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Circulating MicroRNA-1 for the early prediction of unstable angina and the value of second coronary angiography in type 2 diabetes patients with chest pain and no ST-T changes on electrocardiograms

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Abstract

Background To investigate the early diagnostic value of circulating microRNA-1 for unstable angina (UA) in type 2 diabetes patients presenting with chest pain as the main symptom and without ST-T changes on electrocardiograms and to assess the predictive value of microRNA-1 for the need for a second coronary angiography (CAG) in these UA patients.

Methods All hospitalized patients with chest pain undergoing first-time CAG demonstrated no ST-T changes on initial and pre-CAG electrocardiograms and normal initial high-sensitivity cardiac troponin I levels. Plasma microRNA-1 levels were measured via quantitative reverse transcription polymerase chain reaction within 3 h of chest pain onset. UA patients were followed up for 5 years through phone calls and outpatient visits. The endpoints included second CAG and all-cause mortality.

Results A total of 127 patients were enrolled, with 62 in the UA group and 65 in the non-UA group. MicroRNA-1, low-density lipoprotein cholesterol, and diabetes duration were significantly greater in the UA group than in the non-UA group. The area under the receiver operating characteristic curve for microRNA-1 in diagnosing UA was 0.811. Binary logistic regression analysis indicated that microRNA-1 and low-density lipoprotein cholesterol were predictive factors for UA. No UA patients were lost to follow-up or died during the follow-up period. Patients who underwent a second CAG had significantly higher microRNA-1 levels than those who did not. Binary logistic regression revealed that microRNA-1 and hemoglobin a1c were predictive factors for second CAG in UA patients during follow-up. Cox regression analysis revealed that microRNA-1 was an independent risk factor for a second CAG during follow-up in UA patients. Using the Youden index, the optimal cut-off value of microRNA-1 ($2^{-\Delta\Delta Ct}$) for UA diagnosis was determined to be 1.510, which stratified UA patients into high- and low-expression groups. Kaplan-Meier survival analysis revealed

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that the microRNA-1 high-expression group had a significantly greater proportion of second CAG than did the microRNA-1 low-expression group.

Conclusions For type 2 diabetes patients with acute chest pain, no ST-T changes on electrocardiograms, and negative high-sensitivity cardiac troponin I, microRNA-1 may offer early diagnostic value for UA and could serve as an independent risk factor for the need for a second CAG within 5 years.

Keywords MicroRNA-1, Unstable angina, Type 2 diabetes, Chest pain, Value

Introduction

Chest pain is a common symptom in emergency departments, with highly variable causes and severities. Acute coronary syndrome (ACS), a life-threatening cardiovascular condition, often manifests as chest pain [1]. High-sensitivity cardiac troponin I (hs-cTnI) is an important biomarker for the early diagnosis of acute myocardial infarction (AMI). However, some cases of non-ST-segment elevation ACS show no dynamic changes on electrocardiograms (ECGs) and have negative hs-cTnI values, making early identification of unstable angina (UA) a challenge [2]. Patients with type 2 diabetes (T2D) presenting with chest pain have increased cardiovascular risk and are more likely to undergo coronary angiography (CAG). However, the severity of chest pain varies significantly in this population, and low-risk patients may not require excessive diagnostic and therapeutic interventions [3, 4]. Accurately assessing and managing chest pain by rapidly identifying high-risk patients while avoiding resource overuse in low-risk patients is of clinical importance.

Muscle-specific microRNA-1 (miRNA-1), which is highly expressed in both cardiac and skeletal muscles, is closely associated with cardiovascular diseases and their progression. Its stable presence in blood makes it a potential biomarker [5–7]. Studying changes in miRNA-1 in T2D patients with chest pain may provide new insights into the early diagnosis of coronary artery disease (CAD) [8]. This study aimed to explore the diagnostic value of miRNA-1 in the early prediction of UA in patients with T2D presenting with chest pain without ST-T changes on ECGs and negative hs-cTnI and to evaluate the predictive value of miRNA-1 for a second CAG in these UA patients.

