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ORIGINAL RESEARCH

Serum miR-130a-3p and miR-326: Correlation with Airway Inflammation and Prognostic Implications in Pediatric Bronchial Asthma

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Objective: To investigate the relationship between serum microRNA (miR)-130a-3p, miR-326 and airway inflammation in children suffering from bronchial asthma.

Methods: A retrospective study was conducted on 122 children suffering from bronchial asthma. According to the disease progression, 122 cases were divided into a remission group of 73 cases and an attack group of 49 cases. Total 50 healthy children received physical examinations during the same period were taken as the control group. These cases were graded as a well-controlled group of 91 cases and a poorly controlled group of 31 cases following 8 weeks of symptomatic treatment. The interaction was analyzed using a multiplication model (by calculating the odds ratio and other indicators of the occurrence of outcomes under different combinations of factor exposures). ROC curve was used to analyze the predictive value. Multivariate Logistic regression was used to analyze the influencing factors.

Results: Serum miR-130a-3p and miR-326 levels were negatively correlated with serum IL-4, TGF-β1 and CKLF-1 levels (all $P < 0.001$), but positively correlated with serum IL-10 levels ($r = 0.673$ and 0.768; both $P < 0.001$). The poorly controlled group had much lower levels of serum miR-130a-3p and miR-326 than the well-controlled group ($t = 13.637$ and 8.482; both $P < 0.05$). The sensitivity and specificity of the combination of the two factors in predicting poor prognosis were 81.25% and 85.00%, which had certain clinical value. The elevation of serum levels of IL-4, TGF-β1 and CKLF-1 increased the risk of asthma and poor prognosis, but IL-10, miR-130a-3p and miR-326 were protective factors ($P < 0.05$).

Conclusion: The decreased levels of serum miR-130a-3p and miR-326 were related to airway inflammation in children with bronchial asthma. The interaction between the two miRNAs may increase the risk of poor prognosis in children. Detection of the two miRNAs can provide important reference for clinicians to judge the prognosis of children.

Keywords: airway inflammation, bronchial asthma, miR-130a-3p, miR-326, prognosis

Introduction

Bronchial asthma is the most common chronic disease in children. As a chronic inflammatory state of the respiratory tract, bronchial asthma is characterized by airway hyperresponsiveness, changes in airway wall structure, and restricted airflow.[1] Unfortunately, the incidence rate of children suffering from bronchial asthma has increased significantly. According to statistics, almost half of asthma patients have persistent asthma before the age of 6, and about 15% of patients may suffer from severe asthma in childhood. Early diagnosis of bronchial asthma is beneficial for reducing the risk of chronic obstructive pulmonary disease.[2] Previous clinical diagnosis based on lung function testing, bronchial experiments, etc., had poor ability to predict disease development and prognosis due to low specificity.[3] Therefore, it is of great significance to further study the pathogenesis of bronchial asthma and find new biomarkers and therapeutic targets.

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MiRNA is a small molecule nucleotide that can regulate the transcription of target genes. Studies have found that abnormal function or expression of miRNA targets is associated with lung diseases. These abnormalities can participate in the pathogenesis of lung diseases by targeting inflammation related genes, affecting the differentiation, proliferation, and apoptosis of inflammatory cells.[4,5] Airway inflammation is a key mechanism in the occurrence and development of bronchial asthma. Previous studies have confirmed that miRNAs may be involved in the progression of airway inflammation, with miR-21 being associated with asthma. MiR-21 plays a positive regulatory role on Th2 cytokines in the early immune mechanism of asthma, and knocking out miR-21 can reduce eosinophil infiltration.[6,7] In addition, miRNAs can also participate in the pathogenesis of diseases such as asthma by regulating DNA methyltransferase and histone deacetylase.[8] Research has found[9] that miR-130a-3p can affect the levels of inflammatory factors and airway remodeling related factors by targeting autophagy associated protein 7 (ATG7). This indicates that miR-130a-3p exerts certain role in asthma by the regulation of pulmonary macrophage activation and airway remodeling. Recent studies have shown[10] that autophagy is associated with inflammation in asthma, which may develop into a new approach for treating asthma. MiR-326 is a conserved miRNA. In vitro studies have confirmed[11] that miR-326 is downregulated in airway smooth muscle cells, which can inhibit inflammation and promote autophagy, making it a new target for asthma treatment. MiRNA mainly participates in physiological and pathological processes of organisms by regulating gene expression. MiRNAs can interact with each other directly or indirectly, such as through synergistic or antagonistic effects. During the interaction process, one miRNA can affect the expression level, functional activity, and regulation of target genes of another miRNA. This interaction plays an important role in the onset, development, and prognosis of diseases.[12]

MiR-130a-3p and miR-326 have unique advantages over existing biomarkers used in current asthma treatment, such as eosinophil count, IgE level, etc. Firstly, as non-coding RNAs, they can regulate the expression of multiple target genes, thereby affecting the pathophysiological process of asthma at a broader level.[13] Secondly, the expression level of miRNA is relatively stable and easy to detect in blood, which provides a more convenient and accurate means for the diagnosis and prognosis of asthma. Moreover, by regulating the expression of these miRNAs, it is possible to develop miRNA-based therapies that could provide new therapeutic options for patients with asthma. However, the specific mechanism of the interaction between miR-130a-3p and miR-326 is still not fully understood. This study speculated that there was a significant interaction between the expression levels of these two miRNAs in children with asthma, and this interaction may increase the risk of poor prognosis in patients.

To further elucidate the hypotheses behind the interactions between these miRNAs, future studies are needed to delve-in their target genes, signaling pathways, and interactions with other biomolecules. This will help us to more fully understand the pathogenesis of asthma and provide a theoretical basis for the development of more effective treatment strategies. In addition, miRNA-based therapies may have unique advantages in addressing specific challenges faced by pediatric populations, such as developmental considerations or variability in treatment response. Since miRNA plays an important role in physiological processes such as cell proliferation, differentiation and apoptosis, it is possible to achieve individualized treatment for pediatric patients by regulating the expression of miRNA, thereby improving the therapeutic effect and reducing side effects.[14]

In this study, the relationship between the content of serum miR-130a-3p and miR-326 in children with bronchial asthma was explored, as well as their interactive effects on the prognosis.

Materials and Methods

General Materials

The determination of sample size in this study was based on the data of previous studies and formula calculation. The sample size needed for statistically significant differences in serum miR-130a-3p and miR-326 levels between children with bronchial asthma and healthy children was estimated. The sample size estimation formula $n = 2[(Z_{\alpha/2} + Z_{\beta})\sigma/\delta]^2$ was used to compare the means of two samples. Among them, $Z_{\alpha/2}$ is quantile of two-sided standard normal distribution (when α is 0.05, $Z_{\alpha/2} = 1.96$); Z_{β} is the quantile of one-sided standard normal distribution (when the degree of confidence $1-\beta$ is 0.8, $Z_{\beta} = 0.84$); σ is the population standard deviation and δ is the difference between the two population means.

