Clinical, Hemato-Immunological Characteristics of *Mycobacterium tuberculosis* Patients with and without HIV-1 Infection: Responses to Six Month Tuberculosis Treatment

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ABSTRACT

Comprehensive clinical management of tuberculosis (TB)/human immunodeficiency virus (HIV) patients is a challenge in endemic areas. Clinical, hematological and immunological parameters are important for better patient management and further understanding of TB/HIV interactions. We characterized symptoms of TB, and hematological, immunological, HIV RNA load (VL), and interferon-gamma (IFN-γ) responses to *Mycobacterium tuberculosis* (*Mtb*)-specific antigens (ESAT-6/CFP-10), in five clinical groups before and after TB treatment (TT). Adults of both sexes (n=224) included 132 TB cases [HIV-TB+=80; HIV+TB+=52], 67 latent TB infected (LTBI) [HIV-TST+=43; HIV+TST+=24], and 25 controls (HIV-TST-). Cough and weight loss were the common symptoms in HIV-TB+. There was a >70% overlap of cough with CD4+<200 cells/µl in HIV+TB+ patients, indicating late onset of symptoms or late presentation of the patients. There were lower CD4+, total lymphocyte count (TLC), hemoglobin (Hgb), and body mass index (BMI), but higher leukocyte count (WBC) and neutrophil values, in HIV-TB+ (P<0.05), which all normalized after TT. HIV coinfection (HIV+TB+) further perturbed the immunohematological values, and no significant increase in CD4+ count, decrease in viral load (VL), or normalization of any hematological values after TT was observed. IFN-γ production was elevated in HIV-TST+ but impaired in HIV-TB+ (P = 0.003), and severely impaired in HIV+TB+ (P = 0.07) and HIV+TST+ (P = 0.002). In addition, IFN-γ production was reconstituted after TT in HIV-TB+ (P = 0.02) but not in HIV+TB+ patients, which was correlated with CD4+ counts (r = 0.76, P = 0.006). In conclusion, the distinctive pattern of CD4+, TLC, Hgb, WBC, neutrophils, and BMI in TB and TB/HIV patients shows they may serve as markers for response to TB therapy and for prognosis. Likewise, the distinctive profile of IFN-γ during active TB and LTBI confirms the central role of IFN-γ in controlling *Mtb* infection, and its potential to serve as a correlate of protective immunity and response to therapy. The higher mortality and the impaired responses of CD4+, VL and IFN-γ to TT in TB/HIV patients indicates severe immunosuppression at diagnosis, and emphasizes the need for early intervention, supporting early initiation of HAART for TB/HIV patients. *Biomed. Int.* 2012; 3: 22-33. ©2012 Biomedicine International, Inc.

Key words: HIV, immunohematological, IFN-γ, tuberculosis
INTRODUCTION

*Mycobacterium tuberculosis* (*Mtb*) causes severe morbidity and mortality next to HIV/AIDS. Ethiopia, with a population of 79.3 million, ranks 7th among the 22 highest TB burden countries in the world. Although host immunity against *Mtb* is not fully understood, the CD4+ cell mediated cellular immune response plays a central role. Likewise, neutrophils, which are recruited early into infected sites in response to inflammatory signals, are known to induce microbicidal activities as well as granuloma formation during *Mtb* infection. However, TB disease is known to perturb a diverse set of blood cells. Increases in total white blood cell (WBC) and neutrophil counts as well as a decrease in total lymphocyte count (TLC) and in CD4+ cells have been found. In addition, decreases in hemoglobin (Hgb) and in body mass index (BMI) have been reported during TB disease. However, whether TB induces depletion of CD4+ T cells and whether alteration of WBC populations is associated with immune dysfunction specific to TB is not well understood.

In this study, we assessed the correlation between the dynamics of CD4+ T cells and the level of IFN-γ production upon stimulation with *Mtb* antigens during TB disease and treatment.

