Plasmatic MicroRNA Signatures in Elderly People with Stable and Unstable Angina

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Summary

We aimed to investigate the distinctive miRNA profiles in the plasma of elderly patients with unstable angina (UA) and stable angina (SA), and to find more effective markers of UA in elderly people. We compared miRNA expression levels in plasma samples from 10 elderly patients with UA and 10 elderly patients with SA by using microarray-based miRNA chip, and then performed validation with Real-time PCR. Mir-1202, mir-1207-5p, and mir-1225-5p showed a statistically significant down-regulation ($P < 0.05$), while mir-3162-3p showed an up-regulation ($P < 0.05$) during validation. Among all single miRNAs, miR-3162-3p showed the highest discriminatory power in the diagnosis of elderly patients with UA (AUC: 0.79, 95% CI: 0.675-0.905). The discriminatory power of a panel of three miRNAs (mir-3162-3p/mir-1225-5p/mir-1207-5p) was highest with an AUC of 0.91 (95% CI: 0.84-0.98), followed by mir-3162-3p/mir-1225-5p (AUC: 0.833, 95% CI: 0.732-0.934) and mir-3162-3p/mir-1207-5p (AUC: 0.817, 95% CI: 0.712-0.922). In conclusion, multi-miRNA panel could provide higher diagnostic value for the diagnosis of elderly patients with UA.

Key words: Circulating miRNA, Acute coronary syndrome (ACS), Elderly patients, Diagnostic value, Biomarker

Acute chest pain is the most common cause for admission of patients to the emergency department, as about half of these patients are diagnosed with cardiac disease. Among these patients, 50% are due to acute coronary syndrome (ACS), including stable angina (SA), unstable angina (UA), and myocardial infarction. Myocardial infarction can be diagnosed by the elevated plasmatic levels of cardiac-specific protein troponin. However, there are no established circulating biomarkers that may distinguish SA and UA currently, and the latter, as a kind of ACS, is more likely to develop into myocardial infarction and result in an increase in mortality. More attention should be paid to the elderly, as myocardial infarction in them is often characterized as atypical, sudden onset, rapid progression, and high mortality, and is likely to be misdiagnosed. Thus, the identification of novel non-invasive biomarkers of SA and UA in elderly people is a compelling need.

MicroRNAs (miRNAs) are endogenous, small, 22-nucleotide-long, non-coding RNAs involved in the regulation of gene expression, and are remarkably stable to be reliably quantified in circulating blood. A lot of studies have shown that miRNAs play crucial roles in the pathophysiologic process of cardiovascular disease, including endothelial dysfunction, inflammation, and angiogenesis. Circulating miRNAs are increasingly examined as potential biomarkers in cardiovascular disease.

Previous studies have not found valuable biomarkers that can distinguish UA from SA. D'Alessandra, et al. reported that miR-337-5p and miR-145 were differentially expressed in UA patients compared to SA, but no discriminating AUC values were observed comparing SA and UA patients. In our previous study, we found that some miRNAs, such as mir-21 and mir-451, were differentially expressed in UA and SA validation cohort, but the study population did not aim to investigate the elderly people. Therefore, the purpose of this study is to search for distinctive miRNA profiles in the plasma of elderly patients with UA and SA, and to find more effective markers of UA in elderly people.

Methods

Study population: All patients aged ≥ 65 years old were
enrolled at Peking University People’s Hospital between August 1, 2015 and August 31, 2016. The derivation cohort included two groups, UA patients and SA patients. Patients with typical UA and angiographically documented CAD were enrolled in the UA group (n = 10). UA was defined according to 2014 AHA/ACC guideline for the management of patients with non-ST-elevation acute coronary syndromes.10) Patients with SA according to ACC/AHA guidelines were enrolled in the SA group (n = 10).9)

The results obtained in the derivation cohort were further analyzed in a validation cohort with 30 UA patients and 30 SA patients, including 36 men and 24 women, that was different from the derivation cohort. The diagnostic criteria of UA and SA in validation cohort consisted with the derivation cohort.

The exclusion criteria were: (1) UA caused by coronary artery dissection and coronary focal spasm; (2) secondary UA related to anemia, fever, tachycardia, etc.; (3) the elevated level of troponin I or creatine kinase; (4) patients with severe hepatic or renal dysfunction, leukemia, malignant, and ongoing inflammatory diseases.

