Low Transcriptomic of PTPRCv1 and CD3E Is an Independent Predictor of Mortality in HIV and Tuberculosis Co-infected Patient

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Abstract

Background: Assessing immunological profiles from HIV-TB co-infection is essential to predict mortality, to facilitate the development of effective assays, the discovery of novel therapeutic targets and to conduct new interventions appropriately.

Methods: Expression levels of 105 immune-related genes were measured at baseline of enrolment from 9 death HIV and TB coinfecting study participants during follow-up and 18 survived groups for 2 years and matched controls, using focused gene expression profiling by dual-color Reverse-Transcription Multiplex Ligation-dependent Probe Amplification assay. Gene expression levels were also measured at six months and 18th month follow-up.

Results: Eleven of the 105 selected genes were differentially expressed between death individuals and survivor matched controls. *IL4δ2* was significantly higher expressed in death groups than survivor matched controls, whereas *CD3E*, *IL7R*, *PTPRCv1*, *CCL4*, *GNLY*, *BCL2*, *CCL5*, *NOD1*, *TLR3* and *NLRP13* had significantly lower expression levels in death groups compared to survivor matched controls. The expression of *PTPRCv1*, *CD3E*, *CCL5*, *IL7R*, *NOD1*, *IL4δ2* and *GNLY* at baseline could accurately predict the mortality from TB-HIV co-infection.

Conclusions: The expression of *PTPRCv1*, *CD3E*, *CCL5* and *IL7R* host genes in peripheral blood of patients with TB-HIV coinfecting can potentially be used as predictor of mortality in Ethiopian setting. Anti-TB treatment might be less likely to restore gene expression in the level expression of death groups. Therefore, other new therapeutic that can restore these gene (*PTPRCv1*, *CD3E*, *IL7R* and *CCL5*) in the death groups at baseline might be needed to save lives.

Introduction

Tuberculosis (TB) remains to be the major global health threat, caused by *Mycobacterium tuberculosis* (MTB), and remains one of the leading causes of infection-related death worldwide. According to the World Health Organization (WHO), there were 10.0 million (range, 8.9–11.0 million) newly TB patients in 2019 1. Globally, HIV-related tuberculosis comprises, 8.2% of newly tuberculosis cases but contributes a disproportionate around 15% of tuberculosis deaths in 2019 1. WHO recommended developing an effective diagnostic and treatment for latent TB infection to achieve the reduction of death from Mtb by 35% of the 2015 death at 2020 2. The cumulative reduction between 2015 and 2019 was only 14%, less than half of the first milestone of the End TB Strategy 1. After adjusting for age, sex, residence, WHO stage, and bodyweight, People living with HIV and TB co-infection had 40% higher mortality than those without TB 3. Moreover, HIV and TB co-infection has an adjusted 2.08 times higher risk of mortality compared to TB patients 4. The underlying causes of mortality remain poorly characterized. In HIV-related tuberculosis, early mortality has been associated with proxy for severe immunosuppression 5, increases immune activation 6-8, and failure to recover cellular immune responses to *Mycobacterium tuberculosis* 9, lack of effective immunity at baseline 10.

Prediction of death from HIV and TB co-infection is a major challenge. Moreover, current available diagnostic tools cannot be predict which developed active tuberculosis and HIV infected patients will be survive or death. Understanding of immune system and assessing of contributors for mortality could facilitate the development of effective assays, the discovery of novel therapeutic targets and enable appropriate risk stratification to target new interventions appropriately, and aid monitoring of treatment responses. Importantly, impact of host gene expression for risk of mortality in HIV and TB co-infected individuals has little or never been studied. Therefore, our study was focused on host gene expression for assessing mortality risk of TB-HIV co-infected individuals. Therefore, we aimed to assess host gene expression in whole blood for the risk of death in HIV-TB co-infected individuals using focused gene expression profiling by dual-colour Reverse-Transcription Multiplex Ligation-dependent Probe Amplification (dcRT-MLPA).

