Diagnostic biomarkers of latent tuberculosis infection in children: a protocol for systematic review

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Until the Covid-19 pandemic, tuberculosis was the global leading cause of death from a single bacterial pathogen [1]. Diagnosing any form of TB, latent or active, in a timely manner is the first step in mitigating the spread of the disease. However, there is no gold standard in diagnosing latent tuberculosis. Our review process aims to summarize and analyze the evidence of any diagnostic biomarkers for LTBI in children and the clinical relevance of diagnostic biomarkers. Following the Cochrane Collaboration Methodology, we will start by conducting literature searches on sources found on PubMed to scope out all relevant studies. We will include randomized controlled trials, where human participants are under the age of 18 and do not have HIV co-infection; observational studies; and in-vitro experiments (human cells). We will include studies that focus on LTBI diagnostic biomarkers in children with the tuberculin skin tests compared with, but not limited to, QuantiFERON-TB Gold In-Tube test and T-SPOT TB tests. Abstract selection, data extraction, and risk of bias assessment (according to the types of studies) will be conducted by two independent reviewers. We will exclude reviews and editorial letters. The pooled outcomes will be reported using the sensitivity and specificity of diagnostic biomarkers and odds ratio or risk ratio of LTBI cases. We will assess sources of heterogeneity and publication bias. By following this protocol, we will systematically assess and synthesize the existing evidence about biomarkers designed to diagnose LTBI among children without co-infection. Understanding the mechanisms of these biomarkers will provide more insight into better biomarkers of LTBI, particularly among children.

Keywords: Infant; child; pediatrics; pediatric; paediatric; infant; baby; babies; newborns; neonat; toddler; child; preschool; schoolchild; boy; boys; girl; girls; pre-school; adolescence; diagnosis; diagnos; biomarkers; biomarker; molecular pathology; molecular; antibodies; antibod; cytokines; inflammatory mediator; interferon-gamma release test; interferon-gamma release tests; IGRA; T-SPOT; Latent Tuberculosis; Latent Tuberculos; LTBI; Mycobacterium tuberculosis, humans; sensitivity and specificity

Introduction

The United Nations, which oversees the World Health Organization, defines children as any person below the age of 18 [2]. This stage of a person's life encompasses various physical and biological changes, including susceptibility to tuberculosis disease. Children are more

susceptible to TB disease because they have fewer and less functional dendritic cells and macrophages, which play a role in the body's immune response [3].

Latent Tuberculosis Infection (LTBI) is a clinical form of tuberculosis in which *Mycobacteria tuberculosis* remains dormant in the body. Therefore, people with LTBI do not display symptoms and can't spread the bacteria to others [4]. This form of tuberculosis affects about 30% of the world's population [4] with about a million children around the world harboring the disease [5], as well. However, LTBI can progress into active tuberculosis if left untreated. In fact, about 10% of LTBI cases develop into the active form [4]. Individuals with active tuberculosis display symptoms of sickness and have the potential to spread the bacteria to those in their surroundings [4]. The risk of LTBI developing into active tuberculosis is highest in infants and then decreases between the ages of five and 10, followed by an increase in susceptibility during adolescence [3]. LTBI is difficult to diagnose among children especially because of the absence of symptoms and appearance of good health—when an individual may actually be carrying the dormant bacteria with them—and also the lack of a gold standard in LTBI diagnostic procedures.

The first step in mitigating the transmission of tuberculosis is implementing proper diagnostic measures to ensure that those with LTBI are identified and receive proper treatment. Currently, the diagnosis of LTBI still relies on the immune response against the antigen of *Mycobacterium tuberculosis* (Mtb). To our knowledge, the diagnostic tools to distinguish the immune response caused by the LTBI from active TB disease remains unavailable. In children, the lack of direct test to diagnose LTBI complicated by children's young age and need for parental consent in studies; children's inherent aversion from phlebotomy; less frequent biological responses to infections like tuberculosis; and recency of Bacille Calmette-Guerin (BCG) vaccination, which may produce false positive results due to the same antigens used in TST testing material and BCG vaccinations [6].

