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ORIGINAL RESEARCH

The Diagnostic Value of Plasma NETs Levels and iCEB in Silent Myocardial Ischemia in Maintenance Hemodialysis Patients

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Objective: This study evaluated the diagnostic value of plasma Neutrophil extracellular traps (NETs) levels and the index of cardiac electrophysiological balance (iCEB) in identifying silent myocardial ischemia (SMI) in maintenance hemodialysis (MHD) patients.

Methods: This cross-sectional observational study involved patients receiving MHD treatment. Data were collected on coronary angiography performed in our hospital from February 2023 to February 2024. Patients diagnosed with myocardial ischemia via coronary angiography but without obvious symptoms were grouped as the SMI group, while those without SMI were grouped as the control group. Plasma NETs levels were assessed using markers indicative of NETs components including double-stranded DNA (dsDNA), circulating free DNA (cfDNA) and myeloperoxidase, while iCEB (QT/QRS) and electrocardiographic findings were obtained. Additionally, echocardiographic parameters, inflammatory markers, and cardiac biomarkers were analyzed. Receiver operating characteristic (ROC) analysis were employed to evaluate the diagnostic accuracy of plasma NETs levels and iCEB in identifying SMI.

Results: A total of 114 patients were included, with 79 participants in the control group and 35 participants in the SMI group. The SMI group exhibited significantly elevated levels of NETs associated components (dsDNA(37.89±4.55 vs 31.64±5.32, P<0.001), cfDNA(11.27±2.03 vs 8.91±1.84, P<0.001), MPO-DNA(23.69±4.01 vs 17.52±3.41, P<0.001)), as well as higher iCEB compared to the control group(56.45±7.67 vs 45.89±6.23, P<0.001). Furthermore, electrocardiography findings, echocardiographic parameters, inflammatory markers, and cardiac biomarkers showed significant differences between the two groups. The ROC analysis demonstrated the potential diagnostic accuracies of NETs levels and iCEB, with an area under the curve (AUC) of 0.908, sensitivity of 0.987, and specificity of 0.829 for identifying SMI.

Conclusion: The study highlights the combined diagnostic value of plasma NETs levels and iCEB in identifying SMI in MHD patients, providing valuable insights into potential early detection and risk stratification strategies for this population.

Keywords: silent myocardial ischemia, hemodialysis, neutrophil extracellular traps, index of cardiac electrophysiological balance, diagnostic value, cardiovascular complications

Introduction

Myocardial ischemia, characterized by insufficient blood supply to the heart, remains a critical concern in patients undergoing hemodialysis.[1] Hemodialysis patients experience a significantly higher risk of cardiovascular complications, including MI, due to various factors such as accelerated atherosclerosis, endothelial dysfunction, and chronic inflammation.[2,3] Furthermore,

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a substantial proportion of these patients may remain asymptomatic despite the presence of significant MI, which poses a diagnostic challenge for clinicians.[4,5]

Silent myocardial ischemia (SMI) represents a condition in which structural and functional abnormalities of the heart are present without overt clinical symptoms or signs of heart failure, such as dyspnea, fatigue, or edema.[6] This phenomenon poses a diagnostic challenge due to the lack of characteristic symptoms, thereby necessitating the utilization of comprehensive screening and diagnostic modalities to identify and characterize this condition.[7] The diagnosis of SMI typically involves the assessment of cardiac imaging modalities, including echocardiography and magnetic resonance imaging, to detect structural abnormalities such as left ventricular hypertrophy, dilation, or dysfunction, along with the evaluation of biomarkers indicative of myocardial stress and injury.[7,8] Additionally, electrocardiographic and electrophysiological parameters play a crucial role in the detection of subtle electrical abnormalities and arrhythmias associated with SMI.[6]

Neutrophil extracellular traps (NETs) have emerged as an intriguing area of study in the context of cardiovascular disease.[9,10] These web-like structures, composed of DNA, histones, and proteins released by activated neutrophils, play a role in host defense but have also been implicated in the pathophysiology of atherosclerosis and ischemic heart disease.[11] Recent research has highlighted the potential of plasma NETs levels as biomarkers for various cardiovascular conditions, including MI.[12,13]

In parallel, the index of cardiac electrophysiological balance (iCEB) has gained attention as a non-invasive electrocardiographic marker with potential diagnostic value in MI.[14–16] The iCEB reflects the heterogeneity of myocardial repolarization and has been associated with an increased risk of ventricular arrhythmias and sudden cardiac death.[17] Its application in the context of SMI in patients undergoing MHD represents a promising avenue for early detection and risk stratification.

NETs have been implicated contributing to the understanding of the inflammatory processes and endothelial dysfunction that underlie MI. On the other hand, iCEB reflects the heterogeneity of myocardial repolarization, providing valuable insights into the electrical alterations associated with SMI. Despite the individual promise of plasma NETs levels and iCEB, their combined diagnostic value in SMI among hemodialysis patients remains inadequately explored.[18] Therefore, the integration of plasma NETs levels and iCEB provides a more comprehensive evaluation of the complex pathophysiological processes underlying SMI, offering a more nuanced understanding of the interplay between inflammatory, electrophysiological, and endothelial mechanisms in this condition.

In this study, we aimed to present the investigation into the combined diagnostic value of plasma NETs levels and iCEB in SMI in patients undergoing MHD. By elucidating their interplay and integration, we endeavor to contribute to the advancement of early detection and risk stratification strategies for MI in this vulnerable patient population.

Materials and Methods

Study Design and Participants

This was a cross-sectional, observational study. Patients who were undergoing regular hemodialysis at the Affiliated Taizhou People's Hospital of Nanjing Medical University were selected as study participants. The study complied with the principles of the Declaration of Helsinki and was approved by the ethics committee of the Affiliated Taizhou People's Hospital of Nanjing Medical University (batch number: KY 202319201). Informed consent was obtained from all patients. Baseline characteristics, including age, gender, presence of diabetes, hypertension, and duration of hemodialysis were collected for all participants. The patient selection flowchart is shown in Figure 1.

The inclusion criteria were as follows:(a) age over 18 and under 70; (b) stable hemodialysis for >3 months; (c) regular hemodialysis 3 times/week for 4h each time; (d) Patients who have had CAG, CTA or electrocardiography. (e) The patient had no significant chest pain or subjective symptoms related to myocardial ischemia. (f) Cardiac enzyme and troponin analysis results were normal. The exclusion criteria were as follows: (a) severe electrolyte disturbance; (b) Patients with heart failure, severe infections; Patients use ARNI; (c) Patients with a previous history of malignant tumors or autoimmune diseases; (d) lack of complete data.

