Heme Oxygenase-1 and Neopterin Plasma/Serum Levels and their role in Diagnosing Active and Latent TB among HIV/TB Co-Infected Patients

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Research article

Keywords: Latent tuberculosis infection (LTBI), Active tuberculosis,Biomarker

Posted Date: September 23rd, 2019

DOI: https://doi.org/10.21203/rs.2.14803/v1

Version of Record: A version of this preprint was published at BMC Infectious Diseases on July 27th, 2021. See the published version at https://doi.org/10.1186/s12879-021-06370-7.

Abstract

Background: Tuberculosis(TB) diagnosis in the presence of HIV co-infection remains challenging. Heme oxygenase 1(HO-1) and neopterin have been validated as potential biomarkers for TB diagnosis. Infection of macrophages with Mycobacterium tuberculosis (M .tb) causes the production of HO-1 and neopterin and previous studies have shown these to be markers of immune activation. This study was conducted to determine the levels of HO-1 and neopterin and their utility in the diagnosis of TB among individuals enrolled in the Community Health and Social Network of Tuberculosis(COHSONET) study and the Kampala TB Drug Resistance Survey(KDRS). Methods: A total of 210 participants were enrolled in a study of a diagnostic method aimed at determining the levels of HO-1 and neopterin and determine their diagnostic accuracy as biomarkers in TB diagnosis from March to May 2019. M. tb culture was performed on sputum to confirm active TB(ATB) and QuantiFERON TB gold test to confirm latent TB infection(LTBI). ELISAs were performed to determine the levels of HO-1 and neopterin. Data analysis was done using Kruskal Wallis and Receiver Operating Characteristic curves to determine the diagnostic accuracy. Results: HO-1 levels among ATB/HIV patients, LTBI/HIV patients and TB negative individuals were 10.7ng/ml (IQR: 7.3-12.7ng/ml), 7.5ng/ml (IQR: 5.4-14.1ng/ml), 3.3ng/ml (IQR: 2.0-7.1ng/ml) respectively. Neopterin levels among ATB/HIV patients, LTBI/HIV patients and TB negative individuals were 11.7ng/ml (IQR: 5.219.4ng/ml), 8.8ng/ml (IQR: 2.4-19.8ng/ml), and 5.9ng/ml (IQR: 3.410.2ng/ml) respectively. HO-1 showed a sensitivity of 78.57% and a specificity of 71.43% with area under the curve(AUC) of 0.839 when used to diagnose ATB. HO-1 showed AUC of 0.79, sensitivity of 70% and specificity 70% when used to diagnose LTB. Neopterin showed a sensitivity of 61.43% and a specificity of 74.29% with AUC 0.71 when used to diagnose ATB. Neopterin as a biomarker in LTB diagnosis showed AUC of 0.56 which was not significant. Conclusion: HO-1 and neopterin are valuable diagnostic biomarkers for ATB and LTB which could be further utilized to develop less costly rapid diagnostic tools to overcome current TB diagnostic challenges.

Background

In 2016, more than two billion people globally were estimated to be latently infected with *Mycobacterium tuberculosis*. In 2017, 10 million people fell ill with tuberculosis (TB), and 1.6 million died from the disease (including 0.3 million among people with HIV) and TB is the leading cause of death among HIV positive individuals globally(1). In Uganda, the prevalence of Latent tuberculosis infection (LTBI) was estimated to be 49% among adults (2, 3). LTBI is diagnosed using the Tuberculin Skin Test (TST) and Interferon Gamma Release Assays (T-Spot and QuantiFERON TB gold test). However, the above diagnostic tests have shown challenges, for example, TST is associated with false positives or negatives especially among immunocompromised individuals like HIV/TB co-infected individuals with impaired cell-mediated immunity (4).

QuantiFERON test is technology-intensive, requires expertise and difficult to implement in resource-limited settings. More so, all these tests cannot tell whether one has a current, cleared, progressed to active

infection as they are based on infection with *Mtb* and give a positive result for both latently and actively infected patients. Hence the difficulty in distinguishing ATB from LTBI during TB diagnosis(5)

As a result, biomarkers such as *M. tb* thymidylate kinase (TMKmt) antigen (6), LAM (7), Heme oxygenase 1(HO-1 (8) and neopterin (9) have been studied. Studies have shown Heme oxygenase 1(HO-1) and neopterin to successfully distinguish LTBI from ATB (10). HO-1 is a key stress enzyme that is highly expressed in the lung tissue during *Mtb* infection and an anti-oxidant that degrades heme to iron, biliverdin, and carbonmoxide (11). Neopterin is a product of guanosine triphosphate and is produced by human macrophages upon stimulation with the Th1 cell-derived cytokine interferon-gamma more so neopterin has been shown to be a marker of immune activation during *Mycobacterium tuberculosis* infection (12).

