CA-125 IN THE DIAGNOSIS AND THERAPEUTIC MONITORING OF TUBERCULOSIS IN HIV POSITIVE PATIENTS

Kenneth Akwue

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DECLARATION

I, Kenneth Akwue, declare that this Research Report is my own work. It is being submitted for the degree of Master of Science in Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

KENNETH AKWUE

Signature: 

Date: 27 May 2021

Place: Johannesburg, South Africa

The Human Research Ethics Committee (Medical)
Clearance certificate: M191082

Dedicated

To
The Lord Jesus Christ
for salvation and reconciliation

My
Teachers
for patiently imparting knowledge

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ABSTRACT

The diagnosis of active tuberculosis (TB) in patients with human immunodeficiency virus (HIV) is difficult, and there are few biomarkers of disease for the diagnosis of TB in HIV-infected patients. Serum CA-125 is a host biomarker that is elevated in pulmonary and extra-pulmonary TB. We investigated the use of serum CA-125 in the diagnosis of active TB in HIV-infected patients. CA-125 concentrations were measured in 109 stored serum samples, using the Roche Cobas 6000 autoanalyzer. Samples were from patients with or without active TB and HIV. Samples collected from individuals with active TB were also analysed at two-months post-treatment. In HIV-uninfected individuals, pre-treatment serum CA-125 concentration was significantly higher in those with TB compared to healthy controls. We calculated the diagnostic potential of CA-125 for active TB in HIV negative patients using a receiver operating characteristic (ROC) curve; CA-125 had a sensitivity, specificity, positive and negative predictive values of 82.4%, 94.7%, 93.3% and 85.7%, respectively at a threshold value of 27 U/mL. After two months of treatment, serum CA-125 concentration reduced significantly ($P < 0.0001$). However, in HIV-infected individuals, there was no significant difference in CA-125 concentration between patients with and without active TB; moreover, following two months of TB treatment, serum CA-125 concentration was not statistically different from pre-treatment concentrations. Serum CA-125 concentration has potential for the diagnosis and therapeutic monitoring of TB in HIV negative patients but less so in HIV positive patients. These findings should be confirmed in future prospective studies.

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INTRODUCTION

1.2 Background

1.2.1 The burden of Tuberculosis and HIV

Tuberculosis (TB) is the leading cause of death from a single infectious agent – more than HIV/AIDS (1). Globally in 2019, approximately 1.4 million deaths were due to TB, including 208 000 HIV-infected people (1,2).

Sub-Saharan Africa accounted for 25% of new TB cases in 2019. Additionally, two-thirds of the worldwide TB burden is from just eight countries, one of which is South Africa (1,2). South Africa has one of the highest TB incidence rates, with 360 000 cases reported for 2019 (3). This high TB incidence is closely related to the high prevalence of HIV co-infection - where the annual risk of developing TB is 10% compared to a lifetime risk of only 8 to 10% in HIV seronegative individuals (4,5). In South Africa, more than half of those newly diagnosed with TB in 2019 were HIV seropositive (3). Also, it is estimated that 44% of people with HIV and TB co-infection are unaware of their co-infection and, as such, are not on treatment (6).

1.2.2 TB Diagnosis and Diagnostic Tools

The diagnosis of TB may involve the use of several clinical and microbiological investigations. The chest radiograph is one of the most frequently used tools for the diagnosis of TB (7,8), so too is sputum smear microscopy, using the Ziehl-Neelsen (ZN) stain, or fluorescent microscopy with auramine-rhodamine (9). TB diagnosis also uses techniques such as growth-based detection (Rapid Culture) on solid medium, for example, Lowenstein-Jensen culture, and automated liquid culture systems (BACTEC, MGIT) (10,11). Tuberculin skin test (TST), and interferon-gamma release assays (IGRA) have proved useful in diagnosing latent TB. However, TST is confounded by BCG vaccination, while IGRA cannot differentiate TB disease from latent TB infection (12,13). Molecular techniques (nucleic acid amplification test, NAATs) include the use of GeneXpert MTB/RIF and Ultra, the newer version of this assay, and the line probe assay (LPA) (7,10,14). Additionally, the use of biomarkers to aid the diagnosis of TB has increased in recent

times (15). These biomarkers include urine lipoarabinomannan, indoleamine 2,3-dioxygenase, gene signatures and others (16–18).

However, the diagnosis of TB is more challenging in HIV positive patients (4,9) as HIV interferes with host immune responses. Recommended tests in HIV positive persons that detect TB by measuring host immune responses, therefore prove less useful than HIV negative patients with TB disease alone. Moreover, there is less cavitation with TB/HIV co-infection with reduced transfer of mycobacteria into respiratory secretions and fewer bacilli are expectorated, resulting in a high prevalence of smear-negative TB amongst HIV positive patients (4,9,19).

The radiographic appearance of pulmonary TB (PTB) in HIV positive patients is often atypical, and diagnosis is further complicated by the frequent occurrence of other HIV-related pulmonary diseases such as bacterial pneumonia. Additionally, radiographic patterns are inconsistent and may appear normal in 25-50% of active TB disease cases in patients with HIV (8,20).

A study in a resource-limited setting found that when using expanded clinical case definitions using symptoms as a screening tool, 88% of smear-negative pulmonary- and extra-pulmonary TB were identified (21). However, a Kenyan study noted that clinical screening missed approximately a quarter of laboratory-confirmed TB cases in TB/HIV co-infected individuals and greater than 70% in HIV seropositive gravid women (22).

Newer nucleic acid amplification tests (NAATs) have improved TB detection in smear-negative patients, with the GeneXpert MTB/RIF Ultra sensitivity reported as approximately 70% (19). However, a 2017 study from Uganda of smear-negative patients with presumed TB infection reported a lower sensitivity of 53% for the GeneXpert (23). Also, GeneXpert MTB/RIF is not recommended in monitoring therapeutic response as it does not differentiate between live and dead bacilli (9). An additional NAAT is the LPA for the detection of multi-drug-resistant (MDR-TB). A recent study from India assessed the utility of the LPA for MDR-TB in smear-negative PTB and reported a sensitivity of 68% (24).

There are other challenges of TB diagnosis in HIV infection, especially in high HIV prevalent resource-limited settings. In these settings, TB case detection rates are 20-35% (25), and autopsy studies suggest less than half of TB in TB/HIV co-infected patients who die in hospital are diagnosed before death (3). This low case detection is due to the rapid progression to critical or

symptomatic illness, which gives little time for detection. Furthermore, the assessment of TB therapy response in patients with TB/HIV co-infection is challenging (3,7,12).

Due to the challenges associated with the diagnosis of TB in HIV positive patients, interest in identifying biomarkers for TB diagnosis has grown (15). CA-125 is a host biomarker that has found use in the therapeutic monitoring of ovarian cancer. Interestingly, CA-125 has been suggested as a potential diagnostic biomarker in PTB due to its elevated serum concentration in patients with active TB (6–9).The next section reviews the biochemistry, metabolism, and other characteristics of CA-125.

1.3 CA-125

1.3.1 Historical perspectives on CA-125

Cancer antigen 125 (CA-125) was described in 1981 by Robert Bast and colleagues during the screening of monoclonal antibodies against an ovarian cancer cell line, OVCA433. They named the antibody ovarian cancer antibody 125 (OC125) as it was the 125th of such antibodies. Subsequently, the antigen that reacts against this antibody was named cancer antigen 125 (CA-125). Bast et al. initially developed OC125 for a therapeutic purpose against ovarian cancer; however, this was later discontinued. The antigen CA-125 was later discovered by researchers to be elevated in serum months or even years prior to diagnosing ovarian cancer (28,29).

In the early 1990s, Ian Jacobs found that a combination of CA-125 analysis with vaginal ultrasound improved the specificity of ovarian cancer detection and combined use reduced false-positive results that could arise from using each independently (29).

In 2001 Lloyd and O'Brien's group showed that CA-125 is a large membrane-bound mucin and clarified its structure, metabolism, and functions. Still, the precise molecular structure of this cancer antigen remains elusive (30–32).

1.3.2 Biochemistry and biosynthesis of CA-125

CA-125 is a high molecular weight transmembrane glycoprotein and an epitope of mucin 16 (or MUC16). MUC16 is expressed in the epithelial cells of the ovary, fallopian tube, endometrium,

and mesothelial cells lining the pleura, pericardium, peritoneum, amnion, and foetal coelomic epithelium (33,34).

MUC16 is encoded by the *MUC16* gene and mapped to the short arm of chromosome 19 (19p13.2). The protein is a large, type I, single-pass, transmembrane glycoprotein with an average molecular weight of approximately 3–5 million Da (30,33,35,36).

MUC16 is categorised into two major domains: the larger cleaved extracellular domain named CA-125, and a conserved domain termed MUC16 ecto [**Figure 1.1**]. In these domains are regions, fragments, and a tail (37).

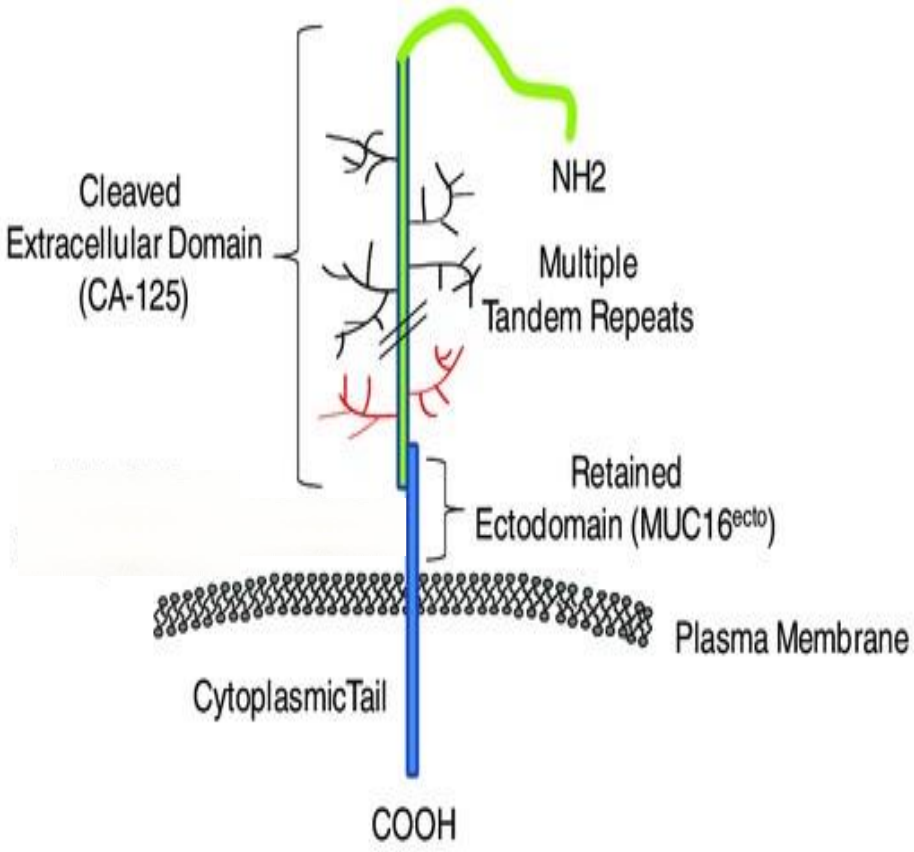

Figure 1.1: Diagram of MUC16 showing CA-125.
Image Attribution: Adapted from – "Schematic Diagram of MUC-16 Structure" by Koneru et al., licensed under CC BY 4.0 (38)

Cleaved extracellular domain

The cleaved extracellular domain (CA-125) consists of:

a) N-terminal region: This is a glycosylated domain of approximately 12,068 amino acids. It contains N- and O-linked glycosylation domains (18,21,22).
b) Tandem repeat region: This region contains 60 glycosylated repeats of a 156 amino acid sequence each. The tandem repeat region interacts with glycoproteins to activate different signalling pathways (18,21,22).

Conserved domain (MUC16 ecto)

The conserved domain, known as MUC16 ecto or the carboxy-terminal domain, is comprised of the:

a) Residual extracellular fragment: This fragment functions as the site of the potential cleavage of MUC16 into CA-125 and MUC16 ecto (18,21,22).
b) Transmembrane region: The single-pass transmembrane region attaches the mucin to the cell membrane resulting in its expression as a cell surface molecule (30).
c) Cytoplasmic tail: Together with the transmembrane region and extracellular portion, the tail constitutes the carboxy-terminal domain. The carboxy-terminal domain contains about 32 amino acids with potential phosphorylation sites. The cytoplasmic tail initiates cytoplasmic phosphorylation, leading to proteolytic cleavage and the extracellular domain release (CA-125) into the circulation (18,21,22).

