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DIAGNOSTIC ACCURACY OF A PANEL OF THREE AUTOANTIBODIES (ANTI-APOA-1, ANTI-MDA-LDL, AND ANTI-OSTEOPONTIN IGG) FOR DETECTING SILENT MYOCARDIAL ISCHEMIA

DIJAGNOSTIČKA TAČNOST PANELA OD TRI AUTOANTITELA (ANTI-APOA-1, ANTI-MDA-LDL I ANTI-OSTEOPONTIN IGG) ZA OTKRIVANJE NEME MIOKARDNE ISHEMIJE

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Summary

Background: Silent myocardial ischemia (SMI) is a significant predictor of adverse cardiac events but remains underdiagnosed. We evaluated the diagnostic accuracy of three autoimmune-inflammation-linked biomarkers – anti-apolipoprotein A-1 (anti-ApoA-1) IgG, anti-malondialdehyde-modified low-density lipoprotein (anti-MDA-LDL) IgG, and anti-osteopontin (anti-OPN) IgG – for detecting SMI.
Methods: In this cross-sectional study, 210 participants were categorised into three groups: SMI (n=70), stable angina (SA, n=70), and healthy controls (n=70). SMI was defined by exercise-induced ST-segment depression on treadmill testing without symptoms. Serum levels of the three antibodies were measured by enzyme-linked immunosorbent assay. Receiver operating characteristic (ROC) analysis determined the area under the curve (AUC), sensitivity, and specificity.
Results: All three biomarkers were significantly higher in the SMI group compared to SA and controls (p<0.001). For SMI vs controls, anti-ApoA-1 IgG showed an AUC of 0.82 (95% CI 0.75–0.89), anti-MDA-LDL IgG an AUC of 0.78 (0.70–0.85), and anti-OPN IgG an AUC of 0.80 (0.73–0.87). A three-marker panel yielded an AUC of 0.91 (0.86–0.96) with a sensitivity of 87.1% and specificity of 84.3%. Anti-ApoA-1 IgG independently predicted SMI presence in multivariate analysis adjusted for age, sex, diabetes, hypertension, and hs-CRP (OR 3.2, 95% CI 1.8–5.6, p<0.001).
Conclusion: Anti-ApoA-1, anti-MDA-LDL, and anti-OPN IgG antibodies were elevated in patients with myocardial

Kratak sadržaj

Uvod: Nema miokardna ishemija (NMI) predstavlja značajan prediktor nepovoljnih srčanih događaja, ali često ostaje neprepoznata. U ovoj studiji procenjivana je dijagnostička tačnost tri biomarkera povezana sa autoimunskim i inflamatornim procesima – IgG antitela na apolipoprotein A-1 (anti-ApoA-1), IgG antitela na malondialdehidom modifikovani lipoprotein male gustine (anti-MDA-LDL) i IgG antitela na osteopontin (anti-OPN) – za otkrivanje neme miokardne ishemije.
Metode: U ovu studiju preseka je uključeno 210 ispitanika podeljenih u tri grupe: NMI (n=70), stabilna angina pektoris (SAP, n=70) i zdrave kontrolne pacijente (n=70). Nema miokardna ishemija je definisana depresijom ST-segmenta izazvanom opterećenjem na testu opterećenja na pokretnoj traci, bez prisustva simptoma. Serumski nivoi tri navedena antitela su određeni metodom imunoenzimskog testa (ELISA). Analiza ROC krive je korišćena za određivanje površine ispod krive (AUC), senzitivnosti i specifičnosti.
Rezultati: Sva tri biomarkera su bila značajno viša u grupi sa NMI u poređenju sa grupom sa SAP i zdravim kontrolnim pacijentima (p<0,001). U poređenju NMI i kontrola, anti-ApoA-1 IgG pokazao je AUC od 0,82 (95% CI 0,75–0,89), anti-MDA-LDL IgG AUC od 0,78 (0,70–0,85), a anti-OPN IgG AUC od 0,80 (0,73–0,87). Panel koji je obuhvatao sva tri biomarkera je dao AUC od 0,91 (0,86–0,96), uz senzitivnost od 87,1% i specifičnost od 84,3%. U multivarijantnoj analizi prilagođenoj za starost, pol, dijabetes, hipertenziju i hs-CRP, anti-ApoA-1 IgG je bio nezavisan prediktor prisustva NMI (OR 3,2; 95% CI 1,8–5,6; p<0,001).

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ischemia, including both silent myocardial ischemia and stable angina, with the highest levels observed in the SMI group. The combined three-marker panel showed strong discrimination between SMI and healthy controls but only moderate discrimination between SMI and stable angina. These findings suggest that the biomarkers may reflect coronary atherosclerotic inflammatory activity and ischemic burden rather than being specific to silent ischemia alone.

