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A novel microRNA miR-1165-3p as a potential diagnostic biomarker for allergic asthma

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Running title: MiR-1165-3p in allergic asthma

Abstract

Context: A further examination of a novel potential miRNA asthma biomarker, miR-1165-3p, which was previously implicated in helper T cells (Th2) in a murine asthma model.

Objective: To determine whether serum miR-1165-3p can serve as a potential diagnostic biomarker for allergic asthma.

Methods: Serum miR-1165-3p was quantified via quantitative real-time PCR (qRT-PCR) in asthmatic and control samples. Serum miR-1165-3p levels were compared between groups and the clinical diagnostic abilities of miR-1165-3p were evaluated. The analyses utilized included a student's *t* test, one-way ANOVA, and the generation of receiver operating characteristic (ROC) curves.

Results: Serum miRNA-1165-3p levels were significantly elevated in asthmatics when compared to the healthy controls. Furthermore, the sensitivity and specificity of serum miR-1165-3p were found to be 83% and 68.2%. Additionally, serum miR-1165-3p levels were also found to be significantly elevated in patients with allergic rhinitis (AR) or allergic bronchopulmonary aspergillosis (ABPA).

Conclusions: This study showed that serum miR-1165-3p can potentially be utilized as a noninvasive biomarker that is able to aid in the diagnosis and characterization of allergic asthma.

Keywords: Asthma, miR-1165-3p, allergic rhinitis (AR), allergic bronchopulmonary aspergillomas (ABPA), biomarker, and Th2

Introduction

Asthma is a common chronic disease affecting approximately 300 million people worldwide. As a heterogeneous disease, asthma is divided into several phenotypes and different subgroups that have varying drug responses, especially following treatment with inhaled corticosteroids (ICS) [1]. When determining treatment, it is difficult to associate a given clinical “phenotype” with a given biological “endotype” [2], so the identification of specific biomarkers that are able to differentiate between asthma phenotypes would facilitate more individualized treatment. One of the great challenges in asthma biomarker discovery has been identifying a marker that can be obtained noninvasively, while still accurately reflecting a patient’s status and potential drug response.

Asthma is influenced by both genetic factors and environmental triggers. Environmental factors modulate asthma progression mainly through epigenetic mechanisms, such as DNA methylation, histone modification, chromatin alterations, and small non-coding RNA regulation [3]. MicroRNAs (miRNAs) are single-stranded, non-coding small RNA molecules that are approximately 20–25 nucleotides in length. Most miRNAs bind to their targets with only partial

complementary, thus a single miRNA can have thousands of potential targets [4]. In patients with inflammatory conditions, miRNA targets are determined based on complex regulatory networks [5]. MiRNAs are produced by a variety of cells, can be secreted into body fluids, and are very stable and difficult to degrade, thus making them attractive disease biomarkers [6, 7]. Collectively, more than 90 differentially expressed asthma miRNAs have been identified [8–10]. However, few studies have verified the efficacy of these miRNAs as circulating diagnostic biomarkers for asthma.

Recently, our research group validated a novel miRNA dubbed miR-1165-3p (5'-AGCAGGCGCAGGGGGUGGUGUGGU-3') in CD4⁺ T cells in a murine asthma model [11]. Considering the pivotal roles of CD4⁺ T cells in the development of asthma and its high evolutionary conservation among different species, we hypothesized that miR-1165-3p could be a novel noninvasive biomarker for asthma. The aims of this study were to determine if miR-1165-3p is differently expressed in asthma patient sera when compared to healthy control subjects and whether serum miR-1165-3p can be used to diagnose and further characterize asthma.

Materials and methods

Patient selection and clinical data

A total of 53 asthmatic patients (14–80 years) were recruited from the Department of Respiratory and Critical Care Medicine at the First Affiliated Hospital of Nanjing Medical University from September to November 2017. A total of 22 healthy subjects (18–23 years, students at the Nanjing Medical University) without a current or previous asthma or allergic diseases diagnosis were also recruited. To further validate the efficacy of miR-1165-3p, an additional 15 allergic rhinitis (AR) patients (6–60 years) were recruited from the Otorhinolaryngology Department of the First Affiliated Hospital of Nanjing Medical University and 10 allergic bronchopulmonary aspergillosis (ABPA) patients (26–58 years) were recruited from the Department of Respiratory and Critical Care Medicine at the First Affiliated Hospital of Nanjing Medical University from September to November 2017. Anyone with clinical or laboratory signs of infection were excluded. Written informed consent was obtained from all of the subjects and this study was approved by the ethics committee of the First Affiliated Hospital of Nanjing Medical University (2017-SR-298).

