



Safety and efficacy of the C-Tb skin test to diagnose *Mycobacterium tuberculosis* infection, compared with an interferon γ release assay and the tuberculin skin test: a phase 3, double-blind, randomised, controlled trial

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Summary

Background Targeted screening and treatment of *Mycobacterium tuberculosis* infection substantially reduces the risk of developing active tuberculosis. C-Tb (Statens Serum Institute, Copenhagen, Denmark) is a novel specific skin test based on ESAT-6 and CFP10 antigens. We investigated the safety and diagnostic potential of C-Tb compared with established tests in the contact-tracing setting.

Methods Negative controls, close contacts, occasional contacts, and patients with active pulmonary tuberculosis were enrolled at 13 centres in Spain. We compared C-Tb with the QuantiFERON-TB Gold In-Tube ([QFT] Qiagen, Hilden, Germany) interferon γ release assay (IGRA) and the purified protein derivative (PPD) RT 23 tuberculin skin test ([TST] Statens Serum Institute). All participants older than 5 years were tested with QFT. Some participants in the negative control group received C-Tb without the TST to test for potential interactions between C-Tb and PPD RT 23. The rest were randomly assigned in blocks of ten and tested with both C-Tb and TST, with five in each block receiving injection of C-Tb in the right arm and the TST in the left arm and five vice versa. The primary and safety analyses were done in all participants randomly assigned to a group who received any test. This trial is registered with ClinicalTrials.gov, number NCT01631266, and with EudraCT, number 2011-005617-36.

Findings From July 24, 2012, to Oct 2, 2014, 979 participants were enrolled, of whom 263 were negative controls, 299 were occasional contacts, 316 were close contacts, and 101 were patients with tuberculosis. 970 (99%) participants completed the trial. Induration sizes were similar for C-Tb and TST, but TST positivity was affected by BCG vaccination status. We found a strong positive trend towards C-Tb test positivity with increasing risk of infection, from 3% in negative controls to 16% in occasional contacts, to 43% in close contacts. C-Tb and QFT results were concordant in 785 (94%) of 834 participants aged 5 years and older, and results did not differ significantly between exposure groups. The safety profile of C-Tb was similar to that for the TST.

Interpretation C-Tb delivered IGRA-like results in a field-friendly format. Being unaffected by BCG vaccination status, the C-Tb skin test might provide more accurate treatment guidance in settings where the TST is commonly used.

Funding Statens Serum Institut.

Introduction

Latent infection with *Mycobacterium tuberculosis* is defined as a state of persistent immune response to *M tuberculosis* antigens without evidence of clinically manifested active tuberculosis.¹ Treatment of latent tuberculosis infection has an important role in tuberculosis control, and is a central pillar in the WHO's End TB Strategy.² Although treatment of latent tuberculosis infection lowers the risk of developing active tuberculosis, there is a trade-off between benefits and risk of harm to the individual. The guiding principle, therefore, is that clinicians should only test for latent tuberculosis if they intend to treat the patient if the result is positive.²⁻⁵ Systematic testing and treatment should be offered to contacts of individuals with infectious

pulmonary tuberculosis, and to other high-risk populations, such as people living with HIV and candidates for immunosuppressive treatment (eg, before TNF- α -blocker treatment is started). Systematic testing also has an important role in tuberculosis control and surveillance in high-risk settings, such as hospitals and prisons, and in the management of contacts of patients with multidrug-resistant tuberculosis.^{2,3}

Since its introduction in 1908, the tuberculin skin test (TST) has been the standard method to test for infection with *M tuberculosis*.⁶ Tuberculin purified protein derivative (PPD), a precipitate of species-non-specific antigens obtained from filtrates of sterilised, concentrated cultures of *M tuberculosis*, is injected intradermally on the forearm⁷ and the diameter of induration (palpable,

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Research in context

Evidence before this study

We searched PubMed and Google Scholar for papers published up to March 10, 2016, then up to June 15, 2016, with the terms “IGRA”, “C-Tb”, “Quantiferon”, “specific skin test”, and “tuberculin”. We identified many publications for interferon γ release assays (IGRAs) and the tuberculin skin test (TST), which we curated, guided by review and meta-analysis articles and the findings from the broader searches. Pooled estimates on diagnostic performance and predictive potential of comparator tests were pulled from the meta-analysis. The risk of bias from these estimates was interpreted, taking into account the background epidemiology, including force of infection with *Mycobacterium tuberculosis*.

Added value of this study

We assessed the diagnostic potential of C-Tb for detection of latent infection with *M tuberculosis* in contacts at risk of developing active tuberculosis. C-Tb was safe, detected a similar number of infections and had very high concordance with IGRA, along with similar specificity in negative controls. Previous BCG vaccination did not compromise the specificity of C-Tb, which was in contrast to the TST.