According to the previous pilot experiment, the standard deviation of serum miR-130a-3p in healthy children and children with bronchial asthma was estimated to be σ1 and σ2, respectively, and the mean value was taken as the estimated value of the overall standard deviation σ. The difference δ between the two population means was set according to the expected difference. The sample size of each group was calculated by substitution into the formula. Taking into account the possible loss of follow-up, the sample size was appropriately expanded, and 122 children with bronchial asthma and 50 healthy children were finally selected as the study subjects. The selected period was from March 2020 to July 2023. The inclusion process was shown in Figure 1. Children who met the criteria were divided into a remission group of 73 cases (the diagnosis criteria for remission period[15] are disappearance of symptoms and signs with or without treatment, recovery of lung function to pre-acute levels for more than 3 months) and an attack group of 49 cases according to the development of their condition. A total of 43 males and 30 females aged 4–11 years (average age of (6.42 ± 2.16) years) formed the remission group. The disease duration was 8–24 months, with an average duration of (16.94 ± 4.30) months. The attack group was consisted by 28 males and 21 females, aged 3–11 years, with an average age of (6.85 ± 2.62) years. The disease duration was 8–24 months, with an average duration of (17.43 ± 3.61) months. Another 50 healthy children received physical examinations at our hospital during the same period were chosen as the control group (The data of these children were collected from the blood samples collected during the physical examination, and the collection and detection process was consistent with that of the children with asthma to ensure the comparability of the data. These healthy children were selected because they had certain similarities in age and living environment with asthmatic children and could be used as a valid control). The control group included 31 males and 19

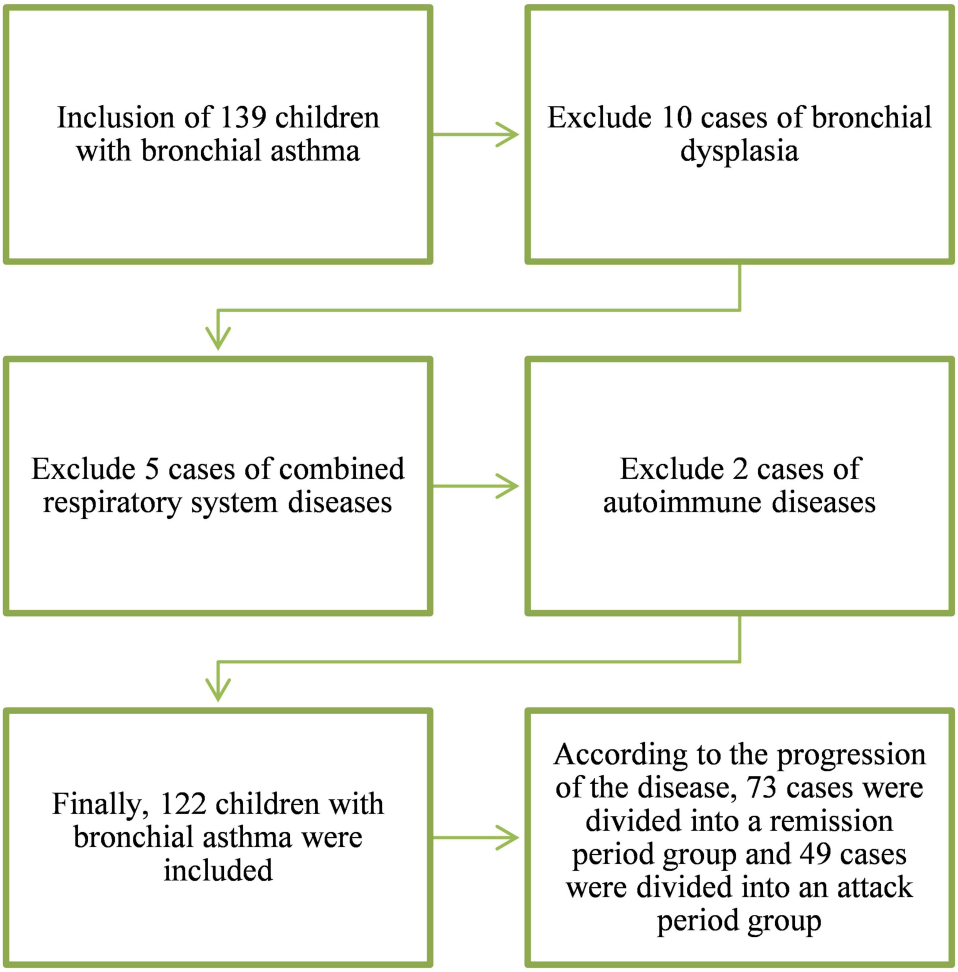

Figure 1 Selection process of general materials.

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females, aged (4–12) years, with an average age of (6.59 ± 2.28) years. There existed no significant difference in the basic data between two groups ($P > 0.05$).

Inclusion and Exclusion Criteria

Inclusion criteria: (1) All patients met the diagnostic criteria for pediatric bronchial asthma;[16] (2) The patients did not receive glucocorticoid treatment within 48 hours before participating in the study; (3) The children were in a quiet environment, with stable vital signs and able to cooperate well; (4) The children without treatment such as immunomodulators and biological agents that may affect the levels of inflammatory factors was received during the study. Exclusion criteria: (1) Patients with bronchial dysplasia; (2) Patients who had undergone cardiac surgery in the past; (3) Patients with combined respiratory system diseases (such as pneumonia and chronic obstructive pulmonary disease); (4) Patients with combined autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis; (5) Patients with severe liver and kidney dysfunction or malignant tumors; (6) Patients had a history of acute infection within 1 month before enrollment.

Detection of Serum miR-130a-3p and miR-326

PCR was used to detect the levels of serum miR-130a-3p and miR-326. 10 mL of elbow vein blood was collected on an empty stomach from each subject and stored in a vacuum anticoagulant collection tube. After centrifugation, the supernatant was collected and mononuclear cells were separated to prepare a cell suspension. Mononuclear cells were obtained from peripheral blood by the way of density gradient centrifugation. miRNAs extracted from mononuclear cells were converted into cDNA through reverse transcription reaction (RT). The synthesized cDNA was transferred to a fluorescence quantitative PCR instrument for qPCR reaction. Each miRNA sample contained a PCR master mix, including 7.2 μL of nuclease free water, 1 μL of 10 × PCR buffer, 0.2 μL of dNTPs, and 0.1 μL of AmpliTaq DNA polymerase. Then, specific miRNA PCR primers and 1 μL of synthesized cDNA were added to the PCR reaction tube in turn. The forward and reverse sequences of the PCR primer for miR-130a-3p were 5'-GGCAGTGCAATGTTAAAAG -3', and 5'- GGCAGTGCAATGTTAAAAG-3'. The forward and reverse primer sequence of miR-326 were 5'-GTGCAGGTCCGAGGT-3', and 5'-GCGCTAGCTTTATCAGA-3'. The fluorescence signal was analyzed using software to determine the expression levels of miR-130a-3p and miR-326.