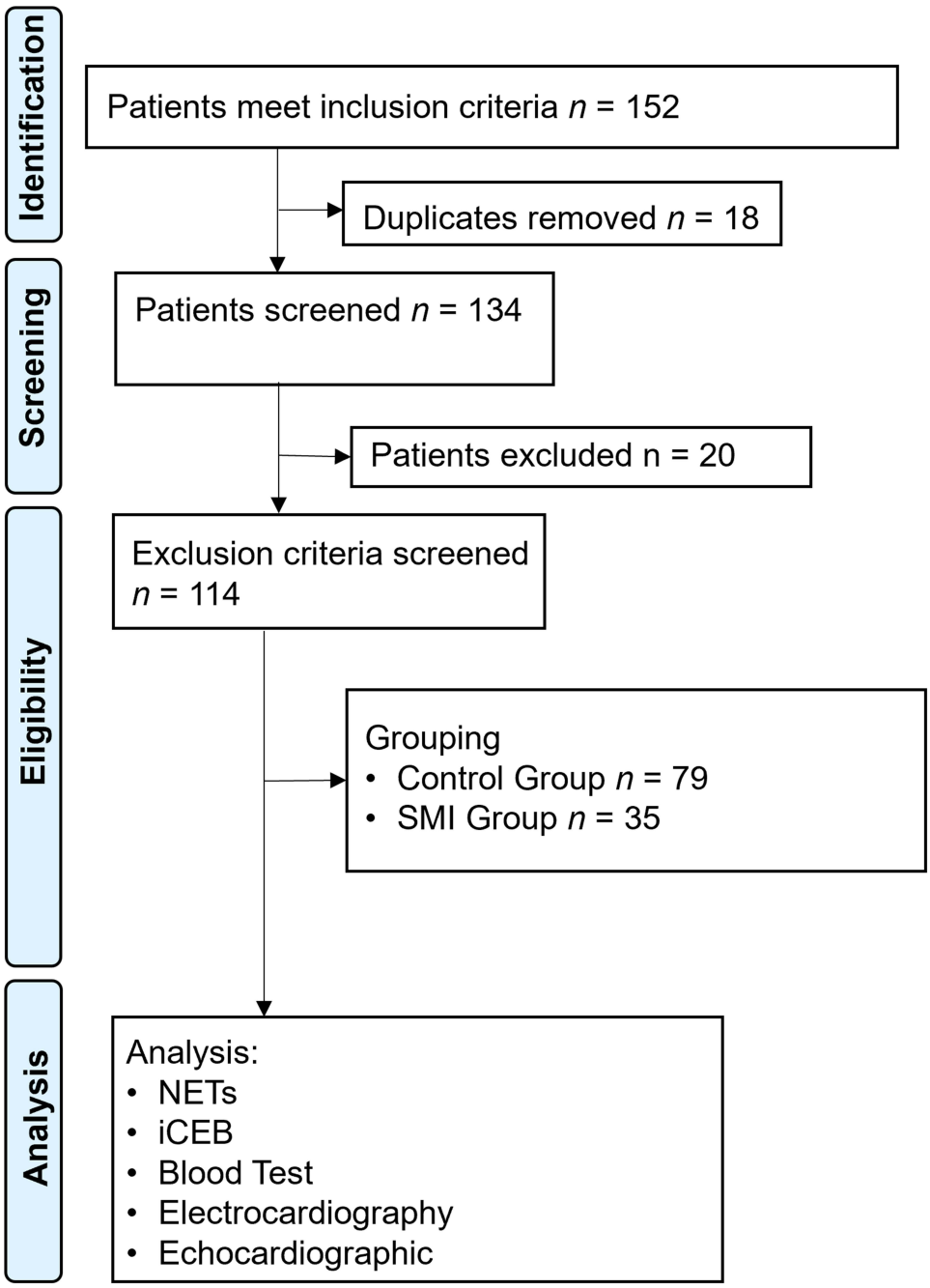

Figure 1 Patient selection flowchart.
Abbreviations: NETs: Neutrophil extracellular traps; iCEB: index of cardiac electrophysiological balance; SMI: silent myocardial ischemia.

Silent Myocardial Ischemia Diagnosis

Silent myocardial ischemia, also referred to as asymptomatic myocardial ischemia, is defined as the presence of objective evidence of myocardial ischemia without the accompanying typical symptoms.[19] Objective evidence includes ≥70% stenosis in the left anterior descending, left circumflex, or right coronary artery, or ≥50% stenosis in the left main coronary artery as indicated by computed tomography angiography (CTA);[20] ≥50% stenosis of any major coronary artery (left anterior descending, left circumflex, or right coronary artery) as shown by coronary angiography (CAG);[21] or ST-segment depression of >0.1 mv in a horizontal or declining pattern with a time interval between two ischemic episodes >1 minute. Moreover, patient exhibited no chest pain, upper extremity, jaw, or epigastric discomfort, or atypical

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symptoms such as dyspnea or diaphoresis. All participants underwent both CTA and CAG to ensure comprehensive diagnostic coverage and maximize sensitivity and specificity in detecting AMI in hemodialysis patients.

All participants were categorized into control group (without SMI) or SMI group according to whether patients had SMI.

The Index of Cardiac Electrophysiological Balance (iCEB)

iCEB utilized in this study was calculated using the formula: iCEB = QTc/QRS. Where QTc represents the corrected QT interval, and QRS denotes the duration of the QRS complex. The iCEB reflects the heterogeneity of myocardial repolarization and has been associated with an increased risk of ventricular arrhythmias and sudden cardiac death, thereby serving as a non-invasive electrocardiographic marker for identifying subtle alterations in cardiac electrophysiology associated with SMI in MHD patients.

Neutrophil Isolation and NETs Detection

The levels of Plasma NETs were determined by using markers indicative of NETs components, such as double-stranded DNA (dsDNA), circulating free DNA (cfDNA), myeloperoxidase.[22] The approach involved utilizing PMA-induced isolated neutrophil granulocytes as positive controls in measurements with a flow cytometer (Attune™ NxT, Invitrogen, USA), and the neutrophil preparation was conducted using the density gradient method with Dextran (5%, D1860, Invitrogen, USA) and Percoll (63% and 72%, D1860, Invitrogen, USA) solutions starting from an EDTA blood collection tube. The isolated neutrophils were induced by the addition of PMA (100×, 23210, Pierce, USA) and 2% BSA (bovine serum albumin, 23210, Thermo Scientific, USA), followed by a four-hour incubation at 37 degrees Celsius. After centrifugation, the supernatant was treated with antibodies and DNA stain as per the described procedure. In this study, the use of PMA-induced neutrophils as a positive control may introduce a certain bias, as PMA induction can lead to non-physiological conditions for NET formation. Additionally, while quantifying dsDNA, cfDNA, and MPO-DNA complexes, despite following strict standard operating procedures, there may still be technical limitations such as inter-sample variability and antibody specificity.

The concentration of dsDNA and cfDNA was measured using the Quant-iT™ PicoGreen® dsDNA Assay Kit by Thermo Fisher Scientific. In summary, the samples were dissolved in Tris-EDTA buffer with the fluorescent dye PicoGreen, which binds DNA in equimolar amounts. Following incubation, the emitted fluorescence was measured at 520 nm (excitation at 480 nm) to calculate the DNA concentration from a standard curve generated using commercial solution K562 DNA from Promega, with an initial concentration of 10 ng/μL.