HIV coinfection is suggested to alter blood cell populations further and to change the Th1/Th2 balance, which influences the course of pulmonary TB, clinical presentation and clinical signs and symptoms, leading to misdiagnosis or delay in diagnosis of TB. Discontinuation and failure of TB treatment and drug resistant TB are also more prevalent in cases of TB/HIV coinfection. Moreover, quality of life of TB/HIV coinfected patients is reported to be lower than for HIV infected patients without active TB. Furthermore, although it is known that TST results can be affected by several confounding factors, diagnosis and treatment of latent TB still depend largely on TST. As reported by others, TST reactivity is also affected by higher viremia and lower CD4+ count in patients infected with HIV. The severity of TB infection could be exacerbated in Africa where dual infection is high. In Ethiopia, the HIV infection rate among TB patients was estimated to be 19% (30% in urban and 15% in rural areas), and 19% in smear-positive and 26% in smear-negative pulmonary TB patients. Earlier studies done in Ethiopia also showed that TB affects all occupational groups, but most severely affects the age group from 20 to 30, which is primarily dying of AIDS. Comprehensive management of TB/HIV coinfected patients is a real challenge in such settings as Ethiopia where TB/HIV coinfection is endemic and resources including well-trained health care personnel and advanced laboratory technologies are limited. Thus, although locally established epidemiological, clinical, and immunohematological indices are helpful for accurate differential diagnosis and better patient management, there are insufficient data related to TB/HIV coinfection in Ethiopia.

The aim of this study was to characterize immunological (CD4+ count), virological (HIV RNA level) and hematological (WBC, TLC, neutrophil, and Hgb) parameters. In addition, presentation of symptoms and BMI were analyzed. Furthermore, we measured IFN-γ production to the *Mtb* specific antigen early secretory antigenic target-6/culture filtrate protein-10 (ESAT-6/CFP-10). These analyses were performed on five clinical groups including pulmonary TB patients [(HIV+TB+, HIV-TB+)]; tuberculin skin test positive (TST+) subjects [(HIV+TST+, HIV-TST+)], and apparently healthy controls (HIV-TST-), before and after TB treatment.
MATERIALS AND METHODS

Study population

This descriptive and longitudinal study was conducted from April 2007 to January 2009 at St Peter Specialized Referral TB Hospital, Akaki and Kality Health Centers, Addis Ababa, Ethiopia. Two hundred and twenty-four adults of both sexes were enrolled from the health facilities after informed and written consent was obtained. These included: 132 pulmonary TB cases [(HIV+TB+=52; HIV-TB+=80)]; 67 latent TB infected (LTBI) groups [(HIV+TST+=43; HIV-TST+=24)]; and 25 controls (HIV-TST-). After enrolment, HIV-TB+ and HIV+TB+ patients were scheduled for a 6-month follow-up visit. Subjects with diabetes mellitus, chronic bronchitis, on steroid therapy, previously or currently on ART or TB treatment, or who were pregnant, were excluded at enrolment.

At recruitment, all subjects were interviewed using a standard questionnaire, and detailed physical and clinical examinations, anthropometric and demographic data were recorded. Twenty ml heparinized venous blood was collected from all subjects at diagnosis and at 6-month follow-up. Samples were processed within three hours at the National HIV Referral Laboratory of the Ethiopian Health and Nutrition Research Institute (EHNRI), Addis Ababa.

Laboratory tests

Laboratory examinations of blood were performed by automated machines. Hematological values were determined using Cell Dyn, Abbott Laboratories, Abott Par IC JI 60064, US; CD4+ T cell counts were determined using BD FACSCalibur (Becton Dickinson, San Jose, USA); and plasma HIV RNA load was assessed using the NucliSens EasyQ NASBA diagnostic 2007/1 (Organon, Teknika), which quantifies HIV-1 with a linear dynamic range from 50 to 3,000,000 copies /ml. Measurement of IFN-γ in the 7th day whole blood culture supernatant stimulated with Mtb specific antigen (ESAT-6/CFP-10) were measured by xMAP multiplex technology (Luminex, Austin TX, USA), using Biosource reagents (Biosource, Camarillo, USA), and analyzed using STarStation v2.0 software (Applied Cytometry Systems, United Kingdom) as described previously. HIV-1 infection was screened using rapid tests: Determine HIV1/2 (Abbott Laboratories, Japan), Capilus HIV-1/2 (Trinity Biotech, Ireland); and Unigold HIV-1/2 Recombinant/Synthetic (Trinity Biotech, Ireland).

