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Transcriptomics in TB: the immune response and diagnosis

Akul Singhania[1], **Robert J. Wilkinson**[2,3,4], **Marc Rodrigue**[5], **Pranabashis Haldar**[6], and **Anne O'Garra**[1,7]

[1]Laboratory of Immunoregulation and Infection, The Francis Crick Institute, London NW1 1AT, United Kingdom

[2]Laboratory of Tuberculosis, The Francis Crick Institute, London NW1 1AT, United Kingdom

[3]Department of Medicine, Imperial College London, London W2 1PG, United Kingdom

[4]Wellcome Centre for Infectious Diseases Research in Africa, University of Cape Town, Observatory 7925, Republic of South Africa

[5]Medical Diagnostic Discovery Department, bioMérieux SA, 69280 Marcy l'Etoile, France

[6]Respiratory Biomedical Research Centre, Institute for Lung Health, Department of Infection Immunity and Inflammation, University of Leicester, Leicester LE3 9QP, United Kingdom

[7]National Heart and Lung Institute, Imperial College London, London W2 1PG, United Kingdom

Abstract

Blood transcriptomics in tuberculosis have revealed an IFN-inducible signature that diminished upon successful treatment, promising improved diagnostics and treatment monitoring, essential to eradicate tuberculosis. Sensitive radiography revealing lung abnormalities and blood transcriptomics have demonstrated heterogeneity in active tuberculosis patients and exposed asymptomatic latent individuals, suggesting a continuum of infection and immune states. Here, we describe the immune response to *M. tuberculosis* infection revealed using transcriptomics, and differences between clinical phenotypes of infection that may inform temporal changes in host immunity associated with evolving infection. We also review the diverse reduced blood transcriptional gene signatures that have been proposed for tuberculosis diagnosis and identification of at-risk asymptomatic individuals, and suggest novel approaches for developing such biomarkers for clinical use.

Tuberculosis (TB) remains a major health problem worldwide and is the leading cause of mortality from a single infectious agent, with 1.67 million reported deaths in 20161. The complexity of the immune response upon airborne transmission of the causative agent *Mycobacterium tuberculosis* and during progressive disease remains poorly characterised

Correspondence should be addressed to AOG (Anne.OGarra@crick.ac.uk).
ORCID IDs: 0000-0001-9845-6134 (AOG); 0000-0002-6941-3618 (AS)

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and understood2,3. Confirmation of active TB is based on the combination of symptoms and pathology (radiographically or histologically identified), as well as microbiological evidence of infection in sputum, typically by culture, which can take up to 6 weeks, and/or a nucleic acid amplification test (eg. GeneXpert® MTB/RIF assay)1,4 (Table 1). However, a sputum sample from patients can be hard to obtain, and although bronchoalveolar lavage can be used as a substitute, this is prohibitive in countries with limited resources and difficult in children4,5. Furthermore, *M. tuberculosis* can disseminate from the lung and cause disease throughout the body. Thus, alternative tests are required to improve and support the diagnosis of TB.

It is estimated that one fourth of all individuals worldwide have been infected by *M. tuberculosis*. The majority of infected individuals generate an effective immune response to possibly eliminate or control the infection and remain clinically asymptomatic, termed latent TB infection (LTBI), which is not transmissible. A small proportion of about 5–15% of latent individuals, however, go on to develop active TB disease at some stage during their lifetime1. Current diagnosis for LTBI, involves testing reactivity to mycobacterial antigens, determined by a tuberculin skin test (TST), or an *M. tuberculosis*-specific interferon-γ (IFN-γ) release assay (IGRA), which can demonstrate whether a T cell mediated immune response has been elicited in response to the infection6, but both tests have poor prognostic value. These tests cannot determine whether the infection has been cleared, whether the individual is controlling the infection or may have subclinical disease, or whether the individual will go on to develop active TB (Fig. 1). Thus, these methods incompletely capture the spectrum of infectious states observed after exposure to *M. tuberculosis* infection.

Heterogeneity of LTBI was recognised by epidemiological differences in the risk of TB between recent and remote infection7. Recent studies in HIV-TB coinfection demonstrated heterogeneity in a cohort of 35 asymptomatic LTBI individuals with HIV-1 co-infection, using combined positron emission and computed tomography (PET-CT) where they identified ten individuals with pulmonary abnormalities suggestive of subclinical active disease who were substantially more likely to progress to clinical disease8. These findings challenge the classical view that divides TB into two states -latent infection or active disease- and give promise for the identification of biomarkers predictive of progression8. Progression from LTBI to active TB disease can be clinically subtle and individuals with subclinical TB have been reported to transmit the organism to others9. Earlier identification of active TB in individuals with undiagnosed disease is needed to initiate early treatment essential to limit onward transmission. A means of screening of high-risk populations to identify people with early disease, or to identify those with latent infection at high-risk of developing TB, is essential so as to apply prophylactic therapy for preventing TB.

The dynamic relationships that exist between the proposed states of latent TB or subclinical TB, and immune factors that influence possible transition between states, are not known. Although protective factors have been described, including IL-12, IFN-γ and TNF-α, our understanding of the early phase of *M. tuberculosis* infection or progression to disease in humans is very limited 2,10–13,14,15. Risk factors responsible for a large proportion of TB cases in the general population3, include HIV-coinfection16, anti-TNF therapy15,17,

vitamin D deficiency18, protein energy malnutrition19–21, pregnancy22 and intercurrent viral infections23,24. A better understanding of the early immune response to *M. tuberculosis* infection in individuals who control the infection after recent contact, remain sublclinical or go on to develop disease, would greatly advance the development of improved diagnostics, to detect early infection and predict progression to disease.

Blood transcriptomics elucidate the host response in tuberculosis

Blood transcriptomic profiling has provided an unbiased analysis and comprehensive overview of host factors perturbed upon infection and in active TB. A whole blood transcriptional signature dominated by IFN-inducible genes was identified in active TB patients, and not present in healthy controls and the majority of individuals with LTBI25. This IFN-inducible gene signature included genes downstream of both IFN-γ and type I IFN, and was diminished upon successful treatment25,26. This transcriptomic signature has been recapitulated in several studies worldwide with independent clinical cohorts26–36, and in meta-analyses combining several of these cohorts37–40. An under-abundance of a type II IFN response in the transcriptional blood signature in TB patients, with downregulation of *IFNG*, as well as *TBX21* has also been found40.

