The evolving research agenda for paediatric tuberculosis infection

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Following exposure to tuberculosis and subsequent infection, children often progress to tuberculosis disease more rapidly than adults. And yet the natural history of tuberculosis in children, as a continuum from exposure to infection and then to disease, is poorly understood. Children are rarely diagnosed with tuberculosis infection in routine care in international settings and few receive tuberculosis infection treatment. In this Personal View, we review the most up-to-date knowledge in three areas of childhood tuberculosis infection—namely, pathophysiology, diagnosis, and treatment. We then outline what is missing in each of these three areas to generate a priority research agenda. Finally, we suggest potential study designs that might answer these questions. Understanding of pathophysiology could be improved through animal models, laboratory studies assessing the immunological responses of blood or respiratory samples to *Mycobacterium* spp in vitro, as well as investigating immune responses in children exposed to tuberculosis. Identification of children with sub-clinical disease and at high risk of progression to clinically overt disease, would allow treatment to be targeted at those most likely to benefit. Optimisation and discovery of novel treatments for tuberculosis infection in children should account for mechanisms of action of tuberculosis drugs, as well as child-specific factors including pharmacokinetics and appropriate formulations. To conduct these studies, a change in mindset is required, with a recognition that the diagnosis and treatment of tuberculosis infection in children is a necessary component in addressing the overall tuberculosis epidemic. Collaboration between stakeholders will be required and funding will need to increase, both for research and implementation. The consequences of inaction, however, will lead to further decades of children suffering from what should increasingly be recognised as a preventable disease.

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Background

The conventional conceptual framework of tuberculosis pathogenesis in children is linear and unidirectional, with two distinct dichotomous clinical states: tuberculosis infection and tuberculosis disease. Children are exposed to *Mycobacterium tuberculosis* via aerosol droplets. Some exposed children develop tuberculosis infection, defined as immunological evidence of sensitisation to *M tuberculosis*, and some of these children progress to tuberculosis disease, defined as the presence of symptoms and signs of tuberculosis, usually together with radiological or microbiological evidence of disease. Young children are at particularly high risk of progressing from infection to disease, and are also more likely than adults to develop severe, debilitating forms of disease, such as tuberculous meningitis.[1] Worldwide, nearly 70 million children have tuberculosis infection,[2] and each year about 1 million children progress to tuberculosis disease.[3] A quarter of these children die, representing one of the top ten causes of mortality in children younger than 5 years.[4]

To address this high mortality, we must identify and treat children with tuberculosis infection in addition to children with tuberculosis disease.[5] WHO, along with almost every national tuberculosis guideline, advocates the provision of isoniazid preventive therapy to young children, following exposure to infectious tuberculosis.[6] Despite these recommendations, tuberculosis infection treatment is rarely implemented in high tuberculosis-burden settings.[7]

Failures in implementation of preventive therapy can result from the perception that many well children require treatment to prevent each case, and that treatment duration is long.[8,9] We hope that biomarkers will be discovered that can predict progression to tuberculosis disease. We also anticipate that research will lead to improvements in the management of tuberculosis infection in children. A better framework of feasible and achievable research in paediatric tuberculosis infection would provide direction to researchers and funders and allow for the rational development of a research agenda. Between Sept 27–28, 2017, 165 global participants attended a 2-day workshop in Dubai with the aim of arriving at consensus on the priorities required to better understand, diagnose, and treat tuberculosis infection. The meeting was supported by the Division of AIDS, National Institute of Allergy and Infectious Diseases and hosted at the Harvard Medical School Center for Global Health Delivery–Dubai. One session focused on children, and presentations were given with subsequent discussion from all attending participants. Themes were then taken forward amongst several paediatric investigators after the meeting, and over the course of multiple conference calls and cycles of written feedback consensus was arrived at for research priorities. In this Personal View we review the most up-to-date knowledge in three areas of childhood tuberculosis infection—namely, pathophysiology, diagnosis, and treatment. We then outline what is missing in each of these three areas to generate a priority research agenda. Finally, we suggest potential study designs that might answer these questions.

