

New tools - new challenges: The diagnosis of tuberculosis in the immunocompetent and immunosuppressed child- a short review

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Abstract

INTRODUCTION

In 1993, WHO declared tuberculosis (TB) a global emergency. Increased efforts to identify infectious cases and administer directly observed therapy (DOT) have been made in many countries (1) to combat this disease that causes over 2 million deaths annually. But despite of these efforts - and in part fuelled by the HIV-epidemic -, the global epidemic has continued to spiral out of control (2). A new and extensive plan to Stop TB was published by WHO and the Stop TB campaign earlier this year (3), and new guidelines for the diagnosis and treatment of tuberculosis in the UK were published by NICE in March 2006 (4). In the UK, particularly in London, cases of active tuberculosis have risen steadily (fig 1) and some boroughs in London now fulfil the criteria to be declared a TB-endemic area by WHO standards (>40 cases/100 000 population). It is therefore timely to review the latest developments to secure a diagnosis of TB in children, as clinicians will be confronted with diagnostic dilemmas more frequently.

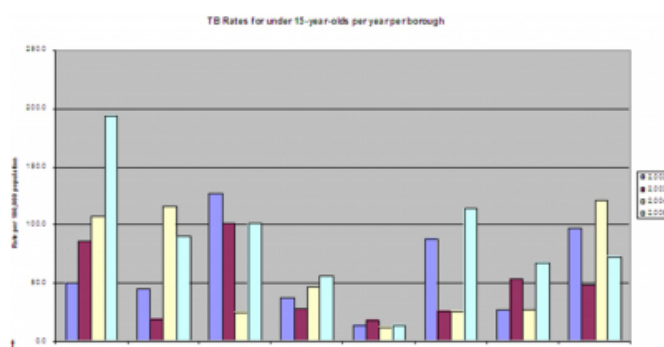
Figure 1



Graph provided by Health Protection Agency, Communicable Disease Surveillance Centre, 2002.

Figure 2

Figure 1: Incidence of Tuberculosis in the UK



WHY IS IT SO DIFFICULT TO MAKE THE DIAGNOSIS OF TB IN CHILDREN?

The diagnosis of tuberculosis in children has always been a problem, because the “gold standard” of isolation of the organism, *M.tuberculosis* (M.Tb) in smear or culture is rarely achieved (5). Why is that? Bacillary burden is much lower in children and cavitating lung disease is extremely rare in primary infection. Children do not produce good sputum samples and the yield from gastric washings is low. The diagnosis is therefore often based on a jigsaw of corroborating evidence, pieced together from clinical signs and symptoms, CXR changes consistent with the diagnosis, maybe a positive skin test and a high index of suspicion as a result of an adult index case in the family or recent travel to TB endemic countries. Matters have potentially improved with the arrival of blood-based tests that can measure whether the host has been exposed to M.Tb in the past.

HOW DO THE NEW TB-DIAGNOSTIC TESTS WORK?

There are currently two blood-based licensed tests available

in the UK, called the Quantiferon Gold (6) test and the T Spot TB (7).

The principle underlying both tests is the ability of TB-specific T cells to secrete of a key cytokine, interferon-gamma (IFN γ), when they are stimulated with specific TB-antigens. In other words, these tests look for a “footprint” that has been left in the immune system by an acute or a previous infection with M.Tb. The immune response can be measured in either a whole blood sample (3ml) using the Quantiferon Gold test or in peripheral blood mononuclear cells (PBMC) prepared from whole blood (minimum 4 ml), using the Elispot technique.

In both whole blood and PBMC tests, the samples are incubated over night with the TB-specific antigens and then coupled with a detection system that can visualise the interferon-gamma produced by antigen-experienced T cells (effector T cells) after the stimulation. The read-out is either the presence and numbers of spots (Elispot) or the levels of IFN γ measured by ELISA technique using colorimetric indicators. Both systems use a positive and a negative control sample to make sure the test is valid. In the literature, these assays are also referred to as Interferon-gamma-release assays (IFNGRA).

In theory, results can be available within 24 hours, if the test is set up immediately for the individual patient. Blood for the preparation of PBMC needs to be processed within 4 hours of sampling, which can be a challenge in an afternoon-clinic setting, but the test is readable 24 hours later. The whole blood approach is more “user-friendly”, as the blood/plasma can be stored following the incubation, which makes the processing timetable a lot more flexible. However, results are not necessarily available as quickly, as it is cumbersome and expensive to run Elisa's for single patients and often samples from several patients are accumulated to make it more cost-effective. Both tests require a sophisticated laboratory set-up and skilled people to perform the assays. The new tests are therefore more costly than the Mantoux skin test, but might give us more useful answers, and as to which of the two assays will be more useful in clinical practice, the jury is still out.

