Antibody Responses to Mycobacterial Antigens in Childhood Tuberculosis: Challenges and Potential Diagnostic Value

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Abstract

The identification of easily detectable biomarkers for active tuberculosis (TB) is a global health priority. Such biomarkers would be of particular value in childhood TB, which poses greater diagnostic challenges than adult TB. Serum antibodies can be detected by simple formats that provide extremely rapid results. However, attempts to develop accurate serodiagnostic tests for TB have been unsuccessful. Whereas antibody responses to mycobacterial antigens in adult TB have been studied extensively and reviewed, the same cannot be said for serologic data in pediatric populations. Here we appraise studies on serological responses in childhood TB and discuss findings and limitations in the context of the developing immune system, the age range, and the spectrum of TB manifestations. We found that the antibody responses to mycobacterial antigens in childhood TB can vary widely with sensitivities and specificities ranging from 14% to 85% and from 86% to 100%, respectively. We conclude that the limitations in serodiagnostic studies of childhood TB are manifold thereby restricting the interpretation of currently available data. Concerns about the methodology used in published studies suggest that conclusions about the eventual value of serodiagnosis cannot be made at this time. However, the available data suggest a potential adjunctive value for serology in the diagnosis of childhood TB. Despite the difficulties noted in this field there is optimism that the application of novel antigens and the integration of those factors which contribute to the serological responses in childhood TB can lead to useful future diagnostics.
Introduction

Active tuberculosis (TB) is a major cause of morbidity and mortality in children, especially in resource-limited countries, where children under the age of 15 years account for approximately 15 to 20% of the disease burden (19, 39). Infection with *Mycobacterium tuberculosis* in children is generally the consequence of household transmission from an adult incident case. Thus, it is not surprising that 75% of the estimated annual 1 million pediatric TB cases occur in the 22 high-burden countries (69). While adult TB is commonly due to reactivation, pediatric TB is typically a primary disease. In addition, there are considerable differences in host immune responses between adults and young children. The results are a more atypical clinical manifestation with a paucity of classical signs and symptoms in pediatric TB, resulting in considerably higher challenges to establish TB diagnosis than in adults.

In young children TB frequently disseminates and can be rapidly progressive early in life before immune competency is fully developed (36). Therefore, diagnostic delay quickly leads to increased morbidity and mortality, and rapid diagnosis becomes particularly important. However, the differences in disease manifestation of pediatric compared to adult TB result in reduced sensitivities for TB diagnostic tests. For example, cavitary disease is uncommon in children while up to 30% have extrapulmonary manifestations indicative of early disease dissemination (38). The yield of sputum smear microscopy, the most commonly used rapid test for adult TB, is 10-15%, and often less than 10%, in childhood TB which is substantially less than the yield in adults (about 50%) (17, 42). Even culture, the gold standard test for adult TB, detects a maximum of 30-40% of pediatric TB cases and in most settings the detection rate is below 20% (17, 42, 53). A recent study evaluating nucleic acid detection with the WHO endorsed test Xpert® MTB/RIF (Cepheid, CA, USA) in South African children demonstrated
improved sensitivity (13%) of this rapid method compared to sputum microscopy (6%), although mycobacterial culture remained slightly superior (16%) (42). To complicate matters further, young children often do not cough and even when they do, they are frequently unable to provide a sputum sample (70). Alternative specimens such as induced sputum or gastric aspirates are more difficult to collect and do not have a higher sensitivity (70). Plausibly, the low yield of specimens originating from the respiratory tract may also be due to the fact that much of the pediatric TB is lymphohematogenous rather than pulmonary parenchymal disease. Furthermore, unless children have significant peripheral lymphadenopathy, sampling of extrapulmonary tissue is commonly not feasible. Therefore, the optimal diagnostic test for pediatric TB should provide rapid results and utilize an easily accessible specimen independent from the site of disease, such as blood or urine.

The amplifying power of the systemic immune responses can potentially detect infection with *M. tuberculosis* at a low antigen-threshold and distant from the site of infection. Assays that detect *M. tuberculosis* infection by measuring interferon gamma release of circulating lymphocytes in response to *M. tuberculosis*-specific antigens (IGRAs) are more accurate than the Tuberculin skin-test (TST) (25). However, they require cell culture techniques that are not feasible in most resource-limited settings. The value of IGRAs in detecting LTBI and TB in children has been recently reviewed (60). In comparison to TST, IGRAs show similar sensitivity in detecting TB in children (70 – 90%) (15). However, the sensitivity of IGRAs for TB diminishes in low-income countries (40-80%), a discrepancy that has yet to be explained (15). There is insufficient data to adequately assess the performance of IGRAs in comparison to TST in children less than 5 yrs old. The studies that do exist show reduced rates of sensitivity and higher rates of indeterminate IGRAs in children under 5 yrs old (34). Most importantly, just as in
Detection of serum antibodies (Abs) is an attractive diagnostic option because it does not require a specimen from the site of disease and can be scaled up into robust and inexpensive formats requiring little laboratory infrastructure. Furthermore, Abs can be detected by simple “dip-stick” formats that provide extremely rapid results and thus could serve as point-of-care tests applicable in all settings (11, 46, 59). In contrast to IGRAs, Ab detection assays based on a variety of mycobacterial antigens can distinguish between LTBI and TB (reviewed in (62)). However, TB serology has suffered from decades of unsuccessful attempts to develop accurate tests for TB in adult as well as pediatric populations (63-65). Despite these setbacks there are continuing efforts to discover new mycobacterial antigens for potential serodiagnostic tests. Many mycobacterial antigens have been evaluated for the serodiagnosis of adult TB and that literature has been extensively reviewed elsewhere (1, 33, 62, 68). In contrast, data in pediatric populations are limited and, to our knowledge, have not been reviewed previously. The objective of this review is to appraise and discuss studies that have evaluated Abs to mycobacterial antigen in pediatric populations. We searched the electronic databases PubMed and Web of Science for articles in the English language published from 1980-2012. Keywords used included “tuberculosis”, “Mycobacterium tuberculosis”, “immunology”, “serology”, “diagnosis”, “serodiagnosis”, “antigen”, “antibody”, “immunoglobulin” which were referenced to all forms of “children”, “pediatric”, and “infants”. Additional studies were identified by searching the reference lists of primary studies and review articles. In contrast to systematic reviews and meta-analysis, we have not limited our search to certain study designs or enrollment numbers but address study limitations in the text. We limited our review to published studies that focused on the evaluation of serum Ab responses for diagnostic purposes.
The developing immune system in children

