

Expression of microRNA-214 and galectin-3 in peripheral blood of patients with chronic heart failure and its clinical significance

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Abstract. Expression of microRNA (miR)-214 and galectin-3 (Gal-3) in peripheral blood of patients with chronic heart failure (CHF) and its clinical significance were investigated. A total of 50 cases of CHF patients, diagnosed and treated in Shanghai Xuhui Central Hospital from January 2017 to March 2018, were the study group and 30 healthy subjects who underwent physical examination during the same period were the control group. Concentration of serum Gal-3 was detected by ELISA and the expression of miR-214 in serum was detected by RT-qPCR. The expression of miR-214 and Gal-3 in the peripheral blood of CHF patients were analyzed. The diagnostic and predictive values of efficacy were analyzed by ROC curve analysis, and the correlation between miR-214 and Gal-3 was analyzed by Pearson's correlation analysis. The serum expression levels of miR-214 and Gal-3 in the observation group were significantly higher than those in the control group, with statistically significant difference ($P < 0.05$). Pearson's correlation analysis revealed that the expression levels of miR-214 and Gal-3 were positively correlated in the peripheral blood of CHF patients ($r = 0.712$, $P < 0.05$). The area under curve (AUC) of miR-214 and Gal-3 for CHF diagnosis was 0.916 and 0.852, respectively ($P < 0.05$). The AUC for predicting the efficacy

of miR-214 and Gal-3 was 0.874 and 0.897, respectively ($P < 0.05$). In conclusion, it is speculated that miR-214 and Gal-3 are involved in the occurrence and development of CHF, which is of guiding significance for the clinical diagnosis and monitoring of CHF.

Introduction

As one of the serious and end-point events of various common heart diseases (1), chronic heart failure (CHF) is the leading cause of death among the elderly worldwide. Studies have shown that 915,000 patients with first-episode heart failure (HF) were diagnosed in the United States in 2012 (2,3), and ~20% of people >40 years of age will develop HF (4). An Italian study (5) has shown that 81.1% of 1,623 subjects aged 65-84 years had preclinical HF. The high cost of health care brought by HF is a heavy burden on patients and society (6). The above research data indicate that the high incidence, high prevalence and high mortality of HF is still a social problem, and its diagnosis and treatment are increasingly valued by society.

Galectin-3 (Gal-3) and microRNA (miR) have attracted increasing attention in recent years. Studies have found that Gal-3 can cause myocardial fibrosis (7), and the higher the concentration of Gal-3, the more severe the myocardial fibrosis (8). Cardiac fibrosis is a decisive factor in the progression of cardiovascular disease to HF. In 2012, the American College of Cardiology Foundation recommended Gal-3 as a fibrosis biomarker (9). However, Gal-3 also has limitations in the diagnosis of CHF. The pathophysiological mechanism and biological half-life in the human body are not fully understood, and they are also expressed in various immune diseases or inflammation (10). Moreover, the specificity is low leading to different diagnostic results. Therefore, seeking a new diagnostic marker has attracted increasing attention. As a short non-coding RNA with a length of ~22 nucleotides, miR's main function is to affect the stability or inhibit the translation of mRNA by binding all or part of the non-translation region of its downstream target gene mRNA 3' terminal, and participates in the physiological and pathological processes of cells in the body. Recent studies

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have found that numerous heart diseases are regulated by miRs (11,12). Circulating miR is expected to be used for the diagnosis and prognosis in HF, and several miRs are involved in important mechanisms that lead to HF, such as hypertrophy and fibrosis (13). In 2008, Lawrie *et al* (14) presented for the first time that miR could stably exist in human serum, and circulating miR has great potential in being a biochemical marker of cardiovascular diseases (15). miR-214 is a member of miR family. The study by van Rooij *et al* (16) showed that the level of miR-214 in patients with dilated cardiomyopathy with HF was significantly higher than that in healthy subjects, suggesting that its clinical significance could be studied according to its expression in the peripheral blood serum of patients with CHF.

