

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

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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.

Active tuberculosis (TB) is a major cause of morbidity and mortality in children, especially in resource-limited countries, in which 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 1 million annual 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 and adult TB result in reduced sensitivities for TB diagnostic tests. For example, cavitory 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 to 15%, and often less than 10%, in childhood TB, an amount 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 to 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) in South African children demonstrated improved sensitivity (13%) of this rapid method compared to that of sputum microscopy (6%), although mycobacterial culture remained slightly superior (16%) (42). To com-

plete 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 many of the pediatric TB cases are 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 gamma interferon 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 latent TB infection (LTBI) and TB in children has been recently reviewed (60). In comparison to the TST, IGRAs show similar sensitivity in detecting TB in children (70 to 90%) (15). However, the sensitivity of IGRAs for TB diminishes in low-income countries (40 to 80%), a discrepancy that has yet to be explained (15). There are insufficient data to adequately assess the

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performance of IGRAs in comparison to the TST in children less than 5 years old. The studies that do exist show reduced rates of sensitivity and higher rates of indeterminate IGRAs in children under 5 years old (34). Most importantly, just as in adults, neither the TST nor IGRAs can distinguish TB disease from the asymptomatic state of infection, LTBI (reviewed in reference 41).

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 can 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 reference 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, 32, 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 to 2012. Keywords used included “tuberculosis,” “*Mycobacterium tuberculosis*,” “immunology,” “serology,” “diagnosis,” “serodiagnosis,” “antigen,” “antibody,” and “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 M (IgM), 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 also receiving 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 involve IgG2, are even

lower in infants, rendering them particularly susceptible to infections by encapsulated bacteria, such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, 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 reaching adult levels at age 6, and IgA not reaching adult levels until the teenage years (51).

SEROLOGIC STUDIES EVALUATING THE DIAGNOSTIC VALUE OF ANTIBODY 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), and 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 ranged from 50% to 100%, with part of the variability being due to the different isotypes tested (22, 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 references 62 to 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 case evaluated, a recent history of *Mycobacterium bovis* BCG vaccination, and how cutoff 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, 23, 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, nonspecific 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 ranging from 40% to 97% (3, 4, 23, 48, 74). Several other studies evaluated a combination of antigens or antigen complexes, either in the form of commercially available kits or in the form of in-house enzyme-linked immunosorbent assays (ELISA), while others evaluated single antigens, such as mycobacterial pro-

TABLE 1 Studies evaluating accuracy of commercially available serodiagnostic tests for TB in children^a

Antigen (assay)	Isotype	Age (yr)	Subject group (no. of subjects)	Sensitivity	Specificity	Comments	Reference
A60 (Anda-TB)	IgG, IgM	0–11	TB (31) (14 culture+), healthy/TST+ (16), other diseases (198)	IgG, 71% for culture+ TB and 65% for culture– TB; IgM, 19%	IgG, 100%; IgM, 100%	Small subgroups of TB cases when categorized by more-narrow age groups	22
A60 (Anda-TB)	IgG, IgM	2–12	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)	IgG, 14%; IgM, 24%	IgG and IgM, 94–100%; 74% in old TB; 91% in adenitis due to other mycobacteria	No information on <i>M. tuberculosis</i> culture results	67
A60 (Anda-TB)	IgG, IgM, IgA	1–12	TB (208) (culture or clinically confirmed), probable TB (244), healthy (93), healthy/TB contacts (15), other diseases (53)	IgG, 32–48% depending on cutoff value; IgA, 36–38%; IgM, 55–57%; IgM and/or IgA, 82%	IgG, 87–97% depending on cutoff value; IgM and/or IgA, 92%	No categorization and comparison of results according to more-narrow age ranges; much higher IgM responses than in other studies with A60	29
A60, 38 kDa (Anda-TB and Pathozyme-TB Complex)	A60, IgG and IgM; 38 kDa, IgG	1–12	TB (42) (35 pulmonary, 7 lymphadenitis), healthy (22)	IgG (A60), 29%; IgM (A60), 71%; IgG (38 kDa), 45%	IgG (A60), 86%; IgM (A60), 50%; IgG (38 kDa), 73%	No comment on how TB cases were diagnosed and no culture results given; no analysis according to more-narrow age groups	66
PPD, HSP60, 38 kDa, 16 kDa (in-house ELISA, Pathozyme-Myco G, TB Complex Plus)	IgG, IgA, IgE	0–15	TB (34) (poorly defined), healthy (46) (32 TST+, 14 TST–)	IgG (PPD), 38%; IgA (PPD), 27%; IgE (PPD), 32%; IgG (HSP60), 38%; IgG (38 kDa and LAM), 20%; IgG (38 kDa and 16 kDa), 20%	IgG (PPD), 96%; IgA (PPD), 93%; IgE (PPD), 77%; IgG (HSP60), 96%; IgG (38 kDa and LAM), 100%; IgG (38 kDa and 16 kDa), 100%	Unclear how cutoff values were defined; rationale for testing IgE not addressed	3
A60, 38 kDa, 16 kDa, LAM (5 commercial tests) ^b	IgG, IgA, IgM	0–18	TB (81) (25 culture+, 31 pulmonary, 41 lymphadenitis, 40 other extrapulmonary); TST+ (30); healthy and other diseases (82)	IgG (38 kDa), 0% for <10 yrs and 42% for >10 yrs; IgG (38 kDa and 16 kDa), 14% for <10 yrs and 36% for >10 yrs; IgG (38 kDa and LAM), 13% for <10 yrs and 27% for >10 yrs; IgA (38 kDa and LAM); 3% for <10 yrs and 15% for >10 yrs; IgM (38 kDa and LAM), 24% for <10 yrs and 50% for >10 yrs; IgG (A60), 0% for <10 yrs and 25% for >10 yrs	Low specificity for all 5 assays tested; no clear value given	Mixtures of antigens tested in commercial kits; all subjects previously BCG vaccinated	24
16 kDa, 38 kDa (Pathozyme-TB Complex Plus)	IgG	0–15	TB (32) (poorly defined); 24 pulmonary, 8 extrapulmonary, healthy (20), other diseases (20)	25% (60% in culture+ TB, 18% in culture– TB)	90%	TB cases poorly defined; no information on age distribution	52
TBLG (TBLG-Ab ELISA kit)	IgG, IgA	≤12	TB (23) (all culture+), healthy (24)	Low IgG and IgA responses in cases and controls, with no significant differences	No values given	Low sample numbers; no information on age distribution	54

