Review Article
The value of miR-219a-5p in the diagnosis of non-tuberculosis mycobacteria pulmonary disease and the prediction of respiratory disease recurrence

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Abstract: To explore the value of miR-219a-5p in the diagnosis of non-tuberculosis mycobacteria (NTM) pulmonary disease and the prediction of respiratory disease recurrence. 69 patients with NTM pulmonary disease admitted to Weifang Second People's Hospital from June 2016 to June 2018 were recruited as the research group (RG). At the same time, 65 cases who visited the hospital for physical examinations were recruited as the control group (CG). The expression of miR-219a-5p in the peripheral blood was measured in the RG and CG before the treatment (T0), at 4 months after the treatment (T1), at 8 months after the treatment (T2), and at 12 months after the treatment (T3). The clinical significance of miR-219a-5p on NTM pulmonary disease was analyzed. miR-219a-5p showed a low expression in the NTM pulmonary diseases (P < 0.050). When the cut-off value was 0.905, the diagnostic sensitivity and specificity of miR-219a-5p for the NTM pulmonary diseases were 69.57% and 92.31%, respectively (P < 0.001). A Spearman's correlation coefficient analysis showed that the expression levels of miR-219a-5p in the RG were positively correlated with the treatment time (r=0.461, P < 0.001). The predictive sensitivity and specificity for the adverse reactions were 60.00% and 79.59%, respectively, P < 0.050. The predictive sensitivity and specificity for the recurrent respiratory diseases were 85.71% and 80.49%, respectively, P < 0.001. miR-219a-5p showed a low expression in NTM pulmonary disease, which has a good predictive value for the development of NTM pulmonary disease, adverse reactions in the treatment process, and the prognosis of respiratory disease recurrence, and it may be a potential diagnosis and treatment target for NTM pulmonary disease in the future.

Keywords: miR-219a-5p, NTM pulmonary disease, respiratory disease, diagnosis

Introduction

Non-tuberculous mycobacteria (NTM) refers to all mycobacteria except mycobacterium tuberculosis and mycobacterium leprae, also known as environmental mycobacteria [1]. NTM easily causes pathological changes in tissues and organs, of which lung invasion is the most common [2]. NTM is considered to be mostly a saprophytic parasitic fungi, which exists in the natural environment and can be transmitted through water, soil, and other media [3]. Government data indicate that NTM pulmonary disease is becoming more and more serious worldwide, chiefly affecting the elderly [4, 5]. Although NTM pulmonary infection is extremely common at present, its incidence is relatively rare. Data indicate that the inci-

dence rate among NTM pulmonary infection patients is about 10.0%-20.0% [5, 6]. However, once NTM pulmonary disease develops, it causes an extremely serious cough, expectoration, systemic poisoning, and other conditions. In the process of pathological changes, NTM pulmonary disease may invade the skin, lymph nodes, bone joints, urinary system, and other organs and tissues, causing systemically spread diseases. In more serious cases, it may directly threaten life and health [7, 8]. Currently, a long-course combined chemotherapy regimen is usually established in the treatment of NTM pulmonary disease, with a course of treatment of 12 months or more and a very long treatment cycle [9]. In addition, a series of dynamic changes and the adverse reactions of chemotherapy may develop in sputum bacte-

ria during chemotherapy, which has a great influence on the rehabilitation of patients [10]. Therefore, clinically, it is very important to prevent the development of NTM pulmonary disease and to make an early diagnosis.

At present, the diagnostic methods for NTM pulmonary diseases are extremely complicated. The main diagnostic basis is a bacteriological examination. It is also necessary to combine PCR restriction endonuclease fragment length polymorphism analysis, PCR-nucleic acid probe hybridization, and other molecular biological techniques, imaging techniques, clinical examination techniques, and other examination methods [11]. Researchers at home and abroad are constantly working to find serum markers of NTM pulmonary disease to improve its early diagnosis rate. With the deepening of research in recent years, microRNAs may be the breakthrough in this effort. MicroRNAs are a kind of evolutionarily conservative non-coding small molecule RNA, which has the function of regulating gene expression at the translation level. They have been shown to have an important influence on many tumor, autoimmune, and infectious diseases [11-13]. Among them, miR-219a-5p has been shown in previous studies to have an abnormal expression in non-small cell lung cancer [14], but its role in NTM pulmonary disease has not been clearly defined. We suspected that miR-219a-5p might have an important clinical significance for NTM pulmonary diseases, so we carried out this experimental analysis to verify our conjecture, aiming to provide new ideas for the clinical diagnosis and treatment of NTM pulmonary diseases in the future.