Materials And Methods

Ethics statement

All study participants requested if provided written, informed consent at enrollment. This study was obtained ethical clearance from the Scientific and Ethics Research Office of the Ethiopian Public Health Research Institute (Ref: E.H.N.R.I. 6.13/01) and the London School of Hygiene & Tropical Medicine Ethics Review Committee (Ref: 7174). All study procedures were conducted according to the Declaration of Helsinki.

Study design and population

This matched case control and an observational cohort study was part of the previous our study 11. For the assess host gene expression in whole blood for the risk of death, 9 active TB with HIV (HIV+TB+) coinfectd as death group during follow-up were included in this study (Figure-1). All of the death groups were deathbed between 3- 6 month follow up from enrolment and they were not HAART treated. For each death group, two matched controls that remained survived during 2 years follow-up were selected and matched by age at enrolment, sex (≤ 18 years, 19–25 years, 26–35 years, or ≥ 36 years), and, year of enrolment 12, CD4+ count and viral load. All cases and controls were treated for TB treatment at recruitment time according to the national guideline 13.

Laboratory Procedure

RNA was extracted spin column based using the Paxgene RNA extraction kit (PreAnalytiX, Qiagen) according to the manufacturer's instructions. After the Paxgene tubes briefly centrifuged at 4000 rpm for 10 minutes, the pellet of cells was lysed using lysing Buffer (Buffer BR1). The unwanted protein in lysed pellet were removed using proteinase K. Ethanol-precipitated nucleic acids were loaded onto a spin column. Unwanted DNA was digested using RNase-free DNase (Qiagen). Finally, purified RNA was eluted

with RNase-free elution buffer (BR5 buffer) and quantified using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, USA).

Dual-color Reverse-Transcription Multiplex Ligation-dependent Probe Amplification (DcRT-MLPA) was performed as described in detail previously 14. Briefly, for each target-specific sequence, a specific reverse transcription (RT) primer was designed located immediately downstream of the left and right-hand half-probe target sequence. Complementary DNA (cDNA) was generated from RNA using an RT primer mix. Subsequently, MMLV reverse transcriptase was inactivated by heating at 98°C for 2 minutes and cDNA was incubated overnight at 60°C with a mixture of customized left and right-hand half-probes to hybridize with the target cDNA. Annealed half-probes were ligated using ligase-65 enzyme and subsequently amplified by PCR (33 cycles of 30 sec at 95°C, 30 sec at 58°C, and 60 sec at 72°C, followed by 1 cycle of 20 min at 72°C). Primers and probes were from Sigma-Aldrich Chemie (Zwijndrecht, The Netherlands) and MLPA reagents from MRC-Holland (Amsterdam, The Netherlands). PCR amplification products were 1:10 diluted in HiDi formamide-containing 400HD ROX size standard, denatured at 95 °C for 5 min, cooled on ice and analyzed on an Applied Biosystems 3730 capillary sequencer in GeneScan mode (Base Clear, Leiden, The Netherlands).

Trace data were analyzed using GeneMapper software 5 package (Applied Biosystems). The areas of each assigned peak (in arbitrary units) were exported for further analysis in Microsoft Excel spreadsheet software. Data were normalized to GAPDH and signals below the threshold value for noise cutoff in GeneMapper (log2 transformed peak area 7.64) were assigned the threshold value for noise cutoff. Finally, the normalized data were log2 transformed for statistical analysis.

RT primers and half-probes were designed by Leiden University Medical Centre (LUMC, Leiden, The Netherlands) 15,16 and comprise sequences for 4 housekeeping genes and 105 selected genes to profile the innate and adaptive immune response 14.

Statistical analysis

A non-parametric two tailed Wilcoxon rank-sum (Mann-Whitney) test was used to compare two unpaired data sets after assessment of normality of the data using the Kolmogorov Smirnov test. Non-parametric Receiver Operator Characteristic (ROC) curves was used to determine the potential predictor of mortality in HIV+TB+ patients. Spearman rank correlation was used to measure the degree of association between mortality and single host gene expression. The statistical significance level used was $P < 0.05$ and all P values are two-tailed. All data analysis was performed using Inter cooled STATA version 16.0 (College Station, Texas, USA).