Methods

Study design and population

This retrospective study included patients with acute chest pain and T2D who presented to the Third Affiliated Hospital of Soochow University from January to December 2019. All hospitalized patients with chest pain undergoing their first CAG demonstrated no ST-T changes on both initial and pre-CAG ECGs, with initial hs-cTnI levels within the normal range. Patients were classified into UA and non-UA groups on the basis of diagnostic criteria from established guidelines [2, 9]. The diagnoses were confirmed by two senior specialists, with a third specialist involved in case of disagreements. The exclusion criteria were as follows: [1] AMI; [2] aortic dissection; [3] pulmonary embolism; [4] heart failure; [5] severe liver or kidney dysfunction; [6] chronic or progressive inflammatory diseases; and [7] malignancies. The clinical indications for secondary CAG in UA patients include: [1] Recurrent or persistent chest pain unresponsive to standard antianginal therapy; [2] Dynamic ECGs changes (e.g., new ST-segment depression ≥ 0.5 mm or T-wave inversion) or ischemia confirmed by non-invasive stress testing (e.g., exercise tolerance test); [3] Hs-cTnI levels exceeding the 99th percentile upper reference limit; [4] Non-invasive imaging evidence suggestive of coronary ischemia, such as echocardiographic wall motion abnormalities or reversible perfusion defects on nuclear myocardial perfusion imaging.

Sample collection and processing

Venous blood samples were collected from the study participants within 3 h of chest pain onset. Five milliliters of blood was collected into an EDTA-containing tube, immediately centrifuged at 3000 rpm for 10 min to extract the plasma, and stored at -80 °C until analysis. General information on patients, including sex, age, body mass index (BMI), personal and medical history, and laboratory parameters such as total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides, estimated glomerular filtration rate (eGFR), hs-cTnI, fasting plasma glucose (FPG), hemoglobin a1c (HbA1c), diabetes duration (DD), and history of percutaneous coronary intervention (PCI), was collected. Additionally, information on medications used by patients before their clinical visit, including antiplatelet agents, angiotensin-converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs), β-blockers, statins, and antidiabetic drugs, was documented. The first measurement data were used for statistical analysis.

Clinical follow-up

UA patients were followed up by phone or outpatient visits, with the follow-up endpoints being the occurrence of second CAG and all-cause mortality within 5 years.

Detection of circulating miRNA-1

Circulating miRNA-1 was detected as previously reported [10]. Total RNA was extracted from plasma via the mirVana PARIS Kit (Applied Biosystems, USA). Reverse transcription was performed via the miScript Reverse Transcription Kit (Applied Biosystems, USA). According to the manufacturer's protocol (Applied Biosystems, USA), miRNA-1 expression was quantified via TaqMan (Applied Biosystems, USA) quantitative reverse transcription polymerase chain reaction (qRT−PCR). The expression level of miRNA-1 was calculated via the $2^{-\Delta\Delta Ct}$ method and converted into $\log2^{-\Delta\Delta Ct}$ values. Cel-miRNA-39 was spiked in as an exogenous control at a final concentration of 10 nM. The formula used was as follows: ΔΔCt = experimental group ($Ct_{miRNA-1}$ - $Ct_{cel-miRNA-39}$) - control group ($Ct_{miRNA-1}$ - $Ct_{cel-miRNA-39}$). Each sample was tested in triplicate, and the average value was calculated. Plasma miRNA-1 levels were independently assessed and analyzed by two researchers who were blinded to the clinical characteristics of the patients.

Statistical analysis

Data analysis was performed via SPSS 27.0 software (Inc., IBM, USA). Categorical variables are expressed as counts and percentages, whereas continuous variables are represented as medians (interquartile ranges) [M (Q_L, Q_U)]. The comparison of categorical variables was performed via Pearson's chi-square test, continuity-corrected chi-square test, or Fisher's exact test. The Mann−Whitney test was used to compare continuous variables between two groups. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of circulating miRNA-1, and the area under the ROC curve (AUC) was calculated. An AUC < 0.5 indicated no diagnostic value, and an AUC of 1.0 indicated an ideal diagnostic test. The Hanley and McNeil test was used to compare multiple diagnostic tests. To identify predictors of UA, binary logistic regression was performed, followed by internal validation using bootstrap resampling ($n = 1000$ iterations) to assess the stability of the miRNA-1 odds ratio (OR) and its 95% confidence interval (CI) in the multivariable model. Spearman rank correlation analysis was used to assess the correlation between multiple factors. Cox regression analysis was used to identify independent risk factors for a second CAG in the UA group. Kaplan−Meier survival analysis was used to evaluate the impact of miRNA-1 expression on the occurrence of second CAG in the UA group. All hypothesis tests were two-sided, and a P value < 0.05 was considered to indicate statistical significance.

