Journal of Biorepository Science for Applied Medicine

Dovepress

open access to scientific and medical research

Open Access Full Text Article

REVIEW

Biomarker discovery for diagnosis and treatment of tuberculosis: a role for biobanking?

This article was published in the following Dove Press journal:
Journal of Biorepository Science for Applied Medicine
5 October 2015
Number of times this article has been viewed

Carmen C Swanepoel[1,2]
Candice I Snyders[1]
Shafieka Isaacs[1]
Emmanuel A Abayomi[1,2]
Ravnit Grewal[1,2]

[1]Faculty of Medicine and Health Sciences, Division of Haematology, Stellenbosch University, Tygerberg, [2]National Health Laboratory Services, Tygerberg Hospital, Cape Town, South Africa

Abstract: The identification and validation of tuberculosis (TB) biomarkers is urgently needed, especially since TB is the second most common infectious cause of death worldwide, despite the fact that it is curable. TB biomarkers, whether host or pathogen specific, would provide prognostic information about pathogenic processes, current health status, and future disease risk of the patient. Thus, a need exists for research development of cost-effective, accurate, and rapid diagnostic assays, in particular, a TB point-of-care test to differentiate between active disease and latent infection and to detect drug resistance in human immunodeficiency virus (HIV)-infected and -uninfected individuals as well as children. Obtaining access to high-quality biospecimens with the associated clinical data in statistically relevant numbers is of the utmost importance; however, it can be a major challenge. Biobanks play an important role as a tool/link that would aid in the collection, validation, and storage of human and/or bacterial specimens. As the ideal sample type for a reliable biomarker is currently unknown, and volumes are not clearly defined, it becomes important to ensure that various sample types are collected, handled correctly, and stored appropriately in order to be fit for this purpose, as new technologies develop over time. Generally, sample collection processes are not standardized, and clinical data capturing is poor; all of which would have an impact on biomarker validation studies. Biobanks can address these shortfalls by ensuring the tight application of standardized protocols, quality control, and address the effects of preanalytical and storage variation on a broad range of sample types. This review gives an overview of global TB challenges, with regard to diagnosis and treatment; needs in the identification and validation of TB biomarker; and the role of open-access and certified biobanks as an essential component in the development of novel TB drugs and diagnostic tests for both public health and personalized medicine.

Keywords: biobanks, tuberculosis, biomarkers, latent and active TB

Introduction

Tuberculosis (TB) remains one of the major infectious causes of death globally, despite the fact that it is curable. In 2013, an estimated 9.0 million people developed TB and 1.5 million died from the disease, 360,000 of whom were human immunodeficiency virus (HIV)-positive.[1] Additionally, the emergence of multidrug-resistance TB, extensively drug-resistance TB, and totally drug-resistant TB just adds to the problems related to worldwide TB control.[1,2]

Programs such as the Grand Challenges in Global Health initiative aim to reduce the global TB incidence through prevention as it is more focused and achievable. Thus, to ensure global TB control, there is a need to increase the rates of early case detection as well as treatment. Each year, TB is slowly declining, and it is estimated

Correspondence: Carmen C Swanepoel
Faculty of Medicine and Health Sciences, Division of Haematology, National Health Laboratory Services, University of Stellenbosch, PO Box 19063, Tygerberg 7505, South Africa
Tel +27 21 938 4103
Fax +27 21 938 4609
Email carmens@sun.ac.za

that 37 million lives were saved between 2000 and 2013 through effective diagnosis and treatment. Figure 1 illustrates the global estimates of TB incidence rates in high-income countries compared to high-disease-burden countries.[1,3]

In 2013, the World Health Organization (WHO) reported that the treatment success rate continued to be high at 86% among all new TB cases using the current recommended 6-month regimen of four first-line drugs: isoniazid, rifampicin, ethambutol, and pyrazinamide. On the other hand, the treatment success rate for multidrug-resistance TB in the last 20 months were much lower.[1,3]

Unfortunately, the inability to detect and treat all infectious cases in a timely manner is still a major problem, despite improvements in global TB control program performance and a decrease in the rate of disease incidence. However, advancement toward elimination of TB has been slow.[4]

Based on these observations, awareness has been refocused on research and development in areas such as diagnostics, therapeutics, vaccines, and most importantly biomarker discovery.[4,5]

Mycobacterium tuberculosis (M. tuberculosis) infection in humans is transmitted by the inhalation of infected aerosol droplets produced through the coughing of persons with active pulmonary disease. The *M. tuberculosis* bacilli within the droplets get phagocytozed by alveolar macrophages. Subsequently, a localized inflammatory response follows, which recruits mononuclear cells from surrounding blood vessels, and these cells serve as hosts for the bacteria and become granulomas.[6] Depending on how the host immune system responds, this granuloma may resolve or progress to more serious complications, such as massive necrosis of the lung tissue or may develop into chronic lesions.[7] Upon rupturing of these lesions, bacilli spill into the atmosphere through coughing by the host.[6]

In most cases, the infection is contained and rarely progresses directly to active disease, resulting in a latent infection. In latent infection, an immune response is elicited within the host, which contains the *M. tuberculosis* bacilli in a dormant state, in which active replication and tissue damage are prevented and no clinical symptoms or signs of active TB disease are observed. However, reactivation can occur depending on the weakening of immunity due to age, undernutrition, chronic diseases such as alcoholic liver disease, diabetes, carcinoma, silicosis, HIV coinfection, and use of immunosuppressive drugs and anti-inflammatory treatments.[8]

