Title:
Xpert-Ultra to detect and quantify HIV-associated *Mycobacterium tuberculosis* blood stream infection: a cohort study.

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Abstract

[300 words]

Background

Mycobacterium tuberculosis bloodstream infection (MTBBSI) is a leading cause of death in people living with HIV. Tools to rapidly identify and quantify MTBBSI could facilitate both diagnosis and research for this critical condition.

Methods

We developed a method to detect *M. tuberculosis* in blood using lysis-wash steps and the Xpert MTB/RIF Ultra platform, testing it on biobanked blood from participants hospitalized with HIV-associated TB. We assessed diagnostic yield (proportion of cases detected, with unavailable test results coded as negative) against a microbiological reference, both as a function of markers of critical-illness and compared with other rapid-diagnostics. Quantitative blood Xpert-Ultra results were evaluated as a disease biomarker by assessing association with disease phenotype defined by principal component analysis of 32 host-response markers, and prognostic value compared to other tuberculosis biomarkers models to predict 12-week mortality.

Findings

Of 582 participants, 447 (77%) had tuberculosis; 165 out of 447 were blood Xpert-Ultra positive giving diagnostic yield 0·37 (95%CI 0·32 to 0·42). Diagnostic yield increased with lower CD4 cell count or haemoglobin, and outperformed urine lipoarabinomannan testing in participants with elevated venous lactate. Quantitative blood Xpert-Ultra results predicted mortality better than other tuberculosis biomarkers including blood culture, and urine lipoarabinomannan or urine-Xpert. Both blood Xpert-Ultra positivity and cycle threshold were strongly associated with a principal component of clinical phenotype capturing markers of inflammation, tissue damage and organ dysfunction.

Interpretation

Xpert-Ultra testing of pre-processed blood could be used as a rapid diagnostic test in critically ill patients with suspected HIV-associated tuberculosis, while also giving additional prognostic information compared with other available markers. A dose-response relationship between quantitative blood Xpert-Ultra results, host-response phenotype, and mortality risk adds to evidence that suggests MTBBSI bacillary load is causally related to outcomes.

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Research in context

Evidence before this study

To identify research studies reporting use of nucleic acid amplification technology (NAAT) on peripheral blood as a tuberculosis diagnostic, we searched PubMed and Scopus, without language restriction from database inception to 12 December 2020, using the terms "tuberculosis" AND ("blood" OR "mycobacteraemia" OR "blood stream infection" OR "bacteraemia" OR "bacillaemia") AND ("NAAT" OR "PCR" OR "Xpert") AND "diagnosis" (full systematic review and meta-analysis in appendix). Since the 1990s dozens of uses of NAAT on blood to diagnose TB have been reported, with extreme variation in reported sensitivity (0 to 100%) not discernibly related to plausible biological covariates such as measures of disease spectrum or severity (HIV status, patient setting, prevalence of TB blood culture positive disease) or technical covariates (volume of blood and blood pre-processing methods). Most studies have used 'in house' polymerase chain reaction (PCR) protocols and are poorly reported with high risk of bias. Promising results in smaller studies have not been replicated in larger studies with low risk of bias, or in studies using scalable, commercially available PCR platforms.

Added value of this study

In the largest study to date reporting use of NAAT on blood to diagnose tuberculosis, we show that, with simple pre-processing of blood, the widely available Xpert-Ultra PCR system can be used to detect *M. tuberculosis* bloodstream infection. Highest diagnostic yield was found in the most critically unwell patients. This method also allows quantification of blood bacillary load using cycle threshold values, which in turn gives additional prognostic information. Fundamental axes of host-response also demonstrate a dose-response relationship with blood Xpert-Ultra quantification.

Implications of all the available evidence

Earlier reports that NAAT applied to blood can be used to diagnose TB have now been replicated in a large study using a protocol deliverable in routine clinical laboratories. Quantification of MTBBSI using blood Xpert-Ultra may be valuable as a disease biomarker.

Introduction

[588 words]

Tuberculosis remains the leading reason for hospitalisation and death in people living with HIV (PLHIV) globally.[1] In severe HIV-associated tuberculosis, *M. tuberculosis* blood stream infection (MTBBSI) is both common and independently associated with mortality,[2] and may therefore account for a substantial fraction of deaths in PLHIV. However, MTBBSI has received less research attention than other forms of tuberculosis, at least in part because mycobacterial blood cultures are unavailable in most high-burden settings, and have limited diagnostic value because median time to positivity is longer than median time from admission to death in fatal cases of MTBBSI.[3] As a result, while bloodstream dissemination is a cardinal feature of severe HIV-associated tuberculosis, it is seldom recognised in clinical practice or measured in research studies. The ability to rapidly identify MTBBSI may have diagnostic utility in critically ill PLHIV, and could facilitate research into this neglected condition.

Many patients admitted to hospital with HIV-associated tuberculosis meet Sepsis Related Organ Failure Assessment criteria,[4] and tuberculosis is the most frequent microbiological diagnosis in patients with sepsis in high HIV-burden settings.[5,6] According to the Third International Consensus (Sepsis-3),[7] a "dysregulated host response to infection" is the defining feature of sepsis, but the pathophysiological basis of this immune dysregulation remains incompletely defined. Hallmark host-responses characterising sepsis[8-10] are also found in life-threatening HIV-associated tuberculosis disease, including concurrent inflammatory and immunosuppressive signalling,[4,11] coagulation and endothelial activation,[12,13] innate cell activation and dysfunction,[4,14,15] lymphopenia and apparent exhaustion of T-cells and their cytokine responses.[4,11,16] Microbiological data, in terms of both the causative microbe and pathogen-burden, are notably absent from contemporary definitions of sepsis. By contrast, severity of tuberculosis infection has classically – from animal model[17] and post-mortem studies[18] – been related to mycobacterial load, and specifically the "*number of bacilli reaching the blood stream and multiplying in the tissues*".[19] The absence of tools to directly and systematically measure bacillary load antemortem has been a fundamental limitation of modern clinical studies investigating host response in severe HIV-associated tuberculosis.

Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) testing of sputum can detect most pulmonary tuberculosis in PLHIV,[20] while the next-generation Xpert MTB/RIF Ultra test has further increased sensitivity for sputum smear-negative but culture positive cases.[21] Sputum Xpert testing results in earlier institution of therapy in outpatients with pulmonary tuberculosis, although evidence of impact on mortality is lacking.[22] In critically ill inpatients, sputum-based diagnostics are limited by the frequent inability to obtain sputum.[5,23] This has led to calls to develop and validate rapid diagnostic tests targeted specifically at inpatients with sepsis and suspected MTBBSI,[3] in whom delayed diagnosis is most dangerous.[2,24] Previous attempts at using Xpert MTB/RIF on blood as a rapid diagnostic for MTBBSI reported poor sensitivity,[25,26] likely due in part to PCR inhibition by blood components. Xpert testing of blood remains an attractive target: Xpert is a widely available platform capable of rapid diagnosis of both MTB and rifampicin resistance, blood is a major site of disease in severe HIV-associated tuberculosis, and is a readily accessible, minimally-invasive sample in severely ill patients.

We developed and used a simple pre-processing method to detect and quantify MTB in biobanked blood samples from a large cohort of patients hospitalized with presumed HIV-associated tuberculosis using Xpert-Ultra, with two objectives. First, we assessed the diagnostic utility of blood Xpert-Ultra testing, both in comparison to other rapid diagnostics, and assessing how diagnostic utility relates to markers of critical illness. Second, we assessed quantitative blood Xpert-Ultra results as a potential prognostic and disease biology biomarker, testing the hypothesis that MTBBSI bacillary load has a dose-response relationship with mortality and host-response phenotype, respectively.

Methods

[1135 words]

We evaluated Xpert MTB/RIF Ultra on biobanked whole blood specimens from a well characterized cohort of participants hospitalized with suspected HIV-associated tuberculosis [4]. The parent study recruited adult PLHIV with CD4 count <350 cells/mm^3 admitted to Khayelitsha Hospital (Cape Town, South Africa) between January 2013–October 2016; all had prospectively-planned baseline testing for tuberculosis within 72 hours of admission, and were followed up to 12 weeks [4]. For this analysis we selected patients according to the availability of biobanked blood specimens from the day of recruitment, and excluded patients without at least one test result for the diagnostic reference

standard (defined below). This study was approved by the University of Cape Town Human Research Ethics Committee (HREC 057/2013).

Laboratory procedures

Sample collection occurred on the day of recruitment. If not already obtained by hospital staff, an experienced operator with access to sputum induction facilities attempted collection; all sputum was sent for liquid culture and Xpert MTB/RIF. Urine-Xpert MTB/RIF was performed on a centrifuged urine pellet as previously described [23]. Five mL of whole blood was cultured in Myco/F-Lytic (Becton-Dickinson Biosciences) bottles for ≥42 days. All microbiology tests were performed by the National Health Laboratory Services. Urine LAM (uLAM) testing was performed retrospectively on frozen samples using the Alere Determine™ TB-LAM test. In a random subset of participants, soluble immune mediators were quantified in plasma stored at -80° C using Bio-Plex Pro-TM Human Cytokine Standard 27-Plex kit. Whole blood in EDTA tubes (3-7 mL) were stored at -80° C.

Blood Xpert MTB/RIF Ultra testing

Thawed 3-7 mL EDTA whole blood samples were made up to a volume of 45 mL with sterile red blood cell lysis-buffer [155 mM NH_4Cl; 12 mM $NaHCO_3$; 0·1 mM EDTA], incubated at room temperature for 30 minutes, centrifuged at 3500G for 25 minutes, and the pellet resuspended in 45 mL water. This was re-incubated and centrifuged as above, with ~2·5 mL pellet residual volumes refrozen at -80° C for subsequent batch processing. Thawed samples were resuspended in 12·5 mL volume of sterile water, centrifuged at 3500G for 25 minutes, and residual pellet volume of 0·7 mL mixed with 1·5 mL Xpert-Ultra buffer. This was incubated at room temperature for 15 minutes with shaking, before being transferred to Xpert MTB/RIF Ultra cartridges. A demonstration is provided in the video in the supplementary appendix. Tests were performed blinded to clinical data.

Diagnostic utility analysis

We defined sensitivity as the number of participants with positive result on the index test (blood Xpert-Ultra) divided by total number of participants with: (1) a valid index test result; and (2) tuberculosis diagnosis confirmed by a *strict microbiological reference standard (MRS)* of MTB culture from any site and/or positive Xpert MTB/RIF from any site other than blood.

We defined diagnostic yield as number of participants with positive result on the index test divided by total number of participants who met an *extended microbiological reference standard (ERS)*: any positive MTB culture or Xpert from any site, and/or positive uLAM. Participants with a missing test result due to inability to obtain sample or technical failure of the index test were included as negative results in the numerator. Diagnostic yield is designed to capture information on performance of the index test in a real-world clinical setting.

Positive and negative blood Xpert-Ultra results in patients who were negative by all other tests in the ERS were defined as false positive and true negative respectively; patients with less than two valid test results in the ERS were excluded from this specificity analysis.

To explore the patient groups in which blood Xpert-Ultra testing might be most useful, sensitivity and diagnostic yield were calculated in different strata defined *a priori* by CD4 cell count <100 cells/mm3, haemoglobin <8 g/dL, and venous lactate >2·5 mmol/L. Diagnostic yield was also modelled as a continuous function of CD4 count, haemoglobin and lactate concentration using a LOESS smoothing regression, with confidence intervals derived from 1000 bootstraps.

Blood Xpert MTB/RIF Ultra Cycle threshold (Ct) as a marker of bacillary load

The minimum Ct value of the four *rpoB* probes was extracted using a custom R script from raw text files exported from the Xpert software as a summary Ct value. We used IS1081-IS6110 Ct values to impute blood Xpert-Ultra Ct values for "trace" positive samples, using a restricted cubic spline model with 3 knots to model the relationship between *rpoB* probe and IS1081-IS6110 Ct values (Figure S1, adjusted R^2 0·82).

Relationship between blood Xpert MTB/RIF Ultra and other tuberculosis detection tests

Intersections between tuberculosis detection on rapid diagnostic tests were explored using set-intersection plots. Covariance of qualitative tuberculosis test results (positive versus negative) were compared, including culture results, using factor analysis. Quantitative results including Ct values and time to positivity of cultures were compared using correlation plots and rank-correlation test.