Pathophysiology

An improved understanding of the natural history, pathophysiology, and immune responses associated with

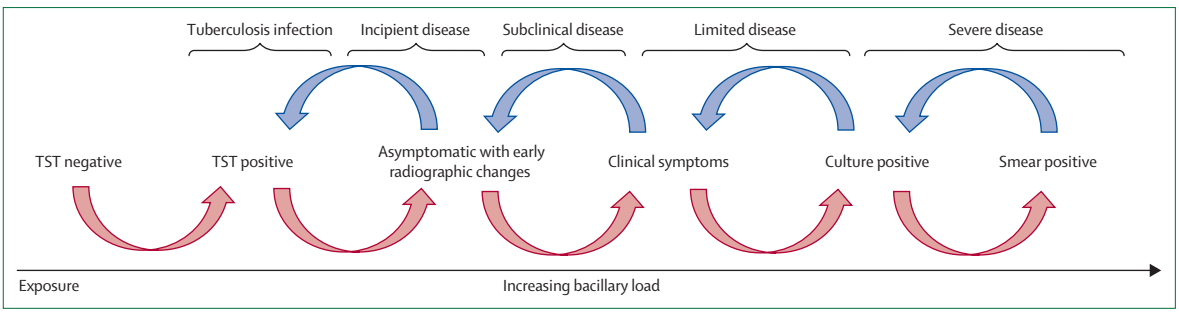

Figure 1: **The dynamic spectrum from *M tuberculosis* exposure to tuberculosis disease in children**
TST=tuberculin skin test. In some children, severe disease can be caused by a low bacillary load that has disseminated widely, such as in miliary tuberculosis or tuberculosis meningitis.

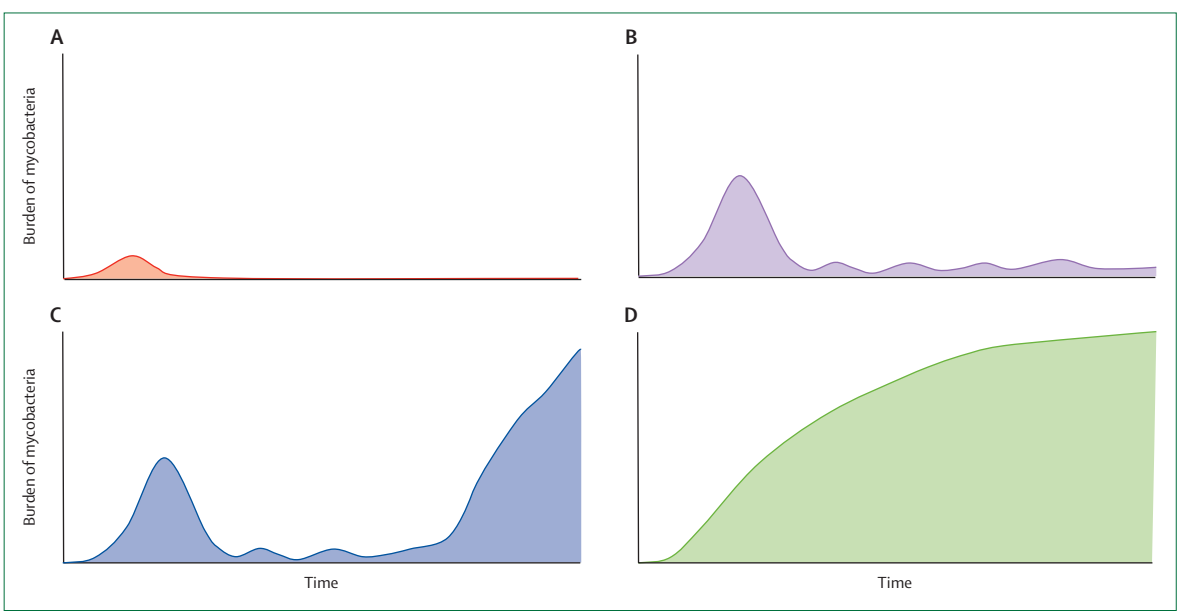

Figure 2: **Four of many potential responses to tuberculosis infection**
TST=tuberculin skin test. IGRA=interferon gamma release assay. (A) Following exposure, organisms are cleared by physical mechanism or by the innate immune system without sensitising the adaptive immune system. This child will be TST or IGRA negative. (B) Following exposure, the organisms breech the physical and innate defenses with local proliferation. The organism is then contained but not cleared by the adaptive immune system with subsequent cycles of proliferation and containment. This child will be TST/IGRA positive but does not have tuberculosis disease. (C) Following exposure, the organisms breech the physical and innate defenses with local proliferation. The organism is then contained but not cleared by the adaptive immune system. At a future time, the organism proliferates without containment and disease results. (D) Following exposure, the organisms breech the physical and innate defenses and then proliferate without effective containment by the adaptive immune system. Rapid progression to tuberculosis disease follows.

