**The effect of antiretroviral therapy and CD4 count on markers of infectiousness in HIV-associated tuberculosis**

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**Running head:** ART and CD4 in HIV-associated TB

**ABSTRACT**

Clinical features of tuberculosis influence infectiousness. This cross-sectional study examined the effect of combination antiretroviral therapy (cART) and CD4 on sputum smear- positivity (SS+) and pulmonary cavitation among 1589 (1185/1589 HIV-positive) miners in South Africa. Proportions SS+ varied non-linearly by CD4 with greatest proportions SS+ (55.3%) in the lowest stratum (<100 cells/µL). Adjusted prevalence ratio (aPR) for SS+; on vs. off cART was 0.90 (95% confidence interval [CI] 0.73-1.11). Proportions with cavitation varied linearly with CD4, with no independent cART effect (aPR 1.17 [95%CI 0.80-1.71]). cART did not independently affect SS+ or cavitation, but may increase infectiousness via CD4 recovery.

**Keywords:** HIV-associated tuberculosis; antiretroviral therapy; CD4 count; infectiousness; pulmonary cavitation

**INTRODUCTION**

Clinical features of tuberculosis vary by degree of immunosuppression and sputum bacillary density has been widely understood to be linearly related to CD4 count, as suggested by some studies1-2. As sputum smear-positivity (SS+) and pulmonary cavitation are associated with tuberculosis transmission3-7, an effect of cART may have clinical and public health implications and has not previously been fully assessed.

This cross-sectional analysis aimed to examine the effect of cART and CD4 count on sputum smear status and radiological features of tuberculosis. This gold-mining workforce in South Africa has tuberculosis case notification rates around 3000/100000/year8 and HIV prevalence estimated at 29% in 20019.

**METHODS**

*Tuberculosis case ascertainment*

Prospective case ascertainment occurred within Thibela TB, a cluster-randomised trial of a tuberculosis prevention intervention10, from February 2006 to June 2011. Participants were individuals treated for tuberculosis at participating mine health services. Data were abstracted from healthcare records using standardised case report forms. Chest radiographs taken closest to the date of starting tuberculosis treatment (“TB treatment start”) were read by investigators, masked to clinical details. All individuals with the necessary data available were included in the analysis.

*Eligibility criteria*

Only the first tuberculosis episode occurring within the study period per individual was included, whether it was a new or retreatment episode. Exclusions were those with a treatment outcome of “not TB”; or non-tuberculous mycobacteria (NTM), but not *M. tuberculosis*, on culture. The analysis of chest radiographs included only those with radiographs taken <60 days before or after TB treatment start.

*Definitions*

“TB treatment start” was the date that tuberculosis treatment started. HIV status was considered negative if there was a negative HIV antibody test result after TB treatment start or <6 months before (with no subsequent positive test); or positive if there was a positive result <=28 days after TB treatment start. cART start dates were used to ascertain cART status at TB treatment start. Those on cART <=90 days were analysed separately due to possible immune reconstitution inflammatory syndrome (IRIS)11. The CD4 count closest to TB treatment start was used and considered missing if measured >365 days from TB treatment start. SS+ was defined as any smear positive (including “scanty”) for acid and alcohol fast bacilli (AAFB) during three months prior to TB treatment start and smear negativity >=1 negative and no positive smears during that time. Tuberculosis treatment outcomes were recorded according to standard World Health Organization definitions12. Site(s) of disease were recorded as in clinical records and categorised as pulmonary, extrapulmonary, disseminated/miliary or combinations thereof.

*Laboratory methods*

Routine laboratories used fluorescence microscopy. Cultures were done using BACTECTM Mycobacterial Growth Indicator Tube 960 system [BD Diagnostics System, Sparks MD]. No additional laboratory investigations were done for this observational analysis.

*Statistical analysis*

Analyses were done using STATA v.12 (STATA Corporation, College Station, Texas). Categorical variables were compared using chi-squared or Fisher’s exact tests and continuous variable distributions using the Kruskal-Wallis test. Multivariable analyses were done using log binomial regression. The effect of cART was examined by comparing those on cART for >90 days with HIV-positive individuals not on cART. Potential confounders considered were age, gender, episode type, number of sputa examined and CD4 count.

*Ethical considerations*

Research Ethics Committees of the University of Kwa-Zulu Natal and the London School of Hygiene and Tropical Medicine approved this study. Ethics committees overseeing the parent study, Thibela TB, approved data collection without individual informed consent.

**RESULTS**

*Study population*

In the complete Thibela TB dataset, 5957 tuberculosis episodes were recorded for 5858 individuals.