1.3.3 CA-125 as a host TB biomarker

Several studies (summarised in Table 1.1 below) have reported higher serum CA-125 in patients with pulmonary and extra-pulmonary TB infection compared to healthy controls (40–43). These data supported by a Spanish study evaluated the use of CA-125 in those with culture-positive PTB compared to those with other lower respiratory tract infections. CA-125 was markedly elevated in the PTB group compared to the other respiratory tract infections group, and concentrations of CA-125 subsequently decreased following 2-4 months post TB treatment. Furthermore, sensitivity and specificity of 68.6% and 77.8% respectively, for detecting PTB at a

diagnostic cut-off concentration of 32.5U/mL was reported. The study, which included HIV positive patients, found no association between HIV infection and elevated CA-125 concentrations (42). Compared to Shah *et al.*, the figures for sensitivity and specificity of GeneXpert were 97.8% and 92.6%, respectively (44).A Turkish study compared CA-125 concentrations in three groups to assess the utility of CA-125 as a TB diagnostic biomarker (40). Smear-positive patients were compared to those with prior TB but no current infection and a healthy control group with no prior PTB infection. Serum CA-125 concentration was significantly higher in those with PTB compared to the other groups. Additionally, they reported CA-125 sensitivity and specificity of 97.6% and 100% respectively, to detect active TB infection. This study recommended a CA-125 threshold value greater than 36.35U/mL as diagnostic for TB. Furthermore, following six months of TB therapy, the CA-125 concentrations in the PTB group had declined. A Korean study also supported elevated serum CA-125 as a biomarker of active TB and its subsequent role in therapeutic response monitoring (26).

More recent studies show similar findings. Mohammed *et al.* (27) found that mean CA-125 values were significantly higher in those with active PTB compared to those with pneumonia or healthy controls. This study also assessed the role of CA-125 relative to the severity of disease and noted that serum concentrations were higher in moderate to advanced PTB. Moreover, an Egyptian study found markedly higher pre-treatment CA-125 concentration in patients with TB infection compared to two months post-treatment (45).

Table 1.1: Summary of prior studies on CA-125 in TB

Author/ Date	Outcome aim(s)	Participants	Results	Conclusion
Yilmaz et al. Respir Med. 2001 (41)	"To investigate the value of CA-125, a tumour marker, in the evaluation of PTB activity."	96 participants – 40 active PTB, 20 inactive PTB, 36 healthy controls	Significantly higher serum CA-125 concentrations in active PTB than other groups CA-125 in active PTB significantly decreased post-therapy	CA-125 can differentiate active and inactive PTB
Fortún et al. Open Respir Med J. 2009 (42)	"Retrospective study to assess the efficacy of CA-125 in differentiating PTB from bacterial pneumonia and other respiratory infections."	89 participants – 35 PTB compared to 54 other lower respiratory tract infections CA-125 concentrations from patients with PTB compared with concentrations from non-TB pulmonary infections	Significantly higher serum CA-125 concentrations in the PTB group compared to other lower respiratory tract infections 2 - 4 months post TB treatment, CA-125 decreased in the PTB group	Serum CA-125 could be used when sputum AFB is negative
Sahin et al. Clin Investig Med. 2012 (40)	"To determine if CA-125 concentrations showed promise as a biomarker for differential diagnosis of active from inactive tuberculosis and in post-treatment follow-up."	42 active TB, 35 inactive TB and 20 healthy controls	Serum CA-125 significantly higher in the active TB compared to other groups Significant decrease in CA-125 after treatment in active TB	Serum CA-125 may be used in the diagnosis and monitoring of PTB

Said et al. Egypt J Chest Dis Tuberc. 2013 (43)	To detect: 1. "The role of Ca-125 in differentiating PTB from other pulmonary infections. 2. "To determine the value of CA-125 was an indicator of response to anti-tuberculous drugs."	88 participants – 27 PTB, 33 other pulmonary infections, & 20 healthy volunteers. Serum CA-125 measured and re-assayed after four months of treatment in the PTB group	Significant increase in CA-125 in PTB compared to other groups CA-125 significantly decreased post TB treatment	Ca-125 may be a useful marker in differentiating PTB from other pulmonary infections and in the assessment of response to anti-tuberculous drugs
Kim et al. Tuberculosis. 2013 (26)	"The clinical significance of serum CA-125 measurements in patients with active PTB."	100 active PTB patients enrolled Serum CA-125 measured pre-treatment (baseline), at 6- & 12-months post-treatment	Thirty-eight patients showed elevated CA-125 which decreased post-treatment Elevated CA-125 is associated with involvement of both bilateral & cavitary pulmonary disease	Serum CA-125 concentration related to the severity of PTB.
Mohammad et al. Egypt J Chest Dis Tuberc. 2016 (27)	"To evaluate the diagnostic value of serum concentration of CA-125 in active PTB and its relation to disease severity."	80 patients – 40 PTB, 20 pneumonia, 20 healthy control	Serum CA-125 was significantly higher in PTB compared to other groups Significantly higher serum CA-125 in moderate & advanced PTB than those with milder disease	Serum CA-125 could be used in diagnosis & assessment of the severity of PTB

Abbreviation: AFB, Acid-fast bacilli.

1.3.4 Functional roles in health and diseases

As more about the biological functions of MUC16, the parent molecule of CA-125, is understood, its use in clinical medicine is increasing. MUC16 is expressed normally in the epithelial lining of several organs, and its functions, physiology and pathology are context-dependent.

Role in normal cells

The physiologic functions of MUC16 can be classified into four broad groups (33,35):

i. Lubrication and hydration
ii. Barrier formation to prevent adhesion
iii. Mucosal defences of epithelial cell layers
iv. Protection of the cell surface from attack by microorganisms

Specific examples of these functions are seen in the ocular surface, nasal/tracheal and reproductive tract epithelia.

Ocular surface epithelia

MUC16 is found on the stratified cells of the conjunctiva and cornea and at the micro-plicae tips of apical cells where it constitutes part of the glycocalyx layer of the tear film surface. It prevents adhesion during sleep and blinking by maintaining the lubrication of cells, and contributes to forming a barrier to pathogens and debris (30,33). Altered mucins, including MUC16, contribute to autoimmune diseases like Sjogren's syndrome with dry eyes(46).

Nasal and tracheal epithelia

MUC16 is part of the mucins found in the mucous gel in both the airway epithelial and respiratory mucus in airway secretions. MUC16 and other mucins serve as a barrier to the entry of pathogens and maintain the integrity of respiratory epithelia (33).

Reproductive tract epithelia

Mucins, with the inclusion of MUC16, are found in the female reproductive tract. MUC16 can be detected in cervical mucus, endometrial tissues and uterine epithelium with enhanced shedding during the female cycle (33).

Implantation and foetal development: A decrease in membrane-associated mucin MUC16 at the apical epithelial layer of the uterus occurs before blastocyst implantation (33,47). MUC16 binds to Siglec-9 inducing immune cell expression of interleukin 10 (IL-10), an anti-inflammatory cytokine that promotes placenta angiogenesis (30,48). These observations have led some to suggest that MUC16 may contribute to foeto-maternal tolerance (33,48). A recent study has shown that MUC16 is an inhibitor of implantation (49).

Pathological roles of MUC16

In addition to physiological functions, MUC16 also has several pathological roles even in benign disease. Some pathological, non-malignant, conditions with elevated CA-125 include uterine fibroids, benign ovarian disease, endometriosis, renal and liver disease, heart failure, and infections of the pleura or peritoneum (40,43,50).

Tyler et al. suggest that serum CA-125 is a novel biomarker for detecting and monitoring preeclampsia based on increased binding of MUC16 to immune cells in preeclampsia (48). A recent systematic review and meta-analysis also demonstrated an increased serum CA-125 in women with preeclampsia (51).

In 2016, *Gunn et al.* (52) reported enhanced binding of antibodies to MUC16 at mucosal sites in chronic HIV infection. They suggested this may be useful when designing next-generation vaccines and monoclonal antibodies to improve mucosal immunity. More recently, a study of 13 cancer biomarkers found CA-125 to be elevated in SARS–CoV-2 infection and the pathophysiological stage of the disease was associated with the measured concentration of the biomarker (53).

Role in tumorigenesis

CA-125 is elevated in several malignancies; these include ovarian, lung, breast, colon, and pancreatic carcinoma (40,43,50,54).

As an epitope of MUC16, CA-125 is involved in immune protection, metastasis, chemotherapy resistance, and survival of cancer cells.

Immunoprotection and cancer metastasis:

The membrane-bound MUC16 acts as a barrier that prevents the formation of a synapse between cancer cells, and the potent anti-tumour immune agent NK cells. The cleaved extracellular domain (CA-125) interacts with the NK cell inhibitory receptor, Siglec-9, leading to the immune cell secretion of IL-10, an immunosuppressive cytokine, which promotes angiogenesis in cancer cells (30,55–57). Lastly, MUC16 (CA-125) has been implicated in immunoprotection by reducing the expression of NK cell-stimulating protein, CD16. Attenuated expression of CD16 reduces NK cells ability to cytolyse cancer cells (55).

Chemotherapy resistance and cancer cell survival:
Boivin *et al.* (58) show that the MUC16 carboxy-terminal domain contributes to Cisplatin resistance and, in particular, MUC16 suppresses apoptosis in ovarian cancer. Although the precise mechanism is not fully understood, research suggests the suppression of apoptosis may involve the extracellular signal-regulated kinase (ERK) signalling pathways (58,60).

Additionally, MUC16 was found to mediate survival, proliferation and chemoresistance in lung cancer via upregulating the expression of testis-specific Y-like protein 5 (TSPYL5), which suppresses the tumour suppressor p53 gene (60,61).

1.3.5 Clinical applications of CA-125

Measurement of serum CA-125 has found clinical use as a potential target for several diagnostic and therapeutic purposes.

An elevated level of CA-125 is used in combination with Human Epididymis Protein 4 (HE4), another ovarian cancer marker, as part of Risk of Ovarian Malignancy Algorithm (ROMA). This combination of biomarkers is used in monitoring residual or recurrent ovarian cancer following treatment (62).

Advances in immunotherapy have increased interest in MUC16 as a potential target for therapy. One of these is the anti-idiotype CA-125 (ACA-125) vaccine (Oregovomab, Abagovomab) which has shown limited benefits in clinical trials (61,63,64). Additional studies have focused on monoclonal antibodies targeting MUC16 ecto (the retained carboxy-terminal domain of MUC16) since it is not cleaved and shed into the circulation like the extracellular domain (CA-125) (39,65).

Table 1.2 below summarises the present and potential clinical applications of CA-125.

Table 1.2: Current & potential applications of CA-125 (MUC16)

Applications	**Disease/ Intervention**	**Uses**		**References**
		Current	**Potential (Predicted)**	
Diagnosis/ Monitoring	Ovarian cancer	Combined biomarker	-	(62)
	Pancreatic cancer	-	Combined biomarker	(66,67)
	Colorectal cancer	-	Combined biomarker	(68)
	Endometrial cancer		Combined biomarker	(69)
	Endometriosis	-	Rule-in test	(70,71)
	Preeclampsia	-	Detection & monitoring	(43,44)
	Tuberculosis	-	Diagnosis & monitoring	(26,42)
Therapy	Anti-idiotype CA-125 Vaccines	-	Anti-idiotype antibodies against CA-125	(61,63,64)
	Monoclonal Antibodies	-	Monoclonal antibodies against carboxy-terminal of MUC16	(39,65)
	Antibody-Drug Conjugates	-	Anticancer agent coupled to antibody targeting MUC16	(72,73)
	Chimeric Antigen Receptor-modified T cells Therapy	-	T cells genetically engineered to express an artificial T cell receptor that recognises MUC16 ectodomain	(38,74,75)

1.4 Problem Statement

The diagnosis of TB in HIV positive patients, who are often smear-negative, is a challenge to clinicians. Due to poor host immune response caused by severe immunosuppression in patients with HIV and TB co-infection, the diagnosis of TB and subsequent therapy initiation is often delayed. Moreover, monitoring treatment response is difficult (8,9,19,76). It has been noted that in HIV positive individuals, the progression from TB infection to active TB disease is rapid, with high rates of extra-pulmonary TB (8,19). Additionally, in the setting of patients with previous PTB infection, chest x-rays and molecular diagnostics like GeneXpert are limited in diagnosing active TB, whilst culture-based methods are limited by the lengthy time to diagnose. These factors may mean that immunocompromised individuals may be commenced on treatment late or even never receive treatment before death (3). As such, a diagnostic test to potentially improve case detection in this population will be of clinical value in determining whether clinicians should initiate TB therapy. The host biomarker CA-125 could assist in the early initiation of treatment in high-risk individuals and possibly ascertain response to treatment.

Furthermore, TB biomarkers could bridge the gap between preclinical and clinical development of candidate TB vaccines (77–79). Biomarkers have been identified that can distinguish active TB from other respiratory diseases. Other biomarkers can identify individuals most at risk of developing active TB from preclinical infection (17,80). More importantly, in TB vaccine research, these biomarkers could help stratify high-risk participants, thereby improving participant selection. In this regard, CA-125, a host biomarker that is elevated in active TB compared to other pulmonary diseases (42), could be used to improve participant selection in clinical trials of candidate TB vaccines.

1.5 Study Aim/Objectives

1.5.1 Aim

This study aimed to evaluate the level of serum CA-125 in HIV positive patients with active TB disease.

We sought to determine the utility of CA-125 as a biomarker for the diagnosis of active TB in HIV positive patients and the role of CA-125 concentration in monitoring treatment efficacy.