Materials and methods

Baseline data

69 patients with NTM pulmonary disease who were admitted to Weifang Second People's Hospital from June 2016 to June 2018 were selected as the study cohort. At the same time, 65 patients who visited the hospital for physical examinations were selected as the control. Among them, the NTM pulmonary disease patients were the RG, and the healthy physical examination patients were the CG. This study was approved by the Weifang Second People's Hospital ethics committee. All the subjects signed an informed consent.

Inclusion and exclusion criteria

Inclusion criteria: Patients who met the clinical manifestations of NTM pulmonary disease and who were diagnosed with NTM pulmonary disease after a series of examinations; Patients who had complete case data; Patients who agreed to cooperate and participate in the investigation with the medical staff in Weifang Second People's Hospital; Their ages ranged from 10-70 years old.

Exclusion criteria: Patients with other respiratory and pulmonary diseases were excluded; Patients with tumors were excluded; Patients with autoimmune deficiency diseases were excluded; Patients with systemic infections were excluded; Patients with organ dysfunction were excluded; Patients with other cardiovascular and cerebrovascular diseases were excluded; Patients with drug allergies were excluded; Patients with low treatment compliance due to having mental disorders were excluded; Patients transferred to another hospital were excluded; Patients with physical disabilities who could not take care of themselves were excluded.

Methods

Treatment: All the patients were treated with the corresponding combined chemotherapy according to their pathological characteristics in our hospital. 4-5 kinds of drugs were combined, including β lactam antibiotics and β lactamase inhibitors, new macrolides, phenazines, quinolones, rifamycins, aminoglycosides, etc. The basic course of treatment was 12 months. After the basic course of treatment was completed, the course of treatment was extended according to each patient's rehabilitation.

Measurement methods: Before treatment (T0), at 4 months after treatment (T1), at 8 months after treatment (T2), and at 12 months after treatment (T3), 5 mL of fasting venous blood samples in both the RG and CG were extracted and placed in EDTA anticoagulation tubes and centrifuged for 10 min (300× g) after 30 min at room temperature to get upper serum. Then the upper serum was stored in a freezer at -80°C for testing. The MEG3 expression in the patients' serum was measured using PCR. Methods: The collected serum was extracted with an EasyPure miRNA Kit to determine the

Table 1. Primer sequences

	Upstream (5'-3')	Downstream (5'-3')
miR-219a-5p	CGCGGCTCCTGATTGTCC	CTCGGCCGGAGCCATAGA
U6	GGGCTCGCTTCGGCAGCACA	TGGTGTCGTGGAGTCG

total RNA. The extracted total RNA was checked for purity, concentration, and integrity using an ultraviolet spectrophotometer and agarose gel electrophoresis. TransScript® miRNA RT Enzyme Mix and 2× TS miRNA Reaction Mix were used to reverse transcribe the serum with total RNA. The steps were carried out strictly in accordance with the kit manufacturer's instructions. Then the PCR amplification experiment was carried out. The PCR reaction system was as follows: cDNA 1 μL, upstream and downstream primers each 0.4 μL, 2× TransTaq® Tip Green qPCR SuperMix 10 μL and Passive Reference Dye (50x) 0.4 μL. In the end, ddH$_2$O was added to complete it to 20 μL. The PRC reaction conditions were as follows: pre-degeneration at 94°C for 30 s, degeneration at 94°C for 5 s, anneal and extension at 60°C for 30 s, for a total of 40 cycles. 3 repeated wells were set up in each sample. The experiment was carried out 3 times. U6 was used as an internal reference and $2^{-\Delta ct}$ was used for the data analysis (**Table 1**).

