Nig. J. Pure & Appl. Sci. Vol. 39 (Issue 1, 2026)
e-ISSN 2756-4045
(C) 2026 Faculty of Physical Sciences and Faculty of Life Sciences, Univ. of Ilorin, Nigeria
www.njpas.com.ng

Redox-Immune Axis and Metallomic Signature as Predictors of Treatment Response in Patients on Anti-Tuberculosis Chemotherapy

[1*]Eworo, R.E., [2]Bebia, G.P., [3]Usoro, U.C., [4]Usoro, N.E. and [5]Esiere, K-U.S.

[1,5]Department of Clinical Chemistry & Immunology, Faculty of Medical Laboratory Science, University of Calabar, Nigeria.

[2]Department of Medical Bacteriology, Virology and Mycology, Un-iversity of Calabar

[3,4]Faculty of Clinical Sciences, University of Calabar, Nigeria

Date Received: 29-11-2025
Date Accepted: 20-05-2026
DOI: https://doi.org/10.48198/NJPAS/25.B53

ABSTRACT

Microminerals play crucial roles in regulating the redox-immune response and Anti-Tuberculosis (ATB) treatment outcomes. This study assessed the levels of copper (Cu), zinc (Zn), iron (Fe), chromium (Cr), selenium (Se) arsenic (As), gamma-glutamyl transferase (GGT), cluster of differentiation 4 (CD4+) and computed metal indices in subjects on ATB chemotherapy. Fifty-five pulmonary tuberculosis (TB) subjects attending Clinics at Infectious Diseases Hospital, Calabar and 40 healthy age-matched controls were recruited into this case-control study. The TB subjects were categorized into those on intensive phase (IPAT) and continuation phase of ATB chemotherapy (CPAT). Trace minerals were determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), CD4+ and GGT by flow cytometry and spectrophotometry respectively. Metal indexes were computed. Data generated were analyzed using SPSS version 27, (IBM). Variation among groups were determined by ANOVA and post hoc analyses. The Receiver Operator Characteristic Curve was utilized to determine the thresholds of metal indexes predictive of redox-immune imbalance. The confidence interval was set to 95% at α =0.05. The GGT, Cu/Zn, and Metal-Immune Score (MIS) were significantly higher in IPAT and CPAT ($p<0.05$) than in controls, while CD4+, and Metallomic Recovery Score (MRS) were significantly higher in controls compared with subjects on IPAT and CPAT ($p<0.05$). The GGT and MIS, levels were significantly lower ($p<0.05$) in controls when compared with MTB and MTB-HIV co-infected subjects. The findings of this study suggest that redox-immune axis and metallomic signature may differentiate TB subjects from healthy controls with high accuracy. These promising findings require validation in independent cohorts.

Keywords: Trace microminerals, Redox-Immune status, Anti-TB chemotherapy

1.0 Introduction

Anti-Tuberculosis (Anti-TB) chemotherapy has been linked with extensive redox and microminerals imbalance and diminished macrophages and T cells responses against *Mycobacterium tuberculosis* (Mtb). An estimated 10.8 million new tuberculosis (TB) cases occurred worldwide in 2024, with 1.25 million mortality rates (Estaji *et al.,* 2025; Cui *et*

Corresponding Author: **Raymond Ekong Eworo**
Department of Clinical Chemistry & Immunology, University of Calabar, PMB 1115, Calabar, Cross River State, Nigeria.
Email:raymondeworo@yahoo.com; raymondeworo@unical.edu.ng; Phone.: +2348069363545

©2026 Creative Common License Attribution 4.0 International

al., 2025). Optimal micromineral levels and composite metallomic signature may reflect oxidative stress level and rate of CD4+ T-cell reconstitution during Anti-TB treatment (Dawi *et al.,* 2026; Stefanache *et al.,* 2023). Whether a Composite Metallomic Index can replace or supplement CD4+cells monitoring in resource-limited settings to track the efficacy of Anti-TB therapy is not fully understood. This study hypothesized that mathematical models combining Selenium, zinc, iron, chromium, arsenic, copper and gamma-glutamyl transferase (GGT) levels at certain thresholds can accurately reflect the immune status (CD4+) and predicts treatment success more cheaply than repeated flow cytometry in a resource-limited setting. During infection, copper levels rise as part of the acute-phase response, while zinc levels typically decrease during active TB due to malnutrition, redistribution to tissues to sequester it from the bacteria, and its consumption by the host's immune cells for antimicrobial responses (Kaushik *et al.,* 2023; Verrall *et al.,* 2025).

Microminerals can be assembled into composite predictive models reflecting different aspects of TB treatment response, such as immune recovery and redox balance (Kaushik *et al.,* 2023). That is, since *M. tuberculosis* requires iron to survive, and the body uses copper to fight it, the balance between these two elements, and the resulting oxidative stress measured by GGT, could be a novel way to track disease severity (Kaushik *et al.,* 2023).