Keywords: silent myocardial ischemia, autoantibodies, apolipoprotein A-I, malondialdehyde-modified low-density lipoprotein, osteopontin, biomarkers, diagnostic accuracy, coronary artery disease, atherosclerosis, enzyme-linked immunosorbent assay

Zaključak: Nivoi anti-ApoA-1, anti-MDA-LDL i anti-OPN IgG antitela su bili povišeni kod pacijenata sa miokardnom ishemijom, uključujući i nemu miokardnu ishemiju i stabilnu anginu pektoris, pri čemu su najviše vrednosti zabeležene u grupi sa NMI. Kombinovani panel od tri biomarkera pokazao je dobru sposobnost razlikovanja između NMI i zdravih kontrola, ali samo umerenu sposobnost razlikovanja između NMI i stabilne angine pektoris. Ovi nalazi ukazuju na to da navedeni biomarkeri mogu odražavati inflamatornu aktivnost koronarne ateroskleroze i opterećenje ishemijom, a ne da budu specifični isključivo za nemu miokardnu ishemiju.

Ključne reči: nema miokardna ishemija, autoantitela, apolipoprotein A-I, malondialdehidom modifikovani lipoprotein male gustine, osteopontin, biomarkeri, dijagnostička tačnost, koronarna arterijska bolest, ateroskleroza, enzimski imunosorbentni test

Introduction

Silent myocardial ischemia (SMI) is defined as objective evidence of myocardial ischemia, typically detected via electrocardiography or advanced imaging, in the complete absence of angina or angina equivalents (1, 2). SMI is a challenging diagnostic problem, with an estimated incidence of 20–50% in patients with coronary artery disease (CAD) (3). The absence of pain, the body's natural alarm signal, often delays diagnosis until a catastrophic event like myocardial infarction or sudden cardiac death (4). The prognostic value of SMI is comparable with that of painful ischemia emphasising the need for effective screening strategies in high-risk populations such as diabetics or hypertensive (5, 6).

Functional testing is heavily relied on by current diagnostic approaches to SMI. Exercise tolerance testing (ETT) is the initial assessment, but it has moderate sensitivity and a high false-positive rate, particularly in women (7). Advanced imaging modalities such as myocardial perfusion scintigraphy or stress cardiac magnetic resonance provide superior accuracy, but they are expensive, time-consuming and not universally available (8). Therefore, a readily available panel of blood-based biomarkers could revolutionise SMI screening and allow early intervention. The etiopathogenesis of SMI remains incompletely understood, but a unique interplay of heightened pain thresholds, autonomic neuropathy and accelerated, low-grade inflammation is involved (9). Unlike stable angina, in which plaque rupture and acute thrombus formation play a major role, SMI is often associated with diffuse endothelial dysfunction and microvascular disease (10). Autoimmune responses to altered self-molecules have thus been proposed as possible mechanisms and biomarkers of vascular injury.

High-density lipoprotein (HDL) is a protective lipoprotein whose primary protein component is apolipoprotein A-1 (ApoA-1). ApoA-1 may be modified and become immunogenic under oxidative and inflammatory stress (11). Anti-ApoA-1 autoantibodies have been reported to neutralise HDL cholesterol efflux and anti-inflammatory functions, rendering HDL dysfunctional rather than cardioprotective, which favours atherogenesis (12). Malondialdehyde (MDA) results from lipid peroxidation and forms adducts with the ApoB-100 moiety of LDL, leading to the formation of MDA-LDL, one of the major antigens in the atherosclerotic plaque (13). Antibodies against MDA-LDL are markers of plaque progression induced by oxidative stress and have been related to unstable carotid disease (14, 15). Osteopontin (OPN) is a matricellular protein with pro-inflammatory and adhesive cytokine-like functions, heavily expressed in foam cells and calcified plaques. Autoantibodies to OPN reflect ongoing tissue remodelling and can modulate OPN's role in immune cell recruitment (16, 17).

We hypothesised that the triad of anti-ApoA-1 IgG, anti-MDA-LDL IgG, and anti-OPN IgG, reflecting HDL dysfunction, oxidative lipid damage, and vascular inflammation, respectively, would serve as a potent diagnostic signature for SMI. This study aimed to determine the serum levels of these novel autoantibodies and assess their individual. It combined diagnostic accuracy for SMI with that of patients with stable angina and healthy controls.

