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Clinical, radiological and laboratory predictors of positive urine lipoarabinomannan in sputum scarce and sputum negative patients with HIV associated TB in two Johannesburg Hospitals

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CLINICAL, RADIOLOGICAL AND LABORATORY PREDICTORS OF POSITIVE URINE LIPOARABINOMANNAN IN SPUTUM SCARCE AND SPUTUM NEGATIVE PATIENTS WITH HIV ASSOCIATED TB IN TWO JOHANNESBURG HOSPITALS

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Medicine in the branch of Internal Medicine.

Johannesburg, 2021

DECLARATION

I, Lior Chernick, declare that this research report is my own work. It is being submitted for the degree Master of Medicine (in the submissible article format with my protocol and an extended literature review) in the branch of Internal 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.

Signed: *Lior Chernick* on this 24th day of March 2021

DEDICATION

To my incredible wife Gina and my boys Ariel and Raphael, the lights of my life, for keeping me grounded and inspired. Thank you for your never-ending support, love, and sacrifice to allow for my pursuits in medicine…

To my parents, Joel and Lynette, for your guidance and upbringing and endless belief in me…

To my patients, whose resilience inspires me daily…

PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS STUDY

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ABSTRACT

Background

Tuberculosis is a major cause of mortality in HIV-infected patients. The diagnosis of TB in patients with low CD4 counts using sputum-based diagnostics is hampered by paucibacillary disease with these patients often being sputum negative or sputum scarce. Urine lipoarabinomannan (LAM) has shown promise in point of care detection of TB in this patient subset but it lacks sensitivity and its exact role in a diagnostic algorithm for TB in South Africa remains to be elucidated.

Methods

This multicentre retrospective record review compared the clinical, radiological and laboratory characteristics of sputum scarce or sputum negative HIV infected patients in two hospitals who underwent urine LAM testing in line with WHO recommendations.

Results

Over a third of patients (35%) had a positive LAM, with a higher yield in sputum scarce patients (42 vs 30%, p = 0.0141). These patients were more likely to have delirium (OR 2.2, 95% CI 1.2 - 3.7), a higher median heart rate (p=0.0135) and a qSOFA score ≥ 2 (OR 3.5, 95% CI 1.6 – 7.6). A positive LAM was significantly associated with the presence of disseminated TB (p < 0.0001) . It was also associated with a clinical diagnosis of TB immune reconstitution syndrome (p=0.0035) and abdominal TB (p<0.0001). Laboratory predictors of a positive LAM included renal dysfunction (p=0.044), severe anaemia (p = 0.0116) and a higher median C-Reactive protein (p=0.0131). Positive LAM results were also noted in 75% of patients with disseminated non-tuberculous mycobacterial infections (p=0.0053).

Conclusion

Urine LAM testing for TB had significant diagnostic utility in HIV infected inpatients that were sputum scarce or sputum negative. A positive LAM was associated with disseminated disease, several markers of severe illness, and the diagnosis of TB IRIS. Disseminated non-tuberculous mycobacterial infection may result in positive urine LAM results. Select use in these patient subsets could maximise yield and improve predictive value, in addition to improving the time to diagnosis.

Keywords: HIV, TB, Lipoarabinomannan, Sputum negative, Sputum scarce

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ABBREVIATIONS

AFB	Acid fast bacilli
ALP	Alkaline phosphatase
ALT	Alanine transferase
ART	Anti-retroviral therapy
BCG	Bacillus Calmette–Guérin
BP	Blood pressure
CD4	Cluster differentiation 4 (T Cell receptor)
CI	Confidence interval
CRP	C-Reactive protein
CXR	Chest X-ray
ELISA	Enzyme-linked immunosorbent assay
EPTB	Extrapulmonary tuberculosis
GFR	Glomerular filtration rate
GGT	Gamma glutamyl transferase
IGRA	Interferon gamma release assay
IQR	Interquartile range
IRIS	Immune reconstitution syndrome
Hb	Haemoglobin
HIV	Human immunodeficiency virus
HIVAN	HIV associated nephropathy
IFN-γ	Interferon gamma
LAM	Lipoarabinomannan
LAMP-PCR	Loop-mediated isothermal amplification polymerase chain reaction

LFA	Lateral flow assay
LFT	Liver function test
LTBI	Latent tuberculosis infection
MAC	*Mycobacterium avium* complex
MAP	Mean arterial pressure
MDR-TB	Multi-drug resistant tuberculosis
MTB	*Mycobacterium tuberculosis*
NAAT	Nucleic acid amplification test
NTM	Non-tuberculous mycobacteria
PJP	*Pneumocystis jirovecii* pneumonia
Plt	Platelet count
PTB	Pulmonary tuberculosis
qSOFA	Quick Severity of Organ Failure Assessment
SA	South Africa
SD	Standard Deviation
SSM	Sputum smear microscopy
TB	Tuberculosis
TBM	Tuberculous Meningitis
TNF	Tumour Necrosis Factor
TST	Tuberculin skin testing
ULN	Upper limit of normal
WCC	White cell count
WHO	World Health Organization
ZN	Ziehl-Neelsen

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CHAPTER 1 - PROTOCOL WITH EXPANDED LITERATURE REVIEW

1.1 Expanded literature review

1.1.1 Tuberculosis

Tuberculosis (TB) describes a wide spectrum of illness caused by the organism *Mycobacterium tuberculosis* (MTB). The majority of infections are acquired via the inhalation of droplet nuclei, making pulmonary tuberculosis (PTB) the most frequent site of initial infection in the general population, with increased prevalence of extrapulmonary TB (EPTB) in certain population subsets [1]. The disease spectrum ranges from latent disease with no clinical symptoms, to active TB along a spectrum of severity, with complex bacterial and host dynamics resulting in the varied host response to infection [2]. In 1993 the World Health Organization (WHO) declared TB a global public health emergency, and TB remains a significant global public health threat [1].

Epidemiology

Over 1 billion people are thought to have latent TB infection (LTBI) globally, although only some of those will go on to develop active disease [3]. Active TB affects approximately 10 million people per year, and as of 2018 it remains the number one cause of death from a single infectious agent worldwide, responsible for an estimated 1.6 million deaths annually. It is a disease of poverty with a skewed distribution towards developing nations, with most high-income countries having an estimated TB incidences of less than ten per 100 000 population per year, while the 30 highest TB burden countries (generally low-middle income) have a combined TB incidence of 183 per 100 000 population per year [2]. South Africa (SA) has the second highest incidence globally of TB with approximately 567 new cases per 100 000 population annually [4]. In South Africa TB remains the number one cause of death with 56 000 deaths in 2017, with more than half of these patients (60%) co-infected with human immunodeficiency virus (HIV), which remains a major risk factor for active TB [5]. Even more concerning is that in resource limited settings there remains a significant proportion (estimated as up to 48%) of patients that remain undiagnosed ante-mortem [6].

Pathogenesis

The natural history of TB infection generally involves inhalation of droplet nuclei 1-5µm in diameter containing MTB bacilli, which can remain aerosolized for several hours after a

patient with PTB coughs [7]. After the bacilli are inhaled, one of three possible clinical outcomes may occur, depending on the immune status of the host. These clinical outcomes are: (i) intact innate or adaptive immunity resulting in clearance of infection, (ii) establishment of LTBI (usually in the lungs or lymph nodes) which remains dormant or (iii) activation (or reactivation) of LTBI that progresses to active disease (this may occur primarily, or after a significant period of dormancy). Active TB disease may in the early stages be sub-clinical and can subsequently disseminate haematogenously or via the lymphatics to various organs to cause EPTB [8]. A complex interplay between MTB bacilli and the host immune system are involved in the above process initiated by small inoculum of MTB, which multiply within alveolar macrophages, using several strategies to survive within the macrophage phagosome and thus avoiding host adaptive T cell responses [1].

Entry of the TB bacilli into macrophages involves interactions with several receptors, including a mannose binding receptor that binds to MTB via lipoarabinomannan (LAM), a lipopolysaccharide anchored to the cell wall of the MTB bacillus that has several other functions in immune evasion by MTB [9]. Thereafter, depending on several host factors including but not limited to: genetics, hypersensitivity to MTB, HIV infection and Cluster differentiation 4 T-cell receptor (CD4) cell count and other causes of immunosuppression, the infection is often contained within a granulomatous reaction involving host macrophages, lymphocytes and various other cells. The immune response of these effector cells is mediated by various cytokines especially tumour necrosis factor (TNF) that often predispose to development of fibrosis, scarring and calcification within the lung parenchyma. Alternatively, if the infection is not fully contained by the host immune response, there is subsequent tissue destruction mediated in part by the host immune response to proliferating bacilli (caseous necrosis), and clinical disease occurs with the possibility of bacillary seeding to extrapulmonary sites of infection [1].

Clinical manifestations

The classical presentation of TB in the immunocompetent adult host is PTB, which is often cavitatory in nature, constitutional symptoms (weight loss, lassitude, loss of appetite and drenching night sweats), a productive cough and haemoptysis. This may be primary (typically occurring 2-8 weeks after infection) or post primary (reactivation of latent disease) with an incubation period of approximately 24 months in high endemic settings [2,10]. The chest X-ray (CXR) appearance can vary depending on primary versus post primary TB, but often

demonstrates ~~upper or lower lobe~~ cavitation and consolidation, hilar lymphadenopathy, and frequently pleural effusions. Extrapulmonary disease is less frequent in the immunocompetent host with lymphadenitis and isolated pleural effusions being the most frequent extrapulmonary sites [10].

Clinical manifestations in HIV positive patients

Tuberculosis is one of the most common HIV associated conditions with prevalence of LTBI in some urban settings in sub-Saharan Africa as high as 80%[11]. It is responsible for up to a quarter of all HIV related mortality[4]. In HIV positive patients with LTBI the annual risk for developing TB is as high as 16% per annum[12]. The presentation also varies depending on severity of the underlying immunosuppression. In early HIV with preserved CD4 counts the presentation tends to mirror that of the immunocompetent host with frequent pulmonary TB with upper lobe cavitation and pleural effusions. At lower CD4 counts < 200 cells/µL the presentation is atypical, with subtle interstitial infiltrates, less cavitation, the presence of pleural effusions, and intrathoracic lymphadenopathy. Sputa in these patients tends to be paucibacillary in nature hampering definitive microbiological diagnosis [13]. There is a significantly increased frequency in these patients of extra pulmonary or *disseminated disease* (defined variably as TB in two or more sites, recovery of MTB from blood or bone marrow, and classic miliary TB) [14,15]. In some case series, this occurs in as many as 40-60 % of TB cases in whom features of PTB are often absent [10]. Disease can be localised to a single organ or result in multiple foci of extrapulmonary disease simultaneously. Examples of EPTB include TB meningitis (TBM), miliary TB via haematogenous spread, TB lymphadenitis, TB pleuritis, and intra-abdominal TB with ascites, intra-abdominal lymphadenopathy and often hepatic and splenic involvement [1]. An often overlooked extrapulmonary site of infection with perhaps an increased prevalence in the setting of HIV is renal or genitourinary tract TB, with a frequency variably described as ranging between 10 and 40% cases of EPTB [1,16–18]. The clinical manifestations in these patients are protean, creating challenges in accurate diagnosis. In addition to this, several other HIV related conditions (infectious and non-infectious) can present with similar signs and symptoms as both PTB and EPTB further confounding definitive diagnosis [19]. There also exists an entity unique to HIV in the form of the TB immune reconstitution syndrome (IRIS), whereby often severe inflammatory symptoms of TB present for the first time post initiation of anti-retroviral therapy (unmasking IRIS) or in patients with known TB, when their symptoms worsen (paradoxical IRIS). TB IRIS carries significant morbidity [20]. Finally, the increased incidence of disseminated tuberculosis carries

with it a significantly increased mortality in HIV associated TB [15] with over 70% of TB related deaths in South Africa occurring in people living with HIV [4].

1.1.2 Diagnostic tools for TB

TB culture

Diagnosis of active TB requires the demonstration of the presence of MTB bacilli. The gold standard (as with most bacterial infections) is culture of MTB from clinical specimens, allowing accurate identification with the added benefit of subsequent phenotypic drug susceptibility testing. Mycobacterial culture is ~~however~~ limited by the slow growth of MTB in culture. Culture of MTB requires an estimated 3-8 weeks on solid culture medium and between 1-3 weeks using automated liquid broth methods [1], making this less useful in the subset of very ill patients (more common in the setting of HIV) who require timeous diagnosis and initiation of treatment. In addition, it requires laboratory infrastructure and expertise.

Sputum smear microscopy

In PTB, diagnosis was traditionally made with the demonstration of mycobacteria using sputum smear microscopy (SSM) with Ziehl-Neelsen (ZN) staining for acid fast bacilli (AFB). Benefits of this diagnostic modality include its ease, low cost, and the need for only a simple laboratory equipment with basic training. In addition, the results are available rapidly, and it remains the most commonly used test in many low income countries with high rates of TB infection [21]. It does, however, suffer from limited sensitivity (50-60%) [22], which drops to as low as 20% in the setting of HIV coinfection [23]. An alternative technique using fluorescence microscopy and auramine staining improves overall sensitivity of SSM by 10% but requires more sophisticated laboratory infrastructure [24].

