

https://dspace.org/

The haematological manifestations of tuberculosis

Bahemia, Imtiaz

2019
https://hdl.handle.net/10539/29737

Downloaded from a DSpace repository. https://dspace.org/

THE HAEMATOLOGICAL MANIFESTATIONS OF TUBERCULOSIS

IMTIAZ BAHEMIA

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirement for the degree of Master of Medicine (MMed)

Johannesburg, 2019

DECLARATION

I, Imtiaz Bahemia, declare that this report is my own unaided work. It is being submitted in the submissible format for the degree of Master of Medicine in the Department of Internal Medicine, Faculty of Health Sciences, University of the Witwatersrand. It has not been presented for any other degree or examination at this or any other University.



08.10.2019

Dr Imtiaz Bahemia

Date

DEDICATION

To my wife and daughter,

For their support throughout this project.

ABSTRACT

Background: The exact haematological changes present in tuberculosis, pulmonary and extra-pulmonary, are still very controversial and have not been adequately investigated. The effect of human immunodeficiency virus (HIV) on these haematological manifestations also remains to be explored since most studies on the haematological effect of tuberculosis were performed in the pre-HIV era.

Objective: To evaluate the haematological manifestations of tuberculosis in an evolving HIV era.

Methods: Prospective study of consecutively recruited patients (n=125) with microbiologically or histologically proven tuberculosis admitted at Chris Hani Baragwanath Academic Hospital between October 2017 and July 2018. Participants were obtained through the National Health Laboratory Service (NHLS). Demographics and relevant medical history were obtained for all participants and their blood results obtained through the NHLS database.

Results: Anaemia was seen in 60.7% of patients with pulmonary tuberculosis (PTB) and 61.1% of extra-pulmonary tuberculosis (ETB) patients ($p = 0.964$). ETB patients had lower white cell counts (WCC) and lower neutrophil counts than PTB patients, 5.59 [3.6-8.1] vs 7.96 [5.27-10.34], $p = 0.002$, and 4.2 [2.1-7.0] vs 5.5 [3.3-8.4], $p = 0.079$, respectively. Leucocytosis was only seen with PTB. Compared to the HIV seronegative patients, HIV co-infected PTB patients had lower WCC, lymphocyte counts, and neutrophil counts, 6.9 [4.8-9.7] vs 9.4 [8.6-11.4], $p = 0.0037$, 0.7 [0.46-1.31] vs 1.51 [1.13-2.05], $p = 0.0051$, and 4.7 [2.8-7.7] vs 8.5 [5.9-10.1], $p = 0.0106$, respectively. Patients co-infected with HIV and tuberculosis had higher levels of WCC, 7.9 [4.6-10.9] vs 5.8 [4.3-8.3], $p=0.0465$, if they were on combination anti-retroviral therapy (cART).

Conclusion: Anaemia is still the most common manifestation of tuberculosis. We have highlighted the reactive nature of PTB haematological abnormalities, as compared to ETB based on the presence of higher white cell counts in both HIV seropositive and HIV seronegative patients. HIV can blunt some of the previously described haematological manifestations of tuberculosis such as neutrophilia and lymphocytosis among patients with PTB but does not significantly alter the haematological presentation of ETB.

ACKNOWLEDGEMENTS

To my supervisors, Professor M. Patel and Professor C. Menezes for their guidance.

To Zvifadzo Matsena-Zingoni for her help with the statistical analysis.

TABLE OF CONTENTS

DECLARATION I

DEDICATION II

ABSTRACT III

ACKNOWLEDGEMENTS V

LIST OF TABLES VIII

ABBREVIATIONS IX

CHAPTER 1: INTRODUCTION AND EXTENDED LITERATURE REVIEW 1

1.1. TUBERCULOSIS – EPIDEMIOLOGY AND DIAGNOSIS 1
1.2. THE HAEMATOLOGICAL MANIFESTATIONS OF TUBERCULOSIS 1
1.3. HUMAN IMMUNODEFICIENCY VIRUS (HIV) AND TUBERCULOSIS 2
1.4. HAEMATOLOGICAL ABNORMALITIES IN PULMONARY TUBERCULOSIS 2
1.5. HAEMATOLOGICAL CHANGES IN DISSEMINATED TUBERCULOSIS (MILIARY TUBERCULOSIS) ... 6
REFERENCES 13

CHAPTER 2: ORIGINAL PROTOCOL – AIMS, OBJECTIVES AND METHODOLOGY 15

2.1. RESEARCH QUESTION 15
2.2. AIM AND OBJECTIVES 15
2.2.1. AIM 15
2.2.2. SPECIFIC OBJECTIVES 15
2.3. METHODS 15
2.3.1. STUDY DESIGN 15
2.3.2. SITE OF STUDY 16
2.3.3. PATIENTS 16
2.3.4. SAMPLE SIZE 16
2.3.5. INCLUSION CRITERIA AND EXCLUSION CRITERIA 16
2.3.5.1. INCLUSION CRITERIA 16
2.3.5.2. EXCLUSION CRITERIA 16
2.4. ENROLMENT AND PROCEDURE 17
2.4.1. IDENTIFICATION OF CANDIDATES 17
2.4.2. DISCHARGED PATIENTS 18
2.5. MEASUREMENTS 18
2.5.1. DEFINITIONS 18
2.5.2. BASELINE PATIENT DATA 19
2.5.3. OTHER DATA 19
2.6. SOURCE OF BIAS 20
2.7. STATISTICAL ANALYSIS 20
2.8. ETHICS 20
2.9. TIMING 21
2.10. FUNDING 21
REFERENCE 21

CHAPTER 3: ARTICLE 22

APPENDICES 50

APPENDIX 1: PATIENT INFORMATION SHEET 50
APPENDIX 2: INFORMED CONSENT 51
APPENDIX 3: POSTER 52
APPENDIX 4: ETHICS CLEARANCE CERTIFICATE 53
APPENDIX 5: DATA COLLECTION SHEET 54
APPENDIX 6: TURNITIN REPORT 57

LIST OF TABLES

CHAPTER 1:

Table 1.1: Studies on the haematological changes in PTB 5

Table 1.2: Studies on the haematological changes in ETB 10

Table 1.3: Summary of known haematological changes in tuberculosis 11

CHAPTER 2:

Table 1: Baseline characteristics of study population (n=125) stratified according to site of tuberculosis. 41

Table 2: Comparison of the proportion of haematological abnormalities of patients with PTB against patients with ETB (PTB vs ETB) 42

Table 3: Haematological parameters of the sample population stratified according to site of tuberculosis (PTB vs ETB). 43

Table 4: Comparison of blood parameters of patients with ETB stratified by cART status – only parameters with significant differences shown (ETB pre cART vs ETB on cART). 44

Table 5: Comparison of blood parameters of all patients in study population stratified by HIV status - only parameters with significant differences shown. 45

Table 6: Blood parameters of patients with PTB stratified by HIV status - only parameters with significant differences shown (PTB HIV+ vs PTB HIV-) 46

Table 7: Clinical features of sample population stratified by mode of diagnosis and HIV sero-status. 47

ABBREVIATIONS

AGXP	Gene Xpert on peritoneal fluid
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
B12	Vitamin B12
BMA/T	Bone Marrow Aspirate & Trephine
cART	Combination anti-retroviral therapy
CD4	Cluster of Differentiation 4
CRP	C-reactive protein
ESR	Erythrocyte sedimentation rate
ETB	Extra-pulmonary tuberculosis
GGT	Gamma-glutamyl transferase
GXP	Xpert MTB/Rif assay
Hb	Haemoglobin
HCT	Haematocrit
IL-1	Interleukin 1
IL-6	Interleukin 6
INR	International normalised ratio
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MPV	Mean platelet volume
Plt	Platelet
PTB	Pulmonary tuberculosis
PTT	Partial thromboplastin time
RCC	Red cell concentration
RCDW	Red cell distribution width
RPI	Reticulocyte production index
TB	Tuberculosis
WCC	White cell count
ZN	Ziehl Neelsen

CHAPTER 1: INTRODUCTION AND EXTENDED LITERATURE REVIEW

1.1. Tuberculosis – Epidemiology and Diagnosis

According to the World Health Organization (WHO), there was an estimate of 10 million new cases of tuberculosis globally in 2017. Locally, tuberculosis had an incidence rate of about 500000 cases in 2017 and accounted for 6.5% of all deaths in 2016.[1, 2]

The diagnosis of tuberculosis relies on a strong clinical suspicion which prompts confirmatory tests such as the Xpert MTB/Rif assay (GXP). This assay is recommended by the WHO for use on a number of specimens such as sputum, cerebrospinal fluid (CSF) and lymph node aspirates but not on stool, urine or blood. The short turnaround time of about 2 hours of the Xpert MTB/Rif assay allows for early diagnosis.[3] However, its sensitivity is dependent on several factors such as the quality of the sample and the site of tuberculosis infection.[3]

1.2. The Haematological Manifestations of Tuberculosis

Extra-pulmonary tuberculosis (ETB), in particular, can prove to be diagnostically challenging, especially when confined to a site such as the bone marrow or abdomen. Haematological changes can provide early clues to the presence of tuberculosis, especially in cases where specimen collection is invasive. Over the years, several different types of blood abnormalities have been described in patients with both pulmonary tuberculosis (PTB) and ETB. One of the earlier publications from 1943, described a spectrum of possible changes including monocytosis, basophilia, leucocytosis, leucopenia, anaemia, "leukemoid reactions" and pancytopenia.[4] Since then, studies have confirmed or disputed these findings: [5-9] pancytopenia has been reported by some authors as an unlikely manifestation of tuberculosis.[10] Some haematological abnormalities present in tuberculosis also have patient outcome implications: lymphopenia has been found to be an independent predictor of mortality in PTB.[11]

On histology, a common observation among tuberculosis patients is the presence of granulomata. However, due to their occurrence in many other conditions such as sarcoidosis, brucellosis, histoplasmosis, lymphomas, Bechet's syndrome, Sjogren's syndrome, myeloma and systemic lupus erythromatosus, granulomata are non-specific for tuberculosis. Taking into account the diagnostic difficulty of ETB, it becomes evident that having a constellation of findings including specific haematological changes can aid in the diagnosis.

1.3. Human Immunodeficiency Virus (HIV) and Tuberculosis

Since most studies on the haematological effect of tuberculosis were performed in the pre-HIV era, there is still some uncertainty about the haematological changes present in patients with tuberculosis co-infected with HIV. With an estimated overall HIV prevalence of 13.1% in 2018 in South Africa, there is a need to re-evaluate the haematological manifestations of tuberculosis in the South African population.[12]

1.4. Haematological Abnormalities in Pulmonary Tuberculosis

It is evident from the literature that the haematological changes in PTB have not been as extensively investigated as those of disseminated tuberculosis. Cytopaenias are rarely seen with PTB but are rather a feature of disseminated tuberculosis.[8, 9] The more common haematological abnormalities associated with PTB are a reactive thrombocytosis and a leucocytosis.[8, 9] A summary of the most relevant studies on the haematological abnormalities in PTB is presented in Table 1.1.

In 1989, Morris *et al.*, studied 265 black South African patients with PTB.[9] The aim of this study was to survey the extent and severity of haematological and biochemical abnormalities and record the haematological changes that occur with treatment of severe PTB. [9] Of note, patients were enrolled using the criteria of Escreet and Cowie for the diagnosis of tuberculosis.[13] Patients with either anaemia (males: haemoglobin (Hb) < 13.0 g/dL, females

Hb < 11.0 g/dL), thrombocytopenia (platelet (Plt) < 150 x 10^9/L), thrombocytosis (Plt > 400 x 10^9/L), leucopenia [White Cell Count (WCC) < 4.0 x 10^9/L] or leucocytosis (WCC > 11.0 x 10^9/L) had a bone marrow aspirate and trephine (BMA/T) study. They reported that 60% of their patients were anaemic, a finding that was more common in males. The majority of these cases (95%) had normochromic and normocytic anaemia. Most patients (94%) also had a high ferritin level. They had no cases with neutropenia or increased eosinophils or basophils. On BMA/T of 37 patients, the most common findings were a mild plasmacytosis and increased iron stores. No granulomas were reported. After 3 months of treatment, most patients had a mean increase in Hb, a decrease in mean WCC and in mean Plt counts. They concluded that body weight, Plt count, WCC, Hb level and ESR are useful indices of severity of disease. [9] The limitations of this study were the use of a diagnostic criteria to select patients for inclusion, no documentation of HIV status of participants and the possibility of dissemination in their patients was not ruled out.