The investigational protocol was approved by the ethics review board of Peking University People’s Hospital. All subjects provided written informed consent at the time of enrollment.

**Plasma samples collection:** Peripheral venous blood samples were collected in EDTA anticoagulant tubes after patients were admitted into the hospital, and processed within 30 minutes. Plasma was prepared by centrifugation at 3000 rpm/minute for 10 minutes, with supernatants transferred into new tubes, and stored at -80ºC until use.

**RNA extraction:** RNA was isolated from plasma using miRNeasy Mini Kit (Qiagen, Valencia, CA), following the protocol as described for plasma samples.10) Briefly, the sample was lysed with 700 μL Qiazol and subsequently mixed with 140 μL chloroform. The organic and aqueous phase was separated by centrifugation at 12,000 g for 15 minutes. The aqueous phase was mixed with 100% ethanol and applied to RNeasy Mini spin column. After being washed several times, RNA was eluted into 25-μL RNase-free water. We used a synthetic Caenorhabditis elegans (C. elegans) miR-39 (cel-mir-39, 50 fmol/sample, synthesized by QIAGEN) as a spiked-in control to normalize for individual RNA-isolation-related variations. 50-fmol cel-mir-39 were introduced into each sample after addition of denaturing Qiazol solution. For each RNA sample, the C. elegans spiked-in miRNAs were measured using TaqMan real-time PCR assays (Applied Biosystems).

**MiRNA array and data analysis:** MiRNA expression profiling of the plasma samples was detected using the Agilent miRNA Microarray system. RNA samples were labeled with the miRNA complete Labeling and Hyb Kit (Agilent 5190-0456) and hybridized on the Human miRNA Microarray (Release 21.0, 8*60K) station. Scanning was performed with the Agilent Scanner G2505C (Agilent Technologies). Feature Extraction software (version 10.7.1.1, Agilent Technologies) was used to read image raw intensity and Genespring software (version 13.1, Agilent Technologies) was employed to finish the basic analysis (OE Biotech’s, Shanghai, China).

**Real-time (RT) PCR:** The protocol of RT-PCR was performed as previously described,10) which was carried out on an Applied Biosystems system at 95ºC for 10 minutes, followed by 40 cycles of 94ºC for 10 seconds and 60ºC for 60 seconds. Values were normalized to cel-mir-39 according to different samples and data were analyzed using the 2-ΔΔCt method.

**Bioinformatics prediction:** Mirbase, Miranda, and TargetScan were used to predict the miRNA target genes, which were classified by Kyoto Encyclopedia of Genes and Genomes pathway analysis. Gene Ontology pathway was then analyzed to evaluate biochemical process, cellular components, and molecular function of target genes involved.

**Statistical analysis:** Differential expression was evaluated using the Volcano Plot method. A cut off of 1.5 was set to determine differentially expressed miRNAs. AUC-ROC with 95% CI was established for miRNAs in discriminating the different subject groups. Combinations of miRNAs were obtained via logistic regression. Continuous clinical variables were analyzed by t-test, while categorical clinical variables were analyzed by Chi-square tests. P < 0.05 was considered statistically significant.

**Results**

**Profiling of circulating miRNAs in screening phase:** Ten patients with UA and ten patients with SA were enrolled in the derivation cohort for miRNA profiling. The average age of the patients was (73.9 ± 5.6) years old. There were no significant differences between the two groups in sex, lipid profile, risk factors, drug administration, etc. The clinical characteristics of the patients were summarized in Table I.

More than 200 miRNAs were detected in all plasma samples of patients. Compared with SA patients, 18 miRNAs were detected to be differentially expressed in plasma of UA patients. Among them, 8 miRNAs were up-regulated and 10 miRNAs were down-regulated significantly (Cluster Analysis seen in Figure 1, fold change > 1.5, P < 0.05).

We selected 6 miRNAs (i.e., mir-21-5p, mir-1202, mir-1207-5p, mir-1225-5p, mir-3162-3p, let-7f-1-3p) for further validation by quantitative RT-PCR in a larger independent patient cohort. These miRNAs were selected based on their expression differences between UA and SA patients (Fold change > 15, P < 0.05), and their abundance in the circulation (16/20).

**Profiling of circulating miRNAs in confirmation step:** A total of 60 patients including 36 men and 24 women with UA and SA were enrolled in the PCR validation cohort (n = 60). The average age of the patients was (73.7 ± 5.4) years old. The clinical characteristics of the patients are summarized in Table II. There were also no significant differences in lipid profile, risk factors and drug administration between the two groups.