Tests for latent TB

Latent tuberculosis infection is defined as “a state of persistent immune response to stimulation by MTB antigens without evidence of clinically manifested active TB” [24]. Its clinical relevance lies in the risk for future active TB and the scope for prophylactic therapy. Tests for latent TB include tuberculin skin testing (TST), which is also known as the Mantoux test, and interferon gamma release assays (IGRAs).

Tuberculin skin testing is performed by the intradermal injection of a standardized dose of a TB related antigen and measuring response to what is a type IV hypersensitivity reaction of

the skin in previously sensitized patients at a standardised timepoint thereafter. It is one of the oldest diagnostic tests for TB, but it is limited by its inability to distinguish latent versus active disease. It is also hampered by false positive results related to prior Bacillus Calmette–Guérin (BCG) vaccination and incorrect administration and interpretation (being a particularly operator-dependant test), and false negative results being related to a number of host factors including HIV positivity, hindering its use in a significant proportion of patients with suspected TB [24].

The use of IGRAs such as QuantiFERON® TB Gold (Cellestis Ltd., Carnegie, Victoria, Australia) measure interferon gamma (IFN-γ) release in whole blood by mononuclear cells in response to TB antigen exposure, which is a surrogate measure of the presence of LTBI. They are recommended by the WHO for the diagnosis of LTBI in specific circumstances, for example prior to the use of certain drugs causing increased risk of reactivation TB such as anti-tumour necrosis factor treatment, prior to organ transplant, in patients living with HIV, and several other scenarios in which there is a high TB exposure or reactivation risk. It is the gold standard diagnosis for LTBI, but again does not distinguish between latent and active TB and suffers from reduced sensitivity in the setting of underlying HIV. In addition they are expensive, and require established laboratory infrastructure and training, making them unsuitable for a resource limited setting [21].

Radiology

Radiology is a valuable component in the armamentarium of diagnostic modalities for both PTB and EPTB, especially in the realm of screening. The most frequently used radiological modality for PTB diagnosis is chest radiography (CXR). Similarly, for EPTB, certain features on abdominal ultrasound are suggestive of the presence of intraabdominal TB but are hampered by poor specificity and sensitivity[25].

Chest Radiography

The WHO recommends CXR as a complementary tool in the diagnosis of PTB in conjunction with microbiological confirmation. given the number of conditions that can mimic PTB radiologically. Chest x-ray in conjunction with clinical assessment can be used to triage those patients who should be tested with microbiological methods (such as with the Xpert MTB/RIF assay) to reduce the number of individuals tested and the associated costs [26]. While an abnormal CXR has fair sensitivity as a screening tool in HIV negative patients, it lacks specificity given the multitude of conditions that can mimic the abnormal appearance[27]. In the

setting of HIV coinfection, up to 9% of patients with confirmed PTB and lower CD4 counts can have a normal CXR, hampering sensitivity in this subset [28]. Unfortunately, given the proportion of patients with HIV presenting with isolated EPTB, its usefulness is further limited as a screening tool for TB in this population.

Typical CXR features of active TB infection include hilar lymphadenopathy, parenchymal consolidation, pleural effusions, cavitation, nodular infiltrates, non-cavitatory infiltrates, and a miliary pattern in haematogenously seeded disease [7,28].

A potential advancement in the use of CXR is the application of artificial intelligence driven computer aided image analysis to the diagnostic process offering a degree of automation using software like CAD4TB. Despite the promise of such technology, there is still a need for further research before such it could be used in routine clinical practice [26].

Abdominal ultrasound

Intra-abdominal TB is a common location for EPTB in patients with HIV, either in isolation or as part of disseminated TB [29]. It may present with abdominal pain, ascites and occasionally jaundice, but it may also be detected in asymptomatic patients when liver function tests (LFT) such as alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) are noted to be elevated [30], often termed an 'infiltrative' or cholestatic liver function test (further defined below in '*Ancillary laboratory findings*'). Sonographic features of intra-abdominal TB include splenic hypoechoic lesions (often termed micro-abscesses), ascites, intra-abdominal lymphadenopathy, hepatomegaly and splenomegaly. Data on the usefulness of such findings is heterogenous and vary vastly in their respective sensitivities (35-82%) and specificities (20-92%) [25].

Radiology as sole modality of diagnosis

While radiological methods are often accessible diagnostic modalities even in resource constrained settings, it should be noted that they are often only suggestive, and not diagnostic of TB. Suggestive radiological findings of TB ideally require microbiological confirmation and are thus useful as screening tools only, given their suboptimal sensitivity and specificity when used in isolation.

Nucleic acid amplification tests

Nucleic acid amplification tests (NAAT) such as the Xpert MTB/RIF (Cepheid, USA) assay have been endorsed by the WHO as rapid diagnostic tools and have dramatically improved the time to diagnosis of patients with PTB to under 3 hours of laboratory time, and as such have become the recommended diagnostic modality especially in patients with HIV and when multi-drug resistant TB (MDR-TB) is a consideration [31]. Studies comparing the Xpert MTB/RIF to SSM in smear positive patients have shown similar specificity (94.4% versus 99.7%) with improved sensitivity (78.7% vs 66.7%), and an improved yield on smear negative patients with a sensitivity of 46.8% [32]. The reduced need to await culture in smear negative patients dramatically improves the time to diagnosis [33]. The newer MTB/RIF Ultra (Xpert Ultra) has shown even more promise in diagnosis of PTB with further improved sensitivity (pooled sensitivity 88%) in both HIV positive and HIV negative patients, albeit at the expense of slightly lower specificity (pooled specificity 96%) [34].While certainly a landmark development in TB diagnostics, the relative cost, need for complex machinery, training, and a reliable power supply limit its usage to larger centralised laboratories, and can preclude point of care testing. This often results in diagnostic delays or inaccessibility in resource limited settings [35]. Furthermore, in settings with high HIV prevalence, patients are often sputum scarce, sputum negative or have extrapulmonary TB, making sputum diagnostics less useful. The original Xpert MTB/RIF assay has been studied in the diagnosis of extrapulmonary disease but the sensitivity is lower than on sputum, and obtaining extrapulmonary samples is not always feasible especially in resource constrained settings [36]. It is not recommended for usage on whole blood samples [31] and it has poor concordance (56%) with mycobacteraemia [37] and as such not in mainstream use to detect this form of disseminated TB, which is common in advanced HIV [15]. This highlights the need for new diagnostic methods which would ideally be reliable, cost effective and available in a point of care format, and applicable to patients with HIV associated TB with the associated high incidence of both sputum scarce and extra pulmonary disease.

There have been advances in attempting to incorporate PCR technology into TB diagnostics, by making PCR more accessible and less cumbersome, requiring fewer steps and thus less equipment. An example of this is Loop-mediated isothermal amplification polymerase chain reaction (LAMP-PCR), which is a form of isothermal PCR. Being an isothermal

amplification process, it does not require a thermal cycler for the various amplification and annealing stages as in conventional PCR[38]. An assay for the diagnosis of pulmonary TB, the Loopamp™, (Eiken Chemical Company, Tokyo, Japan) has been endorsed by the WHO as an alternative to SSM in adults with similar specificity and sensitivity. It is a manual assay that requires less than 1 hour to perform and can be read with the naked eye under ultraviolet light. It is not endorsed to replace the Xpert MTB/Rif assay, is of unclear usage in people living with HIV and is not endorsed for usage in the diagnosis of EPTB [38]. These factors severely limit the applicability of this diagnostic modality in a resource-constrained population with high background HIV prevalence, such as in South Africa.

Ancillary laboratory findings

Haematological abnormalities in patients with TB may include a anaemia of chronic disorders, a mildly elevated white cell count (often including a monocytosis) and occasionally a mild thrombocytosis. Many HIV-negative patients with active tuberculosis have reduced CD4 T lymphocyte counts, which normalize with treatment. Haematuria or sterile pyuria may suggest renal tuberculosis. Hyponatremia with features of inappropriate secretion of antidiuretic hormone is characteristic of tuberculous meningitis but also can occur with isolated pulmonary disease. Hyponatremia in tandem with hyperkalaemia may also suggest associated adrenal insufficiency due to adrenal tuberculosis[3]. Hypercalcemia can also be also seen during pulmonary tuberculosis, usually in the first weeks of therapy[1]. C-reactive protein has also been shown to correlate with disease severity but is poorly specific for TB in isolation [39]. Abnormal liver function tests can be seen in TB of the abdomen involving the liver. These are typically an infiltrative or cholestatic pattern [30] which can be defined using an R-Factor of <2 (calculated by the formula: $\frac{ALT}{ALT\ ULN} \times \frac{ALP\ ULN}{ALP}$)[40]

No single ancillary laboratory test listed above is ~~however~~ in any way pathognomonic for TB, but they do play an ancillary role in supporting the diagnosis and possibly defining the severity of the disease.

Antigen and antibody-based testing

Serology-based testing are common modalities in the diagnosis of numerous infectious diseases, given their robust nature and ease of administration. These tests may be either enzyme-linked immunosorbent assays (ELISA) or a lateral flow assay (LFA). Serodiagnostic

tests in a user-friendly format would be a welcome addition to the TB diagnostic armamentarium, given their ubiquitous use in the setting of other infectious diseases.

Multiple serological assays aimed at the diagnosis of TB have been developed and marketed but unfortunately, they provide inconsistent and imprecise results with highly varied sensitivity and specificity. These disappointing and varied results led to a strong policy statement from the WHO that "there is no evidence that existing commercial serological assays improve patient outcomes, and the high proportions of false-positive and false-negative results adversely impact patient safety. The overall data quality was deemed to be low and it has been strongly recommended that these tests **not** be used for the diagnosis of pulmonary or extra-pulmonary TB"[41].

The only recommended diagnostic test set from this subset of antibody or antigen-based testing is in fact urine-based assay for the detection of the MTB antigen *lipoarabinomannan*. This test, which was developed in its original format in 2005 (Chemogen, Portland, USA), has been the subject of a number of studies and has shown promise as a useful adjunct to the diagnosis of TB, specifically in patients living with HIV [9,42].

1.1.3 Lipoarabinomannan

Microbiology

Lipoarabinomannan (LAM) is a glycolipid component of the mycobacterial outer cell wall. It is one of three major groups of interrelated lipopolysaccharides that are found in all mycobacterial species [9,43]. These molecules attach to the mycobacterial plasma membrane via a glycol-phospholipid anchor and extend to the mycobacterial cell wall surface. The phospholipid anchor is linked to a mannose core which is conserved throughout all mycobacterial species. From this mannose core, various side-chains arise which are variably capped with *mannose residues* creating the significant heterogeneity of LAM molecules across mycobacterial species with a range of diverse properties and functions (*figure 1*) [9,43,44].

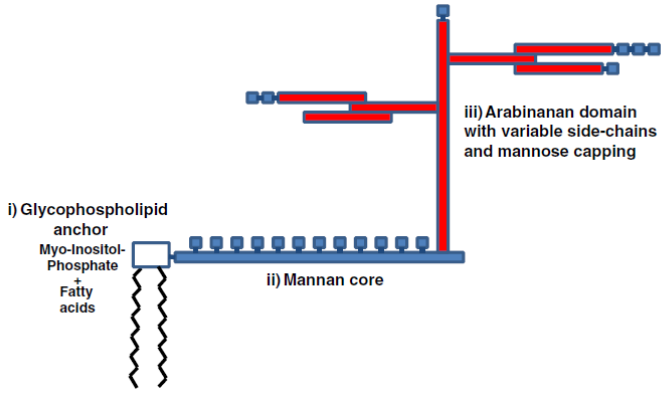


Figure 1 - Lipoarabinomannan structure – adapted with permission under creative commons attribution license from 'Lawn BMC Infectious Diseases 2012, 12:103' [9]

The 3 variants of mannose capping include

i. ManLAM (LAM molecules with mannosylated caps)
ii. PILAM (LAM molecules with phosphoinositol caps)
iii. AraLAM (no specific cap)

ManLAM is found on the more pathogenic mycobacterial species (*M. Tuberculosis*, *M. leprae*, *M. bovis*, ~~MAC~~MAC) and facilitates entry into macrophages via mannose receptors. Macrophages are the preferred intracellular environment for MTB. They also act as virulence factors, resulting in inhibition of phagosome maturation, apoptosis, interferon-γ-signalling in macrophages and interleukin-12 release [9,43–45]

PILAM is found on non-pathogenic species such as *M. smegmatis*, while AraLAM is found on various faster growing mycobacterial species such as *M. chelonae* [43] .

AraLAM and PILAM have a strong pro-inflammatory effect within the human host, in contrast to ManLAM molecules, which have potent immunomodulatory properties as previously described [9,43].