In the present study, the expression of miR-214 and Gal-3 in the peripheral blood of patients with CHF was detected and their diagnostic and efficacy prediction values were studied, providing new clinical diagnostic indicators for CHF.

Patients and methods

General data. A total of 50 cases of CHF patients, diagnosed and treated in Shanghai Xuhui Central Hospital (Shanghai, China) from January 2017 to March 2018, were assigned in the study group, and 30 cases of healthy subjects who underwent physical examination during the same period were assigned in the control group. All test indexes of the healthy subjects were normal. The study was approved by the Ethics Committee of Shanghai Xuhui Central Hospital. Signed informed consents were obtained from the patients or the guardians.

Inclusion and exclusion criteria

Inclusion criteria: Patients diagnosed with heart failure for more than half a year, according to the diagnostic criteria of the ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012 (17); patients treated in the specific hospital; patients with complete clinical data; and patients who were informed and signed an informed consent form.

Exclusion criteria: Patients >80 years of age or <50 years of age; patients with partial absence of clinical data; and patients with myocardial infarction within 3 months, communication disorder, malignant tumor, impaired liver and kidney function, severe infection or mental dysfunction.

Therapy of patients in the observation group. Patients received conventional treatment and were given Enalapril maleate tablets orally, 10 mg/time, 1 time/day; Metoprolol sustained-release tablet (Southwest Pharmaceutical Co., Ltd.) orally, with initial dose of 6.25 mg/time, 2 times/day, and then the weekly dose was doubled, not exceeding, however, the dose of 400 mg/day; Spirinolactone tablet (Beijing zhongxin pharmaceutical factory) orally, 20 mg/time, 1 time/day.

Sample collection and determination

Sample collection. A total of 3 ml of venous blood were taken from the enrolled CHF patients on an empty stomach, on the second day of admission in the morning, and then after 6 months of treatment. Similar blood samples were collected

from the patients of the control group, and were left at room temperature for 30 min and centrifuged at 2,800 x g for 10 min. The supernatant was absorbed and stored in a refrigerator at -80°C for centralized detection.

RT-qPCR detection of miR-214 expression in serum. Total RNA of the collected serum was extracted with TRIzol kit (15596018; Invitrogen; Thermo Fisher Scientific, Inc.). The purity, concentration and integrity of total RNA were detected by UV spectrophotometry and agarose gel electrophoresis. TaqMan Reverse Transcription kit (N8080234; Invitrogen; Thermo Fisher Scientific, Inc.) was used for reverse transcription according to the manufacturer's instructions. The reaction volume was 15 μ l, and the temperature protocol was 16°C for 30 min, 42°C for 30 min, 85°C for 5 min, and 4°C until the end. cDNA was collected for PCR amplification. miR-214 forward, 5'-GATACTCACTTTTTCGCGGTCT-3' and reverse, 5'-GTG CAGGGTCCGAGGT-3'; U6 forward, 5'-CGCTTCGGCAGC ACATATAC-3' and reverse, 5'-CAGGGGCCATGCTAAT CTT-3'. The amplification system of qPCR was as follows: cDNA 1 μ l, forward primer 0.4 μ l, reverse primer 0.4 μ l, 2X TransStart® Green qPCR SuperMix UDG (AQ111-01, Transgen Biotech Co., Ltd.) 10 μ l, Passive Reference Dye (50X) (optional) 0.4 μ l, and nuclease-free water was added for a final volume of 20 μ l. qPCR amplification conditions were as follows: Incubation at 94°C for 10 min, pre-denaturation at 94°C for 5 sec, annealing and extension at 60°C for 30 sec, with a total of 40 cycles. Each sample was set with 3 duplicate wells, and the experiment was carried out 3 times. In this study, U6 was used as the internal reference gene and 2^{- $\Delta\Delta$ C_q} method was used to analyze the data (18).