Information regarding the sex, age and smoking status were collected from all participants. Information pertaining to the general pathogenic condition (clinical stage and asthma severity), control level, complications (especially with AR), medication history (especially a history of ICS usage), fractional

exhaled nitric oxide (FeNO) levels, and Asthma Control Test (ACT) scores were obtained via questionnaire. Severity, clinical stage, and control level were determined based on GINA 2017 and the Guidelines for the Prevention and Control of Bronchial Asthma of China 2016 (Supplementary Tables 1 and 2).

Blood sample and miRNA extraction

Venous blood samples (approximately 5 ml) were collected into drying tubes and allowed to clot. Samples were then centrifugation at 2,000 rpm for 10 min, the serum was collected into no enzyme EP tubes (KG2211; Kirgen, USA) and the samples were stored at -80°C until further use. Serum miRNA was isolated using a Serum/Plasma miRNA Isolation Kit (RE-0111; Foregene, China) according to the manufacturer's instructions. MiRNA purity and quantification were measured at 260/280 nm using NanoDrop Nucleic Acid Quantification (OD-1000; OneDrop, China).

Quantitative real-time polymerase chain reaction (qRT-PCR)

The obtained miRNA was reverse transcribed into cDNA using a Mir-XTM miRNA First-Strand Synthesis Kit (638313; Takara, Japan) in a PCR Thermocycler (4484075; Thermo Fisher Scientific, USA) for 1 h at 37°C. The reaction was terminated by inactivating the enzymes at 85°C for 5 min. The primers utilized included a miR-1165-3p forward primer: AGCAGGCGCAGGGGGTGGTGTGGT (250 nM; Genscript, China) and a

miR-1165-3p reverse mRQ3' primer (250 nM; 638313, Takara, Japan). Human U6 was used as internal reference, with a U6 forward primer: GTGCTCGCTTCGGCAGCACATATATAC (250nM; Genscript, China) and U6 reverse primer: AAAAATATGGAACGCTTCACGAATTTG (250nM; Genscript, China) utilized. The qRT-PCR reaction was performed on an ABI StepOnePlus™ Real-Time PCR System (4376600; Thermo Fisher Scientific, USA) with SYBR® Advantage (639676; Takara, Japan) for 40 cycles at 95° C for 10 s and 60° C for 30 s.

Four technical replicates were performed for each sample and an average cycle threshold (CT) value was obtained. Melting curves were visually analyzed to exclude samples with multiple peaks. Only samples with at least 3 valid replicates and CT values < 30 or > 10 were retained for further analyses. The relative quantification of miR-1165-3p was calculated using the Δ CT method. Since lower Δ CT values represented a higher miR-1165-3p expression, $-\Delta$ CT values were used to reflect the relative expression level.

Enzyme-linked immunosorbent assay (ELISA)

Total serum IgE concentrations were determined using an IgE Human ELISA kit (BMS2097; Thermo Fisher Scientific, USA) according to the manufacturer's instructions. The optical density (OD) for each sample was measured using an ELISA microplate reader (ELX800; BioTek, USA).

Statistical analysis

For continuous variables, results are presented as a mean $\pm$ SD. Categorical variables are indicated by a specific number for each group. Comparisons between two different groups were performed using a student's *t* test and comparisons between three groups were performed using one-way ANOVA. A Spearman's rank analysis was performed to determine correlations between clinical parameters and miR-1165-3p expression levels. Determining correlations between miR-1165-3p expression and age, sex, or smoking history was achieved using a covariance analysis. Receiver operating characteristic (ROC) curves were generated for miR-1165-3p and IgE and the sensitivity, specificity, cut-off value, 95% CI, and z-score were calculated based on the ROC curves. The IgE and miR-1165-3p ROC curves were compared using the MedCalc Software (V7.2.0.2, Belgium). The combined sensitivity and specificity of serum total IgE and miR-1165-3p were analyzed by serial and parallel test. All of the statistical analyses were performed using SPSS software 20.0 (IBM, USA), unless otherwise indicated, and a *p*-value < 0.05 was considered to be statistically significant.

Results

Overview of subjects

A questionnaire was conducted to collect general information and clinical data for patients with bronchial asthma (n = 53) and the healthy controls (n = 22). Additionally, basic information was collected for the AR patients (n = 15) and ABPA patients (n = 10) and is outlined in Table 1. To determine possible correlations between serum miR-1165-3p levels and clinical characteristics in asthma patients, the asthma patients were divided based on severity into the following groups: intermittent attack, mild persistent, moderate persistent, or severe persistent (Supplementary Table 1). The asthma patients were also subgrouped into the categories of clinical remission period, chronic persistent, or acute attack based on clinical stage. The asthma patients were also divided into groups based on the level of asthma control and included well controlled, partially controlled, and uncontrolled (Supplementary Table 2). The number of cases assigned to each asthma subgroup is shown in Table 2.

