Plasma levels of CRP, neopterin and IP-10 in HIV-infected individuals with and without pulmonary tuberculosis

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ABSTRACT

Introduction: Tuberculosis (TB) is a major cause of morbidity and death worldwide, and disproportionally affects people with HIV. Many cases still remain undiagnosed, and rapid and effective screening strategies are needed to control the TB epidemics. Immunological biomarkers may contribute.

Methods: Plasma samples from healthy individuals (n: 12) and from HIV-infected individuals with (n: 21) and without pulmonary TB (n: 122) were tested for C-reactive protein (CRP), neopterin, and interferon-gamma-inducible protein-10 (IP-10). Increased levels of biomarkers and WHO 4-symptom-screening were compared with the presence of pulmonary TB. Survival status at 12 months was recorded. Associations with CD4 count, BMI, haemoglobin, disease severity, and mortality were analysed.

Results: The plasma levels of the biomarkers were significantly higher in TB-positive (n: 21) compared to TB-negative (n: 122) subjects. WHO symptoms, increased neopterin (>10 nmol/L) and CRP (>10 mg/L) showed similar sensitivity and different specificity, with increased CRP showing higher and increased neopterin lower specificity. The three markers were inversely correlated to haemoglobin and to CD4, and CRP levels inversely correlated to BMI. The markers were also significantly higher in individuals with subsequent mortality and in individuals with higher mycobacterial load in sputum according to Xpert results (IP-10 and CRP).

Conclusion: This study showed significant associations of the biomarkers analysed with TB infection and mortality, that could have potential clinical relevance. Biomarker levels may be included in operational research on TB screening and diagnosis.

1. Introduction

Tuberculosis (TB) is a major cause of morbidity and death worldwide, and disproportionally affects people with human immunodeficiency virus (HIV), with a severe impact on mortality and economic growth of highly affected countries [1–4]. Despite several improvements in diagnosis, mostly due to the introduction of rapid tests such as GeneXpert and urine LAM test, that quickly detect presence of nucleic acids or other bioproducts of Mycobacterium Tuberculosis in biological samples [5,6], many cases remain undiagnosed, and rapid and effective screening strategies are still needed to control more successfully the TB epidemics in countries with limited resources [7–9]. Active TB produces an increase in the blood levels of some markers of inflammation or immune response such as C-reactive protein (CRP), interferon gamma, neopterin, complement factor H, interferon gamma inducible protein 10 (IP-10), transthyretin and others. Biomarkers have been studied as diagnostic tools in screening strategies in addition to molecular tests and symptom screening. Although some studies have
evaluated individual markers and panels of serum proteins for the diagnosis of active TB [10–12], none has been included in recommended screening strategies yet. In order to further investigate this issue, we measured the blood levels of some markers of inflammation and immune activation in blood samples from a population of HIV-infected patients that were screened for TB using the WHO symptom screening and the GeneXpert molecular test on sputum within a multicenter study conducted in Mozambique [13].

2. Study population and methods

We used stored blood samples and clinical data from patients participating to a study of intensive TB case finding with symptom screening, conducted in Mozambique within the Disease Relief through Excellent and Advanced Means (DREAM) program of the Community of S. Egidio, an Italian faith-based non-governmental organization. The study, described elsewhere [13], enrolled patients between 2014 and 2016, following informed consent and according to the approval by the National Committee for Health Bioethics of the Mozambican Ministry of Health in 2014 (ref. 36/CNBS/2014). This laboratory substudy obtained an additional specific approval for use of collected samples by the National Committee for Health Bioethics of the Mozambican Ministry of Health in 2018 (IRB0002657, ref. 364/CNBS/18). The samples analyzed here represent residual plasma amounts that were stored at –80°C at the laboratories of the DREAM health centers of Beira and Maputo following routine analyses for the clinical care of patients followed at the two above DREAM clinical sites. We included all available samples of Xpert-positive patients and a casual sample of Xpert-negative individuals. All the patients had a record of survival status at 12 months available. A small number of available plasma samples from healthy laboratory personnel with no declared risk of infection with HIV served as control group in comparative analysis of biomarkers. No additional information regarding the control group was collected.

In accordance with WHO guidelines, active TB was defined by a positive result to a molecular TB assay on sputum (Xpert MTB/RIF Assay system, Cepheid, Sunnyvale, CA, USA) [5], and symptom screen positivity was defined by presence of any of four WHO symptoms (WHO-4SS: current cough, fever, night sweats, weight loss) in the previous 30 days [14]. Demographic and clinical information was collected during routine clinical visits at the DREAM health centers. The three host biomarkers evaluated in plasma samples were CRP, Neopterin, and IP-10. Biomarkers levels were measured according to manufacturer's instructions using the following commercial assays: Human CRP ELISA Kit (Arigo Biolaboratories Corporation, Hsinchu City, Taiwan); Neopterin ELISA (IBL-International GMBH, Hamburg, Germany); Quantikine ELISA Human CXCL10/IP-10 Immunoassay (R&D Systems Europe, Abingdon, UK).