Between the above-mentioned study and 2013, there was minimal work focusing on the blood changes in PTB. In 2013, Amilo *et al.*, then, evaluated the changes in haematologic indices in patients with PTB with or without HIV co-infection in South Eastern Nigeria.[14] Their sample size of 116 included four categories of patients, viz: Category 1: HIV negative patients that tested positive for *Mycobacterium tuberculosis* by ZN stain on sputum, Category 2: HIV positive patients that tested positive for *Mycobacterium tuberculosis* by ZN stain on sputum, Category 3: HIV positive patients not co-infected with tuberculosis, and Category 4: 41 healthy controls.[14] The mean Hb was 11.27 ± 1.62 (mean ± SD) in patients with PTB with HIV co-infection and 11.6 ± 1.96 in patients with PTB but not HIV co-infected.. The results showed no significant difference in the haematological parameters measured between category 1 and 2. In comparison with the control group, HIV negative patients with PTB had significantly lower Hb, WCC, neutrophil count, lymphocyte count and Plt count. However, when co-

infected with HIV, only the difference in Hb remained statistically significant when compared to controls.[14] The main limitation of this study was the small number of patients in each of the categories and failure to document important variables such as ferritin, B_{12} and folate levels.

Kurup *et al.*, evaluated the haematological and biochemistry profile of patients with PTB with or without HIV co-infection between 2013 and 2014 in Guyana.[15] Patients were only included if they had a positive fluorescence microscopy result.[15] Even though the sample size was a significant 316 patients, only 13.9% were HIV positive. The results showed that the majority of patients had a low Hb (80.2% in the HIV negative group and 91.7% in the HIV positive group), normal WCC and normal Plt count. Although this study was a good endeavour to compare the haematological changes of PTB in the HIV era, it was flawed with limitations. The authors failed to define what cut-off was used for a low Hb. Comparisons between the HIV negative and the HIV positive groups were not described clearly but it can be appreciated that HIV positive patients had a significantly lower WCC and Plt count, nonetheless, still within the normal reference range.[15]

One of the most recent studies found in the literature was performed in Chennai, India in 2015.[16] The authors aimed to assess the haematological parameters in PTB.[16] Forty patients with sputum positivity for tuberculosis on ZN stain were included and 37 age and gender matched healthy volunteers were used as control. Their results showed a significantly lower Hb, c-reactive protein (CRP), WCC, red blood cell count (RBC) and Plt count in patients with tuberculosis. This study was prospective in nature and despite being underpowered, showed some similarities to the results of Kurup *et al*.[15, 16] The authors did not document the HIV status of their participants.

TABLE 1.1: STUDIES ON THE HAEMATOLOGICAL CHANGES IN PTB

Authors	*Year*	*Country*	*Study type*	*Aim*	*Relevant results*	*Conclusion*	*Limitations*
Morris *et al.*[9]	1989	South Africa	Prospective, cross sectional	Survey the extent and severity of haematological and biochemical abnormalities in TB	1) Anaemia in 95% of patients 2) High ferritin levels in 94% of patients 3) Mild plasmacytosis and increased iron stores on BMAT of 37 patients 4) No granulomas 5) Increase in Hb, decrease in WCC and Plt after 3 months of treatment	Body weight, Plt count, WCC, Hb and ESR are useful indices of severity of disease	1) Use of a diagnostic criteria to select patients 2) No documentations of HIV status 3) Possibility of dissemination not ruled out
Amilo *et al*[14]	2013	Nigeria	Prospective	Evaluate the changes in haematologic indices in patients with PTB with or without HIV co-infection	1) No statistically significant difference in Hb due to HIV co-infection 2) Similar Hb, higher lymphocytes, lower WCC, neutrophil count in HIV+ group	PTB and HIV have caused haematological deregulation	1. Small sample size 2. Failure to document ferritin, B12, folate
Kurup *et al.*[15]	2013-2014	Guyana	Prospective, observational	Evaluate the hematological and biochemistry profile of patients with or without HIV and TB.	1) Anaemia in 80.2% of HIV-, 91.7% in HIV+ patients 2) Lower WCC, Plt in HIV+ group but within normal range	Haematological and biochemical parameters are important, simple and cheaper methods in analyzing the pattern of health status among PTB patients with or without HIV	1. Definition of anaemia not provided 2. Poor description of comparison of HIV+ and HIV- patients
Rohini *et al.*[16]	2015	India	Prospective	Assess the haematological parameters in PTB patients.	1) Anaemia, RBC, Plt reduced in all patients with TB 2) Higher WBC and ESR in patients with TB	Hb, RBC and Plt count were decreased in TB patients whereas ESR, CRP and WCC were increased compared to controls	1) Small sample size 2) HIV status not documented 3) Discrepancy between text and tables on changes in CRP

BMAT: bone marrow aspiration and trephine, CRP: c-reactive protein, ESR: erythrocyte sedimentation rate, Hb: haemoglobin, HIV+: HIV sero-positive, HIV-: HIV seronegative, Plt: platelet, RBC: red blood cell count, TB: Tuberculosis, WCC: white cell count.

1.5. Haematological changes in Disseminated Tuberculosis (Miliary Tuberculosis)

According to the WHO, miliary tuberculosis is classified as pulmonary due to lung involvement whereas according to the 2014 National tuberculosis management guidelines of South Africa, miliary tuberculosis should be classified as extra-pulmonary.[17, 18] For all intents and purposes, miliary tuberculosis will be referred to as ETB in this study. A summary of the most relevant studies on the haematological abnormalities in ETB is presented in Table 1.2.

Maartens *et al.*, carried out a 10-year retrospective study from 1978 to 1987 at Groote Schuur Hospital, Cape Town, South Africa, to determine the clinical and laboratory characteristics, diagnostic methods and prognostic variables in adults treated for miliary tuberculosis.[8] Patients were included if they had miliary nodules on chest radiograph or evidence of haematogenous dissemination on biopsy or autopsy.[8] The method used to confirm tuberculosis included ZN staining and culture of the following specimens: sputum, gastric lavage, urine, CSF, serosal exudate, bronchial brushings/lavage, pus swab and joint fluid. The sample population (109 participants) included 7 white, 53 of mixed race and 49 black patients. Only 53% of their patients had an anaemia (Hb <13g/dL in males, <11g/dL in females) and as many as 87% had a lymphopenia ($<1.5 \times 10^9$) on peripheral blood. This is in contrast with most other studies in which anaemia was the predominant feature.[10, 19-21] Of note, 6 patients had a pancytopenia, half of which, resolved after treatment. All patients with bone marrow granulomas had a leucopenia and thrombocytopenia on peripheral blood. [8]

Hunt *et al.,* postulated that significant blood abnormalities can be erroneously attributed to tuberculosis and hence overlooked.[10] They argued that pancytopenia in a patient with tuberculosis could be secondary to the myelosuppressive effects of tuberculosis or could actually reflect an underlying haematological disorder.[10] They presented a case report of a

previously fit, 71-year-old woman with generalized malaise, weight loss, ankle swelling and a pancytopenia. Her initial bone marrow biopsy showed focal hyperplasia, abnormal megakaryocytes and Bence Jones protein was also present in her urine. She received a blood transfusion and her white cell count improved spontaneously. On further investigations, she was found to have para-aortic lymph nodes and her liver biopsy revealed giant cell granulomata and her urine cultured *Mycobacterium tuberculosis*. At that point, she was started on a 9-month anti-tuberculous therapy course which successfully resolved her symptoms. Interestingly, post anti tuberculous therapy, despite normal cell counts on peripheral blood, her repeat bone marrow biopsy showed myelodysplastic changes and abnormal immature precursor cells. After around a year, the pancytopenia recurred and she eventually became transfusion dependent. In the opinion of the authors, the transient nature of the peripheral pancytopenia corresponded to a failing but compensating marrow after the added insult of tuberculosis with the transfusion relieving the stress.[10] This case illustrated that a pancytopenia in a patient with tuberculosis might be due to an underlying haematological abnormality.

About 5 years later, Lombard and Mansvelt reviewed the peripheral blood and bone marrow findings on 25 patients known to have tuberculous granulomata on bone marrow examination.[19] This was a study performed at Tygerberg Hospital, Parrow, South Africa.[19] The exclusion criteria were the presence of any underlying disease that could influence the haematological parameters, such as myelodysplasia, lymphoproliferative disease, acquired immunodeficiency syndrome (AIDS) and steroid therapy. The most common abnormalities reported on peripheral blood were a lymphopenia in all patients, an anaemia (mainly normochromic normocytic) in 72% of patients and a thrombocytopenia in 52% of patients. These finding were in part similar to the findings of Maartens *et al.,* and Cucin *et al.*[8, 21] Only 12% of patients had a pancytopenia. On bone marrow biopsies, megaloblastic changes were present in 60% of cases and 41% of patients had a lymphocytosis. All patients with a

lymphocytosis on bone marrow biopsy had a lymphopenia on peripheral blood.[19] The authors concluded that the absence of a peripheral lymphopenia makes tuberculous infiltration of the bone marrow unlikely.[19] The small sample size and AIDS as an exclusion criterion, unfortunately, makes this study poorly generalizable.

A prospective interventional study conducted in New Delhi, India from February 2011 to April 2012 evaluated the haematological and haemostasis parameters in patients with tuberculosis.[20] This was a review of 128 patients with both PTB and ETB.[20] Patients were grouped according to the site of disease as follows: pulmonary, CNS, disseminated and others. The aim of this study was to identify the laboratory parameters contributing to the hypercoagulable state of tuberculosis. Of the 128 patients evaluated, 75.78% had an anaemia (Hb < 13.3 g/dL in males, Hb < 12.0 g/dL in females) and 49.22% had a leucocytosis (WCC > 9.06 x 10^9/L). There was statistically significant improvement in Hb, WCC and Plt counts after two months of treatment. Subset analysis of the different groups was performed on 88 patients and the only parameter that was uniformly abnormal across all groups was a low Hb.[20] There were two main limitations to this study. First, the grouping was unconventional and not based on the WHO classification of tuberculosis.[18] Second, statistically significant improvement in WCC was reported overall (128 patients) but seems to have been lost when all four groups were analysed individually. This raises concern on the validity of the results presented.

In the same year, but in another part of India, Hungund *et al.*, performed a cross sectional study to evaluate the blood and bone marrow findings in tuberculosis, in adults.[22] This was a prospective study of 100 patients with tuberculosis.[22] However, the method of diagnosis of tuberculosis was not documented. They excluded patients with clinical features of drug toxicity from anti-tuberculous drugs and those with primary haematological diseases. All the participants had a bone marrow aspirate and trephine biopsy. They reported that 83% of their participants presented with pulmonary manifestations and 17% with extra-pulmonary

manifestations. About a quarter of their participants (28%) were HIV seropositive. Almost all of their cases (96%) had an anaemia (Hb < 13.5g/dL for male; 12.5g/dL for females). Leucocytosis was present in 34%, and neutrophilia in 35%. Most patients had a normal Plt count (86%). On bone marrow aspirate and trephine analysis, most participants had a normocellular bone marrow, 46% showed hypercellularity and a reactive plasmacytosis was also seen in 46% of cases. Iron stores were also normal in most cases. Only five patients had granulomata while 1 patient stained ZN positive.[22] This study is of questionable value due to several limitations. Fundamental information was not presented. The authors did not specify the mode of diagnosis of tuberculosis, the main organ of involvement, the difference of the haematological changes between PTB and ETB and the differences present in the HIV seropositive subgroup. The reason for performing a bone marrow aspiration and trephine biopsy on 83% of patients that presented with pulmonary manifestations of tuberculosis is not explained making it also ethically questionable.

TABLE 1.2: STUDIES ON THE HAEMATOLOGICAL CHANGES IN ETB.