RT-PCR was performed to verify the differential expression of miRNAs determined by microarray analysis. Mir-1202, mir-1207-5p and mir-1225-5p showed a statistically significant down-regulation in UA patients (P < 0.05), and mir-3162-3p showed up-regulation (P < 0.05). However, mir-21-5p and let-7f-1-3p were not confirmed as
Table I. Clinical Characteristics of the Derivation Cohort for Circulating miRNAs Profiling.

| Baseline data                | SA patients (n = 10) | UA patients (n = 10) | P  |
|-----------------------------|---------------------|---------------------|----|
| Sex M/F                     | 5/5                 | 5/5                 | 1.00 |
| Age (years)                 | 73.9 ± 6.0          | 73.8 ± 5.5          | 0.969 |
| BMI (kg/m²)                 | 25.8 ± 1.1          | 25.6 ± 2.2          | 0.857 |
| SBP (mmHg)                  | 149.4 ± 28.9        | 131.6 ± 23.8        | 0.151 |
| DBP (mmHg)                  | 80.4 ± 11.9         | 73.2 ± 7.9          | 0.131 |
| LVEF (%)                    | 67.1 ± 5.4          | 69.5 ± 7.1          | 0.412 |
| HR (bpm)                    | 69.6 ± 8.3          | 69.2 ± 7.3          | 0.910 |
| PLT (×10¹²/L)               | 188.3 ± 41.7        | 224.2 ± 35.0        | 0.052 |
| AST(U/L)                    | 17.2 ± 2.4          | 19.1 ± 4.9          | 0.289 |
| eGFR(mL/minute/1.73 m²)     | 83.3 ± 7.9          | 84.2 ± 7.6          | 0.795 |
| Lipid profile               |                     |                     |    |
| TG (mmol/L)                 | 1.4 ± 0.5           | 1.5 ± 0.5           | 0.713 |
| TC (mmol/L)                 | 3.9 ± 1.0           | 4.0 ± 1.1           | 0.675 |
| HDL-C (mmol/L)              | 1.0 ± 0.3           | 1.0 ± 0.2           | 0.718 |
| LDL-C (mmol/L)              | 2.3 ± 0.7           | 2.6 ± 0.7           | 0.455 |
| Risk factors                |                     |                     |    |
| Cigarette smoking, %        | 20                  | 30                  | 0.606 |
| Hypertension, %             | 90                  | 90                  | 1.000 |
| Hyperlipemia, %             | 50                  | 40                  | 0.653 |
| Diabetes mellitus, %        | 30                  | 50                  | 0.361 |
| Drug Administration         |                     |                     |    |
| Aspirin, %                  | 50                  | 40                  | 0.653 |
| Clopidogrel, %              | 20                  | 50                  | 0.160 |
| Statin, %                   | 60                  | 50                  | 0.653 |
| Beta-blocker, %             | 50                  | 50                  | 0.639 |
| CCB, %                      | 60                  | 70                  | 0.361 |
| ACEI, %                     | 0                   | 20                  | 0.136 |
| ARB, %                      | 40                  | 40                  | 1.000 |

BMI indicates body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVEF, left ventricular ejection fraction; HR, heart rate; PLT, platelet; TG, triglyceride; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CCB, calcium channel blocker; ACEI, angiotensin- converting enzyme inhibitor; and ARB, angiotensin receptor blocker. All P values represent comparisons between SA patients and UA patients. Comparisons between groups were performed with student’s t test for continuous variables and with Chi-square test for categorical variables. P < 0.05 was considered statistically significant.

significant at this step (Figure 2).

**Diagnostic potential of circulating miRNAs:** To determine the predictive capacity of mir-1202, mir-1207-5p, mir-1225-5p, and mir-3162-3p, we plotted ROC curves and calculated the area under the curve (AUC). Only mir-3162-3p had significant power to differentiate UA patients from SA groups. The AUC-ROC of mir-3162-3p was 0.790 (95% CI: 0.675-0.905, P = 0.003), and the sensitivity and specificity were 76.7% and 70%, respectively. While the AUC-ROC of mir-1202, mir-1207-5p, and mir-1225-5p did not appear statistically significant (Figure 3, Table III).

