Evaluation of the filter paper IP-10 tests in school children after exposure to tuberculosis: a prospective cohort study with a 4-year follow-up

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ABSTRACT

Objectives: The prevalence of active tuberculosis (TB) is low in Finland, but outbreaks do occur. Following exposure national guidelines recommend either tuberculosis skin test or interferon-γ-release assay-testing of asymptomatic children. The aim of this study was to compare QuantiFERON-TB Gold In-Tube test (QFT) and interferon-γ-inducible protein (IP-10) release assay for detection of *Mycobacterium tuberculosis* infection following exposure to TB in a primary school.

Design: A prospective cohort study.

Setting: School children in Helsinki, Finland.

Participants: Two siblings of the index case and 58 classmates exposed to *M tuberculosis*.

Intervention: All the children were screened using the QFT, which was used to guide preventive treatment. All those exposed were followed up through the national TB registry.

Outcome measures: IP-10 was measured in plasma supernatants from the QFT test supernatants and in plasma dried and stored for 1 year on filter paper. IP-10 test results were calculated using preset algorithms for positive and indeterminate tests. The negative predictive values of the tests were assessed.

Results: At an initial screening 2 months after the debut of symptoms in the index case, QFT was positive in two children; 56 tests were negative; one was indeterminate and one was borderline. IP-10 showed a perfect concordance between the dried plasma spot and plasma method; two children were IP-10 positive and two were IP-10 indeterminate. There were two (3%) discordant results between the QFT and IP-10 tests. Four children converted to positive QFT at a 1–3 month follow-up. None of the QFT negative/borderline children developed TB in the 4-year period since exposure.

Conclusions: We demonstrated that IP-10 and QFT perform comparably as screening tools for infection with *M tuberculosis* in a contact investigation. IP-10 determined in dried plasma spots was at par with IP-10 determined in plasma, which further supports the usefulness of this alternative approach.

ARTICLE SUMMARY

Article focus
- Interferon-γ and IP-10 release assays are tests for detection of infection with *Mycobacterium tuberculosis*, little is known about the performance of IP-10 release assays in contact tracing.
- We have recently developed a novel method for IP-10 detection using dried plasma spots.
- In this study, we compare detection rates and predictive values of IFN-γ and IP-10 release assays in a primary school in Finland.

Key messages
- We demonstrated that IP-10 release assays and the IFN-γ release assay (QuantiFERON-TB Gold In-Tube) perform comparably as screening tools in a contact tracing.
- IP-10 determined in dried plasma spots was *at par* with IP-10 determined in plasma, which further supports the utility of this alternative technology.

Strengths and limitations of this study
- We have used a 4-year follow-up to substantiate our findings.
- Our sample size is small but well characterised and closely monitored.

INTRODUCTION

Tuberculosis (TB) remains a global epidemic. Childhood TB is a major cause of childhood morbidity and mortality in endemic regions. The diagnosis of TB in children is difficult owing to diverse clinical presentations combined with paucibacillary infection, making bacteriological confirmation challenging.[1] Children infected with *Mycobacterium tuberculosis* (latent TB infection (LTBI)) are at a risk of developing TB; wherefore, there is a great benefit from preventive therapy.[2]

Interferon-γ-release assays (IGRAs) and interferon-γ-inducible protein (IP-10) release

assays are immunological tests to identify infection with *M tuberculosis*. IGRAs are commercially available as the whole blood and ELISA-based QuantiFERON-TB Gold In-Tube test (QFT) (QIAGEN, Valencia, USA), or the PBMC and Eli-SPOT-based T-SPOT.TB test (Oxford Immunotec, Abingdonm UK). IP-10 is a chemokine produced by monocytes following activation by antigen-specific T cells recognising their specific peptide presented on the monocyte surface. IP-10 is released in 100-fold higher concentrations compared to IFN-γ making it feasible for detection with a lateral flow, and dried blood and dried plasma spot (DPS) technologies.[3]

A series of clinical studies suggest that IP-10 release assays perform with a comparable sensitivity as IGRAs in patients with a confirmed TB and comparable specificity in unexposed controls, and several studies have found that IP-10 is less affected by low CD4 cell count in HIV-infected individuals and by young age.[3–5] Little is yet known about the performance of IP-10 release assays for the diagnosis of LTBI following a recent exposure to *M tuberculosis* in a low-incidence country, nor the predictive values for a positive or negative result.