When active disease occurs in later life, it becomes difficult to ascertain whether it is due to reactivation of latent

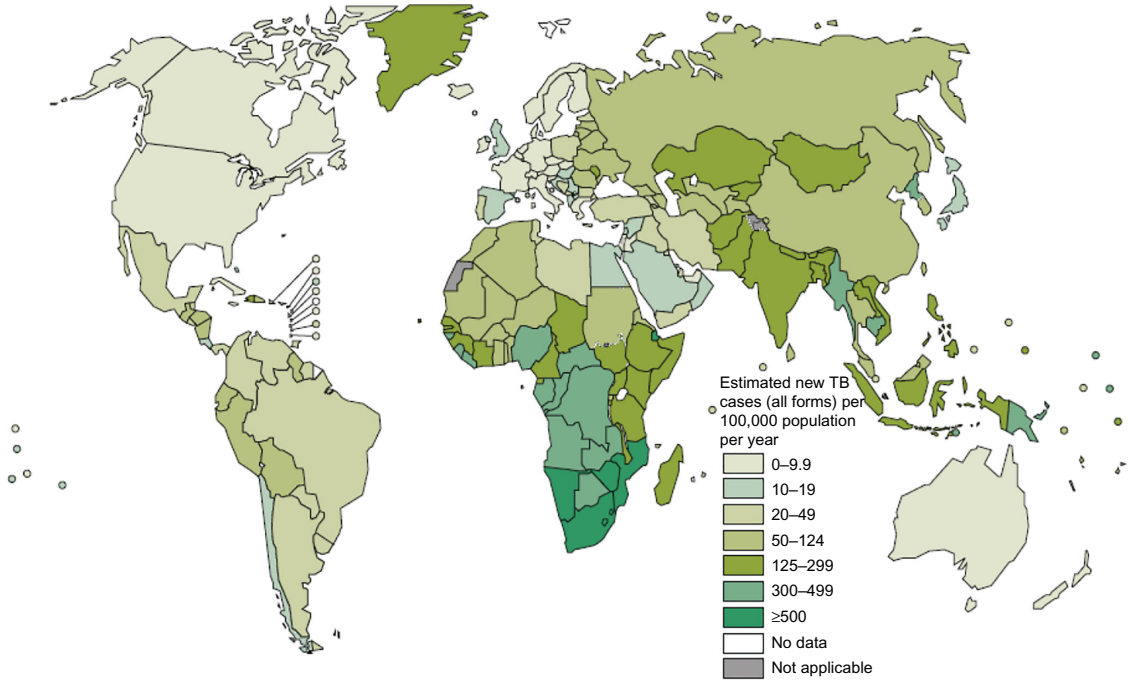

Figure 1 Global TB incidence rates (the number of incident cases relative to population size) in 2013.
Notes: The incidence rate widely varies between countries. The lowest rates were found mostly in high-income countries such as Canada, USA, Japan, New Zealand, and Australia, with incidence rates <10 cases per 100,000 population per year. In contrast, the HBC had rates of 150–300 cases per 100,000 population per year. Rates of >500 per 100,000 population per year were observed in HBC such as Mozambique, South Africa, and Zimbabwe. Reprinted from World Health Organization. *Global Tuberculosis Report 2014*. Geneva, Switzerland: World Health Organization; 2014. Available from: http://www.who.int/tb/publications/global_report/en/. Copyright ©2015.[1]
Abbreviations: HBC, high burden countries; TB, tuberculosis.

M. tuberculosis bacilli or to a new infection with another *M. tuberculosis* strain.[9]

The detection of latent TB, particularly in immune-compromised patients, is important as the risk of developing active TB is very high. So, in these individuals, detection of latent Tb would lead to closer management strategies and therefore early detection of active Tb, so accurate distinction between the two disease states becomes essential. To complicate this matter further, a group of researchers further believe that *M. tuberculosis* infection might be more clinically diverse than the binary distribution of just active disease versus latent disease. Thus, this spectrum can further extend to those individuals clearing the disease, to those who have the active replicating bacterium without clinical symptoms (subclinically active), and to those who have fulminant active disease.[10] This in itself has implications on the identification of suitable diagnostic biomarkers and needs to be taken into account when trying to screen for potential TB biomarkers. Furthermore, TB in children, drug-resistant TB, and sputum smear-negative pulmonary and extrapulmonary TB in adults still remain the greatest diagnostic challenges.[2,11]

TB remains a global dilemma for a number of reasons, one of them being that current gold standard diagnostic strategies and tools are inadequate and laboratory based. Diagnosis to date, especially in a resource-limited setting, still relies heavily on the age-old tubercillin skin test, sputum culture, and light microscopic examination of Ziehl–Neelsen-stained sputum specimens.[12] All are known to have low specificity and sensitivity and are mainly used for definitive diagnoses of suspected pulmonary TB cases. These tests perform poorly in young children[13] and immunocompromised individuals, where obtaining sputum may be difficult, but also fail to detect drug resistance or extra pulmonary disease. The limitations of the present TB diagnostic assays contribute to diagnostic delays. This in turn has implications on the commencement of treatment, which leads to severe consequences for global TB control.

