

Circulating Sulfatide, A Novel Biomarker for ST-Segment Elevation Myocardial Infarction

Gang Li¹, Rui Hu², Yifang Guo¹, Lili He¹, Qingjuan Zuo¹ and Yan Wang¹

Gang Li and Rui Hu contributed equally to this work.

¹Division of Cardiology, Institute of Geriatric Diseases, Hebei General Hospital, Shijiazhuang, Hebei, China

²General Clinical Laboratory, The Second Hospital of Hebei Medical University, Shijiazhuang, Hebei, China

Aims: ST-segment elevation myocardial infarction (STEMI) is an acute inflammatory and thrombotic disease due to coronary artery atherosclerotic lesions. Studies have established the correlation of serum sulfatides with inflammation, thrombogenesis, and atherosclerosis. We observed that serum sulfatides level significantly increased in STEMI patients. In this study, we try to identify the relationship of serum sulfatides level on clinical outcomes of patients in STEMI.

Methods: Serum sulfatides level was monitored in 370 inpatients within 24 h of STEMI onset. On the basis of the level of serum sulfatides that was below 10 $\mu\text{mol/L}$ in the normal population, the patients were divided into two groups with the median value of 15.2 $\mu\text{mol/L}$; low sulfatide group [serum sulfatides level ≤ 15.2 $\mu\text{mol/L}$ ($n=200$)] and high sulfatide group [serum sulfatides level > 15.2 $\mu\text{mol/L}$ ($n=170$)]. Patients' baseline characteristics, in-hospital outcomes, and late major adverse cardiovascular events (MACE) were analyzed. Independent incident for in-hospital death and late adverse events were modeled by multivariate logistic and Cox regression analysis.

Results: Between the two groups, there were no differences in the angiographic characteristics, percutaneous coronary intervention (PCI) results, and in-hospital recovery. However, high serum sulfatides level is positively correlated with increased rate of in-hospital death (OR 0.971; 95% CI 0.926–0.990, $p=0.019$). In addition, this group of patients has more cumulative incidences of target vessel revascularization (TVR) (23% vs. 8%, $p<0.05$) and increased overall MACE (28% vs. 10%, $p<0.05$). Cox regression analysis indicated that high serum sulfatides level contributes to TVR and overall MACE.

Conclusions: Elevated serum sulfatides level positively correlate with in-hospital death and complications (TVR and MACE) in STEMI patients.

Key words: Serum sulfatide, Myocardial infarction, Coronary, Revascularization, Outcome

Introduction

It has been known for decades that ST-segment elevation myocardial infarction (STEMI) is a process of acute inflammatory and thrombotic formation inside the coronary artery, which causes immediate clinical suffering and manifests in short- and long-term complications, such as acute pumping failure, fatal arrhythmia, myocardial rupture, and chronic left

ventricular dysfunction^{1, 2}. Even with immediate percutaneous coronary revascularization (PCI), a large number of patients remain at risk for early electrical/mechanical complications and subsequent major adverse cardiac events (MACE) from myocardial stunning, adverse left ventricular remodeling, culprit lesion restenosis, and *de novo* coronary stenosis³. Early detection and diagnosis are the keys to preventing the progression of STEMI and improving patients' prog-

Address for correspondence: Gang Li, Division of Cardiology, Institute of Geriatric Diseases, Hebei General Hospital, Shijiazhuang, Hebei, China.
E-mail: hrlg2002@163.com

Received: January 9, 2018 Accepted for publication: April 22, 2018

Copyright©2019 Japan Atherosclerosis Society

This article is distributed under the terms of the latest version of CC BY-NC-SA defined by the Creative Commons Attribution License.

nosis and the hospital survival rate^{4, 5}). Currently, traditional methods, including an early electrocardiogram, coronary angiography, and a panel of enzymatic analyses are applied in the diagnosis of acute myocardial infarction, but there is still a lack of efficient early detection of STEMI^{6, 7}). Actually, some biomarkers were identified and may be involved in the process of plaque formation to STEMI. However, their sensitivity and specificity still need to be examined^{8, 9}). Therefore, a novel biomarker is urgently needed for the effective diagnosis and treatment of STEMI.

Sulfatide is a kind of ester, which with sulfuric acid and galactosylceramides at C3 of the galactosyl residue, presents in mammalian serum as a major component of glycosphingolipids in lipoproteins¹⁰). Vast experimental and clinical studies have revealed that serum sulfatides are strongly connected to an inflammatory reaction and thrombogenesis and even the buildup of the extracellular matrix in a damaged vessel¹¹⁻¹³). Given that serum sulfatides contribute to atherosclerosis^{11, 12}) and coronary artery disease (CAD) and have been reported as a novel biomarker for CAD in patients with end-stage renal failure¹⁴), we explored the association of circulating sulfatides level with the outcomes of STEMI in 370 inpatients. Our results indicated that sulfatides might be a valuable biomarker for the early clinical diagnosis and the effective treatment of STEMI as well.

