

The Correlation Between Whole Blood Copper and Zinc Levels and Sepsis-Induced Left Ventricular Systolic Dysfunction (SILVSD) in Patients with Septic Shock: A Single-Center Prospective Observational Study

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Research

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Abstract

Background: The dyshomeostasis of trace elements is associated with multiple organ dysfunction in patients with septic shock. However, it remains unclear whether the change of whole blood copper (Cu) and zinc (Zn) levels influence cardiac function in patients with septic shock. Here, we sought to explore relationship between whole blood Cu and Zn and cardiac dysfunction in septic shock.

Methods: Between April 2018 and March 2020, septic shock patients with sepsis-induced left ventricular systolic dysfunction (SILVSD, left ventricular ejection fraction, LVEF \leq 50%) and with no sepsis-induced myocardial dysfunction (non-SIMD, septic shock alone and LVEF \geq 50%) on an intensive care unit (ICU) in south China and healthy controls were prospectively enrolled. whole blood Cu and Zn levels were measured using flame atomic absorption spectrophotometry.

Results: 86 patients with septic shock including 41 SILVSD and 45 non-SIMD and 25 healthy controls were studied. Whole blood Cu levels were significantly higher and Zn were significantly lower in SILVSD compared with non-SIMD and controls [Cu, (16.34 \pm 1.93) vs. (15.23 \pm 2.07) vs. (14.02 \pm 1.65) μ mol/L, p = 0.009, < 0.001; Zn, (78.45 \pm 12.18) vs. (85.07 \pm 14.80) vs. (94.90 \pm 14.78) μ mol/L, p = 0.029, < 0.001]. Both increased whole blood Cu and reduced Zn were associated with lower LVEF (all p < 0.001) and higher amino-terminal pro-B-type natriuretic peptide (NT-proBNP) (Cu, p = 0.002, Zn, p = 0.001), and had predictive values for SILVSD [Cu, (AUC = 0.666; p = 0.005) ; Zn, (AUC = 0.625; p = 0.039)]. Whole blood Cu levels were increased but Zn were reduced in non-survivors compared with survivors [Cu, (17.20 \pm 2.25) vs. (14.99 \pm 1.49) μ mol/L, p < 0.001; Zn, (71.17 \pm 11.98) vs. (87.67 \pm 11.30) μ mol/L, p < 0.001]. Whole blood Cu and Zn displayed the value of predicting 28-day mortality [Cu (AUC = 0.802, p < 0.001); Zn (AUC = 0.869, p < 0.001)].

Conclusions: Whole blood Cu levels were increased in SILVSD patients and positively correlated with the cardiac dysfunction while Whole blood Zn were reduced and negatively associated with the cardiac dysfunction. Moreover, both whole blood Cu and Zn could distinguish between SILVSD and non-SIMD in septic shock patients and predict 28-day mortality.

Trial registration: ChiCTR1800015709. Registered 16 April 2018, <http://www.chictr.org.cn/edit.aspx?pid=26746&htm=4>.

Introduction

Sepsis, one of the most frequent causes of death in intensive care unit (ICU), is characterized as dysregulated immune response with threatening organ dysfunction(1, 2). Septic shock is a subset of sepsis with circulatory and cellular/metabolic dysfunction associated with a higher risk of mortality(3). Low systemic vascular resistance is one of the most obvious characteristics in patients with septic shock, and the cardiac output is usually reduced in the hypodynamic septic shock(4). Myocardial dysfunction can be seen in up to 30%-80% septic shock patients whose cardiac function is not abnormal prior to septic shock(5).

Sepsis-induced myocardial dysfunction (SIMD) was demonstrated in the study of 20 patients conducted by Parker et al. for the first time(6). SIMD has become one of the major factors of mortality in patients with sepsis(7), and can be divided into left ventricular (LV) systolic/diastolic dysfunction or abnormalities of global/regional wall motion(WMA)(8). Sepsis-induced LV systolic dysfunction (SILVSD) manifests reduced systolic function, cardiomegaly, and lower ejection fraction (EF) to adapt to low systemic vascular resistance caused by vasoplegia(7, 9). SILVSD is characterized by a left ventricular ejection fraction (LVEF) below 50% without LV diastolic dysfunction (LVDD) manifesting E/e' ratios above 15 by transthoracic echocardiography(TTE)(8). Alike B-type natriuretic peptide(BNP), an indirect indicator of heart function, which is released while wall stress is enhanced in sepsis and septic shock, amino-terminal pro-BNP(NT-proBNP) is also increased and elevated NT-proBNP is related to poor prognosis in sepsis and septic shock(10).

Cardiac specific injury biomarkers such as troponin(cTNI, cTNT) and heart-type fatty acid binding protein(HFABP) has been studied in patients with septic shock and elevated TNI and HFABP are related to poor prognosis(11, 12). However, the results of these studies exploring the impact of TNI levels for SIMD show difference. Although Klouche et al. did not discover an association between TNI and SIMD on echocardiography(13), others found that elevated TNI could be a prognostic factor in patients with SIMD(14, 15). Hence, it is vital to look for other more reliable biomarkers with less controversy as prognostic factors for patients with SIMD.

Dysregulation of the normal immune response induced by sepsis can result in various harmful effects such as multi-organ dysfunction syndrome (MODS) including SIMD and even ultimately death in some patients with sepsis or septic shock(16). Recent study identified oxidative injury products such as oxidative lipidomics were involved in the initiation(eicosanoids) and recovery phases (lipoxins and resolvins) of sepsis and systemic inflammatory response(17). Meanwhile, trace elements including copper (Cu) and zinc (Zn) play significant roles in anti-oxidant defence system as they can scavenge oxygen free radicals and prevent oxidative damage. Studies in human patients and animal models shown that lower level of serum Zn was associated with severe pneumonia and sepsis and could increase sensitivity for infection and sepsis(18, 19). As for serum Cu, it was reported that people with high levels of serum Cu were liable to suffer from myocardial infarction(MI), hypertension, cancer, chronic obstructive pulmonary disease(COPD) and infection(20, 21). Alexanian et al. found that high levels of serum Cu in both acute heart failure(AHF) and chronic heart failure(CHF) were associated with LV systolic and diastolic dysfunction, on the contrary, a reduction of serum Zn levels in CHF and AHF was correlated with LVDD(22). However, there are no studies evaluating the influence of the levels of whole blood Cu and Zn on SILVSD patients with septic shock.

Objectives

The current prospective clinical study was planned to explore the change of whole blood Cu and Zn of SILVSD patients with septic shock and the influence with prognosis of SILVSD patients with septic shock.

Methods

Study design and location

A prospective, non-interventional observational, single-center study involved in target sample of 86 patients with septic shock and 25 healthy controls was performed at ICU at Tongde Hospital of Zhejiang Province between April 2018 and March 2020. We report our study following STROBE guidance(23). This study was performed in accordance with the guidelines of the Declaration of Helsinki(24). During the period of recruitment between April 2018 and March 2020, 236 patients received a preliminary diagnosis of septic shock. Firstly, a total of 129 patients were excluded because 24 patients were above 80 years old and the others suffered from past medical history including cardiomyopathy (n = 11), valvular heart disease (n = 5), acute coronary syndrome (n = 34) and chronic heart failure (n = 55). Then, 107 patients with septic shock met the inclusion criteria were divided into non-SIMD group (without SIMD, n = 45) and SIMD group (n = 62) in terms of the diagnostic criteria in TTE. Secondly, in order to investigate the change of whole blood Cu and Zn in SILVSD patients with septic shock, we also excluded patients with LVDD alone (n = 8) and WMA alone (n = 13) from SIMD group. Finally, 41 SILVSD patients with septic shock, 45 non-SIMD patients with septic shock and 25 healthy controls were enrolled in the study. As a result, data from 111 participants were analyzed (Fig. 1).

Diagnosis Criteria

Sepsis and septic shock were diagnosed according to the criteria outlined in the Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016 and the Third International Consensus Definitions for Sepsis and Septic Shock(3), and SILVSD was diagnosed by TTE according to the criteria outlined in SIMD(25). Sepsis is defined as Sequential Organ Failure Assessment (SOFA) score ≥ 2 points consequent to the infection. Adult patients with septic shock can be identified using the clinical criteria of hypotension requiring vasopressor therapy to maintain mean arterial pressure (MAP) 65 mmHg or greater and having a serum lactate level greater than 2 mmol/L after adequate fluid resuscitation(1). SILVSD is diagnosed as a LVEF below 50% without LV diastolic dysfunction manifesting E/e' ratios above 15 by TTE and abnormalities of global/regional wall motion(8).

Inclusion And Exclusion Criteria

Inclusion criteria: patients had suffered from septic shock with or without SILVSD, aged 18–80 years.

Exclusion criteria: age ≥ 80 years or ≤ 18 years, patients with pre-existing cardiomyopathy, valvular heart disease and heart failure, present or suspected acute coronary syndrome within recent two weeks, pregnancy, ventricular outflow tract obstruction were excluded from this study. We also excluded patients with either LVDD alone or abnormalities of wall motion alone on TTE.

In addition, in order to avoid the interference of depurative extracorporeal circulation on leukocyte, hypersensitive C-reactive protein (hs-CRP), plasma troponin T (TNT), N terminal pro brain natriuretic peptide (NT-proBNP), HFABP, procalcitonin (PCT) and whole blood Cu and Zn, all patients did not receive continuous blood purification (CBP) before admission. The experimental procedure, echocardiographic and laboratory results of the study were not concealed to treating physicians and echocardiographers. A group of 25 healthy volunteers free of symptoms, signs and objective evidence of infection and heart disease served as controls. Written informed consents were obtained from healthy volunteers and relatives of all enrolled patients. Demographic and clinical data for septic shock and SILVSD patients were extracted from the electronic medical record. Patients were monitored from ICU admission to day 28 of hospital stay or death.

Measurement

At admission, two 5-ml tubes of venous blood samples that drawn from all patients with septic shock were collected in heparinized tubes with lithium heparin, and one 2-ml venous blood sample was collected in a tube with EDTA-K2. A 5-ml tube of venous blood sample was sent to the Department of Biochemistry at Tongde Hospital of Zhejiang Province (Hangzhou, China) for measuring plasma levels of TNT, NT-proBNP, HFABP and PCT, another 5-ml tube of venous blood sample was sent to DIAN DIAGNOSTICS (Hangzhou, China) for detection of the levels of Cu and Zn in whole blood. And a 2-ml tube of venous blood sample for measuring the counts of blood leukocyte and hs-CRP.

HFABP concentrations were determined by Immunochromatographic assay using the Diagnostic Kit for the Quantitative Determination of Human Heart-type Fatty Acid-Binding Protein Rapid Test (Finecare, Guangzhou, China) in the FS-201 Automatic Immunofluorescence Quantitative Analyzer (Finecare, Guangzhou, China). Plasma concentrations of TNT, NT-proBNP and PCT were determined by Electrochemiluminescence (ECL) assay using the Elecsys Troponin T hs Assay Kit, Elecsys proBNP Immunoassay Kit, Elecsys BRAHMS PCT Assay Kit (Roche) in the cobas e 411 Analyzer for immunoassay tests (Roche Diagnostics, North America). Complete blood cell counts and hs-CRP were determined using BC-6900 Automatic Hematology Analyzer and original matching reagents (Mindray, Shenzhen, China.).

Levels of Cu and Zn in whole blood were measured by Flame Atomic Absorption Spectrophotometry (FAAS) using Bohui Multi-element Detection Reagent for Whole Blood or Serum (improved) (Bohui, Beijing, China) in BH5100T Atomic Absorption Spectrometer (Bohui, Beijing, China). The reference values of whole blood Cu and Zn are in range of 7.12 to 33.80 $\mu\text{mol/L}$ and 67.72 to 111.30 $\mu\text{mol/L}$ respectively in adults. Complete blood cell counts, hs-CRP, plasma TNT, NT-proBNP, HFABP, PCT levels and whole blood Cu and Zn were measured according to the manufacturer's instructions.

Standard Echocardiographic Examination

TTE was often performed at admission using M9 Ultrasound System (Mindray, Shenzhen, China) by an experienced echocardiographer in the critically ill who was not involved in patients care to evaluate LVEF (modified Simpson's rule), abnormalities of global/regional wall motion and E/e' ratios (tissue Doppler imaging).

Statistical analysis

Continuous variables with normal distribution were described as means \pm [standard deviations (SDs)] and analyzed using Independent-Samples *t*-test or One-Way ANOVA, followed by LSD test for multiple groups. Continuous variables with skewed distribution were described as medians [interquartile ranges (IQRs)] and analyzed using the Whitney *U* test. Categorical variables were provided as proportions(%) and tested with the Chi-square test. The correlations between whole blood Cu, Zn and Acute Physiology and Chronic Health Evaluation(APACHE)-II score, SOFA score and LVEF were analyzed with Pearson line correlation, and the correlations between whole blood Cu, Zn and plasma TNT, NT-proBNP, HFABP, and PCT were analyzed with Spearman rank correlation. We used receiver operating characteristic (ROC) and area under the ROC curves (AUC) to evaluate the predictive and prognostic values of whole blood Cu and Zn concentrations. A $p < 0.05$ would be recognized as statistically significant. SPSS software (version 19.0, SPSS Inc., Chicago, IL, USA), GraphPad Software (Prism 8.0.2, GraphPad Software) and MedCalc statistical software (version 11.4.2, MedCalc Software Bvba, Ostend) were used to conduct the statistical analysis.

Results

Patient characteristics

Baseline demographic data, including current age, sex, type of infection, underlying diseases, severity of disease and status of organ's function were collected and shown in Table 1. The demographic data showed no significant difference in age and percentage of male among three groups (SILVSD vs. non-SIMD vs. control): 66.08 ± 12.12 vs. 65.53 ± 13.17 vs. 69.20 ± 10.34 years old (age; $p = 0.335$); 15(60.0%) vs. 29(64.4%) vs. 27(65.9%) (males; $p = 0.888$). The sources of infection which contributed to septic shock in both SILVSD and non-SIMD patients included lung, abdomen, bloodstream, urinary tract and others and none of them showed significant difference ($p = 0.831$), but the major etiologies were pneumonia for both non-SIMD and SILVSD patients. Compared with non-SIMD patients at enrollment, SILVSD patients had significantly higher APACHE-II score and SOFA score and higher total bilirubin (TB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine, TNT, NT-proBNP, HFABP and PCT levels (all $p < 0.05$), but lower LVEF ($p < 0.05$). Furthermore, SILVSD patients were given more dosage of norepinephrine (NE) to maintain MAP than non-SIMD at admission ($p = 0.043$). Patients' underlying diseases including hypertension, diabetes mellitus, COPD, anemia and stroke in the two groups did not show significant difference ($p = 0.867$). And as for leukocyte and hs-CRP, there were not significant difference between SILVSD and non-SIMD (leukocyte: $p = 0.071$; hs-CRP: $p = 0.237$).