Implications of all the available evidence

Identification of latent tuberculosis infection has an important role in tuberculosis control, and is a central pillar in the new WHO End TB Strategy. IGRAs are the most accurate tests for latent tuberculosis infection, but are expensive and labour intensive, which makes them unsuitable for use in settings with restricted resources. Instead, programmes in these settings often rely on the less specific but field-friendly TST to guide treatment decisions. WHO estimates that up to 50 million people are tested with the TST each year, which suggests a substantial degree of overtreatment and preventable adverse drug reactions. C-Tb provided good specificity that was not affected by BCG vaccination status. C-Tb might, therefore, offer more accurate treatment guidance than the TST, which could have substantial effects on morbidity and health expenditure. The similar performance of C-Tb and the QuantiFERON-TB Gold In-Tube IGRA suggests that the accumulated clinical experience with IGRA use for prevention of tuberculosis among contacts can be extrapolated to C-Tb, but further trials are needed to confirm our results.

raised, hardened area) at the site of injection is assessed after 48–72 h.⁶ Important limitations of the TST include the number of false-positive reactions that occur in people infected with non-tuberculous mycobacteria and in people with previous BCG vaccination.⁸ False-positive TST reactions can be overcome to some extent by use of interpretation algorithms that adjust the cutoff value for a positive result dependent on age, BCG vaccination status, HIV infection status, and the individual's risk of developing tuberculosis. Complex TST interpretation algorithms are implemented in many national tuberculosis control programmes.^{9,10} Nevertheless, guidelines vary between countries and settings, and complex TST interpretation algorithms need clinical information that is rarely routinely obtained in a contact investigation.

Interferon γ release assays (IGRAs) are in-vitro diagnostic alternatives to the TST.⁸ These tests are based on the highly immunogenic antigens ESAT-6 and CFP10, which are specific to *M tuberculosis*, and overcome the issues of the interaction with BCG vaccine and infection with non-tuberculous mycobacteria seen with the TST.⁸ Two IGRA tests are commercially available, QuantiFERON-TB Gold In-Tube test ([QFT] Qiagen, Hilden, Germany), which uses whole blood, and the T-SPOT.TB test (Oxford Immunotec, Abingdon, UK), which uses purified peripheral blood mononuclear cells.⁸ IGRAs are more complex and labour intensive than the TST, and need a laboratory infrastructure and skilled staff, but interpretation of the results is more objective and simple.⁸ Nevertheless, IGRAs and the TST have low positive predictive value for the development of active tuberculosis.^{1,8,11,12}

C-Tb (Statens Serum Institute, Copenhagen, Denmark) is a skin test based on recombinant ESAT-6 and CFP10, and is designed to combine the operational advantages of the TST with the specificity of IGRAs for the diagnosis of *M tuberculosis* infection in individuals at risk of developing active tuberculosis. In two phase 2 trials, C-Tb generated indurations of similar sizes to TST and had similar diagnostic specificity and sensitivity to QFT in presumed unexposed individuals and patients with microbiologically confirmed tuberculosis.^{13,14}

We did a phase 3 trial to investigate whether there would be a trend in increased C-Tb test positivity (induration ≥ 5 mm) with increasing risk of tuberculosis (ie, from negative controls, to occasional and close contacts, to people with confirmed tuberculosis). We also compared positivity by BCG vaccination status for C-Tb, QFT, and the TST, and compared safety for C-Tb and the TST.

Methods

Study design and participants

The TESEC06 trial was a combined open comparison of C-Tb positivity compared with QFT in four risk groups and a double-blind, randomised, split-body safety assessment of C-Tb versus TST. To explore whether there was any interaction between C-Tb and PPD, a subgroup of 50 participants in the negative control group received only an injection of C-Tb. We also did a subgroup analysis in participants younger than 18 years. Participants were enrolled at 13 participating centres in Spain. Eligible participants could qualify for four risk groups: negative control, occasional contacts, close

contacts, and positive controls. The negative control group was recruited by advertising to students and staff at the universities where the investigators taught. These volunteers had to have no history of exposure to and no signs or symptoms of tuberculosis. Occasional and close contacts were identified through a routine contact-tracing programme, and all were contacts of patients with smear-positive pulmonary tuberculosis confirmed by culture or nucleic-acid amplification techniques and defined according to local guidelines.^{15,16} Occasional contacts had had frequent sporadic contact with infected individuals in a confined space for between 6 h per week and less than 6 h per day in the previous year (contact tracing circles 2 and 3), and close contacts had had at least 6 h daily contact in a confined space in the past year (contact tracing circle 1). All contacts who met these criteria were invited to participate in the trial. Positive controls were patients randomly selected from tuberculosis registers who had received a diagnosis of tuberculosis in the previous 3 years that was confirmed by culture or nucleic-acid amplification techniques. They were approached by telephone or during non-trial visits to the study centres. Participants in all groups had to be aged between 6 weeks and 65 years.

We excluded people who had been recently vaccinated with any live vaccine or had had the TST in the previous 12 months (to eliminate the potential risk for boosting). Other exclusion criteria were breastfeeding; a positive pregnancy test; the intention of becoming pregnant or unwillingness to use contraception during the trial period; active disease affecting the lymphoid organs except for HIV; current skin conditions that could interfere with the measurement of induration after the C-Tb or TST; conditions with which drawing of blood posed more than minimum risk for the participant; other disorders that investigators deemed unsuitable; participation in another trial that the investigator judged might interfere with this trial; and previous participation in a trial investigating injection with ESAT-6, CFP10, or both. People living with HIV could be included in the trial. No participant underwent HIV testing as part of the trial.

Ethics approval was obtained from local ethics committees and the Clinic Hospital of Barcelona institutional review board. The Spanish Agency of Medicines and Medical Devices approved all versions of the study protocol. All participants gave written informed consent before enrolment. The study was monitored by an independent data safety and monitoring board.