Population characteristics were summarized as medians with interquartile ranges (IQR). The CRP threshold concentration defining a screen positive for TB was set at 10 mg/L, according to previous studies [12]. For neopterin, a cutoff level <10 nmol/L was used to define normal values, for consistency with previous studies [15]. For IP-10, in the absence of established threshold values for screening purposes, we performed exploratory analyses based on different thresholds. Point estimates and 95% CIs were calculated for the sensitivity, specificity, negative and positive predictive value (NPV, PPV) in reference to Xpert results. Differences in sensitivity and specificity were compared with McNemar’s test of paired proportions. Qualitative variables were compared using the chi-square or the Fisher test, and quantitative variables using the Mann–Whitney U test. Correlations between quantitative variables (levels of biomarkers, CD4 cell count, BMI and haemoglobin) were assessed with the Spearman test. For all tests, p values below 0.05 were considered statistically significant. All analyses were performed using the SPSS software, version 22 (IBM Corp, 2013, Armonk, NY, USA).

3. Results

Stored samples were available for 143 patients enrolled in the intensive TB case finding study (21 with a Xpert-positive test result [14.7%] and 122 [85.3%] with two sequential negative Xpert tests) and for 12 controls. The general characteristics of the HIV-infected individuals evaluated for TB are shown in Table 1. The presence of a positive Xpert test on sputum was associated, as expected, with worse demographic and clinical characteristics, and with presence of TB-related symptoms (Table 1). The comparative analysis of the levels of CRP, neopterin and IP-10 according to TB status, showed significantly higher levels of all the three biomarkers in Xpert-positive compared to Xpert-negative subjects. Both groups of HIV-infected subjects, with and without TB, had significantly higher levels of all biomarkers compared to control subjects (Table 2).

The potential diagnostic value (sensitivity, specificity, PPV and NPV) of hypothetical screening strategies for TB based on WHO symptoms or of increased levels of neopterin (>10 nmol/L) and CRP (>10 mg/L) is reported in Table 3. The three strategies showed minor and non-significant differences in sensitivity, but were significantly
Table 2
Levels of biomarkers in each group.

| Panel                        | HIV-positive, Xpert-positive (n: 21) | HIV-positive, Xpert-negative (n: 122) | Controls (n: 12) |
|-----------------------------|-------------------------------------|--------------------------------------|-----------------|
| P value, A vs. B            | <0.001                              | <0.001                               | <0.001          |
| P value, A vs. C            | <0.001                              | <0.001                               | <0.001          |
| P value, B vs. C            | <0.001                              | <0.001                               | <0.001          |

- IP-10 (R: 0.769, p < 0.001)
  - Neopterin (nmol/L, median, IQR): 50.4 (32.6–26.4)
  - CRP (mg/L, median, IQR): 15.7 (6.3–19.2)

4. Discussion

This study showed that levels of IP-10, neopterin and CRP were significantly associated with pulmonary TB and other clinical outcomes in HIV-infected individuals. In HIV-infected persons from a clinical study, the levels of all the three biomarkers were significantly higher in the presence of pulmonary TB, and were also much higher compared to healthy controls. Using commonly accepted thresholds for the definition of increased levels of neopterin (>10 nmol/l) and CRP (>10 mg/l), both markers performed relatively well in terms of sensitivity compared to the traditionally used WHO four-symptom screening panel for pulmonary TB, but had marked differences in specificity, that was particularly poor for neopterin (27.0%, significantly inferior to both symptom screening and CRP), and very good for CRP (95.1%, significantly superior to both symptom screening and neopterin). Overall, neither increased levels of neopterin nor increased levels of CRP appeared to perform substantially better than WHO-4SS in both sensitivity and specificity. Our data, however, confirming that CRP >10 mg/l has better specificity for pulmonary TB compared to symptom screening [12], suggest that this biomarker might deserve further consideration in screening strategies. A major problem, shown by this and other studies, is represented by the lack of clinical or laboratory indexes that may effectively obtain in screening strategies, alone or combined, 100% sensitivity [12,16].

Despite such limitations, biomarkers can still provide useful information, and in this study their levels were strongly linked not only with presence of TB, but also with other indexes of health status, disease severity and mortality. Together with significant positive correlations among the levels of the three biomarkers, we showed significant inverse correlations of all biomarkers with haemoglobin, and a significant inverse correlation between CRP levels and BMI. Even more importantly, the levels of all biomarkers were higher in HIV-infected patients than in controls, and significantly associated with degree of immune deterioration (CD4 cell counts). This should be taken into different in terms of specificity, with CRP >10 mg/L significantly better than both neopterin >10 nmol/L and WHO-4SS, and with neopterin significantly worse than the other two markers. Negative predictive value was high (> 95%) for all the three strategies, while positive predictive value was much more variable, reflecting the observed differences in specificity (Table 3). We also explored the potential diagnostic value of IP-10 using different thresholds but no threshold showed adequate combinations of sensitivity and specificity (data not shown).
account when considering biomarkers as diagnostic tools for TB in HIV-infected patients. It should be also noticed that levels of biomarkers were strongly associated with subsequent mortality, and (for IP-10 and CRP) with mycobacterial load in sputum. The association with mortality, already described by Bedell et al. for CRP [17], indicates a strong prognostic potential that should be further explored. The association with mycobacterial load in sputum, although limited to a small number of cases, and significant only for IP-10 and CRP, is consistent with the results of other studies [18,19], and indicates that even within the group of individuals with pulmonary TB, significant differences can be found in biomarker levels according to severity and, possibly, transmissibility of TB disease.

5. Conclusions

In summary, this study provided a comprehensive evaluation of three commonly used biomarkers of immune activation and inflammation in HIV-infected individuals with and without TB. Despite the limitation of a relatively small sample size, this study showed several significant associations of potential clinical and pathogenetic relevance. The observed findings may represent the basis for subsequent clinical and operational research, particularly in the identification of effective screening strategies for TB, that represent a strong and urgent health priority.

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