1.5.2 Objectives

a. To determine the utility of CA-125 as a marker for active TB disease by comparing CA-125 levels with clinically well controls.
b. To compare the level of CA-125 protein in active TB patients in the presence or absence of HIV.
c. To determine the utility of CA-125 as a marker of TB disease resolution, reflecting the efficacy of TB treatment.

CHAPTER TWO

METHODS AND MATERIALS

2.1 Study Design

This study was an exploratory retrospective case-control study analysing stored samples from a previous study. The Perinatal HIV Research Unit (PHRU), Chris Hani Baragwanath Academic Hospital, Johannesburg, South Africa, carried out the previous research.

We grouped the samples used for this research into two broad categories, TB disease and controls, and then further classified the samples by HIV status. The first two groups were active pulmonary TB disease differentiated by HIV status, whilst the control groups had no active TB, also differentiated by HIV status.

In the active TB groups, serum CA-125 concentrations were measured in samples collected before TB treatment (baseline) and following two months of treatment (after the intensive phase of TB treatment). In the TB negative groups, samples were measured just once as there was no post-treatment timepoint.

2.2 Description of Parent Study

2.2.1 Study Setting

The parent study from which we selected samples for the current research was from South Africa - Hopkins TB collaboration (SoHoT collaboration). Participants for the SoHoT study were recruited by the Perinatal HIV Research Unit (PHRU) at three sites: Matlosana in North West Province, Capricorn in Limpopo Province, and Soweto, Gauteng Province.

Newly diagnosed TB index cases and their households were enrolled in a TB/HIV contact-tracing study. Participants were enrolled in 16 clinics and hospital wards between February and September 2009. Control households were then randomly selected to serve as comparator groups.

2.2.2 Definitions

- TB diagnosis: Defined as TB diagnosis based on clinical evaluation and radiographic findings (with or without sputum smear and culture).
- Household – Households were "defined as all persons sharing the same structure as the index case or living in a separate structure on the same residential plot and sharing meals with the index case" (81).

2.2.3 Enrolment criteria

Eligibility criteria:

- Adults 18 years or older, living with at least one person
- Diagnosed with TB on clinical evaluation and radiographic findings (with or without sputum smear and culture)
- Initiated on TB treatment within 30 days before enrolment
- Resident in Matlosana, Capricorn, or Soweto
- Consenting to home visits

2.2.4 Study procedure

Study teams screened participants for symptoms of TB. Subsequently, the teams visited participants households within two weeks of enrolment and confirmed eligibility criteria. Participants were then administered a verbal questionnaire about their sociodemographic factors, risk factors for TB, and prior TB symptoms. Venous blood was drawn, mycobacterial samples for smear microscopy and culture were collected, and these samples were subsequently processed at the National Health Laboratory Service (NHLS).

2.3 Description of the current study

2.3.1 Population and inclusion

We included 109 residual samples from the SoHoT collaboration in the present study. Table 2.1 below shows the number of samples in each group.

Table 2.1: Samples per study group

Broad Groups	**Sub-groups**	**Sample Description**	**Pre-treatment (baseline) samples**	**Two months post-treatment samples**
Active TB Disease	Group I	HIV positive with Active TB Disease	16	12
	Group II	Active TB disease & HIV Negative	19	18
Control Groups	Group III	TB Negative & HIV Positive (HIV control group)	25	-
	Group IV	TB Negative & HIV Negative (Healthy control)	19	-

2.3.2 Exclusion Criteria

The following samples were excluded from this study:

a. Unlabelled or poorly labelled samples
b. Inadequate samples (less than 300 microliters)

2.3.3 Data extracted

In addition to samples, the following data were also extracted for samples included in this study: age, sex, BMI, TB status, HIV status, and CD4 count.

2.4 Sample Analysis

Serum samples stored between -20 to -70 degrees centigrade were used for this study. They were stored at the Centre for Vaccines and Immunology, National Institute for Communicable Diseases (NICD), Modderfontein, Gauteng. We analysed these samples at the Chemical Pathology laboratory of the University of the Witwatersrand, Johannesburg.

2.5 Description of Methods and Techniques

Selected samples were transported on ice packs for processing at the laboratory. Volumes of 600 microlitres were then aliquoted into Eppendorf tubes.

The samples were processed on the Roche Cobas 6000 autoanalyser (Roche Diagnostics, Mannheim, Germany) using the commercial Elecsys CA 125 II reagent ordered from Roche, South Africa. This reagent contains three different solutions:

- M solution – Streptavidin-coated microparticles (in a transparent cap)
- R_1 solution – Biotinylated anti-CA 125 antibody (in grey cap)
- R_2 solution – Ruthenium-labelled anti-CA 125 antibody (in black cap)

On arrival at the laboratory, tubes were labelled according to the sample laboratory numbers, and the rack number to hold each tube noted. Sample volumes of 300 microlitres were aliquoted into the tubes, placed on the appropriate rack, and sample IDs programmed into the Roche Cobas analyser.

The determination of serum CA-125 concentration was by automated in-vitro quantitative electrochemiluminescence immunoassay (ECLIA). For each batch of samples, assay time is approximately 18 minutes using the *Sandwich Principle* briefly outlined below.

a. First incubation: 300 µL of a serum sample, solutions R_1 and R_2 form a sandwich complex.
b. Second incubation: Microparticle solution (solution M) was added to the above complex and became bound.
c. The analyser then aspirated the reaction mixture into the measuring cell where a voltage is applied through an electrode, inducing chemiluminescent emission. A photomultiplier automatically measured the emission.

The reagent manufacturer defined the normal range of serum CA-125 to monitor ovarian cancer (as part of ROMA) as <35 U/mL, in pre- & postmenopausal women.

2.6 Data Analysis

Categorical data were analysed using Chi-square or Fisher's exact test, while Mann-Whitney test was used to compare continuous variable. We described the CA-125 results in each group by median and interquartile range. We used non-parametric statistics (Kruskal Wallis test) with Dunn post-test for multiple non-parametric groups. We then plotted a Receiver Operating Characteristic (ROC) curve to decide the cut-off level of serum CA-125 to determine TB activity in the HIV positive and negative groups. We used a Wilcoxon matched-pairs signed rank test to compare the pre- and post-treatment values of serum CA-125 in TB positive groups. Spearman correlation coefficient was used for the correlations. Statistical analyses for this study used GraphPad Prism version 8.4.3 software (GraphPad Software), while statistical significance was determined by $P < 0.05$.

2.7 Ethics

The parent study (SoHoT collaboration) was approved by the Human Research Ethics Committee (HREC) of the University of the Witwatersrand (Ethics Reference Number – 160413). Participants gave written informed consent for study participation, and the subsequent use of their samples for TB or lung infection-related research.

For the present study, ethics clearance was also obtained from the Faculty of Health Sciences Research Ethics Committee of the University of the Witwatersrand, Johannesburg. Clearance number M191082.

CHAPTER THREE

RESULTS

3.1 Descriptive statistics

The demographic and clinical characteristics of 78 participants whose samples were included in this study are summarised in Table 3.1 below.

In both the HIV-infected and -uninfected groups, participants' age and body mass index (BMI) differed significantly between those with active TB and those without TB. The BMI was significantly lower among active TB participants than those without TB [Table 3.1].

Table 3.1: Table of participant demographics and clinical characteristics

	HIV Negative			HIV Positive		
	TB Positive	**TB Negative**	**P value**	**TB Positive**	**TB Negative**	**P value**
Samples	18	19	-	16	25	-
Female[♯]	4(22)	9(47)	0.1704[a]	8(50)	20(80)	0.0835[a]
Age[*]	33(25–39)	23(21–30)	<0.0001[b]	43(37–53)	36(27–42)	<0.0001[b]
BMI[*]	18(16–22)	22(18–32)	0.0204[c]	19(15–26)	23(20–31)	0.0244[c]

Abbreviation: BMI, body mass index. The BMI is the weight in kilograms divided by the square of the height in metres, kg/m^2 (calculated during the parent study).
[♯]Values described as n(%).
*Values expressed as median (IQR), where IQR is the interquartile range. Age expressed in years.
[a]*P* values determined by Fisher exact test for gender.
[b]*P* values determined by χ^2 test for age.
[c]*P* values were determined using the Mann-Whitney test.

Table 3.2 below summarises serum CA-125 concentrations in all four groups. Amongst the individuals with active TB, the pre-treatment median CA-125 in HIV-infected persons measured 31.00 U/mL (Interquartile range [IQR]: 5.68 – 128.4 U/mL) whilst, in HIV-uninfected individuals, it measured 70.35 U/mL (IQR: 15.65 – 926.5), respectively. In the TB negative individuals, the pre-treatment value in HIV-infected individuals measured 17.51 U/mL (IQR: 7.02 – 85.50 U/mL) compared to 9.62 U/mL (IQR: 4.75 – 61.25) in HIV-uninfected individuals.

The lowest measured median CA-125 value was in HIV-uninfected TB negative individuals (9.62 U/mL; IQR 4.75 – 61.25 U/mL), followed by the HIV-infected TB negative individuals (17.51 U/mL; IQR 7.02 – 85.50 U/mL). Next was the HIV-infected individuals with active TB (31.00 U/mL; IQR 5.68 – 128.4 U/mL) and the highest value was measured in HIV-uninfected individuals with active TB (70.35 U/mL; IQR 15.65 – 926.5 U/mL) [Table 3.2].

The lowest TB post-treatment value was measured in HIV-infected individuals with active TB (23.89 U/mL; IQR 3.77 333.7 U/mL), whilst the highest value was obtained in HIV-uninfected individuals with active TB (32.84 U/mL; IQR 9.19 – 333.7 U/mL). Table 3.2 below summarises the serum CA-125.

Table 3.2: Serum concentration of CA-125 by HIV sero- and TB-disease status

Groups	Number of Samples	Serum CA-125 Concentration (Median, U/mL)	Serum CA-125 (IQR)
Group I (*TB pos, HIV pos*)			
Pre-treatment	16	31.00	5.68 – 128.4
Post-treatment	12	23.89	3.77 – 52.07
Group II (*TB pos, HIV neg*)			
Pre-treatment	18	70.35	15.65 – 926.5
Post-treatment	18	32.84	9.19 – 333.7
Group III (*TB neg, HIV pos*)	25	17.51	7.02 – 85.50
Group IV (*TB neg, HIV neg*)	19	9.62	4.75 – 61.25

Abbreviations: TB pos, TB positive; TB neg, TB negative; HIV pos, HIV positive; HIV neg, HIV negative.

3.2 Pre-treatment (Baseline) Concentration of Serum CA-125 in all Groups

On comparing the four groups, we found significant differences between the groups (overall p-value; $P < 0.0001$). Then we compared between groups using a post-hoc test.

Amongst the HIV negative groups, HIV-uninfected individuals with active TB had a significantly higher median serum CA-125 compared to TB negative HIV-uninfected individuals (70.35 U/mL compared with 9.62 U/mL; $P < 0.0001$). However, a single outlying sample present amongst HIV-uninfected individuals with active TB, significantly widened the range [Figure 3.1 A].

Following removal of the outlier, the analysis was repeated to determine its effect. However, even on removing the outlier sample, the median CA-125 concentration amongst HIV-uninfected individuals with active TB was seven fold that of HIV-uninfected TB negative individuals (69.67 U/mL compared with 9.62; $P < 0.0001$) [Figure 3.1 B].

Amongst the HIV positive groups, the median CA-125 concentration was elevated in HIV-infected individuals with active TB compared to HIV-infected TB negative individuals. However, there was no significant difference between the HIV-infected individuals with active TB and the HIV-infected TB negative individuals (31.00 U/mL compared to 17.51 U/mL; $P > 0.9999$) [Figure 3.1].

On comparing those with TB, by their HIV-status individuals with TB, serum CA-125 concentrations showed a trend of higher values in the HIV-uninfected compared to HIV-infected individuals (70.35 U/mL compared with 31.00 U/mL; $P = 0.0672$) [Table 3.2]. Once more, we repeated the analysis due to the single outlier in the HIV-uninfected individuals with active TB. The median serum CA-125 concentration in HIV-uninfected individuals remained higher than in HIV-infected individuals (69.67 U/mL compared with 31.00 U/mL; $P = 0.0947$) [Figure 3.1 B].

On comparing TB negative HIV-infected to -uninfected individuals, we found HIV-infected individuals had a significantly higher median CA-125 concentration compared to HIV-uninfected individuals (17.51 U/mL compared to 9.62 U/mL; $P = 0.0381$) [Figure 3.1].