When interpreting serologic data in children, it is helpful to first understand how their immune system develops. Some of the challenges in pediatric serology can be explained by the slow development of the humoral immune responses in infants and young children. In newborns, a rapid increase of antigenic encounters elicits the production of immunoglobulin (Ig) M which later switches to the more protective isotypes IgG and IgA (44). Despite low levels of its own IgG, the infant is initially protected against infection by maternal Abs. Nursing infants have the benefit of receiving also IgA which is secreted into breast milk and provides protection from newly encountered bacteria in the gut. Maternal IgGs are commonly present up to 9 months of age depending on the disease type, but protective effects often decline after 6 months (55). Supporting evidence of a delayed humoral immune response comes from vaccination data in infants. Vaccines that elicit Ab responses to protein antigens, such as the toxins in pertussis and tetanus vaccine, are effective in eliciting an IgG1 subclass response but require multiple doses to increase the duration of protection if given before 6 months of age (56). Antibody responses to many polysaccharide antigens, which typically involves IgG2, are even lower in infants rendering them particularly susceptible to infections by encapsulated bacteria, such as Streptococcus pneumonia, H. influenza and M. tuberculosis (72). Overall, Ab levels comparable to those in adults are not reached until later in life with IgM reaching adult levels at age 2, IgG at age 6, and IgA not until the teenage years (51).

Serologic studies evaluating the diagnostic value of Ab responses to mycobacterial antigens in childhood TB
We found 23 studies evaluating Ab responses to mycobacterial antigens for their diagnostic value in childhood TB. Eight studies assessed commercially available serodiagnostic tests for adult TB (Table 1), the other 15 studies evaluated “in-house” Ab detection assays (Table 2). Overall, Ab responses to mycobacterial antigens in childhood TB varied widely with sensitivities and specificities ranging from 14% to 85% and from 86% to 100%, respectively.

Even when evaluating the same commercially available test such as the Anda-TB® Kit (Anda Biologicals, Strasbourg, France), sensitivities for detecting childhood TB ranged from 14% to 71% and specificities from 50% to 100% with part of the variability being due to the different isotypes tested (23, 24, 29, 66, 67) (Table 1). Such wide ranges in accuracy of serologic assays have also been observed in adult TB, although many of the reasons for this variability differ (reviewed in (62-64)). Several factors influence the accuracy of Ab detection assays for the serodiagnosis of TB in children. Most importantly, and in contrast to adult TB, the age of the child has the strongest impact on Ab responses regardless of antigen evaluated. Other important factors include the Ab isotype evaluated, the kind of antigen tested, how TB cases are defined and whether they are culture confirmed, the type of TB cases evaluated, a recent history of BCG vaccination, and how cut-off values for positive assays are determined. Thus, sensitivity and specificity values in serological studies of childhood TB cannot be interpreted without taking these factors into consideration.

**Mycobacterial antigens evaluated for serodiagnosis in childhood TB**

The rationale for selecting certain mycobacterial antigens for serodiagnostic studies of pediatric TB was typically based on their performance in adult TB. Hence, none of the antigens evaluated as of today was selected based on data in pediatric populations. As in adult TB serology, the earlier studies tested children’s Ab responses to crude mixtures of components
and products of mycobacteria, such as mycobacterial sonicates or purified protein derivative (PPD) (3, 4, 22, 48, 74). Mycobacterial sonicates are obtained from whole cell lysates and PPD, often also referred to as tuberculin, is obtained from filtrates of sterilized, concentrated cultures of *M. tuberculosis* or other mycobacteria. These preparations are mixtures of a large variety of native, non-specific mycobacterial antigens, including proteins, glycolipids and polysaccharides.

Studies assessing Ab responses to these antigen mixtures in children reported sensitivities ranging from 20% to 63% and specificities from 40% to 97% (3, 4, 22, 48, 74). Several other studies evaluated a combination of antigens or antigen complexes either in form of commercially available kits or in form of “in-house” enzyme-linked immunosorbent assays (ELISA), while others evaluated single antigens, such as mycobacterial proteins or glycolipids predominantly in form of “in-house” assays. A few studies also included the evaluation of immune complexes (54, 58).