paediatric tuberculosis would enhance our ability to identify those children most likely to benefit from treatment of infection. It is increasingly clear that the concept of latent infection versus active disease is too simplistic, given the dynamic spectrum from exposure to disease, with children potentially moving in both directions (figure 1).[10,11] The concept of sub-clinical and incipient disease has gained prominence in recent years referring to early disease in asymptomatic people, which is either evident radiologically (sub-clinical) or not (incipient).[12,13] Although most investigation into these early clinical states has been in adults, this concept in not novel in children.[14,15] Children progress more rapidly than adults from exposure to infection to clinically apparent disease, and therefore represent a unique cohort in which to study the pathophysiology of sub-clinical disease and associated host responses. In the interest of clarity, we will use the term sub-clinical to refer to both sub-clinical and incipient states.

The distinction between sub-clinical and clinically apparent disease is likely to be a quantitative difference in bacterial load, rather than a change in the replication state of the mycobacteria.[16] This distinction has implications for novel diagnostics, because the host immunological and metabolic responses might be measurable in sub-clinical disease and similar to clinically apparent disease. One research priority is to identify children with sub-clinical disease who are thought to be at highest risk of

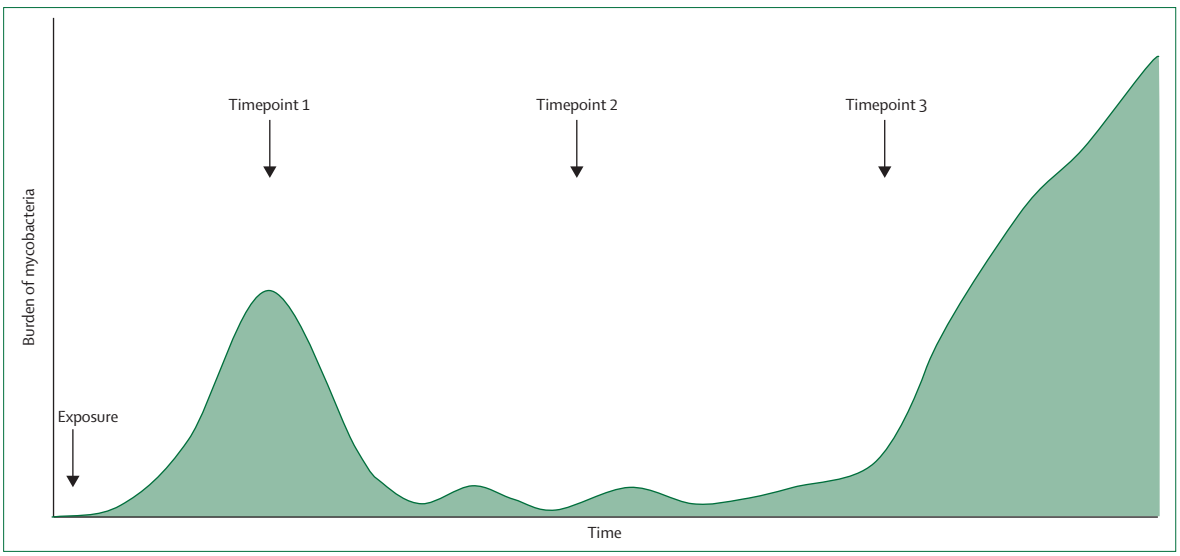

Figure 3: **The natural history following exposure to an infectious tuberculosis case**
A child shown is sampled at three timepoints after exposure. At all points the child is clinically well. However, the stage of tuberculosis infection natural history would be unclear, and the immune responses may be very different between the different time-points. This is a simplified representation and in a high tuberculosis burden setting this situation could be complicated by re-exposure and re-infection.

developing clinically apparent disease in the future, which has been undertaken in adolescent and adults but not in younger children.[17]