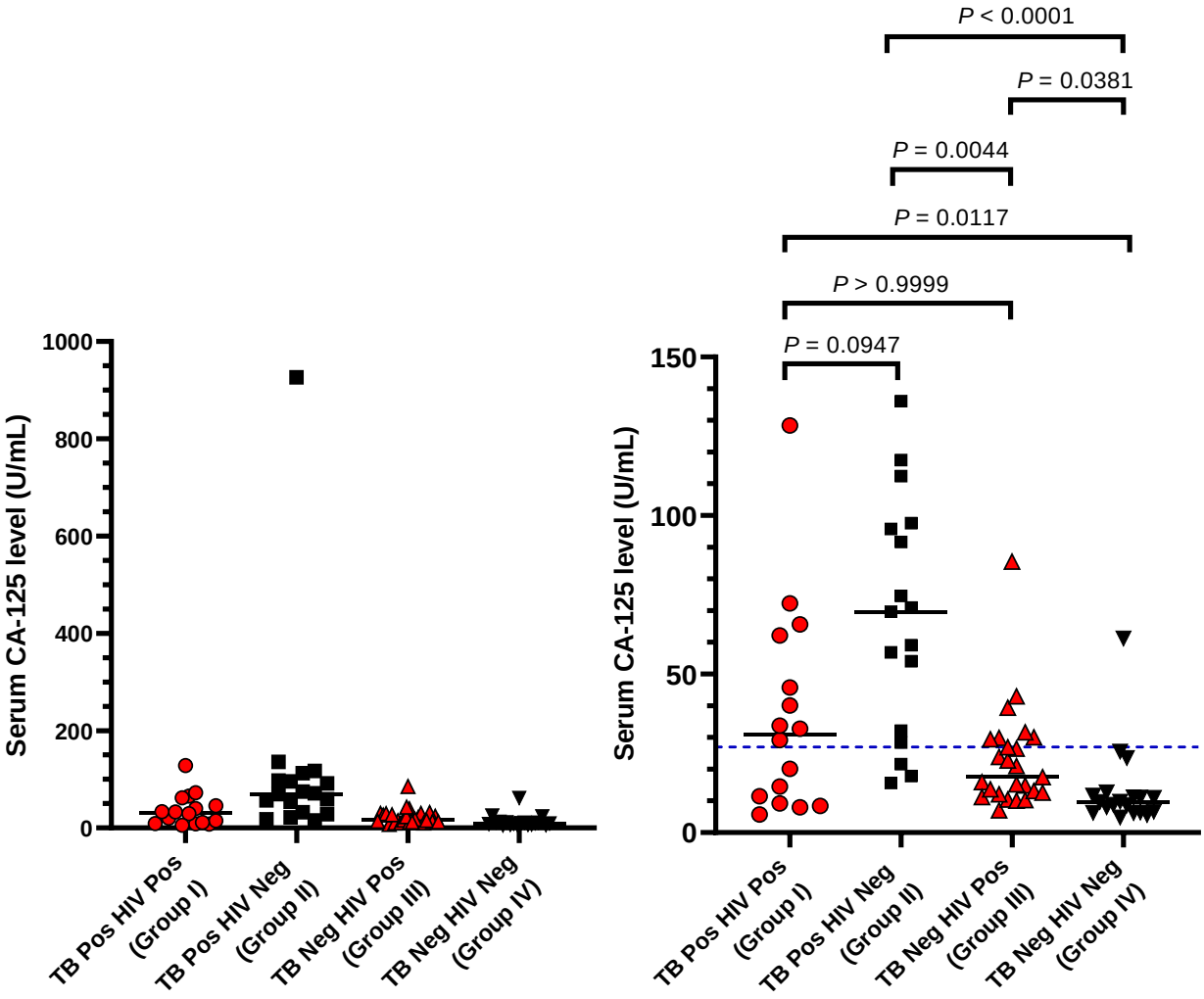

Figure 3.1: Serum CA-125 concentration in the four groups. **A.** With the single outlying sample in Group II present. **B.** After removing the outlier. The significant difference between groups calculated with the Kruskal-Wallis test and Dunn's post hoc test for individual p values. The dotted blue line represents the proposed cut-off (27 U/mL) for using CA-125 to diagnose active pulmonary tuberculosis in HIV negative groups.

3.3 ROC Curves

3.3.1 HIV Negative Groups

For HIV-uninfected individuals, a receiver operating characteristic (ROC) curve for the diagnosis

of TB in HIV negative patients was plotted by comparing Group II (TB Positive) with Group IV (healthy control). Using the ROC curve, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated at 82.4%, 94.7%, 93.3%, and 85.7% respectively, at a diagnostic cut-off of 27 U/mL [Figure 3.2 & bold in Table 3.3]. The blue grid lines in Figure 3.1B show the cut-off from this ROC curve. This ROC curve was plotted without the outlier.

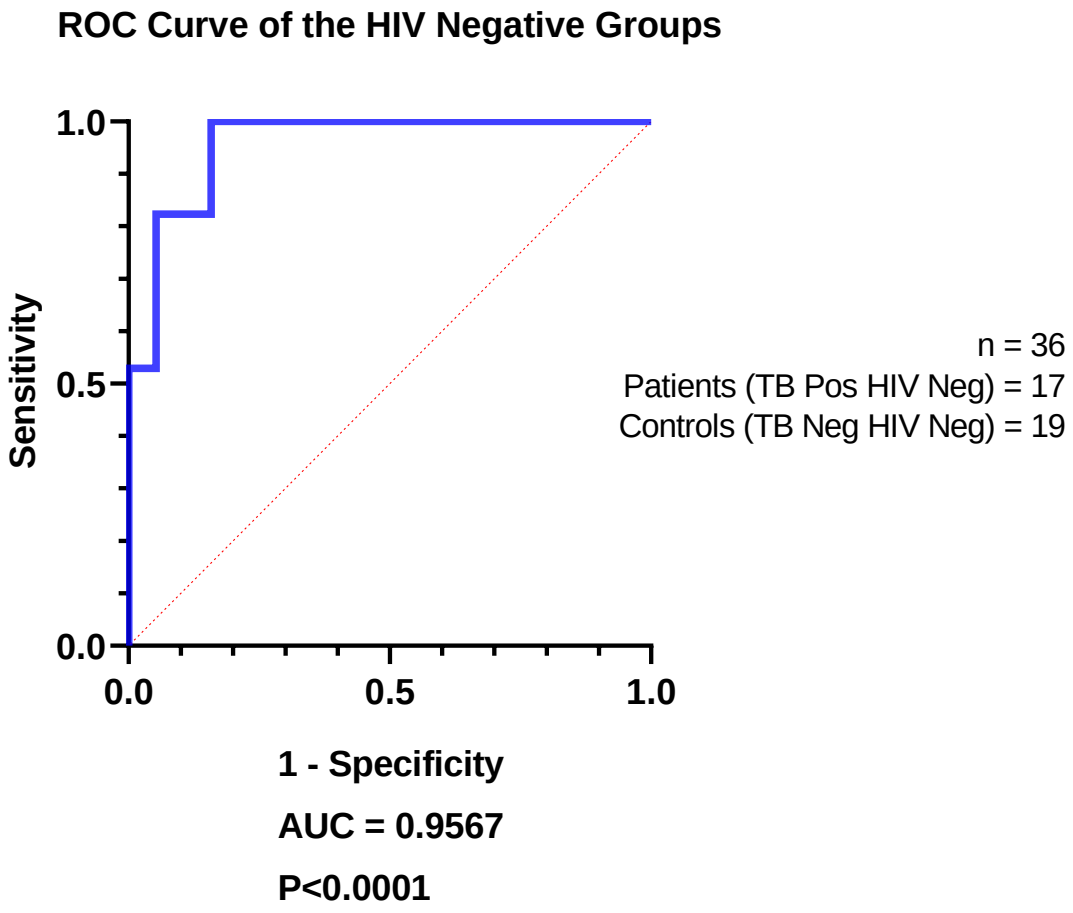

Figure 3.2: Receiver Operating Characteristic (ROC) Curve for the diagnosis of active pulmonary tuberculosis in HIV-uninfected individuals using CA-125 (Groups II & IV only, at enrolment).

Table 3.3: Serum CA-125 for the diagnosis of TB in HIV negative patients

Cut-off value (U/mL)	Sensitivity (%)	95% CI	Specificity (%)	95% CI	PPV (%)	NPV (%)
> 22.6	82.4	59.0 - 93.8	84.2	62.4 - 94.5	82.4	84.2
> 24.5	82.4	59.0 - 93.8	89.5	68.6 - 98.1	87.5	85.0
> 27.0*	**82.4**	**59.0 – 93.8**	**94.7**	**75.4 – 99.7**	**93.3**	**85.7**
> 30.3	76.5	52.7 – 90.4	94.7	75.4 – 99.7	92.9	81.8
> 43.1	70.6	46.9 – 86.7	94.7	75.4 – 99.7	92.3	78.3

*Values in bold represent the cut-off, sensitivity, specificity, PPV, and NPV for diagnosing TB in HIV-uninfected patients.

3.3.2 HIV Positive Groups

Similarly, a ROC curve for TB diagnosis among HIV positive patients was plotted by comparing Group I (TB/HIV co-infection) to Group III (HIV positive). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated at 56.3%, 72.0%, 56.3%, and 72.0% respectively, at a diagnostic cut-off of 28 U/mL [Table 3.4]. [Figure 3.3].

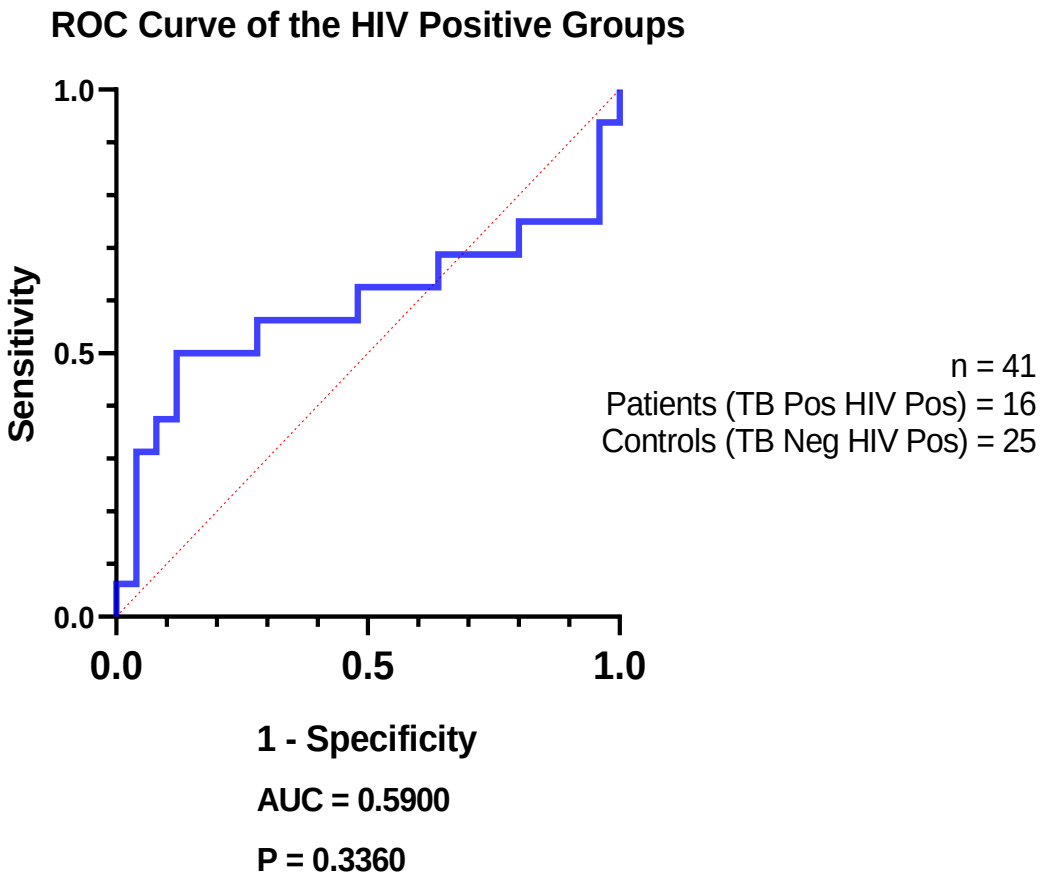

Figure 3.3: Receiver Operating Characteristic (ROC) Curve of the HIV positive groups (Groups I & III only).

Table 3.4: Serum CA-125 for the diagnosis of TB among HIV positive patients

Cut-off value (U/mL)	Sensitivity (%)	95% CI	Specificity (%)	95% CI	PPV (%)	NPV (%)
> 25.2	56.3	33.2 - 76.9	64.0	44.5 – 79.8	50.0	69.6
> 26.7	56.3	33.2 - 76.9	68.0	48.4 – 82.8	52.9	70.8
> 28.1	**56.3**	**33.2 – 76.9**	**72.0**	**52.4 – 85.7**	**56.3**	**72.0**
> 29.3	50.0	28.0 – 72.0	72.0	52.4 – 85.7	53.3	69.2
> 29.7	50.0	28.0 – 72.0	76.0	56.6 – 88.5	57.1	70.4

*Values in bold represent the cut-off, sensitivity, specificity, PPV, and NPV for diagnosing TB in HIV-infected patients.

3.4 Utility of CA-125 as a marker of TB disease resolution and TB therapy efficacy

3.4.1 HIV negative post-treatment

In HIV uninfected individuals with active TB, there was a significant reduction in serum CA-125 concentration following two months of anti TB therapy (median 70.35 U/mL to 32.84 U/mL; *P* = 0.0008) [Table 3.2 & Figure 3.4 A]. The median difference in CA-125 between pre- and post-treatment measured 37.51 U/mL. It is noteworthy that even after removing the outlying sample, this result remained statistically significant (median 69.67 U/mL to 32.84 U/mL; *P* = 0.0017) [Figure 3.4 B].

