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Impact of tuberculosis treatment on CD4 cell count, HIV RNA, and p24 antigen in patients with HIV and tuberculosis

C. Wejse [a,b,*], A. Furtado [a,c], C. Camara [c], M. Lüneborg-Nielsen [d], M. Sodemann [c,e], J. Gerstoft [d], T.L. Katzenstein [d]

[a] Department of Infectious Diseases, Aarhus University Hospital, Brendstrupgaardsvej, 8200 Aarhus N, Denmark
[b] GloHAU, Center for Global Health, School of Public Health, Aarhus University, Aarhus, Denmark
[c] Bandim Health Project, INDEPTH Network, Statens Serum Institut, Bissau, Guinea-Bissau
[d] Department of Infectious Diseases, Rigshospitalet, Copenhagen, Denmark
[e] Department of Infectious Diseases, Odense University Hospital, Odense, Denmark

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SUMMARY

Objectives: To describe HIV RNA levels during tuberculosis (TB) infection in patients co-infected with TB and HIV. Moreover, to examine the p24 antigen profile during TB treatment.
Methods: We examined the changes in CD4 cell count, HIV RNA, and p24 levels during anti-tuberculous therapy in a group of TB/HIV-1 co-infected and HIV-untreated patients from Guinea-Bissau.
Results: A total of 365 TB patients were enrolled, of whom 76 were co-infected with HIV-1 and 19 were dually infected with HIV-1 + HIV-2. No significant changes in CD4, HIV RNA, or p24 levels were found during 8 months of TB treatment. HIV RNA levels correlated well with p24 (Spearman's $R^2 = 0.52$, $p < 0.00001$) and both markers were strong predictors of mortality. Initial HIV RNA levels correlated with a clinical TB severity index – the TBscore (Spearman's $R^2 = 0.23$, $p = 0.02$) – and the TBscore decreased dramatically during TB treatment although HIV RNA levels remained unchanged.
Conclusion: We found no significant changes in CD4, HIV RNA, or p24 antigen levels during 8 months of TB treatment among TB/HIV co-infected individuals, who did not receive antiretroviral treatment. The markers were unaffected by a strong improvement in TBscore and all three markers showed predictive capacity for mortality risk.

1. Introduction

Tuberculosis (TB) and the human immunodeficiency virus (HIV) are among the world's most severe public health threats. We have previously shown that 35% of TB patients in Guinea-Bissau are co-infected with HIV;[1] in South Africa the percentage is estimated to be >60%.[2] The progression of HIV seems to be accelerated by *Mycobacterium tuberculosis* co-infection[3,4], and active TB has been associated with marked increases in HIV RNA levels.[3] Yet, reports on HIV RNA levels during *M. tuberculosis* (MTB) infection vary. Goletti et al.[3] found increased levels at the time of MTB diagnosis among HIV-infected patients receiving no antiretroviral treatment (ART), with lower levels prior to infection as well as after anti-mycobacterial treatment. Likewise, a study from the UK found significant decreases in viral load after treatment of TB irrespective of concurrent ART treatment. Though patients receiving dual therapy had more pronounced decreases in viral load, the HIV-untreated patients also had substantial declines: from 4.91 log HIV RNA at TB diagnosis to 3.97 log HIV RNA at month 12.[5] However, studies from developing countries have not been able to reproduce these findings. Studies from Côte d'Ivoire, South Africa, and Ghana have shown either no change[6,7] or increasing viral loads during anti-TB treatment. A study from Uganda found an increase in viral load among patients with low HIV RNA at baseline and a decrease among patients with high viral load at baseline.[8] Interestingly, various inflammation markers, i.e., sTNF-α, β-2 microglobulin, and neopterin,[6] as well as interleukin 6 (IL-6),[7,9] have been shown to decrease significantly during anti-TB treatment, but in none of these studies were the changes paralleled by changes in viral load or CD4 cell count.

The HIV-1 antigen p24 has been proposed as an alternative marker of HIV progression. p24 has been shown to correlate well with HIV RNA and to have a prognostic value.[10] However, only a few studies have examined the prognostic capabilities of p24 measurements. Furthermore, the p24 antigen profile during TB treatment of HIV co-infected patients not receiving ART has not previously been analyzed.

* Corresponding author.
E-mail addresses: wejse@dadlnet.dk, wejse@skejby.rm.dk (C. Wejse).

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Dean et al. have reported an increase in CD4 count in British HIV-1/TB co-infected patients treated for TB but not receiving ART.[5] There are several reports indicating that the CD4 count is low in active TB, even in the HIV-uninfected,[11–13] but it was only reported recently that CD4 counts in the HIV-uninfected were unaffected by anti-tuberculous therapy.[14]

This study reports changes in CD4 cell count, HIV RNA, and p24 levels during anti-tuberculous therapy lasting 8 months in a group of TB/HIV-1 co-infected patients from Guinea-Bissau.

2. Methods

The study site was the Bandim Health Project, a Health and Disease Surveillance Site (HDSS) located in Guinea-Bissau, West Africa, a member of the INDEPTH network of HDSS. Guinea-Bissau has a poor, urban population of 102 000, with a high TB incidence (470/100 000).[15]

2.1. Study design

We used the framework of a randomized trial of vitamin D supplementation for TB patients, the TBVD trial,[1] for the study of CD4 count, HIV RNA, and p24 levels among HIV-1/TB co-infected patients. Inclusion criteria were a diagnosis of TB either by sputum examination (smear microscopy, no culture was available) or by the World Health Organization (WHO) clinical criteria,[16,17] age $\geq$15 years, and residence in the study area. There were no exclusion criteria. None of the HIV-infected patients received ART, which was not available in Guinea-Bissau at the time. All patients received anti-tuberculous treatment consisting of 2 months of daily observed treatment (DOT) with ethambutol (E), isoniazid (H), rifampin (R), and pyrazinamide (Z), followed by 6 months of H+E.