Following exposure *to M tuberculosis*, several outcomes are possible.[18,19] Organisms can be cleared by immune responses, progress to clinically apparent disease, or reach an equilibrium in which the organism is not cleared, but is contained by the host in a dynamic state that may fluctuate over time. The pathogen might be cleared later, or it might overcome the host defenses and clinical symptoms and signs of disease develop (figure 2). In high tuberculosis-burden settings, the force of infection is so great that re-infection is also common, following either clearance or containment of the organism.[20] In the absence of large-scale longitudinal studies, including repeated sampling of the same individuals, it is therefore difficult to ascertain whether progression to disease occurs following failure of restriction of the initial infection, or from a new exposure. Household contact studies of exposed children of all age groups, with longitudinal follow up and sampling, provide a unique opportunity to define these phenotypes.[21]

Understanding of risk factors for progression to disease following exposure to *M tuberculosis* in children is limited, with few studies exploring this from epidemiological, clinical, or immunological perspectives.[22] Host responses clearly vary with age,[23] and research to better understand the natural history of childhood tuberculosis, accounting for age and exposure, is crucial. Laboratory assays using blood from children of various age groups and stimulated with live mycobacteria or mycobacterial antigens in vitro could provide insight into pathways that are activated or suppressed.[24,25] Animal studies could be used to a greater extent, as few studies have investigated the effect of age on immune response in juvenile animal models. Mouse, guinea pig, rabbit, and non-human primate models could all be explored.

Much of the human interaction with *M tuberculosis* occurs first at a mucosal and then a lymphoid level. Although studies in adults have demonstrated differences in immune responses in lung, pericardial, and mucosal tissue compared with blood, and have highlighted the crucial role of resident innate cells including alveolar macrophages, mucosal associated invariant T (MAIT) cells, Th17 cells, and IL17-producing γδ T cells,[26–36] these studies have not been conducted in children due to ethical and physical challenges of sample collection. Novel in-vitro approaches, including human lung explant studies and alveolar macrophage cultures could be undertaken by paediatric researchers.[27,37] Another challenge is to determine the time of exposure. The child might have been recently exposed, exposed some time ago to the identified source case, or, in a high-burden setting, exposed to other infectious cases in the past. Trying to make sense of the immunological response when the time since exposure is unclear can be challenging (figure 3). The most efficient way of exploring these responses would be to embed basic science studies within cohorts of children who are being recruited to other studies, such as epidemiological studies of household contacts or clinical trials, where the recruitment entry point is a defined exposure. Paediatric treatment trials including SHINE (ISRCTN63579542), TB-CHAMP (ISRCTN92634082), and SURE have already adopted this model, collecting samples for adjunctive biomarker assays.

Diagnosis of infection

The tuberculin skin test (TST) and interferon-gamma release assay (IGRA) assess whether a child mounts an

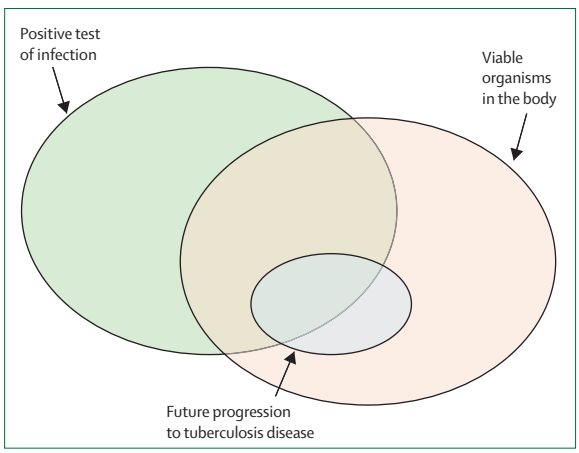

Figure 4: **Conceptual framework for understanding the relationship between true tuberculosis infection, a positive test of infection, and a child at risk of future disease progression**

acquired cellular immune response to *M tuberculosis*.[38,39] However, these tests do not indicate whether a persistent or progressive infection is present, or if the infection has been cleared.[40,41] Moreover, in children with confirmed tuberculosis disease, up to 30% have negative tests,[42] and the sensitivity of these tests is especially poor in young children living in high tuberculosis-burden settings.[43] Moreover, while children with a positive test result are twice as likely to develop disease than those with a negative test, the vast majority of children with a positive test do not develop disease (figure 4).[41] Nonetheless, epidemiological and clinical data suggest that young children with TST or IGRA conversion have increased risk of disease progression, supporting use of these tests to inform decision making around tuberculosis infection treatment. Because the specificity of IGRAs is greater than TST in younger BCG-vaccinated children,[44–47] research is warranted into decreasing the requisite blood volume for, and improving the technical test performance of, IGRA in young children, as well as developing a T cell diagnostic platform that could be used at the point-of-care for tuberculosis infection with small quantities of blood.

