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Immune-based diagnostics for TB in children: what is the evidence?

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SUMMARY

Childhood TB is difficult to diagnose, since disease tends to be paucibacillary and sputum specimens are not easy to obtain in children. Thus, blood-based immune assays are an attractive option. Systematic reviews of serological assays suggest that these tests produce highly inconsistent estimates of sensitivity and specificity, but much of the serology literature is based on adults. In children, there is insufficient evidence to recommend the use of serological tests for active TB diagnosis. Interferon-gamma release assays (IGRA) do not offer substantial improvements in sensitivity over the TST for the diagnosis of active disease. For latent TB infection, the IGRA correlates well with the exposure gradient and seems to have utility in reducing the number of children who undergo preventive therapy due to false-positive TST. Although IGRAs can be used as evidence of TB infection in children, appropriate specimen collection and microbiological confirmation of TB disease should remain a priority.

INTRODUCTION

There is increasing awareness that children account for a substantial part of the global tuberculosis (TB) epidemic.[1] However, the diagnosis of childhood TB is notoriously challenging.[2,3] Microbiological confirmation is often not available due to the paucibacillary nature of disease and difficulty of specimen (especially sputum) collection in younger children. The diagnosis usually relies on non-specific clinical and radiological signs as well as history of exposure (i.e. close contact with a TB case).[4] Furthermore, the consequences of missed diagnosis in children are severe, as untreated children have a high probability of developing active TB, usually within two years of infection.[5]

A rapid and accurate tool for diagnosing childhood TB would be highly beneficial. Much attention has been focused on immune-based assays that do not rely on sputum, but can be done with blood, even finger-prick, specimens. Two classes of immune-based assays are now available (Figure 1): those based on humoral (antibody) immune response and those based on cell-mediated immunity.

Serological tests measure humoral immunity and detect the binding of antibodies to *M. tuberculosis* antigens in serum. Their intended use is for active TB diagnosis (pulmonary and extra-pulmonary). The quick turn-around time and ease of use make them potential replacements for smear microscopy. Rapid test versions are inexpensive, and dozens of commercial kits are on the market. Although no serological test has been approved by regulatory agencies for TB diagnosis and no international guidelines recommend their use, they are aggressively marketed in many parts of the world, especially in developing countries with weak regulatory systems.[6] In some countries (e.g. India), the market for commercial serological tests far exceeds that for conventional microbiological tests (e.g. smear and culture).

Interferon-gamma release assays (IGRA) have been developed to replace the tuberculin skin test (TST) for detection of latent TB infection (LTBI). Their intended use is not for active TB. The TST is widely used in children as evidence of TB infection but requires a 3-dimensional interpretation. It is known to give false-positive results due to the BCG vaccine (especially when vaccination is done post-infancy and when multiple doses are given) and nontuberculous mycobacteria (NTM).[7] The IGRA measures the T-cell response to antigens encoded within the region of difference-1 (RD1) of the *M. tuberculosis* genome, which are absent from all BCG strains and most NTM.[8] Many national guidelines in low-incidence countries have already approved their use in conjunction with the TST for the diagnosis of TB in children.[9–13]

Several free online resources are now available. A web-based, online algorithm (www.tstin3d.com) can be used for interpretation of TST and IGRA results. Another new website on "Evidence-based TB Diagnosis" (www.tbevidence.org) provides additional resources on TB diagnostics, including guidelines, systematic reviews, training materials, and standard operating procedures. A third website, the World BCG Atlas, provides detailed information

