



Age, sex, and nutritional status modify the CD4+ T-cell recovery rate in HIV–tuberculosis co-infected patients on combination antiretroviral therapy[☆]



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SUMMARY

Background: Baseline age and combination antiretroviral therapy (cART) were examined as determinants of CD4+ T-cell recovery during 6 months of tuberculosis (TB) therapy with/without cART. It was determined whether this association was modified by patient sex and nutritional status.

Methods: This longitudinal analysis included 208 immune-competent, non-pregnant, ART-naïve HIV-positive patients from Uganda with a first episode of pulmonary TB. CD4+ T-cell counts were measured using flow cytometry. Age was defined as <24, 25–29, 30–34, and 35–39 vs. ≥40 years. Nutritional status was defined as normal (>18.5 kg/m²) vs. underweight (≤18.5 kg/m²) using the body mass index (BMI). Multivariate random effects linear mixed models were fitted to estimate differences in CD4+ T-cell recovery in relation to specified determinants.

Results: cART was associated with a monthly rise of 15.7 cells/μl ($p < 0.001$). Overall, age was not associated with CD4+ T-cell recovery during TB therapy ($p = 0.655$). However, among patients on cART, the age-associated CD4+ T-cell recovery rate varied by sex and nutritional status, such that age <40 vs. ≥40 years predicted superior absolute CD4+ T-cell recovery among females ($p = 0.006$) and among patients with a BMI ≥18.5 kg/m² ($p < 0.001$).

Conclusions: TB-infected HIV-positive patients aged ≥40 years have a slower rate of immune restoration given cART, particularly if BMI is >18.5 kg/m² or they are female. These patients may benefit from increased monitoring and nutritional support during cART.

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1. Introduction

With expanded access to combination antiretroviral therapy (cART) for people living with HIV (PLWH), the goal of HIV disease management has shifted from delaying death to maintaining a high quality of life. The effectiveness of cART in PLWH is directly linked to its proven capacity to reverse HIV-associated immune deficiency, as measured by the post-treatment rise in CD4+

T-cells.¹ However, immune recovery is seldom complete for PLWH on cART.² The drivers of incomplete immune reconstitution under cART are poorly elucidated. In general, immunity is impacted by age, nutritional status, comorbid health conditions, and lifestyle factors.

The impact of aging on immune function has been described.³ With advancing age, the thymus involutes, contributing to reductions in the population of naïve T-cells and a peripheral T-cell receptor repertoire that is skewed towards memory T-cells.⁴ These functional changes in T-cell populations are compounded by a reduced capacity for generating T-cell precursors to seed the thymus and the production of fewer naïve B-cells in the bone marrow, a lower quality of naïve T-cells produced from the thymus of older individuals, less potent antibodies to foreign antigens, and

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a generalized chronic inflammatory state that develops with advancing age. Malnutrition is a driver of immune suppression and dysregulation⁵ that may interact with age to interfere with immune responsiveness. Micro- and macronutrient deficiencies weaken the immune system partly through accelerated atrophy of the thymic and lymphoid tissue, alteration of T-cell subsets, and the cytokine response.^{6,7}

Some epidemiological studies have associated older age at HIV infection with faster progression to AIDS in the pre-ART era.^{8,9} However, the results of investigations on age-associated differences in the CD4+ T-cell response for HIV-infected adults on highly active antiretroviral therapy (HAART) have been more variable in the context of cART.^{10,11} Some studies have found evidence for a slower rate of CD4+ T-cell recovery or lower magnitude of CD4+ T-cell recovery in older compared to younger HIV-infected adults on HAART.^{12–18} Others have found no age-related differences in CD4+ T-cell recovery following ART initiation,^{19–22} and at least one study has reported a positive association between advanced age and enhanced CD4+ T-cell recovery.²³

In areas of high HIV prevalence, malnutrition and food-insecurity overlap,⁵ even as those infected with HIV make the same survival gains with cART described in resource-affluent settings.²⁴ These nutritional and age-related immune dysregulations may be both accelerated and magnified in HIV-infected populations due to HIV-related immune deficiency,²⁵ age-related decline in thymic output,^{26,27} and the high frequency of comorbid conditions that further aggravate immune alterations.²⁸ Yet, in spite of the biologically plausible expectation that malnutrition and advanced age in HIV-infected persons will unfavorably alter the immune response to cART,²⁹ there is limited empirical evidence that this occurs in PLWH provided cART.

