The diagnostic performance of novel skin-based *in vivo* tests for tuberculosis infection compared to PPD tuberculin skin tests and blood-based in vitro interferon-gamma release assays: A systematic review and meta-analysis

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Abstract

Background

Novel skin-based tests for Tuberculosis (TB) infection may present suitable alternatives to current tests, however, diagnostic performance compared to the PPD-tuberculin skin test (TST) or interferon-gamma release assays (IGRA) needs systematic assessment.

Methods

English (Medline OVID), Chinese (Chinese Biomedical Literature Database and the China National Knowledge Infrastructure), and Russian (e-library) databases were searched up to 15 May 2019 (with updated Russian and English searches on 20 October 2020) "using terms "ESAT6" OR "CFP10" AND "skin test" AND "Tuberculosis" OR "C-Tb" OR "Diaskintest". We included studies reporting performance of index tests alone, or against a comparator. Pooled random-effects estimates are presented where appropriate; total agreement proportion, sensitivity in microbiologically-confirmed tuberculosis and specificity in cohorts with low risk of TB infection. Study quality was assessed with QUADAS-2. (PROSPERO: CRD42019135572).

Findings

29 Diaskintest (N=7,111), five C-Tb (N=2,744), two EC-skintest (N=887), and one DPPD (N=173) studies were reviewed. Tested sub-populations included HIV-infected, children and TB-exposed individuals. Studies were heterogeneous with moderate to high risk of bias. Nine head-to-head studies of index test *vs* TST and IGRA permitted direct comparisons and pooling.

In a mixed TB and non-TB cohort, Diaskintest pooled agreement with IGRA was 88% (95%CI:80-93%) *vs* TST-5mm cut-off 52% (95%CI:42-61%). Diaskintest sensitivity was 91% (95%CI:78-94%) *vs* TST-5mm 88% (95%CI:78-94%), IGRA QuantiFERON 90% (95%CI:79-95%) and TSPOT.TB 91% (95%CI:80-96%). C-Tb agreement with IGRA in active TB was 80% (95%CI:76-84%) vs TST-5mm/15mm cut-off 76% (95%CI:69-82%). C-Tb sensitivity was 75% (95%CI:70-78%) *vs* TST-5mm/15mm 79% (95%CI:68-86%), IGRA 72% (95%CI:63-79%). Specificity, C-Tb 98% (95%CI:94-99%) *vs* TST-15mm 93% (95%CI:90-95%), IGRA 99% (95%CI:80-100%). EC-skintest sensitivity was 86% (95%CI:82-89%).

Interpretation

Novel skin-based tests for TB infection appear to perform similarly to IGRA or TST, however, study quality varied. Evaluation of test performance, patient-important outcomes and diagnostic utility in current clinical algorithms will inform implementation in key populations.

Funding

New Diagnostic Working Group of STOP TB and FIND

Research in context

Evidence before this study

Although diagnosis and treatment of TB infection are key interventions to reduce global TB incidence, at least 30% of those who may benefit from treatment cannot access screening. The widely available skin-based screening test, the PPD-tuberculin skin test (TST), is limited by specificity, operability and dwindling stocks globally, whereas the blood-based interferon gamma release assays (IGRA) are limited by scalability and cost. Recently developed skin tests that elicit a more specific immune response to *Mycobacterium tuberculosis*, have the potential to increase access to more accurate screening tests by utilising existing inexpensive skin testing platforms, however synthesised evidence on diagnostic performance is lacking.

We searched MEDLINE and Embase (Ovid platform), Russian e-library, Chinese Biomedical Literature Database and the China National Knowledge Infrastructure databases on 15 May 2019 and updated the search on 20 October 2020 for all studies using terms "ESAT6" OR "CFP10" AND "skin test" AND "Tuberculosis" OR "C-Tb" OR "Diaskintest".