In this study, we compared QFT to plasma and dried plasma spot-based IP-10 release assays for the detection of LTBI in children, following exposure to TB in a school. We assessed the stability of DPS samples stored for 1 year and determine the negative predictive values (NPV) of the tests in a 4-year follow-up.

MATERIALS AND METHODS
Students and setting
The incidence of TB in Finland is low (6/100 000 inhabitants in 2010) with none to six active cases/year registered among children (<15 years) during the period 2000–2010.[6]

In March 2008, a schoolgirl of African background started to cough, and in June, she was diagnosed with sputum smear-positive TB. As a part of contact tracing the classmates (close contact defined as from 3 to 30 h/week in the same classroom) and other close contacts were examined for symptoms of active TB and investigated with QFT (June). Siblings were examined for symptoms of active TB and QFT tested at the time of diagnosis of the index case and 1 month later. For both siblings and classmates, positive QFT tests were confirmed with the T-SPOT.TB test. Non-positive QFT tests were repeated 3 months later (September). Four years after the contact tracing, the progression rate in the children was evaluated.

This study was approved by the Ethical Committee of the Hospital for Children and Adolescents, University of Helsinki (Nr.164/E7/05).

IFN-γ and IP-10 measurements
The modified version of QFT was performed according to the standard operating procedure (SOP) adopted in our laboratory (described in detail in refs.[7] and [8]).

In brief, stimulation of blood cells was done in tubes of the manufacturer. However, for measurement of IFN-γ levels, we used enzyme immunoassay (EIA) of PeliKine Compact human EIA (Sanquin, Amsterdam, the Netherlands). This results in a steeper calibration curve and a more accurate result interpretation in the cutoff zone.[7] Thereafter, the excess supernatant from the QFT tubes was stored at −80°C and later shipped on dry ice to the research laboratory at the Copenhagen University Hospital, Hvidovre, Denmark. In August 2010, the samples were thawed and 2×25 µl from each sample was pipetted onto Whatman 903 filter paper (Whatman, Maidstone, UK), dried for 4 h and stored at −20°C for a later analysis; plasma samples were refrozen and stored at −80°C. In February 2012, plasma and dried plasma spot samples were analysed for IP-10 using an in-house IP-10 ELISA.[9]

IFN-γ and IP-10 test interpretations
IFN-γ and IP-10 concentrations were analysed according to previously set algorithms. For IFN-γ, two cutoff levels were applied: Samples showing a net reactivity (the reactivity of a sample minus the reactivity of the nil control) <0.35 IU/ml were interpreted as negative. Those showing reactivity between 0.35 and 0.50 IU/ml were interpreted as being borderline and those with a reactivity exceeding 0.50 IU/ml were interpreted as positive. The IP-10 test was considered positive if net reactivity was above 2.3 ng/ml (plasma) and 125 pg/2 DPS discs and deemed indeterminate if the test was negative and a net reactivity of the positive control was below 1.5 ng (plasma) and 71 pg/2 DPS discs.[10]

Statistics
Biomarker concentrations were compared using non-parametric statistics. Test results were compared using the McNemars test and κ statistics. Correlation was assessed using the Persons method. Data were analysed using the GraphPad Prizm V.5.0 for Mac and SAS V.9.3.

RESULTS
Children, setting and QFT results
A total of 67 children were included in the contact investigation; 58 classmates from the school, 7 other close contacts and 2 siblings to the index case (figure 1). Among these seven other close contacts, three had positive QFT and tuberculosis skin test (TST), which were regarded LTBI. These seven samples were not available for IP-10 analysis and they were excluded. A total of 60 individuals were included in the study.