Type I IFN has a deleterious effect in the control of TB in mouse models2,23,41–47, consistent with reports of an IFN-inducible blood transcriptional signature correlating with radiographic lung disease in human TB25 and in non-human primate models48. Varying production of type I IFN by macrophages infected with different strains of *M. tuberculosis* can result from differential activation of the pattern recognition receptors, TLR2, or TLR4 and its downstream MyD88-independent adaptor protein, TRIF49. The cytosolic DNA sensor cGAS has a central role in the detection of mycobacterial DNA50–52 or mitochondrial DNA53 released in the host cytosol, and induction of type I IFN transcription in macrophages. Although *M. tuberculosis* can induce type I IFN in macrophages by these diverse pathways, various studies24,45,54,41–44,47,55 have shown that elevated levels of type I IFN, resulting from either virulent strains of *M. tuberculosis*42,43,49, genetic deletion of type I IFN-regulatory genes such as *tpl-245* or adjuvant44,55 or viral coinfection24, are required to induce detrimental effects to the host upon *M. tuberculosis* infection (Fig. 2). Such high amounts of type I IFN could potentially result from differences in the genetic background41–43,56, the mycobacterial challenge dose or strain42,43,49 or microbiome composition57,58,2. An association between impaired type I IFN signaling and increased resistance to TB has been reported59. Patients with an inherited deficiency in the gene encoding ISG1560 are more susceptible to mycobacterial infections61, although there is some debate as to whether it is the increase in type I IFN which is responsible for the susceptibility to TB.

Various mechanisms account for the adverse effects of type I IFN in TB (reviewed in46), including inhibition of IL-1 and Prostaglandin E2 (PGE2), critical for host defence against *M. tuberculosis* infection44,54,62,63. Another mechanism which may explain the adverse effects of type I IFN on TB is through the induction of IL-10, which suppresses the production of proinflammatory cytokines required for TB control54,64 from *M. tuberculosis*-infected mice. Elevated levels of IL-10 observed in mouse models of TB and

human disease2 contribute to increased bacterial loads2,12,65,66,67. It is tempting to speculate that blockade of IFNαβR signaling, which is currently in clinical trials for autoimmunity68 could be applied to reduce high levels of type I IFN in conjunction with anti-mycobacterial drugs in the treatment of TB, especially in individuals with very severe disease and/or multi-drug resistance TB. The use of biologics as immunemodulators is supported by findings that individuals with mutations in *IL12RB or IFNG14* have been successfully treated with a combination of an anti-mycobacterial drugs and/or IFN-γ or IL-12 respectively.

In some cases, type I IFN may have a protective role against mycobacterial diseases69,70, indicating context-specificity in the pathogenesis of TB46. Low levels of type I IFN are required for the production of IL-12 and TNF54, suggesting that low amounts of type I IFN, in the context of low *M. tuberculosis* burden may be protective against TB. Conversely, high and sustained type I IFN signaling, potentially resulting from different genetic or context-specific effects, including coinfection, may contribute to TB pathogenesis, in part by induction of IL-10 and blockade of the protective factors required to control the mycobacterial infection (Fig. 2).

Blood transcriptomics reveal heterogeneity in LTBI and progression to TB

A longitudinal transcriptomic analysis in cynomolgus macaques48, recapitulating the spectrum of clinical outcomes observed in human TB71,72, reported increased transcriptional activity in innate and adaptive pathways early during infection, including an IFN signature. The blood transcriptome correlated with lung inflammation, as measured by PET-CT at early time points post-infection, and with the extent of disease48,73.

Blood transcriptomics of latent individuals co-infected with HIV who have pulmonary abnormalities suggestive of subclinical active disease, identified an over-abundance of the classical complement pathway and Fcγ receptor 1, and increased amounts of circulating immune complexes in individuals with evidence of subclinical disease8,74. The increased expression of classical complement components in TB may be in response to increased production of immune complexes at the site of disease to allow localized delivery of C1q to inhibit the precipitation of immune complexes and minimize lung damage8,74. This perturbation in the complement pathway was also observed in a cohort of 6,363 healthy adolescents that were followed for 24 months or more75. Individuals (n=44) who ultimately developed microbiologically-confirmed TB disease greater than 6 months after enrolment36 were compared to 106 matched controls who remained healthy during two years of follow up. Transcriptomic analysis of blood collected every six months until diagnosis showed a sequential modulation of immunological processes that preceded the manifestation of TB and subsequent clinical diagnosis36. Type I and II IFN signaling, and genes involved in the complement cascade were observed up to 18 months before diagnosis, while changes in other inflammatory genes were observed closer to disease manifestation36. However, one cannot rule out reinfection, which is prevalent in high TB incidence countries76–83, making it challenging to separate processes arising as a result of reactivation of infection, as opposed to those caused by reinfection. Independent reanalysis of the same dataset suggested heterogeneity of the complement and Fcγ-receptor genes at an individual level74.

Collectively, these studies74,36 suggest that there may be a state consistent with subclinical TB, consisting of a specific increase in IFN response genes and activation of the complement cascade, which can be revealed in blood, in individuals with no other signs of TB disease. Both studies restricted their analysis to the subgroup with IGRA positive (IGRA^{+}) LTBI, assuming that IGRA negative (IGRA^{-}) individuals do not have latent infection. IGRAs have an overall sensitivity of approximately 85% in microbiologically-confirmed active TB, indicating that a proportion of latent infections will be missed using this test alone84.

Although high TB incidence settings have often been referred to as “real world” TB, TB in low incidence settings remains a burden on public health, and both settings need to be addressed in order to eradicate TB. There are clear differences in the priorities, needs and goals for TB control between high TB incidence and low TB incidence settings (Table 4). High-burden, low-income settings have fragile health service frameworks with scarce resources, limited availability of either standard or advanced diagnostics, allowing onward transmission of infection that perpetuates poor TB control. Consideration of TB prevention strategies will be complicated in very high incidence settings by the high risk of re-infection. Low-burden, high-income settings have well-resourced health service frameworks and extensive access to diagnostic tools. A biomarker sampled from an easily accessible part of the body, that identifies latent infection at high risk of TB progression with greater sensitivity and specificity than IGRAs and TST, would greatly advance earlier and more rapid TB diagnosis. Biomarkers of TB risk may best be validated reliably in low incidence TB settings where the risk of re-infection is low, unless study design in high burden TB countries verifies that disease did not arise from reinfection by comparing the *M. tuberculosis* sequence from the index TB case with that of the LTBI contact who seemingly reactivates TB.