In March 2006 in the UK, the National Institute for Clinical Excellence (NICE) published guidelines recommending that that IFNGRA are rolled-out throughout the NHS. These guidelines do not specify which test to use, and currently neither test is widely available, as money has not necessarily

followed the recommendations.

WHAT CAN THE “NEW TB TESTS” CONTRIBUTE AND WHY SHOULD THEY BE BETTER THAN THE SKIN TEST?

Confounding by prior BCG vaccination or environmental mycobacteria gives the Mantoux skin test a low specificity, and this has always been a big draw-back of this investigation (8). Following the completion of the genome sequence for M.Tb, BCG and other mycobacteria it has become possible to design assays with the potential to discriminate between these organisms, based on antigens that are specific to M.Tb (9). These antigens reside in the region of difference 1 (RD1) of the TB genome and were chosen to be the stimulating antigens in the T cell based assays. The results obtained with these assays can therefore discriminate between the effects of BCG vaccination and M.Tb exposure. BCG vaccination can give a positive skin test result, but leads to a negative result in the blood tests. However, M.Tb exposure leads to a skin test result which can be positive to varying degrees or even negative; however it should give a positive blood test result. The blood based tests are therefore more specific than the skin test (10).

WHAT CAN THESE NEW TESTS REALLY DELIVER?

- a) IFNGRA can distinguish BCG vaccination from M.Tb infection in the context of a positive skin test, when TB is in the differential diagnosis.
- b) They can give an indication of previous exposure to M.Tb and existing immunological memory

WHAT ARE THEIR LIMITATIONS?

Neither assay can diagnose active tuberculosis, if used in isolation, as by definition, IFNGRA could be positive in latent or active TB. This is important when dealing with patients in or from TB endemic countries, who may have been infected a long time ago. The clinical information therefore remains vital to their interpretation. The need for bacteriological confirmation also continues to exist, as the results of the IFNGRA are a read-out of the host response but do not tell us anything about the M.Tb strain that might have infected the patient and the drug sensitivities. Therefore there is a continued need to secure a culture-based microbiological specimen, alongside an IFNGRA result.

To date, we do not know enough about the natural evolution of the T cell responses and the time course of this response,

once treatment has been commenced. Several papers have been published trying to establish trends, but none of those refer to children (_{11,12}). The judgement whether an infection is latent or active currently still lies with the paediatrician and a quick and reliable test for the diagnosis of acute tuberculosis continues to be awaited (₁₃).

THE DIAGNOSIS OF TB IN THE HIV-INFECTED CHILD- THE PLOT THICKENS...

HIV is the most significant risk factor for susceptibility to TB. The risk to develop active disease increases from 10% over the life-time in HIV-negative adults to 10% per year in HIV-infected individuals (₁₄). In TB-endemic countries, it's almost a certainty that HIV-infected individuals will develop TB at some stage during their illness. TB can manifest at all CD4 counts and is not just a presentation of profound immunosuppression in the advanced stages of HIV, although it's more commonly seen in advanced stages of immunosuppression. TB is therefore an important potential co-infection in the children that we see in our clinical practice.

As already mentioned, the diagnosis of TB in children is difficult at the best of times. In the context of HIV, the diagnosis becomes even more challenging, since TB can mimic a number of other infections seen in HIV infected patients. For instance, the CXR of miliary TB can look identical to LIP or even *Pneumocystis jirovecii* (PcJ) (Figure 2), and skin tests are likely to be negative in patients with impaired cellular immunity, the hallmark of HIV-infection. Yet it is very important to secure an accurate diagnosis, as the patient will be committed to a 6-months treatment course for tuberculosis, with a large pill-burden, which can potentially interfere with ART.

Figure 3

Figure 2: CXR of miliary TB in HIV-infection



There is no easy answer to the diagnostic dilemma, but it is important to ascertain the appropriate specimens and also consider more disseminated forms of TB in HIV-infected children, such as abdominal TB, tuberculous meningitis (TBM), miliary TB. A thorough history of potential household contacts and travel to TB endemic countries is essential. The use of Bactec culture bottles specific for mycobacteria should be encouraged, as HIV-infected patients have more evidence of dissemination of mycobacteria into the bloodstream (₁₅), which might be picked up in the BacTec bottles - check for availability with your microbiology department. Table 1 summarises the list of investigations in the immunocompromised child with presumed TB.

