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Detection of urine lipoarabinomannan is associated with pro-inflammatory innate immune activation, impaired host defense, and organ dysfunction in adults with severe HIV-associated tuberculosis in Uganda

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Abstract

Background: The immunopathology of disseminated HIV-associated tuberculosis (HIV/TB), a leading cause of critical illness and death among persons-living-with-HIV (PLWH) in sub-Saharan Africa, is incompletely understood. Reflective of hematogenously disseminated TB, detection of lipoarabinomannan (LAM) in urine is associated with greater bacillary burden and poor outcomes in adults with HIV/TB.

Methods: We determined the relationship between detection of urine TB-LAM, organ dysfunction, and host immune responses in a prospective cohort of adults hospitalized with severe HIV/TB in Uganda. Generalized additive models (GAMs) were used to analyze the association between urine TB-LAM grade and concentrations of 14 soluble immune mediators. Whole-blood RNA-sequencing data was used to compare transcriptional profiles between patients with high- vs. low-grade TB-LAM results.

Results: Among 157 hospitalized PLWH, 40 (25.5%) had positive urine TB-LAM testing. Higher TB-LAM grade was associated with more severe physiologic derangement, organ dysfunction and shock. Adjusted GAMs showed that higher TB-LAM grade was significantly associated with higher concentrations of mediators reflecting pro-inflammatory innate- and T-cell activation and chemotaxis (IL-8, MIF, MIP-1β/CCL4, sIL-2Ra/sCD25). Transcriptionally, patients with higher TB-LAM grades demonstrated multifaceted impairment of antibacterial defense including reduced expression of genes encoding cytotoxic and autophagy-related proteins and impaired cross-talk between innate and cell-mediated immune effectors.

Conclusions: Our findings add to emerging data suggesting pathobiological relationships between LAM, TB dissemination, innate cell activation, and evasion of host immunity in severe HIV/TB. Further translational studies are needed to elucidate the role for immunomodulatory therapies, in addition to optimized anti-TB treatment, in this often critically-ill population.

Keywords

tuberculosis; lipoarabinomannan; HIV; biomarkers; Uganda

Introduction

Severe and frequently disseminated tuberculosis (TB) is a leading cause of critical illness and death among persons living with HIV (PLWH) in sub-Saharan Africa (SSA) [1,2]. Despite this, the immunopathological mechanisms that underlie disseminated HIV-associated TB (HIV/TB) are imprecisely understood [3,4].

Lipoarabinomannan (LAM) is a glycolipid component of the mycobacterial cell wall that may promote survival of *Mycobacterium tuberculosis* (MTB) though immunosuppressive modulation of host defense [5]. Reflective of hematogenously disseminated TB, quantitative detection of LAM in urine is associated with greater bacillary burden in adults with HIV/TB [6–8]. The Abbott/Alere Determine TB-LAM test, recommended by the World Health Organization (WHO) as a rapid TB diagnostic for severely ill PLWH with suspected TB, is a semi-quantitative lateral-flow urine LAM assay. In the TB-LAM platform, higher LAM concentrations are reflected by darker (i.e., higher) band grades, increases of which are also

associated with greater bacillary burden and poor clinical outcomes in adults with HIV/TB [9–12].

Here, we determined the relationship between urine detection of TB-LAM, host immune responses, and organ dysfunction in a prospective cohort of adults hospitalized with severe HIV/TB in Uganda. We hypothesized that as a putative marker of MTB dissemination and bacillary burden, higher TB-LAM grade would be associated with dysregulated pro-inflammatory innate immune activation and more severe organ dysfunction.

Methods

We analyzed clinical and biological data from a subset of PLWH with microbiological evidence of TB enrolled in a prospective cohort (RESERVE-U) of adults hospitalized with undifferentiated severe infection (i.e., suspected sepsis) in Entebbe, Uganda from April 12th, 2017 to August 28th, 2019 [13,14]. Patients were included in the parent RESERVE-U study if they fulfilled the following criteria: (1) age ≥18 years, (2) reported a history of fever or had a recorded axillary temperature of ≥37.5°C, (3) had clinical illness severe enough to warrant admission to hospital, and (4) were able to provide informed consent or had a surrogate available to do so [13,14]. Patients were excluded if they presented following trauma or were admitted to a non-medical ward. During the study period, all admissions to the medical wards were screened for eligibility by study staff. Patients were screened on weekdays during daytime hours and were enrolled as close to admission as possible and no longer than 24 hours afterwards.

