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Immunological profiling of tuberculosis-associated immune reconstitution inflammatory syndrome and non-immune reconstitution inflammatory syndrome death in HIV-infected adults with pulmonary tuberculosis starting antiretroviral therapy: a prospective observational cohort study

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Conceived and designed the study and experiments: GPB DW SR RG. Performed the experiments: SR KN. Analyzed the data: SR GPB SLB. Contributed reagents/materials/analysis tools: GPB DW SLB. Wrote the first draft of the manuscript: SR GPB. Contributed to the writing of the manuscript: GPB SR DW RG RRM SLB NT RL APS. Agree with manuscript results and conclusions: GPB SR DW RG RRM SLB NT RL APS KN. Enrolled patients: NT RL. Facilitated study in Botswana: NT APS.

Conflict of Interest

All authors declare no conflict of interest.

Summary

Background—Patients co-infected with advanced HIV and tuberculosis are at risk of tuberculosis-associated immune reconstitution inflammatory syndrome (IRIS) and death soon after initiation of antiretroviral therapy (ART). Tuberculosis-associated IRIS has been associated with quicker recovery of cellular immune responses after ART initiation and early mortality with slower recovery of these responses. We aimed to assess whether patients who have these outcomes have distinct immunological profiles before and after ART initiation.

Methods—We undertook this prospective cohort study at 22 public clinics and the main public hospital in Gaborone, Botswana, in ART-naive adults (aged ≥21 years) with advanced HIV (CD4 cell counts ≤125 cells per μL) and pulmonary tuberculosis. We obtained data for clinical variables and for levels of 29 plasma biomarkers, quantified by Luminex assay. We classified patients as having tuberculosis-associated IRIS, early mortality, or survival without a diagnosis of tuberculosis-associated IRIS (controls), on the basis of outcomes recorded in the 6 months after ART initiation. We used rank-sum or χ^2 tests, and logistic regression with odds ratios (OR) and 95% CIs, to assess the association between variables measured before and 4 weeks after ART initiation with death and tuberculosis-associated IRIS, compared with controls.

Findings—Between Nov 12, 2009, and July 3, 2013, we enrolled 201 participants. 31 (15%) patients left the study before ART initiation, leaving 170 (85%) patients for analysis. Patients with tuberculosis-associated IRIS had reduced pre-ART concentrations of several pro-inflammatory biomarkers, including interleukin (IL)-6 (adjusted OR per 1 log10 increase 0·40 [95% CI 0·18–0·89]). However, patients with early death had increased pre-ART concentrations of inflammatory biomarkers, including monocyte chemoattractant protein-1 (adjusted OR 9·0 [95% CI 1·0–80·0]) and tumour necrosis factor (TNF)-α (7·8 [1·1–55·2]). At week 4 after ART initiation, tuberculosis-associated IRIS was independently associated with greater increases in several inflammatory biomarkers, including IL-6 (adjusted OR 1·7 [95% CI 1·2–2·5]) and TNF-α (1·5 [1·0–2·2]), versus controls. Death was likewise associated with greater increases in systemic inflammatory markers, including granulocyte colony-stimulating factor (adjusted OR 2·8 [95% CI 1·3–6·1]), IL-12p40 (1·8 [1·0–3·4]), and IL-15 (2·0 [1·1–3·7]), versus controls. However, changes in CD4 cell count during ART, which were similar between controls and patients with tuberculosis-associated IRIS (p=0·45), were substantially lower in patients who died (p=0·006).

Interpretation—Distinct immunologic profiles pre- and post-ART initiation characterize advanced HIV/TB patients who experience TB-IRIS and death. Interventions that decrease inflammation while promoting cellular immune recovery on ART among HIV/TB co-infected patients should be considered.

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Introduction

In 2013, HIV-infected patients accounted for 13% of 9·0 million TB cases and 24% of 1·5 million TB-associated deaths worldwide.[1] Initiating antiretroviral therapy (ART) during TB treatment can decrease mortality in HIV-infected individuals.[2] The need for ART is particularly urgent in advanced HIV, as patients with the lowest CD4 T cell counts have improved survival when ART is initiated within the first few weeks of anti-tubercular

therapy.[3-5] Nonetheless, patients with advanced HIV/TB face a persistently high risk of death despite initiating therapy for both diseases. For example, 120 (18%) of 661 patients and 55 (7%) of 783 patients in two trials of ART timing in patients with HIV and tuberculosis died within 48 weeks despite starting both ART and anti-tubercular therapy (M Kendall, Harvard School of Public Health, personal communications).[3,5]

Early ART initiation after TB treatment is also associated with an increased risk of paradoxical TB-immune reconstitution inflammatory syndrome (IRIS)[3-7], characterized by pathologic inflammation after ART initiation in patients concurrently treated for TB.[8-13] While mortality from TB-IRIS is low[14], morbidity can be substantial.[7,14,15] Studies of outcomes in HIV/TB have frequently focused on TB-IRIS while excluding early deaths; as a result, mechanisms of early mortality on ART in HIV/TB are largely unknown. However, failure to recover CD4 counts despite virologic suppression was recently associated with early death after ART initiation in adults with advanced HIV/TB.[16] This is notable as the failed cellular immune recovery seen in early mortality[16] contrasts with the more rapid cellular immune responses often seen in TB-IRIS[8,12,13,17], suggesting that the underlying immunopathogenesis for TB-IRIS and early mortality differ. Understanding risk factors for these conditions is important, because interventions designed to prevent TB-IRIS could increase early mortality if immunologic processes, such as rapid immune recovery, driving TB-IRIS are associated with survival. Given these differences, we hypothesized that patients who later go on to develop TB-IRIS would differ from those who die early after ART initiation with respect to immune activation and immune recovery prior to and early after ART initiation.

We investigated the relation of clinical variables and immunological characteristics before and 4 weeks after ART initiation to risk of non-traumatic death (early mortality) and paradoxical tuberculosis-associated IRIS in adults with advanced HIV and tuberculosis.