Results

Baseline characteristics of the study participants

A total of 127 patients were included, with 62 in the UA group and 65 in the non-UA group. The general data of the two groups are compared in Table 1. The non-UA group consisted of stable angina (22 patients), atrial premature beats (12 patients), ventricular premature beats

Table 1 Baseline characteristics of the study subjects

Variables	UA group ($n = 62$)	Non-UA group ($n = 65$)	Z/χ^2 value	P value
Sex [male, *n* (%)]	45 (72.6)	45 (69.2)	0.172	0.678
Age (years)	64.0 (60.0, 70.0)	65.0 (61.0, 71.0)	0.914	0.361
Smoking [*n* (%)]	18 (29.0)	16 (24.6)	0.316	0.574
BMI (kg/m^2)	23.55 (19.78, 25.91)	23.63 (21.05, 25.71)	0.309	0.758
Hypertension [*n* (%)]	46 (74.2)	49 (75.4)	0.024	0.877
Triglycerides (mmol/L)	1.26 (0.87, 1.98)	1.30 (0.83, 1.73)	0.632	0.527
TC (mmol/L)	3.70 (3.46, 4.57)	4.00 (3.54, 4.56)	0.552	0.581
HDL-C (mmol/L)	1.16 (0.88, 1.43)	1.20 (0.93, 1.68)	1.346	0.178
LDL-C (mmol/L)	3.00 (2.31, 3.74)	2.50 (1.90, 3.38)	2.163	0.031
eGFR (mL/min/1.73 m^2)	70.22 (59.99, 80.15)	73.41 (65.25, 93.26)	1.838	0.066
hs-cTnI (ng/ml)	0.00 (0.00, 0.02)	0.01 (0.00, 0.02)	1.648	0.099
HbA1c (mmol/L)	6.95 (6.20, 7.80)	7.20 (6.30, 8.20)	1.245	0.213
DD (years)	4.0 (3.0, 6.0)	3.0 (2.0, 5.0)	2.313	0.021
FPG (mmol/L)	7.20 (6.80, 7.90)	7.20 (7.00, 7.80)	0.312	0.755
PCI [*n* (%)]	18 (29.0)	5 (7.7)	9.744	0.002
Antiplatelet agents [*n* (%)]	32 (51.6)	40 (61.5)	1.273	0.259
Antidiabetic drugs [*n* (%)]	56 (90.3)	60 (92.3)	0.158	0.691
β-Blockers [*n* (%)]	2 (3.2)	1 (1.5)	0.471	0.613
ACEIs [*n* (%)]	18 (29.0)	28 (43.1)	2.710	0.100
ARBs [*n* (%)]	25 (40.3)	17 (26.2)	2.878	0.090
Statins [*n* (%)]	39 (62.9)	46 (70.8)	0.887	0.346
miRNA-1 ($2^{-\Delta\Delta Ct}$)	1.65 (1.19, 2.04)	1.00 (0.97, 1.19)	6.054	<0.001

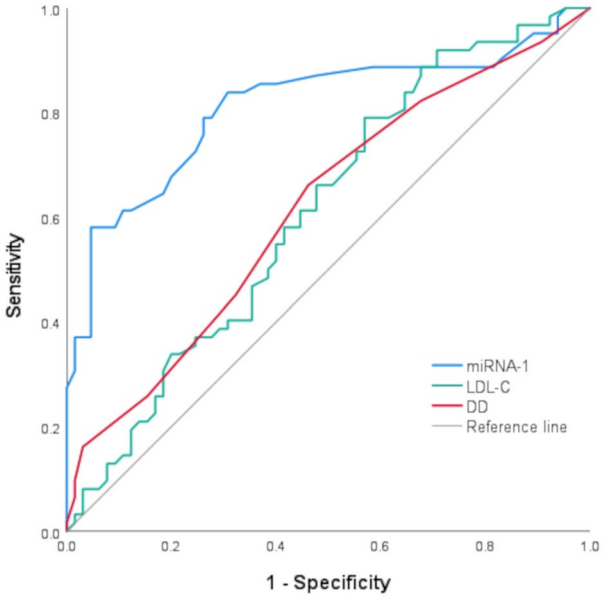

Fig. 1 ROC curves for the early diagnosis of UA with respect to miRNA-1, LDL-C, and DD

