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Diagnostic accuracy of quantitative PCR (Xpert MTB/RIF) for tuberculous pericarditis compared to adenosine deaminase and unstimulated interferon-γ in a high burden setting: a prospective study

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Abstract

Background: Tuberculous pericarditis (TBP) is associated with high morbidity and mortality, and is an important treatable cause of heart failure in developing countries. Tuberculous aetiology of pericarditis is difficult to diagnose promptly. The utility of the new quantitative PCR test (Xpert MTB/RIF) for the diagnosis of TBP is unknown. This study sought to evaluate the diagnostic accuracy of the Xpert MTB/RIF test compared to pericardial adenosine deaminase (ADA) and unstimulated interferon-gamma (uIFNγ) in suspected TBP.

Methods: From October 2009 through September 2012, 151 consecutive patients with suspected TBP were enrolled at a single centre in Cape Town, South Africa. *Mycobacterium tuberculosis* culture and/or pericardial histology served as the reference standard for definite TBP. Receiver-operating-characteristic curve analysis was used for selection of ADA and uIFNγ cut-points.

Results: Of the participants, 49% (74/151) were classified as definite TBP, 33% (50/151) as probable TBP and 18% (27/151) as non TBP. A total of 105 (74%) participants were human immunodeficiency virus (HIV) positive. Xpert-MTB/RIF had a sensitivity and specificity (95% confidence interval (CI)) of 63.8% (52.4% to 75.1%) and 100% (85.6% to 100%), respectively. Concentration of pericardial fluid by centrifugation and using standard sample processing did not improve Xpert MTB/RIF accuracy. ADA (≥35 IU/L) and uIFNγ (≥44 pg/ml) both had a sensitivity of 95.7% (88.1% to 98.5%) and a negative likelihood ratio of 0.05 (0.02 to 0.10). However, the specificity and positive likelihood ratio of uIFNγ was higher than ADA (96.3% (81.7% to 99.3%) and 25.8 (3.6 to 183.4) versus 84% (65.4% to 93.6%) and 6.0 (3.7 to 9.8); *P* = 0.03) at an estimated background prevalence of TB of 30%. The sensitivity and negative predictive value of both uIFNγ and ADA were higher than Xpert-MT/RIF (*P* < 0.001).

Conclusions: uIFNγ offers superior accuracy for the diagnosis of microbiologically confirmed TBP compared to the ADA assay and the Xpert MTB/RIF test.

Keywords: Tuberculous pericarditis, Adenosine deaminase, Interferon γ, Xpert MTB/RIF test, Diagnosis

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Background

Tuberculosis (TB) is a global health priority [1]. In developing countries with dual human immunodeficiency virus (HIV) and TB epidemics there continues to be high TB-related mortality [2]. In immunosuppressed patients, this high mortality can be largely attributed to the increased burden of disseminated and severe forms of extra-pulmonary TB, such as tuberculous pericarditis (TBP) [3]. TBP carries a high case fatality rate (17% to 40% over six months) [3] and accounts for approximately 7% of hospital admissions for acute heart failure in Africa [4]. Despite the burden of disease and associated high mortality, the diagnosis of TBP remains problematic because of the lack of a simple, rapid, accessible and accurate diagnostic test [5]. TBP fluid is known to be paucibacillary with estimated culture and microscope smear-based diagnostic accuracy of only approximately 50% and 5%, respectively [6]. A definitive diagnosis of TBP is, therefore, challenging and often delayed [7]. Recent studies have indicated that the rapid initiation of anti-TB treatment may reduce mortality, making the investigation of new, rapid diagnostic tests for TBP essential [8].

The Xpert MTB/RIF assay is a new quantitative polymerase chain reaction (PCR) test that has been introduced for the rapid diagnosis of *Mycobacterium tuberculosis* (*M. tb*) and rifampicin resistance, providing a result in less than two hours [9]. Xpert MTB/RIF is endorsed by the World Health Organization (WHO) for the diagnosis of pulmonary TB using sputum samples [10]. Validation studies using culture positive sputum samples from pulmonary TB patients show a pooled sensitivity of 98% and 68% in smear-positive and -negative cases, respectively, and an overall pooled specificity of 98% [11]. Except for a few isolated cases, there are no prospective studies of the diagnostic utility of Xpert MTB/RIF test in TBP [12,13].

By contrast, proof-of-principle studies have demonstrated the potential utility of the novel biomarker, unstimulated interferon gamma (uIFNγ) as a diagnostic tool in pericardial and pleural fluid [14,15]. One study found that, when using a diagnostic cut-point of 0.2 IU/ml, pericardial fluid uIFNγ offered a 98% sensitivity and 100% specificity for the diagnosis of TBP. Despite these promising early results, the measurement of uIFNγ has not translated into routine clinical practice partly because of the lack of validation of the original observations [5].

Adenosine deaminase (ADA) level is the current locally available surrogate measure that suggests *M. tb* infection. The South African National Health Laboratory Service (NHLS) reference ranges for normal ADA levels are: 0 to 15 U/L for serum, 0 to 30 U/L for pleural fluid and 0 to 9 U/L for cerebrospinal fluid. Locally available, yet unvalidated data regarding ADA measurements in pericardial fluid suggests an ADA cut-off value of 40 U/L resulted in

a test sensitivity, specificity, positive predictive value, negative predictive value and diagnostic efficiency of 84%, 80%, 91%, 66%, and 83%, respectively [16].

The aim of this study was to assess the diagnostic utility of the new Xpert MTB/RIF test compared to ADA and uIFNγ assays in the diagnosis of TBP in a population with a high burden of TB.