Methods

Participants

We analyzed the data from 370 inpatients (230 males, 140 females; mean age, 67.9 ± 10.3 years) at Hebei General Hospital with a diagnosis of STEMI from March 2009 to February 2013. The median (25th to 75th percentiles) level of serum sulfatides was 15.2 (10.8–21.9) $\mu\text{mol/L}$. The patients were divided into two groups based on their value (15.2 $\mu\text{mol/L}$) of serum sulfatides at admission: below the median ($n=200$) or above the median ($n=170$). The diagnosis of STEMI was made on the basis of standardized criteria²). All of the patients consented to have emergency coronary angiography to define the coronary anatomy.

Our study was performed in conformity with the Declaration of Helsinki. We acquired signed informed consent from every one of the participants, and the Medical Ethics Committee of Hebei General Hospital authorized the study procedures.

Quantitative Analysis of Serum Sulfatide

Once the patient was admitted, peripheral blood samples were collected in the cath lab and a panel of

biochemistry factors, such as glucose, HbA1c, creatine kinase, and other biomarkers, were measured with standardized assays. An aliquot of serum was stored at -80°C for the measurement of sulfatide. In brief, sulfatide was extracted from the serum, and it was then analyzed as lyso-forms using our established method¹⁵). The total lipids, including sulfatides, were extracted from 50 μL of serum with n-hexane: isopropanol (3:2, v/v). After samples were dried, we continued to hydrolyze the dried samples with 0.1 N of NaOH in 90% methanol at 150°C for 30 min to convert sulfatide to lyso-sulfatide, followed by desalting with Mono-tip C18 tips (GL Sciences, Tokyo, Japan). Lastly, all of the specimens were examined with matrix-assisted laser desorption ionization time-of-flight mass spectrometry including delayed ion extraction employing a Voyager Elite XL (6.5 m flight length in the reflector mode) Biospectrometry Workstation (PerSeptive Biosystems, Framingham, MA, USA). A nitrogen laser (337 nm) was implemented for ionization and negative ion mode detection.

Clinical Assessment

Discharged patients were closely followed-up with phone interviews and outpatient clinics. The following data were collected: non-fatal myocardial infarction, target vessel revascularization (TVR), coronary artery bypass grafting, and cardiac-related death in overall mortality. Effects of the administration of lipid-lowering (e.g., statins) and non-lipid-lowering medicines (e.g., aspirin, β -blockers, clopidogrel, angiotensin receptor antagonists, angiotensin-converting enzyme inhibitors, and aldosterone receptor antagonist) were also compared between the groups.

Statistical Analysis

The data are expressed as mean \pm standard deviation. Continuous variables were compared using Student's *t* test. Correlations between serum sulfatides and other continuous variables were examined by univariate analysis. The multivariate logistic regression model identified independent correlates of in-hospital mortality for every patient. Rationale variables chosen for regression analysis involved demographic characteristics, co-morbid illnesses, and angiographic features. Variables were assessed in a backward conditioned multivariate logistic regression model when their univariate *p* values were <0.20 . Statistical significance was established as multivariate $p < 0.05$. The odds ratios and their respective 95% confidence intervals (CIs) from the multivariate logistic regression analysis were employed as estimates of relative risk. Multivariate Cox proportional hazard analysis was applied to identify the independent predictors of TVR and

Table 1. Baseline Characteristics of patients with ST-Segment Elevation Myocardial Infarction Stratified According to Median Level (15.2 $\mu\text{mol/l}$) of Serum sulfatide

	Serum sulfatide = 15.2 $\mu\text{mol/l}$ (<i>n</i> =200)	Serum sulfatide >15.2 $\mu\text{mol/l}$ (<i>n</i> =170)	<i>P</i> Value
Clinical Characteristics			
Age (years)	69.1 \pm 10.2	66.3 \pm 10.5	NS
Female (%)	79 (39.5)	61 (35.9)	NS
Hypertension (%)	110 (55.0)	98 (57.6)	NS
DM (%)	98 (49.0)	86 (50.6)	NS
Smoking (%)	151 (75.5)	123 (72.4)	NS
BMI (kg/m^2)	24.3 \pm 2.3	25.1 \pm 3.0	NS
Previous CAD (%)	33 (16.5)	23 (13.5)	NS
Previous Stroke (%)	19 (9.5)	15 (8.8)	NS
Carotid Stenosis (%)	6 (3.0)	4 (2.4)	NS
PAD (%)	5 (2.5)	3 (1.8)	NS
LVEF(%)	45 \pm 11	43 \pm 12	NS
Biochemical Data			
eGFR (ml/min)	58.1 \pm 19.9	45.9 \pm 17.7	<0.001
TC (mmol/l)	5.2 \pm 1.2	5.2 \pm 1.2	NS
TG (mmol/l)	1.4 \pm 0.8	1.3 \pm 0.8	NS
HDL-C (mmol/l)	1.1 \pm 0.3	1.0 \pm 0.2	NS
LDL-C (mmol/l)	3.2 \pm 1.0	3.2 \pm 1.0	NS
Glucose (mmol/l)	7.3 \pm 3.9	9.9 \pm 4.5	<0.001
HbA1c (%)	6.2 \pm 1.1	6.9 \pm 1.2	NS
BNP (pg/dl)	58.7 \pm 19.7	62.3 \pm 21.2	NS
hs-CRP ($\mu\text{g/dl}$)	153 \pm 87	201 \pm 95	NS
CPK (IU/L)	216 \pm 65	229 \pm 78	NS
CK-MB (IU/L)	25 \pm 17	28 \pm 19	NS
Troponin T (ng/ml)	0.18 \pm 0.09	0.24 \pm 0.10	NS
Peak CPK (IU/L)	1990 \pm 983	3760 \pm 1230	<0.05
Peak CK-MB (IU/L)	175 \pm 68	249 \pm 79	NS
Medication on Admission			
Aspirin, <i>n</i> (%)	30 (15)	27 (16)	NS
Clopidogrel, <i>n</i> (%)	4 (2)	5 (3)	NS
ACEI or ARB, <i>n</i> (%)	58 (29)	53 (31)	NS
B-Blocker, <i>n</i> (%)	20 (10)	15 (9)	NS
Statin, <i>n</i> (%)	34 (17)	27 (16)	NS
Aldosterone receptor antagonist, <i>n</i> (%)	4 (2)	5 (3)	NS

