Original Article

Prognostic value of peripheral blood circular RNAs in patients with acute coronary syndrome

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Background: Acute coronary syndrome (ACS) is a clinical emergency. Although its prognosis has been significantly improved, some patients will have major adverse cardiovascular events (MACE) in the short term. We aimed to analyze the prognostic value of circRNAs in patients with ACS.

Methods: This diagnostic accuracy study enrolled a total of 100 patients with ACS from January 2019 to January 2021. All patients were followed up for 30 days. The expression of circRNAs in peripheral blood was determined using real-time fluorescence quantification PCR (qRT-PCR). 30 patients with MACE were divided into the observation group and 70 patients without MACE were divided into the control group. The general data and the detection results of circRNAs of the two groups were compared, and the influencing factors of MACE in ACS patients were analyzed by logistic regression. Receiver operating characteristic (ROC) curves were generated, and the predictive value of peripheral blood circRNAs for MACE in patients with ACS was evaluated.

Results: The age, sex, hypertension, dyslipidemia, location of coronary artery disease, left ventricular ejection fraction, and Killip grade were not significantly different between the two groups (P>0.05). Type 2 diabetes and smoking history in the observation group were also comparable between the two groups (P>0.05). Logistic regression analysis showed that type 2 diabetes mellitus [odd ratio (OR) 1.314, 95% confidence interval (CI): 1.052–1.437, P=0.002], smoking history (OR 1.227, 95% CI: 1.014–1.385, P=0.001), and the up-regulation of circRNAs in peripheral blood (OR 1.312, 95% CI: 1.028–1.452, P=0.002) were risk factors for MACE in ACS patients. The results of the ROC curve showed that peripheral blood circRNAs could be used as a predictor of MACE in patients with ACS. The best cut-off value was 96.44 ng/μL, the diagnostic sensitivity was 75.71%, the specificity was 100%, and the area under the curve (AUC) was 0.931 (95% CI: 0.884–0.977, P<0.001).

Conclusions: Peripheral blood circRNAs are up-regulated about 3 fold in the peripheral blood of patients with ACS. Abnormal expression is an independent risk factor affecting MACE. Peripheral blood circRNAs can assist in clinical decision-making processes in patients with ACS.

Keywords: Peripheral RNAs; acute coronary syndrome (ACS); adverse cardiovascular events; predictive value

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Introduction

Acute coronary syndrome (ACS) is a common clinical syndrome characterized by acute myocardial ischemia. It has a rapid onset, high disability rate, and high mortality rate. It is prone to major adverse cardiovascular events (MACEs) such as acute heart failure and severe arrhythmia (1,2). In recent years, with the progress and maturity of percutaneous coronary intervention (PCI), the prognosis of ACS patients has been significantly improved, but some patients still develop MACE in a short period of time (2). Therefore, seeking a clinical indicator that can accurately predict the occurrence of MACE in ACS patients is crucial to improving the prognosis of patients. It has been generally acknowledged that some clinical features, including diabetes mellitus and smoking status, are important risk factors for MACE (3,4). However, some patients continue to experience MACE even without these unfavorable characteristics. Moreover, although some studies reported the predictive value of several blood parameters (like C-reactive protein, cholesterol), the reliability of them is still controversial (5,6). Thus, more accurate biomarkers are urgent. Clinical studies have shown that circular RNAs (circRNAs) are closely related to the occurrence and development of cardiovascular diseases (7). There is also a significant association between circRNAs and the pathological process of ACS (8). However, little is known regarding the prognostic impact of circRNAs in patients with ACS. We speculate that circRNAs may serve as a potential predictor of MACE independent of other known prognostic factors. Therefore, this study investigated the predictive value of peripheral blood circRNAs for the occurrence of MACE in ACS patients. We present the following article in accordance with the STARD reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-253/rc).