(6 patients), neurogenic chest pain (6 patients), gastroesophageal reflux disease (6 patients), chest wall or costal cartilage inflammation (4 patients), intercostal neuralgia (2 patients), pleuritis (2 patients), herpes zoster (2 patients), and pneumonia (3 patients).

Diagnostic efficiency of miRNA-1 for detecting UA

Analysis of the ROC curve revealed that miRNA-1, LDL-C, and DD have the potential to predict the occurrence of UA in study subjects (Fig. 1; Table 2). Moreover, the diagnostic efficiency of miRNA-1 was significantly better than that of LDL-C and DD (miRNA-1 vs. LDL-C: Z = 3.165, $P = 0.002$; miRNA-1 vs. DD: Z = 3.074; $P = 0.002$).

Predictive factor analysis for the occurrence of UA

Univariate binary logistic regression analysis included sex, age, smoking, BMI, hypertension, triglycerides, TC, HDL-C, LDL-C, eGFR, hs-cTnI, HbA1c, DD, FPG, and miRNA-1. The results revealed that the levels of miRNA-1, LDL-C, and DD were significantly different. Further multivariate binary logistic regression analysis of variables with $P < 0.15$ from the univariate analysis (LDL-C, eGFR, DD, and miRNA-1) demonstrated that miRNA-1 and LDL-C are predictive factors for UA in the study subjects (Table 3). There was no significant correlation among miRNA-1, LDL-C, and DD (all $P > 0.05$), indicating that there was no substantial effect on the results of the multivariate analysis.

Comparison of the clinical characteristics of secondary CAG in the UA group

The median follow-up period was determined to be 1800.00 days (IQR: 883.25, 1800.00). During the follow-up period, no patients in the UA group were lost to follow-up, and no deaths were reported. A total of 27 patients (43.55%) underwent a second CAG for the following reasons: 20 cases due to recurrent chest pain, 5 cases with dynamic ST-T changes on ECGs (including 3 cases accompanied by recurrent chest pain), 9 cases with elevated hs-cTnI levels (including 8 cases with recurrent chest pain), and 4 cases with noninvasive diagnostic findings suggestive of coronary ischemia. The clinical characteristics of the two groups are shown in Table 4. Differences in miRNA-1 expression, HbA1c, and DD between the second CAG group and the non-second CAG group were statistically significant.

Predictive factors for secondary CAG in the UA group

Univariate binary logistic regression analysis incorporating all variables from Table 1 revealed that HbA1c and DD were significantly different. Further multivariate binary logistic regression analysis of variables with $P < 0.15$ from the univariate analysis (LDL-C, HbA1c, DD, and miRNA-1) revealed that miRNA-1 and HbA1c are predictive factors for second CAG in the UA group during follow-up (Table 5). After 1,000 bootstrap resampling iterations, the 95% CI of miRNA-1 in the multivariable model demonstrated good stability (OR: 1.222–17.770).

Table 2 The early diagnostic value of miRNA-1, LDL-C and DD

Markers	AUC	95%CI	*P* Value	Sensitivity	Specificity	Accuracy	Cut-off Value
miRNA-1	0.811	0.732~0.890	<0.001	0.581	0.954	0.772	1.510
LDL-C	0.611	0.513~0.709	0.031	0.790	0.431	0.606	2.255
DD	0.618	0.521~0.714	0.022	0.661	0.538	0.598	3.500

Table 3 Binary logistic regression analysis of predictive factors of UA

Variables	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
LDL-C	1.445 (1.003~2.082)	0.068	1.549 (1.001~2.397)	0.049
eGFR	0.984 (0.966~1.001)	0.039	0.987 (0.966~1.009)	0.239
DD	1.270 (1.050~1.537)	0.014	1.209 (0.964~1.516)	0.100
miRNA-1	25.697 (7.308~90.365)	<0.001	24.948 (6.828~91.159)	<0.001