Diagnosis and treatment of pulmonary tuberculosis (PTB)

TB diagnosis and treatment were given at the study sites according to the national protocol. Diagnosis of PTB was based on both clinical and bacteriological evidence. At least two sputum smears stained by the Ziehl-Neelsen direct method were required to be positive for Acid Fast Bacilli (AFB) with microscopy. Confirmed TB patients were put on Directly Observed Treatment Short Course (DOTS) with the regimen including rifampicin (R), isoniazid (H), pyrazinamide (Z) and ethambutol (E) for a two month intensive phase, and H and E for a continuation phase for six months.

Mantoux tuberculin skin testing (TST)

A cutaneous test for tuberculin was done for all participants except for active TB patients by an experienced nurse according to the national guidelines. A skin induration of ≥ 10
mm diameter in HIV uninfected, and ≥ 5 mm in HIV-infected, individuals was graded as TST positive (TST+); the remainder were recorded as TST negative (TST-). For further comparative analysis, anergic reaction was defined as TST size “0 mm”.

Definition of clinical and laboratory abnormalities

BMI [weight (kg)/height (m)^2], was defined as normal when the value was between 18.50 and 24.99 kg/m^2; and it was defined as malnutrition when BMI <18.50 kg/m^2. Hgb (<12g/dl), total WBC (<4000/µl), neutrophils (<2200/µl), TLC (<1200/µl) and CD4+ T cell ( <200/µl) were used to define anemia, leucopenia, neutropenia, lymphopenia, and CD4+ T cell lymphocytopenia, respectively. Fever was defined as axillary temperature ≥ 37.5°C.

Statistical analysis

Entered data were double-checked for discrepancies. Descriptive analyses including counts and frequencies for categorical variables, and mean (standard deviation, SD) or median (interquartile range, IQR) for continuous variables, were computed. Results were compared between and within groups using Pearson’s chi square for categorical variables, and non-parametric tests (Mann-Whitney U test or Wilcoxon Rank-Sum test) or parametric tests (Student’s t test or ANOVA) for continuous variables. The level of significance was set at \( p < 0.05 \) and all \( p \) values were two-tailed. Data were analyzed using Intercooled STATA version 10.0 (College Station, Texas, USA).

Ethical considerations

This study was approved by the Research and Ethical Clearance Committee (RECC), EHNRI, and by the National Health Research Ethics Review Committee (NHRERC) of the Ethiopian Science and Technology Agency (ESTA), Ethiopia.

RESULTS

Characteristics of the study participants

A summary of the demographic characteristics and clinical features of the 224 study participants is shown in Table 1. The mean age ± SD of the study population at enrolment was 30.8 ±8.7 years, and 101 (45.1%) were males.

Clinical features

As shown in Table 1, the common symptoms in HIV-TB+ patients in descending order were cough, weight loss, chest pain, fatigue, fever, night sweating, difficulty in breathing, and coughing up blood. The frequencies of these symptoms were similar in the HIV+TB+ group, except that difficulty with breathing was lower in HIV+TB+ than in HIV-TB+ patients \( (p = 0.014) \).

Considering cough for more than two weeks as the common clinical symptom\(^2\), symptoms that were co-expressed with cough in the HIV+TB+ groups were night sweating (90.7%) weight loss (83.7%), fatigue (81.4%), fever (63.3%) and coughing with blood (55.8%). Moreover, there was a more than 70% overlap between the incidence of the symptoms of TB (coughing, coughing up blood, fever, night sweating and fatigue) and immunosuppression (CD4+ < 200 cells/µl) in the HIV+TB+ patients, which might be related to late onset of the symptoms and/or late presentation of the patients to the health facilities.
BCG scar presence and TST responsiveness

A BCG scar was observed in 28.1% of all the study populations. The observed scar frequency was almost 10% lower in TB cases (19.2% in HIV+TB; 21.2% in HIV-TB+), followed by the TST negative (HIV-TST-) groups (32%), while it was highest in the TST positive groups (37.2% in HIV+TST+; 41.7% in HIV-TST+). Scar prevalence was also somewhat lower in the HIV positive than the HIV negative groups (Table 1).

We also observed a lower TST reaction size ($p = 0.008$) and higher frequency of patients with TST ulceration in HIV infected (HIV+TST+) than in HIV uninfected (HIV-TST+) groups. TST reactivity was diminished in subjects with CD4+ T cells below 200 cells/µl and with VL > 100,000 copies/ml (data not shown).