Lipoarabinomannan from any mycobacterial source is somewhat heterogenous with regards to size, pattern of branching of the sidechains, acetylation and phosphorylation of the mannan core and the arabinan side chains with a peak molecular weight of 17.3 kDa. In addition it is heat stable [44].

These characteristics have significant implications with respect to its usage as a potential biomarker of TB although it should be noted that a number of non-tuberculous mycobacteria (NTM) and several other species (Nocardia and Candida) have been found to cross-react with various LAM assays in vitro [46,47] which is of concerned given that disseminated infections caused by these organisms are not uncommon in patients with HIV and low CD4 counts.

Utility as a biomarker of TB

LAM is released by metabolically active and by degrading mycobacteria and is detectable *in vitro* in significant quantities from mycobacterial cultures [9]. It is variably detectable in the urine and with some processing the serum [48] of *some* patients with TB. It is also noted to be immunogenic, generating anti-LAM antibodies in most patients [43,49] and as such has been explored as both a diagnostic modality [9,42] and a possible vaccine target [50,51]

The paradox that free LAM should be more readily detected in urine (owing to higher concentrations) than in serum is likely related to the fact that it is immunogenic and thus exists as antibody-antigen complexes in serum and associates *in vivo* with other molecules especially lipoproteins, preventing glomerular filtration of free LAM [48].

There is significant heterogeneity in reported literature with regards to the detectability, utility, specificity, and sensitivity of LAM as a biomarker of TB. This applies to both serum and urine testing using various assays and across various cohorts of patients. As discussed, serum based assays are not recommended by WHO due to said heterogeneity and poor diagnostic accuracy [41]. As for urine LAM assays, despite promising sensitivity and specificity in the initial trials using the first prototype urine LAM ELISA done in Tanzania by Boehme et al. in 2005 [52], (80% and 99% respectively), most subsequent trials showed significantly less impressive results in both ELISA and LFA formats, with particularly poor sensitivities (6-21% in an HIV negative population, 21-67% in an HIV positive population) despite consistently good specificity (88-100%) [23,53–57]. It needs to be noted that many of the earlier trials used sputum TB culture as the gold standard to assess diagnostic accuracy or urine LAM, and as will be discussed in the following section, urine LAM may be a better representation of disseminated TB as opposed to compartmentalised pulmonary TB which would render sputum TB culture a poor gold standard in isolation.

Within the more recent literature based on WHO recommendations and a recent Cochrane review, there is consensus that the utility of urine LAM as a diagnostic modality is most apparent and pragmatic using an available *lateral flow assay* in the form of the Alere

Determine™ TB LAM Ag, within the population of people living with HIV with advanced immunosuppression defined by a CD4 range below 100 cells/L or those deemed as seriously ill. This has been defined by the WHO based on 4 danger signs: respiratory rate > 30/min, temperature > 39°C, heart rate > 120 beats per min and a patient who is unable to walk unaided[42,58].

Pathophysiology of urinary lipoarabinomannan excretion

Understanding the mechanism of LAM excretion in urine is fundamental to establishing its place in the diagnosis of TB in people living with HIV and can further inform recommendations and utility. Several proposed models exist as illustrated in *figure 2*. Circulating LAM is often complexed with either antibodies or other carrier molecules which would limit filtration in the absence of increased glomerular permeability (*figure 2A)*. It has been suggested that a glomerulopathy such as HIV associated nephropathy (HIVAN) could account for the filtration of bound LAM but no clear association with significantly increased proteinuria, a marker of glomerular dysfunction, has been established in patients with a positive LAM previously [59]. Another possible mode of excretion (*figure 2B)* is that LAM antigenuria is a result of glomerular filtration of some freely circulating LAM in the bloodstream, released from any site of disease by metabolically active or degrading mycobacteria[23,57].

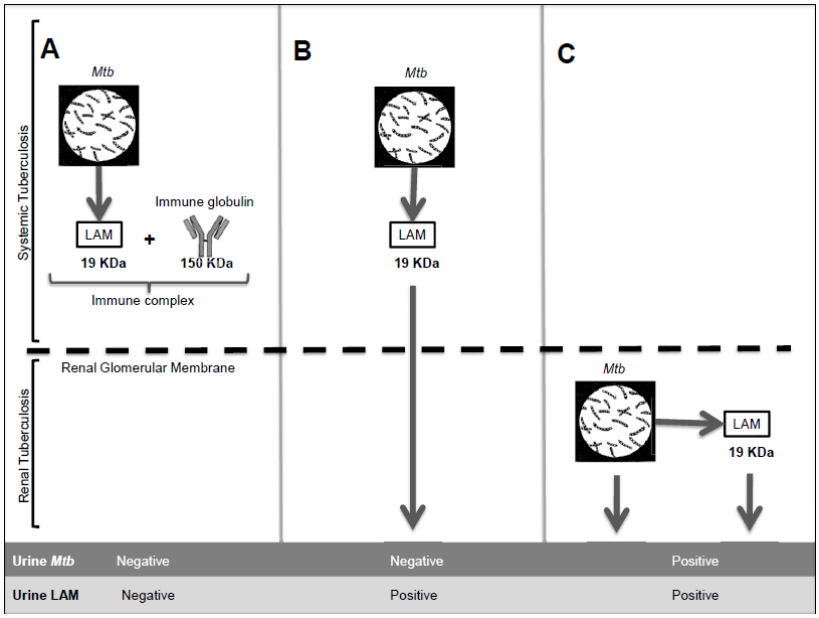


Figure 2 - Proposed methods of excretion of urinary lipoarabinomannan - adapted with permission under creative commons attribution license from 'Wood et al. BMC Infectious Diseases 2012, 12:47' [18]

There is ~~however~~ significant evidence that LAM antigenuria may rather represent involvement of the renal parenchyma and urogenital tract (*figure 2C)* perhaps in isolation or more likely via haematogenously disseminated tuberculosis, as summarized by Lawn and Gupta-Wright [59]:

1. LAM concentration in the urine does not increase during the first weeks of TB treatment as would be expected from the bactericidal effect of anti-tuberculous therapy resulting in increased serum concentrations of lipoarabinomannan from lysed bacteria [18]
2. There is an association of LAM positivity with evidence of whole TB organisms within the urine via either culture or DNA based testing [18]
3. Patients with mycobacteraemia (with possible renal seeding) are more frequently urine LAM positive [60]
4. Post-mortem renal TB in HIV-positive patients is strongly associated with LAM-positive urine [61].

This difference in origin has implications in terms disease severity, and likely patient demographics (for example HIV status and CD4 count) and intensity of care. It also raises further questions as to who ultimately would benefit most from having a urine LAM test performed and how to identify them appropriately.

Commercial diagnostics

Commercial diagnostics for urine LAM originated with a precommercial prototype ELISA (Chemogen, Portland, USA), which was clinically trialled in 2005 in Tanzania [57]. This was superseded by the first readily available commercial ELISA for urine LAM, the Clearview TB ELISA (Inverness Medical Innovations, Waltham, Massachusetts, USA) which used the same polyclonal antibody used in the second generation Chemogen assay [23]. The subsequent lateral flow assay (LFA) format in current use was developed by Alere (Alere Determine™ TB LAM Ag) with studies suggesting good comparative agreement with the ELISA based formats [52] allowing for true point of care testing with results available in approximately 25

minutes with a random urine sample of just 65µl of urine together with the testing strip. This LFA (*figure 3A*) attaches colloidal gold-labelled antibodies to LAM that are captured by immobilised LAM antibodies further along the test strip and form a visual band which is then compared to a reference card (*figure 3B)* for interpretation which is dependent on band visibility and intensity [61]. Pooled sensitivity in the recommended population is between 35-42% and pooled specificity 91-95%, depending on clinical setting and symptoms of disease and with an inverse relationship allowing for improved sensitivity with declining CD4 count [58]. It is this assay currently endorsed by the WHO for use in HIV positive patients with a CD4 count of less than 200 cells/µL with signs and symptoms of TB, advanced HIV, or seriously ill regardless of CD4 count. [42].

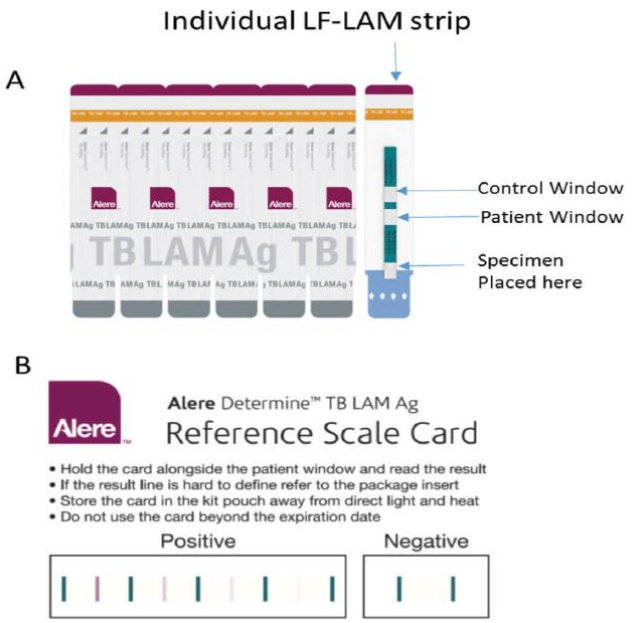


Figure 3 - Alere Determine TB LAM Ag - adapted with permission from Abott (formerly Alere)

Newer urine LAM assays are under exploration, with two promising developments. The first is the FujiLAM™ (Fujifilm, Tokyo, Japan) which is a novel assay that detects LAM on an instrument free platform, with results available in less than 1 hour. This assay combines a

pair of high affinity monoclonal antibodies directed towards MTB-specific epitopes and a patented silver-amplification step (SILVAMP™) that enables the detection of urinary LAM concentrations that are significantly lower than that detected by the Alere LAM assay, reportedly improving the overall sensitivity to 70% while maintaining similar specificity [62]. However, the above study was done retrospectively on banked samples, and real-world utility while certainly promising remains to be established in a prospective trial.

Another promising development was described by Paris et al. using an innovative technique of a copper complex dye within a hydrogel nanocage that captures LAM with very high affinity, displacing interfering urine proteins, and allowing detection of much lower concentrations of LAM, dramatically improving theoretical sensitivity to 95% [63]. Nevertheless, this technology is still at the investigative stage and has yet to be commercialised or adapted to a point of care format and remains to be studied in clinical use.

Evidence for utility and benefit of LAM

Several associations have been identified in patients with a positive urine LAM assay that speak to its utility and benefit.

Perhaps the most significant is the association with increased mortality. This has been shown retrospectively in a review of a number of studies of urine LAM [64], and more recently was identified within the STAMP trial prospectively [65], where in a subset of patients with lower CD4 counts, the inclusion of urine LAM into routine diagnostics had a measurable mortality benefit.

There is a well-established correlation of improved yield and sensitivity of urine LAM in people living with HIV with declining CD4 count, with the highest yield seen in patients with CD4 counts below 50 cells/L, but retained utility in CD4 counts under 100 cells/L [9,56,66–68~~9546465~~66] , and perhaps even below 200 cells/L [56]. This likely represents the increased mycobacterial burden with more disseminated forms of EPTB seen in this patient subset with resultant positive LAM.

This is further supported by an association of urine LAM detection with mycobacteraemia, where disease is overtly demonstrated to haematogenously disseminated with a resultant increased morbidity and mortality as has been demonstrated [60]

Other evidence for urine LAM representing disease dissemination includes evidence for renal TB, a less common and perhaps underappreciated form of disseminated disease in HIV

associated TB, as demonstrated by the recovery of whole TB organisms from urine (mycobacteriuria) [18] and a post mortem study in patients with confirmed renal TB with detectable LAM [61].

As previously described, the presentation of patients with HIV associated TB differs from TB in an HIV negative population especially in more advance HIV disease. In this subset, PTB may be paucibacillary and reliable diagnostic modalities like the MTB/RIF Xpert™ may fail to diagnose a portion of sputum culture positive patients [69]. The diagnosis of TB in these *sputum negative* patients becomes difficult and empiricism is often relied on with regards to initiation of anti-TB therapy with the associated risk of misdiagnosis.

There also exists a population of patients termed *sputum scarce*, where generation of a sputum sample at all, or of adequate quality, is difficult owing to several factors. These include overall poor mobility, presence of abnormal mentation making the ability to follow the steps to produce sputa difficult, and the fact that they may not have clinical PTB but rather EPTB or disseminated TB without significant pulmonary involvement. Diagnosis of this form of TB is often difficult, with confirmatory modalities such as TB blood cultures suboptimal for a timeous diagnosis, as they may require several weeks to obtain a result [1] and obtaining alternative samples for diagnosis from extrapulmonary sites may require invasive testing which is also not always accessible in many resource constrained settings.

It is in people living with HIV who are either *sputum negative* or *sputum scarce* in whom urine LAM testing may have the most significant utility owing to the difficulties in otherwise confirming the presence of disease [70].

Finally it has been suggested that there may be utility in both pre-empting and diagnosing paradoxical or unmasking TB IRIS with the use of a screening urine LAM [71] and use in this population either with or at risk for a TB IRIS remains to be further explored.