Multi-Panel Metal Indices are mathematical combination of trace element concentrations used to reflect oxidative stress, systemic inflammation, and immune recovery during tuberculosis (TB) treatment. Novel composite metal indexes such Metal-Immune Score (MIS), may predict the speed of sputum conversion and immune rebound during Anti-TB chemotherapy. High positive values (+2.0 and above), suggest increased oxidative stress, active disease and suppressed CD4+ cell recovery. Negative values indicate optimal redox balance, superior metabolic homeostasis and robust immune recovery (Chen *et al.,* 2026; Choi *et al.,* 2015; Looney *et al.,* 2021).

The Multi-Metal Inflammatory Index (MMII) tracks the transition from a pro-inflammatory state to an immune-reconstituted state. The MMII decreases with successful Anti-TB treatment (Liu *et al.,* 2025). The Metallomic Recovery Score (MRS), reflects oxidative stress. A low or negative MRS suggest that the patient's "toxic load" is overshadowing their "nutritional shield," leading to oxidative stress and elevated GGT levels (Amaral *et al.,* 2021; Pizzino *et al.,* 2017). The Trace Element Recovery Ratio (TERR), weighs antioxidant capacity against heavy/transition metal burden. Since GGT is often triggered by oxidative stress a rising TERR indicates a reduction in oxidative stress (Sasaninia *et al.,* 2025). The Toxic-Antioxidant Ratio (TAR) measures the balance between toxic metalloids/pro-oxidants and the elements required for immune defense (Cheng *et al.,* 2025). As the index decreases during chemotherapy, it indicates an offsetting of metabolic interference, allowing for CD4 recovery (Luo *et al.,* 2018). The Hepatotoxic Interference Index (HII), focuses specifically on the elements that compete with or deplete the machinery required for liver health and immune signaling. This index serves as an alarm for drug-induced liver injury (DILI) (Mihajlovic and Vinken, 2022). This study assessed the levels of serum Se, Zn, Cu, As, Cr, Fe, GGT, CD4-cells and computed novel metal indices, to determine whether these indices can predict subjects with significantly slower CD4+ recovery, hence can replace or supplement CD4+ monitoring in resource-limited settings and predicts treatment success more cheaply than repeated flow cytometry in pulmonary TB subjects on ATB chemotherapy.

2.0 Materials and Method

Study design

This case-control study was conducted in Calabar Metropolis from July 2024 to August 2025, among pulmonary tuberculosis subjects on Anti-Tuberculosis chemotherapy attending Infectious Disease Hospital, Calabar, categorized into those on intensive phase of Anti-TB (IPAT) ≤ 2 months on Rifampin (RIF), Isoniazid (INH), Pyrazinamide (PZA), and Ethambutol (EMB), and continuation phase of Anti-TB (CPAT) > 2 months on isoniazid (INH) and rifampin (RIF).

Study setting/subject selection

Fifty-five subjects with pulmonary TB cases confirmed by sputum culture and Smear Microscopy Ziehl-Neelsen (ZN)), attending Infectious Diseases Hospital Calabar and 40 apparently healthy age-matched controls from the same geographical location, screened for latent TB using tuberculin skin test (TST), were recruited into this case-control study. The inclusion criteria were adults (20–65 years) diagnosed with MTB, while subjects on mineral supplements, those with chronic kidney disease (CKD), chronic liver disease, alcohol consumption, smoking habit, substance abuse, pregnant women were excluded from the study. Other dietary controls measures and socioeconomic status were not considered, constating a limitation for this study.

Sample collection

Eight milliliters of venous blood was obtained from all the participants after an overnight fast of 8-10 hours. Four milliliters (4 mL) of blood was dispensed into K_2EDTA anticoagulated bottles for the estimation of CD4+ cell count, while 4 mL was dispensed into plain sample bottles, allowed to clot and centrifuged at 3,000 rpm for 5 minutes at room temperature. The sera were separated immediately into aliquots and stored at −20°C until analysis. Three early morning sputum samples (3ml) were collected and taken to the laboratory for analysis.

Laboratory methods

Cluster of Differentiation 4 cell count (CD4+) was determined by flow cytometry using a Sysmex CyFlow Counter. Gamma glutamyl transferase was estimated by Kinetic colorimetric method according to Szasz,[18] using a kit obtained from Agappe Diagnostics Ltd, Kerala, India. Serum Iron, Copper, Zinc, Selenium, Arsenic and Chromium were determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) method. Triple Quadrupole (TQ-ICP-MS/ICP-QQQ) Model from Agilent Technologies. The ICP-MS utilized kinetic energy discrimination (KED) with Helium (He) as a collision gas to remove polyatomic interferences. Rhodium (Rh), was used to correct for drift and matrix effects as internal standard. The method was validated for linearity, precision and accuracy using certified reference materials. Standards were prepared from multi-element stock solutions from certified reference materials, diluted in an acid, and matrix matched to the sample composition as calibration standards (range 0.01–100 µg/L for heavy metals in biological samples) and calibrated (externally) with internal standards to maintain accuracy (internal calibration). Matrix Matching was done by preparing standards in a similar matrix (serum) to minimize matrix-induced bias. Certified Reference Materials (CRMs) from National Institute of Standards and Technology (NIST), were used for quality control, with recovery rates achieved within 85% - 115% of the certified value. Sample replicates were analyzed alongside validation samples to verify both intraday and inter-day precision. The method precision fell within <3%-5% Relative Standard Deviation (RSD), with a Linear Dynamic Range**, s**panning 6 to 9 orders of magnitude, allowing the measurement of major and trace elements in a single run. The LOD was calculated as 3SD of the blank (0.001–0.010 µg/kg for heavy metals), and LOQ as 10SD of the blank (0.005–0.030 µg/kg), depending on the element. The analyzed metals were quantified in a mass spectrometer. The composite metal indexes were derived theoretically based on the biological roles of these elements in immunity, oxidative stress, and nutritional immunity, logarithm transformed to reduce skewness and aggregate risk scores generated (Murdoch and Skaar, 2022).