Blood Xpert MTB/RIF Ultra association with mortality and clinical phenotype

Blood Xpert-Ultra results were assessed for association with 12-week mortality both as a qualitative result (positive or negative, in patients with confirmed HIV-associated tuberculosis) by Fisher's exact test, and by Ct value (in the stratum of participants with a positive result) by logistic regression, and using a LOESS smoothing function. Equivalent analyses of tuberculosis blood culture results, sputum-Xpert and urine-Xpert results were made for comparison. Blood Xpert-Ultra Ct values were categorised by tertile to give an ordinal scale ranging from 0 (negative test) to 3 (positive test with lowest Ct values), and assessed for a linear association with 12-week mortality by Cox proportional hazards regression. Equivalent models were made using qSOFA scores, uLAM results, and ordinal versions of TB blood culture, sputum-Xpert and urine-Xpert results. To formally compare the predictive value of blood Xpert-Ultra for mortality with these variables, nested models were made and compared using likelihood ratio tests, assessing if these variables added predictive value to a models that included blood Xpert-Ultra, and if blood Xpert-Ultra added value to models that included these variables.[27] To allow these nested comparisons, missing observations of sputum and urine based diagnostics were multiple-imputed using Classification and Regression Trees (CART) method in the Multivariate Imputation by Chained Equations (*mice*) package in R, with likelihood ratio statistics averaged across ten imputed datasets.

Association of blood Xpert-Ultra with 16 clinical and 16 immunological variables (selected *a priori* on the basis of known mortality association in this cohort)[4] were assessed; blood Xpert-Ultra results were assessed on an ordinal scale as above, and association with clinico-immunological variables were assessed by rank correlation tests. Q-values representing correction of p-values for multiple-comparisons using Benjamini-Hochberg procedure to limit false discovery rate were derived. Principal components analysis with varimax rotation was performed and the resulting two-dimensional representation of clinico-immunological phenotype related to blood Xpert-Ultra Ct values using both LOESS and linear regression.

All analysis was done using RStudio v1·2·5033, with code available at https://github.com/davidadambarr/blood_xpert_repo . STARD checklist is in the supplementary appendix.

Role of the funding sources

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

[985 words]

Patient inclusion

582 of 659 participants recruited to the parent study had biobanked whole blood available and were included in the current study. All 659 participants in the parent study had been successfully venesected; the 77 missing samples had been used in other studies, and were considered missing completely at random (MCAR) (Figure S2)) and not included in the diagnostic utility denominator. Tuberculosis was confirmed in 424/582 (73%) by the strict microbiological reference standard, and 447/582 (77%) by extended reference. Participants had a median of 6 baseline tuberculosis diagnostic tests with valid results (range 2-11) and therefore no participants were excluded due to incomplete reference standard. Median CD4 cell count was 62 cell/mm^3 (IQR 22-133) and 123/582 (21%) participants died by 12 weeks follow-up (Table S1).

Diagnostic performance for tuberculosis diagnosis

578 of 582 (99%) participants included had a valid blood Xpert-Ultra result (n=4 failed tests), 519 (89%) had a valid uLAM (n=0 failed tests, n=63 no urine sample obtained), and 445 (76%) had a valid sputum-Xpert (n=2 failed tests, n= 135 no sputum sample obtained).

165 of the 447 participants with confirmed tuberculosis by the ERS, were positive on blood Xpert-Ultra testing, giving a diagnostic yield of 0·37 (95%CI 0·32 to 0·42), which was lower than the diagnostic yield of sputum-Xpert and uLAM (0·62 and 0·43 respectively). Blood Xpert-Ultra performed relatively well in the pre-specified subgroups (Table 1), with diagnostic yield similar to uLAM (CD4 count <100 cells/mm^3 and haemoglobin <8 g/dL) or higher than uLAM (patients with venous lactate >2·5 mmol/l and those who died). Sputum-Xpert diagnostic yield had a U-shaped relationship with CD4 count, but out-performed uLAM and blood Xpert-Ultra at almost all CD4 counts (Figure 1). Diagnostic yield of blood Xpert-Ultra approximated that of uLAM across CD4 count range 0-100 cells/mm^3, and for patients with haemoglobin 5-9 g/dL. However, a significant divergence

between the two was seen with rising venous lactate concentrations: blood Xpert-Ultra performed increasingly well, while diagnostic yield of uLAM was lower at higher lactate concentrations (Figure 1). Hyperlactataemia was associated with inability to obtain a urine sample (OR 1.8 for missing urine sample for 1 log increase in venous lactate; 95%CI 1·4 to 4·4, p = 0.003). In 85 participants with lactate >2 mmol/l and a positive blood Xpert-Ultra, 17 had a negative uLAM, and 16 had no urine sample available to test. Combining sputum-Xpert with either uLAM or blood Xpert-Ultra was broadly equivalent (Figure 1, lower panels).

Two blood Xpert-Ultra positive participants were negative on all other available tuberculosis diagnostic tests (specificity 0·98; 95%CI 0·94to1·0) but did have clinical and radiological evidence of tuberculosis (Table S2).

Forty-three participants had rifampicin resistance confirmed by DST; time to detection of rifampicin resistance on these cultures was median 12 days (range 4 to 43 days). Rifampicin resistance was detected on sputum-Xpert in 28/43 (65%) and blood Xpert-Ultra in 10/43 (23%) including 2 not detected by sputum-Xpert.

Covariance of tuberculosis detection modalities

151 of 447 (34%) participants with confirmed tuberculosis (by ERS) were detected by a single positive rapid diagnostic test alone (from the set: sputum-Xpert, uLAM, urine-Xpert, and blood Xpert-Ultra) when the other three tests were negative (Figure 2A). Blood Xpert-Ultra was positive in 111/162 (69%) participants with a positive tuberculosis blood culture, with moderate agreement between the two modalities (Cohen Kappa 0·49 95%CI 0·41 to 0·58) equivalent to agreement between two blood cultures (Figure 2B)). Covariance of positive rapid diagnostic tests was related to compartment sampled, with blood and urine samples having closer agreement to each other relative to sputum diagnostics (irrespective of detection method) (Figure 2C). Correspondingly, blood Xpert-Ultra Ct correlated with blood culture time to positivity (TTP) and urine-Xpert Ct, but not with sputum culture TTP or sputum-Xpert Ct (Figure 2D&E).