Elevated serum miR-1165-3p in asthma patients

To determine whether serum miR-1165-3p could be a potential asthma biomarker, miR-1165-3p expression levels were compared between asthma patients and healthy control subjects. The relative miR-1165-3p levels were significantly higher in asthma patients relative to the healthy controls ($p =$

0.0038; Figure 1A). To preclude any variations in miR-1165-3p expression due to variations in age, sex, smoking history or a history of AR, these factors were examined in conjunction with miR-1165-3p expression. The results showed that no significant differences were present regarding these factors (Table 3) and that serum miR-1165-3p levels were significantly elevated in the asthmatics (Figure 1B).

MiR-1165-3p contributes to asthmatics severity

As a chronic disease, asthma severity can vary between patients. In patients with severe asthma, serum miR-1165-3p levels were significantly increased relative to patients with mild-to-moderate severity ($p = 0.0009$; Figure 2A). Asthma patients can experience frequent exacerbations and remissions. Thus as expected, patients with acute asthma attacks had significantly elevated miR-1165-3p levels when compared to patients with chronic persistent asthma ($p = 0.045$; Figure 2B). However, serum miR-1165-3p levels were found to have no correlation with the control of asthma severity ($p = 0.775$, Figure 2C). ICS is a drug that is commonly used to alleviate asthma severity and attacks. The results showed that in patients medicated with ICS, serum miR-1165-3p levels were significantly lower when compared with patients who do not routinely take ICS ($p = 0.0441$; Figure 2D). AR is a common co-morbidity with asthma and contributes to asthma severity. However, serum miR-1165-3p levels were comparable in the asthmatics with

or without AR ($p = 0.3407$; Figure 2E). Collectively, these findings suggested that elevated miR-1165-3p levels may be correlated with asthma severity.

MiR-1165-3p is not associated with clinical indicators for asthma

To further explore the clinical diagnostic value of serum miR-1165-3p, associations between miR-1165-3p expression levels and clinical indicators were examined. The most important clinical indicators for asthma are FeNO levels, serum total IgE levels, and ACT scores. The results showed that miR-1165-3p expression levels in asthma patients were not significantly correlated with FeNO levels ($p = 0.865$; Figure 3A), serum total IgE levels ($p = 0.401$; Figure 3B), or ACT scores ($p = 0.213$; Figure 3C).

miR-1165-3p as a diagnostic marker for asthma

To examine whether miR-1165-3p can be utilized as a diagnostic biomarker for asthma, 53 asthma patients and 22 control subjects were analyzed. The sensitivity and specificity of serum miR-1165-3p was determined by constructing a ROC curve, with an area under the curve (AUC) value of 0.703 ($p = 0.0051$, $Z = 2.798$; Figure 4A), and a cut-off value of 2.41 was obtained. Additionally, the sensitivity and specificity for serum miR-1165-3p were found to be 83% and 68.2%, respectively, thus indicating the presence of asthma. To assess the diagnostic value of serum miR-1165-3p for characterizing different asthma subgroups, ROC curves were constructed

for the subgroups. For the severe asthma subgroup, an AUC value of 0.76 ($p = 0.007$; $Z = 3.182$) and a cut-off value of 3.54 were obtained (Figure 5A), with a sensitivity and specificity of 91.7% and 56.1%. When examining the ability of miR-1165-3p to determine clinical stage, an AUC value of 0.705 ($p = 0.09$; $Z = 1.693$) was obtained (Figure 5B), thus suggesting that serum miR-1165-3p levels cannot be used to determine clinical stage. When examining the diagnostic value of serum miR-1165-3p in the presence of ICS, an AUC value of 0.652 ($p = 0.0455$, $Z = 2$), a cut off value of 4.28 and a sensitivity and specificity of 87.5% and 44.83% were obtained (Figure 5C). These findings suggest that miR-1165-3p levels can reflect the use of ICS to some extent.