South African context

South Africa (SA) bears the burden of two interrelated epidemics, namely HIV and TB, and multiple interventions have been put in place to try reach ambitious targets of control for each [72,73~~7071~~]. Nevertheless, the current burden of TB remains the number one cause of death in SA[5]. HIV is also associated with significant morbidity in terms of hospital admissions, being responsible for up to two thirds of hospital admissions [74]. This is coupled with a persistently significant number of patients with advanced HIV, with national figures noting that 12.5% of

patients with HIV have a CD4 count of less than 100 cells/L and that 26% of patients have a CD4 count of less than 200 cells/L [75]. From these figures, it is clear that a substantial number of patients admitted to hospitals in SA would fall into the demographic that may benefit the most from urine LAM testing. This patient cohort is also at highest risk for death from TB, with up to 50% diagnosed with TB post mortem [6].

Being a resource constrained setting, the cost of additional testing in SA needs to be considered. In that regard, the Alere Determine TB LAM is both relatively cheap ($3.50 per test) with an efficient turn-around time (25 minutes) and has been proven to be cost effective [76].

Despite this, urine LAM testing is not currently in mainstream use at most public sector hospitals, although policies are underway to increase accessibility.

1.1.4 Role of this study

To be a useful in clinical practice, a given test needs to either add further diagnostic accuracy to that which can be obtained from available clinical, biochemical and radiographic data [77], or to circumvent such investigations to provide a simpler or cheaper means to obtaining an accurate diagnosis.

This study aims to examine clinical, laboratory and radiological parameters of patients in South African hospitals, who are either sputum scarce (unable to produce sputum) or sputum negative (for MTB), with a positive urine LAM, to define in detail the patient profile and TB related parameters that LAM detection is associated with.

This has the potential, by identifying the population it is best suited for usage in, to improve its positive and negative predictive values and allow for cost effective usage in resource constrained settings and specifically the public sector in South Africa.

1.2 Objectives

The specific objectives of this study are, in patients living with HIV and a clinical suspicion of TB:

i. To describe the utility of urine LAM testing via the Alere Determine™ TB LAM in patients ~~that~~ who were unable to produce a sputum sample during their admission (*sputum scarce*) or *sputum negative* (Gene Xpert™ MTB/RIF negative or sputum TB culture negative)

ii. To describe the clinical and demographic characteristics of patients with a positive versus negative urine LAM with specific reference to:

   a. Age
   b. Gender
   c. Vital signs on admission
   d. Presence of danger signs as defined by the WHO[42*]
   e. Calculated qSOFA score [78†]
   f. Any presenting symptoms of TB as per the WHO TB Symptom screen[79‡] (as per available data)
   g. Concurrent diagnoses (as per discharge records)
   h. Use of ART at time of admission
   i. Virological failure as defined by patient being on ART with a HIV viral load (VL) of >1000 copies/ml [80]

iii. To describe the association of radiological characteristics of patients with urine LAM results, specifically:

   a. Chest X-Ray findings deemed suggestive (reticular, nodular, cavitatory, effusion, miliary) or not (alveolar, normal) of TB[28] as assessed by the managing clinicians if

[*] Respiratory rate greater than 30 breaths per minute, temperature more than 39°C, heart rate more than 120 beats/min and any patient who is unable to walk unaided.

[†] Quick Sequential Organ Failure Assessment: Score of 0 to 3 with one point each for abnormal mentation, RR > 20, SBP <100

[‡] Cough greater than 2 weeks, self-reported fever, drenching night sweats, unintentional loss of weight.

captured in the clinical record/database, or characterized by the researchers if not captured, with comparison of the various patterns as appropriate. [28]

b. Abdominal sonographic findings deemed suggestive of TB or renal disease (hepatomegaly, splenomegaly, ascites, intra-abdominal lymphadenopathy, splenic hypoechoic lesions [25] or echogenic/small kidneys)

iv. To describe the association of urine LAM with recovery of MTB from diagnostic samples via microscopy, culture, or PCR-based testing.

v. To describe the relationship of various laboratory parameters associated with disseminated TB in HIV with urine LAM result including:

a. Haemoglobin
b. Platelet count
c. Leukocyte count
d. Alanine Transferase
e. Gamma-glutamyl transferase
f. Alkaline phosphatase
g. Infiltrative/cholestatic LFT (defined above by R-Factor <2) [40*]
h. Protein
i. Albumin
j. Calculated globulin gap
k. C-Reactive protein
l. CD4 count
m. HIV Viral Load
n. Creatinine
o. Calculated GFR based on 4 variable MDRD equation as applied to South African patients [81]
p. The presence or absence of sterile pyuria, haematuria and proteinuria possibly indicative of renal tract TB or a glomerulopathy in patients with a positive urine LAM (if data is available).

vi. To describe urine LAM as a prognostic marker for inpatient death.

* See *section 1.1.2*

1.3 Methodology

1.3.1 Setting

The study will take place at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) and the Helen Joseph Hospital (HJH), both public academic/referral hospitals affiliated with the University of the Witwatersrand.

1.3.2 Population

The study population will consist of HIV positive adult patients (18 years or older) admitted to any department in the abovementioned hospitals, with CD4 cell counts less than or equal to 100 cells/µl, or if above 100 cells/µL at the discretion of the attending infectious disease specialist due to being deemed seriously ill (defined by the WHO based on presence of any of four danger signs: Respiratory rate greater than 30 breaths per minute, temperature more than 39°C, heart rate more than 120 beats/min and any patient who is unable to walk unaided[42]) on whom a urine LAM was conducted , that were either *sputum scarce* or *sputum negative*.

1.3.3 Design

The study will be a multicentre retrospective record review from the abovementioned two hospitals in Johannesburg.

1.3.4 Data Collection

Retrospective data will be collected from two separate sources.

1. From the Helen Joseph Hospital department of infectious diseases database, a database of all patients seen by the infectious disease department, containing demographic, clinical, radiological (in descriptive format) and laboratory data and includes all patients on whom a urine LAM test is done in the hospital. Adult patients (>18 years old) with confirmed HIV, having had a urine LAM test performed while an inpatient in 2017 will be selected from this database and checked for eligibility based on above criteria. Chest X-Ray images will be traced where the record is incomplete if available from hospital records and categorized. Where data is incomplete within the database, hospital records will be used if available. Data from

2017 will provisionally be used, with a view to expanding to prior years if sample size is not met.

2. From records within the department of infectious diseases at Charlotte Maxeke Johannessburg Academic Hospital of sputum negative or sputum scarce patients where the diagnosis of TB was in question and a urine LAM was run independently by an attending infectious disease consultant. Similarly, records/data from 2017 will be used.

Specific data to be collected ~~will include~~ is detailed in the data collection sheet (Appendix A)

Data will be anonymized and transferred to a study database using a spreadsheet package (Microsoft Excel). Once data is collected it will be analysed using a statistics package (GraphPad Prism).

1.4 Data Analysis

1.4.1 Sample size

Minimum target sample size will be calculated based on data from a previous study [70] which found the proportion of sputum scarce patients with a positive LAM to be 31%. Using a 95% confidence interval and Type 1 error value of 0.05:

$n = [(Z)^2 p (1 – p)] / d^2$

$n = [(1.96)^2(0.31)(0.69)]/(0.05)^2$

$n = 308$

1.4.2 Statistical methods

Data will be analysed using GraphPad Prism with significance defined as $p < 0.05$ using 95% confidence intervals.

Distribution of the data will be tested for normality using the Kolmogorov-Smirnov test. Results will be summarized using descriptive statistics, with categorical variables described using frequencies and proportions, and continuous variables using medians with interquartile ranges (IQR) or means with standard deviation (SD) if normally distributed.

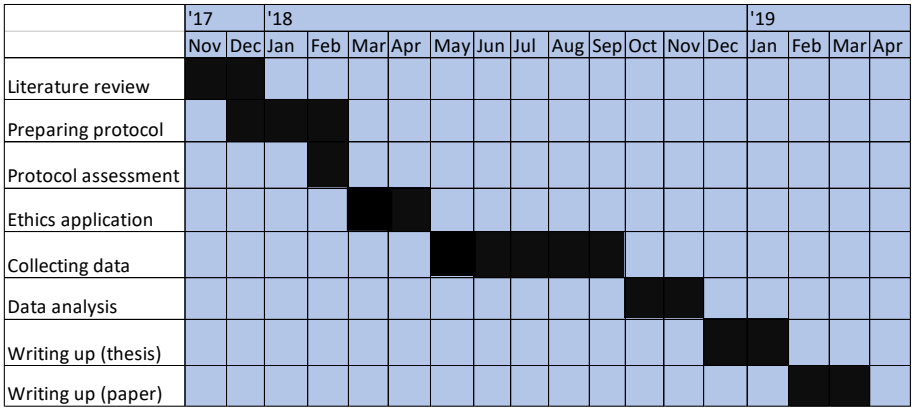
Sub-group univariate analyses will be performed for each parameter comparing LAM positive to LAM negative patients. Categorical variables will be compared using the Chi

Squared test (or Fishers exact test where the expected frequencies were less than 5). The medians of continuous variables will be compared using the Mann-Whitney U-test, and meansd compared if dataset normally distributed using students T Test. Odds ratios will be calculated with 95% confidence intervals.

1.5 Ethics

Ethical approval has been sought from the Human Research Ethics Committee of the University of the Witwatersrand (M180815). Only the primary researchers will have access to the data set, and deidentified data will be used. Permission to conduct this research has been sought from the relevant departmental heads and management of Helen Joseph Hospital and Charlotte Maxeke Johannesburg Academic Hospital. Permission has also been obtained from the infectious disease database gatekeeper at Helen Joseph Hospital. All data that will be collected will remain anonymous and no patient's personal details will be used in the study to maintain confidentiality. As the study is retrospective there will be no physical risk to any patient that is involved.

1.6 Timing



1.7 Funding

Costs for the study will primarily include internet, transport, and printing costs and will be covered by the researcher.

1.8 Limitations

Limitations of this study include the retrospective nature of data collection with associated risk of selection bias. Given the dependence on a prepopulated database, records may be incomplete or inaccurate, and attempts to obtain hospital records may be unsuccessful. Similarly X-Ray films may be lost or missing or misclassified due to interobserver variability. Outcomes data is limited to inpatient death as follow up data will not necessarily be available. There may be some heterogeneity of data accessible from the two study sites. Random urine samples were used in both study settings where it has recently been noted that early morning samples may have an improved sensitivity [82]. Quality of sputum samples used to define patient as sputum negative not specified (as the local laboratory does not routinely do bartlett scoring on specimens submitted for Xpert™) and may misclassify a given patient.

CHAPTER 2 - SUBMISSIBLE ARTICLE

Original research

Clinical, radiological and laboratory predictors of a positive urine lipoarabinomannan in sputum scarce and sputum negative patients with HIV associated TB in two Johannesburg hospitals

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2.1 Abstract

Background

Tuberculosis is a major cause of mortality in HIV-infected patients. The diagnosis of TB in patients with low CD4 counts using sputum-based diagnostics is hampered by paucibacillary disease with these patients often being sputum negative or sputum scarce. Urine lipoarabinomannan (LAM) has shown promise in point of care detection of TB in this patient subset but it lacks sensitivity and its exact role in a diagnostic algorithm for TB in South Africa remains to be elucidated.

Methods

This multicentre retrospective record review compared the clinical, radiological and laboratory characteristics of sputum scarce or sputum negative HIV infected patients in two hospitals who underwent urine LAM testing in line with WHO recommendations.

Results

Over a third of patients (35%) had a positive LAM, with a higher yield in sputum scarce patients (42%), versus sputum negative patients (30%, $p = 0.0141$). These patients were more likely to have delirium (OR 2.2, 95% CI 1.2 - 3.7), a higher median heart rate ($p = 0.0135$) and a qSOFA score ≥ 2 (OR 3.5, 95% CI 1.6 – 7.6). A positive LAM was significantly associated with the presence of disseminated TB ($p < 0.0001$) . It was also associated with a clinical diagnosis of TB immune reconstitution syndrome ($p = 0.0035$) and abdominal TB ($p < 0.0001$). Laboratory predictors of a positive LAM included renal dysfunction ($p = 0.044$), severe anaemia ($p = 0.0116$) and a higher median C-Reactive protein ($p = 0.0131$).

Positive LAM results were also noted in 75% of patients with disseminated non-tuberculous mycobacterial infections ($p = 0.0053$).

Conclusion

Urine LAM testing for TB had significant diagnostic utility in HIV infected inpatients that were sputum scarce or sputum negative. A positive LAM was associated with disseminated disease, several markers of severe illness, and the diagnosis of TB IRIS. Disseminated non-tuberculous mycobacterial infection may result in positive urine LAM results. Select use in these patient subsets could maximise yield and improve predictive value, in addition to improving the time to diagnosis.

