Author's response to reviews

Title: T-cell-based diagnosis of tuberculosis infection in children in Lithuania: a country of high incidence despite high bacille Calmette-Guerin vaccination coverage

Authors:

Edita Hansted (edita.hansted@gmail.com)
Angele Andriuskeviciene (olde@one.lt)
Raimundas Sakalauskas (raimundas.sakalauskas@kmuk.lt)
Rimantas Kevalas (rimantas.kevalas@kmuk.lt)
Brigita Sitkauskiene (brigita.sitkauskiene@kmuk.lt)

Version: 3 Date: 27 March 2009

Author's response to reviews: see over
Response to Reviewers

Reviewer 1: Tae Sun Shim
This manuscript evaluated one of the commercialized IGRAs, T-SPOT.TB, in the diagnosis of TB infection in children in Lithuania where TB prevalence and BCG vaccination rate are high. The results showed that TST and T-SPOT.TB were all positive in confirmed TB patients whereas in patients with high-risk or low-risk for TB, the discrepancy between two tests was huge, mainly due to positive TST/negative T-SPOT.TB results. This discrepancy may be due to false positive TST (possibly due to BCG vaccination), however, the authors did not show any supporting evidence for that.

1. Comment: Clearly, previous studies have shown that the TST does not accurately reflect the risk of latent TB infection in a setting with extensive BCG coverage (ref. 12, 9, 20). All our studied children had BCG vaccination in their early childhood. The specificity of TST may be not adequate due to cross-reactions not only with BCG vaccination, but also with non-tuberculous mycobacteria which appears to be rising, especially among young children (ref. 21).

Even though the studies dealing with IGRA in children are very rare, a similar paper was already published in the journal ‘Diagnostic Microbiology and Infectious Disease’ (Chun et al. 2008;62: 389-94) even though they used QFT-GIT test instead of T-SPOT.TB.

Methods:
The definition of positive result in T-SPOT.TB assay is that ‘the number of spots (test wells – negative control well) should be more than 6’. Hence, ‘#6 times’ should be changed according to the manufacturer’s recommendation.

2. Comment: We thank the Reviewer for this comment. The definition of positive result in T-SPOT.TB assay is now clarified. Please see the corrected version of our manuscript (section “Methods” and “Determination of specific-INF-γ secreting T-cells”).

In the high risk for TB group, were the index cases smear-positive pulmonary TB? If yes, please describe as ‘infectious pulmonary tuberculosis’ not as ‘active TB’.

3. Comment: We apologize for the incorrect description. Now ‘active TB’ is changed to ‘infectious pulmonary TB’

Results:
In the text, the authors mentioned that ‘The numbers of positive T-SOPT.TB subjects based on four different TST cutoff values are presented in Table 3”. However, Table 3 does not contain those results. Please modify.

4. Comment: We thank the Reviewer for this note. In the present version, the title of Table 3 is corrected.

Didn't you have any ‘indeterminate’ results in T-SPOT.TB assay?
5. Comment:
No, we did not.

In order to say that T-SPOT.TB assay is superior to TST in this study, the authors should measure the correlation of two tests (TST and T-SPOT.TB) with the risk of infection by estimating the odds ratio and relating the test results to the likelihood of TB infection as described in other paper (Kang et al., JAMA 2005;293:2756-2761). Or, in high-risk for TB group, the authors should show stronger correlation between T-SPOT.TB results and grade of TB exposure than between TST and grade of TB exposure.

6. Comment: Numbers of children with positive T SPOT according to the defined cut-offs in TST are presented in Table 3. The Kendall’s correlation test showed a significant correlation between TST and T SPOT TB in the “culture-confirmed TB” group, when TST ≥15 mm (r=0.35, p<0.001), - this finding is mentioned in the Results. In other children we found discordance between these tests,- even 51 of the 97 individuals (53%) from the “high risk for TB” and “low risk for TB” groups with a TST reaction ≥10 mm were T SPOT TB negative. The correlation between TST and T SPOT-TB is presented in Figure below (r=0.5, p<0.01). However, we did not include this Figure in the manuscript as we strongly feel that it would be too excessive.
**Reviewer 2:** Claudio Fortis  
**Major Compulsory Revisions**

**Methods**

1) page 4, lines 8-9: the range of age should be reported also for both “high” and “low risk for TB” groups.

*Comment:* The ranges of age for all groups are presented in Table 1. That is why we did not repeat the ranges in the Text.

2) Page 5: Determination of specific-IFN-g secreting T-cells – The individual spots were counted with an automated system or how?

