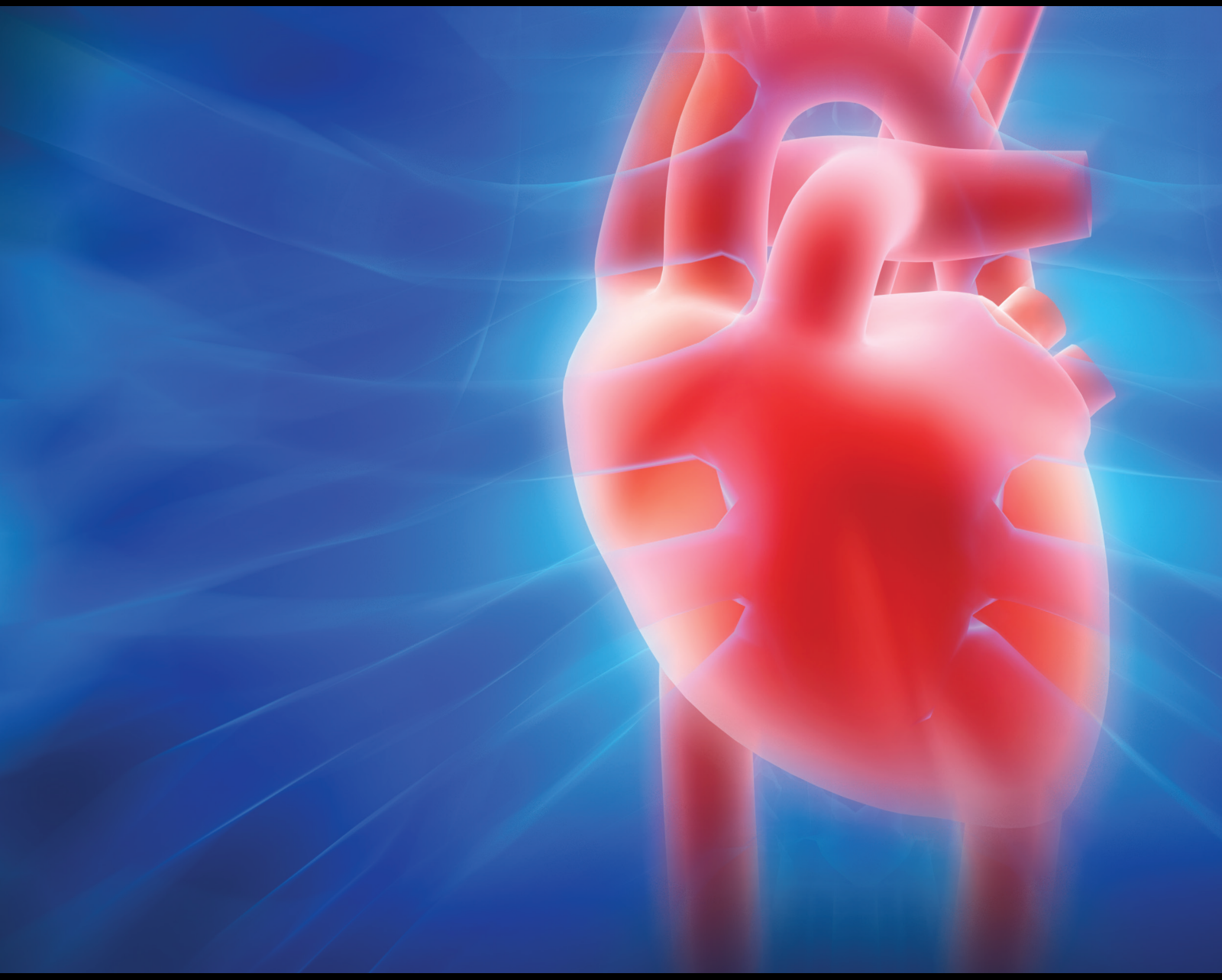
Cardiology Research and Practice

Mechanisms, Diagnosis and Treatment on Aging-Related Heart and Coronary Artery Diseases

Lead Guest Editor: Qian Fan
Guest Editors: Erhe Gao, Xiao-Liang Wang, and Ran Dong



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Research Article

Diagnostic Role of Plasma MicroRNA-21 in Stable and Unstable Angina Patients and Association with Aging

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Guest Editor: Ran Dong

The present study explored the clinical value of plasma microRNA-21 as a novel biomarker for early prediction of stable and unstable angina patients and its relationship with aging. A total of 255 participants, 123 patients with chronic stable angina, 82 patients with unstable angina, and 50 healthy subjects, were included in our study. Stable coronary and unstable coronary patients were confirmed following AHA/ACC clinical protocols. Total RNA was extracted from plasma by using miRNA-based TRIzol reagent. Plasma miR-21 expression levels were determined by real-time polymerase chain reaction. To evaluate the diagnosis accuracy, the receiver operating characteristic (ROC) curves were used. Plasma microRNA-21 concentration levels were significantly elevated in stable and unstable angina patients as compared with control subjects ($P < 0.001$). The area under the ROC curves of circulating microRNA-21 was accurately distinguished in stable angina patients (AUC 0.921) and unstable angina patients (AUC 0.944) from healthy subjects. MicroRNA-21 expression gradually elevated with increasing aging in all the populations. Moreover, the current study also demonstrated that the expression of plasma miR-21 levels was significantly associated with different age groups within healthy subjects and stable and unstable angina patients ($P < 0.001$). This research finding suggested that plasma microRNA-21 may be considered as a suitable new biomarker for early detection of stable and unstable angina patients, and it has a strong correlation with aging.

1. Introduction

Coronary artery disease (CAD) is a major public health problem and remains the leading cause of sudden cardiac death (SCD) all over the world. Atherosclerosis and older age are the two important risk factors for coronary artery disease. Every year, several millions of chest pain patients were admitted into the hospital in both developed and developing countries. Almost 50% of these chest pain cases were of cardiac origin; either stable angina pectoris, unstable angina, acute coronary syndrome, or acute myocardial infarction (AMI) [1].

An early clinical detection and accurate diagnosis of CAD is an important task for physicians to initiate appropriate treatment and subsequently prevent sudden cardiac death. Although coronary angiography (CAG) is the gold standard invasive test for the diagnosis of CAD patients, it has some limitations including high cost, limited availability, and radiation hazards [2]. Therefore, it is a clinical demand to find out new blood-based biomarkers for identification of CAD in the earlier stage.

Microribonucleic acids (miRNAs) are small (≈22 nucleotides), highly specific, endogenous single-stranded, and noncoding ribonucleic acid (RNA) molecules that regulate gene expression. Recent several research groups evidenced that microRNAs have significant impact on various cardiovascular biology and progression of diseases including coronary artery disease (CAD) and atherothrombosis. MicroRNAs which are detectable in various body fluids are defined as circulating microRNAs [3–5].

It has been demonstrated that expressions of several specific circulating microRNA levels were significantly altered in stable angina pectoris patients (miR-19a, miR-133a, miR-149, and miR-208a), unstable coronary artery disease (miR-423, miR-424, and miR-765), and acute coronary syndrome (miR-1, miR-92a, miR-134-5p, and miR-183-5p).

Moreover, plasma miR-208b, miR-499, and miR-223 could be useful as ideal biomarkers for early evaluation of AMI patients [6–12].

Besides, miR-21 has currently received great attention regarding its chronic inflammatory function in coronary atherosclerotic heart disease. Overexpression of miR-21 level significantly promoted abnormal proliferation and migration of vascular smooth muscle cells (VSMCs) and activated the Akt/ERK signaling pathway and aggravated atherosclerosis in a rat model, while knocking down of miR-21 can suppress the activation of VSMCs and reduce atherosclerosis level [13].

Moreover, microRNA-21 expressions in naive CD4+ T cells were amazingly elevated in (65–85 years) older healthy subjects compared with (20–35 years) younger subjects, with higher variance in the geriatric subjects, suggesting miR-21 remarkably controlled immune response and aging [14]. Very recently, it was reported that the expression pattern of circulating microRNA-21 was greatly associated with significant or insignificant coronary stenosis patients [15]. However, the clinical significance of plasma microRNA-21 for coronary artery disease patients and linked with aging is not fully explored. Therefore, the current study investigated the diagnostic potential of circulating microRNA-21 for early detection of stable angina and unstable angina patients and the relationship between plasma miR-21 and aging.

2. Materials and Methods

2.1. Selection of Study Groups. The current study included 255 participants; among them, 123 patients with chronic stable angina and 82 patients with unstable angina were admitted into the cardiology department of Xiangya Hospital and 50 healthy controls from Xiangya health center from March 2016 to August 2017. Chronic stable coronary angina patients (≥50% blocked in one or more than one major coronary artery) were confirmed by invasive coronary angiography. Moreover, stable angina pectoris and unstable angina patients were characterized by following clinical guidelines of ACC/AHA [16, 17]. However, study patients with less than 28 years and more than 84 years old, chronic inflammatory diseases, autoimmune diseases, acute infectious diseases, neoplastic diseases, chronic liver and kidney diseases (creatinine clearance <15 ml/min), implanted coronary stent, prior acute heart attack, and cardiac failure were not enrolled in this research. Healthy controls were well matched with age, gender, and other basic information with patients' group and also free from cardiovascular and any chronic disease.

This study has been followed all the principles outlined by the revised Helsinki Declaration in 2013 for human subjects. All the study groups have given their written informed consent during recruitment to our study. The Ethical Committee of Xiangya Hospital, Central South University (China), approved this study.

2.2. Collection of Blood Samples and RNA Extraction. Cell-and platelet-free plasma were obtained by two-stage centrifugation protocols. Firstly, erythrocytes and other waste products were cleaned by centrifuge at 4°C for 10 min at 1,900 RMP. Secondly, to remove cryoprecipitates and collect fresh plasma, samples were recentrifuged for 10 min at 16,000 RMP at 4°C temperature. After that, all plasma samples were transferred into EP-microtubes and preserved at −80°C. By using TRIzol total RNA extraction reagent, RNA was isolated from plasma following manufactures' guidelines (Invitrogen, Carlsbad, CA, USA). In brief, 250 μL of plasma was mixed with 750 μL of the TRIzol reagent, incubated at room temperature for 5 min, and then mixed with 200 μL chloroform. Then, by centrifuge at 12,000 RMP for 15 min at 4°C, only the upper aqueous phase was collected, and subsequently, RNA was precipitated with adding 500 μL of pure isopropanol. After that, 500 μL of 80% ethanol was added into the RNA samples and cleaned 3 times, and later, RNA samples were diluted with 30 μL DEPC water and kept at 4°C for 10 hours; subsequently, RNA samples' quality and concentration were demonstrated by NanoDrop photometer and stored at −80°C.

2.3. Expression of miR-21 Analysis. Expressions of plasma miR-21 were determined by real-time quantitative reverse-transcription polymerase chain reaction (qRT-PCR). RNA sample was reverse-transcribed to cDNA using a specific RT primer, RiboBio reagent of microRNA-21, with an RT-PCR system according to manufacturer's instructions. Real-time quantitative PCR analysis was carried out with a 7300 Real-Time PCR System (Applied Biosystems, CA, USA) by using Takara qRT-PCR primer synthesis kits followed by the company guidelines. Real-time PCR reactions were performed in triplicate for all the study subjects. MiR-156a was used as the internal control to normalize miR-21 expression. Ct values were measured with SDS 2.3 software. The relative expression of microRNA-21 was calculated through the $2^{-\Delta\Delta CT}$ method (ΔCt microR-21–ΔCt control gene). Those miRNA expressions more than >35 Ct values were not included in this study.

2.4. Analysis of Biochemical Parameters. Fasting glucose, C-reactive protein, total cholesterol, triglycerides, high-density cholesterol, low-density cholesterol, and creatinine levels were investigated with commercial kits. Creatine kinase MB and cardiac troponin I concentrations were measured through Beckman immunoassay.

2.5. Statistics. Statistical analysis was performed with SPSS version 20.0 software, and graphs were generated using GraphPad Prism 6 version software. Student's *t*-test, one-way ANOVA, Mann–Whitney rank test, Kruskal–Wallis test, Dunn's test, and Fischer's exact test or the chi-square test were used for data analysis as appropriate. The specificity and sensitivity of plasma microRNA-21 for diagnosis of stable and unstable CAD patients were measured through the receiver operating characteristic (ROC) analysis. Differences among the groups were considered statistically significant with $P < 0.05$.

Table 1: Clinical information of the participants.

Characteristics	Control subjects ($n = 50$)	Stable angina ($n = 82$)	Unstable angina ($n = 50$)	P_1	P_2	P_3
Gender, male/female (n/n)	30/20	58/24	32/18	0.408	0.671	0.439
Age (yrs)	59.9 ± 7.2	63.7 ± 11.4	65.08 ± 9.1	0.270	0.536	0.331
HR(bpm)	71.02 ± 6.4	74 ± 5.3	75 ± 9.2	0.825	0.798	0.811
SBP (mmHg)	124.9 ± 5.41	135.6 ± 9.25	139.±13.14	0.190	0.476	0.607
DBP (mmHg)	74.8 ± 8.13	77.48 ± 10.25	80.71 ± 9.21	0.731	0.332	0.395
EF (%)	63.4 ± 8.7	59.4 ± 6.05	56 ± 7.21	0.075	0.064	0.332
Risk factors, n (%)						
Current smoking	31 (62)	52 (64)	34 (67)	0.362	0.135	0.544
Hypertension	30 (59)	56 (68)	36 (72)	0.459	0.217	0.709
Hyperlipidemia	17 (33)	39 (48)	27 (54)	0.135	0.082	0.268
Type-2 DM	11 (22)	24 (29)	17 (33)	0.784	0.355	0.759
Family history of CHD	6 (11)	34 (41)	22 (43)	<0.001	<0.001	0.812
Laboratory information						
Fasting glucose (mmol/L)	4.5 ± 0.9	5.34 ± 1.1	5.54 ± 1.1	0.267	0.218	0.530
C-reactive protein (mg/L)	1.2 ± .0.5	8.4 ± 1.5	11.5 ± 7.8	<0.001	<0.001	0.085
Total cholesterol (mmol/L)	4.21 ± 1.1	5.1 ± 1.6	5.48 ± 1.1	0.580	0.466	0.749
Total glyceride (mmol/L)	1.5 ± 0.9	1.63 ± 0.8	1.77 ± 1.6	0.372	0.075	0.214
High-density lipoprotein (mmol/L)	1.41 ± 0.2	1.12 ± 0.2	1.04 ± 0.6	0.628	0.431	0.429
Low-density lipoprotein (mmol/L)	2.9 ± 1.04	3.41 ± 1.8	3.62 ± 1.5	0.221	0.138	0.632
Creatinine (umol/L)	81.7 ± 16.1	84.4 ± 31.1	88.3 ± 42.2	0.536	0.459	0.846
Creatine kinase MB	0	9.08 ± 3.5	11.2 ± 1.7			
Troponin I	0	0.02 ± 0.01	0.04 ± 0.06			

Data are presented as mean ± SD. Abbreviations: HR, heart rate; bpm, beats/minute; SBP, systolic blood pressure; DBP, diastolic blood pressure; EF, ejection fraction; type-2 DM, diabetes mellitus; CHD, coronary heart disease; P_1 (controls and stable angina), P_2 (controls and unstable angina), and P_3 (stable angina and unstable angina).

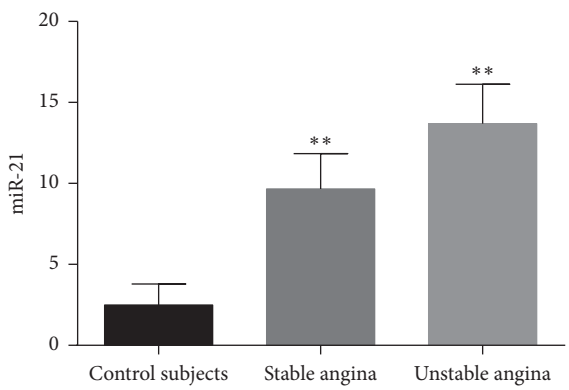

Figure 1: The expression of plasma miR-21 in stable CAD, unstable CAD, and control subjects. Control subjects vs. stable CAD ($P < 0.001$); control subjects vs. unstable stable CAD ($P < 0.001$).

3. Results

3.1. Clinical Information of the Participants. In total, this study included 123 stable angina patients, 82 unstable angina patients, and 50 control subjects. We compared basic and clinical characteristic among different groups. Male and female, age, smoking, hypertension, type-2 DM, HR, SBP, DBP, and EF were not statistically significant ($P > 0.05$). Moreover, family history of CHD and C-reactive protein level between controls with stable and unstable angina patients were highly significant ($P < 0.001$). The detailed information is shown in Table 1.

3.2. Plasma miR-21 Concentration Level in Stable and Unstable Angina Patients and Control Subjects. This study investigated the plasma miR-21 concentration level from stable angina patients, unstable angina patients, and control subjects (Figure 1). We found that plasma miR-21 concentrations were remarkably upregulated in patients with stable angina and unstable angina groups than healthy control groups ($P < 0.001$). Moreover, the expressions of plasma miR-21 level in unstable angina patients were relatively higher than stable angina patients, but the differences were not statistically significant ($P > 0.05$).

3.3. Diagnostic Significance of Plasma MicroRNA-21 Level in Stable and Unstable Angina Patients. Diagnostic accuracy of circulating micoRNA-21 was evaluated through ROC curve analyses. Plasma microRNA-21 was able to strongly separate stable angina patients (AUC 0.921) and unstable angina patients (AUC 0.944) from healthy control subjects (Figures 2(a) and 2(b)). These findings recommended that plasma concentrations of microRNA-21 may be a useful biochemical marker for diagnosis of CAD patients.

3.4. Relationship of miR-21 with Aging. The current study measured circulating miR-21 expression at different age variations among all the study subjects. Circulating microRNA-21 expression gradually elevated with increasing age in all the populations. Plasma microRNA-21 levels were evidently associated with different age groups within healthy

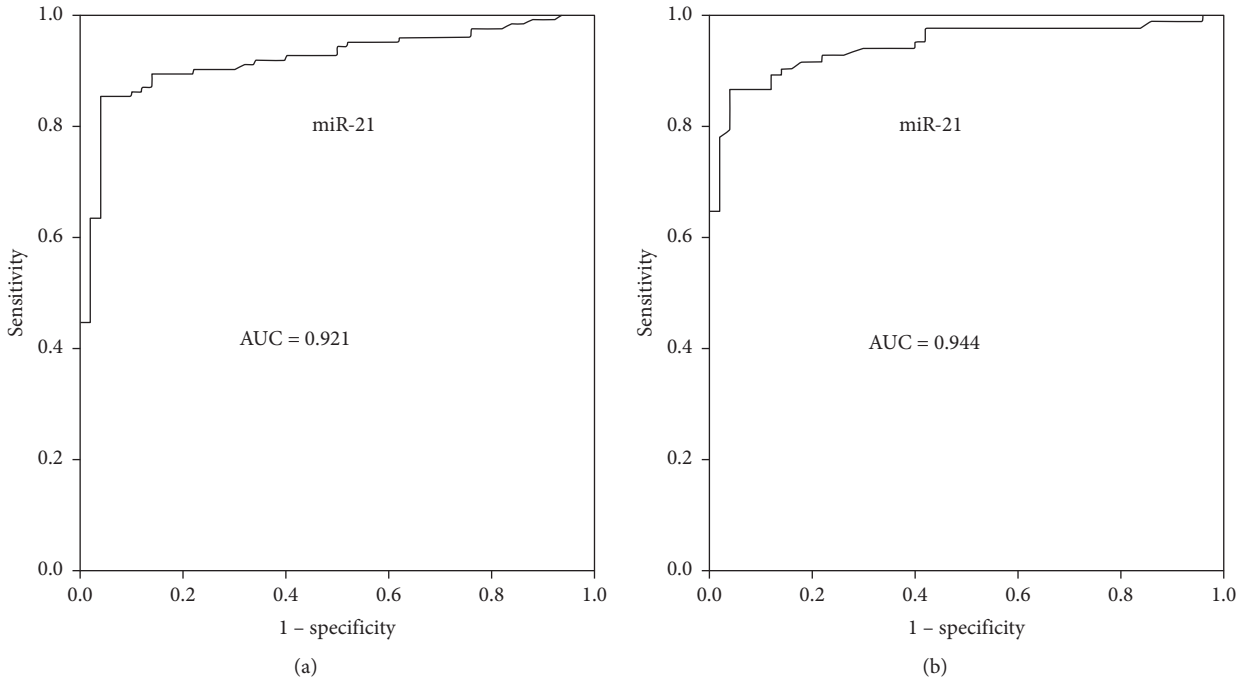

Figure 2: ROC curve analysis to estimate the diagnostic significance of plasma microRNA-21 in stable and unstable CAD patients. (a) Control subjects and stable angina patients (AUC 0.921). (b) Control subjects and unstable angina patients CAD (AUC 0.944).

subjects and stable and unstable angina patients ($P < 0.001$) (Figure 3).

4. Discussion

The present study supported the prospective clinical value of cardiac specific plasma miR-21 as an important blood-based biomarker for early detection of stable and unstable angina pectoris patients. The current study results showed that plasma microRNA-21 was barely detectable in healthy control subjects, but microRNA-21 level was significantly elevated in both stable and unstable angina patients. These results are also in agreement with other research findings in coronary artery disease patients [18, 19]. Moreover, this research investigated the diagnostic importance of plasma miR-21 with ROC analysis. The current study found that AUC values for miR-21 were remarkably increased in stable angina patients (0.921) and unstable angina patients (0.944) with high specificity and sensitivity compared with healthy controls. Taken together, these research results demonstrated that plasma microRNA-21 might be a potential biochemical marker for diagnosis of stable and unstable ischemic heart disease patients.

Sanlialp et al. reported miR-21 expression levels in patients with coronary artery disease were significantly higher than control subjects [20]. A very recent research also demonstrated circulatory microRNA-21 in stable CAD patients was upregulated by 1.90 fold, and AUC was 0.79 and with high sensitivity and specificity and suggested it is a possible diagnostic marker for coronary heart disease [21].

It has been well established that aging is a worldwide problem affecting both developed and developing countries. Aging is a multifactorial process characterized by gradually decrease in physiological and biochemical activities of individual tissues and organs. Geriatric age is the fixed non-modifiable major contributor for atherosclerotic coronary artery disease. Circulating miR-21 can modulate human gene expression and regulate immune system response and directly link to atherosclerosis and aging. Rusanova et al. demonstrated that miR-21 had higher expression in geriatric people than young adults. In addition, expression of plasma cytokine (IL-6, IL-8, IL-10, and TNFα) levels was increased in aged group people (>71 yrs old) compared with young control groups (>18 yrs old). Furthermore, they also found that expression of miR-21, plasma-advanced oxidation protein products (AOPP), and the TNFα/IL-10 ratio were positively correlated in elderly patients compared with younger [22].

Recently, Ahmed et al. revealed that miR-21 levels were prominently increased in both aged mouse (>2 yrs old) and human aged (>78 yrs old) skin as compared with control groups through negative regulation of chromatin remodeller special-AT-rich-sequence-binding protein-1 (SATB1) in keratinocytes and suggested miR-21 remarkably contributed to aging [23].