Added value of this study

Our systematic review synthesised available data on diagnostic performance of four novel skin tests including Diaskintest® (Generium, Russian Federation), C-Tb® (Serum Institute of India), EC-skintest® (Anhui Zhifei Longcom, China), and DPPD® (Creative Biolabs, USA). These were evaluated against a hierarchy of reference standards for TB infection consistent with the 2020 WHO Framework: (1) test agreement with IGRA or TST; (2) test sensitivity in those with microbiologically-confirmed active TB and specificity in those at low risk of TB infection; (3) association between index test result and proximity of exposure among case contacts; (4) predictive value of index test for incident TB; (5) efficacy of preventive therapy based on test result. The search identified 37 studies, of which we meta-analysed 22; only 9 were three-test head-to-head studies of index *vs* TST or IGRA. No longitudinal cohorts were identified, precluding evaluation of predictive ability. There was great heterogeneity in study design, and study quality. Tested sub-populations included HIV-infected, children and TB-exposed individuals. Head-to-head analyses that included three tests permitted simultaneous comparison of the index test *vs* TST or IGRA in the same population under the same study conditions tests, limiting heterogeneity; these enable robust direct comparisons of the performance all three tests and were prioritised over indirect comparisons. Results from these analyses indicate that, across all four novel skin test types, performance is similar to current tests for TB infection. Agreement with IGRA or the TST was similar, approximately 80% or more in individuals with or without active TB. Similarly, sensitivity of the novel skin tests appeared comparable to the TST or IGRA, irrespective of threshold for positivity chosen for the TST or IGRA type. Test specificity is as high as the IGRA (≥98%); shown in two C-Tb studies conducted in TB low-burden settings. The overall comparability of the new skin tests with TST or IGRA suggests the predictive ability of the tests for subsequent disease or benefit from TB preventive therapy would likely be similar.

Implications of all the available evidence

Novel skin-based tests may provide specific and accurate alternatives to current test of TB infection, given similar test performance; these have the potential to improve scale-up of TB prevention programmes and

enhance global TB control, without the need for venepuncture or expensive laboratory facilities. However, our review has also highlighted several limitations in study design and the quality of the evidence that would be useful to address in future studies. Use of the WHO Framework for evaluation of tuberculosis infection tests will help standardise study design. Post-licensure studies should assess test performance in more key populations (e.g. children, immunosuppressed people) and address heterogeneity in study design using head-to-head evaluation of tests. Our review does not inform the relative diagnostic advantage of these newer tests over IGRA or TST when used in current testing algorithms. Patient-important outcomes including implications of false negative or positive results, safety, cost-effectiveness, and qualitative evidence on feasibility, accessibility, patient and provider preference would inform successful implementation and resource planning.

Introduction

Two billion of the world's population are estimated to have TB infection.[1] Progression to active disease can result in transmission of infection and the risk is highest among young children, and in people with immunosuppressive conditions.[2] Strategies for TB control are anchored in screening at-risk populations and offering preventive therapy to those at highest risk of developing active TB disease.[3] Development and validation of accurate, affordable and scalable diagnostic tests for TB infection remain a priority.[3]

Currently the most widely used diagnostic tests are the purified protein derivative (PPD) tuberculin skin test (TST) and interferon gamma release assays (IGRA).[4] However, the TST has relatively low specificity (false positives in those with recent BCG vaccination),[5] lacks sensitivity in immunosuppressed individuals (e.g. HIV infected),[4] requires two clinic visits, and results must be read within the suggested timeframe to be valid. IGRA measure T-cell release of Interferon-gamma (IFNɣ) following stimulation by ESAT-6 and CFP-10 antigens that are specific to the Mycobacterium tuberculosis (*M.tb*) complex.[6] Unlike the TST, IGRAs are not affected by prior BCG vaccination, or by infection with non-tuberculous mycobacteria, with few exceptions.[7] However, IGRA platforms are more expensive to run, requiring specialised facilities and trained personnel.[8] Poor specificity and low testing coverage in areas of high TB prevalence along with global shortages of PPD has limited preventive treatment programmes and underscores the need for alternatives.[9,10]

Newer skin-based tests based on specific *M.tb* antigens have been developed, these combine the simpler skin-test platform with the specificity of IGRA. These include the C-Tb (Serum Institute of India), Diaskintest (Generium, Russian Federation) and the EC-skintest (Anhui Zhifei Longcom, China), which, like IGRA, all utilise recombinant ESAT-6 and CFP-10 antigens, and the DPPD test (Creative Biolabs, USA) which is a recombinant protein based on amino acids from the N-terminus sequence, unique to *M.tb.*[11] All tests use intradermal injection of antigen and, like the TST, are read as induration in mm after 48-72 hours using the method suggested by Mantoux.[12,13] Emerging evidence suggests that compared to IGRA, the tests may have similar specificity[14] and provide more reliable results in children and in HIV-infected cohorts.[15] However, the evidence has not been systematically reviewed.