^a A60, antigen 60, complex consisting of various proteins and the glycolipid lipoarabinomannan; 38 kDa, 38-kDa culture filtrate protein, also known as antigen 5; PPD, purified protein derivative; HSP60, 60-kDa heat shock protein; 16 kDa, 16-kDa heat shock protein; LAM, the glycolipid lipoarabinomannan; TBLG, glycolipid trehalose-6-6-dimycolate; Anda-TB Kit, IgG, IgA, and/or IgM against antigen 60; Pathozyme-TB Complex, IgG against the 38-kDa protein; Pathozyme-TB Complex Plus, IgG against the 38-kDa and 16-kDa proteins (Omega Diagnostics, Alloa, Scotland); Pathozyme MycoG, MycoA, and MycoM, IgG, IgA, and IgM against the 38-kDa protein and LAM (Omega Diagnostics, Scotland); TBLG-Ab ELISA kit, IgG and IgA against TBLG (Kyowa Medex, Tokyo, Japan); TST, tuberculin skin test; TST+, positive TST; TST–, negative TST.

^b Study evaluated Immunozyme Mycobacterium (IgG against A60; Assay Designs, Ann Arbor, MI).

TABLE 2 Studies evaluating mycobacterial antigens with in-house Ab detection assays for serodiagnostic value in childhood TBP^a