Methods

Study population

Between October 2009 and September 2012, consecutive patients with suspected TBP referred to Groote Schuur Hospital in Cape Town for enrolment in the Investigation of Management of Pericarditis in Africa (IMPI Africa) registry [17] were screened for inclusion in this diagnostic study. Inclusion criteria were the presence of a large pericardial effusion amenable to safe pericardiocentesis (greater than 10 mm echo-free space around the heart in diastole), age 18 years or older and the provision of informed consent. Exclusion criteria were pregnancy, anti-TB treatment initiation >1 week prior to pericardiocentesis and refusal or inability to sign consent. Informed consent was obtained from each patient prior to enrolment in the registry and the study protocol conforms to the ethical guidelines of the 2008 Declaration of Helsinki as reflected in *a priori* approval by the human research ethics committee of the University of Cape Town (HREC REF402/2005) (additional data provided in Additional file 1).

Diagnostic sample collection and handling

A minimum of 60 ml of pericardial fluid (PF) was collected for diagnostic testing by means of percutaneous pericardiocentesis. PF was sent to the NHLS for measurement of ADA and lactate dehydrogenase (LDH) levels, differential cell counts and cytology, as well as routine TB diagnosis consisting of concentrated fluorescence smear microscopy and mycobacteria growth indicator tube (MGIT) liquid culture (MGIT 960, BD Diagnostics, Hunt Valley, MD, USA). Drug susceptibility testing was performed on positive culture isolates using the Genotype MDRTBplus assay (Hain Lifescience, Nehren, Germany). In addition, PF samples were stored at −20°C, for later measurement of uIFNγ levels and performance of the Xpert MTB/RIF assay. Investigators performing Xpert MTB/RIF and uIFNγ were blinded to clinical and routine TB diagnostic findings and categorisation (additional data provided in Additional file 1).

Xpert MTB/RIF assay

The Xpert MTB/RIF assay was performed on PF samples using the manufacturer's specifications for sputum samples as previously described (Cepheid, Sunnyvale, CA, USA) [9]. Where possible, Xpert MTB/RIF was performed using both 1 ml of unconcentrated and unprocessed PF as well as 3 to 20 ml of centrifuged (3,000 g × 15 minutes) PF

reconstituted to 1 ml with phosphate buffered saline
(PBS). The fourth generation Xpert MTB/RIF cartridge
was used. The cycle threshold value (C_T-values) indi-
cates the cycle number at which the molecular probe
becomes detectable and is proportional to the amount
of TB-specific starting template. The average C_T-value
for the five TB-specific molecular probes and for the
spore-related positive control (lyophilized *Bacillus
atrophaeus* subsp. *globigii* spores) (SPC) are used as
surrogate markers of bacillary load and PCR inhibition,
respectively. All Xpert MTB/RIF results were available
within two hours from the time of sample processing.
The limit of detection was determined in duplicate by
spiking 0, 50, 75, 100 and 150 H37Rv colony forming
units (CFU) to 1 ml aliquots of PF before dilution with
sample buffer and subsequent Xpert MTB/RIF analysis.
This experiment was repeated twice, thus providing
four replicates for each CFU concentration. Inhibition
was evaluated by comparing the PCR cycle-threshold
(C_T) values of the SPC from unconcentrated and con-
centrated samples.

ADA assay
An adenosine deaminase assay (Diazyme, Poway, CA, USA,
[18]) was performed on 1 to 8 ml PF samples, collected in
serum tubes, according to the manufacturer's specifications
by the National Health Laboratory Services, Groote Schuur,
Cape Town (NHLS GSH). Samples were either processed
immediately or stored (at 2 to 4°C) for processing within
24 hours.

The Diazyme ADA assay is based on the enzymatic
deamination of adenosine to inosine, which is converted
to hypoxanthine by purine nucleoside phosphorylase. The
reagent is used at 37°C ± 0.5°C, using an instrument that
is capable of reading absorbance accurately at 540 nm
to 550 nm. ADA activity was measured as units per litre
(U/L), where one unit of ADA is defined as the amount
of ADA that generates one micromole (μmol) of inosine
from adenosine per minute at 37°C.

uIFNγ assay
uIFNγ levels were measured in duplicate using supernatant
attained from 3 to 20 ml of thawed and centrifuged (3,000 g
for 15 minutes) PF using the InterGam Ultrasensitive Rapid
Immuno-suspension Assay (IRISA; Antrum Biotech, Cape
Town, South Africa; www.antrumbiotech.com; limit of
detection = 5 to 10 pg/ml) following the manufacturer's
instructions and without antigen stimulation.

Diagnostic classification for analysis
All participants who were included had a large pericardial
effusion on echocardiography. Participants were cate-
gorised into the following diagnostic groups based on a
combination of pericardial and non-pericardial sample

culture results, histopathology of pericardial biopsy sam-
ples, basic PF characteristics, and the commencement of
TB treatment as follows: (i) Definite-TB: at least one
M. tb sample positive by liquid culture (either pericardial
or non-pericardial) and/or granulomatous inflammation
on pericardial tissue histology (that is, composite reference
standard); (ii) Probable-TB: not meeting the criteria for
definite-TB, but based on clinical suspicion (symptoms,
imaging, and preliminary fluid analysis) commenced em-
pirically on TB treatment in the absence of an alternative
diagnosis; (iii) Non-TB: no microbiological evidence of
M. tb and an alternative diagnosis is available.

Modelling clinical predictors using multiple imputation
A univariable analysis was used to determined basic clinical
predictors of definite TBP. Thereafter, a set of multivariable
clinical predictors was generated using logistic regression
modelling. Multiple imputation by chained equations was
used to impute missing data prior to model building [19].
Rounded ß-coefficients from the reduced model of signifi-
cant variables were used to generate scores to quantitate
relevant clinical predictors. Receiver operating characteristic
(ROC) curve analysis was performed and three cut-points
were selected for rule-in, Youden's index (the optimal
mathematical balance between sensitivity and specificity)
[20] and rule-out value. Diagnostic accuracy, including
95% CIs, for each cut-point, was assessed. Performance
was also compared against a previously formulated clinical
prediction rule (Tygerberg TB Pericarditis Diagnostic
Index Score (TDIS) of ≥6) [6].