Data are presented as frequency (percentage) for categorical variables and as mean \pm standard deviation or median (interquartile range) for continuous variables.

NS, not significant; CAD, coronary artery disease; DM, diabetes mellitus; PAD, peripheral arterial disease; LVEF, left ventricular ejection fraction; BMI, body mass index; BNP, B-type natriuretic peptide; CK-MB, creatine kinase myocardial isoform; CPK, creatine phosphokinase; eGFR, estimated glomerular filtration rate; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker.

MACE, after modifying for baseline and angiographic variables with unequal distribution. A *p* value <0.05 was deemed to be significant for every one of the analyses. Data were statistically processed with SPSS software version 18.0 (SPSS, Chicago, IL, USA).

Results

High sulfatides decrease estimated glomerular filtration rate (eGFR) but increase serum glucose and peak creatine phosphokinase (CPK) in STEMI patients. The random STEMI patient data were divided into two groups according to their serum sulfatides level. As shown in **Table 1**, the clinical charac-

teristics and medication history of the patients were comparable as indicated by the p values. However, in contrast to the lower sulfatides group, the higher serum sulfatides (above the median group) significantly reduced eGFR ($p < 0.001$) and increased serum glucose ($p < 0.001$) and peak CPK ($p < 0.05$) among all of the 14 biochemical factors that we tested. To further determine the relationship of the sulfatides level with the changes of the serum factors, linear regression analysis was performed. As shown in **Table 2**, a high sulfatides level was strongly correlated with decreased eGFR ($r = -0.118$, $p = 0.043$), increased glucose ($r = 0.235$, $p = 0.001$), and peak CPK ($r = 0.311$, $p = 0.015$), but not with other biomarkers, such as troponin T, BNP, and hs-CRP. These data suggested that increased sulfatides are may be involved in the process of STEMI.

High sulfatides did not affect coronary angiography, interventional treatment, and short-term recovery. As shown in **Table 3**, we also investigated the patients' other in-hospital clinical data, such as the culprit lesion vessel, the severity of overall CAD, the door-to-balloon time, initial TIMI flow, and the therapeutic modalities of PCI in terms of thrombectomy, balloon angioplasty, and endovascular stents. There were no differences between these two groups ($p > 0.05$). In-hospital complications, such as respiratory failure, cardiogenic shock, new atrial fibrillation, and the need for emergency coronary bypass surgery, were also statistically equivalent in these two groups ($p > 0.05$). Even though there were 10 in-hospital deaths in the group with the serum sulfatide levels above the median, and 8 patient deaths in the group with the serum sulfatides below the median, the t test was not statistically significant ($p > 0.05$). However, both univariate regression and multivariate regression analysis identified that the serum sulfatides level and the number of diseased coronary > 1 was positively related to in-hospital death for all of these patients (**Table 4**).

Long-term clinical outcomes. All of the patients discharged after STEMI were medicated under the same protocol. The Kaplan–Meier survival test showed that during a mean follow-up period of 1.6 years (1.63 ± 0.87 years), the patients with a serum sulfatides level above the median had more incidences of TVR at the rate of 23% versus 8% in the low sulfatides group ($p < 0.05$). Therefore, the overall MACE was significantly higher (28% vs. 10%, $p < 0.05$) in the high sulfatides group than it was in the low sulfatides group, but the rates of non-fatal myocardial infarction, novo lesions, cardiac deaths, and all causes of mortality were comparable between these two groups (**Table 5**). Then, we performed multivariate Cox regression analysis. After adjusting for gender, age, smoking, diabetes

Table 2. Spearman Correlation Coefficients (R) Between Serum Sulfatide Levels and Continuous Variables in the Patients with STEMI

