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Baseline Inflammatory and immune activation markers predictive of TB after ART initiation

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Background

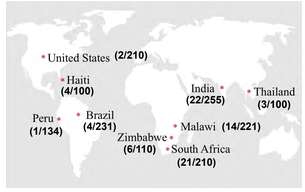
- TB is the leading cause of morbidity and mortality after starting ART in most low- and middle-income countries (LMICs) with high TB burden
- Low baseline CD4 count, clinical disease stage, anemia, and hypoalbuminemia associated with incident TB post-ART initiation in studies from diverse settings
- Finding additional inflammatory and immune activation markers that predict incident TB post-ART could help identify individuals at greatest risk for disease and to a better understanding of immunopathogenesis of TB

Methods

Population

- Sampled from Prospective Evaluation of Antiretrovirals in Resource-Limited Settings (PEARLS) trial (ACTG A5175)
  - 1571 HIV-infected, ART-naïve adults from 9 countries (Figure 1)
  - Baseline CD4 T-cell count <300 cells/mm^3
  - No acute medical illness or severe laboratory derangement (Hgb <7.5 g/dL, AST/ALT >5x upper limit of normal, CrCl <60 mL/min, ANC <750/μL)
  - Randomized to 1 of 3 ART regimens and followed regularly for 96 weeks post-ART initiation

Figure 1. Study Sites



Study Design

- Nested case-control study
  - Cases were participants diagnosed with incident TB within 96 weeks of ART
  - Controls were a random sample of participants not diagnosed with TB during follow up, stratified by study site and treatment arm
- Baseline soluble inflammatory and immune activation markers measured for cases and controls, including:
  - Pro-inflammatory cytokines/chemokines (IFN-γ, IL-6, IP-10, TNF-α)
  - Immune activation markers (EndoCab IgM, lipopolysaccharide, sCD14)
  - Activated T cell markers (CD4/DR+/CD38+, CD8/DR+/38+)
  - General inflammatory markers (CRP, ferritin)

Methods

Analysis

- Performed logistic regression to measure association between biomarkers and incident TB
  - Used establish cutoffs when available or 75th percentile of measured values when not (detectable/not detectable for LPS)
  - Adjusted for age, sex, study site, treatment arm, baseline CD4, baseline log$_{10}$ HIV viral load
- Performed ROC analysis to assess discriminatory value of biomarkers

Results

- 77 (4.9%) diagnosed with TB by 96 weeks
  - 55 pulmonary TB and 22 extrapulmonary TB cases
  - Median time to diagnosis 24 weeks (Figure 1)

Figure 2. Kaplan-Meier curve of time to TB diagnosis for TB cases

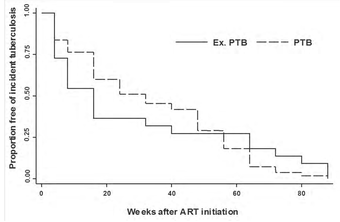


Table 1. Baseline characteristics of cases and controls

Characteristic	Incident TB n = 77	No Incident TB n = 255	p-value[1]
Age, years, median (IQR)	34 (29-39)	35 (29-41)	> 0.95
Female at birth, n (%)	31 (40)	126 (49)	0.16
Baseline CD4, cells/mm^3, median (IQR)	136 (60 – 201)	180 (90 – 231)	0.07
Baseline HIV viral load, log$_{10}$copies/mL, median (IQR)	5.2 (4.6-5.7)	5.0 (4.6-5.5)	0.23
BMI, kg/m^2, n (%)			< 0.01
< 18.5	11 (14)	22 (9)	
≥ 18.5-25	58 (75)	165 (65)	
≥ 25	8 (10)	68 (27)	
HIV treatment regimen[2], n (%)			0.35
EFV+3TC/ZDV	22 (29)	94 (37)	
ATV+DDI+FTC	31 (40)	83 (33)	
EFV+FTC/TDF	24 (31)	78 (31)	
History of TB, n (%)	25 (33)	43 (17)	< 0.01

[1]Baseline characteristics of cases and controls were compared using the non-parametric Mann-Whitney test for continuous variables or Fisher's exact test for discrete variables.
[2]EFV+3TC/ZDC = efavirenz 600 mg daily, lamivudine-zidovudine 150 mg / 300 mg twice daily
ATV+DDI+FTC = atazanavir 400 mg daily, didanosine-EC 400 mg daily, emtricitabine 200 mg daily
EFV+FTC/TDF = efavirenz 600 mg daily, emtricitabine-tenofovir 200 mg / 300 mg daily

Results

Table 2. Unadjusted and adjusted odds ratios of association between selected markers and incident TB within 96 weeks after ART initiation.