Authors	*Year*	*Country*	*Study type*	*Aim*	*Relevant results*	*Conclusion*	*Limitations*
Maartens *et al.*[8]	1978-1987	South Africa	Retrospective	Determine the clinical and laboratory characteristics, diagnostics methods and prognostic variables in adults treated for miliary tuberculosis	1) Anaemia in 53% of patients 2) Lymphopenia in 87% of patients 3) Pancytopenia in 6 patients 4) All patients with granulomas on BMAT had a leucopenia and thrombocytopoenia	Miliary tuberculosis commonly causes hematologic derangements, some of which are helpful prognostically	1) Pre-HIV era
Lombard & Mansvelt[19]	1993	South Africa	Retrospective	Determine whether specific peripheral blood findings are associated with tuberculous infiltration of the bone marrow, as well as evaluate the efficiency of bone marrow cultures in the diagnosis of Tb.	1) Lymphopenia in all patients 2) Anaemia in 72% of patients 3) Thrombocytopenia in 52% of patients 4) Megaloblastic changes in 60% of patients 5) Lymphocytosis in 41% of patients	The absence of peripheral lymphopenia makes tuberculous infiltration of the bone marrow unlikely	1) Small sample size 2) AIDS was an exclusion criterion
Kutiyal *et al.*[20]	2011-2012	India	Prospective, interventional	Study the haematological and haemostasis laboratory parameters to correlate the abnormalities for a hypercoagulable state.	1) Anaemia in 75.78% of patients 2) Leucocytosis in 49.22% of patients 3) Thrombocytopenia in 37.5% of patients 4) Hypoalbuminemia in 75% of patients 5) Elevated ESR in 98.43% of patients	TB does favour a hypercoagulable state. TB can present with varied haematological manifestations	1) Unconventional grouping of patients, not based on WHO classification of TB 2) Overall statistically significant improvement in WCC lost when groups were analysed individually which raises concern on the validity of the results
Hungund *et al.*[22]	2012	India	Prospective, cross sectional	To know the prevalence of blood and bone marrow changes in TB	1) Anaemia in 96% of patients 2) Leucocytosis in 34% of patients 3) Neutrophilia in 35% of patients 4) Normal platelet count in 86% of patients 5) Hypercellular bone marrow in 46% of patients 6) Iron store normal in most cases 7) 5 cases with granulomas and 1 case stained ZN positive	Abnormal haematological findings should prompt the consideration of TB as a differential diagnosis.	1) Mode of diagnosis not specified 2) Main organ involved not specified 3) No comparison between PTB and ETB performed 4) No comparison based on HIV status performed 5) Reason for BMAT on 83% of patients with pulmonary manifestation is not explained and is ethically questionable.

BMAT: bone marrow aspiration and trephine, PTB: pulmonary Tuberculosis, ETB: extra-pulmonary Tuberculosis, ZN: Ziehl Neelsen

Table 1.3: Summary of known haematological changes in tuberculosis

PTB	ETB
Red cells:	*Red cells:*
Normochromic normocytic anaemia	Normochromic normocytic anaemia (e.g. anaemia of chronic disease)
Hypochromic microcytic anaemia	Hypochromic microcytic anaemia
Increased red cell volume distribution width	Leucoerythroblastic anaemia
White cells:	*White cells:*
Leucocytosis (neutrophilia, eosinophilia, monocytosis, lymphocytosis)	Abnormal morphology:
Lymphopenia	Acquired Pelger-Huet anomaly
	Transformed lymphocytes
	Toxic granulation of neutrophils
	Leucocytosis (neutrophilia, eosinophilia, monocytosis, lymphocytosis)
	Leucopenia (neutropenia, lymphopenia)
	Leukemoid reactions (granulocytic, monocytic, lymphocytic, basophilic)
Platelets:	*Platelets:*
Thrombocytosis	Thrombocytosis
	Thrombocytopenia
Haemostasis:	Bleeding, thrombosis
Bone marrow changes:	Fibrosis, necrosis, plasmacytosis
Associations:	Acute leukaemias
	Chronic (granulocytic and lymphocytic leukaemias)
	Hairy cell leukaemia
	Myeloproliferative diseases (polycythemia
	Myelodysplastic syndrome
	Haemophagocytic syndrome
	Lymphoproliferative disorders
	Multiple myeloma
Adverse effects of drugs:	
Isoniazid – thrombocytopenia, sideroblastic anaemia	
Rifampicin – thrombocytopenia, thrombocytosis	

Adapted from Knox-Macaulay HH. Tuberculosis and the haemopoietic system., Baillieres Clin Haematol. 1992;5(1):101-29[23]

As highlighted above, the exact haematological changes present in tuberculosis, pulmonary and extra-pulmonary, are still very controversial and have not been adequately investigated. We hope that this study will be informative and clear the current uncertainties faced by clinicians treating tuberculosis in South Africa. We postulate that several findings such as anaemia and thrombocytosis can still reliably be expected even in HIV co-infected patients. Knowledge of the differences between the haematological abnormalities present in PTB and ETB is also of utmost importance since ETB implies a longer treatment duration.

We aim to describe the haematological manifestations among patients with tuberculosis in an evolving HIV era. Furthermore, there is a need to re-evaluate the relationship of these changes with tuberculosis since they are also seen in haematological malignancies. The early diagnosis of a malignancy may be missed if all abnormalities are wrongly attributed to tuberculosis. A good understanding of the expected haematological derangements in tuberculosis will also be useful when considering appropriate supportive care.

References

1. Statistics South Africa. Mortality and causes of death in South Africa, 2016: Findings from death notification. Pretoria: Statistical release P0309.3 2019.
2. World Health Organization. Global tuberculosis report 2018. Geneva: Licence: CC BY-NC-SA 3.0 IGO.; 2018.
3. Lawn SD, Mwaba P, Bates M, Piatek A, Alexander H, Marais BJ, et al. Advances in tuberculosis diagnostics: the Xpert MTB/RIF assay and future prospects for a point-of-care test. Lancet Infect Dis. 2013;13(4):349-61.
4. Muller GL. Clinical significance of the blood on tuberculosis. New York: The Commonwealth Fund; 1943.
5. Glasser RM, Walker RI, Herion JC. The significance of hematologic abnormalities in patients with tuberculosis. Arch Intern Med. 1970;125(4):691-5.
6. Twomey JJ, Leavell BS. Leukemoid reactions to tuberculosis. Arch Intern Med. 1965;116:21-8.
7. Cameron SJ. Tuberculosis and the blood-a special relationship? Tubercle. 1974;55(1):55-72.
8. Maartens G, Willcox PA, Benatar SR. Miliary tuberculosis: rapid diagnosis, hematologic abnormalities, and outcome in 109 treated adults. Am J Med. 1990;89(3):291-6.
9. Morris CD, Bird AR, Nell H. The haematological and biochemical changes in severe pulmonary tuberculosis. Q J Med. 1989;73(272):1151-9.
10. Hunt BJ, Andrews V, Pettingale KW. The significance of pancytopenia in miliary tuberculosis. Postgrad Med J. 1987;63(743):801-4.
11. Barnes PF, Leedom JM, Chan LS, Wong SF, Shah J, Vachon LA, et al. Predictors of short-term prognosis in patients with pulmonary tuberculosis. J Infect Dis. 1988;158(2):366-71.
12. Statistics South Africa. Mid-year population estimates 2018. Pretoria: Statistical release P0302; 2018.
13. Escreet BC, Cowie RL. Criteria for the diagnosis of pulmonary tuberculosis. S Afr Med J. 1983;64(18):690-1.
14. Amilo GI, Meludu SC, Ele PU, Ezechukwu C, Onyenekwe C, Ifeanyi Chukwu M. Haematologic indices in pulmonary tuberculosis with or without HIV Co-infection in South Eastern Nigeria. Advances in Life Science and Technology. 2013;11:1-7.

15. Kurup R, Flemming K, Daniram S, Marks-James S, Roberts Martin R. Hematological and biochemistry profile and risk factors associated with pulmonary tuberculosis patients in Guyana. Tuberc Res Treat. 2016;2016:6983747.
16. Rohini K, Surekha Bhat M, Srikumar PS, Mahesh Kumar A. Assessment of hematological parameters in pulmonary tuberculosis patients. Indian J Clin Biochem. 2016;31(3):332-5.
17. Department of Health. National tuberculosis management guidelines 2014. Pretoria; 2014.
18. World Health Organization. Definitions and reporting framework for tuberculosis - 2013 revision. Geneva: WHO; 2013.
19. Lombard EH, Mansvelt EP. Haematological changes associated with miliary tuberculosis of the bone marrow. Tuber Lung Dis. 1993;74(2):131-5.
20. Kutiyal AS, Gupta N, Garg S, Hira H. A study of haematological and haemostasis parameters and hypercoagulable state in tuberculosis patients in Northern India and the outcome with anti tubercular therapy. Journal of Clinical and Diagnostic Research. 2017;11(2):OC09-13.
21. Cucin RL, Coleman M, Eckardt JJ, Silver RT. The diagnosis of miliary tuberculosis: utility of peripheral blood abnormalities, bone marrow and liver needle biopsy. J Chronic Dis. 1973;26(6):355-61.
22. Hungund BR, Sangolli SS, Bannur HB, Malur PR, Pilli GS, Chavan RY, et al. Blood and bone marrow findings in tuberculosis in adults - A cross sectional study. Al Ameen J Med Sci. 2012;5(4):362-6.
23. Knox-Macaulay HH. Tuberculosis and the haemopoietic system. Baillieres Clin Haematol. 1992;5(1):101-29.

CHAPTER 2: ORIGINAL PROTOCOL – AIMS, OBJECTIVES AND METHODOLOGY

2.1. Research question

Has HIV changed the haematological picture of tuberculosis?

2.2. Aim and objectives

2.2.1. Aim

To describe the haematological manifestations present in patients with confirmed tuberculosis.

2.2.2. Specific objectives

1. To determine the full blood count (FBC), differential count, smear, iron studies including ferritin, B_{12}, and red cell/serum folate of patients with confirmed tuberculosis.

2. To determine the bone marrow abnormalities in patients with tuberculosis in whom a bone marrow aspirate and trephine biopsy (BMA/T) was performed.

3. To compare the haematological abnormalities between PTB and ETB.

4. To compare the haematological abnormalities between HIV seropositive patients on combination anti-retroviral therapy (cART) and HIV seropositive patients not on cART infected with tuberculosis.

5. To compare the haematological abnormalities between HIV sero-positive and HIV sero-negative patients infected with tuberculosis.

2.3. Methods

2.3.1. Study design

Prospective study on patients with confirmed tuberculosis.

2.3.2. Site of study

Department of Internal Medicine, Chris Hani Baragwanath Academic Hospital (CHBAH), Soweto, Johannesburg, South Africa. CHBAH is a tertiary level facility.

2.3.3. Patients

Adult population (≥18years old)

2.3.4. Sample size

An estimated total of 125 patients will be studied over a period of one year from June 2017 to May 2018. We used anaemia as a variable to determine sample size. Since it was reported at 91.7% in the study by Kurup *et al*.,[1] among HIV seropositive patients with PTB, our expected proportion (p) of patients with anaemia is 91.7%. Using Fisher's sample size calculation formula, our sample size (n) should be 117 patients. The margin of error used was 5%. The collection period will be extended until the required sample size is achieved should the target not be attained.

$$n = \frac{z^2(p)(1-p)}{d^2}$$

$z = 1.96\ (constant) p = expected\ proportion\ (75\%)\ d = 0.05\ (margin\ of\ error)$

2.3.5. Inclusion criteria and Exclusion Criteria

2.3.5.1. Inclusion criteria

- Adult population (≥ 18 years old)
- Microbiological and/or histological evidence of tuberculosis

2.3.5.2. Exclusion criteria

- Pregnancy

- Patients with established chronic kidney disease not related to tuberculosis
- Patients with established chronic liver disease not related to tuberculosis
- Patients with a previous diagnosis of tuberculosis over the last year
- Haemoglobinopathies
- Malaria

2.4. Enrolment and procedure

2.4.1. Identification of candidates

A list of hospitalized patients that have been tested positive for tuberculosis will be obtained on a daily basis from the National Health Laboratory Service (NHLS) laboratory. This will include patients with: 1) positive GXP on sputum, pleural fluid, FNA of lymph node and CSF, 2) positive auramine stain of sputum, pleural fluid, 3) positive Ziehl Neelsen (ZN) on FNA of lymph node, 3) positive culture of *Mycobacterium tuberculosis* on sputum, pleural fluid, FNA of lymph node, CSF, blood and bone marrow aspirate, 4) histological diagnosis of tuberculosis on tissue, lymph node or trephine biopsy. Each patient will then be individually consented to participate in the study after which their demographics and results will be collected on a data collection sheet.

