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Downregulated Circulating Long Non-coding RNA GAS6-AS1 Screens and Predicts Acute Myocardial Infarction

ABSTRACT

Background: Acute myocardial infarction seriously threatens human health and life quality, which needs novel biomarkers to improve its early detection and development prediction. This study aimed to assess the potential of long non-coding RNA GAS6-AS1 in discriminating acute myocardial infarction patients and predicting patients' outcomes.

Methods: The circulating expression of GAS6-AS1 in 83 acute myocardial infarction patients and 62 healthy individuals was evaluated using polymerase chain reaction. The value of GAS6-AS1 in the distinguishing acute myocardial infarction patients was evaluated with receiver operating characteristic analysis, and its prognosis predictive potential was assessed by Kaplan–Meier and Cox analysis. Additionally, the correlation of GAS6-AS1 with patients' critical features was evaluated by Spearman's correlation analysis.

Results: Significant downregulation of GAS6-AS1 was observed in the plasma of acute myocardial infarction patients relative to healthy individuals. Reduced GAS6-AS1 could discriminate acute myocardial infarction patients from healthy controls and indicate patients' unoptimistic prognosis. Moreover, GAS6-AS1 was found to be negatively correlated with the levels of creatine kinase, creatine kinase-myocardial bland, lactic dehydrogenase, hydroxybutyrate dehydrogenase, troponin T, and positively correlated with the ejection fraction of acute myocardial infarction patients.

Conclusion: Changes in circulating GAS6-AS1 in acute myocardial infarction served as a potential diagnostic and prognostic biomarker of acute myocardial infarction.

Keywords: AMI, lncRNA, GAS6-AS1, early detection, prognosis, disease development

ORIGINAL INVESTIGATION

INTRODUCTION

Acute myocardial infarction (AMI) is a major inducing factor of cardiovascular diseases, which are of high morbidity, mortality, and disability.[1-3] AMI is a kind of myocardial ischemic necrosis, which results from the sharp reduction or interruption of blood supply to the coronary arteries. Due to the indirect association of AMI with the degree of coronary stenosis, AMI was mainly caused by local thrombosis and plaque rupture in the coronary artery, and therefore, timely diagnosis and reperfusion therapy are crucial for patients' survival.[4] Clinically, the diagnosis of AMI is based on biochemical indicators, such as myoglobin and cardiac troponin (cTn).[5,6] Myoglobin could show an abnormal increase within 2 hours of onset, but its specificity is unsatisfying.[7,8] While cTnI level showed a relatively high false-positive rate, which was also found to be aberrant in heat failure, arrhythmia, and myocarditis,[9,10] increasing research focused on the exploration of sensitive and specific myocardial indicators, which could help in the early diagnosis of AMI and improve patients' prognoses.

Long non-coding RNAs (lncRNAs) are widely expressed in human tissues and organs, which play critical roles in the physiological and pathological processes of the body. Recently, lncRNAs have become the focus of biological studies, which are able to regulate cellular processes, and further mediate the development of human diseases. In the cardiovascular system, lncRNAs were reported to

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participate in heart development-correlated processes such as hypertrophy, mitochondrial function, and cardiomyocyte apoptosis.[11] Wang et al[12] exhibited a ceRNA-regulating network of myocardial fibrosis after AMI and identified a series of potential biomarker lncRNAs, including lncRNA GAS6-AS1 (GAS6-AS1). GAS6-AS1 has also been revealed to function as the regulator in various human diseases. GAS6-AS1 is located at 13q34 and transcribed in the antisense direction of GAS6, which was reported to be significantly correlated with the risk of hemorrhagic transformation after intravenous thrombolysis.[13] Additionally, GAS6-AS1 was also involved in the pathogenesis and progression of human cancers, such as lung adenocarcinoma, breast cancer, and hepatocellular carcinoma.[14-16] However, whether GAS6-AS1 could serve as a reliable biomarker of AMI in screening the incidence and predicting the development was still unknown.

This study focused on the significance of GAS6-AS1 in AMI, aiming to assess its value in screening AMI occurrence and monitoring disease development.