Methods

Research subjects

This is a diagnostic accuracy study. A total of 100 patients with ACS treated in the Affiliated Hospital of Inner Mongolia Medical University from January 2019 to January 2021 were retrospectively enrolled. There were 42 women and 58 men. The average age was 63.52±6.34 years. The body mass index (BMI) ranged between 20–28 kg/m^2, with an average of 24.62±1.54 kg/m^2. The inclusion criteria were as follows: (I) all patients met the diagnostic criteria for ACS in the basic diagnosis and treatment guidelines for non-ST-segment elevation acute coronary syndrome (2019) (9); (II) coronary angiography (CAG) showed that at least 1 coronary artery stenosis was more than 50%; (III) age >18 years old; (IV) the clinical data were complete; (V) patients signed the consent form. The exclusion criteria were as follows: (I) patients with chronic heart failure, heart valve disease, and other diseases; (II) those with a history of previous cardiac surgery; (III) patients with acute or chronic infectious diseases; (IV) those who took anti-inflammatory drugs and antibiotics 1 month before enrollment; (V) patients transferred to another hospital or withdrew from the research; (VI) patients in a critical state such as multiple organ failure and shock; (VII) patients with Alzheimer's disease and other neurodegenerative diseases; (VIII) patients with rheumatic immune diseases; (IX) patients with a history of surgery or trauma 1 month before enrollment; (X) patients with coagulation dysfunction. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Affiliated Hospital of Inner Mongolia Medical University (No. 2019-048) and informed consent was taken from all the patients.

Study methods

(I) General information was collected by questionnaires, including gender, age, type 2 diabetes, hypertension, smoking history, dyslipidemia, location of coronary artery lesion, left ventricular ejection fraction (LVEF), and Killip classification. Smoking history was defined as cumulative or continuous smoking for more than 6 months. Dyslipidemia was defined as low density lipoprotein cholesterol (LDL-C) ≥4.1 mmol/L or total cholesterol (TC) ≥6.2 mmol/L. (II) Detection of circRNAs in peripheral blood: 5 mL fasting venous blood of the patient was extracted, placed in an EDTA anticoagulant tube, inverted and evenly mixed, and stored in a freezer at −80 or −20 ℃ for examination. Trizol was used to extract RNA from blood according to the requirements of the kit, and a spectrophotometer was used to determine the purity and concentration of total RNA. For qualified samples, linear RNA was removed through Ribonuclease R, fluorescent labeling and cDNA amplification was carried out, and hybridization and cleaning were completed with the Agilent system. Imaging was performed on the Axon Gene Chip Scanner, and the differential expression profiles of circRNAs were analyzed by GenePix Pro 6.0 software analysis. Three

chips were used to detect circRNAs. circRNAs with large differential multiples were verified by real-time fluorescence quantification PCR (qRT-PCR). The cDNA template was obtained by reverse transcription of total RNA and amplified by qRT-PCR (model: 7900). U6 was used as an internal reference to standardize the expression of circRNAs. The 3 miRNA binding sites with the highest matching values were selected by gene software prediction. The qRT-PCR results and the circRNAs consistent with the chip were analyzed by DAVID software. (III) Follow up: all patients were followed up for 30 days. MACEs were defined as a composite of death, myocardial infarction (MI), or any repeat revascularization during follow-up. According to whether MACE occurred or not, 30 patients with MACE were used as the observation group and 70 patients without MACE were used as the control group.

Observation indexes

The general data and the detection results of peripheral circRNAs in the two groups were compared.

Statistical analysis

Normal distribution measurement data were analyzed using SPSS 26.0 software. Comparisons between different groups were mainly performed using the independent sample *t*-test. Count data were analyzed by the Pearson chi square test, and the expected frequency of each cell was less than 5. The continuous correction test is represented by "[n/(%)]". The influencing factors of MACE in patients with ACS were analyzed by logistic regression. Variables with a value of P<0.05 in the univariate analysis were included in the subsequent multivariate analysis. Receiver operating characteristic (ROC) curves were drawn, the area under the curve (AUC) was calculated, and the predictive value of peripheral blood circRNAs for MACE in patients with ACS was determined with an inspection level of α=0.05. A two-sided P value of <0.05 was considered statistical significance.

Results

Comparison of general data between the two groups

There was no significant difference in age, sex, hypertension, dyslipidemia, location of coronary artery disease, LVEF, and Killip grade between the observation group and the control group (P>0.05). Type 2 diabetes mellitus and smoking history in the observation group were significantly different compared with the control group (P<0.05), as shown in *Table 1*.

Comparison of peripheral blood circRNAs between the two groups

The average value of peripheral blood circRNAs in the observation group was 176.25 ng/μL, which was significantly higher than 65.26 ng/μL in the control group. The difference was statistically significant (P<0.001, *Table 2* and *Figure 1*).

Evaluation of the influencing factors of MACE in patients with ACS

The occurrence of MACE as a dependent variable and the risk factors that may lead to the occurrence of MACE (type 2 diabetes mellitus, smoking history, circRNAs) as independent variables were assigned from X1–X3, as shown in *Table 3*.