The index case has two siblings. Both were clinically examined and QFT screened at the time of diagnosis of the index case and 1 month later. The sister (age 13) had symptomatic disease at the initial screening in June and received full TB treatment. Her QFT converted from 0 (June) to 18.34 IU/ml (July). The brother (age 9) was asymptomatic, but received LTBI treatment based on a QFT conversion from 0.0 (June) to 20.4 UI/ml (August).
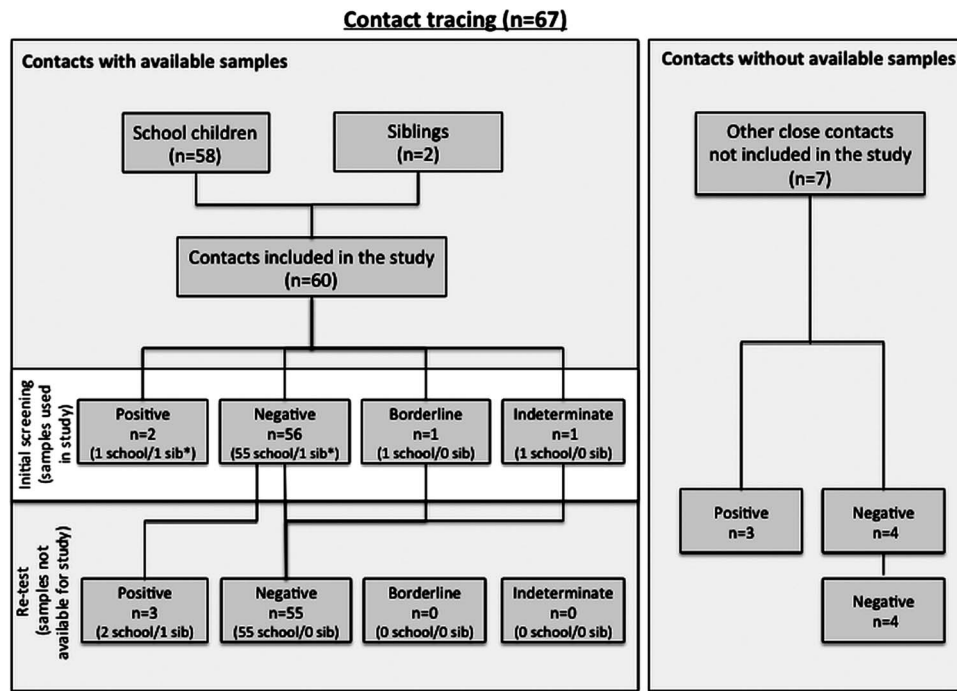
Figure 1 Schematic overview of the contact tracing. Sixty-seven contacts were screened, of which 60 had samples available for the study. Samples from the initial screening (white bar in the middle) were used in the study. *Siblings were screened once before the samples included in this study were obtained. At that time, both tests were negative (see the main text). These samples were not available for the study.

The 58 school children had a median age of 12 years (range 11–14), 40% (25/58) being male. One child had a missing mitogen sample and was classified as a determinate (valid test). At the initial screening (June), the QFT test identified 1 positive, 1 borderline, 55 negative and 1 indeterminate cases. At a retest, 3 months later two children had converted from no reaction (0.00 and 0.02 IU/ml) to strong positive results (6.24 and 10.21 IU/ml IFN-γ). All children with positive tests received LTBI treatment.

IP-10 and IFN-γ levels

Nil and antigen samples from the initial screening were available for 58 school children and 1 sibling. The sibling with symptomatic disease was retested 3 weeks after the treatment was started. This sample was available for the study. Raw values for each biomarker are presented in table 1. Correlation between nil, antigen and mitogen samples pooled (n=179) was very high for IP-10 (r^2=0.98 for IP-10 plasma vs DPS, p<0.0001) and high for IFN-γ vs IP-10 (r^2=0.69 and 0.68 for IP-10 plasma and DPS, respectively, p<0.0001, data not shown).

IP-10 test results and concordance

The two IP-10 tests had a perfect agreement (60/60, k=1.00, p<0.0001) rendering 2 positive, 56 negative and 2 indeterminate (see table 2). The two children who converted to positive QFT at 3 months rescreening were both IP-10 unresponsive at the initial screening. The agreement between QFT and either of the IP-10 tests was 97%, (58/60, k=0.86, p<0.0001 (borderline classified as negative)). Two children were concordant QFT/IP-10 positive. Both were also positive on T-SPOT.TB used as a confirmatory method.