How immune recovery following cART initiation is impacted by nutritional deficits and advanced age at cART initiation in Sub-Saharan Africa, where the burden of HIV lies, is unclear.³⁰ CD4+ T-cell recovery in African PLWH may be materially different from HIV-infected adults in developed countries due to a high frequency of comorbid infections^{30–32} and malnutrition, and lower overall quality of supportive clinical services. Of note, as many as 33% of PLWH in resource-limited, high-HIV prevalence Sub-Saharan Africa settings, have comorbid tuberculosis (TB) disease.³³ Concurrent TB and HIV upregulates immune activation and inflammation, and complicates the diagnosis, presentation, and clinical management of the respective diseases.³⁴ A recent study from South Africa suggested that the CD4+ T-cell recovery rate is comparable for HIV-infected persons with and without TB.³⁵ However, the understanding of age-related differences in immune recovery for HIV and TB co-infected persons is limited. Therefore, the age-related differences in the rate of immune recovery in HIV and TB co-infected adults observed during TB treatment with or without cART were examined in the present study. Secondarily, sex and nutritional status were examined as candidate modifiers of age-associated changes in CD4+ T-cell counts during TB therapy, to inform current understanding of immune recovery in TB and HIV-infected adults on cART. It was hypothesized that immune recovery following ART initiation will decline with increasing age and may be contingent on nutritional status.

2. Methods

2.1. Study design, parent study, and population

This study comprised a secondary analysis of differences in CD4+ T-cell recovery rates among 13–60-year-old HIV and TB co-infected patients during the time of pulmonary TB therapy, with or without cART, in Kampala, Uganda. Details of the design and

findings of the parent study have been reported elsewhere.³⁶ Briefly, an open-label randomized trial of simultaneous cART and TB treatment vs. immediate TB treatment with delayed cART until clinically indicated per standard of care, was implemented between October 2004 and October 2008. At the time, the standard for TB care in HIV infection included immediate TB treatment with ART initiated upon the development of an AIDS-defining opportunistic infection and/or when the absolute CD4+ T-cell count dropped below 200 cells/ μ l. All participants were cART-naïve, non-pregnant, without a history of TB, free of a World Health Organization (WHO) stage IV AIDS-defining condition, and immune competent (i.e. CD4+ T-cell count \geq 350 cells/ μ l) at enrollment. The present analysis included all patients with two or more CD4 assessments ($n = 208$) within the 6 months of follow-up from enrollment through the end of TB therapy.

2.2. Ethical approval

The protocol for the parent study was approved by the institutional review boards of the University of Georgia, Case Western Reserve University, University of California, San Francisco, the Joint Clinical Research Center and the Uganda National Council for Science and Technology. The sponsors of the parent study were not involved in the design or implementation of the present analysis, or in the interpretation of the results.

2.3. Follow-up, measurements, and variable definitions

At enrollment, standardized questionnaires were used to collect socio-demographic and health data. Study participants were evaluated monthly. Microscopic evaluation of TB disease clearance was done for each patient as needed and for all, regardless of indication, at months 0, 1, 2, and 5. HIV-1 RNA levels were measured using the Roche Amplicor assay at months 0, 1, 2, and 5; this assay has a minimum detection level of 400 copies/ μ l.

