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Diagnostic and Prognostic Value of Serum Albumin for Tuberculosis in HIV Infected Patients Eligible for Antiretroviral Therapy: Data from an HIV Cohort Study in India

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ABSTRACT

Introduction: Tuberculosis is difficult to diagnose and it is the leading cause of death in HIV infected individuals in developing countries. There is an urgent need of low-cost diagnostic markers for resource-limited settings. ***Methods:*** The study involved 1571 patients from an HIV cohort study in India with known serum albumin concentrations at the time of becoming eligible for antiretroviral therapy (ART). We investigated the diagnostic accuracy of serum albumin to predict tuberculosis within six months of ART eligibility and the prognostic value in patients who experienced tuberculosis. ***Results:*** The diagnostic accuracy of serum albumin, measured by the area under the receiver operating characteristic curve, to predict tuberculosis was 0.81 (95% confidence interval 0.78-0.83). Serum albumin concentrations <3.2 g/dL were associated with 85% specificity and <4.1 g/dL were associated with negative predictive values >90%, even in settings with high tuberculosis prevalence. Hypoalbuminemia was associated with an increased risk of mortality in patients with tuberculosis. ***Conclusion:*** Serum albumin can be a useful low-cost diagnostic marker for tuberculosis in HIV infected patients eligible for ART. However, we failed to find thresholds to rule out or rule in tuberculosis. If these results are confirmed by other studies, serum albumin could be used to improve the diagnostic accuracy of intensive case finding algorithms for HIV-related tuberculosis. In patients who experience tuberculosis, hypoalbuminemia is associated with poor prognosis.

Introduction

In 2011, 13% of the 8.7 million incident cases of tuberculosis and 30% of the 1.4 million deaths from tuberculosis occurred in HIV infected patients.[1] Tuberculosis is the leading cause of mortality among HIV infected patients living in developing countries.[2,3] Given that tuberculosis is a treatable disease, this high mortality might be explained by the difficulties in diagnosing tuberculosis in HIV infected patients.

Currently, there is not a rapid and accurate diagnostic test for tuberculosis in HIV infected patients. Clinical presentation is often non-specific, and the sensitivity of smear microscopy is poor.[4] The four-symptom screening recommended by World Health Organization (WHO), based on the presence of fever, weight loss, night sweats or cough of any duration,[5] has poor specificity and suboptimal sensitivity, and chest radiology marginally increases the sensitivity of this screening strategy at the expense of specificity.[6] WHO has recently endorsed the implementation of the GeneXpert MTB/RIF assay for national tuberculosis programmes in developing countries.[7] The Xpert MTB/RIF is a diagnostic molecular test with an analytic sensitivity of five genome copies of purified DNA and 131 cfu/ml of *M. tuberculosis* in sputum in less than two hours.[8] Compared to smear microscopy, the Xpert MTB/RIF assay increases the diagnosis of tuberculosis by 13-38%. However, the cost of Xpert assay is 84 times higher than that of LED fluorescent smear microscopy,[9] and two recent studies have shown that the implementation of Xpert MTB/RIF assay had no impact on mortality in both primary health and hospital settings.[10,11] Although the diagnostic accuracy is improved by culture methods, their turnaround time is typically 2-6 weeks, and they might not be feasible in resource-limited settings due to their cost and technical requirements.[2]

Excluding tuberculosis is particularly important in patients who become eligible for HIV treatment before the initiation of antiretroviral therapy (ART). The immunological recovery after the initiation of ART provokes an increased inflammatory response against the high mycobacterial organism load present in HIV infected patients with undiagnosed tuberculosis,[12] unmasking the

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tuberculosis infection and increasing the risk of death due to the strong inflammatory reaction.[3,13]

In this context, low-cost markers able to help clinicians in resource limited-settings to determine the risk of tuberculosis should be welcomed. The potential use of serum albumin as a marker of tuberculosis in HIV infected patients has been recently suggested.[14] The aim of this study is to assess the diagnostic accuracy of serum albumin for tuberculosis in HIV infected patients who became eligible for ART in a cohort study in India. In particular, we wanted to describe positive and negative predictive values that could help clinicians in resource-limited settings to answer this question: How likely is that this ART-eligible patient will have tuberculosis?