ELISA in detection of Gal-3 expression in serum. Serum Gal-3 was detected by Human Galectin-3 enzyme-linked immunosorbent assay (ELISA) kit (cat no. KE00126; ProteinTech Group, Inc.). Specific Gal-3 antibodies were pre-coated on a 96-well microplate adding standard and test samples to the micropores, respectively, and setting up a blank well at the same time. Biotinylated Gal-3 antibody was added to the micropores, and then the micropores were washed thoroughly to remove unbound biotinylated antibody. HRP-labeled avidin was added, the micropores were washed again, and TMB substrate (ProteinTech Group, Inc.) was added for color development. TMB turned blue under catalysis and turned yellow under the action of acid. The absorbance (OD value) was measured by ELISA at a wavelength of 450 nm, and the corresponding concentration was converted using a standard curve.

Observational indexes

Main observational indexes. The expression levels of miR-214 and Gal-3 in the peripheral blood of patients with CHF were compared between the observation and control group. Their correlation and diagnostic value for CHF were also studied.

Secondary observational indexes. miR-214 and Gal-3 expression levels were compared before and after treatment, and regarding the clinical curative effect. Patients were divided into a good curative effect group (significant effect) and a poor curative effect (effective + invalid effect) according to the clinical efficacy. The expression levels of Gal-3 and

Table I. NYHA classification.

Curative effect	Efficacy assessment criteria
Significantly effective	Main clinical symptoms and signs of HF disappeared and cardiac functions improved ≥ 2 levels
Effective	Heart functions improved 1 level
Invalid	Heart functions improved < 1 level

NYHA, New York Heart Association; HF, heart failure.

Table II. Baseline data.

Factors	Observation group (n=50)	Control group (n=30)	t/ χ^2 value	P-value
Age (years)	62.8 \pm 8.5	60.8 \pm 8.4	1.023	0.309
Gender				
Male	27 (54.00)	16 (53.33)	0.003	0.954
Female	23 (46.00)	14 (46.67)		
Smoking history				
Yes	24 (48.00)	13 (43.33)	0.164	0.685
No	26 (52.00)	17 (56.67)		
BMI (kg/m ²)	23.94 \pm 1.99	24.38 \pm 2.03	0.950	0.345
Fasting blood glucose (mmol/l)	5.91 \pm 1.33	5.60 \pm 1.74	1.157	0.250
ALT (U/l)	18.71 \pm 5.81	18.32 \pm 5.87	0.400	0.690
AST (U/l)	21.47 \pm 6.20	20.13 \pm 5.62	1.315	0.191

BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

miR-214 in the two groups after treatment were compared. ROC curve analysis was performed to observe their predictive value in curative effect, and Pearson's correlation analysis was carried out to detect the correlation between miR-214 and Gal-3 expression levels.

Efficacy assessment criteria. After 6 months of treatment, the curative effect of 50 patients was evaluated according to the classification of the New York Heart Association (NYHA), as shown in Table I.

Statistical analysis. SPSS 20.0 software package (Cabit Information Technology Co., Ltd.) was used for the statistical analysis of the collected data. GraphPad Prism 7 (Softhead, Inc.) was used to generate the graphs. Measurement data were expressed as the mean \pm SD. Independent samples t-test was used for their comparisons between two groups, and paired t-test for comparisons between two groups before and after treatment. Chi-square test was used for the comparison of nominal data between two groups. ROC curves were drawn to evaluate the diagnostic and efficacy prediction value of miR-214 and Gal-3 in CHF. Pearson's correlation analysis was carried out for the correlation of miR-214 and Gal-3 expression levels. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Comparison of clinical data. The results revealed that there was no statistical difference in the basic clinical data between the two groups ($P > 0.05$). Details are shown in Table II.