We conducted a systematic review and meta-analysis to assess the performance of newer skin-based recombinant antigen tests compared with currently available tests for TB infection against a hierarchy of *a priori* agreed reference standards that were previously used for evaluation of IGRA performance [16] to determine; (1) test agreement with IGRA or TST; (2) test sensitivity in those with microbiologically-confirmed active TB and specificity in those at low risk of TB infection; (3) association between index test result and proximity of exposure among case contacts; (4) predictive value of index test for incident TB; (5) efficacy of preventive therapy based on test result.

Methods

Search strategy and study selection criteria

The protocol and search strategy were registered on PROSPERO (CRD42019135572, https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=135572. Our report follows PRISMA [17] and the WHO 2020 guidance for evaluating tests for TB infection.[18]

We conducted our search in Russian, English and Chinese on 15 May 2019 using multiple databases including Medline, Embase, e-library (www.e-library.ru), the Chinese Biomedical Literature Database and the China National Knowledge Infrastructure databases and updated Russian and English search on 20 October 2020. Search terms included "ESAT6" OR "CFP10" AND "skin test" AND "Tuberculosis" OR "C-Tb" OR "Diaskintest", and the detailed search terms and strategy are shown in supplementary Table S1. To identify additional studies, the test manufacturers were contacted.

Titles and abstracts were independently reviewed by LF and MK. Russian language studies were reviewed by MK and VN. Discrepancies were discussed with CMD or MXR and resolved by consensus. Further clarification was sought from authors where necessary. Double data extraction was performed by MK, LF and VN. The QUADAS-2[19] tool was used to assess individual study quality. The quality assessment was conducted by MK and verified by LF (for English-language studies) and by MK and VN (for Russian-language studies). Differences between reviewers were resolved by discussion with MXR.

Inclusion and exclusion criteria are described in supplementary Table S2. We used a hierarchy of *a priori* agreed reference standards for TB infection to benchmark test performance (Table S2).

Data analysis

Analyses were conducted in R, version 3·6·2 . Descriptive and quantitative analyses were performed.

Test agreement between the index test and each comparator test was calculated as the agreement proportion (total for negatives and positives), with 95% confidence intervals (CIs) (Clopper-Pearson exact CIs, ensuring valid values at proportions close to 1). Sensitivity in those with microbiologically-confirmed active TB and specificity in those at low risk of TB infection (restricted to studies from low TB burden countries) were calculated where possible.

For outcomes with two or more studies with available data, meta-analyses were performed where appropriate (by example, if studies used the same reference test, e.g. culture-confirmed TB, and/or in the same sub-population, e.g. HIV+, and/or used the same test cut-off for positivity, e.g. TST15mm or 10mm). Univariate random effects models were used for meta-analyses of agreement, sensitivity and specificity estimates (using the 'meta' package in R). [20] Random effects models were chosen (as opposed to fixed effects) to account for heterogeneity of study populations. We applied a continuity correction (0·5) to zero-cells. In addition to pooling agreement for each comparison of a new skin test *vs* TST or IGRA (e.g. two-way head-to-head), we also performed three-way head-to-head comparisons by restricting to studies that compared a new skin test *v*s TST

and IGRA. Meta-analysis of sensitivity and specificity was explored in two ways: (1) including all studies available for each test; and (2) in head-to-head comparisons. Three-way head-to-head analyses permit simultaneous comparison of all three tests in the same population under the same study conditions tests and are prioritised in the report over indirect comparisons.