Materials and Methods

Study design and population

This cross-sectional diagnostic study was conducted at Beijing Anzhen Nanchong Hospital, Capital Medical University & Nanchong Central Hospital, Nanchong City, 637000, Sichuan Province, China, between January 2023 and December 2025. We prospectively recruited 210 subjects categorised into three equal groups (n=70 each):

SMI Group: Asymptomatic patients with documented myocardial ischemia, defined by ≥1 mm horizontal or down-sloping ST-segment depression occurring 80ms after the J-point during a standard Bruce protocol treadmill test (18), without any chest pain or equivalent symptoms.

Stable Angina (SA) Group: Patients with typical exertional angina and angiographically proven significant CAD (≥70% stenosis in at least one major epicardial artery) matched for age and sex to the SMI group (19).

Healthy Control Group: Age- and sex-matched healthy volunteers with no history of cardiovascular disease, normal resting ECG, negative ETT, and no cardiovascular symptoms.

Exclusion criteria were a history of acute coronary syndrome within the prior 3 months, inflammatory or autoimmune diseases, active malignancy, severe hepatic or renal dysfunction (estimated GFR<30 mL/min/1.73m^2), or current infection. The Institutional Review Board approved the protocol (IRB-2021-044), and all participants provided written informed consent. The study conformed to the Declaration of Helsinki.

Sample collection and laboratory measurements

Exercise Treadmill Testing and Interpretation

Treadmill exercise testing was performed using the standard Bruce protocol under continuous 12-lead electrocardiographic monitoring, with blood pressure and symptom assessment at baseline, at each exercise stage, at peak exercise, and throughout recovery. A test was considered diagnostically adequate if the participant achieved at least 85% of the age-predicted maximal heart rate, unless the test was terminated earlier because of diagnostic ischemic ST-segment changes, significant arrhythmia, abnormal blood pressure response, or limiting symptoms.

Myocardial ischemia on ETT was defined as horizontal or downsloping ST-segment depression of ≥1.0 mm, measured 80 ms after the J-point, in at least 2 contiguous leads during exercise or early recovery. Up-sloping ST depression was not considered diagnostic unless it was marked and accompanied by other ischemic features. Patients with baseline electrocardiographic abnormalities that could preclude reliable ST-segment interpretation were excluded from ETT-based classification. These included resting ST-segment depression ≥1.0 mm, left bundle branch block, ventricular paced rhythm, pre-excitation pattern, left ventricular hypertrophy with secondary repolarisation abnormalities, digoxin-associated ST-T changes, or other clinically significant resting repolarisation abnormalities. Participants with uninterpretable or equivocal ETT results were not included in the final analysis.

Silent myocardial ischemia was defined as diagnostic exercise-induced ischemic ST-segment depression in the absence of chest pain, dyspnoea judged to be an anginal equivalent, or other ischemia-related symptoms during exercise or recovery. Stable angina patients met the same objective ischemic ECG criteria or had angiographically proven significant coronary artery disease, but experienced typical exertional angina symptoms consistent with myocardial ischemia.

The Duke Treadmill Score was calculated for each participant undergoing ETT using the standard formula: exercise time in minutes − 5 × maximum ST-segment deviation in millimetres – 4 × treadmill angina index, where the angina index was coded as 0 for no angina, 1 for non-limiting angina, and 2 for exercise-limiting angina. Patients were categorised as low risk (score ≥+5), intermediate risk (score -10 to +4), or high risk (score ≤ -11). Duke Treadmill Score categories were used as an additional risk stratification measure and were compared between groups.

Blood sample collection and processing

Venous blood samples were drawn from an antecubital vein between 8:00 and 9:30 AM after a 12-hour overnight fast to reduce the effect of postprandial metabolic variation. Blood samples (~30 mL) were drawn from each participant and stored in serum separator tubes for biochemical and antibody testing and EDTA tubes for plasma-based hs-CRP measurement. All study participants who enrolled successfully underwent phlebotomy, and none were excluded due to difficult venous access, insufficient sample volume, haemolysis, or poor sample quality. Samples were collected in vacutainer tubes: serum separator tubes (SST) for biochemical and antibody assays and EDTA tubes for plasma extraction. Serum tubes were allowed to clot at room temperature for 30 min and then centrifuged at 4 °C for 15 min at 3000g. Plasma was centrifuged at 4 °C for 20 minutes at 3000g within 30 minutes after collection. The separated serum and plasma were immediately aliquoted into 500 μL cryovials, snap-frozen in liquid nitrogen, and stored at -80 °C until batch analysis at the end of the study. All samples were freeze-thaw cycled once. On the day of collection, a standard biochemical panel, including HbA1c, lipid profile, and renal/hepatic function, was performed at our institution's certified clinical chemistry laboratory.

High-sensitivity C-reactive protein assay

High-sensitivity C-reactive protein (hs-CRP) was measured from thawed EDTA plasma using a particle-enhanced immunonephelometric assay on the BN II System (Siemens Healthcare Diagnostics, Erlangen, Germany). This assay has a lower detection limit of 0.16 mg/L. The intra-assay coefficient of variation (CV) ranged from 2 to 5%, and the inter-assay CV was less than 7% across the measured range.

Enzyme-linked immunosorbent assays (ELISA) for autoantibodies

All three autoantibodies were quantified using previously validated and published in-house indirect ELISA protocols, with testing performed in duplicate by a technician blinded to clinical categorisation.

Anti-Apolipoprotein A-1 (Anti-ApoA-1) IgG ELISA

High binding 96-well microliter plates (Nunc MaxiSorp, Thermo Fisher Scientific) were coated with 100 μL/well of purified, delipidated human ApoA-1 (Merck Millipore, Cat. No. 178422) at 5 μg/mL concentration in phosphate-buffered saline (PBS, pH 7.4) for 18 h at 4 °C. Plates were washed three times with PBS containing 0.05% Tween-20 (PBS-T) and blocked with 2% bovine serum albumin (BSA, fatty acid-free) in PBS for 2 h at 37 °C to minimise non-specific binding. The serum samples were washed and diluted 1:100 in blocking buffer (1% BSA in PBS-T), and then 100 μL of this was added and incubated at 37 °C for 1.5 hours. Following an additional wash step, 100 μL of alkaline phosphatase-conjugated goat anti-human IgG (Fc-specific, Jackson ImmunoResearch, diluted 1:4000) was added and incubated for 1 hour at 37°C. Colour development was performed with 100 μL of p-nitrophenyl phosphate substrate at 37 °C for 25 min, and the reaction was stopped with 50 μL of 3 M NaOH. Optical density (OD) was determined at 405 nm using a BioTek ELx808 microplate reader (BioTek Instruments, Inc., Winooski, VT). Nonspecific binding was determined in uncoated wells. A pool of high-titer positive sera, assigned an arbitrary concentration of 200 arbitrary units/mL (AU/mL) per plate, was used to prepare a seven-point standard curve. The lower limit of quantitation (LLOQ) was 0.2 AU/mL. The intra-assay CV was 6.4%, and the inter-assay CV was 10.8%.

Anti-MDA-modified LDL (Anti-MDA-LDL) IgG ELISA

Native LDL was isolated from pooled fresh human plasma of fasting healthy donors by sequential density ultracentrifugation in the 1.019–1.063 g/mL density range, followed by dialysis against EDTA-containing PBS. MDA-LDL was prepared by incubating native LDL (1 mg/mL) with 0.5 M malondialdehyde-bis-dimethylacetal (Sigma-Aldrich) in acetate buffer (pH 5.5) for 4 hours at 37°C, followed by exhaustive dialysis to remove unreacted MDA. The degree of modification was confirmed by an increase in relative electrophoretic mobility on agarose gels and by a fluorometric assay for thiobarbituric acid-reactive substances. ELISA was performed as above, with plates coated overnight with MDA-LDL at 5 μg/mL in PBS (100 μL/well). Plates were blocked with 2% foetal bovine serum (FBS) in Tris-buffered saline (TBS). Serum samples were diluted 1:100 in TBS containing 1% FBS. Detection was performed with an HRP-conjugated goat anti-human IgG antibody (Thermo Fisher Scientific, diluted 1:5000) and tetramethylbenzidine (TMB) substrate, stopped with 2 N sulphuric acid. Absorbance was read at 450 nm. Results are expressed as AU/mL against a pooled standard curve. The intra-assay CV was 5.5%, and the inter-assay CV was 9.3%.

Anti-osteopontin (Anti-OPN) IgG ELISA

Recombinant human full-length osteopontin (R&D Systems, Cat. No. 1433-OP/CF) was coated on plates at 2 μg/mL in carbonate-bicarbonate buffer (pH 9.6). Blocking was performed using 1% BSA. Serum was diluted 1:100 and incubated overnight at 4 °C to form immune complexes. The detection antibody was a biotinylated mouse anti-human IgG monoclonal antibody (BD Pharmingen, clone G18-145, 2 μg/mL), followed by streptavidin-HRP conjugate (R&D Systems) and TMB substrate. The use of a monoclonal secondary detection antibody results in specific detection of the IgG isotype. A standard curve was prepared in AU/mL using a high-titer serum standard. The intra-assay CV was 7.1%, and the inter-assay CV was 11.5%.

Quality control for immunoassays

Every plate contained a blank well (no serum), a high-standard control, and a low-standard control provided by a pooled normal human serum sample. All controls fell within the pre-specified 2 standard deviation (SD) limits on control charts; otherwise, the plate was rejected and repeated. Cross-reactivity among the three assays was assessed by pre-absorption experiments and found to be negligible (<5% signal inhibition when sera were incubated with heterologous coating antigens).

Outcome definition and sample size calculation

The primary endpoint of this diagnostic accuracy study was the area under the receiver

operating characteristic curve for the ability of anti-ApoA-1 IgG to discriminate patients with silent myocardial ischemia from healthy controls. Anti-ApoA-1 IgG was selected as the primary biomarker for sample size estimation because prior evidence suggested a close association between anti-ApoA-1 autoimmunity and cardiovascular risk, and because it was expected to show the strongest diagnostic performance among the three candidate markers.

Sample size was therefore calculated prospectively for ROC analysis, assuming an AUC of 0.80 for anti-ApoA-1 IgG and testing against the null hypothesis of an AUC of 0.50, with 80% statistical power and a two-sided α level of 0.05. This calculation indicated that a minimum of 54 participants per group was required. To account for potential assay failure, incomplete clinical or ETT data, and the need for covariate adjustment in multivariable logistic regression, the target sample size was increased to 70 participants per group, yielding a total study population of 210 participants. G*Power software (version 3.1.9.7) was used for these calculations.

Statistical analysis

Data normality was tested using the Kolmogorov-Smirnov test. Continuous variables are presented as mean ± SD or median (interquartile range) for non-normal distribution, and categorical variables as frequencies (%). Group differences were calculated using one-way ANOVA with Bonferroni post-hoc correction or by the Kruskal-Wallis test. ROC curves were used to determine the diagnostic accuracy of individual and combined biomarkers (binary logistic regression predicted probabilities) for SMI versus controls and SMI versus SA. The cut-off value was defined as the maximum of the Youden index. Multivariate logistic regression was used to identify independent predictors of SMI, adjusting for age, sex, diabetes, hypertension, and hs-CRP. Statistical analyses were performed with SPSS version 28.0 (IBM Corp.), and $p<0.05$ was considered significant.

To assess the internal validity and potential optimism of the combined diagnostic panel, bootstrap resampling was performed. The three-marker logistic regression model, including anti-ApoA-1 IgG, anti-MDA-LDL IgG, and anti-OPN IgG, was internally validated using 1,000 bootstrap samples drawn with replacement from the original dataset. In each bootstrap sample, the logistic model was refitted, and the area under the ROC curve was used to evaluate its discriminative performance. Model optimism was estimated as the difference between bootstrap performance and performance when the bootstrap-derived model was applied back to the original dataset. The optimism-corrected AUC was calculated by subtracting the mean optimism from the apparent AUC. Calibration was assessed using the Hosmer-Lemeshow goodness-of-fit test and by comparing predicted and observed SMI probabilities across risk strata.

Missing data were assessed for all demographic, clinical, biochemical, ETT, and antibody variables before analysis. Complete data were available for all 210 participants, including hs-CRP, lipid profile, and all three antibody measurements. Therefore, no multiple imputation or other missing-data replacement method was required, and all statistical analyses were conducted using the full study cohort.

Results

Baseline characteristics

A total of 210 participants were enrolled, and complete data were available for baseline clinical characteristics, hs-CRP, lipid profile, ETT

Table I Baseline characteristics of the study population.

Variable	SMI (n=70)	SA (n=70)	Controls (n=70)	p-value
Age (years)	63.5±8.2	64.1±7.8	62.0±8.5	0.29
Male sex, n (%)	46 (65.7)	44 (62.9)	42 (60.0)	0.78
Diabetes Mellitus, n (%)	23 (32.9)	12 (17.1)	6 (8.6)	0.002
Hypertension, n (%)	48 (68.6)	49 (70.0)	22 (31.4)	<0.001
Total Cholesterol (mg/dL)	198.4±38.5	205.9±41.2	185.2±28.9	0.005
HDL-Cholesterol (mg/dL)	42.6±8.9	41.3±9.5	55.1±12.4	<0.001
LDL-Cholesterol (mg/dL)	128.9±32.4	135.8±35.1	110.3±26.1	<0.001
hs-CRP (mg/L)	3.8 (2.0–6.5)	2.5 (1.4–4.8)	1.1 (0.6–2.1)	<0.001

Values are mean ± SD, n (%), or median (IQR). SMI: silent myocardial ischemia; SA: stable angina; hs-CRP: high-sensitivity C-reactive protein. For hs-CRP, post-hoc pairwise comparisons showed: *$p<0.05$ for SMI vs SA; †$p<0.001$ for SMI vs controls; ‡$p<0.01$ for SA vs controls.

Table II Distribution of serum autoantibody levels across study groups.

Biomarker (AU/mL)	SMI (n=70)	SA (n=70)	Controls (n=70)	p-value
Anti-ApoA-1 IgG	0.85 (0.61–1.20)	0.55 (0.38–0.72)	0.40 (0.28–0.55)	<0.001*
Anti-MDA-LDL IgG	0.72 (0.49–0.98)	0.50 (0.32–0.69)	0.39 (0.25–0.52)	<0.001*
Anti-OPN IgG	0.68 (0.44–1.05)	0.41 (0.25–0.61)	0.30 (0.19–0.43)	<0.001*

Values are median (interquartile range). * $p<0.001$ for global comparison; post-hoc tests: SMI > SA > Controls ($p<0.01$ for each inter-group comparison).

Table III Receiver operating characteristic (ROC) analysis for individual biomarkers.

Comparison	Biomarker	AUC (95% CI)	Cut-off (AU/mL)	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
SMI vs controls	Anti-ApoA-1 IgG	0.82 (0.75–0.89)	0.63	71.4 (59.9–80.7)	85.7 (75.7–92.1)	83.3 (72.0–90.7)	75.0 (64.5–83.2)
	Anti-MDA-LDL IgG	0.78 (0.70–0.85)	0.55	67.1 55.5–77.0)	82.9 (72.4–89.9)	79.7 (67.7–88.0)	71.6 (61.0–80.3)
	Anti-OPN IgG	0.80 (0.73–0.87)	0.50	74.3 (63.0–83.1)	78.6 (67.6–86.6)	77.6 (66.3–85.9)	75.3 (64.4–83.8)
SMI vs SA	Anti-ApoA-1 IgG	0.74 (0.66–0.82)	0.72	65.7 (54.0–75.8)	72.9 (61.5–81.9)	70.8 (58.8–80.4)	68.0 (56.8–77.5)
	Anti-MDA-LDL IgG	0.69 (0.60–0.78)	0.61	62.9 (51.1–73.2)	68.6 (57.0–78.2)	66.7 (54.7–76.8)	64.9 (53.5–74.8)
	Anti-OPN IgG	0.72 (0.63–0.80)	0.54	67.1 (55.5–77.0)	71.4 (59.9–80.7)	70.1 (58.3–79.8)	68.5 (57.1–78.0)

interpretation, and serum measurements of anti-ApoA-1 IgG, anti-MDA-LDL IgG, and anti-OPN IgG. No participant was excluded from the final analysis because of missing laboratory or clinical data.

Baseline demographics and clinical profiles are presented in *Table I*. The three groups were well-matched for age and sex. The SMI group exhibited a significantly higher prevalence of diabetes mellitus (32.9%) compared to the SA (17.1%) and control (8.6%) groups ($p=0.002$). Similarly, hypertension was more common in the SMI and SA groups than in controls. Mean LDL-cholesterol was highest in the SA group, while hs-CRP was elevated in both patient groups, with the median peak in the SMI group (3.8 mg/L vs 2.5 mg/L in SA, $p<0.05$).

Duke Treadmill Score analysis demonstrated a higher ischemic risk profile among patients with SMI and stable angina compared with healthy controls. The median Duke Treadmill Score was -4.0 in the SMI group, -6.0 in the stable angina group, and +9.0 in the control group. Based on conventional Duke Treadmill Score categories, 52 patients with SMI were classified as intermediate risk (74.3%), 10 as high risk (14.3%), and 8 as low risk (11.4%). In the stable angina group, 45 patients (64.3%) were classified as intermediate risk, 18 patients (25.7%) as high risk, and 7 patients (10.0%) as low risk. All healthy controls had negative ETT results and were classified as low risk. These findings confirm that the SMI group had objective exercise-induced ischemia despite the absence of anginal symptoms.

Serum levels of autoantibodies

Median serum concentrations of all three autoantibodies were significantly elevated in the SMI group compared to both SA patients and healthy controls (*Table II*, all $p<0.001$). Anti-ApoA-1 IgG levels were 2.1-fold higher in SMI vs controls. Anti-MDA-LDL IgG levels were 1.8-fold higher, and anti-OPN IgG levels were 2.3-fold higher. Notably, the SA group also had significantly higher levels of all three markers compared to healthy controls, but the magnitude of elevation was less than half that seen in SMI.

Diagnostic performance for silent myocardial ischemia

Internal validation of the combined three-marker diagnostic panel was performed using 1,000 bootstrap resamples. For discrimination of SMI from healthy controls, the apparent AUC of the combined model was 0.91. The mean bootstrap optimism was 0.02, yielding an optimism-corrected AUC of 0.89. The bootstrap-corrected sensitivity and specificity were 85.7% and 82.9%, respectively,

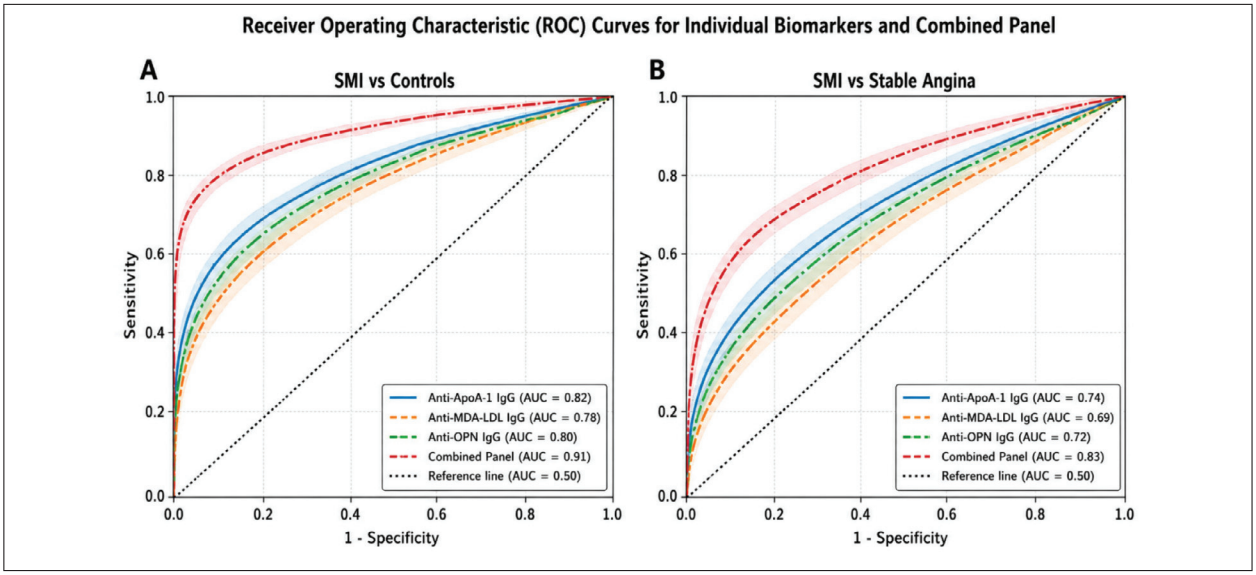

Figure 1 ROC curves for individual biomarkers and the combined diagnostic panel.

Receiver operating characteristic curves showing the diagnostic performance of anti-ApoA-1 IgG, anti-MDA-LDL IgG, anti-OPN IgG, and the combined three-marker panel for distinguishing (A) silent myocardial ischemia (SMI) from healthy controls and (B) SMI from stable angina. The diagonal dotted line represents no discrimination (AUC=0.50). Shaded regions indicate 95% confidence bands.

at the prespecified optimal probability threshold. For discrimination of SMI from stable angina, the apparent AUC was 0.83, with a mean optimism of 0.03 and an optimism-corrected AUC of 0.80. Calibration of the combined model was acceptable, with no significant lack of fit by the Hosmer-Lemeshow test for SMI versus controls (p=0.42) or for SMI versus stable angina (p=0.36).

Because predictive values are prevalence-dependent, PPV and NPV were recalculated for clinically relevant assumed SMI prevalence levels rather than relying only on the enriched study cohort. For the combined three-marker panel, which showed a sensitivity of 87.1% and specificity of 84.3%, the recalculated PPV increased progressively with higher assumed SMI prevalence, from 22.6% at 5% prevalence to 38.1% at 10%, 58.1% at 20%, and 70.4% at 30%. Conversely, NPV remained high across these prevalence scenarios, ranging from 99.2% to 93.8%. These findings suggest that the combined biomarker panel may be particularly useful as a rule-out or risk-stratification tool in asymptomatic or high-risk screening populations. At the same time, positive results would require confirmation by functional or imaging-based cardiac testing.

A combined panel model was constructed using logistic regression. The equation derived was: Logit(p) = -4.15 + (2.10 × anti-ApoA-1) + (1.45 × anti-MDA-LDL) + (1.90 × anti-OPN). The three-marker panel demonstrated a significantly larger AUC for SMI detection (vs controls) of 0.91 (95% CI 0.86–0.96), with a superior sensitivity of 87.1% and specificity of 84.3% (*Figure 1*). For SMI versus SA, the panel AUC was 0.83 (0.76–0.90).

In multivariate logistic regression adjusting for age, diabetes, hypertension, and hs-CRP, anti-ApoA-1 IgG (OR 3.2; 95% CI 1.8–5.6, p<0.001) and anti-OPN IgG (OR 2.5; 95% CI 1.5–4.3, p=0.001) were independent predictors of the SMI state, whereas anti-MDA-LDL IgG was not (p=0.09).

Discussion

This study demonstrates that anti-ApoA-1, anti-MDA-LDL, and anti-OPN IgG are elevated in patients with myocardial ischemia, including both silent myocardial ischemia and stable angina. The highest levels were observed in the SMI group; however, all three markers were also significantly elevated in stable angina compared with healthy controls. Therefore, these biomarkers should not be interpreted as specific markers of silent ischemia alone. Rather, they appear to reflect broader autoimmune-inflammatory activation, oxidative lipid modification, and vascular remodelling associated with coronary artery disease and myocardial ischemia, with quantitatively greater elevations in silent presentations compared with symptomatic ones.

The superior performance of anti-ApoA-1 IgG aligns with the growing paradigm that HDL dysfunction, rather than HDL concentration alone, is a critical driver of vascular risk (20). ApoA-1 is

susceptible to myeloperoxidase-mediated oxidation and nitration, creating neo-epitopes that trigger autoantibody formation (21). These antibodies form immune complexes that further activate the complement cascade and promote foam cell formation in the vessel wall, a process that may be particularly heightened in diffuse coronary microvascular dysfunction, often characteristic of SMI (22, 23). Our OR of 3.2 for anti-ApoA-1 in predicting SMI underscores its potential primacy as a diagnostic and mechanistic marker.

Similarly, the role of anti-MDA-LDL IgG highlights the contribution of enhanced lipid peroxidation in silent disease (24). MDA modifications occur when reactive oxygen species attack polyunsaturated fatty acids in LDL, a process that is amplified by diabetes and metabolic syndrome (25). Antibodies targeting these oxidised epitopes can paradoxically be both atheroprotective and pathogenic, depending on their IgG subclass and titer (26). The significantly elevated levels in our SMI cohort, particularly in people with diabetes, support a model in which an exhausted or maladapted immune response to oxidatively damaged LDL is a hallmark of silent plaque progression (27).

The inclusion of anti-OPN IgG provides a novel window into vascular calcification and immune cell recruitment pathways. OPN promotes smooth muscle cell transdifferentiation and macrophage chemotaxis, central to plaque maturation and destabilisation (28). Its release into circulation and the subsequent immune response against it may signify active, smouldering plaque remodelling occurring without the acute distension of the vessel adventitia that triggers cardiac nociceptive fibres (29). This remodelling pathway may help explain why objective ischemia can occur in the absence of anginal symptoms, although this hypothesis requires confirmation in mechanistic studies.

The diagnostic panel's AUC of 0.91 is clinically promising, outperforming isolated ETT and approaching the performance of some stress imaging protocols (30). An inexpensive serum panel could serve as a gatekeeper, triaging asymptomatic high-risk individuals (e.g., long-standing diabetics) for advanced imaging, thereby optimising resource utilisation. The observed PPV of 83.3% for anti-ApoA-1 IgG at the optimal cut-off suggests potential clinical utility in populations with an intermediate pre-test probability.

Study limitations

This study has several limitations. The cross-sectional design precludes causal inference, and the single-centre setting with a modest sample size may limit generalizability. Although internal bootstrap validation was performed, the absence of an independent external validation cohort means that the diagnostic performance of the biomarker panel requires confirmation in larger multicentre studies. Biomarkers were measured at only one-time point, preventing assessment of longitudinal stability or temporal changes in relation to disease progression or treatment. In addition, SMI was diagnosed using ETT rather than stress imaging, which may have introduced misclassification because ETT is less sensitive and specific than imaging-based approaches. Finally, the study design could not determine whether SMI resulted from epicardial coronary disease, coronary microvascular dysfunction, endothelial dysfunction, or overlapping mechanisms.

Conclusion

Autoantibodies to ApoA-1, MDA-LDL, and OPN represent a distinct serum signature of silent myocardial ischemia, reflecting intertwined pathways of HDL dysfunction, oxidative stress, and vascular inflammation. A multi-marker logistic model combining these three antibodies demonstrates high diagnostic accuracy, highlighting a potential new paradigm for risk-stratifying asymptomatic atherosclerosis. Future prospective studies should evaluate if this panel can predict incident SMI and adverse cardiac events, moving from diagnostic association to prognostic validation.

Authors' contribution

Zhongqing Wang and Bin Sun contributed equally to this work and share first authorship.

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

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