Randomisation and masking

The randomisation schedule was generated centrally by an independent statistician not involved in the trial, by random permutation programmed in SAS version 9.3. 500 consecutive screening numbers were made available for each site. Numbers were assigned in blocks of ten (five numbers for concomitant use of C-Tb in the right

arm and the TST in the left arm, and five numbers for concomitant use of C-Tb in the left arm and the TST in the right arm).

To mask which test was being used in which arm, physicians were provided with test kits that contained two identical vials, one containing C-Tb and one containing the PPD RT 23 (Statens Serum Institute), and instructions about in which arm each test should be administered. The vials and the cardboard box of each kit were all labelled with the same randomisation number. A sealed opaque emergency envelope was also provided for each participant, prepared by the quality assurance department at Statens Serum Institute by staff members not involved in the trial. In the case of an emergency, the envelope could be opened to reveal the allocation of the test agent without unmasking the whole trial. The principal investigator at each site kept the sealed emergency envelopes in a locked cabinet, and a set was also kept in the Department of Regulatory and Medical Affairs at the Statens Serum Institute, under the supervision of a person qualified in pharmacovigilance.

Procedures

QFT testing was planned for all participants aged 5 years and over and before the skin test agents were administered to avoid possible booster responses. The results were interpreted as per manufacturer's recommendations (cutoff for positivity 0.35 IU/L interferon γ).

The C-Tb solution contains recombinant ESAT-6 (dimer) and CFP10 (monomer) antigens derived from *M tuberculosis* and expressed in *Lactococcus lactis*. This and the TST agent were clear, colourless solutions. C-Tb was administered intradermally in 0.1 μg per 0.1 mL dose.¹³ C-Tb and PPD RT 23 were injected according to the Mantoux technique and induration responses were assessed after 48–72 h. Induration was measured transversely to the long axis of the forearm with the ballpoint pen method, from lateral to central on all sides. All skin reactions were measured and agreed by two different investigators and were documented with digital images. With both skin tests, reactions were classified as positive if the induration size was 5 mm or larger (as determined by a receiver operating curve analysis in a separate study).^{13,14} In exploratory analyses, we assessed TST positivity with the alternative cutoffs of 15 mm and 10 mm.

Follow-up and data collection

Participants attended four visits at study centres. At the screening visit (up to 28 days before randomisation), eligibility was checked, informed consent and medical history were obtained, and participants underwent a medical examination and had blood samples taken for safety tests. At baseline, all female participants of childbearing age had pregnancy tests, blood samples were taken for CD4 cell counts in HIV-infected

participants, blood samples for QFT tests were taken in all participants older than 5 years, C-Tb and the TST were administered, and all participants were given diaries. Participants attended follow-up visits on days 2–3 for assessment of skin test reactions, and on day 28 for the safety assessment.

Objectives and outcomes

The primary efficacy outcome was test positivity, defined for C-Tb and the TST as induration 5 mm or larger 48–72 h after intradermal injection. The main efficacy comparator was the QFT. The primary trial objective was to assess whether a trend was seen in the number of C-Tb positive test results with increasing risk of *M tuberculosis* infection (ie, from negative controls to the occasional contacts to close contacts). Secondary outcomes were comparisons of C-Tb, QFT, and TST test positivity, the number of responders (induration >0 mm) among individuals in the negative control group who had received BCG vaccination, and comparisons of the numbers of responders and induration size by risk group, age, sex, and BCG vaccination status.

The safety assessment was based on adverse events after skin testing. Adverse events were classified as injection-site reactions or systemic adverse events (all non-injection-site reactions), and described with Medical Dictionary for Regulatory Activities preferred terms and system organ class levels. As systemic adverse events in participants who received both C-Tb and the TST could not be related to either agent separately, they were ascribed to C-Tb. Laboratory safety parameters included haematology, biochemistry, and measurement of glucose concentrations. The full list of secondary and safety trial objectives is available in the appendix (pp 1–2).

See Online for appendix

Statistical analysis

We calculated that to achieve at least 90% power to detect a trend in C-Tb positivity across the risk groups, assuming a rising number of positive tests from 5%, to 20%, to 40%, and to 95% across groups and 10% dropout, we would need to enrol 950 volunteers (250 negative controls, 300 occasional contacts, 300 close contacts, and 100 positive controls). Numerical data are presented as number (%) of participants, mean (SD), or median (range), dependent on the distribution. Categorical data are presented as number (%) of participants. Differences in positivity between tests and associations with risk groups were assessed with a multivariate logistic regression model and are described with odds ratios (ORs) and 95% CIs. Differences in the number of positive responders in paired analyses were assessed with McNemar's test for marginal heterogeneity, and in unpaired analyses with Fisher's exact test. Agreement between C-Tb and QFT was assessed with Cohen's κ coefficient. Differences in induration size between skin tests were compared with regression analysis and ANOVA. The primary and safety analyses were done in the full analysis set, which included all participants randomly assigned to a group who had received any test. Statistical analyses were done with SAS version 9.3, and graphs were prepared in GraphPad Prism version 6.4. This study is registered with ClinicalTrials.gov, number NCT01631266, and with EudraCT, number 2011-005617-36.