Serum total IgE is a common clinical assistant diagnostic indicator for asthma, especially for eosinophilic asthma. When examining the diagnostic ability of IgE, an AUC value of 0.710 ($p = 0.0024$; $Z = 3.034$) was obtained (Figure 4B). The cut-off value of serum total IgE was 680.99 ng/ml and the sensitivity and specificity were 48.98% and 86.96%. To compare the diagnostic value of miR-1165-3p and serum IgE, ROC curves for each were compared. While the sensitivity of serum miR-1165-3p was higher than that of serum total IgE, the specificity was lower (Figure 4C); thus suggesting that miR-1165-3p may be a potential asthma diagnostic marker. Additionally, when serum miR-1165-3p and total IgE are used jointly, the sensitivity and specificity were 91.3% and 59.3%, as determined by a serial test, and 40.7% and 95.9%, as determined by a parallel test. Consequently, if either serum miR-1165-3p or

serum total IgE were to show a positive outcome, a diagnosis of asthmas could be made, with a sensitivity of 91.3% and a specificity of 59.3%. If both serum miR-1165-3p and serum total IgE are positive, a patient can be deemed asthmatic, with a sensitivity and specificity of 40.7% and 95.9%.

MiR-1165-3p as a Th2-related disease marker

Allergic asthma is a helper T cell (Th2)-dominated disease, with Th2 cells being prevalent in AR and ABPA patients. Therefore, serum miR-1165-3p levels were also examined in AR and ABPA patients. The results showed that miR-1165-3p levels were significantly higher in AR (Figure 6A) and ABPA (Figure 6B) patients relative to the healthy controls, thus suggesting that miR-1165-3p can serve as a marker for Th2-related diseases, including asthma, AR, and ABPA. To further evaluate the diagnostic value of miR-1165-3p for AR and ABPA, ROC curves were generated for AR (Figure 6C) and ABPA (Figure 6D). The diagnostic value of serum miR-1165-3p in diagnosing AR showed an AUC value of 0.642 ($p = 0.166$; $Z = 1.384$). For ABPA, an AUC value of 0.85 ($p = 0.0002$; $Z = 3.726$), cut-off value of 2.41, and sensitivity and specificity of 70% and 100% were obtained.

Discussion

Based on serum miRNA expression in asthma patients, miR-1165-3p was identified as a novel potential noninvasive biomarker for asthma. To our knowledge, miR-1165-3p has not been previously implicated as a potential asthma biomarker. In this study, serum miR-1165-3p levels were found to be increased in patients with asthma when compared with the healthy individuals. Furthermore, the sensitivity and specificity of serum miR-1165-3p as an asthma diagnostic were 83% and 68.2%, respectively. Previous studies have shown that miRNAs can serve as potential biomarkers for different asthma endotypes, such as miR-34/449 being associated with Th2 mediated asthma [12]. Furthermore, miR-223-3p, miR-142-3p, and miR-629-3p have been shown to be closely associated with neutrophil asthma [13]. Herein, serum miR-1165-3p levels were also shown to be significantly elevated in AR and ABPA patients relative to healthy controls. However, serum miR-1165-3p was found to not be efficacious for diagnosing AR. Both AR and ABPA have been shown to be associated with Th2 mediated airway inflammation [14, 15]. ABPA usually occurs secondarily to bronchial asthma, especially in allergic asthma. In an expert consensus issued by the European and American Professional Society, ABPA was regarded as an endotype of asthma. Thus, ABPA is considered an asthma subtype with a unique pathogenesis that is associated with severe asthma [16]. In this study, ABPA patients were also identified as having asthma, which may explain the higher miR-1165-3p specificity when

compared to the asthma patients. Therefore, these findings suggest that miR-1165-3p may in fact serve as a potential Th2 associated asthma biomarker.

In this study, serum miR-1165-3p was shown to be potentially valuable in asthma diagnoses. Consequently, miR-1165-3p was evaluated as an indicator of disease severity, type of inflammation, phenotypic asthma, and treatment responsiveness. Previous studies have reported that asthma medications can affect peripheral blood miRNAs expression in asthmatic patients. For example, Milger *et al.* confirmed that plasma miR-15a-5p/miR-342-3p expression is significantly reduced in patients taking oral steroids when compared to those who were not [17]. Moreover, another study reported that miR-21 and miR-126 expression is closely associated with a decrease in asthmatic symptoms following ICS treatment [18]. Similarly, the present study showed that serum miR-1165-3p levels were lower in asthma patients who had been treated with ICS when compared to those who had not, thus indicating that ICS can influence serum miR-1165-3p levels in asthma patients. Additionally, the sensitivity and specificity of serum miR-1165-3p was compared to serum total IgE, which is a common clinical indicator for asthma [19]. The results showed that serum miR-1165-3p had a lower specificity than serum total IgE, but a higher sensitivity. This finding suggests that serum miR-1165-3p and total IgE may be used in combination to obtain a more efficacious asthma diagnosis.