In regions of the world where the caseload of tuberculosis disease and limited resources makes contact management and delivery of tuberculosis infection challenging, a test that could identify sub-clinical disease (figure 1) would be useful to target tuberculosis infection treatment to those most likely to benefit from it. A recent study in South African infants demonstrated that conversion from negative to very high interferon gamma levels (>4·00 IU/mL) in otherwise asymptomatic infants was associated with a 40 times risk of disease progression compared with non-converters.[48] These results suggest that high IGRA values may be a marker of sub-clinical disease in infants. Although not assessed in young children, a study of 6000 IGRA-positive South African adolescents has identified a 16-gene transcriptomic signature that defined a short-term risk of progression to tuberculosis disease.[17] This signature is being taken forward in a clinical trial to validate decision making around tuberculosis infection treatment in adults (NCT02735590).

The study of candidate biomarkers of sub-clinical disease has the potential to reduce morbidity and mortality from tuberculosis in young children. However, designing studies that could lead to tests able to discriminate between children who ultimately will develop disease and those that will not is challenging. Because most children with tuberculosis infection will not progress to disease, studies need to be large. Also, the standard of care for young children (<5 years) and for children with immunosuppressive conditions is to provide tuberculosis infection treatment.[6] Therefore, observation of children without treatment to identify biomarkers is ethically unacceptable. More recent guidance also suggests to consider treating children 5 years and older with evidence of tuberculosis infection.[49] One option would be to observe children with drug-resistant tuberculosis infection, as most guidelines advocate close observation without treatment. Study of children treated for tuberculosis infection may also be informative, to determine if biomarker changes signify mycobacterial clearance, potentially of use in the evaluation of novel regimens, or treatment shortening trials. Finally, translating the complex laboratory procedures necessary to measure immunological parameters or identify differentially expressed transcripts into affordable true point-of-care tests for widespread use in low resource settings will require collaboration between multiple disparate scientific and commercial partners.

Most efforts have sought to validate diagnostic biomarkers in children that have been discovered in adults. However, accurate diagnostic biomarkers in adults may not translate well into diagnostic accuracy in children, because young children have differently regulated immune systems, have a pre-pubertal hormonal status, more frequently suffer from malnutrition, have received BCG immunisation and other childhood vaccinations more recently, and more often lack the immunological memory conferred from lifetime exposures to microbial and other environmental antigens.[18,19,23,50] Therefore, efforts should not only be directed at biomarker validation, but also at biomarker discovery in young children. Challenges around the timing of sampling in relation to exposure are similar as for that of the immune response (figure 3) and there are significant transcriptomic changes with age, co-infections, nutritional status, recent vaccination, and even time of day.[51]

Treatment of tuberculosis infection

Children with tuberculosis infection are clinically well, and most do not progress to tuberculosis disease. Therefore, unpalatable or intolerable medications would not be acceptable from a risk-benefit perspective. This leaves only a handful of currently available drugs for

tuberculosis infection treatment—ie, the rifamycins, isoniazid, and the fluoroquinolones. When pharmacokinetic parameters and safety profiles are better understood in the youngest children, it may also include delamanid, bedaquiline, and other novel drugs. The ideal tuberculosis infection treatment regimen would be safe, short, well-tolerated, effective, compatible with antiretroviral medications, and affordable, while easy to implement for health services and families. If this ideal is not possible, then it may be appropriate to sacrifice some degree of efficacy for safety, duration, or tolerability. Other options, including easier administration (such as a cutaneous patch) or a very long acting agent (only needed to be given once every week) would also be advantageous.

The evidence base for current treatments of tuberculosis infection has largely been gained from large-scale clinical trials, employing a trial and error approach. A more mechanistic and considered strategy, however, could provide advantages. Several mechanisms can be employed to treat tuberculosis infection, including elimination of mycobacteria; killing enough of the mycobacteria, or weakening them sufficiently to allow the immune system to eliminate or contain them; stimulating the mycobacteria out of their non-replicating state so that they can be eliminated by other drugs or the immune system; stimulating or manipulating the immune system so it is better able to eliminate or contain the mycobacteria; or some combination of the above. All drugs used for the treatment of tuberculosis infection (or presumed infection) at the time of writing act by killing mycobacteria. However, they do this by disrupting different elements of the mycobacterial metabolism or structure.[52] Selecting the best drug or combinations to kill different populations, including metabolically inactive organisms, requires careful consideration, as this might permit more effective or shorter treatment with less toxicity.