Currently, Interferon Gamma Release Assays (IGRA) and Tuberculin Skin Test (TST) are the main diagnostic biomarkers of LTBI. Interferon gamma release assays (IGRAs) marked an impressive feat in this field of TB research when compared to the previous Tuberculin Skin Test (TST) method. TSTs require follow-up appointments for test readings, which may display biases in itself. In addition, administration of the BCG vaccination may lead to false positives[5]. The FDA approved IGRAs—which include QuantiFERON-TB test, QuantiFERON-TB Gold test, QuantiFERON-TB Gold In-Tube, and T-SPOT—are meant to offset the limitations set by TSTs and are the current methods being used to diagnose LTBI [6]. All of these interventions work by measuring the concentration of interferon gamma or IFN-g producing cells in response to antigens that are derived from Mycobacterium tuberculosis. However, as mentioned before, there is no gold standard in LTBI diagnosis. Limitations set by IGRAs include processing blood within 8-30 hours after administration of Mycobacterium tuberculosis derived antigens, requiring a return visit, and incorrectly evaluating the immunological response, absence of indicators to

discern individuals with latent and active tuberculosis, insufficient evidence to support IGRAs interventions on children below the age of five, and high price ranges [7].

Over the course of time, new diagnostic biomarkers to directly diagnose LTBI have been investigated. Several tests have been studied, including molecular diagnostics namely, T7 phages [8], IP10 mRNA expression [9], IL-2, r38 kDa, PPD, and 30 kDa antigens [10]. The combination of different biomarkers also has appeared to provide diagnostic measures in the instance of IP-10, IFN-y, Ferritin, 25-Hydroxyvitamin D via Quantiferon-TB Gold In-Tube tests and CLIA [11]. Although these diagnostic tools show some potential in detecting LTBI, the data is scattered and most studies either use small sample size, conducted in adult patients only, or use technology that is limited to research laboratories. Thus, assessing and summarizing the current biomarkers of LTBI will provide more guidance to the research conducted on this topic and interventions implemented across the world. This is in order to ultimately curb the global burden of LTBI among children.

Assessing and comparing the specificity and sensitivity, in addition to diagnostic odds and risk ratios, will provide more guidance to the research conducted on this topic and interventions implemented across the world. This is in order to ultimately curb the global burden of LTBI among children.

Objectives

This review aims to summarize and analyze the evidence of any diagnostic biomarkers for LTBI in children and the clinical relevance of diagnostic biomarkers.

Methods

This systematic review will follow the Cochrane Collaboration Methodology and Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) reporting guidelines. A PRISMA flow diagram will be used to illustrate the results of literature searches and the study screening and selection process.

Criteria for considering studies for this review

Types of studies. We will include randomized controlled trials (RCTs) and observational studies with a control group.

Types of participants. This review will include studies where participants are humans under the age of 18, or defined as children according to WHO guidelines. Individuals with HIV co-infection will be excluded.

Types of interventions. This review will include studies that use targeted biomarkers designed to diagnose LTBI among children. Examples of biomarkers of interest include, and are not

limited to, QuantiFERON-TB test, QuantiFERON-TB Gold test, QuantiFERON-TB Gold-In Tube, QuantiFERON-TB Gold plus, and T-SPOT TB test. These five are identified under the IGRA category as mentioned previously. We will compare these biomarkers to the Tuberculin Skin Test (TST).

Types of outcome measures. The primary outcomes of interest are sensitivity and specificity of biomarkers and diagnostic odds ratio or risk ratio of LTBI cases.

Search methods for identification of studies

The literature searches will be conducted via Medline (PubMed) in the English language, without any restrictions on the date of publication to retrieve all studies throughout the period of research and work done in this field. The search terms (using a combination of MeSH terms, free text words, and Boolean operators) and search strategies for each database have been defined by two reviewers of this systematic review.