2.3.1. Primary outcome

The absolute CD4+ T-cell count was measured monthly via flow cytometry; this is the primary indicator of immune recovery. Absolute CD4+ T-cell counts (in cells/ μ l) were evaluated as a linear variable with the outcome matrix including all repeated measures of CD4 for each patient given the observational study design.³⁷

2.3.2. Determinants: cART, age, malnutrition

cART status was defined on the basis of randomization to concurrent cART vs. placebo during TB treatment. Baseline age was defined in categories of \leq 24, 25–29, 30–34, 35–39, and \geq 40 years, and was used as an indicator of differences in thymus function at cART initiation. Malnutrition was defined as underweight (body mass index (BMI) $<$ 18.5 kg/m²), or normal (BMI \geq 18.5 kg/m²). Viral load was defined as a time-varying covariate using the lag of log₁₀-transformed RNA values. Time was defined in months and analyzed as a linear variable.

2.3.3. Confounders/modifiers

Baseline factors considered potential confounders in this study included sex (male vs. female), employment (yes vs. no), smoking (current, former vs. never), marital status (never married, divorced/separated/widowed vs. married), and baseline functional status defined using the Karnofsky score (\geq 90 (i.e. able to function normally with minor signs of disease) vs. $<$ 90 (i.e. lower functional status)).

2.3.4. Statistical analyses

Multivariable random effects mixed linear regression models were fitted in SAS software version 9.2 (SAS Institute Inc.). An

unstructured covariance matrix was assumed in order to account for non-independence of repeated CD4+ T-cell measurements within individuals and accounted for potential within-subject differences in the trajectory of CD4+ T-cell change over time by including a random intercept for individual patients. Empirical standard errors were used for all estimations to ensure that significance tests were robust to any misspecification of the covariance matrix.

An extensive array of potential confounders of the relationship between age and change in CD4 values were considered. In addition to specified potential confounders, adjustments were made for cART status, malnutrition, viral load, and time. A lack of information on any confounding covariate was addressed analytically using the missing indicator method. The potential for modification in the association between age and change in CD4 values during follow-up by patient sex and baseline underweight status were examined. For this assessment, differences in likelihood ratio tests between nested models were used to determine the contribution of these factors – included in multivariate models as three-way interactions (age×time×sex or age×time×underweight) – to improvement of the overall model fit and hence the presence of significant heterogeneity.

3. Results

3.1. Sample characteristics

The parent study screened 253 HIV and TB co-infected patients for inclusion. Of these, 45 were excluded from this secondary analysis due to non-repeated CD4+ T-cell assessment and inability to determine whether the patient was allocated concurrent vs. non-concurrent cART in the parent study (Figure 1). The analytic sample included 208 individuals, with slightly more males (57%) than females, all with WHO HIV disease stage 1 or 2 at baseline. The average participant was 31.8 years old and the mean absolute CD4+ T-cell count at enrollment was 573 cells/ μ l. At enrollment, 23.6%, 26.9%, 21.2%, 16.8%, and 11.5% of the sample were aged <25, 25–29, 30–34, 35–39, and \geq 40 years. The majority of participants (64.7%) were employed, 27%

were current drinkers, 11% self-identified as current smokers, and 40.8% had more than 7 years of education (Table 1). There were no differences by age-group with respect to baseline health status indicators including hemoglobin levels, CD4+ T-cell count, log viral load, and BMI. However, the proportion of female patients declined significantly with increasing age category (Table 2). The WHO HIV disease stage did not differ by age category at enrollment (data not shown).