METHODS

Inclusion and Exclusion Criteria

The diagnosis criteria for AMI were based on the 2012 ESC/AHA/ACC guidelines.[17] Specific details are as follows: (a) the cTn level should be 99% higher than the upper limit reference value; (b) significant changes in the ST-T stage or left bundle branch block; (c) obvious symptoms of ischemia in electrocardiogram; (d) coronary thrombosis in angiography; and (e) loss of viable heart muscle or abnormal ventricular wall. Patients who had received anticoagulation or thrombolytic therapy and patients with other comorbidities or organ failures were excluded. Healthy individuals without a history of cardiovascular diseases and thrombolytics were included.

According to the above criteria, a total of 83 AMI patients and 62 healthy individuals were included in this study from June 2019 to June 2020. Approval was obtained from the ethics committee of Beijing JiShuiTan Hospital. The procedures used in this study adhere to the tenets of the Declaration of Helsinki. All participants had signed the informed consent. The AMI patients were followed up for 6 months after corresponding treatments. The cardiovascular events and all cause-induced deaths were defined as the endpoints.

Sample Collection and RNA Extraction

The peripheral blood samples were collected from each participant within 24 hours of their admission. The plasma samples were isolated by centrifugation at 2000 *g* for 10 minutes. Total RNA was isolated from the samples with the TRIzol reagent (Life Technologies, Carlsbad, USA) according to the manufacturer's instructions. The concentration and purification of extracted RNA were evaluated by OD260/OD280 using Nanodrop ND-2000 (Thermo Scientific, Waltham, USA). The extracted RNA was stored at −80°C for the following analyses.

Real-Time Qualitative Polymerase Chain Reaction

Complementary DNA was generated using 1 μg of extracted RNA and the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific). The PCR analysis was conducted with the SYBR Green II mix (Applied Biosystem, Foster City, USA) and the ABI 7500Fast real-time PCR. The primer sequences were: GAS6-AS1, forward 5′-ATGCAAGGACGGAACCACACCT-3′, reverse 5′-GCAGAGGTTTCTGTCTCCATCG-3′; GAPDH forward 5'- TGAAGGTCGGAGTCAACGGATTTGGT-3', reverse 5'-CATGTGGGCCATGAGGTCCACCAC-3'. The expression level was calculated with the $2^{-\Delta\Delta Ct}$ method normalized to GAPDH.

Statistical Analysis

Data were presented as mean ± standard deviation and analyzed with Statistical Package for Social Sciences 26.0 (IBM, Chicago, USA). The difference comparison between the healthy individual group and the AMI patient group was performed with Student's *t*-test. The diagnostic potential of GAS6-AS1 in discriminating AMI patients from healthy individuals was assessed by receiver operating characteristic (ROC) analysis. The outcomes of AMI were summarized by Kaplan–Meier analysis. The prognostic significance of GAS6-AS1 was evaluated by Cox regression analysis. The correlation between GAS6-AS1 and patients' clinicopathological features was evaluated by Spearman's correlation analysis. The statistically significant difference was indicated by $P < .05$.

RESULTS

Basic Characteristics of Study Subjects

The major clinicopathological features of enrolled individuals are listed in Table 1. Healthy individuals included 43 males and 19 females with an average age of 59.24 ± 8.58 years, while the AMI group was composed of 59 males and 24 females with an average age of 60.12 ± 7.63 years (Table 1). The age and gender composition of the 2 groups are matched with no significant difference ($P > .05$). It was found that AMI patients showed a reduced ejection fraction and high-density lipoprotein level and increased levels of white blood cells, low-density lipoprotein, creatine kinase (CK), creatine kinase-myocardial bland (CK-MB), lactic dehydrogenase (LDH), hydroxybutyrate dehydrogenase (HBDH), and troponin T, relative to healthy individuals, and the differences were significant ($P < .05$, Table 1). No significant differences were observed in the age, gender, body mass index, systolic blood pressure, DPB, total cholesterol, triglyceride, and serum creatinine between AMI patients and healthy individuals ($P > .05$, Table 1).

GAS6-AS1 Expression and Its Diagnostic and Prognostic Value in Acute Myocardial Infarction Patients

In the plasma of AMI patients, there was a significant decrease of GAS6-AS1 observed compared with the

HIGHLIGHTS

- Downregulation of GAS6-AS1 could differentiate acute myocardial infarction (AMI) patients from healthy individuals.
- Downregulation of GAS6-AS1 indicates a poor prognosis of patients.
- Circulating GAS6-AS1 was considered as a potential diagnostic and prognostic biomarker of AMI.