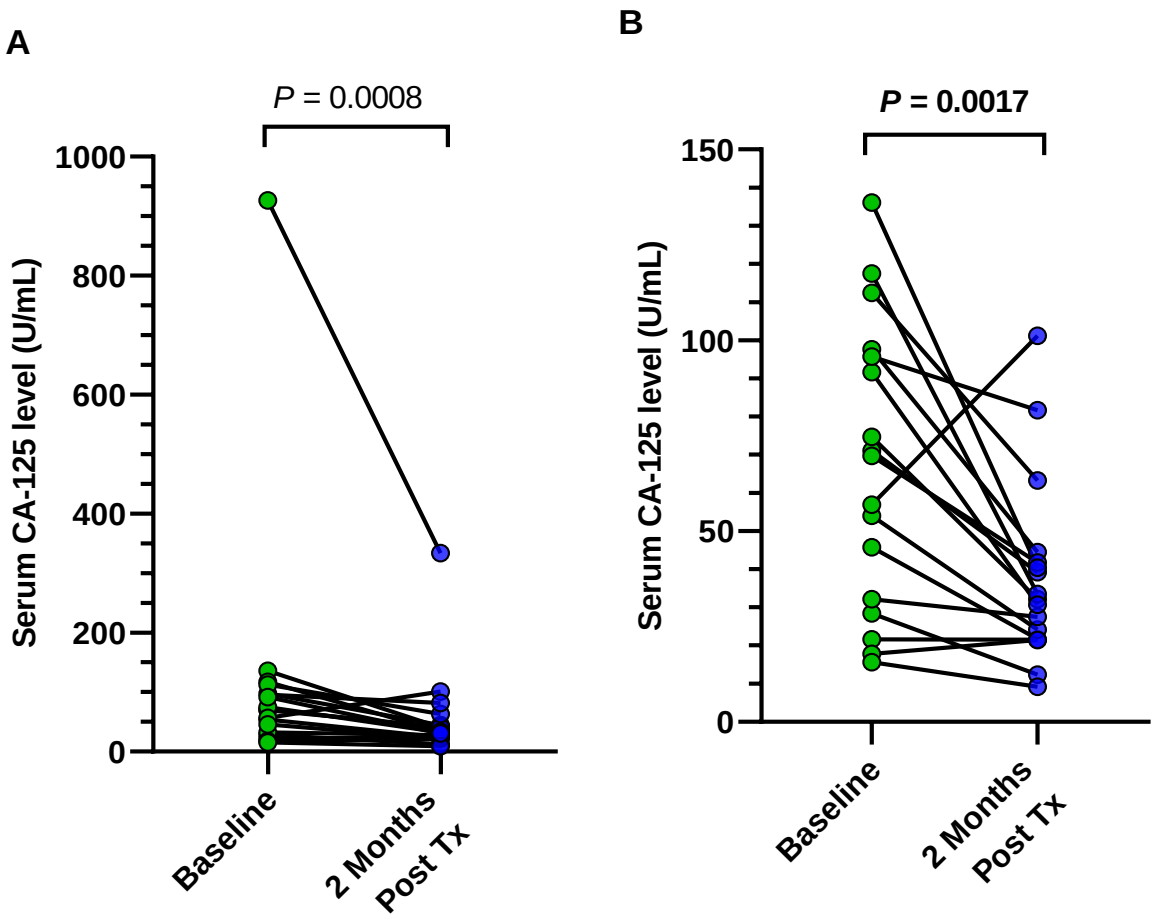

Figure 3.4: The change of serum CA-125 concentrations in active pulmonary TB before and after two months of treatment. A: In samples with the outlier. B: After removing the outlying sample. Samples and their two months post-treatment matched and analysed by the Wilcoxon matched-pairs signed rank test.

3.4.2 HIV positive post-treatment

In HIV-infected individuals with TB, there was no significant change in median CA-125 concentration following two months of anti-TB therapy (31.00 U/mL to 23.89 U/mL; *P* = 0.1099) [Figure 3.5].

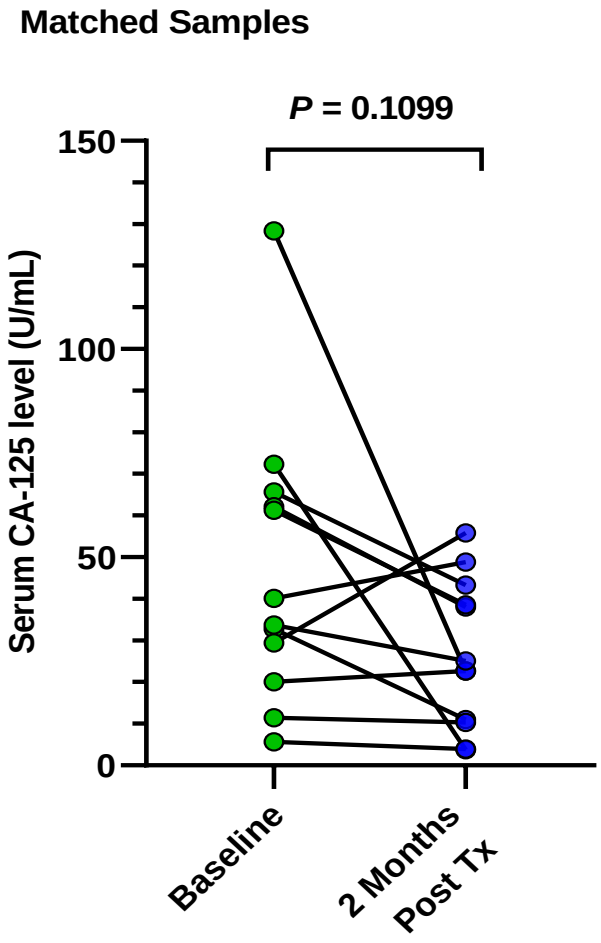

Figure 3.5: The change of serum CA-125 concentrations in TB/HIV co-infection before and after two months of treatment. Samples and their two months post-treatment matched and analysed by the Wilcoxon matched-pairs signed rank test.

3.5 Relationship between CD4 count and CA-125 concentration in the HIV positive groups

3.5.1 In the TB positive group

In HIV infected individuals with TB, there was no correlation between the serum concentration of CA-125 and CD4 count either prior to or following two months of TB treatment, (rs* = 0.06667 & rs = -0.1667 respectively) [Figure 3.6].

*rs: Spearman rank correlation

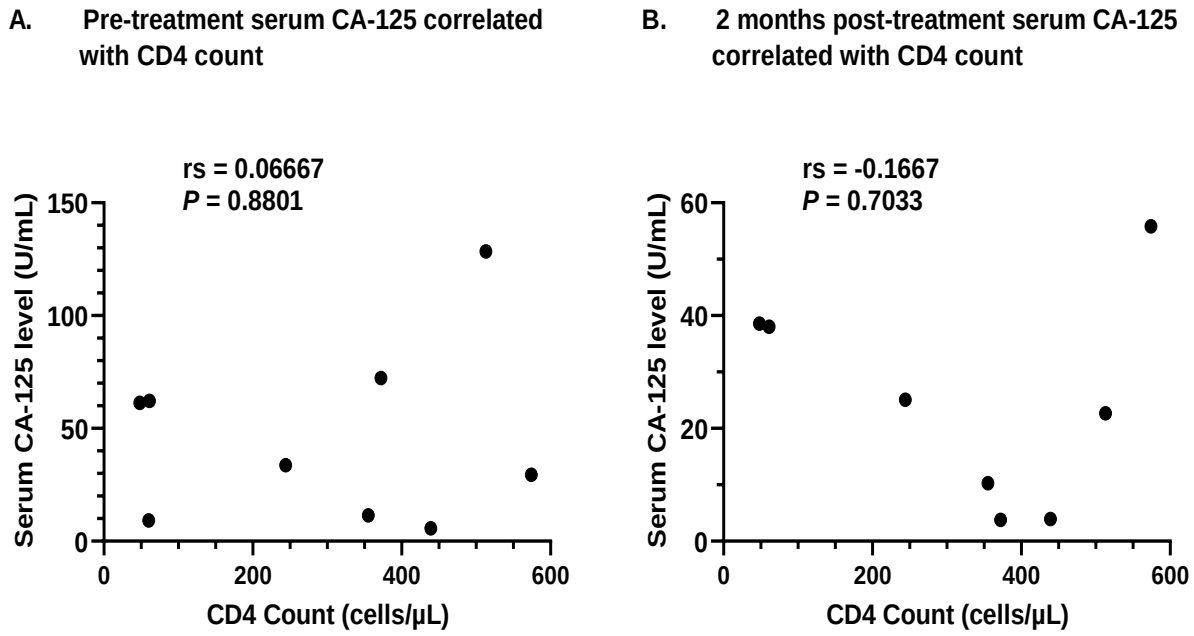

Figure 3.6: Correlation between concentrations of serum CA-125 and CD4 count in the TB positive group **A.** Before TB treatment **B.** After two months of TB treatment.

3.5.2 In the TB negative group

Similarly, in TB negative HIV infected individuals, there was no correlation between the serum concentration of CA-125 and CD4 count (rs = 0.1100) [Figure 3.7].

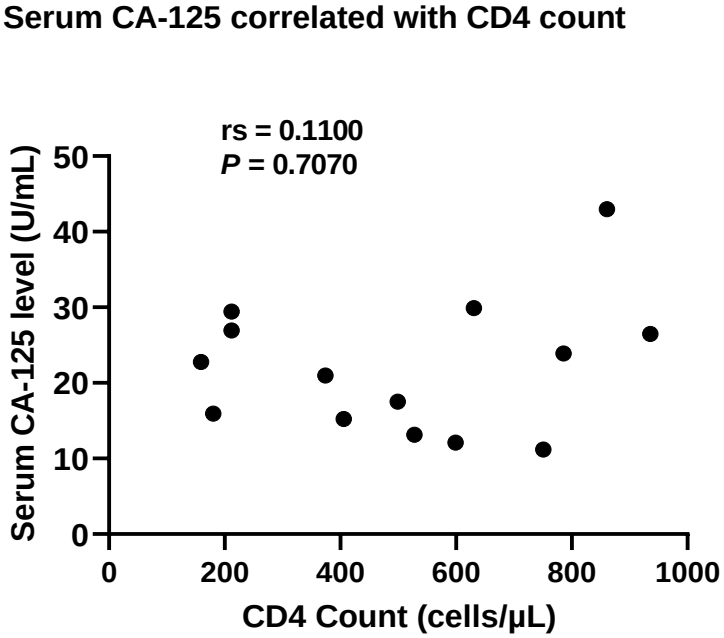

Figure 3.7: Correlation between concentrations of serum CA-125 and CD4 count in HIV-infected individuals without TB

CHAPTER FOUR

DISCUSSION

TB is a global public health concern, given that approximately a quarter of the world population has latent TB infection. Additionally, the immunosuppression from HIV co-infection and limitations with current diagnostic tools has complicated the early diagnosis of PTB in HIV positive patients. These challenges have spurred research evaluating host biomarkers that promise to improve TB diagnosis in persons with HIV. The host biomarker, CA-125, has been reported to be elevated in active PTB compared to clinically well patients and may serve as an additional tool in the TB diagnostic armamentarium. However, there has been no study evaluating the utility of CA-125 in TB diagnosis in HIV positive patients.

First, we analysed CA-125 in healthy HIV negative individuals. The median CA-125 value measured 9.62 U/mL (IQR 4.75 – 61.25), significantly lower than the TB positive group in whom, the median CA – 125 measured 70.35 U/mL (IQR 15.65 – 926.5; $P < 0.0001$). Therefore, the TB positive group had a significantly higher CA-125 compared to healthy controls.

Surprisingly, this pattern differed for HIV-infected individuals. In HIV positive patients without TB the median CA-125 value measured 17.51 U/mL (IQR 7.02 – 85.50), not significantly different compared to patients with TB/HIV co-infection where the median CA-125 measured 31.00 U/mL; (IQR 5.68 – 128.4; $P > 0.9999$). Interestingly, the absence of difference was due to HIV-infected individuals with active TB showing a trend to lower CA125 than HIV-uninfected patients with TB.

Additionally, the CA-125 concentrations in the TB positive groups declined during treatment. There was a no significant decline in median serum CA-125 from a pre-treatment value of 31.00 U/mL to 23.89 U/mL at two months post-treatment in HIV-infected patients with active TB. However, in HIV-uninfected patients with active TB, there was a statistically significant reduction in serum CA-125 value after two months of anti-TB treatment (median 70.35 U/mL to 32.84 U/mL).

In the HIV negative groups, the significantly elevated serum CA-125 concentration in HIV-uninfected patients with active TB compared to TB negative HIV-uninfected patients is similar to results from several studies examining CA-125 concentrations in patients with active PTB compared to healthycontrols. Mohammad et al. (27) show mean serum CA-125

concentrations were significantly higher in the active PTB group compared to healthy controls (65.58 ± 69.77 U/mL compared to 7.14 ± 3.16 U/mL; P = 0.012). Moreover, Azza et al. (43) also observed significantly elevated CA-125 concentrations in those with active PTB compared to healthy controls (93.5 ± 138.9 U/mL compared to 10.5 ± 7.3 U/mL; P = 0.004). Similarly, Sahin et al. (40) noted significantly elevated CA-125 concentrations in active PTB compared to healthy controls (78.88 ± 24.72 U/mL compared to 18.32 ± 2.87 U/mL; P < 0.001). However, since our study stratified the TB and control groups by HIV status, serum CA-125 concentration in HIV positive groups is discussed later.