The bibliographic software Zotero will be used to store, organize, and manage all the references. Study deduplication efforts will also occur in Zotero prior to further deduplication in the Covidence systematic review software.

Data collection analysis

Selection of studies. Titles and abstracts of studies retrieved using the predefined search strategies and those from additional sources will be screened independently by two review authors to identify studies that meet the inclusion criteria defined above. Two reviewers will independently screen studies. The title and abstract of each study will be screened to decide whether the study meets the inclusion and exclusion criteria. Any discrepancies during tiab and full text screenings will be resolved through discussion and, if necessary, a consultation with a third reviewer. The process of tiab, full text screening will be conducted in Covidence. Furthermore, data extraction will be conducted from all the studies that meet the inclusion criteria.

Data extraction and management. A standardized data extraction form will be developed to use on each of the included studies, which will later be used for assessment of study quality and evidence synthesis. Extracted data will include study design, country/study setting, study population and participant demographics (age, sex), details of the biomarkers designed to diagnose LTBI, control group description, the sensitivity and specificity of the biomarkers (including the number of true positive case and true negative case among the population), risk ratio/odds ratio of the LTBI cases among the population. Study authors will be contacted when important data is missing or when more information and details are needed. The extraction of data will be conducted independently by two review authors. Discrepancies will be identified and resolved through discussion. A third author will participate in the discussion when necessary.

Assessment of risk of bias in included studies. We anticipate the inclusion of different types of study designs, that is, RCTs with a controlled group (e.g. a controlled before and after study), observational studies, human experiments, and in-vitro experiments. For each study, we will rate risk of bias as high, low, or unclear based on criteria provided in Cochrane Handbook for systematic reviews of intervention [12], including risk of bias tools for randomized studies and non-randomized studies of interventions. The risk of bias tools will be used appropriately, depending on the study designs. For randomized controlled trials the criteria consists of allocation concealment, blinding of participants, personnel and outcomes, incomplete outcome data, selective outcome reporting, and other sources of bias. Non randomized studies of intervention criteria include the selection of participants, confounding variables, measurement of exposure, blinding of outcome assessments, incomplete outcome data, and selective outcome reporting [12].

Data synthesis and analysis

The summary of sensitivity, specificity, risk ratio, and odds ratio will be reported as forest plots in Revman-2020. The summary of risk of bias also will be reported as a risk of bias graph in Revman-2020. We may conduct a meta analysis to estimate summary measures across studies (among comparable studies).

Dealing with missing data. We will aim to obtain important missing data from authors, if available, and carefully evaluate important numerical data, such as screened, randomized participants as well as intention-to-treat, as-treated, and per-protocol populations. We will investigate attrition rates, for example, drop outs, losses to follow ups, and withdrawals, and critically appraise the issues of missing data and imputation methods.

Subgroup Analysis

If applicable, subgroup analysis will be carried out based on factors that may impact the effect estimates. We plan to conduct subgroup analysis:

1) Age group (pre school children (1-4y), school age children, and adolescents)
2) Type of diagnostic biomarkers (cytokines biomarkers (IGRA, T-SPOT, IL-2, etc), and DNA/molecules)
3) Country setting (region, LMIC/HIC, etc)

Heterogeneity between studies will be assessed using the X^2 test (significance alpha: .05) and the I^2 statistic.

Sensitivity Analysis

Sensitivity analysis will be performed to explore the influence of specific factors on effect size:

1) Accounting for risk and impact of bias (including and removing studies at high risk of bias)
2) Determining the influence of studies with greater sample sizes
3) Considering the influence of methods (study design)

Potential methodological amendments

In the case modifications are required to the present protocol, we will provide a detailed description of what these modifications entailed and a rationale for the need of each change during the publication of the results of the systematic review.

Discussion

This protocol followed the Cochrane methodology for systematic reviews and we intend to complete the review by May 2023. The findings from this research will provide more clarification for the millions of children and families who are affected by LTBI that is likely to turn into the deadly active form.

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