Discordant Early Immune Responses Distinguish TB-IRIS and Death in HIV/TB Co-infection

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Background

Advanced HIV/TB patients are at high risk for both mortality and TB-immune reconstitution inflammatory syndrome (TB-IRIS) despite timely commencement of antiretroviral therapy (ART) following anti-tuberculosis initiation.[1,2,3] Because TB-IRIS and early mortality appear to be related to rapid[4] and minimal[5] immune recovery, respectively, we hypothesized that these two outcomes are characterized by distinct immunologic profiles after ART initiation. We evaluated various immunologic variables to determine factors that distinguish early mortality, TB-IRIS, and non-TB IRIS survivors at baseline and week 4 post-ART initiation among a cohort of advanced HIV/TB co-infected adults in Botswana.

Methods

Study design: Prospective cohort study of ART-naïve, HIV-infected adults with CD4 counts <125/mm^3 and active pulmonary TB at enrollment.

Exposures measured at baseline and week 4 post-ART initiation:
1. HIV- plasma viral load and CD4 counts.
2. Levels of 26 soluble plasma biomarkers by Luminex in all available samples. TNF-β, IL-4, and IL-13 were below the the limit of detection.
3. MTB-specific cellular immune response using fresh peripheral blood mononuclear cells (PBMCs) by Purified Protein Derivative (PPD) stimulation and IFN-γ ELISPOT.

Outcomes determined during monthly follow-ups for 6 months post-ART initiation:
1. Non-traumatic death
2. Paradoxical TB-IRIS assessed retrospectively using INSHI and ACTG guidelines[6,7]
3. Controls were non-TB-IRIS survivors

Analyses
1. Association between pre-ART biomarker levels in TB-IRIS and death vs. controls.
2. Association between change from baseline to week 4 post-ART levels in biomarkers comparing TB-IRIS and death to controls.

Rank-sum tests and logistic regression were used for analysis.

Results

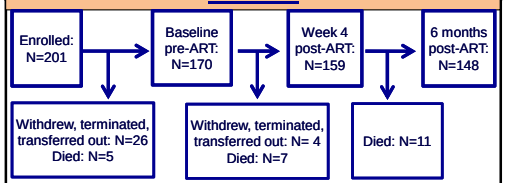

Figure 1. Flow chart of patient enrollment and follow up in study.

Number of patients with baseline data, N	170
Non TB-IRIS survivors (Control), n (%)	120 (70)
Paradoxical TB-IRIS events, n (%)	33 (19) *
Deaths, n (%)	18 (11)
Female sex, n (%)	74 (44)
Age, mean (range in years)	37 (22-73)
Anti-tuberculosis treatment to ART initiation interval, median days (IQR)	28 (20-47)
NVP-based ART, n (%)	24 (14)
Body Mass Index, median (IQR)	19 (17-22)
Viral load, log$_{10}$copies/ml (IQR)	5.5 (5.0-6.0)
CD4 counts, median cells/mm^3 (IQR)	60 (31-90)

Table 1. Baseline characteristics of cohort. *One TB-IRIS event preceded death after ART initiation. IQR= interquartile range; NVP=nevirapine

Results

Biomarker	Control (n=118)	TB-IRIS (n=32)	P value[a]	Death (n=17)	P value[b]
G-CSF	124·1 (86·6-174·1)	88·4 (65·3-132·8)	**0·01**	138·4 (97·7-243·1)	0·29
GM-CSF	34·5 (20·0-51·4)	18·3 (12·2-31·9)	**0·0008**	46·9 (35·5-66·1)	**0·03**
IL-2	2·4 (1·0-7·3)	1·0 (1·0-3·0)	**0·02**	3·7 (1·0-11·4)	0·40
IL-3	3·7 (1·3-9·1)	1·5 (0·7-5·9)	**0·007**	2·8 (1·5-13·2)	0·69
IL-6	14·7 (7·5-28·1)	10·3 (5·0-21·3)	**0·04**	19·8 (11·8-33·2)	0·11
IL-10	18·8 (11·0-32·5)	14·5 (8·3-21·3)	0·07	39·8 (13·1-98·2)	**0·03**
IL-12p40	15·0 (7·4-36·0)	7·4 (7·4-12·0)	**0·002**	20·7 (7·4-27·9)	0·65
IL-12p70	9·8 (5·8-18·7)	6·3 (3·4-12·0)	**0·01**	11·4 (8·1-19·0)	0·49
IL-15	4·3 (1·2-10·0)	1·7 (1·2-4·8)	**0·02**	5·8 (2·5-13·3)	0·27
IL-17a	3·3 (1·6-5·9)	1·4 (0·7-3·8)	**0·005**	4·2 (2·4-6·2)	0·35
MCP-1	548·7 (388·4-779·4)	649·3 (378·2-841·6)	0·71	832·2 (652·3-1331·2)	**0·003**
TNF-α	41·4 (28·9-63·0)	38·2 (27·0-60·5)	0·65	56·6 (44·9-74·8)	**0·02**

Table 2. Baseline, pre-ART, biomarker levels associated significantly with TB-IRIS[a] or deaths[b] compared to controls among patients with advanced HIV/TB initiating ART.