Expression of miR-214 and Gal-3 in the serum of patients in the two groups. By comparing the relative expression of miR-214 and Gal-3 in the serum of patients in the two groups before treatment, it was found that the serum expression of miR-214 in the observation group (1.32 \pm 0.18) was significantly higher than that in the control group (1.03 \pm 0.12), and the expression of Gal-3 in the observation group (6.15 \pm 0.78 ng/ml) was significantly higher than that in the control group (4.78 \pm 0.63 ng/ml) ($P < 0.001$) (Fig. 1).

Diagnostic value of miR-214 and Gal-3 in CHF patients. The expression of the two indexes before treatment was used to draw the ROC curves. The results revealed that the area under curve (AUC) of miR-214 was 0.916 (95% CI, 0.861-0.971), the sensitivity was 92%, the specificity was 74%, Youden index was 72%, and the cut-off value was < 1.165 . The AUC of Gal-3 was 0.852 (95% CI, 0.776-0.927), the sensitivity was 88%, the specificity was 72%, Youden index was 62%, and the cut-off value was < 5.68 (Fig. 2).

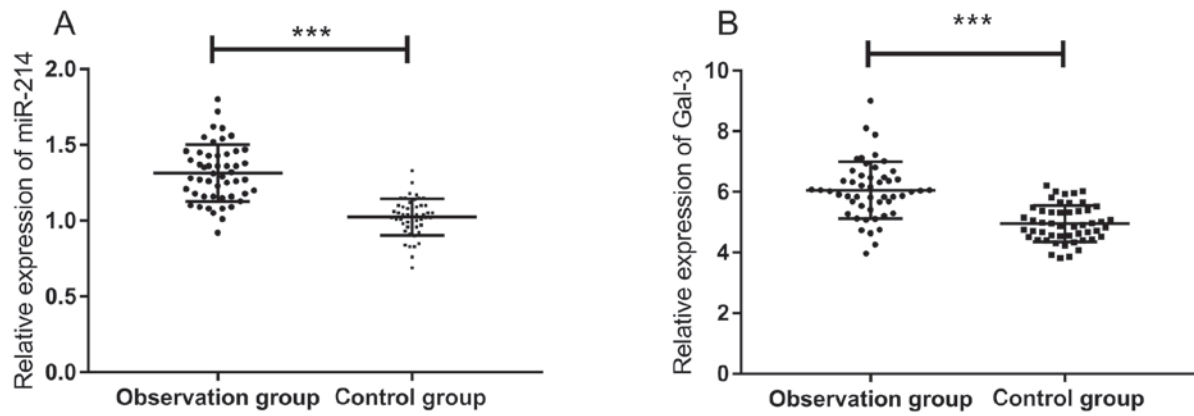


Figure 1. Expression of miR-214 and Gal-3 in the serum of patients in the two groups before treatment. (A) The expression of miR-214 in the observation group (1.32 ± 0.18) was significantly higher than that in the control group (1.03 ± 0.12) before treatment; $***P<0.001$. (B) The expression of Gal-3 in the observation group (6.15 ± 0.78 ng/ml) was significantly higher than that in the control group (4.78 ± 0.63 ng/ml) before treatment; $***P<0.001$. miR-214, microRNA-214; Gal-3, galectin-3.