To further explore the applicability of circulating miRNAs as potential diagnostic biomarkers of elderly patients with UA, we combined mir-3162-3p with mir-1202, mir-1207-5p, or mir-1225-5p to draw the ROC curve, finding that mir-3162-3p/mir-1202, mir-3162-3p/mir-1207-5p, and mir-3162-3p/mir-1225-5p had higher AUC with 0.791, 0.817, and 0.833, respectively. These combinations improved the AUC compared with mir-3162-3p alone (Figure 4A-C, Table IV). We then selected mir-1207-5p and mir-1225-5p, which had better diagnostic power in a previous combination diagnostic test to combine with mir-3162-3p, and found that the AUC-ROC of mir-3162-3p/mir-1225-5p/mir-1207-5p was 0.841, and the sensitivity and specificity were 86.7% and 70%, respectively (Figure 4D). The diagnostic value of a panel of three miRNAs was higher than that of the two miRNAs.

**The predictive target genes of newly identified miRNAs:** Using three databases (Mirbase, Miranda, and Targetscan), the target genes of 4 newly identified miRNAs were predicted, and the target genes are illustrated in Table V.

**Discussion**

ACS is the most common manifestation of coronary artery disease and is the major cause of mortality and morbidity in elderly people. Studies have shown that the mortality increased by 1% when the age increased by 10 years. In people with age < 55 years, 55-64 years, 65-74 years, and 75-84 years, the mortality rates caused by ACS
Figure 1. Cluster Analysis of miRNAs expression in elderly patients with UA and SA (n = 20). The red represents high expression and green represents low expression. UA indicates unstable angina; and SA, stable angina.

Table II. Clinical Characteristics of the Validation Cohort for Circulating miRNAs Profiling

|                     | SA patients (n = 30) | UA patients (n = 30) | P     |
|---------------------|----------------------|----------------------|-------|
| Baseline data       |                      |                      |       |
| Sex M/F             | 18/12                | 18/12                | 1.000 |
| Age (years)         | 72.9 ± 4.6           | 74.5 ± 6.0           | 0.252 |
| BMI (kg/m²)         | 25.3 ± 3.7           | 25.4 ± 4.1           | 0.945 |
| SBP (mmHg)          | 134.4 ± 16.2         | 137.1 ± 18.2         | 0.546 |
| DBP (mmHg)          | 72.6 ± 8.2           | 72.1 ± 7.7           | 0.809 |
| LVEF (%)            | 62.1 ± 19.5          | 64.8 ± 8.9           | 0.506 |
| HR (bpm)            | 67.1 ± 7.6           | 67.5 ± 8.7           | 0.850 |
| PLT (×10¹²/L)       | 184.4 ± 46.8         | 192.0 ± 44.3         | 0.521 |
| eGFR (mL/minute/1.73 m²) | 80.1 ± 9.1          | 76.8 ± 10.7          | 0.208 |
| Lipid profile       |                      |                      |       |
| TG (mmol/L)         | 1.7 ± 1.1            | 1.5 ± 0.7            | 0.607 |
| TC (mmol/L)         | 4.0 ± 0.9            | 4.1 ± 1.1            | 0.712 |
| HDL-C (mmol/L)      | 1.1 ± 0.3            | 1.1 ± 0.3            | 0.896 |
| LDL-C (mmol/L)      | 2.5 ± 0.7            | 2.5 ± 0.8            | 0.783 |
| Risk factors        |                      |                      |       |
| Cigarette smoking, %| 50                   | 40                   | 0.440 |
| Hypertension, %     | 73.3                 | 73.3                 | 1.000 |
| Hyperlipemia, %     | 33.3                 | 33.3                 | 1.000 |
| Diabetes mellitus, %| 50                   | 30                   | 0.595 |
| Drug Administration |                      |                      |       |
| Aspirin, %          | 63.3                 | 65.5                 | 0.862 |
| Clopidogrel, %      | 26.7                 | 31                   | 0.713 |
| Statin, %           | 60                   | 58.6                 | 0.915 |
| Beta-blocker,%      | 46.7                 | 41.1                 | 0.685 |
| CCB, %              | 43.3                 | 44.8                 | 0.909 |
| ACEI, %             | 20                   | 20                   | 1.000 |
| ARB, %              | 26.7                 | 36.9                 | 0.359 |