In our study, the diagnostic threshold of serum CA-125 in HIV negative groups of 27.0 U/mL at sensitivity, specificity, PPV, and NPV of 82.4%, 94.7%, 93.3%, and 85.7%, respectively, is similar to that of Mohammed et al. (27) (21.05 U/mL, 82.5%, 72.5%, 77.3%, and 83.3% respectively). Azza et al. (43) reported a CA-125 diagnostic cut-off level of 34.6 U/ ml at sensitivity, specificity, positive and negative predictive values of 81.4%, 95%, 95.6%, and 79.2% respectively. In comparison, Sahin et al. (40) report a value of 36.35 U/mL as a diagnostic cut-off, with sensitivity, specificity, positive and negative predictive values of 97.6%, 100%, 98.2%, and 100%.

Our result of significantly elevated serum CA-125 in HIV-uninfected individuals with active TB could be useful as a diagnostic biomarker. Since serum CA-125 is elevated in HIV-uninfected individuals with active TB, this biomarker could help high TB burden settings but with low HIV prevalence. Thus, CA-125 may be suitable for TB diagnosis in high burden countries (HBCs) for TB, as defined by WHO, such as Cambodia and Sierra Leone (1,82).

Additionally, our sample type is peripheral blood; the instrument is a widely available high throughput autoanalyser; and our result showed high specificity relative to sensitivity, suitable for high TB prevalence settings. Thus, serum CA-125 could meet the sensitivity and specificity target product profiles (TPP) for new tuberculosis diagnostics. The immunoassay format could potentially be developed further into a lateral flow assay (83). Therefore, CA-125 could be used as a biomarker-based triage test to identify individuals suspected of having TB for a further confirmatory test.

After two months of TB treatment, there was a significant reduction in serum CA-125 concentration in HIV-uninfected individuals with active TB. Therefore, CA-125 could be used to assess therapeutic response to anti-TB therapy in this population. This aspect is potentially promising but requires prospective cohorts to assess treatment successes and failures.

Yilmaz et al. (41) studied 96 participants – 40 active PTB, 20 inactive PTB and 36 healthy controls. Post-treatment CA-125 at the second, fourth, sixth months of anti-TB treatment were measured, and once more at three years after completion of TB therapy. They reported significant and progressively decreasing CA-125 values, 38.4 ± 30.5 U/mL, 16.4 ± 13.2 U/mL, 11.0 ± 7.7 U/mL, and 10.5 ± 7.3 U/mL respectively ($P < 0.0001$).

Similarly, Sahin et al. (40) (42 active TB, 35 inactive TB, and 20 healthy controls) report that CA-125 values significantly declined following four months of TB treatment (78.88 ± 24.72 U/mL to 22.78 ± 8.02 U/mL; $P < 0.001$).

In a study of 80 patients, of whom 27 had active pulmonary TB, 33 with other pulmonary infections, and 20 healthy volunteers, Azza et al. (43) note that CA-125 concentration significantly decreased from 93.5 ± 158.9 U/mL to 22.8 ± 25.6 U/mL (P= 0.001) after four months of anti-TB treatment. It is important to note that their study found significantly higher serum CA-125 in active TB compared to other pulmonary infections (community-acquired pneumonia and acute exacerbation of chronic obstructive pulmonary disease).

Furthermore, CA-125 could find use in TB vaccine clinical research. A major challenge in the development of candidate TB vaccine is the identification of correlates, including - correlate of risk of disease progression, correlate of protection or biomarkers of vaccine immunogenicity. Understanding these correlates could ease the challenges in developing a new TB vaccine (3,70–72). Some TB biomarkers have already found use in defining vaccine immunogenicity in the absence of known correlates of protection. For instance, interferon-gamma (IFN-γ) ELISPOT was used in determining the immunogenicity of the MVA85A TB candidate vaccine during the earlier stages of its development (78,79).

Additionally, a study of transcriptomic bio-signatures suggested biosignatures may help define correlates of risk of disease. The study indicated that prior to TB diagnosis, transcriptomic biomarkers could predict the probability of high-risk individuals developing active TB (84). Should those most at risk of developing TB disease be identified, the size, cost, and duration of clinical trials could be significantly reduced (77–79). In this way, biomarkers offer an efficient way of selecting promising candidate vaccines to progress in clinical trials (77). In this regard, TB biomarkers may be useful to predict the risk of TB disease before the disease becomes evident.

In HIV positive individuals, although CA-125 was elevated in active TB compared to those without active TB, it was not statistically significant.

Furthermore, when comparing HIV-infected patient without active TB and HIV-uninfected patient without active TB, we found that CA-125 is significantly elevated in the HIV positive patients compared to healthy controls ($P = 0.0381$). Thus, independently, HIV significantly elevated serum CA-125. These results suggest that HIV is, to a lesser degree, also important in the elevation of serum CA-125, as is TB. As such, the role of CA-125 in the diagnosis of TB in HIV positive patients is limited (sensitivity = 56.3%, specificity = 72.0%, AUC = 0.5900).

There was no correlation between the serum concentration of CA-125 and CD4 count. Since CD4 count serves as a measure of the severity of HIV infection, this result suggests there is no relationship between the concentration of serum CA-125 and the severity of HIV infection either in HIV-infected individuals with active TB or HIV-infected individuals without active TB. Further research that includes viral load and ARV status of participants is needed to assess better, the relationship between serum CA-125 and HIV severity.

The lower value of serum CA-125 in TB/HIV co-infection compared to TB disease alone may be explained by the fact that HIV is an immunosuppressive disease. Therefore, CA-125, a host biomarker involved in several immunologic pathways (28,30,33), could have been suppressed due to HIV infection. However, one would then expect to see a similar reduction in HIV infection alone compared to healthy controls, which was not observed. A potential explanation could relate to the study enrolment criteria, which allowed a time limit of 30 days between the date of TB treatment start date and study enrolment. Patients with HIV-associated TB may have had higher CA-125 values at diagnosis, which had already decreased by study enrolment. Further studies, including HIV-infected patients, are required to confirm our unexpected finding.

Following two months of anti-TB treatment in HIV-infected individuals with active TB, although CA-125 concentrations did indeed decline following TB therapy, this change was not significant. Similar to the explanation above, our inclusion criterion of enrolling participants who had been initiated on TB treatment within 30 days before enrolment may have missed early timepoints where the decline in CA-125 may have been apparent. Further studies with more frequent serial measures of CA-125 concentrations after initiation of the anti-TB regimen may be necessary to determine the utility of CA-125 as a biomarker to monitor therapeutic efficacy following treatment.

It is also noteworthy that CA-125 is involved in the Warburg Effect in cancer cells (metabolic shift from oxidative phosphorylation to aerobic glycolysis) (86–88). MUC16 activates mTOR, mammalian Target of Rapamycin, which in turn activates the key regulators of the Warburg Effect (86,87). Similarly, *Mycobacterium tuberculosis* infection induces Warburg-like metabolism in macrophages (89–91). Although unproven, the higher concentration of serum CA-125 in HIV-uninfected TB patients could be due to the pathophysiologic role of CA-125 in the Warburg-like metabolism of TB. Further study of these novel metabolic pathways are necessary to determine the role of CA-125 in this shift in the *Mtb* glycolytic metabolism and if this shift accounts for the higher concentration of serum CA-125 in HIV-uninfected TB patients.

4.4 Limitations

This study had several limitations. The 7-day interval between initiation to TB treatment and participant enrolment might have impacted the value of CA-125. Possibly, participants diagnosed earlier in the 7-day interval might have responded very well to treatment, and the value of CA-125 was already in a decline when they were enrolled in the parent study. This interval, we think, may account for the values of CA-125 in the HIV-infected group. Notably, there was no time difference on TB treatment between the HIV-infected and -uninfected groups.

Secondly, the small sample size may have limited our ability to detect the true distribution of CA-125 concentrations in the HIV-infected individuals with active TB. As a nested study, we were limited to the number of available samples rather than enrolling and recruiting participants. Additionally, only two months of TB post-treatment samples were available, and the outcome of treatment was unknown. We did not have information on TB latency as IGRA tests had not been performed. We did not have molecular diagnostic test results as these were not routine during the years of the study.

Thirdly, this study used frozen samples. Like other cancer antigens, CA-125 is sensitive to temperature, freeze/thaw, and sample storage duration. Although samples used in this study were stored at optimal temperature (between -70 to -20 °C), they had undergone freeze/thaw cycles and stored for more than a year. These factors are known to contribute to the reduced value of cancer antigens via degradation. Thus, a prospective study may be needed.

Fourthly, the absence of a comparator respiratory group means we could not compare the results of this study with other respiratory tract infections (RTIs). Though previous studies have shown elevated serum CA-125 concentrations in TB compared to other RTIs like community-acquired pneumonia, none of those studies involved HIV positive patients.

Lastly, there is likely a high proportion of latent TB infection (LTBI) in our control groups without TB disease, since the parent study did not rule out LTBI through diagnostic testing. Thus, our comparison inadvertently may include those with LTBI.

4.5 Strengths

Despite the above limitations, several factors set this study apart from similar research on CA-125. It is the first study to stratify all groups by HIV status and examine the use of CA-125 in the diagnosis of TB in HIV-infected patients.

Furthermore, we used blood samples for this study and samples were processed with a widely available high throughput autoanalyser.

4.5 Recommendations

We recommend prospective studies in the future, with multiple post-treatment follow-up at two and six months, and preferably up to a year. The prospective study will ensure the temperature-sensitive CA-125 is not degraded during storage. Simultaneously, the post-therapy follow-up will enable a more robust comparison of the serial change in serum CA-125 concentrations after therapy.

Furthermore, HIV-infected patients should be classified according to severity to help understand this host biomarker in the pathophysiologic progression of HIV. Participant enrolment and blood collection should occur within seven days of TB diagnosis and before initiation of anti-TB therapy to avoid a decline in serum CA-125 concentration due to robust, effective treatment response.

In addition to CD4 count, further research should include viral load and ARV status of participants to enable an even more detailed comparison. The inclusion of several parameters

for assessing the severity of HIV would clarify the relationship between serum CA-125 and HIV severity.

Lastly, future studies should include a control group of participants with a confirmed bacterial lower respiratory infection to serve as a comparator to pulmonary TB, and where possible, include LTBI as a separate group.

4.6 Conclusion

Serum CA-125 is significantly increased in active PTB in HIV-uninfected individuals, with a reported sensitivity, specificity, PPV, and NPV of 82.4%, 94.7%, 93.3%, and 85.7%, respectively, at a diagnostic threshold of 27.0 U/mL. Therefore, serum CA-125 may be used as a host biomarker to diagnose active PTB in HIV negative patients. Additionally, the biomarker can potentially monitor TB disease resolution and efficacy of therapy in HIV negative patients.

In HIV-infected patients, the use of serum CA-125 concentration to diagnose TB cannot be recommended. Moreover, the biomarker was not sensitive enough to recommend its use for therapeutic monitoring of TB in HIV positive patients. The reason for the trend in TB patients to lower CA-125 in those with HIV compared with those without HIV is unclear, and further study is needed.

REFERENCES

1. World Health Organization (WHO). Global tuberculosis report 2020 . 2020 https://apps.who.int/iris/bitstream/handle/10665/336069/9789240013131-eng.pdf.