A proportion of LTBI individuals, across cohorts from London, South Africa and Leicester have been shown to cluster with active TB patients exhibiting a type I IFN inducible signature similar to that observed in active TB; such individuals were termed LTBI outliers25,40. Modular analysis of co-expressed genes representing distinct biological processes identified an over-abundance of the IFN response, complement system, myeloid and pattern recognition receptors genes in LTBI outliers, similar to that observed in active TB patients40. In addition, a reduced abundance of IFNG and TBX21 was also observed, suggesting a host response evolving towards that of active TB40. Since these LTBI outliers represented static instances of latent infection, transcriptional profiles of individuals exposed to *M. tuberculosis* infection from recent contact with active TB patients, who either remained healthy (n=31) or developed active TB disease (n=9) were evaluated over time in Leicester, a low TB incidence setting with minimal risk of reinfection. Of the contacts who remained healthy, most IGRA^{-ve} individuals showed few perturbations in their modular transcriptional signature over time after exposure. A proportion of the IGRA^{+ve} individuals reflected evidence of profiles similar to that observed in TB, although in most cases this was transient40. In contrast, for the majority of contacts who progressed to TB, a modular signature comparable to that of active TB was observed before a diagnosis was made40. The blood transcriptome thus provides a sensitive approach to characterise between-subject heterogeneity and within-subject variability following TB exposure and provide the

hypothesis of transitions in the host immune response that signal progression of *M. tuberculosis* infection40. It also appears that early events after exposure, measured as patterns of dynamic change in the transcriptional immune response, may influence the fate of infection40,48.

Host transcriptional gene signatures in the diagnosis of tuberculosis

Tackling the global burden of TB depends on the ability to identify active TB at an early stage and identify individuals with latent infection at high risk of developing TB1. Transcriptional blood profiling of the host response might more reliably inform an individual's *M.tuberculosis* infection state and provides a realistic prospect for developing clinical biomarkers that can support both the detection of early active disease and identify at-risk latently infected individuals with sufficient specificity to make large scale screening programmes more cost-effective. A key advantage of developing the blood transcriptome as a biomarker is the ease of blood testing. This is relevant for important groups in which microbiological TB diagnosis is presently constrained by poor sample acquisition capability including pulmonary TB associated with little or no sputum production, typically seen for earlier disease and prior to cavitation; extra-pulmonary TB, where microbiological diagnosis requires examination of samples from the infected tissue site using invasive procedures; paediatric TB, which is paucibacillary and minimally productive of sputum; and HIV associated TB, where pathology leading to sputum production is diminished.

Use of transcriptomics as specific diagnostics for TB rely on the ability to identify commonalities and differences in the host response observed in TB compared to that in other infections and diseases17,31,40,85–87. While TB and sarcoidosis patients show a big overlap in their blood transcriptome, sharing IFN signalling and proinflammatory pathways29,31,88, a subset of differentially regulated genes discriminated between the two pathologies, as well distinguishing TB from lung cancer and pneumonia23. There are also similarities between TB and viral infections which shared two sets of IFN-inducible genes, albeit at different enrichment levels40. While the enrichment level of the complement system and myeloid genes was greater in TB, the IFN-inducible gene set containing pattern recognition receptors and virally induced genes was higher in viral infections40. Conversely, perturbations in cell proliferation, metabolism and haematopoiesis were observed in viral infections but not in TB40.

To develop gene signatures as biomarkers for diagnostic tests for TB, it is necessary to define a small gene set for multiplex testing, with high diagnostic accuracy. Currently there is no consensus on the composition of these published diagnostic signatures. Individual studies have reported distinct sets of genes developed using standard machine learning algorithms, most with similar performance (Tables 2 and 3)35,89–94. These signatures cannot discriminate between TB and other diseases such as pneumonia95,96 and also identify acute viral infections40. This is a potential clinical problem for TB diagnosis in children and some adults, where primary TB can present with clinical and radiological features often indistinguishable from respiratory viral illness97,98. In HIV-coinfected persons, TB quite frequently presents as a rapid onset of non-specific respiratory and systemic illness. Tuberculous meningitis, where the outcome critically depends on early

intervention, requires an average of 3 health care practitioner visits before it is even suspected99. In the context of an LTBI screening programme, the prevalence of intercurrent viral illness in the screened population at the time of testing may be significant, and will present a confounder, lowering the specificity of existing gene signatures for this purpose. To circumvent this problem, a reduced 20 gene signature composed of genes perturbed in TB but not in influenza was developed based on a modular approach (Fig. 3), followed by machine-learning algorithms40. This 20-gene signature captured multiple biological pathways and was able to discriminate between TB and LTBI, albeit with marginally lower sensitivity (Table 3), but importantly did not detect influenza, an example of viral infections40, providing a proof of principle for new approaches to develop reduced signatures. This 20-gene signature was also evident in the majority of healthy individuals, weeks or even months before clinical diagnosis before they progressed to TB, after being in recent contact with TB patients recruited in Leicester, a low TB incidence setting40. This 20-gene signature was minimally enriched in most $IGRA^-$ contacts and only transiently in the $IGRA^+$ group, who did not progress to disease. Other studies75,100 have also identified reduced gene signatures in asymptomatic LTBI individuals and patients with subclinical TB who progressed to active TB (Tables 2 and 3). A 16-gene risk signature of TB in the South African adolescent cohort described above was evident up to six months before clinical presentation with disease75. This 16-gene signature inadvertently detected influenza, indicative of viral infections, with high specificity and sensitivity40. These findings have been expanded in multiple sub-Saharan African cohorts of exposed HIV-negative contacts, where a 4-gene-transcript signature was shown to identify individuals at high risk of developing TB up to two years before the onset of disease100.