BMI indicates body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVEF, left ventricular ejection fraction; HR, heart rate; PLT, platelet; TG, Tri-glyceride; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CCB, calcium channel blocker; ACEI, angiotensin-converting enzyme inhibitor and ARB, angiotensin receptor blocker. All P values represent comparisons between SA patients and UA patients. Comparisons between groups were performed with student’s t test for continuous variables and with Chi-square test for categorical variables. P < 0.05 was considered statistically significant.
were 5%, 8%, 16%, and 32% respectively. At the same time, aging also increases the risk of heart failure, cardiogenic shock, atrial fibrillation, and cardiac perforation. Growing evidences suggest miRNAs as potential diagnostic markers to distinguish patients with ACS from those without ACS. However, there are few studies aiming to investigate elderly ACS patients, especially the early diagnosis of UA. We profiled and replicated some miRNAs as possible early biomarkers to distinguish elderly patients with UA from those with SA earlier. We first found that 4 differentially-expressed miRNAs were associated with elderly UA patients in both derivation and validation cohort, and that most of them were down-regulated in UA patients compared with SA patients. Then, among all single miRNAs, miR-3162-3p showed the best accuracy and had the highest discriminatory power in the diagnosis of elderly patients with UA. Finally, the discriminatory power of a panel of three miRNAs (mir-3162-3p/mir-1225-5p/mir-1207-5p) was highest, with an AUC of 0.91 (95% CI: 0.84-0.98), followed by mir-3162-3p/mir-1225-5p (AUC: 0.833, 95% CI: 0.732-0.934) and mir-3162-3p/mir-1207-5p (AUC: 0.817, 95% CI: 0.712-0.922), which suggested that a multi-miRNA approach provides higher diagnostic value than single miRNAs.

As previous study showed, D’Alessandra, et al. tried to search for distinctive miRNA profiles in plasma of patients with coronary artery disease in comparison to matched controls to identify novel discriminating biomarkers of UA and SA, but failed. They found that none of the considered miRNAs reached an acceptable AUC value to differentiate UA patients from SA patients, ranging from 0.404 (miR-145) to 0.678 (miR-337-5p). In contrast, we succeeded in identifying some miRNAs to distinguish SA from UA, and found that miR-3162-3p alone and miR-3162-3p with other miRNAs could reach an acceptable AUC value. It is probably due to the different groups of study patients we chose, as we aimed to investigate the elderly UA and SA patients.

We used Mirbase, Miranda, and Targetscan to predict the target genes of mir-1202, mir-1207-5p, mir-1225-5p, and mir-3162-3p. These predictions may provide novel insights into the biological functions of these miRNAs in elderly patients with unstable angina.
Figure 4. The ROC curves of different combination of miRNAs in elderly patients with UA and SA. (A) ROC curve analysis of mir-3162-3p/mir-1202. (B) ROC curve analysis of mir-3162-3p/mir-1207-5p. (C) ROC curve analysis of mir-3162-3p/mir-1225-5p. (D) ROC curve analysis of mir-3162-3p/mir-1225-5p/mir-1207-5p. UA indicates unstable angina; and SA, stable angina.

Table III. The Area Under Receiver-Operator Characteristic Curve (AUC) in PCR Validation Cohort (n = 60)

| miRNA         | AUC     | 95%CI      | P      | Cut-off point | Sensitivity | Specificity |
|---------------|---------|------------|--------|---------------|-------------|-------------|
| miR-1202      | 0.482   | 0.334-0.631| 0.813  | 0.000361      | 46.7%       | 60%         |
| miR-1207-5p   | 0.502   | 0.354-0.650| 0.976  | 0.001545      | 56.7%       | 50%         |
| miR-1225-5p   | 0.479   | 0.328-0.630| 0.779  | 0.00007715    | 100%        | 23.3%       |
| miR-3162-3p   | 0.790   | 0.675-0.905| 0.003  | 0.003188      | 76.7%       | 70%         |

The AUC was determined for selected miRNA to distinguish UA from SA cases in the validation cohort. The cut-off values and their corresponding sensitivity and specificity are shown. 95% CI, 95% confidence interval. P < 0.05 was considered statistically significant.