The Multi-Metal Inflammatory Index (MMII) was computed using the formula:

$$MMII = \frac{(Cu \times Cr)}{(Zn \times Se \times Fe)}$$

Metal-Immune Score (MIS**)** was calculated using the formula,

$\log10\{Cu/Zn\} + \log10\{Cr\}/\{Se \times Fe\}$

Standardized using Z score as

$MIS= \{Z_{Cu} + Z_{Fe} + Z_{GGT}\} - \{Z_{Zn} + Z_{Se} + Z_{CD4+}\}$

Where $Z= \frac{x-\mu}{\sigma}$

x = raw score, µ =mean, σ = standard deviation

Metallomic Recovery Score (MRS) was calculated as $\log_{10}$ (Zn x Se x Fe)/(As x Cu x Cr).

Trace Element Recovery Ratio (TERR) was calculated as (Se + Zn)/(Cu + Cr + Fe).

Toxic-Antioxidant Ratio (TAR) was calculated as (As x Cu x Cr)/(Se x Zn x Fe).

Hepatotoxic Interference Index (HII) was calculated as (As + Cr + Cu)/(Zn + Se)

Combined metal index (CMI) was calculated using the formula: CMI= (ZnxSe)/(GGTxAs)

Computation of Cu/Zn and Cu/Se Was conducted by dividing the level of copper by zinc or selenium respectively

Ethical consideration

Ethical approval was obtained from Health Research Ethics Committee, Cross River State Ministry of Health, Calabar. Protocol assigned number REC NO: CRS/MH/HREC/2024/VOL.2/003. Written informed consent was obtained from all participants prior to inclusion in the study. The study was conducted in accordance with the ethical principles for medical research involving human subjects as outlined in the Helsinki Declaration of 1975 (revised 2000).

Statistical analysis

Results were expressed as mean ± standard deviation (SD). The normality of data was tested by the Shapiro-Wilk test. The ANOVA was utilized in comparing multiple-group means and Turkey's post hoc analyses. The Receiver Characteristic Operator Curve (ROC) analysis alongside bootstrapping and leave-one-out cross validation were applied in controlling model overfitting and in determining the performance metrics of the metal indexes in predicting the outcomes in the test subjects. The statistical significance was set at $p < 0.05$.

3.0 Results

Comparison of GGT, CD4+cells, microminerals and micromineral indexes among subjects on IPAT, CPAT and controls is shown in table 1. The levels of GGT, As, Cu, Cu/Zn, Cu/Se, MMII, TAR, HII, MIS, and Se/Zn were significantly lower in controls than in subjects on IPAT and CPAT ($p<0.05$), while CD4+, Fe, Se, Zn, Cr, CMI, TERR and MRS were significantly higher in controls when compared with subjects on IPAT and CPAT ($p<0.05$).

Comparison of GGT, CD4+cells, microminerals and micromineral indexes among subjects with MTB-only, MT-HIV co-infection and controls is represented in Table 2. The GGT, As, Cu, MMII, TAR, MIS, and Se/Zn levels were significantly lower ($p<0.05$) in controls when compared with MTB and MTB-HIV co-infected subjects, while CD4+ cells, Fe, Se, Cr, CMI, Cu/Se, TERR and MRS levels were significantly elevated in controls than in MTB and MTB-HIV co-infection ($p<0.05$).

Receiver Operator Curve of GGT, As, Cu, Cu/Zn, Cu/Se, MMII, TAR, HII, MIS and Se/Zn in subjects with MTB infection is represented in figure 1. The Cu/Se demonstrated AUC95%C.I= 0.963 (0.92–1.00), S.E=0.021, p=< 0.0001, Gini index= 0.926, Max. K-S=0.835, cutoff point of <0.08, sensitivity =100.0%, specificity =83.5%, PPV=88.0%, NPP=100.0%, Leave-one-outcross validation AUCCV=0.957 in predicting MTB subjects with increased oxidative stress. The ROC Analysis & Validation Summary of the variable studied in subjects with MTB is represented in Table 3.