Table 4 Baseline clinical characteristics of the Follow-Up study subjects

Variables	second CAG group (*n* = 27)	non-second CAG group (*n* = 35)	Z/χ^2 Value	*P* Value
Sex [male, *n* (%)]	21 (77.8)	24 (68.6)	0.649	0.420
Age (years)	67.0 (60.5, 70.0)	64.0 (60.0, 66.0)	1.154	0.248
Smoking [*n* (%)]	7 (25.9)	11 (35.0)	0.224	0.636
BMI (kg/m^2)	21.87 (18.86, 26.14)	23.63 (20.27, 25.74)	0.440	0.660
Hypertension [*n* (%)]	21 (77.8)	25 (71.4)	0.321	0.571
Triglycerides (mmol/L)	1.47 (0.94, 2.26)	1.14 (0.85, 1.86)	0.838	0.402
TC (mmol/L)	3.65 (3.45, 4.31)	3.59 (3.93, 4.63)	0.860	0.390
HDL-C (mmol/L)	1.12 (0.82, 1.375)	1.16 (1.05, 1.40)	0.973	0.331
LDL-C (mmol/L)	3.09 (2.49, 3.92)	2.84 (2.18, 3.54)	1.683	0.092
eGFR (mL/min/1.73 m^2)	70.38 (59.18, 80.16)	70.06 (61.74, 78.64)	0.064	0.949
hs-cTnI (ng/ml)	0.00 (0.00, 0.02)	0.00 (0.00, 0.02)	0.400	0.690
HbA1c (mmol/L)	7.60 (6.25, 8.20)	6.40 (6.10, 7.15)	2.961	0.003
DD (years)	5.0 (4.0, 6.5)	4.0 (3.0, 5.0)	2.234	0.025
FPG (mmol/L)	7.20 (6.80, 7.90)	7.20 (7.00, 7.80)	0.491	0.624
PCI [*n* (%)]	10 (37.0)	8 (22.9)	1.487	0.223
miRNA-1 ($2^{-\Delta\Delta Ct}$)	1.87 (1.47, 2.04)	1.26 (1.09, 1.93)	2.201	0.028

Table 5 Binary logistic regression analysis of second CAG during follow-up in the UA group

Variables	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
LDL-C	1.753 (0.989∼3.109)	0.055	1.761 (0.834∼3.719)	0.138
HbA1c	1.967 (1.205∼3.211)	0.007	1.775 (1.020∼3.090)	0.043
DD	1.360 (1.040∼1.778)	0.025	1.305 (0.968∼1.760)	0.081
miRNA-1	2.350 (0.976∼5.654)	0.057	3.008 (1.118∼8.095)	0.029

Table 6 Cox regression analysis of factors influencing secondary CAG in the UA group

Variables	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
LDL-C	1.332 (0.925∼1.918)	0.123	1.155 (0.733∼1.821)	0.535
HbA1c	1.356 (1.078∼1.707)	0.009	1.205 (0.910∼1.595)	0.193
DD	1.198 (1.019∼1.408)	0.029	1.169 (0.968∼1.412)	0.104
miRNA-1	1.849 (1.090∼3.136)	0.023	2.117 (1.191∼3.763)	0.011

There was no significant correlation between miRNA-1 and LDL-C ($P = 0.318$), HbA1c ($P = 0.412$), or DD ($P = 0.841$). HbA1c was not significantly correlated with LDL-C ($P = 0.134$) but was positively correlated with DD ($r = 0.260$, $P = 0.041$), and $r < 0.7$ indicated no substantial impact on the results of the multivariate analysis.

Independent risk factors for second CAG in the UA group

Univariate Cox regression analysis of all the variables shown in Table 1 revealed that miRNA-1, HbA1c, and DD were independent risk factors for a second CAG during follow-up in the UA group (all $P < 0.05$). Further multivariate Cox analysis of variables with $P < 0.15$ from the univariate analysis (miRNA-1, LDL-C, HbA1c, and DD) revealed that only miRNA-1 was an independent risk factor for second CAG during follow-up in the UA group (Table 6).