Outcome measures

Main outcome measures: 1. The expression levels of miR-219a-5p in the peripheral blood of the two groups and the diagnostic value of miR-219a-5p in NTM pulmonary disease. 2. The patients in the RG were followed up for one year. The follow-up was conducted in the form of a hospital review to record the recurrence of any respiratory diseases within one year of the initial prognosis and to analyze the predictive value of miR-219a-5p for the diseases. Secondary outcome measures: Any changes in the miR-219a-5p in the course of chemotherapy and the predictive value of miR-219a-5p for any adverse reactions in the course of the chemotherapy for NTM lung disease.

Statistical methods

SPSS 22.0 was used to analyze and process the data. GraphPad 8 was used to graph the data. The count data were expressed in the form of (%). Chi-square tests were used for the comparisons between the groups. The measurement data were expressed in the form of (mean number ± standard deviation). T tests were used for the inter-group comparisons. Repetitive measurements, analyses of variance, and Bonferroni back-tests were used for the comparisons among multiple time points. Spearman's correlation coefficient was used for the correlation analysis. The diagnostic predictive value was analyzed using an ROC curve. A difference was considered statistically significant when P < 0.050.

Results

Comparison of the baseline data

There were no statistically significant differences in terms of age, BMI, gender, family history, living environment, education levels, respiratory history, or smoking in the two groups (P > 0.050). More details are shown in **Table 2**.

Comparison of miR-219a-5p

The expression levels of miR-219a-5p in the peripheral blood in the RG at T0 were significantly lower than they were in the CG (P < 0.050). More details are shown in **Figure 1**.

The diagnostic value of miR-219a-5p in NTM pulmonary disease

According to the ROC curve analysis, when the cut-off value was 0.905, the diagnostic sensitivity of miR-219a-5p to NTM pulmonary disease was 69.57%, the specificity was 92.31%, the AUC was 0.839, and the 95% CI was 0.768-0.910, P < 0.001. More details are shown in **Figure 2**.

Changes in the miR-219a-5p expression levels during treatment

The expression levels of miR-219a-5p in the peripheral blood in RG were their lowest at T0. There was no difference between T1 and T0 (P > 0.050). It began to rise at T2 and was the highest at T3 (P < 0.050). The Spearman's correlation coefficient analysis showed that the expression level of miR-219a-5p in the RG was positively correlated with the treatment time

Table 2. Comparison of the baseline data between the two groups [n (%)]

|	RG (n=69)	CG (n=65)	x^2 or t	P
Age/years old	52.6±8.9	51.2±9.1	0.899	0.370
BMI (kg/cm^2)	22.62±3.05	22.76±2.76	0.278	0.781
Gender			0.414	0.520
Male	42 (60.87)	36 (55.38)		
Female	27 (39.13)	29 (44.62)		
Family history			0.413	0.521
Yes	5 (7.25)	3 (4.62)		
No	64 (92.75)	62 (95.38)		
Living environment			0.922	0.337
Town	48 (69.57)	50 (76.92)		
Rural	21 (30.43)	15 (23.08)		
Education levels			0.517	0.472
< high school	34 (49.28)	28 (43.08)		
≥ high school	35 (50.72)	37 (56.92)		
Respiratory history			0.922	0.337
Yes	21 (30.43)	15 (23.08)		
No	48 (69.57)	50 (76.92)		
Smoking			1.028	0.311
Yes	42 (60.87)	45 (69.23)		
No	27 (39.13)	20 (30.77)		
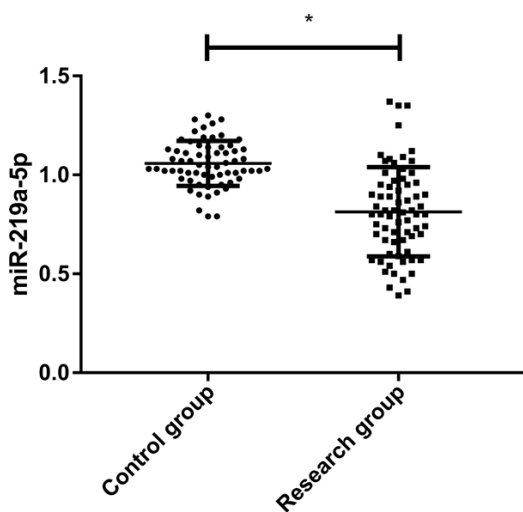
Figure 1. Comparison of miR-219a-5p in the RG and CG at T0, *P < 0.050.