Polymerase chain reaction (PCR)-based diagnostic tools such as the GeneXpert *M. tuberculosis*/rifampin (MTB/RIF, Cepheid Inc., Sunnyvale, CA, USA), which is a cartridge-based automatic nucleic acid amplification assay, has also been recently implemented to determine TB case detection and rifampicin resistance testing from unprocessed sputum samples in <2 hours.[14] This assay endorsed by the WHO in 2011 for use in endemic countries has a higher sensitivity in both pulmonary and extrapulmonary TB; however, the high cost associated with it decreases the popularity of this technology.[15]

In well-developed countries, radiography, advanced imaging techniques, rapid culture methods, as well as nucleic acid amplification tests, which are more sensitive, are used in conjunction with light and light-emitting diode microscopic examinations for the detection of active TB.[2] However, the majority of these technologies are beyond the scope of the world's most heavily affected TB areas in Africa and Southeast Asia.[1]

Thus, there is a great need for more cost-effective, accurate, and rapid methods: first, to accurately diagnose TB, especially where sputum is not obtainable; and second, to differentiate between active disease and latent infection, and detect drug resistance in HIV-infected and HIV-uninfected individuals[11,12] using different sample types. While TB is primarily a disease of the lungs, other organs in the body can also be affected, such as bones, lymph nodes, bone marrow, skin, eyes as well as the gastrointestinal tract and the genitourinary system. Thus samples for diagnostics, other than sputum and blood, may include lymph node aspirate, urine, pleural fluid, gastric washings, cerebral spinal fluid, joint fluid, and bone marrow aspirate, or biopsy material.[7]

In addition, advancement toward a point-of-care test appropriate for field work in the TB setting has been limited, while biomarker discovery remains a major challenge.[16,17]

Tuberculosis biomarkers

A biomarker can be defined as a biological molecule found in blood, other body fluids, or tissues which serve as a measurable indicator to measure normal biological processes, the presence or progress of disease (pathogenesis), or the effects of treatment.[5,18] These markers can either be specific to the host or to the pathogen in infectious disease and can also provide prognostic information about pathogenic processes as well as the current health status and future disease risk of the patient. Respectively, both viral load and CD4+ cell counts in HIV infection are prime examples of a biomarker's usage in initial diagnosis and evaluation of disease state and progression.

Emphasis on biomarker discovery within TB research has became a high priority as seen by numerous initiatives started by the WHO, the European and Developing Countries Clinical Trials Partnership, the Bill & Melinda Gates Foundations, and the Global Alliance for TB Drug Development (TB Alliance).

In order to develop early and specific diagnostic tools, a need exists for biomarkers with application in TB to be able to distinguish at presentation between active TB, latent TB infection, and no disease. Other applications may include

predicting future reactivation risk, monitoring the eradication of latent *M. tuberculosis* infection, or serving as surrogate markers of cure following TB treatment or protective efficacy following TB vaccination in clinical trials.[5] Thus, identifying and validating biomarkers which adhere to most of the abovementioned criteria would be essential in the development of TB treatments and vaccines.

Over the past years, many *M. tuberculosis* and human biomarkers have been studied, with current research focusing on three main areas: biomarkers that will predict treatment, efficacy, and cure of active TB; biomarkers measuring the reactivation of latent TB infection; and biomarkers involved in the induction of protective immune responses by vaccination.[19]

To date, biomarkers are measured either on a transcriptomic, metabolomic, and/or proteonomic level and can be customized to form a biosignature consisting of several biomarkers to give a better predictive value. For transcriptomic measurements, an estimated 50,000–100,000 transcripts can be potential targets, and in most cases, peripheral blood can be used for analyses. Likewise, the proteome consisting of ±1,000,000 proteins within serum or plasma can be used, while biochemicals, metabolites, and volatile compounds can be used for metabolomic analyses.[20]

Currently, a plethora of data are generated from biomarker studies, especially using microarray platforms. Several groups have provided insights in the TB disease condition by comparing gene expression profiles of TB patients versus latent TB individuals, and altered gene expression patterns have been observed in TB patients.[21,22] These profiles shows an indication of chronic immune system activation, increased activation of interferon signaling, as well as proinflammatory signaling through the Janus kinase and signal transducer and activator of transcription pathway. While a state of inflammation, which is to be expected, is reflected with these profiles, it does not reflect disease-specific patterns that are needed for differential diagnosis.[21,22] The latter observation is perfectly illustrated through similar transcriptomic patterns in diseases such as sarcoidosis, eg, which has a similar pathology but different etiology. Results from studies also seem to be inconsistent due to factors such as differences in geographic locations, lack of cohort-specific gene expression comparisons, array platforms, sample treatment, as well as analyses. This highlights once again the challenges that are associated with biomarker studies, whether for TB and/or other disease. To date, with the advances of technology, the scope of transcriptomics also include microRNA (miRNA), which are implicated in modulating signaling pathways associated with inflammation. For example, miR-155 had been identified as a potential diagnostic marker for active TB; however, its role in TB disease development still needs to be elucidated.[23] Likewise, next-generation sequencing, such as RNA sequencing, would also allow a more comprehensive screen of all types of RNA and would enable identification of point mutations and splice variant changes. Recently, Zhang et al[24] performed RNA sequencing using the Solexa platform to examine miRNA expression in the serum of patients with active disease, healthy individuals with latent TB, and those with or without prior Bacillus Calmette–Guérin (BCG) inoculation. They identified 24 miRNAs that were upregulated and six miRNAs that were downregulated in patients with active TB compared with the three groups of healthy controls. On the other hand, potential immunological biomarkers of TB are also present, which are exploited with antigen-induced immune responses (multiplex cytokine measurements) from antigen-stimulated whole blood.[9,25]