Methods

Study Design

We did a prospective cohort study at 22 public clinics and Princess Marina Hospital (the main public hospital) in Gaborone, Botswana, with previously described methods.[16] Eligible patients (aged ≥21 years) were HIV-infected, ART-naive citizens of Botswana with pre-ART CD4 cell counts of 125 cells per μL or less, a new diagnosis of pulmonary tuberculosis, and plans to initiate ART within 2 months of starting standard anti-tuberculosis therapy.[18] Diagnosis of pulmonary tuberculosis required a sputum smear positive for acid-fast bacilli, a positive GeneXpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA), or meeting WHO criteria for smear-negative pulmonary tuberculosis.[18] We included patients with extrapulmonary involvement. We excluded patients who were pregnant, taking steroids or other immune modulators, or had drug-resistant tuberculosis or close contact with drug-resistant tuberculosis at screening. The study sample included all available participants with data and specimens for analysis. For the baseline analysis, we included all enrolled patients who started ART. For the analysis of early responses, we included patients who started ART and had baseline and week-4 plasma available for biomarker analysis.

The institutional review boards of the University of Pennsylvania, Botswana Ministry of Health, and the Princess Marina Hospital approved this study. All patients provided written informed consent.

Data Collection

Clinical data including medical history and non-TB opportunistic illnesses (OIs) were collected at baseline and monthly thereafter.[16] Baseline CD4 counts were obtained from medical records or determined independently at an accredited laboratory in Gaborone. Blood was collected at baseline and week 4 of ART for HIV viral load (NucliSENS Easy Q HIV-1, BioMérieux), plasma, and peripheral blood mononuclear cells (PBMCs). Patients were actively traced by phone or by home visit if they did not return for follow-up, and were deemed lost to follow-up if their vital status was unknown at the 6-month follow-up visit. We defined paradoxical tuberculosis-associated IRIS with a modified version of the International Network for Study of HIV-associated IRIS (INSHI) case definition, by which we adjudicated patients as probable if they met INSHI criteria, or as suspected if criteria for probable disease developed after 3 months of ART or patients developed otherwise unexplained new or worsening respiratory symptoms anytime during the 6 months of follow-up.[19] Furthermore, to assess the strength of our diagnoses of tuberculosis associated IRIS, we retrospectively assessed all INSHI defined cases with the AIDS Clinical Trials Group IRIS definition used by Grant and colleagues (appendix).[20] We used medical records or information from patients' families to assess possible cause of death.

Procedures

A 29-cytokine/chemokine/growth factor magnetic bead Luminex panel (EMD Millipore, Billerica, Massachusetts) was used to measure epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), interferon (IFN)-α, IFN-γ, interleukin (IL)-1RA, IL-1α, IL-1β, IL-2, IL-3, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p40, IL-12p70, IL-15, IL17a, IFN-γ -induced protein(IP)-10, monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1α, MIP-1β, eotaxin, and tumour necrosis factor (TNF)-α in plasma pre-and 4 weeks post-ART initiation. IL-13, IL-4, and TNF-β were below the limit of detection and excluded. Undiluted plasma, previously stored at −80°C, was tested in duplicate, per the manufacturer's protocol on the Bio-Plex2000 Luminex platform (Bio-Rad, Hercules, California). Data were analyzed using a 5-point log-log standard curve with the Bio-Plex Manager software (Bio-Rad, Hercules, California).

We undertook enzyme-linked immunosorbent spot assays of freshly isolated peripheral blood mononuclear cells, as per the manufacturer's protocol [16] (BD Bioscience, San Jose, CA, USA). IFNγ spots in response to 5 μg/mL purified protein derivative (Statens Serum Institute, Copenhagen, Denmark), ionomycin (500 ng/mL) and phorbol 12-myristate 13-acetate (50 ng/mL; Sigma-Aldrich, St Louis, MO, USA), or RPMI 1640 medium containing 10% fetal bovine serum (Lonza, Basel, Switzerland), 1% L-glutamine, and penicillin–streptomycin (Gibco, Life Technologies, Grand Island NY, USA) as a negative control, were enumerated with an ImmunoSpot plate reader (Cellular Technology, Shaker Heights, OH, USA).

Statistical Analyses

Patients who experienced TB-IRIS or early mortality were compared separately to patients who survived without a diagnosis of TB-IRIS (controls) during follow-up. If a patient diagnosed with TB-IRIS died, they were counted as a case of TB-IRIS in the primary analysis because TB-IRIS was the proximal event that may have led to death; such patients were later reclassified as deaths in sensitivity analysis. Continuous variables were summarized using medians and interquartile ranges (IQR) or by means and ranges, depending on distributions. In unadjusted analyses, continuous and categorical variables were evaluated using the Wilcoxon rank sum or the chi square test. In order to assess the distribution of the strength and direction of associations between specific biomarker levels and outcomes, our biomarker analysis emphasized odds ratios (ORs) with 95% confidence intervals (CIs). Specifically, pre-ART plasma biomarker levels were $\log_{10}$ transformed and analyzed using logistic regression to produce ORs relating 1 $\log_{10}$ higher values with each outcome. For early response analyses, where both increases and decreases were possible, ORs were calculated using quartiles of change. In addition, raw biomarker values are presented to facilitate interpretation. In primary analyses p values are unadjusted for multiple comparisons but univariate biomarker comparisons include p values adjusted using the Benjamini-Hochberg method.[21] Tests were two-sided and p values <0·05 were considered statistically significant.