*Comment:* We apologize for the incomplete description. The number of spots in T SPOT TB assay was counted by using manual counting with Tryptan Blue and a haemocytometer according to the manufacturer’s recommendation.

3) Page 6: Statistical analysis is inadequate; agreement between different immunological tests (TST and T SPOT TB) should be assessed by estimating Cohen’s Kappa statistic. Kendall’s correlation test reported at page 7 is not here mentioned. Moreover, the authors write that “comparisons between numbers of spots in T SPOT TB test and TST diameters of induration”, but these data have not been reported in any part of the manuscript. These data should be reported in a separate figure.

*Comment:* We thank the Reviewer for this comment. Agreement between used immunological tests (TST and T SPOT TB) was assessed by Cohen’s Kappa, which was 0.24. Section of Statistical analysis has been improved. Results of TST (induration, mm) in relation to T SPOT TB response are now presented in a separate Figure. Please see also response to Reviewer 1, point No 6.

**Results and Discussion**

5) Table 1 and figure 1: the comparison of the number of spots among the three groups of children was performed considering all the T SPOT TB results, whereas should be performed considering only positive T SPOT TB results.

*Comment:* We appreciate the Reviewer’s opinion; and data in Figure 1 is now presented considering only positive T SPOT TB results.

6) Page 7, lines 21-22, and page 10, lines 14-16: because in the “culture-confirmed TB” group all TST positive children were also T SPOT TB positives, I do not understand what means that “a significant correlation between TST and T SPOT TB” was observed when TST $\geq$ 15 mm

*Comment:* We did not find a correlation between TST (size of induration, mm) and T SPOT TB (number of spots) in the “culture-confirmed TB” group when analyzed data of all subjects from that group. Whereas, in subgroup of patients with TST $\geq$ 15 mm, the Kendall’s correlation test showed a significant correlation between TST and T SPOT TB results.
7) For each group, should be interesting to report in a separate figure box-and-whisker plot of TST induration (in mm) in relation to positive and negative T SPOT TB responses.

Comment: We appreciate Reviewer’s opinion. Separate figure box-and-whisker plot of TST induration (in mm) in relation to positive and negative T SPOT TB responses is now included in the manuscript.

8) Page 8, lines 2-3: it is not clear whether, after 8 weeks of therapy, some positive T SPOT TB results reverted.

Comment: After 8 weeks of anti-TB treatment the number of T SPOT TB spot counts was reduced significantly (it is possible to see in Figure 2); 5/8 positive T SPOT TB results reverted, however, in 3/8 T SPOT TB was still positive.

9) Page 8, lines 3-4, and page 10, last line- page 11, first line: the sentence “results of T SPOT TB in 3 children were still positive because of multidrug-resistant TB form” may not be proved and should be eliminated.

Comment: The sentence is now corrected.

Reviewer 3: Jean-Pierre Zellweger
This paper compares the rate of positivity of TST and T-SPOT.TB between three groups of children (aged 10 to 17, all vaccinated with BCG) a) with active TB, b) after contacts with TB and c) without history of contacts. As expected from other studies, the rate of positivity among children with and without TB contact is much higher using TST than T-SPOT.TB, a fact that is usually interpreted as demonstrating a high rate of false positivity due to prior BCG vaccination or contact with non-tuberculous mycobacteria.

The strong points about this paper are a) the fact that the whole group of children had received BCG vaccination, b) that all children with active TB were positive with both TST and T-SPOT.TB and c) that gives an estimate of the rate of T-SPOT.TB positivity in children living in a country with a high incidence of TB but without history of contact.

The weak point is that the authors do not address the key issue: what is the signification of a negative T-SPOT.TB in individuals with a positive TST?

Claiming that only the contacts with a positive T-SPOT.TB are to be considered as latently infected and needing a preventive treatment may be right, but only if there are evidence that these individuals have a real risk of TB reactivation. Unfortunately, the authors do not discuss this fact and do not mention any of these emerging evidence. The evidence for the risk of reactivation with a positive IGRA test are to be found in Diel,
AJRCCM 2008;177:1164-1170, and in Bakir, Ann Int Med 2008;149:777-86, the evidence for the absence of risk for contacts with a positive TST but negative T-SPOT is in Higushi, Respirology 2007;12:88-92. But there are also studies demonstrating discordant results, for instance Hill, PLoS Medicine 2007;4(6), e192. Furthermore, the authors do not mention other studies, mainly performed with the other available IGRA test (Quantiferon), which demonstrates a fairly high level of negative results even among patients (adults and children) with active TB (see for instance Connell T, PLoS One 2008,3(7):e2624. The authors should also refer to recent overviews of the sensitivity and specificity of the tests, for instance Pai, Ann Int Med 2008;149(3).