Detection of Airway Inflammation Indicators

Enzyme linked immunosorbent assay (ELISA) was used to detect the levels of serum interleukin-4 (IL-4), interleukin-10 (IL-10), transforming growth factor-β1 (TGF-β1), and chemokine like factor-1 (CKLF-1). The blood sample was diluted and the plate holes were pre-coated. 50 μL of capture and detection antibody working solution (Cap/Det Ab) was added to the wells and then followed by 50 μL of standard or test sample. The plate was covered with the film and gently shaken for 10 seconds, followed by incubated at 37°C for 60 minutes. Then, the wells were washed twice to remove unbound substances. The wells were added with 100 μL of biotinylated detection antibody working solution, covered with a film, and then were incubate at 37°C for 90 minutes. After washed twice, appropriate amount of substrate solution was added to the well, avoiding light until the color change stopped. Finally, termination solution was added to terminate the reaction. The absorbance values of each well were measured using an ELISA reader at a wavelength of 450 nm.

Prognostic Evaluation Criteria

Symptomatic treatment should be given to the affected children, including short acting bronchodilators (salbutamol) for rapid symptom relief, long-acting bronchodilators (formoterol) for long-term control, and corticosteroids (budesonide) for controlling inflammatory responses.[17] The children were given symptomatic treatment and followed up for 8 weeks. According to the degree of disease control in children after 8 weeks of treatment, they were divided into uncontrolled (no significant improvement in clinical symptoms, stress medication frequency not less than 2 times a week, asthma test control questionnaire score <20 points), partially controlled (partial relief of clinical symptoms, wheezing after daily activities, relief after rest, asthma test control questionnaire score 20–24 points), and completely controlled (complete relief of clinical symptoms, asthma test control questionnaire score ≥25 points). Then, 31 cases were classified as poorly controlled group with no control, and 91 cases were classified as well controlled group with partial or complete control.

Statistical Analysis

SPSS 24.0 statistical software was employed for data analysis. In this study, independent sample *t*-test was used for measurement data (miR-130a-3p, miR-326, etc. expressed as EQN) comparison. One-way analysis of variance was used for multiple groups. In the study, chi square test was used to compare enumeration data between groups, expressing as [cases (%)]. Pearson correlation analysis was conducted for correlation analysis. A multiplication model was built to analyze the impact of the interaction between serum miR-130a-3p and miR-326 on prognosis. ROC curve was used to analyze the value of miR-130a-3p and miR-326 in predicting poor prognosis in children with asthma. Multivariate Logistic regression was used to analyze the influencing factors of the prognosis of children with bronchial asthma. The statistical results indicated that a difference with $P < 0.05$ was statistically significant.

Results

Changes in Serum miR-130a-3p and miR-326 Levels in Children with Bronchial Asthma

The levels of serum miR-130a-3p and miR-326 among the control group, remission group, and attack group had significant difference ($P < 0.05$). The remission and attack groups had much lower levels of miR-130a-3p and miR-326 than the control group ($P < 0.05$). The attack group had sharply lower levels of serum miR-130a-3p and miR-326 than the remission group ($P < 0.05$, Table 1).

Subgroup Analysis Based on Demographic Factors

In order to further explore the effects of different demographic factors on serum miR-130a-3p and miR-326 levels, subgroup analysis based on age and gender was performed. According to age, the children with bronchial asthma were divided into a young group (3–6 years old) and an older group (7–11 years old). The levels of serum miR-130a-3p and miR-326 in the two groups were compared in the remission and attack stages. The results showed that there were no significant differences in the serum levels of miR-130a-3p and miR-326 between the younger group and the older group during the attack and remission stages ($P > 0.05$). After grouping by gender, there were no significant differences in the serum levels of miR-130a-3p and miR-326 between male and female children in the attack and remission stages ($P > 0.05$). These results indicated that age and gender had no significant effect on serum miR-130a-3p and miR-326 levels in children with bronchial asthma (Table 2).

Analysis of Airway Inflammatory Factors in Children with Bronchial Asthma

There were significant differences in the levels of serum IL-4, IL-10, TGF-β1, and CKLF-1 among the control group, remission group, and attack group ($P < 0.05$). Compared with the control group, the levels of serum IL-4, TGF-β 1, and CKLF-1 were obviously higher in the remission and attack groups, while the level of serum IL-10 was markedly lower ($P < 0.05$). Compared with the remission group, the levels of IL-4, TGF-β1, and CKLF-1 were strongly higher in the attack group, and the serum IL-10 level was much lower ($P < 0.05$, Table 3).

Table 1 Changes in Serum miR-130a-3p and miR-326 Levels in Children with Bronchial Asthma $(\bar{x} \pm s)$

Groups	Cases	miR-130a-3p	miR-326
The control group	50	2.84±0.75	1.23±0.13
The remission group	73	1.96±0.63*	0.89±0.07*
The attack group	49	0.52±0.14*#	0.80±0.08*#
F		201.66	298.42
P		<0.001	<0.001

Notes: *$P<0.05$ compared with the control group; #$P<0.05$ compared with the remission group.

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Table 2 Subgroup Analysis Based on Demographic Factors

Groups	Cases	miR-130a-3p		miR-326	
		Remission Stages	Attack Stages	Remission Stages	Attack Stages
Young group	58	1.92±0.60	0.48±0.12	0.87±0.06	0.78±0.07
Older group	64	2.00±0.65	0.56±0.16	0.89±0.08	0.82±0.09
t		0.704	1.937	1.549	1.360
P		0.483	0.055	0.124	0.176
Male	71	1.90±0.57	0.50±0.18	0.89±0.08	0.76±0.09
Female	51	2.01±0.73	0.51±0.19	0.90±0.10	0.78±0.17
t		0.921	0.298	0.606	0.800
P		0.359	0.767	0.546	0.425

Table 3 Analysis of Airway Inflammatory Factors in Children with Bronchial Asthma($\bar{x} \pm s$)

Groups	Cases	IL-4 (pg/mL)	IL-10 (pg/mL)	TGF-β1 (ng/L)	CKLF-1 (pg/mL)
The control group	50	24.46±4.39	11.96±2.85	102.52±20.17	4.97±0.83
The remission group	73	64.36±10.48*	5.72±1.52*	162.33±25.26*	6.78±1.42*
The attack group	49	75.63±15.52*#	3.50±1.26*#	220.53±29.59*#	8.80±1.79*#
F		305.160	257.010	269.96	92.27
P		<0.001	<0.001	<0.001	<0.001

Note: *P<0.05 compared with the control group; #P<0.05 compared with the remission group.