In adjusted analyses, clinical variables that were associated with either outcome at p≤0·20 were considered potential confounders. Factors that changed the unadjusted association by ≥10% were considered actual confounders and were included in multivariate logistic regression models. In this setting, use of nevirapine (NVP)-based ART was reserved primarily for women. Given this colinearity and that there were more women than NVP users, when both sex and NVP were associated with outcome, we included only sex in the models. Unadjusted sensitivity analyses were conducted after inclusion of all those without TB-IRIS (including the deaths) as controls in the analysis of risk factors for TB-IRIS, and after inclusion of all those who survived (including those with TB-IRIS) as controls in the analysis of risk factors for death. In addition, because biomarker levels on ART were collected at the week-4 visit, we evaluated IL-6 values among patients with “early” (diagnosed at week 4) versus “late” (diagnosed after week 4) TB-IRIS in order to determine if pooling these patients had an affect on the results. Analyses used Stata, 11.0 (College Station, Texas).

Role of Funding Source

The sponsors of the study had no role in study design; data collection, analysis, or interpretation; writing of the manuscript; or the decision to submit the paper for publication. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Cohort Characteristics and Outcomes

A total of 201 subjects were enrolled from November 2009 to July 2013. Prior to ART initiation, 31 (15%) of the 201 patients left the study: 3 withdrew, 10 transferred out, 5 died, and 13 were terminated from the study due to failure to follow study procedures, leaving 170 (85%) for analysis. The median CD4 cell count of included participants was 60 cells per μL (IQR 31–90), nearly three quarters of patients had smears positive for acid-fast bacilli or GeneXpert assays positive for pulmonary tuberculosis, and the median time to ART initiation after tuberculosis diagnosis was roughly 28 days (IQR 20–47; appendix). Thirty-three of 170 (19%) patients experienced paradoxical TB-IRIS following ART initiation; 8 of 33 (24%) were probable and 25 of 33 (76%) were suspected (appendix). All probable and suspected TB-IRIS cases also met the ACTG case definition.[20] Eighteen of 170 patients (11%) died, leaving 120 controls. The median time to tuberculosis associated IRIS onset was about 28 days (IQR 28–56) and to death was about 81 days (IQR 43–93). One (1%) patient was lost to follow-up at week 16. One patient who died had dyspnea and paradoxical TB-IRIS at 4 weeks post-ART initiation, but the IRIS symptoms resolved prior to death, which occurred 2 weeks later. This patient was categorized as an IRIS case and not a death in primary analysis. Clinical details of TB-IRIS and deaths are presented in the Appendix. One (3%) patient with TB-IRIS and 2 (2%) control patients did not have plasma available for analysis, leaving 32 TB-IRIS patients and 118 controls for baseline analyses.

At baseline, TB-IRIS patients were similar to controls with respect to clinical characteristics, including CD4 count and time to ART initiation, except that they had a significantly higher median body mass index (BMI;Table 1).

Figure 1A shows the unadjusted ORs relating TB-IRIS to $\log_{10}$ higher pre-ART biomarker levels relative to controls. Of the 26 biomarkers assessed, 19 (73%) had point estimates for the unadjusted ORs that were lower than 1·0, and 7 of 26 (27%) also had upper limits of the 95% CI that did not cross 1·0, suggesting that lower levels of these 7 biomarkers are associated with an increased risk of TB-IRIS. These biomarkers included plasma concentrations of factors secreted mainly by monocytes or macrophages and endothelial cells such as GM-CSF; soluble markers that are produced by or promote T-helper-1 responses, including IL-2, IL-15, IL-12p40, and IL-12p70; growth factors such as IL-3; and the pro-inflammatory cytokine IL-17a (Figure 1). Associations between these 7 biomarkers and TB-IRIS were essentially unchanged in models adjusting for baseline BMI and NVP (Table 2). Pre-ART concentrations of IL-6 were not associated with tuberculosis-associated IRIS in univariate analysis (Figure 1), but became significantly associated in adjusted analysis (Table 2). After adjustment for multiple comparisons, circulating levels of GM-CSF, IL-3, IL-12p40, IL-12p70, and IL-17a were all significantly lower in TB-IRIS cases (Table 3). Secondary analysis comparing TB-IRIS patients to all those without TB-IRIS produced similar results (appendix). Pre-ART levels of biomarkers were similar among early and late TB-IRIS cases, and pooling of these patients did not change these associations (data not shown).

Prior to ART, patients who died were more likely than controls to be female, to initiate NVP-based ART, to have lower CD4 counts, and to have a non-TB OI. The two groups were similar in terms of time to ART initiation (Table 1).

Figure 1B shows the unadjusted ORs relating death to $\log_{10}$ higher pre-ART levels relative to controls. Of the 26 biomarkers assessed, 25 (96%) had point estimates for the unadjusted ORs that were higher than 1·0, and 5 of 26 (19%) also had lower limits of the 95% CI that did not cross 1·0, suggesting that higher pre-ART levels of these 5 biomarkers are associated with an increased risk of death. These included plasma levels of IL-10, MCP-1, TNF-α, IL-6, and eotaxin (Figure 1B). Elevated pre-ART MCP-1 and TNF-α levels remained significantly associated with death after evaluation of female sex, non-TB OIs, and CD4 count as possible confounders (Table 2). Adjustment for multiple comparisons resulted in non-significant associations between death and MCP-1 and TNF-α (Table 3). Secondary analyses comparing deaths to survivors gave similar results (appendix).

Between baseline and week 4 of ART, 7 (4%) of 170 patients died and 4 (2%) patients did not have plasma collected at week 4 for biomarker analysis. Pre-ART clinical characteristics of the 159 remaining patients were similar to the 170 included overall (data not shown).