It was previously demonstrated that microRNA-21 is critically involved in the regulation of different inflammatory pathways and development of atherosclerosis. Moreover, very recently, it was reported that expression of serum miR-21 levels was notably elevated in atherosclerotic subjects and stroke patients relative to healthy individuals. Likewise, they also found that miR-21 strongly regulated the anti-inflammatory effect of haem oxygenase-1 (HO-1) in aged intracerebral hemorrhagic rats [24]. Besides, plasma

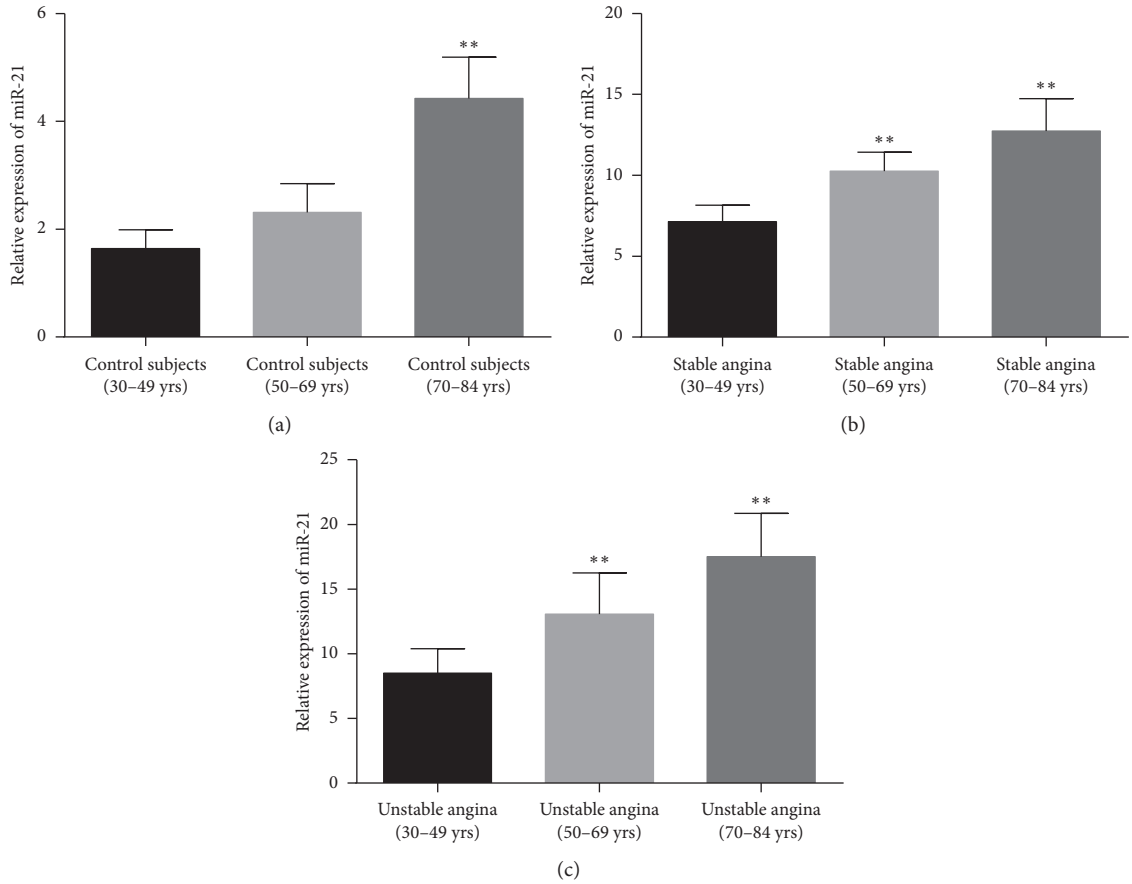

Figure 3: (a) Expression of plasma miR-21 among different age groups within control subjects. (b) Plasma microRNA-21 concentrations within stable angina patients in different age groups. (c) Comparison of circulating microRNA-21 levels within unstable angina patients in variation of ages.

microRNA-21 was also significantly correlated with platelet extracellular vesicles (EVs) in stable coronary artery disease patients [25].

Darabi et al. found that circulating miR-21 levels and matrix metalloproteinase-9 (MMP-9) were significantly higher in acute coronary syndrome (ACS) patients compared with stable coronary artery disease patients. Additionally, expression of miR-21 levels was also positively correlated with MMP-9, high sensitivity C-reactive protein, and aging. The present study also found that C-reactive protein and history of coronary artery disease were significantly higher in stable and unstable angina patients than controls [26].

However, for the first time, this research examined that expression of the plasma miR-21 level was significantly higher in (70–84 years) elderly people as compared with 30–49 years group people, suggesting elevated circulating miR-21 level may be helpful for diagnosis of age-related coronary atherosclerosis.

To decline possible bias from the patient selection, subjects with less than 30 years or more than 84 years were excluded from our study, and the male/female ratio was well balanced between coronary artery disease and healthy controls. However, to minimize the coronary angiography catheter and dye-induced effect on endothelial injury and blood circulation as well as expression of microRNA-21, in this study, all the blood samples were collected from the CAD patients after 72 hours of coronary angiography.

Furthermore, the present study carefully recorded basic data, risk factors, and laboratory information such as HR, SBP, DBP, EF, current smoking, hypertension, hyperlipidemia, type-2 DM, family history of CHD, fasting glucose, C-reactive protein, lipid profiles, creatinine, creatine kinase MB, and troponin I. Statistical analysis demonstrated that clinical characteristics among healthy groups and coronary artery disease patients were not influenced by miR-21 level in plasma, suggesting miR-21 as a potential biochemical biomarker for diagnosis of stable and unstable angina patients.

To avoid possible confounders derived from qRT-PCR methods, the present study used miR-156a as an inner control as compared with other available synthetic endogenous miRNAs because in our previous studies and also in other studies, it was confirmed that miR-156a is highly stable [7, 27, 28]. Furthermore, this study examined every sample in four times; Ct values of miR-21 from 15–35 were added during analysis which strongly suggested these findings are more accurate and reliable.

However, the current research is based on a single center and comparatively smaller size population. Also, this study

did not investigate the pathway of the underline molecular mechanism of miR-21 linked with coronary artery disease and aging. Future studies are required to confirm the clinical value and pathological significance of this biomarker.

5. Conclusion

High level of plasma miR-21 has potential utility as a novel blood-based biomarker for clinical diagnosis of stable and unstable angina patients. Moreover, we also recommended that miR-21 has a strong correlation with aging.

Data Availability

Data used for this current study are accessible on reasonable request from the corresponding author.

Conflicts of Interest

The author declares that there are no conflicts of interest.

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Research Article

The Impact of Statins before High-Risk CABG on Postoperative Multiple Organ Function

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Background. The purpose of this cohort study was to investigate the independent relationship between preoperative statin therapy (PST) and postoperative severe multiorgan failure, measured by the Sequential Organ Failure Assessment (SOFA) maximum greater than 11, in high-risk patients undergoing isolated coronary artery bypass grafting (CABG). *Methods*. The present study is a perspective, single-center, cohort analysis enrolling high-risk patients undergoing CABG from Jan 1, 2018, to Dec 31, 2018, in Beijing Anzhen hospital. *Results*. Among a total of 880 high-risk patients undergoing isolated CABG included in this study, 503 (57.2%) experienced statin therapy before CABG. The SOFA maximum was significantly lower in the PST group compared with the control group (7.8 ± 3.0 v 9.2 ± 3.4, $P < 0.0001$). Multivariate logistic regression analysis demonstrated the incidence of the severe multiorgan dysfunction, measured by SOFA maximum ≥11, was dramatically reduced in the PST group (OR, 0.68, 95% CI 0.50–0.92, $P = 0.013$). Furthermore, preoperative statin therapy (PST) might be associated with a decreased risk of postoperative major adverse cardiovascular and cerebral events and acute kidney injury, but an increased risk of postoperative hepatic inadequacy. *Conclusion*. SOFA maximum was significantly lower in the PST group compared with the control group and the incidence of the severe multiorgan dysfunction was dramatically reduced in the PST group. The findings of this study might shed new light on questions of positive or negative effects of PST on multiple organ function after high-risk CABG, so as to ultimately improve high-risk patient in-hospital outcomes from CABG.

1. Background

Previous studies demonstrated that the incidence of death in coronary artery bypass grafting (CABG) ranges from 2.94 to 32.5 according to different surgical severity and population [1, 2]. Therefore, it is essential to develop prognostic models for accurately identify mortality and morbidity after isolated CABG, especially high-risk CABG. Research data proved that the Sequential Organ Failure Assessment (SOFA) score per se as an independent risk factor for mortality after CABG and SOFA could be regarded as the most effective prognostic model for guiding the use of preventive and early therapeutic strategies to reduce mortality and morbidity for patients undergoing high-risk CABG [2, 3]. Recently, SOFA maximum is recommended to assess multiorgan dysfunction over time and severe multiorgan failure, measured by SOFA maximum greater than 11, predicted an in-hospital mortality of 95% [3, 4].

Preoperative statin therapy (PST) is known to be the most effective medications for cardiac surgical patients with hyperlipidemia [5]. However, the beneficial or detrimental effects of PST on cardiovascular and cerebral vascular, renal, respiratory and liver function in patients undergoing isolated high-risk CABG are still unclear. Results from studies that investigated the effects of PST on postoperative organ dysfunction are also controversial [6–9]. More importantly, previous studies only focused on one single organ, and no studies demonstrated the relationship between PST and

severe multiple organ dysfunction after high-risk CABG. This may be ascribed to the lack of effective and comprehensive prediction models to evaluate postoperative multiple organ dysfunction. The appearance of SOFA maximum can solve this problem.

To fill the above knowledge gap, we systematically assessed the multiorgan function in high-risk patients undergoing isolated CABG in Beijing Anzhen hospital. The purpose of this cohort study was to investigate the independent relationship between PST and postoperative severe multiorgan failure, measured by SOFA maximum greater than 11, in high-risk patients undergoing isolated CABG and also examine the direct correlation between PST and the incidence of cardiac and cerebral vascular, respiratory, liver as well as renal postoperative complications. Recognition of the association and determinants of PST on postoperative multiple organ dysfunction should lead to strategies to improve the prognosis of patients undergoing elective high-risk CABG.

2. Methods

2.1. Study Population and Clinical Data. The present study is a perspective, single-center, cohort analysis enrolling high-risk patients undergoing CABG from Jan 1, 2018, to Dec 31, 2018, in Beijing Anzhen hospital. High-risk was defined as CHD patients with euroscore II of 6% or more. Patients received 20 mg of atorvastatin per day before CABG was included in the PST group. Patients undergoing CABG combined with other open-chest surgeries such as valvular repair or replacement were excluded. The gathered data included the main baseline clinical, echocardiographic, and procedural characteristics. Two reviewers (D.L. and W.Y.) independently extracted the above information. Informed consent was obtained from each patient on the day of admission. The ethical review and informed consent of this study were approved by the institutional ethics committee of Beijing Anzhen Hospital, Capital Medical University.

2.2. Definitions and Study Endpoints. Endpoints were: (1) in-hospital adverse outcomes defined as in the Society of Thoracic Surgeons (STS) national database [10]. The specific definitions are located on the STS website (http://www.sts.org/registries-research-center/sts-national-database/adult-cardiac-surgery-database/data-collection). A composite endpoint of in-hospital major adverse cardiovascular and cerebral events (MACCE) and the STS-defined variables of major morbidity were utilized; (2) in-hospital SOFA maximum: sequential assessment of in-hospital organ dysfunction is a good indicator of prognosis and SOFA, which is assessed in all patients after CABG every day, can help assess organ dysfunction or failure over time and are useful to evaluate morbidity [11]. The highest in-hospital SOFA score, namely SOFA maximum, of greater than 11 predicted a mortality of 95% [11, 12].

2.3. Statistical Analysis. Statistical analysis was performed using the SPSS version 25.0 statistical software (IBM Corporation, Armonk, New York, USA). Baseline characteristics were compared between the patients with major bleeding and without major bleeding. Continuous variables were expressed as mean value ± standard deviation and compared by the Student's *t*-test if normally distributed and otherwise as median (minimum, maximum) and compared by the Wilcoxon rank sum test. Categorical variables are expressed as percentages and were compared by the $\chi 2$ statistic or continuity-correction $\chi 2$ when cell counts were <5 or Fisher's exact test when cell counts were <1.

We used multivariable logistic regression analysis to investigate the association between PST and the incidence of SOFA maximum greater than 11, in-hospital postoperative MACCE, acute kidney injury (AKI), hepatic inadequacy, and infection adjusting for potential confounding factors. Forward stepwise selection was used to identify significant confounding variables. Potential confounders that had been reported in previous studies as important determinants of perioperative outcomes would be offered to the logistic regression models including: age, female gender, body mass index (BMI), diabetes, hypertension, prior MI, prior transient ischemic attacks (TIA) or cerebral vascular accident (CVA), current smoker, hypercholesterolaemia, previous peripheral vascular diseases (PVD), previous atrial fibrillation (AF), previous chronic obstructive pulmonary diseases (COPD), ventricular aneurysm, emergency CABG, decreased left ventricular ejection fraction (LVEF), euroscore II, duration of operation, off Pump CABG, New York Heart Association (NYHA) Functional Classification, and drugs before CABG. And closely associated factors ($P < 0.05$) from the univariate analysis were also included in the multivariable logistic regression analysis ($P < 0.05$ was retention criterion for each factor). Power of the association between risk factors and outcomes was expressed as odds ratio (OR).

All P values are 2-sided. Results were considered to be statistically significant at a $P < 0.05$.

3. Results

3.1. Demographic and Perioperative Characteristics. Among a total of 880 high-risk patients undergoing isolated CABG included in this study, 503 (57.2%) experienced statin therapy before CABG. The mean time of PST was 5.2 months. Baseline, procedural, and discharge data for the patients are shown in Table 1. Compared with the control group, significantly higher proportions of patients with PST had presented with the following clinical characteristics at hospital admission: male sex (69.2% v 46.9%, $P < 0.0001$), moderate and poor LVEF (48.1% v 40.8%, $P = 0.028$), lower euroscore II (8.7 ± 4.4 v 8.8 ± 2.9, $P = 0.033$), hypertension (59.8% v 43.0%, $P < 0.0001$), diabetes (56.1% v 23.6%, $P < 0.0001$), previous MI (16.9% v7.0%, $P < 0.0001$), preoperative angiotensin-converting enzyme inhibitors (ACEI)/angiotensin receptor blockers (ARB) (22.3% v 14.1%, $P = 0.002$), and preoperative beta-blocker therapy (58.6% v 41.4%, $P < 0.0001$). On the contrary, the proportion of patients undergoing CABG without cardiopulmonary bypass was significantly lower in the PST group. Other characteristics were comparable in the two groups.

Table 1: Characteristics of study population.

Variable	Preoperative statin therapy		P-value
	YES ($n = 503$)	NO ($n = 377$)	
Demographics			
Age, mean (SD), y	65.4 (7.6)	64.8 (10.9)	0.349
Male sex, n (%)	348 (69.2)	177 (46.9)	<0.0001
BMI, mean (SD), kg/m2	25.1 (2.9)	24.7 (3.4)	0.061
BMI≥30, n (%)	24 (4.8)	25 (6.6)	0.239
Medical history			
Hypertension, n (%)	301 (59.8)	162 (43.0)	<0.0001
HLP, n (%)	250 (49.7)	112 (29.7)	<0.0001
Diabetes mellitus, n (%)	282 (56.1)	89 (23.6)	<0.0001
Smoker, n (%)	149 (29.6)	91 (24.1)	0.079
COPD, n (%)	51 (10.1)	35 (9.3)	0.731
PVD, n (%)	116 (23.1)	72 (19.1)	0.116
Previous MI, n (%)	85 (16.9)	26 (7.0)	<0.0001
Previous CVA, n (%)	36 (7.2)	18 (4.8)	0.158
Previous AF, n (%)	68 (13.6)	62 (16.4)	0.250
LVEF, mean (SD), %	50.6 (15.1)	53.3 (19.1)	0.018
Moderate and poor LVEF (<50%) n (%)	242 (48.1)	154 (40.8)	0.028
Ventricular aneurysm, n (%)	53 (10.5)	25 (6.6)	0.055
Status			
Urgent CABG, n (%)	21 (4.2)	26 (6.9)	0.053
Euroscore II	8.7 (4.4)	8.8 (2.9)	0.033
NYHA class, n (%)			
II	310 (61.6)	255 (67.6)	0.076
III and IV	193 (38.4)	122 (32.4)	0.076
Off-pump	246 (48.9)	222 (58.9)	0.003
Duration of operation mean (SD), h	4.1 (6.4)	4.7 (1.4)	<0.0001
Medication at discharge			
ACEI/ARB	112 (22.3)	53 (14.1)	0.002
CCB	64 (12.7)	40 (10.6)	0.345
Aspirin	385 (76.5)	274 (72.7)	0.209
Beta-blocker	295 (58.6)	156 (41.4)	<0.0001

CABG: coronary artery bypass grafting; LVEF: left ventricular ejection fraction; BMI: body mass index; MI: myocardial infarction TIA: prior transient ischemic attacks; CVA: cerebral vascular accident; PVD: previous peripheral vascular diseases AF: previous atrial fibrillation; COPD: previous chronic obstructive pulmonary diseases; NYHA : New York Heart Association; ACEI: angiotensin-converting enzyme inhibitors; ARB: angiotensin receptor blockers; CCB: calcium channel blockers.

3.2. In-Hospital Outcomes. In-hospital outcomes for the two groups are shown in Table 2. The SOFA maximum was significantly lower in the PST group compared with the control group (7.8 ± 3.0 v 9.2 ± 3.4, $P < 0.0001$, Table 2). In addition, the proportion of patients with SOFA maximum greater than 11 was also significantly lower in the PST group. With respect to other secondary clinical outcomes, the rate of in-hospital MACCE, especially nonfatal stroke, acute kidney injury (AKI), and noninvasive ventilator, was significantly lower in patients with PST than in controls (Table 2). On the contrary, the rate of hepatic inadequacy postinfection was higher in the PST group (Table 2).

3.3. Multivariate Logistic Regression Analysis on Severe Multiorgan Dysfunction (Primary Endpoint). Multivariate logistic regression analysis on the total patients demonstrated the incidence of the severe multiorgan dysfunction, measured by SOFA maximum ≥11, was dramatically reduced in the PST group (OR, 0.68, 95% CI 0.50–0.92, $P = 0.013$, Table 3). On the contrary, female gender (OR, 1.93, 95% CI 1.43–2.60, $P < 0.0001$), higher euroscore II (OR,1.05, 95% CI 1.01–1.09, $P = 0.012$), hypertension (OR, 1.40, 95% CI 1.02–1.87, $P = 0.021$), previous MI (OR, 1.99, 95% CI 1.30–3.04, $P = 0.002$), NYHA class III and IV (OR, 1.58, 95% CI 1.17–2.13, $P = 0.003$), moderate and poor LVEF (OR, 2.38, 95% CI 1.76–3.21, $P < 0.0001$), emergency CABG (OR, 5.64, 95% CI 3.02–10.56, $P < 0.0001$), off Pump CABG (OR, 1.36, 95% CI (1.46–1.92), $P = 0.044$), and longer duration of surgery (OR, 1.68, 95% CI 1.46–1.92, $P < 0.0001$) (Table 3) were the independent risk factors for severe multiorgan dysfunction.

3.4. Multivariate Logistic Regression Analysis on In-Hospital MACCE. Multivariate logistic regression analysis on the total patients demonstrated PST (OR, 0.60, 95% CI 0.44–0.81, $P = 0.001$) may be associated with a decreased risk of in-hospital MACCE (Table 4). Besides, hypertension, higher euroscore II, current smoker, previous MI, previous TIA or CVA, previous AF, previous COPD, moderate and poor LVEF, NYHA III and IV, and longer duration of surgery were the independent risk factors for in-hospital MACCE (Table 4).

Table 2: In-hospital outcomes.

	PST ($n = 503$)	No PST ($n = 377$)	P value
SOFA Maximum ≥11	24.3% (122/503)	34.2% (129/377)	$P = 0.002$
SOFA maximum	7.8 (3.0)	9.2 (3.4)	$P < 0.0001$
MACCE	27.6% (139/503)	35.0% (132/377)	$P = 0.022$
In-hospital mortality	1.2% (6/503)	1.1% (4/377)	$P = 1.000$
Nonfatal MI	4.6% (23/503)	4.8% (18/377)	$P = 1.000$
Nonfatal stroke	0.6% (3/503)	2.9% (11/377)	$P = 0.011$
New-onset AF	18.1% (91/503)	22.8% (86/377)	$P = 0.090$
New-onset VA	6.6% (33/503)	11.4% (43/377)	$P = 0.015$
Perioperative IABP	13.3% (67/503)	11.9% (45/377)	$P = 0.610$
Perioperative ECMO	0.6% (3/503)	0.0% (0/377)	$P = 0.265$
Reoperation	3.6% (18/503)	1.9% (7/377)	$P = 0.153$
After infection	17.3% (87/503)	9.0% (34/377)	$P < 0.0001$
Pulmonary infection	16.1% (81/503)	9.0% (34/377)	$P = 0.002$
After bloodstream infection	1.2% (6/503)	0.0% (0/377)	$P = 0.040$
AKI	5.4% (27/503)	2.7% (10/377)	$P < 0.0001$
CRRT	2.8% (14/503)	1.3% (5/377)	$P = 0.165$
Hepatic inadequacy	17.7% (89/503)	12.5% (47/377)	$P = 0.047$
Hypoxemia	8.5% (43/503)	6.1% (23/377)	$P = 0.197$
Noninvasive ventilator	2.0% (10/503)	8.0% (30/377)	$P < 0.0001$
Reintubation	2.2% (11/503)	2.4% (9/377)	$P = 1.000$
Tracheotomy	1.0% (5/503)	0.0% (0/377)	$P = 0.075$
ICU stay (Day)	2.7 (3.7)	2.4 (1.8)	$P = 0.130$
Postoperative hospital stay (Day)	8.1 (5.4)	8.0 (3.0)	$P = 0.839$
Cost (RMB)	138,636.1 (62,142.1)	137,345.6 (36,636.8)	$P = 0.720$

MACCE: major adverse cardiovascular and cerebral events; MI: myocardial infarction; AF: previous atrial fibrillation; AKI: acute kidney injury; CRRT: continuous renal replacement therapy; IABP: intra-aortic balloon pump; ECMO: extracorporeal membrane oxygenation; SOFA : Sequential Organ Failure Assessment; VA: ventricular arrhythmias; PST: preoperative statin therapy.

Table 3: Independent risk factors for SOFA maximum greater than 11.

	Maximum SOFA score >11		X^2/t	P value	Multivariate analysis OR (95% CI)	P value
	YES ($n = 251$)	NO ($n = 629$)				
PST	122 (48.6)	381 (60.6)	10.5	0.013	0.68 (0.50–0.92)	0.013
ACE inhibitor or ARB	56 (22.3)	109 (17.3)	2.9	0.104		
Advanced age	66.0 ± 7.7	64.8 ± 9.6	1.8	0.070		
Female gender	130 (51.8)	225 (35.8)	19.1	<0.0001	1.93 (1.43–2.60)	<0.0001
Euroscore II	9.5 ± 3.8	8.5 ± 3.8	3.5	<0.0001	1.05 (1.01–1.09)	0.012
BMI	24.8 ± 3.2	25.0 ± 3.1	0.63	0.531		
Hypertension	149 (59.4)	314 (49.9)	6.4	0.014	1.40 (1.02–1.87)	0.021
Current smoker	61 (24.39)	179 (28.5)	1.6	0.241		
Previous MI	46 (18.3)	65 (10.3)	10.4	0.002	1.99 (1.30–3.04)	0.002
Previous TIA or CVA	9 (3.6)	45 (7.2)	3.9	0.061		
Hypercholesterolaemia	115 (45.8)	247 (39.3)	3.2	0.081		
Previous AF	39 (15.5)	91 (10.3)	0.16	0.675		
Previous COPD	17 (6.8)	69 (11.0)	3.6	0.060		
Ventricular aneurysm	20 (8.0)	51 (8.1)	0.35	0.601		
Moderate and poor LVEF (<50%)	152 (60.6)	244 (38.8)	33.5	<0.0001	2.38 (1.76–3.21)	<0.0001
NYHA III and IV	109 (43.4)	206 (32.8)	8.90	0.003	1.58 (1.17–2.13)	0.003
Emergency CABG	31 (12.4)	16 (2.5)	34.1	<0.0001	5.64 (3.02–10.56)	<0.0001
Off pump	147 (58.6)	321 (51.0)	4.1	0.044	1.36 (1.01–1.82)	0.044
Duration of operation (hours)	4.8 ± 1.5	4.2 ± 0.8	157.2	<0.0001	1.68 (1.46–1.92)	<0.0001

PST: preoperative statin therapy; CABG: coronary artery bypass grafting; LVEF: left ventricular ejection fraction; BMI: body mass index; MI: myocardial infarction; TIA: prior transient ischemic attacks; CVA: cerebral vascular accident; PVD: previous peripheral vascular diseases; DM: diabetes mellitus; NYHA : New York Heart Association; ACEI: angiotensin-converting enzyme inhibitors; ARB: angiotensin receptor blockers.

3.5. Multivariate Logistic Regression Analysis on AKI. Multivariate logistic regression analysis on the total patients demonstrated that PST (OR, 0.25, 95% CI 0.12–0.54, $P < 0.0001$) and ACE inhibitor or ARB may be associated with a decreased risk of postoperative AKI (Table 5). Besides, current smoker, emergency CABG, and longer duration of surgery were the independent risk factors for postoperative AKI (Table 5).

Table 4: Independent risk factors for in-hospital MACCE.

	In-hospital MACCE		X^2/t	P value	Multivariate analysis OR (95% CI)	P value
	YES ($n = 271$)	NO ($n = 609$)				
PST	139 (51.3)	364 (59.8)	5.5	0.022	0.60 (0.44–0.81)	0.001
ACE inhibitor or ARB	48 (17.7)	117 (19.2)	0.3	0.641		
Female gender	117 (43.2)	238 (39.1)	1.3	0.265		
Euroscore II	9.2 ± 5.0	8.6 ± 3.2	26.7	<0.0001	1.04 (1.01–1.08)	0.018
BMI	25.5 ± 3.8	24.7 ± 3.7	1.7	0.193		
Hypertension	178 (65.7)	285 (46.8)	26.8	<0.0001	2.41 (1.78–3.28)	<0.0001
DM	111 (41.0)	260 (42.7)	0.2	0.658		
Current smoker	96 (34.7)	144 (23.6)	13.1	<0.0001	1.60 (1.16–2.21)	0.004
Previous MI	45 (16.6)	66 (10.8)	5.7	0.021	1.57 (1.03–2.40)	0.035
Previous TIA or CVA	29 (10.7)	25 (4.1)	14.2	<0.0001	2.81 (1.60–4.93)	<0.0001
Previous PVD	67 (24.7)	124 (20.4)	2.1	0.157		
Hypercholesterolaemia	110 (40.1)	252 (41.2)	0.05	0.882		
Previous AF	55 (20.3)	75 (12.3)	9.5	0.003	1.67 (1.12–2.45)	0.011
Previous COPD	38 (14.0)	48 (7.9)	8.0	0.001	2.28 (1.42–3.66)	0.001
Ventricular aneurysm	19 (7.0)	59 (9.7)	1.7	0.247		
Moderate and poor LVEF (<50%)	146 (53.9)	250 (41.1)	12.4	0.001	1.79 (1.33–2.42)	<0.0001
NYHA III and IV	115 (42.4)	200 (32.8)	7.5	0.008	1.57 (1.17–2.11)	0.003
Emergency CABG	18 (6.6)	29 (4.8)	1.3	0.258		
Off pump	137 (56.5)	331 (49.8)	1.1	0.306		
Duration of operation (hours)	4.5 ± 1.3	4.3 ± 1.0	1.1	0.003	1.16 (1.02–1.32)	0.022

PST: preoperative statin therapy; CABG: coronary artery bypass grafting; MACCE: major adverse cardiovascular and cerebral events; LVEF: left ventricular ejection fraction; BMI: body mass index; MI: myocardial infarction; TIA: prior transient ischemic attacks; CVA: cerebral vascular accident; PVD: previous peripheral vascular diseases; DM: diabetes mellitus; NYHA : New York Heart Association, ACEI: angiotensin-converting enzyme inhibitors; ARB: angiotensin receptor blockers.

Table 5: Independent risk factors for AKI.

	AKI		X^2/t	P value	Multivariate analysis OR (95% CI)	P value
	YES ($n = 37$)	NO ($n = 843$)				
PST	10 (27.0)	493 (58.5)	14.3	<0.0001	0.25 (0.12–0.54)	<0.0001
ACE inhibitor or ARB	1 (2.7)	164 (19.5)	6.5	0.008	0.14 (0.02–1.01)	0.052
Female gender	17 (45.9)	338 (40.1)	0.5	0.497		
Euroscore II	7.8 ± 1.2	8.8 ± 3.9	17.7	0.112		
BMI	25.5 ± 3.3	24.9 ± 3.1	0.05	0.247		
Hypertension	24 (64.9)	439 (52.1)	2.3	0.134		
Current smoker	25 (67.6)	215 (25.5)	31.6	<0.0001	6.98 (3.40–14.34)	<0.0001
Previous MI	5 (13.5)	106 (12.6)	0.03	0.801		
Previous TIA or CVA	0 (0.0)	54 (6.4)	2.5	0.161		
Previous PVD	8 (21.6)	183 (21.7)	0.01	1.000		
Previous AF	7 (20.3)	123 (12.3)	0.5	0.476		
Previous COPD	5 (13.5)	81 (9.6)	0.6	0.397		
Ventricular aneurysm	19 (7.0)	59 (9.7)	1.7	0.247		
Moderate and poor LVEF (<50%)	15 (53.9)	381 (41.1)	0.3	0.615		
Emergency CABG	6 (16.2)	41 (4.9)	9.0	0.011	3.79 (1.50–9.58)	0.005
Off pump	24 (64.9)	444 (52.7)	2.1	0.178		
Duration of operation (hours)	6.9 ± 1.7	4.2 ± 0.9	44.5	<0.0001	4.47 (3.27–6.12)	<0.0001

PST: preoperative statin therapy; CABG: coronary artery bypass grafting; LVEF: left ventricular ejection fraction; BMI: body mass index; MI: myocardial infarction; TIA: prior transient ischemic attacks; CVA: cerebral vascular accident; PVD: previous peripheral vascular diseases; DM: diabetes mellitus; NYHA : New York Heart Association; ACEI: angiotensin-converting enzyme inhibitors; ARB: angiotensin receptor blockers.

3.6. Multivariate Logistic Regression Analysis on In-Hospital Hepatic Inadequacy. Multivariate logistic regression analysis on the total patients revealed that the independent risk factors for in-hospital hepatic inadequacy were PST (OR, 1.49, 95% CI 1.01–2.18, $P = 0.042$), hypertension, previous MI, previous PVD, and previous COPD. Besides, female gender and off Pump CABG may be associated with a decreased risk of in-hospital hepatic inadequacy (Table 6).

3.7. Independent Risk Factors for Postoperative Infection. Multivariate logistic regression analysis on the total patients revealed that the independent risk factors for postoperative

Table 6: Independent risk factors for In-hospital hepatic inadequacy.

	In-hospital hepatic inadequacy		X^2/t	P value	Multivariate analysis OR (95% CI)	P value
	YES ($n = 136$)	NO ($n = 739$)				
PST	89 (65.4)	414 (56.3)	4.2	0.047	1.49 (1.01–2.18)	0.042
Female gender	35 (25.7)	315 (42.6)	13.7	<0.0001	0.47 (0.31–0.72)	<0.0001
Euroscore II	9.4 ± 5.4	9.0 ± 3.5	2.0	0.050		
BMI	24.2 ± 2.9	25.0 ± 3.1	−2.5	0.357		
Hypertension	93 (68.4)	365 (49.4)	16.6	<0.0001	2.18 (1.48–3.23)	<0.0001
Current smoker	40 (29.4)	200 (27.1)	0.3	0.601		
Previous MI	25 (18.4)	86 (11.6)	4.7	0.035	1.72 (1.05–2.80)	0.030
Previous TIA or CVA	8 (5.9)	46 (6.0)	0.02	1.000		
Previous PVD	40 (29.4)	151 (20.4)	5.4	0.024	1.63 (1.08–2.46)	0.020
Previous COPD	34 (25.0)	47 (6.9)	47.5	<0.0001	4.09 (2.47–6.78)	<0.0001
Ventricular aneurysm	7 (5.1)	71 (9.6)	2.8	0.103		
NYHA III and IV	51 (37.5)	264 (35.7)	0.2	0.698		
Emergency CABG	5 (3.7)	42 (5.7)	0.9	0.413		
Off pump	51 (37.5)	415 (56.2)	16.1	<0.0001	0.59 (0.40–0.87)	0.008
Duration of operation (hours)	4.5 ± 1.4	4.3 ± 1.0	1.8	0.079		

PST : Preoperative statin therapy; CABG: coronary artery bypass grafting; LVEF: left ventricular ejection fraction; BMI: body mass index; MI: myocardial infarction; TIA: prior transient ischemic attacks; CVA: cerebral vascular accident; PVD: previous peripheral vascular diseases; DM: diabetes mellitus COPD: previous chronic obstructive pulmonary diseases; NYHA : New York Heart Association.

Table 7: Independent risk factors for postoperative infection.

	Postoperative infection		X^2/t	P value	Multivariate analysis OR (95% CI)	P value
	YES ($n = 121$)	NO ($n = 759$)				
PST	87 (71.9)	416 (54.8)	12.5	< 0.0001	2.09 (1.42–3.08)	<0.0001
Female gender	42 (34.7)	313 (41.2)	1.8	0.195		
Euroscore II	8.4 ± 3.9	8.8 ± 3.8	−1.1	0.275		
BMI	25.5 ± 3.3	24.8 ± 3.1	2.3	0.024	1.07 (1.01–1.14)	0.024
Hypertension	68 (56.2)	395 (52.0)	0.7	0.433		
DM	70 (57.9)	301 (39.7)	14.2	<0.0001	1.76 (1.17–2.65)	0.007
Previous MI	26 (21.5)	85 (11.2)	10.0	0.003	1.73 (1.03–2.89)	0.038
Previous TIA or CVA	11 (9.1)	43 (5.7)	2.1	0.153		
Previous PVD	22 (18.2)	169 (22.3)	1.0	0.344		
Hypercholesterolaemia	43 (35.5)	319 (42.0)	1.8	0.196		
Previous AF	18 (14.9)	112 (14.8)	0.001	1.000		
Previous COPD	6 (5.0)	80 (10.5)	3.7	0.068		
Ventricular aneurysm	30 (24.8)	48 (6.3)	44.1	<0.0001	4.44 (2.65–7.43)	<0.0001
Moderate and poor LVEF (<50%)	72 (59.5)	324 (42.3)	11.6	0.001	1.82 (1.22–2.72)	0.003
NYHA III and IV	69 (57.0)	256 (33.7)	27.5	<0.0001	2.60 (1.75–3.86)	<0.0001
Emergency CABG	13 (10.7)	34 (44.8)	8.1	0.008	2.02 (1.01–4.04)	0.047
Off pump	63 (52.1)	405 (53.4)	0.07	0.845		
Duration of operation (hours)	4.2 ± 1.0	4.4 ± 1.1	−1.1	0.305		

PST: preoperative statin therapy; CABG: coronary artery bypass grafting; LVEF: left ventricular ejection fraction; BMI: body mass index; MI: myocardial infarction; TIA: prior transient ischemic attacks; CVA: cerebral vascular accident; PVD: previous peripheral vascular diseases; DM: diabetes mellitus; NYHA : New York Heart Association.

infection were PST (OR, 2.09, 95% CI 1.42–3.08, $P < 0.0001$), BMI, DM, previous MI, a history of ventricular aneurysm, moderate and poor LVEF, NYHA III and IV, and emergency CABG (Table 7).

4. Discussion

This is the first study to prospectively explore the independent association between PST and postoperative severe multiorgan failure, measured by SOFA maximum greater than 11, in high-risk patients undergoing isolated CABG. Besides, we also examined the direct correlation between PST and the incidence of cardiac and cerebral vascular, respiratory, liver as well as renal postoperative complications. Our key findings are: (1) the SOFA maximum was significantly lower in the PST group compared with the control group and multivariate logistic regression analysis on the total patients demonstrated the incidence of the severe multiorgan dysfunction, measured by SOFA maximum ≥11, was dramatically reduced in the PST group; (2) PST might be associated with a decreased risk of postoperative MACCE and AKI, but an increased risk of postoperative hepatic inadequacy. Respiratory complications, such as hypoxemia, reintubation, and tracheotomy,

were comparable in the PST and control groups; (3) PST was also independently associated with postinfection.

Although statin is used in a large proportion of patients before surgery, its potential impact on postoperative multiorgan function is still incompletely understood. With respect to renal function, Singh et al. found that PST reduced the CRRT and cardiac mortality significantly but exerted no effects on the incidence of AKI after CABG. [6] Wang et al. also demonstrated that PST may not reduce the risk of AKI in patients following isolated CABG. [13] On the contrary, Layton found that statin therapy immediately before CABG may modestly reduce the incidence of postoperative AKI, particularly in younger CABG patients. [14] Our previous evidence-based study including 59,771 patients also confirmed that PST significantly reduced the risk for postoperative AKI regardless of the types of diagnosis and staging criteria in cardiac surgical patients. [7] In addition, the cardiovascular protective effects of PST have already been well recognized. Knatterud et al. proved that PST delayed the progression of atherosclerosis and further reduced the risk for postoperative cardiovascular events in coronary heart disease patients following revascularization. [15] Furthermore, the uncertain safety of statin on liver function in high-risk CABG remains a major concern, and studies on statin-induced hepatotoxicity after high-risk CABG are sparse. A review preliminarily revealed the hepatotoxicity of statins and other lipid-lowering drugs. [16] They demonstrated that both simvastatin and atorvastatin have been correlated with more than 50 case reports of liver dysfunction and other statins have been implicated in this type of liver dysfunction as well. Another research found the association between dose escalation of atorvastatin and acute liver failure. [17] However, the adverse effects of PST on liver outcomes among cardiac surgical populations still need to be investigated further. Besides, respiratory complications after CABG are common, with an occurrence of 10 to 25% [18]. However, the independent relationship between PST and respiratory complications has not been confirmed because of a paucity of data [8]. Relevant high-quality prospective studies are still essential. Last but not least, neurologic complications, especially stroke, are associated with increased mortality and longer hospitalization [19]. However, previous research found that PST was not associated with a decreased risk for stroke and encephalopathy after high-risk CABG [20, 21]. The current research demonstrated that PST might be associated with a decreased risk of postoperative MACCE and AKI, but an increased risk of postoperative hepatic inadequacy. Respiratory complications, such as hypoxemia, reintubation, and tracheotomy, were comparable in the PST and control groups. More importantly, the incidence of the severe multiorgan dysfunction, measured by SOFA maximum ≥11, was dramatically reduced in the PST group compared with the controls.

Based on the above knowledge, PST has exerted a positive effect on cardiac, neurological, and renal function, and a negative effect on liver function after high-risk CABG. However, the association between PST and postoperative severe multiple organ dysfunction is still unknown. The current study first demonstrated that SOFA maximum was significantly lower in the PST group compared with the control group and the incidence of the severe multiorgan dysfunction, measured by SOFA maximum ≥11, was dramatically reduced in the PST group. The benefits of PST for cardiac dysfunction and mortality after high-risk CABG have been well established [8]. Recently, researchers have demonstrated that PST may benefit not only cardiac but also renal, neurological, and respiratory function. The pathophysiological mechanisms underlying the positive effects of PST on neurological, respiratory, and renal function might be closely related to the non-lipid-lowering activities of statins [22]. First of all, inflammation during CABG is reported to be a potential cause of organ dysfunction [23]. Previous studies proved PST could increase the release of anti-inflammation cytokines and reduce the levels of proinflammatory mediators, such as interleukin-6, interleukin-8, and tumor necrosis factor-α [9, 14]. In addition, ischemia-reperfusion injury as well as endothelial dysfunction are reported to be both independent associated with an increased risk of multiple organ dysfunction in patients undergoing high-risk CABG, especially on-pump surgery [24]. The pleiotropic effects of PST also include improvement in endothelial function and attenuation of reperfusion injury, which can decrease the risk of multiple organ dysfunction directly after CABG and further improve the prognosis of surgery [25, 26]. Recently, a high-quality prospective report confirmed that preoperative high-dose atorvastatin therapy could protect myocardium in patients following coronary revascularization by decreasing the risk of ischemia-reperfusion injury and endothelial damage during surgery [27]. Besides, the beneficial impacts of PST on multiple organ dysfunction might also be attributed to the following activity of statins: antithrombosis [7]. The above positive effects of PST on multiple organ function outweigh its side effects on liver function, leading to the incidence of the severe multiorgan dysfunction, measured by SOFA maximum ≥11, which was dramatically reduced in the PST group.

It is worth mentioning that the current study found that respiratory complications, such as hypoxemia, reintubation, and tracheotomy, were comparable in the PST and control groups. This might be due to the beneficial impacts of PST on respiratory dysfunction that cannot offset other etiologies-induced respiratory dysfunction [28]. Specifically, all of the prolonged mechanical ventilation, hypoxemia, reintubation, and tracheotomy might be regarded as a clinical endpoint of multiconditions including those that have no connection with the beneficial impacts of PST (lipid-lowering activities, anti-inflammation, antithrombosis, and improvement in endothelial function.), such as a prolonged residual anesthesia effect, a major or life-threatening bleeding as well as atelectasis. Those etiologies could offset the organ protective impacts of PST and may make the benefit of PST on respiratory function less noticeable than on a cardiac, neurological, and renal function [29, 30].

This study has several limitations. Firstly, the present study is a perspective, single-center, cohort analysis. High-quality, large-scale, and multicenter randomized controlled trials are required to further confirm the conclusion.

Secondly, although SOFA maximum is recommended to assess multiorgan dysfunction over time and severe multiorgan failure, measured by SOFA maximum greater than 11, predicted an in-hospital mortality of 95%, we still need other organ failure assessments to measure severe multiorgan failure in order to verify each other. Thirdly, we confirmed that PST increase was associated with an increased risk of other endpoints, such as noninvasive ventilator. However, the independence of the correlation still needs further verification. Last but not least, the impact of PST on the long-term multiple organ function requires further examination.

5. Conclusions

This current observational cohort analysis demonstrated that PST might be associated with a decreased risk of postoperative MACCE and AKI, but an increased risk of postoperative hepatic inadequacy. Respiratory complications were comparable in the PST and control groups. In addition, SOFA maximum was significantly lower in the PST group compared with the control group, and multivariate logistic regression analysis on the total patients demonstrated that the incidence of the severe multiorgan dysfunction, measured by SOFA maximum ≥11, was dramatically reduced in the PST group. The findings of this study might shed new light on questions of positive or negative effects of PST on multiple organ function after high-risk CABG, and can ultimately improve high-risk patient in-hospital outcomes from CABG.

Abbreviations

PST: Preoperative statin therapy
CABG: Coronary artery bypass grafting
CHD: Coronary heart disease
MACCE: Major adverse cardiovascular and cerebral events
SOFA: Sequential organ failure assessment
LVEF: Left ventricular ejection fraction
BMI: Body mass index
MI: Myocardial infarction
TIA: Prior transient ischemic attacks
CVA: Cerebral vascular accident
PVD: Previous peripheral vascular diseases
AF: Previous atrial fibrillation
COPD: Previous chronic obstructive pulmonary diseases
NYHA: New York Heart Association
AKI: Acute kidney injury
CRRT: Continuous renal replacement therapy
IABP: Intra-aortic balloon pump
ECMO: Extracorporeal membrane oxygenation
ACEI: Angiotensin-converting enzyme inhibitors
ARB: Angiotensin receptor blockers.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Dr. Jiayang Wang and Prof. Yujie Zhou designed the current study, and drafted the manuscript. Dr. Wen Yuan and Dr. Dong Liu independently extracted the information from the eligible studies. Besides, Dr. Kui Zhang performed the statistical analysis. Dr. Nan Liu participated in the quality assessment.

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Research Article

The Peroxisome Proliferator-Activated Receptor γ Agonist Pioglitazone Protects Vascular Endothelial Function in Hypercholesterolemic Rats by Inhibiting Myeloperoxidase

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Objective. Hypercholesterolemia- (HC-) induced endothelial dysfunction is the first step of atherogenesis, and the peroxisome proliferator-activated receptor γ (PPARγ) has been reported to attenuate atherosclerosis formation; however, the underlying mechanisms are not fully understood. The present study was designed to determine whether myeloperoxidase (MPO) mediates HC-induced endothelial dysfunction and the role of the PPARγ agonist pioglitazone (PIO) in attenuating endothelial dysfunction. *Methods*. Male Wistar rats were fed with normal or high cholesterol diets for 8 weeks. HC rats were randomized to receive dapsone (DDS, the MPO inhibitor) during the last 6 days or PIO for the remaining 4 weeks. Vascular endothelial function was determined by comparing vasorelaxation to ACh, an endothelium-dependent vasodilator, and SNP, an endothelium-independent vasodilator in vascular rings in vitro. The vascular MPO activity, NO_x content, and cGMP level were measured by the MPO activity assay kit, NO assay kit, and cGMP RIA kit. *Results*. Compared with rats fed with normal diet, endothelium-dependent vasodilation, NO_x content, and cGMP level were decreased, and MPO activity was increased in thoracic aortas of rats fed with HC diet. There was a negative correlation between vascular endothelial function, NO_x content or cGMP level, and MPO activity. PIO obviously reduced the MPO activity, increased NO_x content and cGMP level, and improved endothelium-dependent vasodilation function in HC rats, which was essentially the same as that seen with DDS. And, there was a negative correlation between vascular endothelial function, NO_x content or cGMP level, and MPO activity in the HC group and the PIO intervention group. *Conclusion*. MPO might provoke vascular endothelial dysfunction in hypercholesterolemic rats by reducing the NO biological activity and impairing the NO/cGMP/cGK signaling pathway. PIO might inhibit vascular MPO activity and increase NO bioavailability with the net result of reversing endothelial dysfunction.

1. Introduction

Coronary artery disease (CAD) becomes one of the most important diseases that affect longevity and survival quality of aging [1]. Endothelial dysfunction is the first stage in the progression of atherogenesis [2], and hypercholesterolemia is one of the most important causes of endothelial dysfunction [3]. The mechanism of vascular endothelial dysfunction caused by hypercholesterolemia is complex, in which a decrease in the bioavailability of nitric oxide (NO) [4] and impaired NO/cGMP/cGK signaling are considered important contributory mechanisms [5]. Therefore, if the

cause responsible for decreased NO bioavailability in hypercholesterolemia is determined and then blocked, it is thought that vascular endothelial function could be effectively maintained, thereby reducing the occurrence of atherosclerosis.

Myeloperoxidase (MPO) is an oxidase that is stored in azurophilic granules of neutrophils and monocytes, which is released extracellularly during inflammation [6]. MPO plays an important role in the formation and development of many diseases, including atherosclerosis [7]. Studies have shown [8] that MPO is abundantly accumulated in the basement membrane under the vascular endothelium in hypercholesterolemia, and it is speculated that it may lead to endothelial dysfunction by the precipitation of NO. However, the specific mechanism of action of MPO remains to be elucidated.

Upon activation of peroxisome proliferator-activated receptor γ (PPARγ), it plays a crucial role in the regulation of inflammation and improvement of vascular endothelial function [9], thereby regulating MPO gene expression [10]. However, whether PPARγ agonists can restore NO bioavailability by regulating MPO, thereby improving vascular endothelial function and delaying the progression of atherogenesis in hypercholesterolemia, have not been confirmed.

Therefore, the aims of this investigation were as follows: first, to verify that vascular endothelial dysfunction is caused by a decrease in NO bioavailability in hypercholesterolemia, and on this basis, to observe and analyze whether MPO directs endothelial dysfunction in hypercholesterolemia by affecting the vascular NO/cGMP/cGK signaling pathway. We also aimed to further observe whether PPARγ agonists could reverse vascular endothelial dysfunction in hypercholesterolemia and, if possible, to determine whether or not this was related to the regulation of vascular MPO and subsequent restoration of NO bioavailability.

2. Materials and Methods

2.1. Animals. All animal procedures utilized in the investigations conformed to the Guiding Principles in the Use and Care of Animals, published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996) and were approved by the Institutional Animal Care and Use Committee of Capital Medical University.

Healthy male Wistar rats weighing 110.0 ± 10.0 g (SPF grade) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd, China. Animals were maintained in 12 h light-dark cycles, and food and water were available ad libitum. Before conducting the experiment, blood was drawn from the tail of each rat, and baseline plasma lipids were determined using assay kits (Nanjing Jiancheng Bioengineering Institute, China). Then, rats were randomly divided into two different dietary groups: the normal group ($n = 12$) was given a normal diet and the high-cholesterol (HC) group ($n = 60$) was given an HC diet, which comprised 1% cholesterol, 10% egg yolk powder, and 5% lard, for 8 weeks. Four weeks into the HC diet regimen, the rats' blood was redrawn and plasma lipid levels were determined. Thereafter, the rats in the HC group were randomly assigned to five groups: (1) HC group ($n = 16$); (2) HC + dapsone (DDS; the myeloperoxidase inhibitor, Sigma) group ($n = 16$): 100 μmol/kg/day (dissolved in 1 ml/kg DMSO) for the last 6 days, intraperitoneal injection; (3) HC + DMSO (dimethyl sulfoxide) group ($n = 6$): 1 ml/kg/day for the last 6 days, intraperitoneal injection; (4) HC + pioglitazone (PIO, Zhejiang Huayi Pharmaceutical Co., Ltd., China) ($n = 16$): 10 mg/kg/day for the remaining 4 weeks, oral gavage; (5) HC + PIO + DDS group ($n = 6$): PIO (10 mg/kg/day) via oral gavage in the remaining 4 weeks, and DDS (100 μmol/kg/day) via intraperitoneal injection in the last 6 days. At the end of the 8 weeks of the HC diet, the plasma lipid levels were determined again. All serum samples were collected from the 8 h fasted research subjects. Animals were euthanized by a physical method (decapitation, a suggested method for rodents by AVMA Guidelines on Euthanasia). All animals were euthanized with sodium pentobarbital (50 mg/kg, i.p.) to reduce animal anxiety on the guillotine and ensure euthanasia was rapidly accomplished to lessen the animal suffering. The thoracic aortic segments of all rats were collected for the detection of vascular endothelial function, MPO activity, NO content, and cGMP level.

2.2. Measurement of Vascular Endothelial Function. The thoracic aortic segments were excised and placed in ice-cold oxygenated HEPES buffer (mM: NaCl, 144; KCl, 5.8; $MgCl_2 \cdot 6H_2O$, 1.2; $CaCl_2$, 2.5; glucose, 11.1; HEPES, 5; pH 7.38–7.40), and adhering tissues were cleaned off and cut into rings (2 mm length) for the detection of vascular endothelial function. The endothelial function was determined as described previously [11]. Briefly, after the equilibration period, the artery segments were exposed to HEPES buffer containing 60 mM potassium (mM: NaCl, 144; KCl, 60; $MgCl_2 \cdot 6H_2O$, 1.2; $CaCl_2$, 2.5; glucose, 11.1; HEPES, 5; pH 7.38–7.40) until reproducible contractile responses were obtained. After washing with HEPES buffer, segments of thoracic aortas were precontracted with phenylephrine (PE, 10^{-6} mol/L, Sigma). Once a stable contraction was achieved, increasing concentrations of vasodilators were added to the chamber to obtain cumulative concentration-response curves. Endothelium-dependent dilation was measured by acetylcholine (ACh, 10^{-9}–10^{-5} mol/L, Sigma), and endothelium-independent dilation was measured by sodium nitroprusside (SNP, 10^{-10}–10^{-6} mol/L, Sigma).

2.3. Determination of Vascular MPO Activity. MPO activity in thoracic aortic tissue was measured using the MPO assay kit (Nanjing Jiancheng Bioengineering Institute, China) and calculated as U/g protein.

2.4. Detection of Total NO Content in Thoracic Aortic Tissue. NO has a short half-life and is oxidized to form NO_2 and NO_3*in vivo*. Thus, the detection of NO_x ($+NO_2 + NO_3$) concentration has been demonstrated to reflect total NO formation. The NO_x content in thoracic aortic tissue was determined using the NO assay kit (nitrate reductase

method) (Nanjing Jiancheng Bioengineering Institute, China) and calculated as nmol/mg protein.

2.5. Determination of cGMP in Thoracic Aortic Tissue. The cGMP levels in the thoracic aortic tissue were determined by [^{125}I] cGMP radioimmunoassay with commercially available kits (Shanghai Chinese Medicine University, China) and assayed for cGMP in duplicates according to the manufacturer's instructions. The results of duplicate assays were averaged. The cGMP level was calculated as pmol/mg protein.

2.6. Statistical Analysis. Data were analyzed using SPSS19.0 software. Results are presented as mean ± SD. Comparisons between groups were made using one-way analysis of variance (ANOVA) followed by the Bonferroni post hoc test. The relationship was analyzed using linear regression. Differences were considered statistically significant at a value of $P < 0.05$.

3. Results

3.1. Vascular Endothelial Dysfunction in Hypercholesterolemic Rats. After eight weeks of a high cholesterol diet, levels of serum CHO, TG, and LDL-CHO were significantly higher than those in rats being fed a normal diet (Table 1), suggesting that the hypercholesterolemic rat model was successfully built.

After administration of acetylcholine (ACh) across a dose-dependent concentration gradient of 10^{-9}–10^{-5} mol/L, the concentration-dependent vasodilatory response was seen in the thoracic aorta rings of normal diet rats (Figure 1(a)). The concentration-dependent curve induced by ACh in the thoracic aorta rings of hypercholesterolemic rats was severely shifted to the right, and the log EC_{50} of the vascular tone increased from −7.29 ± 0.16 mol/L to −6.61 ± 0.27 mol/L as compared with the normal control group ($P < 0.01$; Figure 1(c)). The maximum vasodilatation decreased from 97.88 ± 9.53% to 50.51 ± 2.44% ($P < 0.01$; Figures 1(b) and 1(d)). After administration of the exogenous NO donor sodium nitroprusside (SNP) at a cumulative concentration of 10^{-10} to 10^{-6} mol/L, there was no significant difference in the vascular tone when comparing both groups (Figures 1(a), 1(b), and 1(e)). SNPs are used to detect endothelium-independent relaxation responses of blood vessels. So, the results suggested evidence of vascular endothelial dysfunction in hypercholesterolemic rats.

3.2. The Vascular NO/cGMP/cGK Signaling Pathway Impaired and MPO Activity Decreased in Hypercholesterolemic Rats. NO is an important signaling molecule that is related to vascular endothelial function and exerts a vasodilatory effect through the NO/cGMP/cGK signaling pathway. To verify whether vascular endothelial dysfunction in hypercholesterolemic rats was associated with impairment of this signaling pathway, this study determined NO_x content and cGMP levels in vascular tissues, which reflected the biological activity of vascular NO at the cGMP level.

As compared with normal diet rats, the content of vascular NO_x in hypercholesterolemic rats had decreased from 9.61 ± 2.47 nmol/mg to 1.09 ± 0.49 nmol/mg ($P < 0.01$; Figure 2(a)), and the cGMP level had decreased from 41.94 ± 5.18 pmol/mg to 21.81 ± 2.11 pmol/mg ($P < 0.01$; Figure 2(b)). This observation suggested that the biological activity of NO in hypercholesterolemic rats had decreased, and the vascular NO/cGMP/cGK signaling pathway was impaired. At the same time, this study found that the vascular MPO activity in hypercholesterolemic rats was approximately 4.5-folds higher than that of normal diet rats (8.37 ± 1.31 U/mg vs. 38.83 ± 5.56 U/mg, $P < 0.01$; Figure 2(c)).

3.3. The MPO Activity of Vascular Tissues Negatively Correlated with Vascular Endothelial Function, NO_x Content, and cGMP Level in Hypercholesterolemic Rats. We analyzed the association of vascular endothelial function and key signaling molecules (NO_x and cGMP) of the NO/cGMP/cGK pathway with MPO activity to explore whether MPO played a role in vascular endothelial dysfunction in hypercholesterolemia. The results showed that the log EC_{50} value of ACh-induced vasodilation positively correlated with MPO activity ($r = 0.797$, $P < 0.01$; Figure 3(a)); i.e., the EC_{50} value negatively correlated with the MPO activity. Similarly, ACh-induced maximum vasodilatation showed a negative correlation with MPO activity ($r = -0.929$, $P < 0.01$; Figure 3(b)). Moreover, vascular NO_x content and the cGMP levels negatively correlated with MPO activity ($r = -0.768$, $P < 0.01$; $r = -0.955$, $P < 0.01$; Figures 3(c) and 3(d)).

3.4. After Inhibiting the MPO Activity, Vascular Endothelial Dysfunction in Hypercholesterolemic Rats Alleviated, and the Impaired Vascular NO/cGMP/cGK Pathway Improved. To further investigate the role of MPO in hypercholesterolemia-induced endothelial dysfunction, the MPO inhibitor dapsone (DDS) was administered to hypercholesterolemic rats. After DDS intervention, the vascular MPO activity in hypercholesterolemic rats decreased from 38.83 ± 5.56 U/mg to 11.92 ± 1.63 U/mg ($P < 0.01$; Figure 2(c)), which did not affect blood lipid levels of treated rats (Table 1). There was no change in the solvent DMSO group (Supplementary Figure 1(a)).

As compared with the hypercholesterolemia group, we found that after DDS was administered to hypercholesterolemic rats, the vasodilation curve had significantly shifted to the left after administration of ACh at a cumulative concentration of 10^{-9} mol/L–10^{-5} mol/L. The vascular tone log EC_{50} value had decreased from −6.61 ± 0.27 mol/L to −6.91 ± 0.11 mol/L ($P < 0.05$; Figure 1(c)), and the maximum vasodilatation increased from 50.51 ± 2.44% to 88.42 ± 3.54% ($P < 0.01$; Figure 1(d)). After administration of SNP at a cumulative concentration of 10^{-10} mol/L–10^{-6} mol/L, there was no significant difference in vascular tone when comparing both groups (Figure 1(e)). In addition, there was no

Table 1: Lipid profile in different groups (mmol/L).

Group	n	0 weeks			8 weeks		
		TC	TG	LDL-CHO	TC	TG	LDL-CHO
Normal	12	1.44 ± 0.21	0.86 ± 0.21	1.44 ± 0.18	1.39 ± 0.16	0.65 ± 0.14	1.26 ± 0.37
HC	16	1.27 ± 0.32	0.82 ± 0.33	1.31 ± 0.27	3.12 ± 0.29**	1.61 ± 0.36**	2.38 ± 0.21**
HC + DDS	16	1.32 ± 0.27	0.99 ± 0.27	1.29 ± 0.32	3.59 ± 0.38**	1.78 ± 0.39**	2.53 ± 0.45**
HC + PIO	16	1.30 ± 0.39	0.77 ± 0.31	1.37 ± 0.29	1.56 ± 0.20##	1.12 ± 0.29##	1.68 ± 0.33##

Values are expressed as mean ± SD. TC: total cholesterol; TG: triglyceride; LDL-CHO: low-density lipoprotein cholesterol; normal: normal diet group; HC: HC diet group; DDS: dapsone; PIO: pioglitazone. $^{**}P < 0.01$ vs. normal diet group; $^{\#\#}P < 0.01$ vs. HC diet group.

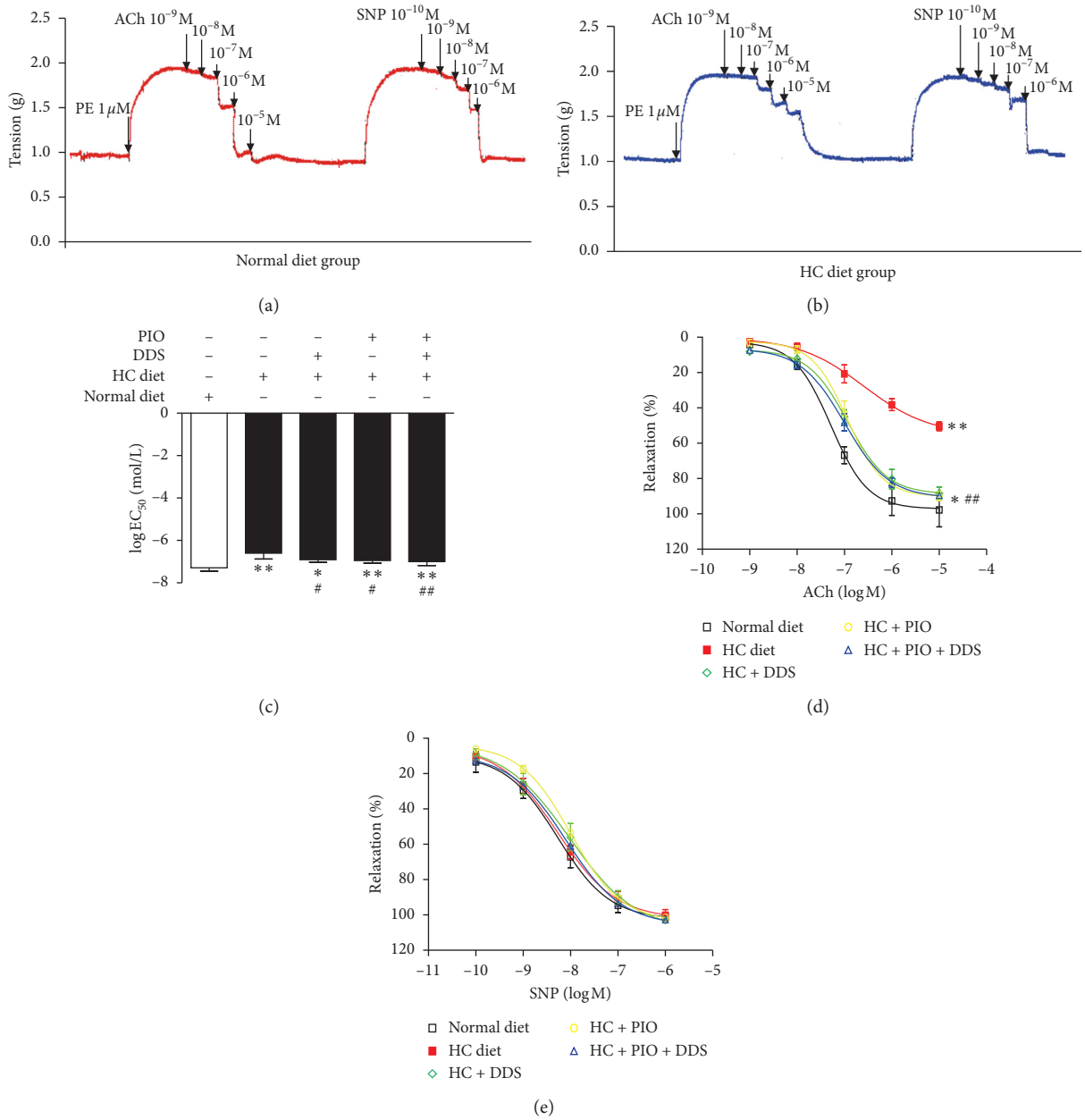

Figure 1: The vasodilatory response in thoracic aortic rings from different groups. The concentration-dependent curve induced by acetylcholine (ACh) and sodium nitroprusside (SNP) in thoracic aorta rings of normal diet rats (a) and hypercholesterolemic (HC) diet rats (b); the log EC_{50} value of vascular tone (c) and the concentration-dependent curve (d) induced by ACh in thoracic aorta rings with different groups; the concentration-dependent curve induced by SNP (e) in thoracic aorta rings with different groups. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. normal diet group; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. HC diet group. $n = 6$–10 rats/group.

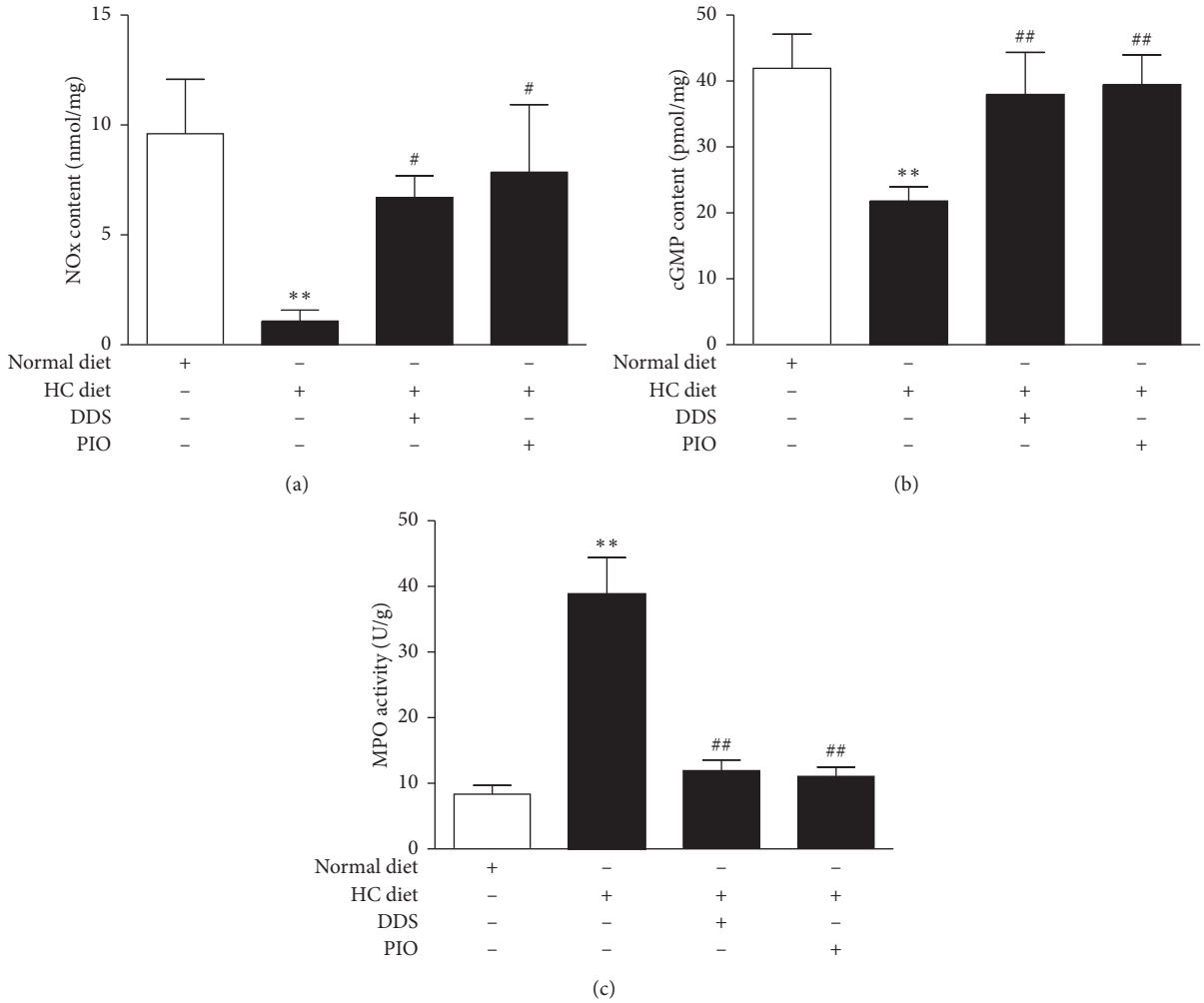

Figure 2: The changes of NO_x content, cGMP level, and MPO activity in thoracic aorta tissue with different groups: (a) NO_x content; (b) cGMP level; (c) MPO activity. $^{**}P < 0.01$ vs. normal diet group; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. HC diet group. $n = 6$–10 rats/group.

change in the solvent DMSO group (Supplementary Figure 1b).

As compared with the hypercholesterolemia group, the vascular NO_x content increased from 1.09 ± 0.49 nmol/mg to 6.71 ± 0.98 nmol/mg ($P < 0.05$) after DDS intervention in hypercholesterolemic rats (Figure 2(a)). The cGMP level increased from 21.81 ± 2.11 pmol/mg to 37.97 ± 6.39 pmol/mg ($P < 0.01$; Figure 2(b)), while the solvent DMSO group remained unaltered (Supplementary Figures 1c and 1d). These observations suggested that MPO might be closely related to vascular endothelial dysfunction in hypercholesterolemic rats and played a key role in impairing the NO/cGMP/cGK signaling pathway by inhibiting NO biological activity.

3.5. After Intervention with the PPARγ Agonist, Vascular Endothelial Dysfunction in Hypercholesterolemic Rats Alleviated, Vascular MPO Activity Decreased, and the NO/cGMP/cGK Signaling Pathway Improved. TZDs (thiazolidinediones, insulin sensitizers) are PPARγ-selective agonists with high affinity, and pioglitazone (PIO) is a key example of one of these agonists. After four weeks of intragastric administration of PIO in hypercholesterolemic rats, blood lipid levels were significantly decreased (Table 1).

As compared with the hypercholesterolemia group, the concentration-dependent vasodilation curve that was induced by ACh had significantly shifted to the left after PIO intervention, and the vascular tone $\log EC_{50}$ value decreased from -6.61 ± 0.11 mol/L to -6.95 ± 0.12 mol/L ($P < 0.05$; Figure 1(c)); meanwhile, maximum vasodilatation increased from $50.51 \pm 2.44\%$ to $89.99 \pm 2.68\%$ ($P < 0.01$; Figure 1(d)), and there was no significant difference observed when comparing changes that were seen after DDS intervention (Figures 1(c) and 1(d)). Moreover, this study found that even after interventions with PIO and DDS, there was no significant difference in ACh-induced vasodilation in hypercholesterolemic rats as compared with that seen after intervention with PIO alone (Figures 1(c) and 1(d)). Thus, there was no more superimposed medication. There was also no significant difference in SNP-induced vasodilation when comparing between groups (Figure 1(e)).

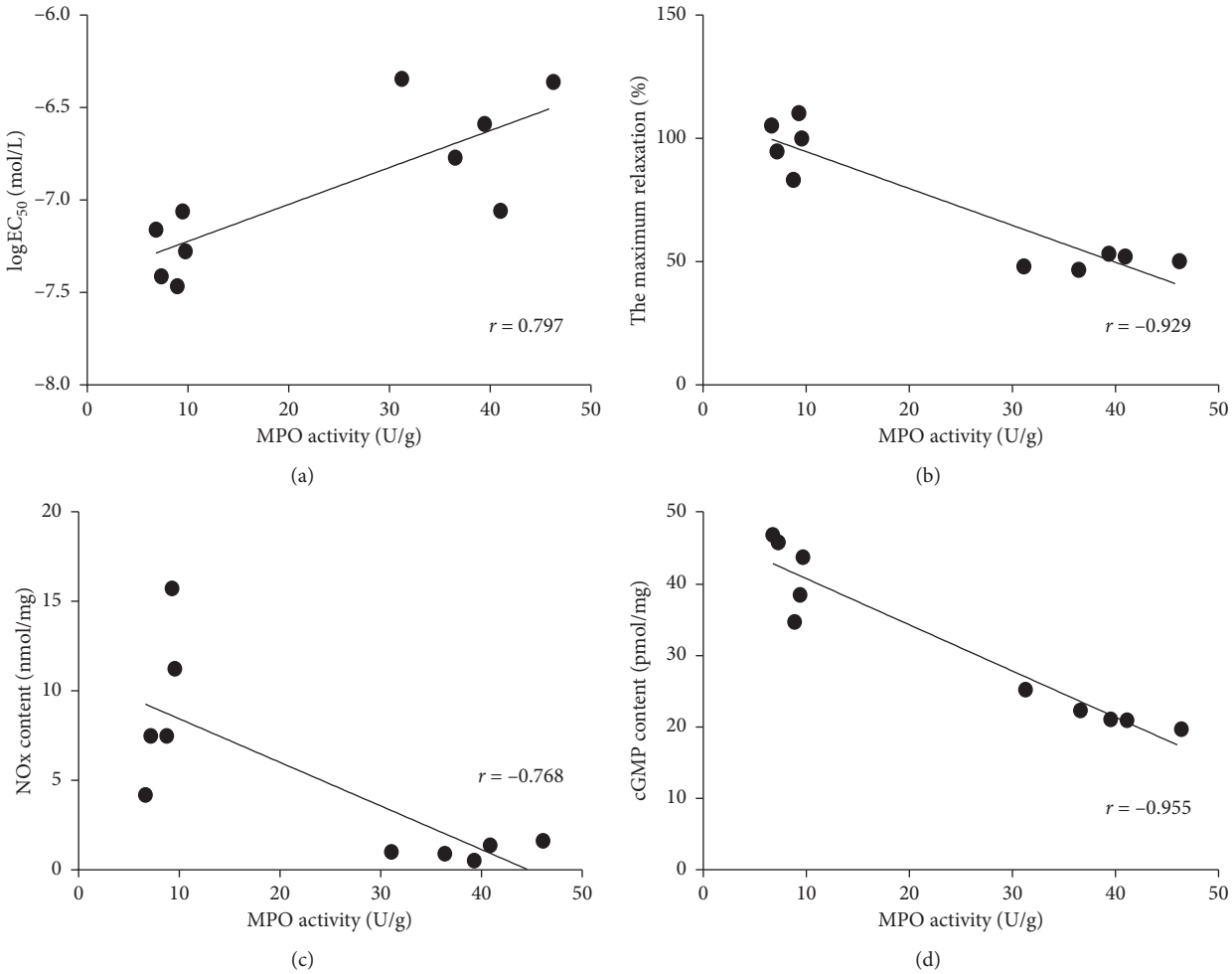

Figure 3: Correlation analysis of MPO activity with vascular endothelial function, NO_x content, and cGMP level in thoracic aorta rings between normal diet and HC diet groups. There was a positive correlation between MPO activity and the $\log EC_{50}$ value of ACh-induced vasodilation (a); ACh-induced maximum vasodilatation (b), NO_x content (c), and cGMP level (d) showed negative correlation with MPO activity.

In addition, when comparing the hypercholesterolemia group, the vascular MPO activity decreased from 38.83 ± 5.56 U/g to 11.05 ± 1.43 U/g ($P < 0.01$; Figure 2(c)) after PIO intervention—an observation that was similar to changes seen after DDC intervention. At the same time, when comparing with the hypercholesterolemic rats, it was found that the vascular NO/cGMP/cGK signaling pathway had also improved after PIO intervention, and the NO_x content increased from 1.09 ± 0.49 nmol/mg to 7.86 ± 3.07 nmol/mg ($P < 0.05$; Figure 2(a)); the cGMP level increased from 21.81 ± 2.11 pmol/mg to 39.47 ± 4.52 pmol/mg ($P < 0.01$; Figure 2(b)), which was essentially the same as that seen with DDS. These observations suggested that PIO could improve vascular endothelial dysfunction in hypercholesterolemic rats and did so by reducing MPO activity and increasing NO bioavailability.

3.6. The MPO Activity of Vascular Tissues Negatively Correlated with Vascular Endothelial Function, NO_x Content, and cGMP Level after Intervention with the PPARγ Agonist. These studies aimed to further confirm the role of PIO in MPO, and its ability to improve vascular endothelial dysfunction in hypercholesterolemic rats. This study conducted an analysis of an association of vascular endothelial function, NO_x content, and cGMP level with vascular MPO activity in the hypercholesterolemia group and the PIO intervention group. The results showed that the $\log EC_{50}$ value of ACh-induced vasodilation positively correlated with MPO activity ($r = 0.675$, $P < 0.01$; Figure 4(a)); i.e., the EC_{50} value of ACh-induced vasodilation negatively correlated with MPO activity. We found that ACh induced a maximum state of vasodilation, which negatively correlated with MPO activity ($r = -0.888$, $P < 0.01$; Figure 4(b)). In addition, NO_x content and cGMP level negatively correlated with MPO activity ($r = -0.748$, $P < 0.01$; $r = -0.899$, $P < 0.01$; Figures 4(c) and 4(d)). It also suggested that, by inhibiting the vascular MPO activity, PIO could maintain the integrity of the vascular NO/cGMP/cGK signaling pathway, thereby protecting vascular endothelial function in hypercholesterolemic rats.

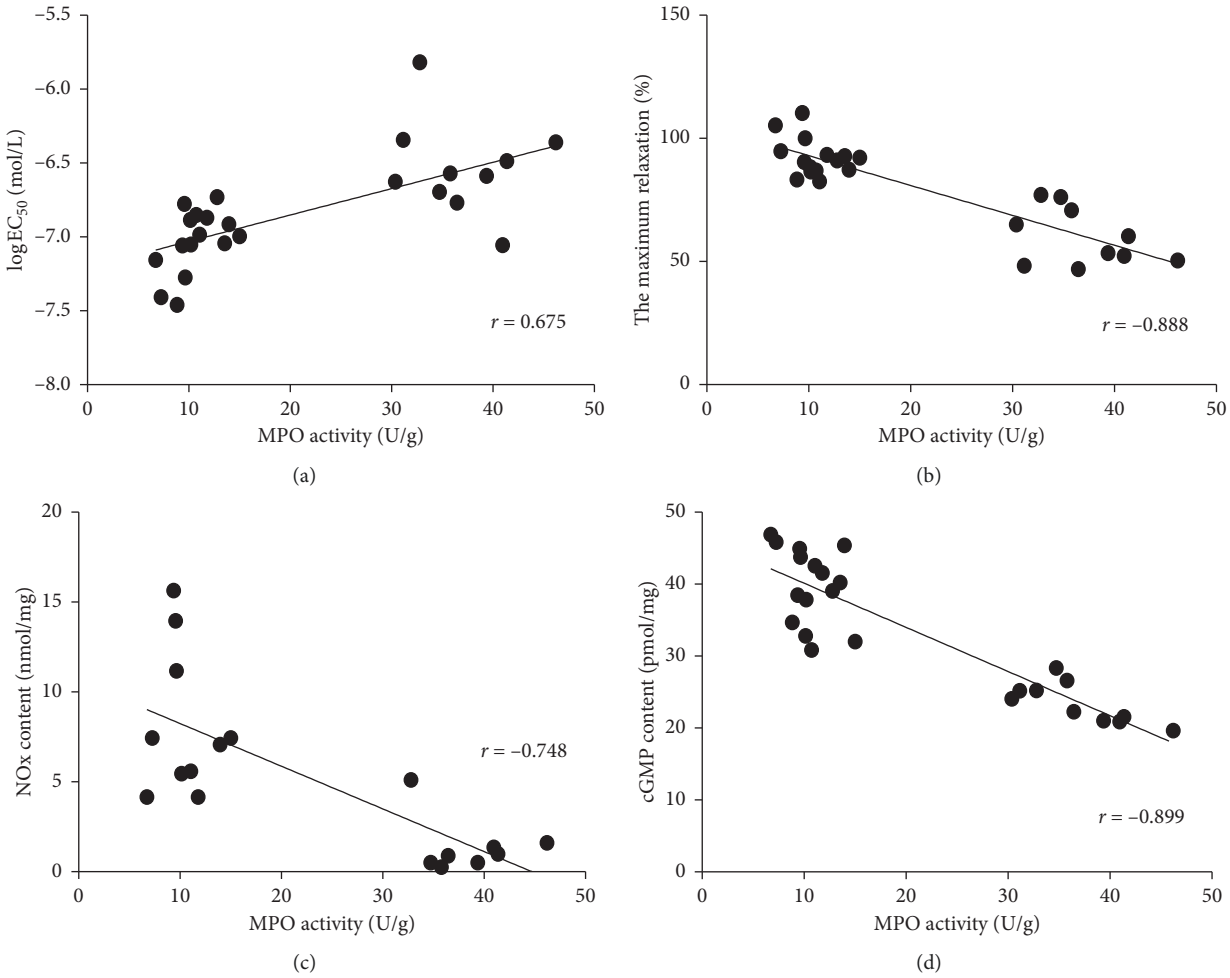

Figure 4: Correlation analysis of MPO activity with vascular endothelial function, NO_x content, and cGMP level in thoracic aorta rings between HC diet rats and after intervention with the PPARγ agonist. There was a positive correlation between MPO activity and the log EC_{50} value of ACh-induced vasodilation (a); ACh-induced maximum vasodilatation (b), NO_x content (c), and cGMP level (d) showed negative correlation with MPO activity.

4. Discussion

Endothelial dysfunction caused by hypercholesterolemia is a prerequisite for the development and progression of atherogenesis and is associated with the occurrence of clinical events (i.e., unstable angina, acute myocardial infarction, sudden coronary death, etc.) in patients already presenting with atherosclerosis [12]. Although the mechanism of vascular endothelial dysfunction in hypercholesterolemia remains unknown, reversing or improving endothelial dysfunction is key to preventing atherosclerosis and subsequent ischemic heart disease.

Vascular endothelial cells regulate vascular tone by secreting an endothelium-derived relaxing factor (EDRF, namely, NO) with a vasodilatory effect, and an endothelium-dependent vasodilatory function that is often used to reflect endothelial function [13]. Some studies have reported that hypercholesterolemia can lead to vascular endothelial dysfunction [14]. In the present study, it was found that following administration of a recognized endothelium-dependent vasodilatory substance, i.e., ACh, the maximum vasodilatation of the thoracic aorta in hypercholesterolemic rats was significantly reduced. However, it was not significantly altered after administration of the endothelium-independent vasodilatory substance SNP. This observation suggests that endothelial dysfunction occurred in hypercholesterolemia, which was consistent with related reports.

NO is a key molecule that mediates endothelial function and causes vasodilation by activating the NO/cGMP/cGK signaling pathway [15]. MPO, which is derived from neutrophils, monocytes, and macrophages, reduces NO production and does so by modifying the NO donors L-Arg [16] and NOS [17]. Baldus et al. [18] reported that MPO circulating in the blood vessels decreases the bioavailability and biological activity of NO. Our study suggested that the vascular NO content in hypercholesterolemic rats was significantly reduced, and its biological activity (reflected by vascular cGMP level) was significantly decreased. Furthermore, this study found that vascular MPO activity in hypercholesterolemic rats was significantly increased, while vascular endothelial function was significantly reduced. Correlation analysis showed a significant negative

correlation between vascular endothelial function, NO content, cGMP levels, and MPO activity. Moreover, after administration of the MPO inhibitor DDS in hypercholesterolemic rats, the vascular NO content and its biological activity were increased, with a concordant improvement in vascular endothelial function. These results suggested that MPO might be involved in vascular endothelial dysfunction in hypercholesterolemia and does so by affecting the NO/cGMP/cGK signaling pathway.

PPARγ is a subtype of PPARs. Among the many synthetic ligands, TZDs are PPARγ-selective agonists with high affinity, including troglitazone, rosiglitazone, and PIO, which are significantly effective in the treatment of type 2 diabetes and cardiovascular complications [19]. PPARγ agonists can alleviate atherosclerosis by improving metabolic risk factors known to cause atherosclerosis and to reduce inflammatory factors in the arterial wall with an improvement in endothelial function seen in diabetic and hypercholesterolemic animals [20]. And, PIO attenuated palmitate-induced ER stress in macrophages, and the effect was reversed in the presence of PPARγ antagonists [21]. Moreover, studies have confirmed that PPARγ agonists can regulate MPO gene expression [10]. In the present work, we found that after PIO intervention in hypercholesterolemic rats, the endothelium-dependent vasodilatory substance ACh directed a significant improvement in the vasodilation of the thoracic aortic rings. By contrast, after administration of the endothelium-independent vasodilatory substance SNP, there was no significant difference in the vasodilatory effect between groups. This indicated that PIO could improve vascular endothelial function in hypercholesterolemic rats. At the same time, PIO could significantly reduce the vascular MPO activity in hypercholesterolemic rats and effectively reverse vascular NO content and cGMP level—observations that were similar to the effect seen with MPO inhibitors. In addition, the MPO activity of vascular tissues negatively correlated with vascular endothelial function, NO_x content, and cGMP level after intervention with the PPARγ agonist. It suggested that PIO might improve vascular endothelial dysfunction in hypercholesterolemia by regulating vascular MPO activity via the NO/cGMP/cGK signaling pathway.

In addition, this study found that after administration of PIO, there was a significant decrease in blood lipid levels in hypercholesterolemic rats; thus, it was believed that the lipid-lowering effect of PIO might be associated with improved endothelial dysfunction. This protective pathway might be different from the abovementioned pathways that were shown to be mediated by regulation of MPO. Numerous clinical data have demonstrated that hypercholesterolemia is one of the most common complications of type 2 diabetes and is the leading cause of atherosclerosis, coronary heart disease, cerebrovascular accident, and ultimately the death of patients presenting with type 2 diabetes. Thus, drugs that can simultaneously treat coronary heart disease, diabetes, and hypercholesterolemia might have additional clinical utility and applications in the management of several conditions. Thus, it is of paramount clinical significance to explore the mechanisms of such drugs in future studies.

5. Conclusions

This study found that (1) MPO might provoke vascular endothelial dysfunction in hypercholesterolemic rats by reducing NO biological activity and impairing the NO/cGMP/cGK signaling pathway and (2) the PPARγ agonist PIO might inhibit vascular MPO activity and increase NO bioavailability with the net result of reversing endothelial dysfunction in hypercholesterolemic rats.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

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Supplementary Materials

Supplementary Figure: the changes of MPO activity, $\log EC_{50}$ value of ACh-induced vasodilation, NO_x content, and cGMP level in the thoracic aorta tissue with the HC + DMSO group: (a) MPO activity; (b) $\log EC_{50}$ value of ACh-induced vasodilation; (c) NO_x content; (d) cGMP content. $^{**}P < 0.01$ vs. normal diet group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. HC diet group; $^{\pm}P < 0.05$, $^{\pm\pm}P < 0.01$ vs. HC diet-group and + DDS group. $n = 6$–10 rats/group. (*Supplementary Materials*)

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Research Article

FibroAtlas: A Database for the Exploration of Fibrotic Diseases and Their Genes

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Background. Fibrosis is a highly dynamic process caused by prolonged injury, deregulation of the normal processes of wound healing, and extensive deposition of extracellular matrix (ECM) proteins. During fibrosis process, multiple genes interact with environmental factors. Over recent decades, tons of fibrosis-related genes have been identified to shed light on the particular clinical manifestations of this complex process. However, the genetics information about fibrosis is dispersed in lots of extensive literature. *Methods*. We extracted data from literature abstracts in PubMed by text mining, and manually curated the literature and identified the evidence sentences. *Results*. We presented FibroAtlas, which included 1,439 well-annotated fibrosis-associated genes. FibroAtlas 1.0 is the first attempt to build a nonredundant and comprehensive catalog of fibrosis-related genes with supporting evidence derived from curated published literature and allows us to have an overview of human fibrosis-related genes.

1. Introduction

Fibrosis is a chronic and progressive process characterized by an excessive deposition of extracellular matrix (ECM) leading to overgrowth, hardening, and/or scarring of various tissues [1]. Fibrotic changes may affect almost all the main tissues and organs, including the skin, kidney, lung, and liver, as well as various vascular disorders [2]. Failure to control the abnormal wound healing responses can lead to considerable tissue remodeling and organ malfunction as seen in late-stage idiopathic pulmonary fibrosis and cardiac fibrosis [2, 3]. Aberrant fibrotic tissue remodeling also may be involved in the tumor initiation and progression, and accelerate chronic graft rejection in recipients of organ transplantation [4]. Fibrosis is one of the major causes of morbidity and mortality. Approximately 45 percent of all-cause mortality in the United States was attributed to fibrotic disorders [1].

Identification of effective therapeutic targets and designation for antifibrotic treatment strategies will depend on the underlying etiology, the severity, and extent of the fibrotic disease. However, the etiology and pathogenesis of fibrosis still remain virtually unknown, which limits our ability to optimally prevent or treat this disease. The natural history and the factors associated with fibrosis progression are highly variable [5]. Currently, lots of studies have indicated that both genetic factors and environmental exposures have been implicated in the formation and progression of fibrosis. For example, rs 35705950, a common polymorphism in the promoter of Mucin 5B (MUC5B), is associated with familial interstitial pneumonia and idiopathic pulmonary fibrosis, which suggests a crucial role of dysregulated MUC5B expression in the pathogenesis of pulmonary fibrosis [6]. Platelet factor 4 (PF4) is identified as a marker for fibrosis, levels of which are elevated in patients

with systemic sclerosis and correlated with the presence and progression of pulmonary arterial hypertension [7]. Studies have suggested that multiple fibrotic diseases are usually triggered by the same irritation and share a number of common pathways, such as transforming growth factor beta (TGF-β), interleukin-6 (IL-6), and integrin-linked kinase signaling [8, 9].

Besides, there is still no database concentrating on fibrosis-associated genes. Therefore, a targeted strategy should be established to collect the magnanimity information about previously reported fibrosis-associated genes. To address the challenge, we create the FibroAtlas database 1.0 (http://biokb.ncpsb.org/fibroatlas/), which identifies 1,439 manual curated fibrosis-related genes by literature mining. FibroAtlas will shed light on the pathogenesis of individual cases, novel biomarkers for diagnosis and prognosis, and personalized therapeutic strategies.

2. Materials and Methods

2.1. Literature Mining and Manual Curation. We have constructed an ontology-based bioentity recognizer to recognize and extract genes in PubMed abstracts. This system compares favorably with current state-of-the-art biomedical annotation systems such as BeCAS [10] and has been evaluated against the CRAFT [11] corpus for gene/protein recognition based on Protein Ontology (PR) [12], which has the precision, F-measure, and recall of 0.959, 0.802, and 0.874, respectively. This system has been used to build AllerGAtlas 1.0 [13] successfully.

Three steps were taken to compile a comprehensive catalogue of human candidate genes related to fibrosis from PubMed abstracts.

First, 227,458 sentences in 114,973 PubMed abstracts including the keywords of "fibrosis," "fibrotic," "fibrotic action," "fibrotic change," or their lexical variants were identified by our bioentity recognizer.

Second, a list of 4,079 human genes with the fibrosis-associated keywords at sentence level co-occurrences were identified and extracted from 62,302 sentences in 10,243 PubMed abstracts by bioentity recognizer based on Protein Ontology (Supplementary material: Table S1.xlsx).

Third, 4,079 candidate genes were manually curated by our experts and 1,439 genes were finally certified as the human fibrosis-associated genes.

The co-occurrences between fibrosis-associated genes/proteins and fibrosis-related disease terminology based on Human Disease Ontology (DO) [14] were identified at sentence level from PubMed abstracts by bioentity recognizer. Furthermore, the genes identified as biomarkers were mined and marked with the terms "biomarker," "biomarkers," "marker," "markers," or "mark," and then these potential biomarkers were manually curated by our experts.

2.2. Gene Annotation. We provided detailed annotations for each fibrosis-related gene to facilitate deeper interpretations for users. NCBI Entrez Gene ID and gene symbol were used for cross links and annotations. The basic gene information including gene symbol, synonyms, gene summary, chromosome, and chromosomal location were supplied to facilitate alignment known splicing sites. Gene ontology (GO) annotations were taken from the AmiGO database [15], and the gene-pathway relations were obtained from the Reactome database [16]. SNPs linked to genes were retrieved by the literature's PMIDs (PubMed Unique Identifier) from the dbSNP database [17]. The public databases such as Ensembl [18], Entrez gene [19], UniProt [20], neXtProt [21], and Antibodypedia [22] were also utilized to map and annotate.

3. Results

3.1. Database Implementation and Service. All identified fibrosis-related genes/proteins, human disease terminology, and their biomarkers were loaded into a local MySQL server. PHP was used to implement the web interface of FibroAtlas on a Windows server. All the data of FibroAtlas are accessible to every user without login or registration.

3.2. Database Search and Navigation. FibroAtlas is a user-friendly interface website to query the database (http://biokb.ncpsb.org/fibroatlas/), which has five components including "Home," "Browse & Download," "Feedback," "FAQ," and "Contact" (Figure 1). In the "Home" page, three main types of navigational queries are available: protein name, nucleotide sequence, and protein sequence. For example, if users submit a gene name in the search box of "Gene Symbol," an autocompleted dropdown list of gene symbols will be displayed to show the possible matches in the FibroAtlas. Users can select one of them and click the "Search" button to jump to the result page. If users search the gene by nucleotide sequence or protein sequence, the sequence match scores from BLAST will be listed. Users can choose the matched gene name and click "continue" to browse result interface (Figure 1(A)). A table containing the queried gene, the supporting literature evidences for related human disease terminology, the role of gene, and the number of evidences will be displayed on the search result page by the search engine (Figure 1(B)). By clicking on the gene hyperlink, users can access the page of gene annotations, which includes a list of SNPs mapped to dbSNP, gene ontology (GO) terms derived from GOA, pathway identifiers derived from Reactome, and the gene description based on UniProtKB, etc. (Figure 1(C)). By clicking on the number of the evidence abstracts or sentences, users can browse a table containing the gene symbol, the PubMed ID, and the manual curated evidences. In addition, to specify individual interested evidence, users can obtain the whole abstract with highlighted names of entities, i.e., the alias names of gene and disease term (Figure 1(D)). Three approaches are supported by the page of "Browse & Download." All the data can be freely downloaded (Figure 1(E)).

3.3. Application Case of the Database. Cardiac fibrosis is an inevitable consequence of chronic myocardial injury and leads to both systolic and diastolic dysfunction in many cardiac pathological conditions [23]. Cardiac fibrosis is a

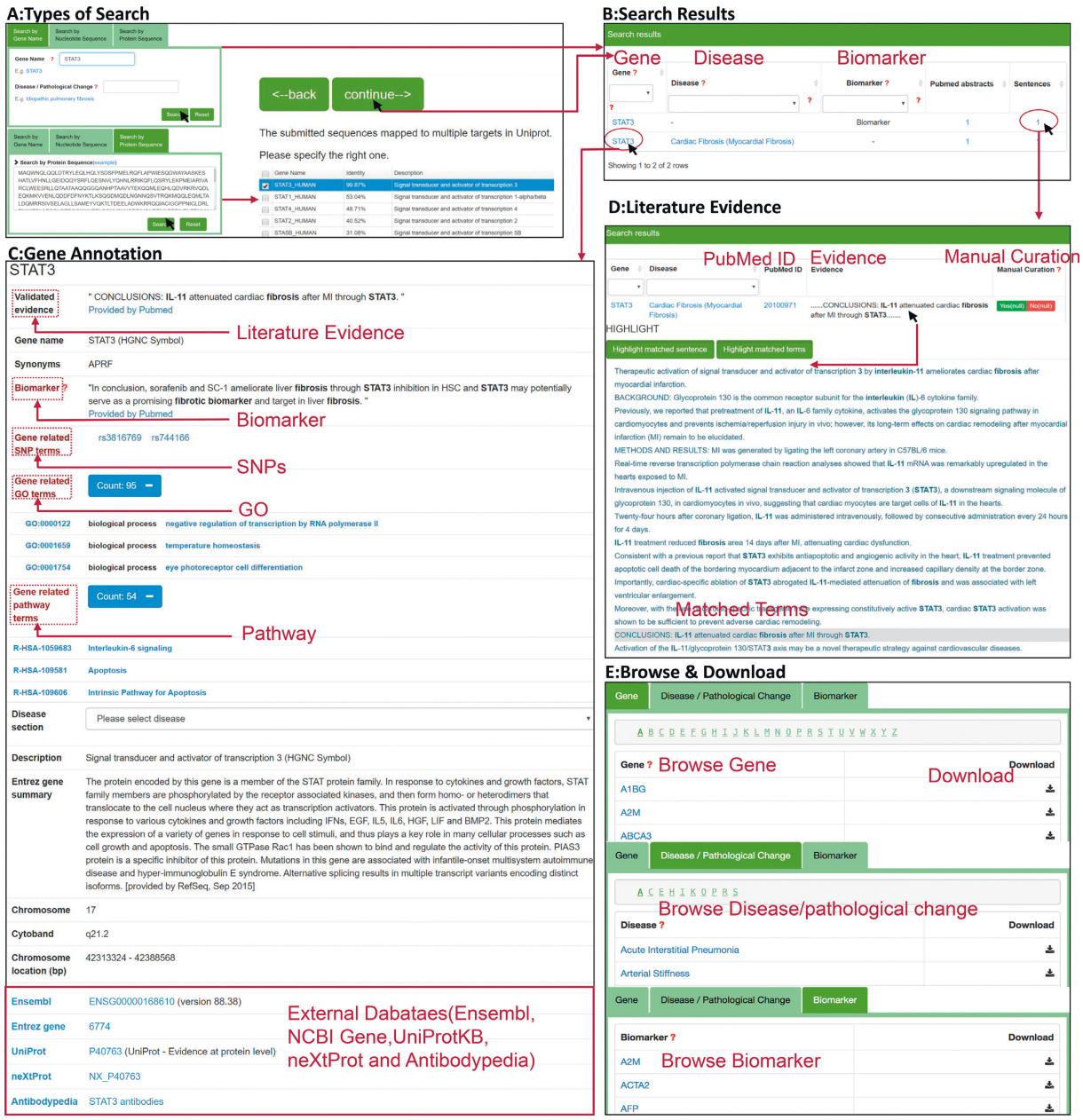


Figure 1: (A) Three main types of queries are supported by the "Home" page: gene symbol query, nucleotide sequence query, and protein sequence query. Users can input the gene symbol such as "STAT3" in the query box. Users can also input a nucleotide or protein sequence, and the sequence similarity identity score from BLAST will be displayed. Choose the matched gene name and click "continue" to scan the set of search results. (B) In the result page, a table including the queried gene, related disease terminology, and supporting evidences is listed. (C) By clicking the gene symbol of "STAT3" in the "search results" interface, users can browse detailed information of "STAT3" and cross links to external databases. (D) By clicking the number of PubMed abstracts or sentences in the "search results" interface, users can scan a table containing the information of gene, associated disease terminology, PubMed ID, evidence, and manual curation. Click the link of evidence in this page to scan the abstract with highlighted keywords. (E) Three approaches for browsing are presented in the "Browse & Download" page. All the data can be downloaded.

common phenomenon in the end stages of diverse cardiac diseases and is a predictive factor for sudden cardiac death [24]. There is an urgent need to unravel the intricate mechanisms underlying the development of cardiac fibrosis, in order to prevent long-term sequelae of cardiac fibrosis. We searched the database with the term of "cardiac fibrosis" and obtained 119 expert curated genes with detailed annotations. Pathway analyses were run on the list of cardiac fibrosis-related genes. The result shows that most of the genes share a number of common pathways and contribute in MAPK signaling pathway, cytokine-cytokine receptor interaction, Hippo signaling pathway, TGF-beta signaling pathway, and mTOR signaling pathway, etc (Figure 2). These results are validated by the literature and suggest that fibrosis

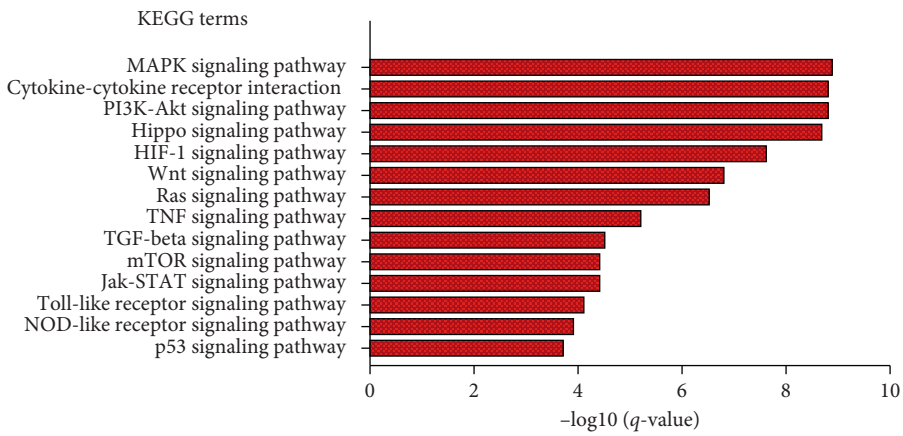
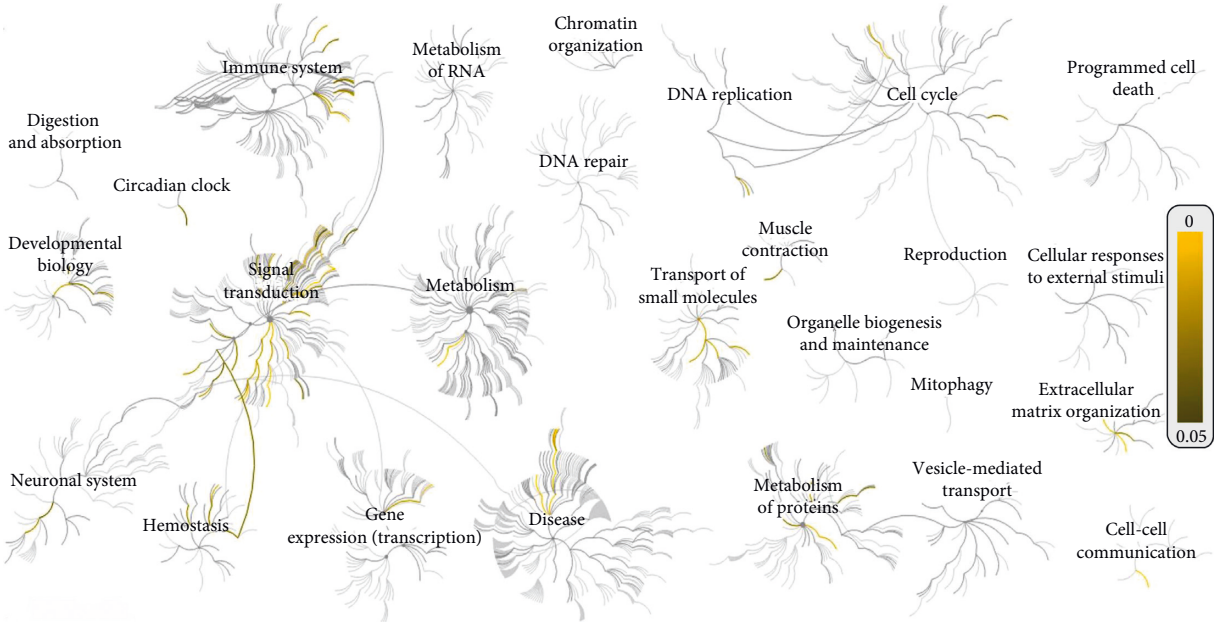
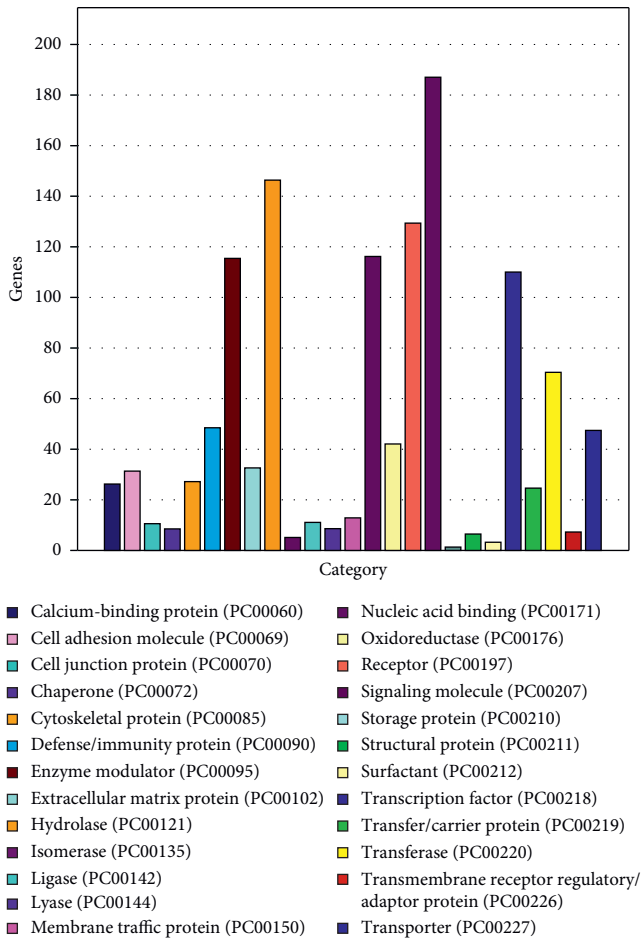


Figure 2: Bioinformatics pathway analysis for cardiac fibrosis-related gene sets with clusterProfiler [29].



(a)

Figure 3: Continued.



(b)

Figure 3: Bioinformatics analysis on the list of human fibrosis-related genes. (a) Biological pathway analysis with Reactome (http://www.reactome.org/). (b) Protein class analysis with PANTHER (http://pantherdb.org/).

arises as a consequence of multiple coactivated pathogenic pathways that affect inflammation and wound repair [25–27]. For example, yes-associated protein (Yap) acts as a transcriptional cofactor in the Hippo signaling pathway by activating the transcription of genes, inactivation of which after MI elicits increased myocyte apoptosis and fibrosis [28]. Furthermore, users can specify the hyperlink of the interested cardiac fibrosis-related genes to find the page with detailed functional annotation of genes, such as gene-related SNPs, pathways, and GO terms.

4. Discussion

Identification of key regulators of cell proliferation and quiescence is a significant step toward potential regenerative therapies [3, 30]. FibroAtlas 1.0 is the first complete and up-to-date gene network aiming to extract the literature on fibrosis-related genes and their function in diseases. FibroAtlas 1.0 (http://biokb.ncpsb.org/fibroatlas/), a powerful and time-saving tool with credible content, can provide accurate information and overview of human fibrosis-related genes. Analysis with Reactome (http://www.reactome.org/) [16] shows a strong tendency for these genes to participate in the pathways of signal transduction, immune system, cell cycle, hemostasis, gene expression (transcription), extracellular matrix organization, metabolism of proteins, developmental biology, neuronal system, cell-cell communication, transport of small molecules, muscle contraction, etc. (Figure 3(a)). The protein class analysis with DAVID (https://david.ncifcrf.gov) [31] reveals that these genes concentrate predominately on the role of signaling molecule, hydrolase, receptor, enzyme modulator, nucleic acid binding, defense/immunity protein, transcription factor, transferase, etc. (Figure 3(b)).

A circulation system is supported by FibroAtlas 1.0. Sign in to give feedback by clicking the green “Yes” or red “No” button to accept or deny the evidence sentences (Figure 4). Our database will be periodically updated based on the results.

In future, we intend to carry out the following work to improve the performance of our database. Firstly, we will continue collecting fibrosis-related genes and replenishing genome-wide association studies data regularly. Second, we want to integrate the PPI information from both HPRD [32] and BioGRID [33] and then extract the direct interactors for fibrosis diseases candidate proteins in fibrosis-related genes.

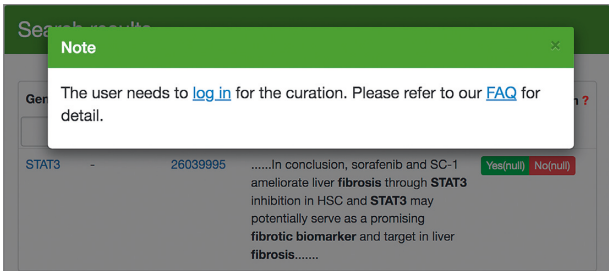


Figure 4: All logged-in users can give their feedback by clicking the "Yes" or "No" button to confirm or reject the evidence phrases.

Finally, to help users to prioritize and select the information, we will further consider the following factors to implement a score for each fibrosis-related gene based on the supporting evidence, such as the number of supporting publications from text mining-based sources, the number of sources that report the association, the animal models and experimental strategies where the association has been studied, and the type of curation of each of these sources. In conclusion, we believe that FibroAtlas 1.0 will become a well-established resource with stable releases and be widely used as it can provide facilities for the research community and allied fields.

Data Availability

The data sets generated during the current study are available in the FibroAtlas 1.0 repository (http://biokb.ncpsb.org/fibroatlas/).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Dong Li and Shuzhen Guo conceived and conducted this work. Data collection, curation, and analysis were performed by Jiale Liu, Yuan Liu, Dezhi Sun, Jinying Liu, Yang Li, Xun Wang, Hao Xu, Lei Tian, and Huimin Zhang. The manuscript was written and revised by Jinying Liu and Dezhi Sun. The website was developed by Lihong Diao and Dezhi Sun. All authors reviewed and approved the submitted manuscript. All authors critically revised and edited the manuscript and approved the final version. Jinying Liu, Dezhi Sun, Jiale Liu, and Hao Xu contributed equally to this work.

Acknowledgments

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Supplementary Materials

Supplementary data are available at Cardiology Research and Practice Online. (*Supplementary Materials*)

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Research Article

Prediction Efficiency of Postoperative Acute Kidney Injury in Acute Stanford Type A Aortic Dissection Patients with Renal Resistive Index and Semiquantitative Color Doppler

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Objectives. This study is aimed to evaluate the efficiency in early prediction of postoperative persistent acute kidney injury (PAKI) after surgery in acute Stanford type A aortic dissection (AAAD) patients by using Doppler renal resistive index (RRI) and semiquantitative color (SQC) Doppler grade, respectively. *Methods*. 84 AAAD patients received Sun's surgical management, and 67 patients were enrolled. RRI and SQC Doppler grade were evaluated by ultrasonography, respectively, at 6 hours after surgery. Serum creatinine (sCr) was recorded before operation and at 24 hours, 48 hours, and 72 hours after operation. AKI grade was evaluated according to the classifications of the Acute Kidney Injury Network (AKIN). PAKI is defined as persistent oliguria and/or sCr elevation after 3 days. RRI and SQC Doppler grade were compared, respectively, between the PAKI and non-PAKI groups. Potential predictors were first tested by univariate logistic regression analysis, and a multivariate model was identified to determine the independent predictive ability of RRI and SQC Doppler grade for the PAKI. Receiver operating characteristic (ROC) analysis was performed to compare the diagnostic accuracy between RRI and SQC Doppler grade in early prediction of PAKI by using AKIN classifications as the reference standard. *Results*. Of a total of 67 patients enrolled during the study period, 21 (31.3%) patients suffered from PAKI and 8 (11.9%) patients required dialysis. There are significant differences in RRI (0.80 ± 0.09 vs. 0.70 ± 0.05, $P = 0.002$) and SQC Doppler grade ($x^2 = 12.193$, $P = 0.007$) between the 2 groups with and without PAKI. Univariate analysis showed that RRI, SQC Doppler grade, length of stay in ICU, time of CPB, and length of stay in hospital were significant predictors of PAKI. RRI and the SQC Doppler grade remained independent predictors of PAKI. Area under the curve (AUC) of RRI was 0.855 (95% CI, 0.74–0.96) with cutoff value 0.725 (sensitivity 90.9% and specificity 71.1%), AUC of SQC Doppler grade was 0.642 (95% CI, 0.49–0.79) with cutoff value grade 2 (sensitivity 50% and specificity 73.3%). *Conclusion*. Both postoperative RRI and SQC Doppler grade are independent predictors for PAKI after surgery in AAAD patients. Both postoperative RRI and SQC Doppler grade can be obtained rapidly by bedside ultrasound, which is a good tool for early prediction for postoperative PAKI.

1. Introduction

The gold standard for the diagnosis of postoperative AKI is serum creatinine (sCr), but it is not used for early detection of AKI [1, 2]. Recent studies provide early diagnosis of AKI with several urine and blood markers [3–6]. Commonly used urine markers included neutrophil gelatinase-associated lipocalin (NGAL), cystatin C, kidney injury molecule 1 (KIM-1), interleukin-18 (IL-18), liver-type fatty acid-binding protein (L-FABP), and other more. Blood markers include cystatin C, uric acid, and monocyte chemoattractant protein-1 (MCP-1). Although these indicators have certain

efficacy while intraoperative monitoring, urine and blood markers cannot be used to predict severe AKI [7].

Postoperative AKI is an ischemia-reperfusion injury, so renal perfusion is associated with AKI [8]. There are several imaging methods to evaluate renal perfusion. Computed tomography angiography (CTA) can evaluate renal perfusion, but the postcontrast AKI carries a risk of more permanent renal insufficiency [9, 10], which is also not suitable for daily bedside operation. Isotope renogram is a very sensitive, simple, and noninvasive method for evaluating renal parenchymal function [11], but it requires the patient to go to the fixed examination room and is also not suitable for the critically ill patients in the postoperative care unit.

Ultrasound is being used increasingly, especially in the intensive care unit (ICU) after operation, which has been recognized by the ICU doctors. The RRI measured by Doppler can be worked as a marker to predict AKI in some kinds of cardiac operations [12–15]. However, some other literatures do not consider RRI to be a good indicator of renal perfusion [16, 17]. RRI as a marker to predict AKI needs to be confirmed in more research studies. Moreover, intrarenal arcuate or interlobar arteries cannot be displayed when critical AKI occurred, RRI was hardly to be obtained, and the clinical application was limited. The color Doppler can intuitively and instantly observe renal perfusion, and semiquantitative evaluation can be acquired rather than an accurately quantitative renal perfusion assessment [18]. But few studies about this indicator are reported. The purpose of this study was to evaluate efficiency of RRI and SQC Doppler for prediction of PAKI after surgery in AAAD patients.

2. Patients and Methods

This study was conducted in accordance with the Declaration of Helsinki. This study was approved by the Ethics Committees of Beijing Anzhen Hospital, Capital Medical University. The committee agreed with using the data obtained from the postoperative patients in the ICU.

2.1. Patients. A prospective, observational study was performed during a nonconsecutive period (September 2017–March 2018) at Beijing Anzhen Hospital, cardiac surgery intensive care unit. Inclusion criteria were as follows: the AAAD patients received Sun's surgical operation with age >18 years and inclusion within 24 hours after ICU admission. Exclusion criteria were as follows: (1) history of chronic kidney dysfunction and preoperative renal injury; (2) pregnancy; (3) death during 72 hours after operation; (4) kidney tumor ($n = 1$); (5) known renal artery stenosis; (6) arrhythmia; and (7) incomplete data. 84 AAAD patients received Sun's surgical operation, and 67 patients were analyzed (Figure 1), including 53 males and 14 females.

2.2. SQC Doppler Grade Evaluation. All ultrasound measurements and evaluations were performed using a 3–5 MHz pulsed wave Doppler probes with GE equipment (Vivid E9, GE Healthcare, Horton, Norway) by one of the investigators. The kidneys were displayed along a longitudinal section with two-dimensional ultrasound at the renal hilum slice. The color Doppler gain was set to minimal background noise. Each renal perfusion was evaluated by color Doppler using the semiquantitative scale (Table 1 and Figure 2) [19].

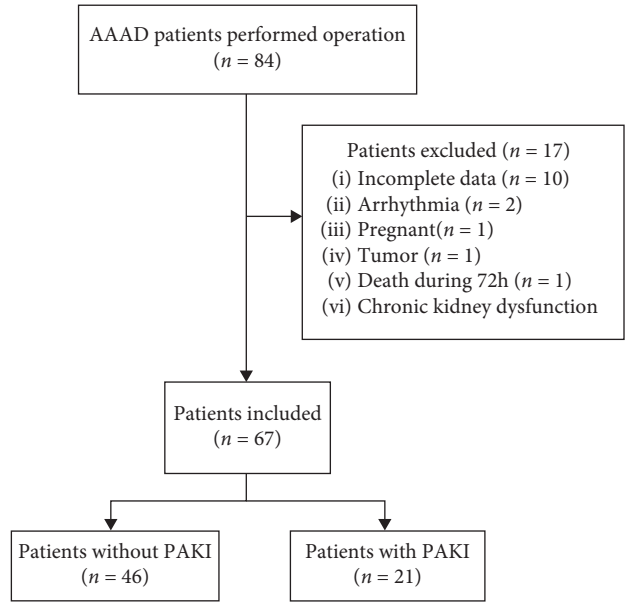


Figure 1: Flowchart of the study (AAAD: acute Stanford type A aortic dissection; PAKI: persistent acute kidney injury).

As the perfusion in the bilateral kidney is diverse in AAAD patients, the renal perfusion grade was defined as the average.

2.3. RRI Measurement. Renal blood flow was located along a longitudinal section showing the renal hilum [18] and interlobar arteries. Sampling any side midinterlobar artery (IRA) with an angle of blood stream <30°. Set to minimal background noise, the Doppler gain can obtain at least three similar continuous waveforms. In each kidney, interlobar artery peak systolic velocity and end diastolic velocity were measured three times at the Doppler spectrum. RRI was calculated by the equation as follows:

$$\text{RRI} = \frac{(\text{peak systolic velocity} - \text{end diastolic velocity})}{\text{peak systolic velocity}}. \quad (1)$$

Because of diversity at the bilateral kidneys perfusion in AAAD patients, RRI was recorded as average.

2.4. Study Design. Ultrasound SQC Doppler grade and RRI measurements were performed at 6 hours after surgery.

We started to measure the RRI and SQC Doppler grade of 14 kidneys in 7 patients by a double-blind method, in order to compare the consistency of the two operators.

sCr was recorded before operation (creatinine was defined as the last known creatinine value before operation) and 24 hours, 48 hours, and 72 hours after operation. AKI was classified into three grades according to the AKIN classification system [20]. PAKI was defined as persistent

Table 1: Semiquantitative color Doppler scale in renal perfusion.

Grade 0	Unidentifiable vessels
Grade 1	Few vessels visible in the vicinity of the hilum
Grade 2	Hilar and interlobar vessels visible in most of the renal parenchyma
Grade 3	Renal vessels identifiable until the arcuate arteries in the entire field of view

oliguria or serum creatinine elevation within at least 3 days [21].

2.5. Statistical Analysis. Quantitative and qualitative data are expressed as median ± standard deviation and numbers (percentages), respectively. The nonparametric test was used for categorical variables, and the t-test was for continuous variables between groups with and without PAKI. Counting data of patient outcome and SQC Doppler grade were analyzed by the x^2 test. Difference of RRI and SQC Doppler grade between the two operators was compared by using the Kendall test. Potential predictors were first tested by univariate logistic regression analysis, to determine the predictive ability of the RRI and SQC Doppler grade for the development of PAKI. A multivariate model was identified applying a p-entry and removed the value of p less than 0.05.

Using AKIN as the standard, receiver operating characteristic (ROC) analysis was performed on RRI and SQC Doppler grade to assess the efficiency of the two markers. All tests were two sided, and a probability value of less than 0.05 was considered statistically significant. SPSS 20.0 (SPSS Inc., IBM, USA) was used to analyze the data.

3. Results

3.1. Operator Consistence. The Kendall coefficient of RRI and SQC Doppler grade between the two operators was 0.827 ($n = 14$, $P \leqq 0.001$) and 0.693 ($n = 14$, $P = 0.027$).

3.1.1. Primary Outcomes. Of a total of 67 patients during the study period, 21 (31.3%) patients suffered from PAKI and 8 (11.9%) patients required dialysis. Base characteristics were similar between the two groups (PAKI and without PAKI) except for preoperative sCr (Table 2). Intraoperative risk factors and postoperative influencing factors were similar between the two groups (PAKI and without PAKI) except for time of CPB and CRRT, length of stay in hospital, and length of stay in ICU (Table 3).

3.1.2. Outcomes of RRI and SQC Doppler Grade

(1) There is a significant difference in postoperative RRI in groups PAKI and without PAKI (0.79 ± 0.09 vs. 0.72 ± 0.06, $P = 0.002$). There is also a significant difference in the SQC Doppler grade in between the two groups ($x^2 = 12.193$, $P = 0.007$) (Table 4).

(2) Since 0 appears in the data of patients without PAKI, we combine SQC Doppler grade 0 and 1 to SQC Doppler grade 1 for univariate analysis. It shows that both RRI and SQC Doppler grade 2 were significant predictors of PAKI; besides, the other three significant predictors were length of stay in ICU, time of CPB, and length of stay in hospital (Table 5).

(3) Similar to univariate regression analysis, we combine SQC Doppler grade 0 and 1 to SQC Doppler grade 1 for multivariate regression analysis. To determine whether RRI and SQC Doppler grade 2 were independent predictors of PAKI, the other three most significant variables in univariate analysis as confounders were analyzed for PAKI prediction in multivariate analysis, in addition to RRI and SQC Doppler grade. RRI remained a significant predictor of PAKI, independent of length of stay in ICU, time of CPB, and length of stay in hospital. Similarly, the SQC Doppler grade is a predictor of PAKI independent of length of stay in ICU, time of CPB, and length of stay in hospital (Table 6).

(4) AUC of postoperative RRI to predict PAKI was 0.855 with cutoff value 0.725 (95% CI, 0.74–0.96) (sensitivity 90.9% and specificity 71.1%) (Figure 3(a)). AUC of the SQC Doppler grade to predict PAKI was 0.642 (95% CI, 0.49–0.79) with cutoff value grade 2 (sensitivity 50% and specificity 73.3%) (Figure 3(b)).

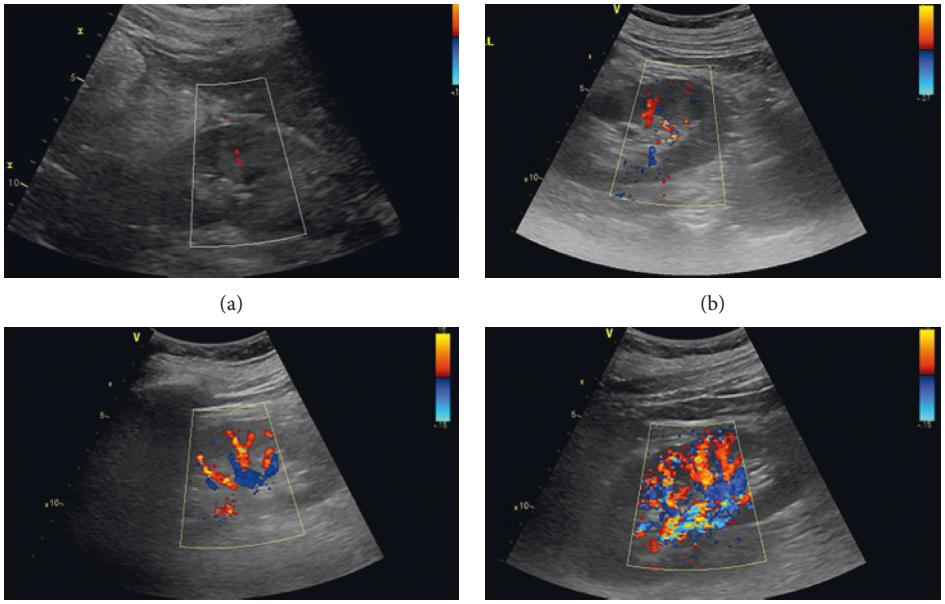
4. Discussion

This prospective study shows that both postoperative RRI and SQC Doppler grade are significant predictors of postoperative persistent AKI after AAAD surgery.

The two operators have good consistence at the RRI calculation and SQC Doppler grade evaluation, and the Kendall coefficient was 0.827 ($n = 14$, $P \leqq 0.001$) and 0.693 ($n = 14$, $P = 0.027$), respectively. Close to the previous study [19], the technology with good reproducibility between the 2 operators avoided technique bias. The accuracy of RRI calculation and SQC Doppler grade evaluation still needs further study in larger scale samples and multiple centers.

The incidence of postoperative PAKI in our study was 31.3%, and 11.9% patients required dialysis, which agrees with the previous study [22].

In this study, postoperative RRI was higher in PAKI patients than that in non-PAKI patients. Univariate and multivariate regression analysis certified that postoperative RRI was an independent predictor for developing of postoperative PAKI. Although preoperative sCr was different between groups with and without PAKI, but by the univariate regression analysis preoperative sCr is not a significant predictor, therefore not included in the multivariate analysis. The result of the AUC of postoperative RRI was 0.855 for predicting PAKI with a cutoff value of 0.725 (95% CI, 0.49–0.79) (sensitivity 90.9%, specificity 73.3%). The cutoff value 0.725 was the same as that of our previous research [23], which indicates that RRI predicts postoperative AKI and has a fairly stable value in AAAD patients in our center. But it was higher than some studies [24, 25] and lower than other literatures [26, 27]. We selected the average RRI in bilateral kidneys because of each kidney perhaps causing by different



(a) (b) (c) (d)

Figure 2: Semiquantitative color Doppler (SQC) grade. (a) Unidentifiable vessels were seen in the kidney; SQC grade was 0. (b) Vessels were seen in the renal hilum, but no vessels were seen in the parenchyma; SQC grade was 1. (c) Interlobar vessels were seen in most of the renal parenchyma; SQC grade was 2. (d) Arcuate arteries were seen in the entire field of view; SQC grade was 3.

Table 2: Base characteristics between groups with PAKI and without PAKI.

	All subjects with operation (n = 67)	Subjects without PAKI (n = 46)	Subjects with PAKI (n = 21)	P value
Male (%)	53 (79.1)	38 (82.6)	15 (71.4)	0.340
Age in years	46.48 ± 10.64	45.61 ± 10.76	48.38 ± 10.38	0.360
BMI (kg/m^2)	27.85 ± 6.35	28.04 ± 7.18	27.45 ± 4.11	0.729
Hypertension (%)	58 (86.5)	39 (84.7)	19 (90.4)	0.709
Diabetes (%)	2 (2.9)	1 (2.0)	1 (4.7)	1.000
Smoking (%)	58 (86.5)	38 (82.6)	20 (95.2)	0.187
Drinking (%)	12 (17.9)	6 (13.0)	6 (28.5)	1.000
Preoperative sCr (μmol/L)	88.1 ± 50.9	78.5 ± 26.6	109.2 ± 79.3	0.021
MAP (mm·Hg)	83.3 ± 12.4	83.8 ± 12.4	83.5 ± 12.5	0.915
HR (beats min^{-1})	96 ± 17	97 ± 17	96 ± 15	0.950
CVP (mm·Hg)	9.4 ± 3.0	9.0 ± 2.0	10.1 ± 4.5	0.155
$PaCO_2$ (mm·Hg)	46.9 ± 8.5	46.5 ± 7.6	47.8 ± 10.4	0.559
PaO_2 (mm·Hg)	158.8 ± 274.0	133.6 ± 57.3	213.9 ± 448.3	0.269
EF (%)	58.4 ± 7.2	59.4 ± 6.6	56.2 ± 8.0	0.086

BMI: body mass index; MAP: mean arterial pressure; HR: heart rate; CVP: central venous pressure; EF: ejection fraction.

perfusion from the true or false lumen in AAAD patients. Furthermore, RRI cannot be evaluated in one of the bilateral kidneys because of poor kidney perfusion in 3 patients. It can result in deviations in the RRI values. Although multiple pathogenic pathways existed between RRI and AKI development [24, 28, 29], in conclusion our study certificated that postoperative RRI with cutoff value 0.725 was an independent predictor for the development of postoperative PAKI in AAAD patients.

In this study, patients below SQC Doppler grade 2 accounted for 71.4% in the PAKI group and 45.7% in the non-PAKI group. Univariate regression analysis certificated that SQC Doppler grade 2 was a significant predictor for postoperative PAKI; our study is the first to certify that SQC Doppler grade was an independent predictor of postoperative PAKI by multivariate regression analysis. In this study, when we set the SQC Doppler grade less than 2 as a predictor for persistent AKI, AUC of SQC Doppler grade to predict PAKI was 0.642 (95% CI, 0.49–0.79) with sensitivity 50% and specificity 73.3%. The precision of SQC Doppler grade in predicting postoperative PAKI was poorer than postoperative RRI, which is in accordance with the recent study [30]. In critically ill patients, persistent AKI is considered to be the result of acute tubular necrosis which is caused by initially reduced renal perfusion [31]. The finding can help solving the problem of rapid, flexible, inexpensive, and noninvasive in patients after surgery at the intensive care unit.

Table 3: Intraoperative and postoperative characteristics between groups with PAKI and without PAKI.

	All subjects (n = 67)	Subjects without PAKI (n = 46)	Subjects with PAKI (n = 21)	P value
Time of CPB (min)	205 ± 43	198 ± 43	221 ± 40	0.037
Time of aortic cross clamping (min)	123 ± 33	125 ± 160	120 ± 23	0.059
Time of DHCA (min)	25.0 ± 11.3	23.3 ± 8.8	28.9 ± 14.8	0.889
Urine output (ml)	1848 ± 1065	1910 ± 958	1714 ± 1285	0.489
Intraoperative drugs				
Dopamine	3.57 ± 2.55	3.48 ± 2.50	3.76 ± 2.60	0.676
Adrenaline	0.025 ± 0.020	0.024 ± 0.021	0.026 ± 0.018	0.802
Outcomes				
Postoperative sCr (μmol/L)	140.32 ± 129.62	116.72 ± 138.56	188.61 ± 94.55	
CRRT (%)	8 (11.9)	2 (4.3)	6 (28.6)	0.009
Length of stay in hospital (days)	14.5 ± 8.4	13.4 ± 1.8	16.8 ± 9.4	0.003
Length of stay in ICU (days)	4.7 ± 3.7	3.8 ± 3.5	6.6 ± 3.5	0.009

CPB: cardiopulmonary bypass; DHCA: deep hypothermic circulatory arrest; CRRT: continuous renal replacement therapy.

Table 4: Comparison of RRI and semiquantitative color Doppler grade between groups with PAKI and without PAKI.

	All subjects (n = 67)	Subjects without PAKI (n = 46)	Subjects with PAKI (n = 21)	P value
RRI				
	0.75 ± 0.08	0.72 ± 0.06	0.79 ± 0.09	0.002
Semiquantitative color Doppler grade				
0	3 (4.4%)	0 (0%)	3 (14.3%)	
1	6 (8.9%)	1 (2.2%)	5 (23.8%)	
2	27 (40.3%)	20 (29.8%)	7 (33.3%)	
3	31 (46.2%)	25 (54.3%)	6 (28.6%)	
				0.007

Table 5: Univariate regression analysis for PAKI.

	OR	OR (95% CI)	P-value
Male	0.377	0.114–1.245	0.109
Age	1.025	0.976–1.078	0.322
BMI	0.997	0.919–1.082	0.944
Hypertension	1.842	0.350–9.709	0.471
Preoperative sCr (μmol/L)	1.106	0.999–1.033	0.063
MAP (mm·Hg)	0.957	0.956–1.039	0.869
HR (beats min^{-1})	0.995	0.964–1.026	0.747
CVP (mm·Hg)	1.138	0.941–1.377	0.182
$PaCO_2$ (mm·Hg)	1.018	0.951–1.081	0.562
PaO_2 (mm·Hg)	1.001	0.999–1.103	0.377
EF (%)	0.958	0.878–1.045	0.330
Time of CPB (min)	1.015	1.002–1.028	0.021
Time of aortic cross clamping (min)	1.000	0.996–1.004	0.972
Time of DHCA (min)	1.037	0.991–1.085	0.112
Dopamine	1.061	0.865–1.301	0.571
Adrenaline	2.241	0.001–1.755	0.950
Urine output (ml)	1.000	0.999–1.000	0.439
CRRT	0.049	0.006–0.429	0.006
Length of stay in hospital (days)	1.085	1.010–1.166	0.025
Length of stay in ICU (days)	1.670	1.256–2.221	<0.001
RRI	6.553	2.454–17.503	<0.001
SQC Doppler grade			
2	27.429	2.912–258.387	0.004
1	1.200	0.360–4.000	0.767

Table 6: Multivariate regression analysis for PAKI.

	OR	OR (95% CI)	*P* value	OR	OR (95% CI)	*P* value
Time of CPB (min)	1.006	0.989–1.023	0.515	1.004	0.991–1.023	0.414
Length of stay in hospital (days)	0.962	0.861–1.073	0.486	0.957	0.848–1.079	0.471
Length of stay in ICU (days)	1.512	1.075–2.218	0.018	1.733	1.204–2.493	0.003
RRI	4.110	1.396–12.013	0.010			
SQC Doppler grade 2				19.380	1.406–267.208	0.027
1				1.798	0.395–8.197	0.448

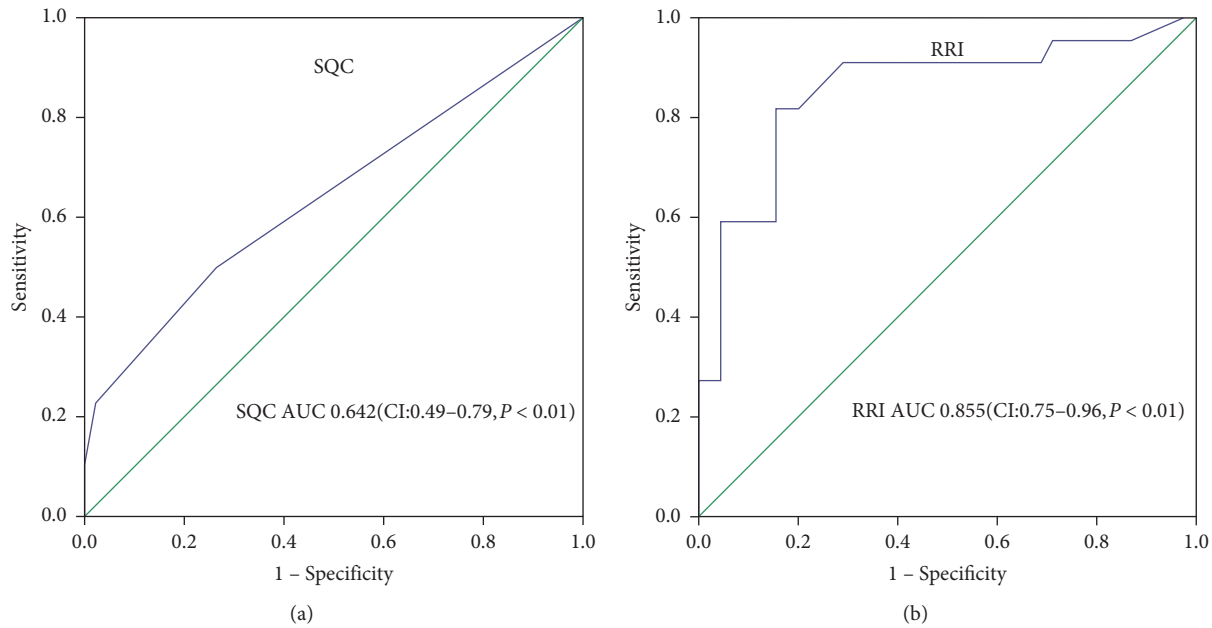


Figure 3: Prediction of postoperative persistent acute renal injury with ROC curves using postoperative RRI (a) and SQC Doppler grade (b).

In conclusion, both postoperative RRI and SQC Doppler grade are independent predictors for PAKI after surgery in AAAD patients. Moreover, both postoperative RRI and SQC Doppler grade can be obtained rapidly by bedside ultrasound, which is an important diagnostic tool in the case of clinical conditions that might impair kidney function [32].

There was no significant difference in demographic and clinical information between the PAKI and without PAKI groups except for preoperative sCr. However, preoperative sCr was not an independent predictor of postoperative AKI by univariate and multivariate analysis in this research. Moreover, to avoid intrarenal hemodynamic changes causing bias, we measured RRI and evaluated the kidney perfusion by ultrasound in the period with mechanical ventilation, supine, during a narrow range of $PaCO_2$. Because the level of breathing, body position and $PaCO_2$ may affect RRI measurement. [33].

We have two strengths: (1) our study is the first to certify SQC Doppler grade as an independent predictor of postoperative PAKI by multivariate regression analysis; (2) the cutoff value 0.725 of postoperative RRI was the same as that of our previous research, which indicates it will have good applicability in AAAD patients in our center.

There are some limitations in our study. (1) The blood flow in the kidney was not easily observed in some obese patients or severe AKI patients. Bias in judgment of the results may exist. (2) The length of the six months study period and the limitation of the small sample, so the statistical results have a large confidence interval. (3) This was a single-center and single-disease study. A further study with wider range of applications, larger sample, and multiple centers is required.

5. Conclusion

Both postoperative RRI and SQC Doppler grade are independent predictors for PAKI after surgery in AAAD patients.

Both postoperative RRI and SQC Doppler grade can be obtained rapidly by bedside ultrasound, which is a good tool for early prediction for postoperative PAKI.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

All authors declare that they have no conflicts of interest.

Authors' Contributions

Huai Qin and Zhanming Fan conceived and designed the experiments; Huai Qin and Yaqiong Liperformed the experiments; Huai Qin, Nan Zhang, and TiezhuWang analyzed the data; Huai Qin and Nan Zhang contributed reagents/materials/analysis tools; and all authors were involved in writing the manuscript.

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Research Article

Predictors for New Native-Vessel Occlusion in Patients with Prior Coronary Bypass Surgery: A Single-Center Retrospective Research

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Objectives. Chronic total occlusion (CTO) is prevalent in patients with prior coronary artery bypass grafting (CABG). However, data available concerning the prevalence of new-onset CTO of native vessels in patients with prior CABG is limited. Therefore, the objective of the study is to determine predictors for new native-vessel occlusion in patients with prior coronary bypass surgery. *Methods*. 354 patients with prior CABG receiving follow-up angiography are selected and analyzed in the present study, with clinical and angiographic variables being analyzed by logistic regression to determine the predictors of new native-vessel occlusion. *Results*. The overall new occlusion rate was 35.59%, with multiple CTOs (42.06%) being the most prevalent (LAD 24.60% and RCA 18.25%, respectively). Additionally, current smoking (OR: 2.67; 95% CI: 2.60 to 2.74; $p = 0.01$), reduced ejection fraction (OR: 1.76; 95% CI: 1.04 to 2.97; $p = 0.04$), severe stenosis (OR: 3.65; 95% CI: 2.55 to 5.24; $p = 0.01$), and diabetes mellitus (OR: 1.86; 95% CI: 1.34 to 2.97; $p = 0.04$) serve as the independent predictors for new native-vessel occlusion. *Conclusion*. As to high incidence of postoperative CTO, appropriate revascularization strategies and postoperative management should be taken into careful consideration.

1. Introduction

Coronary artery disease (CAD), one of the biggest killers, is responsible for approximately 9 million deaths in 2016 [1]. For most CAD patients, they must receive either coronary artery bypass grafting (CABG) or percutaneous coronary intervention (PCI). In some cases, they must receive the combined treatment of CABG and PCI. Clinically, patients treated by CABG therapy tend to experience more complicated conditions and a higher level of severe coronary artery stenosis because they are selected from the total CAD population based on the complexity of coronary heart disease [2]. The mortality and morbidity advantages of CABG in patients with diabetes have been demonstrated by many research studies [3, 4]. A long-term follow-up study showed that CABG shows a superiority in patients with diabetes and multivessel disease over PCI and medication [5]. Similarly, traditional theory holds that CABG is the gold standard in the treatment of the left main coronary artery (LMCA) disease [6].

Despite survival benefits of successful revascularization being firmly established, bypass grafting has several disadvantages. Meta-analysis has demonstrated that patients treated with CABG will experience higher risks in a cerebrovascular accident [7]. Another disadvantage of CABG, as shown by some research studies, is the progression of primary lesions [8, 9]. It was reported in one study that the prevalence of chronic total occlusion (CTO) among CAD patients with and without prior CABG was 89% and 31%, respectively [10], along with a clinical observation of significant increase of CTO. Another study reported that a bypass graft was associated with new native-vessel disease

progression [11]. More importantly, relevant study has shown that native coronary artery CTOs are associated with adverse long-term outcomes [12].

Available evidence has shown that CABG is associated with a high incidence of CTOs, and this may lead to poor prognosis of patients [2].

However, few data regarding the prevalence of new-onset CTOs in native arteries are available, with the relevant risk factors of new-onset CTOs remaining unclear. At present, there is a lack of relevant research on Chinese people, who are the majority of East Asians. Our center, which has the largest number of CABG in China, performs thousands of CABG every year. Therefore, we design this retrospective study to determine incidence and identify independent predictors for postoperative new occlusion in the native vessel.

2. Methods

2.1. Study Population. This study, following the Helsinki Declaration, was approved by the institutional review board and exempted from written informed consent.

All patients were identified from a retrospective review of the institution's database recording detailed information including baseline characteristics, clinical presentation, angiographic data, and medical and surgical treatment. From Jan 2008 to Jan 2017, a total of 5813 patients undergoing CABG were enrolled in the retrospective single-center study, with the following exclusions (1) insufficient data; (2) patients undergoing concomitant valvular or aortic surgery; (3) patients undergoing emergent or urgent surgery; (4) patients with renal failure requiring dialysis; (5) pregnancy; (6) patients with age less than 18 or more than 75 years; (7) without or follow-up angiography less than 1 or more than 5 years; (8) target lesion revascularization within 1 year; and (9) myocardial infarction in recent 3 months. Follow-up angiography analysis was performed after excluding the subjects above.

2.2. Coronary Angiography. A baseline angiography was required for patients within 4 weeks before CABG, and one to five year angiographic follow-up was received for them, along with the performing of diagnostic coronary angiography by experienced and credentialed operators after obtaining written informed consent of angiography. The choice of artery access site (radial or femoral) was made by the interventional physician. Procedures were performed by inserting a 6 or 7 Fr guiding catheter under intravenous administration of 3000–5000 IU heparin, with a requirement of injection of each study vessel with at least 2 orthogonal views. Preoperative coronary angiograms were reviewed by two interventional cardiologists blinded to patient outcomes to determine the presence, location, and length of coronary lesions, along with the conduction of postoperative angiograms by two cardiologists blinded to the baseline interpretation. The major epicardial arteries and major branches (≥2.0 mm in diameter) were assessed, the estimation of which was based on the first onset of angina, prior history of myocardial infarction in the target vessel territory, or comparison with a prior angiogram.

2.3. Surgical Procedures and Perioperative Management. Operative reports available were reviewed. SYNTAX (Synergy between Percutaneous Coronary Intervention with Taxus and Cardiac Surgery) score was calculated with each pre-CABG coronary angiogram as the criterion of surgical intervention. After evaluating the severity of coronary lesions, the procedure and selection of grafting were based on the patient's clinical presentation, angiographic features, and conduit availability. Pharmacologic treatment obtained from electronic medical records was determined by cardiovascular comorbidities. Patients received aspirin on the first postoperative day, along with indefinite continuation of low-dose aspirin and statin treatment.

2.4. Study Outcomes. Qualified readers evaluated angiograms for initial severity of stenosis, morphologic features, location, and occurrence of lesion progression or occlusion using a side-by-side film review, with the primary study outcome as the occurrence of total occlusion in native coronary arteries.

2.5. Definition. CTO is defined as a coronary obstruction with thrombolysis in zero-grade flow of myocardial infarction (TIMI) persisting for at least 3 months. Multivessel coronary disease (MVD) is defined as lesions occurring in the left main coronary artery and or over 50% stenosis of the diameter occurring in at least two main epicardial arteries or the primary branches. Progression was defined as further definite narrowing by 25% [13].

2.6. Statistical Analysis. Results for continuous variables were presented as mean ± standard deviation, whereas discrete parameters are expressed as the counts and percentages. Continuous parameters were compared with one-way ANOVA, along with the comparison of discrete data using the Mann–Whitney U test. The odd ratio (OR) with 95% CI from a logistic regression model was applied to estimate the risks of particular variables on occlusion of the native vessel. All analyses were performed with the Statistical Package for the Social Science (SPSS) software (version 19; Chicago, IL, USA), which were regarded as statistically significant when the critical value $p < 0.05$.

3. Results

3.1. Patient Characteristics. As shown in Figure 1, of 5813 patients undergoing CABG, a total of 354 patients with 938 vessels met the inclusion criteria and were analyzed subsequently, with baseline characteristics of those 354 patients who underwent follow-up angiography being demonstrated in Table 1. Diabetes mellitus (38.16% vs. 49.20%, $p = 0.044$), current smoking (16.67% vs. 47.62%, $p < 0.001$), and lower ejection fraction (59.28% ± 0.07% vs. 56.89% ± 0.1%, $p = 0.010$) are more common for patients who suffered

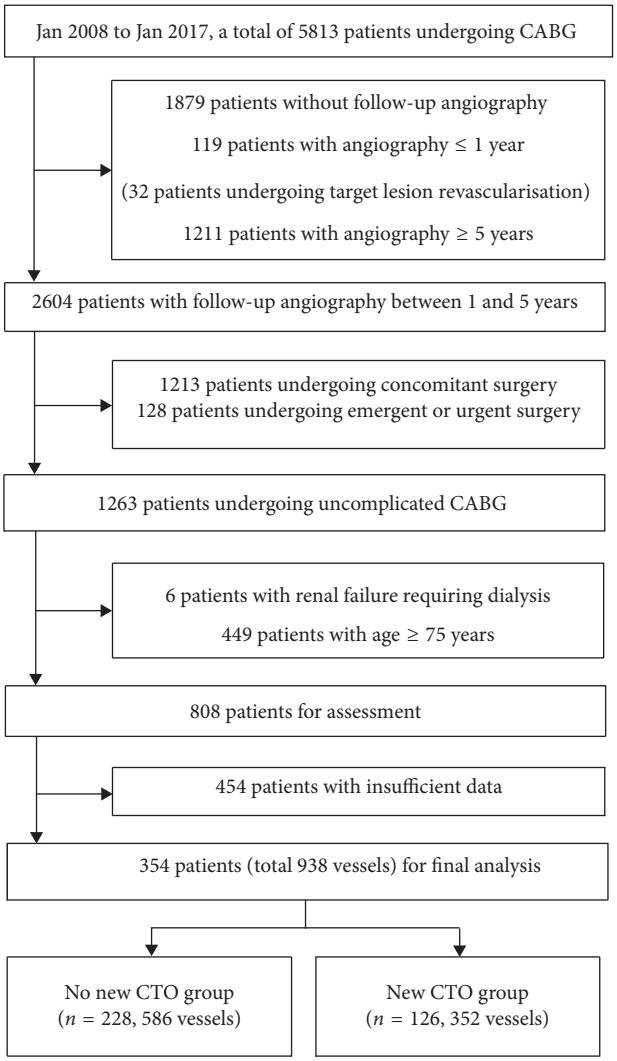

Figure 1: Flow chart of the study.

postoperative new total occlusion of native arteries (new CTO group) than those without new vessel occlusion during the particular follow-up periods (no new CTO group). In addition, most patients were men with multiple cardiovascular risk factors including hypertension and hypercholesterolemia, however without significant difference between the two groups. Of particular concern were overweight (BMI $\geq 24.0\,\mathrm{kg/m^2}$) taking up for in nearly 90% of the patients and obesity (BMI $\geq 28.0\,\mathrm{kg/m^2}$) accounting for approximately one-third of them according to the post-CABG BMI. However, there was no significant difference in the proportion stratified by the BMI category.

3.2. Procedural and Angiographic Characteristics. Procedural and lesion characteristics in both groups are presented in Table 2, finding that 35.04% (124 of all cases) patients with at least one CTO had been treated with CABG, and CTOs occurred in 70.62% (250) of cases after CABG (Table 3). Of these, 35.59% (126) suffered from postoperative new total occlusion of native arteries, with preoperative CTO being more likely presented in RCA (14.97%) and most patients having a single postoperative CTO (74.4%), predominantly in the RCA (30.4%). As shown in Table 3, total CTO distribution was as follows: RCA (21.47%); LAD (19.49%); LCX (10.73%); and multiple distributions (18.08%). The overall new occlusion rate was 35.59%, and multiple CTO (42.06%) was most prevalent, followed by LAD (24.60%) and RCA (18.25%). New CTO distribution is summarized in Table 4, showing that among the new CTO patients, the initial lesion was more severe (stenosis $\geq 70\%$, LAD 90.48% vs. 76.32%; LCX 70.63% vs. 53.95%; RCA 77.78% vs. 57.89%, $p = 0.001$). There was no significant difference in the coronary bypass grafting profiles. The mean follow-up was 37 months, without reaching statistical significance compared to the no new CTO (37.52 ± 17.39 vs. 32.65 ± 15.84, $p = 0.175$). Although not statistically significant, we found a relatively low proportion of free of symptom and a slightly high revascularization rate (predominantly incomplete revascularization) in patients with new CTO. Moreover, disease progression in native vessels is shown in Table 5 as follows: LM (2.38%); LAD (26.60%); LCX (13.70%); and RCA (18.25%).

3.3. Predictors for New Native Coronary Artery Occlusion. Univariate and multivariate analyses were performed to determine the predictors of new native coronary artery occlusion (Table 6). Current smoking (OR: 2.67; 95% CI: 2.60 to 2.74; $p = 0.01$), reduced ejection fraction (OR: 1.76; 95% CI: 1.04 to 2.97; $p = 0.04$), diabetes mellitus (OR: 1.86; 95% CI: 1.34 to 2.97; $p = 0.04$), and initial stenosis $\geq 70\%$(OR: 3.65; 95% CI: 2.55 to 5.24; $p = 0.01$) were associated with an increased risk of new native-vessel occlusion, apart from which, severe stenosis serves as the most powerful predictor among them.

4. Discussion

Revascularization of coronary arteries with severe stenosis has reached a consensus in the area of cardiovascular therapeutics and research. For patients with revascularization indications, aggressive revascularization by CABG or PCI is favorable [14]. In clinical practice, patients presenting with complex coronary atherosclerosis, especially multivessel disease, typically are referred for CABG surgery. The preference has also been well supported by published literatures reporting CTO prevalence and treatment. Previous studies have demonstrated that patients with a CTO undergoing PCI and CABG surgery were 4.6–44.98% and 23–40%, respectively, and patients without a CTO were 36–52.9% and 23.2–28%, respectively [15–18]. Surgical revascularization benefits patients with three-vessel or LMCA disease and CTOs including improvement of angina, left ventricular function, and mortality; however, CABG can accelerate stenosis progression of native vessels [16, 19]. Previously, with more attention being paid to graft patency, we observed new native-vessel occlusion after CABG and identified clinical and angiographic predictors for exacerbation of coronary lesions.

Prevalence of CTO is common in patients with prior CABG, and it was reported up to 50% [17]. Similar to

Table 1: Baseline patient characteristics.

Variables	All ($N = 354$)	No new CTO ($n = 228$)	New CTO ($n = 126$)	p value
Age (yrs.)	61.71 ± 9.47	62.75 ± 7.93	61.80 ± 8.38	0.492
<65 (%)	212 (59.89%)	136 (59.64%)	76 (60.31%)	
65 to <75 (%)	142 (40.11%)	92 (40.35%)	50 (39.68%)	
Sex				0.694
Male	264 (74.58%)	169 (74.12%)	95 (75.40%)	
Female	90 (25.42%)	59 (25.88%)	31 (24.60%)	
BMI (kg/m^2)	26.57 ± 3.88	27.06 ± 2.97	26.59 ± 3.48	0.397
≤18.4	0	0	0	
18.5–23.9	54 (15.25%)	30 (13.16%)	24 (19.05%)	
24.0–27.9	186 (52.54%)	125 (54.82%)	61 (48.41%)	
≥28	112 (31.64%)	72 (31.58%)	40 (31.75%)	
Hypertension	248 (70.06%)	158 (69.30%)	90 (71.43%)	0.532
Hypercholesterolemia	206 (58.19%)	129 (56.59%)	77 (61.11%)	0.217
Diabetes mellitus	140 (39.55%)	87 (38.16%)	62 (49.20%)	0.044
Diet-controlled	10 (7.14%)	6 (6.90%)	4 (6.45%)	
Tablet-controlled	64 (45.71%)	39 (44.82%)	30 (48.39%)	
Insulin treatment	66 (47.15%)	42 (48.28%)	28 (45.16%)	
Smoker				$p < 0.001$
Never	112 (31.64%)	79 (34.64%)	33 (26.19%)	
Former	144 (40.68%)	111 (48.68%)	33 (26.19%)	
Current	98 (27.68%)	38 (16.67%)	60 (47.62%)	
Prior MI	90 (25.42%)	58 (25.44%)	32 (25.40%)	0.990
Prior PCI	68 (19.21%)	47 (20.61%)	21 (16.67%)	0.178
Prior cardiac surgery	2 (0.57%)	2 (0.88%)	0 (0.00%)	0.525
Prior heart failure	36 (10.17%)	21 (9.21%)	15 (11.91%)	0.237
Peripheral vascular disease	74 (20.90%)	45 (19.74%)	29 (23.02%)	0.279
	70 (19.77%)	46 (20.18%)	24 (19.05%)	0.704
Cerebrovascular disease	24 (6.78%)	14 (6.14%)	10 (7.94%)	0.337
Family history of CAD	80 (22.60%)	52 (22.81%)	28 (22.22%)	0.345
Atrial fibrillation/flutter	36 (10.17%)	18 (7.90%)	18 (14.29%)	0.480
				0.104
Presentation of ACS	12 (3.39%)	8 (3.51%)	4 (3.17%)	
CCS class of angina	218 (61.58%)	145 (63.60%)	73 (57.94%)	
Grade 1	110 (31.07%)	68 (29.82%)	42 (33.33%)	
Grade 2	14 (3.69%)	8 (3.51%)	6 (2.78%)	
Grade 3	58.43 ± 0.08%	59.28% ± 0.07%	56.89% ± 0.1%	0.010
Grade 4	11 (3.10%)	3 (1.32%)	8 (6.35%)	
Ejection fraction (%)	31 (8.76%)	14 (6.14%)	17 (13.49%)	
≤40%	312 (88.14%)	211 (92.54%)	101 (80.16%)	
40%–50%				0.404
≥50%	150 (42.37%)	101 (44.30%)	49 (38.89%)	
Left ventricular grade	164 (46.33%)	100 (43.86%)	64 (50.79%)	
Class I	38 (10.73%)	26 (11.40%)	12 (9.52%)	
Class II	2 (0.57%)	2 (0.88%)	0 (0.00%)	
Class III	77.6 ± 20.47	73.93 ± 17.94	79.09 ± 21.30	0.079
Class IV	87.2 ± 22.49	90.5 ± 20.11	85.89 ± 23.33	0.172
Creatinine (μmol/l)	38 (10.73%)	51.19 ± 7.03	46.46 ± 11.85	0.484
GFR (ml/min/1.73 m^2)	2.47 ± 4.27	3.01 ± 4.40	2.26 ± 4.22	0.700
GFR < 60 ml/min/1.73 m^2				
Hs-CRP (mg/L)				

previous studies, we found the morbidity of multiple coronary artery stenosis and total occlusion of native arteries in patients post-CABG are considerable in the current study [8, 11, 13]. In this regard, we favor the reasons that patients with complex coronary atherosclerosis were always recommended for surgery and bypass grafts which accelerate stenosis in native vessels [8, 11, 13, 17]. So far, the underlying mechanism remains unknown, and it is speculated that flow competition between the native vessel and graft contributes this progression [8, 11, 13]. In addition, we also found new occlusion which was more common in non-LAD with the findings consistent with the aforementioned studies [8, 11, 13]. Considering the current surgical practice and previous findings that greater incidence of disease progression in segments bypassed with venous grafts, we speculate that it might be associated with the use of venous grafts [19]. However, no significant difference was found between the type of grafts and the new occlusion in this

Table 2: Procedural and angiographic characteristics of patients with or without a new CTO.

Variables	No new CTO ($n = 228$)	New CTO ($n = 126$)	p value
Preoperative angiogram			
Vessel stenosis at baseline			0.001
Moderate (40%–69%)			
LAD	18 (7.89%)	7 (5.56%)	
LCX	18 (7.89%)	6 (4.76%)	
RCA	22 (9.65%)	9 (7.14%)	
Severe (≥70%)			
LAD	174 (76.32%)	114 (90.48%)	
LCX	123 (53.95%)	89 (70.63%)	
RCA	132 (57.89%)	98 (77.78%)	
Coronary lesion category			0.775
Single-vessel disease	18 (7.89%)	8 (6.35%)	
Double-vessel disease	62 (27.19%)	31 (24.60%)	
Triple-vessel disease	116 (64.47%)	87 (69.05%)	
Left main involvement	102 (44.74%)	48 (38.10%)	0.508
Total no. of patients with ≥1 CTO	83 (36.40%)	44 (34.92%)	0.416
Total of CTO vessel			0.984
LAD	23 (10.09%)	15 (11.91%)	
LCX	15 (6.58%)	7 (5.56%)	
RCA	35 (15.35%)	18 (14.29%)	
Multivessel	8 (3.51%)	3 (2.38%)	
Mean no. of bypass grafts	3.0 ± 0.63	3.06 ± 0.75	0.599
Types of graft			0.460
Internal mammary artery	183 (80.26%)	107 (84.92%)	
Saphenous vein	2.22 ± 0.64	2.25 ± 0.73	
Graft patency			0.522
LIMA	190 (83.33%)	109 (86. 51%)	
SVG-D	195 (85.53%)	96 (76.19%)	
SVG-LCX/OM	185 (81.14%)	93 (73.81%)	
SVG-RCA/PDA	163 (71.49%)	84 (66.67%)	
Postoperative medication			0.902
Aspirin	215 (94.30%)	118 (93.65%)	
Statin	185 (81.14%)	98 (77.78%)	
Follow-up time (Mths)	32.65 ± 15.84	37.52 ± 17.39	0.175
Free of symptom	123 (53.94%)	56 (44.44%)	0.087
Incomplete revascularization	112 (49.12%)	70 (55.56%)	0.125

study. Moreover, the present data do not exclude the possibility that local vascular anatomy is the critical pathogenic factor. Regarding the further therapy, patients presenting with recurrent ischemic symptoms, graft failure, or significant progression of native vessels are eligible to undergo revascularization after surgery. It is established that PCI of native coronary arteries is a preferred revascularization strategy for patients with prior CABG, especially those with patent left internal artery bypass grafts. Reasons are given as follows: for the repeat CABG, technical difficulties, increased mortality, and limited symptomatic improvement are major obstacles, and for graft intervention, the increased risk and worse long-term outcomes than native coronary arteries are also a frustrating problem. Even so, previous CABG was associated with the failure of CTO intervention combined with traditionally low success rates and high complication rates [20]. Despite this, the procedural success rate for CTO-PCI has improved over the years with the development of device, technique, and strategy. However, apart from experienced operators, there are several major drawbacks: higher radiation doses, higher volume of contrast agent administered, more severe complications, higher incidence of repeat revascularization, and lower procedural success rates, as compared with non-CTO-PCI [14, 15, 17, 21, 22]. We thus suggest patients with complex multivessel CAD undergo a hybrid approach, such as two-vessel disease with proximal LAD coronary artery occlusion. The hybrid approach is a potential alternative, namely, PCI, for the nonoccluded artery while leaving the CTO vascularized through minithoracotomy approaches, to avoid following weaknesses: surgery-related progression of native coronary lesions, graft stenosis, low primary success rate, and relatively high risk-benefit ratio of CTO-PCI. Furthermore, the

Table 3: Characteristics of lesion distribution. Preoperative and postoperative analyses of CTO distribution.

Vessel	Pre-CABG ($n = 12$ 35.04%)	Post-CABG ($n = 250$ 70.62%)
LM	0 (0%)	3 (0.85%)
LAD	38 (10.73%)	69 (19.47%)
LCX	22 (6.22%)	38 (10.73%)
RCA	53 (14.97%)	76 (21.47%)
Multi vessels	11 (3.12%)	64 (18.08%)

Table 4: Distribution of new CTO in native vessels.

Vessel	New CTO in native vessels ($n = 126$)
LM	3 (2.38%)
LAD	31 (24.6%)
LCX	16 (12.7%)
RCA	23 (18.25%)
Multivessels	53 (42.06%)

Table 5: Disease progression in native vessels.

Vessel	Overall ($N = 938$)	No new CTO ($n = 586$)	New CTO ($n = 352$)
LM	22 (2.38%)	12 (2%)	3 (0.85%)
LAD	250 (26.6%)	42 (7.11%)	69 (19.49%)
LCX	129 (13.7%)	17 (2.97%)	38 (10.73%)
RCA	171 (18.25%)	37 (6.53%)	40 (11.47%)

Table 6: Predictors for new native-vessel occlusion.

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI	p value	OR	95% CI	p value
DM	1.57	1.01–2.43	0.044	1.86	1.34–2.97	0.04
Current smoking	2.95	1.51–5.75	0.001	2.67	2.60–2.74	0.01
Ejection fraction	2.16	1.19–3.92	0.010	1.76	1.04–2.97	0.04
Creatinine	1.35	0.91–1.97	0.079			
Severe stenosis (≥70%)	3.85	2.16–6.87	0.001	3.65	2.55–5.24	0.01

hybrid approach facilitates PCI for non-bypassed segments to achieve more complete revascularization by reducing the incidence of new native-vessel occlusion.

Recurrence of effort angina increasing over time was frequently observed in patients with prior CABG. Campeau et al. have revealed the proportion of patients without symptoms after the procedure decreased from 72% to 37% in 10 years of follow-up [23]. In the present study, we found a higher proportion of patients with new occlusion complained of recurrent angina which has driven more repeat percutaneous revascularization of native coronary arteries, with accompanying majority incomplete revascularization. Correspondingly, greater follow-up major adverse cardiovascular event (MACE) rates were also found in these patients. According to a previous study, there were pathological differences in CTO patients with and without CABG [24]. CTOs with CABG have extensive calcification which has largely been attributed to blood stasis and low shear stress resulting from competitive flow between the native and bypass graft [25]. It has also been confirmed that calcification makes CTO-PCI difficult, and the success rate for CTO with prior CABG is significantly lower than those without CABG [25, 26]. Therefore, it is difficult or impossible to achieve complete revascularization. Incomplete revascularization results in persistent left ventricular dysfunction which in turn leads to a worse outcome on follow-up (higher mortality) [20, 27].

Previously, a study has reported clinical and angiographic predictors for native coronary vessel occlusion including bypass graft for non-LAD arteries and graft occlusion and no use of aspirin for LADs [11]. Our data suggest that independent risk factors for lesion occlusion also included diabetes, current smoking, initial lesion severity, and lower ejection fraction. The correlation of initial lesion severity with disease worsening is similar to the finding that progression of atherosclerosis with significant stenosis occurs 10 times as frequently in bypassed arteries as in non-bypassed arteries [13]. In addition, CTO-PCI was frequently performed among patients with prior CABG with lower technical success rates compared to patients without prior CABG [25]. In this regard, minimally diseased coronary arteries were recommended not to be bypassed [13]. In conclusion, our data show few predictors of occlusion in native arteries after CABG. Of note, these indicate the clinical importance of risk factor management against subsequent native-vessel occlusion in the postoperative period. It is worth noting that these predictors are also risk factors for general CAD patients except lower ejection fraction. Patients with these predictors tend to have more complexity of coronary heart diseases. For patients with these predictors, appropriate revascularization strategies such as a hybrid approach should be taken into careful consideration to reduce new native-vessel occlusion. Further study is needed to explore the differences of predictors for new native-vessel occlusion between the average CAD patient population and patients with prior CABG.

4.1. Study Limitation. There were several limitations to our study. First, it is a retrospective design. Second, the surgical procedures were performed in a single institution but not by a single surgeon. Our institution does not routinely perform coronary angiography on all patients who have undergone CABG. This means that the study has a bias in the cohort. A prospective study is needed to further explore this issue. An

additional limitation of the study is the heterogeneity bias for operator-related and procedure-related factors.

Data Availability

The data of this study can be obtained from the corresponding author only with a reasonable request. The data are not publicly available because the availability of these clinical data was made under ethical license conditions applied for this study, which contained information that could compromise the privacy of research participants.

Conflicts of Interest

There are no conflicts of interest.

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