Table 1. Baseline Characteristics of Study Subjects

	Healthy Individuals (n = 62)	AMI Patients (n = 83)	*P*
Age (years)	59.24 ± 8.58	60.12 ± 7.63	.569
Gender (male/female)	43/19	59/24	.672
BMI (kg/m^2)	23.37 ± 2.94	24.30 ± 3.88	.119
SBP (mm Hg)	119.00 ± 10.62	120.80 ± 8.78	.463
DBP (mm Hg)	75.29 ± 4.91	76.24 ± 4.79	.244
Ejection fraction (%)	63.22 ± 4.94	54.53 ± 8.19	<.001
White blood cell (×10^9/L)	6.01 ± 1.42	15.08 ± 2.67	<.001
TC (mM)	6.48 ± 1.27	6.38 ± 1.30	.641
TG (mM)	1.78 ± 0.70	1.97 ± 0.89	.162
HDL (mM)	1.28 ± 0.56	1.08 ± 0.38	.014
LDL (mM)	2.52 ± 0.61	2.77 ± 0.61	.013
Scr (μM)	70.69 ± 12.30	71.89 ± 11.19	.543
CK (U/L)	81.34 ± 16.92	1101.84 ± 31.66	<.001
CK-MB (U/L)	14.26 ± 5.32	117.70 ± 13.15	<.001
Troponin T (μg/L)	0.02 ± 0.02	1.77 ± 0.64	<.001
LDH (U/L)	174.11 ± 21.79	762.77 ± 34.72	<.001
HBDH (U/L)	149.71 ± 10.92	507.82 ± 23.89	<.001

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL, high density lipoprotein; LDL, low density lipoprotein; Scr, serum creatinine; CK, creatine kinase; CK-MB, creatine kinase-myocardial bland; LDH, lactic dehydrogenase; HBDH, hydroxybutyrate dehydrogenase.

expression in healthy individuals (*P* < .001, Figure 1A). The result of ROC showed that GAS6-AS1 could discriminate AMI patients from healthy controls with the area under the curve (AUC) of 0.944 (95% CI = 0.908-0.980). The specificity and sensitivity of GAS6-AS1 detecting AMI were 0.855 and 0.940, respectively (Figure 1B).

Additionally, the predictive potential of GAS6-AS1 was also evaluated in AMI patients. According to the mean value of GAS6-AS1 expression in patients' plasma, patients were divided into a high (n = 39) and low GAS6-AS1 group (n = 44). It was observed that patients with lower GAS6-AS1 expression showed a poor prognosis, in other words, those patients were more likely to experience cardiovascular events or deaths (log rank *P* = .040, Figure 1C). Moreover, the multivariate Cox regression analysis demonstrated that GAS6-AS1 acted as a reliable indicator of AMI patients' prognosis with a 95% CI of 0.022-0.562 (*P* = .008), as well as ejection fraction (*P* = .049), CK (*P* = .033), CK-MB (*P* = .011), and LDH (*P* = .042, Table 2).

Association of GAS6-AS1 with Acute Myocardial Infarction Patients' Clinicopathological Features

The correlation of GAS6-AS1 with the differential clinicopathological features of AMI patients was assessed with Spearman's correlation coefficients. A significant positive correlation was observed between GAS6-AS1 and the ejection fraction of AMI patients ($r = 0.690$, $P < .01$, Figure 2A). An insignificant correlation was observed between the GAS6-AS1 expression and AMI patients' white blood cells ($r = -0.080$, Figure 2B), HDL ($r = 0.142$, Figure 2C), and LDL ($r = 0.168$, Figure 2D, $P > .05$). The levels of CK ($r = -0.736$, Figure 2E), CK-MB ($r = -0.673$, Figure 2F), troponin T ($r = -0.740$, Figure 2G), LDH ($r = -0.647$, Figure 2H), and HBDH ($r = -0.583$, Figure 2I) were found to be negatively correlated with GAS6-AS1 ($P < .01$).