Optimising tuberculosis infection treatment also requires optimisation of dosage. Increasing the mg/kg dosage might mean greater efficacy, permit intermittent dosing, or allow shorter treatment. Higher dosage isoniazid (eg, 15–20 mg/kg) has been used to treat multidrug-resistant organisms (resistant to rifampicin and isoniazid) causing both infection and disease.[53,54] Comparing efficacy of this dosage with standard (10 mg/kg) dosing has not been done for the treatment of tuberculosis infection. Investigators are using increasingly high dosages of rifampicin (up to 50 mg/kg) for the treatment of tuberculosis disease in adults (NCT01392911),[55] but these higher dosages have not been evaluated in the treatment of tuberculosis infection.

Host-directed therapies and vaccines that do not aim to kill mycobacteria directly but assist to eliminate organisms should also be assessed. These might include drugs or vaccines that modulate parts of the immune system, inhibit efflux pumps, or wake inactive mycobacteria.[56] The differences between age groups of children also need to be included in these investigations as distinct immunological changes during puberty and early adolescence may alter these responses.

Consideration of tuberculosis infection treatment should extend beyond tolerability and toxicity. Drugs with broad antibacterial activity that are taken for long durations are likely to have other effects. These might be negative, in terms of the promotion of drug resistance in non-mycobacterial bacteria,[57] or disruption of a healthy microbiome.[58] These unintended effects can also be positive, in terms of reduction in other bacterial infections, improved growth, better development, and a reduction in life-threatening infections. These effects are rarely captured in treatment trials and any study of new therapies for tuberculosis infection should analyse these non-specific actions.

Few regimens are recommended to treat drug-susceptible tuberculosis infection, and no regimen is widely recommended to treat drug-resistant tuberculosis infection.[49] It is generally accepted that if a regimen is effective in adults, it will be effective in children.[59] However, the formulation is far more important in children than in adults, and the historical practice of crushing adult tablets should no longer be an acceptable solution. The dosage required to achieve serum concentrations in children equivalent to effective serum concentrations in adults need to be elucidated, as do child-specific safety parameters. In the investigation of rifapentine, a large initial study was conducted to demonstrate efficacy,[60] with recruitment of children continuing after the primary trial had closed to make sure that enough data were available to document safety in the paediatric population.[61] However, there are still gaps in our understanding of the pharmacokinetics and safety of this drug in children younger than 2 years, who have the highest risk of tuberculosis disease progression. Given that children have less concomitant pathology (such as existing lung damage that might act as a site difficult to clear of mycobacteria) and are able to tolerate higher dosages of most tuberculosis medications, it might be possible to treat children with even shorter durations of therapy than adults. Efficacy trials would be required to assess such regimens. However, creative trial designs are needed as the conventional approach used to evaluate new tuberculosis infection treatments is to do a non-inferiority trial comparing the new regimen with an established regimen. Given the rarity of disease endpoints in the absence of any treatment, and even fewer with established regimens, these trials are necessarily large, expensive, and of long duration. Duration response trials have been suggested as one way that treatments of different durations could be assessed.[62]

Conclusion

Even with currently available tools, much can be done to better identify and treat children with tuberculosis infection. However, for most health systems in high burden settings, this remains a low priority. If tuberculosis is to be eliminated, all children with