	Spearman R	P Value
Age (years)	-0.112	0.181
BMI (kg/m ²)	0.022	0.843
LVEF (%)	-0.087	0.323
eGFR (ml/min)	-0.118	0.043
Triglycerides (mmol/l)	0.100	0.245
LDL-C (mmol/l)	-0.028	0.801
HDL-C (mmol/l)	-0.055	0.501
Glucose (mmol/l)	0.235	0.001
HbA1c (%)	0.072	0.365
BNP (pg/dl)	0.030	0.737
hs-CRP (µg/dl)	0.129	0.063
CPK (IU/L)	0.032	0.711
CK-MB (IU/L)	-0.005	0.899
Troponin T (ng/ml)	0.038	0.675
Peak CRP (IU/L)	0.311	0.015
Peak CK-MB (IU/L)	0.157	0.068

STEMI, ST-segment elevation acute myocardial infarction. Other abbreviations as in Table 1.

mellitus, left ventricular ejection fraction, peak CKP level, balloon angioplasty only, bare-metal stent, drug-eluting stent, all medications, triglyceride, serum total cholesterol, low-density lipoprotein (LDL) level, and high-density lipoprotein (HDL) level, we found that TVR and MACE are independent predictors of serum sulfatides. The hazard ratio for sulfatides of TVR was 0.997 (95% CI 0.991–0.999, $p < 0.001$), and overall MACE was 0.995 (95% CI 0.989–0.998, $p < 0.001$), respectively (**Table 6**).

Discussion

The present study showed that a higher level of serum sulfatides (> 15.2 µmol/L) in STEMI patients is an unfavorable correlate for in-hospital death as well as incidences of late TVR and overall MACE. It also suggested that the serum sulfatides level could be used as a marker for the severity of heart injury.

It is well known that STEMI is an acute blockage of the coronary artery in atherosclerotic lesions. Factors, such as high serum lipids, hypertension, and intimal damage of artery, contribute to the development of the disease. Even with the current technical advances in the early diagnosis and treatments, such as PCI, patients with STEMI remain at a high risk for death¹⁶ and many with poor prognoses, such as severe ventricular arrhythmia, cardiogenic shock, or cardiac rupture. Sulfatides are sphingoglycolipids found at the extracellular leaflet of the plasma membrane of most

Table 3. Coronary angiographic findings, PCI results and in hospital outcomes in all patients

	Serum sulfatide = 15.2 $\mu\text{mol/l}$ (<i>n</i> =200)	Serum sulfatide > 15.2 $\mu\text{mol/l}$ (<i>n</i> =170)	<i>P</i> Value
Angiographic Characteristics			
No of diseased vessels			
SVD, <i>n</i> (%)	74 (37)	70 (41)	NS
DVD, <i>n</i> (%)	64 (32)	49 (29)	NS
TVD, <i>n</i> (%)	62 (31)	51 (30)	NS
Treated (culprit) vessels			
RCA, <i>n</i> (%)	66 (33)	63 (37)	NS
LMT, <i>n</i> (%)	2 (1)	1 (1)	NS
LAD, <i>n</i> (%)	110 (55)	85 (50)	NS
LCX, <i>n</i> (%)	22 (11)	13 (12)	NS
Initial TIMI flow			
0, <i>n</i> (%)	134 (67)	121 (71)	NS
1, <i>n</i> (%)	24 (12)	14 (8)	NS
2, <i>n</i> (%)	12 (6)	14 (8)	NS
3, <i>n</i> (%)	30 (15)	21 (18)	NS
Door to balloon time (min)	89.7 \pm 28.5	90.1 \pm 37.2	NS
Interventions			
Balloon angioplasty (%)	194 (97)	168 (99)	NS
Thrombectomy			
Aspiration (%)	134 (67)	109 (64)	NS
Rheolytic (%)	6 (3)	5 (3)	NS
Stenting			
Nil (%)	24 (12)	19 (11)	NS
DES (%)	48 (24)	42 (25)	NS
BMS (%)	124 (62)	102 (60)	NS
DES + BMS (%)	4 (2)	7 (4)	NS
In-hospital complications			
New cardiogenic shock (%)	24 (12)	24 (14)	NS
Respiratory failure (%)	38 (19)	34 (20)	NS
Ventricular arrhythmia (%)	30 (15)	27 (16)	NS
New atrial fibrillation (%)	16 (8)	15 (9)	NS
Emergency CABG (%)	6 (3)	3 (2)	NS
In-hospital Death (%)	8 (4)	10 (6)	NS

SVD, single vessel disease; DVD, double-vessel disease; TVD, triple-vessel disease; RCA, right coronary artery; LMT, left main coronary artery; LAD, left anterior descending artery; LCX, left circumflex artery; TIMI, Thrombolysis in Myocardial Infarction; DES, drug-eluting stent; BMS, bare-metal stent; CABG, coronary artery bypass grafting.

eukaryotic cells. They are expressed predominantly in the myelin sheath of the nervous system, but they are also found on the surface of blood cells and as a component of lipoproteins in blood serum¹². Sulfatides exist in mammalian serum and are synthesized and secreted mainly from the liver as a component of lipoproteins¹³. In addition, they participate in a wide range of cellular processes, including cell adhesion and aggregation, neural plasticity, and immune responses. Importantly, our early study found that changes in cellular and serum circulating sulfatides directly impact cardiovascular diseases in patients with renal

dysfunctions¹⁴. In cardiovascular diseases, sulfatides have tremendous physiological roles in the vascular inflammation linked with the initiation of atherothrombosis or atherosclerosis, particularly after vascular damage¹¹. In events, such as atherosclerosis, angiography, and stent implantation, leukocytes, neutrophils, and monocytes interact with platelets through cell adhesion molecules^{17, 18}. The sulfatides are recognized as native ligands of P-selectin and communicate with several cell adhesion molecules as well. Once neutrophil activation occurs after vascular injury, sulfatides will be released from the activated neutrophils.