Collectively findings to date suggest that there might be a trade-off between achieving a diagnostic TB signature with high sensitivity against LTBI, as well as high specificity against other diseases, and that alternative and complementary approaches, beyond machine algorithms should be considered for signature development. For example, applying a modular approach to inform gene expression changes across the global immune response, observed in TB, but not in LTBI, or other potentially confounding diseases (Fig. 3), followed by machine-learning algorithms, to select the most discriminant genes across multiple differentially expressed modules, may allow identification of a more specific reduced gene signature. Pooling such a signature, with a second characterised by high sensitivity for TB detection over LTBI and healthy controls, and applying combined yet discriminatory algorithms could then allow the development of a test to diagnose TB with greater confidence. Additional use of gene sets that detect and rule out confounding diseases, such as intercurrent viral infections, could be used to supplement these gene sets. The inclusion of the IFN-inducible genes that diminish upon successful treatment, as early as 2 weeks25,26,32,101, may provide added clinical utility for determining optimal treatment duration. The diminished blood transcriptomic signature observed during successful TB treatment could also help in monitoring the response to treatment and in the development of new drugs, since current tests for monitoring drug efficacy such as the early bactericidal assays and 2-month sputum conversion are both time-consming and lack specificity, even when sputum can be obtained101. Such diagnostic biomarkers will need to be carefully tested in a multitude of TB cohorts from distinct geographical locations and optimised for

specificity using cohorts of other infections. New molecular platforms with increasing capacity of multiplexing could be of help in facilitating the use of such tests in the clinic. Furthermore, it is anticipated that different contexts, goals and clinical applications in high-incidence, low-income countries, or low-incidence, high-income countries (Table 4), will dictate the use of a transcriptomic based diagnostic or prognostic, in addition to a tool for monitoring drug treatment.

Conclusions and future perspectives

There is still limited understanding of the complete spectrum of infectious states evident in latently infected individuals. High sensitivity radiographic imaging together with blood transcriptomic signatures have revealed the heterogeneity of latent TB in both humans and non-human primate models. However the events that determine whether an exposed individual will control the infection or go on to develop TB are unknown. It is critical to understand the host response in the lung directly following exposure to *M. tuberculosis* infection to determine how this may influence the outcome of infection. This could be achieved using transcriptomic and complementary immunological approaches in well-defined and carefully curated clinical cohorts, longitudinally profiling blood as well as lung samples (e.g. bronchoalveolar lavage) from individuals exposed to TB. This will advance our knowledge of the local host immune response involved in the control of infection or progression to disease.

Transcriptomic approaches also show promise with respect to the development of biomarkers for diagnosis and prognosis of TB, and for drug treatment monitoring. Biomarker signatures for clinical use would need to be downsized to facilitate a multiplex type test, be rapid and automated, with a turnaround time of 2-3 hours, and inexpensive, to be feasible for implementation testing in a field or bedside setting. This would facilitate effective and early treatment which is essential for the eradication of TB.

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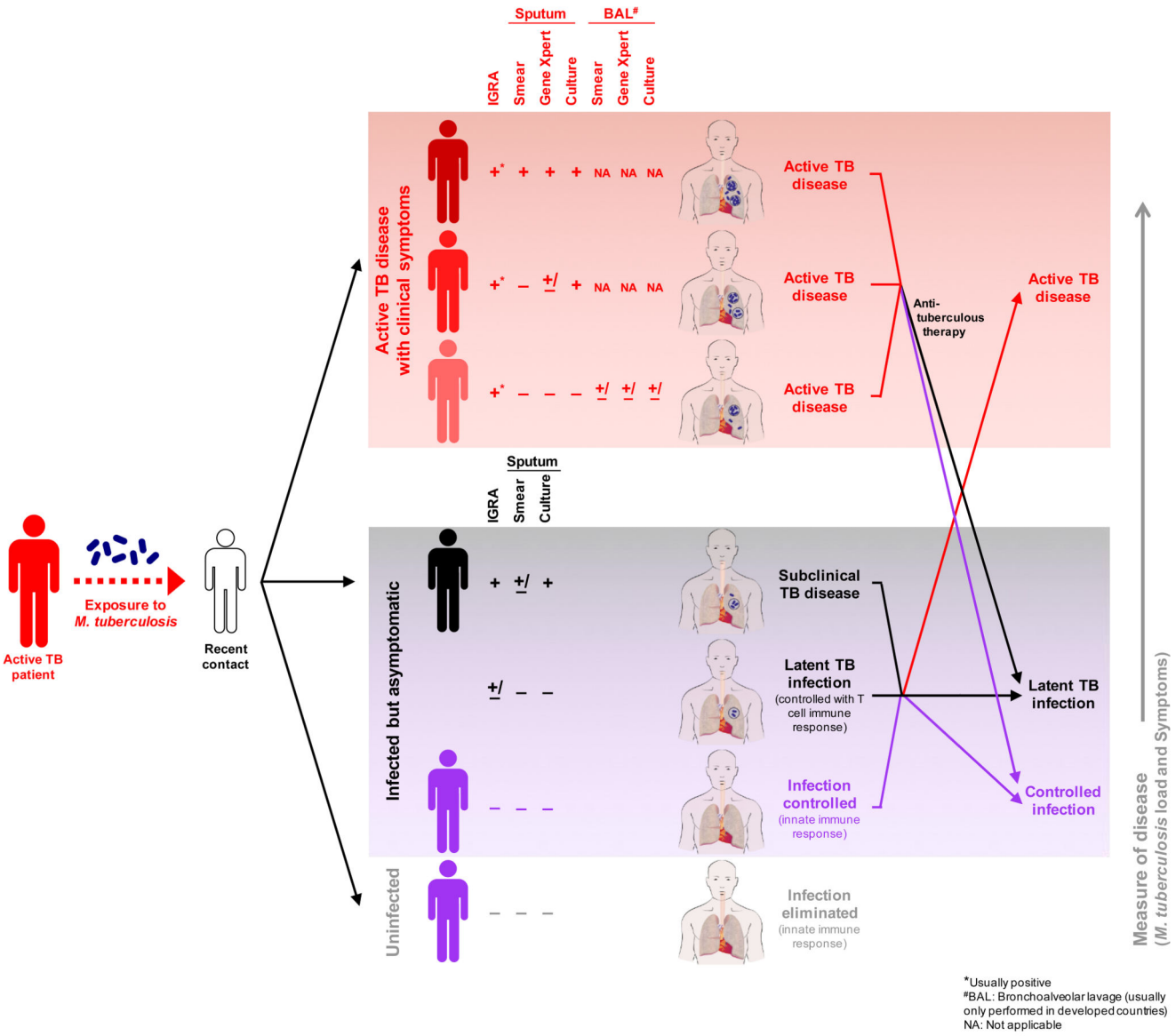

Figure 1. Heterogeneity in outcomes upon exposure to *M. tuberculosis*.