Table 1: Comparison of GGT, CD4+cells, microminerals and micromineral indexes among subjects in the intensive phase, continuation phase of Anti-TB treatment and controls

Parameters	IPAT (n=24)	CPAT (n=31)	Controls (n=40)	F	P	Effect size
GGT (U/l)	42.87±29.03[a]	38.11±21.22	16.43±4.08	18.329	<0.001	0.285
CD4+ (µl/ml)	324.75±109.42	305.16±80.09	948.40±157.44[b]	299.319	<0.001	0.867
As (µg/ml))	0.38±0.14[c]	0.32±0.22	0.04±0.01	51.263	<0.001	0.527
Cu (µg/ml))	1.58±0.22	1.55±0.28	0.48±0.16[d]	336.499	<0.001	0.880
Fe (µg/ml))	0.16±0.08	0.17±0.07	1.47±0.55[e]	150.262	<0.001	0.766
Se (µg/ml))	0.04±0.02	0.06±0.02	0.07±0.04[f]	4.617	0.012	0.091
Zn (µg/ml))	0.28±0.13	0.31±0.17	1.13±0.06[g]	524.536	<0.001	0.919
Cr (µg/ml))	0.01±0.01	0.02±0.01	0.06±0.02[h]	108.806	<0.001	0.703
CMI	1.07±1.45	1.87±1.22	135.29±111.54[i]	39.276	<0.001	0.461
Cu/Zn	6.73±3.12	6.98±4.36	0.43±0.14[j]	55.585	<0.001	0.547
Cu/Se	55.86±21.22[k]	30.56±13.30	12.56±15.18	30.723	<0.001	0.400
MMII	19.63±23.37	21.23±28.81	0.41±0.44[l]	11.555	<0.001	0.442
TERR	0.19±0.09	0.21±0.1	0.65±0.21[m]	95.218	<0.001	0.674
TAR	7.12±7.59[n]	5.88±8.89	0.02±0.01	12.077	<0.001	0.208
HII	7.02±2.65	6.33±2.95	0.49±0.14[o]	95.754	<0.001	0.675
MRS	-0.68±0.40	-0.52±0.43	1.95±0.41[p]	432.311	<0.001	0.904
MIS	1.12±0.39	1.11±0.40	-0.54±0.34[q]	222.990	<0.01	0.829
Se/Zn	0.17±0.13	0.25±0.16	0.06±0.04[r]	25.453	<0.001	0.357

Key: Results expressed as mean±SD, GGT= gamma glutamyl transferase, CD4+- cluster of differentiation 4 cells, As= Arsenic, Cu =Copper, Fe=iron, Se=Selenium, Zn = zinc, Cr = chromium, CMI= combined metal index, Cu/Zn= copper:zinc ratio, Cu/Se= copper:selenium ratio, MMII= Multi-Metal inflammatory Index, TERR= Trace element Recovery Ratio, TAR= the Toxic-Antioxidant Ratio, HII= The hepatotoxic Interference Index, MRS= Metallomic Recovery Score, MIS=Metal-Immune Score, Se/Zn= selenium:zinc ratio. a, c, d, j, l, n, o, q ,r = significantly lower in controls than in IPAT and CPAT, b, e, f, g, h, I, k, m, and p= significantly higher in controls than in IPAT and CPAT

Table 2: Comparison of GGT, CD4+cells, microminerals and micromineral indexes among subjects with MTB-only, MT-HIV co-infection and controls.

Parameters	MTB-only (n=37)	MTB-HIV (n=18)	Controls (n=40)	F	P	Effect size
GGT (U/l)	43.51±28.97[a]	33.90±12.23	16.44±4.08	19.980	<0.001	0.303
CD4+ (µl/ml)	299.00±96.33	341.58±83.55	948.40±154.43[b]	303.548	<0.001	0.868
As (µg/ml)	0.37±0.20[c]	0.29±0.17	0.04±0.01	51.614	<0.001	0.529
Cu (µg/ml)	1.57±0.21	1.54±0.24	0.48±0.16[d]	336.438	<0.001	0.880
Fe (µg/ml)	0. 13±0.05	0.12±0.07	1.47±0.55e	151.219	<0.001	0.767
Se (µg/ml)	0.04±0.02	0.06±0.02	0.07±0.04[f]	4.326	0.016	0.086
Zn (µg/ml)	0.36±0.15	0.17±0.06	1.13±0.06[g]	810.348	<0.001	0.946
Cr (µg/ml)	0.02±0.01	0.02±0.01	0.06±0.02[h]	104.862	<0.001	0.695
CMI	1.67±1.62	1.24±0.61	135.29±111.54[i]	39.275	<0.001	0.461
Cu/Zn	4.99±1.97	10.42±4.04	0.43±0.14[j]	141.184	<0.001	0.754
Cu/Se	48.82±31.41[k]	27.92±10.31	12.55±15.18	25.277	<0.001	0.355
MMI	23.68±31.74[l]	14.58±8.25	0.41±0.44	13.122	<0.001	0.442
TERR	0.24±0.10	0.13±0.05	0.65±0.21[m]	103.778	<0.001	0.693
TAR	7.61±9.63[n]	4.15±4.17	0.02±0.01	14.164	<0.001	0.235
HII	5.47±2.00	8.84±2.86[o]	0.49±0.15	162.766	<0.001	0.780
MRS	-0.65±0.46	-0.47±0.34	1.95±0.40[p]	433.832	<0.001	0.904
MIS	1.14±0.43[q]	1.07±0.31	-0.54±0.35	223.973	<0.01	0.830
Se/Zn	0.12±0.05	0.39±0.11[r]	0.06±0.04	184.460	<0.001	0.800