Table 4. Independent predictors of in-hospital mortality in patients with STEMI undergoing PCI

Variable	Hazard ratio	95%C.I.	<i>P</i> *	<i>P</i> ⁺
Variables in the model				
CAD number	4.558	1.012-20.115	0.032	0.048
1 (reference)	1			
> 1	4.558	1.012-20.115	0.032	0.048
Serum sulfatide	0.971	0.926-0.990	0.048	0.019
Variables not in the model				
Door to balloon time			0.699	
LM involvement			0.107	NS
Cardiogenic shock			0.003	NS
Culprit LAD			0.301	
Culprit LCX			0.852	
Culprit RCA			0.437	
Culprit LM			0.020	NS
TIMI grade			<0.001	NS
Thrombectomy				
Aspiration			0.341	
Rheolytic			0.039	NS

*From univariate regression analysis, ⁺from multivariate regression analysis. CAD, coronary artery disease. Other abbreviations as in Table 3.

Table 5. Medications at discharge and late clinical outcomes in patients with STEMI

	Serum sulfatide = 15.2 µmol/l (<i>n</i> = 192)	Serum sulfatide > 15.2 µmol/l (<i>n</i> = 160)	<i>P</i> Value
Medications at discharge			
Aspirin, <i>n</i> (%)	190 (99)	160 (100)	NS
Clopidogrel, <i>n</i> (%)	188 (98)	158 (99)	NS
ACEI or ARB, <i>n</i> (%)	182 (95)	149 (93)	NS
B-Blocker, <i>n</i> (%)	101 (53)	98 (61)	NS
Statin, <i>n</i> (%)	171 (89)	141 (88)	NS
Aldosterone receptor antagonist, <i>n</i> (%)	61 (32)	56 (35)	NS
MACE			
Non-fatal MI, <i>n</i> (%)	27 (14)	26 (16)	NS
TVR, <i>n</i> (%)	16 (8)	37 (23)	<0.05
De novo lesion, <i>n</i> (%)	29 (15)	27 (17)	NS
Cardiac Death, <i>n</i> (%)	0 (0)	1 (1)	NS
All-Cause Mortality, <i>n</i> (%)	23 (12)	24 (15)	NS
Overall, <i>n</i> (%)	19 (10)	45 (28)	<0.05

MACE, major adverse cardiovascular events; MI, myocardial infarction; TVR, target vessel revascularization. Other abbreviations as in Table 1.

These sulfatides act agonistically as a P-selectin ligand, leading to P-selectin/Mac-1 cross talking and the activation of Mac-1¹⁹⁻²¹. Studies have also demonstrated that sulfatides specifically bind to extracellular matrix components, such as thrombospondin, laminin, and von Willebrand factor¹⁹. The release of sulfatides from the surface of the neutrophils and in combination with an increased expression of P-selectin on the surface of platelets led to the accumulation of cyto-

kines and the extracellular matrix in vessel lesions, resulting in increased vascular wall thickness^{20, 21}.

Previous studies have also shown that sulfatides are involved in the progress of lipid metabolism in the development of atherosclerosis. In a study that implemented a rabbit model for human familial hypercholesterolemia-Watanabe heritable hyperlipidemic (WHHL), Hara and Taketomi showed that the heightened level of sulfatides in lipoprotein and ath

Table 6. Independent predictors of TVR and overall MACE by multivariate Cox regression analysis in all patients

Variable	Hazard ratio	95%C.I.	P Value
TVR			
Serum Sulfatide	0.997	0.991-0.999	<0.001
CAD number			
1 (reference)	1		
>1	2.832	1.701-5.002	<0.001
Overall MACE			
Serum sulfatide	0.995	0.989-0.998	<0.001
CAD number			
1 (reference)	1		
>1	2.816	1.729-4.621	<0.001

Other abbreviations as in Table 5.

erosclerosis plaques is tightly correlated with the progression of atherosclerosis in WHHL rabbits. The serum sulfatides in the WHHL rabbit were notably multiplied 40-fold over the typical rabbit level. In addition, the lipid analysis of the atherosclerosis aorta of WHHL rabbits revealed a substantial buildup of sulfatides at the lesions, but not in the typical aorta¹². All of these studies demonstrated that sulfatides are pathogenic factors for the initiation and progression of cardiovascular diseases.