Results

Whole blood gene expression profiles of 9 death individuals and 18 survivor matched controls TB with HIV positive individuals were analysed by dcRT-MLPA using probe sets for 105 selected genes to profile innate and adaptive immune responses at enrolment (**Supplementary Table 1**). Eleven of the 105 selected

genes were differentially expressed between death individuals and survivor matched controls (Table 1). One gene (*IL4δ2*) was significantly higher expressed in death groups than survivor matched controls, whereas 10 genes (*CD3E*, *IL7R*, *PTPRCv1*, *CCL4*, *GNLY*, *BCL2*, *CCL5*, *NOD1*, *TLR3* and *NLRP13*) had significantly lower expression levels in death groups compared to survivor matched controls.

Non-parametric Receiver Operator Characteristic (ROC) curves to determine the potential risk for death of single genes identified *CCL5*, *PTPRCv1*, *CD3E*, *IL7R*, *NOD1*, *IL4δ2* and *GNLY* with Area Under the Curve (AUCs) of 0.86, 0.86, 0.86, 0.85, 0.78, 0.77 and 0.76 respectively as those genes with the most powerful classifying potential to contributing for risk of mortality among survivors of TB disease with HIV-coinfected individuals (Figure 2). The gene expression profiles of these signature genes are displayed in Figure 3.

After performing the spearman analysis to look the relationship among the expression of genes, the gene expression of *PTPRCv1* had positive correlation with 5 gene expression (*CD3E*, *CCL5*, *IL7R*, *NOD1* and *GNLY*), but negative correlation with *IL4d2* (Figure 4). However, the gene expression of *CD3E* and *IL7R* had not correlation with the expression of *NOD1* and *IL4d2*.

The host gene expression in the survived matched control was increased during and after TB treatment over the follow up time (Figure 5). However, anti-TB treatment may not have any impact on the gene expression at the level of death group.

Table 1. Gene expression profiles differentiating between death groups and survived controls

Gene Symbol	HIV+TB+ death (n=9)	HIV+TB+ survivor as control (n=18)	P-value
T cell subset markers			
CD3E	12.27(10.25-12.27)	13.21(12.68-13.49)	0.0033
IL7R	9.98(9.62-10.58)	12.02(11.59-12.25)	0.0039
PTPRCv1	9.20(8.99-9.95)	10.50(10.03-11.62)	0.0028
Th2/Treg associated genes			
IL4δ2	12.15(9.78-13.17)	7.64(7.64-11.20)	0.0200
CCL4	9.38(9.16-9.72)	10.07(9.39-10.25)	0.0492
Cytotoxicity genes			
GNLY	12.01(11.26-13.42)	13.88(12.81-14.28)	0.0333
Apoptosis/survival			
BCL2	8.27(7.64-8.97)	9.54(8.92-9.89)	0.0458
Myeloid associated genes			
CCL5	13.93(13.14-14.37)	14.70(14.36-14.83)	0.0033
Pattern recognition receptors			
NOD1	7.79(7.64-8.02)	8.32(7.96-8.52)	0.0183
TLR3	9.63(9.45-10.12)	10.40(10.21-10.82)	0.0380
Inflammasome components			
NLRP13	7.64(7.64-7.64)	7.64(7.64-9.32)	0.0177

Median (inter quartile range) gene expression values (peak areas normalized for GAPDH and log2-transformed) are shown at baseline of the death groups and controls, and significant differences between the death groups and controls were determined using Wilcoxon Mann-Whitney test. In pink: genes are indicated that were more highly expressed in the test group compared to the reference/control group. In blue: genes are indicated that had lower expression in the test group compared to the

reference/control group. Only genes whose expression level significantly differed between the two study groups are listed.