Comment: We thank the Reviewer for his opinion. Depending on the Reviewer’s suggestion, the Discussion section has been newly developed, and list of publications has now been updated.

Some minor comments:

Comment: We appreciate the Reviewer’s preference for another Ref. It has now been changed.

Abstract:
Irrational treatment of active TB may increase the risk of MDR-TB, but not preventive treatment (no evidence up to now)

Comment: We agree with the Reviewer, and corrected the sentence.

Results:
The rate of T-SPOT.TB positivity is not similar in both groups of children with and without TB contacts (17.8 and 9.5%). How can the authors claim that they “do not differ”?

Comment: We apologize for this incomplete wording, which now has been changed – “they did not differ significantly”.

Repetition of TST may induce a booster effect. Should be mentioned as a hypothesis.

Comment: We agree with the Reviewer, and have mentioned this in the Discussion.

The reason why children with MDR-TRB still had a high degree of positivity of T-SPOT.TB may be linked with the persistence of a high bacteriological load, not with drug resistance. Please clarify.

Comment: We agree with the Reviewer’s opinion. Please see the corrected version.

Discussion:
Again, the presentation of the data is misleading: according to the results, 60 to 65% of children without active TB had a positive TST (not 70%) and 17.8 and 9.5% had a positive T-SPOT. TB, not 30%. Please clarify

Comment: Indicating that 70% of all studied children had positive TST, and only 30% - positive T SPOT TB, we had in mind all three studied groups, not only children without active TB.

The authors should mention studies using the other IGRA test, where the results are not similar (see Menzies, Ann Int Med 2007;146:340-6)

Comment: We agree with the Reviewer, and corrections have already been made in the Discussion.

Children with negative TST and positive T-SPOT.TB may have false-negative TST due to intercurrent viral infection, for instance
Mentioning a number without the denominator is misleading and not informative: "3" out of the blues does not give any information, 3/52 or 6% is much better
"...in our country with high BCG coverage, where TST is currently the only tool..."

Comment: We thank the Reviewer for his comments. Please see the corrected version of our manuscript.

**Reviewer 4:** Neil Schluger
The manuscript by Hansted et al. describes the results of a study comparing TST and T.SPOT results in a cohort of children ages 10-17 with either confirmed active tuberculosis, or in groups felt to be at high or low risk for latent tuberculosis infection. This report is from Lithuania, a moderate prevalence country for tuberculosis in which BCG vaccination is routinely administered. The authors report that there was excellent correlation between the two tests in patients with active tuberculosis, but that in other groups, correlation was poor, with far more children having positive TST than T.SPOT tests. In addition, the authors report that in a subset of patients with active tuberculosis, the number of spots in the T.SPOT assay declined with successful treatment. The authors conclude from these results that T.SPOT is at least of equal sensitivity to TST and of far greater specificity. The study is consistent with the results of many other studies of this type, and it adds useful and much needed data on the performance of interferon gamma release assays in younger populations. However, the study is also subject to the same limitations affecting many studies of this type, and the manuscript could be strengthened if several points were addressed.
Major comments:
1. At what age do children in Lithuania receive BCG vaccine? Are all routinely vaccinated at birth? Are any children given booster doses of vaccine during the teenage years? If age of vaccination was different among children in the cohort, did the age of vaccination have an effect on the skin test result in the two groups without bacteriologically confirmed active tuberculosis?

Comment: According to the National Tuberculosis program and vaccination calendar in Lithuania (Lithuanian Health Ministry order No.V-646, 1996 03 21) all children should be vaccinated at their birth. Before 1996, all Lithuanian children were revaccinated with BCG before their school, at 7th year. That is why we think some children may have false-positive TST caused by previous BCG vaccination. The weak point of our study is that we could not receive the documentation of each child with the exact date of the last BCG vaccination. However, we do not think that it could influence the results of our study as mean age of studied groups was almost the same.

2. Is there any way that the patients in the “high risk for TB” group could be further stratified to assess the performance of the two tests under consideration? Could the source cases be identified as have 1+, 2+, 3+, of 4+ positive sputum smears, for example? If this was done could it affect the interpretation of the results, i.e. if all the positive T.SPOT tests were in children who were contacts of 4+ smear positive cases, the interpretation of the authors regarding the specificity of the test would be stronger.