Similarly, protein biomarkers have also become a promising target for roles in drug discovery, diagnostics, and therapy monitoring due to the progress in the field of high-throughput proteomics. Here, tools such as high-throughput mass spectrometry (MS) along with advanced bioinformatics have become vital tools that allow for better detection and quantification of potential disease biomarkers as it gives information on the dynamic state of the cell, tissue, and/or organism.[20] Thus, MS profiling, statistical modeling, and bioinformatics have yielded potential serum biomarkers for TB. One such example includes earlier in vitro and in vivo studies, which had shown that *M. tuberculosis* infection of macrophages increased the release of microparticles (MPs) which in turn induced a proinflammatory response from uninfected macrophages.[26] Thus, Hare et al[27] wanted to determine how *M. tuberculosis* infection modulates the protein composition of the MPs, and whether this contribution is due to their proinflammatory characteristics compared to the proteomes of MPs derived from *M. tuberculosis*-infected and -uninfected human THP-1 monocytic cells (monocytic cell line derived from an acute monocytic leukemia patient). These MP proteins were analyzed by MS, and 68 proteins were shown to be statistically significantly abundant and seemed to be associated with immune function, lysosomal/endosomal maturation, vesicular formation, nucleosome proteins, and antigen processing, all of which seem to be potential candidates for TB infection.[27] Despite this potential, the field is much more complex than genomics and transcriptomics as the proteome consists of numerous analytes, undergoes

posttranslational modifications, and forms multimeric complexes, which brings it own challenges and issues for biomarker discovery. Furthermore, analytical challenges that also need to be considered include algorithms for protein identification, difference in spectra readings, different fragmentation methods, as well as accuracy of instruments. Furthermore, the validation of potential disease-related biomarkers in the clinical setting has also proven to be challenging as there seems to be inconsistencies between studies with regard to the biomarkers identified. This observation is not only noted in TB but in most diseases related to biomarker research.[28] Hopefully, larger sample size and standardization of sample collection/treatment protocol may improve these challenges, and this is where the importance of biobanks comes into play.

Of late, metabolic profiling has also been used to classify disease and diagnose the onset of disease using two steps, consisting of gas or liquid chromatography for separation of molecules and nuclear magnetic resonance (NMR) or MS for the detection of these molecules.[20] Metabolic profiles of molecules involved in an organism's metabolism, such as sugars, fatty acids, nucleotides, amino acids, and lipids, have successfully been used in infectious diseases such as TB. One such example is where volatile organic compounds in breath were analyzed via gas chromatography and mass spectroscopy for potential biomarkers of active pulmonary TB derived from the infectious organism (metabolites of *M. tuberculosis*) and from the infected host (products of oxidative stress). From this study, biomarkers of active pulmonary TB with 85% accuracy in symptomatic high-risk subjects had been identified.[29]

However, progress in the development and/or validation of specific TB biomarkers identified has been very slow, and the ones that have been identified thus far lack high predictive values. Another challenge is to ensure that any developments in biomarker discovery translate into diagnostic assays, which can then be implemented in the most diseased burden setting, often resource poor countries.

For certain TB biomarker studies which focuses on the establishment of long-term treatment outcome, one would require biospecimens with the associated clinical and microbiological information from patients who had adequate follow-up. This would include those patients from the point of pre-treatment throughout the 6-month therapy regime as well as the subsequent 18-24 months. However to obtain and maintain these type of samples and adequate numbers for the pursuing of these type of research requires the availability of well-characterized biobanks.[4,30]

Various biospecimens are thus required for testing to identify potential biomarkers for easy characterization of TB in patients. Furthermore, the access and the availability of high quantity and quality biospecimens are essential to studies in order to provide statistical power and be adequate to differentiate in test systems.[7] The demand for biospecimens therefore highlights the importance of biobanks in research and diagnostic testing.[31]

Overview and importance of biobanks as an infrastructure for biomarker identification

Biobanking is more than just the storage of samples and, to date, emerges as a complex science which includes ethical and legislative disciplines as well as the biological and social sciences.[32] Ethics, whether specific for TB research or other diseases, go hand in hand with long-term storage of biospecimens and are one of the foundational prerequisites for the establishment of successful human biobanking. Especially, the task of informed consent in the context of biobanking becomes difficult as future uses of research may not be known at the time of the collection of the biological samples. Therefore, alternative forms of consent such as broad or tiered consent need to be considered, which are options that balances out the progress of science but still allow for participant autonomy.[32] Interestingly, van Schalkwyk et al[33] conducted a qualitative sociological study, where they investigated the views of South African research participants in a TB research project on aspects related to biobanking ethical issues. The aim of the TB study used was to detect potential biomarkers of protective immunity in a high-risk TB population. Thus, interviews were conducted, and participant viewpoints on issues related to sample storage, sample ownership, sample reuse for other studies, community benefit, academic and institutional benefit, as well as research for commercial purposes, were examined. Overall, the outcome highlighted that the participants were in favor of having samples stored and reused for research, while there were concerns with regards to the commercial "for-profit" aspect of biospecimen usage.[33] This highlights once again that biobanks need to consider ethics an important part of their operations, whether private, research, or hospital based.