Exclusions were: second or subsequent episode in the same individual (N=99); missing TB treatment start date (N=50); culture of NTM only (N= 496); inconsistent HIV and CD4 data (N=2) and treatment outcome “not TB” (N=2). HIV status was unknown for 2567 individuals and cART status unknown for 1152/2337 HIV-positive individuals (figure S1).

Among 1589 individuals included (97% male, median age 43 years [interquartile range {IQR} 38, 48], consistent with workforce demographics10), 1185 (74.6%) were HIV-positive and 463 (39.1%) taking cART at TB treatment start. 817/1185 (69.0%) HIV-positive individuals had CD4 counts available (table 1), with median CD4 count 153 cells/µL (IQR 80, 265). The median time on cART was 300 days (IQR 100, 666, N=463), with 109 (23.5%) on cART for ≤90 days. CD4 counts were not associated with duration of cART (Kruskal-Wallis p=0.492 for comparison of median CD4 counts by duration of cART, N=297).

*Clinical features of tuberculosis disease*

The majority (1054/1561, 67.5% with data available) were new tuberculosis episodes. Retreatment episodes were commonest among those on cART for >90 days (186/344, 54.1%). Proportions SS+ varied by HIV/cART status group (table 1).

Among HIV-positive individuals, including all sites of disease, proportions SS+ varied by CD4 count, with 110/199 (55.3%) SS+ in the lowest stratum of CD4 count <100 cells/µL; 74/179 (41.3%) with CD4 count 100-199 cells/µL; 69/148 (46.6%) with CD4 count 200-349 and 30/72 (41.7%) with CD4 >349 cells/µL (table S1; chi-squared p=0.035).

*Factors associated with smear-positivity*

Overall 54.2% (599/1106) of episodes were SS+. cART did not affect proportions SS+ (unadjusted prevalence ratio [PR] 0.97; 95% CI 0.84-1.13 for on cART for >90 days versus not on cART) (table S1).

In multivariable analysis, (N=541 with data available), adjusted prevalence ratio (aPR) for SS+ in those on cART for >90 days versus not on cART was 0.90 (95% confidence interval [CI] 0.73-1.11), adjusted for episode type (new or retreatment), CD4 count and number of sputa examined.

*Effect of CD4 count and cART on sputum smear status among culture-positive, pulmonary tuberculosis episodes*

In this subgroup, 32/564 (5.7%) had miliary/disseminated and 33/564 (5.9%) extrapulmonary in addition to pulmonary disease. Proportions SS+ varied by study group (chi-squared p=0.009) and, among HIV-positive individuals, by CD4 count (chi-squared p=0.005), forming a J-shaped curve, with highest proportions SS+ in the lowest CD4 stratum (figure 1).

On multivariable analysis, aPR for SS+ in those on cART for >90 days versus not on cART (n=273 included), adjusted for episode type, CD4 count and number of sputa examined, was 0.92 (95% CI 0.73-1.15).

*Sputum bacillary density among culture-positive tuberculosis episodes*

Of 1589 individuals included in this analysis, 669 had positive sputum culture and known smear status. HIV-negative individuals were most likely to have “3+” positive smear (38.4%). Among 363 HIV-positive individuals with known CD4 count, “2+” or “3+” smear grade was seen in 52/123 (42.3%) of those with CD4 count <100 cells/µL; 22/112 (19.6%) with CD4 100-199 cells/µL; 20/87 (23.0%) with CD4 200-349 cells/µL and 9/41 (22.0%) with CD4 >=350 cells/µL (chi-squared comparing “scanty” or 1+ vs. 2+ or 3+ in each CD4 stratum p=0.004).

*Association of CD4 count and cART with chest radiograph appearance*

Of 1589 individuals included, 1086/1588 with data available had pulmonary disease. 533/1086 (49.1%) had chest radiographs available; four were excluded because they were smear and culture-negative and 34 because the radiograph was taken >60 days from TB treatment start, leaving 495 individuals in this analysis.

24/495 (4.8%) had extrapulmonary and 37/495 (7.5%) disseminated in addition to pulmonary disease. 318/487 (65.3%) with data available were new episodes; 370/495 (74.8%) were SS+ and 385/423 (91.0%) culture positive. 224/495 (45.3%) were HIV-positive not on cART; 28 (5.7%) on cART for <=90 days; 87 (17.6%) on cART for >90 days and 156 (31.5%) HIV-negative. Pulmonary cavitation was most common among HIV-negative individuals (109/156, 69.6%); vs. 64/224 (28.6%) HIV-positive not on cART; 10/28 (35.7%) on cART <=90 days and 37/87 (42.5%) on cART >90 days (chi-squared p<0.001; table S2).

