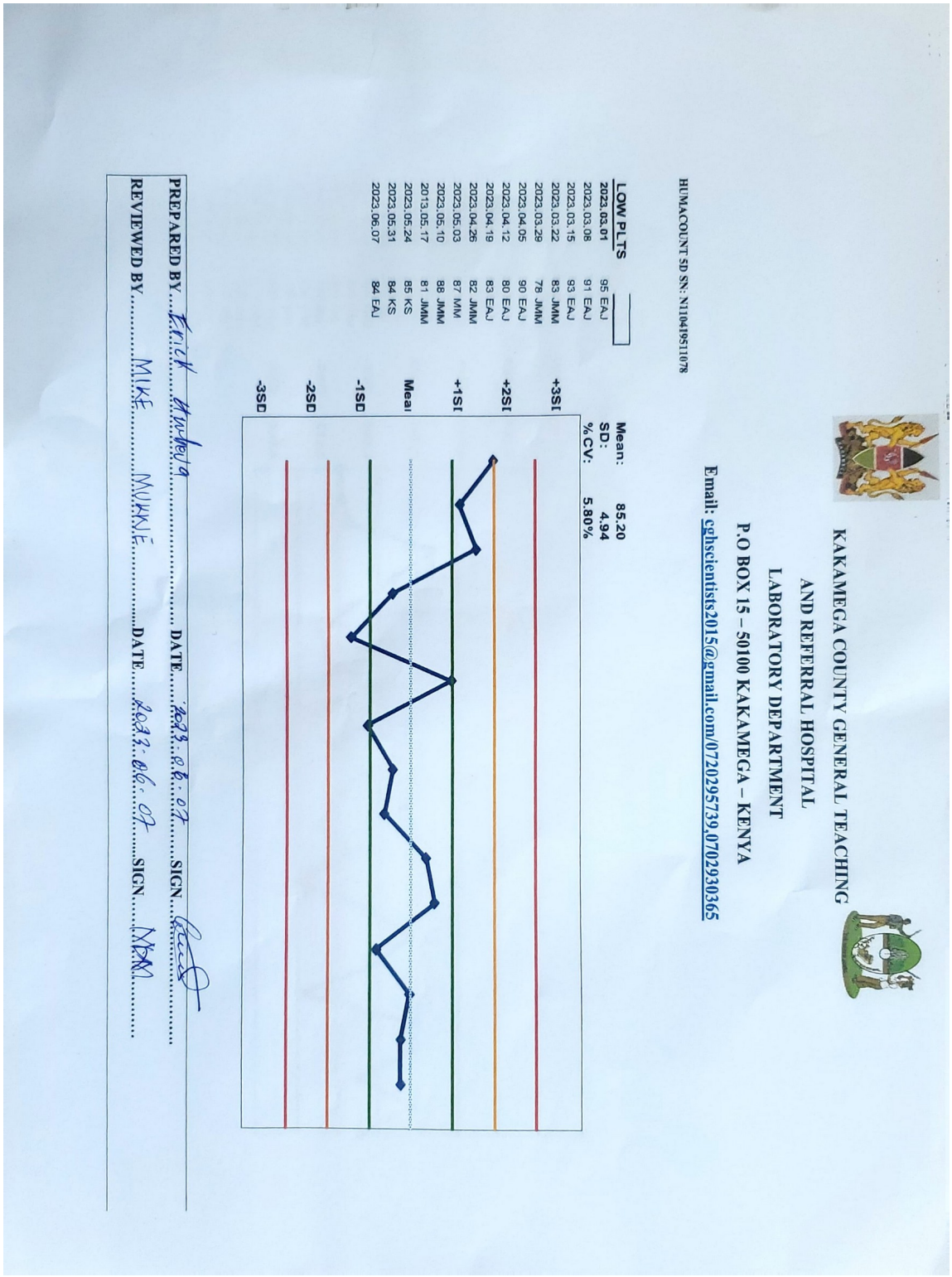
HUMACOUNT 5D SN: N11041951078

HB HIGH		Mean:	15.89
2023.03.01	15.7 EAJ	SD:	0.14
2023.03.08	15.9 EAJ	%CV:	0.90%
2023.03.15	15.9 EAJ		
2023.03.22	15.7 EO	+3SD	16.3
2023.03.29	16 EO		
2023.04.05	16 ZA		
2023.04.12	15.8 ZA	+2SD	16.2
2023.04.19	16.1 EAJ		
2023.04.26	16 ZA		
2023.05.03	15.9 ZA	+1SD	16.0
2023.05.10	16 EAJ		
2023.05.17	16 EO		
2023.05.24	16 ZA	Mean	15.9
2023.05.31	15.9 ZA		
2023.06.07	15.6 EAJ		
2023.06.14	15.8 EO	-1SD	15.7
2023.06.21	15.7 EO		
		-2SD	15.6
		-3SD	15.5