Materials and methods

This study was performed in the district of Anantapur, India. Rural Development Trust (RDT) is a nongovernmental organization that provides free medical care to HIV infected patients. The Vicente Ferrer HIV Cohort Study (VFHCS) is an open cohort study of all HIV infected patients who have attended RDT hospitals.[15,16] Since September 2009, clinical information of the patients has been collected prospectively.

For this study, we selected adults (>15 years) who had a serum albumin determination within seven days before or after becoming eligible for ART from October 1st 2009 to August 1st 2012. ART eligibility was defined according to the CD4 lymphocyte count. Following the Indian HIV guidelines,[17] the CD4 lymphocyte count threshold for starting ART was 250 cells/µl until November 4th 2011, and 350 cells/µl thereafter. The selection of patients from the database was executed on May 27th, 2013.

Serum albumin concentration was calculated using the automated analyser Hitachi 902 (Roche Diagnostics, Indianapolis, IN, USA). Acid fast bacilli staining of sputum and chest radiograph were performed on all patients. Analysis of cerebrospinal fluid, pleural fluid or ascitic fluid was performed if there were signs of neurological involvement, pleural fluid in the chest radiograph or ascites, respectively. In smear-negative patients referring important weight loss, an abdominal ultrasound was performed for investigating signs of abdominal tuberculosis.[18,19] In accordance with WHO recommendations for the definition of tuberculosis case and the locally available standard of care,[20] the diagnosis of tuberculosis was based on the presence of acid fast bacilli on sputum smear, caseating or necrotizing granuloma in clinical specimens, and clinical presentation suggestive of tuberculosis along with supportive findings in the chest radiograph, abdominal ultrasound and/or laboratory results from biological fluids.[21] Disseminated tuberculosis was defined when there were signs of tuberculosis infection in two different sites. Enumeration of the CD4 lymphocyte count was performed using the FACSCalibur system (Becton Dickinson Biosciences, CA, USA).[22]

The primary objective of the study was to assess the diagnostic accuracy of serum albumin for the diagnosis of tuberculosis within six months of becoming eligible for ART. Patients who did not complete six months of follow-up or who had a previous episode of tuberculosis within one year before ART eligibility were excluded from the study.

Statistical analysis was performed using Stata Statistical Software (Stata Corporation. Release 11. College Station, Texas, USA). The accuracy of the test was measured using the area under the receiver operating characteristic (ROC) curve calculated by the non-parametric method suggested by DeLong.[23] Kaplan-Meier curves were used to describe the cumulative incidence of tuberculosis according to different serum albumin levels and the prognostic value of serum albumin concentration in patients with tuberculosis. The study was approved by the ethical committee of the RDT Institutional Review Board.

Results

During the study period, 3388 patients became eligible for ART and serum albumin was measured in 1947 (57%) of them. Thirty-two patients who had a previous episode of tuberculosis, and 344 patients who did not complete six months of follow-up were excluded from the analysis. Of 1571 patients included in the final analysis, 838 (53%) were diagnosed with tuberculosis within six months. Baseline characteristics of the patients and serum albumin concentrations by subgroups are presented in Table 1. Of the patients, 40% were women; the median age was 35 years (interquartile range [IQR], 30-42) and the median CD4 lymphocyte count was 120 cells/µl (IQR, 66-185). The overall median serum albumin was 3.4 g/dL (IQR 2.8-4). Patients who experienced tuberculosis had lower serum albumin concentrations (median 3 g/dL, IQR 2.6-3.5) than patients who did not have tuberculosis (median 3.9 g/dL, IQR 3.4-4.3) (Fig. 1). Patients with abdominal, disseminated, pulmonary or pleural tuberculosis had lower serum albumin concentrations than patients with tuberculous lymphadenitis, meningitis and other forms of tuberculosis. The cumulative incidence of tuberculosis by serum albumin concentrations is presented in Fig. 2. The risk of tuberculosis increased gradually with lower serum albumin concentrations (Log-rank test $p< 0.001$).