Overall, cavitation was strongly associated with SS+, with positive smears in 183/220 (83.2%) of those with and 187/275 (68.0%) without cavitation (chi-squared p<0.001). However, stratifying by HIV status, this association was seen among HIV-negative (32/47 [68.1%] SS+ without vs. 98/109 [89.9%] with cavities) but not HIV-positive (155/229 [68%] SS+ without vs. 85/111 [76.6%] with cavities) individuals. PR for SS+ in those with versus without cavitation in the HIV-negative group was 1.32 (95% CI 1.08-1.62, p=0.008) and in the HIV-positive group 1.13 (95% CI 0.98-1.29, p=0.09, p-value for interaction between HIV status and cavitation was 0.055). Combining HIV-positive and -negative individuals (n=495), pulmonary cavitation was associated with higher sputum bacillary density, with “3+” smears in 48/275 (17.5%) without and 80/220 (36.4%) with cavitation (Fisher’s exact test comparing each smear grade in those with vs. without cavitation, p<0.001). The proportions with cavitation varied by study group (chi-squared p<0.001) and linearly by CD4 count (chi-squared p=0.001) (table S3; figure 1).

Adjusting for CD4 count and episode type, including 254 HIV-positive individuals with known CD4 count, PR for cavitation among those on cART for >90 days, versus those not on cART was 1.17 (95% CI 0.80-1.71)(table S3).

**DISCUSSION**

In this mining workforce, in HIV-associated tuberculosis, high bacillary density was seen in the highest and lowest CD4 count strata. Proportions with cavitation in pulmonary disease increased linearly with CD4 counts. cART did not have an independent effect on sputum smear positivity or pulmonary cavitation, but may increase the proportions with pulmonary cavitation and high bacillary density via its effect on CD4 count, thus having an overall effect of increasing infectiousness.

Although the increase in proportions with smear-negative tuberculosis disease in HIV-positive versus –negative individuals is well documented13-14, with contrasting findings on the effect of CD4 count1-2, 20-22, there is little published information on associations of cART with factors associated with tuberculosis infectiousness. Our data, including associations between HIV, cART, CD4 count and bacillary density suggest different mechanisms of smear positivity depending on CD4 count. At low CD4 counts, smear-positivity must be via a mechanism other than cavitation, probably multibacillary, disseminated disease in the very immunosuppressed, consistent with the observation that mycobacteraemia occurs more frequently at lower CD4 counts23.

Median CD4 counts were lower among those not on cART than those taking cART and many individuals were excluded because of unknown or undocumented HIV status. This suggests poor testing coverage and late initiation of cART, perhaps due to poor linkage to care, and needs addressing in this setting. Time on cART was not strongly associated with increasing CD4 counts, which is unexpected and possibly related to the relatively short durations of cART use seen in this population.

Limitations of this study include missing data, due to reliance on routine records and lack of virological monitoring to confirm effective cART. CD4 counts used here were not updated, potentially leading to residual confounding after adjusting for CD4 counts in multivariable models. Another factor particular to this population is silicosis, but there is no evidence that silicosis affects sputum smear status. As routine and parent study data were used for the study, we were unable to perform additional microbiological investigations, such as blood cultures, which could have enhanced interpretation of the results

These findings are relevant largely to settings of high HIV prevalence, where cART use means that large numbers of individuals survive, but with incomplete immune reconstitution, especially those starting cART at very low CD4 counts24. These individuals are at high risk for tuberculosis and represent a source of transmission to others, albeit with shorter duration of infectiousness25. This is particularly relevant to congregate, including nosocomial, settings, where the immunosuppressed would be exposed to those with infectious tuberculosis. .

Additional factors, not measured in this analysis, are important in determining transmission risk, such as duration of infectiousness25, cough and aerosol production26-27. However, relationships between cART, CD4 count and clinical features of HIV-associated tuberculosis can add to the understanding of tuberculosis epidemiology in high HIV-prevalence.

These data also underline the importance of infection control for tuberculosis and may be of use in mathematical modelling of the effect of cART on tuberculosis epidemiology in settings of high HIV prevalence and in predicting and planning for the impact of new diagnostic tests such as Xpert MTB/RIF, which rely on bacillary density in sputum specimens.

This study must be viewed in the context of other work demonstrating reduced tuberculosis incidence and prevalence among HIV clinic patients and in populations with high HIV prevalence28-30, attributable to the reduced risk of active tuberculosis with higher CD4 counts and time on cART31-32. Ultimately, a well-implemented combination of evidence-based measures is likely to be required to control HIV-associated tuberculosis.