To assess heterogeneity, we planned to stratify data and analyses by TB status (microbiologically-confirmed TB, under investigation for TB, no TB), age (children vs. adults), HIV status and previous BCG vaccination. Where feasible, results were pooled within these strata, and statistical heterogeneity assessed using the I^2 statistic. A lower I^2 value was interpreted as low between-study heterogeneity and consequently higher reliability of pooled estimates.

To assess 'dose-response' association along a gradient of exposure, we compared the proportion of positive index tests (with 95%CIs) in each contact group according to proximity from a source case.

Although not pre-specified in the protocol, sensitivity assessments were identified at analysis and performed on full data (Tables S11-S14, S20-S22, S24).

Role of funding source

The work was conducted in collaboration with the New Diagnostic Working Group of STOP TB and FIND; these funding entities gave input into study design, data collection, analysis, interpretation and manuscript writing. MK, LF, and MXR had full access to all of the data included in the study, LF and YH verified the statistical code, and MK and MXR accept responsibility for the decision to submit for publication.

Results

We identified 1,466 original articles, 427 in Russian language, 1,039 English, and none in Chinese, once duplicates were removed (PRISMA, Figure 1). We included 37 studies for qualitative synthesis (29 Diaskintest (Generium, Russia), five C-Tb (Serum Institute of India), two EC-skintest (Anhui Zhifei Longcom), one DPPD (Creative Biolabs, USA). Twenty-two studies were included in quantitative synthesis; 15 Diaskintest, five C-Tb, and two EC-skintest.

All 29 Diaskintest studies (7,111 participants) were conducted in Russia (Table 1). All were cross-sectional assessments under routine clinical practice, and cohorts recruited prospectively or constructed retrospectively. [21–48] None conducted randomised comparisons. Four studies [25,27,32,40] performed head-to-head comparisons of Diaskintest *vs* TST^{5mm} and IGRA in the same study. Study populations enrolled in studies included HIV-infected individuals [33,38,44,49] (346 adults, 23 children)) and children under 18 (N=3,803) (Table 1).[21,25–32,34–36,41,48] Approximately half of the HIV-infected cohorts had a CD4 count lower than 200 cells/mm^3. [33,38] Proportion BCG vaccinated was reported in 4 studies, and ranged from 93-100%.[25,27,28,44] Diaskintest threshold for positivity varied and included any skin induration ($Diaskintest^{AI}$) according to national guidance [50] or 5mm ($Diaskintest^{5mm}$). Studies used PPD-L TST previously shown bioequivalent to PPD-RT23;[51,52] ≥5mm (TST^{5mm})

induration denoted positivity, and 1-5 mm indeterminate.[50] IGRA used included T-SPOT.TB and QFT. Results are reported for QFT unless otherwise stated.

Of the five C-Tb studies (N=2,744), three were conducted in a high TB-incidence country (South Africa)[15,53,54] and two in low TB-incidence countries (Spain, UK) (Table 1).[12,55] All were designed as prospectively conducted diagnostic accuracy studies of C-Tb *vs* IGRA and/or TST; all five conducted three-test head-to-head comparisons within the same tested cohort.[15,53–55] Sub-populations tested included HIV+ (N=499),[15,54] children (including <5 years) (N=920),[54] and TB exposed/contacts (N=615).[54,55] Their characteristics are given in Table 1. All included QFT IGRA as comparators. In all five studies, the threshold for positivity was stratified depending on the sub-population tested; TST^{5mm} for HIV+ and TST^{15mm} for BCG vaccinated populations, reported aggregated (shown as $TST^{5mm/15mm}$ cut-off) or disaggregated. By contrast, the manufacturer-recommended 5mm threshold for C-Tb positivity was consistently used.

Two studies[56,57] (n = 887) conducted in China provided data for assessment of EC-skintest sensitivity in individuals with active TB (Table1). A study in Brazil [11] (n=173) assessed DPPD performance *vs* the TST^{5mm} in HIV+ and TST^{10mm} in HIV-uninfected individuals with microbiologically-confirmed TB, and in healthy individuals (Table 1), all of whom were BCG-vaccinated. We did not identify studies that followed up participants for risk of incident TB or evaluated effectiveness of preventive treatment. Table S3 in supplement summarises studies available for assessment of each review objective.