To facilitate the interpretation of ROC curve, we described the accuracy of multiplicative inverse (1/x) of serum albumin levels for the diagnosis of tuberculosis in Fig. 3. The area under the ROC curve was 0.81 (95% CI, 0.78-0.83). To explore the utility of serum albumin in clinical practice, we described the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of several thresholds of serum albumin concentrations (Table 2).[24] No threshold was useful to rule out or rule in tuberculosis. However, serum albumin concentrations <3.2 g/dL yielded 85% specificity for tuberculosis and serum albumin concentrations <3.8 g/

Table 1. Baseline characteristics of patients and serum albumin concentrations by subgroups

	N (%)	Median Alb (IQR)
Gender		
Male	948 (60.3)	3.4 (2.8-4)
Female	623 (39.7)	3.5 (2.8-4)
Age (years)		
<30	496 (31.6)	3.5 (2.9-4.1)
30-35	338 (21.5)	3.5 (2.9-4.1)
35-40	302 (19.2)	3.4 (2.9-3.9)
>40	435 (27.7)	3.2 (2.7-3.8)
CD4 count (cells/µl)		
<100	657 (41.8)	3.2 (2.6-3.7)
100-200	590 (37.6)	3.5 (2.9-4)
>200	324 (20.6)	3.9 (3.2-4.3)
Tuberculosis		
No	733 (46.7)	3.9 (3.4-4.3)
Yes	838 (53.3)	3 (2.6-3.5)
Organs involved*		
Abdomen	144 (17.2)	3 (2.6-3.5)
Disseminated	40 (4.8)	2.8 (2.6-3.2)
Lymphadenitis	121 (14.4)	3.2 (2.8-3.7)
Meningitis	122 (14.6)	3.2 (2.8-3.7)
Pleura	104 (12.4)	3 (2.5-3.2)
Pulmonary	290 (34.6)	2.9 (2.3-3.4)
Others	17 (2)	3.3 (2.8-3.6)

*Only in patients who experienced tuberculosis. Alb, serum albumin concentration (g/dL); IQR, interquartile range.

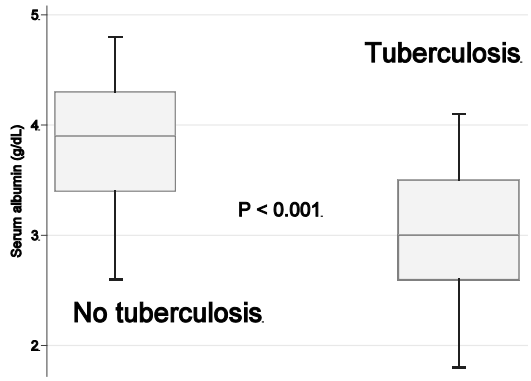

Fig. 1. Box-plots of serum albumin concentrations in 838 patients who experienced tuberculosis within six months of becoming eligible for antiretroviral therapy and in 733 patients who did not experience tuberculosis. Boxes represent median and 25-75 percentiles. Whiskers represent 5 and 95 percentiles.

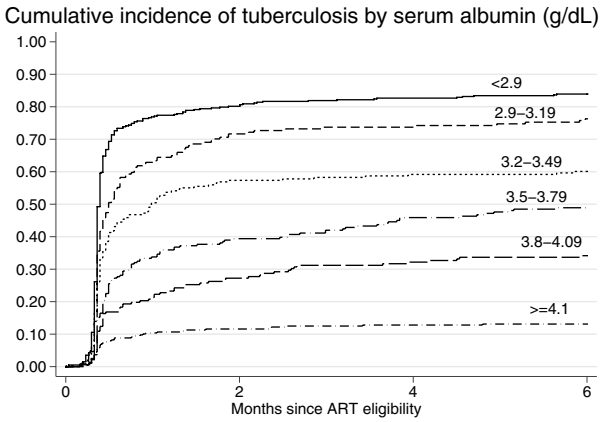

Fig. 2. Kaplan-Meier curves of incident tuberculosis by serum albumin concentrations at baseline

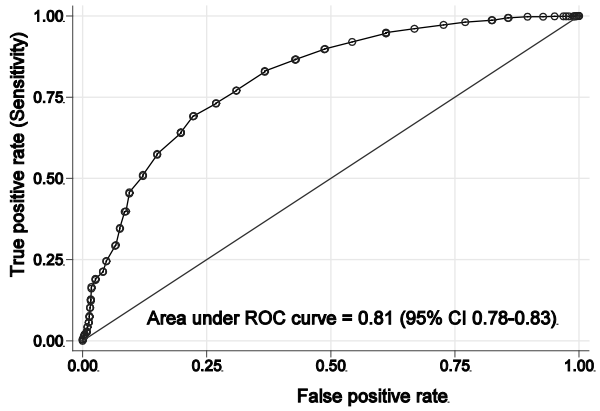

Fig. 3. Area under receiver operating characteristic (ROC) curve of the multiplicative inverse (1/x) of serum albumin for the diagnosis of tuberculosis