LTBI is diagnosed using TST and QuantiFERON TB gold test, however, there is no rapid diagnostic test to accurately distinguish LTBI from ATB. The sensitivity of TST is reduced for persons with impaired cell-mediated immunity as a reaction to PPD is impaired including individuals with HIV infection(13) and yet diagnosing latent tuberculosis infection is important for the overall control of the disease.

More importantly, offering antituberculous treatment to individuals with LTBI significantly decreases their risk of developing active tuberculosis(14). HIV infection increases the risk of reactivation of latent TB infection as infection with HIV is the most powerful known risk factor predisposing for *Mycobacterium tuberculosis* infection(15) and progression to active disease, which increases the risk of latent TB reactivation.

A study done in India in 2013 on plasma heme oxygenase 1 level to distinguish ATB from LTBI showed that plasma levels were elevated in those with ATB compared to individuals with latent infection and the healthy control (8). However, HO-1 levels in ATB and LTBI have not been studied in several other settings including Uganda. Another study also in India in 2004, on serum neopterin levels in HIV infected patients with and without tuberculosis showed serum neopterin levels to be highest in HIV positive individuals with ATB and lowest in healthy controls (10). However, these two soluble markers have not been studied together rather independently Therefore, this study aimed at determining the Plasma levels of HO-1 and neopterin and their role in distinguishing LTBI from ATB among patients and also determined the diagnostic accuracy of HO-1 and neopterin in diagnosing tuberculosis compared to the sputum culture and QuantiFERON TB gold test.

Materials And Methods

Study Subjects

Archived samples collected by Community Health and Social Network of Tuberculosis (COHSONET) study and Kampala TB Resistance Survey study from 70 patients with active TB (ATB), 70 individuals with LTBI and 70 healthy donors were used in this study. Diagnosis of ATB and LTB was based on

sputum culture positivity and QuantiFERON TB gold test ELISA positivity respectively by the above parent studies. De-identified data coded with participant identification numbers were extracted.

Study Design: A study of diagnostic method nest in Social Network of Tuberculosis (COHSONET) study and Kampala TB Resistance Study

Measurement of Plasma HO-1 and neopterin

HO-1 and neopterin levels in the samples were determined using the human heme oxygenase 1 ELISA kits (Xpress Biotech International) and human neopterin ELISA kits (Express Biotech International) respectively after optimizing by running different sample dilutions.

HO-1 ELISA

Sandwich ELISA was used as the method. The anti-ho-1 antibody was pre-coated onto the plate and the biotin-conjugated anti-HO-1 antibody was used as the detection antibodies. The standards, test samples, and detection antibody were added to wells subsequently and washed with wash buffer. HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer. TMB substrates were added followed by a stop solution. The color change was determined spectrophotometrically at a wavelength of 450nm. The concentration of HO-1 in the sample was determined by comparing the optical density of samples to the standard curves.

Laboratory procedure

In order to determine the level of HO-1 enzyme in the samples, a sandwich ELISA was done in the Immunology laboratory, Makerere University, using commercially obtained kits (Express Biotech International Cat No. XPEH3234). HO-1 kits and the samples were removed from the refrigerator and allowed to attain room temperature. The standard solution was serially diluted to obtain different dilutions. The HO-1 ELISA protocol was optimized by first running known samples before running the test samples The plated wells were washed using 350 µl phosphate-buffered saline (PBS) in each well two times with a soaking time of 1 minute and then blotted to dry. 0.1ml of 20ng/ml, 10ng/ml, 5ng/ml, 2.5ng/ml, 1.25ng/ml, 0.625ng/ml, 0.313ng/ml standard solutions were aliquoted into the standard wells. 0.1ml of the sample (in duplicate) was into the sample wells. The plate was sealed with a cover and incubated at 37ºC for 90 minutes. The cover was removed and contents discarded then washed plate two times using wash buffer. 0.1 ml Biotin-detection antibody working solution into the above wells and plate incubated at 37 ºC for 60 minutes. The cover was removed and washed three times with wash buffer.

Following washing, 0.1 ml of SABC working solution into each well, covered and incubated at 37 ºC for 30 minutes. The plate was washed five times with wash buffer. Then 90 µl of TMB substrate was added into each well, the plate covered and incubated at 37 ºC in the dark for 20 minutes. Then 50 µl of stop solution was added into each well and mixed thoroughly and the colour changed from blue to yellow immediately. The plate was read at 450nm

Neopterin ELISA

Competitive ELISA was used as the method. The microtiter plate was pre-coated with neopterin. During the reaction, neopterin in the sample or standard competes with a fixed amount of neopterin on the solid phase supporter for sites on the Biotinylated Detection Antibody specific to neopterin. Excess conjugate and unbound sample or standard were washed from the plate and HRP-Streptavidin(SABC) was added to each well and incubated. Then TMB substrate was added to each well followed by a stop solution. The color change was determined spectrophotometrically at a wavelength of 450nm. The concentration of neopterin in the sample was determined by comparing the optical density of samples to the standard curves

Laboratory procedure

To determine the concentration levels of neopterin in the samples, a competitive ELISA was performed using a commercially prepared neopterin kit (Cat No. XPEH3413). Neopterin kits and the samples were removed from the refrigerator and allowed to attain room temperature. The standard solution was serial diluted and the protocol optimized. The plate was washed two times before adding standard, sample and controls wells then 50 µl of sample and standard solution was added to the wells. Immediately 50 µl of Biotin-detection antibody to each well and incubated for 45 minutes at 37 ºC. The plate was then washed three times using a phosphate buffer. 100 µl SABC working solution was added to each well and incubated for 30 minutes at 37 ºC, followed by washing the plate five times. 90 µl of TMB substrate was added to each well and incubated for 20 minutes at 37 ºC. 50 µl stop solution was added to the wells. The plate was read at 450nm.