Relative to controls, patients who experienced TB-IRIS had similar increases in CD4 cell count and purified protein derivative-specific immune responses, whereas those who died had minimal immune recovery despite virological control during ART (appendix). Each quartile increase in CD4 cell count from baseline to week 4 of ART was associated with an approximate 60% reduction in the odds of death (Table 4). TB-IRIS was associated with significantly greater increases in G-CSF, IL-6, IL-8, IL-17a, and TNF-α compared to controls at week 4 of ART (Figure 2 and appendix). Although GM-CSF decreased in both TB-IRIS patients and in controls, the decrease in controls was greater than in TB-IRIS patients (Figure 2 and appendix). P values for these biomarkers increased somewhat after adjustment for multiple comparisons (appendix). In adjusted analyses, increases in IL-6, TNF-α, IL-8, and G-CSF remained independently associated with TB-IRIS (Table 4). Additionally, increases in IL-6 levels in the 17 (53%) of 32 patients with early TB-IRIS (median 14·5pg/ml [IQR:-0·40-25·1]) were greater than the increases in the 15 (47%) patients with late TB-IRIS (median −2·5pg/ml [IQR: −5·0-5·6] (p=0·01). The increase in IL-6 concentration, from baseline to week 4 post-ART initiation, in the early TB-IRIS patients was also significantly greater than in controls (median −3·0 pg/ml [IQR: −10·8-8·3], p=0·0006 and Benjamini-Hochberg corrected p=0·02; adjusted OR: 2·7 [CI: 1·5-4·9]).

Biomarkers also increased relative to controls in those who died (Figure 2 and appendix). P values for associations between increases in biomarkers and death and TB-IRIS increased slightly to after adjustment for multiple comparisons (appendix). In a logistic regression model, increases in G-CSF, IL-3, IL-12p40, IL-15, and IL-1RA from baseline were significantly and independently associated with death (Table 4).

Sensitivity analyses reclassifying the patient who died after diagnosis of TB-IRIS did not meaningfully change the results (appendix). Reclassification of one lost to follow-up patient as having died also had minimal effect (data not shown).

Discussion

In this prospective cohort study from Botswana, substantial differences in pre-ART biomarker levels were observed in individuals who subsequently experienced early mortality or paradoxical TB-IRIS, compared to patients with uncomplicated immune recovery. Furthermore, we found that both TB-IRIS and early mortality patients experience rapid increases in immune activation and inflammation early on ART, but differed markedly in terms of the magnitude of early cellular immune recovery.

While numerous studies have evaluated biomarker levels in patients with TB-IRIS[8-12,22-24], to our knowledge, no previous reports have compared cellular immune responses and biomarker profiles among patients who died or experienced paradoxical TB-IRIS to internal controls. A major strength of our study therefore is a novel design that places risk factors for TB-IRIS in the context of risk factors for death. Other strengths include a large number of paradoxical TB-IRIS cases and non-IRIS controls relative to published immunologic reports[8-11,20], comprehensive assessment of relevant biomarkers before and soon after ART initiation, and the high HIV/TB burden setting.

Limitations include possible misclassification of TB-IRIS, a limited number of deaths and lack of data on precise causes of death, and study of circulating biomarkers rather than those at the site of infection. Major bias from IRIS misclassification is unlikely as our event adjudication was blinded to biomarker data and was consistent with definitions used by other groups.[7,20]Given many IRIS cases were mild, it is possible some incident respiratory symptoms were attributable to the heterogenous course of TB and not to overt inflammation. Mixing “true” IRIS cases with these patients likely would bias towards the null in comparisons with controls, however, such that we may have underestimated true differences. Furthermore, we did not investigate in detail if patients experienced IRIS associated with non-TB OIs. Study of circulating biomarkers is unlikely to have substantially biased our results, as responses at the site of infection are generally positively correlated with those observed in the periphery.[25] Additionally, while the incidence of early mortality and paradoxical TB-IRIS observed here suggest generalizability to similar settings[14,26], the findings may be less generalizable to central nervous system infections, where IRIS is more often fatal.[27] Multiple testing is another limitation, and further study in other settings is needed. Nonetheless, a focus on odds ratios enabled an evaluation of the strength and direction of the associations shown in Figures 1A and 1B, which strongly suggest systematic differences between these patient groups.

Our analysis of early and late TB-IRIS cases, and strong associations between cytokine increases at time of TB-IRIS onset reported by others[8,9,11,12,22,23], suggests we may have underestimated changes on ART by collecting samples at week 4 and not at time of event. These data indicate that, among this superficially homogenous group, patients who suffer early mortality have profound immunologic dysfunction at the time of ART initiation. Other studies have observed that high pre-ART inflammatory cytokine levels are associated with mortality in HIV[28], and levels of TNF-α and other cytokines generally decrease during TB treatment.[29] Although we do not have biomarker levels at the time of TB diagnosis, autopsy studies demonstrating overwhelming TB in advanced HIV/TB patients on TB therapy[30], and

studies linking elevated MCP-1 with TB dissemination[31], suggest that TB may have been more advanced at presentation in those who died. In contrast, in adjusted analyses patients who experienced TB-IRIS had lower levels of several circulating inflammatory markers, including IL-6 and the Th1/2/17 cytokines IL-2, IL-3, IL-12, IL15, and IL-17a. Goovaerts et al. also recently reported that lower pre-ART IL-6 and G-CSF levels were associated with an increased risk of paradoxical TB-IRIS.[22]

While our data superficially conflict with results relating higher pro-inflammatory cytokine levels to TB-IRIS in a report by Narendran et al., this finding may be related to a shorter time to ART initiation and more advanced HIV-disease stage among the IRIS cases in that study.[10] Andrade et al. recently demonstrated that the distribution of circulating biomarkers, along with *M tuberculosis* sputum load, decreases with time spent on anti-tubercular therapy prior to ART initiation.[32] Because time to ART initiation and antigen burden are also associated with TB-IRIS risk[23,32,33], studies demonstrating higher pre-ART biomarker levels in TB-IRIS patients who started ART earlier than those who did not develop the syndrome[10,23] may not inform this association when examined in populations where time to ART is relatively uniform. The findings of lower pre-ART biomarkers seen in the TB-IRIS patients in this study and in Goovaerts et al. are notable in this regard, as time to ART initiation in both cohorts was very similar among TB-IRIS patients and non-IRIS controls.[22] Inclusion of patients with unmasking IRIS, due to an undiagnosed, untreated OI at the time of ART initiation, also could explain the higher pre-ART cytokine levels found in those with IRIS in other reports.[20,28]