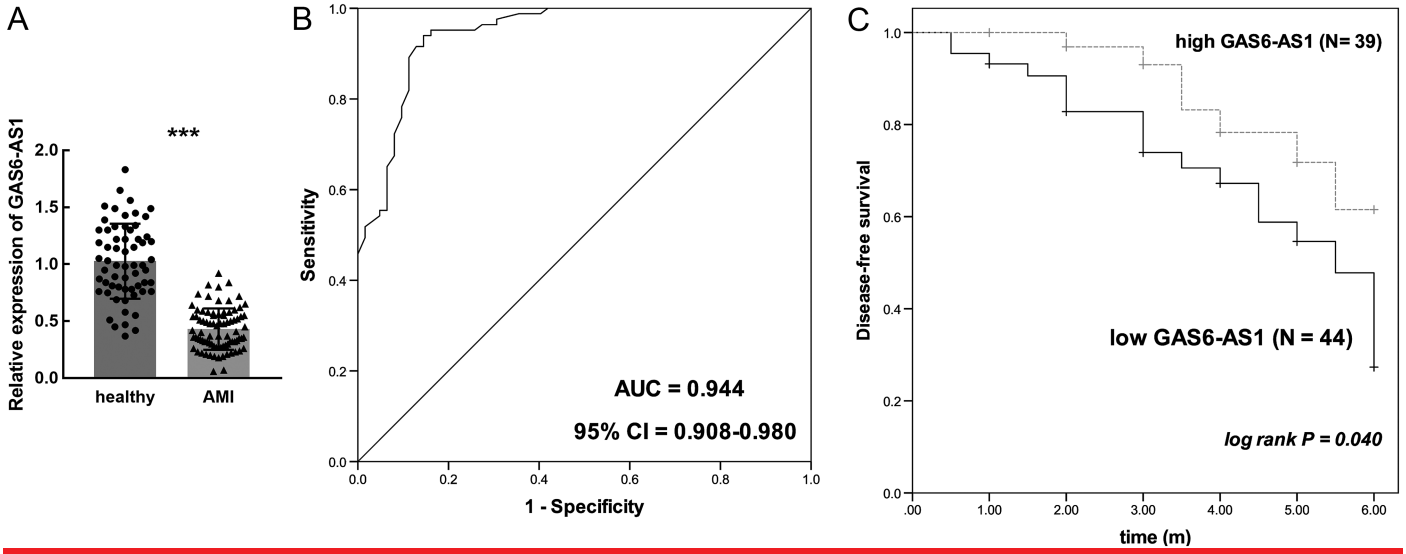

Figure 1. The expression level of GAS6-AS1 and its significance in the diagnosis and prognosis of AMI. (A). GAS6-AS1 was significantly downregulated in AMI compared with healthy individuals. ***$P < .001$. (B). ROC curve indicated that GAS6-AS1 could discriminate AMI patients from healthy individuals with an AUC of 0.944. (C). Kaplan–Meier curve based on the average expression of GAS6-AS1 in AMI showed that patients with lower GAS6-AS1 expression possess a worse prognosis, and the difference was significant. Log rank *P* = .040. AMI, acute myocardial infarction; ROC, receiver operating characteristic; AUC, area under the curve.

Table 2. Multivariate Cox Regression Models for Poor Prognosis of AMI Patients

	95% CI	HR	*P*
GAS6-AS1	0.022-0.562	0.111	.008
Ejection fraction	0.096-0.996	0.310	.049
CK	1.088-7.336	2.825	.033
CK-MB	1.411-13.471	4.359	.011
Troponin T	0.607-8.319	2.247	.226
LDH	1.043-10.490	3.308	.042
HBDH	0.783-4.946	1.967	.150
White blood cell	0.813-5.538	2.121	.125
HDL	0.228-1.553	0.595	.289
LDL	0.862-7.697	2.575	.90

HR, hazard ratio; CK, creatine kinase; CK-MB, creatine kinase-myocardial bland; LDH, lactic dehydrogenase; HBDH, hydroxybutyrate dehydrogenase; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

DISCUSSION

Acute myocardial infarction is one of the leading causes related to death worldwide, threatening human lives and life quality. A noninvasive and reliable biomarker for the early detection of AMI is a key procedure to minimize the ischemic damage to the myocardium. Long non-coding RNAs are newly discovered disease development regulators, which mediate a wide spectrum of biological processes and serve as potential biomarkers in human diseases. Several lncRNAs have been demonstrated to be stably and detectably expressed in human body fluids, including urine, saliva, plasma, and serum.[18-21] During the course of this study, a number of abnormally expressed lncRNAs were reported to be potential biomarkers for AMI. For example, Wang et al[22] identified 3 peripheral blood mononuclear cell-derived lncRNAs, HT19, MALAT1, and MIAT related to the risk factors, and revealed their ranking significance in the diagnosis of AMI. Herein, we evaluated the expression of GAS6-AS1 in the plasma of AMI patients and healthy individuals and observed its significant downregulation in AMI. GAS6-AS1 is the antisense RNA of GAS6, of which the genetic variations have been demonstrated to indicate cardiovascular risk and predict cardiovascular events.[23] In previous studies, the dysregulation of GAS6-AS1 was reported to relate to the development and onset of human diseases. For instance, the upregulation of GAS6-AS1 in acute myeloid leukemia was