	Study suggestions
Understanding pathophysiology	
Define immuno-phenotypes most likely to progress to tuberculosis disease (sub-clinical tuberculosis in particular) to identify children and adolescents who would benefit most from tuberculosis infection treatment	Large household contact studies and large prospective longitudinal analyses of immuno-phenotypes;* bio-banking of samples from ongoing tuberculosis household contact studies in children
Investigate if previous tuberculosis infection and containment protects against disease development in the face of further exposure and, if so, whether treatment of tuberculosis infection might impair this immunity	Studies in animal models through exposure, infection testing, and re-exposure
Explore reasons for persistently negative tests of infection (IGRA and TST)	In children heavily exposed to tuberculosis, compare innate immune responses in children with persistently negative IGRA or TST results and those with positive test results
Understand the effect of age on mycobacterial immune responses	Juvenile animal studies to define ontogeny of mycobacterial immune responses; in-vitro human studies exploring ontogeny of mycobacterial immune responses
Understand the role of mucosal immunity in tuberculosis infection following exposure to determine role of alternative vaccine routes	Animal and in-vitro human studies of mycobacterial mucosal immunity in children of different ages*
Identification of tuberculosis infection	
Technical improvement of IGRAs to diagnose tuberculosis infection in young children	Technical research to reduce requisite blood volumes and decrease incidence of indeterminate results among young children
Improvement of access to a diagnostic platform of tuberculosis infection in children	Technical research to develop a point-of-care diagnostic platform for detection of *Mycobacterium tuberculosis* infection that minimises blood volume
Validation in young children of a diagnostic biomarker of sub-clinical disease discovered in older children or adults	Large prospective observational study of young children for development of tuberculosis disease with longitudinal collection of biological specimens*
Biomarker discovery in young children for diagnostic biomarkers of sub-clinical tuberculosis	Validation studies of diagnostic biomarkers of subclinical tuberculosis disease*
Tuberculosis infection treatment	
Explore the shortest, effective regimen for treatment of drug-susceptible tuberculosis infection	Randomised cluster non-inferiority study; prevention of infection trials, either with novel vaccines or BCG re-vaccination; duration of randomisation trials
Explore the shortest, effective regimen for treatment of drug-resistant tuberculosis infection	Randomised cluster superiority study; prevention of infection trials, either with novel vaccines or BCG re-vaccination; duration of randomisation trials
Obtain pharmacokinetic data on all regimens for all ages of children	Embed pharmacokinetic studies in all cohorts and trials; conduct rifapentine pharmacokinetic studies in children younger than 2 years
Palatability and acceptability of proposed new formulations or regimens	Nested social science evaluations within a randomised study
Explore non-specific effects of current and novel anti-tuberculosis treatment, both positive and negative, including impact on the microbiome	Nested within randomised cluster superiority or non-inferiority study
Explore role of host directed therapies, with a focus on different effects with age	Prevention of infection trials – either with novel vaccines or BCG re-vaccination; trials of host-directed therapies in addition to antimicrobial agents
Implementation research outcomes evaluation with robust monitoring and evaluation	Step wedge design within routine implementation settings to evaluate cost, coverage, and sustainability

TST=tuberculin skin test. IGRA=interferon-gamma release assay. *These studies would address more than one research need, and would ideally be embedded within vaccine trials, diagnostic biomarker studies, or household contact studies with a common entry point of household exposure.

Table: **Research priorities for improved management of paediatric tuberculosis infection**

tuberculosis infection will need to be reached, diagnosed, and treated. There is a pressing need to better understand the pathophysiology and natural history of tuberculosis infection in children from birth to adulthood, to develop tests that better identify those at the highest risk of disease progression, to target the delivery of tuberculosis infection treatment to those most likely to benefit from it, and to develop and implement treatments that are shorter and easier to deliver. Much can be learnt from studies in adults, but for many questions, research needs to be conducted in children specifically. One of the most efficient methods of investigating these questions is to embed basic science research into already established clinical cohorts and trials, with the collection of relevant specimens and key data elements during the conduct of such studies. An overview of key research priorities and suggested study designs that could be undertaken to address them are outlined in the table.

This body of research will require the development of novel partnerships between disparate stakeholders, including academics of multiple disciplines, industry, policy-makers, regulators, funders, and the communities affected by tuberculosis. Innovative strategies are required to maximise the scientific output from clinical studies, which will require significant increases in funding. The consequences of ongoing inaction, however, will lead to future generations continuing to suffer from what should increasingly be regarded as a preventable condition.

Contributors

JAS, BK, DAL, ACH, RR, and FA defined the scope of the Personal View. JAS produced the first draft and all figures. All authors gave critical input and all authors approved the final version.

Declaration of interests

DAL and Oregon Health and Science University (OHSU) have financial interests in ViTi, Inc, a company that is developing biomarkers of tuberculosis progression in children, and therefore may have a commercial interest in the contents of this Personal View. This potential

individual and institutional conflict of interest have been reviewed and managed by the OHSU Conflict of Interest in Research committee. ViTi had no role in the decision to publish or preparation of the Personal View. The other authors declare no competing interests.

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