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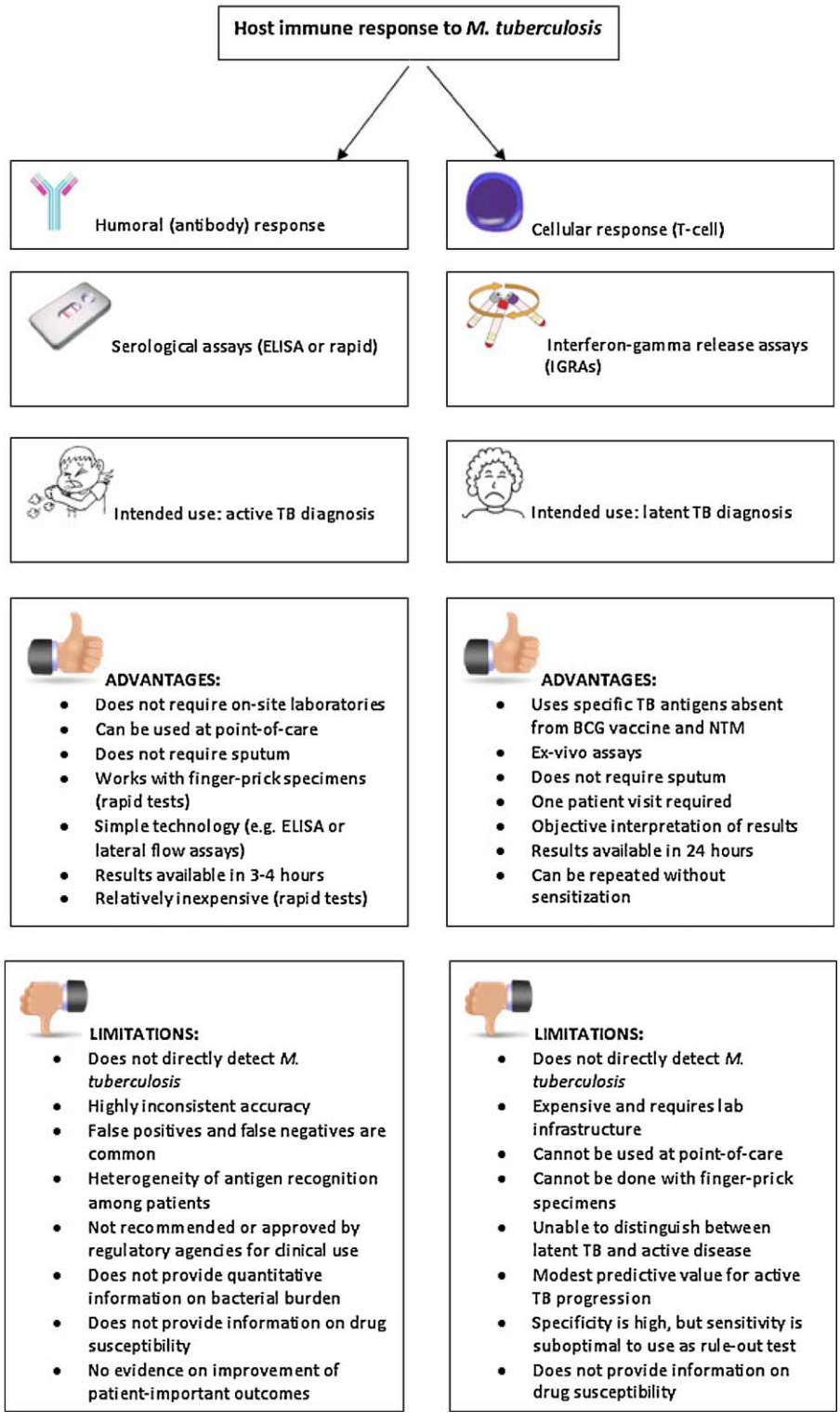

Figure 1. Immune-based assays for TB and their advantages and limitations.

on BCG policies and practices in countries across the world (www.bcgatlas.org). Lastly, the Child TB Subgroup of the Stop TB Partnership's New Diagnostics Working Group has created a website focusing on childhood TB (www.ndwgchildsubgroup.org).