Laboratory parameters and response to the six month TB treatment

We also characterized the immunohematological, virological, BMI, and IFN-γ production responses to Mtb-specific antigen (ESAT-6/ CFP-10) in the five clinical groups at enrollment. Moreover, the responses of these baseline parameters after six months of TB treatment in pulmonary TB patients were investigated. Overall, a total of 15/155 (9.7%) patients died within the first six months of follow-up of whom 8/52 (15.4%) were HIV+TB+, 3/80 (5.0%) were HIV-TB+ and 4/43 (9.3%) were HIV+TST+. Of the HIV+TB+ patients who died, 87.5% had CD4+ T cells below 200/µl, 66.7% were anemic and 37.5%, had malnutrition (data not shown). We further investigated the risk factors for early mortality of the pulmonary TB patients using univariate logistic regression. As shown in Table 2, CD4+ cells <100/µl (OR: 6.3, 95% CI 1.7 - 23.5); CD4+ % < 14 (OR: 3.4, 95%CI 1.0 - 12.1), TLC < 1200 cells/µl (OR: 7.6, 95% CI 1.0 - 63.7), and WHO-stages III plus IV (OR: 5.6 95% CI 1.1 - 28.3, $p = 0.03$), were significantly associated with early mortality.

Hematological, virological and immunological values and response to TB treatment

The summary of the immunohematological (CD4+ count, WBC, TLC, neutrophil, and...
Hgb), virological (HIV RNA level), and BMI at diagnosis and after six months of TB treatment are shown in Table 3. At diagnosis and relative to controls, Hgb (g/dl) ($p < 0.0001$), CD4 cells/µl ($p < 0.0001$), and TLC (p = 0.02) were lower, and WBC and neutrophil counts ($p < 0.0001$; $p < 0.00001$) were higher, in cases with TB disease (HIV-TB+). We observed lower Hgb ($p = 0.018$) in HIV-TST+ than controls but no difference in any of the leukocyte subpopulations.

In response to TB treatment, increases in CD4+ cells ($p = 0.05$), CD4% ($p = 0.22$), Hgb ($p = 0.003$), and in TLC ($p = 0.0006$), but decreases in total WBC ($p = 0.08$) and neutrophil ($p < 0.001$) counts, were observed in the HIV uninfected TB patients (Table 3). However, re-

### Table 2: Risk factors associated with early deaths of active pulmonary patients (n=11) occurring within the first six months of follow-up.

<table>
<thead>
<tr>
<th>Characteristics (variables)</th>
<th>Early deaths n (%)</th>
<th>Crude OR (95% CI)</th>
<th>OR or p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>7/80 (8.8)</td>
<td>2.0 (0.5-7.4)</td>
<td>0.28</td>
</tr>
<tr>
<td>Female</td>
<td>4/72 (5.6)</td>
<td>(0.45)</td>
<td></td>
</tr>
</tbody>
</table>

| Age (years)                 |                     |                   |              |
| 15-34                       | 4/35 (11.4)         | 1.2               |              |
| 35-60                       | 7/117 (6.0)         | (0.27)            | 1.6 (0.5-5.8)| 0.45         |

| CD4+ count/µl               |                     |                   |              |
| ≥100                        | 4/80 (5.0)          | 9.2               |              |
| <100                        | 7/30 (23.3)         | (0.002)           | 6.3 (1.7-23.5)| 0.002        |

| CD4+ %                      |                     |                   |              |
| ≥14                         | 5/82 (6.1)          | 4.0               |              |
| <14                         | 6/53 (18.2)         | (0.04)            | 3.4 (1.0-12.1)| 0.01         |

| TLC                          |                     |                   |              |
| ≥1200                       | 1/44 (2.3)          | 5.1               |              |
| <1200                       | 8/53 (15.1)         | (0.02)            | 7.6 (0.9-63.7)| 0.03         |

| BMI                          |                     |                   |              |
| ≥18.5                       | 6/75 (8.0)          | 0.12              |              |
| <18.5                       | 5/77 (6.5)          | (0.72)            | 0.8 (0.2-2.7)| 0.56         |

| Hgb                          |                     |                   |              |
| ≥12                         | 2/56 (3.6)          | 1                 |              |
| <12                         | 7/41 (17.1)         | (0.03)            | 5.5 (1.1-28.3)| 0.40         |

| WHO staging                 |                     |                   |              |
| I and II                    | 7/48 (14.6)         | 0.5               |              |

### Table 3: BMI and laboratory parameters of the latent TB infected populations (HIV+TST+ and HIV-TST+) and of the pulmonary TB patients (HIV+TB and HIV+TB+) with and without HIV coinfection at diagnosis and after six months of anti-TB treatment.