Blood Xpert MTB/RIF Ultra association with mortality and clinico-immunological phenotype

A positive blood Xpert-Ultra result was associated with an odds ratio for 12-week mortality of 2·39 (95%CI 1·5 to 3·9) compared with 2·08 for TB blood culture (95%CI 1·3 to 3·4) and 2·08 for urine-Xpert

(95%CI 1·2 to 3·6) (Figure 3A). Blood Xpert-Ultra Ct value also correlated with mortality, showing a larger effect size than other quantitative TB markers (Figure 3A): predicted probability of death was 0·25 (95%CI 0·16 to 0·36) with blood Xpert-Ultra Ct of 28, rising to 0.57 (95%CI 0·42 to 0·70) with Ct of 22. Blood Xpert-Ultra was more predictive of mortality than any of: TB blood culture, urine-Xpert, sputum-Xpert, uLAM, qSOFA score, or these five variables combined as a multivariable predictor (figure 3B&C).

In participants with confirmed tuberculosis, 31/32 of the clinic-immunological variables remained significantly correlated with blood Xpert-Ultra after correction for multiple testing (Figure S3 & S4). Markers of acute inflammation, innate cell chemotaxis, coagulation system activation, lymphocyte counts, and interleukin-1 receptor antagonist were most strongly associated with blood Xpert-Ultra positivity.

A substantial proportion of variance in clinico-immune markers was captured in 2 principal components (PC): variance in T-cell associated mediators on PC1, markers of acute inflammation on PC2, and blood cell counts loading partially on both axes (Figure 4A). Twelve-week mortality risk mapped closely to this 2-dimensional representation of participant phenotype: the quarter of participants with below average PC1 score and above average PC2 score had an odds ratio for mortality of 11 compared to the quarter of participants with above average PC1 and below average PC2 scores (upper-left PCA quadrant versus lower-right PCA quadrant, figure 4B: odds ratio 95%CI 4·7 to 29; p <0.001 Fisher's exact test). In turn, blood Xpert-Ultra positivity was associated with a 0·3 standard deviation (s.d.) decrease in PC1 score, and a 1·1 s.d. increase in PC2 score (p=0·003 and p<0.001 respectively, figure 4C). Within the stratum of participants with a positive blood Xpert-Ultra, Ct value was not significantly related to PC1, but showed strong negative correlation with PC2 score (r = -0·49, p <0.001 figure 4C): 68 of 75 (91%) participants with blood Xpert-Ultra Ct value below the median had a PC2 score above the mean.

Discussion

[785 words]

Detection of *M. tuberculosis* blood stream infection using the described Xpert MTB/RIF Ultra protocol gives more prognostic information in severe HIV-associated tuberculosis than previously available markers of tuberculosis dissemination. Accordingly, blood Xpert-Ultra has greatest diagnostic utility in critically ill patients: compared with Alere urine-LAM, blood Xpert-Ultra had similar diagnostic yield in participants with low CD4 counts, and higher yield in participants with raised venous lactate and those who died. Blood Xpert-Ultra cycle threshold value has a robust dose-response relationship with disease phenotype and mortality risk – giving evidence that blood bacillary burden has an important, and potentially causal, roll in HIV-associated tuberculosis outcomes.

We optimized the pre-processing of blood requiring only low-cost reagents, combined with the WHO endorsed Xpert MTB/RIF Ultra platform and report higher sensitivity (38%) than previous studies.[25,26] The method for pre-processing blood is accessible in low-resource settings for use where Xpert platform is available. This is in contrast to previous studies using 'in-house' PCR protocols that are not scalable to routine clinical labs. Ease of blood sampling in clinical settings, same-day results, rifampicin-resistance reporting and prognostic value, are additional benefits.

MTBBSI is common, under-recognized, and related to high mortality-risk when initiation of anti-tuberculosis therapy is delayed, compared to other forms of HIV-associated tuberculosis [4,28]. TB blood culture is unavailable in most settings with a high burden of HIV-associated tuberculosis, and, irrespective of availability, prolonged time-to-detection decreases its utility in critically ill patients. Blood Xpert-Ultra could replace TB blood culture in the clinical setting as the tests have equivalent sensitivity for MTBBSI detection. The extra pre-processing steps required may be a consideration, but are offset by a short time-to-detection and direct results without identification steps required for positive blood cultures. The ability to rapidly identify patients with MTBBSI strengthens the rationale for developing a dedicated evidence base for treatment of this critical condition.

Markers of dissemination and MTBBSI in HIV-associated tuberculosis have been linked to adverse clinical status, sepsis, and mortality by our group and others [4,29,30].
In this analysis of participants with advanced HIV-associated tuberculosis, blood Xpert-Ultra was more strongly associated with mortality than other bacillary load and dissemination markers (TB blood culture, uLAM, urine-Xpert, and sputum-Xpert). Risk of mortality strongly correlated with a low-dimensional representation of host-response phenotype, which in turn closely corresponded

with blood Xpert-Ultra results. Even within the stratum of participants with a positive blood Xpert-Ultra test, we found that clinical phenotype and mortality risk correlated with Ct values as a quantitative read-out of MTBBSI. This robust dose-response relationship strengthens the evidence that blood bacillary load is a major determinant of outcome in HIV-associated tuberculosis.

This insight resonates with classical understandings of TB pathophysiology, which held bacillary load to be the major determinant of disease severity.[17,19] It suggests that bacillary load is a fundamental variable for interrogating "dysregulated" host-response in HIV-associated tuberculosis, the leading cause of sepsis in high HIV burden settings. Modern studies of host response in severe HIV-associated tuberculosis and sepsis acknowledge that microbial load may be an important latent variable but have lacked the tools to measure it.[13,31] We found the blood bacillary load dose-response relationship was most strongly seen with a host-response component capturing innate-cell, pro- and anti-inflammatory, and tissue damage signals, rather than the other major axis of variation in clinical phenotype which appeared to represent T-cell dysfunction. We speculate that antimicrobial and host-directed interventions targeting each of these pathophysiological components could be synergistic. For example, anti-inflammatory strategies should be combined with optimized antimicrobial killing. Further, blood Xpert-Ultra has potential as a pharmacodynamic assay to facilitate much-needed interventional trials for MTBBSI.

