Revista Interdisciplinar de Estudos em Saúde

OPEN ACCESS

ARTIGO DE REVISÃO

https://doi.org/10.33362/ries.v14i2.3590

Laboratory diagnosis of pediatric tuberculosis: *a review of routine tests and promising new biomarkers*

Diagnóstico laboratorial da tuberculose pediátrica: *uma revisão dos testes de rotina e novos biomarcadores promissores*

Diagnóstico de laboratorio de la tuberculosis pediátrica: *una revisión de pruebas de rutina y nuevos biomarcadores prometedores*

Andrea Alice Silva [1]
Thalia Medeiros [2]
Natalia Fonseca do Rosário [3]
Alice Ramos-Silva [4]
Clemax Couto Sant'Anna [5]
Claudete Aparecida Araújo Cardoso [6]
Fabiana Rabe Carvalho [7*]

Recebido em: 20 ago. 2024
Aceito em: 23 jun. 2025

ABSTRACT: Despite substantial efforts for the early diagnosis of tuberculosis (TB) in children, the paucibacillary characteristic of the disease and clinical manifestations resembling other pulmonary childhood infections contribute to pediatric TB remaining a significant challenge for global public health. Overall, for children and adolescents, rapid molecular methods, culture and antigen detection in a lateral flow format, constitutes the main technology to help detect active TB and to evaluate drug resistance. In addition, with the rapid technological development, other methodologies are becoming promising for diagnosing pediatric TB. Furthermore, the recent advances in early diagnosis have shown a positive impact on the

[1] Doutora em Biologia Celular e Molecular. Universidade Federal Fluminense. ORCID: https://orcid.org/0000-0001-5856-6128. E-mail:aasilva@id.uff.br
[2] Doutora em Patologia. Universidade Federal Fluminense. ORCID: https://orcid.org/ 0000-0002-5642-4027. E-mail: thaliamedeiros@id.uff.br
[3] Doutora em Patologia. Universidade Federal Fluminense. ORCID: https://orcid.org 0000-0001-5109-085X /. E-mail: nataliarosario@id.uff.br
[4] Doutora em Ciências Farmacêuticas. Universidade Federal do Rio de Janeiro. ORCID: https://orcid.org/0000-0001-6037-8206. E-mail: aliceramosoliveiradasilva@gmail.com
[5] Doutorado em Doenças infecciosas e parasitárias. Universidade Federal do Rio de Janeiro. ORCID: https://orcid.org/0000-0001-8732-8065. E-mail: clemax01@gmail.com
[6] Doutorado em Ciências da Saúde. Universidade Federal de Minas Gerais. ORCID: https://orcid.org/. E-mail: claudetecardoso@id.uff.br
[7]* Doutorado em Ciências Médicas. Universidade Federal Fluminense. ORCID: https://orcid.org/0000-0002-4713-233X. E-mail: fabianarc@id.uff.br

timing and effectiveness of TB treatment in children. In this way, this review aimed to summarize the laboratory tests available for children while providing insights into new biomarkers (inflammatory mediators, liquid biopsy and extracellular vesicles) in the evolving landscape of pediatric TB diagnosis.

Keywords: Tuberculosis. Diagnosis. Children. Biomarkers. Laboratorial tests.

RESUMO: Apesar dos esforços substanciais para o diagnóstico precoce da tuberculose (TB) em crianças, a característica paucibacilar da doença e as manifestações clínicas semelhantes a outras infecções pulmonares da infância contribuem para que a TB pediátrica permaneça um desafio significativo para a saúde pública global. No geral, para crianças e adolescentes, métodos moleculares rápidos, cultura e detecção de antígenos em formato de fluxo lateral constituem as principais tecnologias para ajudar a detectar a TB ativa e avaliar a resistência aos medicamentos. Além disso, com o rápido desenvolvimento tecnológico, outras metodologias estão se tornando promissoras para diagnosticar a TB pediátrica. Ademais, os avanços recentes no diagnóstico precoce demonstraram impacto positivo no tempo e na eficácia do tratamento da TB em crianças. Assim, esta revisão teve como objetivo resumir os testes laboratoriais disponíveis para crianças, fornecendo também perspectivas sobre novos biomarcadores (mediadores inflamatórios, biópsia líquida e vesículas extracelulares) no cenário em evolução do diagnóstico da TB pediátrica.

Palavras-chave: Tuberculose. Diagnóstico. Crianças. Biomarcadores. Exames laboratoriais.

RESUMEN: A pesar de los esfuerzos sustanciales para el diagnóstico temprano de la tuberculosis (TB) en niños, la característica paucibacilar de la enfermedad y las manifestaciones clínicas que se asemejan a otras infecciones pulmonares infantiles contribuyen a que la TB pediátrica siga siendo un desafío importante para la salud pública mundial. En general, para niños y adolescentes, los métodos moleculares rápidos, el cultivo y la detección de antígenos en formato de flujo lateral constituyen la principal tecnología para ayudar a detectar la TB activa y evaluar la resistencia a los medicamentos. Además, con el rápido desarrollo tecnológico, otras metodologías están mostrando ser prometedoras para el diagnóstico de la TB pediátrica. Asimismo, los avances recientes en el diagnóstico temprano han mostrado un impacto positivo en el tiempo y la efectividad del tratamiento de la TB en niños. Por lo tanto, esta revisión tuvo como objetivo resumir las pruebas de laboratorio disponibles para niños y proporcionar perspectivas sobre nuevos biomarcadores (mediadores inflamatorios, biopsia líquida y vesículas extracelulares) en el panorama evolutivo del diagnóstico de la TB pediátrica.

Palabras clave: Tuberculosis. Diagnóstico. Niños. Biomarcadores. Pruebas de laboratorio.

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by bacteria of the *Mycobacterium complex*. The infection is spread by air, through the sputum of *Koch's bacilli* expelled by infected individuals (Seddon *et al*., 2014), thus agglomeration is the main risk factor for transmission (Lima *et al.*, 2019). TB is still a serious global public health problem, especially in

children. About 1.1 million of children (aged 0–14 years) become ill with TB every year and in 2021, 16% died from the disease (Global Tuberculosis Report 2023). However, national TB programs usually report less than half of these numbers and, in addition to this scenario, the World Health Organization (WHO) recently discussed the negative global impact of the coronavirus disease 2019 (COVID-19) pandemic on case notification and access to treatment (Global Tuberculosis Report 2023). It is important to mention that there was an increase 21.8% in the number of extrapulmonary TB (EPTB) cases in children <5 years-old in 2021 (Brazil, 2023). Of note, the high mortality rate in this age group is predominantly observed among patients without diagnosis or with an incomplete treatment (Jenkins *et al.*, 2017; Dodd *et al.*, 2017). Importantly, the rate of neonatal vaccination has been decreasing in Brazil in the last few years (Brazil, 2023). These data demonstrate the slow progress towards to what was proposed by the "End TB Strategy" implemented by the World Health Assembly of 194 Member States in 2014, which included the goals of cover treatment in ≥ 90% of children in contact with TB patients and increase vaccination rate (Program the End TB strategy, 2015)

Nowadays, the presumptive diagnosis of pulmonary pediatric TB is performed based on clinical/laboratory criteria: clinical symptoms + history of TB contact + chest radiography + tuberculin skin tests (TST) and/or Interferon-Gamma Release Assays (IGRA) and/or Nucleic Acid Amplification Test (if available) + nutritional status (Graham *et al.*, 2015; WHO, 2023). Nevertheless, the early diagnosis is affected by the absence of clinical symptoms or the presence of nonspecific symptoms which may also be seen in other respiratory infections, particularly among children under five years old (Maphalle *et al.*, 2022). These clinical manifestations include evening fever, weight loss, persistent or productive dry cough, and night sweats (Graham *et al.*, 2015). Other diseases such as non-tuberculous bacterial pneumonia (e.g. *Mycobacterium conspicuum*), systemic mycoses (e.g. *Paracoccidioides brasiliensis* and *Pneumocystis jirovecii*) or other lung diseases/neoplasms are important for differential diagnosis (Committee on Infectious Diseases, 2021). Besides absence of symptoms, the chest X-ray could be atypical, and the laboratory diagnosis can provide false-negative or inconclusive results mainly due to inadequate sample collection (CDC TB, 2020).

In pregnant women, congenital infection can occur through hematogenous dissemination or through aspiration/ingestion of amniotic fluid containing *Koch's bacilli* (Li *et*

al., 2019). Due to the immaturity of the Th1 immune system, TST and IGRA assays are generally negative in this group (Vanden *et al.*, 2013). In addition to routine laboratory evaluation for suspected cases of congenital infection, the assessment of the placenta is essential (Shao *et al*, 2021). Considering all difficulties for pediatric TB diagnosis, this review aimed to summarize the laboratory tests currently available and to describe the perspectives and new promising biomarkers to enhance early *Mycobacterium Tuberculosis* (*Mtb*) detection.

DEVELOPMENT

Current laboratory diagnosis for pediatric TB endorsed by WHO

Cartridge-based rapid molecular tests (TRM-TB)

In recent years, there has been an incessant search for a pediatric sample that is easily collected in a non-invasive way and with high accuracy for TB diagnosis (WHO, 2023). In addition, molecular biology techniques have been widely applied for the detection of genetic material from pathogenic bacteria, individually or simultaneously detecting target sequences in multiple samples (Kay *et al.*, 2020). Advantages of this type of assay include an operator-friendly characteristic and fast results (approximately 120 minutes), which makes this technique an important tool in TB diagnosis, affecting the clinical decision-making and epidemiological control (Brazil, 2023). Moreover, these techniques can also be used for the diagnosis of EPTB (WHO, 2023).