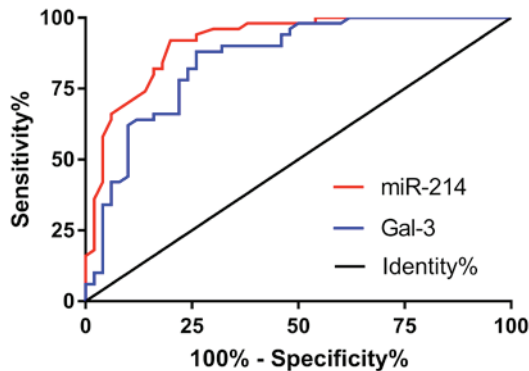


Figure 2. ROC curves of miR-214 and Gal-3 in CHF diagnosis. Red line is the ROC curve of miR-214. AUC, 0.916 (95% CI, 0.861-0.971); sensitivity, 92%; specificity, 74%; Youden index, 72%; cut-off value, <1.165 . Blue line is the ROC curve of Gal-3. AUC, 0.852 (95% CI, 0.776-0.927); sensitivity, 88%; specificity, 72%; Youden index, 62%; cut-off value, <5.68 . miR-214, microRNA-214; Gal-3, galectin-3; CHF, chronic heart failure; AUC, area under curve.

Relative expression of miR-214 and Gal-3 before and after treatment. The changes in the relative expression of miR-214 and Gal-3 in the observation group, before and after treatment were compared. The results revealed that the expression of miR-214 (1.17 ± 0.14) and Gal-3 (5.31 ± 0.54 ng/ml) in the serum of patients in the observation group was significantly decreased after treatment, and there was a significant difference compared with that before treatment ($P<0.001$) (Fig. 3).

Association of the expression of miR-214 and Gal-3 with clinical efficacy. The recent clinical efficacy was assessed. After treatment, there were 19 patients with significantly effective, 27 patients with effective, and 4 patients with invalid effect. According to clinical efficacy, the patients were divided into a group with good efficacy (significant effect, 19 patients) and a group with poor efficacy (effective + invalid effect, 31 patients). By comparing the expression of miR-214 and Gal-3 after treatment, it was found that the expression of miR-214 in the group with good efficacy (1.173 ± 0.097) was significantly lower than that in the group with poor efficacy (1.400 ± 0.179) after treatment, and the expression of Gal-3 in the group with

good efficacy (5.352 ± 0.608 ng/ml) was significantly lower than that in the group with poor efficacy (6.487 ± 0.839 ng/ml) after treatment ($P<0.001$) (Fig. 4). Subsequently, ROC curve analysis of miR-214 and Gal-3 expression after treatment in the group with good efficacy and the group with poor efficacy showed that the AUC of miR-214 was 0.874 (95% CI, 0.771-0.976), with sensitivity of 77.42%, specificity of 100%, Youden index of 77.42%, and cut-off value of >1.315 . The AUC of Gal-3 was 0.897 (95% CI, 0.812-0.982), with sensitivity of 77.42%, specificity of 94.74%, Youden index of 72.16%, and cut-off value of >6.03 (Fig. 5).

Correlation between miR-214 and Gal-3 expression. Pearson's correlation analysis was used to analyze the correlation between miR-214 and Gal-3 in serum of patients before treatment, and it was found that miR-214 and Gal-3 expression levels were positively correlated ($r=0.712$, $P<0.05$). The scatter plot in Fig. 6 shows that serum miR-214 level increased significantly with the increase of Gal-3.

Discussion

CHF has a high incidence and mortality. In recent years, studies have shown that, although the survival rate has improved, the mortality rate of HF remains $\sim 50\%$ within 5 years after diagnosis, and the prognosis is even worse (19). Therefore, it is of great significance to improve diagnosis, treatment guidance and curative effect prediction of CHF. Gal-3 is currently recognized as an inflammatory factor that promotes cardiac fibrosis. With the further research on the inflammatory mechanism of HF, it has been confirmed that inflammatory factors play a crucial role in the occurrence and development of HF (20). Relevant studies have supported that miR-214 and Gal-3 are upregulated in patients with CHF (16,21), which is closely related to the pathophysiological process of HF and is expected to be a new biomarker for HF.