Multivariate analysis of risk factors for MACE in patients with ACS

Multivariate analysis showed that type 2 diabetes, smoking history, and peripheral blood circRNAs were independent risk factors for MACE in ACS patients (OR =1.314, 1.227, 1.312, P<0.05), as shown in *Table 4*.

Predictive value of peripheral blood circRNAs for MACE in patients with ACS

The results of the ROC curve showed that peripheral circRNAs could be used as predictors of MACE in patients with ACS, and the best cut-off value was 96.44 ng/μL. The diagnostic sensitivity was 75.71%, the specificity was 100%, and the AUC was 0.931 (95% CI: 0.884–0.977, P<0.001), as shown in *Figure 2*.

Discussion

ACS is a clinical syndrome in which unstable atherosclerotic plaques in the coronary artery erode or rupture, resulting in thrombosis and incomplete or complete occlusion of the coronary artery (10). ACS is characterized by nausea and vomiting, angina pectoris, irritability, pale complexion, and tachycardia, among others. Untimely treatment can cause

Table 1 Comparison of the general data of the two groups

General information	Observation group (n=30)	Control group (n=70)	χ^2/t	P
Age	62.62±5.62	60.59±4.85	1.828	0.071
Gender			0.031	0.860
Female	17 (56.67)	41 (58.57)		
Male	13 (43.33)	29 (41.43)		
Type 2 diabetes			7.369	0.007
Yes	20 (66.67)	26 (37.14)		
No	10 (33.33)	44 (62.86)		
Hypertension			0.124	0.725
Yes	14 (46.67)	30 (42.86)		
No	16 (53.33)	40 (57.14)		
Smoking history			5.738	0.017
Yes	25 (83.33)	41 (58.57)		
No	5 (16.67)	29 (41.43)		
Dyslipidemia			0.429	0.513
Yes	19 (63.33)	49 (70.00)		
No	11 (36.67)	21 (30.00)		
Coronary artery disease			2.970	0.396
Left backbone	6 (20.00)	21 (30.00)		
Arteriae coronaria dextra	10 (33.33)	19 (27.14)		
Left cyclotron branch	4 (13.33)	15 (21.43)		
Left anterior descending	10 (33.33)	15 (21.43)		
LVEF (%)	54.26±5.11	55.62±6.05	1.077	0.284
Killip classification			3.705	0.157
II	4 (13.33)	16 (22.86)		
III	20 (66.67)	32 (45.71)		
IV	6 (20.00)	22 (31.43)		

LVEF, left ventricular ejection fraction.

Table 2 Comparison of peripheral blood circRNAs in the two groups

Group	Circular RNAs (ng/μL)
Observation group (n=30)	176.25±19.62
Control group (n=70)	65.26±4.34
t	46.092
P	0.000

CircRNAs, circular RNAs.

complications such as ventricular septal defect, ventricular aneurysm formation, and ventricular free wall embolism, threatening the lives and safety of patients (11,12). PCI can effectively open the occluded artery in patients with ACS and reduce mortality (13). However, clinical studies have confirmed that ACS patients are prone to cardiovascular adverse events such as myocardial infarction in the short term (14). The occurrence of MACE will not only reduce the therapeutic effect of PCI, but also cause death in

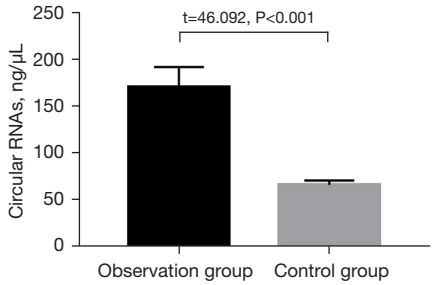

Figure 1 Comparison of peripheral blood circular RNAs between the two groups.

Table 3 Assignment of factors affecting MACE in ACS patients

Group	Risk factors	Assignment
X1	Type 2 diabetes	No =0, yes =1
X2	Smoking history	No =0, yes =1
X3	Circular RNAs	Continuous variable

MACE, major adverse cardiovascular event; ACS, acute coronary syndrome.