Role of funding source

The funder of the study was involved in the study design, data analysis, data interpretation, and writing of the report. The funder had no role in data collection. The

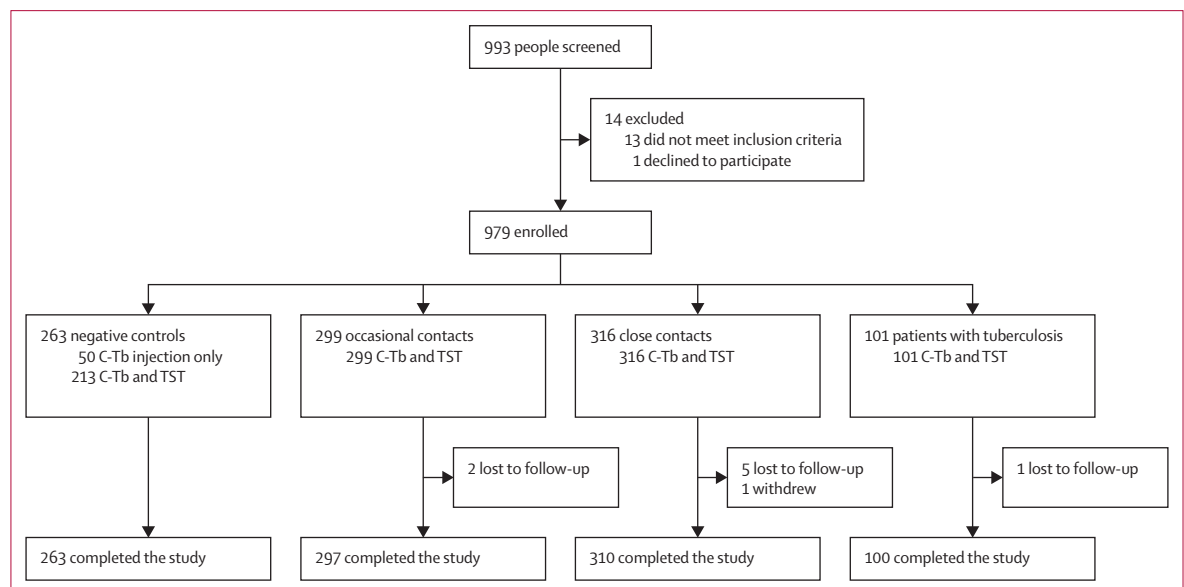


Figure 1: Trial profile
TST=tuberculin skin test.

corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

From July 24, 2012, to Oct 2, 2014, 993 volunteers were screened, of whom 979 were enrolled. 970 (99%) of 979 completed the trial (figure 1). Some of the patients with active tuberculosis were still taking treatment and some had completed treatment within the 3 years before the study (median time since diagnosis 392 days, IQR 143–577). Close and occasional contacts had similar mean ages. Negative controls were significantly younger and people with tuberculosis were significantly older than contacts (table 1). There were more girls and women in the study (519 [54%] of 979) than boys and men. Most participants were white (898 [91%] of 979); 366 (37%) of 979 were BCG-vaccinated and 104 (11%) of 979 had unknown BCG vaccination status (table 1). The overall mean body-mass index was 24.3 (SD 5.0) kg/m² and was similar across the four risk groups. 214 (22%) of 979 participants reported known HIV status (seven [1%] were HIV positive, six of the patients with active tuberculosis and one participant in the close contact group), all of whom were adults with CD4 cell counts greater than 1000 cells/μL.

C-Tb results were available for 977 (99%) of 979 participants, QFT results for 940 (96%) of 979, and TST results for 928 (95%) of 979 (table 2). 936 participants had determinate and four (in the two contact groups) had indeterminate QFT test results. 13 occasional contacts and 26 close contacts did not have blood taken for QFT testing, of whom 38 (98%) were younger than 18 years. The two participants with missing C-Tb results had negative QFT results.

701 (72%) of the 977 participants who received C-Tb skin tests were classified as non-responders and 276 (28%) as responders (induration >0 mm). Very few negative controls and occasional contacts had responses to C-Tb, whereas almost half of the close contacts and 69% of the people with active tuberculosis had responses (figure 2). In a pooled analysis of responders from all four exposure groups, induration size followed a normal distribution (mean 19.0 [SD 8.7] mm, data not shown). More responders to the TST than to C-Tb were seen in all exposure groups (figure 2) and the distribution was skewed to the left, showing a greater overlap with non-responders and a lower mean induration size (15.2 [7.6] mm, data not shown).

C-Tb test positivity, defined as induration 5 mm or larger, showed a significant ($p < 0.0001$) increasing trend with increasing risk of *M tuberculosis* infection from nine (3%) of 262 in the negative control group, to 49 (16%) of 299 among occasional contacts, to 136 (43%) of 316 among close contacts (tables 2, 3). A similar trend was observed for QFT (tables 2, 3). C-Tb and QFT positivity were highly concordant in negative controls

and occasional and close contacts, showing agreement in 785 (94%) of 834 participants across these groups (McNemar $p = 0.57$ and Cohen's κ coefficient 0.83 (95% CI 0.79–0.88; appendix p 3). In an assessment of the two contact groups, C-Tb and QFT positivity did not differ (appendix p 4). By contrast, C-Tb was classified as positive in fewer patients with active tuberculosis than was QFT ($p = 0.003$, table 2).