Biobanks range from smaller research-related sample collections that exist in freezers in individual laboratories within an academic institution to larger collections such as private or national biobanks or those associated with clinical trials, consortia, or initiatives. Various categories of biobanks exist depending on their purpose and design and can range

from disease-orientated biobanks, population-based biobanks to twin cohort studies.[34]

For biobanks to be efficient and reliable and to ensure that that they are compliant with quality standards, biobanks need to adopt and implement best practices and guidelines on infrastructure, biospecimen handling, recommendations on informatics and data management, and recommendations on ethical, legal, and social issues. Key biobanking-related infrastructural issues include the availability of constant power, liquid nitrogen, dry ice, as well as capable transport logistics. All of these factors need to be taken into account to ensure proper preservation and protection of valuable biospecimens.[32,35]

Sample integrity is of utmost importance as investigators have difficulty finding high-quality biopecimens with associated clinical data, as evidence shows that 30% fail quality assessment following molecular testing. Likewise, cancer researchers only obtain 39% samples of sufficient numbers, while 47% of samples were of satisfactory quality.[36] Given these statistics, one can conclude that any variables that can be introduced during processes such as biospecimen collection, processing, storage, and analysis are all sources of bias in research that can lead to distorted results. In turn, these effects due to low-quality samples cause researchers to question their findings. This highlights the fact that sample integrity especially from a biobank perspective becomes critical. This also emphasizes the vital role biobanks play to address these shortfalls by ensuring the tight application of standardized protocols, quality control, and address the effects of preanalytical and storage variation on a broad range of sample types.

A number of studies have provided evidence for the role of human genetics in susceptibility to TB. These observations come from twin studies,[37,38] observation of differences in susceptibility between ethnic groups, animal models, and segregation analysis. Numerous candidate gene and genome-wide studies have been conducted to search for genes underlying this susceptibility to TB.[37,38] However, inconsistencies have been observed across these studies and may partially be explained by the complexity of TB as a phenotype, differences across populations in both population genetic parameters as well as patterns of *M. tuberculosis* strain variation, and other cofactors such as HIV, age, sex, and other genes.[37,38] Thus, future studies are needed to examine these factors as well as the immune response underlying TB pathogenesis. This highlights once again how biobanks are imperative for TB host genomic research, as it would require larger studies in diverse populations.[39]

Therefore, biobanking of high-quality and well-annotated biospecimens in statistically relevant numbers would provide an essential resource, as this would facilitate cutting-edge scientific research on the biological causes of disease and aid in accelerating the discovery of vaccines, drugs, and diagnostics. In the setting of *M. tuberculosis* as an example, these biobanks would complement TB research as an independent source, which would aid in the identification and validation of surrogate TB biomarkers as well as the validation of new diagnostic assays.[5,32]

Overview of known research and private TB biobanks or TB biobank initatives

To date, only a small number of organized biobanks specifically associated with *M. tuberculosis* exist, as most biological material collections are stored either in hospitals or in clinical and research laboratories in conjunction with other infectious disease types.[7] For biobanks to be classified as organized, procedures and technologies for collection, annotation, processing and long-term storage of biospecimens must be established and fully compliant with quality standards and conduct procedures in accordance with international and national biobanking best practices and guidelines such as International Society for Biological and Environmental Repositories and National Cancer Institute.[7,40]

In addition, to conduct efficient research, high volumes of quality biospecimens need to be achieved in order to give statistical power for meaningful conclusions and must be sufficient to provide reference intervals for newly identified biomarkers. For example, according to the guidelines as set by the Expert Panel on Theory of Reference Values of the International Federation of Clinical Chemistry, a minimum sample size of 120 reference subjects are required for the estimation of reference intervals.[7]

A number of organizations that have nationwide or translational collections develop large consortia, and networks have increased over the years and are changing the landscape of biobanking of international collaboration and aid in research development. These include large networks and consortia such as the Public Population Project in Genomics and Society (P3G) (http://p3g.org/), the Biobanking and Biomolecular Resources Research Infrastructure (http://bbmri.eu/), as well as the H3Africa Biobank program (http://h3africa.org/) which was funded by the National Institutes of Health. Other population-based biobank models include the UK Biobank (http://www.ukbiobank.ac.uk/) as well as the Swedish National Biobank (http://bbmri.se/en/).[39]

Over the years, initiatives have been launched to facilitate biomarker discovery; however, as no ideal sample type has been identified as a reliable TB biomarker, it is advisable that many different sample types are collected.[7,31] Examples include the TB Immunology Group Research Tissue Bank in the UK whose biospecimen collection ranges from blood, sputum, urine, pleural fluid, pleural biopsies, pericardial fluid, ascitic fluid, cerebral spinal fluid, bronchoalveolar lavage specimens, transbronchial lung biopsies, open lung biopsies, renal, adrenal, liver, gastric/bowel, laryngeal, muscle, bone marrow, lymph node to skin biopsies. These samples will be used to study the physiopathology of TB.[7] Likewise, the TB Alliance or TBA established in 2000 is another initiative which aims to lead the search for new TB regimens and catalyze global efforts for new TB regimens. An example includes the REMox TB clinical trials which focus on moxifloxacin treatment (http://clinicaltrials.gov/ct2/show/NCT00864383). In addition, a biobank that collects and stores whole blood, RNA of *M. tuberculosis* strains, sputum, and urine from study participants with TB undergoing treatment in the trial has been established.