Statistical analysis
Sensitivity, specificity, positive (LR+) and negative (LR-)
likelihood ratios, and positive predictive values (PPV)
and negative predictive values (NPV) for all diagnostic
tests are presented with 95% CIs. Demographic, clinical
and microbiological characteristics of different groups were
compared using χ^2 and Wilcoxon rank-sum tests as appro-
priate. Diagnostic sensitivity and specificity of individual
and/or combinations of tests were compared using the χ^2
and Fisher's exact tests as appropriate. The Spearman cor-
relation coefficient (R_s) was used to evaluate the association
between Xpert MTB/RIF-generated PCR cycle-threshold
(C_T) values and liquid culture time-to-positivity. All statis-
tical tests were two sided at α = 0.05. STATA IC, version 10
(Stata Corp, College Station, TX, USA) was used for all stat-
istical analyses. The STARD criteria were used for analysis
and reporting of this study [21].

Results
Clinical characteristics
Figure 1 shows the study flow chart. Of the 175 pa-
tients screened, 24 patients were excluded due to having
pericardial effusions that were not amenable to safe

Figure 1 Screening, recruitment and diagnostic classification of patients with suspected tuberculous pericarditis. TB = tuberculosis; uIFNγ = unstimulated interferon gamma; TDIS: Tygerberg diagnostic index score; PF: pericardial fluid. *Insufficient clinical or diagnostic data acquired at baseline assessment.
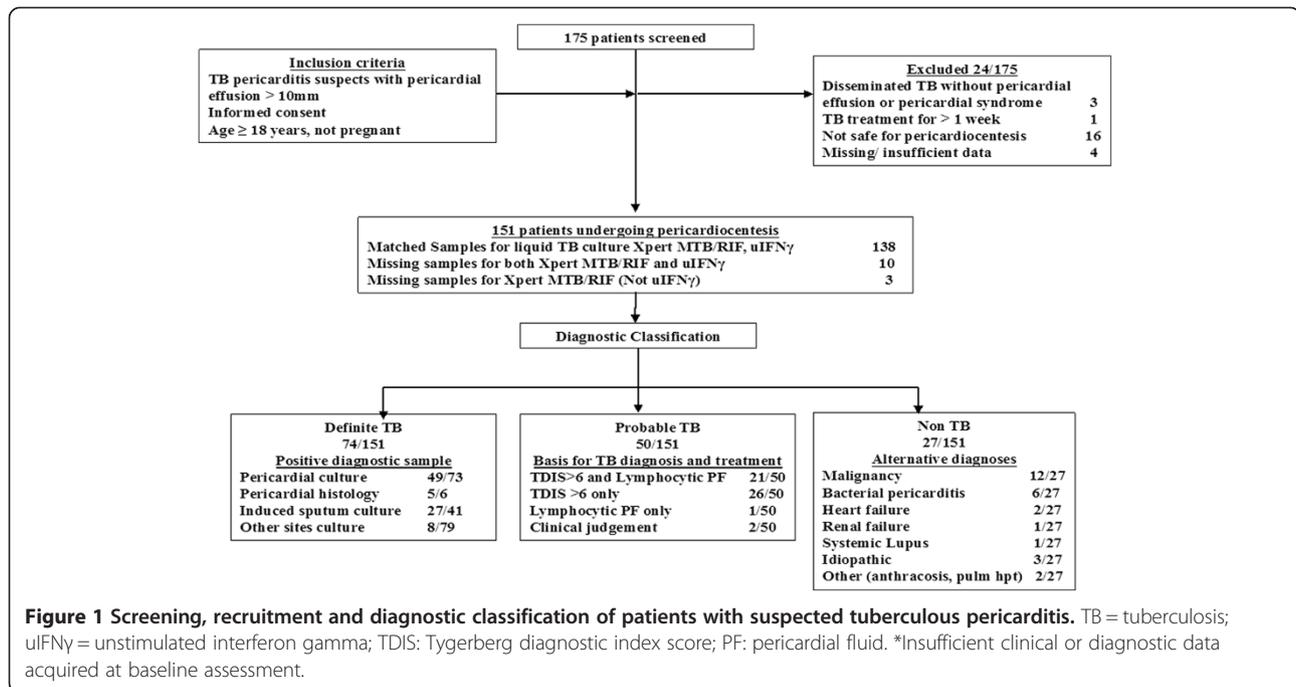
pericardiocentesis (n = 16), missing information (n = 4), absence of a pericardial effusion (n = 3) and prolonged TB therapy (n = 1). Of the remaining 151 patients, 49.0% (74/151), 33.1% (50/151) and 17.9% (27/151) were classified as definite-, probable- and non-TB, respectively. Only 1/74 definite-TB patients was PF smear-positive.

Tables 1A and B show the clinical characteristics of patients with suspected TBP stratified by final diagnostic group. Of these patients, 74% (105/151) were HIV-infected with a median (interquartile range (IQR)) CD4 count of 139 (81 to 249); 9/151 participants refused HIV testing or had an unknown HIV status. Only 18% (18/98) of HIV-infected patients were on anti-retroviral therapy at enrolment. Non-TB participants were significantly older, less likely to be HIV-infected, and more likely to have severe shortness of breath despite significantly smaller pericardial effusions than those with definite and probable TB. In contrast, definite-TB and probable-TB patients had similar clinical characteristics.