Cytokines such as IL-6, GM-CSF, IL-15, and IL-12, primarily produced by monocytes/macrophages and dendritic cells, mediate immunologic control of *M tuberculosis* by stimulating T and natural killer cell responses that activate macrophages.[34-36] Low pre-ART levels of these cytokines in TB-IRIS patients may therefore be indicative of abnormal innate immune responses, which may lead to impaired pathogen clearance and high antigen loads at ART initiation, as hypothesized.[32,37] However, cytokine levels of those with TB-IRIS in this study, where timing of ART initiation was similar across groups, could also have been lower due to less severe disease (e.g., due to a lower pathogen burden) at the time of ART initiation. Regardless of the mechanism, these data, and data on the generally favorable pulmonary TB-IRIS prognosis reported by others[6,14,23,38], indicate that an innate immune defect, if present, does not result in abnormally low protective cellular immune responses prior to ART or decreased patient survival thereafter. Given these data, we would argue that the innate and cellular immune systems may actually be particularly functional in these patients.

The divergent immunologic profiles of those with early mortality and TB-IRIS are further highlighted by the analysis of early changes after ART initiation. In adjusted analyses, we, like others[9-11,22,23], noted a vigorous upsurge in pro-inflammatory cytokines such as IL-6 and TNF-α in those who experienced TB-IRIS. The finding that these patients developed incident symptoms despite starting out with lower IL-6 levels suggests that the clinical worsening after ART initiation characteristic of TB-IRIS is more reflective of the change in, and perhaps not the absolute levels of, circulating cytokine levels. Patients who died also

experienced increases in the pro-inflammatory cytokine IL-6 but, in striking contrast, this was not accompanied by early CD4 recovery.

Taken together, these findings have several implications for future research and clinical care. Several randomized trials are evaluating or have recently evaluated immunomodulatory therapies at the time of ART initiation in advanced HIV/TB as a way to prevent TB-IRIS. Our data suggest that immunomodulatory therapies that inhibit inflammation without suppressing adaptive immune recovery should be prioritized. The increases in inflammation seen in both those who died and experienced TB-IRIS raises the possibility that, if inflammation hinders immune recovery, as one large study from Uganda has suggested,[39] selective anti-inflammatory therapies could decrease risk of both outcomes, moving patients towards the middle of the immune recovery spectrum. Although corticosteroids may decrease inflammation without affecting pathogen-specific immune function in HIV/TB-IRIS[24], they also may impair T-cell recovery and proliferation, as well as induce T-cell death.[38,40] Measurement of immunologic profiles prior to and early after ART initiation may also be useful as a means of stratifying risk of severe TB-IRIS and death and should be further investigated (panel).

In conclusion, this study urges caution in treatment of patients with advanced HIV/TB as a homogenous group. The relatively inverse relationship between pre-ART cytokine levels in TB-IRIS and early mortality, and the contrasting degrees of CD4 cell recovery, suggests that interventions that seek to prevent TB-IRIS could inadvertently increase risk of death. Further study is needed to identify ways to predict and improve outcomes in these individuals.

Panel: Research in Context

Systematic Review—We searched PubMed for studies published before Dec 19, 2014, with no restrictions, for studies describing tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS) or early mortality among HIV/TB patients starting antiretroviral therapy (ART) with the search terms “HIV” AND “Tuberculosis” AND “immune reconstitution inflammatory syndrome” AND “ART”, and then by the addition of the phrase: AND “death”. Our search identified several papers including [2-14,16,20,22-24,28,32,37]. Relevant studies,[8-13,16,17,22-24,28,32] investigated circulating biomarkers or cellular immune responses before and after ART initiation and assessed the association with TB-IRIS or death. We identified no study that compared immunologic profiles of patients with paradoxical TB-IRIS or death to non-IRIS survivors within a single cohort at risk for both outcomes. Furthermore, although many studies, including key studies,[8,12,13,17,24] assessed TB (purified protein derivative)-specific immune response and its association with TB-IRIS, no studies other than our previously published report[16] assessed immune response (CD4 cell count or purified protein derivative-specific responses) during ART and its association with early mortality in patients co-infected with HIV and TB.

Interpretation—To date, research attention has been primarily focused on the immunologic correlates of paradoxical TB-IRIS in advanced HIV/TB patients.[8-13,22,23]

Early mortality is an adverse outcome for which these patients are at similar risk.[2,3,5] However, previous studies have exclude patients who die soon after ART initiation. Thus, in this prospective cohort study, we compared immunologic risk factors before and early after ART initiation between patients who had either TB-IRIS or early mortality and surviving, non-TB-IRIS controls. Although patients with TB-IRIS had significantly lower pre-ART concentration of several inflammatory biomarkers than controls, in line with one other study[22], pre-ART concentration of the biomarkers in those who died were remarkably higher, similar to findings from Boulware and colleagues' study.[28] Soon after ART initiation, inflammatory markers of both the TB-IRIS and early mortality groups increased rapidly, but the two groups were distinguished by sharply divergent recovery of the adaptive immune system.