Upon contact with an active TB patient (red), an individual with recent (white) exposure to *M. tuberculosis* can manifest a range of infectious states. The majority of the exposed individuals will remain asymptomatic with the possible scenarios: remain uninfected or eliminate the bacteria (purple); become infected but control the bacteria either by innate immune responses (purple) or by *M. tuberculosis* antigen-specific T cell response as detected by the IGRA test (gradation from purple to black); develop subclinical TB and show pulmonary abnormalities by advanced radiographic approaches, and a transient blood signature (black). A small proportion of exposed individuals will progress to active TB (red) and further represent a spectrum of infection states based on the *M. tuberculosis* load as measured in sputum by a smear test (indicative of high bacterial load); *M. tuberculosis* culture or nucleic acid amplification test (GeneXpert®); or if negative in sputum, measured

in BAL, when possible (indicative of lower bacterial load) and may manifest different degrees of symptoms (different degrees of red). Adapted from Pai et al., 2016 (Ref. 3)

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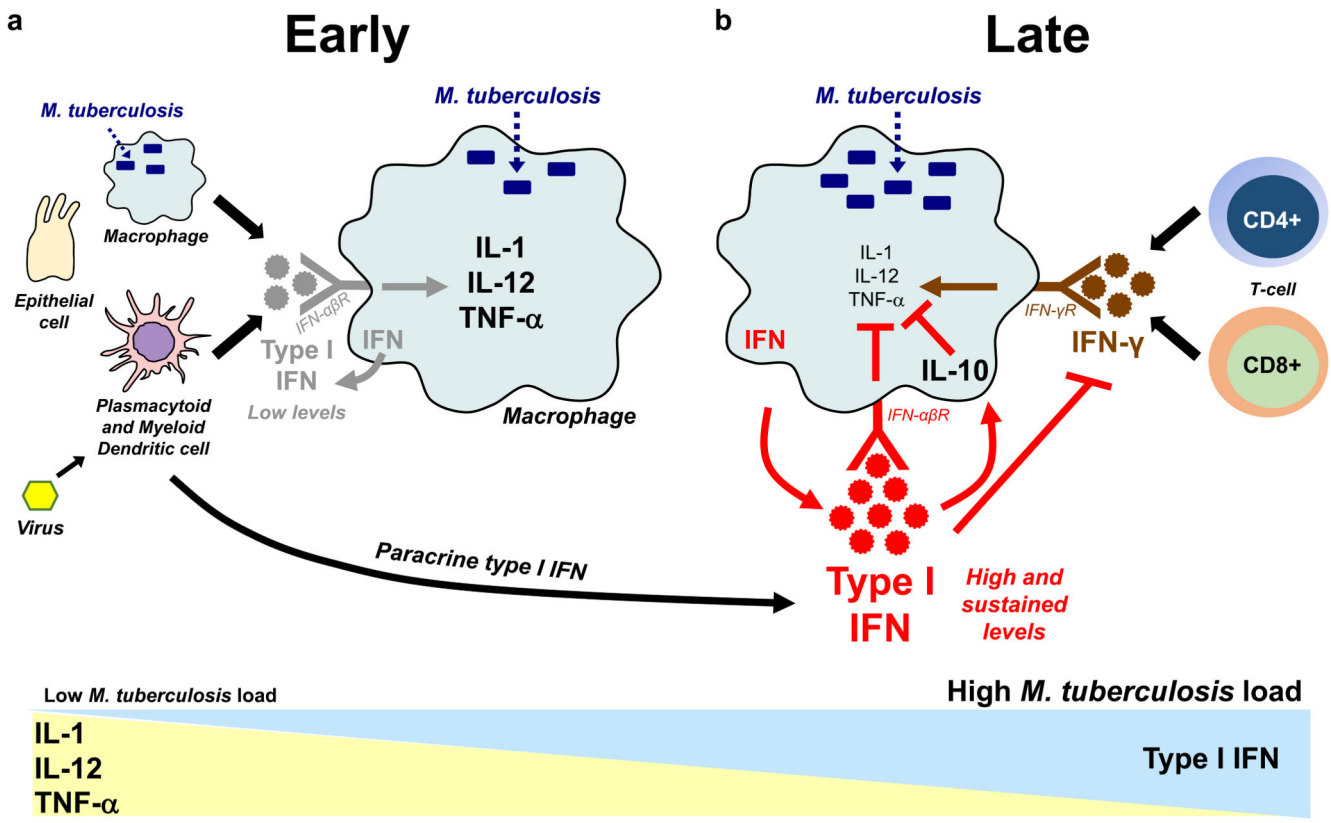

Figure 2. The immune response to *M. tuberculosis* infection.

The immune response generated in the host upon exposure to *M. tuberculosis* is complex and remains incompletely understood, with limited information about host factors that determine control versus progression. The cytokines IL-12, IL-1 and TNF, produced by innate immune cells, as well as IFN-γ produced by T cells, are protective against TB. Upon infection with *M. tuberculosis*, resident lung alveolar macrophages can become infected. **(a)** Early and low levels of type I IFN from macrophages, inflammatory monocytes and myeloid dendritic cells (DCs) and other innate immune cells at low mycobacterial loads can induce IL-1, IL-12 and TNF. **(b)** High and sustained levels of type I IFN from the macrophage and other sources (e.g. paracrine type I IFN produced by DCs upon infection with virus), can be harmful and lead to the production of the suppressive cytokine IL-10 leading to the inhibition of the production of IL-1, IL-12 and TNF by macrophages and DC, and inhibition of their activation by IFN-γ. Thus in the context of low mycobacterial loads type I IFN may be protective, whereas high mycobacterial loads and increased and sustained levels of type I IFN may result in disease progression.