Key: Results expressed as mean±SD, GGT= gamma glutamyl transferase, CD4+- cluster of differentiation 4 cells, As= Arsenic, Cu =Copper, Fe=iron, Se=Selenium, Zn = zinc, Cr = chromium, CMI= combined metal index, Cu/Zn= copper:zinc ratio, Cu/Se= copper:selenium ratio, MMII= Multi-Metal Inflammatory Index, TERR= Trace element Recovery Ratio, TAR= the Toxic-Antioxidant Ratio, HII= The hepatotoxic Interference Index, MRS= Metallomic Recovery Score, MIS=Metal-Immune Score, Se/Zn= selenium:zinc ratio. a, c, d, l, n, q, r = significantly lower in controls than in MTB and MTB-HIV coinfection, b, e, f, h, i, k, m, and p= significantly higher in controls than in MTB and MTB-HIV co-infection, g=significantly higher in MTB than MTB-HIV, j= significantly higher in MTB than in MTB-HIV and controls, o = significantly lower in MTB than MTB-HIV, lower in control than MTB and MTB-HIV, k = significantly higher in MTB than in MTB-HIV

Table 3: ROC Analysis & Validation Summary of the variable studied in subjects with MTB

Biomarker / Ratio	Optimal Cutoff	AUC (95% CI)	S.E	p-value	Gini Index	Max KS Index	Sens. (%)	Spec. (%)	PPV (%)	NPV (%)	LOOCV AUCCV
GGT	21.00	0.621 (0.47–0.77)	0.076	0.0410	0.242	0.282	41.5%	86.7%	78.6%	55.3%	0.598
As	> 0.15	0.999 (0.99–1.00)	0.003	< 0.0001	0.998	0.982	98.2%	100.0%	100.0%	97.4%	0.998
Cu	> 1.00	1.000 (1.00–1.00)	0.000	< 0.0001	1.000	1.000	100.0%	100.0%	100.0%	100.0%	1.000
CU/Zn	< 0.33	0.993 (0.98–1.00)	0.007	< 0.0001	0.986	0.941	100.0%	94.1%	95.3%	100.0%	0.991
Cu/Se	< 0.08	0.963 (0.92–1.00)	0.021	< 0.0001	0.926	0.835	100.0%	83.5%	88.0%	100.0%	0.957
MMII	< 3.15	0.994 (0.98–1.00)	0.006	< 0.0001	0.988	0.970	97.1%	100.0%	100.0%	96.1%	0.992
TAR	> 1.00	0.998 (0.99–1.00)	0.003	< 0.0001	0.996	0.970	97.1%	100.0%	100.0%	96.1%	0.997
HII	> 40.00	0.785 (0.68–0.89)	0.054	< 0.0001	0.570	0.518	61.8%	90.0%	87.5%	67.5%	0.769
MIS	<1.00	0.996 (0.99–1.00)	0.004	< 0.0001	0.992	0.971	100.0%	97.1%	97.6%	100.0%	0.994
Se/Zn	< 0.40	0.916 (0.84–0.99)	0.038	< 0.0001	0.832	0.771	94.1%	83.0%	86.5%	92.3%	0.904

GGT= gamma glutamyl transferase, As= Arsenic, Cu =Copper, Cu/Zn= copper:zinc ratio, Cu/Se= copper:selenium ratio, MMI= Multi-Metal Index, TAR= the Toxic-Antioxidant Ratio, HII= The hepatotoxic Interference Index, MIS=Metal-Immune Score, Se/Zn= selenium:zinc ratio, S.E=standard error, AUC = area under the curve, p= significance level, C.I confidence interval, PPV= positive predictive value, NPV= negative predictive value, sen=sensitivity, spec=specificity

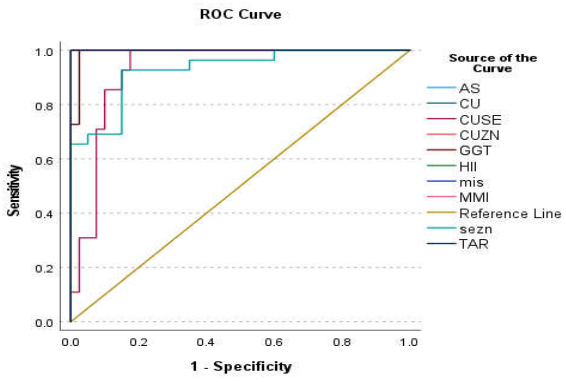

Figure 1: Receiver operator curve of GGT, As, Cu, Cu/Zn, Cu/Se, MMI, TAR, HII, MIS and Se/Zn in subjects with MTB infection.

4.0 Discussion

The significantly elevated levels of GGT, Cu/Zn, MMII, TAR, HII, MIS, and Se/Zn in subjects on IPAT and CPAT compared with controls, suggest metabolic alteration in the levels of these metals due to effect of anti-TB chemotherapy as well as ongoing disease activities which are responsible for inflammation and oxidative stress. During Anti-TB Chemotherapy, the first-line anti-TB drugs, specifically Isoniazid (INH), Rifampicin (RIF), and Pyrazinamide (PZA) are potent metabolic stressors. The metabolism of Isoniazid produces toxic intermediates (like hydrazine), while Rifampicin is a powerful inducer of GGT. As the patient recovers, GGT levels typically normalize alongside the improvement in immune markers (CD4+ cells). This finding aligns with that of a previous study by Grosu *et al.,* (2026), who observed a normalization in pro-oxidants following reduction in oxidative stress and immune recovery with treatment. Similarly, Sankar *et al.,* (2024), had reported the toxicity associated with metabolism of Isoniazid.