It has been known that HDL-C and LDL-C levels decreased to below their baseline once patients have STEMI. But our study was designed to observe the serum sulfatide changes in STEMI patients. The STEMI patients were divided into two groups based on their value (15.2 $\mu\text{mol/L}$) of serum sulfatides upon admission. Patients' baseline characteristic data were collected, including clinically significant history, biochemical panel, and medication history. Among others, patients' LDL-C and HDL-C are in the same range, which established the comparability in two groups of patients. We found the correlations between a high level of serum sulfatides and the incidences of in-hospital death, late TVR, and overall MACE in patients with STEMI, but not with HDL-C and LDL-C levels. In future studies, we are going to seek to discover the relevant mechanisms.

In our prior study, we found a strong correlation among the levels of serum sulfatides and the risk of CVD in end-stage renal failure patients^{14,22}. Although the changes of serum sulfatides in the current study seem to be contradictory to our prior study, we believe end-stage renal failure and STEMI are different pathological processes, even patients with end-stage renal failure undergoing maintenance hemodialysis frequently present higher CVD risk and lower cholesterol level^{23,24}. In patients with end-stage renal failure,

we think that high CVD risk is caused by many factors, including hemodialysis, blood lipids, inflammation, vascular endothelial damage, and hypotension. Meanwhile severe blood lipid loss may partly contribute to low serum sulfatides in patients. However, in patients with atherosclerosis, we and other studies found a positive correlation between serum sulfatide levels and human atherosclerotic lesions formation^{12,20}. Current study suggested that sulfatides also are involved in the late stage of severe coronary atherosclerosis, even the prognosis of STEMI. We are glad that this clinical study revealed more prospective on the function of sulfatides.

About the relationship between serum sulfatides and renal function in coronary heart disease (CHD) patients. Recently, Charoen reported their Mendelian randomization study of the influence of eGFR on coronary disease. They observed that eGFR (<60 mL/min) is significantly negatively associated with CHD²⁵, which is consistent with some other studies of eGFR-CHD association²⁶. More interestingly, Sidana et al. found that in 207 patients with partial nephrectomy, most patients developed creatine kinase elevations >2000 IU/L, whereas others had a greater decline in eGFR²⁷. The present study results suggested that patients with serum sulfatides level above the median were significantly associated with decreased eGFR and increased CPK. The significant inverse correlation between serum sulfatides and eGFR may shed light on understanding sulfatides in the development of CVD. Higher serum sulfatides cause lower eGFR, which directly impacts the heart function. This may explain why high sulfatide levels lead to more incidences of TVR and overall MACE over the long-term. The pathophysiological mechanism between a high level of serum sulfatides and decreased eGFR in the STEMI patients should be fur-

ther explored.

In the current study, hs-CRP was not recognized as a crucial and free marker for late TVR and, in turn, overall MACE. A significant restriction of hs-CRP is its deficient specificity. Raised hs-CRP levels as a systemic acute phase reactant are frequently noted with conditions, including infections, except atherosclerosis²⁸). Serum sulfatides are raised in the acute phase of cardiovascular disease but not in common acute inflammatory diseases in which hs-CRP levels are increased^{11, 14, 19}). Therefore, serum sulfatides may be useful prognostic biomarkers, even in STEMI patients complicated by acute inflammatory diseases.

We clearly know that this study has limitations. This was a single-center study with a relatively limited number of patients in each group; however, the statistical significance indicates the important role of sulfatides in STEMI patients. Further large-scale multi-center studies are needed to validate the prognostic value of serum sulfatides after STEMI.

Conclusions

The incidences of in-hospital death, late TVR, and, in turn, overall MACE in patients with STEMI were significantly associated with high level of serum sulfatides. Although additional clinical studies are needed, the sulfatide-dependent pathway could be a new target for the prevention and/or treatment of STEMI.

Acknowledgment

This work was supported by the National Natural Science Foundation of China (81370316) and (81601858).

Conflict of Interest

The authors report no relationships that could be construed as a conflict of interest.