2.2 Background

South Africa (SA) has the second highest global incidence of tuberculosis (TB), with an estimated 567 new cases per 100 000 population annually [4]. Tuberculosis remains the number one cause of death, with more than half of these patients being co-infected with human immunodeficiency virus (HIV) [5]. It is most concerning that in resource limited settings such as SA there remain a significant proportion (up to 48%) of patients coinfected with advanced HIV and TB that remain undiagnosed ante-mortem [6]. This highlights the difficulty in accurately diagnosing TB in people living with HIV, as a result of the atypical and often nonspecific presentation of disease [10]. This is especially the case in those with lower CD4 counts (<200 cells/μL), who comprise a substantial proportion (26%) of all patients living with HIV in SA [75] and who are at highest risk for HIV related mortality.

Sputum based diagnosis (namely the Xpert™ MTB/Rif or newer Xpert™ MTB/Rif Ultra assay) are endorsed by the World Health Organization (WHO) [31] and recommended in local guidelines as first line diagnostic modalities [79] in place of sputum smear microscopy where available.

Sputum in patients with advanced HIV disease maybe either negative [13] owing to paucibacillary disease [10], or unavailable based on lack of pulmonary symptoms or the inability to produce sputum [70], hampering these sputum-based diagnostic methods.

The frequency of extrapulmonary TB (EPTB) in this population has been shown to be between 40-60% of all cases of TB [10]. A significant proportion of these EPTB cases are disseminated TB (defined as TB in two or more sites, recovery of MTB from blood or bone marrow, or radiological evidence of miliary TB) which carries a high mortality and a risk of significantly delayed diagnosis if culture based methods are relied upon [14,15].

In these populations of people living with HIV with suspected TB, termed *sputum scarce* and *sputum negative* respectively, conventional sputum based diagnostic methods frequently fail, with resultant delayed diagnosis and consequent increased mortality. Modalities such as chest radiography or abdominal sonography are often not suggestive of TB [28] or of inadequate sensitivity and specificity [25] in this population. Definitive means to establish diagnosis of TB such as blood culture, fine needle aspirate, bronchoalveolar lavage or tissue biopsy are invasive, not always appropriate and the results of these testing modalities are not always available timeously. As such, many of these patients are treated empirically with the associated risk of misdiagnosis [83]. Therefore, alternative diagnostic modalities are needed.

The Alere Determine™ TB LAM, a point of care urine-based assay, has been recommended by the WHO for a specific subset of patients with HIV and suspected TB has the potential to fill this gap. The patient subset with the highest level of evidence includes hospitalised patients with CD4 counts of less than 200 cells/µL (previously 100 cells/µL at the time of *this* study), or deemed seriously ill by predefined criteria[42].

This test allows for true point of care testing with results available in approximately 25 minutes with a random (or if available early morning [82]) urine sample of just 65µl of urine together with the testing strip. This lateral flow assay (*figure 1A*) attaches colloidal gold-labelled antibodies to LAM that are captured by immobilized LAM antibodies further along the test strip. These form a visual band which is then compared to a reference card (*figure 1B)* for interpretation dependent on band visibility and intensity [61]. Pooled sensitivity and specificity in the recommended population are 45% and 92% respectively with sensitivity inversely proportional to CD4 count [84].

It detects a form of lipoarabinomannan (LAM), a glycolipid component of the mycobacterial outer cell wall. It is one of three major groups of interrelated lipopolysaccharides that are found in all mycobacterial species and released by metabolically active or degrading mycobacteria and easily detectable *in vitro* in mycobacterial cultures [9,43].

There is debate as to whether LAM is filtered via the glomerulus in the setting of a possible glomerulopathy from active TB elsewhere or the prevailing understanding that LAM excretion in urine likely represents haematogenous or other dissemination to the renal parenchyma or urogenital tract in context of extrapulmonary or more likely disseminated TB [59]. This form of EPTB is likely underrecognized and may be subclinical or suggested by sterile pyuria or unexplained haematuria [16–18].

The relatively poor sensitivity and limited recommended population are perhaps the major contributors towards its underappreciation and underuse as a diagnostic tool. The fact that at present it is not uniformly available at public sector hospitals also contributes to its underuse, and lack of large-scale roll-out is possibly linked to the abovementioned factors. It should be noted ~~however~~ that HIV associated hospital admissions are responsible for up to two thirds of all public sector hospital admissions [74] and the target population of advanced immunosuppression to which this test is applicable is up to 25% of the HIV positive population [75]. Given the abovementioned prevalence, mortality and underdiagnosis of TB in

this very population, urine LAM has the potential to be a valuable addition in the timely diagnosis of TB.

Earlier diagnosis, coupled with the measurable mortality benefit that incorporation of urine LAM testing showed in patients with lower CD4 cell counts (as demonstrated in the STAMP trial) underpin its potential utility in the South African setting [65] It has also been shown to be cost effective with a per unit cost of approximately $3.50 per test [76,84]

The aim of this study was thus to identify parameters associated with a positive urine LAM using the Alere Determine™ TB LAM assay to further clarify its optimal usage in an inpatient setting in SA and possibly improve its predictive value and diagnostic yield.

2.3 Objectives

This study set out to retrospectively examine clinical, laboratory and radiological parameters to better define the patient profile and TB associated parameters that LAM detection is associated with. This was done in patients living with HIV admitted to public academic/referral hospitals in SA, who were either *sputum scarce* or *sputum negative*. In addition, it set out to determine which, if any, were predictive of a positive urine LAM with a view to identifying its place within a diagnostic algorithm, specifically in a patient population where other diagnostic tools and algorithms often fail, such as sputum scarce or sputum negative patients.

2.4 Methods

2.4.1 Design

This was a multicentre retrospective record review of adult patients admitted to the Helen Joseph Hospital (HJH) and Charlotte Maxeke Johannesburg Academic hospital (CMJAH) in Johannesburg, South Africa in 2017. The eligible study population consisted of HIV positive patients who were either sputum scarce (unable to produce sputum) or sputum negative (Xpert™ MTB/Rif negative) and had a urine LAM (Alere Determine™ TB LAM Ag) performed on a random urine sample in keeping with WHO recommendations for its use.

Patients were excluded if they were HIV negative, did not meet WHO criteria for performing a urine LAM (CD4 count < 100 or meeting WHO criteria for seriously ill* regardless of CD4 count)

2.4.2 Data collection

Records were obtained using a database detailing all patients on whom a urine LAM had been performed kept by the relevant department of infectious disease at each hospital. Records were screened for the above eligibility criteria and included if met. Clinical, laboratory and radiological data was retrieved from the same databases, and where incomplete, hospital patient records were reviewed as available to complete records. Data was extracted using manual data collection forms and then captured in spreadsheet form using Microsoft Excel. A total of 363 records were screened, of which 342 met inclusion criteria. It should be noted that given the retrospective nature of the data, some records were incomplete but were included for analysis on available data. All data obtained was anonymized and given a study number at capture with removal of identifiers such as name, date of birth and hospital file number.

2.4.3 Data Parameters

Clinical data extracted included demographics, including age, gender and clinical data such as vital signs on admission, presence of danger signs as defined by the WHO* [42], calculated qSOFA score†[78], symptoms of TB as per WHO screening tool‡ [85], site of TB if diagnosed, concurrent diagnoses, use of antiretroviral therapy (ART) at time of admission and the presence of virologic failure (VF) as defined by patient being on ART with a HIV viral load >1000 copies/ml [80].

Chest X-Ray findings were recorded in descriptive format deemed suggestive (reticular, nodular, cavitatory, effusion, miliary) or not (alveolar, normal) of TB having been characterised by the attending clinical team or the primary investigator if description not entered but film available [28].

Abdominal sonographic findings (where performed and available) suggestive of TB or renal disease (hepatomegaly, splenomegaly, ascites, intra-abdominal lymphadenopathy, splenic hypoechoic lesions [25] or echogenic/small kidneys) were recorded in descriptive format.

Any microbiology pertaining to the site and diagnosis of TB was obtained and results recorded, with a positive result being based on microscopy, culture or PCR based results. This included, but was not limited to, sputum Xpert™ MTB/Rif if done (required by

* Respiratory rate greater than 30 breaths per minute, temperature more than 39°C, heart rate more than 120 beats/min and any patient who is unable to walk unaided

† Score of 0 to 3 with one point each for abnormal mentation, RR > 20, SBP <100

‡ Current cough, self-reported fever, drenching night sweats, unintentional loss of weight.

eligibility criteria to be negative) andTB blood culture results including time to positivity. testing.

Routine laboratory investigations were recorded including haemoglobin (Hb), platelet count, white cell count (WCC), alanine transferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), presence of infiltrative/cholestatic liver function test (defined by R-Factor <2) [40*], protein, albumin C-Reactive protein (CRP), CD4 count, HIV viral load, creatinine and calculated glomerular filtration rate (GFR) [81†].

Where available, urine results possibly indicative of renal tract TB (sterile pyuria defined as urine leukocyte count >10000 cells/µL in the absence of positive bacterial culture and any associated haematuria) were documented. Similarly, proteinuria (protein: creatinine ratio) indicative of a possible glomerulopathy in patients with a positive urine LAM was recorded if available.

Patient outcomes were recorded as inpatient death or survival to hospital discharge if available.

2.4.4 Statistics

Sample Size

Target sample size of 308 was based on data from a previous study [70] which found the proportion of sputum scarce patients with a positive LAM to be 31%. Using a 95% confidence interval and Type 1 error value of 0.05. Eligible records yielded a final sample size of 342.

Data Analysis

Data was analysed using GraphPad Prism with significance defined as $p < 0.05$ using 95% confidence intervals.

The data was shown to be non-normally distributed using the Kolmogorov-Smirnov test. Results were summarized using descriptive statistics, with categorical variables described using frequencies and proportions, and continuous variables using medians and interquartile ranges. Sub-group univariate analyses were performed for each parameter comparing LAM positive to LAM negative patients. Categorical variables were compared using the Chi

* (ALT X Upper Limit of Normal of ALP) ÷ (ALP x Upper Limit of Normal ALT)
† Based on 4 variable MDRD equation as applied to South African patients

Squared test (or Fishers exact test where the expected frequencies were less than 5). The medians of continuous variables were compared using the Mann-Whitney U-test. Odds ratios were calculated with 95% confidence intervals.

2.4.5 Ethics

Ethical approval for the research was attained from the University of Witwatersrand human research ethics committee (ethics clearance certificate number M180815).

2.5 Results

2.5.1 Demographics

A total of 342 patients met the study criteria being admitted to the study hospitals in 2017. These patients were HIV positive, sputum negative or scarce, and had a urine LAM done at the discretion of the managing team in keeping with general WHO recommendation. Of these, 35% (n = 121) were LAM positive. There were no significant differences in patient demographics (Table 1) between the two groups with respect to gender with 53% female in the LAM positive group and 48% female in LAM negative group (p = 0.3412). Similarly, no significant difference was determined in the ages of patients between the two hospitals. The median age was 38 years in each group (IQR 32 - 43 and 32 - 46 respectively).

2.5.2 Patient outcomes

Regarding patient outcomes (Table 1), data was available for 301 patients (88%) of whom 46 (5%) [illegible] group.

TABLE 1

Patient Demographics and Outcomes	N	LAM positive	LAM negative	*p* value
		n (%)	n (%)	
Patients	342	121 (35)	221 (65)	
Demographics				
Median Age, Years (IQR)		38 (32-43)	38 (32-46)	0.5411
Male		57 (47)	116 (52)	0.3412
Female		64 (53)	105 (48)	
Outcomes data	301	106 (35)	195 (65)	
Inpatient death		21 (20)	25 (13)	0.1074

IQR, interquartile range

[#] Limited records with relevant data available

[*] Statistically significant

2.5.3 HIV parameters

With regards to HIV related parameters (Table 2), the median CD4 count was lower in the LAM positive group (22 cells/µL, IQR 7 - 42) compared to the LAM negative group (26 cells/µL, IQR 10 - 55) but this difference was not significant (p = 0.0944). A significantly larger proportion (80%) in the LAM positive group had a CD4 below 50 cells/µL (p = 0.0439). Patients in the LAM positive group were more likely to be on ART at time of admission with an odds ratio (OR) of 1.7 (95% CI 1.01 – 3.0). There was no difference in HIV viral load between the two groups, with a median of log 5.2 copies/ml in both groups. Similarly, there was no significant difference in the proportion of patients with virological failure[*] between the groups (64% vs 60%, p = 0.6356).

[*] Defined as being on cART on admission with an HIV VL > 1000 copies/ml

TABLE 2

HIV Related Parameters	N	LAM positive	LAM negative	*p value*	Odds ratio (95% CI)
		n (%)	n (%)		
ART data available	296[#]	104 (35)	192 (65)		
Currently on ART		63 (61)	89 (46)	0.0194*	1.8* (1.1-2.8)
CD4 count					
Available data	326[#]	115 (35)	211 (65)		
Median, cells/µL (IQR)		22 (7-42)	26 (10-55)	0.0944	
< 50 cells/µL		92 (80)	147 (70)	0.0439*	1.7* (1.01-3.0)
HIV VL					
Available data	268[#]	97 (36)	171 (64)		
Median, log copies/ml (IQR)		5.2 (3.5-5.8)	5.2 (3.4-5.9)	0.9895	
Virological failure					
Available data	134[#]	56 (42)	72 (58)		
VL > 1000 copies/ml on ART		36 (64)	47 (60)	0.6356	1.2 (0.6-2.3)

ART, anti-retroviral therapy; VL, viral load; CD, cluster differentiation; IQR, interquartile range; CI, confidence interval

[#] number of records with relevant data available

* Statistically significant

2.5.4 Clinical presentation

With regards to presenting symptoms from the WHO TB symptom screen (Table 3), patients with a history of drenching night sweats or loss of weight were significantly more likely have a positive LAM, 41% vs 25% (p = 0.0037) and 54 vs 42% (p = 0.039) respectively with respective odds ratios of 2 (95% CI 1.3 – 3.2) and 1.6 (95% CI 1.03 to 2.5). Presence of a cough or a self-reported fever had no significant association.

Reduced patient mobility was associated with a positive LAM (63% vs 34%, p < 0.0001) with an OR of 3.3 (95% CI 1.9 – 5.8). Similarly, 41% of patients in the LAM positive group

TABLE 3

Clinical features	N	LAM positive	LAM negative	*p* value	Odds ratio (95% CI)
		n (%)	n (%)		
TB symptoms	342	121	221		
Cough		56 (46)	106 (48)	0.7657	0.9 (0.6-1.5)
Fever		39 (32)	56 (25)	0.1736	1.4 (0.9-2.3)
Night sweats		49 (41)	56 (25)	0.0037*	2.0 (1.3-3.2)
Weight loss		65 (54)	93 (42)	0.039*	1.6 (1.03-2.5)
Mobility	223#	80	143		
Immobile		50 (63)	48 (34)	<0.0001*	3.3 (1.9-5.4)
Mentation	260#	95	165		
Confused		39 (41)	40 (24)	0.0045*	2.2 (1.2-3.7)
Vital signs					
MAP	338#	121	217		
Median, mmHg (IQR)		83 (73-91)	86 (75-95)	0.0398*	
MAP < 65		10 (8)	8 (4)	0.0723	2.4 (0.9-6)
HR	338#	121	217		
Median, b.p.m (IQR)		110 (96-123)	105 (90-115)	0.0135*	
HR > 110		57 (47)	71 (33)	0.0089*	1.8 (1.2-2.9)
Temperature,	328#	119	209		
Median, °C (IQR)		37 (36.5-37)	37 (36 - 37)	0.2335	
RR	225#	80	145		
Median, b.p.m (IQR)		20 (18-24)	20 (18-22)	0.0823	
qSOFA score	225#	80	145		
0	89	26 (29)	63 (71)	0.0075*	
1	107	36 (34)	71 (66)		
2	27	16 (59)	11 (41)		
3	2	2 (100)	0 (0)		
qSOFA ≥ 2	29	18 (23)	11 (8)	0.0014*	3.5 (1.6-7.6)

MAP, mean arterial pressure; HR, heart rate; RR, respiratory rate; qSOFA, quick sequential organ failure assessment

* Statistically significant

Number of records with relevant data available

had abnormal mentation on admission versus 24% of those who were LAM Negative (p = 0.0045) with an OR of 2.2 (95% CI 1.3 – 3.7).

With regards to vital signs on admission: Patients in the LAM positive group had a significantly lower median systolic blood pressure (BP) of 109 mmHg (IQR 99-120) vs 112 mmHg (IQR 100-126, p = 0.0199) a lower median mean arterial pressure (MAP) of 83 mmHg (IQR 73-91) vs 86 mmHg (IQR 75-95), a higher median heart rate (HR) of 110 bpm (IQR 96 – 123), vs 105 bpm (IQR 90 - 115, p = 0.0135) and were more likely to have a HR greater than 110 bpm (OR = 1.8, CI 1.2 to 2.9). There was no significant difference in median temperature or the presence of documented fever, or respiratory rate. Of note was an

increasing proportion of patients in the LAM positive group directly correlated with increasing qSOFA score namely 29%, 34%, 59% and 100% respectively for qSOFA equal to 0, 1, 2 and 3 respectively (p = 0.0075). In addition, a qSOFA score of greater than or equal to 2 had an OR of 3.5 (95% CI 1.6 to 7.6) for being LAM positive.

2.5.5 TB diagnosis

Of those diagnosed with TB (n = 168), 72% (n = 121) were LAM positive (Table 4). Of those that were LAM positive, for more than half (52%), a urine LAM was the only microbiological modality of TB confirmation that was positive.

Grouping patients as either sputum scarce or sputum negative, patients were significantly more likely to be LAM positive in the sputum scarce group (42% vs 30%, p = 0.0141), perhaps reflecting the negative predictive value of a negative Xpert™ MTB/Rif result.

Of those patients diagnosed with disseminated TB (as defined above), 83% were LAM positive (p < 0.0001) with an OR of 13 (95% CI 6 – 27.8).

There was also a significant correlation within the LAM positive group with mycobacteraemia with an OR of 8.4 (95% CI 3.4 – 20.4). There was no significant difference in the median time to positivity of mycobacterial blood culture between patients that were LAM positive (21 days, IQR 16 – 28) vs those that were LAM negative (22 days, IQR 15 – 27, p = 0.7759).

With regard to other sites of involvement (often more than one), a positive urine LAM was significantly associated with pulmonary involvement based on CXR findings (excluding miliary TB) with 79% (n = 22) of patients with suspected pulmonary involvement but sputum negative or scarce having a positive LAM (p < 0.0001). Of patients with abdominal TB based on sonography or supporting ascitic fluid results, 73% (n = 40) had a positive LAM (p < 0.0001). Of those with miliary TB based on CXR (n = 10), 70% were LAM positive (p = 0.0376). Bone marrow involvement based on a positive bone marrow aspirate culture or supportive trephine histology with ZN staining was predictive of a positive LAM in 100% (n = 7) of patients with TB bone marrow (p = 0.0006). Also, of note is that the diagnosis of TB immune reconstitution inflammatory syndrome (IRIS) at any site (based on recorded discharge diagnosis) had an OR of 13.5 (95% CI 2.2 – 152) with 88% (n = 7) of patients with a TB IRIS having a positive LAM (p = 0.0035).

TABLE 4

TB diagnosis	N	LAM positive (n = 121)	LAM negative (n = 221)	*p* value	Odds ratio (95% CI)
		n (%)	n (%)		
Sputum results, n (%)					
Sputum scarce	156	66 (42)	90 (58)	0.0141*	1.7 (1.3-2.7)*
Sputum negative	186	55 (30)	131 (70)		
TB diagnosis, n (%)	168	121 (72)	47 (28)		
Microbiology (PCR/Culture) positive	48	34 (71)	14 (29)	0.8279	0.9 (0.4-1.9)
Microbiology (PCR/Culture) negative	120	87 (73)	33 (27)		
LAM sole diagnostic modality[¶]	87 (52)				
Site involved, n (%)					
Disseminated disease[§]	52	43 (83)	9 (17)	<0,0001*	13.0 (6.0-27.8)
Miliary[†]	10	7 (70)	3 (30)	0,0376*	4.5 (1.2-16.1)
Pulmonary[†]	28	22 (79)	6 (21)	<0,0001*	8.0 (3.1-19.2)
Abdominal,	55	40 (73)	15 (27)	<0,0001*	6.8 (3.5-12.5)
Lymph node	10	6 (60)	4 (40)	0.1753	2.8 (0.7-9.0)
Mycobacteraemia	33	27 (82)	6 (18)	<0,0001*	8.4 (3.3-20.3)
Median time to positivity, days (IQR)		21 (16-28)	22 (15-27)	0.7759	
Bone Marrow	7	7 (100)	0 (0)	0,0006*	∞ (3.4-∞)
CNS	18	7 (39)	11 (61)	0.7491	1.7 (0.5-3.1)
Pleural	5	1 (20)	4 (80)	0.6597	0.5 (0.0-2.8)
Pericardial	1	0 (0)	1 (100)	>0,9999	0 (0.0 - 16.4)
Spinal	1	1 (100)	0 (0)	0.3538	∞ (0.2-∞)
IRIS	8	7 (88)	1 (12)	0,0035*	13.5 (2.2 - 152.8)
No anatomical localization	42	42 (100)	0 (0)		

PCR, polymerase chain reaction; CNS, central nervous system; IQR, interquartile range; IRIS, immune reconstitution inflammatory syndrome

* Statistically significant

[¶] Of those with final TB diagnosis, urine LAM was the only microbiological diagnostic method found to be positive

[§] TB in two or more sites, recovery of MTB from blood or bone marrow, or radiological evidence of miliary TB

[†] Diagnosis based on chest radiography

No significant correlation was demonstrated with TB involvement of the central nervous system (tuberculomas or TB meningitis), lymphadenitis, pleura, pericardium or spine (Pott's disease), although sample sizes were small for all these sub-analyses and thus did not reach significance.

It should also be noted that of those patients ~~that~~ who were LAM positive, 35% (n = 42) had no clearly diagnosed anatomical localisation of disease based on available clinical information, microbiology results and available radiology.

2.5.6 Investigations

With regards to haematological parameters (Table 5), patients in the LAM positive group had a significantly lower median haemoglobin of 9.1 g/dL (IQR 7 – 11) vs 10.6 g/dL (IQR 8.5 – 15, $p < 0.0001$). In addition, the presence of a severe anaemia as per the WHO definition [86] (Hb < 8 g/dL for males & <7 g/dL for females) had an OR of 2 (95% CI 1.2 – 3.5) for having a positive LAM. There was no significant difference in median white cell count (WCC) between patients that were LAM positive (6×10^9 cells/L, IQR 3,9 – 9,1) and those that were LAM negative (5.1×10^9 cells/L, IQR 3.2 – 8) nor any difference in the presence of a leucocytosis (WCC > 11×10^9 cells/L) or a leukopenia (WCC < 4×10^9 cells/L). Similarly, there was no significant difference in median platelet count between the groups, 240×10^9 cells/L (IQR 141 – 347) vs 263×10^9 cells/L (IQR 177 - 336) or the presence of clinically significant thrombocytosis (Plt > 450×10^9 cells/L) or thrombocytopenia (Plt < 100×10^9 cells/L).

With regards to renal function, patients in the LAM positive group had a lower median GFR 73 ml/min/1.73m² (IQR 44-103) vs 85 ml/min/m² (IQR 56-109) which approached statistical significance ($p = 0.068$) and a significantly higher proportion had abnormal renal function, defined by an eGFR of < 60 ml/min/1.73m², 41% vs 30% ($p = 0.044$) with an OR 1.6 (95% CI 1.0 to 2.5).

Regarding urine parameters there was no difference in the presence of sterile pyuria ($p = 0.6851$) between the two groups. There

TABLE 5

Laboratory markers	N	LAM positive	LAM negative	*p value*	Odds Ratio (95% CI)
		n (%)	n (%)		
WCC (×10⁹ cells/L)	339[#]	119	221		
Median (IQR)		6 (3.9-9.1)	5.1 (3.2-8)	0.1263	
> 11		20 (17)	26 (12)	0.1948	1.5 (0.8-2.8)
< 4		31 (26)	79(36)	0.0683	0.6 (0.4-1)
Hb (g/dL)	341[#]	120	221		
Median (IQR)		9.1 (7-11)	1.6 (8.5-12)	<0,0001*	
Severe anaemia[†]		30 (25)	31 (14)	0.0116*	2 (1.2-3.5)
Platelets (×10⁹ cells/L)	340[#]	119	221		
Median (IQR)		240 (141-347)	263 (177-336)	0.3306	
< 100		14 (12)	21 (10)	0.5126	1.3 (0.6-2.6)
> 450		10 (8)	20 (9)	0.8411	0.9 (0.4-2)
GFR (mL/min/1.73m²)	340[#]	119	221		
Median (IQR)		73 (44-103)	85 (56-109)	0.068	
< 60		49 (41)	67 (30)	0.044*	1.6 (1-2.5)
Liver function tests (g/dL or IU/L)					
Available data	334[#]	119 (36)	215 (64)		
Median Albumin		24 (19-47)	25 (20-43)	0.474	
< 30		94 (79)	150 (70)	0.0688	1.6 (1-2.7)
Available data	328[#]	117	211		
Median GGT		85 (45-162)	68 (40-133)	0.0805	
GGT > ULN		72 (62)	105 (50)	0.0404*	1.6 (1-2.5)
Available data	327[#]	115	212		
Median ALT		36 (20-66)	17 (17-52)	0.0174*	
ALT > ULN		51 (44)	75 (35)	0.1115	1.5 (0.9-2.3)
Infiltrative LFT[‡]		39 (34)	61 (19)	0.3355	1.3 (0.8-2.1)
CRP (mg/L)	317[#]	115	202		
Median (IQR)		125 (54-200)	91 (23-157)	0.0131*	
>100		72 (63)	94 (47)	0.0059*	1.9 (1.2-3.1)
Urinalysis					
Available data	103[#]	47	56		
Sterile pyuria[§]		15 (32)	20 (36)	0.6851	0.8 (0.4-1.8)
Available data	59[#]	31	28		
Median PCR, g/µmol (IQR)		0.157 (0.115-0.379)	.138 (0.057-0.261)	0.1491	
Nephrotic proteinuria (> 3.5g/µmol)		8 (26)	2 (7)	0.0838	4.5 (0.9-22.4)

WCC, white cell count; Hb, haemaglobin; GFR, glomerular filtration rate; GGT, gamma-gutamyl transferase; PCR, protein:creatinine ratio
ULN, upper limit normal; LFT, liver function tests; ALT, alanine-amino transferase; CRP, C-reactive protein

* Statistically significant

[#] Number of records with relevant data available

† Hb < 8g/dL for males and <7g/dL for females

‡ Defined by R factor < 2 calculated with the formula (ALT × ALP ULN) ÷ (ALP × ALT ULN)

§ >10000 cells/µL in the absence of bacterial culture

was a higher proportion of patients in the LAM positive group with nephrotic range proteinuria (26% vs 7%), trending toward statistical significance (p = 0,0838).