Data Analysis

Median values with interquartile ranges (IQR) were used as measures of central tendency. HO-1 and neopterin levels were compared among the study groups using the Kruskal-Wallis test with Dunn's multiple comparisons. Receiver Operator Characteristics (ROC) curves were designed to test the diagnostic accuracy of HO-1 and neopterin to distinguish LTBI from ATB. Univariate linear regression analysis was performed to assess the odds ratios (OR) of the associations between HO-1, neopterin, ATB and LTBI. Correlation between neopterin and HO-1 plasma levels with QuantiFERON -TB Gold test in diagnosing LTBI was measured using Bland-Altman Plot for percentage agreement. The statistical analysis was done using Graphpad Prism version 8.1

Results

Participant baseline characteristics

A total of 210 archived participants' plasma/serum samples were included in this study. The participants were stratified into three groups; 70 (33.33%) of the participants were active TB patients based on positive sputum culture, 70 (33.33%) of the participants had latent TB infected patients diagnosed by

QuantiFERON TB Gold test and 70 (33.33%) were TB negative individuals. One hundred eighteen (56.2%) of the participants were males. Details of participant demographic characteristics stratified by the study group are summarized in Table 1 below.

HO-1 levels in serum/plasma

The median concentration levels of HO-1 among ATB/HIV co-infected patients, LTBI/HIV co-infected patients, and TB negative individuals were 10.7ng/ml (IQR: 7.3-12.7ng/ml), 7.5ng/ml (IQR: 5.4-14.1ng/ml) and 3.3ng/ml (IQR: 2.0-7.1ng/ml) respectively Fig 2. HO-1 levels were significantly higher among ATBI/HIV patients compared to TB negative individuals (P-value <0.0001). HO-1 levels among LTBI/HIV co-infected patients were significantly higher compared to TB negative individuals (P-value <0.0001). There was no significant difference in HO-1 levels among ATBI/HIV patients compared to LTBI/HIV patients (P-value =0.267). However, there was a significant difference in HO-1 levels across the three study groups (P-value <0.0001)

Neopterin levels in serum/plasma

The median concentration levels of neopterin among ATB/HIV co-infected patients, LTBI/HIV co-infected patients, TB negative individuals were 11.7ng/ml (IQR: 5.2-19.4ng/ml), 8.8ng/ml (IQR: 2.4-19.8ng/ml) and 5.9ng/ml (IQR: 3.4-10.2ng/ml) ng/ml respectively. Neopterin levels were significantly higher in ATBI/HIV patients compared to TB negative (P-value <0.0001) and LTBI/HIV patients (P-value = 0.0256). There was no significant difference in neopterin concentrations among LTBI/HIV patients when compared to TB negative individuals (P-value = 0.219). However, there was a significant difference in the median concentrations of neopterin across the three study groups (P-value <0.0002)

Diagnostic accuracy of HO-1

To explore the possibility of using HO-1 and neopterin as a possible diagnostic biomarker for ATB, and LTBI, receiver operating characteristics (ROC) curves were used to assess the diagnostic accuracy of HO-1 in the diagnosis of TB

In testing HO-1 as a marker of active TB, HO-1 had an area under the curve (AUC) of 0.833 (95% CI, 0.76 – 0.9) (Fig 4). A highest diagnostic accuracy for HO-1 was obtained by using a cut off value of > 6.38ng/ml, with a sensitivity of 78.57% (95% CI, 67.1% - 87.5%) and a specificity of 71.43% (95% CI, 59.4% - 81.6%). Negative predictive value (NPV) and positive predictive value (PPV) were also computed from the above sensitivity and specificity at a prevalence of TB of 43.75% (70/210). NPV was 86.96% and PPV was 57.89%

In exploring HO-1 as a marker in latent TB diagnosis, HO-1 levels had an area under the curve AUC of 0.79(95% CI, 0.72%-0.87%)with a sensitivity of 70% (CI, 57.9%-80.4%) and a specificity of 70%(CI, 57.9%-80.4%) obtained using >5.94ng/ml as the cut off. NPV and PPV were also computed from the above sensitivity and specificity at a prevalence of TB of 43.75% (70/210). NPV was 82.35% and PPV was 53.85%