Of the 14 studies evaluating sensitivity of Diaskintest, risk of bias was high in 5 (35.7%) studies where test assessors were not blinded to TB culture results, [22,33,37,38,44] and unclear in at least one of the four risk of bias criteria in 12 (85.7%) studies as information on patient selection or blinding was not presented. [21,22,32,33,35,37,38,40,43–46] Of those evaluating Diaskintest concordance, 11/13 (84.6%) had high risk of bias in the reference standard criterion as assessors of reference standard (TST) were not blinded to index test results, [25,27–31,34,42,44,47,49] whereas for the index test criterion one had high risk of bias as index test assessors were not blinded to reference standard results [44] and the remaining 12 (92.3%) were classed as unclear as this information was not provided. [21,25–31,34,42,47,49] Of all Diaskintest studies, patient selection bias was unclear for 23 out of 29 (79.3%) studies as reporting of patient selection was incomplete. [21–28,30,33,37–40,42,44,45,47–49] One C-Tb study scored high on a risk of bias criterion because not all participants received the same reference standard (IGRA or TST). [54] Four out of five (80.0%) C-Tb studies and one EC-skintest study had conflict of interest concerns, as studies either did not report disclosures or were directly affiliated with the test manufacturer. [12,15,53,54] In addition, for EC-skintest studies, it was unclear whether patient selection was random or consecutive. [56,57] Applicability concerns and risk of bias were low for the DPPD study. [11] (See Table S31 in supplement for QUADAS-2[19] results).

In two studies that conducted head-to-head comparisons of the Diaskintest, IGRA and TST in HIV-uninfected children under investigation for TB or with clinically diagnosed TB, pooled test agreement of $Diaskintest^{AI}$ with IGRA was 88% (95% CI, 80-93%) and appeared considerably higher than agreement between TST^{5mm} and IGRA which was 52% (95% CI, 42-61%) or between $Diaskintest^{AI}$ and TST^{5mm} (55%, 95% CI 46-64%) (Figure

2a).[25,27] In two studies comparing all three tests (Figure 3) in HIV uninfected adults with active TB, pooled sensitivity for Diaskintest^{5mm} was 91% (95% CI, 82-96%); TST^{5mm} 88% (95% CI, 78-94%) and IGRA; 90% (95% CI, 79-95%) for QFT and 91% (95% CI, 80-96%) for TSPOT.TB.[32,40] Only Diaskintest^{5mm} studies could be pooled for sensitivity as no Diaskintest^{AI} studies fulfilled inclusion criteria for the analysis. Considering all studies where at least two-way test comparisons were possible, pooled agreement of Diaskintest^{AI} with IGRA was 94% (95%CI, 90-97; $I^2 = 57.0\%$) in four studies in participants with any TB status (Supplement Table S4).[25–27,32] By contrast, agreement between Diaskintest^{AI} and TST^{5mm} demonstrated considerable heterogeneity; pooled agreement was not estimated except in children with active TB (97% ; 95%CI, 96-98%) (Supplement Table S5); [29,31,32,35] agreement between Diaskintest^{5mm} and TST^{5mm} is shown in Table S6. Pooled estimates of Diaskintest sensitivity in two-way comparisons were 67% and 88% in HIV-uninfected adults for Diaskintest^{AI} and Diaskintest^{5mm}, respectively.[22,32,36,37,40,43–46] Highly variable methods and sub-populations precluded meaningful meta-analysis for most risk groups; sensitivity estimates from individual studies ranged from 40%-71% in HIV-infected adults[33,38] and from 92% to 100% in uninfected children, [21,32,36] (Supplement Table S7-S9). Specificity was not estimated for Diaskintest as TB infection had not been excluded in enrolled populations and studies were conducted in a high-burden setting. Proportion test positive appeared to vary by exposure gradient and was higher in contacts proximal to a source case (Supplement Table S10).[23,24,40,48] Full Diaskintest results are in supplementary section 3 (Tables S4-S14 and Figure S1).