During the 6 months of TB therapy, the CD4+ T-cell count increased by approximately 2 CD4+ T-cells each month. However, among patients on TB therapy without cART, a net decline of 2.5 CD4+ T-cells per month was observed, whereas patients on concomitant TB therapy and cART gained approximately 8 CD4+ T-cells each month in the overall group. Overall, and within treatment strata, nutritional status was not independently associated with CD4+ T-cell recovery, although underweight patients gained more cells per month in comparison to patients of normal weight at enrollment. Each log increment in HIV RNA viral load was associated with a net deficit in monthly absolute CD4+ T-cells recovered, but this association was not statistically significant overall or within strata of concurrent vs. delayed cART. Over the study period, women on average gained more CD4+ T-cells per month compared to men in the overall sample. The magnitude and direction of sex-associated change in CD4+ T-cells differed by cART status. Among study participants on concurrent cART and TB treatment, women gained nearly 24 more CD4+ T-cells per month compared to men. On the other hand, among patients on TB treatment without cART, women in comparison to men lost approximately 7 CD4+ cells/ μ l per month (Table 3).

3.2. Effect of age, ART, and interactions with sex and BMI

In the entire sample, cART use during TB treatment was associated with an average gain of 16 CD4+ T-cells per month. Overall, the CD4+ T-cell recovery rate was higher for three of the four younger age categories in comparison to patients aged \geq 40 years, but these age-associated differences were not statistically significant. However, the magnitude and direction of age-associated differences in immune recovery varied based on

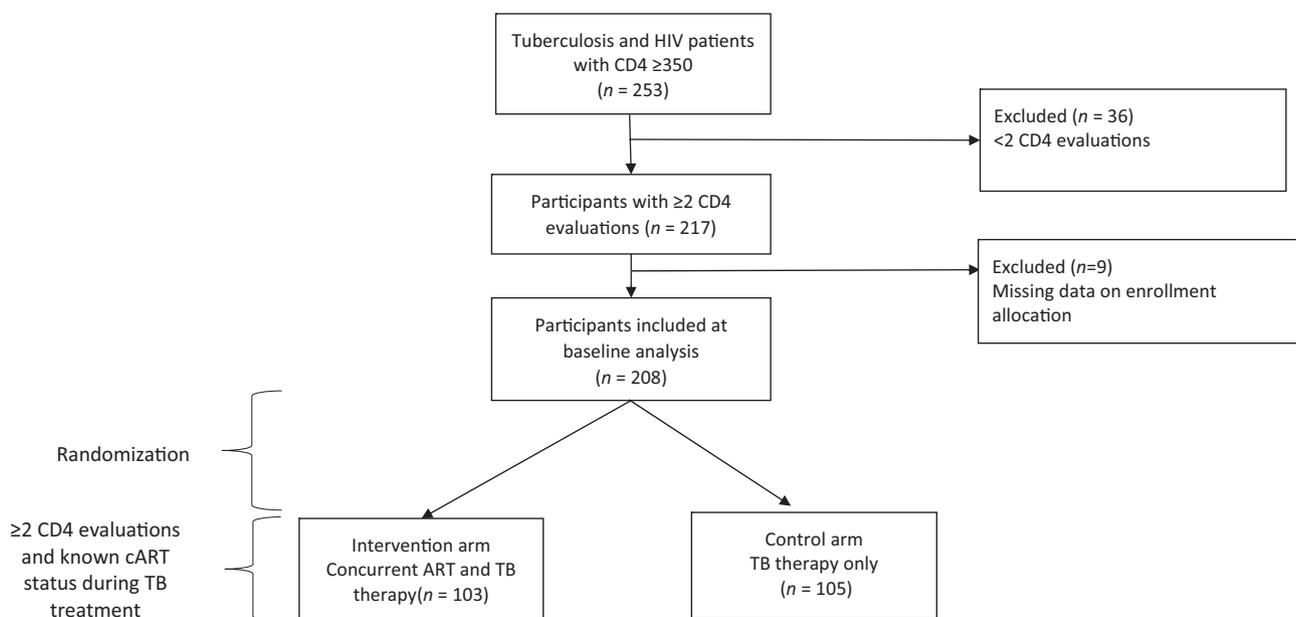


Figure 1. Secondary analysis study of a randomized clinical trial on TB therapy only versus concurrent antiretroviral and TB therapy in HIV (CD4 \geq 350 cells/ μ l) and TB co-infected participants in Kampala, Uganda, 2004–2008.