(r=0.461, P < 0.001). More details are shown in **Figure 3**.

The predictive value of miR-219a-5p for adverse reactions

There were 20 cases with adverse reactions in the RG, for an adverse reaction rate of 28.99%.

The expression levels of miR-219a-5p in RG before treatment were selected as the prediction index for the ROC curve analysis. The results showed that the miR-219a-5p expression levels of the patients with adverse reactions were significantly lower than the levels of the patients without any adverse reactions (P < 0.050). When the cut-off value was 0.705, the predictive sensitivity of miR-219a-5p to the adverse reactions of patients was 60.00%, and the specificity was 79.59%, P < 0.050. The AUC was 0.704, and the 95% CI was 0.576~0.832, P=0.008. More details are shown in **Figure 4**.

The predictive value of miR-219a-5p for the recurrence of respiratory diseases

The 69 patients were followed up for one year. All the 69 patients were successfully followed up, for a follow-up success rate of 100.0%. During the one-year follow-up, 28 patients suffered from recurrent respiratory diseases, for a recurrence rate of 40.58%. The patients' miR-219a-5p expression levels at T3 were selected as the prediction index for the ROC curve analysis. The results showed that the expression levels of miR-219a-5p in the recurrent patients were significantly higher than they were in the non-recurrent patients (P < 0.050). When the cut-off value was 1.015, the predictive sensitivity of miR-219a-5p to respiratory disease recurrence was 85.71%, the specificity was 80.49%, the AUC was 0.894, and the 95% CI was 0.820-0.968, P < 0.001. More details are shown in **Figure 5**.

Discussion

NTM pulmonary disease is a disease with an extremely high potential incidence worldwide, with many infected persons [15]. Although some related studies showed that NTM pulmonary disease can be cured effectively by timely treatment after its onset, once the best treatment period is missed in the early stages, the patients are prone to systemic infectious complications, with great harm [1]. Therefore, it is of great significance to fully understand the pathogenesis of NTM pulmonary disease for the early clinical screening and treatment of NTM pulmonary disease in the future. However,
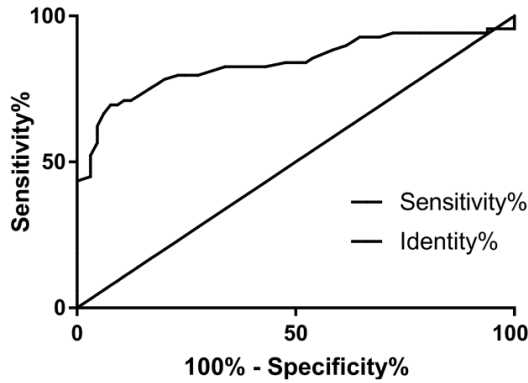
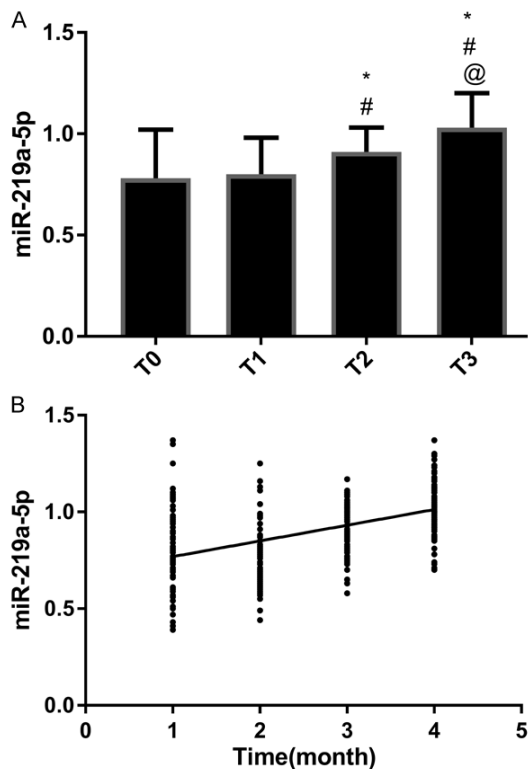**Figure 2.** The ROC curve of miR-219a-5p for the diagnosis of NTM pulmonary disease.