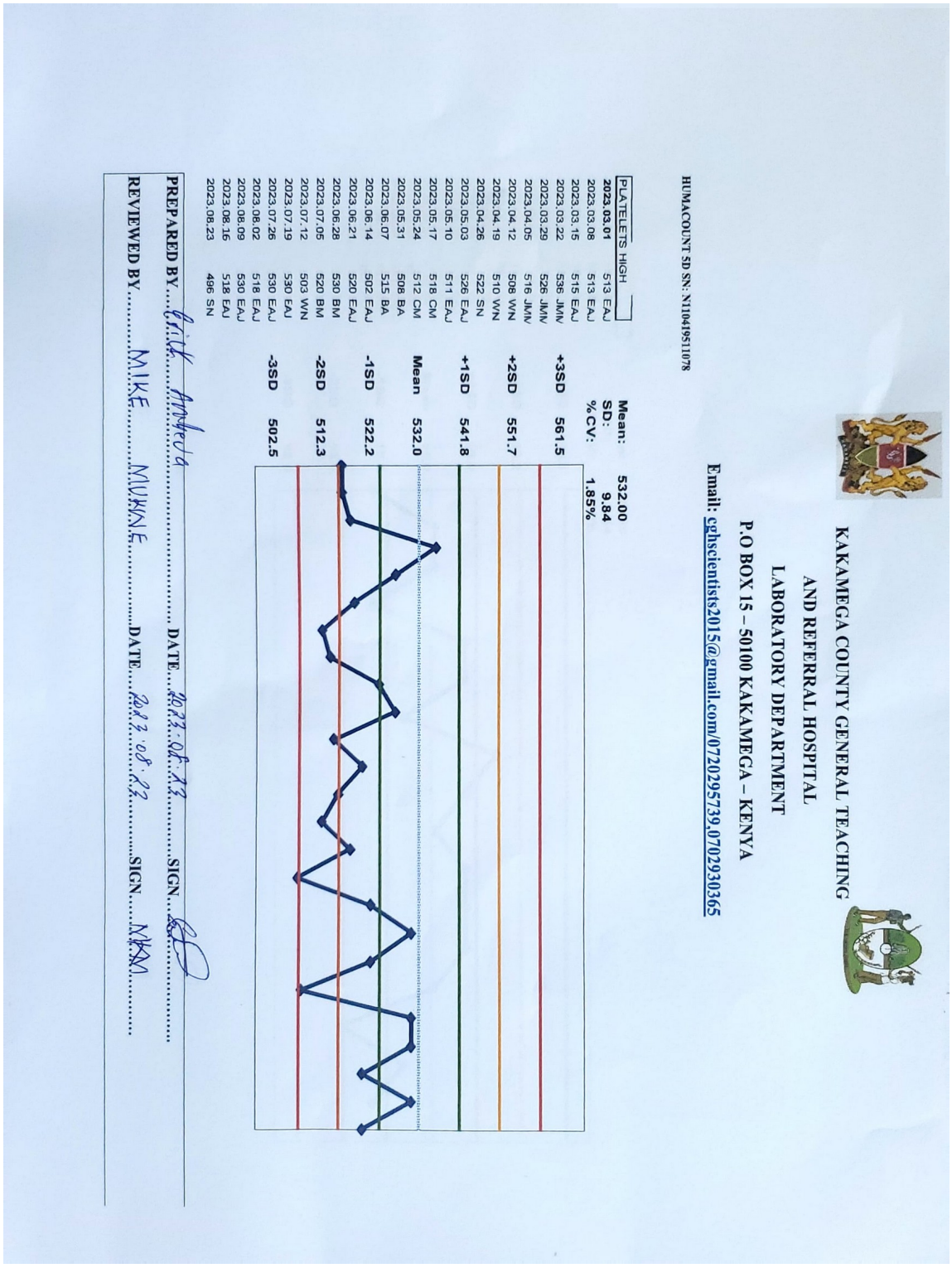
PREPARED BY..Eunice Kavuha Va..... DATE.....2023.06.21..... SIGN.....

REVIEWED BY.....MIKE MUKWE..... DATE.....2023.06.21..... SIGN.....