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**FIGURE CAPTIONS**

Figure 1: Proportions of culture-positive, pulmonary tuberculosis episodes with positive sputum smears, by study group (1a) and by CD4 count for 302 individuals where CD4 count known (1b). Proportions with lung cavitation seen on chest radiograph, by study group including 495 tuberculosis episodes (1c) and by CD4 count including 281 episodes in HIV-positive individuals with known CD4 count (1d)

**TABLES**

Table 1: Clinical features of tuberculosis and HIV disease in four study groups (totals with data available given for each variable)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Variable | HIV+ not on cART(n=722) | HIV+ on cART <=90 days(n=109) | HIV+ on cART >90 days(n=354) | HIV negative(n=404) | Total(n=1589) |
|  |  |  |
| Male (n/N, %) | 690/720 (95.8) | 104/109 (95.4) | 348/354 (98.3) | 398/404 (98.5) | 1540/1587 (97.0) |
|  |  |  |  |  |  |
| Median age1 (years; IQR) | 42 (37, 47) | 43 (38, 47) | 44 (38, 48) | 46 (40, 50) | 43 (38, 48) |
|  |  |  |  |  |  |
| Episode type (new/retreatment) (n) | 711 | 107 | 344 | 399 | 1561 |
|  | New episode (n [%]) | 522 (73.4) | 73 (68.2) | 158 (45.9) | 301 (75.4) | 1054 (67.5) |
|  |
| Site of disease (n) | 704 | 106 | 329 | 385 | 1524 |
|  | Pulmonary (n [%]) | 382 (54.3) | 55 (51.9) | 212 (64.4) | 312 (81.0) | 961 (63.1) |
|  | Extrapulmonary | 143 (20.3) | 27 (25.5) | 66 (20.1) | 57 (14.8) | 293 (19.2) |
|  | Pulmonary and extrapulmonary | 38 (5.4) | 10 (9.4) | 16 (4.9) | 5 (1.3) | 69 (4.5) |
|  | Miliary/disseminated | 141 (20.0) | 14 (13.2) | 35 (10.6) | 11 (2.9) | 201 (13.2) |
|  |
| CD4 count2 (n)  | 520 | 67 | 230 | - | 817 |
|  | Median (IQR) | 129 (69, 220) | 189 (92, 295) | 225 (109, 323) | - | 153 (80, 265) |
|  |
| CD4 count (cells/µL) (n) | 520 | 67 | 230 | - | 817 |
|  | <100 (n [%]) | 205 (39.4) | 20 (29.9) | 47 (20.4) | - | 272 (33.3) |
|  | 100-199 | 169 (32.5) | 16 (23.9) | 57 (24.8) | - | 242 (29.6) |
|  | 200-349 | 100 (19.2) | 21 (31.4) | 84 (36.5) | - | 205 (25.1) |
|  | >=350 | 46 (8.9) | 10 (14.9) | 42 (18.3) | - | 98 (12.0) |
|  |
| Days between TB treatment start and CD4 count measurement [median (IQR)] |
|  |  | 24 (8, 98) | 38 (15, 115) | 36 (11, 115) | - | 28 (9, 105) |
|  |
| Smear status (n) | 515 | 72 | 234 | 285 | 1106 |
|  | Positive (n [%]) | 267 (51.8) | 28 (38.9) | 118 (50.4) | 186 (65.3) | 599 (54.2) |
|  |
| Smear grade (n) | 515 | 72 | 234 | 285 | 1106 |
|  | Negative (n [%]) | 248 (48.2) | 44 (61.1) | 116 (49.6) | 99 (34.7) | 507 (45.8) |
|  | Scanty | 13 (2.5) | 1 (1.4) | 5 (2.1) | 4 (1.4) | 23 (2.1) |
|  | 1+ | 123 (23.9) | 17 (23.6) | 57 (24.4) | 59 (20.7) | 256 (23.2) |
|  | 2+ | 56 (10.9) | 5 (6.9) | 22 (9.4) | 36 (12.6) | 119 (10.8) |
|  | 3+ | 75 (14.6) | 5 (6.9) | 34 (14.5) | 87 (30.5) | 201 (18.2) |
|  |
| Culture status (n) | 443 | 65 | 207 | 233 | 948 |
|  | Positive (n [%]) | 336 (75.9) | 44 (67.7) | 140 (67.6) | 176 (75.5) | 696 |

1 Kruskal-Wallis test comparing age distribution between HIV-positive and HIV-negative groups, p<0.001

2Kruskal-Wallis test comparing CD4 count distributions in HIV+ on cART >90 days versus HIV+ not on cART, p<0.001

IQR = interquartile range

**FIGURES**