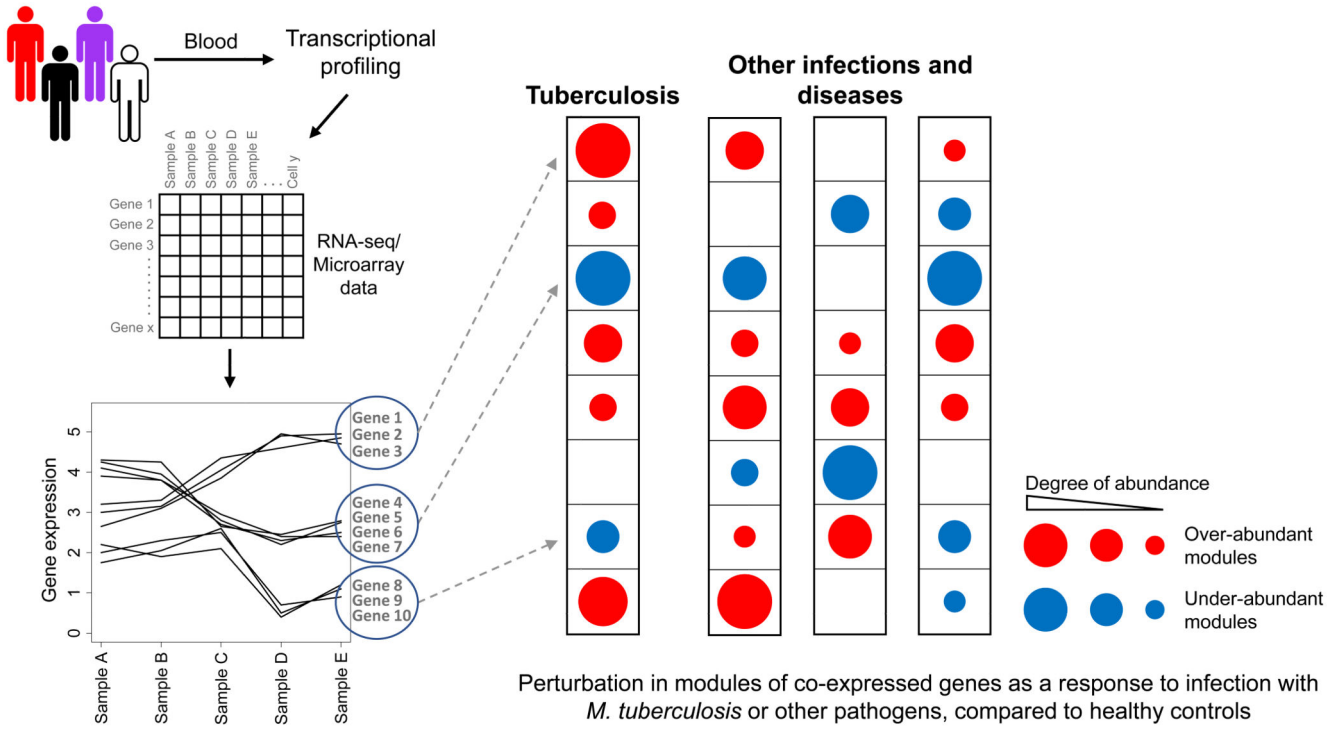

Figure 3. Modular host gene signatures in tuberculosis and in other infections and diseases.

Modular approaches can be utilized to tease out subtle differences between TB and other diseases and infections, by profiling blood from patients using transcriptomics approaches, such as RNA-sequencing, to capture the entire transcriptome. Each gene within the transcriptome is expressed at a particular level across each individual sample, and genes involved in similar biological pathways are co-ordinately expressed. These groups of co-ordinately expressed genes constitute individual modules that represent discrete biological pathways and can be identified using unbiased approaches such as weighted gene co-expression network analysis (WGCNA). Perturbation as a response to infection with *M. tuberculosis* or other pathogens, can be measured within each module of co-expressed genes, compared to healthy controls. Using such an approach, modular signatures can be identified for TB and other infections and diseases, to inform on the immune response, and this information can also be utilized to develop reduced gene signatures that are more specific to TB to develop biomarkers for diagnosis.

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Table 1
Diagnostics for TB currently in clinical use

Type of measurement	Objective	Tests available	Sample type	Measure	Advantages	Disadvantages
Detect presence of bacteria	To confirm active tuberculosis	Smear microscopy	Expectorated sputum Bronchoalveolar lavage (in developed countries)	Presence of mycobacteria	Simple, rapid and inexpensive Highly indicative in high tuberculosis incidence areas Allows identification of highly infectious patients	Operator dependent and labour intensive Poor sensitivity Difficult in extra-pulmonary, pediatric, and HIV co-infected tuberculosis Cannot distinguish viable from nonviable organisms
		Bacterial culture	Expectorated sputum Bronchoalveolar lavage (in developed countries)	Confirmation of *M. tuberculosis* Evaluation of drug sensitivity	High sensitivity and specificity Enables determination of phenotypic and genotypic drug sensitivity	Culture not successful in all cases (70% in pulmonary TB and <50% in all forms of extra-pulmonary TB) Results can take up to 6 weeks or more
		Nucleic acid amplification tests (eg. GeneXpert® MTB/RIF assay)	Expectorated sputum Bronchoalveolar lavage (in developed countries)	Direct detection of *M. tuberculosis* Evaluation of certain drug sensitivities	High sensitivity and specificity Rapid turnaround time (~2 hours)	Requires sputum that can be hard to obtain from 30% of adults and most children Expensive for resource-poor settings Cannot distinguish viable from nonviable organisms
Detect host response to infection	To confirm history of *M. tuberculosis* infection	Tuberculin skin test (TST)	Skin sensitization	Memory response to mycobacterial antigens	Relatively simple test Cheap	Cannot distinguish active from latent disease Cannot distinguish remote from recent infection Cannot distinguish from other mycobacteria or BCG Operator dependent and subjective assessment of induration size

Type of measurement	Objective	Tests available	Sample type	Measure	Advantages	Disadvantages
		Interferon gamma release assay (IGRA)	Blood	Memory response to *M. tuberculosis* antigen	Specific for *M. tuberculosis*	Cannot distinguish active from latent disease Cannot distinguish remote from recent infection Expensive Can be practically challenging