Table IV. The Area Under Receiver-Operator Characteristic Curve (AUC) in PCR Validation Cohort (n = 60)

| miRNA         | AUC     | 95%CI      | P      | Cut-off point | Sensitivity | Specificity |
|---------------|---------|------------|--------|---------------|-------------|-------------|
| miR-3162-3p/miR-1202 | 0.791   | 0.677-0.905| < 0.001| 0.002618      | 76.7%       | 70%         |
| miR-3162-3p/miR-1207-5p | 0.817   | 0.712-0.922| < 0.001| 0.001429      | 76.7%       | 70%         |
| miR-3162-3p/miR-1225-5p | 0.833   | 0.732-0.934| < 0.001| 0.0011        | 90%         | 66.7%       |

The AUC was determined for selected miRNA to distinguish UA from SA cases in the validation cohort. The cut-off values and their corresponding sensitivity and specificity are shown. 95% CI, 95% confidence interval. P < 0.05 was considered statistically significant.

and mir-3162-3p. The predicted target genes of mir-1202 were Janus kinases1 (JAK1), Janus kinases2 (JAK2), ATP binding cassette transporter-A1 (ABCA1), and Platelet-derived growth factor (PDGF). JAK1 and JAK2 are predominant in cardiac myocytes, and JAK signaling-linked STAT has been shown to be associated with the protective responses to ischemic injury. ABCA1 facilitates the formation of high density lipoprotein and plays anti-
inflammatory and anti-atherosclerotic roles, showing inverse correlation with coronary vascular disease. In contrast, PDGF stimulates smooth muscle cell proliferation and vasoconstriction. Koizumi, et al. found that PDGF played a role in coronary plaque instability in STEMI patients. Mir-1202 was probably involved in inflammation and atherosclerosis. The decreased mir-1202 maybe increased the release of PDGF, which was involved in the ischemia pathophysiological process.

The predicted target gene of mir-1207-5p and mir-1225-5p was a disintegrin and metalloproteinase with thrombospondin type 1 motifs (ADAMTS). ADAMTS family are circulating plasma enzymes responsible for processing procollagens and von Willebrand factor, and involved in cleavage of proteoglycans. Numerous studies have shown that ADAMTS were key participants in the pathogenesis of vascular diseases, including atherosclerosis, aneurismal change, and restenosis. ADAMTS proteases contributed to the plaque destabilization by weakening the fibrous cap, and expressed higher in coronary atherosclerotic plaques of ACS patients than SA patients. Mir-1207-5p and mir-1225-5p expressed lower in elderly patients with UA might increase the expression of ADAMTS, thus leading to the formation of vulnerable plaque.

IL-23 receptor (IL-23R), which is located on T-helper 17 cells, was one of the putative target genes of mir-3162-3p. IL-23R played an important role in the IL-23/IL-17 inflammatory signal transduction pathway, which was essential to atherosclerosis. As an important molecule of the IL-23 pathway, studies have showed that IL-23R played critical roles in atherosclerosis and atherosclerosis-related diseases. Zhang, et al. found IL-23R rs6682925T/C polymorphism acted as a risk factor in process of atherosclerosis. Mir-3162-3p may be an important factor involved in the formation of atherosclerosis by modulating IL-23R.

SMAD3, as an important mediator of transforming growth factor-β signaling, was another predicted target gene of mir-3162-3p. Kobayashi et al. reported that SMAD3 mice had enhanced neointimal hyperplasia and decreased matrix deposition in response to femoral artery injury, showing the protective effect in the vasculature. Turner et al. found that SMAD3 knockdown in human arterial smooth muscle cells increased cell viability, which was consistent with an anti-proliferative role of SMAD3. The increased mir-3162-3p in UA elderly patients might down-regulate SMAD3, resulting in the development of atherosclerosis.

There are several limitations in our study. The sample size was small, which may lead to low test efficiency. To assess the potential of plasmatic miRNAs as diagnostic biomarkers in elderly patients with UA, multicenter large-scale studies will be required. Besides, the function of miRNAs and their regulative effects on pathways involved in UA patients should be investigated in further study.

In summary, we identified some miRNAs in plasma as biomarker for differential diagnosis between UA patients and SA patients using a screening-confirmation model. Our study demonstrated that a multi-miRNA panel could provide higher diagnostic value for the diagnosis of elderly patients with UA.

Disclosures

Conflicts of interest: There were no potential conflicts of interest in this study, including related consultancies, shareholdings, and grant funding.

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Table V. The Target Genes of Newly Identified miRNAs

| miRNA     | Target genes            |
|-----------|-------------------------|
| mir-1202  | JAK1, JAK2, ABCA1, PDGF |
| mir-1207-5p | ADAMTS          |
| mir-1225-5p | ADAMTS          |
| mir-3162-3p | IL-23R, SMAD3     |
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