Antigen	Isotype	Age (yr)	Subject group (no. of subjects)	Sensitivity	Specificity	Comments	Reference
PPD	IgG, IgM	0–14	TB (163) (49 culture+), healthy/not TST tested (17), healthy/TST+ (38), healthy/TST– (20), other diseases (38)	IgG, 51% for culture+ TB (63% for smear+ TB, 36% for smear– TB); 28% for culture– TB; lower sensitivities with similar high specificity of IgM	IgG, 98% in diseases other than TB and 88% in TST+ and 90% in TST– household contacts	No categorization and comparison of results according to more-narrow age ranges	4
Ag5	IgG	1–14	TB (21) (all smear+ and culture+), healthy TB contacts (19) (all controls BCG vaccinated)	86%	100%	No categorization and comparison of results according to more-narrow age ranges; high sensitivity likely due to evaluating only smear+ cases	2
Mycobacterial sonicates Old tuberculin, PPD; 30 kDa	IgG	<5 yrs 0–12	TB (31), healthy (129) TB (72) (no info on culture results), healthy (188) (83 BCG vaccinated and 0–5 yrs old, 104 BCG vaccinated and 6–12 yrs old)	21%	40%	No description of ELISA method; small sample size	48
LOS, DAT, PGLTb1	IgG	0–18	TB (12) (7 pulmonary, 5 lymphadenitis), healthy TST+ (8), TB contacts (7), other diseases (26)	34% for culture+ TB, 33% for culture– TB	86%	No description of ELISA method; small sample size	58
16 kDa	IgG, IgM, IgA	0–14	TB (74) (29 culture+, 70 pulmonary, 4 extrapulmonary), healthy TB contacts (49), other diseases (149)	IgG, 34% for culture+ and culture– TB; IgA, 19%; IgM, 3%; IgG and/or IgA, 43%	For all isotypes, 95% for subjects with other diseases	No description of ELISA method; small sample size	30
16 kDa, 30 kDa	IgG, IgM, IgA	<15	TB (26) (9 smear+ and/or culture+, 3 lymphadenitis, 14 other extrapulmonary TB), healthy TST– (61)	IgG and/or IgM and/or IgA (30 kDa), 83%; IgG and/or IgM and/or IgA (16 kDa), 73%	For all isotypes and both proteins, 98%	Graphs appear to be not consistent, with specificity claimed for all isotypes combined; no information given on age distribution	47
ES-31	IgG	No age information	TB (70) (no information on how TB was diagnosed; 30 pulmonary, 20 meningitis, 10 pleural, 10 lymphadenitis), healthy (50), other diseases (105)	81%	93%	Poor description of ELISA method; no age information; no information on how TB was diagnosed	6
PPD, 38 kDa	Salivary IgA	0–15	TB (31) (TB cases poorly defined), healthy/TST+ (53), healthy/TST– (43)	Salivary IgA (38 kDa), 26%; salivary IgA (PPD), 8%	Salivary IgA (38 kDa), 88% for TST+ and 93% for TST–; salivary IgA (PPD), 94% for TST+ and 95% for TST–	TB cases poorly defined	23
ESAT6, PGLTb1	IgG	<18	Two separate studies described: for anti-PGLTb1 Abs, TB (65) (10 culture+, 32 pulmonary, 13 lymphadenitis, 16 meningitis, 4 GI), controls (27) (not specified); for anti-ESAT6 Abs, TB (83) (29 culture+, 37 pulmonary, 19 lymphatic, 16 CNS, 11 GI), controls (27) (not specified)	IgG (ESAT6), 49%; IgG (PGLTb1), 53%	IgG (ESAT6), 96%; IgG (PGLTb1), 91%	No description of ELISA method; no information on controls; no information on age distribution	21
Ag85 complex	IgG	0–18	Suspected TB (83) (64 pulmonary [17 culture+, 19 meningitis [3 culture+]]; healthy, mostly BCG vaccinated (32)	59%	72%	No description of ELISA method; TB cases poorly defined; no information on age distribution	20
Ag85 complex, Ag85A, Ag85B, Ag85C, ESAT-6, CFP10	IgG	<18	TB (88) (36 pulmonary, 20 lymphadenitis, 11 abdominal, 20 meningitis, 1 military), healthy (25), other diseases (17)	IgG (Ag85 complex), 56%; IgG (85A), 32%; IgG (85B), 65%; IgG (85C), 90%; IgG (CFP10), 30%; IgG (all Ags and ESAT 6), 67%	IgG (Ag85 complex), 72%; IgG (85A), 24%; IgG (85B), 64%; IgG (85C), 92%; IgG (CFP10), 80%; IgG (all Ags and ESAT 6), 80%	No information on age distribution	31

^a PPD, purified protein derivative; Ag5, 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, fractions 4 and 5 of polar lipids belonging to the lipo-oligosaccharide family; DAT, 2,3-diacetyl trehalose, formerly also referred to as 2,3-diacetyl-trehalose-2'-sulfate (SLIV) antigen; PGLTb1, glycolipid triglycosyl phenol phthiocerol dimycoserolate 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; smear+, smear positive; smear–, smear negative.

teins or glycolipids, predominantly in the 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 Immunozyne Mycobacterium (Assay Designs, Ann Arbor, MI), 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 masses, 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 nonspecific native mycobacterial antigens, which may 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 ranging from 50% to 100% (22, 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 protein, previously also referred to as antigen 5 (Ag5), is an approximately 38-kDa protein present in the culture filtrates of *M. tuberculosis* and *M. bovis* (43). The 16-kDa protein belongs to the family of heat shock proteins and is a nonspecific cytosolic mycobacterial protein that elicits Ab responses in early mycobacterial infection and disease. These two nonspecific mycobacterial proteins have frequently been evaluated in serodiagnostic studies of adult TB and have been found to lack sensitivity and specificity (reviewed in references 62 and 64). Seven studies assessed Ab responses to the 38-kDa and/or 16-kDa protein in childhood TB, either in the form of the Pathozyme-TB tests (3 studies; Table 1) or in the form of an in-house ELISA (4 studies; Table 2) (24, 52, 66). The reported sensitivities and specificities of these studies ranged from 25% to 45% and from 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, and 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 an in-house ELISA included (i) antigen 5 (Ag5)/the 38-kDa protein (2), (ii) the 30-kDa antigen, a culture filtrate protein, more commonly known as antigen 85B (47, 74), (iii) fractions 4 and 5 of polar lipids belonging to the lipo-oligosaccharide family (LOS)