To compare diagnostic accuracy between diagnostic tests and basic clinical predictors, given the demographic and clinical differences, a multivariate logistic regression model was developed to generate a quantitative estimate for the predictive value of clinical findings. Additional file 1: Table S1 in the online supplementary materials shows the results of the univariate and multivariate analyses. A set of the following basic clinical predictors: age ≤50 years, HIV-infection and the presence of night sweats offered the best predictive utility for TBP. Table 2 compares the diagnostic accuracy measures for the previously reported Tygerberg diagnostic index score ≥6 and the quantified

clinical predictors of this cohort, using both a ROC-selected rule-in cut-point of >6.1 and Youden's rule-out cut-pointof >3.5.

Xpert MTB/RIF

Of the 151 patients who underwent pericardiocentesis, 9% (13/151) were not tested by the Xpert MTB/RIF assay because of missing samples. The range of PF volume used for a concentrated Xpert MTB/RIF was 3 to 20 ml. Spiking experiments in PF demonstrated that the Xpert MTB/RIF assay was detected in 100% of samples spiked with ≥75 CFUs/ml of PF [see Additional file 1: Figure S1, 4/4 replicates detected for 75, 100 and 150 CFUs/ml]. Table 3 shows that, overall, when comparing matched samples, the concentration of PF did not significantly increase the number of positive Xpert MTB/RIF results (1 ml unconcentrated: 41% (48/117) versus 20 ml concentrated 53% (62/117), $P = 0.07$). However, a higher number of indeterminate Xpert MTB/RIF results occurred when using concentrated versus unconcentrated (1 ml) PF samples (10.4% (14/134) versus 2.1% (3/138), $P = 0.005$). No difference in the mean C_T-value ± SD Xpert MTB/RIF Spore (*Bacillus globigii*) positive control C_T-values were noted between concentrated versus unconcentrated PF samples (26.7 ± 2.4 versus 26.4 ± 2.2, $P = 0.4$). No association between Xpert MTB/RIF-generated C_T-values and PF liquid culture time-to-positivity (in days) was detected ($R_s = 0.199$, $P = 0.3$, Figure 2).

Overall, the sensitivity (95% CI) of PF Xpert MTB/RIF was 63.8% (52.4 to 75.1) (Table 2). The sensitivity was higher in HIV-positive compared to HIV-negative patients

Table 1 Baseline demographic and clinical (A), echocardiographic and biochemical (B) characteristics of patients referred with suspected TB pericarditis

A.

Baseline feature	All study patients Number = 151	Definite-TB Number = 74	Probable-TB Number = 50	Non-TB Number = 27
Demographics				
Age (median, IQR)	34 (29 to 42)	33 (27 to 38)*	33 (28 to 37)*	52 (34 to 60) *P <0.001
Male (number, %)	93 (62)	48 (65)	32 (64)	13 (48)
HIV positive (number, %)[a]	105 (74)	59 (80)*	41 (82)*	5 (28) *P < 0.001
CD4 count (median, IQR)[a]	139 (81 to 249)	131 (70 to 206)*	153 (81 to 271)*	301 (229 to 424) *P = 0.04
ARV therapy (number/Number, %)	18/98 (18)	10/54 (19)	7/39 (18)	1/5(20)
Presenting clinical features				
NYHA Class I – II (number/Number, %)	77/134 (58)	39/66 (59)*	35/47 (75)*	3/21 (14) *P < 0.001
NYHA Class III – IV (number/Number, %)	57/134 (41)	27/66 (41)*	12/47 (25)*	18/21 (86) *P < 0.001
Systolic blood pressure (mean, SD) (number = 145)	113 (17)	113 (17)	112 (16)	115 (22)
Diastolic blood pressure (mean, SD) (number = 145)	72 (14)	72 (15)	72 (13)	73 (14)
Heart rate (mean, SD) (number = 146)	111 (20)	114 (22)	108 (16)	111 (21)
Serum biochemical data				
Haemoglobin g/dl (mean, SD)	9.6 (2.1)	9.4 (2.1)*	9.3 (1.7)*	10.9 (2.3) *P = 0.004
Creatinine, μmol/L (median, IQR)	73 (59 to 90)	72 (58 to 86)	77 (61 to 92)	72 (61 to 81)
Total WCC × 10^9/L (median, IQR)	6.5 (4.8 to 9.3)	6.5 (4.4 to 8.2)*	5.8 (4.8 to 7.2)*	10.5 (7.1 to 15.2) *P <0.001
B.				
Echocardiographic features				
Size of effusion (mm) (mean, SD) (number = 126)	36 (14)	36 (14)*	38 (13)*	29 (14) *P <0.001
Tamponade (number, %) (number = 141)	94 (67)	50 (72)	32 (64)	12 (55)
Routine pericardial fluid analyses				
ADA IU/L (median, IQR) (number = 142)	51 (34 to 75)	59 (45 to 86)*	51 (34 to 77)*	17 (11 to 27) *P <0.001
Total protein g/L (median, IQR) (number = 148)	60 (52 to 68)	59 (53 to 68)	63 (56 to 68)	56 (48 to 62)
Lactate dehydrogenase (median, IQR) (number = 135)	1419 (867 to 2305)	1553 (999 to 2800)*	1093 (725 to 1613)*	884 (442 to 2305) *P = 0.02
Total PF WCC × 10^9/l (Median, IQR) (number = 136)	2.1 (1.1 to 3.3)	2.0 (1.2 to 3.0)	2.2 (1.2 to 2.9)	3.0 (0.7 to 8.9)
Lymphocyte predominance (number/Number, %)[b]	52/107(49)	29/56 (52)*	22/34 (65)*	1/17 (6) *P <0.001

[a]Nine patients refused testing or had unknown HIV status, and four HIV-infected patients had no CD4 cell count data. [b]Multiple invalid results. *P values indicate significant differences between patient groups. ADA, adenosine deaminase; ARV, anti-retroviral therapy; IQR, interquartile range; NYHA, New York Heart Association; PF, pericardial fluid; SD, standard deviation; WCC, white cell count.