SEROLOGICAL TESTS

Serological tests vary in a number of features, including antigen composition [commonly used antigens are 38 kDa, Ag 60, and lipoarabinomannan (LAM)], antigen source (e.g. native or recombinant), chemical composition (e.g. protein or lipid), extent of purification of the antigen(s), and immunoglobulin detected. The majority are based on the enzyme-linked immunosorbent assay (ELISA) technique, and rapid versions use various immunochromatographic formats, with lateral flow being the most popular.

The development of serological tests for TB diagnosis has been attempted for decades. Three systematic reviews by Steingart and colleagues have synthesized the extensive literature evaluating

Table 1
Findings from four systematic reviews and meta-analyses of serological tests

Review	Total studies (# of paediatric studies)	Principal findings
Commercial tests for pulmonary TB[16]	68 (0)	Sensitivity varied widely from 10-90%; Specificity varied widely from 47-100%
Commercial tests for extrapulmonary TB[15]	21 (0)	Sensitivity varied widely from 0-100%; Specificity varied widely from 59-100%
In-house tests for pulmonary TB[14]	254 (1)	No antigen has sufficient sensitivity to replace smear microscopy; multiple antigens have higher sensitivity than single antigens
In-house tests for childhood TB[21]	13 (13)	Sensitivity varied widely from 26-88%; Specificity was >90% in all but one study

commercial and in-house serological tests for pulmonary and extrapulmonary TB.[14–16] These reviews found highly variable sensitivity and specificity in adults and a paucity of data in children, likely due to the inclusion of only studies with microbiologically-confirmed cases. The systematic review of in-house tests[14] identified just a single article on serodiagnosis in children. Imaz and colleagues assessed an ELISA test measuring IgG, IgM and IgA antibodies to the recombinant 16-kDa antigen.[17] The sensitivity ranged from 3-34%. Furthermore, all three systematic reviews discussed shortcomings in study design and methodological quality, especially the use of case-control designs and lack of blinding, elements that may result in exaggerated estimates of test accuracy.[18,19]

In 2005, the WHO/Special Programme for Research and Training in Tropical Diseases (TDR) performed an evaluation of 19 commercially available rapid diagnostic TB tests (i.e. test result available in <15 minutes). This evaluation found poor and highly variable accuracy. In comparison with culture plus clinical follow-up, sensitivity and specificity values were 1–60% and 53-99%, respectively.[20]

In a review focused exclusively on TB serodiagnosis in children, Lagrange and colleagues described 13 studies evaluating ELISA-based serological tests.[21] In most studies, TB diagnosis was based on clinical and radiological features and treatment response. Except for one study, the specificity of tests containing a variety of antigens was >90%. The sensitivity was inconsistent, ranging from 26-88%. The studies in this review did not involve children with HIV infection. In a recent study, Stavri and colleagues found that the sensitivity of an in-house ELISA in children co-infected with TB/HIV was only 11%.[22]

Thus far, the evidence from published studies and systematic reviews suggests that currently available serological tests have no role in the diagnosis of childhood TB (Table 1). However, extensive work is ongoing to develop improved serodiagnostics, especially those that can be deployed in a point-of-care (POC) format. Recently, a group including representatives from *Medecins Sans Frontieres* (MSF), Treatment Action Group, Partners in Health and other agencies, developed minimum test specifications that must drive the development of any new POC test for TB.[23]

INTERFERON-GAMMA RELEASE ASSAYS (IGRAS)

Two IGRAs are currently available: QuantiFERON-TB Gold In-Tube (QFT-GIT, Cellestis Ltd., Carnegie, Australia) and T-SPOT.*TB* (Oxford Immunotec Ltd., Abingdon, UK). The QFT-GIT is a newer version of the QuantiFERON-TB Gold (QFT-G). The evidence on adult IGRA studies has been summarized elsewhere.[24,25]

One of the challenges in evaluating IGRAs and, indeed, all new diagnostics in children is the lack of an adequate gold standard for childhood TB. Since IGRAs are intended for LTBI, which has no gold standard in adults or children, a hierarchy of reference standards has been proposed (Figure 2). In the absence of randomized trials on the efficacy of preventive therapy based on IGRA results, predictive value for progression to active disease is one of the most important reference standards for IGRAs.