With the TST, significantly more participants were classified as having positive results than with C-Tb (appendix p 4). Most participants with positive TST and negative C-Tb, QFT, or both, results were in the negative control or occasional contact groups (table 2), which was expected because of the risk of PPD

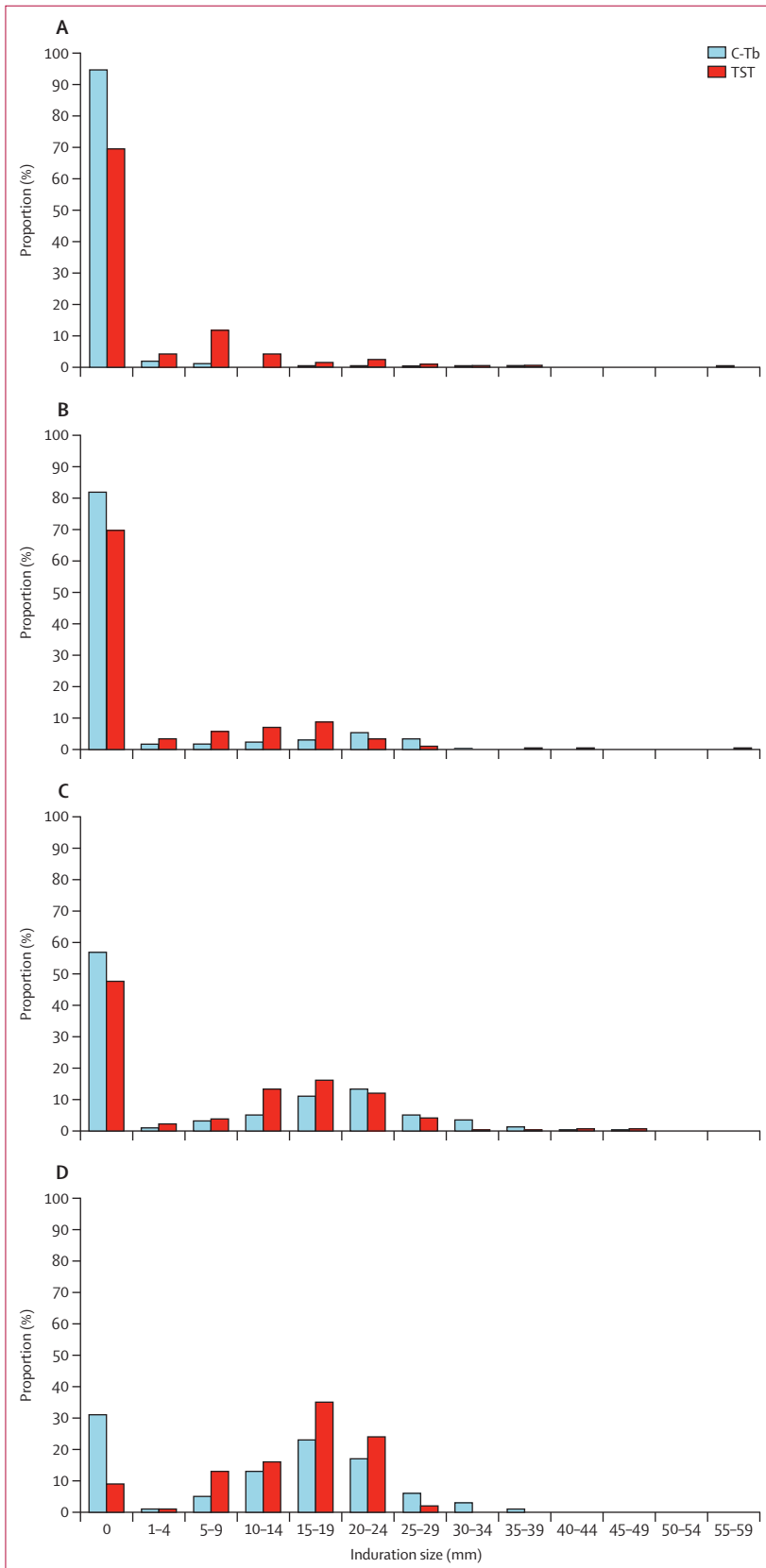
	Negative controls (n=263)	Occasional contacts (n=299)	Close contacts (n=316)	Patients with tuberculosis (n=101)
Age (years)				
Mean (SD)	24.1 (7.9)	31.5 (14.3)	32.9 (17.7)	37.3 (11.2)
>17	259 (98%)	262 (89%)	239 (76%)	98 (97%)
5–17	4 (2%)	25 (8%)	54 (17%)	3 (3%)
<5	0	12 (4%)	23 (7%)	0
Sex				
Female	188 (72%)	150 (50%)	146 (46%)	40 (40%)
Male	75 (29%)	149 (50%)	170 (54%)	61 (60%)
Country of birth				
Spain	263 (100%)	287 (96%)	267 (85%)	78 (77%)
Other*	0	12 (4%)	49 (16%)	23 (23%)
BCG status				
Vaccinated	108 (41%)	101 (34%)	113 (36%)	44 (44%)
Unvaccinated	154 (59%)	167 (56%)	151 (48%)	37 (36%)
Unknown	1 (<0.5%)	31 (10%)	52 (17%)	20 (20%)

*Includes Latin-American, European, Asian, and African countries.