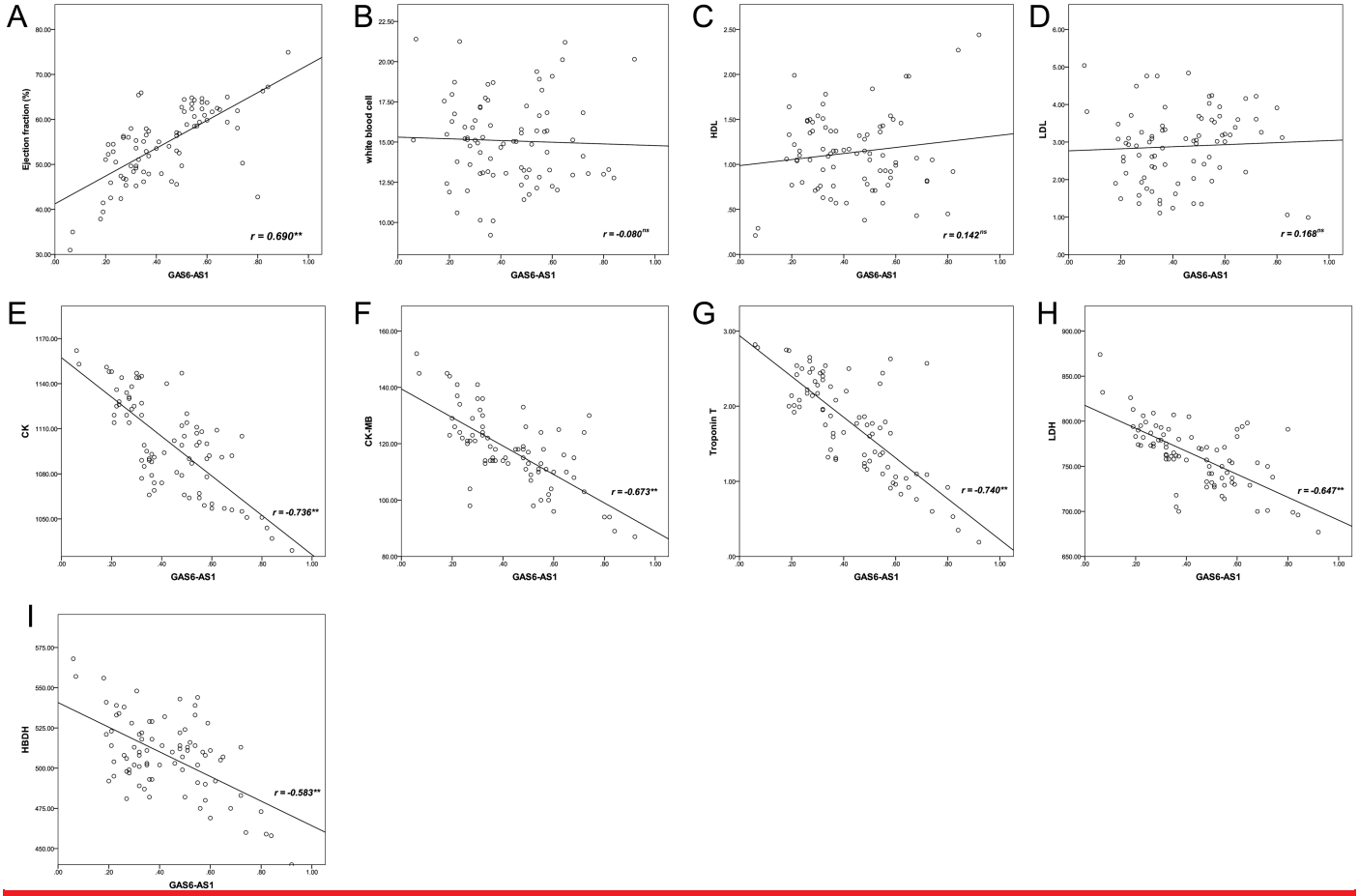

Figure 2. Correlation of GAS6-AS1 with the ejection fraction (A), white blood cell (B), HDL (C), LDL (D), CK (E), CK-MB (F), troponin T (G), LDH (H), and HBDH (I) of AMI patients evaluated by Spearman's correlation analysis. HDL, high-density lipoprotein; LDL, low-density lipoprotein; CK, creatine kinase; CK-MB, creatine kinase-myocardial bland; LDH, lactic dehydrogenase; HBDH, hydroxybutyrate dehydrogenase; Ns, not significant, $P > .05$; **$P < .01$.

suggested to alleviate tumorigenesis and suppressed disease progression.[24,25] In breast cancer, GAS6-AS1 could enhance cell aggressiveness and promote tumor development.[15,26]

Otherwise, the potential of GAS6-AS1 in disease screening and monitoring was also disclosed. The downregulation of GAS6-AS1 could predict the poor prognosis of non-small cell lung cancer patients and correlate with lymph node metastasis and advanced metastasis stage.[27] It was also identified as a prognosis-correlated biomarker of papillary renal cell carcinoma from the lncRNA signatures.[28,29] Moreover, the downregulation of GAS6-AS1 was found to predict the poor prognosis of breast cancer patients, which is consistent with our results.[30] GAS6-AS1 showed dramatic significance in discriminating AMI patients from healthy individuals with high sensitivity and specificity. Additionally, the lower expression of GAS6-AS1 in AMI patients was found to correlate with patients' worse outcomes. Thus, the significant diagnostic and prognostic value of GAS6-AS1 in AMI have been illustrated according to the above results.

A notable elevation was observed in the cardiac enzymes of AMI patients, including CK, LDH, and HBDH, which were released due to cardiomyocyte necrosis and also indicated the development risk of patients' conditions.[31] The expression of GAS6-AS1 was indicated to be negatively correlated with the level of cardiac enzymes. Additionally, the enrolled AMI patients possessed a reduced ejection fraction, which was positively correlated with GAS6-AS1. Both the increasing levels of cardiac enzymes and the decreasing ejection fraction are risk factors for disease occurrence and development. Hence, the significant association of GAS6-AS1 with these factors suggested the potential involvement in the onset and progression of AMI.