As a hot research field in recent years, miR has attracted the attention of numerous scholars. The main role of miR is to bind to the downstream target gene 3'-UTR, leading to degradation of mRNA, to inhibit its translation and transcription (22,23), so as to change the expression of the target gene. Normally,

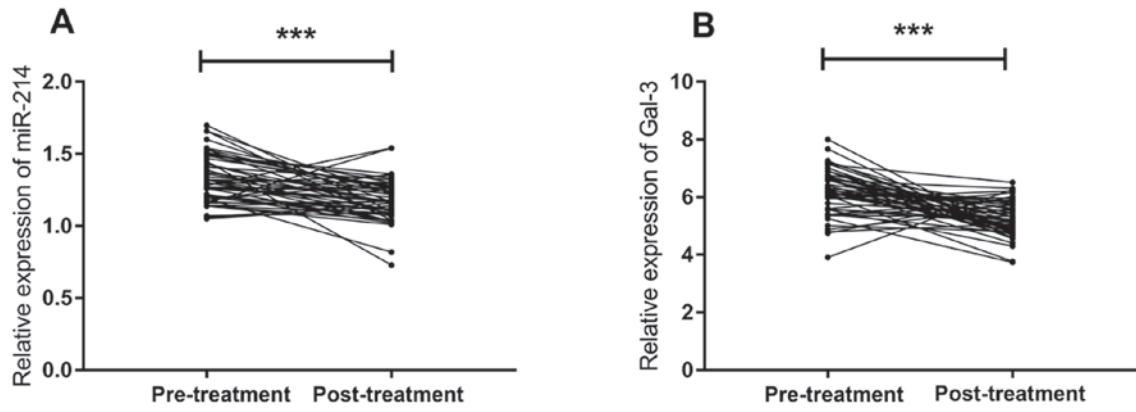


Figure 3. Relative expression of miR-214 and Gal-3 in patients before and after treatment. (A) After treatment, the serum miR-214 level in the observation group (1.17 ± 0.14) was significantly lower than that before treatment (1.32 ± 0.18); $***P<0.001$. miR-214, microRNA-214; Gal-3, galectin-3. (B) After treatment, the serum Gal-3 level in the observation group (5.31 ± 0.54 ng/ml) was significantly lower than that before treatment (6.15 ± 0.78 ng/ml); $***P<0.001$. miR-214, microRNA-214; Gal-3, galectin-3.