Table 1
Baseline socio-demographic, immunologic, and health status of 208 HIV and pulmonary tuberculosis co-infected Ugandans

	Mean	SD
Age, years	31.8	7.9
Baseline BMI, kg/m ²	19.8	2.5
Viral load	127 708	195 021
Log viral load	4.53	0.87
Absolute CD4 cell count, cells/ μ l	572.3	243.4
CD4 percent	30.1	9.93
Absolute CD8 cell count, cells/ μ l	877	476
Hemoglobin, g/dl	12.1	1.8
Baseline alanine aminotransferase	31.4	22.3
CD4:CD8 ratio	0.8	0.5
	<i>n</i>	%
Randomized to HAART	103	49.5
Age categories, years		
<25	49	23.6
25–29	56	26.9
30–34	44	21.2
35–39	35	16.8
40+	24	11.5
Underweight (BMI \leq 18.5 kg/m ²)	69	33.2
Marital status		
Single/never married	30	14.6
Currently married	98	47.6
Divorced/separated/widowed	78	37.8
Sex		
Male	118	56.7
Female	90	43.3
Employed	134	64.7
Educational status		
0–P5 education	57	30.1
P6–P7 education	55	29.1
S1–S3 education	36	18.9
S4–S6 education	42	21.8
Above secondary education	18	9.4
Behavioral risk factors		
Current alcohol drinker	56	27.2
Smoking status		
Never	146	70.2
Former	40	19.2
Current	22	10.6
Baseline health status		
Abnormal indication on general physical examination	130	62.5
Normal blood pressure	157	75.5
Karnofsky score \geq 90	83	39.9
Presence of a comorbid diagnosis	82	40.0
HIV WHO stage		
1	4	2.1
2	188	97.9

SD, standard deviation; BMI, body mass index; HAART, highly active antiretroviral therapy; WHO, World Health Organization.

concurrent cART status (p -value = 0.027 from the likelihood ratio test of differences in nested models with and without the age \times time \times ART interaction; data not shown). Similarly, stratification by cART identified a marginally significant qualitative age-associated interaction in CD4+ T-cell recovery (Table 3, age \times time \times ART interaction; p -value = 0.058). Among patients on

concurrent cART and TB therapy, the absolute CD4+ T-cell count increased from enrollment through the end of 6 months of TB therapy by an average value of 8 CD4+ T-cells/ μ l per month, and patients in the younger age strata all gained more CD4+ T-cells than those \geq 40 years. The magnitude of CD4+ T-cells recovered in patients on cART and TB treatment increased with younger age at enrollment. Conversely HIV-infected patients on TB therapy alone lost an average of 2.5 CD4+ T-cells/ μ l monthly, and the age-associated change in CD4 values was variable with a slower rate of recovery noted for only one of the four younger categories vs. the oldest age category.

The age-associated rate of immune recovery during the 6 months of TB treatment was dependent on baseline nutritional status (p -value age \times time \times BMI: $<$ 0.0358) and sex (p -value age \times time \times sex: $<$ 0.0001) among patients on concurrent TB and cART. Specifically, a faster rate of CD4+ T-cell count recovery was noted for patients in the four younger age strata compared to the oldest age category among female patients and among normally nourished patients at enrollment. As in the overall sample, the magnitude of CD4+ T-cells recovered during TB therapy was inversely proportional to patient age at enrollment. Conversely, the association between age and CD4+ T-cell recovery was variable and non-significant for male patients and for patients underweight at enrollment (Table 4).