There were two discordant results. One Finnish-born girl had QFT borderline results (0.45 IU/ml IFN-γ); it is noteworthy that she also had the highest spontaneous IFN-γ production in the cohort (0.83 IU/ml). This girl had IP-10 levels of 1.26 ng/ml and 26 pg/2 DPS spots (plasma and DPS samples, respectively, which is interpreted as IP-10-test negative; figure 2A). She was further tested with T-SPOT.

Table 1	IFN-γ and IP-10 levels in plasma and dried plasma spots		
	Nil	**Antigen**	**Mitogen**
IFN-γ (IU/ml)	0.04 (0.02–0.11)	0.04 (0.02–0.12)	9.57 (5.52–10.00)
IP-10 plasma (ng/ml)	0.16 (0.05–0.44)	0.27 (0.10–0.53)	9.80 (4.84–13.19)
IP-10 DPS (pg/2 DPS discs)	4.2 (0.00–14.3)	3.3 (0.00–14.6)	280.9 (148.1–381.2)
DPS, dried plasma spot; IP-10, interferon-γ-inducible protein.			

Table 2 Test concordance

		IP-10 (plasma or dried plasma spots)			
		Positive	Negative	Indeterminate	
QFT	Positive	2	0	0	2
	Borderline	0	1	0	1
	Negative	0	55	1	56
	Indeterminate	0	0	1	1
		2	56	2	60

IP-10, interferon-γ-inducible protein; QFT, QuantiFERON-TB Gold In-Tube test.

TB and tuberculin skin test, both tests being negative. This child was not offered any treatment.

Another child was IP-10 indeterminate but QFT negative. IFN-γ levels in response to mitogen were 1.4 IU/ml, and IP-10 levels were 1.27 ng/ml and 43 pg/2 DPS discs, in plasma and DPS samples, respectively (figure 2B).

Follow-up
No new cases were found among the contacts during the following 4 years; the total follow-up time of this study has been 240 person years. None of the seven contacts with a positive test and no symptoms of active TB (four contacts included in the study and the three other close contacts) had recurrent TB.
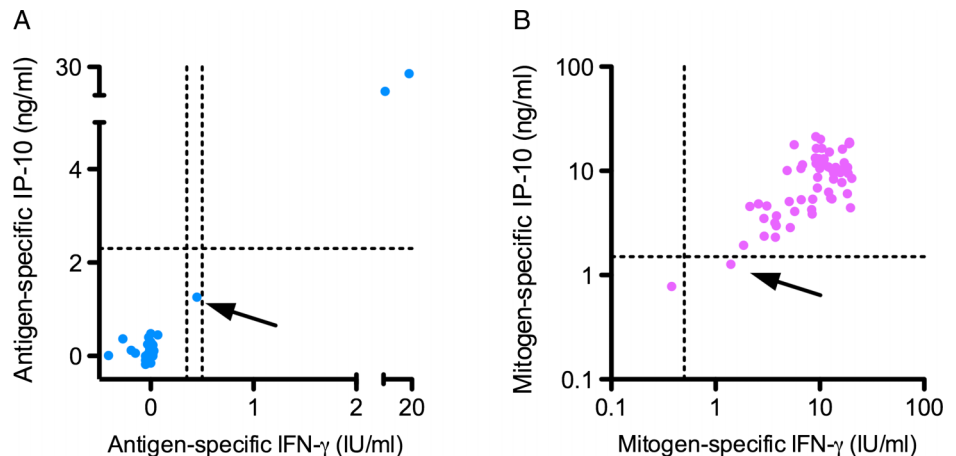
DISCUSSION
In this study, we compared the QFT and the IP-10 tests for the diagnosis of LTBI in a cohort of 6th grade school children from an urban setting in Finland. IP-10 was detected in both plasma samples and dried plasma spots and analysed using pre-set test algorithms; the two IP-10 methods were 100% concordant. Two children were deemed IP-10 and QFT positive. The QFT test showed 97% agreement with both the IP-10 tests. The children were followed up for 4 years and no new cases were identified, rendering a very high negative predictive value for both QFT and IP-10 tests.