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Table 2

Blood transcriptional reduced gene signatures proposed for TB diagnosis

Gene List from 8 published studies**	Frequency of gene in each proposed published** signature	Singhania et al. 2018**	Suliman et al. 2018**	Zak et al. 2016**	Maertzdorf et al. 2016**	Roe et al. 2016**	Sweeney et al. 2016**	Kaforou et al. 2013** (TB vs. LTBI)	Kaforou et al. 2013** (TB vs. Other Diseases)
DUSP3	3	-	-	-	-	-	DUSP3	DUSP3	DUSP3
FCGR1A	3	-	-	FCGR1A	FCGR1A	-	-	FCGR1A	-
GBP5	3	-	-	GBP5	GBP5	-	GBP5	-	-
SEPT4	3	-	SEPT4	SEPT4	-	-	-	-	SEPT4
ANKRD22	2	-	-	ANKRD22	-	-	-	ANKRD22	-
BATF2	2	-	-	BATF2	-	BATF2	-	-	-
FCGR1B	2	-	-	FCGR1B	-	-	-	FCGR1B[#]	-
FCGR1C	2	-	-	-	FCGR1C	-	-	FCGR1C	-
GAS6	2	-	GAS6	-	-	-	-	GAS6[#]	-
GBP1	2	-	-	GBP1	GBP1	-	-	-	-
GBP6	2	-	-	-	-	-	-	GBP6	GBP6
LHFPL2	2	-	-	-	-	-	-	LHFPL2	LHFPL2
S100A8	2	-	-	-	S100A8	-	-	S100A8	-
SCARF1	2	SCARF1	-	SCARF1	-	-	-	-	-
SERPING1	2	-	-	SERPING1	-	-	-	-	SERPING1
AAK1	1	-	-	-	-	-	-	-	AAK1
ALDH1A1	1	-	-	-	-	-	-	-	ALDH1A1[#]
APOL1	1	-	-	APOL1	-	-	-	-	-
APOL4	1	APOL4	-	-	-	-	-	-	-
ARG1	1	-	-	-	-	-	-	-	ARG1
ARHGEF9	1	ARHGEF9	-	-	-	-	-	-	-
ARNTL2	1	ARNTL2	-	-	-	-	-	-	-
BACH2	1	BACH2	-	-	-	-	-	-	-
BDH1	1	BDH1	-	-	-	-	-	-	-
BLK	1	-	BLK	-	-	-	-	-	-
BTN3A1	1	-	-	-	-	-	-	-	BTN3A1
C19ORF12	1	-	-	-	-	-	-	-	C19ORF12
C1QB	1	-	-	-	-	-	-	C1QB	-
C1QC	1	-	-	-	-	-	-	C1QC	-
C4ORF18	1	-	-	-	-	-	-	C4ORF18	-
C5	1	-	-	-	-	-	-	C5	-
CALML4	1	-	-	-	-	-	-	-	CALML4
CASC1	1	-	-	-	-	-	-	-	CASC1

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CCDC120	1	CCDC120	-	-	-	-	-	-	-
CCR6	1	-	-	-	-	-	-	CCR6	-
CD177	1	-	-	-	-	CD177	-	-	-
CD1C	1	-	CD1C	-	-	-	-	-	-
CD274	1	-	-	-	CD274	-	-	-	-
CD74	1	-	-	-	-	-	-	-	CD74
CD79A	1	-	-	-	-	-	-	CD79A	-
CD79B	1	-	-	-	-	-	-	CD79B	-
CD96	1	-	-	-	CD96	-	-	-	-
CERKL	1	-	-	-	-	-	-	-	CERKL
CLC	1	-	-	-	-	CLC	-	-	-
CNIH4	1	-	-	-	CNIH4	-	-	-	-
COL4A4	1	COL4A4	-	-	-	-	-	-	-
CREB5	1	-	-	-	-	-	-	-	CREB5
CTSB	1	CTSB	-	-	-	-	-	-	-
CXCR5	1	-	-	-	-	-	-	CXCR5	-
CYB561	1	-	-	-	-	-	-	-	CYB561[#]
DHRS9	1	-	-	-	DHRS9	-	-	-	-
EBF1	1	-	-	-	-	-	-	-	EBF1
ETV7	1	-	-	ETV7	-	-	-	-	-
FAM20A	1	-	-	-	-	-	-	FAM20A	-
FAM26F	1	-	-	-	FAM26F	-	-	-	-
FBXL5	1	-	-	-	FBXL5	-	-	-	-
FLVCR2	1	-	-	-	-	-	-	FLVCR2	-
GBP2	1	-	-	GBP2	-	-	-	-	-
GBP4	1	-	-	GBP4	-	-	-	-	-
GJA9	1	-	-	-	-	-	-	-	GJA9
GNG7	1	-	-	-	-	-	-	GNG7	-
HLA-DPB1	1	-	-	-	-	-	-	-	HLA-DPB1
HM13	1	-	-	-	-	-	-	-	HM13[#]
HP	1	-	-	-	-	HP	-	-	-
HS.131087	1	-	-	-	-	-	-	-	HS.131087
HS.162734	1	-	-	-	-	-	-	-	HS.162734
ICAM1	1	ICAM1	-	-	-	-	-	-	-
ID3	1	-	-	-	ID3	-	-	-	-
IFITM3	1	-	-	-	IFITM3	-	-	-	-