2. World Health Organization (WHO). Tuberculosis. 2020 https://www.who.int/news-room/fact-sheets/detail/tuberculosis

3. World Health Organization (WHO). Tuberculosis profile: South Africa. 2020 https://worldhealthorg.shinyapps.io/tb_profiles/?_inputs_&entity_type=%22country%22&lan=%22EN%22&iso2=%22ZA%22

4. Mendelson M. Diagnosing tuberculosis in HIV-infected patients: challenges and future prospects. Br Med Bull. 2007;81–82(1):149–65. https://pubmed.ncbi.nlm.nih.gov/17470475/

5. Corbett EL, Watt CJ, Walker N, Maher D, Williams BG, Raviglione MC, et al. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. Arch Intern Med. 2003 May 12;163(9):1009–21. https://jamanetwork.com/journals/jamainternalmedicine/article-abstract/215525

6. UNAIDS. Global HIV & AIDS statistics - 2020 fact sheet. 2020 https://www.unaids.org/en/resources/fact-sheet

7. Ryu YJ. Diagnosis of pulmonary tuberculosis: recent advances and diagnostic algorithms. Tuberc Respir Dis (Seoul). 2015 Apr 1;78(2):64–71. http://dx.doi.org/10.4046/trd.2015.78.2.64

8. Skoura E, Zumla A, Bomanji J. Imaging in tuberculosis. Int J Infect Dis. 2015 Mar 1;32:87–93. https://www.sciencedirect.com/science/article/pii/S120197121401724X

9. Méndez-Samperio P. Diagnosis of tuberculosis in HIV co-infected individuals: current status, challenges and opportunities for the future. Scand J Immunol. 2017 Aug 1;86(2):76–82. http://doi.wiley.com/10.1111/sji.12567

10. Caulfield AJ, Wengenack NL. Diagnosis of active tuberculosis disease: from microscopy to molecular techniques. J Clin Tuberc Other Mycobact Dis. 2016 Aug 1;4:33–43. https://doi.org/10.1016/j.jctube.2016.05.005

11. Gupta AK, Singh A, Singh S. Diagnosis of tuberculosis: nanodiagnostics approaches. NanoBioMedicine. 2020 Jan 1;261–83. https://doi.org/10.1007/978-981-32-9898-9_11

12. Sharma SK, Vashishtha R, Chauhan LS, Sreenivas V, Seth D. Comparison of TST and IGRA in diagnosis of latent tuberculosis infection in a high TB-burden setting. PLoS One. 2017 Jan 6;12(1):e0169539. https://dx.plos.org/10.1371/journal.pone.0169539

13. Carranza C, Pedraza-Sanchez S, de Oyarzabal-Mendez E, Torres M. Diagnosis for latent tuberculosis infection: new alternatives. Front Immunol. 2020 Sep 10;11. https://doi.org/10.3389/fimmu.2020.02006

14. Gaude G, Vishwanath S. Molecular diagnosis of tuberculosis with emphasis on Xpert Mycobacterium tuberculosis assay – Clinical review. J Clin Sci. 2020;17(4):93. https://www.jcsjournal.org/text.asp?2020/17/4/93/298456

15. Yerlikaya S, Broger T, MacLean E, Pai M, Denkinger CM. A tuberculosis biomarker database: the key to novel TB diagnostics. Int J Infect Dis. 2017;56:253–7. http://dx.doi.org/10.1016/j.ijid.2017.01.025

16. Adu-Gyamfi CG, Snyman T, Hoffmann CJ, Martinson NA, Chaisson RE, George JA, et al. Plasma indoleamine 2, 3-dioxygenase, a biomarker for tuberculosis in human immunodeficiency virus-infected patients. Clin Infect Dis. 2017 Oct 1;65(8):1356–63. https://academic.oup.com/cid/article/65/8/1356/3868841

17. Petruccioli E, Scriba TJ, Petrone L, Hatherill M, Cirillo DM, Joosten SA, et al. Correlates of tuberculosis risk: predictive biomarkers for progression to active tuberculosis. Eur Respir J. 2016 Dec 1;48(6):1751–63. http://ow.ly/OzCL304ezfk

18. Correia-Neves M, Fröberg G, Korshun L, Viegas S, Vaz P, Ramanlal N, et al. Biomarkers for tuberculosis: the case for lipoarabinomannan. ERJ Open Res. 2019 Feb 1;5(1):00115–2018.http://ow.ly/FyCs30n4uFE

19. Padmapriyadarsini C, Narendran G, Swaminathan S. Diagnosis & treatment of tuberculosis in HIV co-infected patients. Indian J Med Res. 2011;134(12):850–65. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3284094/

20. Reid MJ, Shah NS. Approaches to tuberculosis screening and diagnosis in people with HIV in resource-limited settings. Lancet Infect Dis. 2009 Mar 1;9(3):173–84. https://doi.org/10.1016/S1473-3099(09)70043-X

21. Wilson D, Nachega J, Morroni C, Chaisson R, Maartens G. Diagnosing smear-negative tuberculosis using case definitions and treatment response in HIV-infected adults. Int J Tuberc Lung Dis. 2006;10(1):31–8. https://www.ingentaconnect.com/content/iuatld/ijtld/2006/00000010/00000001/art00007#

22. Modi S, Cavanaugh JS, Shiraishi RW, Alexander HL, McCarthy KD, Burmen B, et al. Performance of clinical screening algorithms for tuberculosis intensified case finding among people living with HIV in western Kenya. Fernandez-Reyes D, editor. PLoS One. 2016 Dec 9;11(12):e0167685. https://dx.plos.org/10.1371/journal.pone.0167685

23. Meyer AJ, Atuheire C, Worodria W, Kizito S, Katamba A, Sanyu I, et al. Sputum quality and diagnostic performance of GeneXpert MTB/RIF among smear-negative adults with presumed tuberculosis in Uganda. PLoS One. 2017 Jul 7;12(7):e0180572. https://dx.plos.org/10.1371/journal.pone.0180572

24. Singh BK, Sharma SK, Sharma R, Sreenivas V, Myneedu VP, Kohli M, et al. Diagnostic utility of a line probe assay for multidrug resistant-TB in smear-negative pulmonary tuberculosis. PLoS One. 2017 Aug 22;12(8):e0182988. https://dx.plos.org/10.1371/journal.pone.0182988

25. Achkar JM, Lawn SD, Moosa MS, Wright CA, Kasprowicz VO. Adjunctive tests for diagnosis of tuberculosis: serology, ELISPOT for site-specific lymphocytes, urinary lipoarabinomannan, string test, and fine needle aspiration. J Infect Dis. 2011;204(Suppl 4):S1130–S1141. https://academic.oup.com/jid/article/204/suppl_4/S1130/1009277

26. Kim ES, Park KU, Song J, Lim HJ, Cho YJ, Yoon H, et al. The clinical significance of CA-125 in pulmonary tuberculosis. Tuberculosis. 2013 Mar 1;93(2):222–6. https://www.sciencedirect.com/science/article/pii/S147297921300005X

27. Mohammad OI, Okab AA, El Behisy MM, Sudhom FG. Value of CA-125 in diagnosis and assessment of severity of active pulmonary tuberculosis. Egypt J Chest Dis Tuberc. 2016 Jan 1;65(1):205–9. https://www.sciencedirect.com/science/article/pii/S0422763815300856

28. Das S, Batra SK. Understanding the unique attributes of MUC16 (CA125): potential implications in targeted therapy. Cancer Res. 2015 Nov 15;75(22):4669–74. https://cancerres.aacrjournals.org/content/canres/75/22/4669.full.pdf

29. Schmidt C. CA-125: A biomarker put to the test. J Natl Cancer Inst. 2011;103(17):1290–1. https://academic.oup.com/jnci/article/103/17/1290/2516773

30. Felder M, Kapur A, Gonzalez-Bosquet J, Horibata S, Heintz J, Albrecht R, et al. MUC16 (CA125): tumor biomarker to cancer therapy, a work in progress. Mol Cancer. 2014 May 29;13(1):1–15. http://www.molecular-cancer.com/content/13/1/129

31. Yin BW, Lloyd KO. Molecular cloning of the CA125 ovarian cancer antigen: identification as a new mucin, MUC16. J Biol Chem. 2001 Jul 20;276(29):27371–5. https://www.jbc.org/content/276/29/27371.short

32. Yin BW, Dnistrian A, Lloyd KO. Ovarian cancer antigen CA125 is encoded by the MUC16 mucin gene. Int J Cancer. 2002 Apr 10;98(5):737–40. https://onlinelibrary.wiley.com/doi/full/10.1002/ijc.10250

33. Haridas D, Ponnusamy MP, Chugh S, Lakshmanan I, Seshacharyulu P, Batra SK. MUC16: molecular analysis and its functional implications in benign and malignant conditions. FASEB J. 2014 Oct 2;28(10):4183–99. https://onlinelibrary.wiley.com/doi/abs/10.1096/fj.14-257352

34. Chugh S, Gnanapragassam VS, Jain M, Rachagani S, Ponnusamy MP, Batra SK. Pathobiological implications of mucin glycans in cancer: sweet poison and novel targets. Biochim Biophys Acta - Rev Cancer. 2015;1856(2):211–25. http://dx.doi.org/10.1016/j.bbcan.2015.08.003

35. Perez BH, Gipson IK. Focus on molecules: human mucin MUC16. Exp Eye Res. 2008;87(5):400–1. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2586928/

36. Davies JR, Kirkham S, Svitacheva N, Thornton DJ, Carlstedt I. MUC16 is produced in tracheal surface epithelium and submucosal glands and is present in secretions from normal human airway and cultured bronchial epithelial cells. Int J Biochem Cell Biol. 2007 Jan 1;39(10):1943–54. https://www.sciencedirect.com/science/article/pii/S1357272507001690

37. Golubovskaya V. CAR-T cell therapy: from the bench to the bedside. Cancers (Basel). 2017 Oct 31;9(12):150. https://www.mdpi.com/2072-6694/9/11/150/htm

38. Koneru M, O'Cearbhaill R, Pendharkar S, Spriggs DR, Brentjens RJ. A phase I clinical trial of adoptive T cell therapy using IL-12 secreting MUC-16 ecto directed chimeric

antigen receptors for recurrent ovarian cancer. J Transl Med. 2015 Mar 28;13(1):1–11. https://doi.org/10.4161/2162402X.2014.994446

39. Aithal A, Junker WM, Kshirsagar P, Das S, Kaur S, Orzechowski C, et al. Development and characterisation of carboxy-terminus specific monoclonal antibodies for understanding MUC16 cleavage in human ovarian cancer. PLoS One. 2018;13(4):1–18. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0193907

40. Sahin F, Yildiz P. Serum CA-125: Biomarker of pulmonary tuberculosis activity and evaluation of response to treatment. Clin Investig Med. 2012 Aug 4;35(4):E223–8. https://cimonline.ca/index.php/cim/article/view/17151

41. Yilmaz A, Ece F, Bayramgürler B, Akkaya E, Baran R. The value of CA125 in the evaluation of tuberculosis activity. Respir Med. 2001;95(8):666–9. https://www.sciencedirect.com/science/article/pii/S0954611101911217

42. Fortún J, Méndez R, Martínez A, Norman F. Ca-125 : A useful marker to distinguish pulmonary tuberculosis from other pulmonary infections. Open Respir Med J. 2009;3:123–7. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2788741/

43. Said AF, Mohamed BI, El-Sharkawy E, Al-Sherif M. Role of cancer antigen 125 in active pulmonary tuberculosis. Egypt J Chest Dis Tuberc. 2013 Jul 1;62(3):419–24. https://www.sciencedirect.com/science/article/pii/S0422763813001751

44. Shah W. To determine diagnostic accuracy of gene xpert and sputum Ziehl-Neelsen staining taking sputum culture as gold standard. In: European Respiratory Journal. European Respiratory Society (ERS); 2016. p. PA2779. https://erj.ersjournals.com/content/48/suppl_60/PA2779

45. Rizk E, Rakha EB, El-Rahman AA, Elshabrawy WO, Saudy N. Pulmonary tuberculosis: could tumor markers CA 15-3 and CA 125 be useful for diagnosis and evaluation of response to therapy? Comp Clin Path. 2018 Jan 1;27(1):107–14. https://link.springer.com/article/10.1007/s00580-017-2559-3

46. Portal C, Gouyer V, Gottrand F, Desseyn JL. Ocular mucins in dry eye disease. Exp Eye Res. 2019;186(April):107724. https://www.sciencedirect.com/science/article/pii/S0014483519302714

47. Gipson IK, Blalock T, Tisdale A, Spurr-Michaud S, Allcorn S, Stavreus-Evers A, et al. MUC16 is lost from the uterodome (pinopode) surface of the receptive human endometrium: in vitro evidence that MUC16 is a barrier to trophoblast adherence. Biol Reprod. 2008 Jan 1;78(1):134–42. https://academic.oup.com/biolreprod/article-lookup/doi/10.1095/biolreprod.106.058347

48. Tyler C, Kapur A, Felder M, Belisle JA, Trautman C, Gubbels JA, et al. The mucin MUC16 (CA125) binds to NK cells and monocytes from peripheral blood of women with healthy pregnancy and preeclampsia. Am J Reprod Immunol. 2012 Jul 1;68(1):28–37. http://doi.wiley.com/10.1111/j.1600-0897.2012.01113.x

49. Liu L, Wang Y, Chen X, Tian Y, Li TC, Zhao L, et al. Evidence from three cohort studies on the expression of MUC16 around the time of implantation suggests it is an inhibitor of implantation. J Assist Reprod Genet. 2020 May 1;37(5):1105–15. https://link.springer.com/article/10.1007/s10815-020-01764-z

50. Rabesalama SS, Mandeville KL, Raherison RA, Rakoto-Ratsimba HN. Isolated ovarian tuberculosis mimicking ovarian carcinoma: case report and literature review. African J Infect Dis. 2011;5(1):7–10. https://www.ajol.info/index.php/ajid/article/view/66508

51. Bellos I, Pergialiotis V, Loutradis D, Papapanagiotou A, Daskalakis G. Serum CA-125 levels in preeclampsia: a systematic review and meta-analysis. Int J Clin Pract. 2019 Oct 28;73(10). https://onlinelibrary.wiley.com/doi/abs/10.1111/ijcp.13380

52. Gunn BM, Shansab M, Schneider JR, Bastian AR, Fahrbach KM, SmithIV AD, et al. Enhanced binding of antibodies generated during chronic HIV infection to mucus component MUC16. Mucosal Immunol. 2016 Nov 1;9(6):1549–58. https://www.nature.com/articles/mi20168