dL yielded 90% sensitivity for tuberculosis. As predictive values depend on the prevalence of tuberculosis, we calculated the PPV and NPV of several thresholds of serum albumin concentrations in settings with 1%, 5%, 10%, 20%, 30% and 40% prevalence of tuberculosis (Fig. 4). While the NPV of serum albumin concentrations <3.8 g/dL or <4.1 g/dL was above or near 90% even in settings with high tuberculosis prevalence, the PPV of the test was less satisfactory, especially in settings with low tuberculosis prevalence.

The prognostic value of serum albumin concentrations in patients with tuberculosis is presented in Fig. 5. The mortality of patients with tuberculosis increased gradually with lower serum albumin concentrations (p = 0.0013).

The reagent cost of performing one serum albumin measurement was 1 Indian rupee (0.017 USD in July 2013).

Discussion

The results of this study show that the concentration of

Table 2. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of several cut-offs of serum albumin concentration for the diagnosis of tuberculosis in a setting with 53% tuberculosis prevalence.

Albumin (g/dL)	Study group %	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
<2.3	11.2	18.7 (16.1-21.5)	97.4 (96-98.4)	89.2 (83.7-93.4)	51.2 (48.5-53.8)
<2.6	18.7	29.2 (26.2-32.4)	93.5 (91.4-95.1)	83.6 (78.9-87.7)	53.6 (50.8-56.4)
<2.9	25.3	39.9 (36.5-43.3)	91.3 (89-93.2)	83.9 (79.9-87.4)	57 (54.1-59.9)
<3.2	37.7	57.5 (54.1-60.9)	85 (82.2-87.5)	81.4 (78-84.5)	63.6 (60.5-66.7)
<3.5	51.6	73.2 (70-76.1)	73.1 (69.8-76.3)	75.7 (72.6-78.6)	70.4 (67.1-73.7)
<3.8	70.6	89.7 (87.5-91.7)	51.3 (47.6-55)	67.8 (65-70.6)	81.4 (77.5-84.8)
<4.1	82.4	96.1 (94.5-97.3)	33.2 (29.7-36.7)	62.2 (59.5-64.8)	88 (83.6-91.6)

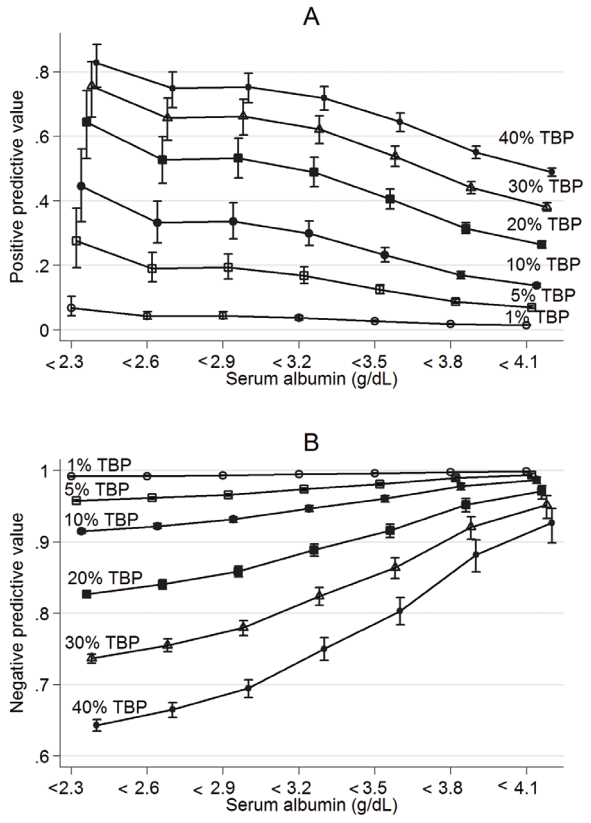

Fig. 4. Positive (A) and negative (B) predictive value of serum albumin concentration for the diagnosis of tuberculosis in settings with 40%, 30%, 20%, 10%, 5% and 1% tuberculosis prevalence (TBP) among HIV-infected patients