The elevated copper-to-zinc (Cu/Zn) ratio observed in subjects on Anti-TB may suggest sequestration of zinc in tissues to limit bacterial growth, malnutrition, oxidative stress. This observation aligns with a previous study by Schneider *et al.,* (2020), Which reported elevated Cu/Zn to be associated with malnutrition, and disrupted immune status during chronic disease. A declining Cu/Zn ratio during Anti-TB treatment may reflect a positive treatment response. This observation is in line with those of Modi *et al.,*

(2025); Moideen *et al.,* (2025); and Sepehri *et al.,* (2017), who observed elevated levels of Cu/Zn to affect oxidative status. Similarly, Talreja *et al.,* (2023); Kianifar *et al.,* (2024), demonstrated that macrophages sequester high concentrations of Cu within phagosomes to intoxicate and kill Mycobacterium Tuberculosis, while limiting the availability of Zn in the blood to starve the bacteria.

Elevated levels of multi-metal inflammatory indexes (MMII, TAR, HII, MIS) in TB subjects on Anti-TB treatment suggest chronic inflammation, immune disfunction, and significant oxidative stress. This finding is similarly reported by Molla *et al.,* (2024); Dong *et al.,* (2024), who observed Anti-Tuberculosis induced hepatoxicity in pulmonary TB patients. Elevated levels of MMII and MIS in subjects on IPAT and CPAT, suggests a high cumulative inflammatory burden. This observation is similar to that of a previous study by Jumaar *et al.,* (2025), which reported significant inflammatory burden in pulmonary tuberculosis patients during Anti-TB treatment.

Trace Metal Recovery Ratio/Score, measuring the rising levels of Zn, Fe, and Se are vital in assessing immune recovery and decreasing oxidative stress following successful therapy. A high Metallomic Recovery Score correlates with a favorable therapeutic response, reversal of oxidative damage, and recovery of immune system. This finding is in line with that of a previous study by Moideen *et al.,* (2025), who demonstrated alteration in trace minerals and elevated inflammatory patterns in pulmonary TB patients. The extremely abnormally low levels Fe, Zn, Cr and As, in MTB and MTB-HIV coinfected subjects may be due to elevated systemic inflammation, oxidative stress, and severe malnutrition associated with the double burden of MTB and MTB-HIV co-infection in these subjects.

Elevated MIS in MTB than in MTB-HIV suggest an active, poorly controlled MTB/MTB-HIV, high oxidative stress, and a high risk of treatment failure. This finding aligns with that of a previous study Nriagu and Skaar, (2015), who reported trace element as disease modifiers.

The multi-metal inflammatory index (MMII), showed AUC95% C.I=0.994(0.98–1.00), S.E= 0.006, p< 0.0001, Gini index= 0.988, Max K-S index= 0.970, sensitivity=97.1%, specificity=100.0%, PPV=100.0%, NPP=96.1% and Loocv AUCCV=0.992. The MMII tracks the transition from a pro-inflammatory state to an immune-reconstituted state. The MMII decreases with successful anti-TB treatment. The LOOCV parameters (AUC_{CV}) mimic the native empirical AUC margins closely. This tight correlation indicates that the classification thresholds are highly stable and not merely artifacts of localized overfitting. However, the near perfect AUC observed in most metal indices in this study may also not be solely as a result of perfect discriminatory ability of the models, hence, they require validation in an independent, prospective cohort.

Limitation of the study

This study is limited by its small subgroup sample sizes, lack of validation cohort, potential batch effects, inflammatory markers like IL-6, CRP were not measured to validate the indices, no test for overfitting was conducted. The composite indices are unit dependent and there is lack of standardization across laboratories.

Conclusion

The findings of this study suggest that composite metallomic indices may differentiate TB patients from healthy controls and reflect redox-immune recovery status during Anti-TB chemotherapy with high accuracy. Optimal micronutrient levels may serve as a therapeutic lever in improving TB treatment outcomes by supporting a stronger immune recovery. However, these promising findings require validation in an independent cohort before clinical applications.

Conflict of interest

The authors have no conflict of interest to declare

References

Amaral, E. P., Vinhaes, C. L., Oliveira-de-Souza, D., Nogueira, B., Akrami, K. M., and Andrade, B. B. (2021). The Interplay

Between Systemic Inflammation, Oxidative Stress, and Tissue Remodeling in Tuberculosis. *Antioxidants and Redox Signaling*, *34*(6), 471–485. https://doi.org/10.1089/ars.2020.8124

Chen, X., Wang, Q., Zhang, H., Wang, Y., and Zhu, H. (2026). Metal homeostasis as a therapeutic lever: advancing metalloimmunology to remodel the tumor microenvironment and enhance cancer immunotherapy. *Theranostics*, *16*(3),1350–1373. https://doi.org/10.7150/thno.121988