Three studies provided suitable head-to-head data for agreement comparisons between C-Tb, IGRA and TST. Pooled test agreement between C-Tb and IGRA was 80% (95% CI, 76-83%), similar to that between IGRA and TST$^{5mm/15mm}$ (75%; 95% CI, 64-83%) and C-Tb and TST$^{5mm/15mm}$ (79%, 95% CI 75-83%) (Figure 2b).[15,53,54] In four head-to-head studies[15,53–55] (Figure 4), pooled sensitivity for C-Tb was 75% (95% CI, 70-78%), similar to that for TST^{15mm} (77%; 95% CI, 66-85%) and aggregated TST$^{5mm/15mm}$ (78% ; 95% CI, 68-86%). In the same four studies, sensitivity for TST^{5mm} was 83% (95% CI, 75-88%) and for IGRA 72% (95% CI, 63-79%); however, confidence intervals overlapped. Evaluation of specificity was possible in two studies that evaluated all three tests in low-burden settings (Figure 5).[12,55] Pooled specificity estimates for C-Tb (98%, 95% CI 94-99%) and IGRA (99%, 95% CI 80-100%) were similarly high, but slightly lower for TST^{15mm}, 93% (95% CI, 90-95%); the analysis was not possible for TST^{5mm} due to insufficient data. C-Tb results from studies that only compared two tests are shown in supplement section 4 (Tables S15-S22, Figure S2, Figure S3). These showed pooled agreement of C-Tb with TST to be similar, 81% (95% CI, 76-85%) at TST^{5mm} in HIV-infected and 76% (95% CI, 71-81%) at TST^{15mm} in HIV-uninfected (Table S15).[15,53–55] Test agreement among individuals without TB was reported in two studies. In one study,[55] C-Tb and IGRA agreement ranged from 92% to 97% across sub-populations with different levels of TB exposure, while it was 78% and 81% in HIV-infected and uninfected individuals, respectively, in the second study.[54] Agreement between C-Tb and the TST^{5mm} in these two studies was 83% and 87% respectively (Supplement Table S15). A dose-response association between C-Tb test positivity and proximity to a source-case was demonstrated. (Figure S3).[55]

Two studies evaluated sensitivity of the EC-skintest. [56,57] Sensitivity at the ≥5mm induration threshold ranged from 77% (95%CI: 55-92%) to 87% (95%CI:83-90%), with a pooled estimate of 86% (95%CI: 82-89%) (supplementary section 5). Test specificity or agreement with TST or IGRA was not estimated. [56,57] For DPPD,

agreement with the TST in active TB was 60% in HIV-infected individuals. [11] In HIV-uninfected individuals, agreement was 100% in active TB and 56% in healthy BCG-vaccinated controls. Sensitivity was 89% in HIV-infected and 100% in HIV-uninfected.[11] Test specificity was not estimated. Results for the EC-skintest and DPPD are in supplementary section 6.

We conducted sensitivity analyses which included: (1) classification of indeterminate Diaskintest results first into the positive results group and then into the negative results group for test agreement and test sensitivity objectives; (2) inclusion of clinical diagnosis of TB instead of only microbiologically-confirmed cases (from studies already included in data synthesis that report test performance in microbiologically-confirmed as well as clinically-diagnosed cases (3) inclusion of groups with 'unknown' HIV status in the HIV- and HIV+ groups separately, to create composite groups for test agreement and sensitivity objectives for C-Tb. Results did not vary considerably and did not alter conclusions (Supplementary Tables S11-14 (Diaskintest), S20-22 (C-Tb), S24 (EC-skintest), S27 and S28 (DPPD)).

All C-Tb studies and one EC-skintest study [56] provided safety data (Tables S29 and S30 in supplementary). However, adverse events were not classified consistently using the same grading system across studies. For C-Tb, injection site reactions were seen in 30·9% (853/2264) of participants which was similar to TST (827/2819, 29·3%) in the same studies. Other reported adverse events for C-Tb were infection-site pruritis (20·3%), pain (16·0%), rash (4·5%) and vesicles (2·5%). For EC, 4·9% (7/144) participants experienced mild pain and 12·5% (18/144) mild itching at injection site. None of the included Diaskintest or DPPD studies reported safety data.