The Relationship Between Serum miR-130a-3p, miR-326 Levels and Airway Inflammation

Pearson correlation analysis confirmed that serum miR-130a-3p level was negatively correlated with serum IL-4, IL-10, TGF-β 1, and CKLF-1 levels ($P < 0.05$, Table 4 and Figure 2A, C and D), while was positively correlated with the serum IL-10 level ($P < 0.05$, Table 4 and Figure 2B). The serum miR-326 level was negatively correlated with serum IL-4, TGF-β 1, and CKLF-1 levels ($P < 0.05$, Table 4 and Figure 2E, G and H), while it was positively correlated with the serum IL-10 level ($P < 0.05$, Table 4 and Figure 2F).

Changes in Serum miR-130a-3p and miR-326 Levels in Patients with Different Prognoses

Compared with the poorly controlled group, the levels of miR-130a-3p and miR-326 in the serum of patients in the well-controlled group were significantly increased ($P < 0.05$, Table 5).

Table 4 The Relationship Between Serum miR-130a-3p, miR-326 Levels and Airway Inflammation

Indicators	miR-130a-3p		miR-326	
	r	*P*	*r*	*P*
IL-4	−0.643	<0.001	−0.781	<0.001
IL-10	0.673	<0.001	0.768	<0.001
TGF-β1	−0.723	<0.001	−0.723	<0.001
CKLF-1	−0.575	<0.001	−0.583	<0.001

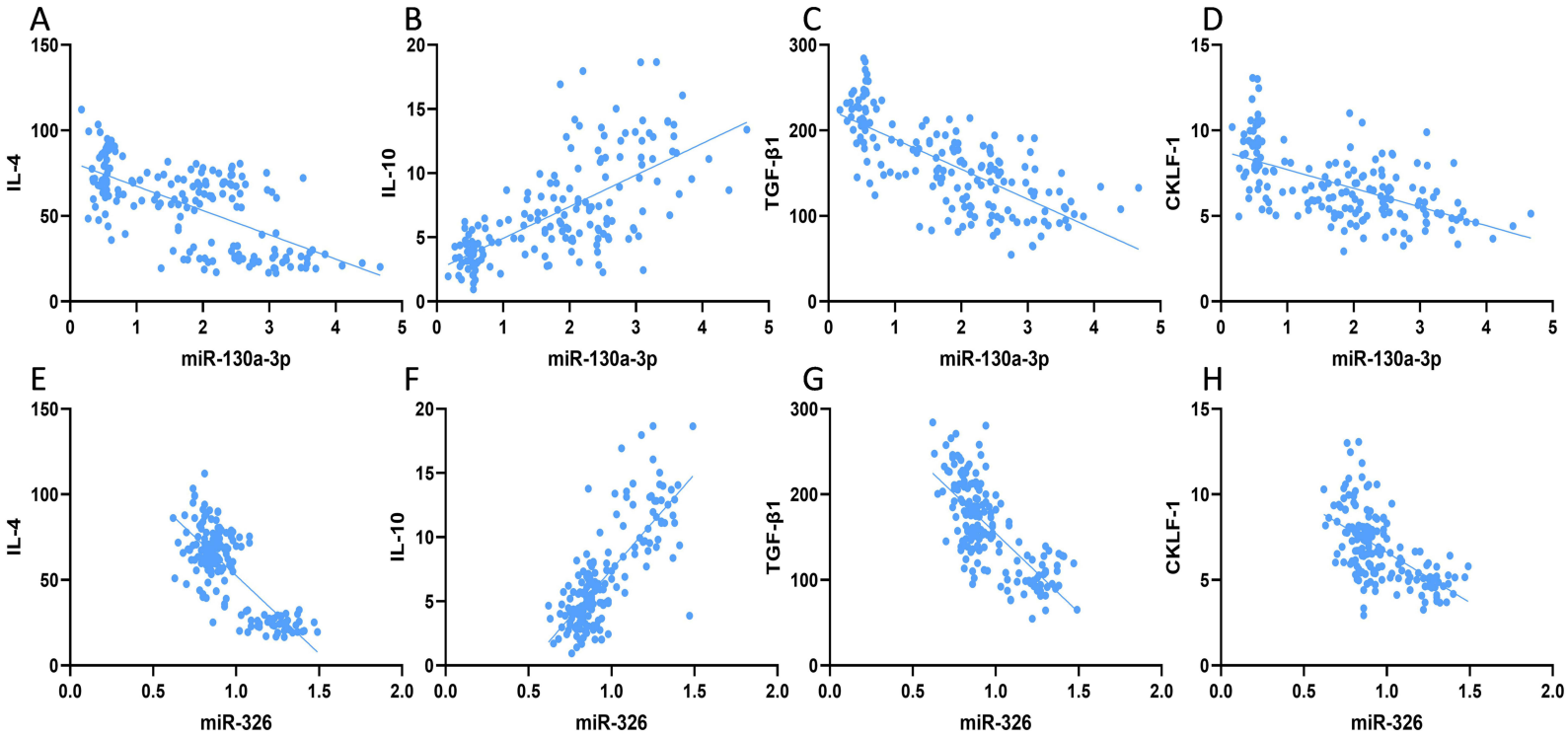

Figure 2 Correlation between serum miR-130a-3p, miR-326 levels and airway inflammation indicators. (**A**): Correlation between serum miR-130a-3p and IL-4; (**B**): Correlation between serum miR-130a-3p and IL-10; (**C**): Correlation between serum miR-130a-3p and TGF-β1; (**D**): Correlation between serum miR-130a-3p and CKLF-1; (**E**): Correlation between serum miR-326 and IL-4; (**F**): Correlation between serum miR-326 and IL-10; (**G**): Correlation between serum miR-326 and TGF-β1; (**H**): Correlation between serum miR-326 and CKLF-1.