This study is the first to show that patients with TB-IRIS have linked recovery of markers of innate and adaptive immune function, whereas those who died have increases in inflammation without recovery of cellular immune responses that are known to be crucial to the control of *Mycobacterium tuberculosis*. Findings from this study have several implications for future research and clinical care. First, immunomodulatory therapies that inhibit inflammation without suppressing adaptive immune recovery should be prioritized as interventions in HIV/TB. Second, future studies should evaluate the ability of immune markers to risk-stratify patients for early outcomes during ART. If accurate, such strategies could help direct different TB-IRIS and early mortality prevention interventions to those at greatest risk. Third, triaging care of advanced HIV/TB patients using assessment of CD4 cell count in the initial weeks of ART initiation could have benefits in resource-limited settings and needs further evaluation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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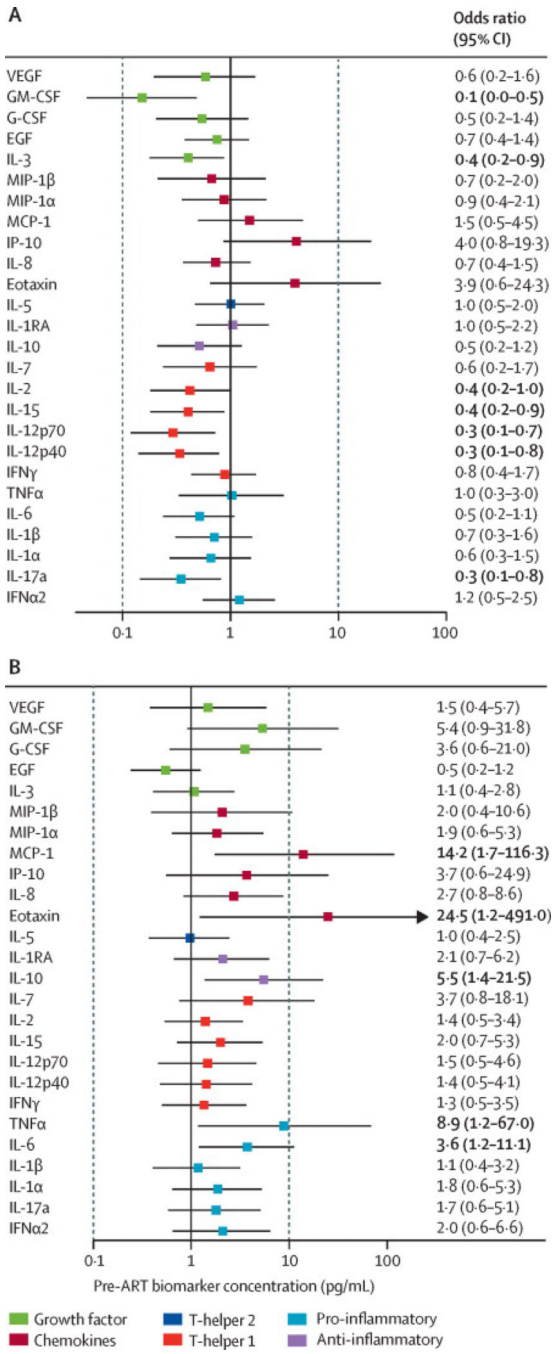

Figure 1.

Unadjusted ORs relating 1 log10 increases in baseline biomarker concentration to risk of outcomes after initiation of antiretroviral therapy in patients with advanced HIV and tuberculosis who had TB-IRIS (A) or early death (B), compared with controls.

Data are OR (95% CI). Data in bold show markers that were significantly associated with outcome. OR=odds ratio. VEGF=vascular endothelial growth factor. GM-CSF=granulocyte-macrophage colony-stimulating factor. EGF=epidermal growth factor. MIP=macrophage inflammatory protein. MCP=monocyte chemoattractant protein. IP= IFN-γ induced protein.

IL=interleukin. IFN=interferon. TNF=tumour necrosis factor. G-CSF=granulocyte colony-stimulating factor.

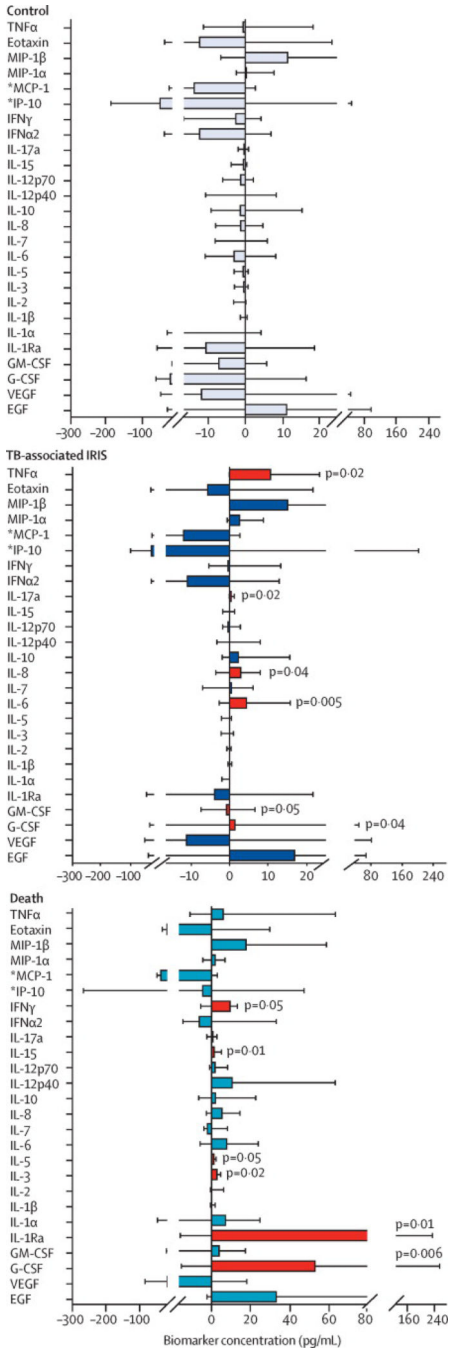

Figure 2.

Early changes in biomarker concentration after initiation of antiretroviral therapy in controls, patients with TB-IRIS, and patients who died.