Characteristic	Overall	TB Case	TB Control	Adjusted OR[1] (95% CI)
Prior history of TB				
No	264 (80%)	52 (68%)	212 (83%)	1
Yes	68 (20%)	25 (32%)	43 (17%)	1.09 (0.53-2.26)
BMI, kg/m^2				
[18.5-25)	223 (67%)	58 (75%)	165 (65%)	1
< 18.5	33 (10%)	11 (14%)	22 (9%)	0.93 (0.35-2.44)
≥ 25	76 (23%)	8 (10%)	68 (27%)	0.37 (0.14-0.97)
Albumin, g/dL				
≥ 3.5	254 (77%)	40 (53%)	214 (84%)	1
< 3.5	77 (23%)	36 (47%)	41 (16%)	5.24 (2.69-10.20)
Hemoglobin, g/dL				
≥ 12 (f) or ≥ 13 (m)	143 (43%)	19 (25%)	124 (49%)	1
< 12 (f) or < 13 (m)	187 (57%)	56 (75%)	131 (51%)	2.16 (1.07-4.35)
CRP, mg/dL				
Q1-Q3 (1.47-11.72)	217 (70%)	35 (50%)	182 (76%)	1
Q4 (> 11.72)	91 (30%)	35 (50%)	56 (24%)	4.12 (2.18-7.77)
Ferritin, ng/mL				
≤ 150 (f) or ≤ 200 (m)	213 (64%)	38 (49%)	175 (69%)	1
> 150 (f) or > 200 (m)	119 (36%)	39 (51%)	80 (31%)	1.72 (0.92-3.21)
IFN-γ, pg/mL				
Q1-Q3 (5.86-49.65)	219 (66%)	49 (64%)	170 (67%)	1
Q4 (> 49.65)	113 (34%)	28 (36%)	85 (33%)	0.76 (0.42-1.39)
IL-6, pg/mL				
Q1-Q3 (9.17-49.88)	219 (66%)	50 (65%)	169 (66%)	1
Q4 (> 49.88)	113 (34%)	27 (35%)	86 (34%)	0.80 (0.45-1.42)
TNF-α, pg/mL				
Q1-Q3 (13.53 – 27.66)	219 (66%)	41 (53%)	178 (70%)	1
Q4 (> 27.66)	113 (34%)	36 (47%)	77 (30%)	1.66 (0.94-2.95)
IP-10, pg/mL				
Q1-Q3 (610.78-2922.43)	219 (66%)	38 (49%)	181 (71%)	1
Q4 (> 2922.43)	113 (34%)	39 (51%)	74 (29%)	1.89 (1.05-3.39)
EndoCab IgM, MMU/mL				
Q1-Q3 (29.5-71.5)	233 (70%)	50 (65%)	183 (72%)	1
Q4 (> 71.5)	99 (30%)	27 (35%)	72 (28%)	1.19 (0.76-2.13)
LPS, pg/mL				
< detection	192 (62%)	45 (63%)	137 (61%)	1
≥ detectable limit	120 (38%)	26 (37%)	94 (39%)	2.39 (1.13-5.06)
sCD14, pg/mL				
Q1-Q3 (487100-2800000)	223 (67%)	45 (58%)	178 (70%)	1
Q4 (> 2800000)	109 (33%)	32 (42%)	77 (30%)	1.40 (0.79-2.46)
CD4/DR+/38+, % CD4 lymphocytes[2]				
Q1-Q3 (16.3 – 35.3)	90 (75%)	14 (64%)	76 (78%)	1
Q4 (> 35.3)	30 (25%)	8 (36%)	22 (22%)	2.60 (0.51-13.21)
CD8/DR+/38+, % CD8 lymphocytes[2]				
Q1-Q3 (36.1 – 56.9)	90 (75%)	14 (64%)	76 (78%)	1
Q4 (> 56.9)	30 (25%)	8 (36%)	22 (22%)	1.85 (0.47 – 7.31)

[1] Adjusted odds ratio represents the independent risk of the added marker characteristic when adjusting for age, sex, treatment, study site, baseline CD4l count and baseline HIV viral load. Bold numbers signify p <0.05.
[2] Only measured where viable peripheral blood mononuclear cells were collected, which excludes populations from Thailand and India.

Results

- ROC analysis
  - Baseline model incorporating readily accessible clinical data (patient age + gender + study site + prior history of TB + BMI group (>18.5, 18.5-24.9, ≥ 25) + CD4 count): AUC 0.81 (95% CI: 0.76-0.86)
  - Additional of hypoalbuminemia or upper-quartile CRP to baseline model resulted in improvement in discrimination (AUC 0.84, 95% CI: 0.79-0.89)
  - Additional of anemia to baseline model resulted in more limited improvement improvement in discrimination (AUC 0.83, 95% CI: 0.78-0.88)

Limitations, Conclusions, and Future Directions

Limitations

- Lack of established cutoffs for inflammatory and immune activation markers and relatively healthy patient population with certain exclusion criteria

Conclusions

- In patients from diverse, predominately LMIC settings, significant association found between established TB risk factors (hypoalbuminemia, anemia) and incident TB post-ART
- Less established factors, including CRP, IP-10, and LPS, also associated with incident TB and may serve as accessible soluble markers of risk for incident TB among people starting ART in LMICs
- In ROC analysis, adding albumin or CRP to routinely available measures improves discrimination for incident TB within 96 weeks

Future Directions

- Establish optimal cutoffs and create treatment decision algorithms based on risk status, evaluate biomarkers in predicting non-TB outcomes, and stratify risk by tuberculosis skin test (TST) or interferon-gamma release assay (IGRA)

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