Comment: We appreciate the Reviewer’s suggestion. However, such data (sputum smear results of contacts) is not available, because those patients were treated in different Hospitals (or even different cities).

3. Is there any follow-up information available about children in the high risk group (or low-risk group) who were not treated for latent infection? Did any of them develop active tuberculosis? If so, how was active tuberculosis defined? Did any of the children who developed active tuberculosis have either a positive TST or T.SPOT test? Did either test more accurately predict the development of active tuberculosis?

Comment: No, we did not perform any follow-up visits.

Minor comments:
1. The cases of active tuberculosis in group 1 are listed as bacteriologically confirmed. Were all cases pulmonary cases?

Comment: Yes, certainly.

Reviewer 5: Satoshi Mitarai
Major comments;
The authors evaluated the clinical usefulness of T SPOT TB test for the diagnosis of latent tuberculosis (TB) infection among children under high TB prevalent and BCG vaccinated setting. As the authors describe, it is very important to diagnose latent TB accurately to prevent the development and further dissemination of TB. It is clear that any ex vivo test must avoid the affection of BCG vaccination to diagnose TB infection accurately. It means that the test result should correlate with the risk of TB infection. The methodology/design employed in this study may be appropriate, however, the results obtained indicate no clear difference between the groups of high and low risk for TB infection. It means the T SPOT TB test is not useful to differentiate the recent TB infection, which could be the major target of chemoprophylaxis, under the current setting. If the authors would conclude that the T SPOT TB is useful to diagnose latent TB infection, the difference between risks ought to be demonstrated. If the test is positive for many individuals among low risk group, how we can utilise it for decision making for chemoprophylaxis. It may be an issue of policy for chemoprophylaxis. If all the IGRA positive individuals who do not have active TB receive anti-TB chemoprophylaxis whether the infection is new or old, it may be useful to eliminate the false-positives by TST. This point should be discussed to clarify the point of discussion.

Comment: We thank the Reviewer for the opinion. Please see newly developed version of our manuscript (Discussion section)

The authors describe that three children were infected by MDR-TB. Is it practical to implement INH prophylaxis under such high TB resistant setting?

Comment: Usually, chemophylaxis of TB infection in children in Lithuania is performed by isoniazid and rifampin for 3 months, because of high prevalence of resistance TB. Three children in our study had MDR TB confirmed by BACTEC. They were treated with the first line drugs (Isoniazid, Rifampin, Pyrazinamide and Ethambutol) only for the first month until we have got results from the bacteriological lab. According to the M.tuberculosis resistance, treatment was individualized and prolonged. We are using IUATLD recommendations (www.umdnj.edu/globaltb).

Minor comments;
Page 3, line 12
Which category is indicated as the incidence of drug resistance among new TB cases? Is it any resistance to first line anti-TB drugs, or any designated drug?

Comment: The incidence of drug resistance among new TB cases is indicated when the resistance to the first line of anti-TB drugs was identified.

Page 3, line 15
Although the authors describe that TST has poor specificity and sensitivity, TST normally has high sensitivity despite of low specificity, especially among BCG vaccinated population.
Comment: We agree with the Reviewer’s comment, TST has high sensitivity and low specificity.

Page 3, line 20 and others
Interferon-gamma is normally abbreviated to “IFN-#”. In the manuscript, “INF-#” is also seen. Please unify the term.

Comment: We apologize for typos, which is now corrected.

Page 4, line 7
I am not convinced of the definition of “children”. A man of 17 years-old could be categorised into child?

Comment: The age of studied children was 10-17 y.o. According to the Convention on the Rights of the Child, 20 of November, 1989, Part I, Article 1, “a child means every human being below the age of eighteen years”. People under 18y.o. are defined as children in Lithuania (Lithuanian Heath Ministry and Lithuanian Parlament lawful; http://www3.lrs.lt/pls/inter3/dokpaieska.showdoc_l?p_id=19848&p_query=&p_tr2=)

Page 4, line 17
Please describe the definition of “TB-sick” in detail. Does it mean smear positive patient? If so, what was the average positivity of the group? It must be surely important for the readers to recognise the risk of TB infection.

Comment: In our study “TB-sick” is person who has infectious TB case (smear positive patient). Please see also response to Reviewer 4, point No 2.

Page 5, line 7
The spell of pyrazinamid must be pyrazinamide.

Page 6, line 7
“Student’s test” must be “Student’s T test”.

Comment: We apologize for these mistakes, what have now been corrected.