Figure 4

Table 1: Recommended investigations in HIV-infected children with suspected tuberculosis (not in order of preference)

Contact history, including previous TB treatment in carers, as this could be a reason for drug-resistant strains!
Travel history
History of signs and symptoms incl. weight chart
BCG status
CXR/CT chest)
Other radiological investigations, depending of presumed site of infection
Sputum/gastric washings
Mycobacterial blood culture (BacTec) with routine investigations of constitutional symptoms
Exclusion of active (viral) infections such as CMV, Pcj
Skin test
Interferon-gamma-release assays such as T Spot, Quantiferon where available
Liaison with TB services re contact tracing, and drug sensitivities/resistance patterns of strains in members likely to have been the index case

Good communication with the TB services caring for presumed adult contacts and index cases is also essential, since positive mycobacterial cultures and information on drug susceptibility are often available from these contacts. Multi-drug resistant TB (MDR) is still rare in children in the UK, but we have seen cases already. Adjustments to drug therapy might be necessary in light of resistance patterns of strains isolated from adult household contacts. This is particularly important because of the low yield of bacteriological confirmation of TB in our paediatric population- no bug, no resistance/susceptibility information!!!

CAN THE NEW DIAGNOSTIC TOOLS HELP IN HIV-INFECTED CHILDREN WITH PRESUMED TB?

Data published up till now indicate that these blood-based tests are more likely to be positive in HIV-infected children than the skin test, which is encouraging and could be extremely helpful. A large study of children in South Africa by Liebeschutz et al (¹⁶) showed that 73 % of HIV-infected children with confirmed TB had a positive T spot, as compared to 35% in the skin test. However, no CD4T cell

counts were measured in this study, so we can not be sure of the level of immunosuppression in the children. Another recent paper by Dheeda et al (¹⁷) showed again good performance of the T spot assay in HIV-infected adults with a variety of CD 4 counts, as compared to the skin test, but this study only mentioned the responses to the non-specific stimulus, PHA, and not the specific antigens. No data are published on the use of the Quantiferon test in HIV as yet, but several studies are ongoing. If the blood based tests turn out to be quite specific, they will at least help us to detect the “footprint” of exposure to M.Tb, although it is up to our clinical judgement to decide, if this represents latent or active infection in our patients, as outlined above.

In the context of HIV though, a positive IFNGRA carries very important information, as any HIV-positive child with evidence of previous exposure is likely to activate tuberculosis in the future. These children might benefit from chemoprophylactic treatment to eradicate the latent infection, if they are living in non-TB endemic areas and their risk of re-infection can be considered very low. In TB endemic countries there is probably little benefit in treating latent infection with prophylactic 2-drug treatment, as re-infection can easily occur. This question is the subject of larger research projects, mainly in TB- endemic countries currently. Earlier trials of chemoprophylaxis in HIV-infected adults have not shown lasting benefits (¹⁸), but the outcome may be different when patients receive ART, and this issue needs to be reconsidered. A recent paper by Zar et al (¹⁹) showed significantly reduced morbidity from TB in HIV-infected children in the 2 years following continued INH prophylaxis, compared to placebo.

In summary, we must consider TB as a potential pathogen in virtually all of our HIV-infected patients, independent of their CD4 counts, as many have been exposed at home or in the country of origin, and we need to pursue confirmation of the diagnosis with all tools available.

WHAT CAN RESEARCH CONTRIBUTE TO THIS FIELD?

It is important to establish whether differences in the levels of production of IFNg in either Elispot or Elisa will allow us to distinguish between latent or active TB, and this is currently a subject of research. It would be extremely useful to have cut-off values that could make the test more discriminative between active TB and latent infection. Unfortunately, the heterogeneity of the human immune response makes this a difficult task. Larger studies in

children of different ages are needed for us to develop a feel for the magnitude of immune responses that we might expect in active versus latent infection in both immunocompetent and immunocompromised hosts. The progression and longevity of these responses is also extremely interesting, as not only would such data provide clues about antigen-specific immunological memory, but could help in daily practice. To date, we are not yet in a position to make recommendations of this nature, but it would be helpful to pool data from our experience with IFNGRA in different centres in order to assemble cohorts of children with multiple time points and different age groups.

Similar questions are raised within the context of HIV and TB diagnostics, since the performance of T cell-based assays is likely to be influenced by immune status, which in turn is influenced by the ART the child might be receiving.

Current research is trying to address some of these dynamics, but to date, again, recommendations for the reliability of the T cell-based assays for TB can not yet be made, except that they appear to perform better than the skin tests and give more reliable results in patients with levels of CD4 counts above 10 %. More data are being gathered in this field.

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