Figure 3. The changes in the miR-219a-5p expression levels during the treatment in the RG. A. The changes of the miR-219a-5p expression level during the treatment in the RG. * stands for comparison with T0, P < 0.050; # stands for comparison with T1, P < 0.050; @ stands for comparison with T2, P < 0.050. B. The correlation between the miR-219a-5p expression level and treatment time in the RG during treatment.

it is of great significance to the future clinical diagnosis and treatment of NTM pulmonary diseases to explore the clinical significance of miR-219a-5p in NTM pulmonary diseases in this study.
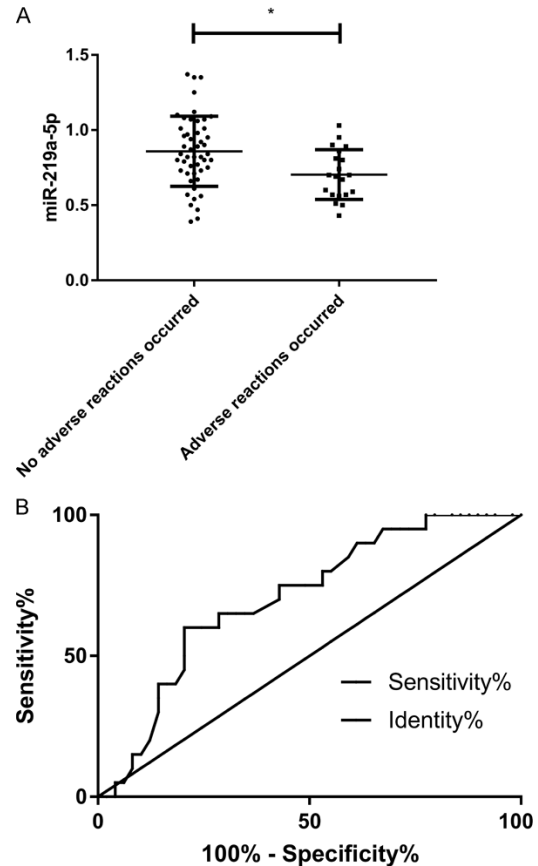

Figure 4. The predictive value of miR-219a-5p for adverse reactions. A. Difference in the miR-219a-5p expression levels in patients with and without adverse reactions, *P < 0.050. B. The ROC curve of miR-219a-5p for predicting adverse reactions.

The results of this experiment showed that miR-219a-5p is low expressed in the peripheral blood of NTM pulmonary disease patients, suggesting that miR-219a-5p may be involved in the development and progression of NTM pulmonary disease. This is also consistent with the study of Xiao et al. [16] on the expression of miR-219a-5p in ischemia-reperfusion injury, which supports the results of this experiment. MiR-219a-5p is a member of the miRNA family that is highly correlated with tumors. Previous studies have confirmed that miR-219a-5p inhibits the migration and epithelial-mesenchymal transition of breast cancer cells through myocardial related transcription factors [17] and inhibits the migration and invasion of osteosarcoma cells by targeting EYA2 [18]. However, the influence mechanism of NTM pulmonary disease is still unclear. NTM is a type of parasitic fungi, but it can only be parasitic in living cells. It is usually transmitted through
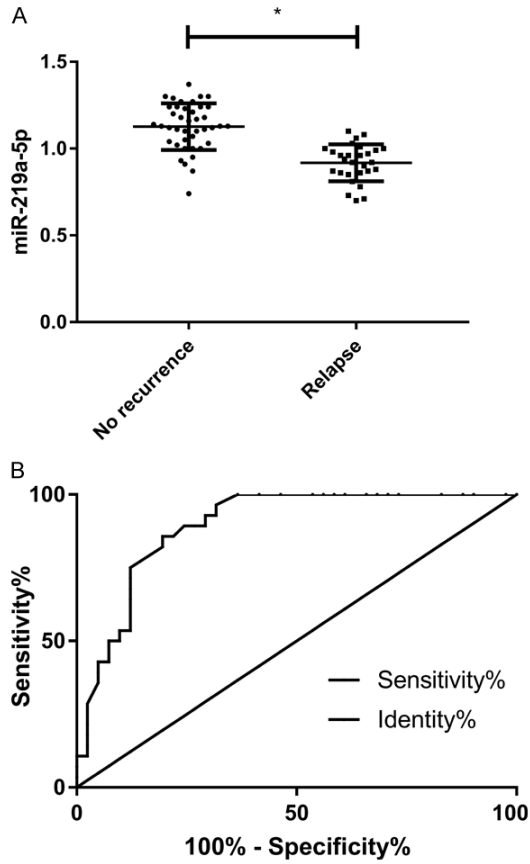
Figure 5. The predictive value of miR-219a-5p for the recurrence of respiratory diseases. A. Comparison of the miR-219a-5p expression levels in the patients with recurrent respiratory diseases and the patients without recurrent respiratory diseases, *P < 0.050; B. The ROC curve of miR-219a-5p for predicting the recurrence of respiratory diseases.