Discussion

In our previous study, at baseline, there was no significant difference between host gene expression of HAART eligible and ineligible HIV+TB+ groups 11. However, the prevalence of mortality among HAART ineligible groups was higher than eligible groups 17. Assessing immunological profiles contributors' for mortality from HIV-TB co-infection is essential to prediction of mortality, to facilitate the development of effective assays, the discovery of novel therapeutic targets and enable appropriate risk stratification to target new interventions appropriately, and aid monitoring of treatment responses. Here, we used a focused gene expression profiling platform, dcRT-MLPA 15, targeting innate and adaptive immune response genes, to analyze RNA expression levels of 105 pre-selected genes in peripheral blood of TB-HIV-co-infected individuals and identified biomarkers with excellent predictor capacity between 9 death groups and 18 survivor matched controls. The expression levels of 8 single genes (*CCL5*, *PTPRCv1*, *CD3E*, *IL7R*, *NOD1*, *IL-4δ2* and *GNLY*) could accurately predict which active TB with HIV coinfecting patients will be death with AUCs ≥ 75 , indicating these interrelating biomarkers are strongly associated with TB-HIV-coinfected related mortality.

The lower expression of T-cell associated genes (*PTPRCv1*, *CD3E* and *IL7R*) in these death groups may reflect reduce responsiveness of TCR signaling which may represent an HIV-induced evasion mechanism in individuals with weak immune system 18-20. Given the fact that HIV infection accelerates decreasing of the expression of all investigated T cell subsets which leads to HIV progression. However, our result shows lower expression of *PTPRCv1* cells was associated with an increased risk of dying which is similar with previous report 21. This lower expression may related to depletion of naive T cells 22,23, and a relative expansion of immature and activated CD4+ cells 24,25. Lower gene expression of *CD3E* in death groups suggests lack of preTCR-associated intrinsic kinase activity which may play critical role in transmitting signal across T cell membrane 26. Previous study demonstrated that low *CD3E* expression was a predictor of poor survival 27. This may correlated with the infiltration levels of CD8+ T cells to the infection cite that leads deletion of T-cells and with the increase of pathological stage 28. Lower expression of *IL7R* were also reported in AIDS patients elsewhere previously 29. These findings shows impaired T-cell functions and immune failure described in TB/HIV patients that can predict the mortality. The function of *IL-7R* has improved the functionality of tolerized CD8 T cells their ignorance to self-antigens and promote CD8 T cell cytotoxicity to self-antigens which protects autoimmune 30.

Our finding shows, the gene expression of *CCL5* (Myeloid associated gene) was amongst the strongest differentially expressed genes between death groups and survived matched controls, with potential discriminatory power between death groups and survived matched controls. This is consistent with the expression profiles of soluble *CCL5*/*RANTES* from plasma 31. T cells preferentially used *CCR5* interactions with ligands (*CCL5* and *CCL4*) for early migration into *Mtb*-infected lungs and granuloma

formation 32, which indicates CCL5 contributes to early, protective, Mtb-specific immunity 33, but may also important role in immunopathogenesis during TB 34.

The survived matched control had increased host gene expression over the follow up time (Figure 5), which is similar with our previously finding ‘most gene expression were normalized at the end of anti-TB treatment of TB patients to levels observed in latent TB infected and uninfected control subjects during HIV infection’ 11. This finding shows clearing of the TB bacilli from the infection body and restore gene expression due to anti-TB treatment. However, anti-TB treatment may be less likely to clear of the TB bacilli and restore gene expression in these death group. Moreover, HAART have not any impact on the level of gene expression during HIV infection. Therefore, other therapeutic that can restore these gene (PTPRCv1, CD3E, IL7R and CCL5) might be needed to save lives.