DIAGNOSIS OF ACTIVE TB

High-incidence settings

The TST is used in the diagnostic work-up for active TB in children as a marker of recent infection. Several studies have assessed the performance of the IGRA for this purpose, using culture-confirmed TB as the reference standard. Three studies found that the sensitivity of the ELISPOT assay, ranging from 53-81%, was not high enough to rule out TB in children.[26–28] However, the two studies from South Africa showed that the ELISPOT performed better than the TST in immunocompromised children. In a study by Liebeschuetz and colleagues, the sensitivity ranged from 73-85% in children who were HIV-infected, malnourished or <3 years, while for TST the sensitivity decreased to <51% in these groups.[27] Davies and colleagues enrolled younger children (median 20 months), and all confirmed cases were HIV-positive.[26] The sensitivity of the ELISPOT was 67%, compared to 33% for TST.

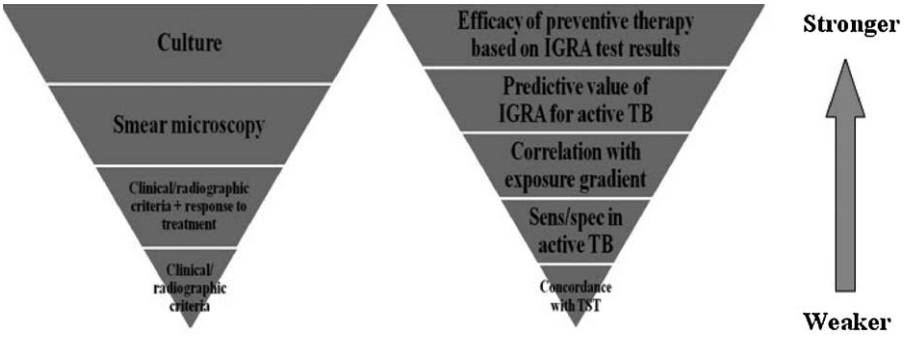

Figure 2. Proposed hierarchy of reference standards for evaluation of IGRAs in active TB (left) and latent TB infection (right).

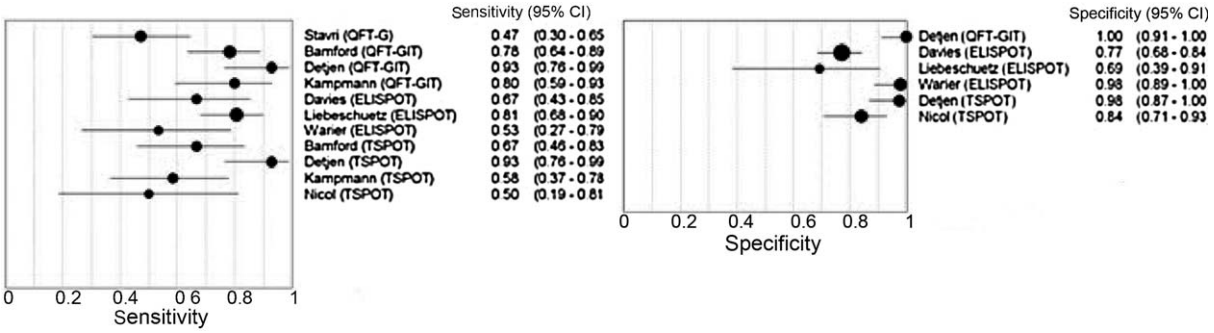

Figure 3. Forest plots of sensitivity (left) and specificity (when available) for eight studies on IGRAs for confirmed active TB in children.