All enrolled participants in the RESERVE-U study underwent rapid testing for HIV; for PLWH, urine and spontaneously expectorated sputum samples, if obtainable, were tested for MTB using the Determine TB-LAM (Abbott/Alere, Lake Bluff, IL, USA) and Xpert MTB/RIF Ultra (Cepheid, Sunnyvale, CA, USA) platforms, respectively. For TB-LAM, the intensity of any visible band on the test strip was examined by two study laboratory technicians independently; discordant findings were reviewed by a third laboratory technician whose interpretation was recorded as the accepted result. Results were graded (1–4) using band intensities on the manufacturer's post-2014 reference card scale; results were considered positive using the grade 1 cutoff.

In serum samples collected at RESERVE-U enrollment, we quantitated, using Luminex immunoassays (Luminex, Austin, TX, USA), 14 soluble immune mediators reflective of immunopathological domains that may contribute to infection-related organ dysfunction, including among patients with severe HIV/TB [3,13,15–18]. These included: innate/adaptive immune activation (IL-6, IL-8, IL-10, IFN-γ, IP-10/CXCL10, MIP-1α/CCL3, MIP-1β/CCL4, TNF-α, MIF, sTNFR1, sIL-2RA/sCD25), endothelial dysfunction (angiopoietin [Ang]-1, Ang-2), and fibrinolysis (PAI-1). From whole-blood samples collected in PAXgene blood RNA tubes from a consecutive subset of patients, RNA was isolated and purified using PAXgene blood RNA kits (Qiagen, Hilden, Germany), after which RNA sequencing libraries were prepared using the NEBNext Ultra RNA Library Prep Kit (NEB, Ipswich, MA, USA). Sequencing libraries were multiplexed and analyzed using a 2×150 paired-end

configuration on the Illumina HiSeq 4000 platform (Illumina, Inc., San Diego, CA, USA) [13].

As we did not assume a linear association between TB-LAM grade (analyzed as an ordinal independent variable) and $\log_{10}$-transformed immune mediator concentrations (analyzed as a continuous dependent variable), we created generalized additive models (GAMs) using the *mgcv* R-package. PLWH with pulmonary TB (sputum Xpert Ultra positive) and negative TB-LAM testing were included as a "grade zero" control group. Models were estimated by applying a second-order ordinal smoothing penalty to TB-LAM grade (*ordPens* R-package), with optimal smoothing parameters selected via restricted maximum likelihood. All models were adjusted for age and sex, as well as use of antiretroviral therapy (ART) and trimethoprim-sulfamethoxazole prophylaxis where indicated. We also explored the relationship between TB-LAM grade, clinical severity, and immune mediator concentrations using unsupervised methods, including correlation matrices, force-directed correlation networks, and principal components analysis (PCA).

For transcriptional analyses, we performed differential gene expression using the *DESeq2* R-package, comparing patients with high-grade LAM positivity (grades 3 or 4) to those with low-grade positivity (grades 1 or 2) [19]. To infer relative abundance of immune cell subsets between patients with high- vs. low-grade LAM positivity, we performed digital cytometry deconvolution using the CIBERSORTx platform and LM22 hematopoietic gene signature reference matrix [20].

Each participant or their surrogate provided written informed consent. Study protocols were approved by ethics committees at Columbia University (AAAR1450), Uganda Virus Research Institute (GC/127/17/02–06/582), and Uganda National Council for Science and Technology (HS2308). RNA-sequencing data are available in the NIH/NCBI Sequence Read Archive (PRJNA794277).

Results

Among 301 adults enrolled in the parent RESERVE-U cohort, 52.2% (157/301) were PLWH, of whom 29.9% (47/157) were included in our analyses: 40 with positive urine TB-LAM including a documented band grade and 7 with positive sputum Xpert MTB/RIF Ultra testing and negative TB-LAM results (Figure S1 and Tables S1–S2 in Supplement). Of these 47 patients, median age was 32 years (IQR 27–40) and 55.3% (26/47) were male. All 47 patients had World Health Organization (WHO) stage 3 or 4 disease, 61.7% (29/47) were receiving anti-retroviral therapy (ART), and 40.4% (19/47) were being treated for active TB prior to admission. A substantial proportion of patients had evidence of organ dysfunction, with 25.5% (12/47), 31.9% (15/47), and 25.5% (12/47) meeting criteria for shock, acute respiratory failure, or reduced consciousness, respectively (Table S1 in Supplement). At 30-days after hospital discharge, 40.4% (19/45) of patients had died.