It is expected that a subgroup of patients will be missed if only the above method is used to enrol patients since the confirmatory test might have been done at a different facility. To overcome this shortcoming, posters will be placed in the medical wards of the hospital to inform doctors of the study to encourage them to contact the investigator to enrol their eligible patients. Messages will also be placed on the WhatsApp® groups of the medical registrars of the hospital to recruit patients.

2.4.2. Discharged patients

For patients that have been discharged before the availability of the confirmatory test for tuberculosis, their records would be accessed through the archives of the hospital. This would be a retrospective review of records for which informed consent is not required.

2.5. Measurements

2.5.1. Definitions

The normal reference ranges set out by the WHO for Hb, neutrophil count, lymphocyte count and Plt count will be used.

Anaemia: Hb < 13 g/dL (males), Hb < 12 g/dL (females)

Polycythemia: Hb >18.5 g/dL ± haematocrit (HCT) > 0.52 L/L (males), Hb >16.5 g/dL ± HCT > 0.48 L/L (females)

Leucopenia: WCC < 4 x 10^9/L

Leucocytosis: WCC > 11 x 10^9/L

Neutropenia: neutrophil count < 2 x 10^9/L

Neutrophilia: neutrophil count > 7.5 x 10^9/L

Lymphopenia: lymphocyte count < 1 x 10^9/L

Lymphocytosis: lymphocyte count > 4 x 10^9/L

Monocytosis: monocyte count > 0.8 x 10^9/L

Monocytopenia: monocyte count <0.2 x 10^9/L

Clinical thrombocytopenia: Plt count < 100 x 10^9/L

Thrombocytosis: platelet count > 450 x 10^9/L

2.5.2. Baseline patient data

2. Unique participant number, location in hospital, age, gender.

3. Relevant medical history: HIV status, CD4 count, HIV viral load, type and duration of cART, diabetes mellitus, haematological malignancy, chemotherapy, blood/blood product transfusion and other comorbidities.

4. Method of diagnosis of tuberculosis: 1) GXP on sputum/tracheal aspirate (TA), bronchial washings, lymph node aspirate, CSF, pleural fluid and tissue. 2) Auramine stain on sputum, bronchial washings. ZN stain on lymph node aspirate, trephine and tissue. 3) Culture of sputum/tracheal aspirate, bronchial washings, lymph node aspirate, CSF, pleural fluid and tissue. 4) Histology.

5. Site of tuberculosis: pulmonary or extra-pulmonary.

2.5.3. Other Data

- Blood results: WCC, red cell concentrate (RCC), Hb, HCT, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RCDW), Plt count, mean platelet volume (MPV), neutrophil count, lymphocyte count, monocyte count, eosinophil count, blast count, smear, reticulocyte production index (RPI), international normalised ratio (INR), activated partial thromboplastin time (PTT), iron, ferritin, transferrin, B_{12}, folate, electrolytes, urea, creatinine, calcium, magnesium, phosphate, Erythrocyte Sedimentation Rate (ESR).
- Radiological findings, chest X-ray or any other form of imaging available.
- Bone marrow aspirate and trephine results including adequacy, M:E ratio, evaluation of megakaryocytes, erythropoiesis, leucopoiesis, plasma cells, macrophages, iron stores,

cellularity, fibrosis, lymphoid aggregates, malignant infiltrates and presence of granulomata or AFBs.

- History of transfusion of blood products.
- History of treatment with corticosteroids.

2.6. Source of Bias

Known confounding variables such as HIV status, diabetes, steroid therapy, chemotherapy, cART, anti-tuberculous therapy and transfusion of blood products will be documented.

2.7. Statistical Analysis

Data will be captured using Microsoft Excel® and transferred onto Stata® for statistical analysis. A statistician will be consulted. Descriptive data, such as age, will be presented using means and standard deviations or medians and interquartile ranges depending on the distribution. Continuous data will be compared using the t-test if normally distributed or the Mann-Whitney test if not normally distributed. Comparison of categorical data will be performed using the Chi-square test. A p-value of < 0.05 will be considered as being statistically significant.

2.8. Ethics

Informed consent will be obtained from all participants admitted to hospital.

Hospital approval will be obtained from the (1) Clinical Head of Internal Medicine and (2) CEO of CHBAH. Approval from the NHLS will be obtained for access to laboratory results. Ethics approval will be obtained from the Human Research Ethics Committee (HREC) (Medical) of the University of the Witwatersrand.

2.9. Timing

Expected start: 01.07.2017

Expected end: 30.06.2019

	Jan 17-Feb 17	Mar 17 – Apr 17	May 17	Jun 17 – Sep 17	Oct 17-Jul 18	Aug 18-Sep 18	Oct 18-Mar 19	Apr 19 – Jun 19
Literature review	2 months							
Preparing protocol		2 months						
Protocol assessment			1 month					
Ethics application				4 months				
Collecting data					10 months			
Data analysis						2 months		
Writing up – thesis							6 months	
Writing up - paper								3 months

2.10. Funding

The only cost implications of this study include stationery and printing which will be self-funded.

Reference

1. Kurup R, Flemming K, Daniram S, Marks-James S, Roberts Martin R. Hematological and biochemistry profile and risk factors associated with pulmonary tuberculosis patients in Guyana. Tuberc Res Treat. 2016.

CHAPTER 3: ARTICLE

Title: The haematological manifestations of tuberculosis

Authors:

Bahemia IA[a*]

Patel M[b]

Matsena-Zingoni Z[c]

Menezes CN[d]

Affiliations:

[a] Department of Internal Medicine, Chris Hani Baragwanath Academic Hospital and Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.

[b] Division of Haematology, Department of Internal Medicine, Chris Hani Baragwanath Academic Hospital and Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.

[c] School of Public Health, University of the Witwatersrand, Johannesburg, South Africa.

[d] Division of Infectious Diseases, Department of Internal Medicine, Chris Hani Baragwanath Academic Hospital and Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.

Corresponding author:

Dr IA Bahemia

Department of Medicine, Chris Hani Baragwanath Academic Hospital

Johannesburg, South Africa

Tel: +27 795427967

E-mail: imt7@hotmail.com

Total electronic word count: 3850

Abstract word count: 327 words

Keywords: HIV, pulmonary, disseminated, blood, changes, tuberculosis

Intended journal of publication: South African Medical Journal (SAMJ)

ABSTRACT

Background: The exact haematological changes present in tuberculosis, pulmonary and extra-pulmonary, are still very controversial and have not been adequately investigated. The effect of human immunodeficiency virus (HIV) on these haematological manifestations also remains to be explored since most studies on the haematological effect of tuberculosis were performed in the pre-HIV era.

Objective: To evaluate the haematological manifestations of tuberculosis in an evolving HIV era.

Methods: Prospective study of consecutively recruited patients (n=125) with microbiologically or histologically proven confirmed tuberculosis admitted at Chris Hani Baragwanath Academic Hospital between October 2017 and July 2018. Participants were obtained through the National Health Laboratory Service (NHLS). Demographics and relevant medical history were obtained for all participants and their blood results obtained through the NHLS database.

Results: Anaemia was seen in 60.7% of patients with pulmonary tuberculosis (PTB) and 61.1% of extra-pulmonary tuberculosis (ETB) patients (p=0.964). ETB patients had lower white cell counts (WCC) and lower neutrophil counts than PTB patients, 5.59 [3.6-8.1] vs 7.96 [5.27-10.34], p = 0.002, and 4.2 [2.1-7.0] vs 5.5 [3.3-8.4], p = 0.079, respectively. Leucocytosis was only seen with PTB. Compared to the HIV seronegative patients, HIV co-infected PTB patients had lower white cell count (WCC), lymphocytes count, and neutrophil counts, 6.9 [4.8-9.7] vs 9.4 [8.6-11.4], p = 0.004, 0.7 [0.46-1.31] vs 1.51 [1.13-2.05], p = 0.005, and 4.7 [2.8-7.7] vs 8.5 [5.9-10.1], p = 0.011, respectively. Patients co-infected with HIV and tuberculosis had higher levels of WCC, 7.9 [4.6-10.9] vs 5.8 [4.3-8.3], p = 0.047 if they were on combination anti-retroviral therapy (cART).

Conclusion: Anaemia is still the most common manifestation of tuberculosis. We have highlighted the reactive nature of PTB haematological abnormalities, as compared to ETB based on the presence of higher white cell counts in both HIV seropositive and HIV seronegative patients. HIV can blunt some of the previously described haematological manifestations of tuberculosis such as neutrophilia and lymphocytosis among patients with PTB but does not significantly alter the haematological presentation of ETB.

INTRODUCTION

According to the World Health Organization (WHO), there was an estimate of 10 million new cases of tuberculosis globally in 2017. In South Africa, tuberculosis had an incidence rate of about 500000 cases in 2017 and accounted for 6.5% of all deaths in 2016.[1, 2] Haematological changes can provide early clues to the presence of tuberculosis, especially in cases where specimen collection is invasive. Over the years, several different types of blood abnormalities have been described in patients with both pulmonary tuberculosis (PTB) and extra-pulmonary tuberculosis (ETB). The more common haematological abnormalities associated with PTB are a reactive thrombocytosis and a leucocytosis.[3, 4]

ETB can prove to be diagnostically challenging, especially when confined to a site such as the bone marrow or abdomen. One of the earlier publications from 1943, described a spectrum of possible changes including monocytosis, basophilia, leucocytosis, leucopenia, anaemia, "leukemoid reactions" and pancytopenia.[5] Since then, studies have confirmed or disputed these findings[3, 4, 6-8] : pancytopenia has been reported by some authors as an unlikely manifestation of tuberculosis.[9] Some haematological abnormalities present in tuberculosis also have patient outcome implications: lymphopenia has been found to be an independent predictor of mortality in PTB.[10]

Since most studies on the haematological effect of tuberculosis were performed in the pre-HIV era, there is still some uncertainty about the haematological changes present in patients with tuberculosis co-infected with HIV. With an estimated overall HIV prevalence of 13.1% in 2018 in South Africa, there is a need to re-evaluate the haematological manifestations of tuberculosis in the South African population.[11] This study was proposed to re-evaluate the haematological manifestations of tuberculosis and determine the impact of HIV on these manifestations.

METHODS

Patients admitted to the department of Internal Medicine at Chris Hani Baragwanath Academic Hospital (CHBAH) with microbiologically or histologically proven confirmed tuberculosis, were prospectively and consecutively recruited between October 2017 and July 2018. The Department of Internal Medicine at CHBAH has 693 general medical beds, and admits approximately 3000 patients per month.[12] It serves patients from Soweto and the rest of the Gauteng province.[12] A list of hospitalized patients that tested positive for tuberculosis was obtained from the National Health Laboratory Service (NHLS) laboratory. This included patients with: 1) positive Xpert® MTB/RIF (GXP) on sputum or pleural fluid, FNA of lymph node, ascitic fluid or CSF, 2) positive auramine stain of sputum, pleural fluid, 3) positive Ziehl Neelsen (ZN) on FNA of lymph node, 4) positive culture of *Mycobacterium tuberculosis* on sputum, pleural fluid, fine needle aspirate (FNA) of lymph node, CSF, blood and bone marrow aspirate, 5) histological diagnosis of tuberculosis on tissue, lymph node or trephine biopsy. Exclusion criteria were: pregnancy, established chronic kidney disease not related to tuberculosis, established chronic liver disease not related to tuberculosis, haemoglobinopathies and malaria. Written informed consent was obtained from all participants that were admitted to the hospital. For discharged patients, their records were accessed through the archives of the hospital. This study was approved by the Human Research Ethics Committee (HREC) (Medical) of the University of the Witwatersrand (M170633).