Similarly, Stavri and colleagues reported a sensitivity of 47% for the QFT-G, higher than the 39% for TST in TB/HIV-infected children.[22]

In younger children (median 18 months) with milder disease from a community setting, Nicol and colleagues reported a sensitivity of 50% for TSPOT, compared with 80% for TST.[29] However, the difference was not statistically significant, possibly due to having only ten confirmed cases. In general, specificity estimates were higher than sensitivity for these IGRA studies (Figure 3), although the tests cannot distinguish between latent and active disease.[30]

Low-incidence settings

Detjen and colleagues found near-perfect specificity of the IGRAs in children with NTM or other respiratory infections: 100% for QFT-GIT and 98% for TSPOT, both of which were significantly higher than the TST (58%, $p < 0.001$).[31] On the other hand, the TST had perfect sensitivity, compared to 93% for both IGRAs. Two studies from the UK also concluded that the sensitivity of IGRAs was not better than the TST at cutoff >15 mm. Kampmann and colleagues found the sensitivity to be 83%, 80% and 58% for TST, QFT-GIT and TSPOT, respectively.[32] Using retrospective data from six TB centers across the country, Bamford and colleagues reported sensitivities of 82% for TST, 78% for QFT-GIT and 66% for TSPOT.[33] In both UK studies, the sensitivity increased to >90% when the combined IGRA or TST result was used to diagnose definite TB. This figure dropped to 67-75% when probable cases were included, which would better represent the accuracy in routine clinical practice.

Overall, because IGRAs cannot distinguish between LTBI and active disease, they will always have poor specificity for active TB in high-incidence settings. Furthermore, given their suboptimal sensitivity, they cannot be used to rule out active TB. This is especially true in HIV-infected individuals where IGRA sensitivity tends to be lower.[34] Further work is needed to determine the added value of IGRAs, beyond conventional tests such as smears and chest x-rays. It is also important to determine if a negative IGRA (perhaps in combination with negative smears or chest x-rays) can be used to rule out TB disease in young children.

DIAGNOSIS OF LATENT TB INFECTION (LTBI)

High-incidence settings

In the absence of a reference standard for LTBI, an exposure gradient is often used, though this is still imperfect since not everyone who is exposed will become infected. Nakaoka and colleagues found that most children were TST-positive/QFT-GIT negative in the low-risk group, while most children were TST-negative/QFT-GIT positive in the high-risk group.[35] Another study by Hansted and colleagues reported that the TST was positive in 65% of the low-risk contacts and 60% of the high-risk contacts.[36] For the TSPOT, these figures were 10% and 18%, indicating better specificity. Two separate studies conducted in the Gambia by Adetifa and Hill evaluated both in-house and commercial IGRAs in child contacts.[37,38] For the TST and IGRAs, the likelihood of a positive result increased with sleeping proximity to the index case, but the TST showed the strongest correlation with this exposure gradient.

For the diagnostic performance in household contacts ≤ 5 years, Okada and colleagues reported that the positivity rates for TST and QFT-G were significantly different (24% vs 17%, $p = 0.007$).[39] In this younger age group, there was no trend for increasing age and positive results ($p = 0.22$ for TST and $p = 0.97$ for QFT-G). However, a study by Tsiouris and colleagues that included older children with household exposure found that age was correlated with positive tests ($p = 0.007$ for TST and $p = 0.011$ for QFT-GIT).[40] In multivariable analysis, Dogra and colleagues also showed that increasing age was associated with both TST and QFT-GIT positivity, but only age >8 years reached statistical significance for the QFT-GIT (OR = 5.92, 95% CI: 1.02, 34.39).[41]

Low-incidence settings

Chun and colleagues reported QFT-GIT positivity rates of 7% in casual contacts and 19% in close contacts.[42] Among controls with no known exposure, only 2% were positive on QFT-GIT, while 65% were TST positive. Similarly, in children without risk factors for TB, Lighter and colleagues found that none were positive on QFT-GIT and 27% were positive on TST.[43] However, two studies by Connell and colleagues found a higher proportion of TST-positive results than IGRA-positive results in child contacts.[44,45] One of these studies that compared TST, QFT-GIT and TSPOT showed that age was significantly associated with TST induration ($p = 0.03$) but not IFN-γ response.[45] Likewise, in a younger cohort of children (median 54 months) Bianchi and colleagues found no correlation between age and the QFT-GIT ($p = 0.773$).[46]