Table 1: Baseline characteristics

	Negative controls (n=263)	Occasional contacts (n=299)	Close contacts (n=319)	Patients with tuberculosis (n=101)
C-Tb skin test				
Positive	9 (3%)	49 (16%)	136 (43%)	68 (67%)
Negative	253 (96%)	250 (84%)	180 (57%)	32 (32%)
Not done	1 (<0.5%)	0	0	1 (1%)
QuantIFERON-TB Gold In-Tube interferon γ release assay				
Positive	10 (4%)	57 (21%)	122 (42%)	82 (81%)
Negative	253 (96%)	227 (82%)	166 (57%)	19 (19%)
Indeterminate	0	2 (<1%)	2 (<1%)	0
Not done	0	13	26	0
Tuberculin skin test				
Positive	46 (22%)	80 (27%)	162 (51%)	90 (90%)
Negative	167 (78%)	219 (73%)	154 (49%)	10 (10%)
Not done	50 (19%)	0	0	1 (1%)

Table 2: Results by tuberculosis test



cross-reactivity with the BCG vaccine. To investigate this potential interference, we separated participants by BCG vaccination status. The number of positive results with the TST were similar to those for C-Tb and QFT in unvaccinated negative controls and contacts, but the number of responders to the TST in these groups was significantly higher among vaccinated participants (figure 3). When a 15 mm cutoff was used for the TST in BCG vaccinated participants but the 5 mm cutoff was maintained in HIV-infected participants, the number of positive results decreased and became similar to the numbers for C-Tb and QFT (appendix p 5). After this adjustment, the TST was concordant with C-Tb in 831 (90%) of 927 participants and with QFT in 784 (89%) of 885 participants, and TST specificity in BCG vaccinated participants rose from 62% to 92% ($p < 0.0001$), which is similar to that for C-Tb and QFT (both 96%, $p = 0.096$; appendix p 5).

Whether there was an interaction between C-Tb and PPD RT 23 was assessed by comparing positivity in negative controls who received only C-Tb ($n = 50$) with those who received C-Tb and the TST concomitantly ($n = 103$). The numbers of positive results were low in both subgroups (none vs four [4%], $p = 0.30$), which suggests that C-Tb responses were not affected by PPD RT 23 administered at the same time.

121 (12%) participants were younger than 18 years, among whom 35 (29%) were younger than 5 years and 21 (18%) were BCG vaccine recipients (appendix p 6). 114 (94%) of 121 children were in the occasional contact or close contact group. 28 (33%) of 86 children aged 5–17 years and eight (23%) of 35 younger than 5 years had positive C-Tb results. We saw concordance between C-Tb and QFT in 79 (95%) of 83 children aged 5–17 years (appendix p 6). Although the study was not powered to assess the effect of age on test positivity, a gradual negative effect on skin test reactivity (magnitude of induration response) with decreasing age was hypothesised. However, when we compared the magnitude of response in children aged 0–4 years with that in those aged 5–17 years, we found no evidence of an effect of age on TST or C-Tb induration size.

In the 28-day follow-up period, 12 participants (three occasional contacts and nine close contacts, including two children aged 1 year and 6 years) were diagnosed as having active tuberculosis. Diagnoses were based on clinical symptoms in five and on microbiological findings in seven participants. Ten of the 12 participants had positive C-Tb, QFT, and TST tests. The other two participants, both adults, had negative results in all three tests. None of the 12 participants reported symptoms at the time of enrolment.

Figure 2: Distribution of induration sizes after C-Tb and the TST
(A) Negative controls. (B) Occasional contacts. (C) Close contacts. (D) Patients with tuberculosis. TST=tuberculin skin test.

565 injection-site reactions were reported by 341 (35%) participants and 550 systemic adverse events were reported by 317 (32%) participants. The most common injection-site reactions were pruritus, haematoma, and pain (table 4). Injection-site haematoma and pain were associated with C-Tb significantly more often than with the TST. The most common systemic adverse events were headache and nasopharyngitis, which were reported by 137 (14%) and 39 (4%) participants, respectively. 31 (6%) systemic adverse events were deemed to be certainly or possibly related to the skin tests. No systemic adverse events led to withdrawal from the trial. 559 (99%) injection-site reactions and 523 (95%) systemic adverse events were mild to moderate. Only one participant, in the positive control group, reported one serious adverse event (a transient increase in the concentrations of liver enzymes), but this was deemed to be unrelated to skin testing. No participants died during the trial.

Discussion

In this phase 3 trial, we explored the association between C-Tb test positivity and risk of infection with *M tuberculosis* in a contact-tracing setting. C-Tb induration sizes were similar in magnitude to those after the TST, but because C-Tb was unaffected by BCG vaccination status, separation of responders from non-responders was clearer than with the TST. C-Tb was safe, highly concordant with QFT, and positive results were strongly associated with risk of infection.

The strength of this large contact-tracing trial is that assessment of C-Tb and the TST was masked and done in parallel in the target population for the tests. Positive results with TST and QFT were highly associated with *M tuberculosis* exposure, with findings being similar to those in other large contact-tracing trials in low endemic areas.^{11,12,17} C-Tb and QFT were highly concordant in the negative control and the two contact groups, which suggests similar clinical performance to QFT.^{13,14} C-Tb seems to resolve the issue of specificity that is associated with the TST,⁸ but, owing to the high concordance between C-Tb and QFT, C-Tb is unlikely to improve positive predictive value for developing tuberculosis.^{11,12} The 5% discordance between C-Tb and QFT suggests that combining these tests might increase the diagnostic sensitivity, which could be beneficial in immunocompromised high-risk people, such as those living with HIV, in whom QFT is suboptimum.¹⁸

We included participants with current and previous microbiologically confirmed active tuberculosis as positive controls.^{19–21} This approach has proven useful for assessment of diagnostic potential in case-control trials,²¹ but around 20% of patients with tuberculosis have negative results with the TST, IGRAs, or both.^{8,20} In our trial, 14% fewer C-Tb tests were positive than QFT tests among patients with tuberculosis, which conflicts with the findings in previous studies, where