the respiratory tract by dust, droplets, and other substances, and then spread to alveolar sites [19]. NTM has no significant effect on the patient's body at the initial stage of parasitism. However, when the body's immune ability decreases, NTM develops corresponding pathological changes, causing a series of diseases [20]. Therefore, we suspected that the mechanism of miR-219a-5p in NTM pulmonary disease may be related to changes in the patients' immune function. We speculated that miR-219a-5p has a certain inhibitory effect on NTM. The expression of miR-219a-5p was inhibited when the immune function of the patients changes, thus increasing the activity of NTM and triggering the development of diseases. However, since this experiment was not conducted in vitro, it is still impossible to confirm whether our conjecture is correct. This will be

pursued in-depth as our research focus in the future. However, the ROC curve analysis showed that miR-219a-5p has a good diagnostic value for NTM pulmonary diseases, suggesting that the detection of miR-219a-5p may become an early screening indicator for NTM pulmonary diseases in the future. At present, the gold standard is the bacterial culture method for the diagnosis of NTM pulmonary disease in the clinic. The strain identification is completed through biochemical identification or sequencing after the strain is obtained by culturing a patient's sputum [21]. However, this method requires a very high level of laboratory biosafety and is difficult to popularize in some areas where medical technology is relatively backward. Moreover, the culture and detection cycle needs 6-8 weeks, which is not conducive to the early treatment of NTM pulmonary disease patients. At this time, miR-219a-5p has extremely significant advantages as a diagnostic marker for NTM pulmonary diseases. Not only is the test convenient, it can be completed just by taking a patient's peripheral blood sample, and the test results are also objective and accurate, reducing the possibility of detection errors caused by human factors. Moreover, the storage period of the blood sample is longer, which is also more beneficial for doctors to recheck the sample at any time. In addition, in subsequent experiments, it has a better predictive value for the adverse reactions and the prognosis of recurrent respiratory diseases in the treatment processes of patients by analyzing their expression levels of miR-219a-5p in different time periods, which further verifies the future clinical application value of miR-219a-5p. In addition, we found that patients' miR-219a-5p levels gradually increased with the treatment time during the treatment process. It also verified that miR-219a-5p has a close relationship with the development of the patients' conditions. We suspected that miR-219a-5p may also become a potential treatment target for NTM pulmonary diseases in the future. However, due to the constraints of this experiment, we need to conduct further experiments to verify this.

This experiment explored the clinical application value of miR-219a-5p in NTM pulmonary diseases. Due to the insufficient experimental conditions, there are still many deficiencies. For example, the mechanism of miR-219a-5p on

NTM pulmonary disease still needs to be verified using basic experiments. However, the included patient data were limited, so it was not possible to comprehensively analyze whether miR-219a-5p has expression differences in different NTMs. In addition, due to the short time period of this experiment, the effect of miR-219a-5p on the long-term prognosis of patients with NTM pulmonary disease cannot be determined. We will conduct a more comprehensive and in-depth analysis and discussion on the above deficiencies as soon as possible to obtain more representative experimental results for clinical reference.

To sum up, miR-219a-5p has a low expression in NTM pulmonary disease, and it has a good predictive value for the development of NTM pulmonary disease, adverse reactions in the treatment process, and the prognosis of respiratory disease recurrence, and it may be a potential diagnostic and treatment target for NTM pulmonary disease in the future.

Disclosure of conflict of interest

None.

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