Procedures

Demographics and relevant medical history were obtained for all participants. The NHLS database was accessed to obtain their laboratory results. The following were collected: full blood count, differential count, reticulocyte production index (RPI), international normalised ratio (INR), activated partial thromboplastin time (PTT), iron, ferritin, transferrin, B_{12}, folate,

electrolytes, urea, creatinine, calcium. If performed, bone marrow aspirate and trephine results were also collected.

Definitions

The modified WHO grading system for blood parameters was used: anaemia: Hb < 11 g/dL, polycythemia: Hb >18.5 g/dL +/- HCT > 0.52 L/L (males), Hb >16.5 g/dL +/- HCT > 0.48 L/L (females), leucopenia: WCC < 4 x 10^9/L, leucocytosis: WCC > 11 x 10^9/L, neutropenia: neutrophil count < 2 x 10^9/L, neutrophilia: neutrophil count > 7.5 x 10^9/L, lymphopenia: lymphocyte count < 1 x 10^9/L, lymphocytosis: lymphocyte count > 4 x 10^9/L, monocytosis: monocyte count > 0.8 x 10^9/L, monocytopenia: monocyte count < 0.2 x 10^9/L, clinical thrombocytopenia: platelet (Plt) count < 100 x 10^9/L, thrombocytosis: Plt count > 450 x 10^9/L.

Statistical analysis

Data was captured using Microsoft Excel® and transferred onto Stata® 12.0 for statistical analysis. Age and other non-normally distributed variables were presented using medians and interquartile ranges. Normally distributed parameters such has Hb were presented using means and standard deviations. Continuous data was compared using the t-test if normally distributed, Hb as an example, or the Mann-Whitney test if not normally distributed as in the case of white cell count (WCC). Comparison of categorical data such as leucopenia was performed using the Chi-square test. A p-value of < 0.05 was considered as being statistically significant.

The blood results of the following groups of patients were then compared: PTB vs ETB, PTB co-infected with HIV vs ETB co-infected with HIV (PTB HIV+ vs ETB HIV+), PTB on cART vs ETB on cART, PTB HIV seronegative vs ETB HIV seronegative (PTB HIV- vs ETB HIV-), PTB on cART vs PTB pre cART, ETB on cART vs ETB pre cART, TBHIV+ vs TBHIV-, PTB

co-infected with HIV vs PTB HIV seronegative (PTB HIV+ vs PTB HIV-), ETB co-infected with HIV vs ETB HIV seronegative (ETB HIV+ vs ETB HIV).

RESULTS

In this study, data on 125 patients were collected over a period of 1 year. The baseline characteristics of the study population are illustrated in Table 1. About 90% of patients were in the age group 18-60 years. PTB was diagnosed through sputum GXP or pleural fluid GXP. ETB was diagnosed on lymph node GXP, CSF GXP, ascitic fluid GXP, tissue or bone marrow trephine. In our sample, 26.4% were cigarette smokers and 16.1% consumed alcohol. Among patients that were HIV seronegative, 32% had other identifiable risk factors for tuberculosis. These included diabetes mellitus, smoking, malignancy or alcohol use.

i) PTB vs ETB

Most patients with anaemia were normocytic (68%) and either hypochromic (57.3%) or normochromic (41.3%). Table 2 and 3 illustrates the differences in the haematological profile between the PTB and ETB groups. Patients with PTB had a significantly higher WCC than with ETB (7.96 [5.27-10.34] vs. 5.59 [3.6-8.1] p=0.002). Leucocytosis was only seen in patients with PTB, this was mainly due to high neutrophil counts. Neutrophils were found to be higher among PTB patients approaching statistical significance (5.5 [3.3-8.4] vs. 4.2 [2.1-7.0], p = 0.079) but these were still within the normal range. No statistically significant difference in neutropenia or neutrophilia was present between the two groups. All other blood results, including haemoglobin and platelet count were similarly distributed between the two groups. One patient in the PTB group had a platelet count above 1000 x 10^9/L. Patients with tuberculous meningitis showed no cytopaenias besides anaemia. Polycythemia was an incidental finding on one male PTB patient (Hb = 18.5 g/dL, HCT = 0.562).

ii) PTB HIV+ vs ETB HIV+

Our sample consisted of 80.8% seropositive patients, 76.4% with PTB and 91.6% with ETB. Among our HIV seropositive patients (76.4%), we observed anaemia in 60.8% of patients, 67.6% in the PTB and 63.6% in the ETB group but this difference was not statistically significant. WCC was again statistically significantly higher in the PTB HIV+ group (6.77 [4.8-9.7] vs. (5.27 [3.39-8.77] p= 0.033). Protein and albumin levels were lower among patients with PTB HIV+, but the difference was only statistically significant for protein levels ([71.6 ±+/- 12.8 vs. 77.6 +/- 11.1, p = 0.035] and [25.9 +/-6.4 vs. 28.5+/-7.4, p=0.099], respectively). No difference in neutrophil count, lymphocyte count, platelet count or electrolytes were observed between these two groups.

iii) PTB HIV- vs ETB HIV-

In HIV seronegative patients, the only abnormalities were a higher WCC and neutrophil count in the PTB group (9.9 [8.6-11.4] vs. 5.8 [5.7-7.2], p=0.026) and (8.51 [5.85-10.13] vs. 3.75 [3.05-4.45], p=0.030). All other measured parameters were similarly distributed between the HIV seronegative PTB and ETB groups.

iv) PTB on cART vs ETB on cART

Among HIV seropositive patients on cART, WCC was similarly distributed between the PTB and ETB groups. The following parameters were lower among patients with ETB on cART compared to patients with PTB on cART: Hb (9.6 ± 2.3 vs. 11.3 +/- 2.3, p=0.043), albumin (25.4 ± 7.4 vs. 32.9 ± 8.9, p= 0.020), protein (71.4 ± 11.0 vs. 80.1 ± 5.2, p=0.310), AST (56 [30-91] vs 27 [24-40], p=0.049), total bilirubin (9 [6-17] vs 6 [4-6], p= 0.071), conjugated bilirubin (5 [4-12] vs. 3[2-3], p=0.002). The rest of the blood parameters were not different between the two groups.

v) PTB pre cART vs ETB pre cART

In the 64 HIV seropositive patients pre cART, we observed statistically significantly higher levels of WCC in the PTB group (6.1 [4.8-8.4] vs. 4.8 [3.0-7.3] p = 0.043). All other parameters were similarly distributed between these two groups.

vi) *EFFECT OF CART*

When compared to the pre cART group, patients on cART (combined PTB and ETB) had higher levels of WCC and platelet count (7.9[4.6-10.9] vs. 5.8 [4.3-8.3], p = 0.047) and (316 [217-436] vs. 233 [119-318], p = 0.020), respectively. The effect of cART was then assessed among patients with PTB and ETB separately. No statistically significant haematological changes were seen between PTB pre cART and PTB on cART. Of note, PTB patients on cART demonstrated lymphopenia in 73.3%. The significant differences attributable to cART in patients with ETB were a higher haemoglobin 11.3 ± 2.3 vs 9.3 ± 2.2 (p = 0.026) and higher albumin levels 32.9 ± 8.9 vs 26.4 ± 5.8 (p = 0.028). This is illustrated in Table 4.

vii) EFFECT OF HIV

Table 5 illustrates the significant differences in blood results between HIV seropositive and HIV seronegative patients. Hypoalbuminaemia was present in 80.8% of our study population, being present in 88.1% of HIV seropositive patients and 50% of HIV seronegative patients. Neutrophilia was present in 32.7% of patients with PTB and 26.8% if co-infected with HIV.

viii) PTB HIV+ vs PTB HIV-

In Table 6, we can observe the significant differences attributable to HIV on the patients diagnosed with PTB. The PTB HIV+ group had statistically significantly lower white cell counts, haemoglobin, neutrophils, lymphocytes, monocytes and albumin. However, despite these parameters being lower in the PTB HIV+ group, they were still within the normal range.

Thirty-six patients (28.8%) were diagnosed with ETB, most of whom (91.7%) were HIV seropositive. Lymphopenia was common among HIV seropositive patients in this group at 73.9%.

ix) ETB HIV+ vs. ETB HIV-

Lymphopenia was observed in 80% on patients in the ETB HIV+ group. Levels of lymphocytes in the upper ranges of normal were seen in patients with ETB that were HIV seronegative. We did not observe any difference between the results of the ETB HIV+ vs ETB HIV- groups.

x) Bone marrow aspirates and trephine

Only six patients had a bone marrow aspirate and trephine biopsy done. Four of the patients had a diagnosis of PTB and the remaining two, ETB, based on the method of initial diagnosis. Four patients had a bicytopenia and two patients a pancytopenia. Overall, no features of tuberculosis were observed on any bone marrow aspirates. Plasmacytosis was not seen on any of the samples. Four bone marrow trephine specimens had evidence of tuberculosis with granulomata in all four and acid fast bacilli observed in two. All of these patients either had a pancytopenia or a bicytopenia in their peripheral blood. All the patients exhibited a peripheral lymphopenia and half of them had a blood monocytopenia. The presence of bone marrow granulomata was associated with thrombocytopenia in all cases. There was no evidence of a neoplastic infiltrate in any of the trephine biopsies.

xi) Nutritional parameters

Strangely, no patient exhibited iron, B_{12} or folate deficiency.

xii) Haemostatic changes

There was no statistically significant difference in the INR or PTT between patients with PTB vs. ETB. One PTB patient had bleeding in the form of mild haematemesis. His blood results showed a mildly deranged INR and liver function tests (LFT). One ETB patient presented with epistaxis. He had a Plt count of 38, a normal INR and mildly deranged LFTs. His BMA/T confirmed infiltration with tuberculosis. Thrombotic manifestations were not observed in our patient population.

xiii) Clinical features

The clinical features of our patients are illustrated in Table 7. Patients with ETB were statistically significantly more likely to have lymphadenopathy ($p = 0.011$). No statistically significant difference in pallor, wasting, clubbing, hepatomegaly or splenomegaly was observed.

DISCUSSION

To our knowledge this was the first South African study to highlight the haematological manifestations of tuberculosis in the HIV era. A total of 125 patients were reviewed in this study. The median age was 37 years (IQR 30-47), 61.6% were males, 38.4% females with a male to female ratio of 1.6:1. In comparison, the global male to female ratio of new cases of tuberculosis in 2017 was 1.8:1.[2] The majority of our patients (75%) were in the working age group which is in line with the study of Murray, who pointed out that the heaviest burden of tuberculosis falls on the most productive members of society.[13]

Anaemia is still the most common manifestation and that is irrespective of mode of diagnosis, HIV status or use of anti-retroviral therapy. There was no statistically significant difference in the degree of anaemia between PTB and ETB. At 60.7% in the PTB group, our reported frequency of anaemia was similar to a study in the pre-HIV era by Morris *et al*.,[4] but much lower than the 76% reported by Baynes *et al*.[14] It is noteworthy that these studies used different definitions of anaemia. While Morris *et al.,* used Hb < 13 g/dL for males and < 11 g/dL for females, Baynes *et al*., used Hb <14.3 g/dL in males and Hb < 12.1 g/dL for females.[4, 14]

Similar to Amilo *et al*., anaemia was significantly more severe in our patients co-infected with HIV.[15] Among our ETB patients, anaemia occurred in 61.1%, which is the approximate average value of previously published studies. Lombard and Mansvelt reported anaemia at 72% in their cohort of patients with miliary tuberculosis and Maartens *et al*., observed anaemia in 52% of their patients.[3, 16]. Both studies used similar cut-offs for anaemia (Lombard and Mansvelt: < 13 g/dL for males and < 11.5 g/dL for females, Maartens *et al.*,: < 13 g/dL for males and < 11 g/dL for females). The mechanism of anaemia is probably similar to the anaemia of other chronic disorders. Tuberculosis, through the release of IL-6, stimulates

hepcidin synthesis which in turn causes internalisation and degradation of ferroportin in reticuloendothelial cells and duodenal enterocytes.[17] The net effect is reduced iron availability for erythropoiesis. On the other hand, most of our patients exhibited high levels of ferritin, and low iron level which is thought to be related to IL-1 activation by monocytes. Patients with both PTB and ETB showed a significantly higher degree of anaemia if co-infected with HIV.