Myeloperoxidase-DNA (MPO–DNA) complexes were identified using an ELISA assay. Specifically, 5 μg/mL of anti-MPO monoclonal antibody from Abcam was coated onto a 96-well plate overnight at 4°C and after blocking with 1% bovine serum albumin, serum samples were added to distinct wells (100 μL per well) for a 2-hour incubation at 37°C. A horseradish peroxidase–labeled anti-human DNA monoclonal antibody was subsequently added to each well, followed by a 2-hour incubation at room temperature. The plate was then washed and the peroxidase substrate was added, followed by a 2N sulfuric acid stop solution. The optical density (OD) of each well was measured at a wavelength of 405 nm (OD405), with 490 nm used as a reference. The results were expressed as mean OD405/490 values.

Blood Test

A 3 mL sample of fasting antecubital venous blood was drawn from the patients. After serum separation, hemoglobin, troponin I, brain natriuretic peptide (BNP), and N-terminal pro-brain natriuretic peptide (NT-proBNP) were measured using an automatic biochemistry analyzer (AU5800). Additionally, enzyme-linked immunosorbent assay (ELISA) was used to detect the levels of C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α).

Electrocardiography

Measurements were taken using a standard 12-lead MECG-200 electrocardiograph with a paper speed of 25 mm/s, standardized so that (10 mm = 1 mV), and recordings were made with filter settings. Patients rested for 5 to 10 minutes

before and after the examination. Collected parameters included QT interval, QTc interval, QRS duration, PR interval, and the rate of T wave abnormalities.

Echocardiographic

A Hitachi Aloka ProSound F75 color Doppler ultrasound diagnostic system with a UST-52105 probe, operating at frequencies between 1.0 to 5.0 MHz, equipped with a DAS-RSl workstation, was utilized. The Cardiac mode was activated to acquire dynamic images of the parasternal long-axis view of the left ventricle to measure the left atrial diameter and calculate the left ventricular mass index. Dynamic images of the apical four-chamber and two-chamber views were collected, and the modified biplane Simpson method was used to measure the left ventricular ejection fraction. The PW/TDI mode was initiated to record dual-spectrum static images in the standard apical four-chamber view, documenting the E/e′ and E/A ratios.

Statistical Analysis

All statistical analysis were performed with R program. Differences between subgroups of patients were analyzed by Student's *t*-test (unpaired, two-tailed). $P < 0.05$ were considered to be statistically significant. Spearman correlation analysis was conducted to explore the preliminary associations between the occurrence of silent myocardial ischemia and various parameters, applying Bonferroni correction to adjust for multiple comparisons. Logistic regression analysis was used to assess the relationship between the occurrence of SMI and various parameters, adjusting for potential confounders. Receiver Operating Characteristic (ROC) analysis was employed to evaluate the diagnostic accuracy of plasma NETs levels and iCEB in predicting silent myocardial ischemia. The variables included in the ROC analysis were plasma NETs levels and iCEB. A multivariate logistic regression model was constructed using the nnet package to incorporate all relevant parameters and predict silent myocardial ischemia. To ensure the generalizability of the model, we used ten-fold cross-validation to test the model's predictive performance on unknown data.

Results

Baseline Data

The baseline characteristics of the study participants are presented in Table 1. The control group (n=79) and the SMI group (n=35) were comparable with no statistically significant differences observed in age (59.23±7.45 vs 60.17±6.92, t=0.653, P=0.516), gender distribution (M/F: 41/38 vs 20/15, $\chi2$=0.099, P=0.753), diabetes prevalence (13/66 vs 8/27, $\chi2$=0.304, P=0.581), hypertension prevalence (28/51 vs 14/21, $\chi2$=0.065, P=0.799), duration of hemodialysis (65.15 ±9.63 vs 68.29±8.41 months, t=2.504, P=0.098), and hemoglobin levels (10.89±1.57 vs 10.45±1.22 g/dL, t=1.606, P=0.112).

Plasma NETs Levels

Plasma NETs levels can be detected by the markers in Table 2 because NETs are composed of DNA, histones, and various granule proteins such as myeloperoxidase and citrullinated histone H3, as shown in the markers in the table.[23–25]

Table 1 Baseline Characteristics of Study Participants

Characteristic	Control Group (n=79)	SMI Group (n=35)	t/χ^2	P
Age (years)	59.23±7.45	60.17±6.92	0.653	0.516
Gender (M/F)	41 (51.90%) / 38 (48.10%)	20 (57.14%) / 15 (42.86%)	0.099	0.753
Diabetes (n, %)	13 (16.46%)	8 (22.86%)	0.304	0.581
Hypertension (n, %)	28 (35.44%)	14 (40.00%)	0.065	0.799
Duration of Hemodialysis (months)	65.15±9.63	68.29±8.41	2.504	0.098
Hemoglobin (g/dL)	10.89±1.57	10.45±1.22	1.606	0.112

Abbreviation: SMI, silent myocardial ischemia.

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Table 2 Plasma NETs Levels

Parameter	Control Group (n=79)	SMI Group (n=35)	t	P
dsDNA (ng/mL)	31.64±5.32	37.89±4.55	6.416	<0.001
cfDNA (ng/mL)	8.91±1.84	11.27±2.03	5.882	<0.001
MPO-DNA Complexes (ng/mL)	17.52±3.41	23.69±4.01	7.924	<0.001

Abbreviations: NETs, Neutrophil extracellular traps; SMI, silent myocardial ischemia; dsDNA, double-stranded DNA; cfDNA, circulating free DNA; MPO-DNA, myeloperoxidase-DNA.

When neutrophils are activated, they release NETs, which are involved in the immune response and have been implicated in various pathological conditions, including cardiovascular diseases.

The plasma NETs levels in the study participants are presented in Table 2. dsDNA was markedly higher in the SMI group (37.89 ng/mL±4.55) than in the control group (31.64 ng/mL±5.32), with a notable difference indicated by a t-value of 6.416 and a highly significant P <0.001. Furthermore, the levels of cfDNA were significantly elevated in the SMI group (11.27 ng/mL±2.03) compared to the control group (8.91 ng/mL±1.84), with a substantial difference demonstrated by a t-value of 5.882 and a highly significant P <0.001. Likewise, the MPO-DNA complexes were notably higher in the SMI group (23.69 ng/mL±4.01) in comparison to the control group (17.52 ng/mL±3.41), indicating a significant difference with a t-value of 7.924 and a highly significant P <0.001.

Electrocardiography Findings

iCEB and electrocardiography findings for the study participants are presented in Figure 2 and Table 3, respectively. The SMI group displayed a significantly higher iCEB (56.45 ms±7.67) compared to the control group (45.89 ms±6.23), indicating a substantial difference with a t-value of 7.169 and a highly significant P <0.001. Additionally, electrocardiography findings revealed that the SMI group exhibited markedly prolonged PR interval (168.75 ms±15.32), QRS duration (102.36 ms±11.45), QT interval (380.55 ms±30.89), and QTc interval (440.22 ms±25.46) compared to the control group,

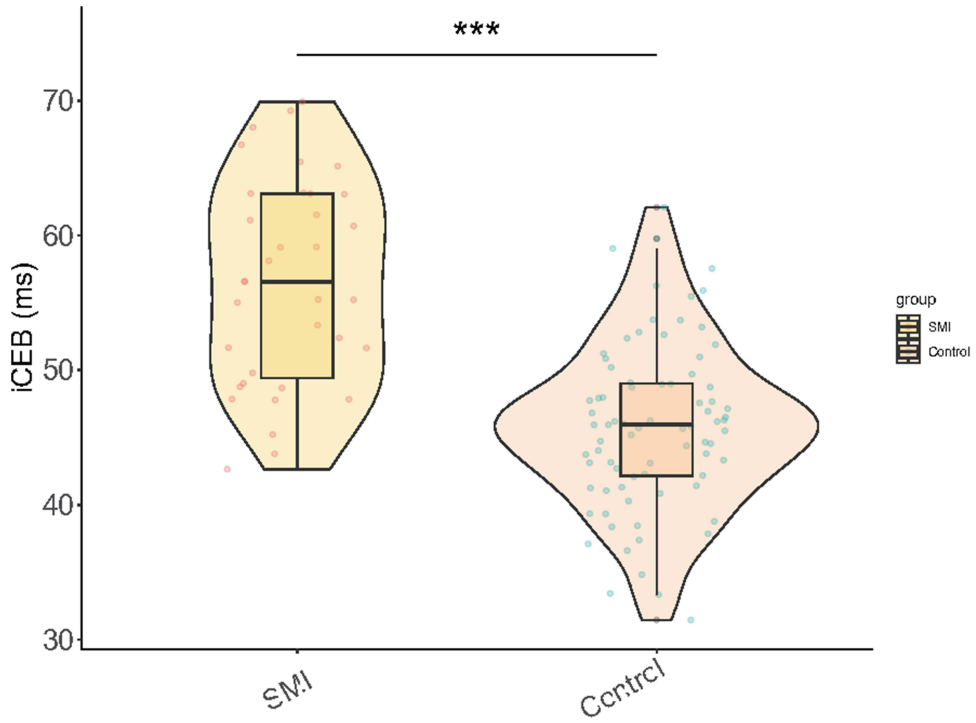

Figure 2 Index of Cardiac Electrophysiological Balance ***:P<0.001.
Abbreviations: iCEB: index of cardiac electrophysiological balance; SMI: silent myocardial ischemia.

Table 3 Electrocardiography Findings

Parameter	Control Group (n=79)	SMI Group (n=35)	t/χ2	P
PR Interval (ms)	155.23±12.45	168.75±15.32	4.595	<0.001
QRS Duration (ms)	92.14±9.63	102.36±11.45	4.605	<0.001
QT Interval (ms)	360.21±25.67	380.55±30.89	3.408	0.001
QTc Interval (ms)	420.36±20.78	440.22±25.46	4.054	<0.001
T Wave Abnormality (%)	15 (18.99%) / 64 (81.01%)	16 (45.71%) / 19 (54.29%)	7.453	0.006

Abbreviation: SMI, silent myocardial ischemia.

as evidenced by the statistically significant differences with P <0.001 for PR interval, QRS duration, and QTc interval, and a P-value of 0.001 for QT interval. Moreover, T wave abnormalities were more prevalent in the SMI group (45.71%) than in the control group (18.99%), with a notable difference indicated by a χ2 value of 7.453 and a P-value of 0.006.

Echocardiographic Parameters

The echocardiographic parameters for the study participants are presented in Table 4. The SMI group demonstrated significantly lower left ventricular ejection fraction (55.78%±6.55) compared to the control group (62.48%±5.36), signifying a substantial difference with a t-value of 5.319 and a highly significant P <0.001. Furthermore, the left ventricular mass index was notably higher in the SMI group (105.28 g/m^2±10.57) in comparison to the control group (95.36 g/m^2±8.45), demonstrating a significant difference with a t-value of 4.899 and a highly significant P <0.001. Similarly, the E/A ratio and E/e′ ratio were significantly lower in the SMI group (1.12±0.15 and 10.21±2.05, respectively) compared to the control group (1.25±0.17 and 8.56±1.42, respectively), with P <0.001 for both parameters, indicating notable differences. Additionally, the left atrial diameter was significantly larger in the SMI group (3.52 cm±0.53) than in the control group (3.21 cm±0.45), with a t-value of 2.971 and a P-value of 0.004, signifying a notable difference.

Inflammatory Markers

As shown in Figure 3, the levels of inflammatory markers displayed notable differences between the control and SMI groups. Specifically, C-reactive Protein levels were higher in the SMI group compared to the control group (4.32±1.64 vs 3.55±1.57 mg/L, t=2.348, P=0.022) (Figure 3A), as were the levels of Interleukin-6 (18.45±4.26 vs 15.78±4.36pg/mL, t=3.066, P=0.003) (Figure 3B) and Tumor Necrosis Factor-α (15.67±3.84 vs 13.23±3.55pg/mL, t=3.211, P=0.002) (Figure 3C).

Cardiac Biomarkers

The analysis of cardiac biomarkers revealed significant differences between the control and SMI groups. Troponin I levels were notably higher in the SMI group compared to the control group (0.28±0.12 vs 0.21±0.08 ng/mL, t=3.214, P=0.002) (Figure 4A), as were the levels of NT-proBNP (146.32±35.71 vs 120.56±17.89pg/mL, t=4.049, P<0.001) (Figure 4B) and BNP (55.32±12.45 vs 45.25±6.87pg/mL, t=4.49, P<0.001) (Figure 4C). These findings emphasize the presence of increased myocardial stress and dysfunction, reflected by the elevated levels of cardiac biomarkers in the SMI group, highlighting their potential utility in identifying SMI in this patient population.

Table 4 Echocardiographic Parameters

Parameter	Control Group (n=79)	SMI Group (n=35)	t	P
Left Ventricular Ejection Fraction (%)	62.48±5.36	55.78±6.55	5.319	<0.001
Left Ventricular Mass Index (g/m^2)	95.36±8.45	105.28±10.57	4.899	<0.001
E/A Ratio	1.25±0.17	1.12±0.15	4.026	<0.001
E/e′ Ratio	8.56±1.42	10.21±2.05	4.323	<0.001
Left Atrial Diameter (cm)	3.21±0.45	3.52±0.53	2.971	0.004

Abbreviation: SMI, silent myocardial ischemia.

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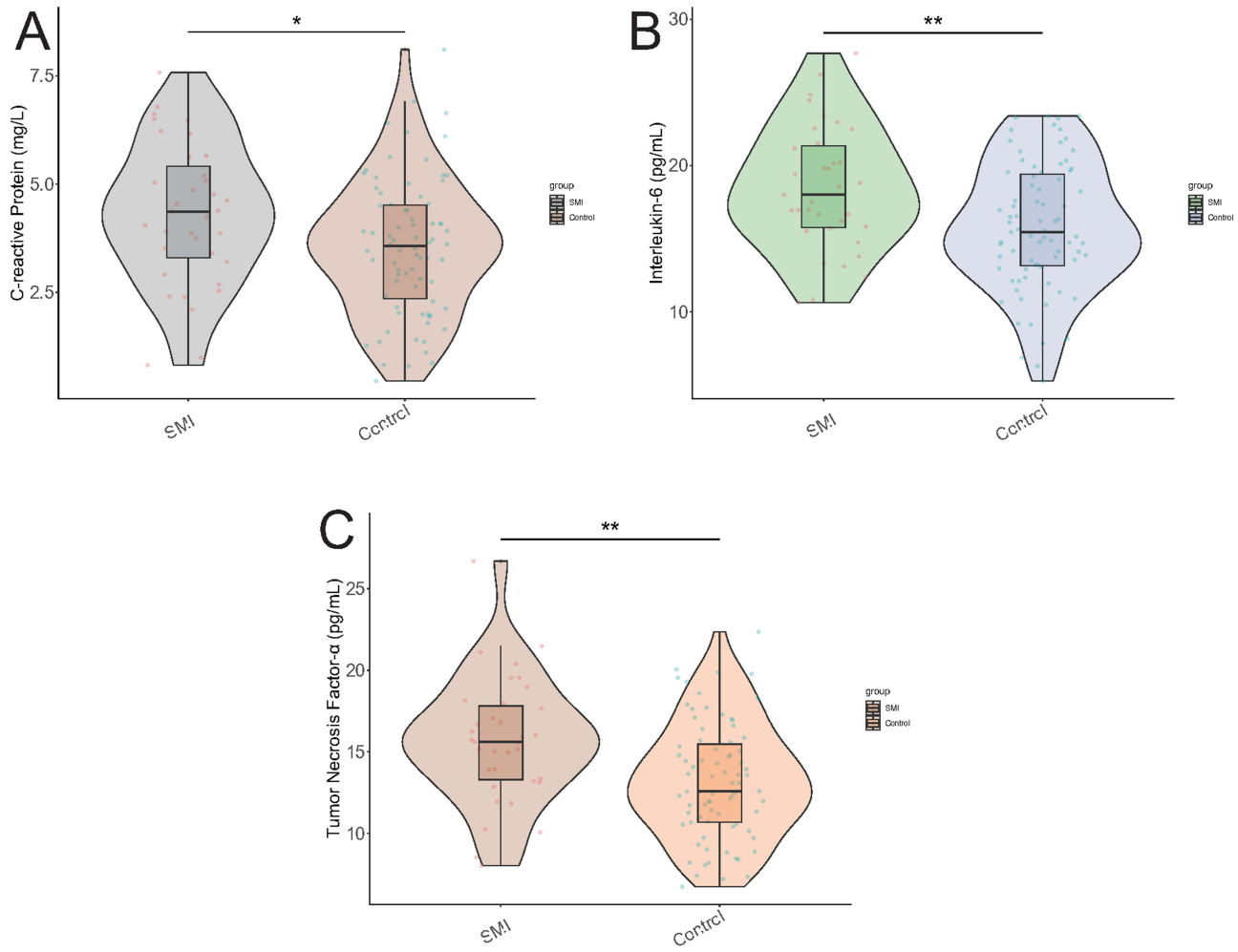

Figure 3 Inflammatory Markers *: P<0.05; **: P<0.01. (**A**) C-reactive protein (**B**) Interleukin-6 (**C**) Tumor Necrosis Factor-α.
Abbreviation: SMI, silent myocardial ischemia.

Spearman Correlation Analysis of the Associations Between the Occurrence of SMI and Various Parameters

Spearman correlation analysis was conducted to assess the associations between the occurrence of SMI and various parameters. As presented in Table 5, revealed several significant associations between various parameters in the study cohort. Positive correlations were observed for dsDNA (r=0.496, P<0.001), cfDNA (r=0.589, P<0.001), MPO-DNA Complexes (r=0.623, P<0.001), iCEB (r=0.592, P<0.001), PR Interval (r=0.426, P<0.001), QRS Duration (r=0.422, P<0.001), QT Interval (r=0.327, P<0.001), QTc Interval (r=0.383, P<0.001), T Wave Abnormality (r=0.277, P=0.003), Left Ventricular Mass Index (r=0.450, P<0.001), E/e′ Ratio (r=0.424, P<0.001), Left Atrial Diameter (r=0.288, P=0.002), C-reactive Protein (r=0.220, P=0.019), Interleukin-6 (r=0.276, P=0.003), Tumor Necrosis Factor-α(r=0.299, P=0.001), Troponin I (r=0.338, P<0.001), NT-proBNP (r=0.437, P= P<0.001), and BNP (r=0.464, P= P<0.001). Negative correlations were observed for Left Ventricular Ejection Fraction (r=−0.477, P<0.001) and E/A Ratio (r=−0.339, P<0.001). Moreover, a total of 20 correlation tests were performed. According to the Bonferroni correction, the original significance level of 0.05 was divided by the number of tests, 20, resulting in a corrected significance level of 0.0025. After Bonferroni correction, we found that the correlations for dsDNA, cfDNA, MPO-DNA Complexes, iCEB, PR Interval, QRS Duration, QT Interval, QTc Interval, Left Ventricular Mass Index, E/e′ Ratio, Troponin I, NT-proBNP, BNP, Left Ventricular Ejection Fraction, and E/A Ratio remained significant (P values were all less than 0.0025). However, the

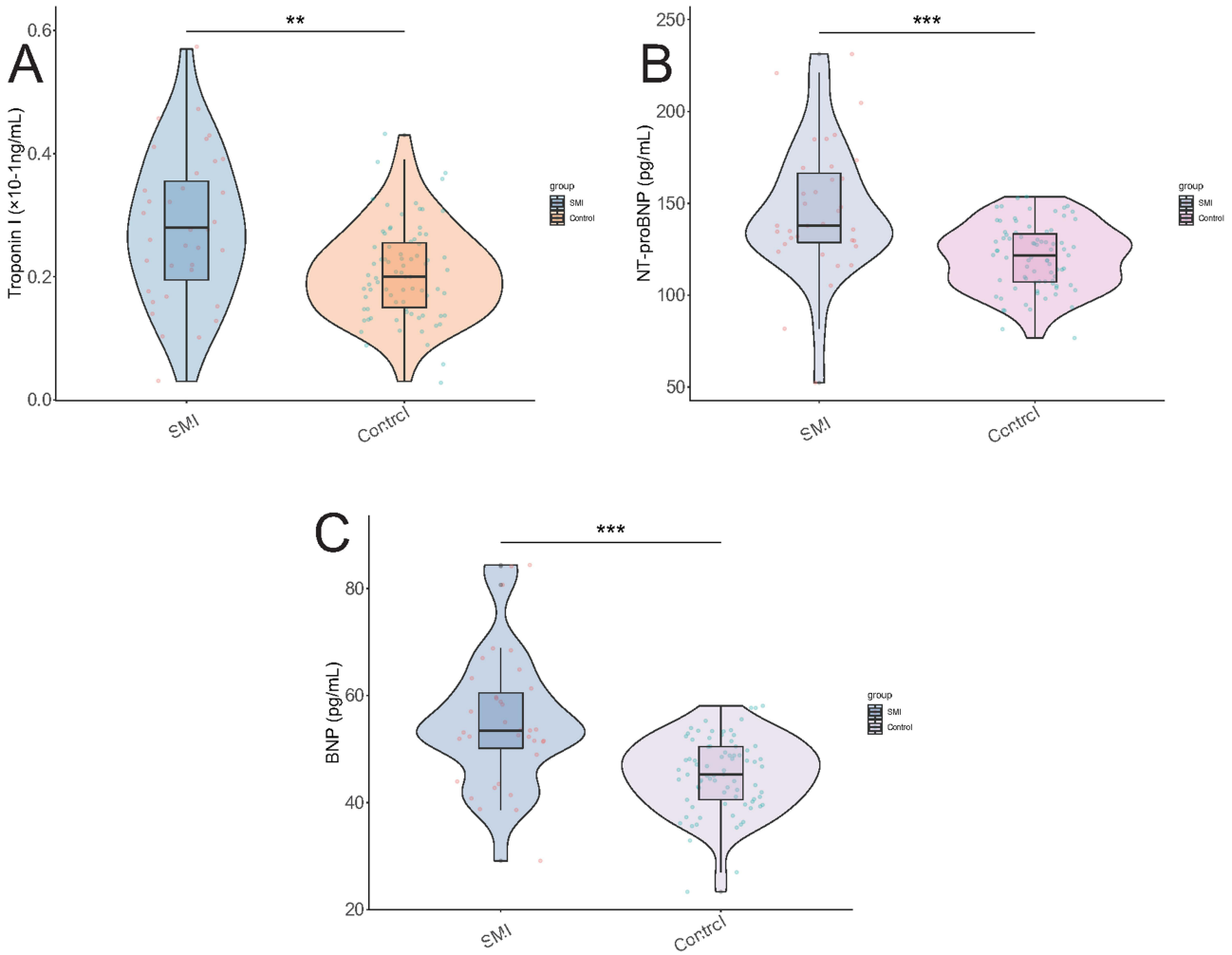

Figure 4 Cardiac Biomarkers **: P<0.01; ***: P<0.001. (**A**) Troponin I (**B**) NT-proBNP (**C**) BNP.
Abbreviations: SMI, silent myocardial ischemia; NT-proBNP, N-Terminal Pro-Brain Natriuretic Peptide; BNP, Brain Natriuretic Peptide.

correlations for C-reactive Protein, Interleukin-6, T Wave Abnormality, and Left Atrial Diameter became non-significant after correction. But we still considered them to show differences.

Logistic Regression Analysis of NETs and Electrocardiography Parameters

In a logistic regression analysis investigating the combined diagnostic value of plasma NETs levels and the iCEB in silent myocardial ischemia in maintenance hemodialysis patients, several factors were evaluated for their association with the occurrence of silent myocardial ischemia (Table 6). Univariate regression analysis revealed significant associations (p <0.001) between dsDNA levels (OR 1.285, 95% CI 1.165–1.443), cfDNA levels (OR 1.85, 95% CI 1.457–2.448), MPO-DNA Complexes levels (OR 1.579, 95% CI 1.355–1.908), iCEB (OR 1.238, 95% CI 1.149–1.354), PR Interval (OR 1.081, 95% CI 1.044–1.125), QRS Duration (OR 1.105, 95% CI 1.057–1.164), QT Interval (OR 1.027, 95% CI 1.012–1.044), QTc Interval (OR 1.04, 95% CI 1.02–1.064), and T Wave Abnormality (OR 3.593, 95% CI 1.512–8.71). However, in the multivariate regression analysis, after adjusting for confounding variables, only dsDNA levels (OR 1.017, 95% CI 1.009–1.025, p <0.001), MPO-DNA Complexes (OR 1.034, 95% CI 1.021–1.046, p <0.001), iCEB (OR 1.009, 95% CI 1.001–1.017, p = 0.024), QRS Duration (OR 1.007, 95% CI 1.003–1.011, p <0.001), QTc Interval (OR 1.004, 95% CI 1.002–1.006, p <0.001), and T Wave Abnormality (OR 1.140, 95% CI 1.025–1.267, p = 0.015) remained significantly associated with silent myocardial ischemia. Notably, cfDNA levels (OR 1.025, 95% CI 1.000–1.052, p = 0.054), PR Interval (OR 1.004, 95% CI 1.000–1.008, p = 0.046), and QT Interval (OR 1.001, 95% CI

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Table 5 The Spearman Associations Between the Occurrence of SMI and Various Parameters

Parameter	r	P
dsDNA (ng/mL)	0.508	<0.001
cfDNA (ng/mL)	0.509	<0.001
MPO-DNA Complexes (ng/mL)	0.603	<0.001
iCEB (ms)	0.567	<0.001
PR Interval (ms)	0.404	<0.001
QRS Duration (ms)	0.430	<0.001
QT Interval (ms)	0.314	<0.001
QTc Interval (ms)	0.382	<0.001
T Wave Abnormality (%)	0.277	0.003
Left Ventricular Ejection Fraction (%)	−0.477	<0.001
Left Ventricular Mass Index (g/m^2)	0.450	<0.001
E/A Ratio	−0.339	<0.001
E/e' Ratio	0.424	<0.001
Left Atrial Diameter (cm)	0.288	0.002
C-reactive Protein (mg/L)	0.220	0.019
Interleukin-6 (pg/mL)	0.276	0.003
Tumor Necrosis Factor-α (pg/mL)	0.299	0.001
Troponin I (×10-1ng/mL)	0.338	<0.001
NT-proBNP (pg/mL)	0.437	<0.001
BNP (pg/mL)	0.464	<0.001

Abbreviations: SMI, silent myocardial ischemia; dsDNA, double-stranded DNA; cfDNA, circulating free DNA; MPO-DNA, myeloperoxidase-DNA; Iceb, index of cardiac electrophysiological balance; NT-proBNP, N-Terminal Pro-Brain Natriuretic Peptide; BNP, Brain Natriuretic Peptide.

Table 6 Logistic Regression Analysis of NETs and Electrocardiography Parameters and the Occurrence of Asymptomatic Myocardial Ischemia

Factor	Univariate Regression			Multivariate Regression		
	OR	95% CI	P	OR	95% CI	P
dsDNA (ng/mL)	1.285	1.165–1.443	<0.001	1.017	1.009–1.025	<0.001
cfDNA (ng/mL)	1.850	1.457–2.448	<0.001	1.025	1.000–1.052	0.054
MPO-DNA Complexes (ng/mL)	1.579	1.355–1.908	<0.001	1.034	1.021–1.046	<0.001
iCEB (ms)	1.238	1.149–1.354	<0.001	1.009	1.001–1.017	0.024
PR Interval (ms)	1.081	1.044–1.125	<0.001	1.004	1.000–1.008	0.046
QRS Duration (ms)	1.105	1.057–1.164	<0.001	1.007	1.003–1.011	<0.001
QT Interval (ms)	1.027	1.012–1.044	<0.001	1.001	0.999–1.003	0.317
QTc Interval (ms)	1.040	1.020–1.064	<0.001	1.004	1.002–1.006	<0.001
T Wave Abnormality (%)	3.593	1.512–8.71	0.004	1.140	1.025–1.267	0.015

Abbreviations: NETs, Neutrophil extracellular traps; dsDNA, double-stranded DNA; cfDNA, circulating free DNA; MPO-DNA, myeloperoxidase-DNA; iCEB, index of cardiac electrophysiological balance.

0.999–1.003, p = 0.317) did not exhibit statistically significant associations in the multivariate analysis. These findings underscore the potential diagnostic value of dsDNA levels, MPO-DNA Complexes, iCEB, QRS Duration, QTc Interval, and T Wave Abnormality in assessing silent myocardial ischemia in this maintenance hemodialysis patient population. However, it should be clarified that multivariate logistic regression analysis may not perfect fit these factors, for the reason that dsDNA, cfDNA, and MPO-DNA Complexes are all biomarkers of NETs and its multicollinearity diminished the performance of the logistic regression model.

Table 7 ROC Analysis of NETs and Electrocardiography Parameters

Parameter	Sensitivities	Specificities	AUC
dsDNA (ng/mL)	0.886	0.722	0.862
cfDNA (ng/mL)	0.829	0.722	0.818
MPO-DNA Complexes (ng/mL)	0.800	0.810	0.877
iCEB (ms)	0.914	0.671	0.855
PR Interval (ms)	0.686	0.810	0.753
QRS Duration (ms)	0.714	0.785	0.769
QT Interval (ms)	0.514	0.848	0.696
QTc Interval (ms)	0.686	0.797	0.739
T Wave Abnormality (%)	0.457	0.810	0.634

Abbreviations: ROC, Receiver Operating Characteristic; NETs, Neutrophil extracellular traps; dsDNA, double-stranded DNA; cfDNA, circulating free DNA; MPO-DNA, myeloperoxidase-DNA; iCEB, index of cardiac electrophysiological balance.

ROC Analysis of NETs and Electrocardiography Parameters

The ROC analysis, displayed in Table 7, demonstrated the diagnostic accuracies of various parameters in identifying SMI in patients undergoing MHD. The analysis revealed significant areas under the curve (AUC) for dsDNA, cfDNA, MPO-DNA Complexes, iCEB, PR interval, QRS duration, and QTc interval, with AUC values ranging from 0.634 to 0.877, indicating their potential as discriminatory markers for SMI.

Moreover, while the AUC of NETs levels alone was notably high, the integration of plasma NETs levels with iCEB provides additional insights into the pathophysiological mechanisms underlying SMI. NETs have been implicated contributing to the understanding of the inflammatory processes and endothelial dysfunction that underlie MI. On the other hand, iCEB reflects the heterogeneity of myocardial repolarization, providing valuable insights into the electrical alterations associated with SMI. When considered together, elevated plasma NETs levels and higher iCEB may represent a convergence of inflammatory and electrophysiological perturbations in hemodialysis patients with SMI. Therefore, the integration of plasma NETs levels and iCEB provides a more comprehensive evaluation of the complex pathophysiological processes underlying SMI, offering a more nuanced understanding of the interplay between inflammatory, electrophysiological, and endothelial mechanisms in this condition.

To avoid the impact of multicollinearity, we used neural network algorithm to establish the prediction model. After Ten folds cross validation, the AUC values for the prediction model on the basis of all NETs and iCEB showed the AUC being 0.908 with sensitivities and specificities of 0.987 and 0.829 (Figure 5). These findings highlight the significance of these parameters as potential diagnostic tools and emphasize their capacity to enhance early detection and risk stratification strategies for this high-risk patient population.

Discussion

The identification of SMI in patients undergoing MHD presents a considerable clinical challenge due to the high prevalence of cardiovascular complications and the complex interplay of various contributing factors such as accelerated atherosclerosis, endothelial dysfunction, and chronic inflammation.[26–28] Importantly, a substantial proportion of these patients may remain asymptomatic despite the presence of significant MI, highlighting the urgent need for more sensitive and specific diagnostic tools to detect and characterize this condition.[29,30] In this context, the investigation into plasma NETs levels and iCEB as potential biomarkers for SMI holds notable promise, as both parameters have been individually implicated in the pathophysiology of cardiovascular diseases and offer valuable insights into the underlying mechanisms of MI. Through the comprehensive analysis of plasma NETs levels, iCEB, electrocardiography findings, echocardiographic parameters, inflammatory markers, and cardiac biomarkers, this investigation provides valuable insights into the complex pathophysiological mechanisms underlying SMI and underscores the potential of these biomarkers in enhancing the diagnostic capabilities in this vulnerable patient population.

Neutrophil extracellular traps (NETs) are fibrous nets of DNA released by neutrophils during a specific form of cell death known as NETosis, playing an important role in host defense mechanisms.[31–33] However, when dysregulated, they

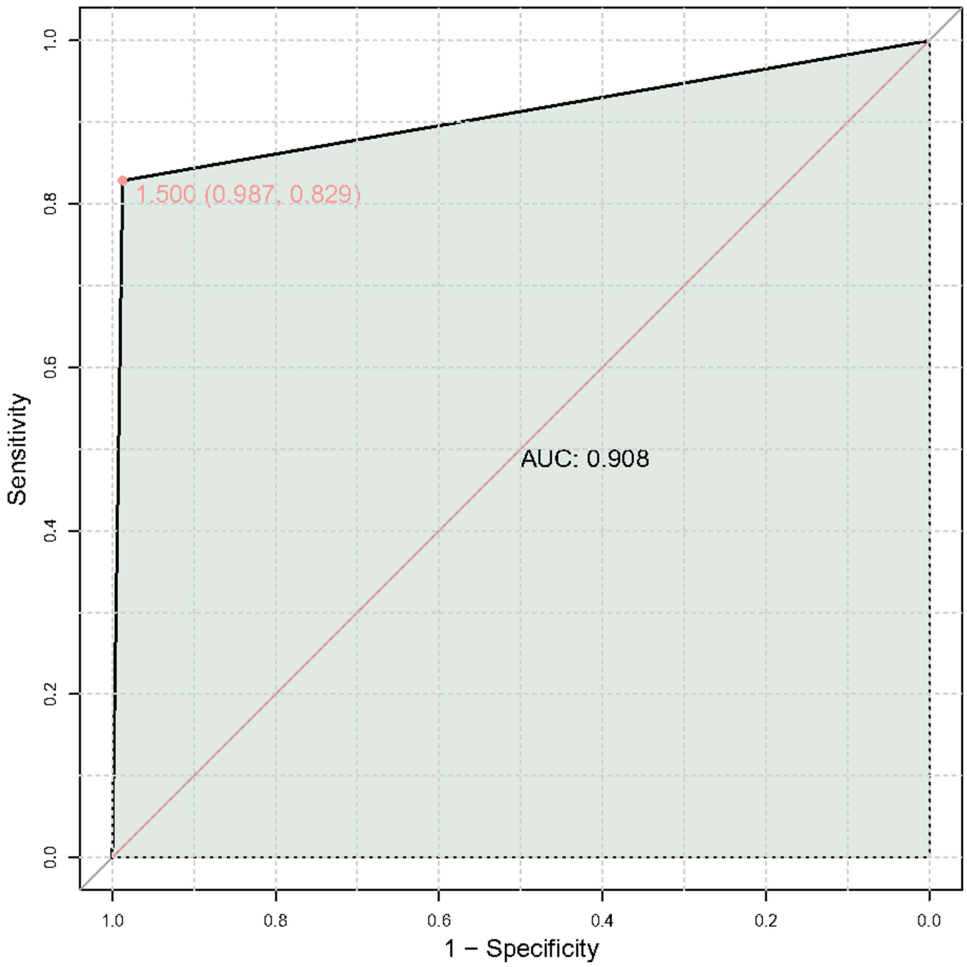

Figure 5 The ROC analysis of multiple logistic regression on the basis of all NETs and iCEB for SMI in patients undergoing MHD.
Abbreviations: ROC, Receiver Operating Characteristic; NETs, Neutrophil extracellular traps; iCEB, index of cardiac electrophysiological balance; SMI, silent myocardial ischemia; MHD, maintenance hemodialysis; AUC, Area Under the Curve.

can also contribute to pathological processes.[34,35] In this study, the plasma NETs levels (including dsDNA, cfDNA, and MPO-DNA complexes) in patients with SMI were significantly higher than those in the control group, suggesting that NETs may serve as a potential diagnostic biomarker for SMI. Chronic inflammation and oxidative stress are key drivers of NET formation.[36,37] In various conditions, including cardiovascular diseases, persistent inflammatory responses promote continuous NET release by neutrophils.[38,39] In maintenance hemodialysis (MHD) patients, elevated NETs levels may reflect ongoing inflammatory processes or endothelial dysfunction, which could be one of the driving forces behind cardiovascular disease.[40] In our study, we also found that levels of inflammatory markers (CRP, IL-6, and TNF-α) and cardiac-specific markers (Troponin I, NT-proBNP, and BNP) were significantly elevated in the SMI group. This further supports the presence of SMI and indicates the role of inflammation and myocardial stress in the progression of the disease. In the context of hemodialysis patients, who are predisposed to chronic inflammation and endothelial injury, elevated NETs levels may serve as an important biomarker for underlying SMI.

Moreover, electrocardiographic examinations showed that the SMI group had significantly prolonged parameters such as iCEB, PR interval, QRS duration, QT interval, and QTc interval, along with a higher incidence of T wave abnormalities. These changes may reflect the presence and severity of myocardial ischemia. Echocardiographic results indicated that the left ventricular ejection fraction in the SMI group was significantly lower than that in the control group, while the left ventricular mass index was higher, and the E/A ratio and E/e′ ratio were reduced, with an increase in left atrial diameter. These changes in echocardiographic parameters may reflect alterations in cardiac structure and function in patients with SMI. Elevated iCEB in

the context of SMI entail intricate pathophysiological mechanisms that reflect alterations in cardiac electrical properties and the predisposition to arrhythmogenic risk.[41] The iCEB, calculated as the ratio of QT interval duration to QRS complex duration on an electrocardiogram (ECG), serves as a surrogate marker for the heterogeneity of myocardial repolarization.[42,43] Myocardial ischemia disrupts the normal process of myocardial repolarization, which is crucial for the timely and synchronized recovery of myocardial cells after each heartbeat.[44] Ischemia alters the transmural dispersion of repolarization, leading to prolonged QT intervals and potentially to elevated iCEB, as a reflection of this dispersion.[45] Ischemia-induced heterogeneity in myocardial repolarization across different regions of the heart increases the vulnerability to developing re-entrant arrhythmias.[46] Such heterogeneity can be exacerbated by electrolyte imbalances, autonomic nervous system imbalances, or structural heart disease, all common in patients with kidney disease or undergoing hemodialysis.[46] Chronic kidney disease (CKD) and its associated dialysis treatments have complex interactions with cardiovascular complications.[47] CKD patients, due to changes in their internal environment such as electrolyte imbalances, metabolic acidosis, and the accumulation of uremic toxins, are prone to cardiovascular abnormalities.[48] Especially when these patients undergo hemodialysis, repeated hemodynamic changes, fluid volume fluctuations, and inflammation that may occur during dialysis increase cardiac load, potentially leading to myocardial ischemia.[49] Additionally, endothelial dysfunction in CKD patients is another critical factor; it not only promotes the progression of atherosclerosis but also increases the risk of vascular inflammation and thrombosis, all of which are potential mechanisms leading to SMI. Since hemodialysis patients are at risk for electrolyte imbalances and other metabolic disturbances that can affect cardiac electrophysiology, iCEB offers a non-invasive means of detecting subtle electrical changes that may signal underlying SMI.

The ROC analysis provides valuable insights into the diagnostic accuracies of various parameters in identifying SMI in patients undergoing MHD. The high sensitivity and specificity of NETs levels and iCEB, as well as their significant AUC values, emphasize their potential as discriminatory markers for SMI. Notably, the robust diagnostic accuracy of NETs levels, as evidenced by its high AUC, sensitivities, and specificities, underscores its promising utility as a diagnostic tool for identifying SMI in this high-risk patient population. Furthermore, the substantial AUC value obtained from the multiple logistic regression based on all NETs and iCEB underscores the combined diagnostic value of these biomarkers in enhancing the early detection and risk stratification strategies for SMI in MHD.

A diagnostic strategy combining NETs levels with iCEB not only aids in the early identification of patients at high risk but also provides crucial information for prognostic assessment. The study found that elevated NETs levels in conjunction with increased iCEB in maintenance hemodialysis (MHD) patients are associated with a higher incidence of cardiovascular events, suggesting that these markers may not only reflect the presence of SMI but also indicate a poorer prognosis. The clinical relevance of the combined biomarker approach in detecting silent myocardial ischemia extends beyond merely identifying the condition. Elevated levels of neutrophil extracellular traps (NETs) and increased iCEB may also indicate poorer prognosis for end-stage renal disease (ESRD) patients undergoing hemodialysis, regardless of whether silent myocardial ischemia is documented. Further investigation into the prognostic value of these markers could inform risk stratification strategies and guide preventive interventions aimed at improving long-term outcomes for this vulnerable patient population.

This study is not without limitations. The cross-sectional, observational study design may introduce inherent biases, and the relatively small sample size warrants further validation in larger, prospective studies. Additionally, the generalizability of the findings to broader hemodialysis populations and diverse clinical settings requires careful consideration. Future research endeavors should focus on validating the diagnostic and prognostic implications of plasma NETs levels and iCEB in SMI through larger, multicenter studies with long-term follow-up data. The exploration of potential therapeutic implications based on the integration of these biomarkers into clinical practice represents a promising avenue for future investigation.

Conclusion

In conclusion, the findings of this study highlight the combined diagnostic value of plasma NETs levels and iCEB in identifying and characterizing SMI in MHD. By elucidating the synergistic effects of these biomarkers and their interplay with clinical and biochemical parameters, this investigation contributes to the advancement of diagnostic capabilities in the context of SMI in patient undergoing MHD, laying the foundation for further research and clinical translation in this critical area of cardiovascular medicine.

Data Sharing Statement

The datasets used during the present study are available from the corresponding author upon reasonable request.

Ethics Approval and Consent to Participate

This was a cross-sectional, observational study. Patients who were undergoing regular hemodialysis at the Affiliated Taizhou People's Hospital of Nanjing Medical University were selected as study participants. The study complied with the principles of the Declaration of Helsinki and was approved by the ethics committee of the Affiliated Taizhou People's Hospital of Nanjing Medical University (batch number: KY-202319201). Informed consent was obtained from all patients.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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