Kaplan–Meier analysis of the effect of miRNA-1 on second CAG in the UA group

The diagnostic threshold for miRNA-1 expression ($2^{-\Delta\Delta Ct}$ = 1.510) in UA was established through Youden index optimization, enabling stratification of the cohort into distinct high- and low-expression groups. A comparison of the clinical characteristics between the two groups is shown in Table 7. The proportion of patients who underwent a second CAG in the high-expression miRNA-1 group was significantly greater than that in the low-expression miRNA-1 group (55.6% vs. 26.9%, $\chi^2 = 5.034$, $P = 0.025$). Kaplan-Meier survival analysis revealed that the probability of avoiding a second CAG was significantly lower in the miRNA-1 high-expression group than in the low-expression group (Fig. 2).

Table 7 Comparison of the clinical characteristics of UA patients in different miRNA-1 groups

Variables	high-expression group (*n* = 36)	low-expression group (*n* = 26)	Z/χ^2 value	*P* value
Sex [male, *n* (%)]	25 (69.4)	20 (76.9)	0.424	0.515
Age (years)	65.0 (60.0, 70.5)	64.0 (60.0, 67.0)	1.059	0.289
Smoking [*n* (%)]	10 (27.8)	8 (30.8)	0.066	0.798
BMI (kg/m^2)	23.05 (19.56, 25.54)	23.79 (20.20, 26.09)	0.792	0.428
Hypertension [*n* (%)]	27 (75.0)	19 (73.1)	0.029	0.864
Triglycerides (mmol/L)	1.18 (0.90, 1.98)	1.31 (0.85, 1.98)	0.250	0.803
TC (mmol/L)	3.93 (3.45, 4.55)	3.65 (3.52, 4.57)	0.493	0.622
HDL-C (mmol/L)	1.14 (0.93, 1.38)	1.17 (0.84, 1.43)	0.050	0.960
LDL-C (mmol/L)	2.86 (2.36, 3.19)	3.52 (2.27, 4.09)	1.427	0.154
eGFR (mL/min/1.73 m^2)	69.89 (57.90, 78.59)	74.76 (66.20, 82.69)	1.619	0.105
hs-cTnI (ng/ml)	0.00 (0.00, 0.02)	0.00 (0.00, 0.02)	0.527	0.598
HbA1c (mmol/L)	7.00 (6.20, 7.70)	6.95 (6.10, 8.10)	0.164	0.869
DD (years)	4.5 (3.0, 6.0)	4.0 (3.0, 5.0)	0.245	0.806
FPG (mmol/L)	7.20 (6.75, 7.50)	7.25 (6.90, 8.10)	0.858	0.391
PCI [*n* (%)]	13 (36.1)	5 (19.2)	2.088	0.148
Antiplatelet agents [*n* (%)]	19 (52.8)	13 (50.0)	0.047	0.829
Antidiabetic drugs [*n* (%)]	31 (86.1)	25 (96.2)	3.956	0.387
β-Blockers [*n* (%)]	1 (2.8)	1 (3.9)	1.392	0.999
ACEIs [*n* (%)]	11 (30.6)	7 (26.9)	0.097	0.756
ARBs [*n* (%)]	15 (41.7)	10 (38.5)	0.064	0.800
miRNA-1 ($2^{-\Delta\Delta Ct}$)	1.98 (1.75, 2.12)	1.12 (0.90, 1.20)	6.679	<0.001