4. Discussion

In this sample of immune-competent TB and HIV co-infected adult men and women followed through the end of anti-TB therapy with or without cART, an age-associated differential rise in CD4 values during 6 months of TB therapy was found among patients on concurrent anti-TB and cART, but not in patients on TB treatment without cART. For patients on simultaneous TB and ART therapies, the average monthly CD4+ T-cell gain was inversely proportional to age at enrollment. This inverse association between age at enrollment and the absolute number of CD4+ T-cells recovered was particularly strong and statistically significant among female HIV and TB co-infected adults whose BMI was \geq 18.5 kg/m² at enrollment.

In line with the study hypothesis, age \geq 40 years at ART initiation was associated with a sub-optimal immune recovery rate relative to younger age-groups. The results of the present study confirm the age-related sub-optimal immune recovery observed in adult HIV-infected patients from West Africa and South Africa,^{35,38} as well as similar findings in several developed country settings.^{14,16–18} None of these prior studies investigated HIV and TB co-infected patients. Thus our results corroborate these previous reports and suggest that co-infection with TB may not alter the age-associated absolute CD4+ T-cell recovery deficits reported previously.

These data confirm the previously reported age-associated immune recovery deficits in mostly immune-compromised HIV-infected adults and extend this observation to immune-competent

Table 2
Baseline description of HIV and pulmonary tuberculosis co-infected study participants by age at enrollment

	<25 years <i>n</i> = 49	25–29 years <i>n</i> = 56	30–34 years <i>n</i> = 44	35–39 years <i>n</i> = 35	40+ years <i>n</i> = 24	<i>p</i> -Value ^a
Absolute CD4+ T-cell, mean (SD)	572 (250)	558 (248)	627 (251)	561 (243)	524 (206)	0.6854
BMI, mean (SD)	19.9 (2.7)	20.0 (2.7)	19.6 (2.5)	19.7 (2.0)	20 (2.8)	0.8077
Log viral load, mean (SD)	4.39 (0.85)	4.58 (0.85)	4.64 (0.85)	4.64 (0.79)	4.36 (1.1)	0.6671
Female, <i>n</i> (%)	29 (59.2)	33 (58.9)	14 (31.8)	9 (25.7)	5 (20.8)	$<$ 0.0001
cART, <i>n</i> (%)	24 (49.0)	32 (57.1)	20 (45.5)	17 (48.6)	10 (42.7)	0.4088
Hemoglobin, g/dl, mean (SD)	11.7 (1.7)	11.9 (1.6)	12.3 (1.8)	12.4 (1.7)	11.7 (2.1)	0.3479

SD, standard deviation; BMI, body mass index; cART, combination antiretroviral therapy.

^a p -Values are based on the t -test of difference in means for continuous variables and the Chi-square test of difference in proportions for categorical variables.

Table 3

Monthly change in absolute CD4 cell count among HIV and tuberculosis co-infected Ugandan adults during 6 months of tuberculosis treatment in relation to age, concurrent cART, sex, nutritional status, time, and viral load^a

	Entire sample (n=208) ^b	TB therapy only, no ART (n=105)	Concurrent ART and TB therapy (n=103)
	Risk difference (95% CI)	Risk difference (95% CI)	Risk difference (95% CI)
Time (per month increment)	1.87 (−6.7, 10.5)	−2.5 (−11.2, 6.1)	8.2 (−5.0, 21.5)
BMI <18.5 vs. ≥18.5 kg/m ²	8.3 (−5.0, 27.0)	8.7 (−10.8, 28.1)	3.9 (−12.6, 20.4)
Female vs. male sex	10.7 (−3.72, 23.9)	−3.32 (−21.5, 15.0)	23.7 (−3.0, 50.4)
Viral load (per log ₁₀ increment)	−3.54 (−7.7, 0.6)	−3.3 (−8.2, 1.6)	−2.0 (−7.2, 3.2)
Punctuated ART vs. standard of care	16.3 (4.8, 27.8)	N/A	N/A
Age categories, years	<i>p</i> -Value age×time: 0.362	<i>p</i> -Value age×time: 0.495	<i>p</i> -Value age×time: 0.378
<25	16.1 (−2.18, 34.4)	−2.9 (−23.9, 18.0)	33.2 (4.25, 62.2)
25–29	12.4 (−3.3, 28.0)	−1.8 (−20.7, 17.1)	21.8 (−1.0, 44.6)
30–34	12.7 (−3.8, 29.3)	9.2 (−11.8, 30.2)	4.34 (−19.2, 27.9)
35–39	−5.3 (−27.1, 16.5)	−21.7 (−51.0, 7.7)	4.91 (−36.5, 46.3)
40+	Ref.	Ref.	Ref.
	When the <i>p</i> -value associated difference in likelihood ratio test between nested models was <0.05, stratification was done by levels of the effect modifier	Interaction: age×time×ART; <i>p</i> -value=0.0575	