Table 1
Baseline Characteristics

Characteristics	Control (n = 25)	non-SIMD (n = 45)	SILVSD (n = 41)	p-value	p-value non-SIMD vs. SILVSD
Age(years)	66.08 ± 12.12	65.53 ± 13.17	69.20 ± 10.34	0.335	0.158
Male (n,%)	15(60.0%)	29(64.4%)	27(65.9%)	0.888	0.891
APACHE-II score		34.20 ± 7.91	39.56 ± 10.87		0.010
SOFA score		14.82 ± 2.32	16.46 ± 3.12		0.007
Death in 28 days (n, %)		13(28.9%)	17(41.5%)		0.222
Sources of infection					0.831
Pneumonia(n, %)		19(42.2%)	17(41.5%)		
Peritonitis(n, %)		11(24.4%)	12(29.3%)		
CRBSI(n, %)		8(17.8%)	5(12.2%)		
Urinary tract infection(n, %)		4(8.9%)	6(14.6%)		
Other(n, %)		3(6.7%)	1(2.4%)		
Underlying diseases					0.867
Hypertension(n, %)		13(28.9%)	16(39.0%)		
Diabetes mellitus(n, %)		9(20.0%)	10(24.4%)		
COPD(n, %)		6(13.3%)	5(12.2%)		
Anemia(n, %)		2(4.4%)	3(7.3%)		
Stroke(n, %)		4(8.9%)	2(6.1%)		
PaO ₂ /FiO ₂ (mmHg)		209.60 ± 57.37	197.52 ± 65.41		0.364

Values are number (proportion), mean ± standard deviation (SD) or medians [interquartile ranges (IQRs)]. SIMD: sepsis-induced myocardial dysfunction; SILVSD: sepsis-induced left ventricular systolic dysfunction; APACHE: acute physiology and chronic health evaluation; SOFA: sequential organ failure assessment; CRBSI: catheter related bloodstream infection; COPD: chronic obstructive pulmonary disease; PaO₂:arterial partial pressure of oxygen; FiO₂: fraction of inspiratory oxygen; BUN: blood urea nitrogen; TB: total bilirubin; AST: aspartate aminotransferase;ALT: alanine aminotransferase; NE: norepinephrine; MAP: mean arterial pressure; TNT: troponin T; NT-proBNP: amino-terminal pro-B-type natriuretic peptide; HFABP: Heart-type fatty acid binding protein; hs-CRP: hypersensitive C-reactive protein; PCT: procalcitonin; LVEF: left ventricular ejection fraction.

Characteristics	Control (n = 25)	non-SIMD (n = 45)	SILVSD (n = 41)	p-value	p-value non-SIMD vs. SILVSD
Mechanical ventilation(n, %)		31(68.9%)	33(80.5%)		0.218
BUN(mmol/L)		9.76 ± 2.69	11.28 ± 3.52		0.026
Creatinine(μmol/L)		113.98 ± 48.38	135.32 ± 42.64		0.034
TB(mmol/L)		27.02 ± 10.09	32.83 ± 14.46		0.032
Albumin(g/L)		34.74 ± 8.43	32.29 ± 9.17		0.200
AST(U/L)		46.15 ± 16.99	49.20 ± 15.37		0.387
ALT(U/L)		38.04 ± 15.21	42.86 ± 16.75		0.156
MAP(mmHg)		72.17 ± 10.32	69.55 ± 12.49		0.290
Lactate(mmol/L)		4.43 ± 1.61	5.20 ± 2.20		0.066
Dosage of NE(μg/kg/min)		0.39 ± 0.08	0.43 ± 0.10		0.043
Leukocyte(10 ⁹ /L)		18.48 ± 4.45	20.59 ± 6.18		0.071
hs-CRP(mg/L)		168.63 ± 69.83	185.51 ± 60.86		0.237
TNT(μg/L)	0.01(0.01, 0.01)	0.01(0.04,0.01)	0.78(1.39,0.39)	< 0.001	0.008
NT-proBNP(pg/mL)	117(100, 205)	1993(3468, 861)	6152(13794, 3334)	< 0.001	< 0.001
HFABP(ng/mL)	8.4(5.2, 12.0)	21.30(29.65, 11.45)	77.60(102.80, 45.75)	< 0.001	< 0.001
PCT(ng/L)	0.05(0.05, 0.05)	11.73(25.20, 3.16)	23.60(55.81, 15.14)	< 0.001	< 0.001
LVEF(%)	61.44 ± 8.48	58.84 ± 8.11	34.54 ± 4.44	< 0.001	< 0.001
Values are number (proportion), mean ± standard deviation (SD) or medians [interquartile ranges (IQRs)]. SIMD: sepsis-induced myocardial dysfunction; SILVSD: sepsis-induced left ventricular systolic dysfunction; APACHE: acute physiology and chronic health evaluation; SOFA: sequential organ failure assessment; CRBSI: catheter related bloodstream infection; COPD: chronic obstructive pulmonary disease; PaO ₂ :arterial partial pressure of oxygen; FiO ₂ : fraction of inspiratory oxygen; BUN: blood urea nitrogen; TB: total bilirubin; AST: aspartate aminotransferase;ALT: alanine aminotransferase; NE: norepinephrine; MAP: mean arterial pressure; TNT: troponin T; NT-proBNP: amino-terminal pro-B-type natriuretic peptide; HFABP: Heart-type fatty acid binding protein; hs-CRP: hypersensitive C-reactive protein; PCT: procalcitonin; LVEF: left ventricular ejection fraction.					

Increased whole blood Cu and reduced Zn and the correlation with LVEF and NT-proBNP in SILVSD patients

Whole blood Cu and Zn concentrations and *Levels of* LVEF and plasma NT-proBNP at admission were examined in 41 patients with SILVSD, 45 non-SIMD patients and 25 healthy controls. Whole blood Cu concentrations in patients with SILVSD were higher than those in non-SIMD patients and healthy controls [(16.34 ± 1.93) µmol/L vs. (15.23 ± 2.07) µmol/L, $p = 0.009$; (16.34 ± 1.93) µmol/L vs. (14.02 ± 1.65) µmol/L, $p < 0.001$], and non-SIMD patients also showed increased levels of Cu compared with healthy controls [(15.23 ± 2.07) µmol/L vs. (14.02 ± 1.65) µmol/L, $p = 0.013$] (Fig. 2A). Whole blood Zn levels in patients with SILVSD were lower than those in non-SIMD patients and healthy controls [(78.45 ± 12.18) µmol/L vs. (85.07 ± 14.80) µmol/L, $p = 0.029$; (78.45 ± 12.18) µmol/L vs. (94.90 ± 14.78) µmol/L, $p < 0.001$], and non-SIMD patients also showed reduced levels of Zn compared with healthy controls [(85.07 ± 14.80) µmol/L vs. (94.90 ± 14.78) µmol/L, $p = 0.005$] (Fig. 2B). Correlation analysis showed that whole blood Cu was negatively correlated with LVEF ($r = -0.545$, $p < 0.001$), but it was positively correlated with plasma NT-proBNP ($r = 0.466$, $p = 0.002$) (Fig. 2C). While whole blood Zn was positively correlated with LVEF ($r = 0.594$, $p < 0.001$), it was negatively correlated with plasma NT-proBNP ($r = -0.503$, $p = 0.001$) (Fig. 2D).

Whole blood Cu and Zn concentrations in SILVSD patients correlated with APACHE-II score, SOFA score, plasma TNT, HFABP and PCT

Pearson correlation analysis showed that whole blood Cu was positively correlated with APACHE-II score and SOFA score (APACHE-II : $r = 0.379$, $p = 0.015$; SOFA: $r = 0.354$, $p = 0.023$) (Fig. 3A). On the contrary, whole blood Zn was negatively correlated with APACHE-II score and SOFA score (APACHE-II : $r = -0.313$, $p = 0.046$; SOFA: $r = -0.395$, $p = 0.011$) (Fig. 3B). *Spearman* rank correlation analysis showed that whole blood Cu was positively correlated to plasma TNT, HFABP and PCT (TNT: $r = 0.607$, $p < 0.001$; HFABP: $r = 0.391$, $p = 0.012$; PCT: $r = 0.396$, $p = 0.010$) (Fig. 3A), in contrast, whole blood Zn was negatively correlated with plasma TNT, HFABP and PCT (TNT: $r = -0.692$, $p < 0.001$; HFABP: $r = -0.475$, $p = 0.002$; PCT: $r = -0.575$, $p < 0.001$.) (Fig. 3B).

Predictive values of whole blood Cu and Zn for SILVSD

ROC curve analysis was applied to compare the predictive value of whole blood Cu and Zn between septic shock and SILVSD. The AUC for Cu (AUC = 0.666; 95% CI, 0.556–0.764; $p = 0.005$) was comparable to that of Zn (AUC = 0.625; 95% CI, 0.514–0.727; $p = 0.039$) (Fig. 4). These findings suggested that whole Cu could distinguish between SILVSD and septic shock and could perform at least as well as whole Zn for prediction of SILVSD. When the cut-off points for whole blood Cu and Zn were set at 14.2 µmol/L and 84.11 µmol/L, they had sensitivity of 87.80% and 73.13% and specificity of 46.67% and 53.33% respectively.

Predictive values of whole blood Cu and Zn for 28-day mortality

All recruited patients were monitored for 28 days after enrollment or until death. Whole blood Cu levels at admission were significantly higher in non-survivors ($n = 30$) than in survivors ($n = 56$) [$(17.20 \pm 2.25) \mu\text{mol/L}$ vs. $(14.99 \pm 1.49) \mu\text{mol/L}$, $p < 0.001$) but Zn concentrations were significantly lower in non-survivors than survivors [$(71.17 \pm 11.98) \mu\text{mol/L}$ vs. $(87.67 \pm 11.30) \mu\text{mol/L}$, $p < 0.001$) (Fig. 5A1 and A2). The performance of Cu and Zn for predicting 28-day mortality was evaluated by ROC curve analysis between hospital deaths and survivors for all enrolled patients. Whole blood Cu displayed the value of predicting 28-day mortality (AUC = 0.802; 95% CI, 0.702–0.880; $p < 0.001$) as well as Zn (AUC = 0.869; 95% CI, 0.779 – 0.932; $p < 0.001$) (Fig. 5B). When the cut-off points for whole blood Cu and Zn were set at 16.45 $\mu\text{mol/L}$ and 79.15 $\mu\text{mol/L}$, they had sensitivity of 73.33% and 83.33% and specificity of 85.71% and 83.93% respectively.

Discussion

In the current study, we shown that whole blood Cu levels were significantly higher in SILVSD patients with septic shock compared with non-SIMD patients with septic shock independently of age, sex, type of infection and underlying diseases such as hypertension, diabetes mellitus, COPD, anemia and stroke, whereas whole blood Zn were significantly lower in SILVSD patients with septic shock compared with non-SIMD patients with septic shock. And, in SILVSD patients, whole blood Cu was negatively correlated with LVEF ($r = -0.545$, $p < 0.001$), but was positively correlated with plasma NT-proBNP, whole blood Zn was positively correlated with LVEF, but was negatively correlated with plasma NT-proBNP. Secondly, whole blood Cu levels were positively correlated with APACHE-II score, SOFA score and plasma levels of TNT, HFABP and PCT, but whole blood Zn levels were negatively correlated with APACHE-II score, SOFA score and plasma levels of TNT, HFABP and PCT. Finally, although there were no significant difference in mortality at day28 between SILVSD and non-SIMD patients, the whole blood Cu levels were higher in non-survivors compared with survivors, but whole blood Zn were lower in non-survivors patients compared with survivors, furthermore, whole blood Cu and Zn were predictive of 28-day mortality.

A study performed by Ayoglu et al(26). revealed that serum Cu and Zn levels were in the normal range in patients with sepsis and systemic inflammatory response syndrome(SIRS), which is almost consistent with the discovery that the levels of whole blood Cu and Zn were in the normal range in patients with septic shock in our research. However, in our study, the levels of Cu in SILVSD patients with septic shock were higher than non-SIMD patients with septic shock while the levels of whole blood Zn were lower than non-SIMD patients with septic shock. LVSD could be contribute to the differences of the whole Cu and Zn levels between SILVSD and non-SIMD group according to the previous study of Alexanian et al(22). In their research, compared with healthy volunteers, patients with either AHF or CHF had higher serum Cu and lower serum Zn that were correlated with LVDD, moreover, further analysis indicated that increased serum Cu and decreased serum Zn were significantly correlated with LVSD in patients with AHF, which could support the results in our study.

Compared with researches investigated the correlation between serum Zn and sepsis or septic shock(27, 28), studies on the relationship between serum Cu and sepsis or septic shock are fewer but have shown

that serum Cu levels are elevated in patients with sepsis(26). Idris et al. and Manuel et al. found that elevated serum Cu concentrations existed in septic patients(29, 30). Cu is vital for synthesis of collagen, antioxidant response, iron transportation, acts as a cofactor for oxidative metalloenzymes and can result in anaemia, leukopenia and pancytopenia(31, 32), but the potential mechanisms of higher serum Cu in sepsis remain unclear absolutely. On the contrary, studies have shown that Zn is related to nutritional immunity and involved in the synthesis of acute phase proteins and acts as a hepatoprotective agent, or a differentiation signal for innate immune cells. Production of inflammatory cytokines such as tumor necrosis factor- α (TNF- α), interleukin-1 (IL-1) and IL-6 can upregulate zinc import proteins (mainly ZIP14) and metallothionein (MT) in the liver, which can redistribute Zn from blood into the liver during sepsis(27). Finally, septic shock is subset of sepsis with circulatory and cellular/metabolic dysfunction associated with higher risk of mortality(1), so we assume that the whole blood Cu concentrations of patients with septic shock are higher than those of healthy volunteers but the whole blood Zn concentrations are lower than those in our study.