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Table 5 Changes in Serum miR-130a-3p and miR-326 Levels in Patients with Different Prognoses ($\bar{x} \pm s$)

Groups	Cases	miR-130a-3p	miR-326
The well controlled group	91	2.57±0.84	1.18±0.27
The poorly controlled group	31	0.50±0.12	0.76±0.09
t		13.637	8.482
P		<0.001	<0.001

The Impact of the Interaction Between Serum miR-130a-3p and miR-326 on Prognosis

Serum miR-130a-3p and miR-326 both below the mean were considered as exposure. Taking miR-130a-3p and miR-326 as references, the risk of poor control in patients was 11.195 times higher than that in the control group when miR-130a-3p and miR-326 were both exposed ($P < 0.05$, Table 6).

Analysis of the Value of miR-130a-3p and miR-326 in Predicting Poor Prognosis in Children with Asthma by ROC Curve

ROC curve analysis showed that the optimal cut-off value of serum miR-130a-3p for predicting poor prognosis in children with asthma was 1.89, with a sensitivity of 62.50% and a specificity of 75.00%. The optimal cut-off value of miR-326 was 0.93, the sensitivity was 53.13%, and the specificity was 95.00%. The sensitivity and specificity of the combination of the two factors in predicting poor prognosis of children with asthma were 81.25% and 85.00%, which had certain clinical value (Table 7 and Figure 3).

Analysis of the Influencing Factors of the Prognosis of Children with Bronchial Asthma by Multivariate Logistic Regression

Logistic regression analysis was performed with the prognosis of children with asthma after 8 weeks of treatment as the dependent variable (poorly controlled = 1, well controlled = 0) and serum levels of IL-4, TGF-β1, CKLF-1, IL-10, miR-130a-3p, and miR-326 as independent variables. The results showed that increased serum levels of IL-4, TGF-β1 and CKLF-1 increased the risk of asthma and poor prognosis, while IL-10, miR-130a-3p and miR-326 were protective factors ($P < 0.05$, Table 8).

Table 6 The Impact of the Interaction Between Serum miR-130a-3p and miR-326 on Prognosis

Interaction Term		The well Controlled Group (n=91)	The Poorly Controlled Group (n=31)	*OR* Value	95% CI	*P* value
Low miR-130a-3p	Low miR-326					
-	-	68	14	1.000	—	—
-	+	2	1	1.518	0.945~2.116	0.020
+	+	18	6	11.195	5.156~18.342	<0.001
+	-	3	10	5.504	2.589~9.144	0.007

Table 7 Analysis of the Value of miR-130a-3p and miR-326 in Predicting Poor Prognosis in Children with Asthma by ROC Curve

Indicators	AUC	95% CI	Sensitivity	Specificity	Youden Index	Cutoff Value
MiR-130a-3p	0.733	0.617–0.849	62.50	75.00	0.375	1.89
MiR-326	0.798	0.694–0.903	53.13	95.00	0.481	0.93
Combination of the two	0.881	0.802–0.960	81.25	85.00	0.663	/

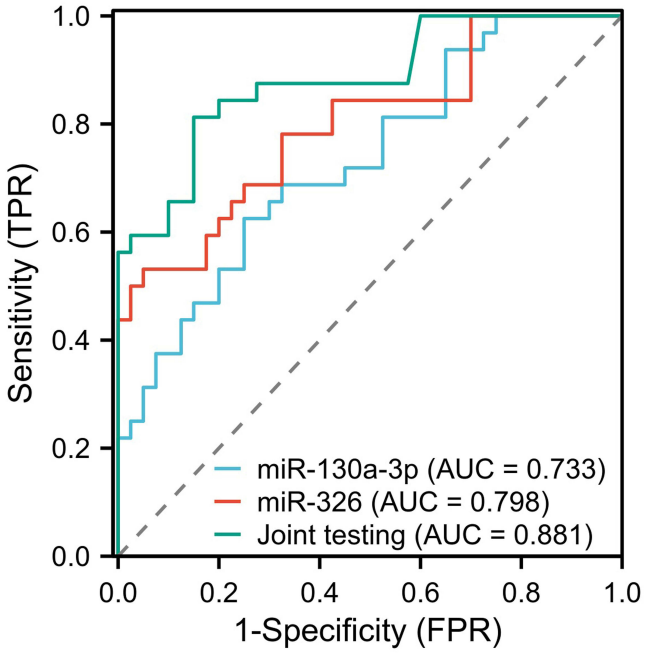

Figure 3 ROC curve analysis of the value of miR-130a-3p and miR-326 in predicting poor prognosis in children with asthma.

Discussion

Asthma is a heterogeneous disease and one of the most common chronic diseases in children, with the symptoms such as wheezing, shortness of breath, chest tightness, and cough. The limitation of expiratory airflow over time and intensity can lead to serious consequences such as chronic bronchitis, pulmonary fibrosis, bronchiectasis, and emphysema due to long-term recurrent attacks and infections.[18] The onset of asthma has a close connection to the airway immune inflammatory mechanism. Continuous chronic inflammation of the airway and damage to the airway epithelium can lead to airway remodeling, including processes such as epithelial shedding, subepithelial fibrosis, and angiogenesis. These changes not only affect the structure of the airway, but also further exacerbate airway hyperresponsiveness and stenosis.[19] In addition, cytokines are an important influencing factor of asthma. They can promote the infiltration of macrophages and monocytes and increase the high reactivity of respiratory mucosa, resulting in bronchial obstruction and exacerbation of asthma symptoms.[20] Although some progress has been made in the treatment of bronchial asthma, there are still some patients with poor disease control. Therefore, it is of great significance to conduct in-depth research on the pathogenesis of bronchial asthma and search for new biomarkers and therapeutic targets.

Table 8 Analysis of the Influencing Factors of the Prognosis of Children with Bronchial Asthma by Multivariate Logistic Regression

Indicators	B value	SE	Wald value	*P* value	OR	95% *CI*
IL-4	1.295	0.352	13.535	<0.001	3.651	1.831~7.279
TGF-β1	1.068	0.330	10.474	<0.001	2.910	1.523~5.557
CKLF-1	1.135	0.263	18.624	<0.001	3.111	1.859~5.207
IL-10	−1.227	0.299	16.811	<0.001	0.293	0.163–0.527
miR-130a-3p	−0.795	0.279	8.126	0.004	0.452	0.262–0.780
miR-326	−0.447	0.112	15.916	<0.001	0.640	0.155~0.429