(74.6% (61.7 to 84.2) versus 21.4% (7.6 to 47.6), P <0.001; see Additional file 1: Table S2), corresponding to higher bacillary loads in the PFs of HIV-positive patients (median (IQR) time-to-positivity (days) of liquid TB culture samples HIV-positive: 21 (17 to 29) versus HIV-negative: 25 (12 to 38), P <0.001]. Sensitivity did not decrease significantly when definite- and probable-TB patients were combined (P = 0.09, see Additional file 1: Table S3). Overall, Xpert MTB/RIF specificity (95% CI) was 100% (85.6 to 100) when using the composite reference standard, but only 69.0% (59.2 to 78.7) when the microbiological reference was used (P <0.001, see Additional file 1: Table S4). All positive PF Xpert MTB/RIF were rifampicin-sensitive giving a specificity (95% CI) for rifampicin resistance of 100% (88 to 100). Sensitivity could not be calculated.

uIFNγ and ADA

Of the 151 patients enrolled, 6.6% (10/151) and 5.9% (9/151) were not subjected to uIFNγ and ADA assays, respectively. The optimal cut-points for uIFNγ and ADA levels to maximise diagnostic accuracy were determined using the ROC-curve shown in Figure 3. uIFNγ and ADA had similar areas under the ROC-curve (AUROC uIFNγ: 0.96 versus AUROC ADA: 0.91, P = 0.33). For uIFNγ, both the Youden's index (cut-point that correctly classifies the most number of patients) and optimal rule-out cut-point was 44 pg/ml, while for ADA the cut-point currently utilised in routine clinical practice is >35 IU/l [22] compared to a ROC selected optimal rule-in cut-point which is 107 IU/L. These cut-points are used for the diagnostic accuracy analyses presented in Table 2.

Table 2 Diagnostic accuracy measures of Xpert MTB/RIF and the biomarkers uIFNγ and ADA using ROC-selected cut-points (definite-TB for sensitivity and non-TB for specificity calculations)

Diagnostic test[a]	Sensitivity (95% CI) (n/N)[c]	Specificity (95% CI) (n/N)[c]	Positive likelihood ratio, LR + (95% CI)	Negative likelihood ratio, LR- (95% CI)	Positive predictive value, PPV (95% CI)	Negative predictive value, NPV (95%CI)
Xpert MTB/RIF	63.8% (52.4 to 75.1) 44/69[* *1 *2]	100% (85.6 to 100) 26/26[*4 *5]	n/c	0.36 (0.33 to 0.39)	100% (98.0 to 100)	86.6% (84.0 to 88.7)
uIFNγ (Intergam) (Youden's, rule-in and rule-out cut-points: ≥44 pg/ml)[c]	95.7% (88.1 to 98.5) 67/70[* *3] P <0.001	96.3% (81.7 to 99.3) 26/27[*6]	25.8 (3.6 to 184)	0.045 (0.023 to 0.09)	91.7% (88.1 to 94.3)	98.1% (96.8 to 98.9)
ADA (rule-in cut-point: >107 IU/ml)	15.7% (9.0 to 26.0) 11/70 [*1]P <0.001	96% (80.5 to 99.3) 24/25	3.93 (0.21 to 72.5)	0.88 (0.85 to 0.91)	62.7% (51.4 to 72.8)	72.7% (69.7 to 75.4)
ADA (cut-point in current clinical use: ≥35 IU/ml) [5]	95.7% (88.1 to 98.5) 67/70 [*1 *3]P < 0.001	84% (65.4 to 93.6) 21/25 [*4]P = 0.03	6.0 (3.7 to 9.8)	0.051 (0.026 to 0.10)	71.9% (67.3 to 76.1)	97.9% (96.4 to 98.7)
Tygerberg score ≥6	85.3% (75.9 to 81) 58/68 [*2]P = 0.004 [*3]P = 0.04	77.3% (56.6 to 89.9) 17/22 [*5]P = 0.01 [*6]P =0.04	3.75 (2.52 to 5.59)	0.19 (0.15 to 0.24)	61.7% (56.9 to 66.2)	92.5% (90.0 to 94.3)
Clinical predictors (rule-in cut-point: >6.1)	60.8% (49.4 to 71.1) 45/74 [*3]P <0.001	96.3% (81.7 to 99.3) 26/27	16.4 (2.25 to 119.9)	0.41 (0.38 to 0.44)	87.6% (82.4 to 91.4)	85.1% (82.5 to 87.5)
Clinical predictors (Youden's and rule-out cut-point: >3.5)[b]	91.9% (83.4 to 96.2) 68/74 [*2]P <0.001	81.5% (63.3 to 91.8) 22/27 [*5]P = 0.02	4.96 (3.34 to 7.36)	0.10 (0.07 to 0.14)	68.0% (63.3 to 72.4)	95.9% (94 to 97.2)
Xpert MTB combined with uIFNγ (with uIFNγ if Xpert MTB/RIF negative)	97.1% (89.9 to 99.2) 66/68	100% (86.7 to 100) 25/25	n/c	0.03 (0.01 to 0.08)	100% (98.7 to 100)	98.8% (97.7 to 99.4)
Xpert MTB combined with ADA (with ADA if Xpert MTB/RIF negative)	98.4% (91.7 to 99.7) 63/64	100% (85.7 to 100) 23/23	n/c	0.02 (0.002 to 0.11)	100% (98.7 to 100)	99.3% (98.4 to 99.7)