53. Wei X, Su J, Yang K, Wei J, Wan H, Cao X, et al. Elevations of serum cancer biomarkers correlate with severity of COVID-19. J Med Virol. 2020 Jun 3;jmv.25957. https://onlinelibrary.wiley.com/doi/abs/10.1002/jmv.25957

54. Yates JA, Collis OA, Sueblinvong T. Red snappers and red herrings: pelvic tuberculosis causing elevated CA 125 and mimicking advanced ovarian cancer. A case report and literature review. Hawai'i J Med Public Heal. 2017;76(8):220–4. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5551276/

55. Bhatia R, Gautam SK, Cannon A, Thompson C, Hall BR, Aithal A, et al. Cancer-associated mucins: role in immune modulation and metastasis. Cancer Metastasis Rev. 2019 Jun 15;38(1–2):223–36. https://link.springer.com/article/10.1007/s10555-018-09775-0

56. Gubbels JA, Felder M, Horibata S, Belisle JA, Kapur A, Holden H, et al. MUC16 provides immune protection by inhibiting synapse formation between NK and ovarian tumor cells. Mol Cancer. 2010 Jan 20;9(1):1–14. https://link.springer.com/articles/10.1186/1476-4598-9-11

57. Saraiva M, O'garra A. The regulation of IL-10 production by immune cells. Nat Rev Immunol. 2010;10:170–81. https://doi.org/10.1038/nri2711

58. Boivin M, Lane D, Piché A, Rancourt C. CA125 (MUC16) tumor antigen selectively modulates the sensitivity of ovarian cancer cells to genotoxic drug-induced apoptosis. Gynecol Oncol. 2009 Dec 1;115(3):407–13. https://www.sciencedirect.com/science/article/pii/S0090825809006349

59. Comamala M, Pinard M, Thériault C, Matte I, Albert A, Boivin M, et al. Downregulation of cell surface CA125/MUC16 induces epithelial-to- mesenchymal transition and restores EGFR signalling in NIH:OVCAR3 ovarian carcinoma cells. Br J Cancer. 2011 Mar 15;104(6):989–99. https://www.nature.com/articles/bjc201134/

60. Lakshmanan I, Salfity S, Seshacharyulu P, Rachagani S, Thomas A, Das S, et al. MUC16 regulates TSPYL5 for lung cancer cell growth and chemoresistance by suppressing p53. Clin Cancer Res. 2017 Jul 15;23(14):3906–17. https://clincancerres.aacrjournals.org/content/clincanres/23/14/3906.full.pdf

61. Aithal A, Rauth S, Kshirsagar P, Shah A, Lakshmanan I, Junker WM, et al. MUC16 as a novel target for cancer therapy. Expert Opin Ther Targets. 2018 Aug 3;22(8):675–86. https://www.tandfonline.com/doi/abs/10.1080/14728222.2018.1498845

62. Kim B, Park Y, Kim B, Ahn HJ, Lee KA, Chung JE, et al. Diagnostic performance of CA125, HE4, and risk of ovarian malignancy algorithm for ovarian cancer. J Clin Lab Anal. 2019 Jan 1;33(1):e22624. http://doi.wiley.com/10.1002/jcla.22624

63. Sabbatini P, Harter P, Scambia G, Sehouli J, Meier W, Wimberger P, et al. Abagovomab as maintenance therapy in patients with epithelial ovarian cancer: a phase

III trial of the AGO OVAR, COGI, GINECO, and GEICO-the MIMOSA study. J Clin Oncol. 2013 Apr 20;31(12):1554–61.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5795662/

64. Battaglia A, Fossati M, Buzzonetti A, Scambia G, Fattorossi A. A robust immune system conditions the response to abagovomab (anti-idiotypic monoclonal antibody mimicking the CA125 protein) vaccination in ovarian cancer patients. Immunol Lett. 2017 Nov 1;191:35–9.
https://www.sciencedirect.com/science/article/abs/pii/S016524781730353X

65. Rao TD, Fernández-Tejada A, Axelrod A, Rosales N, Yan X, Thapi S, et al. Antibodies against specific MUC16 glycosylation sites inhibit ovarian cancer growth. ACS Chem Biol. 2017 Aug 18;12(8):2085–96.
https://pubs.acs.org/doi/abs/10.1021/acschembio.7b00305

66. Wang Z, Tian Y. Clinical value of serum tumor markers CA19-9, CA125 and CA72-4 in the diagnosis of pancreatic carcinoma. Mol Clin Oncol. 2014 Mar 28;2(2):265–8.
https://www.spandidos-publications.com/mco/2/2/265

67. Meng Q, Shi S, Liang C, Xiang J, Liang D, Zhang B, et al. Diagnostic accuracy of a CA125-based biomarker panel in patients with pancreatic cancer: a systematic review and meta-analysis. J Cancer. 2017;8(17):3615–22.
https://www.jcancer.org/v08p3615.htm

68. Gao Y, Wang J, Zhou Y, Sheng S, Qian SY, Huo X. Evaluation of serum CEA, CA19-9, CA72-4, CA125 and ferritin as diagnostic markers and factors of clinical parameters for colorectal cancer. Sci Rep. 2018 Dec 1;8(1):1–9.
https://www.nature.com/articles/s41598-018-21048-y

69. Huang GQ, Xi Y-Y, Zhang C-J, Jiang X. Serum human epididymis protein 4 combined with carbohydrate antigen 125 for endometrial carcinoma diagnosis: a meta-analysis and systematic review. Genet Test Mol Biomarkers. 2019 Aug 1;23(8):580–8.
https://www.liebertpub.com/doi/10.1089/gtmb.2019.0046

70. Hirsch M, Duffy JM, Davis CJ, Nieves-Plana M, Khan KS. Diagnostic accuracy of cancer antigen 125 for endometriosis: a systematic review and meta-analysis. BJOG An Int J Obstet Gynaecol. 2016 Oct 1;123(11):1761–8.
http://doi.wiley.com/10.1111/1471-0528.14055

71. Hirsch M, Duffy JM, Deguara CS, Davis CJ, Khan KS. Diagnostic accuracy of cancer antigen 125 (CA125) for endometriosis in symptomatic women: a multi-center study. Eur J Obstet Gynecol Reprod Biol. 2017 Mar 1;210:102–7. https://www.sciencedirect.com/science/article/abs/pii/S0301211516310545

72. Leipold D, Mallet WG. Case study: an antibody-drug conjugate targeting MUC16 for ovarian cancer. In: Phillips G (eds) antibody-drug conjugates and immunotoxins cancer drug discovery and development. Springer, New York, NY; 2013. p. 221–39. https://doi.org/10.1007/978-1-4614-5456-4_13

73. Chen Y, Clark S, Wong T, Chen Y, Chen Y, Dennis MS, et al. Armed antibodies targeting the mucin repeats of the ovarian cancer antigen, MUC16, are highly efficacious in animal tumor models. Cancer Res. 2007 May 15;67(10):4924–32. https://cancerres.aacrjournals.org/content/canres/67/10/4924.full.pdf

74. Li T, Wang J. Therapeutic effect of dual CAR-T targeting PDL1 and MUC16 antigens on ovarian cancer cells in mice. BMC Cancer. 2020 Dec 20;20(1):678. https://bmccancer.biomedcentral.com/articles/10.1186/s12885-020-07180-x

75. Zhu X, Cai H, Zhao L, Ning L, Lang J. CAR-T cell therapy in ovarian cancer: from the bench to the bedside. Oncotarget. 2017;8(38):64607–21. https://www.oncotarget.com/article/19929/text/

76. Kaforou M, Wright VJ, Oni T, French N, Anderson ST, Bangani N, et al. Detection of tuberculosis in HIV-infected and -uninfected African adults using whole blood RNA expression signatures: a case-control study. PLoS Med. 2013 Oct 22;10(10):e1001538. https://dx.plos.org/10.1371/journal.pmed.1001538

77. Kaufmann SH, Weiner J, Maertzdorf J. Accelerating tuberculosis vaccine trials with diagnostic and prognostic biomarkers. Expert Rev Vaccines. 2017;16(8):845–53. https://doi.org/10.1080/14760584.2017.1341316

78. Kaufmann SH, Fortune S, Pepponi I, Ruhwald M, Schrager LK, Ottenhoff TH. TB biomarkers, TB correlates and human challenge models: new tools for improving assessment of new TB vaccines. Tuberculosis. 2016;99:S8–11. http://dx.doi.org/10.1016/j.tube.2016.05.010

79. Fletcher HA, Dockrell HM. Human biomarkers: can they help us to develop a new

tuberculosis vaccine? Future Microbiol. 2016 Jun 1;11(6):781–7. https://www.futuremedicine.com/doi/10.2217/fmb.16.15

80. Bloom CI, Graham CM, Berry MP, Rozakeas F, Redford PS, Wang Y, et al. Transcriptional blood signatures distinguish pulmonary tuberculosis, pulmonary sarcoidosis, pneumonias and lung cancers. Rengarajan J, editor. PLoS One. 2013 Aug 5;8(8):e70630. https://dx.plos.org/10.1371/journal.pone.0070630

81. Shapiro AE, Variava E, Rakgokong MH, Moodley N, Luke B, Salimi S, et al. Community-based targeted case finding for tuberculosis and HIV in household contacts of patients with tuberculosis in South Africa. Am J Respir Crit Care Med. 2012 May 15;185(10):1110–6. https://www.atsjournals.org/doi/full/10.1164/rccm.201111-1941OC

82. World Health Organization (WHO). Use of high burden country lists for TB by WHO in the post-2015 era (discussion paper). 2015. https://www.who.int/tb/publications/global_report/high_tb_burdencountrylists2016-2020.pdf

83. World Health Organization (WHO). High-priority target product profiles for new tuberculosis diagnostics: report of a consensus meeting. Geneva; 2014. https://apps.who.int/iris/bitstream/handle/10665/135617/WHO_HTM_TB_2014.18_eng.pdf

84. Zak DE, Penn-Nicholson A, Scriba TJ, Thompson E, Suliman S, Amon L, et al. A prospective blood RNA signature for tuberculosis disease risk. Lancet. 2017;387(10035):2312–22. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5392204/

85. Dawany N, Showe LC, Kossenkov A V., Chang C, Ive P, Conradie F, et al. Identification of a 251 gene expression signature that can accurately detect M. tuberculosis in patients with and without HIV co-infection. Torrelles JB, editor. PLoS One. 2014 Feb 25;9(2):e89925. https://dx.plos.org/10.1371/journal.pone.0089925

86. Shukla SK, Gunda V, Abrego J, Haridas D, Mishra A, Souchek J, et al. MUC16-mediated activation of mTOR and c-MYC reprograms pancreatic cancer metabolism. Oncotarget. 2015;6(22):19118–31. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4662479/

87. Yu L, Chen X, Sun X, Wang L, Chen S. The glycolytic switch in tumors: how many players are involved? J Cancer. 2017;8(17):3430–40. https://www.jcancer.org/v08p3430.htm

88. Kornberg MD. The immunologic Warburg effect: evidence and therapeutic opportunities in autoimmunity. Wiley Interdiscip Rev Syst Biol Med. 2020;1–17. https://onlinelibrary.wiley.com/doi/full/10.1002/wsbm.1486

89. Escoll P, Buchrieser C. Metabolic reprogramming of host cells upon bacterial infection: why shift to a Warburg-like metabolism? FEBS J. 2018 Jun 14;285(12):2146–60. https://onlinelibrary.wiley.com/doi/abs/10.1111/febs.14446

90. Shi L, Salamon H, Eugenin EA, Pine R, Cooper A, Gennaro ML. Infection with Mycobacterium tuberculosis induces the Warburg effect in mouse lungs. Sci Rep. 2015 Dec 10;5(1):18176. www.nature.com/scientificreports

91. Gleeson LE, Sheedy FJ, Palsson-McDermott EM, Triglia D, O'Leary SM, O'Sullivan MP, et al. Cutting edge: Mycobacterium tuberculosis induces aerobic glycolysis in human alveolar macrophages that is required for control of intracellular bacillary replication. J Immunol. 2016 Mar 15;196(6):2444–9. http://www.jimmunol.org/content/196/6/2444

APPENDIX A

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

Private Bag 3 Wits, 2050
Fax: 027117172119
Tel: 02711 7172076

Reference: Mrs Sandra Benn
E-mail: sandra.benn@wits.ac.za

02 December 2019
Person No: 1894968
PAG

Dr K Akwue
4 Memeh Street
321251
Nigeria

Dear Dr Kenneth Akwue

Master of Science in Medicine: Approval of Title

We have pleasure in advising that your proposal entitled *CA-125 in the diagnosis and Therapeutic monitoring of Tuberculosis in HIV Positive patients* has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

APPENDIX B

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Kenneth Akwue et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M191082

NAME: (Principal Investigator)	Kenneth Akwue et al
DEPARTMENT:	Pathology CLS and NHLS
PROJECT TITLE:	CA-125 in the diagnosis and therapeutic monitoring of tuberculosis in HIV positive patients
DATE CONSIDERED:	Ad hoc
DECISION:	Approved unconditionally
CONDITIONS:	Lab Study
SUPERVISOR:	Faheem Seedat
APPROVED BY:	Dr C Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	08/11/2019

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 October and will therefore be due in the month of October each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

11/11/2019
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES