Antigens of *Mycobacterium tuberculosis* with reference to diseases diagnosis and special emphasis on lipoarabinomannan

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Tuberculosis (TB) is a contagious and notorious disease globally. There are several tests available for the detection of TB, but they have severe limitations. There is no reliable test present that quickly can detect TB at an early stage and also discern between different stages of the disease. Detection of TB is the major problem. Resolving it may lead to initiation of early treatment and thus controlling further spread. Methods to detect TB are continuously evolving to achieve rapid, cheaper, sensitive, and specific results. Here, we review *Mycobacterium tuberculosis* lipoarabinomannan (LAM) as a diagnostic marker, which is present in the sputum and body fluids, including urine and blood. Thus, it could be an innovative approach in the diagnosis of childhood TB using urine as a sample. There is a need for developing better diagnostic tools to detect TB and using LAM as a diagnostic marker, we can overcome the shortcomings of the present tools and techniques. The application of rapid LAM test has the potential to evolve with innovative approaches being attempted to increase the sensitivity of TB detection.

Keywords. Antigens, diagnostic marker, lipoarabinomannan, *Mycobacterium tuberculosis*, tuberculosis.

Tuberculosis: the major killer of microbial infection

MYCOBACTERIUM TUBERCULOSIS (MTB) is the causative agent of tuberculosis (TB), an airborne infections disease. MTB has been one of the most ubiquitous pathogens across the globe for thousands of years. It was discovered by the German physician Robert Koch in 1882. TB generally affects the lungs with symptoms of coughing, chest pain, anorexia and fever. The reason for its severity is the release of microscopic droplets from a TB patient through sneezing, coughing or speaking, which will subsequently spread the disease to a healthy person[1]. The size of droplets is up to 5 μm, and it may contain 1–3 bacilli. The infection is characterized by either latent or active TB. Most of the infected subjects remain asymptomatic, i.e. latent TB, and infection prevails in a quiescent state without any clinical symptoms of the disease. Epidemiological studies of TB in both developing and developed countries report that 5–10% of latent subjects may develop active TB during their lifespan[2]. Active TB is the condition of an infected subject, when the immune system is unable to find or defend against MTB. Active TB is one of the disastrous and is of great concern due to high human mortality in the world.

About 23% of the world population is reported to be infected with MTB, which represents almost 1.4 million deaths every year[3]. Unfortunately, identification of cases is one of the most fragile step, and up to 40% of TB subjects are either not diagnosed or reported on time to medical care. This is to some extent inevitable due to impediments to existing diagnostic methods[4]. Multi-drug resistant (MDR), extensively drug-resistant TB and progressing pestilence of co-infection of human immunodeficiency virus (HIV)-TB further exacerbate disease management. In 1993, the World Health Organization (WHO), Geneva, Switzerland, had declared TB as a global public health emergency. Even after decades of persistent global efforts, TB is still among the top ten causes of human mortality worldwide[5].

TB has been associated with destitution and poverty as well as the lack of proper health services, malnutrition, social disruption and inadequate living conditions. HIV infection leading to acquired immune deficiency syndrome (AIDS) is one of the strongest risk factors for TB.

In 2019, 10 million people were infected with TB globally, and there was an estimated 1.2 million TB deaths among HIV-negative and 208,000 deaths among HIV-positive subjects[6]. In 2014 the 'post-2015 global TB strategy' was announced by the World Health Assembly, a decision-making body of WHO, to eradicate the global TB epidemic with targets to reduce its mortality by 95% and taking down TB cases by 90% by 2035 (ref. 7). Detection of the disease in the early phase and providing initial treatment to patients is the first step of this scheme. Therefore, prevention of disease transmission is significant and requires early diagnosis, along with appropriate medical treatment.

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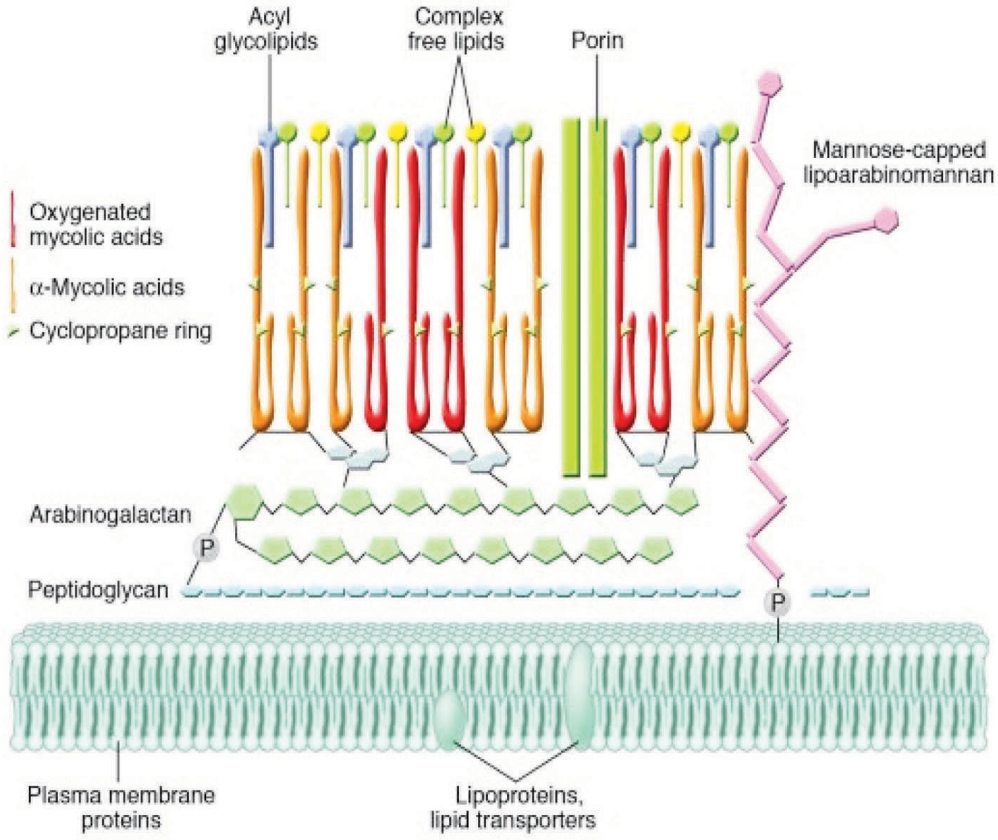