Table 4 Multivariate analysis of the risk factors for developing MACE in patients with ACS

Risk factors	Wald	P	OR	95% CI
Type 2 diabetes	4.452	0.002	1.314	1.052–1.437
Smoking history	4.596	0.001	1.227	1.014–1.385
Circular RNAs	4.496	0.002	1.312	1.028–1.452

MACE, major adverse cardiovascular event; ACS, acute coronary syndrome; OR, odds ratio; CI, confidence interval.

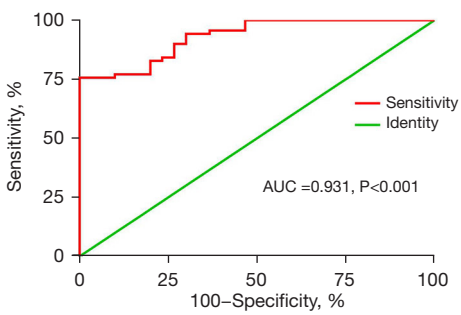

Figure 2 ROC curve of MACEs for ACS patients. AUC, area under the curve; ROC, receiver operating characteristic; MACE, major adverse cardiovascular events; ACS, acute coronary syndrome.

patients with severe conditions (15). Therefore, seeking a reliable clinical index to evaluate the prognosis of ACS patients is of high concern in clinical practice.

This study shows that type 2 diabetes, smoking history, and peripheral blood circRNAs are risk factors for MACE in ACS patients. The reasons may be as follows: (I) for type 2 diabetes, abnormal glucose fluctuation induces oxidative stress in ROS-induced cells, triggering a cascade of inflammatory reactions, aggravating the degree of damage to vascular endothelial cells, and promoting the progression of atherosclerosis (16). Hyperglycemia also affects cardiomyocyte repair and ventricular remodeling, increasing the incidence of MACE (17). (II) Smoking history: a large amount of oxygen inhalation will lead to the increase of local oxLDL in the coronary artery, participating in the oxidative stress injury of the coronary artery, aggravating the degree of damage to vascular endothelial cells, and increasing the incidence of MACE (18). (III) Peripheral blood circRNAs: circRNAs are closed circular noncoding RNAs that are stably expressed in eukaryotic cells (19). By reverse splicing and exon activity, circRNAs produce covalent circRNAs which is basically composed of exons. CircRNAs have functions in linear RNA generation regulation, protein translation, and gene expression in a variety of ways. For example, they can regulate the processes of cardiomyocyte apoptosis, myocardial fibrosis, and ventricular hypertrophy through different mechanisms, so as to increase the incidence of cardiovascular diseases (20,21). In recent years, clinical studies have confirmed that there is a certain correlation between the abnormal expression of circRNAs and the occurrence and development of cardiovascular diseases, and circRNAs can be used as markers for the diagnosis and treatment of cardiovascular diseases (22). CircRNAs can act as miRNA sponges, affecting the normal biological functions of miRNAs by reducing their activity or binding with them, thereby inducing cardiovascular disease (23,24). The 5 miRNAs with high matching values with circRNAs are miR-16, miR-211, miR-204, miR-194, and miR-541. The above abnormally expressed miRNAs play an important regulatory role in the occurrence and development of cardiovascular diseases. In the study of Ding *et al.* (25), it was confirmed that there was a significant difference in the expression of circRNAs between patients with heart failure and the control group. A total of 109 circRNAs were highly expressed in the plasma of patients with heart failure, and the expression profiles of plasma circRNAs changed significantly. This study showed that the AUC of peripheral blood circRNAs for predicting MACE

in ACS patients was 0.756. Therefore, circRNAs have good predictive value for MACE. Because circRNAs can maintain high stability in plasma, serum, and other cellular environments for a long time, this provides guaranteed laboratory detection of circRNAs. Therefore, it is worth using peripheral blood circRNAs as a specific index to evaluate and predict the prognosis of ACS patients.

This study has several limitations. First, as a retrospective study, selection bias may be inevitable. Second, as a single institution study without external validity evidence, the generalizability of our findings may be limited. Third, the sample size was small and statistical significance may be hard to be obtained. A multicenter, prospective study is warranted to assess the feasibility of our results.

In conclusion, the high expression of peripheral blood circRNAs in patients with ACS will increase the incidence of MACE. Peripheral blood circRNAs have high prognostic value in patients with ACS, and are therefore worthy of reference and promotion.

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Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-253/rc

Data Sharing Statement: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-253/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-253/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Affiliated Hospital of Inner Mongolia Medical University (No. 2019-048) and informed consent was taken from all the patients.

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