The performance of IGRAs has also been evaluated in school outbreaks. Ewer and colleagues found that the odds of a positive test increased significantly across the exposure gradient for both TST and ELISPOT, but the ELISPOT correlated better ($p < 0.05$).[47] Higuchi and colleagues reported on the performance of the QFT-G in two outbreaks in Japan. In a high school, only four of the TST-positive students had positive QFT-G results, three of whom were close contacts.[10] For the outbreak in a primary school, QFT-G positivity was significantly higher for close contacts than casual contacts (10% vs 2%, $p = 0.02$).[11] The QFT-G had higher specificity that was unaffected by the BCG vaccine and reduced the number of children given INH by 95% in the two outbreaks. None of the students had developed active TB in the >3 years post exposure.

The IGRA has also been used to confirm TST-positive results in school-based screenings. Winje and colleagues described perform-

Table 2
Summary of evidence from 14 studies evaluating IGRAs and TST for latent TB infection (NR = not reported)

Author, year, country	Study size (age range)	IGRA type	Indeterminate results (%)	IGRA positive (%)	TST positive (%)	Kappa with TST
Connell, 2006, Australia[44]	106 (0-18 yrs)	QFT-G	17/101 (17)	20/84 (24)	NR	0.30
Higuchi, 2009, Japan[11]	313 (8-12 yrs)	QFT-G	3/313 (1)	9/310 (3)	200/306 (65)	NR
Okada, 2008, Cambodia[39]	217 (0-5 yrs)	QFT-G	22/217 (10)	33/195 (17)	47/195 (24)	0.63
Bianchi, 2009, Italy[46]	336 (31-82 mos)	QFT-GIT	2/336 (1)	60/334 (18)	58/336 (17)	0.53
Chun, 2008, Korea[42]	227 (0-16 yrs)	QFT-GIT	17/227 (7)	16/210 (8)	98/227 (43)	0.19-0.53
Dogra, 2007, India[41]	105 (1-12 yrs)	QFT-GIT	0	11/105 (10)	10/105 (10)	0.73
Lighter, 2009, USA[43]	207 (0-18 yrs)	QFT-GIT	3/207 (1)	31/204 (15)	116/207 (56)	0.17
Nakaoka, 2006, Nigeria[35]	207 (1-14 yrs)	QFT-GIT	NR	65/192 (34)	57/193 (30)	0.24-0.50
Tsiouris, 2006, South Africa[40]	184 (5-15 yrs)	QFT-GIT	0	61/184 (33)	80/184 (43)	0.56
Ewer, 2003, UK[47]	535 (11-15 yrs)	ELISPOT	0	147/535 (27)	155/535 (29)	0.72
Hill, 2006, Gambia[38]	718 (0-14 yrs)	ELISPOT	0	232/718 (32)	225/693 (32)	0.62
Hansted, 2009, Lithuania[36]	120 (10-17 yrs)	TSPOT	0	36/120 (30)	84/120 (70)	NR
Adetifa, 2010, Gambia[37]	285 (0-14 yrs)	QFT-GIT TSPOT	QFT-GIT 2/245 (1) TSPOT 0	QFT-GIT 72/215 (33) TSPOT 71/215 (33)	57/215 (27)	QFT-GIT 0.52 TSPOT 0.54
Connell, 2008, Australia[45]	100 (0-19 yrs)	QFT-GIT TSPOT	QFT-GIT 3/100 (3) TSPOT 5/100 (5)	QFT-GIT 29/97 (30) TSPOT 25/95 (26)	46/95 (48)	QFT-GIT 0.50 TSPOT 0.51

ing the QFT-GIT in Norway, where no TB case has been identified for several years through the nationwide screening of 9th graders (14-15 years) using the TST.[48] Students who have a positive TST (>6 mm) are followed for three years with chest radiographs. Among the TST-positive students, only 9% had positive QFT-GIT results. Using the IGRA as a diagnostic aide for false-positive TST results due to BCG or NTM could help cut the costs associated with preventive therapy or active follow-up in low-incidence settings. A summary of IGRA performance for diagnosis of LTBI is presented in Table 2.