Diagnostic accuracy of neopterin

In exploring neopterin as a marker for active TB diagnosis, area under the curve AUC was 0.71 (95% CI, 0.63-0.8) and a diagnostic accuracy obtained using a cut-off>9.675ng/ml with a sensitivity of 61.43% (95% CI, 49%-72.8%) and a specificity of 74.29% (95% CI, 64.2%-84%).NPV and PPV were also computed from the above sensitivity and specificity at a prevalence of TB of 43.75% (70/210). NPV was 74.28% and PPV was 54.43%

In exploring neopterin as a marker in latent TB diagnosis, HO-1 levels had an area under the curve AUC of 0.56 (95% CI, 0.46-0.66) which was not significant (Fig 5B).

a) Correlation between neopterin and HO-1 plasma levels with QuantiFERON-TB Gold test We tested neopterin and QuantiFERON TB Gold test level of agreement using Kappa test for agreement: There was a fair Kappa agreement of 0.27 and a percentage agreement of 63.57% between neopterin and QuantiFERON TB Gold test in diagnosing latent TB. (Table 2)

b) We tested HO-1 and QuantiFERON TB Gold test level of agreement in LTBI

There were a 0.2 Kappa agreement and a percentage agreement of 60% between HO-1 and QuantiFERON TB Gold test in diagnosing latent TB (Table 3)

Discussion

We report data to support the fact that heme oxygenase 1 (HO-1) and neopterin concentration levels could potentially identify patients with active tuberculosis, latent tuberculosis, and TB negative individuals. While the current diagnostic methods for tuberculosis can't distinguish between active and latent tuberculosis, previous studies have shown several biomarkers and there use in distinguishing active from latent tuberculosis such as LAM (16), *Mycobacterium tuberculosis* TMK antigen (6) neopterin, C-reactive protein (17) and these studies have shown some of the biomarkers to be promising diagnostic markers. However, no study has been carried out to clinically test the diagnostic potential of HO-1 and neopterin in our population. Therefore, in this study, we aimed to determine and compare the median levels of HO-1 and neopterin in plasma/serum of active TB/HIV co-infected patients, latent TB/HIV co-infected patients and the diagnostic accuracy of these markers in diagnosing active tuberculosis and latent tuberculosis

Interestingly, there was a statistical difference in the median serum/plasma heme oxygenase 1 (P-value<0.0001) and neopterin (P-value<0.0002) levels among active TB patients, latent TB patients, and TB negative individuals. These findings are in agreement with findings from study by Michael Eyeshot et., al (2016) assayed for urinary neopterin to distinguish active tuberculosis from latent *Mycobacterium tuberculosis* infection, which revealed that neopterin levels were significantly higher in patients with active tuberculosis 374.1 micromol/mol compared to patients with latent infection was 142.1 micromol/mol (P<0.01) (17). Another study on Plasma heme oxygenase-1 levels distinguish latently or

successfully treated human tuberculosis from active disease showed that Individuals with active pulmonary displayed significantly higher systemic levels of HO-1 5.8ng/ml [IQR: 3.2–11.6] ng/mL and 3.45ng/ml [2.0–4.5] ng/mL, respectively; P<0.01) than either individuals with latent TB or healthy controls, who expressed only marginal concentrations of the HO-1 1.3ng/ml [0.78–1.5] ng/mL and 1.4 [1.0–1.9] ng/mL, respectively; P=0.49 This was similar to our findings in which HO-1 levels were highest among ATB/HIV 11.7ng/ml (5.2-19.4)ng/ml compared to latently infected patient and TB negative individuals who expressed lower concentrations of 8.8ng/ml (2.4-19)ng/ml and 5.9ng/ml (3.4-10.2)ng/ml respectively.

Our study also carried out receiver operator characterization of HO-1 as a biomarker for TB diagnosis. HO-1 showed good diagnostic accuracy with a sensitivity of 78.5% and specificity of 71.43% for active TB diagnosis and a sensitivity of 70% (CI, 57.9%-80.4%) and a specificity of 70% for latent TB diagnosis. A similar study carried out in Southern India by Andrade et al (2013) demonstrated that HO-1 had the highest discriminatory power with a 23.5% higher specificity in distinguishing active from LTBI compared to SAA (94.9% vs. 71.4%, respectively) and 48.8% higher specificity compared to CRP (94.9% vs. 46.1%, respectively (8). The difference in higher diagnostic accuracy compared to our study could be attributed to the sample size difference in different study groups whereby the number of active TB individuals was much more than the latently infected group and the healthy donors.

Receiver operator characterization of neopterin was also done as a potential biomarker for TB diagnosis. Neopterin demonstrated to be a fair biomarker for active TB diagnosis with a sensitivity of 61.43% and a specificity of 74.29% for ATB diagnosis. However, neopterin presented as a poor diagnostic marker for LTBI. Generally, HO-1 showed a better diagnostic ability for active TB and latent TB diagnosis compared to neopterin. When the agreement between neopterin, HO-1 and QuantiFERON TB Gold test using Kappa test for agreement in patients with latent TB, neopterin showed a fair agreement while HO- showed a slight agreement with QuantiFERON TB gold test.