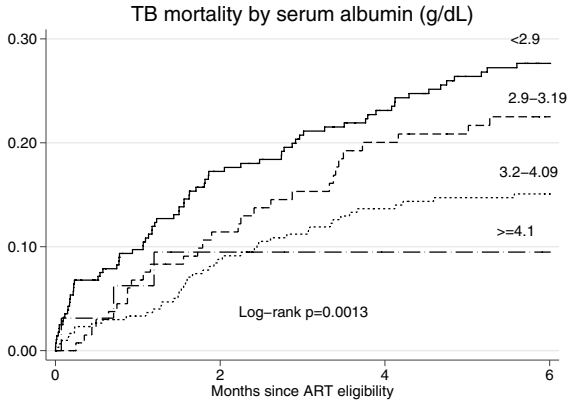

Fig. 5. Cumulative incidence of mortality in 838 HIV-infected patients with tuberculosis (TB) by serum albumin concentrations

serum albumin can be a useful diagnostic marker for tuberculosis in HIV infected patients. The diagnostic accuracy of serum albumin, measured by the area under the ROC curve, was higher than the diagnostic accuracy of models including the WHO symptom screening, CD4 lymphocyte count, and body mass index, which was 0.74 (95% CI, 0.69-0.80) in a South-African HIV clinic.[25] Given the low cost of serum albumin and the low sensitivity of the WHO symptom screening, these data suggest that serum albumin could be useful to improve the diagnostic accuracy of WHO intensified tuberculosis case finding algorithms to determine the risk of tuberculosis before initiating isoniazid preventive therapy in resource-limited settings.[5]

The study shows that the diagnostic utility of serum albumin in the common clinical practice is not straightforward. Serum albumin concentrations <3.2 g/dL were associated with 85% specificity, and serum albumin concentrations >3.8 g/dL were associated with good negative predictive values even in settings with high tuberculosis prevalence. However, we failed to find a threshold to rule out or rule in tuberculosis, so the decision of whether to start anti-tuberculous treatment should be made taking into account other factors associated with an increased risk of tuberculosis such as compatible symptoms, low CD4 lymphocyte count, chest radiology findings, or the prevalence of tuberculosis in the area. In a previous study, low serum albumin concentrations were not associated with other HIV-related infections such as pneumonia, oral candidiasis, Kaposi sarcoma or chronic diarrhoea.[14] Serum albumin is reduced in several conditions such as

malnutrition, chronic inflammation, or liver problems.[26] Therefore, ruling out causes of these conditions is likely to increase the positive predictive value of low serum albumin concentrations.

In accordance with previous studies,[14,27,28] we found that hypoalbuminemia was associated with mortality in patients with tuberculosis. Patients with serum albumin <3.2 g/dL had a six month mortality above 20%, indicating these patients will need extra care to reduce the risk of death.

The study has some limitations. A big proportion of patients eligible for ART (43%) were not included in the study because their serum albumin concentration was not measured at the moment of ART eligibility. It is possible that patients having serum albumin concentration measured were more likely to have tuberculosis, which could partially explain the high tuberculosis prevalence found in our study. In addition, we did not perform sputum culture for tuberculosis; so some patients with pulmonary tuberculosis might have been misclassified as not having tuberculosis. However, the definition of tuberculosis case used in this study reflects the "real life" situation of patients treated in resource-limited settings. Serum albumin concentrations might be less useful in patients with suspicion of tuberculous lymphadenitis or meningitis, because these forms of tuberculosis had higher serum albumin concentrations than patients with disseminated, pulmonary or abdominal tuberculosis.

Conclusion

The results of this study indicate that serum albumin concentration can be a useful diagnostic and prognostic marker for tuberculosis in HIV infected patients eligible for ART. Serum albumin concentrations <3.2 g/dL were associated with 85% specificity and an increased risk of mortality in patients with tuberculosis. Serum albumin concentrations >4.1 g/dL were associated with good negative predictive values and a reduced risk of mortality in patients with tuberculosis. However, we could not find thresholds to rule out or rule in tuberculosis, so the decision of whether or not initiate antituberculous treatment in HIV infected patients should not be based on serum albumin concentrations alone.

Acknowledgements

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Ethical issues

The study was approved by the ethical committee of the RDT Institutional Review Board.

Competing interests

The authors declare that they have no competing interests.

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