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MiRNA is a short non-coding RNA with regulatory function. It can regulate mRNA containing complementary sequences after transcription through RNA induced silencing complex and can be used as a potential biomarker for disease. MiRNAs are involved in various physiological and pathological processes, including cell proliferation, differentiation, apoptosis, immune regulation, etc. In disease states, the expression levels of miRNA often exhibit abnormalities, which can serve as potential targets for disease diagnosis, prognosis assessment, and treatment.[21] MiR-130a-3p and miR-326 are both members of miRNAs. A study has found that the expression level of miR-326 in airway smooth muscle cells is significantly reduced, which might play an important role as a target in the development of asthma.[22] Inhibition of miR-326 expression may activate the nuclear transcription factor κB (NF-κB) signaling pathway, thereby promoting the development of pneumonia, suggesting a possible relationship between miR-326 levels and inflammatory response.[23] A study has found that the levels of miR-130a-3p in the bronchial epithelium of patients with bronchial lung cancer are significantly reduced,[24] which is similar to the conclusion of our present study. Our present study analyzed the changes in serum miR-130a-3p and miR-326 levels in different disease conditions. As a result, it was found that with the progression of the disease, the levels of serum miR-130a-3p and miR-326 significantly decreased, suggesting that they might be involved in the development of bronchial asthma. Analyzing the relevant reasons, it might be that miR-130a-3p could target immune related genes, such as certain cytokine receptors or immune cell surface markers, to affect the activation, proliferation, and differentiation of immune cells, thereby regulating the immune imbalance of endothelial cells.[25] MiR-326 can regulate the differentiation and function of helper T cell 1 (Th1) and helper T cell 2 (Th2), affect immune response, and thus participate in the pathogenesis of bronchial asthma.[21]

Airway inflammation is an important characteristic of asthma, and inflammation is the immune system's response to injury. Under normal circumstances, inflammation has certain benefits for the host. When asthma occurs, abnormal immune responses caused by non-pathogenic stimuli can lead to chronic inflammatory reactions related to the pathogenesis of the disease, and abnormal immune activation may cause long-term occurrence of asthma.[26,27] The results of this study found that compared with the control children, the levels of serum IL-4, TGF-β1, and CKLF-1 were much higher in the acute attack period, while the level of IL-10 was sharply lower. The above results all indicated that children with bronchial asthma had severe airway inflammation reactions and detecting the levels of serum airway inflammation indicators might help to assess the condition of children with asthma. In addition, the results of this study found a significant correlation between serum miR-130a-3p and miR-326 levels and airway inflammatory factors. The reason for this may be that ATG7 is a key protein in autophagy, which is a process of intracellular degradation and reuse of damaged or excess organelles. In children with bronchial asthma, miR-130a-3p may interfere with normal cellular function, including activation and clearance of inflammatory cells, by downregulating the expression of ATG7 and affecting autophagy. Changes in autophagy process may exacerbating the inflammatory response in asthma by affecting the balance between the release of inflammatory factors and cell apoptosis.[28] In addition, miR-130a-3p may directly act on genes related to inflammatory mediators, promoting the release of inflammatory mediators, which may be related to exacerbating airway inflammation.[29] Research has found[30] that miR-130a-3p may regulate key molecules in inflammation related pathways and inhibit the release of inflammatory mediators. Relevant data shows[31,32] that miR-326 can participate in regulating the differentiation of helper T cells (Th), promoting the polarization of Th2 cells, leading to increased secretion of Th2 cytokines, and exacerbating airway inflammation. In addition, miR-326 can affect the apoptosis and repair process of airway epithelial cells and disrupt the barrier function of airway epithelium, thus making the airway more susceptible to inflammatory stimuli. The results of multivariate Logistic regression analysis showed that increased serum levels of IL-4, TGF-β1 and CKLF-1 increased the risk of asthma onset and poor prognosis, while IL-10, miR-130a-3p and miR-326 were protective factors ($P < 0.05$). The results further support the conclusion of this study that serum miR-130a-3p and miR-326 levels are correlated with airway inflammatory factors, and both of them have an effect on the prognosis of children with asthma. Combined with the results of previous studies, we can speculate that miR-130a-3p and miR-326 may affect the development and prognosis of asthma by regulating the expression of these inflammatory factors. Specifically, serum miR-130a-3p and miR-326 levels were negatively correlated with IL-4, TGF-β1 and CKLF-1 levels, and positively correlated with IL-10 levels. This means that miR-130a-3p and miR-326 may inhibit the expression of IL-4, TGF-β1 and CKLF-1 by inhibiting the expression of IL-4, TGF-β1 and CKLF-1 and promoting the expression of IL-10, which has an impact on the development and prognosis of asthma.

The occurrence and development of diseases are usually caused by the interaction of multiple factors. The interaction analysis effect can be used to determine the degree to which changes in a factor at different levels depend on other factors, thereby further assessing the impact of different factors on patient prognosis.[33] Previous studies have found that serum miR-130a-3p and miR-326 levels may be associated with the prognosis of cancer patients.[34,35] The results of this study found that the risk of poor control in patients exposed to miR-130a-3p and miR-326 was higher than the sum or product of the risk of poor control when miR-130a-3p and miR-326 were exposed alone. The above results indicated that combined exposure enhanced the risk of poor disease control. Perhaps due to poor disease control, patients with bronchial asthma had a higher airway inflammatory response, which could increase vascular permeability, cause respiratory mucosal edema, airway remodeling and irreversible airflow limitation, thereby further exacerbating the progression of the disease. Further ROC curve analysis showed that miR-130a-3p and miR-326 both had certain predictive value for the prognosis of children with bronchial asthma, and the critical values were 1.89 and 0.93, respectively. The previous study has shown that miRNAs regulate gene expression by complementary pairing and binding to target miRNAs and then affect cell function and disease progression.[36] In asthma, when the levels of miR-130a-3p and miR-326 are lower than the above critical value, it may lead to imbalance in the expression of related target genes, increase the activation of inflammatory cells, aggravates airway inflammation, and then affects the prognosis of children with asthma. Although eosinophil count and IgE level can reflect the inflammatory state of asthma to a certain extent, they are affected by many factors, such as infection, allergy, etc., and their stability is relatively poor.[37] As non-coding RNA, miR-130a-3p and miR-326 can regulate the expression of multiple target genes and affect the pathophysiological process of asthma at a wider level. It has shown that in some asthma patients, abnormal changes in serum miRNA levels can still occur when eosinophil count and IgE levels are normal, suggesting the potential risk of the disease.[38] In addition, the expression level of miRNA is relatively stable and easier to detect in blood, which provides a more accurate basis for the early diagnosis and prognosis of asthma.

The results of this study have potential applications in clinical risk assessment and workflow. In terms of risk assessment, serum miR-130a-3p and miR-326 levels can be included in the existing asthma risk assessment tools, and a more accurate risk assessment model can be constructed to help doctors more accurately judge the risk of asthma attack and prognosis in children combined with traditional indicators such as symptoms and lung function. In terms of personalized treatment, gene therapy and other means can be explored to improve the expression level of miR-130a-3p in children with low levels. For example, gene vectors targeting miR-130a-3p were designed and introduced into children to promote the expression of miR-130a-3p, thereby regulating related target genes and improving airway inflammation and immune imbalance. For children with low levels of miR-326, small molecule drugs that can mimic the function of miR-326 can be developed to alleviate asthma symptoms by regulating Th1/Th2 cell balance and inhibiting inflammatory response. At the same time, combined with other clinical characteristics of the child, such as age, allergy history, pulmonary function status, etc., more targeted treatment plans should be formulated.