Although blood Xpert-Ultra Ct value correlated with tuberculosis blood culture TTP, the former was more robustly associated with risk of mortality. Possible explanations for this include that TTP has more stochastic variation than Ct, that TTP reflects lag-time for the fastest -growing bacilli in the sample rather than directly reporting overall bacilli numbers [32], or that Xpert-Ultra captures non-culturable bacilli. By extension, blood Xpert-Ultra Ct may be a more useful microbial load biomarker than TTP in future studies of severe HIV-associated tuberculosis.

Limitations of our analysis include retrospective use of biobanked samples: sample volumes varied, and may not have been optimal for the blood Xpert-Ultra protocol, which could have introduced noise and reduced sensitivity respectively. Storage at -80^{o}C may also have reduced sensitivity. The reported sputum diagnostic yield may be higher than clinical practice because of sputum induction in those patients unable to produce spontaneous sputum.

In conclusion, we report the use of Xpert-Ultra on whole blood and show utility as a novel rapid-diagnostic and prognostic biomarker for critically ill patients with HIV-associated tuberculosis. Blood Xpert-Ultra is a useful tool for characterizing MTBBSI, demonstrating dose-response association between blood bacillary load, and adverse clinical phenotype.

Contributors

GrM and DB conceived the study; CS, GaM, MS, LB, RJW, MN contributed to study design. GrM and CS conceived the parent study (KDHTB); CS, AW, and DB contributed clinical data acquisition; GrM and RB provided clinical oversight. NS, AB, CS contributed to laboratory data acquisition. DB , AB and MS developed the blood pre-processing protocol; MS and MN provided laboratory oversight. RJW provided laboratory facilities for storage and processing of samples. LB and DB performed data analysis with input from all coauthors and oversight from GrM, GaM and CS. LB wrote the manuscript and all co-authors reviewed and contributed to the manuscript.

Declaration of interests

All authors declare no conflict of interest.

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Table 1. **Sensitivity & diagnostic yield in whole cohort and subgroups**

A: Sensitivity

Strata	index test	Valid test, n	Confirmed TB,* n	True positive, n	Sensitivity	CI 95
Whole cohort	Alere LAM	519	375	171	0.46	0·40 to 0·51
	blood Xpert Ultra	578	423	161	0.38	0·33 to 0·43
CD4<100	Alere LAM	328	253	144	0.57	0·51 to 0·63
	blood Xpert Ultra	371	287	144	0.50	0·44 to 0·56
Haemoglobin<8	Alere LAM	173	142	87	0.61	0·53 to 0·69
	blood Xpert Ultra	204	168	78	0.46	0·39 to 0·54
Lactate>2·5	Alere LAM	117	95	47	0.49	0·39 to 0·60
	blood Xpert Ultra	137	113	57	0.50	0·41 to 0·60
Died by w12	Alere LAM	101	74	39	0.53	0·41 to 0·64
	blood Xpert Ultra	121	89	49	0.55	0·44 to 0·65

B: Diagnostic yield

Strata	index test	n	Confirmed TB,# n	True positive, n	Diagnostic yield	CI 95
Whole cohort	sputum Xpert	582	447	275	0.62	0·57 to 0·66
	Alere LAM	582	447	190	0.43	0·38 to 0·47
	blood Xpert Ultra	582	447	165	0.37	0·32 to 0·42
CD4<100	sputum Xpert	374	300	185	0.62	0·56 to 0·67
	Alere LAM	374	300	152	0.51	0·45 to 0·56
	blood Xpert Ultra	374	300	148	0.49	0·44 to 0·55
Haemoglobin<8	sputum Xpert	205	177	112	0.63	0·56 to 0·7
	Alere LAM	205	177	92	0.52	0·44 to 0·59
	blood Xpert Ultra	205	177	82	0.46	0·39 to 0·54
Lactate>2·5	sputum Xpert	140	122	67	0.55	0·46 to 0·64
	Alere LAM	140	122	52	0.43	0·34 to 0·52
	blood Xpert Ultra	140	122	60	0.49	0·4 to 0·58
Died by w12	sputum Xpert	123	96	53	0.55	0·45 to 0·65
	Alere LAM	123	96	43	0.45	0·35 to 0·55
	blood Xpert Ultra	123	96	51	0.53	0·43 to 0·63

* By strict micro reference standard

By extended micro reference standard

Figure 1. **Diagnostic yield of individual and combination rapid diagnostic tests as a function of clinical variables**