Clinically, unsupervised analyses suggested relationships between TB-LAM grade, physiologic severity, and organ dysfunction. Correlation matrices and force-directed network analysis revealed positive correlations between TB-LAM grade, physiologic severity scores

(quick Sepsis-related Organ Failure Assessment, Universal Vital Assessment, and Modified Early Warning Scores), shock, and reduced consciousness (Figure 1A and Figure S2 in Supplement). When TB-LAM grade and markers of clinical severity were mapped using PCA, a variable correlation axis across the first two principal components was apparent, defined by higher TB-LAM grade, lower systolic blood pressure and oxygen saturation, and shock (Figure 1B).

Of the 47 patients included in our clinical analyses, 46 had serum samples available for immune mediator analyses. Correlation matrices and force-directed network analysis identified positive correlations between TB-LAM grade and concentrations of most mediators (Figure S3A–S3B in Supplement). Negative correlations were observed between TB-LAM grade and MIP-1α/CCL3 and Ang-1, the latter of which promotes endothelial stabilization and has anti-inflammatory properties. Adjusted GAMs showed that higher TB-LAM grade was significantly (smoothing term p-value ≤0.05) and linearly (effective degrees-of-freedom=1) associated with higher concentrations of key pro-inflammatory innate immune mediators, including those related to neutrophil and macrophage activation and chemotaxis (IL-8, MIF, MIP-1β/CCL4) (Figure 1C–E). Higher TB-LAM grade was also significantly and linearly associated with higher concentrations of sIL-2Ra/sCD25, a T-cell activation marker that, in the context of persistent antigenic stimulation, may act as an immune checkpoint protein through consumption of circulating IL-2 (Figure 1F) [21,22]. Similar but non-significant associations were observed for TNF-α, IP-10/CXCL10, sTNFR1, IFN-γ, IL-6, and IL-10 (Figure S4 in Supplement). Results were generally consistent when ART-experienced patients and those with active TB on treatment prior to admission were excluded (Figures S5–S6 in Supplement).

Transcriptionally, 194 genes were differentially expressed between patients with high- vs. low-grade TB-LAM results. Patients with higher TB-LAM grade showed increased expression of genes encoding pathogen recognition receptors (PRRs; including C-type lectins) and calprotectin, with higher inferred quantities of neutrophils and resting (M0) macrophages (Figure 2A–2D). Concomitantly, patients with higher TB-LAM grade showed decreased expression of genes related to T- and B-cell receptor stimulation and signaling, with lower inferred quantities of B-cells, plasma cells, CD4+ and CD8+ T-cells, and activated dendritic cells (Figure 2A–2D). In addition, these patients had decreased expression of the genes encoding key cytotoxic effector molecules essential for anti-bacterial cytolysis (granulysin, granzyme M, macrophage perforin) and antigen processing (MHC class II molecules), as well as those essential to ubiquitination and DNA damage response, processes related to anti-bacterial autophagy (Figure 2A–2C) [23]. Similarly, patients with high TB-LAM grades showed increased expression of arginase-1, which may impair nitric oxide-dependent anti-bacterial killing and modulate anti-mycobacterial T-cell immunity [24,25]. Relevant to mitophagy, patients with high TB-LAM grades also demonstrated increased expression of genes reflecting oxidative stress-related mitochondrial dysfunction, altered NAD+/NADH metabolism and glycolytic reprogramming, and cholesterol transport.

Discussion

In a prospective cohort of adults hospitalized with severe HIV/TB in Uganda, we show that semi-quantitative detection of TB-LAM in urine is associated with pro-inflammatory innate immune activation, impaired antibacterial host defense, and severe organ dysfunction. Our results add to data suggesting pathobiological relationships between LAM, MTB dissemination, innate cell activation, and host immunity in patients with severe HIV/TB co-infection, and highlight the potential role for immunomodulatory therapies, in addition to optimized anti-TB treatment, in this often critically ill population [3,17,23,26,27].