Our finding also shows low gene expressions of NOD1 and NLRP13 in death HIV+TB+ groups compared to survived groups, but less likely predict the mortality. Mekonnen and his colleagues demonstrated, *NOD1* was identified novel genetic associations with TB in Ethiopian populations 35. NOD1 plays important roles in the detection of bacteria and the production of proinflammatory molecules, given that both signaling pathways lead to NF- κ B and MAPK activation 36. NOD1 deficiency mice show increased susceptibility to lung infection, which is associated with reduced neutrophil recruitment to the lungs 37, and associated with the development of inflammatory disorders 38. In the contrast of the other gene expression, the gene expression of IL-4 δ 2 was higher in death HIV+TB+ groups compared to survive matched controls that might be correlated with disease severity 39,40. However, IL-4 δ 2 may has less probable to predict the mortality; [AUC (95%CI) = 0.77 (0.57 - 0.97)].

In conclusion, the expression of PTPRCv1, CD3E, CCL5 and IL7R in peripheral blood can predict the mortality in HIV+TB+ co-infected individuals. In the future, the identified biomarkers could be applied to facilitate the development of effective assays for both tuberculosis and HIV diseases, the discovery of novel therapeutic targets and enable appropriate risk stratification to target new interventions appropriately, and aid monitoring of treatment responses. Future work could determine to completely characterise these signatures in different populations and larger study populations.

Declarations

Data available

All necessary data generated or analyzed during this study are included in this article

Competing interests

The authors declare that they have no conflicting interests

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Figures

Figure 1

Figure 1

Flow diagram of the cohort.

At enrollment 58 HIV+TB+ subjects were enrolled: All HIV+TB+ patients were ATT treated at baseline. LFU = Lost during follow up; HAART+ = Subjects that had received HAART treatment for at least 3 months at 6th month follow up HAART- = Subjects that had not received HAART treatment at the indicated time point. All death group was HAART- until the death date.

Figure 2

Figure 2

Single host genes with discriminatory power to classify death groups from survived groups.

Receiver operator characteristics (ROC) curves showing the accuracies of individual genes in discriminating death groups versus survival groups. AUC = Area under the curve.

Figure 3

Figure 3

Gene expression profiles of signature genes.

Median gene expression levels (peak areas normalized to GAPDH and log2-transformed) of the indicated genes are shown as box-and-whisker plots (5-95 percentiles). Significant differences between study groups were determined using Wilcoxon Mann-Whitney test. Shown are individual genes that were found to have the best discriminatory power to distinguish between death groups versus survival groups. (* = P-value ≤ 0.05 , ** = P-value ≤ 0.01 , *** = P-value ≤ 0.001 , **** = P-value ≤ 0.0001).

Figure 4

	ptprcv1_	cd3e_0m	cc15_0m	il7r_0m	nod1_0m	il4d2_0m	gmly_0m
ptprcv1_	1.0000						
cd3e_0m	0.6205* 0.0007	1.0000					
cc15_0m	0.6137* 0.0009	0.7511* 0.0000	1.0000				
il7r_0m	0.7552* 0.0000	0.7326* 0.0000	0.4660* 0.0164	1.0000			
nod1_0m	0.4609* 0.0178	0.2899 0.1508	0.4550* 0.0195	0.2500 0.2180	1.0000		
il4d2_0m	-0.4391* 0.0248	-0.3850 0.0521	-0.2855 0.1575	-0.2627 0.1947	-0.4975* 0.0097	1.0000	
gmly_0m	0.6978* 0.0001	0.7224* 0.0000	0.7142* 0.0000	0.6656* 0.0002	0.3422 0.0870	-0.3260 0.1041	1.0000

Figure 4**The relationship among the expression of genes.**

Spearman analysis was done to look the relationship among the expression of genes. Upper number indicates the degree of relation and lower number indicates P-value. – sign indicates negative relation and * indicates had relation between the host gene expression.

Figure 5

Figure 5

ATT treatment response transcriptomic profiles.

Median gene expression levels (peak areas normalized to *GAPDH* and log2-transformed) and standard deviation are shown of the indicated genes at baseline (0M) and 6 months after treatment initiation (6M) and (18M) of study groups.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTable1.doc](#)