When compared to the above studies, the difference could also be due to cross-reactivity of HO-1and neopterin with HO-1 and neopterin produced as a result of other infections causing immune activation other than tuberculosis. The transitional significance of our research is the possibility of using HO-1 and neopterin to develop low cost, easy to use a rapid diagnostic test which could be employed for TB screening by measuring these biomarkers to identify TB patients as they show significantly different concentration levels at different disease status of tuberculosis. Storage circumstances of the serum/plasma samples used in the study and the number of times these samples had been thawed are factors that could contribute to the levels of the biomarkers in the samples. We also did not consider a group of patients with other infections that affect the systemic levels of these biomarkers.

Conclusions

Findings from our study further reinforce previous observations that there are higher levels of HO-1 and neopterin serum/plasma levels in active tuberculosis and latent tuberculosis compared to TB negative

individuals. Therefore, HO-1 and neopterin could be reliable diagnostic biomarkers for TB. These form alternative tests to replace smear microscopy and overcome the current TB diagnostic challenges since the ELISA assays can easily be translated to a point of care, thereby enabling cheap, easy to use and rapid detection of tuberculosis.

We assessed HO-1 and neopterin in plasma and serum, however, future research should assess HO-1 and neopterin levels in other biological samples including sputum, pleural fluids, lymph node aspirates, cerebral spinal fluid, and bone marrow aspirates. We also recommend that these biomarkers are measured in samples which have not been stored for a very long time or fresh samples as the concentration levels of these markers could have reduced in the samples over the period of storage

List Of Abbreviations

Nconc	concentration of neopterin
Hconc	concentration of heme oxygenase 1
HO-1	heme oxygenase 1
AIDS	Acquired Immune Deficiency Syndrome
ATB	Active Tuberculosis
ATP	Adenosine triphosphate
CI	Confidence intervals
COHSONET	Community Health and Social Network of Tuberculosis
dTTP	deoxythymidine-5’ -triphosphate
ELISA	Enzyme-linked immunosorbent assay
Fig	Figure
HIV	Human Immunodeficiency Virus
IGRA	Interferon-gamma release assay
KDRS	Kampala Drug Resistance Survey
LTB	Latent tuberculosis
LTBI	Latent Tuberculosis Infection
OD	Optical density

PBS	Phosphate Buffered Saline
TB	Tuberculosis
TMB	3,3’,5,5’-Tetramethylbenzidine
TST	Tuberculin Skin Test
WHO	World Health Organization

Declarations

Ethical Approval and consent to participate

Ethical approval was obtained from the School of Biomedical Sciences Higher Degrees Research and Ethics Committee (SBS-606) Makerere University and Uganda National Council of Science and Technology (HS301ES).

Waiver of consent for this analysis was also obtained from the School of Biomedical Sciences Higher Degrees Research and Ethics Committee. Participants provided written informed consent including storage and future use of samples at enrolment in the parent studies. Participant information obtained from the parent studies was coded and identification numbers were used for purposes of confidentiality and privacy.

Consent for publication

Not applicable

Availability of data and materials

The data that support the findings of this study are available from the parent studies but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of parent studies.

Competing interests

Co-author Barbara Castelnuovo is a Section Editor of this journal. The other authors have declared that no competing interests exist

Funding

This work was funded by the EDCTP Reg Net. 2015-1104 TB Node, MAPRONANO ACE Uganda who provided funds for laboratory reagents and logistical support, MakCHS–UC-Berkeley-Yale Pulmonary

Complications of AIDS Research Training (PART) Program, NIH D43TW009607, from the Fogarty International Center which also provided logistical support.

Authors' contributions

Wrote the concept/proposal and Performed the laboratory experiments: EU, BSB, BC, DPK.

Analyzed the data, EU, BSB, BC, DPK, AG.

Contributed reagents/materials/analysis tools: BC, NK, CCW, MJ,

Wrote the paper: EU, BSB, BC, DPK.

Acknowledgments

I would like to acknowledge the Infectious Disease Institute Makerere University and MakCHS–UC-Berkeley-Yale Pulmonary Complications of AIDS Research Training (PART) Program, NIH D43TW009607, from the Fogarty International Center for the technical support and mentorship during my period of study

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Tables

Table 1: Patient baseline characteristics stratified by TB status

Table 1

Table 2: Agreement between neopterin and QuantiFERON TB Gold test using the Kappa test for agreement in patients with latent TB