Table 1 Examples of private and research TB biobanks and initiatives

Biobank initiative	Biobank type	Objective	Sample type	Location
Grand challenge in global health of protective immunity against TB in the context of HIV/AIDS in Africa	Public–private partnership	Study correlates to immune protection for biomarkers discovery	Various	Uganda, The Gambia, Malawi, South Africa, Ethiopia
GSK TB Biobank	Public–private partnership	Predictive surrogate biomarkers	Blood, sputum	Africa and Europe
TB Immunology Research Tissue Bank	Public–private partnership	Collaborative research on TB	Fluid and tissue sample types	Africa and Europe
TBA	Public–private partnership	TB treatment predictive biomarkers	Various	South Africa and India
Catalysis Foundation for Health (TB Diagnostic Biomarker Research)	Nonprofit	TB treatment predictive biomarkers	Various	South Africa, Southeast Asia, USA
CTB2	Independent research	TB treatment predictive biomarkers	Various	South Africa, India, Southeast Asia, USA
WHO/TDR	Public	TB diagnostic biomarkers	Various	South Africa, Peru, Colombia, Vietnam, Kenya, Canada, The Gambia, Uganda, Brazil, Spain, Bangladesh, Zambia
TBRU	Public	Surrogate biomarkers of protective immune response: able to do early-stage clinical trials	Various	USA, Uganda, Brazil, Philippines, South Africa
TBTC	Public	Clinical trials	Human samples	USA, Brazil, Peru, Spain, South Africa, Uganda, Kenya, Vietnam, Hong Kong
National Reference Laboratories (eg, RIVM, the Netherlands; Swiss TPH; National TB Reference Laboratory, Borstel, Germany; Swedish National Institute of Public Health, Stockholm)	Public	Public health	*M. tuberculosis* strains, clinical isolates	South Africa-NHLS (CTB), Lesotho, Uganda
K-RITH	Independent research	Biomarkers, sequencing genomes of multidrug-resistant TB, characterizing the human immune response to TB	Various	South Africa (KwaZulu-Natal)
SUN-IRG	Independent research	Immunology of *M. tuberculosis* infection and host biomarkers	Various	South Africa (Cape Town)
SATVI	Independent research	Clinical trials	Various	South Africa (Cape Town)

Note: Adapted from Tuberculosis, 91(6), Betsou F, Parida S, Guillerm M, Infectious diseases biobanking as a catalyst towards personalized medicine: *Mycobacterium tuberculosis* paradigm, Pages 524–532, Copyright ©2015, with permission from Elsevier.[7]

Abbreviations: CTB2, Consortium for TB Biomarkers; GSK, GlaxoSmithKline; K-RITH, KwaZulu-Natal Research Institute for Tuberculosis and HIV; RIVM, National Institute for Public Health and the Environment; SATVI, South African Tuberculosis Vaccine Initiative; SUN-IRG, Stellenbosch University Immunology Research Group; TB, tuberculosis; TBA, TB Alliance; TBRU, Tuberculosis research unit; TBTC, Tuberculosis Trials Consortium; TPH, Tropical and Public Health Institute; WHO/TDR, World Health Organization Special Programme for Research and Training in Tropical Diseases; CTB, Centre for Tuberculosis; NHLS, National Health Laboratory Services.

TBA was also funded in 2010 by the US Food and Drug Administration to establish a consortium for TB biomarkers (CTB2) in association with the National Institute of Allergy and Infectious Diseases' AIDS Clinical Trials Group and the US Center for Disease Control and Prevention's TB Trials Consortium. They combine their biobanking efforts by collecting high-quality patient specimens in late-stage TB drug clinical trials, where they are linked to detailed (anonymized) clinical documentation; CTB2 aims to enable the discovery and qualification of biomarkers in order to speed up the clinical development of improved TB treatments for both drug-sensitive and multidrug-resistant TB.[7,31,41]

Similarly, the WHO/TDR (World Health Organization Special Programme for Research and Training in Tropical Diseases) also has an extensive collection of well-characterized TB biospecimens from 13 different geographic regions from untreated TB suspect patients. This includes serum, saliva, sputum, and urine samples with associated clinical and biological information from each patient. The WHO/TDR TB Specimen Bank (http:apps.who.int/tdr/svc/disease/tuberculosis/specimen-bank) launched in 2000 is one of the few TB biobanks with an open-access policy, where biospecimens are available at no cost, except for shipping and distribution fees.[7] This biobank always has and still remains instrumental in the development and evaluation of TB diagnostic tests as it has been approached by many private companies and academic researchers over the years and highlights the importance of such infrastructure in TB biomarker discovery.

Within South Africa, a number of biobanks relating to TB specifically are operating on a smaller scale and are either private or research based within an academic institution. This currently limits available information for large-scale research.