2.2. Follow-up

Patients were invited to clinical follow-up examinations after 2, 5, and 8 months of treatment, and a household visit after 1 year, until death or until moving out of the study area. Verbal autopsies were conducted on all deaths; a physician used a standardized questionnaire to obtain information from the closest relative. No traumatic deaths were recorded; all died of causes that could be related to TB or HIV.

2.3. Measurements

The severity of TB was assessed by TBscore, recording signs, symptoms, and anthropometry: cough, chest pain, hemoptysis, dyspnea, night sweating, anemia, tachycardia, lung auscultation findings, fever, body mass index, and mid upper arm circumference, with a maximal score of 13. The TBscore has been validated in another cohort and has been grouped into severity classes as follows: I, 0–5 points; II, 6–7 points; and III, $\geq$8 points.[18]

Blood samples were analyzed for HIV at the National Public Health Laboratory in Guinea-Bissau. Sera were screened using Enzygnost Anti-HIV 1/2 Plus (Behring Diagnostics GmbH, Marburg, Germany) and reactive sera were confirmed with Capillus HIV-1/HIV-2 (Cambridge Diagnostics, Galway, Ireland), as well as ImmunoComb II HIV-1&2 Bispot (Orgenics, Yavne, Israel).

T-lymphocyte subsets were determined at the National Public Health Laboratory, Guinea-Bissau, by flow cytometry (FACStrak; Becton Dickinson, San Jose, CA, USA) with the use of three two-color immunofluorescence reagents, CD45/CD14, CD3/CD4, and CD3/CD8 (Simultest, Becton Dickinson, San Jose, CA, USA). Leukocyte and differential counts were performed manually.

HIV RNA and p24 antigen analyses were performed at the AIDS Laboratory at Rigshospitalet, Copenhagen, Denmark. For the HIV-1 RNA quantification, 100 μl of plasma was suspended in 900 μl phosphate buffered saline (PBS) and the samples were analyzed by COBAS AmpliPrep/COBAS Taqman 48 HIV-1 Test v1.0 and v2.0 (Roche diagnostics, GmbH, Mannheim, Germany), in accordance with the manufacturer's instructions. Due to the reduction in input volume (100 μl vs. 1000 μl routinely used), the lower limit of detection (LLD) was set at 400 copies/ml in v1.0 and 200 copies/ml in v2.0. Due to a higher than expected proportion of patients with an undetectable viral load using v1.0, we chose to re-analyze with v2.0 once this method became available.

p24 was performed using the PerkinElmer p24 Ultrasensitive Assay Protocol (PerkinElmer, Life Sciences, Inc., Boston, MA, USA), in accordance with the manufacturer's instruction, except for the use of the virus disruption buffer as described by Schüpbach et al.[19] According to the manufacturer's instructions, the lower level of detection is run-specific.

2.4. Statistical analyses

p24 and HIV RNA measurements were log-transformed to approximate normal distribution. We expressed variables by their means or medians and standard deviations or range. Pearson's Chi-square test was used to assess statistical differences in proportions between groups, Student's *t*-test to assess differences in means between two groups when normal distribution was present, and the Wilcoxon rank-sum test was used when non-parametric analysis was needed. Linear and logistic regression analyses were used as multivariate models to adjust clinical outcomes for other factors. Cox regression and the Wilcoxon–Breslow–Gehan log rank test for equality of survivor functions were used to analyze mortality, and Kaplan–Meier survival graphs were used to estimate the survival function. A two-sided p-value of < 0.05 was considered significant. Statistical analyses were performed with STATA version 9 software (StataCorp, TX, USA).

3. Results

Recruitment began in November 2003 and ended in December 2005. Follow-up was completed in December 2006. A total of 365 TB patients were enrolled; 76 were co-infected with HIV-1 and 19 were dually infected with HIV-1 + HIV-2. Of these 95 patients, 93 (54 men and 39 women) had samples available for HIV RNA and p24 measurement. Among these, 47 were alive and had samples available at 8 months of follow-up. Due to technical problems on several occasions, CD4+ T-lymphocyte counts were only available for 276 patients at inclusion and for 187 patients at 8 months. The baseline characteristics of the enrolled patients are shown in Table 1.

3.1. Changes in CD4 in HIV-infected and HIV-uninfected TB patients

We found minor changes in CD4 count during TB treatment, with a trend towards a higher CD4 count at the end of treatment both for HIV-infected and for HIV-uninfected patients (Table 2). The mean CD4 count at inclusion for the entire group of patients is displayed in Table 1. At 8 months the mean CD4 counts was 675 cells/μl for the 120 HIV uninfected, and 306 cells/μl for the 35 HIV-1 infected. For the 21 HIV-2 infected it was 327 cells/μl.

Table 2 displays the subgroup of HIV-uninfected patients with both a baseline and a follow-up sample at 8 months.

3.2. Changes in HIV markers over time

Using v2.0 more virus was detected, hence fewer patients had undetectable HIV RNA and more patients had high viral loads