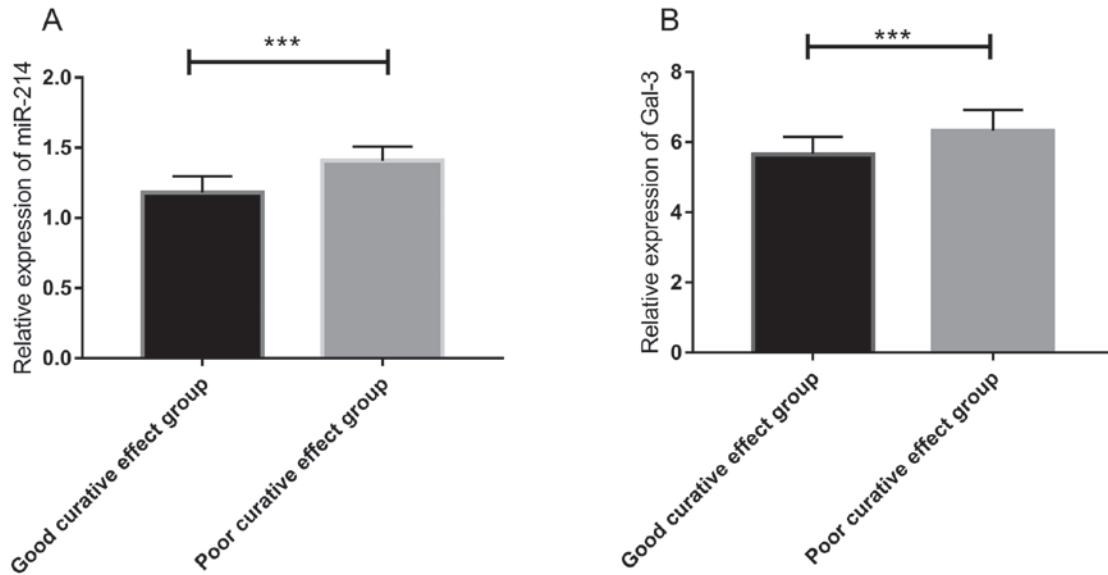


Figure 4. Comparison of miR-214 and Gal-3 expression after treatment between the group with good efficacy and the group with poor efficacy. (A) The relative expression of miR-214 in the good efficacy group (1.173 ± 0.097) was significantly lower than that in the poor efficacy group (1.400 ± 0.179) after treatment; $***P<0.001$. (B) The relative expression of Gal-3 in the good efficacy group (5.352 ± 0.608 ng/ml) was significantly lower than that in the poor efficacy group (6.487 ± 0.839 ng/ml) after treatment; $***P<0.001$. miR-214, microRNA-214; Gal-3, galectin-3.

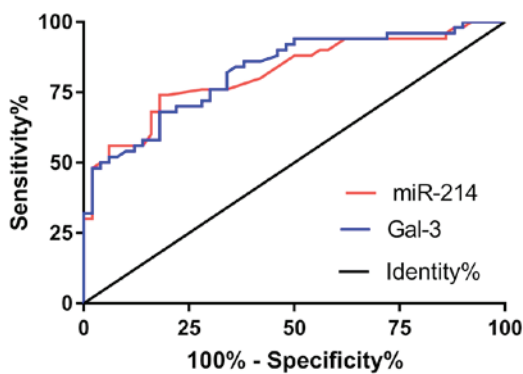


Figure 5. ROC curves of efficacy prediction value of miR-214 and Gal-3. Red line is the ROC curve of miR-214. AUC, 0.874 (95% CI, 0.771-0.976); sensitivity, 77.42%; specificity, 100%; Youden index, 77.42%; cut-off value, >1.315 . Blue line is the ROC curve of Gal-3. AUC, 0.897 (95% CI, 0.812-0.982); sensitivity, 77.42%; specificity, 94.74%; Youden index, 72.16%; cut-off value, >6.03 . miR-214, microRNA-214; Gal-3, galectin-3; AUC, area under curve.

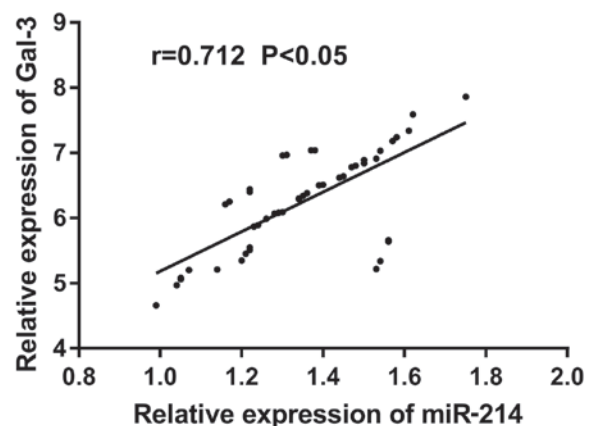


Figure 6. Scatter plot of correlation analysis between miR-214 and Gal-3 in serum of patients. There was a positive correlation between miR-214 and Gal-3 expression levels in serum of patients before treatment, $r=0.712$, $P<0.05$. miR-214, microRNA-214; Gal-3, galectin-3.

Gal-3 is expressed in a small amount in cardiac tissue. In the case of myocardial injury, however, the concentration of Gal-3 increases rapidly, leading to cardiac fibrosis (24), which can provide short- or long-term independent prognostic information for patients with HF (25). The study of Dong *et al* (26) showed that the expression of miR-214 in the infarcted part of rats after 6 h of acute myocardial infarction increased, which could protect cardiomyocytes (27). miR-214 and Gal-3 are involved in the occurrence and development of CHF; however, their clinical efficacy indexes after treatment have not been studied. Therefore, this study further verified the correlation between miR-214 and Gal-3, and their clinical diagnostic and efficacy prediction value for CHF.