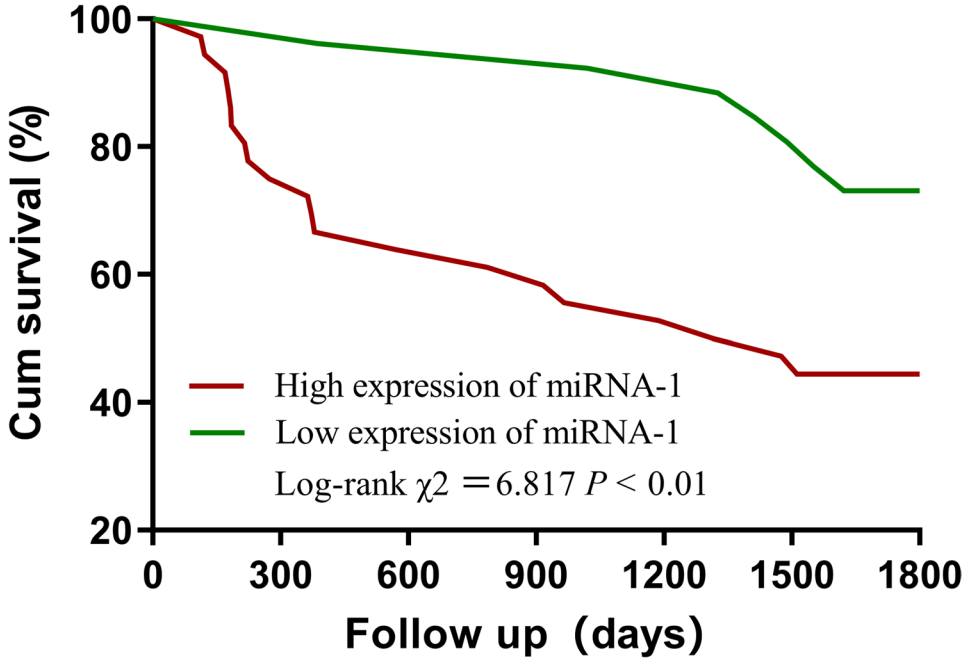

Fig. 2 Kaplan–Meier analysis of second CAG in UA patients by miRNA-1 group

Discussion

This study explored the diagnostic value of miRNA-1 in the early prediction of UA in T2D patients presenting with chest pain without ST-T changes on ECGs and negative hs-cTnI. Additionally, this study explored the role of miRNA-1 in predicting rehospitalization risk among UA patients who required a second CAG, highlighting its potential as a novel biomarker for early risk stratification and targeted intervention. Among clinical populations, 20-40% of patients report chest pain at least once in their lifetime, with an annual incidence of approximately 15.5% [11]. Chest pain is associated with age and sex, with a relatively high prevalence among elderly males [12]. In 2020, data from Chinese Chest Pain Centers revealed 1,869,010

patients with chest pain, among whom 647,472 (33.57%) had ACS, including 241,208 (12.91%) with UA. Non-ACS cardiac chest pain was reported in 498,705 patients (26.68%), non-ACS vascular emergencies were reported in 27,957 patients (1.5%), and other causes were reported in 714,876 patients (38.25%) [13]. These findings highlight the significant proportion of low- to moderate-risk patients with chest pain. Chest pain should be systematically, efficiently, and accurately differentiated, adhering to the principles of "early diagnosis, risk stratification, appropriate triage, and scientific treatment." High-risk lethal chest pain requires rapid diagnosis and immediate rescue measures, moderate-risk chest pain necessitates dynamic evaluation and monitoring, and low-risk chest pain warrants rational triage and early discharge [14, 15].

Previous studies have suggested that miRNA-1 affects insulin sensitivity and glucose metabolism, regulates adipocyte differentiation and function, and participates in various inflammatory pathways, all of which play a central role in the pathogenesis of T2D [16, 17]. Furthermore, miRNA-1 is upregulated during the development of T2D, leading to the dysregulation of genes involved in glucose and lipid metabolism. By modulating key genes associated with inflammation and fibrosis, miRNA-1 contributes to myocardial fat degeneration and the onset of diabetic complications [18–20]. This mechanism may synergize with oxidative stress pathways, where elevated malondialdehyde levels in ACS patients further exacerbate myocardial injury [21]. Dyslipidemia, characterized by high levels of LDL-C and DD, is the primary risk factor for CAD in T2D patients [22]. In this study, the performance of LDL-C aligns with previous findings, while miRNA-1 has demonstrated strong diagnostic value, significantly outperforming LDL-C, DD, hs-cTnI, and other markers, even when DD, a well-established risk factor for UA, did not significantly differ. Moreover, prior research has shown that miRNA-1 is upregulated in T2D patients with non-obstructive CAD compared with healthy individuals, with a more significant increase in those with obstructive CAD, and its high expression is significantly associated with CAD risk [16, 18]. Notably, UA patients included patients with non-obstructive coronary ischemia, such as microvascular dysfunction, endothelial dysfunction, or coronary artery spasm. Furthermore, emerging evidence suggests that inducible nitric oxide synthase may serve as a potential biomarker for myocardial infarction with non-obstructive coronary arteries, even in the absence of CAG [23]. As T2D is an independent risk factor for microcirculatory dysfunction [24], this provides a more comprehensive perspective for the early diagnosis of UA via miRNA-1. It is worth noting that miRNA-1 reached significance only in the multivariable logistic regression model ($P = 0.029$)—not the univariate analysis ($P = 0.057$). This likely reflects the impact of confounder adjustment, which may reveal a stronger predictive effect that the univariate model underestimates. Additionally, 1,000 bootstrap resampling iterations confirmed the stability of the 95% CI for miRNA-1 in the multivariable model.