Characteristic	Active TB/HIV N (%)	Latent TB/HIV N (%)	TB negative N (%)	P-Value	Total
Disease state	70(33.33%)	70(33.33%)	70(33.33%)		210
Sex				0.549	
Female	27 (38.57%)	32(45.71%)	33(47.14%)		92 (43.81%)
Male	43(61.43%)	38(54.29)	37(52.86%)		118 (56.19%)
Age in years Median (IQR)	28 (24-36)	24(21-30)	27(21-38)	0.0029	26 (22-35)
HConc Median (IQR) ng/ml	10.7(7.3-12.7)	7.5(5.4-14.1)	3.3(2.0-7.1)	0.0001	7.2 (4.4-12.3)
NConc Median (IQR) ng/ml	11.7(5.2-19.4)	8.8(2.4-19)	5.9(3.4-10.2)	0.0002	8.1 (3.5-16.7)

Agreement	Expected Agreement	Kappa	Standard Error	Z	Prob>Z
63.57%	50.00%	0.2714	0.0717	3.78	0.0001

Table 3: Kappa test for agreement between HO-1 and QuantiFERON TB Gold test in patients with latent TB

Agreement	Expected Agreement	Kappa	Standard Error	Z	Prob>Z
60.00%	50.00%	0.2000	0.0828	2.42	0.0079

Figures

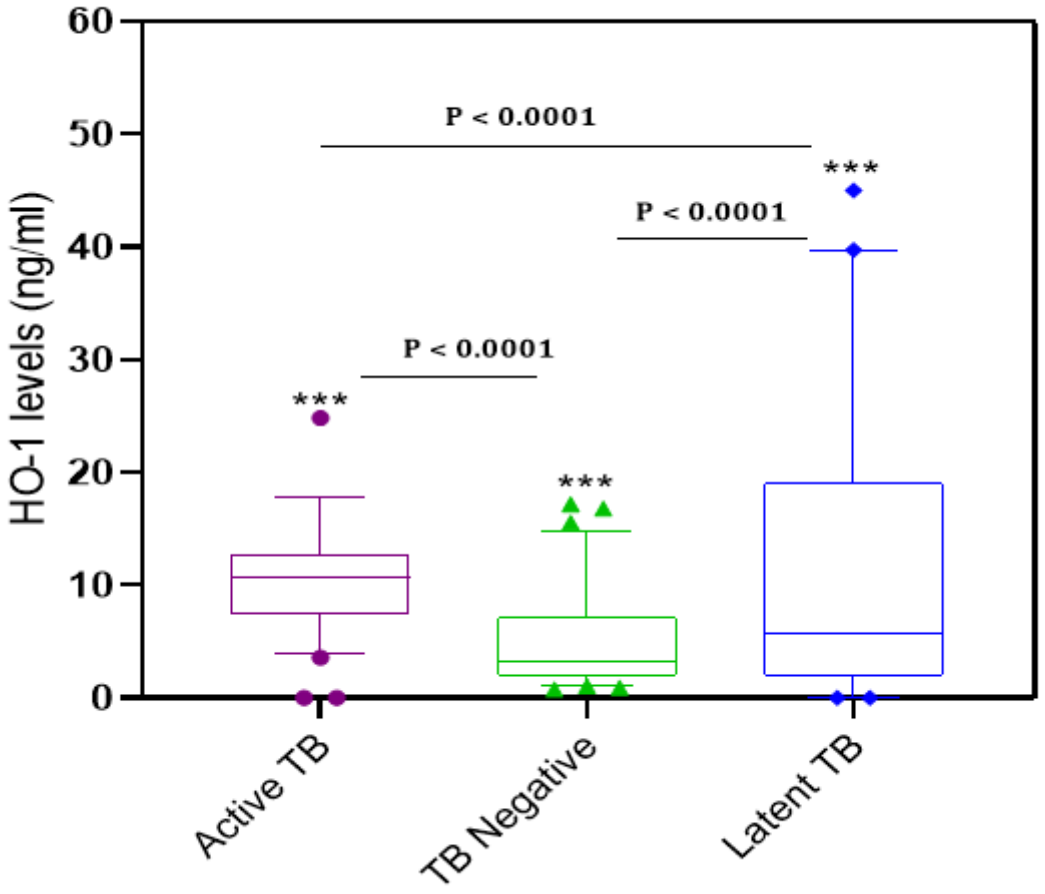

Figure 1

Concentration HO-1 levels in plasma/serum among active TB patients, latent TB patients, and TB negative individuals. The boxes show median and interquartile ranges, whiskers show the 5th and 95thpercentiles, dots represent outliers.