With regards to liver function testing, patients in the LAM positive group had a lower median albumin of 24 g/L (IQR 19 – 28) vs 25 g/L (IQR 20-31, p = 0.0474), with a higher proportion of those that were LAM positive having an albumin of < 30g/L, tending towards statistical significance, (79% vs 70%, p = 0.0688). Regarding hepatic enzyme levels, patients in the LAM positive group had a non-significantly higher median GGT (p = 0.0805) of 85 U/L (IQR 45 – 162) vs 68 U/L (IQR 40 – 133). In addition, they were more likely to have a GGT above the upper limit of normal (ULN) defined as greater than 68 U/L, with an OR 1.6 (95% CI 1.02 – 2.5), with 62% in the LAM positive group having an abnormal GGT versus 50% in the LAM negative group (p = 0.0404). Patients in the LAM+ group also had a significantly higher median alanine amino-transferase (ALT) of 36 (IQR 20 – 66) U/L versus 27 U/L (IQR 17 – 52) with p = 0.0174. There was no significant difference between the two groups with regards to the presence of an infiltrative LFT defined by and R-Factor of < 2 as defined above, 34% vs 29% (p = 0.3355).

With regards to inflammatory markers, patients who were LAM positive had a significantly higher median CRP of 125 mg/L (IQR 54 – 354) vs 91 mg/L (IQR 23 – 157) with p = 0.0131. The presence of a CRP > 100 mg/L had an OR of 1.9 (95% CI 1.2 to 3.1) for a positive LAM.

2.5.7 Radiology

~~Regarding classification of chest x-ray findings, the~~ The presence of nodular and miliary infiltrates were associated with a positive LAM with odds ratios of 2.5 (95% CI 1.2 – 5.3) and 4.3 (95% CI 1.2 – 15.6) respectively (Table 6). Of those patients with a miliary pattern on CXR (n=10), 70% had a positive LAM (p = 0.0394). Of note, 32% of patients in the LAM positive group had a normal CXR. There were no specific associations with any other CXR patterns.

Regarding abdominal sonography for features of abdominal TB or renal disease, a positive LAM was associated with the presence of splenic micro-abscesses with an odds ratio of 3.9 (95% CI 1.7 – 9.3) and intra-abdominal lymphadenopathy with an odds ratio of 3.2 (95% CI 1.4 to 7.3). No significant associations were noted with the presence of splenomegaly, hepatomegaly, ascites, or renal parenchymal abnormalities.

TABLE 6

Radiology	N	LAM positive	LAM negative	*p* value	Odds ratio (95% CI)
		n (%)	n (%)		
Chest radiography †	307	111	196		
Normal	104	35 (34)	69 (66)	0.5136	0.8 (0.5-1.4)
Interstitial infiltrate	75	25 (33)	50 (67)	0.5583	0.8 (0.5-1.5)
Nodular infiltrate	30	17 (57)	13 (43)	0.0138*	2.5 (1.2-5.3)
Alveolar infiltrate	18	0 (0	18 (100)	0.0005*	0 (0-0.3)
Consolidation	39	17 (44)	22 (56)	0.3011	1.4 (0.7-2.8)
Cavitation	17	6 (35)	11 (65)	0.9999	1 (0.4-2.9)
Effusion	14	2 (14)	12 (86)	0.094	0.3 (0.1-1.1)
Hilar lymphadenopathy	21	9 (43)	12 (57)	0.4921	1.4 (0.6-3.1)
Miliary pattern	10	7 (70)	3 (30)	0.0394*	4.3 (1.2-15.6)
Abdominal sonography ‡	142	56	86		
Normal	46	15 (33)	31 (67)	0.2491	0.6 (0.3-1.4)
Ascites	21	9 (43)	12 (57)	0.7282	1.2 (0.5-2.9)
Splenic microabscesses	21	19 (66)	10 (34)	0.0013*	3.9 (1.7-9.3)
Splenomegaly	14	7 (50)	7 (50)	0.3943	1.6 (0.6-4.4)
Lymphadenopathy	29	18 (62)	11 (38)	0.0052*	3.2 (1.4-7.3)
Hepatomegaly	24	12 (50)	12 (50)	0.2454	1.7 (0.7-3.9)
Hepatic lesion/s	6	2 (33)	4 (67)	0.9999	0.8 (0.1-3.4)
Echogenic kidneys	15	5 (33)	10 (67)	0.7818	0.7 (0.3-2.2)

* Statistically significant

† As characterized by clinician or researcher

‡ As per sonography report

2.5.8 Concurrent diagnoses

Selected HIV associated diagnoses were considered to identify possible screening populations with the potential for a significant yield (Table 7). No significantly increased frequency of LAM positivity was noted in patients with an isolated positive serum cryptococcal latex agglutination assay (CLAT) cryptococcal meningitis or community acquired pneumonia. A negative association was noted in patients with *Pneumocystis jirovecii* pneumonia (PJP) with 11% patients with PJP having a positive LAM versus 89% LAM negative ($p < 0.0001$). Given that lymphoproliferative disorders are often misdiagnosed as TB [83], it was significant that in patients with a final diagnosis of lymphoma (n = 8) none had a positive LAM ($p = 0.0053$).

Also, of significance was that 75% of patients with disseminated non-tuberculous mycobacterial infection (n = 9) had a positive LAM ($p = 0.0053$) with an OR of 5.8 (95% CI 1.6 to 20.2)

TABLE 7

Selected Concurrent Diagnoses	N	LAM positive (n = 121) n (%)	LAM negative (n = 221) n (%)	*p* value	Odds ratio (95% CI)
Cryptococcal disease					
CLAT†	16	8 (50)	8 (50)	0.2103	1.9 (0.7-5.1)
CCM	52	14 (27)	38 (73)	0.166	0.6 (0.3-1.2)
CAP	46	11 (24)	35 (76)	0.0804	0.5 (0.3-1.1)
PJP	54	6 (11)	48 (89)	<0,0001*	0.2 (0.1-0.4)
NTM (disseminated)	12	9 (75)	3 (25)	0.0053*	5.8 (1.6-20.3)
NTM (pulmonary)	1	1 (100)	0 (0)	0.3538	∞ (0.2- ∞)
Lymphoma	13	0 (0)	13(100)	0.0053*	0 (0-0.5)
AKI	70	30 (43)	40 (57)	0.1424	1.5 (0.9 - 2.5)

CCM, cryptococcal meningitis; CAP, community acquired pneumonia; PJP, pneumocystis *jirovecci pneumonia;*
NTM, non-tuberculous mycobacteria; AKI, acute kidney injury
† Isolated cryptococcal antigen detection on serum without meningitis
* Statistically significant

2.6 Discussion

~~The results of this~~ This study confirms the utility of urine LAM as a clinically useful diagnostic modality in ~~this study population~~ HIV positive hospitalized patients who are sputum scarce or negative. It is notable that over one third (35%, n = 121) of patients in this group of HIV positive sputum scarce or sputum negative inpatients with a CD4 count under 100 cells/µL or deemed to be severely ill regardless of CD4 count, had a positive urine LAM. This utility is further illustrated by the fact that of the 168 patients diagnosed with TB in the study population, being either sputum negative or sputum scarce, in 52% (n = 87) a urine LAM was the only microbiological confirmatory modality of TB. This highlights that sputum based diagnostics may be negative or not feasible in this population and that other diagnostic samples were often not available or negative for TB. A higher proportion of those who were sputum scarce (42%) were LAM positive as compared to the sputum negative group (30%) in keeping with the negative predictive value of a sputum GXP.

As demonstrated in other studies [66,67], the yield (and sensitivity) of urine LAM testing is inversely proportional to CD4 count with a higher yield in this study in patients with CD4 counts less than 50 cells/µL (80% versus 20%). While HIV viral load or the presence of virologic failure were not significantly different between those who were LAM positive versus those who were LAM negative, patients in the former group were more likely to be on ART, perhaps reflecting those with a TB IRIS, in whom a urine LAM was positive in 88% of suspected TB IRIS patients with an OR of 13.5.

In this study, patients that were urine LAM positive had a higher mortality of 20% vs 13% with similar figures when limiting this to those with a diagnosis of TB, but this did not meet criteria for statistical significance (p = 0.107) although it is in keeping with the findings from other studies [67]. This is of interest, given that the recent STAMP trial [65] showed a mortality benefit in the subset of lower CD4 counts by incorporating urine LAM into the diagnostic algorithm for TB.

Interesting clinical features with a positive predictive value for a positive urine LAM included patients with reduced mobility and confusion, a unique subset in whom sputum-based diagnostics would be difficult to obtain, once again highlighting the usefulness of urine LAM testing. Other notable clinical features associated with a positive LAM included a significantly lower median blood pressure and a higher median heart rate, suggesting an overall sicker patient group. A qSOFA score [78] showed a significant correlation with proportional increases in LAM yield relative to a higher score for a given patient. This, together with the higher mortality, speaks to a sicker cohort of patients, in whom the urgency of confirmation of diagnosis to guide targeted management is clear.

With regards to the nature of the underlying TB diagnosis, several results from this study are pertinent. Firstly, there was a significant association of urine LAM with more disseminated forms of TB, namely mycobacteraemia, TB involving the bone marrow, in whom 100% (n = 7) of patients were LAM positive, radiologically diagnosed miliary TB, and TB involving two or more sites based on available clinical data. This group had a cumulative odds ratio for a positive LAM of 13.5. Secondly with regards to the diagnosis of mycobacteraemia, 82% of patients had a positive LAM, the importance of which is amplified when the 25 minutes it takes to run a LAM is contrasted with the median blood culture time to positivity of 21 days. Thirdly, it is useful in confirming abdominal sonographic features of tuberculosis which in isolation are only of modest sensitivity (63%) and specificity (68%) in isolation [25]. Finally, it should be noted that in 33% of patients with a positive LAM no clear site of disease was identified based on available clinical parameters, suggesting subclinical disease in some with an associated screening utility, and in those with symptoms it implies a possible subset of patients where all other diagnostic modalities clearly fail.

Analysis of laboratory parameters associated with a positive urine LAM included the presence of a severe anaemia as per the WHO classification (less than 7 g/dL for females and 8 g/dL for males [86]) with an OR of 2, and the presence of renal dysfunction defined as a GFR

of less than 60 ml/min/1.73m² with an OR of 1.6. Regarding liver function testing, which is often used as a surrogate for the presence of abdominal TB in high endemic areas, urine LAM positivity was significantly associated with an abnormal GGT, and higher median ALT, but no significant association was found with an infiltrative/cholestatic LFT picture defined by an R-factor (as defined previously) of less than 2 [40]. Lastly, patients with a positive LAM had a higher median CRP with values greater than 100 mg/L having an OR of 1.9.

Various urinary parameters were available for a subset of patients, and no association was found between patients with a positive LAM and sterile pyuria, a marker of renal TB. An increased proportion of patients with nephrotic range proteinuria in the LAM positive group approached statistical significance (p = 0.0838), possibly representing an increase in the glomerular filtration of LAM from active sites of disease elsewhere. Alternatively, it may be coincidentally related to the high prevalence of HIV associated nephropathy (HIVAN) in this population with advanced HIV and lower CD4 counts [87]. It should also be mentioned that only a small proportion (17 – 30%) of patients included in the study had urinalysis data, and specific indications like the presence of renal dysfunction or dysuria will likely have existed to prompt such testing in the first place.

With all the above findings, a suggested diagnostic algorithm for usage of urine LAM in this population is suggested in *figure 4* below.