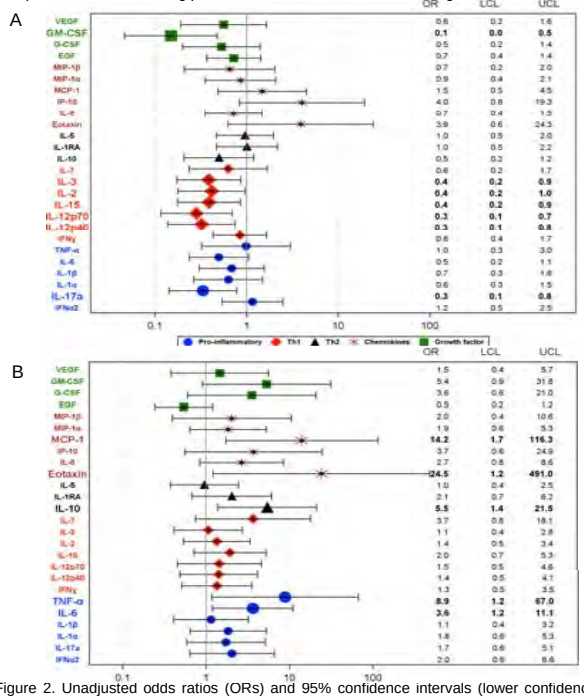

Figure 2. Unadjusted odds ratios (ORs) and 95% confidence intervals (lower confidence limit [LCL] and upper confidence limit [UCL]) are shown relating baseline levels of biomarkers to (A) TB-IRIS and (B) death compared to controls.

Biomarker	TB-IRIS AOR (95% CI)#	Death AOR (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)
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)**

Table 3. Adjusted odds ratio (AOR) relating $\log_{10}$ transformed baseline biomarker levels to TB-IRIS or death. #TB-IRIS associations were adjusted for BMI and NVP. *Model included pre-ART CD4 count, female sex, and baseline OI. **Independent association between biomarker and outcome.

Parameters	Control (n=116)	TB-IRIS (n=33)	P value[a]	Death (n=10)	P value[b]
CD4 count median cells/μL (IQR); n					
Change from baseline	70 (33-130); 116	93 (24-164); 33	0·45	21 (-18- 44); 10	**0·006**
Week 4	132 (85-223); 116	150 (96-242); 33	0·38	60 (39-83); 10	**0·005**
PPD median SFU/10^6 PBMCs (IQR); n					
Change from baseline	120 (-60-847); 92	337 (0-1783); 19	0·22	-13 (-88-482); 7	0·38
Week 4	662 (105-1600); 96	1277 (317-2160); 21	0·06	298 (21-1063); 8	0·20

Table 4. Change from baseline to week 4 post-ART initiation in cellular immunologic parameters among TB-IRIS[a] and deaths[b] vs. controls with paired data available for analysis.

Results

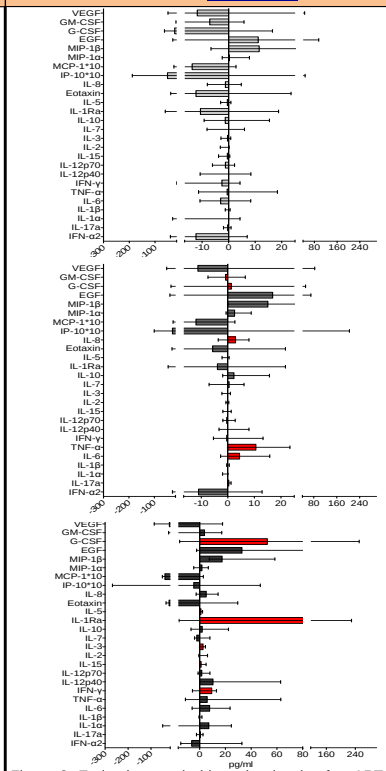

Figure 3. Early changes in biomarker levels after ART initiation among controls, TB-IRIS patients, and deaths. Bars indicate median change from baseline to week 4 post-ART initiation and interquartile ranges of plasma biomarkers measured by Luminex assay. Red bars indicate p<0.05 comparing TB-IRIS or death to controls.

Biomarker	TB-IRIS AOR (95% CI)#	Death AOR (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)**
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)

Table 5. Adjusted odds ratios (AOR) per quartile change in immunologic variable from baseline to week 4 post-ART initiation with TB-IRIS and death. #TB-IRIS associations were adjusted for BMI and NVP. *Model included pre-ART CD4 count, female sex, and baseline OI. **Independent association between biomarker and outcome.

Conclusion

	TB IRIS	Early Mortality
Baseline	• Lower levels of systemic inflammation vs. controls	• Higher levels of systemic inflammation vs. controls
Week 4 post ART	• Increasing levels of systemic inflammation • Increasing CD4 counts and TB-specific immune response	• Increasing levels of systemic inflammation • Reduced or failure to reconstitute CD4 counts and TB-specific immune response

Distinct inflammatory biomarker profiles and cellular immune responses characterize early mortality and TB-IRIS pre- and post-ART initiation. Interventions that decrease inflammation without inhibiting adaptive immune function hold promise in treatment of advanced HIV/TB.

References: 1. Havlir DV et al. NEJM 2011; 2. Blanc FX et al. NEJM 2011; 3. Luetkemeyer AF et al. JAIDS 2014; 4. Bourgarit et al. AIDS 2006; 5. Ravimohan et al JID 2013; 6. Meintjes G et al. Lancet ID 2008; 7. Grant PM et al. Plos One 2010.

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