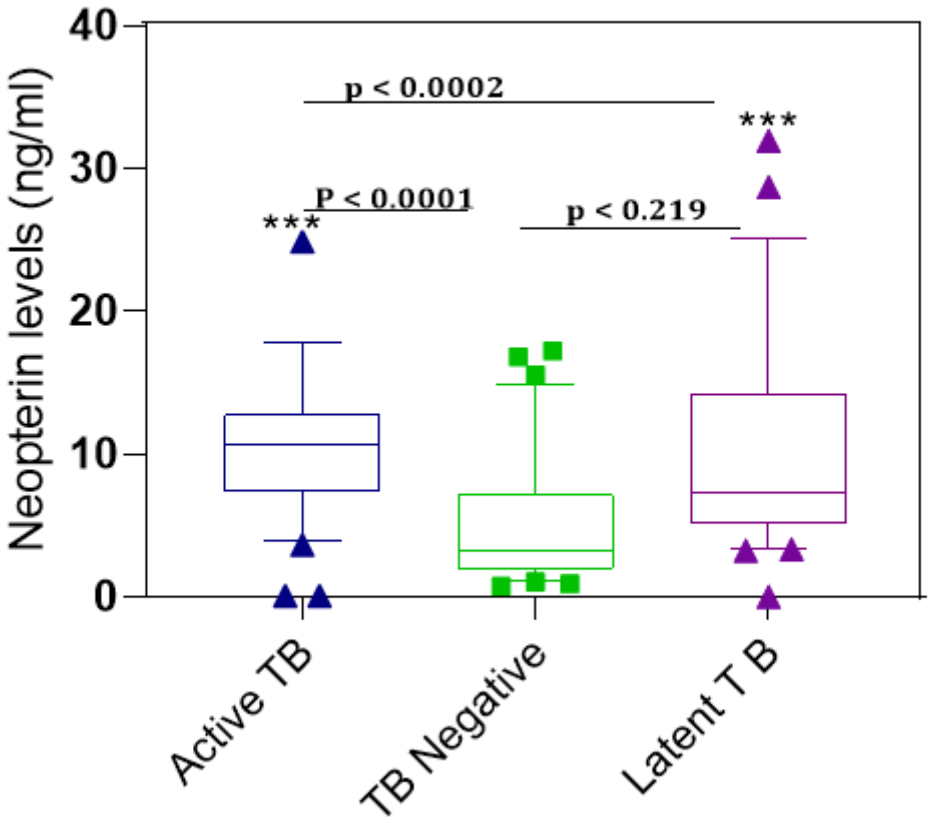

Figure 2

Concentration of neopterin levels (ng/ml) in plasma/serum among active TB patients, latent TB patients, and TB negative individuals. The boxes show median and interquartile ranges, whiskers show the 5th and 95thpercentiles, dots represent outliers.