[a]Denominators other than 74 definite- and 27 non-TB patients indicate missing test results. Thus, 5/74 definite- and 1/27 non-TB patients did not have an Xpert MTB/RIF performed; 4/74 definite-TB did not have a uIFNγ (QFT-kit) test performed and 4/74 definite- and 2/27 non-TB did not have an ADA level measurement. Diagnostic accuracy measures presented in this table are for Xpert MTB/RIF performed on 1 ml unconcentrated PF, uIFNγ (both the QFT-kit and Intergam-kits) performed on the supernatants on 3 to 20 ml of centrifuged PF, and ADA performed on 1 to 8 ml of unprocessed PF.
[b]Youden's index (the cut-point allowing the most correctly classified) and optimal 'rule-out' (the cut-point with the lowest LR-) cut-points for the uIFNγ (both the QFT-kit and Intergam-kits) were the same (QFT: 0.18 IU/l, Intergam: 44 pg/ml) respectively. [c]P-values compare diagnostic tests for the indicated diagnostic accuracy measure, for example, sensitivity (specific proportions compared indicated by * and corresponding number symbol).
ADA: adenosine deaminase; CI: confidence interval; n/c: not calculable as denominator equals zero; PF, pericardial fluid; uIFNγ: unstimulated interferon gamma. The reference standard was culture and/or histopathology pathognomonic of TB[†]. TB prevalence = 30% used for predictive value estimates.

Table 3 Comparison of Xpert MTB/RIF assay test characteristics and diagnostic accuracy when using unconcentrated and concentrated pericardial fluid samples

Pericardial fluid type	Indeterminate rate (number/total number of assays performed)	Internal positive control[a] C_T-value (median, IQR)	Xpert MTB/RIF positive tests in definite and probable-TB patients
Unconcentrated (1 ml)	2.1% (0.3 to 4.6) 3/138	26.2 (25.1 to 27.4)	41.0% (32.1 to 49.9) 48/117
Concentrated/centrifuged pellet (20 ml)	10.4% (5.3 to 15.6) 14/134	26.2 (25.1 to 27.6)	52.9% (43.9 to 62.0) 62/117
P-value[b]	0.005	0.9	0.07

A median of 20 ml of pericardial fluid was centrifuged at 3,000 × g for 15 minutes with the pellet re-suspended in sterile PBS, followed by Xpert MTB. [a]Internal positive control: to evaluate potential PCR inhibition an unrelated fragment of fungal DNA (*Bacillus globigii*) is co-amplified in the reaction; [b]*P*-values compare unconcentrated and concentrated pericardial fluid samples for particular Xpert. CI, confidence interval; IQR: Interquartile range; TB, tuberculosis,

The overall sensitivity (95% CI) of uIFNγ was 95.7% (88.1 to 98.5), which was similar to ADA using the clinical cut-point (Table 2). However, the specificity (95% CI) of uIFNγ was 96.3% (81.7 to 99.3) versus only 84% (65.4 to 93.6) for ADA at the clinical cut-point (*P* = 0.1). Similarly, although the sensitivity of the biomarkers uIFNγ and ADA was similar for both HIV-positive and -negative patients, the specificity of ADA (clinical cut-point) was lower in HIV-positive patients (*P* <0.001, Additional file 1: Table S2).

Comparative diagnostic accuracy of routine and new same-day diagnostic tools

We further interrogated the potential clinical utility of routine (that is, ADA assay) and new same-day diagnostic tools (that is, uIFNγ and Xpert MTB/RIF) by comparing positive (LR+) and negative (LR-) likelihood ratios (Table 2) and positive (PPV) and negative (NPV) predictive values at different prevalence rates of TB (TB prevalence = 30% in Table 2, TB prevalence of 10%, 30%, and 50% presented in Additional file 1: Table S5). With 100% specificity, the LR + and PPV (irrespective of TB prevalence) for Xpert MTB/RIF was excellent, but sensitivity was suboptimal

compared to other biomarkers and clinical predictors and thus LR- was only 0.49. Compared to ADA (clinical cut-point 35 IU/ml) and clinical predictors, the biomarker uIFNγ (cut-point 44 pg/ml) offers better rule-in utility with higher sensitivity, LR+, and in high TB prevalence settings (prevalence = 50%) a PPV of 96.9% (95.1 to 98.1) [see Additional file 1: Table S5]. Both ADA (clinical cut-point 35 IU/ml) and uIFNγ (cut-point 44 pg/ml) with sensitivities >95% offer excellent rule-out utility with low LR- and NPV just below 95% in high TB prevalence settings (prevalence = 50%, Additional file 1: Table S5).

Xpert MTB/RIF in combination with pericardial fluid biomarkers

Table 2 shows the diagnostic accuracy of using PF Xpert MTB/RIF together with the biomarkers ADA and uIFNγ. Performing a PF Xpert MTB/RIF followed by either ADA or uIFNγ offered equivalent excellent diagnostic accuracy with sensitivity and specificities >97%.