Predictive value

Two studies have evaluated the predictive value of a positive IGRA result for the development of active disease in children. Bakir and colleagues found that the incidence rate among ELISPOT-positive children was 20.5/1000 person-years.[49] Children with positive ELISPOT results had 3.4 times higher risk of developing active TB than ELISPOT-negative children ($p = 0.04$). For the TST, the incidence rate was 16.6/1000 person-years. Children with positive TST results had 2.7 times higher risk of active TB than TST-negative children, though this was not statistically significant ($p = 0.13$). The number needed to treat TST-positive children was significantly higher than that for ELISPOT (61% vs 42%, $p < 0.0001$) to prevent a similar number of cases. In another study, del Corral and colleagues evaluated an in-house culture filtrate protein 10 (CFP-10) based IGRA among adult and child household contacts.[50] Children < 5 years accounted for 22% of the incident cases that occurred during follow-up. No significant difference for TB development was found between positive and negative IGRA results (HR = 1.82, $p = 0.16$). However, a significant trend for increasing IFN-γ response and incident TB was observed ($p = 0.007$).

Overall, although evidence is limited, the results show that IGRAs have modest predictive value, perhaps of the same magnitude as the TST. The data also suggest that a vast majority of IGRA (or TST) positive individuals will not progress to active disease, which means that we still do not have highly predictive biomarkers to help target those who might benefit from preventive therapy.[51]

RISK FACTORS FOR INDETERMINATE RESULTS

The IGRA includes both positive and negative controls as part of its testing procedure, which is an improvement over the TST. A high response to the nil or low response to the mitogen will give an indeterminate result. Lucas and colleagues found that African children with co-morbidities such as helminthic infections, malaria or hepatitis were more likely to have indeterminate results (OR = 4.7, $p < 0.0001$).[52] Similarly, Haustein and colleagues reported an indeterminate rate of 35% in a tertiary-care hospital.[53] In multivariable analysis, immunosuppressive conditions and young age were significantly associated with an indeterminate QFT-GIT result. Other studies have also reported that younger children are significantly more likely to have invalid tests.[33,45] Several have found a significant relationship between increasing age and IFN-γ response to the mitogen.[42–44]

Another study by Bergamini and colleagues reported that the association between age and indeterminate results was significant for the QFT assays (OR = 0.79, 95% CI: 0.69-0.90 for QFT-G and 0.68, 95% CI: 0.52-0.90 for QFT-GIT) but not for TSPOT (OR = 0.89, 95% CI: 0.67-1.18).[54] However, the TSPOT is more complex, and others have shown that this assay is prone to technical errors.[32,45] One study found that while the QFT-GIT gave more indeterminate results than the TSPOT ($p < 0.0001$), the number of failed TSPOT tests at the processing stage resulted in similar proportions of missing results between the two IGRAs.[52]

The majority of indeterminate tests have been due to insufficient mitogen response, suggesting that a lower threshold for the positive control may be necessary in children. Furthermore, the need for venous blood can be problematic in younger ages. One study found that the blood draw failed in 17% of the children.[40] Until the required blood volume can be lowered, IGRAs may not always be feasible in community-based settings. Of the two commercial tests, the QFT-GIT requires a smaller blood volume (3 ml), and the in-tube format makes it easier to use in the field. At this time, a finger-prick version of IGRAs is unlikely to be developed, given the need for an incubation step.