Consistent with *in vitro* studies, our results suggest that following recognition by PRRs, LAM may induce a pro-inflammatory response driven by mediators of neutrophil and macrophage activation and chemotaxis [5]. Concomitantly, we observed evidence of multifaceted impairment of antibacterial host defense in patients with higher TB-LAM grades. This included reduced expression of genes encoding cytotoxic and autophagy-related proteins and impaired cross-talk between innate and cell-mediated immune effectors, with lower MHC class II and T-cell receptor gene expression and fewer activated dendritic cells (DCs) and CD4+ and CD8+ T-cells. These observations are consistent with those from *in vitro* and murine studies suggesting that LAM plays a key role in MTB evasion of host immunity through manipulation of innate and adaptive pathways integral to intracellular pathogen clearance [5,23,27]. For example, *in vitro* experiments suggest that engagement of mannose capped-LAM by DC C-type lectins may inhibit DC maturation (thereby blunting T-cell activation), with infection of migratory DCs potentially contributing to hematogenous and lymphatogenous bacterial dissemination [23,27–29]. LAM-related immune evasion may be particularly relevant in the context of HIV-coinfection, in which LAM-specific antibody titers, which have been associated with MTB dissemination risk, are reduced [30–32]. Future studies are needed to more precisely elucidate the role of LAM in the pathobiology of severe and disseminated HIV/TB, including the preventive and therapeutic potential of LAM-directed agents (e.g., monoclonal antibodies, conjugate vaccines) and treatment strategies targeting mechanisms of LAM-related immune evasion [26].

Considering TB-LAM grade as a putative marker of MTB dissemination and bacillary burden, our observations are consistent with clinical studies from South Africa, in which more extensive MTB dissemination was associated with exaggerated pro-inflammatory innate immune activation [3,17,18]. While qualitative TB-LAM testing improves TB treatment initiation and mortality among severely ill PLWH with suspected TB in SSA, a semi-quantitative approach to testing, if validated further, could stratify this population into more homogenous subgroups that may benefit from differential MTB-directed (e.g., dose-optimized anti-TB agents) and immunomodulatory (e.g., therapeutics targeting pro-inflammatory host responses) treatment strategies [33,34].

Our study has limitations. First, our findings are derived from a single center. Second, given our limited sample size, including among the TB-LAM-negative control group, some of our GAMs may have been underpowered. Third, considering a pooled specificity of 87% (95% credible interval 78–93%) for Determine TB-LAM among hospitalized PLWH, we cannot exclude the possibility of false positive results. Fourth, while soluble mediators were

included based on immunopathologic relevance, there may be other protein biomarkers that better reflect the pathobiology of severe HIV/TB. Lastly, we were unable to perform mycobacterial cultures or quantify viral loads or CD4 counts due to study site resource limitations, and cannot precisely exclude the contribution of uncontrolled HIV viremia to our findings.

