DIAGNOSTIC ACCURACY TRIAL OF A NOVEL BLOOD-BASED ASSAY FOR IDENTIFICATION OF TB IN PEOPLE LIVING WITH HIV

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Background

- A triage test for active tuberculosis on a non-sputum sample is one of the highest priority WHO target product profiles.
- Triage tests would be beneficial for patients that are difficult to diagnose, including people living with HIV and children.
- The minimum requirements for ruling out disease in such a test have been outlined by WHO through a modified Delphi consensus process as 90% sensitivity and 70% specificity against a confirmatory test. The ideal test should furthermore maintain a sensitivity above 80% in patients with HIV co-infection.
- Chest X-ray is widely used for screening purposes but is limited by the infrastructure and instrumentation need and its lack of specificity for TB. TST and IGRA testing have reduced sensitivity in people living with HIV and do not distinguish active tuberculosis from latent infection. Detection of C-reactive protein on lateral flow tests has been suggested for use as triage test for HIV-associated active pulmonary TB (ATB), but performance evaluations show limited specificity.
- In 2016, Sweeney et al published a bioinformatically derived 3-gene mRNA signature obtained from analysis of 14 publicly available microarray datasets from whole blood samples from patients with active tuberculosis, LTBI, other diseases and healthy controls. The 3-gene signature (GBP5, DUSP3 and KLF2) was shown to discriminate between active TB and other diseases with AUC = 0·84 [95% CI 0·8-0·95] and with no confounding effect by HIV status or BCG vaccination.
- Cepheid (Sunnyvale, CA, USA) has developed a prototype for direct analysis of the Sweeney et al 3-gene signature in peripheral blood.
- This trial is a first feasibility to evaluate the performance of Cepheid's novel 3-gene Xpert prototype ("Xpert MTB Host Response", or Xpert-MTB-HR-Prototype) on preserved blood samples from PLHIV.

Figure 1: The TB-score values across participants with and without TB as defined by CMRS, further subdivided by Xpert MTB/RIF result on first sputum, is depicted in Figure 1(A). Figure 1(B) shows the same for CRP

Figure 1(A): Box plot of TB score with clinical classification

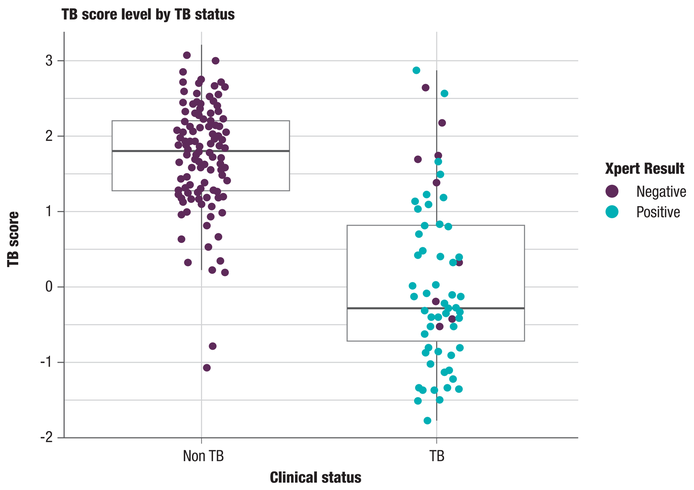


Figure 1(B):

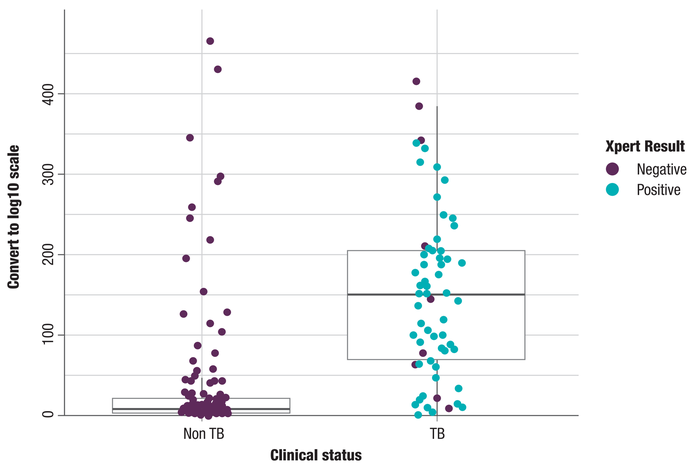
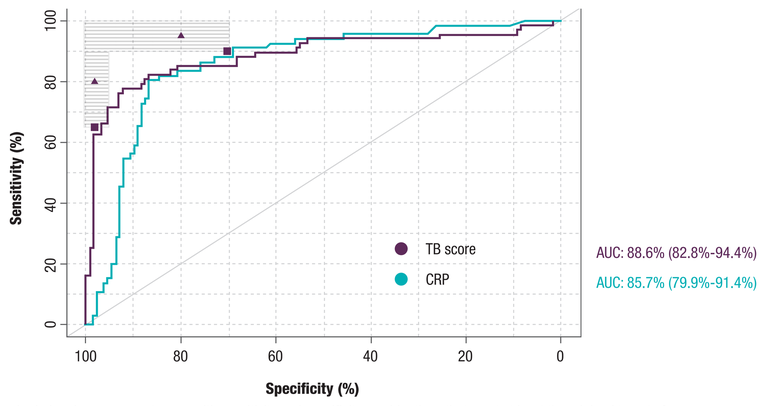