References

- 1) Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Makuc DM, Marcus GM, Marelli A, Matchar DB, Moy CS, Mozaffarian D, Mussolino ME, Nichol G, Paynter NP, Soliman EZ, Sorlie PD, Sotodehnia N, Turan TN, Virani SS, Wong ND, Woo D, Turner MB. Heart disease and Stroke statistics–2012 update: a report from the American Heart Association. *Circulation*, 2012; 125: e2-e220.
- 2) O’Gara PT, Kushner FG, Ascheim DD, Casey DE Jr, Chung MK, de Lemos JA, Ettinger SM, Fang JC, Fesmire FM, Franklin BA, Granger CB, Krumholz HM, Linderbaum JA, Morrow DA, Newby LK, Ornato JP, Ou N, Radford MJ, TamisHolland JE, Tommaso CL, Tracy CM, Woo YJ, Zhao DX, Anderson JL, Jacobs AK, Halperin JL, Albert NM, Brindis RG, Creager MA, DeMets D, Guyton RA, Hochman JS, Kovacs RJ, Kushner FG, Ohman EM, Stevenson WG, Yancy CW. 2013ACCF/AHA guideline for the management of ST-elevation myocardial infarction: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol*, 2013; 61: e78-e140.
- 3) Perers E, Caidahl K, Herlitz J, Karlson BW, Karlsson T, Hartford M. Treatment and short-term outcome in women and men with acute coronary syndromes. *Int J Cardiol*, 2005; 103: 120-127.
- 4) Yusuf S, Reddy S, Ounpuu S, Anand S. Global burden of cardiovascular diseases: part I: General considerations, the epidemiologic transition, risk factors, and impact of urbanization. *Circulation*, 2001; 3: 2746-2753.
- 5) Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Barker-Collo S, Bartels DH, Bell ML, Benjamin EJ, Bennett D, Bhalla K, Bikbov B, Bin Abdulhak A, Birbeck G, Blyth F, Bolliger I, Boufous S, Bucello C, Burch M, Burney P, Carapetis J, Chen H, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahodwala N, De Leo D, Degenhardt L, Delossantos A, Denenberg J, Des Jarlais DC, Dharmaratne SD, Dorsey ER, Driscoll T, Duber H, Ebel B, Erwin PJ, Espindola P, Ezzati M, Feigin V, Flaxman AD, Forouzanfar MH, Fowkes FG, Franklin R, Fransen M, Freeman MK, Gabriel SE, Gakidou E, Gaspari F, Gillum RF, Gonzalez-Medina D, Halasa YA, Haring D, Harrison JE, Havmoeller R, Hay RJ, Hoen B, Hotez PJ, Hoy D, Jacobsen KH, James SL, Jasrasaria R, Jayaraman S, Johns N, Karthikeyan G, Kassebaum N, Keren A, Khoo JP, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Lipnick M, Lipshultz SE, Ohno SL, Mabweijano J, MacIntyre MF, Mallinger L, March L, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM, McAnulty JH, McDermott MM, McGrath J, Mensah GA, Merriman TR, Michaud C, Miller M, Miller TR, Mock C, Mocumbi AO, Mokdad AA, Moran A, Mulholland K, Nair MN, Naldi L, Narayan KM, Nasseri K, Norman P, O’Donnell M, Omer SB, Ortblad K, Osborne R, Ozgediz D, Pahari B, Pandian JD, Rivero AP, Padilla RP, Perez-Ruiz F, Perico N, Phillips D, Pierce K, Pope CA 3rd, Porrini E, Pourmalek F, Raju M, Ranganathan D, Rehm JT, Rein DB, Remuzzi G, Rivara FP, Roberts T, De León FR, Rosenfeld LC, Rushton L, Sacco RL, Salomon JA, Sampson U, Sanman E, Schwebel DC, Segui-Gomez M, Shepard DS, Singh D, Singleton J, Sliwa K, Smith E, Steer A, Taylor JA, Thomas B, Tleyjeh IM, Towbin JA, Truelsen T, Undurraga EA, Venketasubramanian N, Vijayakumar L, Vos T, Wagner GR, Wang M, Wang W,