Cheng, Y. F., Zhao, Y. J., Chen, C., and Zhang, F. (2025). Heavy Metals Toxicity: Mechanism, Health Effects, and Therapeutic Interventions. *Medical Communications,* *6*(9), e70241. https://doi.org/10.1002/mco2.70241

Choi, R., Kim, H. T., Lim, Y., Kim, M. J., Kwon, O. J., Jeon, K., Park, H. Y., Jeong, B. H., Koh, W. J., and Lee, S. Y. (2015). Serum Concentrations of Trace Elements in Patients with Tuberculosis and Its Association with Treatment Outcome. *Nutrients*, *7*(7), 5969–5981. https://doi.org/10.3390/nu7075263

Cui, K., Zhao, X., Liu, W., & Bai, L. (2025). Global, regional, and national burden and trends of multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis in adolescents and adults aged 15-49 years from 2010 to 2021: insights from the global burden of disease study 2021. *BMC Medicine*, *23*(1), 445. https://doi.org/10.1186/s12916-025-04269-7

Dawi, J., Affa, S., Misakyan, Y., Gonzalez, E., Affa, S., & Venketaraman, V. (2026). Glutathione-Mediated Redox Regulation of Immune Dysfunction in COVID-19 and Tuberculosis. *Antioxidants (Basel, Switzerland)*, *15*(2), 214. https://doi.org/10.3390/antiox15020214

Dong, H., Feng, J., Chang, X., Wu, S., Tang, G., Liang, F., Tang, H., Dong, Y., Fang, W., Hu, J., & Wang, W. (2024). Predictive value of systemic immune-inflammatory biomarkers for drug-induced liver injury in hepatitis B virus surface antigen positive tuberculosis patients: A retrospective observational study. *Medicine*, *103*(45), e40349. https://doi.org/10.1097/MD.0000000000040349

Estaji, F., Kamali, A., & Keikha, M. (2025). Strengthening the global Response to Tuberculosis: Insights from the 2024 WHO global TB report. *Journal of Clinical Tuberculosis and other Mycobacterial Diseases*, *39*, 100522. https://doi.org/10.1016/j.jctube.2025.100522

Grosu, I. A., Dobrin, M. E., Marginean, C., Esanu, I. M., Melinte, O. E., Stavarache, I. E., Dumitrache-Rujinski, S., Cioroiu, I. B., Crisan-Dabija, R. A., Vicol, C., & Trofor, A. C. (2026). Integrated Approach of Hematological Parameters and Glutathione as Predictors of Pulmonary TB Evolution: A Comprehensive Review. *Journal of Clinical Medicine*, *15*(3), 1017. https://doi.org/10.3390/jcm15031017

Jumaar, C., Malefane, L., Jacobs, S., Sanni, O., Louw, E., Baines, N., Payne, C., Schulz, S., Lombard, C., Feyasa, M., Maree, D., Windvogel, S., Strijdom, H., Botha, B., Allwood, B., & Maarman, G. J. (2025). Delineating the Significance of Several Inflammatory Markers in a Lung Tuberculosis Cohort During the Active and Post-Tuberculosis Stages of the Disease: An Observational Study in Cape Town, South Africa (2019 to 2024). *Infectious Disease Reports*, *17*(3), 52. https://doi.org/10.3390/idr17030052

Kaushik, S. R., Sahu, S., Guha, H., Saha, S., Das, R., Kupa, R. U., Kapfo, W., Deka, T., Basumatary, R., Thong, A., Dasgupta, A., Goswami, B., Pandey, A. K., Saikia, L., Khamo, V., Das, A., & Nanda, R. K. (2023). Low circulatory Fe and Se levels with a higher IL-6/IL-10 ratio provide nutritional immunity in tuberculosis. *Frontiers in Immunology*, *13*, 985538. https://doi.org/10.3389/fimmu.2022.985538

Kianifar, F., Vahid Dastjerdi, A. R., Mohammadi Maram, M., Bagtash, K., Zamanian, M. Y., Eslami, M., & Farazi, A. (2025).

Serum zinc level and its association with pulmonary tuberculosis. *Immunopathologia Persa,* 11, e40537. https://doi.org/10.34172/ipp.2025.40537

Liu, J., Chen, K., Tang, M., Mu, Q., Zhang, S., Li, J., Liao, J., Jiang, X., & Wang, C. (2025). Oxidative stress and inflammation mediate the adverse effects of cadmium exposure on all-cause and cause-specific mortality in patients with diabetes and prediabetes. *Cardiovascular Diabetology*, *24*(1), 145. https://doi.org/10.1186/s12933-025-02698-5

Looney, M. M., Lu, Y., Karakousis, P. C., & Halushka, M. K. (2021). Mycobacterium tuberculosis Infection Drives Mitochondria-Biased Dysregulation of Host Transfer RNA-Derived Fragments. *The Journal of Infectious Diseases*, *223*(10), 1796–1805. https://doi.org/10.1093/infdis/jiaa596

Luo, X., Wu, F., Ma, J., Xiao, H., & Cui, H. (2018). Immunological recovery in patients with pulmonary tuberculosis after intensive phase treatment. *The Journal of International Medical Research*, *46*(9), 3539–3551. https://doi.org/10.1177/0300060518773258