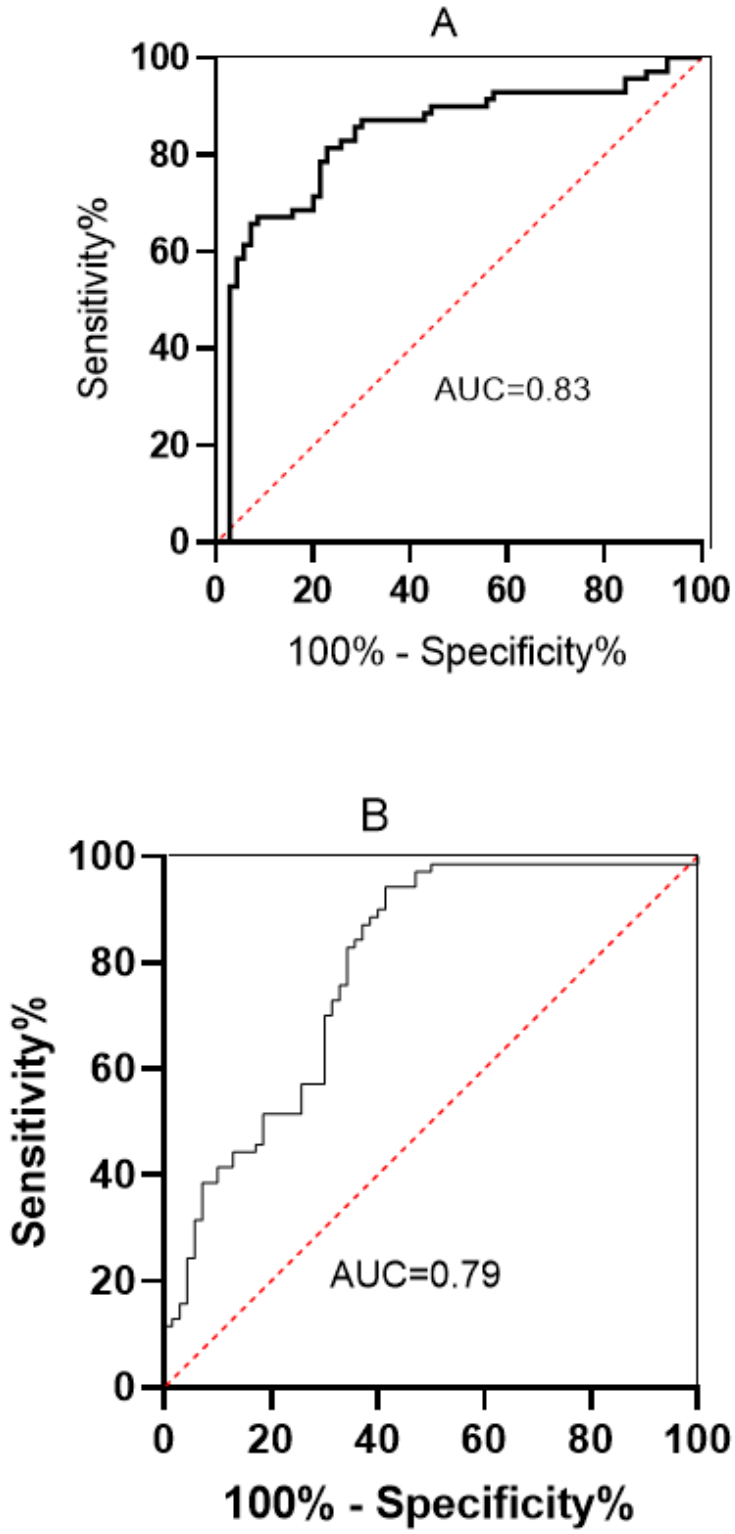

Figure 3

A: Receiver operating characteristic curves for HO-1 as a diagnostic biomarker for active TB diagnosis, B: Receiver operating characteristic curves for HO-1 as a diagnostic biomarker latent TB diagnosis

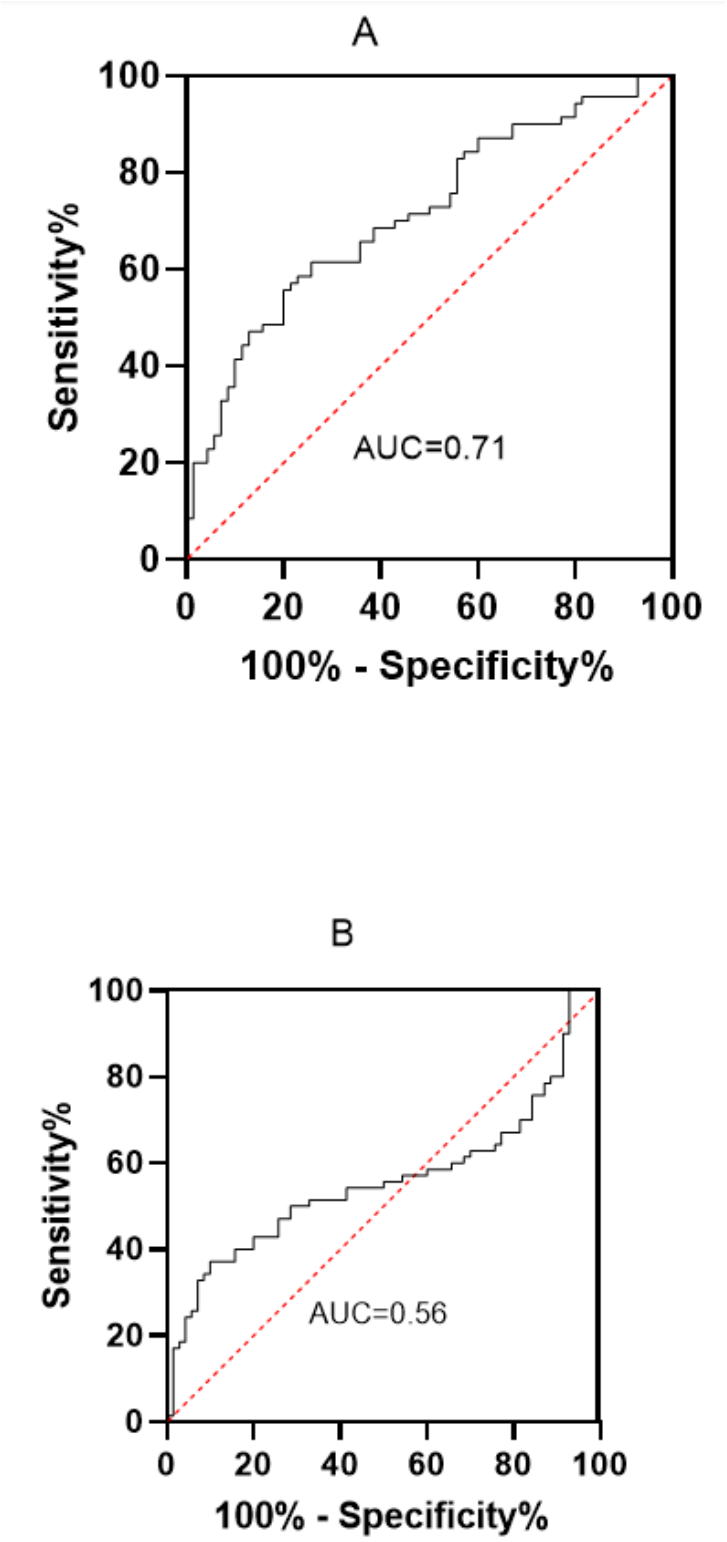

Figure 4

A: Receiver Operating Characteristic curve for neopterin as a diagnostic biomarker for active TB diagnosis. B: Receiver Operating Characteristic curve for neopterin as a diagnostic biomarker latent TB diagnosis.