C-Tb and QFT had similar sensitivity (Aggerbeck H, unpublished).^{13,14}

PPD comprises more than 100 different antigens and can induce responses from a larger pool of specific T cells than C-Tb,²² and, therefore, it could be speculated that C-Tb responses would be smaller and less consistent than TST responses. The size of induration after injection with C-Tb and the TST, however, indicates that the C-Tb response is robust and that interpretation of test results by health-care providers already familiar with the TST will be similar. C-Tb separated responders from non-responders more clearly than the TST in this study, including in BCG vaccine recipients. While this separation was also seen with the TST in participants not vaccinated with BCG, differentiation was less clear in those who had received the BCG vaccine. This well known effect of BCG on TST results can be overcome to some extent by increasing the induration cutoff in BCG vaccinated individuals without HIV infection. This

	C-Tb skin test	Tuberculin skin test	QuantiFERON-TB Gold In-Tube IGRA
Patients with tuberculosis vs negative controls	47.6 (21.2–106.8)	28.2 (13.2–60.4)	90.2 (37.5–217.2)
Close contacts vs negative controls	17.8 (8.7–36.6)	3.5 (2.3–5.4)	18.6 (8.6–40.0)
Occasional contacts vs negative controls	4.6 (2.2–9.8)	1.2 (0.8–1.9)*	6.8 (2.8–13.4)
Patients with tuberculosis vs occasional contacts	10.2 (6.0–17.4)	23.0 (11.2–47.0)	14.7 (8.2–26.0)
Close contacts vs occasional contacts	3.8 (2.6–5.7)	2.9 (2.0–4.1)	3.0 (2.0–4.4)
Patients with tuberculosis vs close contacts	2.7 (1.6–4.3)	8.0 (4.0–16.2)	4.9 (2.8–8.4)

Multivariate logistic regression analysis adjusted for BCG vaccination status, sex, and age. For all, $p < 0.0001$ unless otherwise stated. IGRA=interferon γ release assay. *Not significant.

Table 3: Odd ratios (95% CIs) for test positivity across risk groups

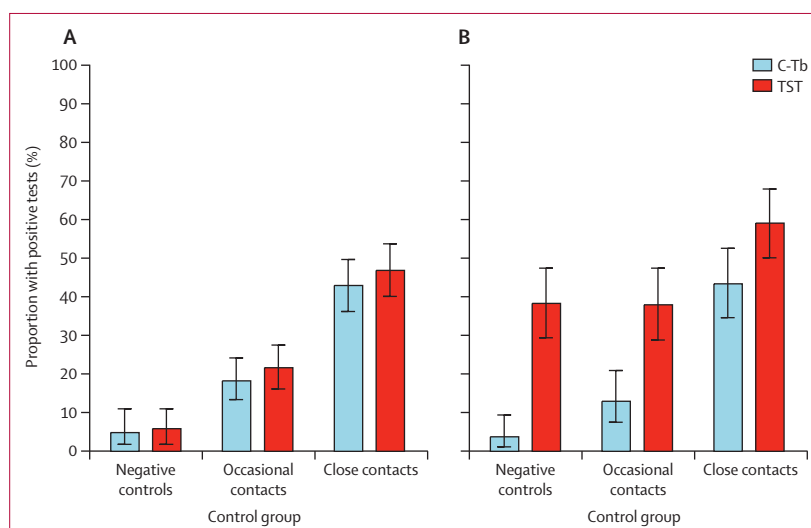


Figure 3: Association between positive skin test results and risk of infection in BCG vaccination status (A) Not BCG vaccinated. (B) BCG vaccinated. TST=tuberculin skin test.

	C-Tb skin test (n=978)	TST (n=929)
All	347 in 288 (29%)	218 in 182 (20%)
Pruritus	126 in 126 (13%)	134 in 134 (14%)
Haematoma	133 in 133 (14%)	18 in 18 (2%)*
Pain	41 in 41 (4%)	32 in 34 (3%)*
Vesicles	17 in 17 (2%)	13 in 13 (1%)
Rash	13 in 13 (1%)	13 in 13 (1%)
Haemorrhage	11 in 11 (1%)	3 in 3 (<0.5%)
Induration	2 in 2 (<0.5%)	1 in 1 (<0.5%)
Discolouration	1 in 1 (<0.5%)	1 in 1 (<0.5%)
Oedema	1 in 1 (<0.5%)	0
Exfoliation	0	1 in 1 (<0.5%)
Erythema	0	1 in 1 (<0.5%)
Urticaria	1 in 1 (<0.5%)	0
Anaesthesia	1 in 1 (<0.5%)	0
Paraesthesia	0	1 in 1 (<0.5%)

Data are shown as the number of injection-site reactions and the total number (%) of participants affected. All participants shown received both skin tests and, therefore, the number of reactions may be greater than the number of participants. Injection-site reactions are described with the Medical Dictionary for Regulatory Activities preferred terms. TST=tuberculin skin test. *p<0.05 for C-Tb vs the TST.

Table 4: Injection-site reactions in the full analysis set

adjustment, however, also increases the complexity of the diagnostic algorithm because further clinical information is needed that might be difficult to obtain, especially in resource-restricted settings (eg, HIV testing).^{10,13} Under ideal phase 3 trial conditions, though, the adjusted cutoff increased overall TST concordance to 89% with QFT and 90% with C-Tb. TST specificity in individuals previously vaccinated with BCG also improved from 62% to 92%, bringing it in line with that for QFT and C-Tb (both 96%).