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<table>
<thead>
<tr>
<th>Parameter</th>
<th>HIV+TST</th>
<th>HIV+TST+</th>
<th>Before treatment (n=38)</th>
<th>After treatment (n=54)</th>
<th>Before treatment (n=44)</th>
<th>After treatment (n=50)</th>
<th>Controls (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>21.2 ± 5.0</td>
<td>21.4 ± 2.6</td>
<td>18.9 ± 3.1*</td>
<td>18.9 ± 2.0*</td>
<td>20.1 ± 3.4*</td>
<td>21.4 ± 2.0*</td>
<td></td>
</tr>
<tr>
<td>Hgb (g/dl)</td>
<td>12.6 ± 2.7*</td>
<td>16.4 ± 1.8*</td>
<td>18.8 ± 3.0*</td>
<td>13.8 ± 2.1*</td>
<td>13.4 ± 2.2*</td>
<td>16.0 ± 2.3*</td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>3972 ± 1806</td>
<td>4686 ± 1575</td>
<td>4854 ± 1599*</td>
<td>438 ± 883(=60)</td>
<td>682.4 ± 312*</td>
<td>5320 ± 1595(=21)</td>
<td>4547 ± 1497</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>2117 ± 1286</td>
<td>2333 ± 1494</td>
<td>3265 ± 1504*</td>
<td>1860 ± 772(=60)</td>
<td>48.0 ± 285*</td>
<td>2655 ± 129(=19)</td>
<td>2599 ± 1170</td>
</tr>
<tr>
<td>TLC</td>
<td>1474 ± 849</td>
<td>1975 ± 1483</td>
<td>1137 ± 789*</td>
<td>1597 ± 381(=7)</td>
<td>1477 ± 88*</td>
<td>2112 ± 908(=21)</td>
<td>1667 ± 559</td>
</tr>
<tr>
<td>CD4+ cells/µl</td>
<td>26.7 ± 10.2*</td>
<td>44.8 ± 6.7*</td>
<td>13.7 ± 5.8*</td>
<td>19.0 ± 9.1(=9)</td>
<td>37.1 ± 9.4*</td>
<td>40.0 ± 6.0(=27)</td>
<td>45.7 ± 6.5</td>
</tr>
<tr>
<td>CD4+ %</td>
<td>274 ± 200*</td>
<td>787 ± 274</td>
<td>173.7 ± 181*</td>
<td>316.9 ± 569(=9)</td>
<td>473 ± 254*</td>
<td>6667 ± 223(=30)</td>
<td></td>
</tr>
<tr>
<td>RNA/log-copies</td>
<td>4.1 ± 3.6</td>
<td>NA</td>
<td>5.0 ± 5.2*</td>
<td>5.0 ± 5.6 (n=8)</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
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</table>

**a**: number of subjects; values presented as mean ± standard deviation (SD); WBC, TLC and neutrophils counts are (x1000 /µl of blood); NA, not applicable.

Significant difference ($P < 0.05$) from that of the controls (t test).

Significant difference ($P < 0.05$) between values before and after treatment (paired t test).

n=38 for CD4%; n=40 for CD4+cells/µl; n=39 for HIV RNA

p<: n=25 for CD4+cells/µl

n=50 for CD4%; n=50 for CD4+cells/µl; n=44 for HIV RNA.
Response to TB treatment in HIV coinfected TB patients (HIV+TB+) did not improve CD4+ cells, CD4%, Hgb, TLC, WBC or neutrophil values. Likewise, despite the lower CD4+ count ($p = 0.003$) and elevated VL level ($p = 0.02$) in HIV+TB+ than in HIV+TST+ patients at enrolment, there was no significant increase in either CD4+ count or VL level after TB treatment in the HIV+TB+ patients with no HAART (Table 3).