Currently, the widespread clinical application of miRNA-1 faces significant challenges. Beyond the well-recognized limitations of prolonged detection time and high costs, a critical barrier to clinical translation is the lack of standardized protocols for data normalization [25, 26]. For instance, variations in pre-analytical factors and the absence of consensus on endogenous controls or exogenous spike-ins across studies introduce substantial inter-laboratory variability, complicating result comparability and clinical validation. The authors recommend accelerating its clinical translation and prioritizing its use in challenging cases. Additionally, efforts should be made to develop efficient, rapid, and cost-effective detection methods for broader clinical adoption. In summary, this study supports the clinical application of miRNA-1 in patients with T2D presenting with chest pain but without abnormal ST-T changes or hs-cTnI elevation on ECGs.

This study has several limitations. First, its retrospective nature could have introduced inherent biases and limitations in data collection and analysis. Additionally, the study cohort was from a single-center setting, and the specificity of the study population led to a relatively small sample size, which may limit the generalizability of the findings to a broader patient population. Among UA patients in follow-up, only 27 patients underwent a repeat CAG due to clinical indications. Despite stable bootstrap-validated 95% CI for miRNA-1 (1,000 iterations), the small sample size increases the risk of overfitting in the multivariable analysis. Most significantly, the absence of an independent prospective validation cohort restricts the extrapolation of results beyond the derivation dataset. While acknowledging the operational challenges associated with prospective multicenter replication, we emphasize the critical need for external validation across heterogeneous populations to enhance methodological rigor and evidence robustness. Therefore, future evaluation in larger prospective clinical studies will be necessary to confirm the results of this study. Second, previous studies have shown that miRNA-1 is significantly downregulated in asymptomatic T2D patients with acute heart failure, and this study cannot draw conclusions for such patients [27]. Third, to avoid confounding effects from malignancies, cancer patients were excluded, meaning that the results are applicable only to non-cancer patients [28].

Conclusions

In T2D patients with acute chest pain, no ECGs ST-T changes, and negative hs-cTnI, miRNA-1 may offer early diagnostic value for UA and could serve as an independent risk factor for the need for a second CAG within 5 years.

Abbreviations

ACS	Acute coronary syndrome
hs-cTnI	High-sensitivity cardiac troponin I
AMI	Acute myocardial infarction
UA	Unstable angina
miRNA-1	microRNA-1
CAG	Coronary angiography
T2D	Type 2 diabetes
ROC	Receiver operating characteristic
AUC	Area under the ROC curve
DD	Diabetes duration
PCI	Percutaneous coronary intervention
HbA1c	Hemoglobin a1c
FPG	Fasting plasma glucose
eGFR	estimated glomerular filtration rate
HDL-C	High-density lipoprotein cholesterol
LDL-C	Low-density lipoprotein cholesterol
BMI	Body mass index
CAD	Coronary artery disease
ACEIs	Angiotensin-converting enzyme inhibitors
ARBs	Angiotensin receptor blockers

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by TS, CH, and JZ. The first draft of the manuscript was written by XPZ and QW. TS, QW, and XPZ take responsibility for the integrity of the data and the accuracy of the data analysis. TS and XPZ commented on previous versions of the manuscript. LY supervised the study. All authors read and approved the final manuscript.

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Data availability

The datasets generated and/or analyzed during the current study are not publicly available due [REASON WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study adhered to the Declaration of Helsinki and was approved by the Ethics Committee of the Third Affiliated Hospital of Soochow University (Approval No. 2022–102). Informed consent was obtained from all the participants. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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