Discussion

Our review identified four novel skin-based tests for TB infection, Diaskintest, C-Tb, EC-skintest and DPPD. Sub-populations tested include HIV-infected, children and TB-exposed individuals. To limit heterogeneity and allow direct comparisons between index tests with IGRA and TST under the same study conditions, we restricted analyses to studies that conducted head-to-head assessments of all three tests. Results from these analyses indicate that, across all four novel skin test types, performance may be similar to current tests for TB infection. Agreement with IGRA or TST was similar, approximately 80% or more in individuals with or without active TB. Similarly, sensitivity of the novel skin tests appeared comparable to TST or IGRA, irrespective of threshold for positivity chosen for comparator tests. Test specificity could only be assessed for C-Tb and is as high as that of IGRA (≥98%); as shown in two C-Tb studies conducted in TB low-burden settings.

Test agreement between Diaskintest or C-Tb and TST appeared to vary between groups, depending on characteristics of the group tested (e.g. HIV-infected vs uninfected, children vs adults), and consequently varied according to the threshold for TST positivity used. However, test performance was similar for a given threshold for TST positivity since stratified thresholds were applied; this maximises TST specificity for that sub-population[58] . The trend and estimates are consistent with published literature on agreement of IGRA with TST. The US National Health and Nutrition Examination Survey found test agreement between IGRA and TST^{10mm} in 6,064 individuals was 97% in the US-born population and 81·6% in non-US-born likely previously TB-exposed individuals.[59] Estimates of IGRA and TST agreement using a 5mm cut-off from a number of smaller studies in

HIV-infected individuals ranged between 66 and 89%.[60–62] We did, however, note the trend to higher proportions of TST+:Index test- discordant pairs relative to TST-:Index test+ pairs in reviewed studies but a more equal distribution of discordant results for IGRA vs Index for the C-Tb and Diaskintest studies, which may suggest greater agreement of Diaskintest and C-Tb with IGRA than with the TST, however confidence intervals around the estimates overlapped. C-Tb or Diaskintest sensitivity in culture-confirmed TB disease in our head-to-head analyses was similar to IGRA sensitivity reported in previous reviews, where estimates range from 80-93% [63,64] and also dependent on subgroup tested. In one large prospective observational study in England,[65] comparable sensitivity of IGRA and the BCG-adjusted TST (e.g. 10mm subtracted from TST measurement in those with previous BCG vaccination) was shown, which is similar to findings on C-Tb in the head-to-head analysis.

We have presented the first most comprehensive assessment of currently available novel skin tests for TB infection. However, the quality of included studies varied, particularly for Diaskintest studies.
A considerable proportion of Diaskintest studies were not primarily designed to evaluate test performance. In these studies, Diaskintest was performed in TB dispensaries (facilities responsible for all TB care at a regional level) for indications outlined in the national recommendations which include; annual TB screening of schoolchildren to determine those in need of vaccination;[66] initial screening to determine those who require investigation for active disease ; for TB diagnosis; or to monitor treatment response.[50] As a result, there are a number of concerns which affect the quality of the studies. Notably, clinical and test procedures across settings are inconsistent, and reporting often insufficient. Ascertainment of TB was inadequate; the diagnosis often pragmatically made on clinical and/or radiological findings rather than microbiologically-confirmed. Although Russian national TB guidelines define Diaskintest positivity as induration of any size,[50] more than a third of studies used the 5mm cut-off,[28,32,33,35,40,42,45,46,48] making comparison between studies and products difficult. Incorporation bias is a risk in studies that selected study participants based on TST-positivity or had followed Russian national TB recommendations and used Diaskintest for TB diagnosis. There are also concerns that are common across the index test studies. Potential conflicts of interest are possible with many of the included studies given many were industry-led and/or funded studies. Studies often did not stratify TST cut-off according to history of BCG vaccination, HIV infection or other immunosuppression, which may influence test agreement, especially with the TST. [56] In others, there was a risk of bias because participants in the same study received different reference standards.[54] TB infection had not been ruled-out in populations tested with Diaskintest, EC-skintest, or DPPD; therefore specificity could not be evaluated. While the data on C-Tb, and especially on EC-skintest and DPPD studies is limited, they were performed under trial conditions, enabling rigorous evaluation of test performance. In 2020 the WHO released a Framework for evaluation of new tests for TB infection;[18] it is envisaged this will standardise study design and improve the quality of future studies.