As far as the difference in whole blood Cu concentrations between non-SIMD group and SILVSD group is concerned, LVSD (LVEF \leq 50%) probably plays an important role and is correlated with the change of whole blood Cu concentrations. Firstly, elevated whole blood Cu in SILVSD patients with septic shock probably represents a significant elevation of ceruloplasmin (Cp), which binds about 95% of the circulating blood Cu(33). Cp can not only delivery Cu to cells, but oxidize iron (Fe^{2+} to Fe^{3+}) that is incorporated into transferrin (TRF). The TRF- Fe^{3+} -compound can scavenge reactive oxygen species (ROS) because it exerts antioxidant glutathione–peroxidase activity(33). It is reported that IL-1 and IL-6 can upregulate the synthesis of Cp in the liver(34), which supports our finding that a significant positive correlation exists between whole blood Cu and NT-proBNP, TNT, PCT and HFABP that reflects cardiac function and myocardial injury in septic shock(11). We speculate that this also may explain the phenomenon that elevated serum Cu concentrations existed in septic patients to some extent. Secondly, hypoxia can stimulate the raised release of hypoxia-inducible factor (HIF) that promotes elevated synthesis of Cp in the liver(35) and macrophages and consequently result in elevated serum Cu levels under hypoxia condition. Finally, although serum Cu can reflect Cp metabolism, there are up to 40% additional Cu that loosely bind and dissociate from Cp under excessive oxidative stress because the structure and function of Cp can be modulated by ROS(36). The free Cu can deteriorate the cardiac systolic and predict worse prognosis in patients with LVSD added to septic shock because it participates the ROS production by its pro-oxidant activity(37, 38). This is consistent with our finding that the raised whole blood Cu levels were negatively correlated with cardiac systolic function (lower LVEF). Therefore, raised whole blood Cu concentrations in SILVSD based on septic shock may be fraction of a systemic mechanism induced by inflammation and hypoxia. This mechanism that is activated by cardiac systolic function can deteriorate cardiac systolic function that has been impaired by septic shock.

Our finding that whole blood Zn concentrations were lower in patients with LVSD is supported by some of previous researches investigated the role of Zn in cardiac systolic dysfunction(39, 40). We found in the present study that whole blood Zn was significantly lower in SILVSD based on septic shock than in non-

SIMD subjects and positively correlated with LVEF and NT-proBNP. There are several possible mechanisms that are implicated in this correlation. Firstly, during sepsis or septic shock, hypozincemia is one of the characteristics of acute phase reaction (APR), and as mentioned above, one of main reasons of hypozincemia is redistribution of Zn from blood to liver pro facilitated by raised ZIP14 and MT which bind Zn from plasma and tissues leading to lower Zn bioavailability(41). Secondly, raised production of inflammation in LVSD further results in MT and consequently reduce Zn levels, which is in line with our finding that a significant negative correlation exists between whole blood Zn and NT-proBNP, TNT, PCT and HFABP that reflects cardiac function and myocardial injury in septic shock. Finally, besides inflammatory response and sepsis or septic shock, cardiac systolic dysfunction also aggravates hypozincemia in sepsis. In SILVSD patients with septic shock, lower cardiac output (CO) due to LVSD and hypoperfusion usually coexist, which can reduce renal perfusion and further lead to hyperadrenergic state along with increased concentrations of cortisol, angiotensin II and aldosterone(42), Zn can redistribute to the damaged sites selectively under hyperadrenergic state(42). This supports our finding that the reduced whole blood Zn concentrations were positively correlated with cardiac systolic function (lower LVEF) and NT-proBNP. Furthermore, studies have found that coupled within tracellular and intramitochondrial Ca^{2+} accumulation in cardiac dysfunction, intracellular Zn^{2+} was raised because of increased Zn^{2+} entry and release of inactive Zn from MT-1 induced by nitric oxide under excessive catecholamine state. Hence, there was a significant reduction of whole blood Zn in SILVSD based on septic shock.

There are some previous studies that investigated the relationship between levels of serum Cu and Zn and prognosis in sepsis or septic shock and demonstrated that the higher mortality was correlated with higher serum Cu and lower serum Zn(29, 43), but this correlation was not be found in the research of Ayoglu et al. in Turkey(26). In contrast, although the 28-day mortality was not significant difference between non-SIMD and SILVSD groups, compared with survivors with septic shock, we discovered that the increased levels of whole blood Cu and the reduced levels of whole blood Zn existed in non-survivors with septic shock, which is consistent with previous studies(29, 43). After analysis of the correlations between whole blood Cu and Zn and APACHE-II score and SOFA score by Pearson line correlation, we found whole blood Cu was positively correlated with APACHE-II score and SOFA score and whole blood Zn was negatively correlated with APACHE-II score and SOFA score. Therefore, we believe that higher Cu concentration and lower Zn concentration may be associated with severity of MODS at admission and later in patients with septic shock.

Besides, as mentioned above, SILVSD patients also manifested higher levels of whole blood Cu that were positively associated with APACHE-II score and SOFA score and lower levels of whole blood Zn that were negatively correlated with APACHE-II score and SOFA score than non-SIMD patients. Cu and Zn could also distinguish SILVSD and non-SIMD in our study subjects (Cu: AUC = 0.666; 95% CI, 0.556–0.764; $p = 0.005$, and Zn: AUC = 0.625; 95% CI, 0.514–0.727; $p = 0.039$). It is reported that multiple indicators including LVEF, TNT and NT-proBNP have been applied to provide improved predictive accuracy to myocardial dysfunction in septic shock(44–46). Concomitant research of echocardiographic indicators (LVEF) and biomarkers (NT-proBNP or TNT) has not provided convincing results(47, 48). Therefore, the combination

of whole Cu and Zn with traditional biomarkers including NT-proBNP and LVEF could be performed for the accurate prediction of SILVSD based on septic shock.

We have also investigated the prognostic value of Cu and Zn. A significant increase in Cu levels at admission and decrease in Zn levels were found in non-survivors compared with survivors. A cut-off level of 16.45 $\mu\text{mol/L}$ and 79.15 $\mu\text{mol/L}$ of Cu and Zn had a sensitivity of 73.33% and 83.33% and a specificity of 85.71% and 83.93% for prediction of 28-day mortality with an AUC of 0.802 and 0.869 respectively. These results were in agreement with previous researches(37, 40). Excessive Cu in the human organism has been recognized in cell damage in sepsis and HF because it is involved in production of ROS and results in deterioration of the inflammation and cardiac systolic dysfunction. Previous studies had shown that hypozincemia was associated with lymphopenia, thymic atrophy and dysfunction of cell-and antibody-mediated immunity(49, 50), which could decrease lots of pre-B and pre-T cells lead to the immune paralysis in both sepsis and cardiac dysfunction(51, 52). Studies revealed that elevated TNT was related to higher short-term or long-term mortality in patients with sepsis or septic shock(53, 54). So, excessive Cu and hypozincemia have been indicators of poor prognosis in critically ill patients. It is reported that HFABP, a small cytoplasmic protein, can not only diagnose SIMD, but predict the prognosis of sepsis and septic shock. However the AUC of HFABP for evaluation of the outcome of sepsis is less than 0.7(55). Webb et al. indicated that elevated serum PCT levels were correlated with in-hospital mortality in septic patients(56). Therefore, the combination of whole Cu and Zn with traditional biomarkers including TNT, PCT and HFABP may be performed for more prognostic value of SILVSD based on septic shock.

However, our study has several limits. First, in the current study, although the statistics difference in whole blood Cu levels and Zn levels was significant between SILVSD group and non-SIMD group, there is a little regret without control group of heart dysfunction without infection or sepsis. Second, the whole blood Cu and Zn concentrations were used in this study since they have been applied in most hospitals in our area for several years and are reliable indicators for clinical staff. Besides, several studies have convinced that there is good relationship between levels of trace elements and those serum concentrations (57, 58). Finally, the sample size of this study is small so the current findings need confirming in further researches. Since the disease in this study has a prolonged course and complex cellular/metabolic mechanisms, studying a single time point at admission also limits our findings.

Conclusions

In the present study, whole blood Cu and Zn concentrations were different significantly between SIMD patients with septic shock and SILVSD, and were correlated with important clinical variables. Whole blood Cu and Zn concentrations were also associated with cardiac dysfunction and outcome of septic shock, and existed predictive and prognostic values for SILVSD patients with septic shock. There are indications that patients' whole blood Cu and Zn seem to have potential to be used as biomarkers or even as new sights into novel therapies.

Abbreviations

AHF: Acute heart failure; ALT: Alanine aminotransferase; APACHE-II: Acute physiology and chronic health evaluation II; APR: Acute phase reaction; AST: Aspartate aminotransferase; AUC: Area under curve; BNP: Brain natriuretic peptide; BUN: Blood urea nitrogen; CBP: Continuous blood purification; CHF: Chronic heart failure; CO: Cardiac output; COPD: Chronic obstructive pulmonary disease; Cp: Ceruloplasmin; CRBSI: Catheter related bloodstream infection; Cu: Copper; ECL: Electrochemiluminescence; EF: Ejection fraction; FAAS: Flame Atomic Absorption Spectrophotometry; HFABP: Heart-type fatty acid binding protein; HIF: Hypoxia-inducible factor; hs-CRP: Hypersensitive C-reactive protein; ICU: Intensive care unit; IL-6: Interleukin-6; IL-1: Interleukin-1; IQRs: Interquartile ranges; LV: Left ventricular; LVDD: Left ventricular diastolic dysfunction; LVEF: Left ventricular ejection fraction; MAP: Mean arterial pressure; MI: Myocardial infarction; MODS: Multi-organ dysfunction syndrome; MT: Metallothionein; NE: Norepinephrine; NT-proBNP: Amino-terminal pro-B-type natriuretic peptide; PCT: Procalcitonin; ROC: Receiver operating characteristic; ROS: Reactive oxygen species; SDs: Standard deviations; SIMD: Sepsis-induced myocardial dysfunction; SIRS: Systemic inflammatory response syndrome; SIVLSD: Sepsis-induced left ventricular systolic dysfunction; SOFA: Sequential organ failure assessment; TB: Total bilirubin; TNF- α : Tumor necrosis factor- α ; TNI: Troponin I; TNT: Troponin T; TRF: Transferrin; TTE: Transthoracic echocardiography; WMA: Wall motion abnormality; ZIP: Zinc import protein; Zn: Zinc.

Declarations

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Authors'contributions

JBM, XGG and SJH conceived and designed the study. JBM, MHH and WZ carried out the recruitment and clinical management. GPH and WZ performed the TTE. JBM and MHH carried out the statistical analysis. JBM, XGG and SJH drafted the manuscript. All authors have contributed significantly to the final version of this manuscript and have approved submission to this journal. All authors have read and approved the final manuscript.

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Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author upon reasonable request.

Ethical Approval

This study was approved by the Ethics Committee of Tongde Hospital of Zhejiang Province (approval no. [2018]016). Every potential participant was evaluated and their relatives were informed about the procedures as well as the risks involved with participation in this study at the initial interview, and a full past medical history was taken.

Consent for publication

Consent for publication in a scientific journal was obtained from all study participants and/or their legal guardians.

Competing interests

All authors have nothing to disclose.

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Figures

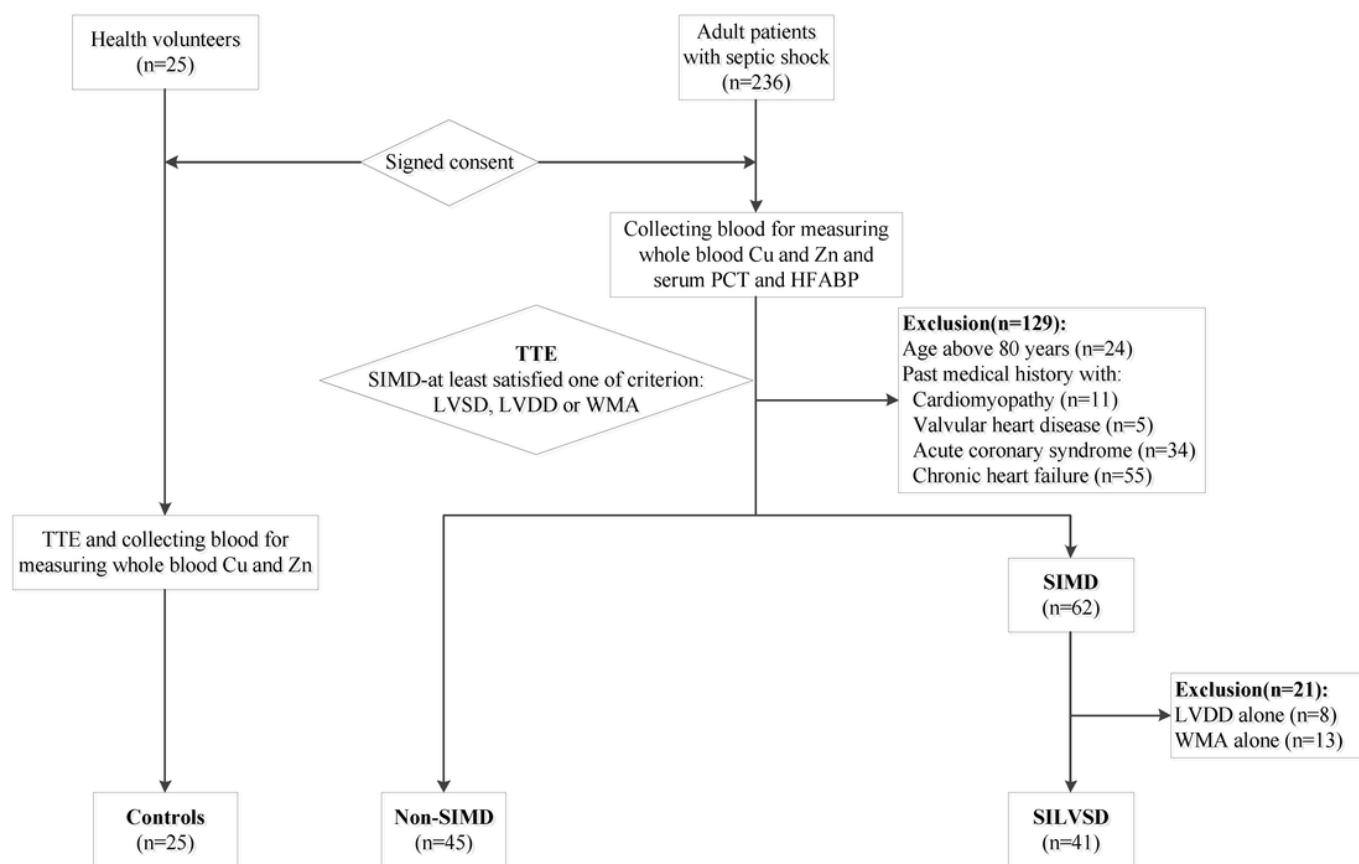


Figure 1

Diagram of the study. Cu: copper; Zn: zinc; PCT: procalcitonin; HFABP: heart-type fatty acid binding protein; TTE: transthoracic echocardiography; LVSD: left ventricular systolic dysfunction; LVDD: left ventricular diastolic dysfunction; WMA: wall motion abnormalities; SIMD: sepsis-induced myocardial dysfunction; SILVSD: sepsis-induced left ventricular systolic dysfunction; Cu: copper; Zn: zinc; LVEF: left ventricular ejection fraction; hs-CRP: hypersensitive C-reactive protein; TNT: troponin T; NT-proBNP: amino-terminal pro-B-type natriuretic peptide; HFABP: Heart-type fatty acid binding protein; PCT: procalcitonin.