In summary, the levels of serum miR-130a-3p and miR-326 in children with bronchial asthma are significantly decreased, which are closely related to airway inflammation. The interaction between the two miRNAs may increase the risk of poor prognosis in children. Detection of the levels of the two miRNAs can provide important reference for clinicians to judge the condition and prognosis of children. However, this study has certain limitations. Firstly, although some confounders (eg, coexisting conditions and recent infections) were controlled for by strict inclusion and exclusion criteria, the retrospective study design may have resulted in other potential confounders (eg, environmental exposures, family history of allergies) not being fully captured or adjusted. Secondly, the small sample size may limit statistical power, and the fact that our findings have not been validated in independent cohorts is one of the limitations of our study. Due to the nature of the retrospective study and the limitation of sample size, the results of the study may be subject to some bias. In the future, prospective studies including larger independent cohorts are needed to further verify the relationship between serum miR-130a-3p, miR-326 levels and airway inflammation and prognosis to ensure the reliability and universality of the research results. Longitudinal study can conduct long-term follow-up of children with asthma and detect serum miR-130a-3p and miR-326 levels at different disease stages and treatment time points, so as to observe their changes more accurately.

The future research directions: Future research can be explored in the direction of miRNA targeted therapy. Based on the relationship between miR-130a-3p and miR-326 and the condition and prognosis of asthma in this study, targeted drugs against these two miRNAs can be developed. For example, designing molecules that can up-regulate the

expression levels of miR-130a-3p and miR-326 can improve airway inflammation and prognosis in patients with asthma by regulating the related target genes. Longitudinal studies can also be carried out to conduct long-term follow-up of children with asthma. Also, the levels of serum miR-130a-3p and miR-326 can be detected at different disease stages and treatment time points. Thus, we can observe their changes more accurately, clarify the dynamic relationship between the changes of miRNA levels and disease progression and treatment effect and provide stronger support for clinical treatment. It is necessary to further study the interaction between miR-130a-3p and miR-326 with other biomarkers to construct a more complete pathogenesis network of asthma and lay the foundation for precision treatment of asthma.

Data Sharing Statement

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

Ethics Approval and Consent to Participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 helsinki declaration and its later amendments or comparable ethical standards.

This study was approved by The Ethics Committee of Suizhou Central Hospital. Written informed consent was obtained from the study participants parents/guardians prior to study commencement for the participation in the study, and all methods were carried out in accordance with relevant guidelines and regulations.

Consent for Publication

The patients from the study participants parents/guardians prior to study commencement participating in the study all agreed to publish the research results.

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Disclosure

The authors declare that they have no competing interests.

References

1. Qian K, Xu H, Chen Z, et al. Advances in pulmonary rehabilitation for children with bronchial asthma. *Zhejiang Da Xue Xue Bao Yi Xue Ban*. 2023;52(4):518–525. doi:10.3724/zdxbyxb-2023-0081
2. Kang Y, Bai M, Deng L, et al. MiRNA-21 regulates bronchial epithelial cell proliferation by activating Tgfβ1/smad signaling pathway and its correlation with asthma severity in children. *Iran J Public Health*. 2021;50(10):1973–1982. doi:10.18502/ijph.v50i10.7497
3. Starczewska-Dymek L, Bozek A, Jakalski M. The usefulness of the forced oscillation technique in the diagnosis of bronchial asthma in children. *Can Respir J*. 2018;2018:7519592. doi:10.1155/2018/7519592
4. Lobera ES, Varela MA, Jimenez RL, et al. miRNA as biomarker in lung cancer. *Mol Biol Rep*. 2023;50(11):9521–9527. doi:10.1007/s11033-023-08695-9
5. Kumazoe M, Ogawa F, Hikida A, et al. Plant miRNA osa-miR172d-5p suppressed lung fibrosis by targeting Tab1. *Sci Rep*. 2023;13(1):2128. doi:10.1038/s41598-023-29188-6
6. Wang Z, Song Y, Jiang J, et al. MicroRNA-182-5p attenuates asthmatic airway inflammation by targeting NOX4. *Front Immunol*. 2022;13(1):853848. doi:10.3389/fimmu.2022.853848
7. Hur J, Rhee CK, Lee SY, et al. MicroRNA-21 inhibition attenuates airway inflammation and remodelling by modulating the transforming growth factor β-Smad7 pathway. *Korean J Intern Med*. 2021;36(3):706–720. doi:10.3904/kjim.2020.132
8. Perry MM, Lavender P, Kuo CS, et al. DNA methylation modules in airway smooth muscle are associated with asthma severity. *Eur Respir J*. 2018;51(4):1701068. doi:10.1183/13993003.01068-2017
9. Shi J, Chen M, Ouyang L, et al. miR-142-5p and miR-130a-3p regulate pulmonary macrophage polarization and asthma airway remodeling. *Immunol Cell Biol*. 2020;98(9):715–725. doi:10.1111/imcb.12369
10. Dong H, Yang W, Li W, et al. New insights into autophagy in inflammatory subtypes of asthma. *Front Immunol*. 2023;14:1156086. doi:10.3389/fimmu.2023.1156086
11. Zhang H, Yan HL, Li XY, Guo YN. TNFSF14, a novel target of miR-326, facilitates airway remodeling in airway smooth muscle cells via inducing extracellular matrix protein deposition and proliferation. *Kaohsiung J Med Sci*. 2020;36(7):508–514. doi:10.1002/kjm2.12197
12. Chen X, Qu J, Yin J. TLHNMDA: triple layer heterogeneous network based inference for MiRNA-disease association prediction. *Front Genet*. 2018;9:234. doi:10.3389/fgene.2018.00234