Error bars show median and interquartile range for change in plasma biomarkers from baseline to week 4 after ART initiation (appendix). Red bars show biomarkers that were significantly associated with TB-IRIS or death compared with controls by Wilcoxon rank-sum tests. TB=tuberculosis. IRIS=immune reconstitution inflammatory syndrome. TNF=tumour necrosis factor. MIP=macrophage inflammatory protein. MCP=monocyte

chemoattractant protein. IP= IFN-γ induced protein. IFN=interferon. IL=interleukin. GM-CSF=granulocyte-macrophage colony-stimulating factor. G-CSF=granulocyte colony-stimulating factor. VEGF=vascular endothelial growth factor. EGF=epidermal growth factor. *We transformed MCP-1 and IP-10 values by a factor of 1/10 to enable plotting with other biomarkers.

Table 1

Baseline characteristics of advanced HIV/TB co-infected patients initiating ART in Botswana

Parameter	Control	TB-IRIS[#]	P value[a]	Deaths	P value[b]
n	**120**	**33**		**17**	
Female sex, n (%)	49 (41)	14 (42)	0·87	11 (65)	0·06
Age in years, mean (range)	36 (22-73)	37 (22-59)	0·98	39 (27-56)	0·21
Time between anti-tubercular therapy and ART initiation (days)	27 (18-47)	30 (21-44)	0·54	29 (22-49)	0·59
NVP-based ART, n (%)	12 (10)	7 (21%)	0·08	5 (29)	**0·02**
Presence of extrapulmonary TB at baseline, n (%)	6 (5)	2 (6)	0·81	1 (6)	0·88
Presence of non-TB OI at baseline n (%)	10 (8)	5 (15)	0·24	5 (29)	**0·01**
CD4+ T cell count, median cells/μl (IQR)	64 (32-94)	61 (36-86)	0·79	34 (14-66)	**0·02**
HIV viral load, $\log_{10}$copies/ml (IQR), n	5·5 (4·9-6·0), 116	5·5 (5·0-5·9), 32	0·77	5·7 (5·5-5·9), 17	0·24
BMI, median (IQR); n	19·0 (16·4-21·8); 119	20·4 (18·3-22·9); 33	0·08	19·3 (17·7-24·5); 15	0·66
BMI<19 kg/m^2, n (%)	61 (51)	10 (30)	**0·03**	7 (47)	0·74
AFB smear negative TB	30 (25)	6 (18)	0·41	6 (35)	0·37
PPD SFU/10^6 PBMC, median (IQR), n	266 (12-860); 98	318 (64-1508); 20	0·19	68 (35-370); 12	0·54

ATT=anti-tubercular therapy; IQR=interquartile range; NVP=nevirapine; ART=anti-retroviral therapy; OI=opportunistic infection; BMI=Body Mass Index.

AFB= acid-fast bacilli; PPD=purified protein derivative; SFU=spot forming units; PBMC= peripheral blood mononuclear cells.

[#]Includes one patient who died and had TB-IRIS.

[a]P value comparing controls (non-TB-IRIS survivors) to TB-IRIS patients.

[b]P value comparing control patients to deaths. Ns for HIV viral load, BMI, and PPD-specific responses are mentioned in line with the parameter to indicate missing data.

Table 2

Relation of baseline biomarker concentrations to TB-associated IRIS and early mortality in patients with advanced HIV and tuberculosis starting antiretroviral therapy

Biomarker	TB-IRIS OR (95% CI)[#]	Death OR (95% CI)[*]
GM-CSF	0·15 (0·05-0·48)[**]	3·0 (0·49-18·9)
IL-2	0·41 (0·18-0·96)[**]	1·4 (0·54-3·4)
IL-3	0·39 (0·17-0·86)[**]	1·1 (0·41-2·8)
IL-12p40	0·32 (0·14-0·75)[**]	1·4 (0·48-4·2)
IL-12p70	0·28 (0·12-0·69)[**]	1·4 (0·46-4·6)
IL-15	0·39 (0·18-0·86)[**]	2·0 (0·72-5·3)
IL-17a	0·33 (0·14-0·78)[**]	1·7(0·58-5·1)
IL-6	0·40 (0·18-0·89)[**]	2·8 (0·93-8·4)
IL-10	0·5(0·21-1·2)	3·5(0·89-13·5)
MCP-1	1·5 (0·49-4·5)	9·0 (1·0-80·0)[**]
TNF-α	0·99 (0·32-3·0)	7·8 (1·1-55·2)[**]
Eotaxin	5·4 (0·78-36·5)	3·6 (0·58-22·5)
GCSF	0·53 (0·20-1·4)	3·6 (0·61-21·0)
IP-10	4·0 (0·84-19·3)	2·9 (0·50-16·8)

Log_{10} transformed baseline values of biomarkers that were associated with TB-IRIS or death at p<0.10 in unadjusted analyses (Table 3) were used to determine association with paradoxical TB-IRIS and death in logistic regression model.

[#]TB-IRIS associations are adjusted for BMI and NVP.

[*]Models included pre-ART CD4 count, female sex, and presence of baseline OI.

[**]Independent association between biomarker and outcome. CI=confidence interval.

Table 3

Baseline biomarker concentrations associated with TB-associated IRIS and early mortality in patients with advanced HIV and TB starting antiretroviral therapy