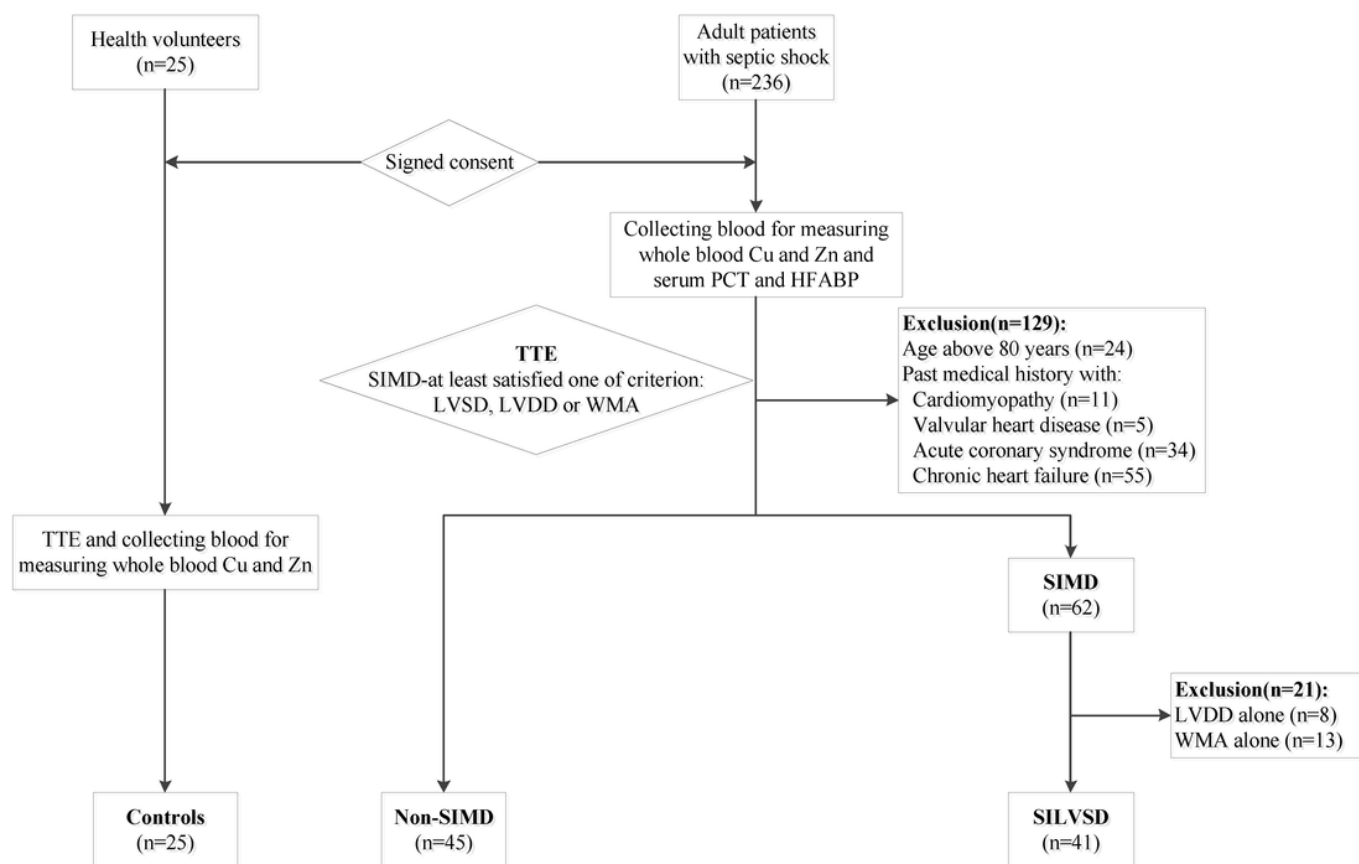


Figure 1

Diagram of the study. Cu: copper; Zn: zinc; PCT: procalcitonin; HFABP: heart-type fatty acid binding protein; TTE: transthoracic echocardiography; LVSD: left ventricular systolic dysfunction; LVDD: left ventricular diastolic dysfunction; WMA: wall motion abnormalities; SIMD: sepsis-induced myocardial dysfunction; SILVSD: sepsis-induced left ventricular systolic dysfunction; Cu: copper; Zn: zinc; LVEF: left ventricular ejection fraction; hs-CRP: hypersensitive C-reactive protein; TNT: troponin T; NT-proBNP: amino-terminal pro-B-type natriuretic peptide; HFABP: Heart-type fatty acid binding protein; PCT: procalcitonin.

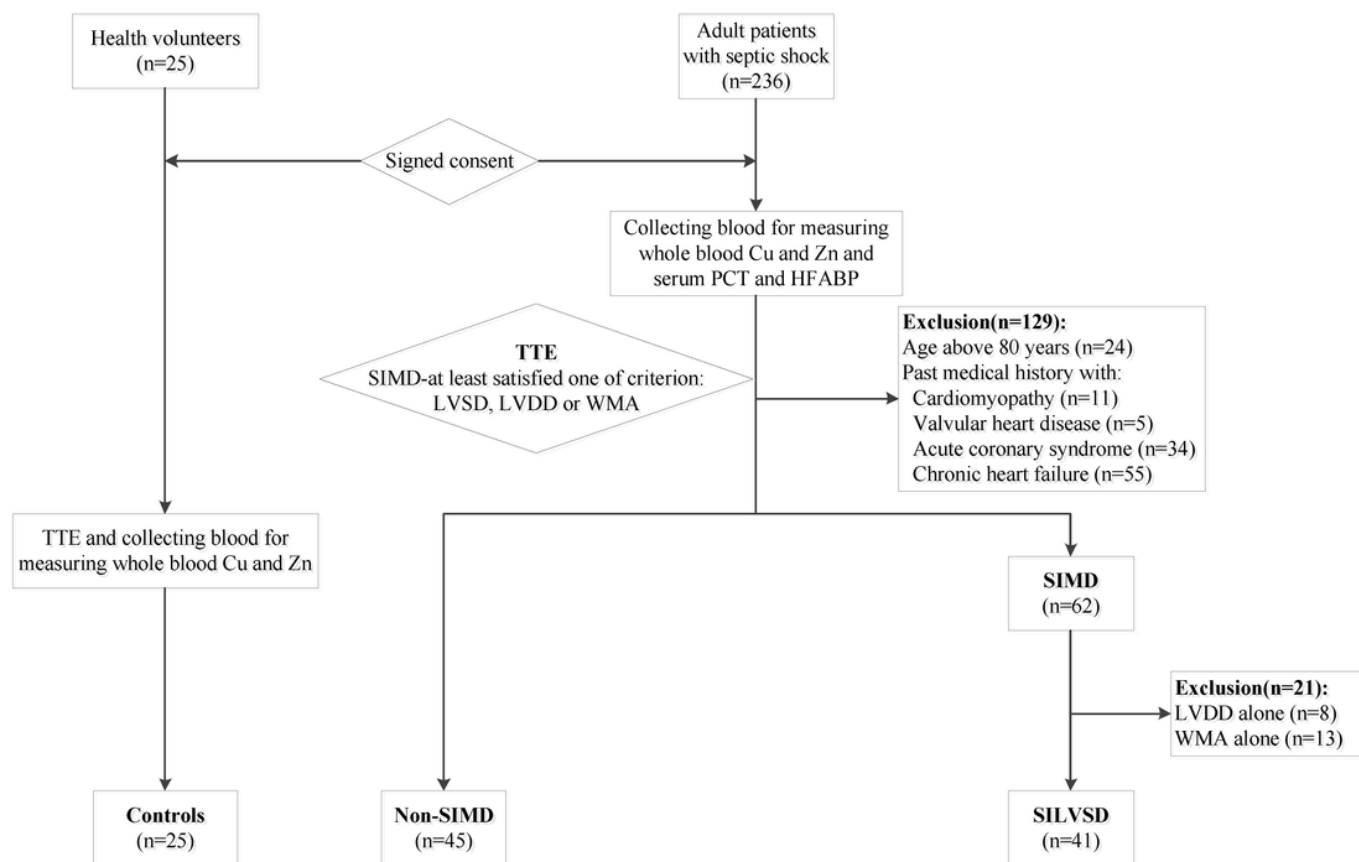


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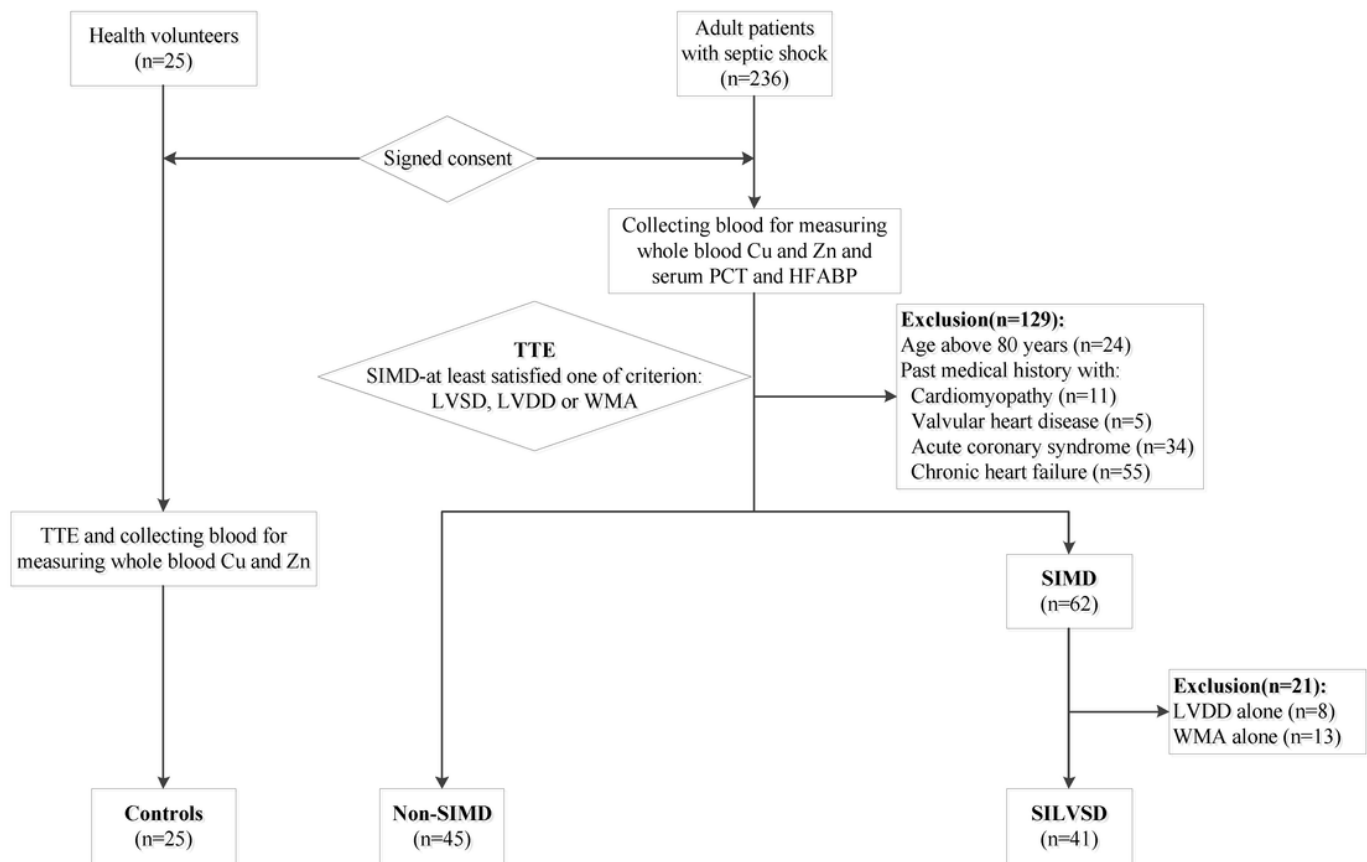


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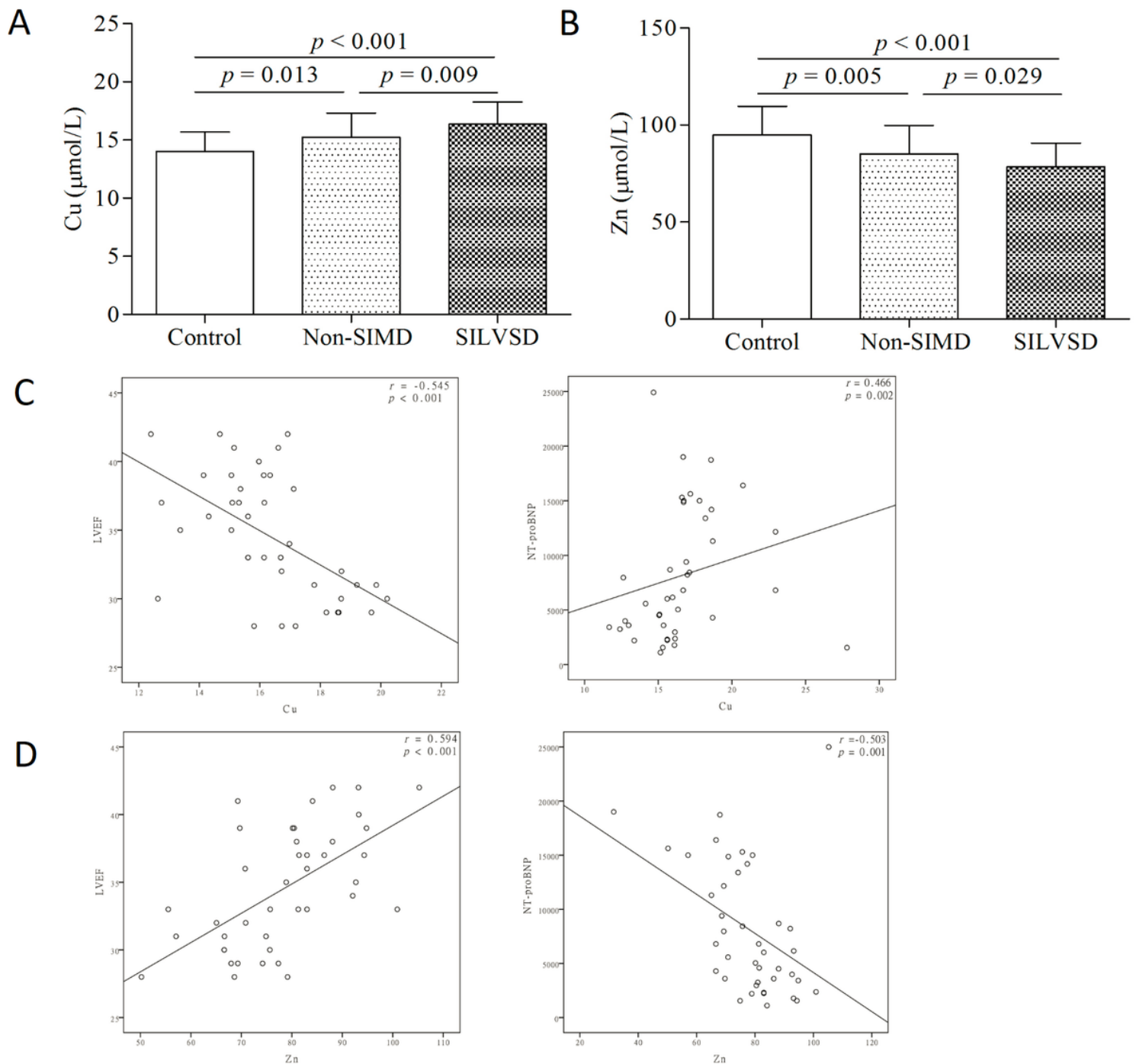


Figure 2

Whole blood Cu, Zn and LVEF levels of SILVSD patients with respect to non-SIMD and control. The bars represent the levels of whole blood Cu (A) and Zn (B) of healthy controls ($n = 25$), non-SIMD patients ($n = 45$), and SILVSD patients ($n = 41$). (C) The curves were plotted by Cu values at admission of 41 SILVSD patients to their respective LVEF and NT-proBNP. (D) The curves were plotted by Zn at admission of 41 SILVSD patients with septic shock to their respective LVEF and NT-proBNP. Data are presented as mean \pm SD. Each circle represents an individual patient. Cu: copper; Zn: zinc; SIMD: sepsis-induced myocardial

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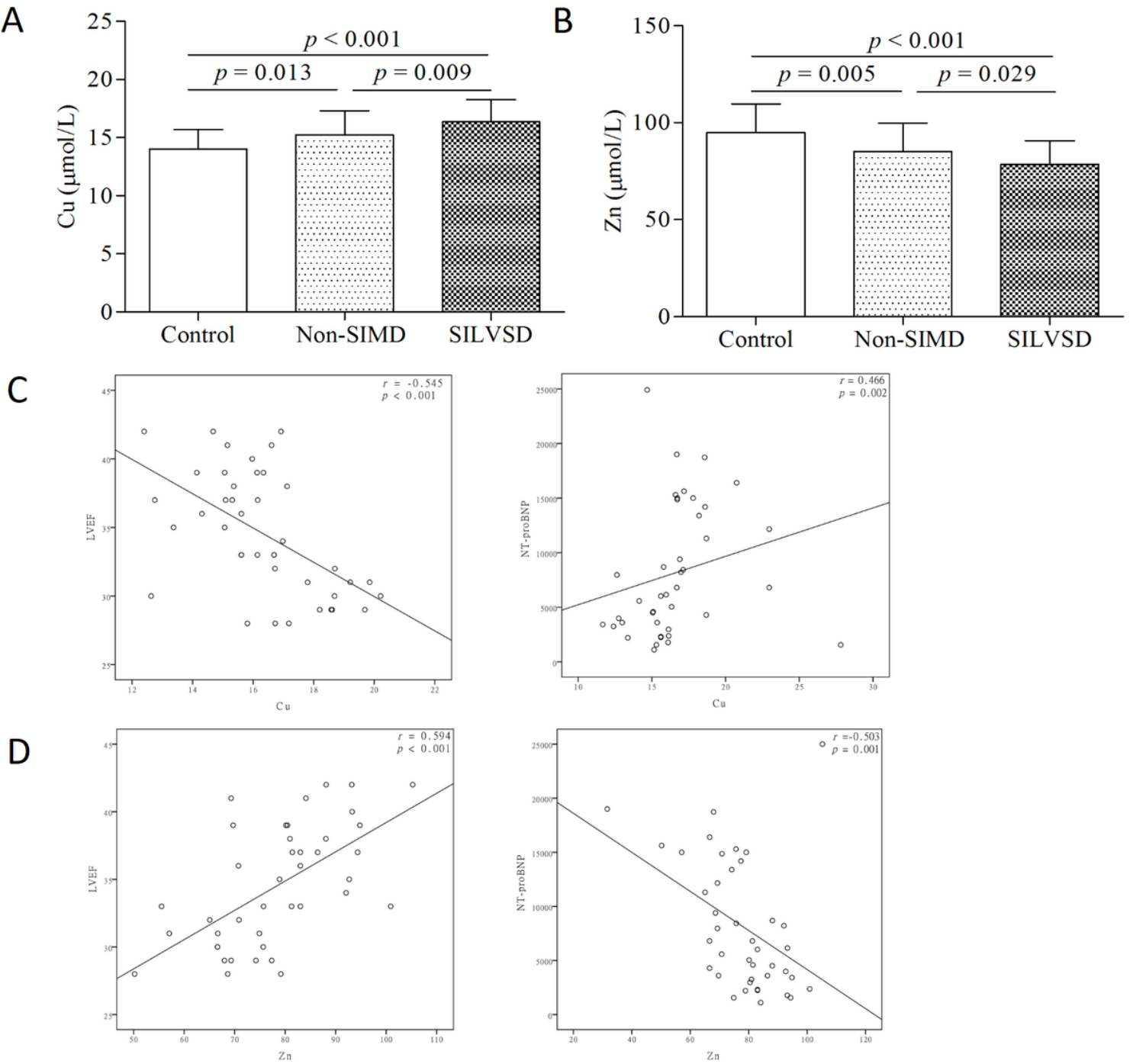


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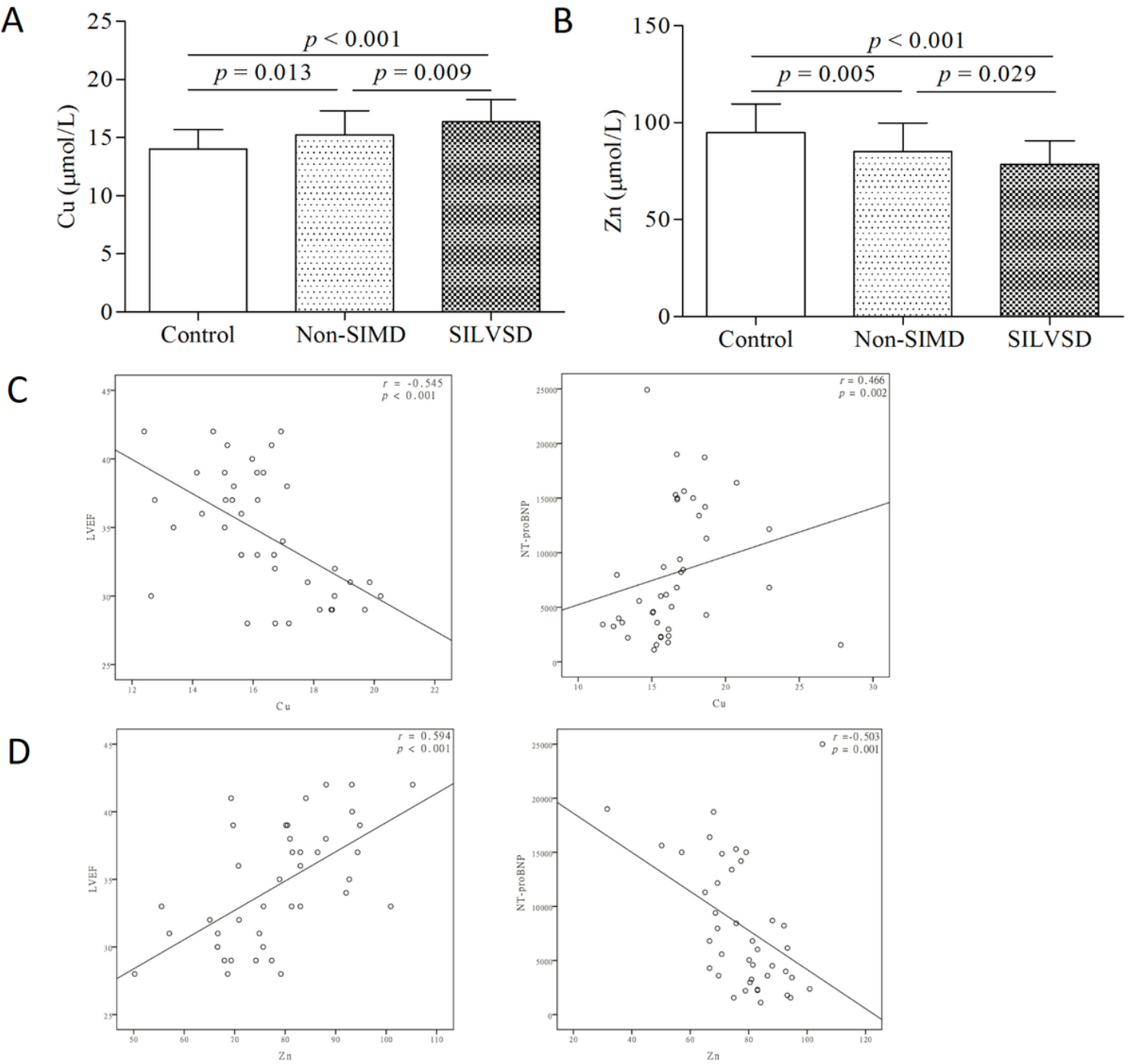


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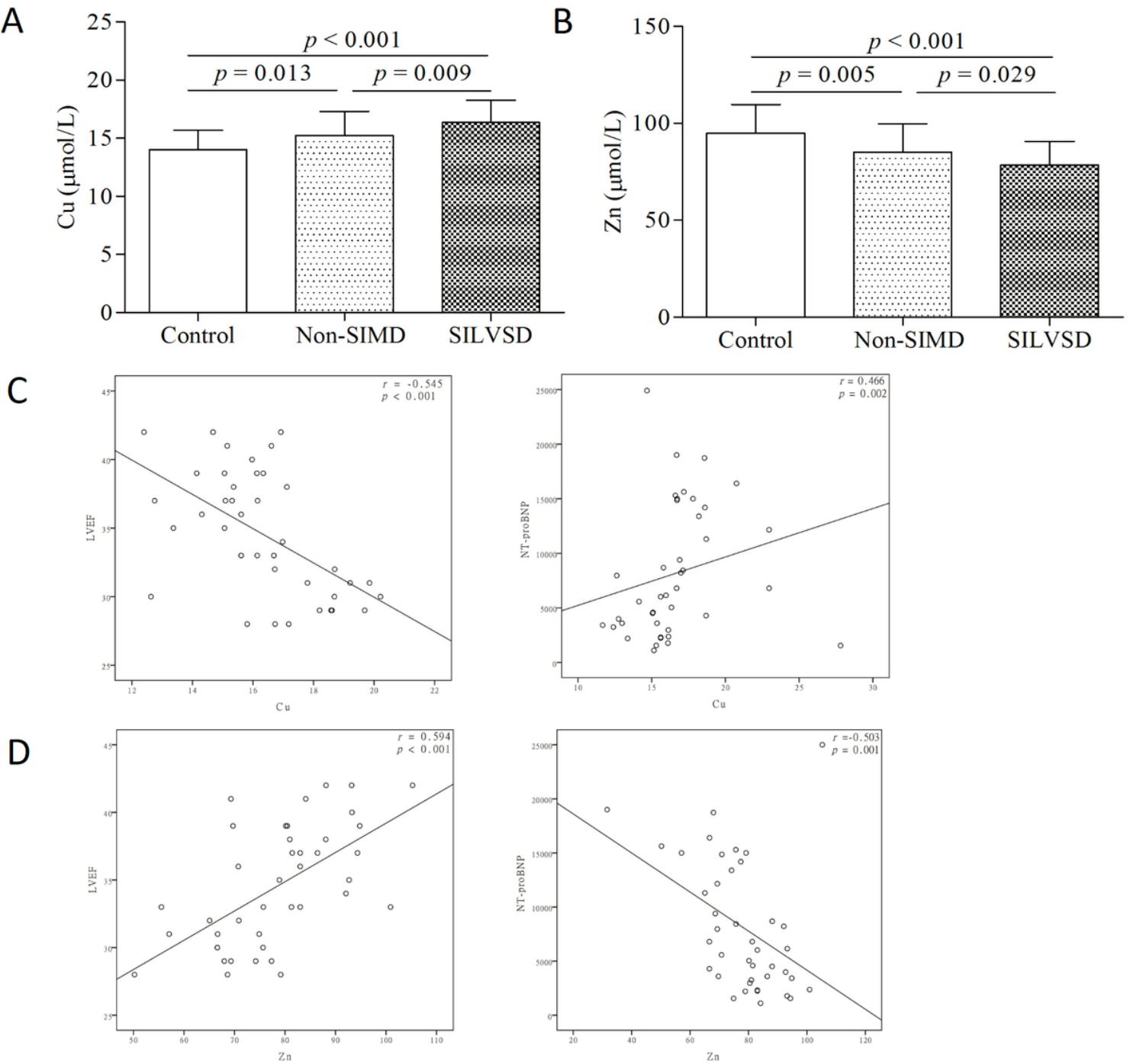


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Whole blood Cu, Zn and LVEF levels of SIVLD patients with respect to non-SIMD and control. The bars represent the levels of whole blood Cu (A) and Zn (B) of healthy controls (n = 25), non-SIMD patients (n = 45), and SILVSD patients (n = 41). (C) The curves were plotted by Cu values at admission of 41 SILVAD

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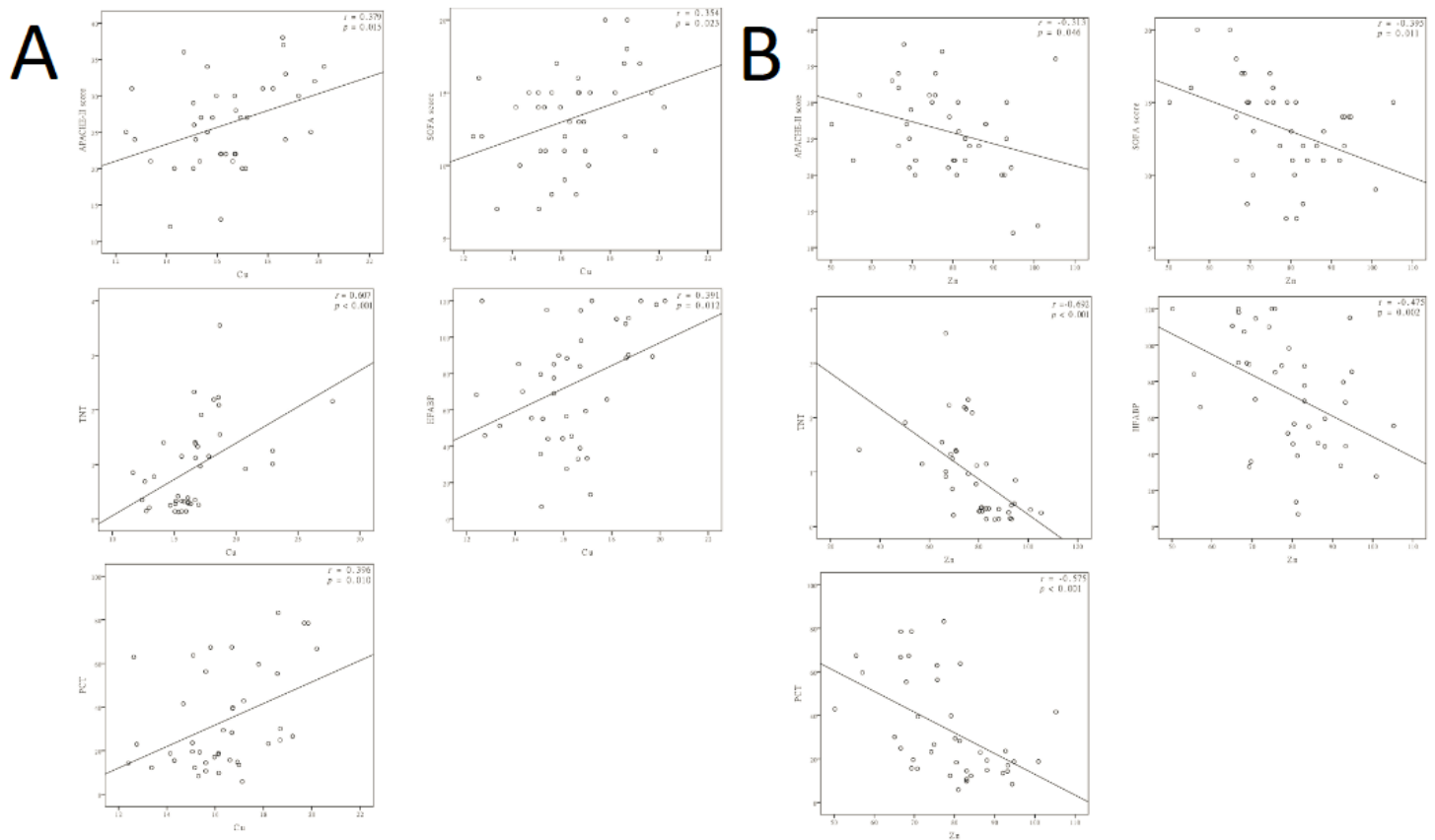


Figure 3

Correlation analysis of whole blood Cu and Zn concentrations with APACHE-II score, SOFA score, TNT, HFABP and PCT levels. (A) The curves were plotted by Cu values at admission of 41 SILVAD patients to their APACHE-II score, SOFA score, TNT, HFABP and PCT respectively. (B) The curves were plotted by Zn at admission of 41 SILVAD patients to their APACHE-II score, SOFA score, TNT, HFABP and PCT respectively. Each circle represents an individual patient. Cu: copper; Zn: zinc; APACHE: acute physiology and chronic health evaluation; SOFA: sequential organ failure assessment; TNT: troponin T; HFABP: Heart-type fatty acid binding protein; PCT: procalcitonin; SILVSD: sepsis-induced left ventricular systolic dysfunction.

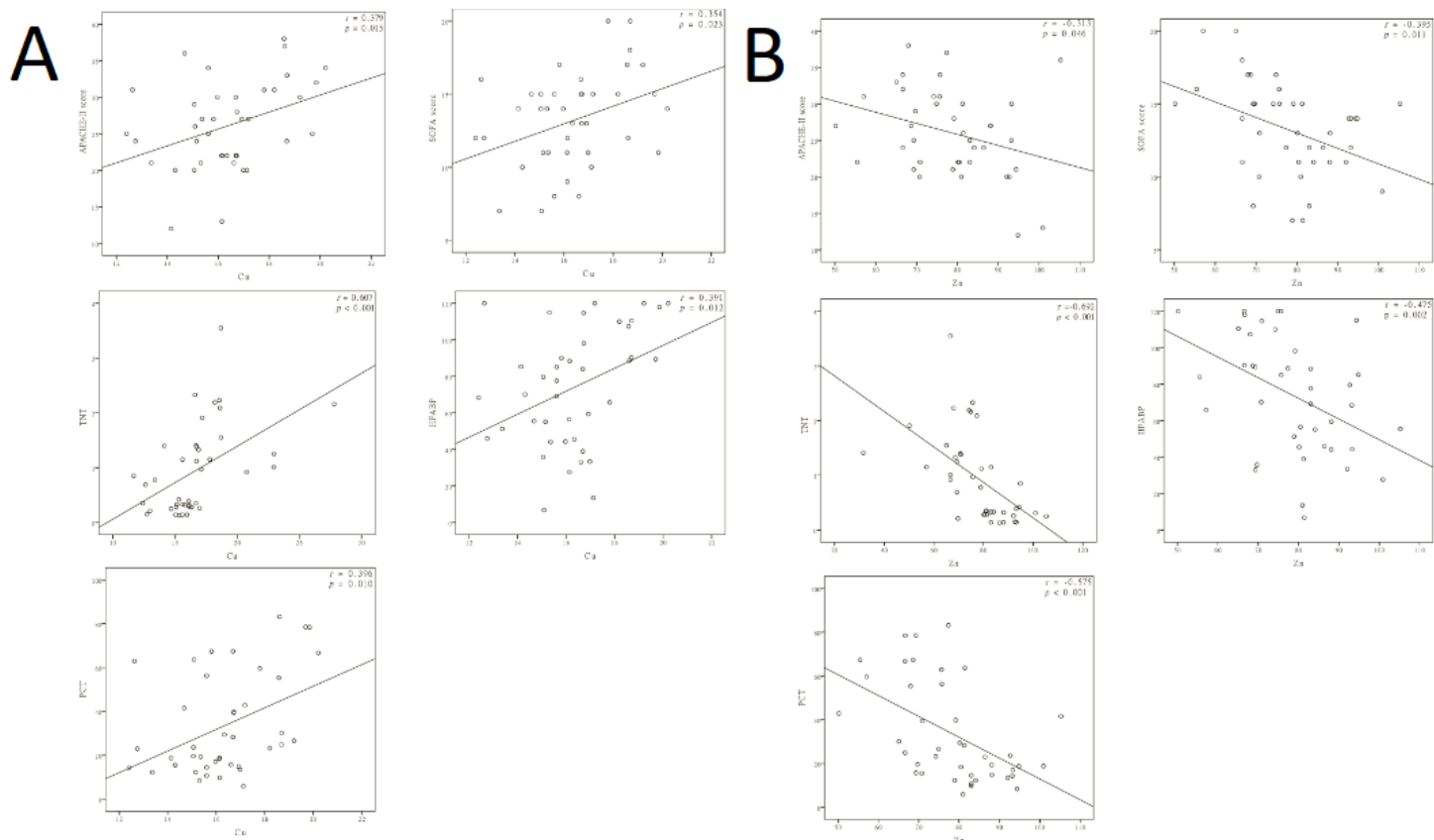


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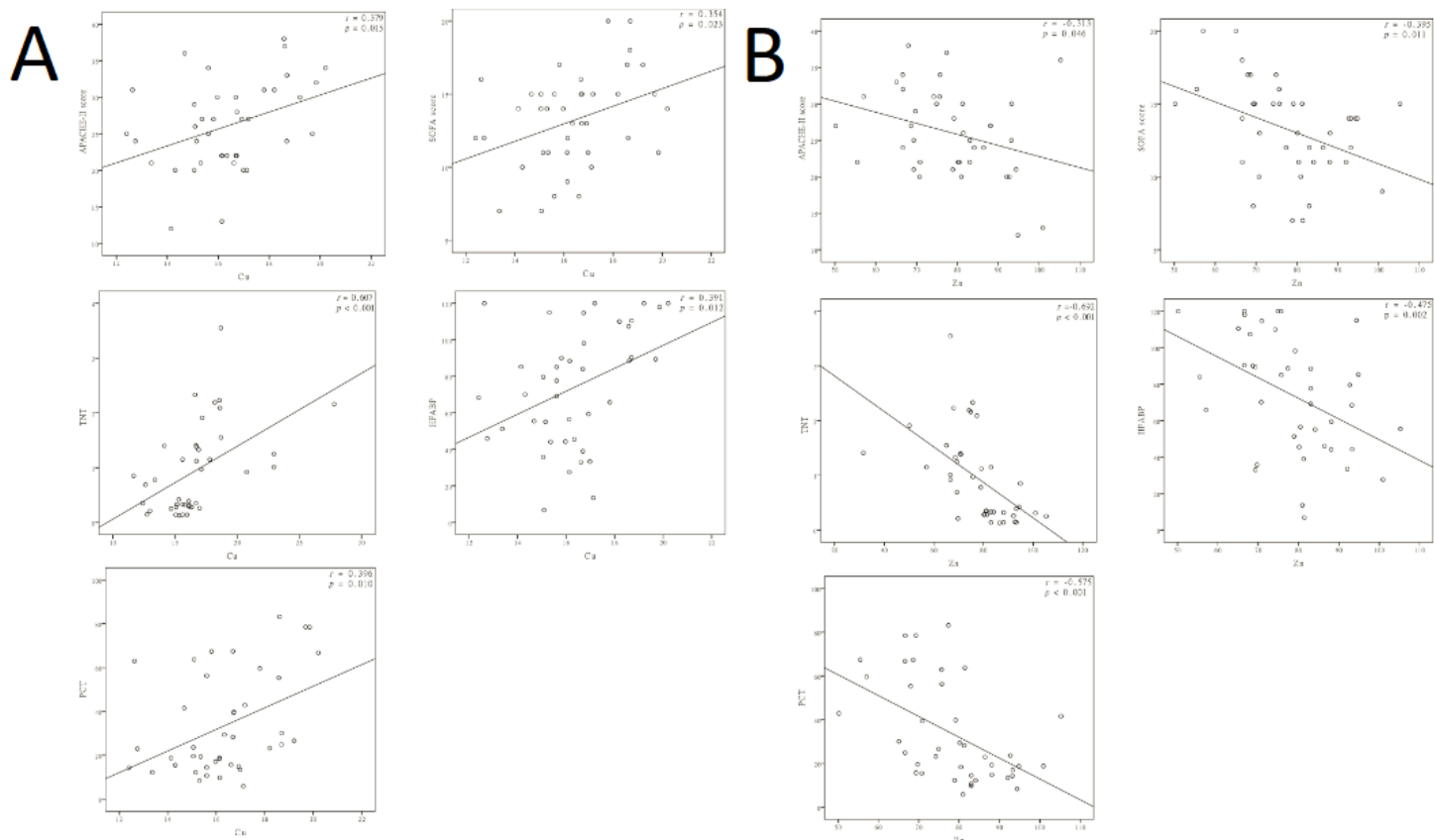


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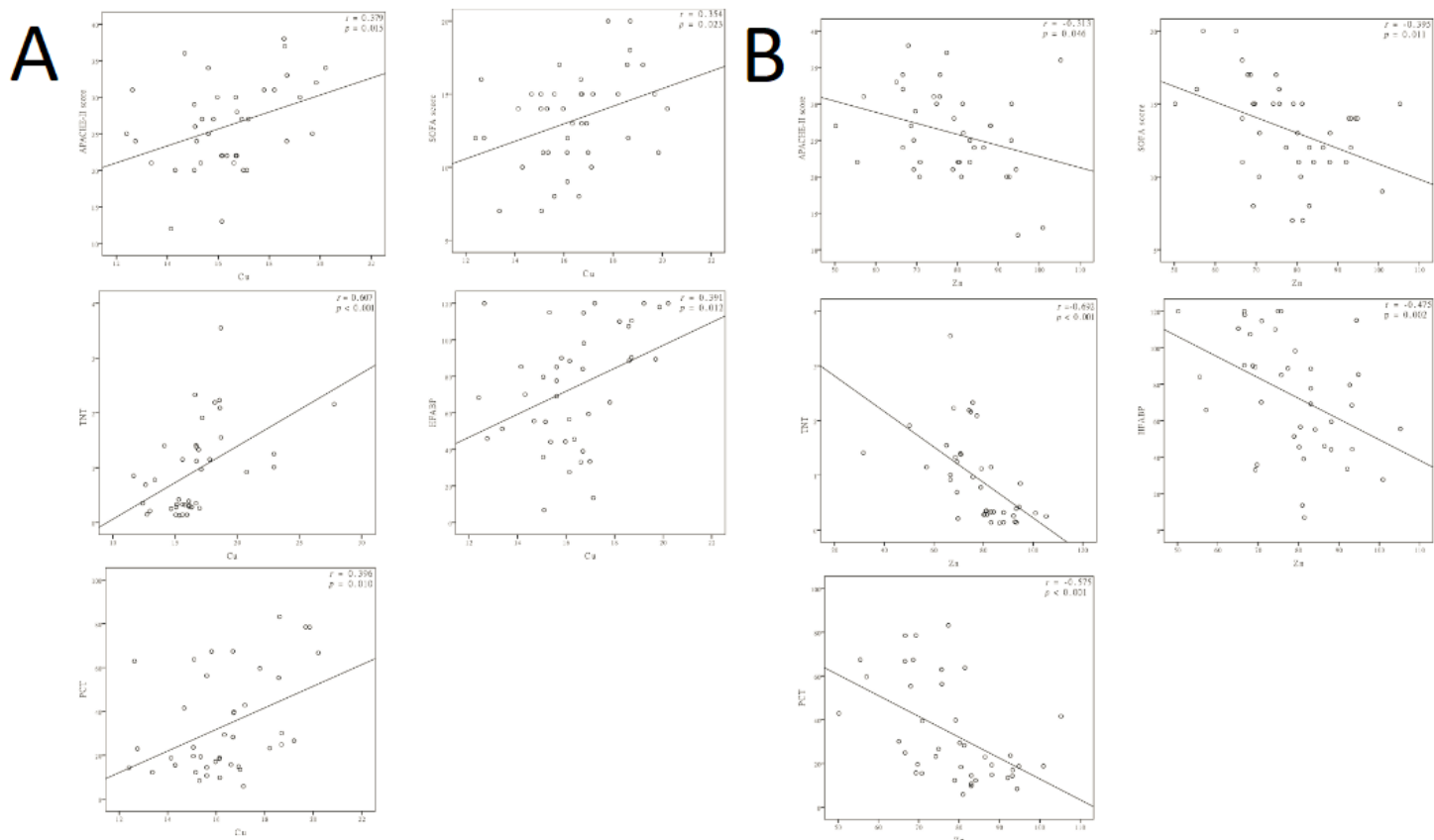


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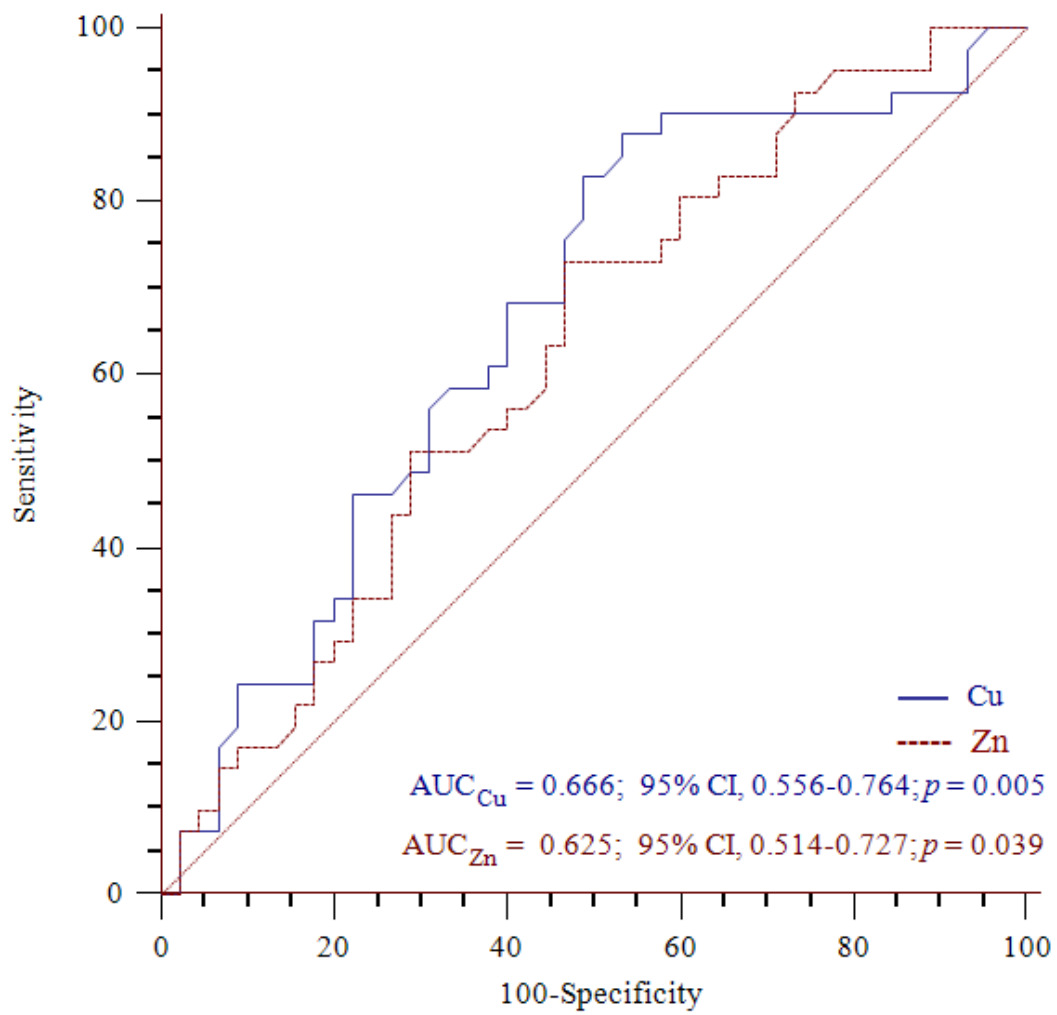


Figure 4

ROC curves for the prediction of SILVSD. ROC curves present sensitivity and specificity of Cu and Zn for predicting SILVSD. Cu: copper; Zn: zinc.

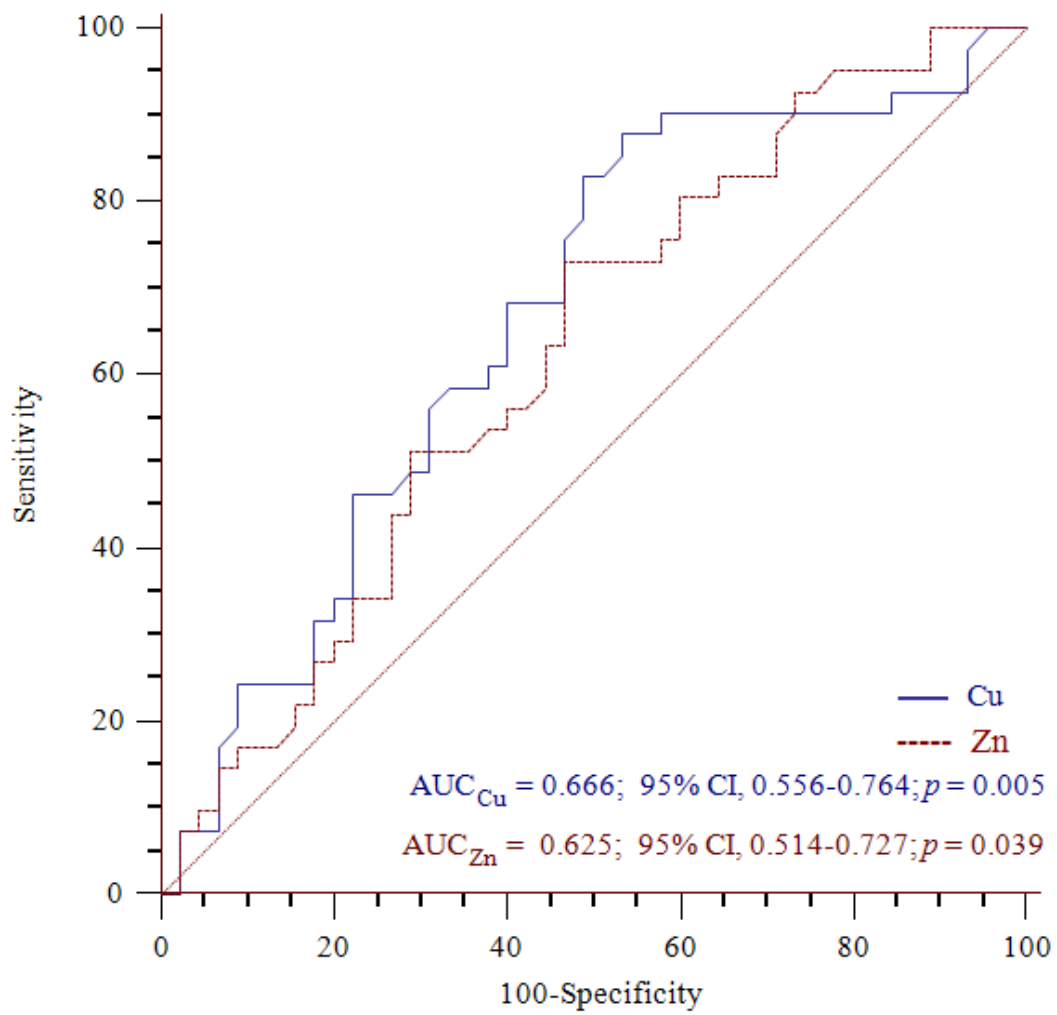


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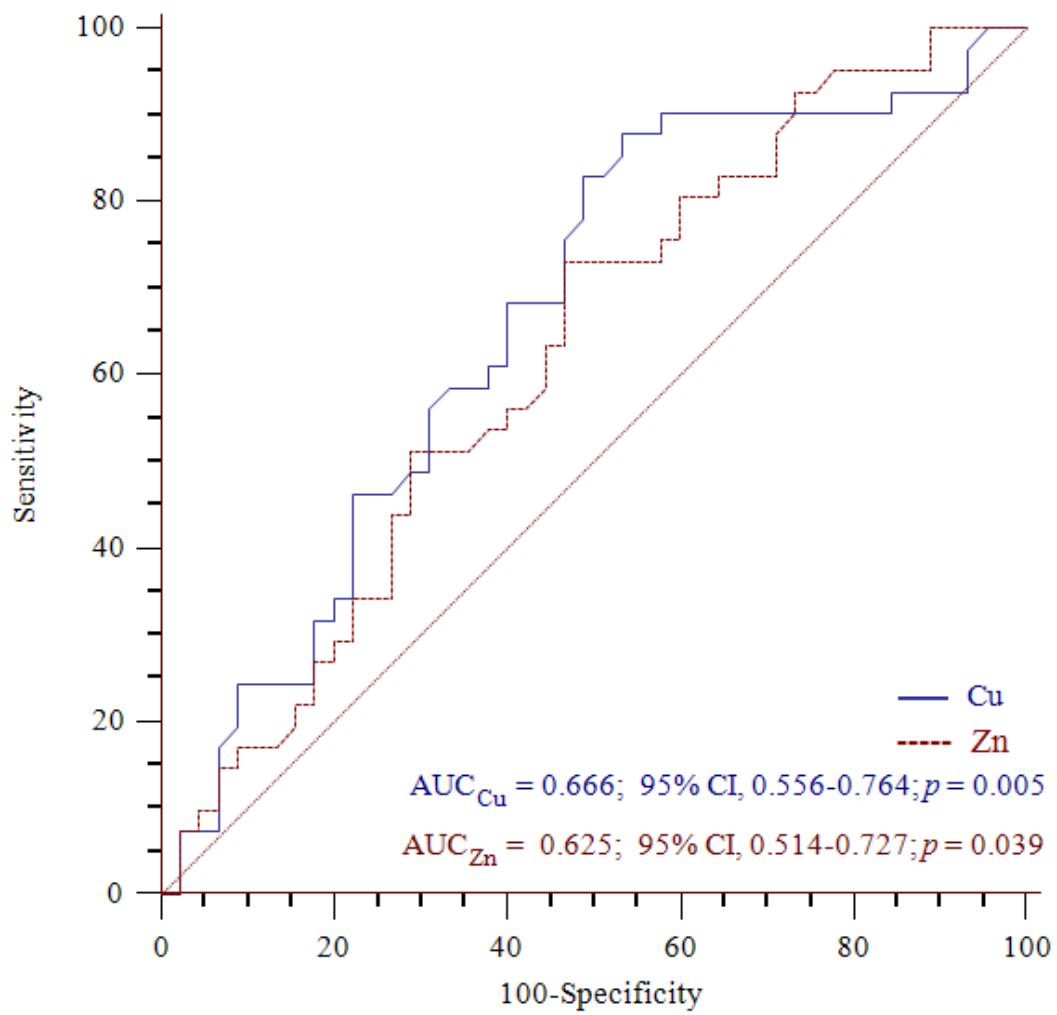


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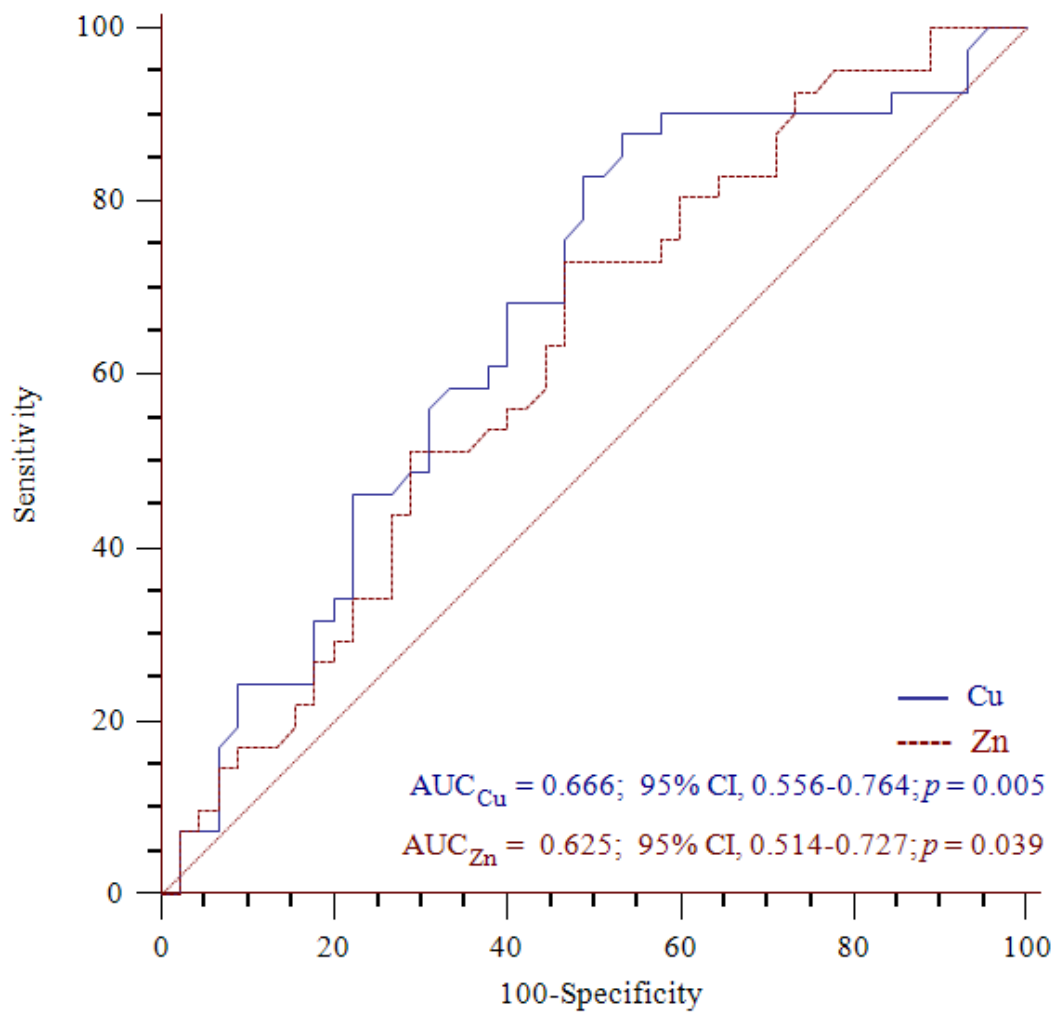


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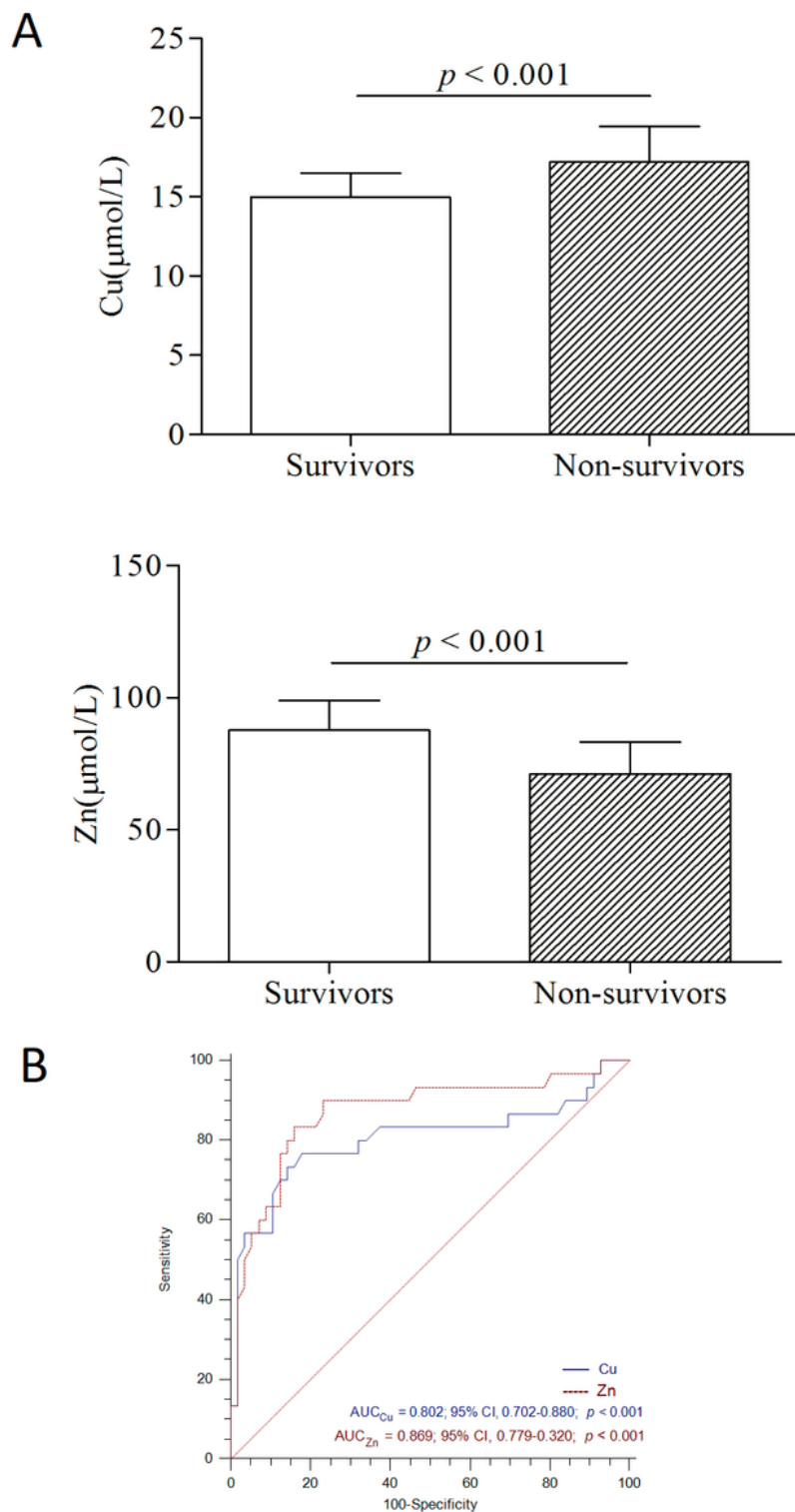


Figure 5

ROC curves for the prediction of 28-day mortality. (A) Cu and Zn levels were compared between non-survivors (n = 30) and survivors (n = 56). (B) ROC curves present sensitivity and specificity of Cu, and Zn for predicting 28-day mortality. Cu: copper; Zn: zinc.

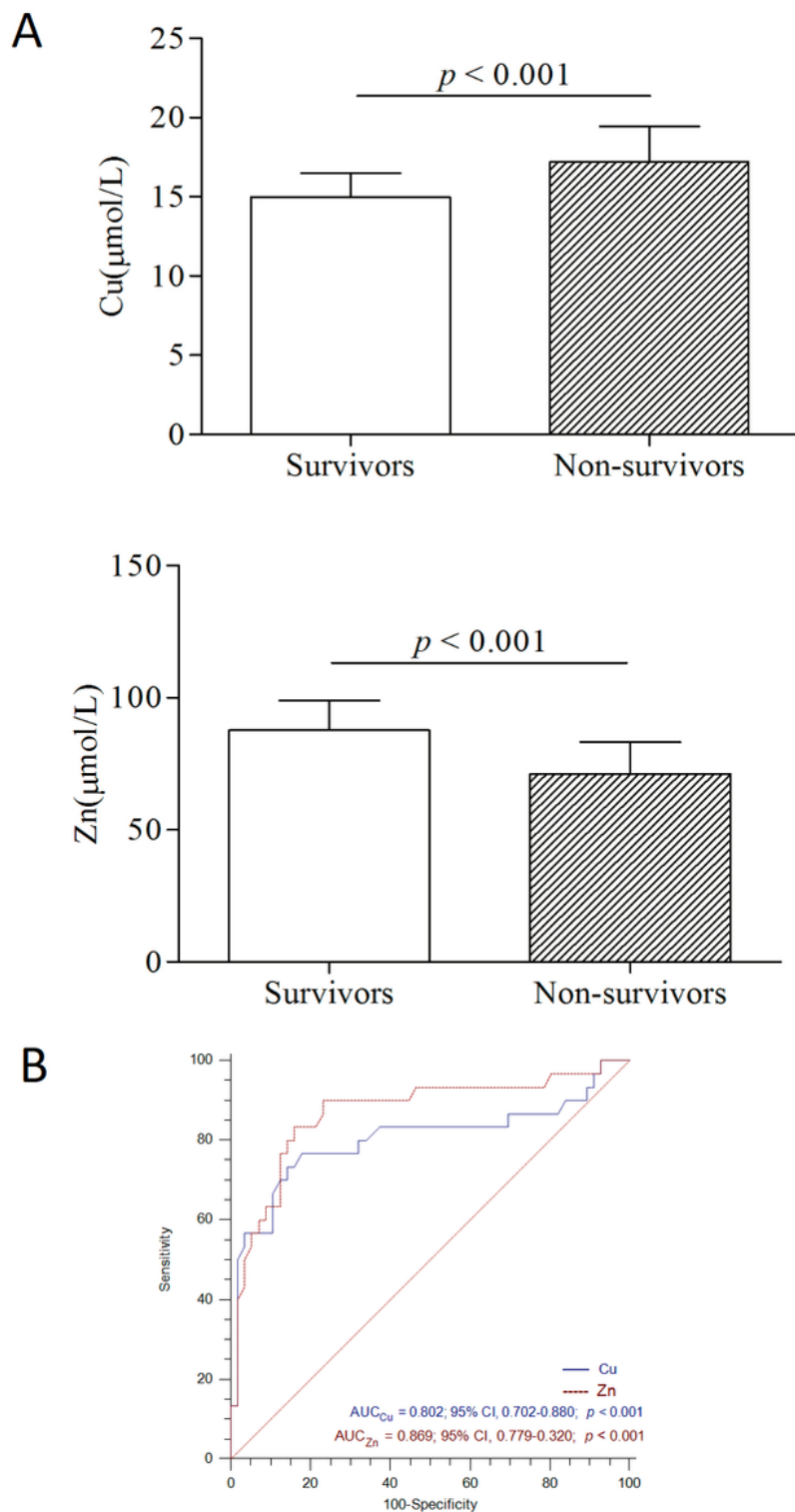


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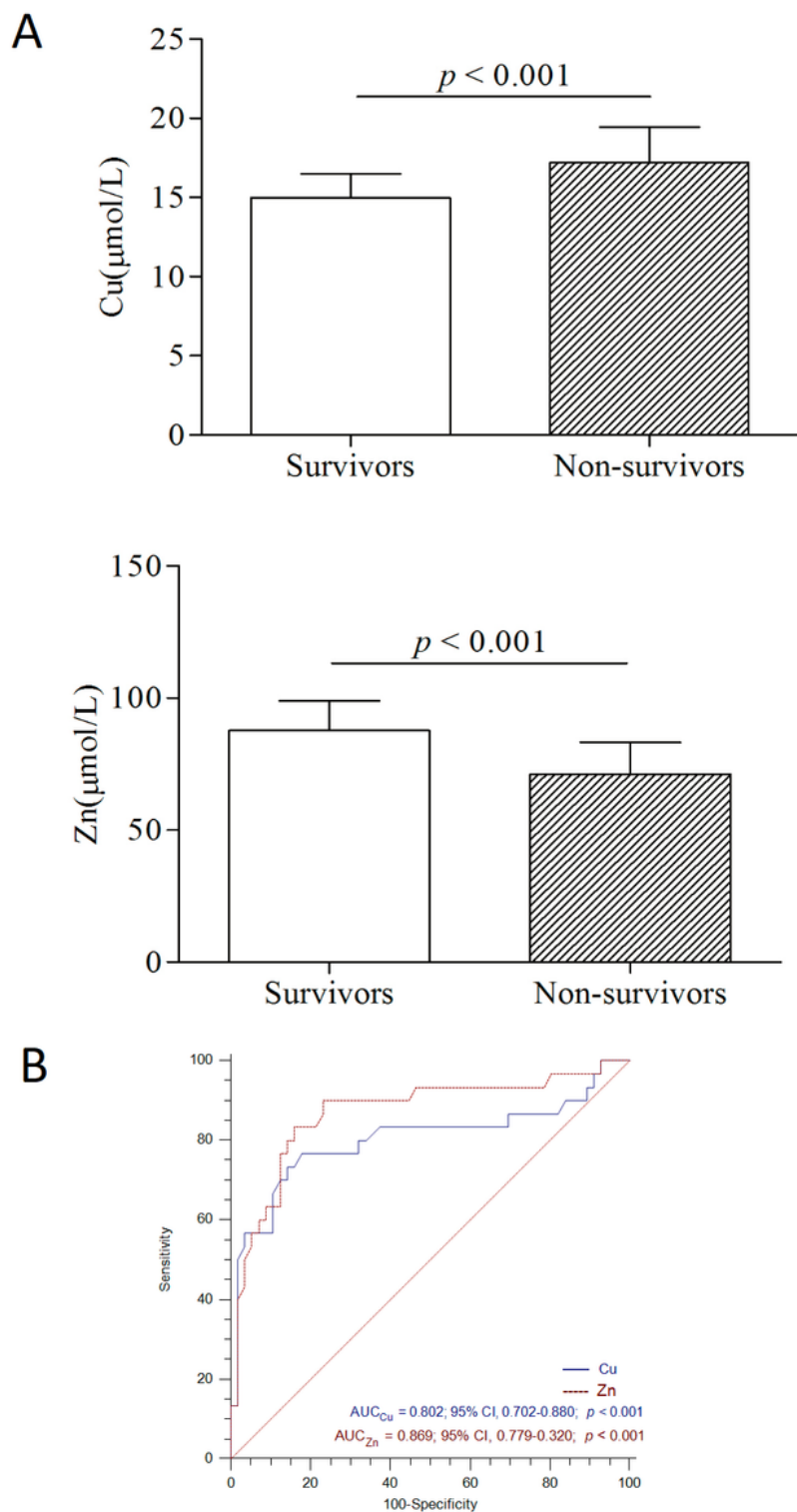


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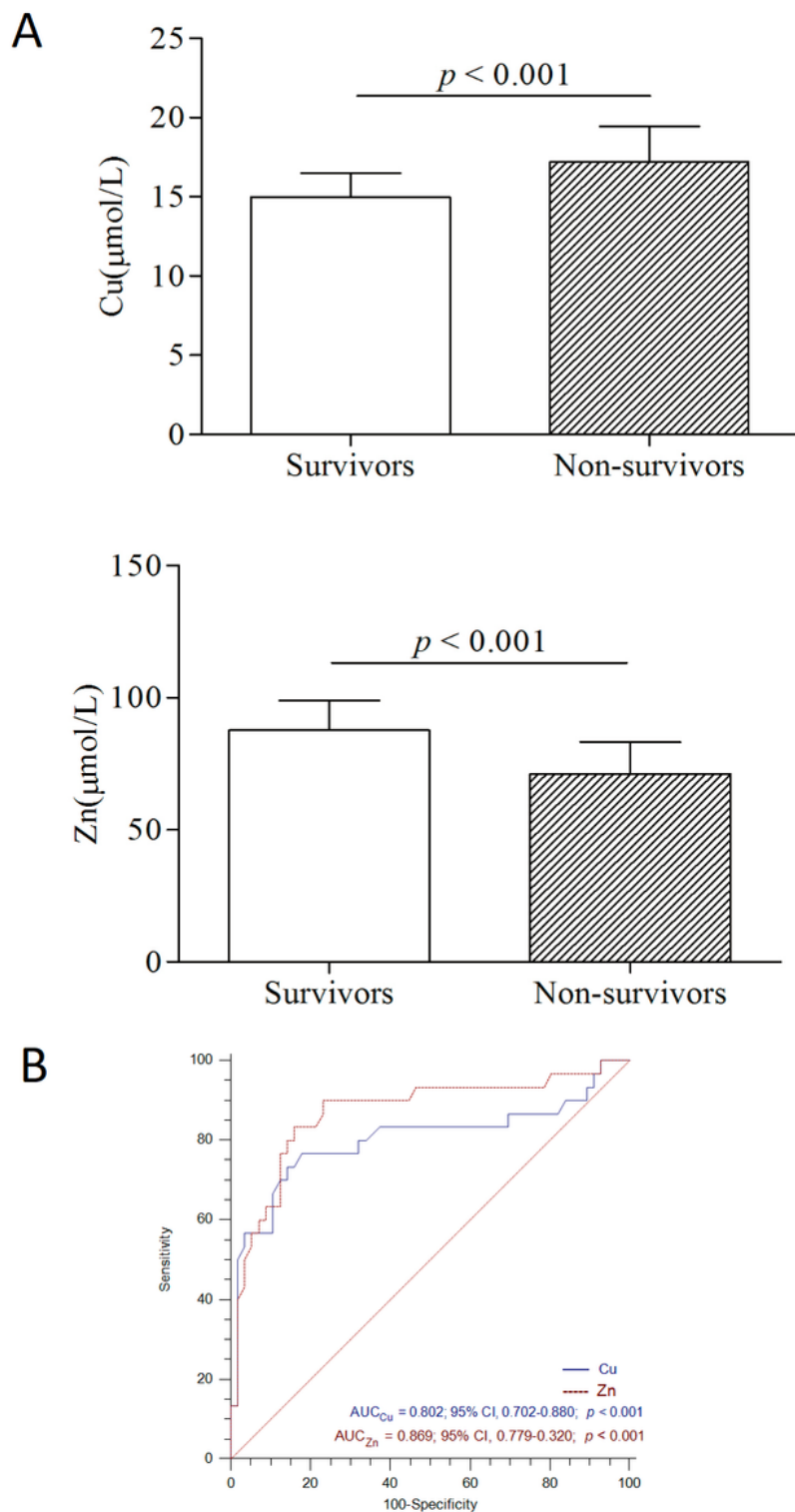


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