Gene List from 8 published studies**	Frequency of gene in each proposed published** signature	Singhania et al. 2018**	Suliman et al. 2018**	Zak et al. 2016**	Maertzdorf et al. 2016**	Roe et al. 2016**	Sweeney et al. 2016**	Kaforou et al. 2013** (TB vs. LTBI)	Kaforou et al. 2013** (TB vs. Other Diseases)
IGJ	1	-	-	-	-	IGJ	-	-	-
IMPA2	1	-	-	-	-	-	-	-	IMPA2
KCNC4	1	KCNC4	-	-	-	-	-	-	-
KLF2	1	-	-	-	-	-	KLF2	-	-
LIMK1	1	LIMK1	-	-	-	-	-	-	-
LOC100133800	1	-	-	-	-	-	-	-	LOC100133800
LOC196752	1	-	-	-	-	-	-	-	LOC196752
LOC389386	1	-	-	-	-	-	-	-	LOC389386
LOC728744	1	-	-	-	-	-	-	LOC728744	-
MAK	1	-	-	-	-	-	-	-	MAK
MAP7	1	-	-	-	-	-	-	-	MAP7[#]
MIR1974	1	-	-	-	-	-	-	-	MIR1974
MPO	1	-	-	-	-	-	-	MPO	-
ORM1	1	-	-	-	-	-	-	-	ORM1
P2RY14	1	-	-	-	P2RY14	-	-	-	-
PAIP2B	1	PAIP2B	-	-	-	-	-	-	-
PCNXL2	1	-	-	-	PCNXL2	-	-	-	-
PDK4	1	-	-	-	-	-	-	-	PDK4
PGA5	1	-	-	-	-	-	-	-	PGA5
PPPDE2	1	-	-	-	-	-	-	-	PPPDE2
PRDM1	1	-	-	-	-	-	-	-	PRDM1
RBM12B	1	-	-	-	-	-	-	-	RBM12B
RNF19A	1	-	-	-	-	-	-	-	RNF19A
RP5-1022P6.2	1	-	-	-	-	-	-	-	RP5-1022P6.2
SMARCD3	1	-	-	-	-	-	-	SMARCD3	-
SMYD5	1	SMYD5	-	-	-	-	-	-	-
SPHK1	1	SPHK1	-	-	-	-	-	-	-
STAT1	1	-	-	STAT1	-	-	-	-	-
TAP1	1	-	-	TAP1	-	-	-	-	-
TMCC1	1	-	-	-	-	-	-	-	TMCC1
TMEM25	1	TMEM25	-	-	-	-	-	-	-
TRAF4	1	TRAF4	-	-	-	-	-	-	-
TRAFD1	1	-	-	TRAFD1	-	-	-	-	-
TRIM47	1	TRIM47	-	-	-	-	-	-	-
UGP2	1	-	-	-	-	-	-	-	UGP2
USP54	1	USP54	-	-	-	-	-	-	-

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VAMP5	1	-	-	-	-	-	-	VAMP5	-
VEGFB	1	VEGFB	-	-	-	-	-	-	-
VPREB3	1	-	-	-	-	-	-	-	VPREB3
ZNF296	1	-	-	-	-	-	-	ZNF296	-

Abbreviations: TB, tuberculosis; LTBI, latent TB infection

[#] the gene appears twice in the signature

Table 3

Accuracy of proposed blood transcriptional reduced gene signatures in diagnosing adult TB

Study	Type of signature	Number of genes	Classification	Accuracy
Singhania et al. 2018	TB vs. LTBI/Other diseases	20	TB vs. LTBI	AUC 0.92-1
			TB vs. Other diseases	AUC 0.74-0.79
Suliman et al. 2018	Risk of TB progression	4	Risk of TB progression within a year of TB diagnosis	AUC 0.66
Zak et al. 2016	Risk of TB progression	16	Risk of TB progression in the 12 months preceding TB diagnosis	AUC 0.779; Sensitivity 66.1%, Specificity 80.6%
Maertzdorf et al. 2016	TB vs. Healthy individuals	4, 15	TB vs. Healthy individuals	AUC 0.98
Roe et al. 2016	TB vs. Healthy individuals/Other febrile infections	5	TB vs. Healthy individuals and other febrile infections	AUC 0.951
Sweeney et al. 2016	TB vs. LTBI/Healthy individuals/Other diseases	3	TB vs. LTBI	AUC 0.88
			TB vs. Healthy individuals	AUC 0.9
			TB vs. Other diseases	AUC 0.84
Kaforou et al. 2013	TB vs. LTBI	27	TB vs. LTBI	Sensitivity 95%, Specificity 90%
	TB vs. Other diseases	44	TB vs. Other diseases	Sensitivity 93%, Specificity 88%

Abbreviations: TB, tuberculosis; LTBI, latent TB infection; AUC, area under the curve

Table 4

TB in high and low incidence settings

	Setting	
	High incidence, low income country	**Low incidence, high income country**
Context	Paucity of healthcare resources and infrastructure. Requirement for automated, point of care tests to support investigation and TB management	Extensive access to diagnostic tools within a well organised healthcare framework
Goals	To reduce onward transmission of infection by early identification of active TB	Progress toward TB elimination through TB prevention programmes and early identification of active TB
Clinical applications	TB diagnostic used alone or in conjunction with sputum microbiology for pulmonary TB (samples and resource permitting) to inform early initiation of TB treatment Screening tool in active case finding programmes to identify individuals with possible active TB for treatment or further investigation	TB diagnostic for supporting diagnosis of difficult cases As a screening tool to identify individuals with latent TB infection at significant risk of developing TB Screening tool for active case finding programmes in underserved populations
	Test requirements	
	Key features	**Comments**
All	Sampling from easily accessible site Point of care or rapid inexpensive laboratory-based hardware, with automation	Blood offers a readily accessible, minimally invasive tissue compartment for universal sampling Automated platforms supporting rapid detection of specified reduced gene signatures are in development
TB diagnostic	High specificity to avoid inappropriate TB diagnosis	A highly specific transcriptional signature that effectively discriminates from confounding illnesses may have lower sensitivity that risks missing TB. This can be overcome by use as a follow-on test after ruling in the possibility of TB with a highly sensitive transcriptional signature developed for active case finding A biomarker that comprises a combination of gene sets and algorithms in a multiplex assay to achieve high sensitivity and high specificity in one test
Screening in active case finding	High sensitivity to avoid missing early cases of active TB	A highly sensitive test may not be sufficiently specific to discriminate from confounding illness but can effectively rule out TB in screening programmes A biomarker that comprises a combination of gene sets and algorithms in a multiplex assay to achieve high sensitivity and high specificity in one test
Screening in latently infected populations	High specificity to improve cost-effectiveness of targeted chemopreventative therapy	Transcriptional signatures with a higher specificity than TST or IGRAs for identifying individuals at risk of TB progression may be insufficiently sensitive to identify latent infection. In this context, they may be developed for use in two-step screening programmes after TST or IGRA