cART, combination antiretroviral therapy; TB, tuberculosis; ART, antiretroviral therapy; CI, confidence interval; BMI, body mass index; N/A, not applicable.

^a All effects were estimated from mixed general linear models using PROC Mixed in SAS version 9.2. Risk differences represent the average monthly change in absolute CD4 values in relation to shown predictors. Age effects were derived from the following model: [CD4it] = $\alpha + \beta_1 \text{time} + \beta_2 \text{age} + \beta_3 \text{time} \times \text{age} + \beta_4 \text{sex} + \beta_5 \text{baseline underweight status} + \beta_6 \text{lag viral load} + \beta_7 \times \text{marital status} + \beta_8 \text{employment} + \beta_9 \text{smoking status}$. The BMI effect was derived from the following model: [CD4it] = $\alpha + \beta_1 \text{time} + \beta_2 \text{baseline underweight status} + \beta_3 \text{time} \times \text{baseline underweight status} + \beta_4 \text{sex} + \beta_5 \text{age} + \beta_6 \text{lag viral load} + \beta_7 \times \text{marital status} + \beta_8 \text{employment} + \beta_9 \text{smoking status}$.

^b The sample size in each column is based on unique individuals included in multivariate analyses; numbers within age strata are derived from the baseline file.

HIV-infected populations. Of note, there was no age-related difference in CD4+ T-cell count at enrollment, thus excluding the presence of lower CD4+ T-cell counts in older persons with HIV at the beginning of cART as an alternative explanation for this finding. The pattern of association observed is in line with previous reports that the CD4+ T-cell immune recovery rate given cART may be more effective in younger PLWH, in part due to relatively more preserved thymic function.¹⁴

The study finding of significant age-related differences in immune recovery is not corroborated by investigations among HIV-infected adults from Uganda^{30,31} and Barbados.³⁹ Important methodological differences were noted between these latter studies and the present study that may in part explain this inferential divergence. The present study included HIV and TB co-infected adults and was adjusted for major known confounders/modifiers of immune function including nutritional status and several socio-demographic factors. Further, coincident TB infection was controlled for by design, allowing the derivation of more thoroughly adjusted estimates of effect compared to the previous reports.^{30,31}

Neither sex nor nutritional status was an independent predictor of overall immune recovery in this study. However, both factors modified the age-associated differentials in immune recovery rate. The observation of age-related differences in immune recovery among nutritionally adequate but not underweight adults at ART initiation is noteworthy and suggests that any age-related immune

recovery advantage conferred by preserved thymic function in younger HIV-infected adults is over-ridden by immune dysregulation due to nutritional impairment. The significance of stronger age-related deficits in immune recovery among women is less clear and warrants further elucidation in future studies. Female sex may be a surrogate indicator of greater compliance or generally better health-seeking behavior or health status at cART initiation, which impacts favorably on the immune recovery rate during TB therapy. Several African studies have associated female sex with a greater magnitude of immune recovery and greater overall adherence to ART,^{40,41} suggesting that sex differentials in adherence to cART may be important.