Moreover, there was a higher proportion of HIV+TB+ patients with abnormal laboratory values at diagnosis including CD4+ lymphopenia (24.9%), lymphopenia (73.7%), malnutrition (50%), anemia (57.9%), leucopenia (31.6%), and neutropenia (21.1%).

BMI and response to TB treatment

As shown in Table 3, the BMI of HIV-TB+ patients was lower than that of controls ($p < 0.0001$). Moreover, although the mean BMI in HIV-TB+ patients at diagnosis was as low as that in HIV+TB+ ($p = 0.82$), it reached normal values in HIV-TB+ but not in HIV+TB+ patients after TB treatment.

IFN-$\gamma$ production in response to ESAT-6/CFP-10 mycobacterium antigen

To study differences in immune reactivity against $Mtb$ antigens and to determine whether TB treatment influences these responses, we measured IFN-$\gamma$ production upon ESAT-6/CFP-10 stimulation. Compared to HIV-TST-, there was lower IFN-$\gamma$ production in HIV-TB+ ($p = 0.0002$), and there was significantly lower IFN-$\gamma$ production in HIV-TB+ than in HIV-TST+ ($p = 0.003$). Moreover, the level of IFN-$\gamma$ was markedly lower during co-infection with HIV in the LTBI groups (HIV+TST+ vs. HIV-TST+; $p = 0.002$), as well as in the active TB cases (HIV+TB+ vs. HIV-TB+; $p = 0.07$) (Figure 1A). After six months of TB treatment, the level of IFN-$\gamma$ in HIV-TB+ patients doubled relative to the values at diagnosis ($p = 0.02$), although it remained half that in the LTBI groups. In the HIV+TB+ on TB treatment without HAART, however, there was no significant increase in IFN-$\gamma$ compared to values at baseline ($p = 0.33$) (Figure 1B).

Correlation between CD4+ T cell count recovery and IFN-$\gamma$ production

We also assessed whether the CD4+ cell dynamics in the HIV-TB+ patients before and after treatment correlates with IFN-$\gamma$ production upon ESAT6 stimulation (immune function specific to TB). We observed a strong correlation between the recovery of the absolute counts of CD4+ cells and IFN-$\gamma$ production after 6 months of TB treatment ($r=0.76$, $p = 0.006$, $n=11$) (Figure 2), which might confirm that the decrease in the level of IFN-$\gamma$ observed in this study is associated with the depletion of the CD4+ cells from the periphery.

DISCUSSION

Comprehensive management of TB patients has become a challenge since the emergence of HIV/AIDS. In this study, we characterized immunohematological profiles, level of IFN-$\gamma$ production specific to $Mtb$ antigens and nutritional status in five clinical groups.

Clinical features

As in previous reports from elsewhere and from Ethiopia, cough was the most frequent symptom of HIV-TB+ patients in this study. However, we observed no difference in occurrence of TB symptoms between HIV infected and uninfected TB; that could be due to
the inclusion criteria, since all subjects were expected to have shown symptoms of TB prior to enrolment as TB cases. In agreement with Ong et al. we observed a strong overlap between the occurrence of major TB symptoms and immunosuppression in HIV+TB+ patients. This could be related to the poor immune function of patients in whom the immunopathological mediated onset of symptoms is expressed at an advanced disease stage; or it could be due to late presentation of the patients to health facilities. As delay in TB diagnosis and initiation of treatment could result in a higher death rate in TB/HIV patients16, our data emphasize the need for early intervention for TB patients infected with HIV.

The 28.1% BCG scar prevalence in this study might indicate previous BCG immunization coverage. The lower BCG scar prevalence in TB cases was comparable with a previous report from Ethiopia and could be related to variations in BCG vaccination status,
scar visibility, or scar failure among the groups. The somewhat lower BCG scar frequency in HIV positive than HIV negative groups could be due to waning of the scar owing to diminished immunity associated with HIV infection. Collectively, our data emphasize the need for careful interpretation of BCG when used as an indicator of previous BCG vaccination coverage in a population.