Discussion

The performance of the new WHO-endorsed, Xpert MTB/RIF assay has recently been reported for some types
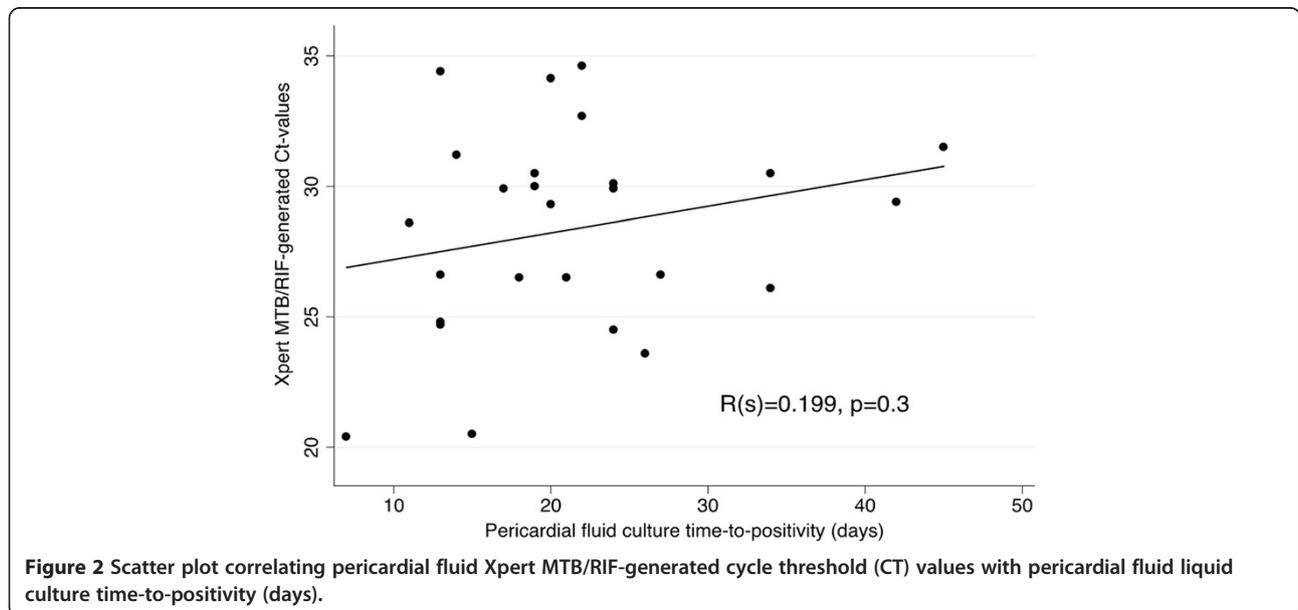
Figure 2 Scatter plot correlating pericardial fluid Xpert MTB/RIF-generated cycle threshold (CT) values with pericardial fluid liquid culture time-to-positivity (days).
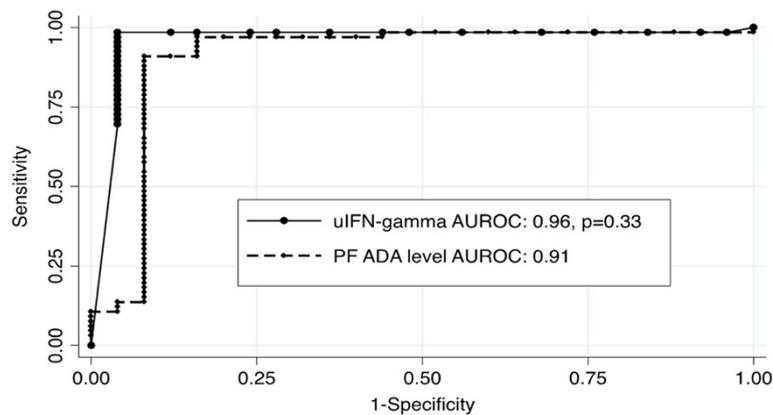
Figure 3 Comparison of receiver operator characteristics (ROC) curves for the pericardial fluid biomarkers unstimulated interferon gamma (uIFN-gamma) and adenosine deaminase (ADA). AUROC, area under the receiver operator characteristics curve. The point sensitivity versus specificity for the Xpert MTB/RIF is indicated on the graph as a solid black triangle.

of extra-pulmonary TB such as TB lymphadenitis [23], pleural TB [24], and TB meningitis [25]. However, there are no comprehensive data about TBP to guide clinical practice. Here we report on the first large comprehensive study of Xpert MTB/RIF for the diagnosis of pericardial TB [5,10]. It is also the first study to compare Xpert MTB/RIF to several alternative diagnostic assays, including ADA and IFN-γ, and to evaluate test performance outcomes in a TB and HIV-endemic setting.

The key findings of our study are that: (1) uIFNγ offers superior accuracy for the diagnosis of microbiologically confirmed TBP compared to the new Xpert MTB/RIF test and the established ADA assay; (2) PF Xpert MTB/RIF could bacteriologically confirm a TB diagnosis (and allow for drug susceptibility testing) in two thirds of patients with suspected TBP; (3) PF uIFNγ offered better rule-in diagnostic utility compared to ADA in current clinical use, while both tests could rapidly rule-out TBP; (4) PF Xpert MTB/RIF, when combined with either ADA or uIFNγ, offers >97% sensitivity and specificity for TBP diagnosis; and (5) concentration of PF samples prior to Xpert MTB/RIF testing increased the number of 'indeterminate' tests without significantly improving diagnostic yield.

Xpert MTB/RIF testing is undergoing phased implementation in a number of high burden settings for routine diagnosis of pulmonary TB [26,27]. There is limited information on the diagnostic utility of the test in extra-pulmonary cases of TB, and, in particular, Xpert MTB/RIF performance has only been evaluated in a very small number of PF samples [13]. Our study is the largest systematic evaluation to date, and the first to examine Xpert MTB/RIF level of detection in PF and explore the effects of concentrating larger volumes of PF on Xpert MTB/RIF performance. Importantly, Xpert MTB/RIF testing could microbiologically confirm TB and allow drug susceptibility testing in almost two thirds of culture-positive