APPENDIX XIX: Levey-Jennings Control Chart-Platelets Low



APPENDIX XX: Levey-Jennings Control Chart-Platelets High



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LABORATORY DEPARTMENT
P.O BOX 15 – 50100 KAKAMEGA – KENYA
Email: cghscientists2015@gmail.com/0720295739,0702930365

HUMACOUNT 5D SN: N11041951078

PLATELETS HIGH			
2023.03.01	513 EAJ	Mean:	532.00
2023.03.08	513 EAJ	SD:	9.84
2023.03.15	515 EAJ	%CV:	1.85%
2023.03.22	536 JMN		
2023.03.29	526 JMN	+3SD	561.5
2023.04.05	516 JMN		
2023.04.12	508 WN	+2SD	551.7
2023.04.19	510 WN		
2023.04.26	522 SN		
2023.05.03	526 EAJ	+1SD	541.8
2023.05.10	511 EAJ		
2023.05.17	518 CM		
2023.05.24	512 CM	Mean	532.0
2023.05.31	508 BA		
2023.06.07	515 BA		
2023.06.14	502 EAJ	-1SD	522.2
2023.06.21	520 EAJ		
2023.06.28	530 BM		
2023.07.05	520 BM	-2SD	512.3
2023.07.12	503 WN		
2023.07.19	530 EAJ		
2023.07.26	530 EAJ	-3SD	502.5
2023.08.02	518 EAJ		
2023.08.09	530 EAJ		
2023.08.16	518 EAJ		
2023.08.23	496 SN		

PREPARED BY.....Erick Amayia.....DATE.....2023.08.23.....SIGN.....

REVIEWED BY.....MIKE.....MUKHALE.....DATE.....2023.08.23.....SIGN.....MKM.....

APPENDIX XXI: Sample filled PTB consent form

KAR - CH - 001
29/04/2023.

APPENDIX III: INFORMED CONSENT FORM (PULMONARY TUBERCULOSIS PATIENTS)

EVALUATION OF HEMATOLOGICAL CHANGES AND THEIREFFECT ON TREATMENT OUTCOMES IN NEWLY DIAGNOSED PULMONARY TUBERCULOSIS PATIENTS

Dear participant,

My name is EDWIN MWAKISO.....and this is a consent form, which provides information that you need to understand this study. You are free to ask questions and to freely accept or decline to participate in this study.

Jina langu ni EDWIN MWAKISO.....na hii ni fomu ya kukubali, inayokupa habari unayohitaji kuhusu utafiti nitakaofanya. Kama una maswali, uko na haki ya kuuliza na pia haki ya kukataa kushiriki utafiti huu.

What you need to know?

Pulmonary tuberculosis is a disease caused by the bacterium called *Mycobacterium tuberculosis*. The disease can be spread from one person to another through infectious droplet nuclei generated through sneezing, coughing or talking. The bacterium causes significant alterations in normal body processes.

Kifua kikuu ni ugonjwa unaosababishwa na bakteria inayoitwa Mycobacterium tuberculosis. Ugonjwa huu unaweza kuambukizwa kutoka mtu mmoja hadi mwingine kupitia kupiga chafya, kukohoa au kuongea. Bakteria hii huadhiri utendakazi wa viungo kwa mwili.

Study purpose

The purpose of this study is to document any hematological changes associated with Mycobacterium tuberculosis infection and how treatment of the same can be used to augment anti-tuberculous therapy for patients.

Lengo la utafiti huu ni kuchunguza mabadiliko ya damu ambayo yanahusiana na maambukizi ya Mycobacterium tuberculosis na jinsi ambavyo mabadiliko haya yanaweza kushughulikiwa ili kuboresha matibabu ya wagonjwa.

What I will do

To get this information, I will be collecting blood samples at respective intervals of treatment during the six months course period.

Kupata ujumbe huu, nitatoa damu muda baada ya muda wakati wa matibabu katika muda wa miezi sita.

What are the Potential Benefits?

The direct benefit from this study is that patients on care will be able to receive optimized and individually tailored treatment interventions as informed by results of hematological analysis and sputum smear conversion.

Utafiti huu utasaidia wagonjwa kupata matibabu yaliyo boreshwa kama vile tutakavyo arifiwa na matokea ya uchunguzi wa damu na kikohozi.

What are the risks? There are No foreseen risks in this study.

Utafiti huu hauna madhara yeyote

Privacy, confidentiality and right to participate/Decline

I will protect your privacy, no name or any other personal identifier will be included anywhere during the data collection period, on reports or publication. Participant data will be serialized throughout the study

Majina au habari nyingine yeyote kuhusu mgonjwa haitatumika mahali popote. Ila, nambari maalum kuwatambulisha wahusika katika utafiti itatumika. Uko na haki ya kubali au kukataa kushiriki.

The above has been explained to me and I agree to take part in the study. I understand that I am free to participatein this study and that declining will have No consequences for me. *Nimeelezwa masuala ya utafiti huu na nakubali kushirika. Niko huru kushiriki au kutoshiriki bila matatizo yeyote.*	If you agree to participate, tick"YES," if you do Not agree, tick 'NO' *Kama unakubali kushiriki, weka alama kwa "NDIO". Kama unakataa, weka alama kwa "HAPANA".*	
	YES: *NDIO:*	NO: *HAPANA:*