**TST reactivity**

In agreement with Chadha et al. there was moderate variation in TST reactivity related to BCG scar, gender, age, and BMI. Our data re-confirm the need for careful interpretation of TST results during epidemiological studies or for treatment of LTBI.

**Hematological and nutritional profiles**

Similar to previous reports we observed alteration of hematological (Hgb, TLC, neutrophil, WBC) and BMI values in TB patients, which were reversed to the level of healthy controls after six months of TB treatment. However, co-infection with HIV (HIV+TB+) led to different baseline immunohematological profiles from those of HIV-TB+ patients. In particular, none of the laboratory and BMI values were normalized after TB treatment. The difference in levels of Hgb, TLC, neutrophils, and WBC during active TB before and after TB treatment indicate that these measures can serve as prognostic markers. Previous reports have shown the value of monocyte, neutrophil and total WBC counts in monitoring TB treatment outcomes.

**Immunological and virological profiles**

As in previous reports from Ethiopia, we observed lower CD4+ cell counts in the healthy control groups (HIV-TST-) than in East African and Dutch populations. The implication of the lower baseline CD4+ count in healthy Ethiopians for disease progression during HIV infection has been discussed by others. However, as the rate of CD4+ decline in HIV infected Ethiopians is slower despite the lower baseline values, and is similar to that in other African populations, the lower CD4+ count did not directly correlate with lower survival time during HIV infection. Our observation of lower CD4+ cell counts in the
HIV negative TB patients (HIV-TB+), which was normalized after TB treatment, was in agreement with others. Thus, in areas where TB/HIV is endemic and monitoring of HIV/AIDS and response to ART is dependent on CD4+ counts, the effect of TB on the prognostic value of CD4+ cells should be taken into account.

The CD4+ counts were low and the viral load elevated in HIV+TB+ patients at diagnosis, and there was no significant recovery in either of these measures after TB treatment. This could be related to persistent activation of the immune system despite TB treatment, which would boost viral replication. Similarly, higher level of plasma immune activation markers including neopterin, beta2M and sTNFalpha-RI were reported in TB/HIV patients despite effective clearance of M. tuberculosis infection. Our data and another report strengthen the recent WHO recommendation for early initiation of HAART for TB/HIV patients despite CD4+ counts.

IFN-γ production to ESAT-6/CFP-10

As reported by others, ESAT-6/CFP-10-induced production of IFN-γ was lower in the HIV negative TB patients but higher in HIV negative LTBI groups. This might reflect the predominant role of IFN-γ in immunity against Mtb infection. However, although the level of IFN-γ was normalized in response to TB treatment in the HIV-TB+ patients, it remained lower than that of the LTBI after six months of continuous TB treatment. We further investigated whether the CD4+ count dynamics before and after TB treatment in HIV-TB+ cases is associated with immune functions specific to TB. The strong positive correlation between the level of IFN-γ and the number of CD4+ counts following TB treatment (Figure 2) could reflect the predictive value of higher CD4+ count for elevated IFN-γ levels, indicating an improvement in immune function specific to TB.

Similar to other reports, we found the lowest level of IFN-γ production in response to ESAT-6/CFP-10 stimulation in HIV+TB+ patients at diagnosis, and this was not reconstituted after TB treatment. This might reflect the degree of immunodeficiency (exhaustion of immune system) in TB+HIV+ patients at the time of diagnosis, and persistent immune activation and persistent elevated plasma viral load despite TB treatment and clearance of the bacilli. This further strengthens the recommendation for early initiation of HAART in TB/HIV patients.

In conclusion, the distinctive CD4+, TLC, WBC, neutrophil, Hgb, and BMI profiles in TB patients before and after TB treatment indicates their potential as prognostic markers for patient monitoring and response to TB therapy. Likewise, the distinctive pattern of IFN-γ in TB disease cases and LTBI reconfirms the central role of IFN-γ in controlling M. tuberculosis infection as well as its potential to serve as a correlate of protective immunity. The higher early mortality, and the impaired responses of CD4+, VL and IFN-γ to TB treatment in TB/HIV patients, strengthen the recommendation for early initiation of HAART for TB/HIV patients. Finally, studies aimed at assessing whether the alteration of peripheral leukocyte subpopulations during TB and TB/HIV is associated with host immunological deterioration or improvement are warranted.

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