In summary, our data suggest that urine TB-LAM grade is associated with pro-inflammatory innate immune activation, impaired antibacterial host defense, and severe organ dysfunction in adults with severe HIV/TB in Uganda. Future studies are needed to further elucidate the immunopathology of severe and disseminated HIV/TB in high-burden settings, with the goal of developing effective pathogen- and host-directed treatment strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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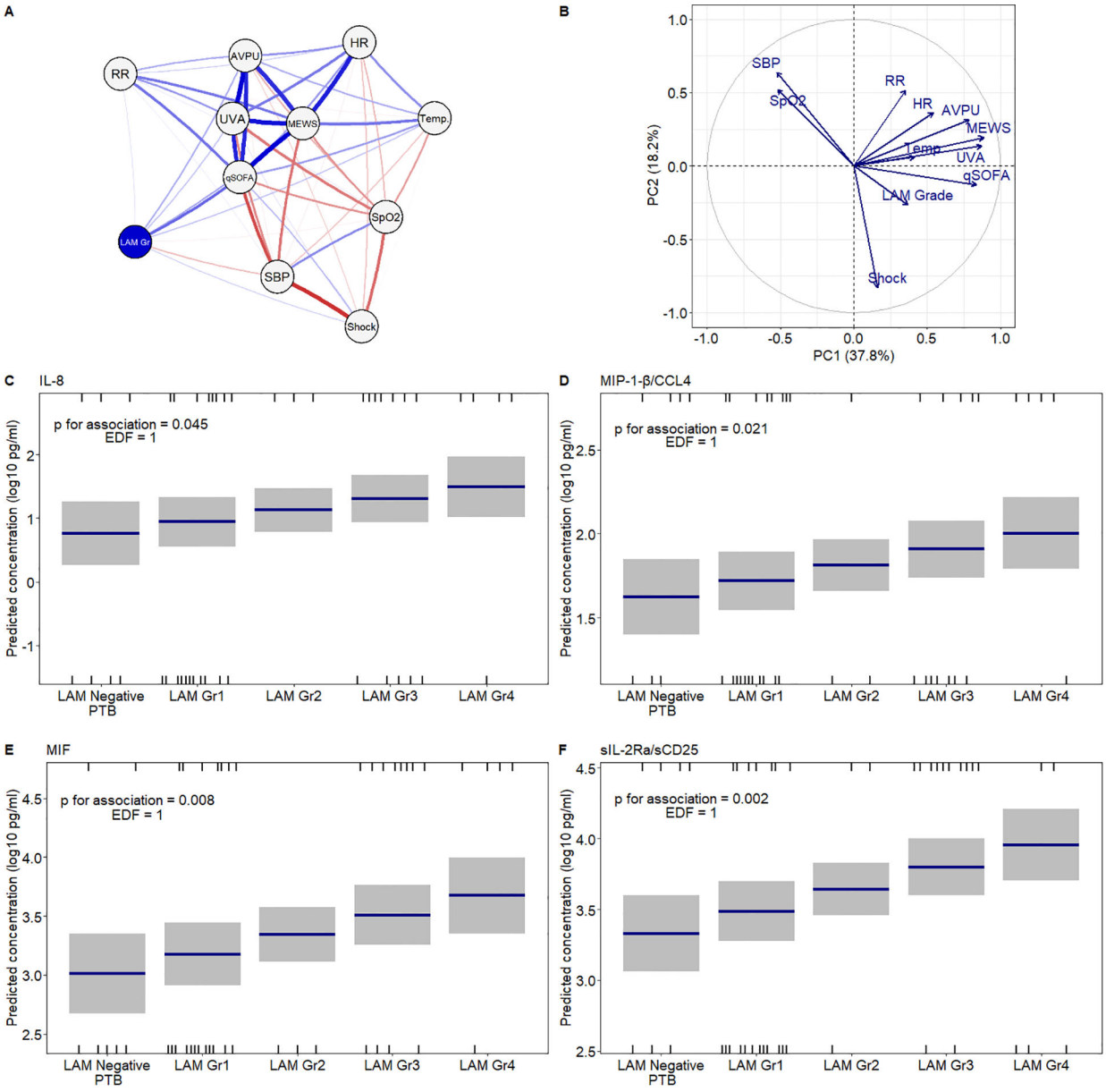

Figure 1: Relationships between urine TB-LAM grade, clinical severity, and soluble immune mediators.

(A) Force-directed correlation network showing relationships between clinical severity variables and TB-LAM grade; network structured on weighted correlations with each variable set as a network node and between-variable correlations indicated by weighted edges (blue edges indicate positive correlation, red edges indicate negative correlation, edge width indicates strength of correlation based on Spearman correlation coefficient) (N=47). **(B)** Principal components variable correlation plot showing relationships between clinical severity variables and TB-LAM grade (N=47); positively correlated variables are located in the same quadrant while negatively correlated variables are in opposite quadrants (e.g., LAM grade and systolic blood pressure [SBP] and peripheral oxygen saturation [SPO2] are located in opposite quadrants, suggesting positive correlations between higher LAM

grade and lower SBP and SPO2; AVPU: alert, responsive to voice, responsive to pain, unresponsive mental status assessment (coded 1–4, respectively), LAM Gr: TB-LAM grade (0–4; grade 0 reflects sputum Xpert MTB/RIF positive, TB-LAM negative group); MEWS: Modified Early Warning Score, qSOFA: quick-Sepsis Related Organ Failure Assessment, UVA: Universal Vital Assessment; shock defined as systolic blood pressure ≤90 mmHg despite administration of ≥1 liter of intravenous fluid, RR: respiratory rate, HR: heart rate. **(C-F)** Associations between TB-LAM grade and soluble immune mediator concentrations; predicted concentrations generated from generalized additive models adjusted for age, sex, and use of anti-retroviral therapy and trimethoprim-sulfamethoxazole prophylaxis (N=46); rug marks on top and bottom of plot indicate observations with positive and negative residuals, respectively.