13. Rad SG, Orang FN, Shadbad MA. MicroRNA networks in prolactinoma tumorigenesis: a scoping review. *Cancer Cell Int*. 2024;24(1):418. doi:10.1186/s12935-024-03529-5
14. Goodarzi V, Nouri S, Nassaj ZS, Bighash M, Abbasian S, Hagh RA. Long non coding RNAs reveal important pathways in childhood asthma: a future perspective. *J Mol Histol*. 2023;54(4):257–269. doi:10.1007/s10735-023-10131-y
15. Horie T. Outline of pharmacotherapy of adult bronchial asthma based on Japanese asthma guideline 2015. *Nihon Rinsho*. 2016;74(10):1658–1663.
16. Comité Nacional de Neumonología; Comité Nacional de Alergia. Comité Nacional de Emergencia y Cuidados Críticos; Comité Nacional de Familia y Salud Mental. Guía de diagnóstico y tratamiento: asma bronquial en niños ≥ 6 años. Actualización 2021 [Guideline on diagnosis and treatment: bronchial asthma in children ≥ 6 years old. Update 2021]. *Arch Argent Pediatr*. 2021;119(4):S123–S158. doi:10.5546/aap.2021.S123
17. Somashekar AR, Ramakrishnan KG. Evaluation of asthma control in children using childhood- asthma control test (C-ACT) and asthma therapy assessment questionnaire (ATAQ). *Indian Pediatr*. 2017;54(9):746–748. doi:10.1007/s13312-017-1167-2
18. Agache I, Eguiluz-Gracia I, Cojanu C, et al. Advances and highlights in asthma in 2021. *Allergy*. 2021;76(11):3390–3407. doi:10.1111/all.15054
19. Li M, Wang ZN, Yang LF, et al. TLR4 antagonist suppresses airway remodeling in asthma by inhibiting the T-helper 2 response. *Exp Ther Med*. 2017;14(4):2911–2916. doi:10.3892/etm.2017.4898
20. Shimoda T, Obase Y, Nagasaka Y, Nakano H, Kishikawa R, Iwanaga T. Lung sound analysis and airway inflammation in bronchial asthma. *J Allergy Clin Immunol Pract*. 2016;4(3):505–511. doi:10.1016/j.jaip.2016.02.001
21. Lin J, Feng X, Zhang J. Circular RNA circHIPK3 modulates the proliferation of airway smooth muscle cells by miR-326/STIM1 axis. *Life Sci*. 2020;255(1):117835. doi:10.1016/j.lfs.2020.117835
22. Zhou G, Duan Y, Lu C, et al. Knockdown of circ-UQCRC2 ameliorated lipopolysaccharide-induced injury in MRC-5 cells by the miR-326/PDCD4/NF-κB pathway. *Int Immunopharmacol*. 2021;97(1):107633. doi:10.1016/j.intimp.2021.107633
23. Liang Y, Miao Y, Xiang J. Circular RNA circESPL1 knockdown alleviates lipopolysaccharide (LPS)-induced lung cell injury via sponging miR-326 to regulate MAPK14. *Int Immunopharmacol*. 2022;112:109146. doi:10.1016/j.intimp.2022.109146
24. Yu XF, Wang J, OUYang N, et al. The role of miR-130a-3p and SPOCK1 in tobacco exposed bronchial epithelial BEAS-2B transformed cells: comparison to A549 and H1299 lung cancer cell lines. *J Toxicol Environ Health A*. 2019;82(15):862–869. doi:10.1080/15287394.2019.1664479
25. Gu M, Liu K, Xiong H, You Q. MiR-130a-3p inhibits endothelial inflammation by regulating the expression of MAPK8 in endothelial cells. *Heliyon*. 2024;10(2):e24541. doi:10.1016/j.heliyon.2024.e24541
26. Duecker RP, Alemdar O, Wimmers A, et al. MicroRNA profiling of the inflammatory response after early and late asthmatic reaction. *Int J mol Sci*. 2024;25(2):1356. doi:10.3390/ijms25021356
27. Rybka-Fraczek A, Dabrowska M, Grabczak EM, et al. Inflammatory phenotypes of cough variant asthma as response predictors to anti-asthmatic therapy. *J Inflamm Res*. 2022;Volume 15:595–602. doi:10.2147/JIR.S343411
28. Snyder-Talkington BN, Dong C, Sargent LM, et al. mRNAs and miRNAs in whole blood associated with lung hyperplasia, fibrosis, and bronchiolo-alveolar adenoma and adenocarcinoma after multi-walled carbon nanotube inhalation exposure in mice. *J Appl Toxicol*. 2016;36(1):161–174.
29. Zhang M, Zhao S, Xu C, et al. Ablation of lncRNA MIAT mitigates high glucose-stimulated inflammation and apoptosis of podocyte via miR-130a-3p/TLR4 signaling axis. *Biochem Biophys Res Commun*. 2020;533(3):429–436. doi:10.1016/j.bbrc.2020.09.034
30. Ding Y, Hou Y, Liu Y, Yu T, Cui Y, Nie H. MiR-130a-3p alleviates inflammatory and fibrotic phases of pulmonary fibrosis through proinflammatory factor TNF-α and profibrogenic receptor TGF-βRII. *Front Pharmacol*. 2022;13:863646. doi:10.3389/fphar.2022.863646
31. Pattnaik B, Negi V, Chaudhuri R, et al. MiR-326-mediated overexpression of NFIB offsets TGF-β induced epithelial to mesenchymal transition and reverses lung fibrosis. *Cell mol Life Sci*. 2023;80(12):357. doi:10.1007/s00018-023-05005-1
32. Zuo Y, Xiong C, Gan X, et al. LncRNA HAGLR silencing inhibits IL-1β-induced chondrocytes inflammatory injury via miR-130a-3p/JAK1 axis. *J Orthop Surg Res*. 2023;18(1):203. doi:10.1186/s13018-023-03661-4
33. Kronek LP, Reddy A. Logical analysis of survival data: prognostic survival models by detecting high-degree interactions in right-censored data. *Bioinformatics*. 2008;24(16):i248–53. doi:10.1093/bioinformatics/btn265
34. Jing J, Zhao X, Wang J, Li T. Potential diagnostic and prognostic value and regulatory relationship of long noncoding RNA CCAT1 and miR-130a-3p in clear cell renal cell carcinoma. *Cancer Cell Int*. 2021;21(1):68.
35. Fan Z, Bai Y, Zhang Q, et al. CircRNA circ_POLA2 promotes lung cancer cell stemness via regulating the miR −326/ GNB1 axis. *Environ Toxicol*. 2020;35(10):1146–1156. doi:10.1002/tox.22980
36. Yoshioka H, Jun G, Suzuki A, Iwata J. Dexamethasone suppresses palatal cell proliferation through miR-130a-3p. *Int J mol Sci*. 2021;22(22):12453. doi:10.3390/ijms222212453
37. Sharma R, Tiwari A, McGeachie MJ. Recent miRNA research in asthma. *Curr Allergy Asthma Rep*. 2022;22(12)):231–258. doi:10.1007/s11882-022-01050-1
38. Bélanger É, Madore AM, Boucher-Lafleur AM, et al. Eosinophil microRNAs play a regulatory role in allergic diseases included in the atopic march. *Int J mol Sci*. 2020;21(23):9011. doi:10.3390/ijms21239011