In the pediatric population (specifically <10 years-old), the Xpert *MTB*/RIF ULTRA assay (Cepheid Inc., Sunnyvale, CA, USA) demonstrates low sensitivity (66%) for *Mtb* detection but high sensitivity for the detection of multi-resistant strains to rifampicin (95%) (WHO, 2022). In addition, its specificity was considered low (95%) when compared to its predecessor Xpert *MTB*/RIF (> 98% in respiratory samples in adults) (Signorino *et al.*, 2022). Despite the strong recommendations to use the Xpert *MTB*/RIF and Xpert Ultra as initial tests in children with pulmonary involvement, the accuracy is variable depending of the sample type, where sputum (75.3%) or induced sputum (70.4%) present higher sensitivity in comparison to gastric aspirate (64%), nasopharyngeal aspirate (43.7%) and stool (56.1%) (Jayagandan *et al.*, 2022; WHO, 2022; Kay *et al.*, 2022). For children with signs of TB meningitis, cerebrospinal fluid (CSF) samples should be used (Kabir *et al.*, 2021; Dubale *et al.*, 2022; Kamra *et al.*, 2022). Also, recent

research has tested oral and lingual swab samples with the Xpert *MTB*/RIF ULTRA technology, but the results were discouraging in children, due to low with sensitivity (22%) despite the high specificity (100%) (Cox *et al.*, 2022). Importantly, the trace results should be considered positive because of the paucibacillary nature of TB disease in children. In these cases, screening for rifampicin resistance will also provide an indeterminate result (WHO, 2022; Cepheid). Furthermore, it is important to emphasize that the presence of *Mtb* DNA may be residual from previous infections. Therefore, this assay is not recommended for TB treatment control, and the confirmation of active TB in reinfected patients should be confirmed by culture assay. In these cases, resolution of clinical symptoms and weight gain can be used as indicators of improvement (Costantini *et al.*, 2020; WHO 2022).

Recently, Xpert *MTB*/XDR was made available; making it possible to evaluate mutations associated with resistance to isoniazid, fluoroquinolones, second-line injectable antibiotics (amikacin, kanamycin and capreomycin) and ethionamide (Cepheid). According to our knowledge, the accuracy of this test was not investigated in children. In addition, other technologies, such as slide-PCR gel droplet microarray - based on genotypic detection of multiple genes in a single droplet of DNA sample, line probe assay, and Nucleic Acid Amplification Tests are available for diagnosis; however, due to the higher cost per sample in relation to Xpert *MTB*, they are mainly used to detect mutations associated with multidrug-resistance (MDR) (Günther *et al.*, 2022). Importantly, new techniques using coated beads demonstrated to improve the detection rate of the *Mtb* complex in saliva due to increased mycobacterial DNA concentration (Hansen *et al.*, 2022). This highlights the potential use of saliva for *Mtb* DNA detection. Moreover, other molecular techniques have also been studied for the diagnosis of TB in children, such as Loop-mediated isothermal amplification (TB-LAMP), which presented a moderate sensitivity (76.5%) in children with bacteriologically confirmed with PTB and EPTB (Inbaraj *et al.*, 2023).

Lateral-flow urinary lipoarabinomannan assay (LF-LAM)

The lateral-flow urinary lipoarabinomannan assay (LF-LAM) for the detection of glycolipids from the *Mtb* cell wall (lipoarabinomannan) have been used for TB diagnosis. These glycolipids have important immunomodulatory properties, activating a series of cellular and humoral events linked to the innate immune response (Correia-Neves *et al.*, 2019). This test

is recommended by WHO mainly for the diagnosis of active TB in HIV-positive patients with strong evidence of signs and/or symptoms of TB (WHO, 2023). In this regard, studies have demonstrated an inverse correlation between CD4+ cells count (≤100 cells/mm3) and LF-LAM accuracy, showing the assay's efficiency in children living with HIV and severely immunocompromised (sensibility 57.9% and specificity 96%). Despite being a child-friendly test and providing results in 25 min, this test has a low sensibility and specificity, 48.3% and 60.8% respectively, for HIV-negative children (Marais *et al.*, 2014; Seid *et al.*, 2022). Importantly, the combination of LF-LAM and Xpert *MTB*/RIF assays has been considered promising for the diagnosis of pediatric TB, with sensitivity comparable to culture (Simieneh *et al.*, 2022, WHO 2022).

It is important to mention that more studies have been using different technologies to validate the use of urinary LAM for TB diagnosis in the HIV-negative population. For example, Magni and colleagues (2020), in a multicentric study, identified that urinary LAM was able to distinguish TB infection in non-HIV patients using three monoclonal antibodies (average sensitivity of 90% and specificity of 73.5%), including in a group of 19 children. Similar results were also observed in a posterior study using ELISA and mass spectrometry approach, even for smear negative individuals (Amin *et al.* 2021).

Interferon gamma release assay (TB-IGRA and T-SPOT-TB)

The Interferon gamma release assay (IGRA) measures the production of interferon gamma (IFN-γ) by T cells stimulated with purified or synthesized *Mtb* antigens (WHO, 2023). IGRA was included in the Unified Health System of Brazil in July 2022 and is especially recommended for (i) people living with HIV with a T-CD4+ count >350 cells/mm^3; (ii) children in contact with of active TB cases (2-10 years-old); (iii) people who are candidates for stem cell transplants and (iv) patients in treatment with immunobiologicals and/or immunosuppressants (Brazil, 2023). Advantages of this assay includes high specificity and sensitivity (98% and 96%, respectively) in adults, does not cross-react with the BCG vaccine and single visit of patient to perform the test. On the other hand, it has a high economic cost, longer time to process, and impossibility to use for treatment control (CDC, 2023; Kay *et al.*, 2018; Ahmed *et al.*, 2020; Brazil, 2023). Furthermore, high rates of indeterminate results,

mainly in children <5 years old, and limited data on immunocompromised patients increases the risk of diagnostic failure (Kay *et al.*, 2018; WHO, 2023; Hirabayashi *et al.*, 2023).

The T-SPOT-TB is an immunoenzymatic method that quantifies the number of effector T cells (spots) producing IFN-γ after being stimulated with *Mtb*-specific antigens (WHO, 2023). In children >10 years-old, this test presents a sensitivity of 60% and a specificity of 75% (Girit *et al.*, 2019; Ahmed *et al.*, 2020). The assays recommended by WHO are the T-SPOT-TB (Oxford Immunotec, UK), QuantiFERON-TB Gold Plus (Qiagen, USA) and Wantai TB-IGRA (Wantai, China) (WHO, 2023). However, few studies were performed in children and the performance for ILTB diagnosis is directly related to TB incidence (Campbell *et al.*, 2021; Jia *et al.*, 2022). In 293 South African children with suspected active TB, the sensitivity of T-SPOT-TB was similar between children in different age ranges (around 81-85%) (Liebeschuetz *et al.*, 2004).

Tuberculin skin tests (TST)

Widely used in clinical routine, the tuberculin skin test (TST) is the main assay used to detect latent TB infection (LTBI) in asymptomatic patients. However, due to its moderate accuracy (sensitivity of 77% and specificity of 97%), clinical assessment (medical history, physical examination, chest radiograph) is necessary to confirm LTBI diagnosis (Brazil, 2023). The induration should be carried out between 48-72 hours after application, and it is considered positive when >5 mm (Gilani *et al.*, 2024; Brazil, 2023). Importantly, when a TST is positive in adolescents and adults, a diagnosis of LTBI should be considered for asymptomatic patients in contact with active TB with normal chest x-ray. TST can result in a false negative in young children (<6-8 weeks) due to immunological immaturity or false-positive results due to cross-reactivity with a group of non-tuberculous mycobacteria (N*MTB*). Still, even with the limitations, it is recommended to use the TST in low- and middle-income countries for the diagnosis of TB infection in children (Muñoz *et al.*, 2015; Lewinsohn *et al.*, 2017; Brazil, 2022; Hirabayashi *et al.*, 2023). In the scoring system proposed by the Brazilian Ministry of Health (the main guideline for diagnosing PTB in children), TST interpretation (PPD - purified protein derivative RT-23) is of great importance (Pedrozo *et al.*, 2010; Carvalho *et al.*, 2020; Brazil, 2023).

In 2022, three new antigen-based skin tests cy-Tb (Serum Institute of India, India), c-TST (Anhui Zhifei Longcom Biopharmaceutical Co. Ltd, China) and Diaskintest (Jsc Generium,

Russian Federation) were recently recommended by WHO due to higher specificity in comparison to TST. However, the efficacy of these tests has not been investigated so far (WHO 2023).

Culture

Culture is the gold standard for diagnosing TB. Unlike the tests above mentioned, culture is capable of identifying the pathogenic species of the *Mtb* complex, in addition to make it possible to perform sensitivity tests for anti-TB drugs, possibly detecting resistance (WHO, 2023). For this reason, it is mandatory that all initial samples to be sent to confirm the diagnosis by culture, regardless of using a second method of analysis (CDC 2023). It is important to mention that a positive *Mtb* culture confirm the diagnosis of TB disease in childhood; however, a negative result does not rule out the when the patient presents clinical symptoms (CDC 2023).

Studies demonstrate that the sensitivity of this test in children varies between 30-50% (Detjen *et al.*, 2015; DiNardo *et al.*, 2016; Click *et al.*, 2022). However, according to the American Thoracic Society, Centers for Disease Control and Prevention, and Infectious Diseases Society of America, culture should be performed on respiratory samples in all children suspected of having PTB (Lewinsohn *et al.*, 2017). Due to the delay in obtaining the final result (~6 weeks), other faster tests (e.g. TRM-TB) can be carried out in parallel, in an attempt to confirm the clinical diagnosis and prevent disease spread. Besides, the use of liquid culture media and automated systems can decrease the result release time to 5-13 days (Silva *et al.*, 2021). Recently, Nguyen and colleagues (2022) observed discordant results between Xpert *MTB*/RIF assay and liquid culture for *Mtb* detection. Patients with a low sputum bacterial load presented positive results for Xpert and negative results for liquid culture (Nguyen *et al.*, 2022). Thus, these data should be considered when screening for TB in the pediatric population.

In addition to culture, the analysis by acid-fast-bacilli (AFB) smear is routinely performed in clinical laboratories. This technique consists in a microscopic analysis of colonies stained using the Ziehl Neelsen method, allowing the counting of bacilli/field. In children, this method presents a low positivity rate (<20%) due to paucibacillary characteristic of pediatric TB (Khan *et al.*, 1995). Nevertheless, a systematic review conducted by Tiemersma and

colleagues (2011) observed a weighted mean of lethality of 70% in smear-positive untreated HIV-negative individuals in 10 years, showing a good predictive value for mortality using this test (Tiemersma *et al.*, 2011). However, there is a group of bacteria that share the characteristic of resistance to acids, making clinical evaluation an essential point for defining the diagnosis (Reynolds *et al.*, 2009).

Adenosine deaminase (ADA)

The adenosine deaminase (ADA) is present mainly in lymphocytes and is involved in stimulation of cellular immunity and regulation of antibody production (Passos *et al.*, 2018; Yap *et al.*, 2021). In adults with extrapulmonary (pleural, abdominal, peritoneal and miliary), the ADA immunoenzymatic assay presents sensitivity and specificity of 89% and 58% respectively, at a cut-off value of 40 U/L (Han *et al.*, 2021; Na *et al.*, 2022; Zhou *et al.*, 2022). In patients with pleural effusion living in endemic areas, the ADA assay shows a positive predictive value of 98%, when associated with the presence of clinical symptoms and lymphocytosis (Jiménez *et al.*, 2003). In cerebrospinal fluid (CSF) of children with TB meningitis (6 months to 11 years), ADA demonstrated higher specificity (90%) and sensitivity around 66.6% (cut-off at 10 U/L) in comparison to other types of meningitis (Rana *et al.*, 2004). In addition, possible interferents such as jaundice and hemolysis can influence on the accuracy of the test (Delacour *et al.*, 2010). In children and adolescents with suspected pleural TB, high concentrations of ADA (>40U/L) guarantee higher sensitivity (~90%) but with lower specificity (~ 58%) (Na *et al.*, 2022; Lunelli *et al.*, 2022). The recommended laboratory tests for pediatric TB diagnosis are summarized in **Table 1**.

Perspectives on pediatric TB diagnosis

In pediatric diseases, a rapid diagnosis is essential to initiate and monitoring for treatment. In this regard, research for the implementation of new technologies for TB diagnosis is being relentlessly performed to solve this issue. Molecular detection and drug resistance, aerosol capture technologies, new IFN-γ release assays, biomarker-based assays, computer-aided detection for digital chest radiography and other artificial-intelligence tools are new methods in development (WHO, 2023). However, despite technological advances, there is still no precise test that can be performed with easily collected samples and higher accuracy for the pediatric population (Chiang *et al.*, 2023).

Table 1 - Recommended laboratory tests for pediatric Tuberculosis diagnosis.

Tests	Clinical presentation	Recommended age	Accuracy (sens. and spec.)	Advantages	Disadvantages	Reference
TST (PPD RT-23)	LTBI	> 3 months	77% - 97%	Lower cost; no laboratory analysis required; widely available	Trained technician; requires a second visit; low sensitivity in immunosuppression	Brazil, 2023 Ghanaie *et al.* 2021
IGRA	LTBI	2-10 years-old	> 90% - 98%	Single visit; easy interpretation; highest specificity and sensitivity; no cross-reactivity (BCG or N*MTB*)	Trained technician; affected by sample interferents (e.g. hemolysis) and/or transportation; high cost	Kay *et al.*, 2018 Ahmed *et al.* 2020, Brazil 2022
Culture	PTB, EPTB	<15 years-old	50% - 100%	Species identification and typing of bacteria; evaluation of drug susceptibility; treatment-response monitoring; various sample types	↑ rate of contamination; trained technician; results in ~6 weeks	DiNardo *et al.*, 2016 Click *et al.*, 2022
T-SPOT	PTB, EPTB	>10 years-old	60% - 75%	Fewer FP than TST; enables individual counting of IFN-γ producing cells	Trained technician; requires cell isolation; high cost and largest laboratory structure; no widely available	Girit *et al.*, 2019
TRM-TB	PTB, EPTB	< 10 years-old	66% - 95%	↑ specificity; results in ~2h; identification of multi-resistant strains; operator-friendly; various sample types	Higher cost; affected by presence of residual *Mtb* DNA	Signorino *et al.*, 2022
		> 10 years-old	90% - 95%			WHO, 2023
LF-LAM	PTB, EPTB	< 15 years-old	51% - 89%	Single visit; operator-friendly; POC; lower cost	Sensitivity is suboptimal in immunocompetent individuals; not evaluate drug resistance	Seid *et al.*, 2022 WHO 2022; Chiang *et al.*, 2023
		< 4 years-old	48% - 61%			Marais *et al.*, 2014; WHO 2022; Chiang *et al.*, 2023
ADA	EPTB	<14 years-old	89% - 58%	Single visit; easy collection	Trained technician; affected by sample interferents (e.g. hemolysis) and/or transportation; high cost	Na *et al.*, 2022; Zhou *et al.*, 2022

Note: PTB: pulmonary TB; EPTB: extrapulmonary TB; TST: tuberculin skin tests; IGRA: Interferon-Gamma Release Assays; T-SPOT: interferon gamma release assay; TRM-TB:Molecular Rapid Test for Tuberculosis; LF-LAM: lateral-flow urinary lipoarabinomannan assay; ADA: adenosine deaminase; sens: sensibility; spec: specificity; N*MTB*:non-tuberculous mycobacteria

Fonte: Dados da pesquisa (2024).

Immune mediators

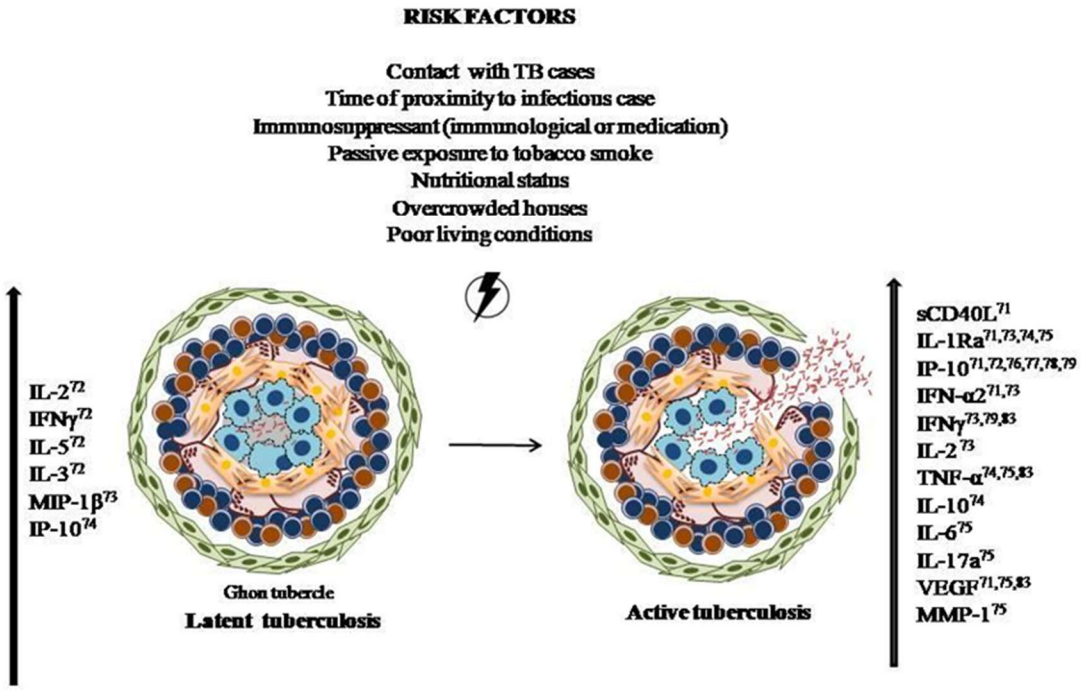
During *Mtb* infection, the interaction between innate and adaptive immune responses leads to the activation of alveolar macrophages and dendritic cells (DCs) which leads to the secretion of several proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin (IL)-1 (Stek *et al*; 2018; Cicchese *et al.* 2018; Rodo *et al.*, 2019; Vinhaes *et al.* 2021; Mayer-Barber *et al.* 2023). The consequences of this response are antigen presentation to lymphocytes and the formation of granulomas, which intend to restrict the *Mtb* growth and dissemination (Ernst, 2012; O'Garra *et al.* 2013; McNab*et al.* 2015) but leads to a persistent inflammation with a consequent tissue damage (Amaral *et al.* 2021). Granulomas are typical of infections caused by Koch's bacillus. Its circular characteristic is formed by the encapsulation of the bacillus by inflammatory cells (alveolar macrophages, monocytes, lymphocytes), and subsequent formation of the primary nodule (*Ghon's tubercle*) retaining viable mycobacteria (LTBI) which can progress to reactivation or cavitation (characteristic of secondary TB), through endogenous or exogenous triggers, particularly in children (Cruz-Knight; Blake-Gumbs., 2013).

Several pediatric studies have focused on the accuracy of inflammatory mediators to detect the TB infection, as well as to discriminate active TB from LTBI. Several biomarkers have been studied, using immunological methods, for the diagnosis of TB in childhood; however, some data is still controversial. Studies have observed that IFN-γ, IP-10, IL-2, TNF-α, IL-1ra, IL-13, IFN-αand the vascular endothelial growth factor (VEGF) present high sensitivity and specificity to distinguish TB-infected and non-infected children (Armand *et al.* 2014; Tebruegge *et al.*, 2015; Sudbury *et al.*,2019; Manyelo *et al.* 2022).

Additionally, some of these biomarkers seem to be capable to distinguish the forms of the disease. In a previous study, our group have demonstrated that the combinatory analysis of VEGF and IL-1Ra showed a high area under the curve (AUC of 0.99) on the discrimination of active TB and LTBI in children <5 years-old (Martins *et al* 2022). Moreover, the combinations of TNF-α/IL-1ra and TNF-α/IL-10 correctly classified children with active TB and LTBI (Tebruegge *et al.*, 2015) and simultaneous analysis of IP-10/IFN-γ/ferritin/25(OH)D also showed a good diagnostic performance (Comella-del-Barrio *et al.* 2019). IP-10, considered a downstream marker with the advantage of being expressed at higher levels than IFN-γ, is one

of the most studied biomarkers to differentiate active TB from LTBI, but some studies have demonstrated that IP-10 could not distinguish these TB status (Whittaker *et al.* 2008; Petrone *et al.* 2015; Jenum*et al.* 2016). Figure 1 summarizes the immune mediators involved in *Mtb* infection that could be used for TB diagnosis in children.

Figure 1. Childhood pulmonary tuberculosis (TB): risk factors and inflammatory biomarkers involved in active and latent TB infection.



Note: IL, interleukin; IFNγ, interferon-gamma; MIP, macrophage inflammatory proteins; IP-10, interferon-gamma inducible protein 10; sCD40L, soluble CD40L; VEGF, vascular endothelial growth factor; IFN-α2, interferon alpha-2; TNF, tumor necrosis factor; MMP-1, matrix metalloproteinase-1.
Fonte: Authors' own work (2024).

We have also evaluated immunoglobulins (Ig)in an attempt to distinguish TB and LTBI in children; especially those <5 years-old, who are more prone to disease progression. In this regard, a good performance was found for the anti-Mce1A (mammalian cell entry) protein IgG in children and adolescents (Schmidt *et al.*, 2021). The Mce1Aprotein can promote a proinflammatory response in stimulated macrophages (Xue *et al.* 2007). In another study, a longitudinal analysis demonstrated a decrease in IgM levels against cardiolipin, sulfatide, mycolic acid and Anti-Mce1A IgG after TB treatment in children and adolescents, showing that the antibody response to *Mtb* cell wall products can be a useful tool to monitor the response

to therapy (Dos Santos *et al.*, 2020).Moreover, antibody detection in saliva has shown to be valuable for PTB diagnosis (Estévez *et al.*, 2020; Khambati *et al.*, 2021), but few studies were performed in the pediatric population. In children under 15 years-old, the detection of Ig against specific *Mtb* antigens have demonstrated a sensitivity and specificity of 64.7% and 81.8%, respectively (Araujo *et al.*, 2004).

Omics-based biomarkers

The combination of "omics" technologies such as genomics, proteomics, metabolomics and lipidomics using the mass spectrometry technique were able to provide important information about the *Mtb* molecular composition, which helps to understand the pathogenicity and evasion mechanisms in addition to identify new biomarkers for TB diagnosis (Galagan2014). Thus, in recent years, the application of proteomics to elucidate the molecular mechanisms and metabolism pathways, especially in the expression of small and long-non-coding RNAs (lncRNAs) and transcription factors, has become a technological advance for the diagnosis of pediatric TB (Vaezipour *et al.*, 2022; Sholeye *et al.*, 2022). For instance, one study analyzing lncRNAs and mRNAs in PBMCs of TB patients receiving primary therapy showed several dysregulated biological processes, including the immune modulation that could be related to disease progression (Li *et al.*, 2023).

A recent pan-genome analysis demonstrated a high protein similarity (87.5%) between mycobacteria of the *Mtb* complex (Silva-Pereira *et al.*, 2023). However, despite the protein similarity, the presence of the lipid phthiocerol dimycocerosates in the *Mtb* cell wall is related to increased virulence and the immune evasion (Augenstreich *et al.*, 2020). Besides, different regions of the granuloma demonstrated distinct cellular components, with a concentration of neutrophilic proteins in the caseum region and proteasome in cellular regions, suggesting an intense activity of DCs and macrophages (Seto *et al.*, 2019). This fact may be associated with the lipid changes found in the cell wall of*Mtb* carrying the Mce1 mutation compared to wild-type *Mtb* which, according to Queiroz and colleagues (2015), could induce in different host responses to infection, demonstrating that lipids play an important role in TB development (Queiroz *et al.*, 2015; Schmidt *et al.*, 2020).

Interestingly, researchers recently identified 15 core genes able to differentiate active TB from LTBI with AUC ≥ 0.80 in children <15 years-old using bioinformatics analysis and gene

expression profile data. In this study, the accuracy analysis showed haptoglobin, and adenylate cyclase 3with greater sensitivity (>80%) (Shao *et al.*, 2021). Other studies have already observed an increase in haptoglobin in plasma samples from children with TB (Pavan *et al.*, 2013) and the Hp 2-2 genotype has already been described as a predictor of mortality 6.1 times greater when compared among those with Hp 1-1 genotype (Kasvosve *et al.*, 2000).

The metabolomic assessment of biochemical responses to pathophysiological stimuli or genetic modifications has also been used for TB differential diagnosis. Dutta e colleagues (2020) observed a combined metabolite analysis in plasma samples using integrated metabolomics and transcriptomics assays and found that gamma-glutamylalanine, gamma-glutamylglycine, glutamine, and pyridoxate were associated with a successful response to TB treatment in the pediatric population with an AUC of 0.86 (Dutta *et al.*, 2020). In addition, a metabolomics analysis in serum samples and *Mtb*-stimulated whole blood cultures indicated that leucine and kynurenine as potential biomarkers to detect TB infection in children (Magdalena *et al.*, 2022).Another study showed that the kynurenine/tryptophan ratio also presented moderated accuracy (AUC of 0.67 and sensitivity of 81.5%) for diagnosing TB in a pediatric population with a median age of 8.5 years-old (Tornheim *et al.*, 2022).The same group evaluated participants <15 years-old with TB by transcriptome analysis, and found 25 genes differentially expressed during treatment. Among them were the DEFA3 and PRRG4were also present among younger children with TB and exposed household contacts (Tornheim *et al.*, 2020). In urine samples, distinct metabolic fingerprints were observed in children <7 years-old with suspect TB (Comella-Del-Barrio *et al.*, 2021). Overall, this growing evidence shows that omics-based studies have a promising role in TB diagnosis and monitoring of treatment in the pediatric population; however, currently there is no consensus on the genes or molecules that should be investigated.

Extracellular vesicles

The analysis of cell-derived components can be also performed within extracellular vesicles (EVs), which can be found in different biological samples (Poulet *et al.* 2019). These nanoparticles, released by cells at early stages of stress, are being extensively researched as sensitive detectors of cellular injury (Abels; Breakefield, 2016). EVs are usually classified according to their size and biogenesis mainly in exosomes, microparticles (or microvesicles)

and apoptotic bodies; however, different types of EVs have been described in the recent literature (e.g. oncosomes, migrasomes, exopheres) (Jeppesen *et al.* 2023). Importantly, since EVs can mediate several biological mechanisms, including the cell-cell comunication, these structures are the focus of studies in different pathological contexts, such as cancer (Urabe *et al* 2020), cardiovascular disease (Burger e Touyz, 2012), renal disease (Medeiros *et al.* 2020), autoimmune and inflammatory disease (Lu *et al.* 2021) and infections, such as COVID-19 (Medeiros *et al.* 2022).

It is known that bacteria produce membrane-derived vesicles that can promote the trafficking of several molecules between cells and contribute to the pathogenesis of infections in humans. Besides, EVs mediate important roles to ensure bacterial survival and dissemination such as DNA transfer, biofilm production and resistance to antibiotics (Brown *et al.* 2015). Interestingly, studies have shown that the number of M. tuberculosis-derived EVs (*Mtb*-EVs) is elevated in low-iron conditions (Prados-Rosales *et al.* 2014), but these EVs present lower protein expression and immunogenic capacity (Schirmer *et al.* 2022). At the same time, the host cellular response to nocive stimuli can also lead to EV secretion, which are involved in defense mechanisms, especially in the immune response (Li *et al.* 2023). However, studies have demonstrated that there is a difference between the cargo of *Mtb*-EVs in comparison with those released by the infected host cells. The cargos of bacterial EVs include proteins, lipoproteins, lipids, carbohydrates, nucleic acid, toxins and metabolites (Li *et al.* 2023). Meanwhile, the EVs released by infected cells are rich in both mycobacterial and host RNA, lipids such as lipomannan and other lipoproteins, in addition to actively secreted proteins (Schorey, Chen; McManus, 2021).

As mentioned, *Mtb*-EVs have an important immunomodulatory effect, carrying antigens and interacting with immune cells such as DCs, macrophages and lymphocytes (Sun, Pi; Xu, 2021). For this, LAM, (especially the arabinomanan portion) acts a potent antigen (Ziegenbalg *et al.* 2013). Moreover, EVs released from infected cells can also promote intercellular communication. In this context, Alvarez-Jiménez and colleagues (2018) have reported that EVs produced by infected neutrophils carry ligands for toll-like receptors (TLRs) 2/6 and stimulate the expression of co-modulatory proteins and the production of proinflammatory cytokines by macrophages. In the same study, they found that these

macrophages are more efficient in eliminate *Mtb* through autophagy induction (Alvarez-Jiménez *et al.* 2018). Posteriorly, other studies have also identified that EVs released by *Mtb*-stimulated macrophages contribute to increased *Mtb* control (Cheng; Schorey, 2019; García-Martínez *et al.* 2019).

Despite the important effects promoted by *Mtb*-EVs to activate the immune response and fight the infection, they can also contribute to immune evasion through the suppression of IFN production, for example (Singh *et al.* 2011). Some may suggest that the type of the response promoted by EVs depend on their cargo and at which point of the infection they are secreted, but this requires further investigation. For example, Li *et al.* (2018) identified that serum EVs from infected mice, collected at 14 days after infection, but not at 7 and 21 days, affected endothelial permeability, chemokine (C-C motif) ligand2 (CCL-2) expression and gene regulation in vitro (Li *et al.* 2018). Nevertheless, the interaction with the immune response shed light to EVs as interesting candidates for vaccine and therapy. In this context, Cheng Schorey (2013) reported that mice treated intranasally with EVs from *Mtb*-infected macrophages developed a protective immune response, demonstrated by higher levels of pulmonary and splenic antigen-specific CD4+/CD8+ cells. Additionally, EVs derived from mesenchymal stem cells (MSC-EVs), which are known to promote beneficial effects in pathological conditions, has also been investigated in TB (Yan; Xu; Li, 2022) and rifampicin-loaded MSC-EVs present an antibacterial activity in vitro (Li *et al.* 2023).

In humans, EV detection in biological samples can be used as diagnostic and prognostic biomarkers. Since *Mtb* components can be found in EVs isolated from infected patients (Kruh-Garcia *et al.* 2014), they could be useful for TB diagnose using different types of samples and detection methods. In this context, it is important to mention that recommendations from the International Society for Extracellular Vesicles (ISEV) must be considered for study design and data interpretation. Since the number of studies in the EV field significantly increased in the last decade (Allan *et al.* 2020), we can find several methods for EV isolation and detection in the literature. Thus, in order to ensure data reproducibility to increase the quality of evidence, ISEV publishes guidelines such as the “Minimal information for studies of extracellular vesicles (MISEV)”, which is constantly updated since 2014 to promote standardization of sample

collection, reagent preparation, EV isolation, EV detection among other relevant parameters/methods of EV-based studies (Welsh *et al.* 2023).

Thus, considering the of EVs role as biomarkers in TB, studies have shown that total circulating EVs are significantly increased in TB patients and that they can induceIFN-γ expression in peripheral blood mononuclear cells (PBMC) and macrophage activation (Dornelas *et al.* 2021), in addition to promote cell death in a dose-dependent manner (Javadi *et al.* 2022). Furthermore, Dahiya and colleagues (2019) have found LAM and the culture filtrate protein-10 (CFP-10) from *Mtb* within urinary EVs from patients with pulmonary TB (PTB) and EPTB. EVs were also isolated from pleural effusion in TB patients (Luo *et al.* 2020). In pediatric TB, studies are scarce. A recent study has reported the presence of LAM and LprG protein in circulating EVs of children with TB with a diagnostic good accuracy (AUC >0.9) using a nanoparticle point-of-care immuno-based assay while the analysis of urinary LAM showed a poor performance in the cohort of HIV-infected children (74% vs. 37.5%) (Zheng *et al.* 2022). These results encourage new research to develop accessible methods to stablish EVs as biomarkers in TB disease.

Lastly, to identify the cargo of EVs in human samples, proteomic-based studies have been performed and some studies demonstrate different regulation of protein expression in active TB and LTBI (Ayra *et al.* 2020; Mehaffy *et al.* 2020). Recently, Zhou and colleagues (2022) identified the upregulation of genes related to the immune response in EVs from TB patients. Also, the analysis of miRNA, especially in exosomes isolated from TB patients, could also provide potential diagnostic markers (Alipoor *et al.* 2019; Lu *et al.* 2021). Thus, the analysis of EV content could be also useful for the differential diagnosis of malignancy (Luo *et al.* 2020).

CONCLUSION

This review summarizes the current laboratory diagnosis for pediatric TB and its future perspectives. Although this evaluation continues to be carried out mainly by clinical and radiological criteria, recent research focused on the analysis of different biofluids and cell-derived components has increased the possibility of new biomarkers for TB in children. Although promising, future studies should focus on the standardization of methodologies that

facilitate the collection of child-friendly samples and its application in clinical day-to-day practice.

CONTRIBUIÇÕES DOS AUTORES

Study design and planning: Carvalho, F. R.; Silva, A. A. **Manuscript drafting or review:** Carvalho, F. R.; Silva, A. A.; do Rosário, N. F.; Ramos-Silva, A.; Sant´Anna, C. C.; Cardoso, C. A. A. **Approval of the final version:** Carvalho, F. R.; Silva, A. A.; do Rosário, N. F.; Ramos-Silva, A.; Sant´Anna, C. C.; Cardoso, C. A. A. **Public responsibility for the content of the article:** Carvalho, F. R.; Silva, A. A.; do Rosário, N. F.; Ramos-Silva, A.; Sant´Anna, C. C.; Cardoso, C. A. A.

CONFLITO DE INTERESSE

The authors declare no conflict of interest.

APOIO FINANCEIRO

Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ - processes E-26/205.996/2022, E-26/205.997/2022).

DECLARAÇÃO DE IA GENERATIVA NA ESCRITA CIENTÍFICA

The authors declare that no generative AI was used in scientific writing.

REFERENCE

ABELS, E. R.; BREAKEFIELD, X. O. Introduction to Extracellular Vesicles: Biogenesis, RNA Cargo Selection, Content, Release, and Uptake. **Cellular and Molecular Neurobiology**, [S. l.], v. 36, n. 3, p. 301–312, abr. 2016.

AHMED, A. *et al.* Interferon-γ Release Assays in Children <15 Years of Age. **Pediatrics**, [S. l.], v. 145, n. 1, jan. 2020. Disponível em: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9301964/. Acesso em: 05 fev. 2024.

ALIPOOR, S. D. *et al.* Serum Exosomal miRNAs Are Associated with Active Pulmonary Tuberculosis. **Disease Markers**, [S. l.], v. 2019, p. 1907426, 2019.

ALLAN, D. *et al.* Mesenchymal stromal cell-derived extracellular vesicles for regenerative therapy and immune modulation: Progress and challenges toward clinical application. **Stem Cells Translational Medicine**, [S. l.], v. 9, n. 1, p. 39-46, jan. 2020.

ÁLVAREZ-JIMÉNEZ, V. D. *et al.* Extracellular Vesicles Released from Mycobacterium tuberculosis-Infected Neutrophils Promote Macrophage Autophagy and Decrease Intracellular Mycobacterial Survival. **Frontiers in Immunology**, [S. l.], v. 9, p. 272, 2018.

AMARAL, E. P. *et al.* The Interplay Between Systemic Inflammation, Oxidative Stress, and Tissue Remodeling in Tuberculosis. **Antioxidants & Redox Signaling**, [S. l.], v. 34, n. 6, p. 471–485, 20 fev. 2021.

AMERICAN ACADEMY OF PEDIATRICS. Committee on Infectious Diseases. **Red Book**: 2021–2024 Report of the Committee on Infectious Diseases. Itasca: American Academy of Pediatrics, 2021. Disponível em: https://publications.aap.org/redbook/book/347/Red-Book-2021-2024-Report-of-the-committee-on1. Acesso em: 05 fev. 2024.

AMIN, A. G. *et al.* Urine lipoarabinomannan in HIV uninfected, smear negative, symptomatic TB patients: effective sample pretreatment for a sensitive immunoassay and mass spectrometry. **Scientific Reports**, [S. l.], v. 11, n. 1, p. 2922, 03 fev. 2021.

ARAUJO, Z. *et al.* Study of the antibody response against Mycobacterium tuberculosis antigens in Warao Amerindian children in Venezuela. **Memórias do Instituto Oswaldo Cruz**, Rio de Janeiro, v. 99, n. 5, p. 517–524, ago. 2004.

ARMAND, M. *et al.* Cytokine responses to quantiferon peptides in pediatric tuberculosis: a pilot study. **Journal of Infection**, [S. l.], v. 68, n. 1, p. 62–70, jan. 2014.

ARYA, R. *et al.* Serum Small Extracellular Vesicles Proteome of Tuberculosis Patients Demonstrated Deregulated Immune Response. **Proteomics Clinical Applications**, [S. l.], v. 14, n. 1, p. e1900062, jan. 2020.

AUGENSTREICH, J. *et al.* Phthiocerol Dimycocerosates From Mycobacterium tuberculosis Increase the Membrane Activity of Bacterial Effectors and Host Receptors. **Frontiers in Cellular and Infection Microbiology**, [S. l.], v. 10, p. 420, 2020.

BRASIL. Ministério da Saúde. Secretaria de Vigilância em Saúde. **Boletim Epidemiológico de Tuberculose**: número especial. Brasília, DF: Ministério da Saúde, mar. 2023. Disponível em: https://www.gov.br/saude/pt-br/centrais-de-conteudo/publicacoes/boletins/epidemiologicos/especiais/2023/boletim-epidemiologico-de-tuberculose-numero-especial-mar.2023. Acesso em: 05 fev. 2024.

BROWN, L. *et al.* Through the wall: extracellular vesicles in Gram-positive bacteria, mycobacteria and fungi. **Nature Reviews Microbiology**, [S. l.], v. 13, n. 10, p. 620–630, out. 2015.

BURGER, D.; TOUYZ, R. M. Cellular biomarkers of endothelial health: microparticles, endothelial progenitor cells, and circulating endothelial cells. **Journal of the American Society of Hypertension**, [S. l.], v. 6, n. 2, p. 85–99, 2012.

CAMPBELL, J. I.; SANDORA, T. J.; HABERER, J. E. A scoping review of paediatric latent tuberculosis infection care cascades: initial steps are lacking. **BMJ Global Health**, [S. l.], v. 6, n. 5, p. e004836, 20 maio 2021.

CARVALHO, R. F. *et al.* Diagnosis of pulmonary tuberculosis in children and adolescents: comparison of two versions of the Brazilian Ministry of Health scoring system. **Revista do Instituto de Medicina Tropical de São Paulo**, São Paulo, v. 62, p. e81, 2020.

CENTERS FOR DISEASE CONTROL AND PREVENTION (CDC). **Tuberculosis (TB)**: TB Guidelines. Atlanta: CDC, 2020. Disponível em: https://www.cdc.gov/tb/publications/guidelines/default.htm. Acesso em: 05 fev. 2024.

CEPHEID. **Enabling Access to Molecular Diagnostic Testing Everywhere**. [S. l.: s. n.], [20--?]. Disponível em: https://www.cepheid.com/en-US.html. Acesso em: 05 fev. 2024.

CHENG, Y.; SCHOREY, J. S. Extracellular vesicles deliver Mycobacterium RNA to promote host immunity and bacterial killing. **EMBO Reports**, [S. l.], v. 20, n. 3, p. e46613, mar. 2019.

CHIANG, S. S. *et al.* Clinical standards for drug-susceptible TB in children and adolescents. **The International Journal of Tuberculosis and Lung Disease**, [S. l.], v. 27, n. 8, p. 584-598, 01 ago. 2023.

CICCHESE, J. M. *et al.* Dynamic balance of pro- and anti-inflammatory signals controls disease and limits pathology. **Immunological Reviews**, [S. l.], v. 285, n. 1, p. 147–167, set. 2018.

CLICK, E. S. *et al.* Performance of Xpert MTB/RIF and Mycobacterial Culture on Multiple Specimen Types for Diagnosis of Tuberculosis Disease in Young Children and Clinical Characterization According to Standardized Research Case Definitions. **The Pediatric Infectious Disease Journal**, [S. l.], v. 41, n. 8, p. 671–677, 01 ago. 2022.

COMELLA-DEL-BARRIO, P. *et al.* A Model Based on the Combination of IFN-γ, IP-10, Ferritin and 25-Hydroxyvitamin D for Discriminating Latent From Active Tuberculosis in Children. **Frontiers in Microbiology**, [S. l.], v. 10, p. 1855, 2019.

CORREIA-NEVES, M. *et al.* Lipoarabinomannan in Active and Passive Protection Against Tuberculosis. **Frontiers in Immunology**, [S. l.], v. 10, p. 1968, 2019.

COSTANTINI, L.; MARANDO, M.; GIANELLA, P. Long-Term GeneXpert Positivity after Treatment for Pulmonary Tuberculosis. **European Journal of Case Reports in Internal Medicine**, [S. l.], v. 7, n. 10, p. 001737, 2020.

COX, H. *et al.* Oral Swab Specimens Tested With Xpert MTB/RIF Ultra Assay for Diagnosis of Pulmonary Tuberculosis in Children: A Diagnostic Accuracy Study. **Clinical Infectious Diseases**, [S. l.], v. 75, n. 12, p. 2145–2152, 19 dez. 2022.

CRUZ-KNIGHT, W.; BLAKE-GUMBS, L. Tuberculosis: an overview. **Primary Care**, [S. l.], v. 40, n. 3, p. 743–756, set. 2013.

DAHIYA, B. *et al.* Detection of Mycobacterium tuberculosis lipoarabinomannan and CFP-10 (Rv3874) from urinary extracellular vesicles of tuberculosis patients by immuno-PCR. **Pathogens and Disease**, [S. l.], v. 77, n. 5, p. ftz049, 01 jul. 2019.

DELACOUR, H. *et al.* Analytical performances of the Diazyme ADA assay on the Cobas® 6000 system. **Clinical Biochemistry**, [S. l.], v. 43, n. 18, p. 1468–1471, dez. 2010.

DETJEN, A. K. *et al.* Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in children: a systematic review and meta-analysis. **The Lancet Respiratory Medicine**, [S. l.], v. 3, n. 6, p. 451–461, jun. 2015.

DINARDO, A. R. *et al.* Culture is an imperfect and heterogeneous reference standard in pediatric tuberculosis. **Tuberculosis**, Edinburgh, v. 101S, p. S105–8, dez. 2016.

DODD, P. J. *et al.* The global burden of tuberculosis mortality in children: a mathematical modelling study. **The Lancet Global Health**, [S. l.], v. 5, n. 9, p. e898–906, set. 2017.

DORNELAS MOREIRA, J. *et al.* Circulating microparticles from subjects with active pulmonary tuberculosis infection modulate immune response. **European Journal of Immunology**, [S. l.], v. 51, n. 5, p. 1282–1284, maio 2021.

DOS SANTOS, D. C. M. *et al.* Serological biomarkers for monitoring response to treatment of pulmonary and extrapulmonary tuberculosis in children and adolescents. **Tuberculosis**, Edinburgh, v. 123, p. 101960, jul. 2020.

DUBALE, M. *et al.* Stool-based Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in children at a teaching and referral hospital in Southwest Ethiopia. **PLoS One**, [S. l.], v. 17, n. 5, p. e0267661, 2022.

DUTTA, N. K. *et al.* Integration of metabolomics and transcriptomics reveals novel biomarkers in the blood for tuberculosis diagnosis in children. **Scientific Reports**, [S. l.], v. 10, n. 1, p. 19527, 11 nov. 2020.

ERNST, J. D. *et al.* Meeting report: The International Conference on Human Immunity to Tuberculosis. **Tuberculosis**, Edinburgh, v. 92, n. 5, p. 440–444, set. 2012.

ESTÉVEZ, O. *et al.* Identification of candidate host serum and saliva biomarkers for a better diagnosis of active and latent tuberculosis infection. **PLoS One**, [S. l.], v. 15, n. 7, p. e0235859, 2020.

GALAGAN, J. E. Genomic insights into tuberculosis. **Nature Reviews Genetics**, [S. l.], v. 15, n. 5, p. 307–320, maio 2014.

GARCÍA-MARTÍNEZ, M. *et al.* Extracellular vesicles released by J774A.1 macrophages reduce the bacterial load in macrophages and in an experimental mouse model of tuberculosis. **International Journal of Nanomedicine**, [S. l.], v. 14, p. 6707–6719, 2019.

GHANAIE, R. M. *et al.* Diagnosis of latent tuberculosis infection among pediatric household contacts of Iranian tuberculosis cases using tuberculin skin test, IFN- γ release assay and IFN-γ-induced protein-10. **BMC Pediatrics**, [S. l.], v. 21, p. 76, 2021.

GILANI, B.; SERGENT, S. R. **Interferon Test**. Treasure Island: StatPearls Publishing, 2024. Disponível em: http://www.ncbi.nlm.nih.gov/books/NBK560585/. Acesso em: 05 fev. 2024.

GİRİT, S. *et al.* Screening for Latent Tuberculosis in Children With Immune-mediated Inflammatory Diseases Treated With Anti-tumor Necrosis Factor Therapy: Comparison of Tuberculin Skin and T-SPOT Tuberculosis Tests. **Archives of Rheumatology**, [S. l.], v. 35, n. 1, p. 20–28, mar. 2020.

GRAHAM, S. M. *et al.* Clinical Case Definitions for Classification of Intrathoracic Tuberculosis in Children: An Update. **Clinical Infectious Diseases**, [S. l.], v. 61, supl. 3, p. S179-187, 15 out. 2015.

GÜNTHER, G. *et al.* Clinical Evaluation of a Line-Probe Assay for Tuberculosis Detection and Drug-Resistance Prediction in Namibia. **Microbiology Spectrum**, [S. l.], v. 10, n. 3, p. e0025922, 29 jun. 2022.

HAN, X. F. *et al.* Factors associated with negative pleural adenosine deaminase results in the diagnosis of childhood pleural tuberculosis. **BMC Infectious Diseases**, [S. l.], v. 21, n. 1, p. 473, 25 maio 2021.

HANSEN, J. *et al.* Lipobiotin-capture magnetic bead assay for isolation, enrichment and detection of Mycobacterium tuberculosis from saliva. **PLoS One**, [S. l.], v. 17, n. 7, p. e0265554, 2022.

HIRABAYASHI, R. *et al.* Utility of interferon-gamma releasing assay for the diagnosis of active tuberculosis in children: A systematic review and meta-analysis. **Journal of Infection and Chemotherapy**, [S. l.], dez. 2023. DOI: 10.1016/j.jiac.2023.12.007.

INBARAJ, L. R. *et al.* TB-LAMP (loop-mediated isothermal amplification) for diagnosing pulmonary tuberculosis in children. **Cochrane Database of Systematic Reviews**, [S. l.], v. 9, p. CD015806, 07 set. 2023.

JAVADI, A. *et al.* Extracellular vesicles from serum samples of mycobacteria patients induced cell death of THP-1 monocyte and PBMC. **BMC Pulmonary Medicine**, [S. l.], v. 22, n. 1, p. 57, 09 fev. 2022.

JAYAGANDAN, S. *et al.* Evaluation of Xpert MTB/RIF Assay on Stool Samples for the Diagnosis of Pulmonary Tuberculosis among the Pediatric Population. **Journal of Laboratory Physicians**, [S. l.], v. 15, n. 3, p. 329–335, set. 2023.

JENKINS, H. E. *et al.* Mortality in children diagnosed with tuberculosis: a systematic review and meta-analysis. **The Lancet Infectious Diseases**, [S. l.], v. 17, n. 3, p. 285–295, mar. 2017.

JENUM, S. *et al.* Added Value of IP-10 as a Read-Out of Mycobacterium tuberculosis: Specific Immunity in Young Children. **The Pediatric Infectious Disease Journal**, [S. l.], v. 35, n. 12, p. 1336–1338, dez. 2016.

JEPPESEN, D. K. *et al.* Extracellular vesicles and nanoparticles: emerging complexities. **Trends in Cell Biology**, [S. l.], v. 33, n. 8, p. 667–681, ago. 2023.

JIA, J. *et al.* Prevalence of Latent Tuberculosis Infection Among Healthy Young Children and Adolescents and a Two-step Approach for the Diagnosis of Tuberculosis Infection in Chengdu, China. **The Pediatric Infectious Disease Journal**, [S. l.], v. 41, n. 1, p. 6–11, jan. 2022.

JIMÉNEZ CASTRO, D. *et al.* Diagnostic value of adenosine deaminase in nontuberculous lymphocytic pleural effusions. **European Respiratory Journal**, [S. l.], v. 21, n. 2, p. 220–224, fev. 2003.

KABIR, S. *et al.* Xpert Ultra Assay on Stool to Diagnose Pulmonary Tuberculosis in Children. **Clinical Infectious Diseases**, [S. l.], v. 73, n. 2, p. 226–234, 15 jul. 2021.

KAMRA, E. *et al.* Diagnosis of urogenital tuberculosis by multiplex-nested PCR targeting mpt64 (Rv1980c) and IS6110: comparison with multiplex PCR and GeneXpert® MTB/RIF. **Letters in Applied Microbiology**, [S. l.], v. 75, n. 4, p. 857–868, out. 2022.

KASVOSVE, I. *et al.* Haptoglobin polymorphism and mortality in patients with tuberculosis. **The International Journal of Tuberculosis and Lung Disease**, [S. l.], v. 4, n. 8, p. 771–775, ago. 2000.

KAY, A. W. *et al.* Interferon-γ Release Assay Performance for Tuberculosis in Childhood. **Pediatrics**, [S. l.], v. 141, n. 6, p. e20173918, jun. 2018.

KAY, A. W. *et al.* Xpert MTB/RIF Ultra assay for tuberculosis disease and rifampicin resistance in children. **Cochrane Database of Systematic Reviews**, [S. l.], v. 9, n. 9, p. CD013359, 06 set. 2022.

KHAMBATI, N. *et al.* Host-Based Biomarkers in Saliva for the Diagnosis of Pulmonary Tuberculosis in Children: A Mini-Review. **Frontiers in Pediatrics**, [S. l.], v. 9, p. 756043, 2021.

KHAN, E. A.; STARKE, J. R. Diagnosis of tuberculosis in children: increased need for better methods. **Emerging Infectious Diseases**, [S. l.], v. 1, n. 4, p. 115–123, 1995.

KRUH-GARCIA, N. A. *et al.* Detection of Mycobacterium tuberculosis peptides in the exosomes of patients with active and latent M. tuberculosis infection using MRM-MS. **PLoS One**, [S. l.], v. 9, n. 7, p. e103811, 2014.

LEWINSOHN, D. M. *et al.* Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: Diagnosis of Tuberculosis in Adults and Children. **Clinical Infectious Diseases**, [S. l.], v. 64, n. 2, p. e1–33, 15 jan. 2017.

LI, C.; LIU, L.; TAO, Y. Diagnosis and treatment of congenital tuberculosis: a systematic review of 92 cases. **Orphanet Journal of Rare Diseases**, [S. l.], v. 14, n. 1, p. 131, 10 jun. 2019.

LI, G. *et al.* Long non-coding RNA expression in PBMCs of patients with active pulmonary tuberculosis. **Frontiers in Microbiology**, [S. l.], v. 14, p. 1257267, 2023.

LI, L. *et al.* Activation of endothelial cells by extracellular vesicles derived from Mycobacterium tuberculosis infected macrophages or mice. **PLoS One**, [S. l.], v. 13, n. 5, p. e0198337, 2018.

LI, Y. *et al.* The functions and applications of extracellular vesicles derived from Mycobacterium tuberculosis. **Biomedicine & Pharmacotherapy**, [S. l.], v. 168, p. 115767, dez. 2023.

LIEBESCHUETZ, S. *et al.* Diagnosis of tuberculosis in South African children with a T-cell-based assay: a prospective cohort study. **The Lancet**, [S. l.], v. 364, n. 9452, p. 2196–2203, 18 dez. 2004.

LIMA, S. V. M. A. *et al.* Spatial and temporal analysis of tuberculosis in an area of social inequality in Northeast Brazil. **BMC Public Health**, [S. l.], v. 19, p. 873, 04 jul. 2019.

LU, G. *et al.* Two Small Extracellular Vesicle sRNAs Derived From Mycobacterium tuberculosis Serve as Diagnostic Biomarkers for Active Pulmonary Tuberculosis. **Frontiers in Microbiology**, [S. l.], v. 12, p. 642559, 2021.

LU, M. *et al.* The Role of Extracellular Vesicles in the Pathogenesis and Treatment of Autoimmune Disorders. **Frontiers in Immunology**, [S. l.], v. 12, p. 566299, 2021.

LUNELLI, M. *et al.* Pleural tuberculosis: experiences from two centers in Brazil. **Jornal de Pediatria**, Rio de Janeiro, v. 98, n. 6, p. 621-625, nov./dez. 2022.

LUO, P. *et al.* Metabolic characteristics of large and small extracellular vesicles from pleural effusion reveal biomarker candidates for the diagnosis of tuberculosis and malignancy. **Journal of Extracellular Vesicles**, [S. l.], v. 9, n. 1, p. 1790158, 14 jul. 2020.

MAGDALENA, D. *et al.* Targeted metabolomics analysis of serum and Mycobacterium tuberculosis antigen-stimulated blood cultures of pediatric patients with active and latent tuberculosis. **Scientific Reports**, [S. l.], v. 12, n. 1, p. 4131, 08 mar. 2022.

MAGNI, R. *et al.* Lipoarabinomannan antigenic epitope differences in tuberculosis disease subtypes. **Scientific Reports**, [S. l.], v. 10, n. 1, p. 13944, 18 ago. 2020.

MANYELO, C. M. *et al.* Validation of host cerebrospinal fluid protein biomarkers for early diagnosis of tuberculous meningitis in children: a replication and new biosignature discovery study. **Biomarkers**, [S. l.], v. 27, n. 6, p. 549–561, set. 2022.

MAPHALLE, L. N. F. *et al.* Pediatric Tuberculosis Management: A Global Challenge or Breakthrough? **Children**, Basel, v. 9, n. 8, p. 1120, 27 jul. 2022.

MARAIS, B. J. Urine lipoarabinomannan testing in children with tuberculosis. **The Lancet Global Health**, [S. l.], v. 2, n. 5, p. e245-246, maio 2014.

MARTINS, C. DE P. *et al.* Vascular endothelial growth factor (VEGF) and interleukin-1 receptor antagonist (IL-1Ra) as promising biomarkers for distinguishing active from latent tuberculosis in children and adolescents. **Tuberculosis**, Edinburgh, v. 134, p. 102205, maio 2022.

MAYER-BARBER, K. D. Granulocytes subsets and their divergent functions in host resistance to Mycobacterium tuberculosis - a "tipping-point" model of disease exacerbation. **Current Opinion in Immunology**, [S. l.], v. 84, p. 102365, out. 2023.

MCNAB, F. *et al.* Type I interferons in infectious disease. **Nature Reviews Immunology**, [S. l.], v. 15, n. 2, p. 87–103, fev. 2015.

MEDEIROS, T. *et al.* Exploring Urinary Extracellular Vesicles and Immune Mediators as Biomarkers of Kidney Injury in COVID-19 Hospitalized Patients. **Diagnostics**, Basel, v. 12, n. 11, p. 2600, 27 out. 2022.

MEHAFFY, C. *et al.* Identification of Mycobacterium tuberculosis Peptides in Serum Extracellular Vesicles from Persons with Latent Tuberculosis Infection. **Journal of Clinical Microbiology**, [S. l.], v. 58, n. 6, p. e00393-20, 26 maio 2020.

MUÑOZ, L.; STAGG, H. R.; ABUBAKAR, I. Diagnosis and Management of Latent Tuberculosis Infection. **Cold Spring Harbor Perspectives in Medicine**, [S. l.], v. 5, n. 11, p. a017830, 08 jun. 2015.

NA, F. *et al.* Performance of adenosine deaminase in detecting paediatric pleural tuberculosis: a systematic review and meta-analysis. **Annals of Medicine**, [S. l.], v. 54, n. 1, p. 3129–3135, dez. 2022.

NGUYEN, H. V. *et al.* Discordant results of Xpert MTB/Rif assay and BACTEC MGIT 960 liquid culture to detect Mycobacterium tuberculosis in community screening in Vietnam. **BMC Infectious Diseases**, [S. l.], v. 22, n. 1, p. 506, 31 maio 2022.

O'GARRA, A. Systems approach to understand the immune response in tuberculosis: an iterative process between mouse models and human disease. **Cold Spring Harbor Symposia on Quantitative Biology**, [S. l.], v. 78, p. 173–177, 2013.

PASSOS, D. F. *et al.* Adenosine signaling and adenosine deaminase regulation of immune responses: impact on the immunopathogenesis of HIV infection. **Purinergic Signalling**, [S. l.], v. 14, n. 4, p. 309–320, 01 dez. 2018.

PAVAN KUMAR, N. *et al.* Circulating biomarkers of pulmonary and extrapulmonary tuberculosis in children. **Clinical and Vaccine Immunology**, [S. l.], v. 20, n. 5, p. 704–711, maio 2013.

PEDROZO, C. *et al.* Efficacy of the scoring system, recommended by the Brazilian National Ministry of Health, for the diagnosis of pulmonary tuberculosis in children and adolescents, regardless of their HIV status. **Jornal Brasileiro de Pneumologia**, [S. l.], v. 36, n. 1, p. 92–98, 2010.

PETRONE, L. *et al.* Blood or Urine IP-10 Cannot Discriminate between Active Tuberculosis and Respiratory Diseases Different from Tuberculosis in Children. **BioMed Research International**, [S. l.], v. 2015, p. 589471, 2015.

POULET, G.; MASSIAS, J.; TALY, V. Liquid Biopsy: General Concepts. **Acta Cytologica**, [S. l.], v. 63, n. 6, p. 449–455, 2019.

PRADOS-ROSALES, R. *et al.* Role for Mycobacterium tuberculosis membrane vesicles in iron acquisition. **Journal of Bacteriology**, [S. l.], v. 196, n. 6, p. 1250–1256, mar. 2014.

QUEIROZ, A. *et al.* Comparative metabolic profiling of mce1 operon mutant vs wild-type Mycobacterium tuberculosis strains. **Pathogens and Disease**, [S. l.], v. 73, n. 8, p. ftv066, nov. 2015.

RANA, S. V. *et al.* Adenosine deaminase levels in cerebrospinal fluid as a diagnostic test for tuberculous meningitis in children. **Indian Journal of Clinical Biochemistry**, [S. l.], v. 19, n. 2, p. 5–9, jul. 2004.

REYNOLDS, J.; MOYES, R. B.; BREAKWELL, D. P. Differential staining of bacteria: acid fast stain. **Current Protocols in Microbiology**, [S. l.], nov. 2009. DOI: 10.1002/9780471729259.mca03hs15.

RODO, M. J. *et al.* A comparison of antigen-specific T cell responses induced by six novel tuberculosis vaccine candidates. **PLoS Pathogens**, [S. l.], v. 15, n. 3, p. e1007643, mar. 2019.

SCHIRMER, S. *et al.* Immunogenicity of Mycobacterial Extracellular Vesicles Isolated From Host-Related Conditions Informs About Tuberculosis Disease Status. **Frontiers in Microbiology**, [S. l.], v. 13, p. 907296, 2022.

SCHMIDT, C. M. *et al.* Serum anti-Mce1A immunoglobulin detection as a tool for differential diagnosis of tuberculosis and latent tuberculosis infection in children and adolescents. **Tuberculosis**, Edinburgh, v. 120, p. 101893, jan. 2020.

SCHOREY, J. S.; CHENG, Y.; MCMANUS, W. R. Bacteria- and host-derived extracellular vesicles - two sides of the same coin? **Journal of Cell Science**, [S. l.], v. 134, n. 11, p. jcs256628, 01 jun. 2021.

SEDDON, J. A.; SHINGADIA, D. Epidemiology and disease burden of tuberculosis in children: a global perspective. **Infection and Drug Resistance**, [S. l.], v. 7, p. 153–165, 2014.

SEID, G. *et al.* Value of urine-based lipoarabinomannan (LAM) antigen tests for diagnosing tuberculosis in children: systematic review and meta-analysis. **IJID Regions**, [S. l.], v. 4, p. 97–104, set. 2022.

SETO, S. *et al.* Proteomic Profiling Reveals the Architecture of Granulomatous Lesions Caused by Tuberculosis and Mycobacterium avium Complex Lung Disease. **Frontiers in Microbiology**, [S. l.], v. 10, p. 3081, 2019.

SHAO, M. *et al.* Screening of potential biomarkers for distinguishing between latent and active tuberculosis in children using bioinformatics analysis. **Medicine**, Baltimore, v. 100, n. 5, p. e23207, 05 fev. 2021.

SHAO, Y.; HAGEMAN, J. R.; SHULMAN, S. T. Congenital and Perinatal Tuberculosis. **NeoReviews**, [S. l.], v. 22, n. 9, p. e600–605, set. 2021.

SHOLEYE, A. R. *et al.* Tuberculous Granuloma: Emerging Insights From Proteomics and Metabolomics. **Frontiers in Neurology**, [S. l.], v. 13, p. 804838, 2022.

SIGNORINO, C. *et al.* Diagnostic accuracy of Xpert ultra for childhood tuberculosis: A preliminary systematic review and meta-analysis. **Pediatric Allergy and Immunology**, [S. l.], v. 33, supl. 27, p. 80–82, jan. 2022.

SILVA, D. R. *et al.* Diagnosis of tuberculosis: a consensus statement from the Brazilian Thoracic Association. **Jornal Brasileiro de Pneumologia**, [S. l.], v. 47, n. 2, p. e20210054, 2021.

SILVA-PEREIRA, T. T.; SOLER-CAMARGO, N. C.; GUIMARÃES, A. M. S. Diversification of gene content in the Mycobacterium tuberculosis complex is determined by phylogenetic and ecological signatures. **Microbiology Spectrum**, [S. l.], p. e0228923, 17 jan. 2024.

SIMIENEH, A. *et al.* Combination of Xpert® MTB/RIF and DetermineTM TB-LAM Ag improves the diagnosis of extrapulmonary tuberculosis at Jimma University Medical Center, Oromia, Ethiopia. **PLoS One**, [S. l.], v. 17, n. 2, p. e0263172, 2022.

SINGH, P. P. *et al.* Exosomes released from M. tuberculosis infected cells can suppress IFN-γ mediated activation of naïve macrophages. **PLoS One**, [S. l.], v. 6, n. 4, p. e18564, 14 abr. 2011.

STEK, C. *et al.* The Immune Mechanisms of Lung Parenchymal Damage in Tuberculosis and the Role of Host-Directed Therapy. **Frontiers in Microbiology**, [S. l.], v. 9, p. 2603, 2018.

SUDBURY, E. L. *et al.* Mycobacterium tuberculosis-specific cytokine biomarkers for the diagnosis of childhood TB in a TB-endemic setting. **Journal of Clinical Tuberculosis and Other Mycobacterial Diseases**, [S. l.], v. 16, p. 100102, ago. 2019.

SUN, Y. F.; PI, J.; XU, J. F. Emerging Role of Exosomes in Tuberculosis: From Immunity Regulations to Vaccine and Immunotherapy. **Frontiers in Immunology**, [S. l.], v. 12, p. 628973, 2021.

TEBRUEGGE, M. *et al.* Mycobacteria-Specific Cytokine Responses Detect Tuberculosis Infection and Distinguish Latent from Active Tuberculosis. **American Journal of Respiratory and Critical Care Medicine**, [S. l.], v. 192, n. 4, p. 485–499, 15 ago. 2015.

TIEMERSMA, E. W. *et al.* Natural history of tuberculosis: duration and fatality of untreated pulmonary tuberculosis in HIV negative patients: a systematic review. **PLoS One**, [S. l.], v. 6, n. 4, p. e17601, 04 abr. 2011.

TORNHEIM, J. A. *et al.* Transcriptomic Profiles of Confirmed Pediatric Tuberculosis Patients and Household Contacts Identifies Active Tuberculosis, Infection, and Treatment Response Among Indian Children. **The Journal of Infectious Diseases**, [S. l.], v. 221, n. 10, p. 1647–1658, 27 abr. 2020.

TORNHEIM, J. A. *et al.* The Kynurenine/Tryptophan Ratio Is a Sensitive Biomarker for the Diagnosis of Pediatric Tuberculosis Among Indian Children. **Frontiers in Immunology**, [S. l.], v. 12, p. 774043, 2021.

URABE, F. *et al.* Extracellular vesicles as biomarkers and therapeutic targets for cancer. **American Journal of Physiology-Cell Physiology**, [S. l.], v. 318, n. 1, p. C29–39, 01 jan. 2020.

VAEZIPOUR, N. *et al.* Towards Accurate Point-of-Care Tests for Tuberculosis in Children. **Pathogens**, [S. l.], v. 11, n. 3, p. 327, 08 mar. 2022.

VANDEN DRIESSCHE, K. *et al.* Immune vulnerability of infants to tuberculosis. **Clinical and Developmental Immunology**, [S. l.], v. 2013, p. 781320, 2013.

VINHAES, C. L. *et al.* An Inflammatory Composite Score Predicts Mycobacterial Immune Reconstitution Inflammatory Syndrome in People with Advanced HIV: A Prospective International Cohort Study. **The Journal of Infectious Diseases**, [S. l.], v. 223, n. 7, p. 1275–1283, 08 abr. 2021.

WELSH, J. A. *et al.* Minimal information for studies of extracellular vesicles (MISEV2023): From basic to advanced approaches. **Journal of Extracellular Vesicles**, [S. l.], v. 13, n. 5, p. e12451, maio 2024.

WHITTAKER, E.; GORDON, A.; KAMPMANN, B. Is IP-10 a better biomarker for active and latent tuberculosis in children than IFNgamma? **PLoS One**, [S. l.], v. 3, n. 12, p. e3901, 2008.

WORLD HEALTH ORGANIZATION (WHO). **Global Tuberculosis Report 2023**. Geneva: WHO, 2023. Disponível em: https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2023. Acesso em: 05 fev. 2024.

WORLD HEALTH ORGANIZATION (WHO). **The End TB Strategy**. Geneva: WHO, [2015?]. Disponível em: https://www.who.int/teams/global-tuberculosis-programme/the-end-tb-strategy. Acesso em: 05 fev. 2024.

WORLD HEALTH ORGANIZATION (WHO). **WHO consolidated guidelines on tuberculosis**: module 5: management of tuberculosis in children and adolescents. Geneva: WHO, 2023. Disponível em: https://www.who.int/health-topics/tuberculosis. Acesso em: 05 fev. 2024.

XUE, L. J. *et al.* Mammalian cell entry protein of Mycobacterium tuberculosis induces the proinflammatory response in RAW 264.7 murine macrophage-like cells. **Tuberculosis**, Edinburgh, v. 87, n. 3, p. 185–192, maio 2007.

YAN, K.; XU, G.; LI, Z. MicroRNA-20b carried by mesenchymal stem cell-derived extracellular vesicles protects alveolar epithelial type II cells from Mycobacterium tuberculosis infection in vitro. **Infection, Genetics and Evolution**, [S. l.], v. 101, p. 105292, jul. 2022.

YAP, J. Y. *et al.* Intrinsic Defects in B Cell Development and Differentiation, T Cell Exhaustion and Altered Unconventional T Cell Generation Characterize Human Adenosine Deaminase Type 2 Deficiency. **Journal of Clinical Immunology**, [S. l.], v. 41, n. 8, p. 1915–1935, nov. 2021.

ZHENG, W. *et al.* Diagnosis of paediatric tuberculosis by optically detecting two virulence factors on extracellular vesicles in blood samples. **Nature Biomedical Engineering**, [S. l.], v. 6, n. 8, p. 979–991, ago. 2022.

ZHOU, R. *et al.* Diagnostic performance of adenosine deaminase for abdominal tuberculosis: A systematic review and meta-analysis. **Frontiers in Public Health**, [S. l.], v. 10, p. 938544, 2022.

ZIEGENBALG, A. *et al.* Immunogenicity of mycobacterial vesicles in humans: identification of a new tuberculosis antibody biomarker. **Tuberculosis**, Edinburgh, v. 93, n. 4, p. 448–455, jul. 2013.