- Watt K, Weinstock MA, Weintraub R, Wilkinson JD, Woolf AD, Wulf S, Yeh PH, Yip P, Zabetian A, Zheng ZJ, Lopez AD, Murray CJ, AlMazroa MA, Memish ZA. Global and regional mortality from 235 causes of death for 20 agegroups in 1990 and 2010: A systematic analysis for the global burden of disease study 2010. *Lancet*, 2013; 3: 2095-2128.
- 6) Chen C, Lei W, Chen WJ, Zhong J, Gao XX, Li B, Wang H, Huang CX. Serum TGF- β 1 and SMAD3 levels are closely associated with coronary artery disease. *BMC Cardiovasc Disord*, 2014; 14: 18.
 - 7) Pharithi RB, Meela M, Kropmans T, Ward F, Conway M, Newell M. Magnetic resonance myocardial perfusion imaging in the diagnosis of functionally significant obstructive coronary artery disease: A systematic review protocol. *Syst Rev*, 2014; 3: 53.
 - 8) Poredos P, Spirkoska A, Lezaic L, Kuhn P, Meinertz T, Thiagarajan P. Patients with an Inflamed Atherosclerotic Plaque have Increased Levels of Circulating Inflammatory Markers. *J Atheroscler Thromb*, 2017; 24: 39-46.
 - 9) Shimabukuro M. Adiponectin and T-Cadherin: a Tree for Biomarkers in ST-Elevation or Non-ST-Elevation Myocardial Infarction. *J Atheroscler Thromb*, 2017; 24: 788-790.
 - 10) Merten M, Beythien C, Gutensohn K, Kuhn P, Meinertz T, Thiagarajan P. Sulfatides activate platelets through P-selectin and enhance platelet and platelet-leukocyte aggregation. *Arterioscler Thromb Vasc Biol*, 2005; 25: 258-263.
 - 11) Inoue T, Taguchi I, Abe S, Li G, Hu R, Nakajima T, Hara A, Aoyama T, Kannagi R, Kyogashima M, Node K. Sulfatides are associated with neointimal thickening after vascular injury. *Atherosclerosis*, 2010; 211: 291-296.
 - 12) Hara A, Taketomi T. Characterization and changes of glycosphingolipids in the Aorta of the Watanabe hereditary hyperlipidemic rabbit. *J. Biochem. (Tokyo)*, 1991; 109: 904-908.
 - 13) Kyogashima M, Tamiya-Koizumi K, Ehara T, Li G, Hu R, Hara A, Aoyama T, Kannagi R. Rapid demonstration of diversity of sulfatide molecular species from biological materials by MALDI-TOF MS. *Glycobiology*, 2006; 16: 719-728.
 - 14) Hu R, Li G, Kamijo Y, Nakajima T, Aoyama T, Inoue T, Node K, Kannagi R, Kyogashima M, Hara A. Serum sulfatides as a novel biomarker for cardiovascular disease in patients with end-stage renal failure. *Glycoconj J*, 2007; 24: 565-571.
 - 15) Li G, Hu R, Kamijo Y, Nakajima T, Aoyama T, Inoue T, Node K, Kannagi R, Kyogashima M, Hara A. Establishment of a quantitative, qualitative and high throughput analysis of sulfatides from small amounts of sera by MALDI-TOF MS. *Anal Biochem*, 2007; 362: 1-7.
 - 16) Kojima S, Matsui K, Ogawa H, Kumamoto Acute Coronary Events (KACE) Study Group. Temporal trends in hospitalization for acute myocardial infarction between 2004 and 2011 in Kumamoto. *Japan Circ J*, 2013; 77: 2841-2843.
 - 17) Zarbock A, Polanowska-Grabowska RK, Ley K. Platelet-neutrophil-interactions: linking hemostasis and inflammation. *Blood Rev*, 2007; 21: 99-111.
 - 18) Shimazawa M, Kondo K, Hara H, Nakashima M, Umemura K. Sulfatides, L- and P-selectin ligands, exacerbate the intimal hyperplasia occurring after endothelial injury. *Eur J Pharmacol*, 2005; 520: 118-126.
 - 19) Kyogashima M. The role of sulfatide in thrombogenesis and haemostasis. *Arch Biochem Biophys*, 2004; 426: 157-162.
 - 20) Li G, Hu R. Association between serum sulfatide and carotid intima media thickness in patients with familial hypercholesterolemia. *Glycoconj J*, 2014; 31: 587-592.
 - 21) Li G, Hu R, Gu J, Wu H.Z. Relationship between carotid artery atherosclerosis and sulfatide in hypertensive patients. *Genetics and Molecular Research*, 2015; 14: 4840-4846.
 - 22) Li G, Hu R, Kamijo Y, Nakajima T, Aoyama T, Ehara T, Shigematsu H, Kannagi R, Kyogashima M, Hara A. Kidney dysfunction induced by protein overload nephropathy reduces serum sulfatide levels in mice. *Nephrology (Carlton)*, 2009; 14: 658-662.
 - 23) Sarnak MJ, Levey AS, Schoolwerth AC, Coresh J, Culleton B, Hamm LL, McCullough PA, Kasiske BL, Kelepouris E, Klag MJ, Parfrey P, Pfeffer M, Raij L, Spinosa DJ, Wilson PW. Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. *Circulation*, 2003; 108: 2154-2169.
 - 24) Bologna RM, Levine DM, Parker TS, Cheigh JS, Serur D, Stenzel KH, Rubin AL. Interleukin-6 predicts hypoalbuminemia, hypocholesterolemia, and mortality in hemodialysis patients. *Am J Kidney Dis*, 1998; 32: 107-114.
 - 25) Charoen P, Nitsch D, Engmann J, Shah T, White J, Zabaneh D, Jefferis B, Wannamethee G, Whincup P, Mullick Cassidy A, Gaunt T, Day I, McLachlan S, Price J, Kumari M, Kivimaki M, Brunner E, Langenberg C, Ben-Shlomo Y, Hingorani A, Whittaker J, Pablo Casas J, Dudbridge F. Mendelian Randomisation study of the influence of eGFR on coronary heart disease. *Sci Rep*, 2016; 6: 28514.
 - 26) Welt FG, Rogers C. Inflammation and restenosis in the stent era. *Arterioscler Thromb Vasc Biol*, 2002; 22: 1769-1776.
 - 27) Sidana A, Walton-Diaz A, Truong H, Siddiqui MM, Miao N, Shih J, Mannes A, Bratslavsky G, Linehan WM, Metwalli AR. Postoperative elevation in creatine kinase and its impact on renal function in patients undergoing complex partial nephrectomy. *Int Urol Nephrol*, 2016; 48: 1047-1053.
 - 28) Hayashida K, Kume N, Murase T, Minami M, Nakagawa D, Inada T, Tanaka M, Ueda A, Kominami G, Kambara H, Kimura T, Kita T. Serum soluble lectin-like oxidized low-density lipoprotein receptor-1 levels are elevated in acute coronary syndrome: A novel marker for early diagnosis. *Circulation*, 2005; 112: 812-818.