Adverse events were for C-Tb similar to that for TST except for injection-site haematomas, which were reported seven times more often at C-Tb injection sites. Haematomas were mostly small (a few mm) and transient, but were nonetheless clearly associated with C-Tb. Previous studies have also shown haematomas to be the most frequent injection-site reaction related to C-Tb testing (Aggerbeck H, unpublished).^{13,14} In a joint analysis of seven completed trials of C-Tb that involved 2957 participants, haematoma at the C-Tb injection site was seen in 172 (6%), compared with in 25 (1%) of 2826 at the TST site. 195 (99%) of 197 instances of haematoma were seen in trials done in Europe, and two were seen in trials done in Africa. Haematomas were mainly mild (99%) and the rest were moderate. Of note, haematomas were almost exclusively (92%) reported in participants with negative C-Tb test results, which suggests under-reporting of haematomas in individuals with induration and underestimation of the true prevalence of

haematomas in BCG vaccine recipients who have been tested with the TST. Future studies are needed to assess the real frequency of haematomas associated with C-Tb.

Our study has several limitations. C-Tb was developed as a tool to guide treatment for latent tuberculosis infection in people at risk of developing active tuberculosis. Ten participants who had positive C-Tb and QFT results and two who had negative C-Tb and QFT results developed active tuberculosis during follow-up, which corresponds to 2% of 615 contacts. Most of these contacts probably had insipient active tuberculosis at the time of enrolment, despite the absence of symptoms, and the follow-up period was too short to assess the predictive potential of C-Tb. On the basis of the high concordance between tests, we assume that C-Tb has similar positive predictive value to QFT, but we cannot exclude the possibility that the individuals who progressed would be among the 5% with positive QFT and negative C-Tb results. Although a randomised controlled intervention trial would be needed to confirm that progression occurs in this discordant subgroup, we think it would be unlikely because C-Tb and IGRA are based on the same antigens, detect the same type of immune response, and probably have very similar positive and negative predictive capabilities. Although we present data on the diagnostic performance of C-Tb in children and people living with HIV, this study was not powered to test effects in these subgroups. These questions have been formally addressed in a phase 3 trial being done in South Africa (NCT1642888).

The ambitious goals of WHO's End TB Strategy have led to a renewed focus on screening for and treatment of latent tuberculosis infection in individuals at risk.^{9,23} The guidelines for the programmatic management of latent tuberculosis infection now recommend extending these activities to resource-limited countries.^{9,24} Owing to good specificity, IGRAs are recommended in high-income and upper-middle-income countries, but not in middle-income and low-income countries with incidence of tuberculosis greater than 100 per 100 000 of the general population, where skin testing is recommended instead.⁹ The rationale for this restriction is that false-positive TST responses are less frequent in individuals who received BCG vaccination at birth,²⁵ which is common in low-income countries, and the skin test format provides operational and financial advantages, including low equipment, laboratory, and infrastructure requirements, low reagent costs, and limited skill requirements. By contrast, IGRAs are expensive to implement on a large scale in settings with restricted resources.²⁶

The C-Tb was designed to provide high specificity in a field-friendly format. It improves on the specificity of the TST in settings where BCG coverage is high, which suggests that if C-Tb were available worldwide, it could have a substantial effect on morbidity and health expenditure.²⁷ Furthermore, as the manufacturing process for C-Tb is modern and simple compared with that for PPD,

the periodic shortages that have been seen for the TST²⁸ seems less likely to occur with C-Tb.

Our data in this double-blind, randomised clinical trial show that C-Tb was safe, detected similar numbers of people with *M tuberculosis* infection with very high concordance to QFT in individuals aged 5 years and older, and showed good specificity in negative controls. By contrast, TST specificity was compromised in people who had received BCG vaccination. The similar results for C-Tb and QFT suggest that the accumulated clinical experience gained with IGRA use for tuberculosis prevention can be extrapolated to C-Tb. Nevertheless, further trials are needed to confirm our findings and to determine the cost-effectiveness and the potential of C-Tb to predict later development of active tuberculosis.

Contributors

HA, STH, BB, and IK designed the trial and STH, BB, and IK oversaw the trial conduct and JAC coordinated the trial and communication between sites. RVG, JIV, JAM, AP, LLA, MLdS-G, FS, JAR-P, AN-J, XM-L, MVT, VLF, JPM, AM, NC, JMM, LR, and AO coordinated individual trial sites and participated in the inclusion of trial participants. MR, HA, JAC, and PA participated in the statistical analysis. MR, HA, STH, BB, and IK, JA, and PA interpreted the data. MR wrote the first draft and HA, RVG, JIV, JAM, AP, LLA, MLdS-G, FS, JAR-P, AN-J, XM-L, MVT, VLF, JPM, AM, NC, JMM, LR, AO, and PA reviewed the report. All authors approved the final version of the Article before submission.

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Declaration of interests

MR, HA, BB, IK, and PA are employees of Statens Serum Institut, Copenhagen, Denmark, which is a governmental not-for-profit research organisation that holds all intellectual property rights on the use of the ESAT-6 and CFP10 proteins in interferon γ release assays and C-Tb. The other authors declare no competing interests.

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