*Biomarker	Control (n=118)	TB-IRIS# (n=32)	P value[a]	P_{corr} [a]	Death (n=17)	P value[b]	P_{corr} [b]
EGF	146·9 (55·6-235·3)	92·7 (46·3-201·5)	0·17	0·28	78·6 (38·7-156·5)	0·10	0·36
VEGF	123 (76·1-181·7)	106·2 (65·4-160·2)	0·21	0·30	173·3 (97·4-219·6)	0·28	0·50
G-CSF	124·1 (86·6-174·1)	88·4 (65·3-132·8)	**0·01**	**0·04**	138·4 (97·7-243·1)	0·29	0·50
GM-CSF	34·5 (20·0-51·4)	18·3 (12·2-31·9)	**0·0008**	**0·02**	46·9 (35·5-66·1)	**0·03**	**0·20**
IL-1RA	78·7 (39·8-153·7)	90·4 (34·2-200·0)	0·74	0·74	115·5 (50·8-160·2)	0·25	0·50
IL-1α	20·1 (9·4-57·3)	9·4 (9·4-44·7)	0·20	0·30	44·2 (9·4-109·5)	0·32	0·52
IL-1β	1·2 (0·8-3·9)	0·8 (0·8-3·2)	0·16	0·28	1·6 (0·8-1·9)	0·77	0·80
IL-2	2·4 (1·0-7·3)	1·0 (1·0-3·0)	**0·02**	**0·07**	3·7 (1·0-11·4)	0·40	0·58
IL-3	3·7 (1·3-9·1)	1·5 (0·7-5·9)	**0·007**	**0·04**	2·8 (1·5-13·2)	0·69	0·75
IL-5	2·8 (1·5-6·5)	3·0 (1·1-4·8)	0·62	0·70	3·1 (0·5-7·7)	0·97	0·97
IL-6	14·7 (7·5-28·1)	10·3 (5·0-21·3)	**0·04**	**0·12**	19·8 (11·8-33·2)	0·11	0·36
IL-7	21·0 (14·1-29·4)	15·8 (12·2-22·7)	0·14	0·28	23·0 (19·0-47·5)	0·15	0·36
IL-8	16·8 (9·6-28·6)	14·6 (6·8-20·8)	0·15	0·28	22·2 (11·9-38·8)	0·14	0·36
IL-10	18·8 (11·0-32·5)	14·5 (8·3-21·3)	0·07	0·17	39·8 (13·1-98·2)	**0·03**	**0·20**
IL-12p40	15·0 (7·4-36·0)	7·4 (7·4-12·0)	**0·002**	**0·03**	20·7 (7·4-27·9)	0·65	0·74
IL-12p70	9·8 (5·8-18·7)	6·3 (3·4-12·0)	**0·01**	**0·04**	11·4 (8·1-19·0)	0·49	0·66
IL-15	4·3 (1·2-10·0)	1·7 (1·2-4·8)	**0·02**	**0·07**	5·8 (2·5-13·3)	0·27	0·50
IL-17a	3·3 (1·6-5·9)	1·4 (0·7-3·8)	**0·005**	**0·04**	4·2 (2·4-6·2)	0·35	0·54
IFN-α	53·3 (29·8-98·6)	71·8 (31·1-110·0)	0·36	0·49	57·4 (35·4-91·4)	0·55	0·68
IFN-γ	18·8 (9·2-35·9)	16·3 (5·0-34·9)	0·56	0·66	20·2 (9·3-43·4)	0·64	0·74
IP-10	3390 (2298-4267)	4185 (2827-5931)	0·06	0·16	3935 (2964-5203)	0·12	0·36
MCP-1	548·7 (388·4-779·4)	649·3 (378·2-841·6)	0·71	0·74	832·2 (652·3-1331·2)	**0·003**	**0·08**
MIP-1α	13·3 (5·7-22·9)	10·4 (6·8-15·4)	0·41	0·53	19·7 (8·2-25·0)	0·14	0·36
MIP-1β	66·9 (47·1-112·2)	63·7 (39·1-106·9)	0·45	0·56	99·2 (49·3-114·3)	0·51	0·66
Eotaxin	146·5 (119·6-191·8)	180·0 (132·9-220·0)	0·08	0·17	189·8 (140·6-258·7)	0·06	0·31
TNF-α	41·4 (28·9-63·0)	38·2 (27·0-60·5)	0·65	0·71	56·6 (44·9-74·8)	**0·02**	**0·20**

*Biomarker expressed as median pg/ml (interquartile range).

[#] Includes one patient who had TB-IRIS and died.

[a] P and Benjamini-Hochberg corrected p(corr) value comparing controls to TB-IRIS patients.

[b] P and pcorr value comparing controls to deaths.

Table 4

Association between changes in biomarker concentrations from baseline to week 4 after ART initiation and TB-associated IRIS and early mortality in patients with advanced HIV and TB

Biomarker	TB-IRIS OR (95% CI)[#]	Death OR (95% CI)[*]
IL-6	1·7 (1·2-2·5)[**]	1·8 (0·94-3·3)
TNF-α	1·5 (1·0-2·2)[**]	1·2 (0·67-2·1)
IL-8	1·4 (1·0-2·0)[**]	1·7 (0·91-3·1)
G-CSF	1·5 (1·0-2·1)[**]	2·8 (1·3-6·1)[**]
IL-3	1·1 (0·80-1·6)	2·3 (1·1-4·6)[**]
IL-12p40	1·2 (0·89-1·8)	1·8 (1·0-3·4)[**]
IL-15	1·3 (0·92-1·9)	2·0 (1·1-3·7)[**]
IL-1RA	1·1 (0·76-1·6)	2·2 (1·1-4·4)[**]
GM-CSF	1·3 (0·93-1·9)	1·3 (0·71-2·2)
IL-10	1·3 (0·93-1·9)	1·4 (0·77-2·4)
IFN-γ	1·4 (0·98-2·0)	1·7 (0·91-3·2)
IL-5	1·0 (0·71-1·4)	1·7 (0·91-3·2)
IL-12p70	1·2 (0·81-1·6)	1·8 (0·93-3·3)
CD4	1·2 (0·85-1·8)	0·38 (0·17-0·85)[**]
PPD	1·2 (0·78-1·9)	0·75 (0·37-1·5)

Change from baseline to week 4 post-ART levels of biomarkers that were associated with TB-IRIS or death at p<0.10 in unadjusted analyses (appendix) were stratified into quartiles and assessed for association with either outcome in logistic regression models.OR and CI indicated are per quartile change in each biomarker.

[#]TB-IRIS associations are adjusted for BMI, NVP, and pre-ART levels of respective biomarker.

[*]Model included pre-ART CD4 count, female sex, presence of baseline OI, and pre-ART levels of respective biomarker.

[**]Independent association between biomarker and outcome. CI=confidence interval.