Polycythemia has not been described in the literature as a manifestation of tuberculosis. We observed polycythemia in one PTB patient and the most likely aetiology was a strong smoking history.

In our study population, PTB was associated with significantly higher white cell counts than ETB. This was a consistent finding across the following groups: PTB HIV+ vs ETB HIV+, PTB HIV- vs ETB HIV-, PTB pre cART vs ETB pre cART. Leucocytosis was only observed among patients with PTB. This supports the previously reported findings in the literature.[3, 4] The PTB group on cART had similar white cell counts to the PTB HIV seronegative group. This suggests that anti-retroviral therapy restores the body's ability to mount an immune response to tuberculosis.

Overall, co-infection with HIV was associated with lower levels of white cell count across all PTB groups. For example, lower white cell counts, neutrophil counts and lymphocyte counts were seen in the PTB HIV+ group compared to the PTB HIV- group. This is also in agreement with other studies in the literature.[15, 18] This could explain why the 59.6% of our PTB patients had lymphopenia as our cohort consisted of a majority of HIV seropositive patients. We observed lymphopenia in only 18.1% of our HIV seronegative patients. Lymphopenia has classically been associated with ETB and not PTB. In the subset PTB HIV+, 70.7% of patients had lymphopenia demonstrating the compounding effect of HIV on lymphopenia in patients with PTB. However, even PTB patients on cART demonstrated lymphopenia at 73.3%.

Among ETB patients, lymphopenia was highly prevalent, at 73.9% of all ETB patients and at 80% in the ETB HIV+ group. Levels of lymphocytes in the upper ranges of normal were seen in patients with ETB that were HIV seronegative. In comparison, Maartens *et al*., reported lymphopenia in 87% of their patients with milliary tuberculosis using a cut-off of $<1.5 \times 10^9$/L.[3] Lymphopenia in tuberculosis is thought to be a reaction to the mycobacterial infection and not due to an underlying immunodeficiency.[19] T4 lymphocytes are recruited at the areas of granuloma formation.[20] Lymphopenia is of clinical relevance as it has been established as an independent predictor of mortality in patients with PTB.[10] Morris *et al.*, reported a very small number of patients with lymphocytosis in their cohort of PTB patients. Our results support this finding with the absence of lymphocytosis in all of our patients.

It appears that cART also has significant effects on neutrophils. Neutrophilia was present in 32.7% of patients with PTB and 26.8% if co-infected with HIV. This is lower than the 40% reported by Morris *et al*.[4] The average neutrophil counts of PTB patients was lower in the HIV seropositive group than in the HIV seronegative group. Higher levels of neutrophils occurred among patients on cART than those not on cART but this was not statistically significant. It seems that HIV impairs the neutrophilic response to tuberculosis and cART is useful in its restoration.

All of our six patients with evidence of tuberculosis on bone marrow were HIV seropositive. While they all exhibited a lymphopenia, half of them demonstrated monocytopenia. These results are in accordance with those of Lombard and Mansvelt who described lymphopenia and monocytopenia in their 25 HIV seronegative patients.[16] Cucin *et al.*, reported monocytosis in 22% of their patients with miliary tuberculosis.[21] In contrast, monocytosis was more common with PTB than ETB (17.8% vs 8.3%) in our study. In comparison with previous studies that demonstrated plasmacytosis in some cases, none of our patients had this finding but this might be due to the small number of bone marrow aspirates performed.[4, 22]

Thrombocytosis has been reported as a haematological manifestation of pulmonary tuberculosis. Our cohort consisted of 15.7% of PTB patients with thrombocytosis with one patient having a platelet count above 1000 x 10^9/L. Thrombocytosis was also observed by Baynes *et al*., and was reported at a similar proportion (12.5%) by Kutiyal *et al*.[23, 24]

No significant differences were seen in platelet counts in the group comparisons. Clinical thrombocytopenia was relatively uncommon in our sample. It was present at 11.2% in the PTB HIV+ group and at 13.9% in the ETB HIV+ group. Of note, it was uniformly present in all four patients who had bone marrow infiltration with granulomata. Cucin *et al.*, and Maartens *et al.*, have previously reported the correlation between thrombocytopenia and the presence of granulomata in the bone marrow.[3, 21]

The absence of cytopaenias among patients with tuberculous meningitis suggests that tuberculous meningitis is more of a localised disease. Patients diagnosed through tissue biopsies, through GXP on lymph node aspirates or bone marrow trephine biopsies showed more cytopaenias.

Hypoalbuminaemia was evident in 80.8% of our study population, being present in 88.1% of the HIV seropositive patients and 50% of the HIV seronegative patients. This is similar to the study by Kutiyal *et al*., who observed hypoalbuminaemia in 75% in their series.[24] Some studies among Western Europeans have suggested that the active inflammatory process in tuberculosis might cause folate depletion.[25] However, in contrast with Line *et al*., low serum folate levels (<4.5 nmol/L) were not observed in our patients.[26]

Lymphadenopathy, present in 61.1% of ETB patients, was the only clinical sign that was statistically significantly higher compared to PTB patients. This was much higher than the 21% reported by Maartens *et al.*[3] Hepatomegaly, on the other hand, was seen in 12% of ETB patients which was lower than previous studies.[3, 16] Hepatomegaly and splenomegaly were

similarly distributed among PTB, PTB HIV+, ETB and ETB HIV+ groups. The absence of any thrombotic manifestations in our sample was unexpected since tuberculosis and HIV have both been described as pro-coagulable states.[27, 28]

Limitations

The strength of this single centre study was that it was prospective, however, the total sample size was 125 patients and this meant that all sub-group analyses performed were on much smaller sample groups. While some sub-groups such as the PTB probably had numbers large enough for relevant analysis, others, such as the ETB subgroup did not. We could not establish the effect of co-trimoxazole prophylaxis on our patients since a very small number were on this therapy.

CONCLUSION

Anaemia is still the most common manifestation of tuberculosis. We have highlighted the reactive nature of PTB haematological abnormalities, as compared to ETB based on the presence of higher white cell counts in both HIV seropositive and HIV seronegative patients. Co-infection with HIV was associated with lower levels of haemoglobin, white cell count, neutrophil count and lymphocyte count among patients with PTB. The end result is that HIV seems to blunt the reactive response to PTB to some extent. Further studies are required to evaluate the effect of HIV on ETB.

Table 1: Baseline characteristics of study population (n=125) stratified according to site of tuberculosis.

Characteristic	n	PTB	ETB
Participants	125	89 (71.2%)	36(28.8%)
Gender – Male	77	57(64.0%)	20(55.6%)
Female	48	32 (36.0%)	16 (44.4%)
Age*	37 (30-47)	38 (31-50)	35 (25-39)
HIV seropositive	101(80.8%)	68 (76.4%)	33 (91.7%)
CD4 count*	100	74 (17-188)	37 (14-75)
VL*	86	124450 (277-587000)	75100 (12100-709000)
On combination antiretroviral therapy (cART)	37	27	10
Method of diagnosis:			
Sputum GXP	88		
Lymph node aspirate GXP	15		
CSF GXP	12		
Tissue biopsy	3		
Pleural fluid GXP	5		
Bone marrow trephine	4		
AGXP	1		

*Median + IQR, IQR = interquartile range, PTB = pulmonary tuberculosis, ETB = extra pulmonary tuberculosis, HIV = Human Immunodeficiency Virus, VL = HIV viral load, cART = combination anti-retroviral therapy, GXP = Xpert MTB/Rif assay, AGXP – Xpert MTB/Rif assay on peritoneal fluid

Table 2: Comparison of the proportion of haematological abnormalities of patients with PTB against patients with ETB (PTB vs ETB)

	Total	PTB	ETB	p	HIV+	HIV-	p
n	125	89	36		101	24	
Anaemia*	76 (60.8%)	54 (60.7%)	22 (61.1%)	0.96	67 (67.3%)	9(37.5%)	0.010
Polycythemia	1	0	1		1 (1%)		
Leucopenia*	25 (20%)	13 (14.6%)	12 (30%)	0.01	24 (23.8%)	0	
Leucocytosis	19 (15.2%)	19 (21.3%)	0	0.003	13 (12.9%)	10 (41.7%)	0.137
Thrombocytopenia	15 (12%)	10 (11.2%)	5 (13.9%)	0.67	12 (11.9%)	3 (12.5%)	0.933
Thrombocytosis	17(13.6%)	13 (14.6%)	4 (11.1%)	0.50	10 (9.9%)	0	
n	76	52	24		63	13	
Neutropenia*	11(14.5%)	6 (11.5%)	5 (13.9%)	0.28	11 (17.4%)	0	
Neutrophilia	22 (28.9%)	17 (32.7%)	5 (20.8%)	0.22	16 (25.4%)	6 (46.2%)	0.042
n	75	52	23		62	13	
Lymphopenia	48 (64%)	31 (59.6%)	17 (73.9%)	0.34	45 (72.6%)	3 (23.1%)	0.001
n	76	52	24		63	13	
Monocytopenia	14 (18.4%)	9 (17.3%)	5 (20.8%)	0.71	14 (22.2%)	0	
Monocytosis	11 (14.5%)	9 (17.3%)	2 (8.3%)		3 (4.8%)	2 (%)	0.160
Anaemia only**	54 (43.2%)	41 (46.1%)	13 (36.1%)		45 (44.6%)	9 (37.5%)	
Bicytopenia	17 (13.6%)	12 (13.5%)	5 (13.9%)		16 (15.8%)	1 (4.2%)	
Pancytopenia	6 (4.8%)	2 (2.2%)	4 (11.1%)		6 (5.9%)	0	

HIV+ = HIV seropositive, HIV- = HIV seronegative, PTB = pulmonary tuberculosis, ETB = extra-pulmonary tuberculosis, *Grade 1-4, **from grade 1

Table 3: Haematological parameters of the sample population stratified according to site of tuberculosis (PTB vs ETB).

	n	PTB	ETB	p value
WCC (x 10^9/L)*	125	7.96 (5.27-10.34)	5.59 (3.58-8.1)	0.002
RCC (x 10^{12}/L)**	125	3.577 ± 0.88	3.82 ± 0.98	0.789
Hb (g/dL)**	125	10.3 ± 2.7	10.2 ± 2.7	0.816
HCT (L/L)**	125	0.324 ± 0.77	0.324 ± 0.084	0.996
MCV (fL)*	125	86.0 (80.9-90.3)	85.6 (76.3-93.6)	0.840
Plt (x 10^9/L)*	125	262 (144-393)	262 (189-402)	0.817
Neutrophils (x 10^9/L)*	76	5.5 (3.3-8.4)	4.2 (2.1-7.0)	0.079
Lymphocytes (x 10^9/L)*	76	0.82 (0.49-1.49)	0.73 (0.42-1.13)	0.365
Monocytes (x 10^9/L)*	76	0.45 (0.27-0.78)	0.37 (0.21-0.58)	0.213
Eosinophils (x 10^9/L)*	29	0.05 (0.01-0.13)	0.02 (0-0.1)	0.287

PTB = pulmonary tuberculosis, ETB = extra-pulmonary tuberculosis, WCC = white cell count, RCC = red cell count, Hb = haemoglobin, HCT = haematocrit, MCV = mean corpuscular volume, Plt = platelet count, *median + IQR, **mean +/- standard deviation.

Table 4: Comparison of blood parameters of patients with ETB stratified by cART status – only parameters with significant differences shown (ETB pre cART vs ETB on cART).

	n	ETB HIV+	Pre cART	On cART	p
Hb (g/dL)**	33	10.0 ± 2.4	9.3 ± 2.2	11.3 ± 2.3	0.026
HCT(L/L)*	33	0.318 (0.264-0.359)	0.280 (0.253-0.354)	0.325 (0.318-0.440)	0.019
Total bilirubin (µmol/L)*	28	8.5 (5.5-11)	10 (6-13)	6 (4-6)	0.006
Conjugated bilirubin (µmol/L)*	28	3.5 (3-6)	6 (3-6)	3 (2-3)	0.005
B12 (pg/ml)*	13	684 (424-858)	766.5 (621-937)	291 (262-381)	0.011
Calcium (mmol/L)**	26	2.06 ± 0.16	2.03 ± 0.12	2.19 ± 0.24	0.042
Albumin (g/L)**	28	28.5 ± 7.4	26.4 ± 5.8	32.9 ± 8.9	0.028
AST (U/L)*	28	42.5 (29-76.5)	51 (37-79)	27 (24-40)	0.014

ETB HIV+ = ETB co-infected with HIV , Pre cART = not on combination anti-retroviral therapy, on cART = on anti-retroviral therapy. Hb = haemoglobin, AST = aspartate transaminase, *median + IQR, **mean +/- standard deviation.