IMPACT OF NEW GUIDELINES

To date, one study has reported on the clinical impact of new guidelines that incorporate the IGRA. Taylor and colleagues compared management decisions under the local Newcastle guidelines with the UK National Institute for Health and Clinical Excellence (NICE) guidelines, which call for a two-step process in which the IGRA is used to confirm a positive TST.[13] Over 18 months, a change in clinical decision was observed for 22% of the children using the QFT-G. The NICE guidelines would have recommended an 'inform and advise' decision for 18 children who were treated with

INH, while one untreated child would have been given INH. In total, 85% fewer children would be treated under the new guidelines. However, two probable TB cases would have been missed, both of whom were close contacts and had abnormal chest x-rays.

CONCLUSION

Current evidence on immune-based tests for childhood TB indicates that a proper diagnosis remains difficult in this population. There are insufficient data on serological tests and a lack of studies in children that have used culture-confirmed TB as the reference standard. Adult studies on serological assays show poor and inconsistent performance and this is likely to be the case in children as well. Therefore, currently available serological tests should not be routinely used for diagnosis of childhood TB. In fact, the World Health Organization (WHO) is considering a strong negative policy recommendation to curb the abuse of serological tests for TB. Further research is needed to develop better versions of serological assays, especially point-of-care tests that are rapid and accurate.

IGRAs have suboptimal sensitivity for active TB and therefore cannot be used in isolation to rule out TB disease in children—this is especially true in HIV-infected children. For the diagnosis of LTBI, there is high agreement between the IGRAs, but much discordance (mostly TST-positive/IGRA-negative) between the IGRA and TST. The high specificity of IGRAs may be useful in reducing the number of low-risk children who need preventive therapy, although longitudinal studies will help determine whether this is due to a false-positive TST or false-negative IGRA result. While IGRAs may be used to help support a diagnosis of TB in combination with the TST and other investigations, they should not be a substitute, or obviate the need, for appropriate specimen collection.[9]

Children are an important group to target for new diagnostics. While considerable efforts are being made to develop new biomarkers and diagnostics for TB,[55] much of the work is being done in adults. Children are often excluded from clinical studies because of the perceived and real difficulty in making a definite diagnosis. Increased efforts should be made to develop an accurate and practical reference standard for childhood TB. Furthermore, there is also the need for studies that go beyond the test accuracy paradigm. Whether or not a new test is recommended for widespread use depends on the trade-offs among several factors: the quality of evidence, impact on patient-important outcomes, uncertainty about values and preferences, risk of complications, and feasibility in resource-limited settings.

KEY POINTS

- It is difficult to evaluate new diagnostics in children due to the challenge of establishing the reference standard
- Serological tests produce inconsistent estimates of test accuracy and have little or no role for the diagnosis of childhood TB
- The IGRA cannot distinguish between latent and active disease
- IGRAs have suboptimal sensitivity for active TB and cannot be used alone to rule out disease reliably
- While IGRAs can be used as evidence of TB infection in children, they cannot replace conventional tests for microbiological confirmation

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CONFLICTS OF INTEREST

No financial conflicts. MP is a consultant for the Foundation for Innovative New Diagnostics (FIND), a non-profit agency that works with several industry partners for new diagnostics development. He also consults for the Bill & Melinda Gates Foundation and co-chairs the Stop TB Partnership's New Diagnostics Working Group. KRS is co-chair of the Subgroup on Evidence Synthesis for TB Diagnostics of the Stop TB Partnership's New Diagnostics Working Group. None of these agencies had any involvement in this publication.

Research Directions

- Increased efforts should be made to develop an accurate and practical reference standard for childhood TB
- Improved serodiagnostic tests are needed, especially for point-of-care use
- More longitudinal studies are necessary to validate the predictive value of IGRAs in children
- Further research should focus on evaluating the incremental value of new tests, their impact on clinical decision-making, their cost-effectiveness in programmatic settings, and their effect on patient-important outcomes

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