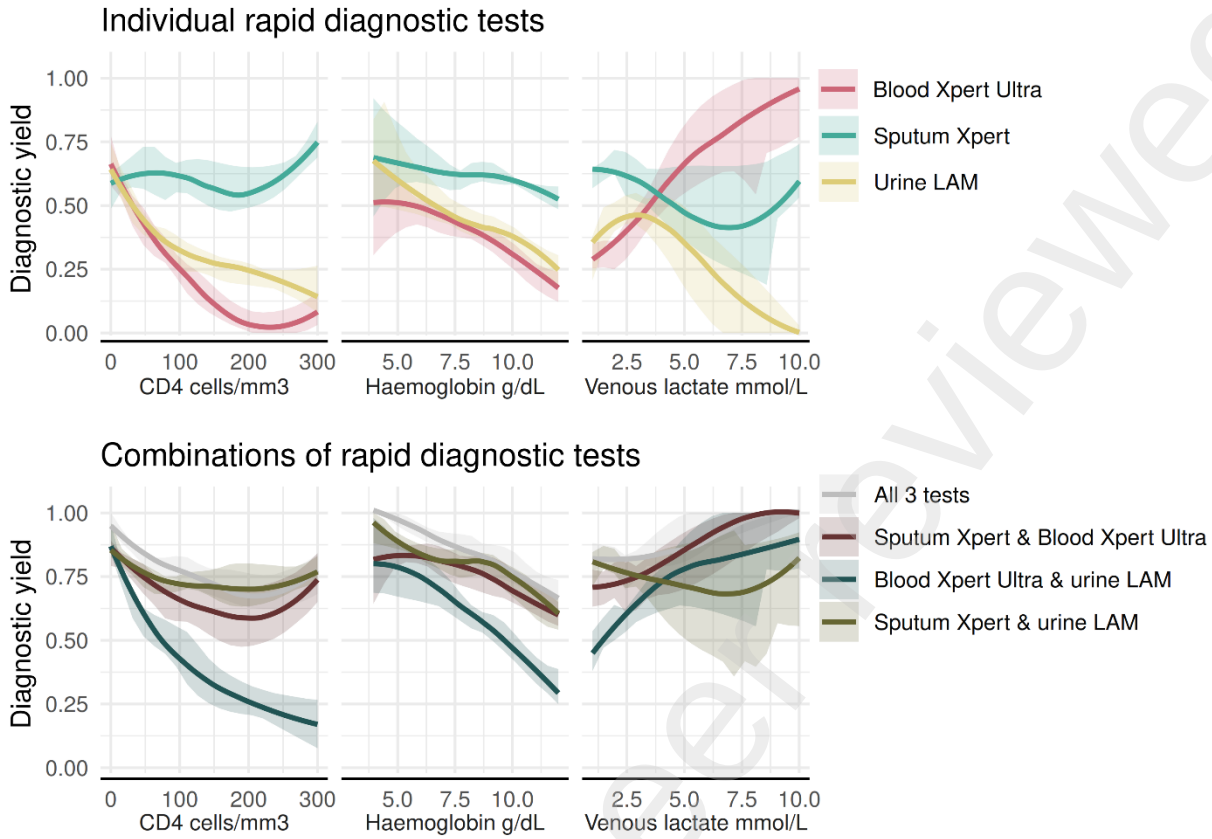

Figure 1 notes:

Individual test (top row panels) and combinations of these tests (bottom row panels) diagnostic yield modelled as a function of 3 *a priori* specified patient variables: CD4 cell count (left column), haemoglobin (middle column), and venous lactate (right column). Plotted lines show a LOESS smoothing function fit to the proportion of tuberculosis cases identified by the diagnostic tests by different levels of the patient variables. Shaded areas indicated 95% confidence intervals derived from 1000 bootstrapped resampling and refitting of the LOESS models

Figure 2. **Qualitative & quantitative associations between tuberculosis detection modalities**

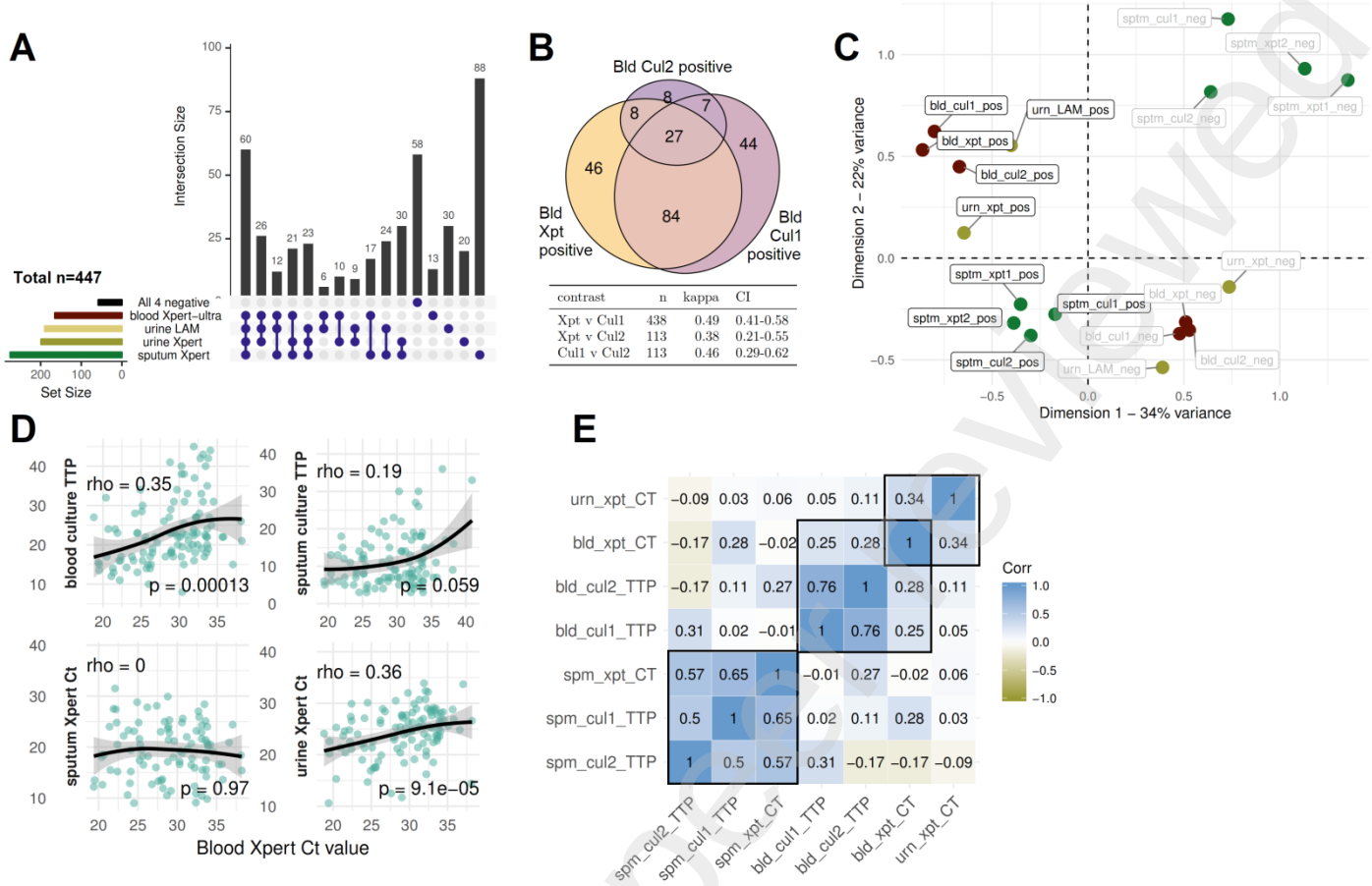

Figure 2 notes:

A. Intersections between sets defined by positive rapid-test results. Total set size (number of patients with positive sputum Xpert, positive urine Xpert, positive blood Xpert Ultra, and number of patients with all 4 tests negative) shown by horizontal coloured bars. Intersections of these sets are indicated by the connected blue dots; number of patients in each of the possible intersections are shown with vertical bars. These figures are based on a single test result as described in main text. **B**. Intersections between *M. tuberculosis* blood stream infection diagnostics. Blood Xpert Ultra was positive in 111/162 (69%) of patients who had tuberculosis recovered from their first blood culture. 113 patients also had a second tuberculosis blood culture performed, of which 50 were positive; blood Xpert Ultra was positive in 35/50 of these. Agreement (measured by Cohen's Kappa) between blood Xpert Ultra and blood culture 1 was the same as agreement between pairs of blood cultures in the same patient. **C**. Factor analysis describing the main dimensions of variation seen for qualitative test results (positive or negative) including where two samples were sent for the same test. The first dimension of variation (capturing 34% of total variance in test results between patients) separates patients with predominantly positive and negative test results; the second dimension of variation (explaining 22% variance) separates patients by compartment yielding positive results, with blood and urine diagnostics separating from sputum based diagnostics. Categories further from the origin are less frequent (higher variance) than those near the origin. **D**. Correlation between quantitative read-outs from tests (sputum and blood culture time to positivity, TTP; urine and sputum Xpert Ct values) and blood Xpert Ultra Ct value. Fitted line and shaded 95% confidence interval from LOESS smoothing function; Rho and p value from Spearman's

rank test. **E**. Pearson's correlations between time to positivity of cultures (TTP) and Ct values from blood, urine and sputum (bld, urn, spm) samples, ordered using hierarchical clustering.

Figure 3. **The association of blood Xpert ultra with 12-week mortality and comparison to other diagnostic tests as predictors of mortality.**

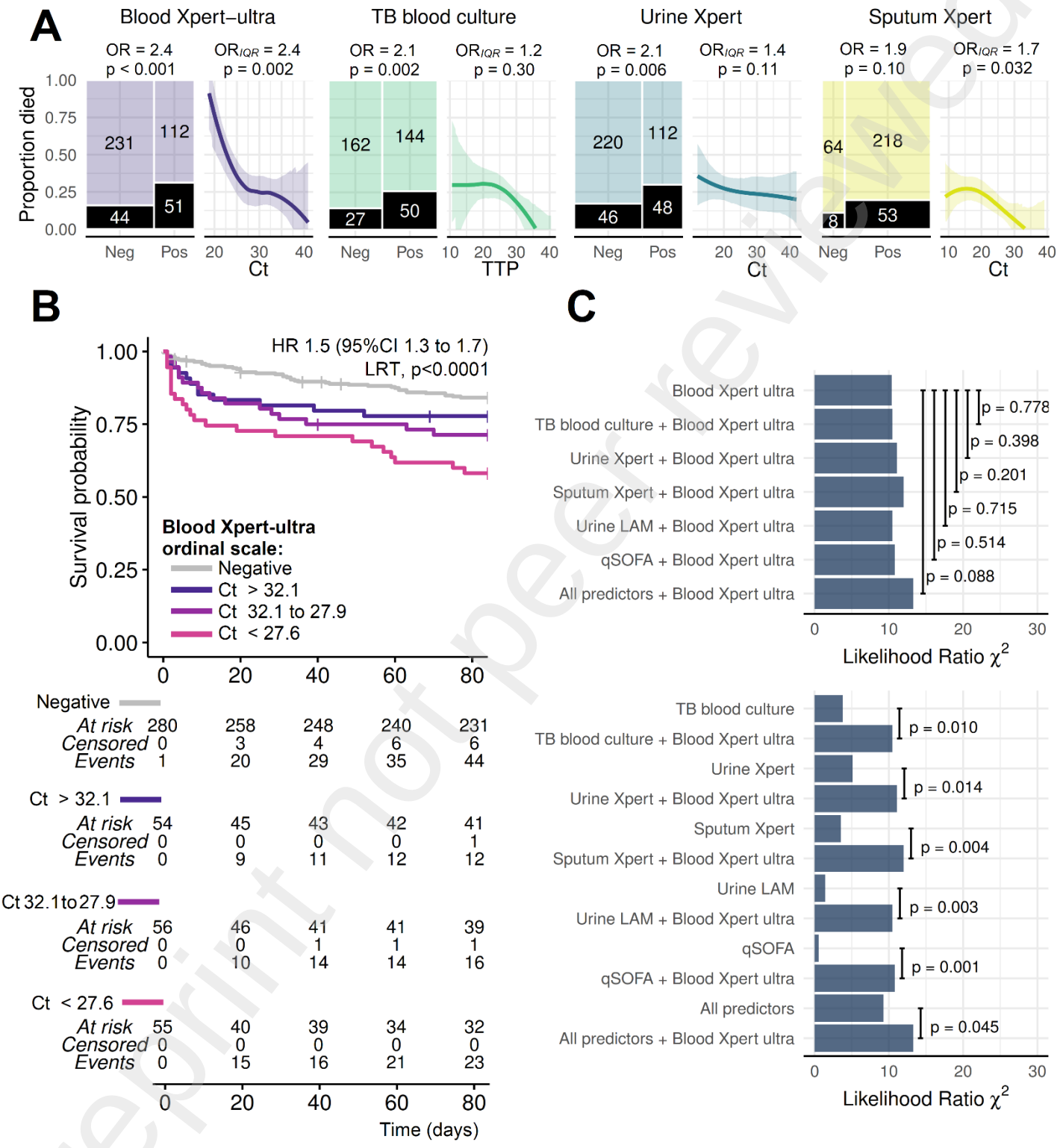

Figure 3 notes:

A. Mosaic plots of 2x2 cross-tabulation of test result (positive or negative) and mortality for the 4 diagnostics, blood Xpert-ultra, TB blood culture, urine Xpert, and sputum Xpert; y-axis indicates proportion who died by day 84 follow-up, area of each cell proportional to numbers in cross-tabulation category; OR = odds ratio with p-value from Fisher's exact test. Also shown are estimated proportions who died by Ct (Xpert tests) or time to positivity (blood culture) for those patients with positive tests; a LOESS smoothing function fit is shown by line with 95% confidence intervals for the fit derived from 1000 bootstraps; OR_{IQR} indicates the odds ratio for a decrease in Ct or TTP of one inter-quartile range ("the inter-quartile effect size") with associated p-value derived by logistic regression.
B. Kaplan-Meier plot showing survival by blood Xpert-ultra result on an ordinal scale, where positive results are further categorised by Ct value cut-offs based on observed tertiles, giving four ordinal categories. HR = hazard ratio for a one unit increase in ordinal category, with 95% confidence interval from a Cox proportional hazards model fit to this data; LRT p value = likelihood ratio test of model fit. Goodness-of-fit test for violation of proportional hazards assumption, p>0·05. **C**. Predictive value of blood Xpert-ultra result (on ordinal scale as per B.) formally compared to other variables from the set {TB blood culture, urine Xpert, sputum Xpert, urine LAM, qSOFA score} using likelihood ratio tests. The addition of each of these variables to blood Xpert-ultra results, either individually or in combination, did not significantly improve model fit over blood Xpert-ultra alone, as indicated by p-values from likelihood ratio tests with 1 degree of freedom in top panel. By contrast, addition of blood Xpert-ultra result to each of these variables as an individual predictor or when all were combined did improve model fit in all cases, as indicated by p-values from likelihood ratio tests in lower panel.

Figure 4. **Major axes of covariance in clinic-immunological variables & their relationship to blood Xpert Ultra results**

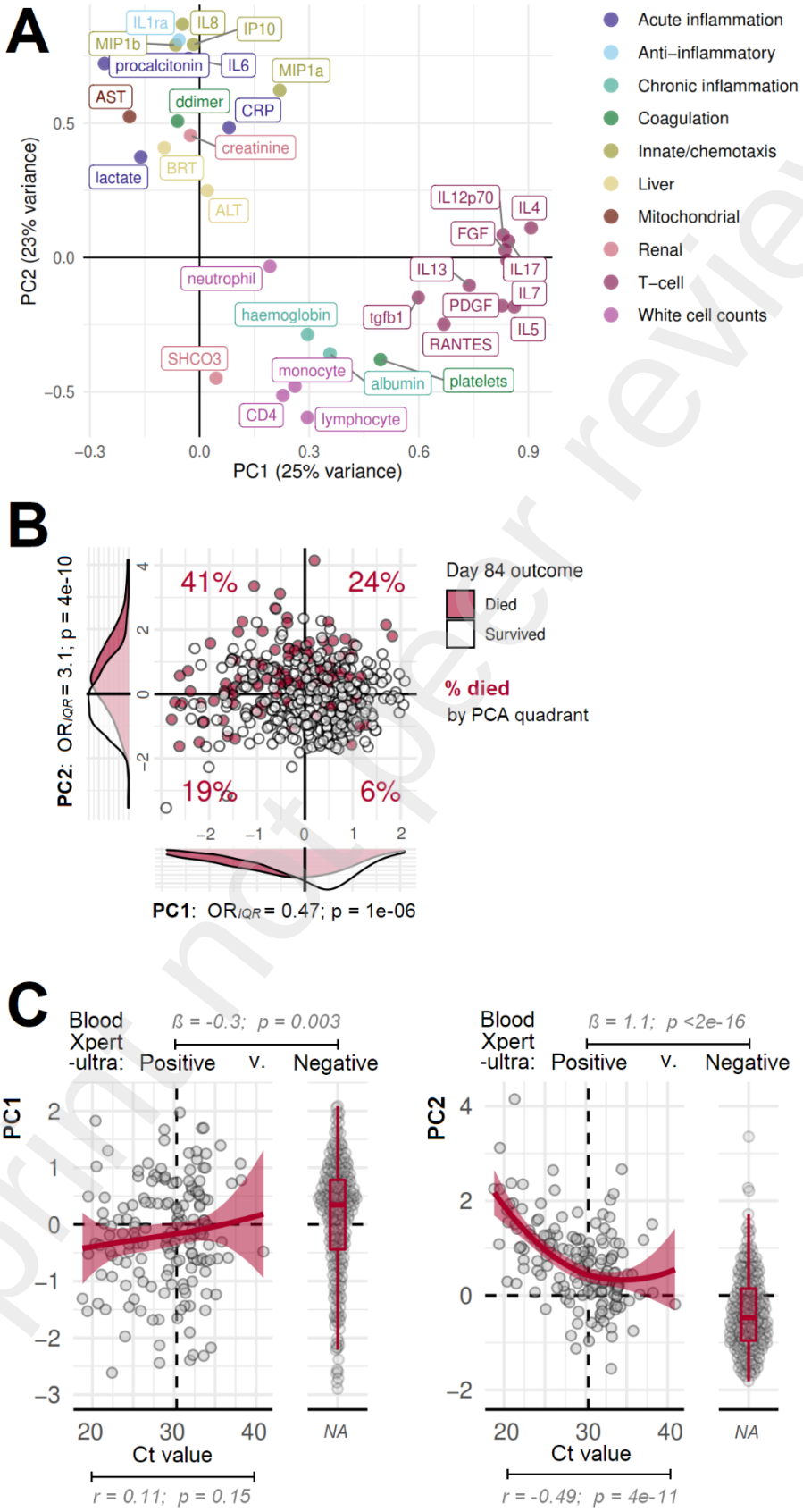

Figure 4 notes:

Principle components analysis of 32 clinic-immunological variables using varimax rotation performed on n=447 patients with confirmed tuberculosis. **A**. Loadings of the 32 variables on first two principal components (PC1, PC2) which together capture 48% of total variance. **B**. Individual patients' PC1 and PC2 scores, by day 84 outcome. Density histograms show distributions of patients' PC1 and PC2 scores by day 84 outcome; OR_{IQR} indicates the odds ratio for mortality associated with a one interquartile-range increase in PC score ("the inter-quartile effect size") with associated p-value derived by logistic regression. Scatter-plot shows mortality outcome mapped onto the two-dimensional space defined by PC1 and PC2 scores;. **C**. Distribution of PC1 and PC2 scores by blood Xpert-ultra Ct value, and in patients with a negative blood Xpert-ultra result. A LOESS fit regressing PC score on Ct value is shown in the strata of patients with a positive blood Xpert-ultra, with 95%CI indicated by red shaded area, as well as a correlation coefficient and associated p-value for a linear regression. Distribution of PC score in blood Xpert-ultra negative patients is shown, with β coefficient from regressing PC score on blood Xpert-ultra result (indicating the average difference in PC score between blood Xpert-ultra positive and negative patients).