In the present study, however, adherence to both TB and ART was similarly high for men and women because all were given directly observed therapy, but underweight status was more prevalent in men than women at enrollment. This study group has previously reported sex differences in adiposity, TB-associated lean tissue wasting, and relationships to mortality among Ugandan adults.⁴² The relative contribution of sex-related differences in metabolic dysregulation and of sex-related behavioral differences such as adherence driving the observed age-related differentials in CD4+ T-cell recovery deserves further elucidation in future studies to inform effective intervention strategies.

This study is subject to important limitations that should lead to cautious interpretation of the findings. First, the observational design of this study limits the ability to conclude that advanced age

Table 4

Nutritional status- and sex-dependent age-related monthly increases in absolute CD4 counts in tuberculosis and HIV co-infected patients on concurrent antiretroviral therapy during 6 months of tuberculosis treatment in Kampala, Uganda^a

	Baseline nutritional status		Sex	
	BMI <18.5 kg/m ² (n=36)	BMI ≥18.5 kg/m ² (n=67)	Female (n=50)	Male (n=53)
	Risk difference (95% CI)	Risk difference (95% CI)	Risk difference (95% CI)	Risk difference (95% CI)
Age categories, years	Age×time: <i>p</i> =0.954	Age×time: <i>p</i> <0.001	Age×time: <i>p</i> =0.006	Age×time: <i>p</i> =0.795
<25	−1.5 (−30.9, 28.0)	56.0 (22.8, 83.4)	52.8 (18.7, 86.9)	14.6 (−15.6, 44.7)
25–29	17.3 (−16.6, 49.2)	50.2 (21.0, 73.2)	53.7 (25.8, 81.4)	5.2 (−19.6, 30.0)
30–34	0.1 (−41.1, 41.2)	36.7 (8.2, 57.2)	47.0 (11.6, 82.4)	6.5 (−19.0, 32.1)
35–39	14.6 (−21.6, 50.8)	30.1 (−0.3, 60.4)	26.5 (−21.7, 74.8)	−0.2 (−32.3, 31.9)
40+	Ref.	Ref.	Ref.	Ref.
	<i>p</i> -Value age×time×BMI: 0.036		<i>p</i> -Value age×time×sex: <0.001	

^a Estimates were derived from linear mixed models implemented via SAS PROC Mixed. The model assumes an unstructured covariance for within-subject repeated measures of CD4 values. All estimates are adjusted for time, patient sex, and lag viral load.

at enrollment leads to poor immune recovery. Second, this analysis included a small sample of HIV and TB co-infected patients on concurrent ART and TB therapy. Consequently between 10 and 32 patients were in the five age-strata specified. Third, the significant association between advanced age and lower immune recovery rate noted in this study was evident in sub-group analyses. However, given smaller sample sizes in sub-group analyses, one would have expected less power to detect significant associations; this did not materialize in this study. Future studies are needed from resource-limited settings, with careful consideration of nutritional status and patient socio-demographic factors, to confirm or refute these findings. The strengths of this study include the rigorous control for important confounders, evaluation of effect modification, and the use of a sample of HIV and TB infected adults that allowed the simultaneous contrasting of the differential impact of age on immune responses in the context of TB treatment alone versus concomitant TB and ART.

In conclusion, it was found that given cART, the recovery of CD4+ T-cells was age-dependent and that younger patients recovered their CD4+ T-cells at a higher rate than their older counterparts. Moreover, the rate of immune recovery was affected by gender and nutritional status. These findings suggest that age, sex, and nutritional status may be important determinants of health outcomes in HIV and TB co-infected adults. As a result, therapeutic approaches may need to be tailored in accordance with these factors. The data from this study suggest that increased monitoring and nutritional support as adjunct therapy should be considered for HIV-positive patients with TB and that those starting cART at an older age may benefit from this intervention. The mitigation of malnutrition in TB and HIV co-infected patients, which is a modifiable risk factor, may enhance immune recovery in adult PLWH on cART.

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