An example of a large TB research study is the South African Tuberculosis Vaccine Initiative with its associated biobank, which is a TB vaccine clinical research facility that was launched in 2001 at the University of Cape Town. To date, it is the largest dedicated TB vaccine research group on the African continent and has conducted more than 16 clinical trials over the last 14 years (http://www.satvi.uct.ac.za).[42]

Another example includes the KwaZulu-Natal Research Institute for Tuberculosis and HIV and its associated biobank, which were launched in 2009 and focuses on basic science research in TB and HIV and how to translate theses scientific findings into novel diagnostic tools to help control TB and HIV. This initiative involves collaboration with the Howard Hughes Medical Institute, the University of KwaZulu-Natal, and public sector support through the Technology Innovation Agency, the biotechnology investment arm of the South African government (http://www.k-rith.org/).

Likewise, the Stellenbosch University Immunology Research Group, a specialist Tuberculosis Immunology Group at Stellenbosch University, was established in 2002 (http://www.sun.ac.za/english/faculty/healthsciences/Molecular_Biology_Human_Genetics/immunology). This group, funded by the Bill & Melinda Gates Foundation, TBA, and European and Developing Countries Clinical Trials Partnership, focuses on the immunology of *M. tuberculosis* infection and, in particular, host biomarkers, including diagnostic markers, markers of TB treatment response, and markers of protective immunity against *M. tuberculosis*.

Other examples of private and research TB biobanks and initiatives are summarized in Table1.

Conclusion

Despite high TB treatment success rates, major efforts are needed to ensure that all TB cases are detected, notified, and treated. This is where laboratory confirmation of TB and drug resistance comes into play as this is the key to ensure that TB is correctly diagnosed. However, despite rapid progress and promising evidence over the last decade in TB vaccine development and TB biomarker research, there is still a need for a more accurate, inexpensive point-of-care TB diagnostic test that is applicable in high burden countries, where TB and HIV is endemic. The success of any new diagnostic test will depend on the ability to obtain high-quality materials in adequate numbers and further support for the vital role that TB or infectious disease biobanks can play. Identification and validation of these TB biomarkers will assist in the stratification of patients, predict risk of disease progression, facilitate rapid diagnosis and treatment of TB patients, and allow preventive measures for latently infected patients, as well as speed up clinical trials with novel drug and vaccine candidates. Thus, it is clear that further investigation is required which would combine the efforts of experimental research, clinical studies, computational biology, and biobanking in order to aid in the control of the TB pandemic.

Author contributions

CCS and RG initiated and coordinated the article writing with the input of all authors. All authors contributed to the concept and design as well as drafting of this work. All authors read and approved the final paper and declare that they have no competing interests. All authors agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