Limitations of this review are (1) heterogeneity precluded meta-analyses and assessment for a number of objectives. Head-to-head analyses limited bias, however, were only possible for a small subset of included studies. Meta-regression to adjust for various study-level factors (sub-populations, study design, etc) was not feasible given the low number of studies for which quantitative data could be extracted; (2) a limited number of studies evaluated test performance in sub-populations e.g. HIV-infected or children; (3) low study quality / high

risk of bias warrants careful interpretation of findings particularly for the Diaskintest studies (issues discussed above). In addition, many studies are at high risk of bias due to potential conflicts of interest given these were industry-led and/or funded studies (not uncommon in early evaluation phases); (4) longitudinal studies were not identified; predictive utility was not assessed. However, given similar performance with IGRA or TST, results are expected to be comparable;[67] (5) safety data was reported in 6 studies; (6) Diaskintest and C-Tb studies are overrepresented in this review which may skew conclusions; however, trends observed when EC-skintest and DPPD are compared to the IGRA or TST are similar. Further studies evaluating the performance of EC and DPPD tests are required. Although not a review objective, none of the studies evaluated novel skin tests (C-Tb, EC-skintest, Diaskintest, DPPD) against each other, although indirect comparisons suggest similar performance. Strengths of this review include a search strategy conducted in three languages using representative international and national medical literature databases and contacting test manufacturers and authors to ensure inclusion of as many studies and additional data as possible thus offering a comprehensive qualitative review of the landscape of novel skin tests. All known novel skin tests for TB infection with published performance data were included in this systematic review. Study objectives covered a breadth of internationally recognised reference standards for TB infection, allowing comparisons to previous IGRA reviews.[18] Where feasible, we restricted analyses to studies that conducted head-to-head analysis of all three tests; this reduced the influence of variation in study conditions on results. The goal of the review is to facilitate a critical assessment of the utility of the novel skin tests for TB infection for use in current testing algorithms. Our study results are thus important for researchers, clinicians, patient groups as well as policy-makers.

Overall, diagnostic performance of novel skin tests for TB infection appears comparable with IGRA or the TST with regards to concordance and test accuracy, and could offer more accessible and as reliable alternatives to current tests. However, this inference is based on a few studies that reported head-to-head results of a novel index test compared to IGRA or the TST. Variations in study design and quality precluded assessment of review objectives and quantitative synthesis considering all included studies. Our review also does not inform the relative diagnostic advantage of these newer tests over IGRA or TST when used in current testing algorithms for TB infection. To further inform local policy and practice, high quality real-world evidence from post-licensure studies is needed on the effectiveness of the tests when used as alternatives to IGRA and TST within current screening algorithms and the resultant impact on the cascade of care. Endorsement for use in current guidelines for TB infection should consider each test separately since these are all at different phases of evaluation and licensure. Future evaluation studies should particularly focus on inclusion of varied populations of people most at risk of TB that are under-represented in current studies, and address patient-important outcomes to provide insight into the utility and optimum implementation of these tests.

Contributors

Conceptualisation: MXR, CMD, AK; Funding acquisition: MXR, CMD; Methodology: MXR, CMD, MK Data curation: MK, LF, VN; Data visualisation and analysis: MK, LF; Access and verification of data: LF, YH, Writing - original draft preparation: MK, LF; Writing - review and editing: MK, LF, VN, MXR, CMD, RG, YH, AK, AM, DMC; Overall supervision: MXR.

Declaration of interests

The authors declare no competing interests.

Data sharing statement

All raw data is presented in the manuscript or its supplementary files; additional information including data for three-test head-to-head comparisons is available upon request from study authors after review and approval of a proposal. The study protocol will be stored at the UCL data repository and accessible upon request DOI: 10.5522/04/13607216. These data will be available for 10 years following study publication date.

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