The present study demonstrated the diagnostic and prognostic significance of GAS6-AS1 in AMI, providing novel opportunities for using these molecular biomarkers and therapeutic targets. On the other hand, this study also suggested some obvious advantages of lncRNAs in serving as biomarkers. Long non-coding RNAs were stable enough to be detected in the blood samples and analyzed by highly sensitive methods like polymerase chain reaction (PCR), which could improve the diagnostic efficiency and specificity. In addition, the changes in lncRNA expression levels also imply the mechanism underlying the disease onset and development, although the mechanism of GAS6-AS1 involved in AMI development has not been disclosed in the present study. Therefore, this study also confirmed the worth of lncRNAs in the research of heart disease diagnosis and treatment.

The obtained results were based on a relatively small sample size of AMI patients, which might limit the significance of GAS6-AS1. Therefore, further larger sample size and clinical validation are needed. The other potential limitation of this study was that GAS6-AS1 detected was in an unknown source in the blood, which was considered to be associated with cell apoptosis and necrosis. Previously, studies have demonstrated that the stability of lncRNAs or other non-coding RNAs might result from the protection from some microparticles, such as exosomes.[32] Therefore, the source of GAS6-AS1 and its specific role in the biological function of cardiomyocytes are critical directions for our future investigations.

Taken together, circulating GAS6-AS1 served as diagnostic and prognostic biomarker of AMI that discriminates AMI patients and predicts poor prognosis. Future studies would focus on the biological function of GAS6-AS1 and its underlying mechanism.

Data Availability Statement: All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Ethics Committee Approval: Approval was obtained from the Ethics Committee of Beijing JiShuiTan Hospital, China (approval No.: 20190402) on May 12, 2019. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

Informed Consent: All participants had signed the informed consent.

Peer-review: Externally peer-reviewed.

Author Contributions: Z.H.W. and M.Q.Z. made substantial contributions to conception and design, acquisition of data, analysis and interpretation of data and draft of the manuscript. Y.F. revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

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