Figure 2: ROC curve for Xpert-MTB-HR-Prototype test and laboratory-based CRP test against a comprehensive microbiological reference standard



The shaded regions represent areas with sensitivity and specificity combinations that meet at least the minimal target of one of the TPPs (triage or non-sputum diagnostic). The triangles represent the optimal, the square the minimal targets. The black line represents the TB-score, the red line the CRP.

Methods

- We evaluated the accuracy of an early-prototype cartridge-assay ("Xpert MTB Host Response", or Xpert-MTB-HR-Prototype) of this 3-gene signature on biobanked blood-samples (https://www.finddx.org/specimen-bank/) from inpatients (>18 years) living with HIV, independent of CD4 count, in a South African district hospital and a Peruvian referral hospital between February 2016 and August 2017 against a comprehensive microbiological reference standard (CMRS) and against Xpert® MTB/RIF on first sputum alone (as the most likely confirmatory assay used in high-burden settings).
- Participants were adults with TB symptoms, able to produce sputum. Participants with presumed only extra-pulmonary disease were excluded.
- The samples were preserved in temperature-controlled freezers at -80°C from collection until testing.
- We depict results based on performance targets set by WHO in comparison with a laboratory-based CRP assay.

Ⓐ **The index test;** the early Xpert-MTB-HR-Prototype, evaluates mRNA levels of three differentially expressed genes (GBP5, DUSP3 and KLF2). For the testing, one aliquot per patient of 380 µL thawed PAXgene-blood was centrifuged. The pellet was resuspended with a lysis buffer (Cepheid) and vortexed. The lysate was then transferred to the Xpert-MTB-HR-Prototype cartridge and subsequently analyzed on a GeneXpert instrument.

Ⓑ **Comparator test;** serum CRP levels were measured using the Multigent CRP Vario assay on Abbott Architect C8000 at Quest Diagnostics. The Multigent CRP Vario assay is a latex immunoassay.

Ⓒ **Analysis;** all samples had complete data on the reference standard testing. The diagnostic performance of the obtained TB-score was evaluated with a ROC (Receiver Operating Characteristic) curve analysis using the diagnostic categories of "Definite TB" and "Not-TB" as binary reference standard for the classifier. In the primary analysis, the categories "Definite TB" and "Not-TB" were defined by the CMRS. In a secondary analysis, the categories "Definite TB" and "Not-TB" were defined based on the Xpert MTB/RIF result on the first sputum as a reference standard. Three separate analyses were performed for each of the two different reference standards.

- Sensitivity and specificity were calculated at the threshold value that maximized the Youden index.
- To evaluate if the test fulfills the WHO requirements for a triage test, specificity was calculated at the closest threshold value corresponding to 90% sensitivity for "minimal target". The same analysis was performed for a sensitivity of 95% for "optimal target".
- While the specificity was set by WHO at 98% in the TPP, we decided to set it at 95% in our analysis as we recognize the limitations of the reference standard particularly in PLHIV. We restricted this analysis to a comparison against a CMRS only.
- Confidence intervals reported were calculated based on the Wilson method.

Patient Characteristics			N (total = 201)
Age	Median (IQR)		36 (31-43)
Sex	Female (%)		130 (65.0)
	Male (%)		71 (35.0)
TB status	TB+	S-C+ (% of culture positive)	23 (34.3)
		S+C+	44 (65.7)
	TB – (% of all)		134 (66.7)
CD4 Count (cells/mm3)	<200 (% with CD4 count)		58 (30.0)
	≥200 (% with CD4 count)		133 (70.0)
	Unknown (% of total)		10 (5.0)
	Median (IQR)		375 (154 – 596)
History of BCG	Positive history of vaccination (% of total)		177 (88.1)
	Negative history of vaccination (% of total)		4 (2.0)
	Unknown history of vaccination and scar indeterminate (% of total)		20 (10.·0)
Prior history of TB	Positive (% of total)		128 (63.7)
	Negative (% of total)		73 (36.3)
QuantiFERON result	Positive (% with result)		78 (38.8)
	Negative (% with result)		95 (47.3)
	Indeterminate (% with result)		26 (12.9)
	Not obtained (% of total)		2 (1.0)
Site of Study	South Africa		195 (97.0)
	Peru		6 (3.0)

Table 1: Patient characteristics

All but 20 patients had more than three symptoms suggestive of TB.