A number of commercially available tests for TB serodiagnosis have been evaluated in children, mostly based on their performance in adult TB (Table 1). Specifically, these include the Anda-TB® Kit (Anda Biologicals, Strasbourg, France) which is the most frequently evaluated test for childhood TB. The Anda-TB® Kit is available for testing IgG, IgA and IgM responses to antigen 60 (A60), a highly immunogenic lipopolysaccharide-protein complex found primarily in the cytoplasm but also in the cell wall fractions of mycobacteria (12, 13). Another assay, the Immunozyme Mycobacterium® (Assay Designs, Ann Arbor, MI, USA) measures only IgG against A60. The A60 complex has about 30 components, of which some have been identified by monoclonal Abs (14). These components include LAM, a glycolipid of the mycobacterial cell wall, and proteins of various molecular weights, such as 65, 40, 38, 35, 19 and 14 kDa. The majority of the highly antigenic A60 complex proteins are found in most mycobacteria. Thus, similar to studies with PPD or mycobacterial sonicates, studies with A60-based assays evaluate
serological responses to a broad mixture of mainly non-specific native mycobacterial antigens which could result in considerable cross-reactivity. Five studies have evaluated IgG, IgA and IgM responses to A60 in children using the Anda-TB® Kit with reported sensitivities ranging from 14% to 71% and specificities from 50% to 100% (23, 24, 29, 66, 67) (Table 1).

Other commercial tests evaluated in childhood TB include the Pathozyme-TB Complex® and the Pathozyme-TB Complex Plus® (Omega Diagnostics, Alloa, Scotland) which measure IgG responses against the 38 kDa protein alone (Pathozyme-TB Complex®) or against both the 38 kDa and 16 kDa proteins combined (Pathozyme-TB Complex Plus®). The 38 kDa, previously also referred to as antigen 5 (Ag5), is an about 38 kDa protein present in the culture filtrates of *M. tuberculosis* and *M. bovis* (43). The 16 kDa protein belongs to the family of heatshock proteins and is a non-specific cytosolic mycobacterial protein that elicits Ab responses in early mycobacterial infection and disease. These two non-specific mycobacterial proteins have frequently been evaluated in serodiagnostic studies of adult TB and have been found to lack sensitivity and specificity (reviewed in (62, 64)). Seven studies assessed Ab responses to 38 kDa and/or 16 kDa in childhood TB, either in form of the Pathozyme-TB tests (3 studies; Table 1), or in form of “in-house” ELISA (4 studies, Table 2) (24, 52, 66). The reported sensitivities and specificities of these studies ranged from 25% to 45% and 73 to 90%, respectively. Further commercial assays evaluated included the Pathozyme Myco G® Myco A® and Myco M® (Omega Diagnostics, Alloa, Scotland) measuring IgG, IgA or IgM responses, respectively, to the 38 kDa protein and the glycolipid LAM, and the TBGL-Ab ELISA kit® (Kyowa Medex, Tokyo, Japan) measuring IgG and IgA against the glycolipid cell wall antigen trehalose-6-6-dimycolate (TBLG). Sensitivities and specificities reported for these studies varied as widely as those with other commercial tests (Table 1).
Ab responses to further mycobacterial antigens evaluated via “in-house” ELISA included:

i) antigen 5 (Ag5)/38 kDa protein (2); ii) the 30 kDa antigen, a culture filtrate protein, more commonly known as antigen 85B (47, 74); iii) fraction 4 and 5 of polar lipids belonging to the lipo-oligosaccharide family (LOS) (58); iv) 2,3-diacyl trehalose, formerly also referred to as SLIV antigen (DAT) (58); v) the glycolipid antigen triglycosyl phenol phthiocerol dimycocerosate (PGLTb1) (58); vi) the 16 kDa heatshock protein (30, 47); vii) the 60 kDa heatshock protein (HSP60) (3); viii) the excretory-secretory antigen (ES-31) (6); ix) the early secretory antigen 6 (ESAT-6) (21, 31); x) the antigen 85 complex consisting of the proteins Ag85A, 85B and 85C (Ag85 Complex) (20, 31); and xi) the culture-filtrate protein 10 (CFP10) (31). Of these antigens only ESAT-6 and CFP10 are M. tuberculosis complex specific while the other antigens can be found in several other mycobacteria. As in adult TB, Ab responses to ESAT-6 and CFP10 were lower than against several other antigens in pediatric TB (31, 57, 71). Sensitivities and specificities reported for the reported studies varied as widely (Table 2) influenced by the factors discussed in detail. Of note, most investigators evaluating “in-house” Ab detection assays used the same groups of cases and controls to determine cut-off values and estimate sensitivity and specificity values (30, 31, 47, 74). In addition, many studies lacked a description of their assays making data interpretation not possible (3, 6, 20, 21, 48).

Influence of age on serologic responses to mycobacterial antigens

The maturation of the humoral immune system in infants and young children results in age dependent differences in Ab responses to various antigens. Despite this well-known effect only a limited number of serodiagnostic studies have analyzed and compared data according to more narrow age groups. As can be anticipated, those that did, have, in general, found lower IgG responses in very young children, especially infants, compared to children over 5 yrs old (23, 24). For example, among BCG vaccinated children without TB, those older than 5 yrs had
significantly higher IgG reactivity to A60, a complex consisting of non-specific mycobacterial antigens, than those less than 5 yrs old (23). In concordance with these data, another study testing serological responses in children with TB via commercially available kits containing antigens such as 38 kDa, 16 kDa and LAM found that IgG responses were significantly higher in children over 5 yrs than under 5 yrs old (24). Also, IgA responses to these antigens were significantly higher in children 10 yrs and older compared to younger children (24). In contrast, IgM reactivity to several antigens varied widely in other studies with no significant differences between age groups and considerable overlap between TB cases and controls (23, 24). Overall, except for IgM, the Ab responses to all mycobacterial antigens evaluated in young children were much lower than those reported in adults. A few studies assessing Ab responses in children also included adult TB cases, allowing for comparison of responses using the same methods in the same lab. In such studies, IgG and IgA responses were significantly higher in adults than in children with TB regardless of the antigens tested (7, 54).

When evaluating the influence of age on Ab responses in young children the type of antigen must also be taken into consideration. Pilkington et al. studied the development of IgG responses to mycobacterial antigens in BCG unvaccinated children from the United Kingdom whose age ranged from 0 to over 10 yrs old (45). They tested IgG and IgG subclass responses to mycobacterial sonicates for a variety of slow and fast growing mycobacteria, including *M. tuberculosis*, to the lipopolysaccharide cell wall antigen LAM, and to non-specific 65-70 kDa heat shock proteins present in a variety of bacteria and mycobacteria. While IgG responses to mycobacterial sonicates were elevated during the first month of life, likely reflecting the transfer of maternal Abs, they decreased to almost undetectable levels between 1 and 23 months, and only started increasing after 24 months, with a slower continued increase into the first decade of life. Interestingly, when IgG responses to mycobacterial sonicates and LAM were compared,
they correlated strongly and significantly, suggesting that the predominant response to crude mycobacterial antigen preparations reflects mainly the response to LAM, or potentially to the mycobacterial capsular polysaccharide arabinomannan (AM). This, as well as the relationship between age and IgG response, accords with the observation that IgG2 accounted for almost all the IgG responses to mycobacterial sonicates. In contrast, IgG response to the 70 kDa heat shock protein of *M. tuberculosis* started rising in infants as early as 6 months of age. These data combined with data from the vaccine field demonstrate that the age influence on IgG responses in infants and young children varies according to antigen tested and likely reflect the differences in IgG subtype responses with delayed rise in IgG2 compared to other subtypes in infants and children under 2 yrs old.

**Spectrum of Ig isotype responses to mycobacterial antigens**

Most serological studies in children found that IgG, although often not the predominant isotype, was the most specific response against mycobacterial antigens. Studies including IgM evaluation frequently also found higher levels of IgM than IgG. However, most of those studies indicated little diagnostic value of IgM due to considerable overlap between pediatric TB cases and controls (4, 23, 24, 67). The overlap of IgM responses to mycobacterial antigens in TB cases and controls is likely to reflect the response to initial infection with mycobacteria, including environmental organisms, in early childhood regardless of progression to disease.

Typically relatively low IgA responses were seen in children with TB. Studies reporting higher IgA responses reported those in older rather than younger children (24). Nevertheless, some studies documented an increase in sensitivity when including IgA with other isotype testing. For example, Imaz et al. found a poor correlation between IgG and IgA responses to the
16 kDa protein (30). Combining the assays increased the overall sensitivity from 34% for IgG and 19% for IgA to 43% for both with only limited reduction in specificity. On the other hand, Gupta et al. found 55% sensitivity for IgM, 36% for IgA, and 33% for IgG against A60 in definite pediatric TB cases (29). They found a good correlation between IgG and IgA responses without major increase in sensitivity when combining these isotype responses. In contrast, when combining IgM and IgA detection assays the sensitivity increased to 72% with a reduction in specificity from the upper 90s to 92%.

Ab responses according to type of TB

With a few exceptions (30), the majority of studies found higher Ab responses to mycobacterial antigens in culture-confirmed than in non-confirmed TB cases. This is not surprising as in most settings only about 20% of childhood TB cases are culture-positive and establishing TB diagnosis in the remaining suspected cases is very challenging (17, 42, 53). Therefore, while culture-positive cases can be considered confirmed cases, culture-negative cases are probable and possible cases presenting a heterogeneous and potentially "overdiagnosed" group that could also include non-TB cases. It is also plausible that culture-negative cases present a more paucibacillary disease stage with resulting lower Ab responses in comparison to culture-positive cases. Several studies support of such causality. One pediatric study compared sensitivity between smear-positive (smear+) and smear- culture-confirmed cases, and found higher sensitivities for IgG against PPD in smear+ than smear- cases (63% versus 36%, respectively) (4). Another study evaluated IgG responses to Ag5 only in smear+ culture-confirmed cases and reported considerably higher sensitivities (86%) than other studies (2). Furthermore, commercially available kits testing IgG and IgA responses to 38 kDa and 16 kDa proteins, the glycolipid LAM, and the antigen complex A60 have shown significantly higher
sensitivities in cavitary compared to non-cavitary childhood TB (24). The higher Ab levels found in smear+ compared to smear- and in cavitary compared to non-cavitary childhood TB are consistent with data in adult TB and could reflect a more advanced stage of the disease with a potentially higher mycobacterial burden and possibly more inflammatory responses (reviewed in (62, 64) and (73)). However, when considering such causality one has to keep in mind that children under 10 yrs old rarely develop cavitary lesions that classically lead to smear-positivity (37). Thus, age could be a strong confounder contributing to the higher Ab responses in smear-positive and cavitary childhood TB.

The clinical presentation of childhood TB varies according to age with higher rates of dissemination in early childhood to a more adult-like presentation in adolescents (16, 26, 35, 37, 40, 50, 61), which is likely a reflection of the maturity of the immune system. Data indicate that Ab responses in children, just as in adults, vary according to clinical manifestations. Some studies evaluating Abs in different types of childhood TB found that responses in TB lymphadenitis and pulmonary TB were not significantly different (20, 29), while other studies found significantly lower Ab responses in TB lymphadenitis compared to pulmonary TB (24). However, Ab responses were usually lower in meningeal and pleural TB (20, 29). Furthermore, consistent with the data in children lower Ab responses to mycobacterial antigens have also been described in adults with pleural TB (49). On the other hand, the low Ab response in children with TB meningitis could also be due to the fact that this clinical manifestation is common in children under two yrs old (16). Nevertheless, Ab detection assays could have adjunctive value in detecting these forms of extrapulmonary TB due to the extremely low yield for culturing *M. tuberculosis* from cerebrospinal or pleural fluid. For example, Dayal et al. detected serum IgG responses against the mycobacterial glycolipid PGLTb1 and the protein ESAT6 in 7/16 (44%) and 9/16 (56%) children with TB meningitis, respectively, in contrast to a
positive cerebrospinal fluid culture in only 2/16 (13%). Thus, local Ab detection in such cases
might have additional diagnostic value.

Influence of BCG vaccination on Abs to mycobacterial antigens in children

Serological data in children reveal that the influence of BCG vaccination on Abs against
mycobacterial antigens depends on several factors, the type of antigen tested, the isotype
being evaluated, the timing between vaccination and Ab testing, and the children’s age at the
time of testing. A study assessing Ab responses of infants pre- and post BCG vaccination at age
1-2 mos found a significant increase in IgM against PPD in the first serum samples obtained 2
mos post vaccination and an increase in IgG against PPD 4 mos post vaccination with
continued increase until 15 mo after vaccination (5). Nevertheless, studies found a relatively
low impact of prior BCG vaccination on Ab responses in children under 2 yrs old. For example,
IgG reactivity against Ag60 was relatively low in children under 2 yrs old, regardless of prior
BCG vaccination, and the difference between vaccinated and unvaccinated children was
negligible (23). In contrast, the difference of IgM reactivity to Ag60 between vaccinated and
unvaccinated children under 2 yrs old was larger and significant (23). In general, although a few
studies have observed mild to moderate differences in Ab responses in children according to
history of BCG vaccination, a higher number of studies testing a variety of mycobacterial
antigens have not seen any effect regardless of isotype and age group evaluated (2, 24, 29,
74).

Limitations of and aspects to consider in serological studies of childhood TB
Several major study limitations must be taken in consideration when evaluating the data published on serological responses in childhood TB. First, most studies evaluated children with a broad age range (up to 0 – 14 yrs old), and with few exceptions, did not analyze their data according to more narrow age groups. Such subgroup analysis is necessary in serological studies of children because a part of the humoral immune response does not mature to adult levels until about 5 yrs old (51). Infants less than 2 yrs old have considerably lower IgG responses, especially to polysaccharide antigens, than children over 2 yrs old (reviewed in (72)). Furthermore, during the first year of life, serum IgG is likely to reflect the maternal Abs transferred in utero. In addition, the clinical presentation of TB varies according to age with higher rates of dissemination in early childhood to a more adult-like presentation in adolescents (16, 26, 35, 37, 40, 50, 61). Taking each of these aspects into account, it would be most sensible to categorize children into age groups such as: i) less than 1 yr; 1-2 yrs; 2-5 yrs; over 5-10 yrs; and over 10 yrs old when conducting future TB serology studies.

Second, due to the tremendous challenges in establishing a diagnosis, TB case definitions in children require a thorough description of symptoms, radiologic imaging and diagnostic test results. Many methodological issues for conducting and reporting studies on diagnostics for pediatric TB have been identified recently (18). Stringent criteria for clinical case definitions of intrathoracic pediatric TB were defined and published by an expert panel this year (28). According to a variety of clinical and diagnostic criteria the authors propose to categorize pediatric TB into “confirmed”, “probable”, “possible” and “unlikely” TB cases. Many of the studies evaluating the potential value of Ab detection assays in children lack a detailed description on how TB was defined and some lacked even information on the proportion of culture-confirmed cases. Thus, the groups evaluated in serologic studies included varying proportions of more or less likely TB cases. This considerable heterogeneity of TB groups, in addition to the wide age
range of children being evaluated, complicates the comparison and interpretation of serological data in childhood TB tremendously.

Third, many reported studies evaluating “in-house” Ab detection assays lacked a description of their assays (3, 6, 20, 21, 48, 58). Furthermore, the use of crude mycobacterial antigen mixtures in Ab detection assays by many investigators introduced considerable data variability. In addition, several investigators used the same groups of cases and controls to determine cut-off values and estimate sensitivity and specificity values (30, 31, 47, 74). This approach, although valid in pilot studies, limits the reproducibility of data and is likely to have contributed to the wide variability of reported Ab responses. Also, the majority of studies analyzed and presented their data in mean values of Ab titers despite a large variation in Ab responses that were not normally distributed. As a result, reported values of cases and controls were often driven by a few subjects with high Ab titers complicating the interpretation of sensitivity and specificity values given. Lastly, studies rarely described the inclusion of immunocompromised children, and if they did, the numbers were as low as 2-3 subjects without data described separately (58). Thus, Ab responses in overtly immunocompromised children, such as those infected with HIV, are unknown.

Although some Ab responses in infants and very young children are generally lower than in older children and adults, several Ab detection assays for the diagnosis of other infectious diseases during childhood exist. Many of these serologic tests are based on detecting disease specific IgM and/or rising IgG titers for the diagnosis of acute infections such as rubella, measles or hepatitis B (8-10). In infants and neonates, IgM detection utilizing capture ELISAs or immunoblots has proven useful for the diagnosis of measles and congenital toxoplasmosis,
respectively (27, 32). In contrast, most serological studies in childhood TB used indirect ELISAs for Ab detection, which might not be the optimal method for all age groups.

Conclusions

Serological responses to mycobacterial antigens in childhood TB vary widely with sensitivities and specificities ranging from 14% to 85% and from 86% to 100%, respectively. This wide variability is driven by several factors that have not been properly integrated into the study design and data analysis. Most importantly, the children’s age has the strongest impact on Ab responses regardless of antigen evaluated, and the majority of studies did not categorize their analysis by more narrow age groups. Other important factors with an impact on accuracy of serodiagnostic tests for childhood TB are the Ab isotype evaluated, the kind of antigens tested, how TB cases are defined, the type of TB cases evaluated, and how cut-off values for positive assays are determined. Given all the problems identified with the published studies the conclusion from this review is that no conclusion can be made at this time about the eventual value of serodiagnosis in childhood TB.

In general, studies demonstrated a stronger humoral immune response to TB in children 5 years and older and those with evidence of definite or more extensive disease, such as culture-positive and or smear-positive TB. Nevertheless, given the extremely low sensitivity of microbiologic confirmation in pediatric TB, particularly in young children, many Ab detection assays could have a potential adjunctive value in the TB diagnosis of most children. Such value is supported by the generally higher values in specificity estimates in childhood compared to adult TB and the existence of sensitive serologic assays for other childhood diseases regardless of age.
In summary, given all the difficulties in diagnosing pediatric TB, serology remains a very attractive diagnostic approach. Future studies with immunodominant mycobacterial antigens in age defined subgroups are needed to establish the usefulness of serology in diagnosis and move the field forward. Currently, there is no data supporting the necessity that such antigens need to be M. tuberculosis-specific but further studies to assess this are warranted. A critical concern in the design of future serodiagnostic studies is to appreciate and integrate the basic differences in immune responses and pathophysiology of TB between adults and children. Until such studies are done the potential of serodiagnosis in pediatric TB will remain uncertain.

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Table 1. Studies evaluating accuracy of commercially available serodiagnostic tests for TB in children

<table>
<thead>
<tr>
<th>Antigen (Assay)</th>
<th>Isotype</th>
<th>Age (yrs)</th>
<th>Subjects (n)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Comments</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>A60 (Anda-TB®)</td>
<td>IgG, IgM</td>
<td>0 - 11</td>
<td>TB (31) [culture+ (14)] Healthy/TST+ (16) Other diseases (198)</td>
<td>IgG: 71% for culture+ TB 65% for culture- TB IgM: 19%</td>
<td>IgG: 100% IgM: 100%</td>
<td>Small subgroups of TB cases when categorized by more narrow age groups</td>
<td>(23)</td>
</tr>
<tr>
<td>A60 (Anda-TB®)</td>
<td>IgG, IgM</td>
<td>2 - 12</td>
<td>TB (29) [no culture info] Healthy/TST- (28) Other Diseases/TST- (53) Healthy/BCG vaccinated (9) Healthy/recent TST+ (35) Old TB (23) Adenitis due to other mycobacteria (11)</td>
<td>IgG: 14% IgM: 24%</td>
<td>IgG and IgM: 94%-100%; 74% in old TB; 91% in adenitis due to other mycobacteria</td>
<td>No information on Mtb culture results</td>
<td>(67)</td>
</tr>
<tr>
<td>Test</td>
<td>Sample Description</td>
<td>Range</td>
<td>TB (n) [Diagnosis]</td>
<td>Healthy (n)</td>
<td>IgG (A60): %</td>
<td>IgM (A60): %</td>
<td>IgG (38 kDa): %</td>
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<td>--------------</td>
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</tr>
<tr>
<td>A60 (Anda-TB®) IgG, IgM, IgA</td>
<td>1 - 12</td>
<td>TB (208) [culture or clinically confirmed] Probable TB (244) Healthy (93) Healthy TB contacts (15) Other diseases (53)</td>
<td></td>
<td></td>
<td>IgG: 32-48% depending on cut-off value</td>
<td>IgG: 32%</td>
<td>IgA: 36-38%</td>
</tr>
<tr>
<td>A60 38 kDA (Anda-TB® &amp; Pathozyme-TB Complex®) IgG &amp; IgM (A60); IgG (38 kDa)</td>
<td>1 - 12</td>
<td>TB (42) [pulmonary (35), lymphadentitis (7)] Healthy (22)</td>
<td></td>
<td>IgG (A60): 29%</td>
<td>IgM (A60): 71%</td>
<td>IgG (38 kDa): 45%</td>
<td></td>
</tr>
<tr>
<td>PPD HSP60 38 kDa 16 kDa (In-house ELISA &amp; Pathozyme - Myco G® &amp; TB Complex Plus®) IgG, IgA, IgE</td>
<td>0 - 15</td>
<td>TB (34) [poorly defined] Healthy (46) [32 TST+, 14 TST-]</td>
<td></td>
<td>IgG (PPD): 38%</td>
<td>IgA (PPD): 27%</td>
<td>IgE (PPD): 32%</td>
<td>IgG (HSP60): 38%</td>
</tr>
</tbody>
</table>

(29)

(66)

(3)
<table>
<thead>
<tr>
<th>Test</th>
<th>Antigen(s)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Additional Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>A60</td>
<td>IgG, IgA, IgM</td>
<td>0% for &lt; 10 yrs, 42% for &gt; 10 yrs</td>
<td>Low specificity for all 5 assays tested, no clear value given</td>
<td>Mixtures of antigens tested in commercial kits (24)</td>
</tr>
<tr>
<td>38 kDa</td>
<td>[38 kDa]: 14% for &lt; 10 yrs, 36% for &gt; 10 yrs</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>16 kDa</td>
<td>[38 kDa+16 kDa]: 27% for &gt; 10 yrs</td>
<td></td>
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<td></td>
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<tr>
<td>LAM</td>
<td>[38 kDa+LAM]: 13% for &lt; 10 yrs, 27% for &gt; 10 yrs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 - 18</td>
<td>IgG (38 kDa): 0% for &lt; 10 yrs, 42% for &gt; 10 yrs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TB (81)</td>
<td>IgG (38 kDa): 0% for &lt; 10 yrs, 42% for &gt; 10 yrs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[culture+ (25/81); pulmonary (31); lymphadenitis (41); other extrapulmonary (40)]</td>
<td>IgG (38 kDa+LAM): 13% for &lt; 10 yrs, 27% for &gt; 10 yrs</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TST+ (30)</td>
<td>IgA (38 kDa+LAM): 3% for &lt; 10 yrs, 15% for &gt; 10 yrs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy &amp; other diseases (82)</td>
<td>IgM (38 kDa+LAM): 24% for &lt; 10 yrs, 50% for &gt; 10 yrs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All subjects previously BCG vaccinated</td>
<td>A60 (IgG): 0% for &lt; 10 yrs, 25% for &gt; 10 yrs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TB (81)</td>
<td>IgG (38 kDa): 0% for &lt; 10 yrs, 42% for &gt; 10 yrs</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>IgG (38+16 kDa): 14% for &lt; 10 yrs, 36% for &gt; 10 yrs</td>
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<td></td>
</tr>
<tr>
<td>IgG (38 kDa+LAM): 13% for &lt; 10 yrs, 27% for &gt; 10 yrs</td>
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<td></td>
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<tr>
<td>IgA (38 kDa+LAM): 3% for &lt; 10 yrs, 15% for &gt; 10 yrs</td>
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<td></td>
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<tr>
<td>IgM (38 kDa+LAM): 24% for &lt; 10 yrs, 50% for &gt; 10 yrs</td>
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<td></td>
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<tr>
<td>A60 (IgG): 0% for &lt; 10 yrs, 25% for &gt; 10 yrs</td>
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<tr>
<td>TB cases poorly defined; No information on age distribution (52)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>TBGL</td>
<td>IgG</td>
<td>90%</td>
<td>Low sample numbers; No information on age distribution (54)</td>
<td></td>
</tr>
<tr>
<td>(TBGL-Ab®ELISA kit)</td>
<td>0 - 15</td>
<td>TB (32) [poorly defined; pulmonary (24), extrapulmonary (8)]</td>
<td>Low specificity for all 5 assays tested, no clear value given</td>
<td>Mixtures of antigens tested in commercial kits (24)</td>
</tr>
<tr>
<td>IgG: 25%</td>
<td>IgG: 90%</td>
<td>[Sensitivity 60% in culture+, 18% in culture-TB]</td>
<td></td>
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<tr>
<td>Healthy (20)</td>
<td>Healthy (24)</td>
<td></td>
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<tr>
<td>Other diseases (20)</td>
<td></td>
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<tr>
<td>TB (23) [all culture+]</td>
<td>Low IgG and IgA responses in cases and controls with no significant differences.</td>
<td>No values given</td>
<td></td>
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<tr>
<td>Healthy (24)</td>
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<tr>
<td>Low sample numbers; No information on age distribution (54)</td>
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</tbody>
</table>
1: Country where samples were obtained; Antigens (in chronological order): A60: Antigen 60, complex consisting of various proteins and the glycolipid lipoarabinomannan (LAM); 38 kDa: 38 kDa culture filtrate protein, also known as antigen 5; PPD: purified protein derivative; HSP60: 60 kDa heatshock protein; 16 kDa: 16 kDa heatshock protein; LAM: glycolipid lipoarabinomannan; TBGL: glycolipid trehalose-6-6-dimycolate; Commercially available assays: Anda-TB ® Kit (IgG, IgA and/or IgM against antigen 60 (A60); Pathozyme-TB Complex® (IgG against 38 kDa) and Pathozyme-TB Complex Plus® (IgG against 38 kDa and 16 kDa; Omega Diagnostics, Alloa, Scotland); Pathozyme MycoG ®, MycoA ® and MycoM ® (IgG, IgA and IgM against 38 kDa and LAM; Omega Diagnostics, Scotland); X: study evaluated Immunozyme Mycobacterium® (IgG against A60; Assay Designs, Ann Arbor, MI, USA); Pathozyme-TB Complex Plus®, Pathozyme MycoG®, MycoA® and MycoM®; TBGL-Ab ELISA kit ® (IgG and IgA against TBGL; Kyowa Medex, Tokyo, Japan); TST: Tuberculin skin-test
Table 2. Studies evaluating mycobacterial antigens with “in-house” Ab detection assays for serodiagnostic value in childhood TB

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Isotype</th>
<th>Age (yrs)</th>
<th>Subjects (n)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Comments</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPD</td>
<td>IgG, IgM</td>
<td>0 – 14</td>
<td>TB (163) [culture+ (49/163)] Healthy/not TST tested (17) Healthy/TST+ (38) Healthy/TST- (20) Other diseases (38)</td>
<td>IgG: 51% for culture+ TB (63% for smear+; 36% for smear- TB); 28% for culture- TB Lower sensitivities with similar high specificity of IgM</td>
<td>IgG: 98% in other diseases than TB; 88% in TST+ and 90% in TST- household contacts</td>
<td>No categorization and comparison of results according to more narrow age ranges</td>
<td>(4)</td>
</tr>
<tr>
<td>Ag51</td>
<td>IgG</td>
<td>1 - 14</td>
<td>TB (21) [all smear+ and culture+] Healthy TB contacts (19) [all controls BCG vaccinated]</td>
<td>IgG: 86%</td>
<td>IgG: 100%</td>
<td>No categorization and comparison of results according to more narrow age ranges; High sensitivity likely due to evaluating only smear+ cases</td>
<td>(2)</td>
</tr>
<tr>
<td>Mycobacterial sonicates</td>
<td>IgG</td>
<td>&lt; 5 yrs</td>
<td>TB (31) Healthy (129)</td>
<td>IgG: 21%</td>
<td>IgG: 40%</td>
<td>No description of ELISA method</td>
<td>(48)</td>
</tr>
<tr>
<td>Old Tuber-culin PPD 30 kDa</td>
<td>IgG</td>
<td>0 - 12</td>
<td>TB (72) [no info on culture results] Healthy [188] [BCG vaccinated 0-5 yrs old [83], BCG revaccinated 6-12 (104)]</td>
<td>IgG (PPD): 50% IgG (30-kDa): 36%</td>
<td>IgG (PPD): 97%</td>
<td>No comment on how TB cases were diagnosed (74)</td>
<td></td>
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<tr>
<td>LOS DAT PGLTb1 IgG 0 - 18</td>
<td>TB (12) [pulmonary (7), lymphadenitis (5)] Healthy TST+ (8) TB Contacts (7) Other diseases (26)</td>
<td>IgG: 34% for culture+ TB, 33% for culture- TB</td>
<td>IgG: 86%</td>
<td>No description of ELISA method; Small sample size (58)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 kDa IgG, IgM, IgA</td>
<td>0 - 14</td>
<td>TB (74) [culture+ (29/74); pulmonary (70), extrapulmonary (4)] Healthy TB Contacts (49) Other Diseases (149)</td>
<td>IgG: 34% for culture+ and culture- TB IgA: 19% IgM: 3% IgG and/or IgA: 43%</td>
<td>All isotypes: 95% in other diseases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>Isotype</td>
<td>Age</td>
<td>Sample Description</td>
<td>Specificity</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>16 kDa</td>
<td>IgG, IgM, IgA</td>
<td>&lt; 15</td>
<td>TB (26) [smear and/or culture+ (9), lymphadenitis (3), other extrapumonary TB (14)] Healthy TST- (61)</td>
<td>IgG and/or IgM and/or IgA (30 kDa): 85% IgG and/or IgM and/or IgA (16 kDa): 73% All isotypes, both proteins: 98%</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>30 kDa</td>
<td>IgG</td>
<td>No age info</td>
<td>TB (70); no info how TB was diagnosed [pulmonary (30), meningitis (20), pleural (10), lymphadenitis (10)] Healthy (50) Other diseases (105)</td>
<td>IgG: 81% IgG: 93%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPD 38 kDa</td>
<td>Salivary IgA</td>
<td>0 - 15</td>
<td>TB (31) [TB cases poorly defined] Healthy/TST+ (53) Healthy TST- (43)</td>
<td>Salivary IgA (38kDa): 26% for TST+; 93% for TST- Salivary IgA (PPD): 8% for TST+; 95% for TST-</td>
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</tbody>
</table>

Graphs appear not consistent with specificity claimed for all isotypes combined; No info given on age distribution.

Poor description of ELISA method; No age information; No info on how TB was diagnosed.

TB cases poorly defined.

TB cases poorly defined.
<table>
<thead>
<tr>
<th>Ag85 Complex</th>
<th>IgG</th>
<th>0 - 18</th>
<th>Suspected TB [83] (Pulmonary [64; 17/64 culture+]; meningitis [19; 3/19 culture+])</th>
<th>IgG: 59%</th>
<th>No description of ELISA method; TB cases poorly defined; No information on age distribution (20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESAT6 PGLTb1</td>
<td>IgG</td>
<td>&lt; 18</td>
<td>Two separate studies described For anti-PGLTb1 Abs: TB (65) [culture+ (10/65); pulmonary (32), lymphadenitis (13), meningitis (16); GI (4)] Controls (27) [not specified] For anti-ESAT6 Abs: TB (83); culture+ (29/83) [pulmonary (37); lymphatic (19); CNS (16); GI (11)] Controls, not specified (27)</td>
<td>IgG (ESAT6): 49% IgG (PGLTb1): 53%</td>
<td>IgG: 69% IgG (PGLTb1): 91%</td>
</tr>
<tr>
<td>Ag85 Complex</td>
<td>IgG</td>
<td>Age &lt;18</td>
<td>TB(88) [pulmonary (36), lymphadenitis (20), abdominal (11), meningitis (20); military (1)]</td>
<td>Healthy (25)</td>
<td>Other diseases (17)</td>
</tr>
</tbody>
</table>

1: Country where samples were obtained; Antigens (in chronological order): PPD: purified protein derivative; A5: Antigen 5, 38 kDa culture filtrate protein, also known as 38 kDa; 30 kDa: 30 kDa culture filtrate protein, more commonly known as antigen 85B; LOS: fraction 4 and 5 of polar lipids belonging to the lipo-oligosaccharide family; DAT: 2,3-diacyl trehalose, formerly also referred to as SLIV antigen; PGLTb1: glycolipid triglycosyl phenol phthiocerol dimycocerosate glycolipid antigen; HSP60: 60 kDa heat shock protein 60; ES-31: excretory-secretory antigen; ESAT6: Early secretory antigen 6; Ag85 Complex: Antigen 85 complex consisting of the proteins Ag85A, 85B and 85C; CFP10: culture-filtrate protein 10; ATT: antituberculous therapy; TST: Tuberculin skin-test.