There exists debate in the literature as to the relevance of the finding of urine LAM in patients with confirmed disseminated non-tuberculous mycobacterial (NTM) disease, namely whether it represents a confounder for the MTB result related to the NTM infection directly [46], or given the high prevalence of TB in this population, perhaps the presence of concurrent undiagnosed TB [88]. In this study, of the 12 patients with confirmed disseminated NTM infections, 75% (n = 9) had a positive urine LAM, which was statistically significant (p = 0.0053). These included 8 patients with disseminated *Mycobacterium avium* complex (MAC) and one patient with disseminated *Mycobacterium kansasii.* Of interest in respect to this debate, was that in one of these patients concurrent disseminated tuberculosis was confirmed on a blood culture. The balance though does seem to suggest that disseminated NTM infection can cause a positive urine LAM and this should be kept in mind when interpreting a urine LAM result.

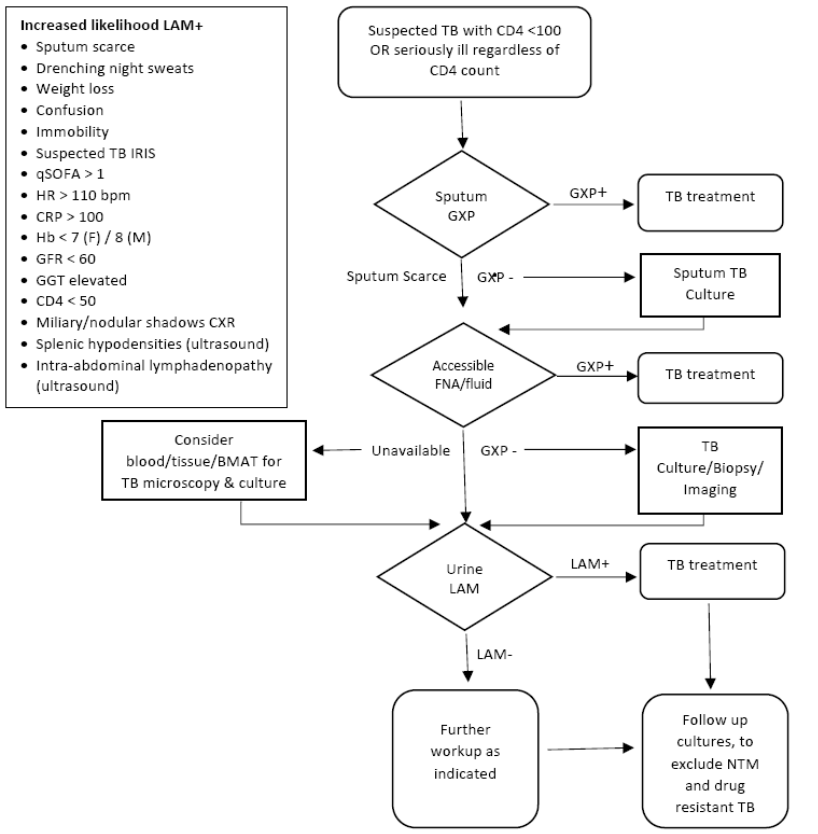

Figure 4 - A suggested algorithm for usage of urine LAM

2.7 Limitations

The limitations of the study include the retrospective nature of the data obtained with the associated risk of information and misclassification bias. Classification of CXR patterns by either the managing clinical team or the researcher may have been subject to interobserver variability bias. In classifying patients as sputum negative, the quality of the sputum was not known, which may have resulted in the erroneous inclusion of sputum positive patients. The diagnosis of TB-IRIS was based on the discharge diagnosis as per the clinical records and meeting of the formal case criteria48 was not always confirmed. Similarly, cases of VF were

defined based on a detectable HIV VL in a patient already on ART, but the duration of ART use was not available. Several records were incomplete, and thus only the available data was analysed. Cases defined as having abdominal TB based on sonography alone may have been misclassified, given the differentials of sonographic features such as splenic microabscesses[25]. It has been reported with an earlier generation LAM ELISA assay that Candida species may cause a false-positive LAM[47]. This cannot be excluded as a confounder on untested specimens, but no urine samples in this study that were cultured and also tested positive for urine LAM demonstrated Candida. Small samples in several subanalyses limited

2.8 Conclusion

Urine LAM is a simple, easy to perform point of care test and takes only 25 minutes to establish a result. This study demonstrates that it had significant utility in hospitalised patients with CD4 counts of less than 100 cells/µL or in patients who are seriously ill, who were either sputum scarce or sputum negative, with a higher yield in the former. In this cohort of patients, the definitive diagnosis of TB is both elusive and often delayed with dire consequences. A positive result predicted a sicker subset of patients with a possibly higher mortality, in whom a mortality benefit of including the test in diagnostic algorithms has been previously demonstrated. A positive urine LAM result was also strongly associated with several disseminated and extrapulmonary forms of the disease, specifically mycobacteraemia, bone marrow involvement, and abdominal TB, in whom diagnosis in resource limited settings is often difficult or delayed. It was also helpful and accurate in establishing the diagnosis of a TB IRIS where suspected. Several laboratory associations were established including the presence of a severe anaemia, renal dysfunction, and abnormalities on liver function testing, and an increased yield in those patients who were immobile or confused, with these possibly guiding its rational usage in a diagnostic algorithm (figure 4), with potential to improve its positive and negative predictive values. Possible false positive results may be encountered in patients with disseminated NTM infections including MAC and *Mycobacterium kansasii*.

Given all the above it is a useful test to confirm the diagnosis of TB in a cohort of patients in whom this can be notoriously difficult, and as such should be included in the routine care of such patients.

CHAPTER 3 - REFERENCE LIST

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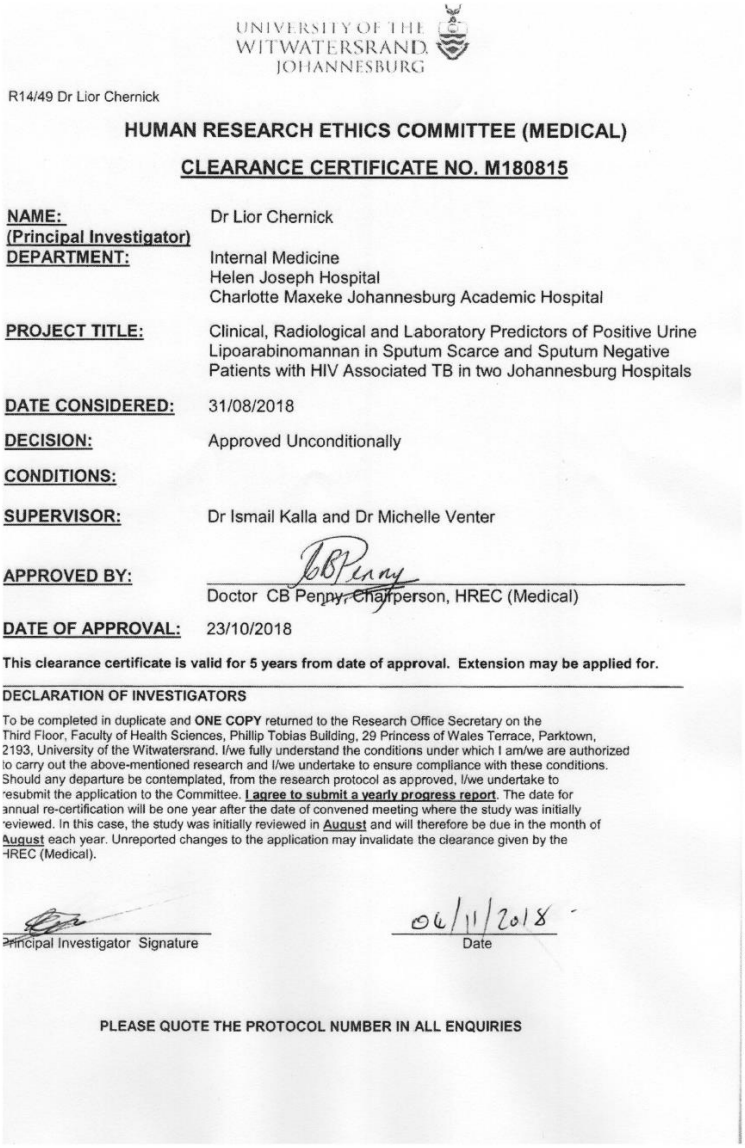
APPENDICES

A. Ethics clearance



UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R14/49 Dr Lior Chernick

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M180815

NAME: (Principal Investigator)	Dr Lior Chernick
DEPARTMENT:	Internal Medicine Helen Joseph Hospital Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	Clinical, Radiological and Laboratory Predictors of Positive Urine Lipoarabinomannan in Sputum Scarce and Sputum Negative Patients with HIV Associated TB in two Johannesburg Hospitals
DATE CONSIDERED:	31/08/2018
DECISION:	Approved Unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Ismail Kalla and Dr Michelle Venter
APPROVED BY:	Doctor CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	23/10/2018

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

Principal Investigator Signature

06/11/2018
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

B. Institutional Permission Letters

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

Gauteng Department of Health
Helen Joseph Hospital
Enquiries: Dr. M.R. Billa
Chief Executive Officer
Tel :(011) 489-0306/1087
Fax :(011) 726-5425
E mail: Raymond.Billa@gauteng.gov.za
Date: 01 August 2018

Dr.M.R.Billa
Chief Executive Officer
Helen Joseph Hospital

Dear Dr. M.R Billa

STUDY: Clinical ,Radiological and Laboratory predictors of positive urine lipoarabinomannan in sputum scarce and sputum negative patients with HIV associated TB in Johannesburg Hospitals.

RESEARCHERS: Dr. Lior Chernick

Above the study was discussed at the Research Committee meeting. We recommend that permission be granted for Helen Joseph Hospital to be used as a site for the above research, However , since this is individual /Patients.
Upon completion of the study, copy thereof should be submitted to Helen Joseph Hospital.
It is duty of the researcher to collect the data to the relevant department after the Research Committee approved the study.
Thank you

Dr. Murimisi Mukansi
CHAIRPERSON
DATE: 07/08/2018

Approved

Dr. M.R. BILLA
CHIEF EXECUTIVE OFFICER
DATE: 08.08.2018

1

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

Enquiries:
Ms. N. Mzila
Office of the Clinical Director
Email:Nolwazi.Mzila@gauteng.gov.za
Tell: (011): 488-4812
02 August 2018

Dear Dr. L. Chernick

STUDY TITTLE: Clinical, Radiology and Laboratory Predictors of Positive Urine Lipoarabinomannan in Sputum Scarce and Sputum Negative Patients with HIV Associated TB in Johannesburg Hospitals.

Permission to conduct the above mentioned study is provisional approved. Your study can only commence once Ethics approval is obtained. Please forward a copy of your Ethics Clearance Certificate as soon as the study is approved by the Ethics Committee for the CEO's office to give you the final approval to conduct the study.

Supported / ~~not supported~~

pp
Dr. M.I. Mofokeng
Clinical Director
DATE: 02/08/2018

Approved / not approved

Ms. G. Bogoshi
Chief Executive Officer
DATE: 06.08.2018

C. Permission for use of copyrighted images

Abbott

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DESCRIPTION OF MATERIAL
Alere Determine™ TB Lam Ag – 2 reference images on page 5

ACKNOWLEDGMENT
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Denise Duax
Authorized Representative

Date: 21/02/2021

Abbott-47229

D. Data collection sheet

Study number: _____

1) Urine LAM: Pos □ Neg □ Grade____/4

2) Demographics

a) Age: ___

b) Gender: Male □ Female □

c) ART: Yes □ No □

3) Clinical

a) Symptoms TB:

i) Cough

ii) Night sweats

iii) Loss of weight

iv) Fever

b) Examination

i) HR: ____

ii) RR: ____

iii) BP: ____

iv) T: ____

v) Delirium: ____

vi) qSOFA: 0 1 2 3

c) Diagnosis:

i) Definite TB*

ii) Probable TB†

iii) Not TB

d) Outcome:

i) Discharge □ Death □

* Microbiological confirmation including TB microscopy, culture, PCR or LAM+

† Empiric diagnosis by managing clinical team based on clinical features, physician gestalt and supportive radiology

ii) Other diagnoses:_____

4) TB Microbiology

a) Sputum submitted: Yes □ No □

b) Sputum Xpert MTB/RIF: Pos □ Neg □

c) Microbiological confirmation of TB:

i) Yes □ No □

(1) Sputum culture: Pos □ Neg □

(2) TB Bactec: Pos □ Neg □

(3) Other sample: Pos □ Neg □

(a) Site: _____

(b) Culture □ PCR □

5) Radiology

a) Chest X-Ray

i) Suggestive pulmonary TB

(1) Nodular infiltrate

(2) Reticular-nodular infiltrate

(3) Cavitation

(4) Hilar lymphadenopathy

(5) Pleural effusion

(6) Other:_____

ii) Suggestive Miliary TB

iii) Not suggestive TB

b) Abdominal US

i) Suggestive TB: Yes □ No □

(1) Lymphadenopathy

(2) Splenic hypodensities

(3) Ascites

6) Laboratory Parameters

WCC	
Hb	
Platelets	
Creatinine	
Protein	
Albumin	
ALT	
ALP	
GGT	
CD4	
VL (log)	
Urine leukocytes (IF culture negative)	

7) Notes

1

2