Table 5: Comparison of blood parameters of all patients in study population stratified by HIV status - only parameters with significant differences shown.

Parameter	n	HIV seropositive	HIV seronegative	p value
WCC (x 10^9/L)*	125	6.1 (4.4-9.3)	9.4 (8.0-11.2)	0.001
RCC (x 10^{12}/L)**	125	3.7 ± 0.8	4.1 ± 1.1	0.046
Hb (g/dL)**	125	9.9 ± 2.5	11.5 ± 3.0	0.009
HCT (L/L)*	125	0.31 (0.26-0.36)	0.37 (0.31-0.42)	0.005
Neutrophils (x 10^9/L)*	76	4.7 (2.5-7.6)	7.5 (5.1-9.4)	0.014
Lymphocytes (x 10^9/L)*	76	0.69 (0.39-1.18)	1.51 (1.13-1.93)	0.001
Monocytes (x 10^9/L)*	76	0.38 (0.21-0.59)	0.77 (0.48-0.89)	0.005
Bicarbonate (mmol/L)*	125	20 (17-21)	20.5 (18.5-23.5)	0.027
Calcium (mmol/L)**	85	2.04 ± 0.19	2.15 ± 0.17	0.046
Albumin (g/L)**	104	26.7 ± 6.8	33.0 ± 8.5	0.001
AST (U/L)*	104	53.5 (31-87)	21.5 (17-53)	0.001

WCC = white cell count, RCC = red cell count, Hb = haemoglobin, HCT = haematocrit, MCV = mean corpuscular volume, AST = aspartate transaminase, *median + IQR, **mean +/- standard deviation.

Table 6: Blood parameters of patients with PTB stratified by HIV status - only parameters with significant differences shown (PTB HIV+ vs PTB HIV-)

Parameter	n	HIV seropositive	HIV seronegative	P value
WCC (x 10^9/L)	89	6.9 (4.8-9.7)	9.9 (8.6-11.4)	0.004
Hb (g/dL)	89	9.9 ± 2.6	11.4 ± 2.7	0.030
HCT (L/L)	89	0.305 (0.257-0.358)	0.359 (0.321-0.417)	0.007
Neutrophils (x 10^9/L)	52	4.7 (2.8-7.7)	8.5 (5.9-10.1)	0.011
Lymphocytes (x 10^9/L)	52	0.7 (0.46-1.31)	1.51 (1.13-2.05)	0.005
Monocytes (x 10^9/L)	52	0.39 (0.24-0.56)	0.80 (0.48-0.98)	0.006
ALT	73	31 (14.5-53)	20 (11-33)	0.058
AST	73	64 (34.5-96.5)	22 (18-65)	0.006
GGT	73	77.5 (47-156)	54 (35-84)	0.055
Albumin	73	25.5 (22-30)	32 (28-41)	0.002

WCC = white cell count, RCC = red cell count, Hb = haemoglobin, HCT = haematocrit, MCV = mean corpuscular volume, ALT = alanine transaminase, AST = aspartate transaminase, GGT = gamma-glutamyltransferase, *median + IQR, **mean +/- standard deviation.

Table 7: Clinical features of sample population stratified by mode of diagnosis and HIV sero-status.

Clinical feature	PTB	PTB HIV+	PTB HIV-	ETB	ETB	ETB HIV-
Temperature**	36.8 (36.5-37.4)	36.9 (36.5-37.5)	36.9 (36.5-37.2)	36.7 (36.4-37.2)	36.9 (36.4-37.2)	37.0 (36.0-38.0)
Pallor*	34 (38.2%)	31 (45.6%)	3 (14.3%)	13 (33.3%)	12 (36.4%)	1 (33.3%)
Lymph-adenopathy*	30 (33.7%)	23 (33.8%)	7 (33.3%)	22 61.1%)	22 (66.7%)	0
Wasting*	50 (56.2%)	41 (60.3%)	9 (42.9%)	22 (61.1%)	22 (66.7%)	0
Clubbing*	7 (7.9%)	6 (8.8%)	1 (4.8%)	3 (8.3%)	2 (6.0%)	1 (33.3%)
Hepatomegaly*	14 (15.7%)	12 (17.6%)	2 (9.5%)	4 (12.0%)	4 (12.0%)	0
Splenomegaly*	1 (1.1%)	1 (1.5%)	0	2 (6.0%)	2 (6.0%)	0
Ascites*	3 (3.3%)	2 (3.0%)	1 (4.8%)			
Bleeding manifestations*	1 (1.1%)	1 (1.5%)	0	1	1 (3.0%)	0
Thrombotic manifestations*	0	0	0	0	0	0

** Median + IQR, IQR = interquartile range, *number of patients, PTB = Pulmonary tuberculosis, PTB HIV+ = PTB co-infected with HIV, PTB HIV- = PTB and HIV seronegative, ETB = extra-pulmonary tuberculosis, ETB HIV+ = ETB co-infected with HIV, ETB HIV- = PTB HIV- = ETB and HIV seronegative.

REFERENCES

1. Statistics South Africa. Mortality and causes of death in South Africa, 2016: Findings from death notification. Pretoria: Statistical release P0309.3 2019.
2. World Health Organization. Global tuberculosis report 2018. Geneva: Licence: CC BY-NC-SA 3.0 IGO.; 2018.
3. Maartens G, Willcox PA, Benatar SR. Miliary tuberculosis: rapid diagnosis, hematologic abnormalities, and outcome in 109 treated adults. Am J Med. 1990;89(3):291-6.
4. Morris CD, Bird AR, Nell H. The haematological and biochemical changes in severe pulmonary tuberculosis. Q J Med. 1989;73(272):1151-9.
5. Muller GL. Clinical Significance of the blood on tuberculosis. New York: The Commonwealth Fund; 1943.
6. Glasser RM, Walker RI, Herion JC. The significance of hematologic abnormalities in patients with tuberculosis. Arch Intern Med. 1970;125(4):691-5.
7. Twomey JJ, Leavell BS. Leukemoid reactions to tuberculosis. Arch Intern Med. 1965;116:21-8.
8. Cameron SJ. Tuberculosis and the blood-a special relationship? Tubercle. 1974;55(1):55-72.
9. Hunt BJ, Andrews V, Pettingale KW. The significance of pancytopenia in miliary tuberculosis. Postgrad Med J. 1987;63(743):801-4.
10. Barnes PF, Leedom JM, Chan LS, Wong SF, Shah J, Vachon LA, et al. Predictors of short-term prognosis in patients with pulmonary tuberculosis. J Infect Dis. 1988;158(2):366-71.
11. Statistics South Africa. Mid-year population estimates 2018. Pretoria: Statistical release P0302; 2018.
12. Chris Hani Baragwanath Hospital. Department of Internal Medicine [Available from: https://www.chrishanibaragwanathhospital.co.za/]
13. Murray CJ. World tuberculosis burden. Lancet. 1990;335(8696):1043-4.
14. Baynes R, Flax H, Bothwell T, Bezwoda W, MacPhail A, Atkinson P, et al. Haematological and iron-related measurements in active pulmonary tuberculosis. Scand J Haematol. 1986;36(3):7.
15. Amilo GI, Meludu SC, Ele PU, Ezechukwu C, Onyenekwe C, Ifeanyi Chukwu M. Haematologic indices in pulmonary tuberculosis with or without HIV co-infection in South Eastern Nigeria. Advances in Life Science and Technology. 2013;11:1-7.

16. Lombard EH, Mansvelt EP. Haematological changes associated with miliary tuberculosis of the bone marrow. Tuber Lung Dis. 1993;74(2):131-5.
17. Ganz T, Nemeth E. The hepcidin-ferroportin system as a therapeutic target in anemias and iron overload disorders. Hematology Am Soc Hematol Educ Program 2011. 2011:538-42.
18. Kurup R, Flemming K, Daniram S, Marks-James S, Roberts Martin R. Hematological and biochemistry profile and risk factors associated with pulmonary tuberculosis patients in Guyana. Tuberc Res Treat. 2016: Article ID 6983747.
19. Beck J, Potts H, Kardjito T, Greange J. T4 lymphopenia in patients with active pulmonary tuberculosis. Clin Exp Immunol. 1985;60:49-54.
20. Onwubalili J, Edwards A, Palmer L. T4 lymphopenia in human tuberculosis. Tubercle. 1987;68:195-200.
21. Cucin R, Coleman M, Eckardt J, Silver R. The diagnosis of miliary tuberculosis: Utility of peripheral blood abnormalities, bone marrow and liver needle biopsy. Journal of Chronic Diseases. 1973;26:355-61.
22. Badenhorst CJ, Fourie J, Steyn K, Jooste PL, Lombard CJ, Bourne L, et al. The haematological profile of urban black Africans aged 15-64 years in the Cape Peninsula. East Afr Med J. 1995;72(1):19-24.
23. Baynes R, Bothwell T, Flax H, McDonald T, Atkinson P, Chetty H, et al. Reactive thrombocytosis in pulmonary tuberculosis. J Clin Pathol. 1987;40(6):676-9.
24. Kutiyal AS, Gupta N, Garg S, Hira H. A study of haematological and haemostasis parameters and hypercoagulable state in tuberculosis patients in northern india and the outcome with anti tubercular therapy. Journal of Clinical and Diagnostic Research. 2017;11(2):OC09-13.
25. Cameron S, Horne NW. The effect of tuberculosis and its treatment on erythropoiesis and folate activity. Tubercle. 1971;52(1):37-48.
26. Line DH, Seitanidis B, Morgan JO, Hoffbrand AV. The effects of chemotherapy on iron, folate, and vitamin B12 metabolism in tuberculosis. Q J Med. 1971;40(159):331-40.
27. Hodkinson KE, Mahlangu JN. Deep-vein thrombosis in the era of high HIV and tuberculosis prevalence: A prospective review of its diagnosis and treatment in a quaternary centre. S Afr Med J. 2017;107(10):859-63.
28. Saif MW, Greenberg B. HIV and thrombosis: a review. AIDS Patient Care STDS. 2001;15(1):15-24.

APPENDICES

Appendix 1: Patient information sheet

Patient information sheet

The haematological manifestations of tuberculosis.

Hello,
I am a Medical Registrar (Specialist in Training) from the University of the Witwatersrand. I am investigating the different changes in the blood of patients with tuberculosis.
Since your doctors have diagnosed you with tuberculosis, I would like to invite you to participate to this study.

Why am I doing this study?
Current information indicates that the presence of some abnormalities in the blood can suggest the presence of tuberculosis. In order to prove or disprove this concept I am collecting information on patients that have been diagnosed with tuberculosis. I would like to analyse the blood abnormalities and compare them with several factors such as other medical conditions that you may have besides tuberculosis to determine the changes that are truly from tuberculosis. The Human Research Ethics Committee (Medical) of the University of the Witwatersrand has approved this study.

What would I like from participants in this study?
Following your consent (giving us permission), I would ask you a few questions on your medical conditions, read through your file, perform a physical examination (non-invasive) and take down the results of the blood tests that have already been done on you. The information gathered will not affect your treatment in any way.
All results and information will remain confidential. If you would like any further information, you may contact me on the number below.

Will you have to undergo any procedure if you participate?
No, I will not be taking more blood samples from you or subject you to any procedure. You will not be harmed in any way if you participate in this study.

May you change your mind about participating in this study?
Participation is completely voluntary and will not affect your treatment. You may change your mind at any time without any reason and your information will then not be used.
Please remember that you will remain anonymous. If you feel comfortable about participating in this study, please read and sign the attached consent form.