SUBGROUP	N	SENSITIVITY % (CI)	SPECIFICITY % (CI)
CD4			
≥200	129	75.0 (50.5 – 89.9)	94.7 (88.9 – 97.5)
<200	56	93.8 (79.9 – 98.3)	66.7 (46.7 – 82.0)
SYMPTOMS			
≤3	19	100 (70.1 - 100)	80.0 (49.0 – 94.3)
>3	176	86.4 (73.3 – 93.6)	90.2 (83.9 – 94.2)
LTBI (IN NON TB)			
NON-LTBI	82	-	93.9 (86.5-97.4)
LTBI	46	-	89.1 (77.0-95.3)

Table 2: Xpert-MTB-HR-Prototype subgroups analyses at optimal TB-score cut-point against Xpert MTB/RIF on first sample as reference standard

Abbreviations: CI – confidence interval; LTBI – latent tuberculosis infection

Considering the implementation of the Xpert-MTB-HR-Prototype as a triage test at a sensitivity of 90.6% (specificity 85.9%) followed by an Xpert MTB/RIF as a confirmatory test, 68 out of 196 patients (excluding 5 subclinical cases) would have a positive result with the triage test (48 true positive and 20 false positive cases) and would have to be confirmed by Xpert MTB/RIF on the first sputum. The algorithm of Xpert-MTB-HR-Prototype followed by Xpert MTB/RIF would miss 19 cases identified by CMRS compared to 14 cases missed with a strategy of Xpert MTB/RIF alone on all presumed TB patients. This strategy would utilize 196 Xpert-MTB-HR-Prototype cartridges and 69 Xpert MTB/RIF cartridges. In comparison the CRP-test (at a sensitivity of 90.4%; specificity 76.8%), would miss the same number of cases but utilize 81 Xpert MTB/RIF cartridges in addition to the 196 CRP tests.

Results

The accuracy of the Xpert-MTB-HR-Prototype was evaluated against Xpert MTB/RIF, which is the most likely confirmatory assay in high-burden, resource-limited settings, and parallelly against a comprehensive microbiological reference standard (CMRS) consisting of multiple cultures.

Of 201 patients included, 67 were culture-positive for *Mycobacterium tuberculosis*. With Xpert MTB/RIF as diagnostic reference, AUC for the Xpert-MTB-HR-Prototype was 0.94 [95% CI, 0·89-0·98] with 86% specificity at 91% sensitivity. With the CMRS as reference, AUC was 0·89 (CI 0·83-0·94) for the Xpert-MTB-HR-Prototype with a specificity of 55·8% (CI 47·2-64·1) at 91% sensitivity.

Considering Xpert-MTB-HR-Prototype as a triage test (at nearest upper value of sensitivity to 90%), the corresponding specificity was 55·8% (CI 47·2-64·1).

Comparing to Xpert MTB/RIF as a confirmatory test, Xpert-MTB-HR-Prototype specificity was 85·9% (CI 79·3-90·7). Considering Xpert-MTB-HR-Prototype as a stand-alone diagnostic test, at a specificity near 95%, the test achieved a sensitivity of 65·7% (CI 53·7-75·9).

CRP performed similarly in the triage use-case against the CMRS but substantially poorer against Xpert MTB/RIF as reference standard and in a diagnostic use-case.

Discussion

- To achieve global end TB goals, Cepheid's novel Xpert-MTB-HR-Prototype may make an important contribution for patients whose disease can be difficult to diagnose due to their limited ability of producing a sputum sample, including HIV-positive patients and children.
- This trial supports the technical feasibility of the Sweeney et al 3-gene signature as a non-sputum triage test for Cepheid's GeneXpert platform.
- Performance was demonstrated in a cohort of HIV positive patients and implicates a potential benefit for other patient groups where a non-sputum sample is favorable.

Given that we tested an early prototype of the cartridge, further improvements in its diagnostic performance are conceivable with refinements in its development.

Conclusion

This is the first accuracy trial of a novel blood-based host-marker assay, here we show the value of the Xpert-MTB-HR prototype for diagnosis of TB in a vulnerable and very difficult to diagnose population living with HIV.

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