In the present study, we collected serum of CHF patients and healthy subjects and detected the expression of miR-214 and Gal-3 in the serum of the two groups. It was found that the serum levels of miR-214 and Gal-3 in the observation group were higher than those in the control group, with significant difference. Studies on mice by Yu *et al* (28) and Martínez-Martínez *et al* (29) have found that myocardial fibrosis would not occur in the absence of Gal-3. In addition, the upregulation of miR-214 could reduce endothelial cell proliferation and angiogenesis during the transition from hypertrophic heart rhythm to HF (30). This suggested that these two indexes are expected to be potential diagnostic indexes for CHF. Therefore, ROC curve analysis was performed on the expression of miR-214 and Gal-3 in the observation and control groups. The results revealed that the AUC of the expression of miR-214 and Gal-3 was 0.874 and 0.897, respectively. This indicates that CHF patients and healthy subjects can be well distinguished by detecting the expression of miR-214 and Gal-3, and therefore can be used as potential diagnostic indicators for patients with CHF. Although the above studies revealed that miR-214 and Gal-3 could be used as clinical diagnostic indexes of CHF, there was no further study conducted on the clinical efficacy assessment after treatment of CHF patients. According to the clinical efficacy in the observation group, patients were divided into a group with good efficacy and a group with poor efficacy, and the association of miR-214 and Gal-3 expression with efficacy was investigated. The results revealed that the relative expression of miR-214 and Gal-3 in the group with good efficacy were significantly lower than those in the group with poor efficacy, which suggests that the expression of miR-214 and Gal-3 before treatment may be a potential predictive index of the clinical efficacy of patients after treatment. For this reason, ROC curves were drawn, and it was found that the AUC of miR-214 and Gal-3 was 0.818 and 0.825, respectively, which indicates that the expression of the two indexes before treatment could be used as a predictor of the clinical efficacy of patients after treatment.

Pearson's correlation analysis was used to analyze the correlation between miR-214 and Gal-3 expression in serum of patients, and it was found that the expression level of miR-214 was positively correlated with the expression level of Gal-3 ($r=0.712$, $P<0.05$). The scatter plot revealed that the expression level of Gal-3 increased with the increase of miR-214 expression, suggesting that both of them participate in the development of CHF and may be considered prognostic indexes of CHF. Gal-3 has the effect of promoting cardiac

fibrosis. Furthermore, studies have found that miR-214 could regulate the proliferation of fibroblasts (31). Combined with the results of this study, it is suggested that the two indexes have a synergistic effect on promoting cardiac fibrosis. However, as this study did not investigate the patient's survival, whether miR-214 and Gal-3 can become prognostic indicators of CHF needs further investigation.

Although the clinical significance of miR-214 in the occurrence and development of a variety of heart diseases has been recognized, the mechanism of its abnormal expression has not been clarified. In addition, miR is very sensitive to temperature, and therefore will be inevitably partially decomposed due to changes in the surrounding environment, causing deviations in experimental data. The pathophysiological mechanism and biological half-life of Gal-3 in the human body are not fully understood, which along with the low specificity, are responsible for deviations in the diagnostic results. At present, this study is still in the initial stage, and further exploration is needed to apply these results into practice. The stability and durability of miR-214 and Gal-3 expression need further investigation.

In conclusion, it is speculated that miR-214 and Gal-3 are involved in the occurrence and development of CHF and are expected to be potential indicators for the diagnosis and efficacy prediction of CHF.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

RH, KL, LL and LZ conceived and designed the study, and drafted the manuscript. RH, KL, LZ and HZ collected, analyzed and interpreted the experimental data. RH revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Shanghai Xuhui Central Hospital (Shanghai, China). Signed informed consents were obtained from the patients or the guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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