Important contact information:
For additional information you may contact me, Dr. I Bahemia, on telephone number 0795427967.
Any concerns or complaints can be made to the Human Research Ethics Committee.

Human Research Ethics Committee (Medical) **HREC (Medical) contact details:**
Prof P Cleaton Jones, Tel 011 717 2301, email peter.cleaton-jones1@wits.ac.za
Ms Z Ndlovu/ Mr Rhulani Mkansi/ Mr Lebo Moeng Administrative Officers 011 717 2700/2656/1234/1252 zanele.ndlovu@wits.ac.za; Rhulani.mkansi@wits.ac.za; and Lebo.moeng@wits.ac.za.

Appendix 2: Informed consent

Informed consent form

I, (full name)_____, have read the information sheet provided. I understand what my participation in the study entails and **voluntarily consent** to the use of the collected information. I understand that all the information related to the study is anonymous and cannot be linked to me. I understand that I have the right to withdraw from the study at any time. I also understand that my withdrawal will not affect my treatment.

Participant	**Witness**
Name:	Name:
_____	_____
Signature:	Signature:
_____	_____
Date:	Date:
_____	_____

Appendix 3: Poster

CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

DEPARTMENT OF MEDICINE
P.O. BERTSHAM
2013
TEL: +27 11 933 0377
FAX: +27 11 933 9449

Participants needed.

Have you diagnosed tuberculosis on your patient?
We are looking for adults with confirmed tuberculosis.

We are conducting a study on the haematological manifestations of tuberculosis at Chris Hani Baragwanath academic hospital.

THE INCLUSION CRITERIA ARE:

- Adult population (≥18years old)
- Microbiological and/or histological evidence of tuberculosis

THE EXCLUSION CRITERIA ARE:

- Pregnancy
- Patients with established chronic kidney disease not related to tuberculosis
- Patients with established chronic liver disease not related to tuberculosis
- Patients with a previous diagnosis of tuberculosis over the last year
- Haemoglobinopathies
- Malaria

This study has been reviewed and approved by the Health Research Ethics Committee (Medical) of the University of the Witwatersrand.

If you have patients that qualify for this study, please contact me, Dr. I Bahemia, on telephone number 0795427967.

Contact Imtiaz Bahemia 0795427967, WhatsApp, SMS or phone
Contact Imtiaz Bahemia 0795427967, WhatsApp, SMS or phone
Contact Imtiaz Bahemia 0795427967, WhatsApp, SMS or phone
Contact Imtiaz Bahemia 0795427967, WhatsApp, SMS or phone
Contact Imtiaz Bahemia 0795427967, WhatsApp, SMS or phone
Contact Imtiaz Bahemia 0795427967, WhatsApp, SMS or phone
Contact Imtiaz Bahemia 0795427967, WhatsApp, SMS or phone
Contact Imtiaz Bahemia 0795427967, WhatsApp, SMS or phone
Contact Imtiaz Bahemia 0795427967, WhatsApp, SMS or phone
Contact Imtiaz Bahemia 0795427967, WhatsApp, SMS or phone
Contact Imtiaz Bahemia 0795427967, WhatsApp, SMS or phone
Contact Imtiaz Bahemia 0795427967, WhatsApp, SMS or phone

Appendix 4: Ethics clearance certificate

R14/49 Dr Imtiaz Bahemia et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170633

NAME: (Principal Investigator)	Dr Imtiaz Bahemia et al
DEPARTMENT:	Internal Medicine Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	The Haematological Manifestations of Tuberculosis
DATE CONSIDERED:	30/06/2017
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Moosa Patel
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	04/08/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in June and will therefore be due in the month of June each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

Date 24/08/2017

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 5: Data collection sheet

DATA COLLECTION SHEET

All information contained in this questionnaire is strictly confidential.

Participant number:	**Date of data collection:**

DEMOGRAPHICS

Age	**Gender** ☐ M ☐ F

METHOD OF DIAGNOSIS OF TUBERCULOSIS

☐ **Sputum/TA GXP**	☐ **Sputum/TA culture**	☐ **Bronchial washings**	
☐ **Blood culture**	☐ **LN GXP** ☐ **LN culture**	☐ **Tissue GXP**	☐ **Tissue culture**
☐ **CSF GXP**	☐ **CSF culture**	☐ **Pleural fluid GXP**	☐ **Pleural fluid culture**

☐ **Histology – Tissue biopsy** (not BM)

☐ **Histology – BMT**

Extra-pulmonary TB	☐ Yes	☐ No

Currently on TB treatment?	☐ **Yes**	☐ **No**	**Duration:**

Regimen: RIF ☐ INH ☐ PZA ☐ EMB ☐ MFX ☐ LVX ☐ ETH ☐ TER ☐ CYS ☐ CFZ ☐ LNZ ☐

KAN ☐ AMI ☐ STR ☐ CAP ☐ PAS ☐

RELEVANT MEDICAL CONDITIONS

List medical problems that other doctors have diagnosed

RVD ☐ Y ☐ N if yes, CD4: VL: on cART? ☐ Y ☐ N Duration:

Regimen: TDF ☐ ABC ☐ AZT ☐ D4T ☐ DDI ☐ 3TC/FTC ☐ EFV ☐ NVP ☐ LP/R ☐ ATZ ☐ RAL ☐ ETR ☐ DRV ☐

Co-trimoxazole prophylaxis: ☐ Y ☐ N

Diabetes Melitus ☐ Y ☐ N

Malignancy ☐ Y ☐ N Type:

if yes, on chemotherapy? ☐ Y ☐ N Stage: Grade:

Last chemo date:

History of:

Constitutional symptoms ☐ Y ☐ N

Weight loss ☐ Y ☐ N

Fever ☐ Y ☐ N

Night sweats ☐ Y ☐ N

Bleeding ☐ Y ☐ N

Thrombosis ☐ Y ☐ N

Type of chemotherapy:

Cyclophosphamide ☐ BCNU ☐ Bleomycin ☐ Doxorubicin ☐ DTIC ☐

Etoposide ☐ Prednisone ☐ Rituximab ☐ Vincristine ☐ Vinblastine ☐

Other ☐ Y ☐ N Specify:

HABITS

Smoking ☐ Y, pack year history: ☐ N **Alcohol** ☐ Y ☐ N units/week

Haematological Manifestations of Tuberculosis, Bahemia IA, Patel M. & Menezes CN 2017- Data collection sheet

CLINICAL FINDINGS
General: Pallor ☐ Jaundice ☐ Lymphadenopathy ☐ Wasting ☐ **BP:** / **Temp:**
Chest: Normal breath sounds ☐ Bronchial breathing ☐ Wheezes ☐ Crackles ☐ Absent breath sounds ☐
CVS: Normal heart sounds ☐ Loud P2 ☐ Murmur present ☐ Y ☐ N specify:
Abdomen: Hepatomegaly ☐ Splenomegaly ☐ Ascites ☐ Scrotum/Testes: Atrophy ☐ Enlarged ☐
CNS: Focal signs ☐ Meningism ☐ Y ☐ N **Fundi:** Normal ☐ Abnormal ☐ Specify:
Skin: normal ☐ Abnormal ☐ specify:
Bleeding manifestation: ☐ Y ☐ N
Thrombosis manifestation: ☐ Y ☐ N

RESULTS			
Blood Results			**Bone Marrow Aspirate**
Date			Date
Barcode			Adequacy: Poor ☐ Acceptable ☐ Good ☐ Excellent ☐
WCC (x 10^9/L)			M:E ratio: Normal ☐ Myeloid hyperplasia ☐ Erythroid hyperplasia ☐
RCC (x 10^{12}/L)			Megakaryocytes: Adequate ☐ Decreased ☐ Increased ☐
Hb (g/dL)			Erythropoiesis: Normal ☐ Decreased, normoblastic ☐ Decreased normomegaloblastic ☐
HCT (L/L)			Leucopoiesis: Normal☐ Decreased ☐ Increased ☐ Dysplasia ☐ Transformed ☐
MCH (pg)			Plasma cells: Normal ☐ Increased ☐
MCHC (g/dL)			Macrophages: Normal ☐ Increased ☐
RCDW (%)			Iron store: Normal ☐ Increased ☐ Decreased ☐
MCV (fL)			Cellularity: Normocellular ☐ Hypocellular ☐ Hypercellular ☐
PLT (x 10^9/L)			Evidence of nutritional abnormalities Y ☐ N ☐
MPV (fL)			Evidency of myelodysplasia Y ☐ N ☐
Neutrophils (x 10^9/L)			Evidence of haemophagocytosis Y ☐ N ☐
Lymphocytes (x 10^9/L)			**Bone Marrow Trephine**
Monocytes (x 10^9/L)			Date
Eosinophils (x 10^9/L)			Adequacy: Poor ☐ Acceptable ☐ Good ☐ Excellent ☐ Cellularity: Normocellular ☐ Hypocellular ☐ Hypercellular ☐
Blasts (x 10^9/L)			
Smear			Fibrosis: Y ☐ N ☐
			Necrosis Y ☐ N ☐
			Lymphoid aggregates Y ☐ N ☐
RPI			Malignant infiltrate Y ☐ N ☐
INR			Granulomas: Present ☐ Absent ☐
PTT (s)			AFBs: Present ☐ Absent ☐
Iron (umol/L)			
Ferritin (ng/ml)			
Date			
Transferrin (g/L)			

B12 (pg/ml)			Date		
Folate (ng/ml)			Total bilirubin (umol/L)		
ESR (mm)			Conj. Bilirubin (umol/L)		
Sodium (mmol/L)			Protein (g/L)		
Potassium (mmol/L)			Albumin (g/L)		
Chloride (mmol/L)			GGT (U/L)		
Bicarbonate (mmol/L)			ALP (U/L)		
Urea (mmol/L)			AST (U/L)		
Creatinine (umol/L)			ALT (U/L)		
Calcium (mmol/L)					
Magnesium (mmol/L)					
Phosphate (mmol/L)					

RCC transfusion?	☐ Yes	☐ No
Date		
PLT transfusion?	☐ Yes	☐ No
Date		
Corticosteroid therapy	☐ Yes	☐ No
Duration		

Appendix 6: Turnitin report

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

Ttp://www.bbraun.com
Division of Haematology, Department of Medicine, Chris Hani Baragwanath Academic Hospital, University of the Witwatersrand, Johannesburg
Chris Hani Road, Diepkloof, Soweto. Tel: +27 11 9339377, Fax: +27 11 9339449, email: moosa.patel@wits.ac.za

19 June 2019

The Chair

Postgraduate Studies Committee

Faculty of Health Sciences

University of the Witwatersrand

Re: Turn-it-in report: Dr Imtiaz Bahemia – MMed: 'The Haematological manifestations of Tuberculosis'. Student number: 0503419D

As the supervisor, I have reviewed the Turn-it-in report of Dr Bahemia's MMed dissertation. The report identifies a similarity index of 13%. This is well within the acceptable limits of similarity. Much of this similarity relates to standardized factual information. The other information which bears a similarity has been appropriately referenced.

Thank you

Yours sincerely



Moosa Patel MBChB, FCP(SA), MMed(Wits), FRCP(Lond.), PhD(Wits)

Professor and Head of Clinical Haematology, Department of Medicine, Chris Hani Baragwanath Academic Hospital and the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

0503419d:0503419D_Bahemia_Imtiaz.pdf

ORIGINALITY REPORT

13%	6%	10%	6%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	E.H. Lombard, E.P.G. Mansvelt. "Haematological changes associated with miliary tuberculosis of the bone marrow", Tubercle and Lung Disease, 1993 Publication	1%
2	Knox-Macaulay, H.H.M.. "Tuberculosis and the haemopoietic system", Bailliere's Clinical Haematology, 199201 Publication	1%
3	I.A. Bahemia, A. Muganza, R. Moore, F. Sahid, C.N. Menezes. "Microbiology and antibiotic resistance in severe burns patients: A 5 year review in an adult burns unit", Burns, 2015 Publication	<1%
4	B. J. Hunt, V. Andrews, K. W. Pettingale. "The significance of pancytopenia in miliary tuberculosis.", Postgraduate Medical Journal, 1987 Publication	<1%
5	link.springer.com Internet Source	<1%