cases, which is higher than in other body cavity fluids, including pleural, non-sputum biological fluids such as urine, and similar to performance in induced sputum specimens [13,28,29]. Preliminary level of detection experiments suggest that the Xpert MTB/RIF assay could reliably detect PF samples spiked with ≥75 cfus/ml of H37Rv, which is lower than the 131 cfu/ml limit of detection found in spiked sputum samples [30]. Further studies with more replicates are required to confirm this finding. However, the diagnostic yield from PF was not improved by centrifugation of larger volumes and concentration only increased the number of 'indeterminate' test results, although this was not the result of an increase in PCR inhibition. The increased error rate may have resulted from reaction failure secondary to large amounts of pelleted blood and other inflammatory proteins found in pericardial exudates. Methods to further digest these proteins or the addition of a PCR-friendly blood lysis buffer may help to decrease error rates [31,32]. Interestingly, unlike in sputum and pleural samples, no correlation was found between Xpert MTB/RIF-generated C_T-values and liquid culture time-to-positivity using PF [33]. However, the sensitivity of Xpert MTB/RIF was found to be significantly higher in HIV-positive versus negative patients, and this was due to the higher bacillary loads, as measure by liquid culture time-to-positivity (TTP), found in the PF of HIV-positive versus -negative TBP. This sensitivity difference may impact on the utility of Xpert MTB/RIF in low HIV prevalence settings.

Proof-of-principle studies in TB pericarditis have demonstrated the potential utility of using uIFNγ PF levels for diagnosis of TB pericarditis [6,14,34]. Although it can be easily measured, it is not routinely performed due to its high cost and the kits only being available in a 96-well format, which would lead to a considerable wastage of unused wells [5,35]. However, the recent availability of a low-cost assay (Intergam, Antrum Biotech, Cape

Town, South Africa) which is tested in this study may allow for more widespread use of uIFNγ for the diagnosis of TBP in clinical practice. In this study, using ROC-curve analysis, we demonstrate an optimal cut-point of 44 pg/ml, and show that with this cut-point of uIFNγ we could detect almost all definite-TB cases (missing only three cases) and incorrectly classified only one non-TB case.

Are the findings of this study generalisable to other settings, and does either Xpert MTB/RIF or uIFNγ testing potentially offer utility beyond existing same-day diagnostic tools, such as smear microscopy, PF ADA measurements and/or basic clinical information? In this study we compare the utility of Xpert MTB/RIF, uIFNγ or ADA, alone or in combination across different TB prevalence rates, focusing on the diagnostic priorities of rapid rule-in and rule-out, as well as bacteriologically confirmed diagnosis. In a high prevalence setting (TB prevalence >30%), Xpert MTB/RIF and uIFNγ outperforms ADA and basic clinical predictors for rapid rule-in (highest LR + and PPV). However, both ADA and uIFNγ offer equivalent rapid rule-out utility, outperforming Xpert MTB/RIF and clinical predictors. Combining Xpert MTB/RIF testing followed by ADA or uIFNγ in Xpert-negative PF maximised both sensitivity and specificity to >97% for TBP diagnosis. This may offer the best diagnostic approach in high burden settings, especially where drug-susceptibility testing is desirable, but the cost of a two test algorithm will remain a key consideration in resource-poor conditions where TB is endemic. Xpert MTB/RIF currently costs approximately US$20/test, while ADA measurement is less than US$0.1/test. Intergam kits are not currently commercially available so the cost is unknown but likely to be only slightly more than smear microscopy. Prospective studies of the cost-effectiveness of diagnostic options are needed before it can be considered for clinical practice.

Our study had a number of important limitations. This study did not optimise PF sample volumes or processing beyond comparing two volumes and a simple centrifugation step thought applicable to resource-limited settings. The use of different volumes or alternative processing methods may have improved Xpert MTB/RIF sensitivity and/or decreased the high indeterminate rate found. A low number of replicates were performed in limit of detection experiments and these findings should be confirmed in further studies. The study was conducted in a high TB and HIV burden setting, which may limit the generalisability of the findings. Performance may differ in a low TB burden setting and where HIV co-infection rates and, hence, bacterial load, are lower, such as Europe and the US. However, the use of diagnostic accuracy measures that are less affected by prevalence, such as LRs, and generating estimates across varying TB prevalence rates helps to highlight potential performance differences between low and high burden settings and,

hence, improve generalisability. Whilst this is the largest study that has comprehensively evaluated several diagnostic strategies and tools in the same prospective cohort, the sample size was limited in the non-TB group. The small number of non-TB patients reflects the high burden of infectious and HIV-related disease in the South African environment [27]. Although the use of a combined reference standard may introduce a minor degree of selection bias, this consideration is outweighed by the avoidance of misclassification bias when using a culture only reference (data provided in the online supplementary materials).

Conclusions

In conclusion, uIFNγ offers superior accuracy for the diagnosis of microbiologically confirmed TBP compared to the new Xpert MTB/RIF test and the established ADA assay, performed using available Xpert MTB/RIF testing protocols without fluid-specific optimisation beyond simple centrifugation. These data suggest that the uIFNγ assay may be the optimal first line test for the diagnosis of TB pericarditis, and merits consideration for implementation in clinical practice. Furthermore, PF Xpert MTB/RIF, when combined with either ADA or uIFNγ, offers high sensitivity and specificity for TBP diagnosis. Studies are needed to test the utility and cost-effectiveness of a two-test strategy, which may be preferred in HIV-positive patients where biomarker specificity may be reduced. Collectively, these data suggest that a biomarker-oriented approach may be feasible and accurate for the diagnosis of suspected TBP in a high TB and HIV prevalence setting.

Additional file

> **Additional file 1: Table S1 Table S2-5 and Figure S1.**

Authors' contributions
SP, JGP, KD and BMM contributed to the conception and design of the study, the acquisition of data, analysis and interpretation of data, and drafting of the manuscript. ZK, RM, GT, UG, and MN contributed to the acquisition and interpretation of data and drafting of the manuscript. All authors read and approved the final version of the manuscript.

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