References

1. World Health Organization. *Global Tuberculosis Report 2014*. Geneva, Switzerland: World Health Organization; 2014. Available from: http://www.who.int/tb/publications/global_report/en/. Accessed February 24, 2015.
2. Lawn SD, Zumla AI. Tuberculosis. *Lancet*. 2011:378–372.
3. World Health Organization. *Multidrug and Extensively Drug-Resistant TB (M/XDR-TB): 2010 Global Report on Surveillance and Response*. Geneva: World Health Organization; 2010.
4. McNerney R, Maeurer M, Abubakar I, et al. Tuberculosis diagnostics and biomarkers: needs, challenges, recent advances, and opportunities. *J Infect Dis*. 2012;205:S147–S158.
5. Wallis R, Pai M, Menzies D, et al. Biomarkers and diagnostics for tuberculosis: progress, needs, and translation into practice. *Lancet*. 2010;375:1920–1937.
6. Russell DG, Barry CE, Flynn JL. Tuberculosis: what we don't know can, and does, hurt us. *Science*. 2010;328:852–856.
7. Betsou F, Parida S, Guillerm M. Infectious diseases biobanking as a catalyst towards personalized medicine: *Mycobacterium tuberculosis* paradigm. *Tuberculosis*. 2011;91(6):524–532.
8. Lienhardt C. From exposure to disease: the role of environmental factors in susceptibility to and development of tuberculosis. *Epidemiol Rev*. 2001;23:288–301.
9. Zumla A, Atun R, Maeurer M, et al. Viewpoint: scientific dogmas, paradoxes and mysteries of latent *Mycobacterium tuberculosis* infection. *Trop Med Int Health*. 2011;16:79–83.
10. Barry CE 3rd, Boshoff HI, Dartois V,et al. The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. *J Immunol*. 2013;190(2):669–677.
11. Cuevas LE. The urgent need for new diagnostics for symptomatic tuberculosis in children. *Indian J Pediatr*. 2011;78:449–455.
12. Storla DG, Yimer S, Bjune GA. A systemic review of delay in the diagnosis and treatment of tuberculosis. *BMC Publ Health*. 2008;8:15.
13. Cuevas L, Browning R, Bossuyt P, et al. Evaluation of tuberculosis diagnostics in children: 2 methodological issues for conducting and reporting research evaluations of tuberculosis diagnostics for itrathoracic tuberculosis. *J Infect Dis*. 2012;205(Suppl 2):S209–S215.
14. Boehme C, Nabeta P, Hillerman D, et al. Rapid molecular detection of tuberculosis and rifampin resistance. *N Engl J Med*. 2011;363:1005–1015.
15. World Health Organization (WHO). *Policy Statement: Automated Realtime Nucleic Acide Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: Xpert MTB/RIF System*. Geneva: World Health Organization; 2010.
16. Weyer K, Carai S, Nunn P. TB diagnostics-what does the world really need? *J Infect Dis*. 2011;204(Suppl 4):S1196–S1202.
17. McNerney R, Daley P. Towards a point-of care test for active tuberculosis: obstacles and opportunities. *Nat Rev Microbiol*. 2011;9:204–213.
18. Walzl G, Ronacher K, Hanekom W, Scriba TJ, Zumla A. Immunological biomarkers of tuberculosis. *Nat Rev Immunol*. 2011;11:343–354.
19. Wallis RS, Kim P, Cole S, et al. Tuberculosis biomarkers discovery: developments, needs, and challenges. *Lancet Infect Dis*. 2013;13(4):362–372. doi: 10.1016/S 1473-3099(13)70034-70033.
20. Maertzdorf J, Weiner J III, Kaufmann SHE. Enabling biomarkers for tuberculosis control. *Int J Tuberc Lung Dis*. 2012;16(9):1140–1148.
21. Maertzdorf J, Repsilber D, Parida SK, et al. Human gene expression profiles of susceptibility and resistance in tuberculosis. *Genes Immun*. 2011;12:15–22.
22. Jacobsen M, Repsilber D, Gutschmidt A, et al. Candidate biomarkers for discrimination between infection and disease caused by *Mycobacterium tuberculosis*. *J Mol Med*. 2007;85:613–621.
23. Wu J, Lu C, Diao N, et al. Analysis of microRNA expression profiling identifies miR-155 and miR-155* as potential diagnostic markers for active tuberculosis: a preliminary study. *Hum Immunol*. 2012;73:31–37.
24. Zhang H, Sun Z, Wei W, et al. Identification of serum microRNA biomarkers for tuberculosis using RNA-seq. *PLoS One*. 2014;9(2):e88909.
25. Chegou NN, Detjen AK, Thiart L, et al. Utility of host markers detected in quantiferon supernatants for the diagnosis of tuberculosis in children in a high-burden setting. *PLoS One*. 2013;8(5):e64226.
26. Walters SB, Kieckbusch J, Nagalingam G, et al. Microparticles from mycobacteria-infected macrophages promote inflammation and cellular migration. *J Immunol*. 2013;190(2):669–677.
27. Hare NJ, Chan B, Chan E, Kaufman KL, Britton WJ, Saunders BM. Microparticles released from mycobacterium tuberculosis-infected human macrophages contain increased levels of the type I interferon inducible proteins including ISG15. *Proteomics*. 2015;15(13).
28. Al-Tarawneh SK, Border MB, Dibble CF, Bencharit S. Defining salivary biomarkers using mass spectrometry-based proteomics: a systematic review. *OMICS*. 2011;15(6):353–361.
29. Phillips M, Cataneo RN, Condos R, et al. Volatile biomarkers of pulmonary tuberculosis in the breath. *Tuberculosis (Edinb)*. 2007;87(1):44–52.
30. Walzl G, Ronacher K, Djoba Siawaya J, Dockrell H. Biomarkers for TB treatment response: challenges and future strategies. *J Infect*. 2008;57:103–109.
31. Nahid P, Kim S, Evans CA. Clinical research and development of tuberculosis diagnostics: moving from silos to synergy. *J Infect Dis*. 2012;15:205(Suppl 2):S159–S168.
32. Abayomi A, Christoffels A, Grewal R, et al. Challenges of biobanking in South Africa to facilitate indigenous research in an environment burdened with human immunodeficiency virus, tuberculosis, and emerging noncommunicable disease. *Biopreserv Biobank*. 2013;11(6):347–354.
33. van Schalkwyk G, de Vries J, Moodley K. "It's for a good cause, isn't it?" – exploring views of South African TB research participants on sample storage and re-use. *BMC Med Ethics*. 2012;13:19.
34. Hewitt R. Biobanking: the foundation of personalized medicine. *Curr Opin Oncol*. 2011;23:112–119.
35. ISBER best practices for biorepositories: collection, storage, retrieval, and distribution of biological materials for research. *Biopreserv Biobank*. 2012;10(2):79–161.
36. Massett HA, Atkinson NL, Weber D, et al. Assessing the need for a standardized cancer HUman Biobank (caHUB): findings from a national survey with cancer researchers. *Natl Cancer Inst J Monogr*. 2011;2011(42):8–15.
37. Hill AV. Immunogenetics and genomics. *Lancet*. 2011;357:2037–2041.
38. Hill AV. Aspects of genetic susceptibility to human infectious diseases. *Annu Rev Genet*. 2006;40:469–486.
39. Branković I, Malogajski J, Morré S. Biobanking and translation of human genetics and genomics for infectious diseases. *Appl Translat Genomics*. 2014;3:30–35.
40. National Cancer Institute, NIH. NCI best practices for biospecimen resources [Internet]. Rockville, MA: National Cancer Institute, NIH; 2010. Available from: http://biospecimens.cancer.gov/bestpractices/2011-NCIbestpractices.pdf. Accessed June, 2013.
41. Nahid P, Saukkonen J, Mackenzie WR, et al. CDC/NIH Workshop. Tuberculosis biomarker and surrogate endpoint research roadmap. *Am J Respir Crit Care Med*. 2011;184:972–979.
42. Geldenhuys H, Veldsman A, Tameris M, et al. Analysis of time to regulatory and ethical approval of SATVI TB vaccine trials in South Africa. *SAMJ*. 2013;103(2):85–89.