Mihajlovic, M., & Vinken, M. (2022). Mitochondria as the Target of Hepatotoxicity and Drug-Induced Liver Injury: Molecular Mechanisms and Detection Methods. *International journal of molecular sciences*, *23*(6), 3315. https://doi.org/10.3390/ijms23063315

Modi, P., Krishnagopal, S. N., Mittal, R., Ahirwar, S. S., Singh, S., Chourasia, N., Tripathi, A., Shukla, A. K., & Kotnis, A. (2025). Low Serum Levels of Zinc and Copper Are Associated With Tuberculosis. *Cureus*, *17*(9), e93613. https://doi.org/10.7759/cureus.93613

Moideen, K., Gunasekaran, H., Loukrakpam, B., Ahamed, S. F., Nathella, P. K., Rajendran, A., Bethunaickan, R., & Babu, S. (2025). Altered serum mineral concentrations among pulmonary tuberculosis and its association with Vitamin D, adipokines and inflammatory cytokines. *Frontiers in Nutrition*, *12*, 1666416. https://doi.org/10.3389/fnut.2025.1666416 4

Molla, Y., Wubetu, M., & Dessie, B. (2021). Anti-Tuberculosis Drug Induced Hepatotoxicity and Associated Factors among Tuberculosis Patients at Selected Hospitals, Ethiopia. *Hepatic Medicine: Evidence and Research*, *13*, 1–8. https://doi.org/10.2147/HMER.S290542

Murdoch, C. C., & Skaar, E. P. (2022). Nutritional immunity: the battle for nutrient metals at the host-pathogen interface. *Nature reviews. Microbiology*, *20*(11), 657–670. https://doi.org/10.1038/s41579-022-00745-6

Nriagu JO, Skaar EP. Introduction. In: Nriagu JO, Skaar EP, editors. Trace Metals and Infectious Diseases Cambridge (MA): MIT Press; 2015. Chapter 1. Available from: https://www.ncbi.nlm.nih.gov/books/NBK569690/

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., & Bitto, A. (2017). Oxidative Stress: Harms and Benefits for Human Health. *Oxidative Medicine and Cellular Longevity*, 8416763. https://doi.org/10.1155/2017/8416763

Sankar, J., Chauhan, A., Singh, R., & Mahajan, D. (2024). Isoniazid-historical development, metabolism associated toxicity and a perspective on its pharmacological improvement. *Frontiers in Pharmacology*, *15*, 1441147. https://doi.org/10.3389/fphar.2024.1441147

Sasaninia, K., Mohan, A. S., Badaoui, A., Glassman, I., Yoon, S., Karapetyan, A., Kolloli, A., Kumar, R., Ramasamy, S., Subbian, S., & Venketaraman, V. (2025). Glutathione Depletion Exacerbates Hepatic *Mycobacterium Tuberculosis* Infection. *Biology*, *14*(2), 131. https://doi.org/10.3390/biology14020131

Schneider, T., Caviezel, D., Ayata, C. K., Kiss, C., Niess, J. H., & Hruz, P. (2020). The

Copper/Zinc Ratio Correlates With Markers of Disease Activity in Patients With Inflammatory Bowel Disease. *Crohn's & Colitis 360*, *2*(1), otaa001.https://doi.org/10.1093/crocol/otaa001

Sepehri, Z., Mirzaei, N., Sargazi, A., Sargazi, A., Mishkar, A. P., Kiani, Z., Oskoee, H. O., Arefi, D., & Ghavami, S. (2017). Essential and toxic metals in serum of individuals with active pulmonary tuberculosis in an endemic region. *Journal of Clinical Tuberculosis and other Mycobacterial Diseases*, *6*, 8–13. https://doi.org/10.1016/j.jctube.2017.01.001

Stefanache, A., Lungu, I. I., Butnariu, I. A., Calin, G., Gutu, C., Marcu, C., Grierosu, C., Bogdan Goroftei, E. R., Duceac, L. D., Dabija, M. G., Popa, F., & Damir, D. (2023). Understanding How Minerals Contribute to Optimal Immune Function. *Journal of Immunology Research*, *2023*, 3355733. https://doi.org/10.1155/2023/3355733

Szasz, G. A. (1969). Kinetic Photometric Method for Serum γ-Glutamyl Transpeptidase. *Clinical Chemistry.* 15(2):124–136

Talreja, S. D., Manjrekar, P. A., M S, R., Yalla, D. R., & Sowndarya K, S. K. (2023). Pre-Post Assessment of Serum Zinc and Copper Status in Uncomplicated Pulmonary Tuberculosis Patients Receiving Directly Observed Treatment Short Course. *Current Research in Nutrition and Food Science Journal*, 11(2), 822-829. https://doi.org/10.12944/crnfsj.11.2.31

Verrall, A. J., Houghton, L., Apriani, L., Atmaja, H. E., van Laarhoven, A., Ussher, J. E., Ruslami, R., Sharples, K., McAllister, S., van Crevel, R., Hill, P. C., & Alisjahbana, B. (2025). Micronutrient status and risk of Mycobacterium tuberculosis infection in Indonesian tuberculosis case contacts. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *119*(4), 346–353. https://doi.org/10.1093/trstmh/trae140