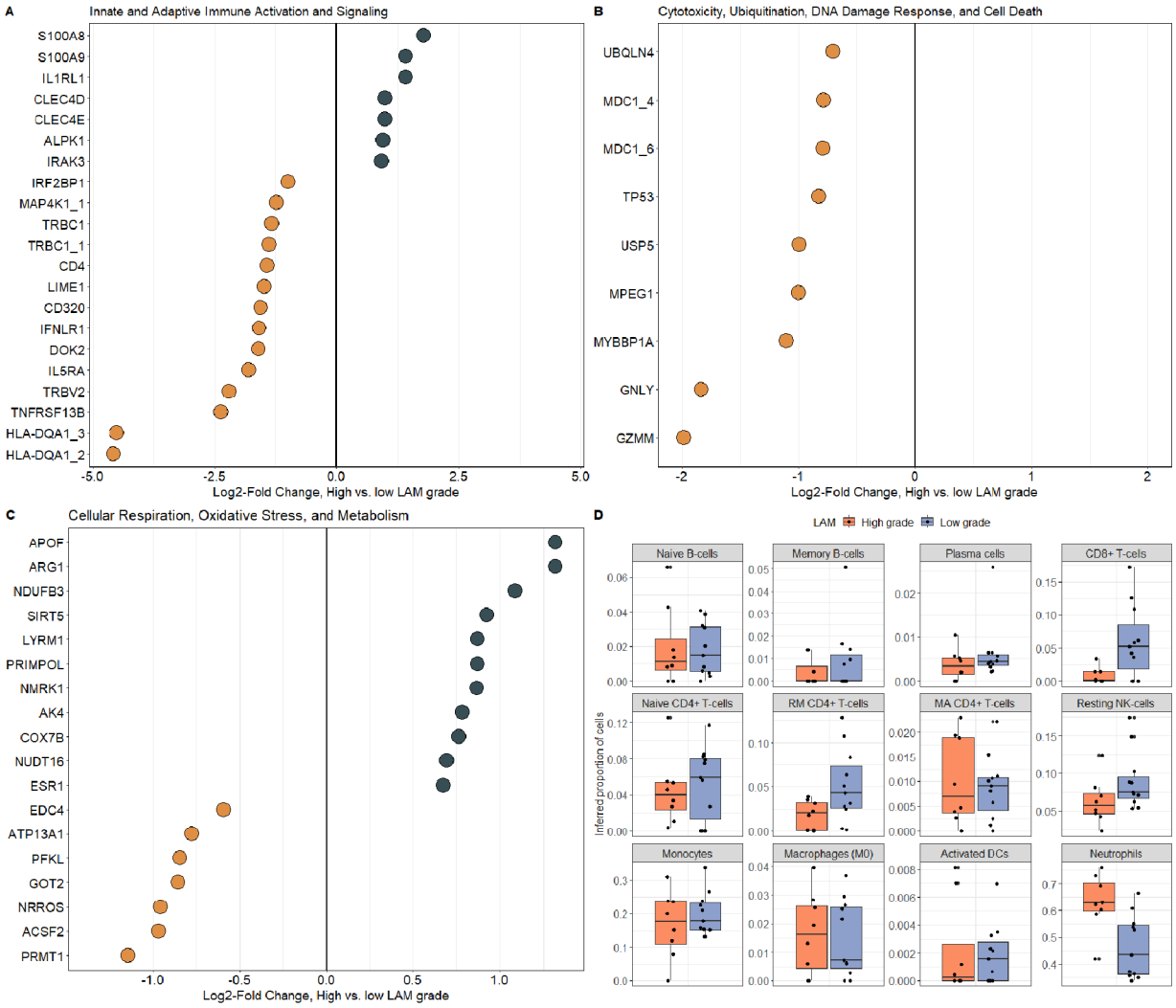

Figure 2: Key differentially expressed genes and inferred immune cell abundance between patients with high-grade vs. low-grade TB-LAM results.

(A-C) Differentially expressed genes between groups defined by $\log_2$-fold change ≥|0.26| and Benjamini-Hochberg-adjusted p-value ≤0.05; high-grade TB-LAM result defined as grade 3 or 4 (N=8), low-grade result defined as grade 1 or 2 (N=11). (**B**) Inferred abundance of key immune cell subsets between patients with high-grade (N=8) and low-grade TB-LAM results (N=11); relative immune cell fractions inferred using LM22 hematopoietic gene signature reference matrix in CIBERSORTx platform [19]; RM: resting memory, MA: memory activated; DC: dendritic cells.