(58), (iv) 2,3-diacetyl trehalose, formerly also referred to as 2,3-diacetyl-trehalose-2'-sulfate (SLIV) antigen (DAT) (58), (v) the glycolipid antigen triglycosyl phenol phthiocerol dimycoserate (PGLTb1) (58), (vi) the 16-kDa heat shock protein (30, 47), (vii) the 60-kDa heat shock 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) 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 those against several other antigens in pediatric TB (31, 57, 71). Sensitivities and specificities for the reported studies varied 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 cutoff 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 years old (22, 24). For example, among BCG-vaccinated children without TB, those older than 5 years had significantly higher IgG reactivity to A60, a complex consisting of nonspecific mycobacterial antigens, than those less than 5 years old (22). In concordance with these data, another study testing serological responses in children with TB via commercially available kits containing antigens, such as the 38-kDa protein, the 16-kDa protein, and LAM, found that IgG responses were significantly higher in children over 5 years than under 5 years old (24). Also, IgA responses to these antigens were significantly higher in children 10 years and older than in 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 (22, 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 years 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 nonspecific 65- to 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 the response 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 the antigen tested and likely reflects the differences in IgG subtype responses, with a delayed rise in IgG2 compared to other subtypes in infants and children under 2 years old.

SPECTRUM OF IMMUNOGLOBULIN 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, 22, 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 them 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 a 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 a 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%.

ANTIBODY 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 nonconfirmed 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 may also include non-TB cases. It is also plausible that culture-negative cases present a more paucibacillary disease stage with resulting lower Ab responses compared to culture-positive cases. Several studies support such causality. One pediatric study compared sensitivity between smear-positive (smear+) and smear-negative (smear-) culture-confirmed cases and found higher sensitivities for IgG against PPD in smear+ cases than in 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 cavitory than in noncavitory childhood TB (24). The higher Ab levels found in smear+ cases than in smear- cases and in cavitory cases than in noncavitory childhood TB are consistent with data in adult TB and may reflect a more advanced stage of the disease with a potentially higher mycobacterial burden and possibly more inflammatory responses (reviewed in references 62, 64, and 73). However, when considering such causality, one has to keep in mind that children under 10 years old rarely develop cavitory lesions that classically lead to smear positivity (37). Thus, age may be a strong confounder contributing to the higher Ab responses in smear-positive and cavitory childhood TB.

The clinical presentation of childhood TB varies according to age, with higher rates of dissemination in early childhood and 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 than in 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 may also be due to the fact that this clinical manifestation is common in children under two years old (16). Nevertheless, Ab detection assays may 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. (21) 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 ANTIBODIES 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 before and after BCG vaccination at an age of 1 to 2 months found a significant increase in IgM against PPD in the first serum sam-

ples obtained 2 months postvaccination and an increase in IgG against PPD 4 months postvaccination, with a continued increase until 15 months after vaccination (5). Nevertheless, studies found a relatively low impact of prior BCG vaccination on Ab responses in children less than 2 years old. For example, IgG reactivity against Ag60 was relatively low in children less than 2 years old, regardless of prior BCG vaccination, and the difference between vaccinated and unvaccinated children was negligible (22). In contrast, the difference in IgM reactivity to Ag60 between vaccinated and unvaccinated children less than 2 years old was larger and significant (22). 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 into 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 to 14 years 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 years of age (51). Infants less than 2 years old have considerably lower IgG responses, especially to polysaccharide antigens, than children more than 2 years old (reviewed in reference 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 and 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 year, (ii) 1 to 2 years, (iii) 2 to 5 years, (iv) 5 to 10 years, and (v) over 10 years 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 of how TB was defined and some lacked even information on the proportion of culture-confirmed cases. Thus, the groups evaluated in serologic studies included various 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 consider-

able data variability. In addition, several investigators used the same groups of cases and controls to determine cutoff 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 to 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 those 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 or congenital toxoplasmosis, respectively (27, 33). 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 antigen tested, how TB cases are defined, the type of TB case evaluated, and how cutoff 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 may 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 than in 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 are 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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