This is the first study to describe the performance of IP-10 in a contact-tracing investigation in school children in a low-endemic setting. We applied IP-10 cutoffs and test algorithms for plasma and DPS estimated in cohorts of cases with active TB and healthy controls.[10] As expected, IP-10 from dried plasma spots and plasma samples correlated well, and a good agreement between the test results was obtained.[9] [10] The DPS samples had been stored in sealed plastic bags with a desiccant for 12 months at −20°C before analysis, apparently without a loss of IP-10 signal. This finding adds to previous experiments of IP-10 stability in dried form on filter paper, where it was shown that there was no loss of IP-10 signal after 4 weeks of storage at temperatures up to +37°C.[9]

IP-10 and QFT performed similar, albeit 2 of the 60 paired samples were discordant. These findings are in line with previous studies in adults and children without any active TB or comorbidity.[11–15] One child was QFT borderline/IP-10 negative; this 12-year-old girl was TST and T-SPOT.TB negative at both initial and repeat tests 3 months later. Based on the complete test panel, no treatment was initiated. This child remains asymptomatic. Most probably, this child was not infected and the QFT was false positive, if the interpretation for positivity is taken strictly as instructed. We and others have shown earlier that the QFT assay could have high variation around the cutoff area, thus cautioning not to misinterpret clinical conversions and reversions when sequential samples are analysed.[16–18] IP-10 is expressed in 100-fold higher levels making detection of low-level responses possible with a simpler technology such as the filter paper assay, but also with a higher accuracy

Figure 2 Correlation plots: (A) Antigen-specific IFN-γ vs IP-10 (plasma) release, Lines denotes cutoff for the borderline 0.35–0.50 IU/ml and positive test 0.50 IU/ml and 2.3 ng/ml for IFN-γ and IP-10, respectively. (B) Mitogen-specific IFN-γ vs IP-10 (plasma) release lines denote cutoffs for indeterminate tests 0.5 IU/ml and 1.5 ng/ml for IFN-γ and IP-10, respectively. Arrows denote discordant responders.

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compared to IFN-γ.[3] [9] Further studies are needed to compare the reproducibility of the IP-10 and QFT ELISAs at concentrations in the range of the diagnostic cutoff.

Another discordant result was in a child who had a negative QFT and an indeterminate IP-10 test result. The IP-10 response to mitogen was only 0.23 ng/ml below the cutoff for an indeterminate test.[10] Notably, the current cutoff for indeterminate tests was established on a small set of data, and a larger material is needed to validate this cutoff.

Predictive value

One of the key questions in the field is how well IGRAs differentiate between people who will develop TB and those who will not, that is, the 'predictive value' of the IGRAs. In this study, none of the 56 children with negative QFT and IP-10 progressed to active TB at a 4 years follow-up, rendering a NPV of 100%. To our knowledge, this is the first assessment of the negative predictive value of IP-10. This finding supports recent reports demonstrating a very high negative predictive value of the IFN-γ-based IGRAs (reviewed by Diel et al[19]).

LIMITATIONS

There is no gold standard for *M tuberculosis* infection wherefore our assessments of QFT and IP-10 detection rates might be imprecise. Studies in patients with active TB suggest that both tests do not have perfect sensitivity and there is a risk of underestimation of the number of truly infected children.[3] [20] The long follow-up period brings strength to the assessment of the predictive values, but the low number of observations increases the risk of type-2 error. IFN-γ was measured using the SOP developed as HUSLAB. This method ensures a higher degree of accuracy in the IFN-γ measurements and has been fully optimised for the QFT interpretation algorithm.[7] [17] This modified algorithm classifies responses between 0.35 and 0.50 as borderline responses. One child fell into this category, and was subjected to further testing with the T-SPOT.TB and TST. Both these tests as well as the IP-10 test were found to be negative. This finding underlines the importance of interpreting IGRA responses based on both the test result per se and importantly also on the basis of the biomarker concentration.

CONCLUSION

We demonstrated that IP-10 and QFT perform comparably as screening tools for infection with *M tuberculosis* in a contact tracing. Both tests demonstrated 100% NPV. IP-10 determined in dried plasma spots was at par with IP-10 determined in plasma, which further supports the utility of this alternative technology.

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