Figure 1. Cell-wall envelope of *Mycobacterium tuberculosis* showing lipoarabinomannan[15].

TB causing pathogen: *Mycobacterium tuberculosis*

MTB of family Mycobacteriaceae is a pathogenic bacterium of TB. The bacteria in this cohort were named so because of their mould-like (myco: fungus) pellicular growth pattern in liquid medium[8]. The genome size of MTB is about 4 million base pairs and contains ~4000 genes[9]. Due to the presence of mycolic acid, MTB has an unusual waxy coating on its cell surface. This coating makes the cells impervious to Gram staining and hence acid-fast dye is used to identify MTB by microscopy[10].

Due to the presence of thick peptidoglycan, MTB is categorized as acid-fast and Gram-positive. The bacterium divides in about 18–20 h and stays in the human alveolar macrophages. TB infection may occur either due to the activation of already existing latent bacilli or by new bacilli. In general, an equilibrium is established between active and latent bacilli; however, deviation of this equilibrium may cause an acute infection[11]. According to the dynamic reinfection hypothesis, non-replicating bacteria may reach the bronchial tree and initiate infection, followed by granuloma formation[12]. The MTB cell wall is composed of approximately 60% of lipids. Mycolic acid, cord factor, and wax-D comprise a major fraction of the MTB cell wall[13]. It is also composed of two segments, including the outer part and core of the cell wall (Figure 1). The core of the cell wall is made up of peptidoglycan (PG), covalently attached with arabinogalactan (AG) and mycolic acid, forming the mycolyl arabinogalactan peptidoglycan (mAGP) complex. The different cell wall proteins, including phosphatidylinositol mannosides (PIMs), lipomannan (LM) and lipoarabinomannan (LAM) are present in the upper part, which is made up of free lipids[14]. There are many antigens present in MTB. Some of the major antigens are cord factor, total mycolic acid-containing glycolipid (TBGL), sulpholipid-I and LAM[15].

Host–pathogen interaction: role of host immunity

In subjects exposed to MTB, the interaction between host and pathogen is significantly influenced by host immunity. The entry route of tubercle bacilli into the body is through the inhalation of respiratory droplet nuclei[16]. Based on the immune response of the host upon exposure to MTB, the exposed human being may get rid of bacteria, may develop active TB, or the disease may become a chronic infection without clinical manifestations. The prefatory reaction is due to the interplay of specific glycolipids/lipids/carbohydrates and/or peptidoglycans of the MTB cell envelope, with the cells of the innate immune system, e.g. macrophages and dendritic cells[17]. The way in which macrophages and

dendritic cells either activate or suppress distinct antibacterial mechanisms, the pattern of cytokines being secreted and how the antigens interact with the major histocompatibility complex (MHC) directs the profile of the acquired immune response. The acquired immune responses mediated through T-cells perform an especially important role in MTB infection control[18]. However, the profile of the host immune response necessary for effective acquired immunity to MTB antigens is yet to be elaborated. Research on the acquired immune response revolves around the function performed through antigenic peptides and poorly addresses the mycobacterial antigens of a lipoglycan nature. The lipoglycan antigens may have been undervalued and may in fact play a vital role in the overall immune response to the bacterium and as such be important for TB diagnosis. While the significance of T-cell immunity has been well established, the role of humoral immunity has been less considered[19]. Numerous pieces of evidence support the role of antibodies and B-cells in the establishment of an efficient immune response against TB infection[20].

LAM inhibits multiplication of T-cells and bactericidal activities of macrophages[21]. LAM molecules can insert themselves into biological membranes and bind with toll-like receptors (TLRs), affecting signalling events. Mannosylated LAMs (ManLAMs) have been reported to have an immunosuppressive nature by restoring IL-10 production. It inhibits the production of interleukin 12 (IL-12) interfering with TLRs and tumour necrosis factor (TNF). ManLAM additionally modulates MTB-induced apoptosis of macrophages by binding with host mannose receptors. This helps in deactivate host macrophages to allow the bacteria to survive and multiply inside them[22].

Need for an improved novel biomarkers for TB diagnosis

The need for a novel TB biomarker at any stage of disease diagnosis, treatment and prevention is extensively recognized. At present the markers and tests available for TB diagnosis have several limitations for point-of-care (POC) diagnostic purpose. There is a lack of diagnostic biomarkers as well as predictive markers to test the development of latent to active TB[23]. The available tests are not able to differentiate between subclinical progressing infection and non-progressing latent infection[24]. Active TB diagnosis is primarily based on the detection of bacilli in the sputum through 'sputum smear acid-fast staining' and bacilli culture. Microscopy is available; however, the missing sensitivity and specificity remain a problem in several samples. The gold standard for diagnosing TB is still mycobacterium culture, but being time-consuming it may require as many as 6–8 weeks for obtaining the results[25]. The Xpert MTB/RIF test is an automated and cartridge-based system, but the disadvantage is that it is expensive and frequently unavailable in primary-care settings due to unavailability of many reagents, etc. Diagnosis of latent TB is carried out by tuberculin skin test (TST) or the interferon-γ release assays (IGRAs). TST makes use of purified tuberculin derivative (PPD); however, it may be nonspecific. This test is primarily based on skin infiltration through intradermal injection of PPD in a crude aggregate of antigens, where lots of antigens are shared through MTB, *Mycobacterium bovis*, BCG and numerous environmental mycobacteria species[26]. None of the diagnostic tests could credit the development of active TB. This is a major shortcoming, as efficient and accurate detection of those with LTB1 is at higher risk of developing the TB disease in due course of time[27]. Although these tests are helpful in patient management, they provide insufficient predictive value for progression to active TB[24].

Though, there are many strategies and techniques for TB diagnosis, they are not specific and sensitive. Hence novel solutions for TB diagnosis are needed (Table 1). WHO has described the overall performance and operational characteristics of a test appropriate for primary care or at the POC in its high-priority target product profiles (TPPs)[28]. Thus, better biomarkers to predict TB outcomes are the need of the hour. This is a concern for TB research and clinical practice globally[29].

Lipoarabinomannan as a potential diagnostic marker for TB

LAM is one of the major lipoglycans of the mycobacterial cell envelope, which is present inside the body fluids of MTB-infected individuals. Post mycobacterial infections, the LAM molecule is present in many body fluids, making it a potential biomarker to identify TB infection. The immune response against LAM can also serve as a diagnostic tool.

Mycobacteria has a peculiar cell wall with an array of lipid-based molecules that provide a thick waxy surface. This molecule is an important diagnostic tool for detecting TB infection[30]. LAM is a significant structural element of the mycobacterium cell wall and is a prominent mediator of functions that result in successful infection and pathogenicity[17]. Antigenic molecules in LAM are made up of five repeating linked D-arabinofuranose residues. Epitopes on this molecule are arranged on the surface of the mycobacterium cell wall. However, in *Mycobacterium leprae*, these have an inside orientation. LAM is soluble in water, resistant to proteases and boiling and degrades slower than protein molecules because of its polysaccharide nature[31]. It is a glycoconjugate and one of the virulence elements associated with MTB. As one of the wall components, it allows MTB to survive in the host cell by affecting equilibrium of host resistance and immune response. It is being reported as up to 15% of the total mass of bacteria. LAM of mycobacterium has a molecular mass of 17.3 kDa, as reported by several MALDI-MS studies[32]. The LAM molecule is made up of three components: a phosphatidylinositol membrane anchor, a $(1 \rightarrow 6)$-linked mannan backbone of

Table 1. Currently used tuberculosis (TB) diagnosis techniques and their limitations

Testing indication	Currently employed techniques	Limitation of the current techniques	Desirable new techniques	Reference
Diagnosis of latent TB infection (LTBI)	Tuberculin skin test (TST). Interferon-gamma release assay (IGRA)	IGRA and TST are unable to satisfactorily distinguish between latent and active TB. The test is unable to identify those at higher risk of progression to active TB.	A new test that can resolve the spectrum of TB and identify subjects infected with latent TB, who are at higher risk towards progression to active TB and may benefit from preventive therapy.	58
Diagnosis of active pulmonary TB	Sputum smear microscopy (SSM).	Smear microscopy is insensitive and cannot detect drug resistance TB.	A non-sputum-based biomarker test for all kinds of TB is needed, as well as sputum-based replacement test for smear microscopy.	59
	Nucleic acid amplification test (NAAT).	NAAT test is expensive and not easily adaptable at the peripheral level.		
	Culture	Culture is extended performance and takes extended time.		
Test to identify individuals with expected TB, who need confirmatory testing	TB symptoms (e.g., two weeks of cough and irregular weight loss).	Symptoms lack sensitivity and specificity, particularly in HIV-infected subjects and young children.	A simple, inexpensive triage test which is ideal for use by community health workers and could be used as a rule-out test by healthcare providers.	59
	Chest X-ray	Although sensitive, chest X-rays are not specific for TB.		
Diagnosis of extra pulmonary TB (EPTB) and TB in children.	Smear microscopy	Paediatric patients with EPTB often are unable to produce sufficient sputum. Invasive samples are usually necessary. Smear microscopy does not have appropriate sensitivity and specificity.	For all TB types (pulmonary and EPTB), a non-sputum-based biomarker test is required.	59, 60
	NAAT	NAAT tests are cost-effective and not easily adaptable at the peripheral level.		
	Culture	Culture is time-consuming.		

mannopyranose (Manp) and an arabinan chain containing a couple of arabinofuranoside (Araf) residues and a hexa-Araf termini. At the time of infection, the membrane anchor helps facilitates the molecule to attach to the cell wall, and homo polysaccharides function as a carbohydrate skeleton (Figure 2)[33]. There are three major classes of LAM based on the presence and structure of capping. In mannosylated LAMs (Man LAM), the mannosyl groups are present on the D-arabinan group. After mannosyl capping, Man LAM acts as an anti-inflammatory molecule and inhibits the production of TNF-α and IL-12. Such properties of Man LAM facilitate the bacteria to survive in the host cell for a long time[34]. Man LAM is observed in pathogenic mycobacterial species, including MTB, *M. leprae* and *M. bovis*. Phosphoinositol-capped LAMs (PILAM) are present in non-pathogenic *Mycobacterium smegmatis*[35].

Arabinofuranosyl-terminated LAM (Ara LAM), Ara LAM 1 and 3-mannosyl side chains are found in many mycobacterial species. Several lipid additives of the bacterial cell wall, e.g. lipomannan (LM) and phosphatidylinositol mannosides (PIMs) play a role in the synthesis of LAM by the addition of mannopyranosyl to a phosphoinositol. PIMs are taken into consideration as pioneers of LAMs in the biosynthesis pathway[21]. PIMs and LM are synthesized by the addition of mannopyranosyl to a phosphoinositol. Glycosylation of PIMs and LM with arabinan results in the formation of LAM[36]. Mannosyl transferases are involved in the synthesis of PIMs.

The concentration of LAM inside various body fluids can be influenced by other factors, including bacterial load, co-infection with HIV and/or the site of infection[37]. HIV-TB co-infected patients with immune suppression and disseminated TB have been reported with higher LAM concentration in the urine[38]. Compared to host markers, the detection of MTB pathogen markers may be more specific.

Simple antigen detection has the capability to serve as a diagnostic marker for TB because the diagnosis of MTB using DNA-based method is complex and cost-effective, so simple antigen detection for TB is an achievement[39]. There is vast literature available on the immunogenic features of LAM and its antigenic attributes. Hence LAM is abundant and antigenic in nature making it crucial for the diagnosis of TB (Table 2). Our laboratory has been working on the use of LAM as a TB diagnostic marker.

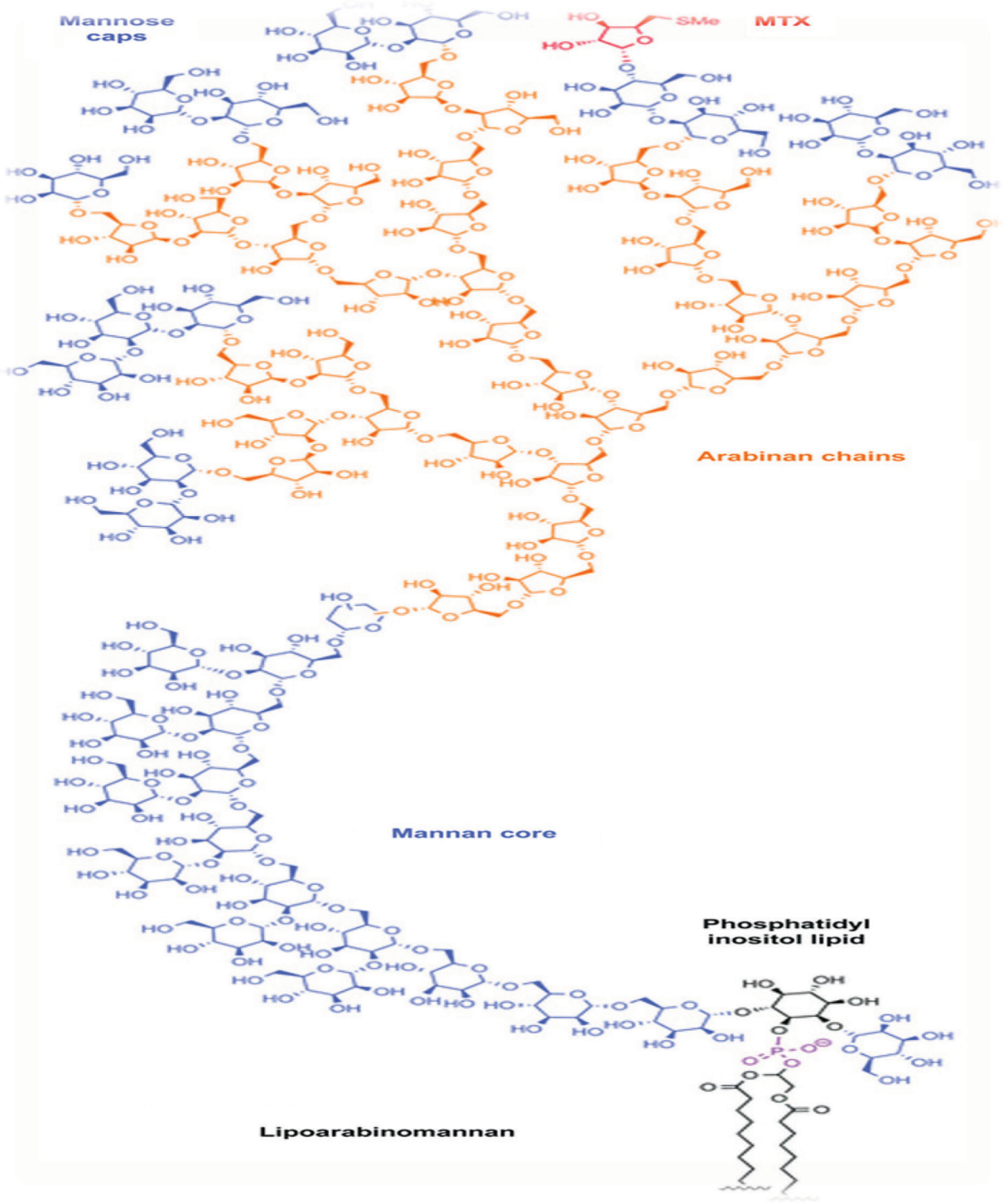

Figure 2. Structure of lipoarabinomannan (LAM) with typical carbohydrate composition of MTB Man LAM[37].

Gaps and unmet needs for new diagnostic biomarkers

Nearly 40% of TB patients are either not diagnosed or reported to the health system, making TB diagnosis one of the difficult steps of the disease control. This is partly due to the available diagnostic tools, which are either ineffective or inaccessible, particularly at the primary care level, where majority of patients seek care for non-specific signs and symptoms like cough and fever[4]. We need more effective strategies and techniques for managing TB. A simple diagnostic test with ease of application at the POC in primary-care settings has been a dream of the TB medical care fraternity. WHO has published TPPs with elaborated specifications

Table 2. LAM as a diagnostic marker for TB

Title of paper	Study type	Sample type	Total no. of samples	Technique applied	Location of sample collection	Sensitivity/ specificity	Year	Outcome	Reference
Diagnostic accuracy of a urine LAM-ELISA for screening ambulatory HIV infected persons for TB.	Cross-sectional study	Sputum, Blood, Urine	422 HIV infected patients included 30 active TB, 18 PTB, 5 ETB and 7 both PTB & ETB.	AFB, mycobacterial culture using BACTEC MGIT 960 system and ELISA.	Tembisa Main Clinic in Ekurhuleni, South Africa.	LAM-ELISA Sensitivity – 32% Specificity – 98%. 27% of TB cases AFB positive.	2011	Sensitivity of the LAM in urine was lower than that reported in previous studies, likely because the participants of that study were ambulatory and some sick persons with lower bacillary burdens of TB.	61
A bispecific antibody-based assay shows potential for detecting TB in resource constrained laboratory settings.	N/A	Serum	21 sample (14 were TB positive and 7 were negative).	Immuno-swab assay, SDS-PAGE, western blot, FACS and sandwich ELISA.	TB samples from TB trials consortium (TBTC) and healthy controls from Fort Collins, CO, USA.	Assay showed sensitivity-64% and specificity – 100%.	2012	The assay might be used as a rapid diagnostic tool in resource constrained laboratory settings, as this assay has all the characteristics of an ideal diagnostic TB test as affordable, more sensitive, specific, user-friendly, rapid, and equipment-free and can be delivered to those in need.	62
The value of serum LAM in the diagnosis of pulmonary TB.	Case-control study	Serum	40 PTB and 20 healthy.	Ziehl-Neelsen (AFB), LAM-ELISA.	Chest and Medical Biochemistry Department, Faculty of Medicine, Menoufia University Hospitals, Egypt.	Sensitivity: ELISA-90% ZN smear-85% Specificity: ELISA 100% ZN smear – 100%.	2014	The LAM test is simple, reliable, and rapid diagnostic test for the PTB. The LAM serum assay test is unlikely to be used alone for definitive TB diagnostic testing.	63
Detection of LAM in urine is an independent predictor of mortality risk in patients receiving treatment for HIV-associated TB in sub-Saharan Africa: a systematic review and meta-analysis.	Meta analysis	Urine	1172 HIV-TB cases, out of them 512 were LAM positive.	N/A	sub-Saharan Africa	N/A	2016	This study has proven that HIV-TB con-infected patients and detectable urinary LAM patients have greater mortality rate as compared to TB patients without detectable urinary LAM.	38

(*Contd*)

Table 2. (*Contd*)

Title of paper	Study type	Sample type	Total no. of samples	Technique applied	Location of sample collection	Sensitivity/ specificity	Year	Outcome	Reference
The diagnostic value of urine LAM antigen in childhood TB.	Cross-sectional study	Urine	Paediatric patients 61 suspected either PTB and ETB, aged 0–14 years.	Microbiological examination (AFB staining, sputum culture) and ELISA.	Child Health Department of Saiful Anwar Hospital in Malang, East, Java, Indonesia	Sensitivity: ELISA – 83% Microbiological test – 33% Specificity: ELISA – 85%, Microbiological test – 60%	2017	Urinary LAM provides a few benefits due to the fact samples are easy to collect compared to sputum collection. Rapid urinary LAM test result is expected to improve childhood TB control as a POC test and should be considered as POC diagnostic test for childhood TB.	49
Diagnosing TB in hospitalized HIV-infected individuals who cannot produce sputum is urine lipoarabinomannan testing the answer?	Multi-centre study	Sputum, Urine	Total patients enrolled 2528 Cohort 1-Sputum producing 2341 samples. Cohort 2-187 sputum scarce.	Sputum culture, chest X-ray, and Alere determine TB LAM Ag lateral flow.	South Africa, Tanzania, Zambia, and Zimbabwe	N/A	2017	This study states that urine LAM testing facilitates rapid diagnosis and positive predictive value in hospitalized HIV patients with scarce sputum. This POC test is a useful diagnostic tool of TB in those patients who cannot produce enough sputum.	64
Detection of LAM in urine and serum of HIV-positive and HIV-negative TB suspects using an improved capture-ELISA and GC/MS.	Cohort study	Urine, Serum	Cohort 1. 25 TB and HIV +ve, Cohort 2. 25 TB +ve HIV –ve, Cohort 3. 25 TB –ve HIV +ve, Cohort 4. 25 TB and HIV –ve.	Capture ELISA, gas chromatography/ mass spectrometry (GC-MS)	Vietnam, South Africa, and Peru	Sensitivity: GC/MS – 99% ELISA – 98% Specificity: GC/MS – 84% ELISA – 92%	2018	The GC/MS and ELISA have a significantly better sensitivity and specificity and confirmed that LAM is present in HIV–ve and TB +ve patients in lower amounts, than HIV +ve/TB +ve.	65
Biomarker for TB: case for lipoarabinomannan.	N/A review	Urine, Serum	N/A	TB LAM (Clearview) ELISA, determine (Alere) TB LAM test.	N/A	Sensitivity: 1. Clearview test: TB-HIV +ve – 51% TB-HIV–ve – 14% 2. Alere test: TB-HIV +ve – 45% TB-HIV–ve ND Specificity: 1. Clearview test: HIV +ve – 94% HIV +ve – 97% 2. Alere test: HIV +ve – 92% HIV–ve ND	2019	A method of detection and quantification of LAM in serum need to be further explored. If increased sensitivity of an Ag test for LAM is achieved, LAM should be investigated as a predictive biomarker of the outcomes following MTB infection as well as a biomarker in TB treatments.	50

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Table 2. (*Contd*)

Title of paper	Study type	Sample type	Total no. of samples	Technique applied	Location of sample collection	Sensitivity/ specificity	Year	Outcome	Reference
LAM in sputum to detect bacterial load and treatment response in patients with pulmonary TB: Analytic validation and evaluation in two Cohorts	Case-control cohort study	Sputum	308 patients, 244 diagnosed as PTB and 64 diagnosed as non-TB.	Smear microscopy, MGIT 960 and LJ culture, LAM-ELISA, and Xpert MTB/RIF.	Manila, Philippines	Sensitivity: LAM-ELISA-70% Xpert MTB/RIF-79.3% Specificity: LAM-ELISA-100% Xpert MTB/ RIF-ND	2019	This study reports that LAM-ELISA can ascertain the LAM concentration in sputum, and LAM in sputum measured by the ELISA may be utilized as a biomarker for bacterial load both before and after TB treatment.	53
Combining urine lipoarabinomannan with antibody detection as a simple non-sputum-based screening method for HIV-associated TB.	Case-control study	Urine, serum	104 (TB cases 74 and randomly selected non-TB 30).	U-LAM, sputum microscopy, culture, Gene Xpert.	Gugulethu township, Cape Town, South Africa	Sensitivity: U-LAM with Ab detection-92%, Sputum microscopy with IgG detection-88%, Xpert-96% Specificity: U-LAM with Ab detection-80%	2019	Combining urine LAM with serum antibody detection could offer easy low-cost technique that meets the necessities for a non-sputum-based test for the screening of HIV-associated tuberculosis.	66
Lateral flow urine lipoarabinomannan assay for diagnosis of active TB in adults with human immune deficiency virus infection: a prospective cohort study.	Prospective cohort study	Urine	280 patients enrolled in which 72 have definitive TB, 65 have probable TB and 143 have no evidence of TB.	Alere Determine TB LAM Ag test (LF LAM test), smear microscopy.	Siriraj Hospital and Chonburi Hospital, Thailand	Sensitivity: LF-LAM Assay-75% Smear microscopy – 61.1% Specificity: LF-LAM Assay-76% Smear microscopy-98.1%	2019	The LF-LAM test performed satisfactorily in the diagnosis of active TB in a few patients with more advanced TB and co-infected with HIV.	67
Novel lipoarabinomannan point-of-care TB test for people with HIV: a diagnostic accuracy study.	Prospective cohort study	Urine	968 hospitals in patients with HIV.	FujiLAM and AlereLAM assay.	FIND specimen bank and the university of Cape Town bank, South African hospitals	Sensitivity: FujiLAM-70.4% AlereLAM-42.3% Specificity: FujiLAM-90.8% AlereLAM-95%	2019	FujiLAM give advanced diagnostic sensitivity, specificity and could transform rapid POC TB diagnosis for hospital in patients with HIV compared to AlereLAM.	68
Point-of-care urine lipoarabinomannan antigen detection for diagnosis of TB in children.	Cohort study	Urine, Expectorated or induced sputum or gastric aspirates from ITTB and needle. Cytological aspirates from LNTB patients	381 children recruited, Cohort 1 – 280 children with presumed ITTB Cohort 2 – 101 children with presumed LNTB.	ZN staining, culture, Xpert MTB/RIF testing, and LAM assay (Alere determine TB LAM Ag).	OPD of the Department of Paediatrics, All India Institute of Medical Sciences (AIIMS), New Delhi, India	LAM assay-sensitivity: ITTB patients-73.2% LNTB patients-76% Specificity: ITTB patients-92% LNTB patients-93%	2019	The LAM assay increased the accuracy of TB detection significantly while in comparison with other reference tests. Urinary LAM testing showed high. Specificity and sensitivity in paediatric TB. LAM assay may also show to be useful as new diagnostic tool for paediatric TB.	57

(*Contd*)

Table 2. (*Contd*)

Title of paper	Study type	Sample type	Total no. of samples	Technique applied	Location of sample collection	Sensitivity/ specificity	Year	Outcome	Reference
Detection of mycobacterial lipoarabinomannan in serum for diagnosis of active TB.	Retrospective case-control study	Serum, sputum	145 subjects with clinical symptoms, 90 confirmed PTB and 55 non-TB.	Single molecule array (Simoa), liquid and solid cultures, AFB and Xpert.	Vietnam, South Africa, and Peru	Sensitivity: 37% in TB + Subjects 47% in TB +/HIV + 60% in TB+/HIV+/smear + Specificity: 100%	2019	Using Simoa, mycobacterial LAM antigen is detectable in serum with high specificity and appropriate sensitivity.	69
Diagnostic accuracy of 3 urine lipoarabinomannan TB assays in HIV-negative outpatients.	Multicentre diagnostic test accuracy study	Urine and Sputum	372 patients included (111 definite TB, 10 TB and 251 not TB).	FujiLAM, AlereLAM, EclLAM, SSM, Xpert and MGIT liquid culture and solid culture on LJ media.	Healthcare centre in Peru, South Africa, Khayelitsha township, DOTS treatment centre in Suburbs of Lima, University of Cape towns.	Sensitivity: Urine LAM Test FujiLAM-53.2% AlereLAM-10.8% EclLAM-66.7% Sputum Test-SSM-61.3% Xpert-76.6% Combine sputum test + FujiLAM SSM + FujiLAM-70.3% Xpert + FujiLAM-82% Specificity: Urine LAM Test-FujiLAM-98.9% AlereLAM-92.3% EclLAM-98.1% Sputum Test-SSM-100% Xpert-100% Combine Sputum test + FujiLAM SSM + FujiLAM-98.9% Xpert + FujiLAM-98.9%	2020	Compared with AlereLAM, FujiLAM was detected five times more in the patients with TB in HIV –ve subjects and had a high positive prognostic value. This has the potential to boost rapid diagnosis of TB at the POC. EclLAM in contestable that further sensitivity gains are possible, that highlights LAM as a potential biomarker.	70
Point-of-care urine LAM tests for TB diagnosis: a status update.	Review article	Urine	N/A	AlereLAM, FujiLAM, sputum culture, microscopy, NAAT and Xpert.	N/A	N/A	2020	Urine LAM is a promising TB diagnostic biomarker. The final objective of a urine LAM test is to achieve high sensitivity and specificity for TB patients.	45

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Table 2. (*Contd*)

Title of paper	Study type	Sample type	Total no. of samples	Technique applied	Location of sample collection	Sensitivity/ specificity	Year	Outcome	Reference
Cost-effectiveness of a novel lipoarabinomannan test for TB in patients with HIV.	Clinical cohort study	Sputum, Urine	1 million patients with HIV.	(i) Sputum Xpert MTB/RIF (ii) Sputum Xpert+ urine AlereLAM (iii) Sputum Xpert+ urine FujiLAM and CD4 count	South Africa, Malawi	N/A	2020	This study found that combining urine FujiLAM to sputum Xpert for TB testing among unselected hospitalized PWH would increase life expectancy and be economical. Additional feasibility studies should examined FujiLAM in clinical practice settings.	71

Abbreviations: TB, Tuberculosis; ELISA, enzyme-linked immunosorbent assay; LAM, lipoarabinomannan; +VE, positive; –VE, negative; IgG, Immunoglobulin-G; US, United States; AFB, Acid-fast bacilli; HIV, Human immunodeficiency virus; PTB, Pulmonary TB; EPTB, Extra-pulmonary TB; MGIT, Mycobacterial growth indicator tube; SDS, Sodium dodecyl sulphate; PAGE, Polyacrylamide gel electrophoresis; FACS, Fluorescence-activated cell sorting; GC, Gas chromatography; MS, Mass spectrometry; POC, Point of care; LJ, Lowenstein Jensen medium; MTB, *Mycobacterium tuberculosis*; RIF, Rifampin; ND, Not determined; U, Urine; LF, Lateral flow; Ag, Antigen; Ab, Antibody; SSM, Sputum smear microscopy; ITTB, Intra thoracic TB; LNTB, Lymph node TB; NAAT, Nucleic acid amplification test; PWH, Patient with HIV; DOTS, Directly observed treatment, short-course and OPD, Outpatient department.

for such a test[40]. Various stakeholders in POC have come forward with unmet issues, which would help in developing newer tools. Researchers have identified the need for developing numerous TB diagnostic tests, in addition to the currently available tools. The assessment for patients is difficult to diagnose for children, TB-HIV co-infected patients, and patients with EPTB with a non-sputum-based biomarker test for detection of active TB, according to the list of triage and screening tests[41,42]. A very sensitive TB test that is applicable for use at lower levels of care and is based on biological samples other than sputum (such as urine, blood, saliva, or inhaled air) would be a practical aid and reduce the time before diagnosis to enable early treatment[43]. A non-sputum-based biomarker may be useful in the diagnosis of latent TB infection that predicts progression to active TB and a test for monitoring drug susceptibility test (DST) at a proper setting[44]. This priority exercise eventually identifies the consecutive test as key priorities.

(i) Smear replacement test: A rapid sputum-based test as a substitute for smear microscopy with or without DST. The smear microscopy test is extensively used for TB diagnosis; however, its sensitivity limitations are well known.

(ii) Non-sputum-based biomarker test: A rapid non-sputum-based test capable of detecting all kinds of TB through the identity of traits of biomarkers or biosignatures.

(iii) Triage test: A triage test that can be utilized as a rule-out test by first-contact healthcare providers and must be simple and affordable. This test is used to determine whether or not someone has TB.

(iv) Rapid DST at microscopic observation centre level.

The second and third tests mentioned above are especially for advanced diagnosis in young subjects, who constitute around 10% of the worldwide TB burden. Eradication of TB cannot be done without identifying subjects with latent infection who are most at risk of developing active TB. WHO has published a consensus file with TPPs for priority diagnostics, with illustrations[40]. At present, there is no standard diagnostic test available that meets all POC TB test TPP requirements. The quick urine LAM test alone comes close because of its ease of sample collection, being cost-effective and having the capability used in decentralized settings[4].

LAM, a potential diagnostic marker of TB

To increase early case detection and address various current gaps in TB control, new diagnostic devices and strategies are being expected in this field. Early diagnosis of TB is an important aspect of biomarker research because initially most of the MTB-infected subjects remain healthy, but have latent TB infection. Current diagnostic tools for TB rely on sputum samples, and have disadvantages and limitations. At present, many diagnostics strategies and tools are under development. WHO has reported on the development of a 'rapid biomarker-based non-sputum test capable of detecting all forms of TB by identifying characteristic biomarkers'[40].

There are two LAM-based commercial assays available, i.e. Abbott determining TB LAM Ag (AlereLAM) and Fujifilm SILVAMP TB LAM Assay (FujiLAM). These tests meet the WHO TPP traits for a biomarker-based, non-sputum TB test, but studies show low sensitivity and specificity[45].

Hence, the main aim of the LAM test is to attain high diagnostic precision of sensitivity and specificity. One predominant problem in non-sputum-based tests is an inferior reference standard, mainly for patients with EPTB and adolescent TB. To overcome these limitations, a microbiological reference standard (MRS) and composite reference standard (CRS) must be taken into consideration with the LAM test[46]. MRS must consider parameter values from extra pulmonary samples and pulmonary samples of the mycobacterial culture, ZN staining and Xpert test to confirm diagnosis TB. CRS is used to make a final diagnosis primarily based on the result of two or more in TB tests. CRS considers chest X-ray, clinical suspicion and treatment initiation for definite tuberculosis[47]. TB diagnosis in children is difficult because clinical presentation is not specific, chest X-ray explication has low accuracy and the collection of sputum samples is challenging[48]. Urine LAM Ag detection test is a rapid and non-invasive alternative for the diagnosis of childhood TB, indicating that urinary LAM has a great diagnostic value for childhood TB[49]. The key factor regarding LAM is that it can diagnose TB in patients and children who are not capable of producing sputum. Therefore, the patients not able to produce sputum must now no longer be excluded from any sort of study. This group is likely advantaged by LAM testing using non-sputum urine samples.

It is necessary to evaluate the innovative diagnosis yield of sputum-based diagnostics combined with urinary LAM diagnostics[45]. If improved sensitivity of a LAM antigen test is achieved, LAM must be explored as a predictive biomarker of the consequences following MTB, and further as a marker to evaluate the efficacy of anti-TB therapies[50]. As mentioned earlier the LAM antigen detection test comes close to the POC TB test TPP needs. So many companies and groups are working on the high sensitivity of LAM detection and LAM Ag as a diagnostic biomarker might provide a new perception in the diagnosis of TB. The urine LAM test has a potential capability[51].

LAM is present in the urine of TB patients. This has been proved by a study where mice were injected with crude antigen extract of H37RV MTB bacterial cell wall. Hence, we can use it as a diagnostic tool for the identification of active TB[52]. There is no technique present to measure the bacterial burden of PTB; however, studies have suggested that LAM-ELISA can identify LAM concentration in sputum samples. So the measured LAM concentration in the sputum may be a good biomarker of bacterial burden before and during treatment[53]. Sputum-based LAM-ELISA could provide a real-time monitoring tool for TB treatment response

in TB therapy. There are several studies regarding LAM antigen as a diagnostic tool for TB, which provide newer insight to this field[54]. One may detect LAM in urine which is a non-invasive sample and is easy to handle.

Use of LAM in disease diagnosis might find a place in disease management and medical care[45]. The development of novel biomarkers, primarily based on non-sputum tests may be essential to eradicate TB from the world. Hence, a novel biomarker for TB tests requires appropriate evaluation and validation prior to global implementation[47].

LAM can be a novel approach in early stages of diagnosis. Further with the innovative tactics, sensitivity of the LAM detection can be improved.

Conclusion and future prospects

TB needs a new diagnostic tool that is capable of detecting infection in the latent phase and in a brief period[55]. There are several reference standard techniques present for TB diagnosis like AFB microscopy, sputum culture and gene Xpert NAAT, but they have shortcomings. New biomarkers for TB diagnosis are important to control disease spread. So, there is an immediate need for a diagnostic tool using a novel, non-invasive method. Another concern is the absence of a non-sputum-based test for children and biomarker test for the development of TB. Increasing investments are important to assist in biomarker discovery, evaluation and validation into clinical tools[56]. Research on TB biomarkers is now gaining interest; however, its effect has been restricted. For the higher established order of recent novel TB biomarkers, significant novel study is required. Funding and interest in biomarker research have increased from basic biomarker studies to fundamental biomarker discovery to clinical applications. Improved detection of lipoglycan biomarker LAM could lead to a breakthrough urine-based LAM antigen detection test.

The detection of TB in paediatric patients is challenging due to insufficient sputum production; however with the aid of urinary LAM Ag test, TB can be diagnosed among children. LAM Ag detection may lead to an early detection of TB in children[57]. LAM is a good biomarker for the diagnosis of TB in adults and children because it is present in most of the body fluids, e.g. sputum, urine and blood.

Hence, we need to explore LAM as a predictive biomarker for MTB infection. A review of the literature reveals that a diagnostic marker could be helpful in detecting a disease in the community. If a disease is detected in the early phase, it would also help in initial treatment and controlling its spread. Advancement in the present LAM tests and unfolding of next-generation assays need to be prioritized. There is a demand for TB biomarker studies at primary, secondary, and tertiary levels.

Conflicts of interest: The authors declare that they have no conflict of interest.

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ACKNOWLEDGEMENTS. P.C. acknowledges the facilities provided and support of intramural project of National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Indian Council of Medical Research (ICMR), Agra, and the facilities provided by the Department of Zoology, Dayalbagh Educational Institute (Deemed to be University), Agra, for her doctoral degree.

Received 15 June 2022; re-revised accepted 10 April 2023

doi: 10.18520/cs/v125/i5/494-507