The findings presented herein suggest that miR-1165-3p may serve as a

potential asthma biomarker or therapeutic target. Additionally, serum miR-1165-3p levels can potentially be utilized as a clinical diagnostic indicator, especially when combined with serum total IgE. Furthermore, serum miR-1165-3p was shown to be of great importance when evaluating the state of illness of an asthma patient. However, this study does have some limitations. First, asthma patients were not divided into different subgroups according to blood eosinophils, sputum eosinophils, or exhaled breath condensate (EBC) [20, 21]. Therefore, serum miR-1165-3p levels were not further analyzed in these different subgroups to investigate the diagnostic value of miR-1165-3p in differentiating between different asthma endotypes or phenotypes. This may also explain why the sensitivity of serum total IgE was lower than in previous reports [22] due to some of the utilized subjects being non-eosinophilic asthmatics. However, AUC values for both the serum miR-1165-3p and total IgE were very similar. Second, the sample sizes utilized herein for both the patients and healthy controls were small, with miR-1165-3p levels found to vary among the healthy controls. To further verify the diagnostic value of serum miR-1165-3p, it may be necessary to further expand the sample size and enroll more asthma patients from different demographics in the future.

The findings in this study suggest that serum miR-1165-3p may be more valuable in Th2-mediated asthma diagnoses. The results herein did not show a correlation between serum total IgE levels, FeNO levels, or miR-1165-3p levels. While both IgE and FeNO have been shown to be influenced by many factors,

such as hyperventilation, smoking, and autoimmune diseases [23–25], their exact mechanisms in asthma are not fully understood. Therefore, we speculate that the lack of correlation between serum total IgE, FeNO, and serum miR-1165-3p may be due to such extraneous factors. Certainly, the relationship and potential interactions between serum miR-1165-3p levels and other biomarkers, such as FeNO and serum total IgE, warrants further investigation.

Conclusion

In summary, this study identified a novel biomarker that is able to distinguish asthma patients from non-asthmatic subjects and reflect clinical characteristics associated with asthma. Herein, miR-1165-3p was shown to be potentially useful in determining asthma pathogenesis, diagnosis, and treatment, especially when combined with serum total IgE. Our future studies will focus on examining the applicability of miR-1165-3p in a larger, more diverse asthma population and analyze whether miR-1165-3p can be useful for the clinical management of asthma.

Clinical significance

1. Serum miR-1165-3p levels were found to be correlated with asthma severity, clinical stage, and medication history.
2. Serum miR-1165-3p levels did not show any correlation with serum total IgE

levels, FeNO levels, or ACT score.

3. Serum miR-1165-3p was shown to be a potential diagnostic biomarker for allergic asthma.

Declaration of conflicts of interest: None.

Acknowledgments

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References

1. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat Med.* 18(5), 716-725 (2012).
2. Corren J. Asthma phenotypes and endotypes: an evolving paradigm for classification. *Discov Med.* 15(83), 243-249 (2013).
3. Ho SM. Environmental epigenetics of asthma: an update. *J Allergy Clin Immunol.* 126(3), 453-465 (2010).
4. Wahid F, Shehzad A, Khan T, Kim YY. MicroRNAs: synthesis, mechanism, function, and recent clinical trials. *Biochim Biophys Acta.* 1803(11), 1231-1243 (2010).
5. Rebane A, Akdis CA. MicroRNAs: Essential players in the regulation of inflammation. *J Allergy Clin Immunol.* 132(1), 15-26 (2013).
6. Chen X, Liang H, Zhang J, Zen K, Zhang CY. Secreted microRNAs: a new form of intercellular communication. *Trends Cell Biol.* 22(3), 125-132 (2012).
7. Mitchell PS, Parkin RK, Kroh EM et al. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci U S A.* 105(30), 10513-10518 (2008).
8. Rebane A, Akdis CA. MicroRNAs in allergy and asthma. *Curr Allergy Asthma Rep.* 14(4), 424 (2014).
9. Perry MM, Adcock IM, Chung KF. Role of microRNAs in allergic asthma: present and future. *Curr Opin Allergy Clin Immunol.* 15(2), 156-162

(2015).

10. Heffler E, Allegra A, Pioggia G, Picardi G, Musolino C, Gangemi S. MicroRNA Profiling in Asthma: Potential Biomarkers and Therapeutic Targets. *Am J Respir Cell Mol Biol*. 57(6), 642-650 (2017).
11. Liu Y, Chen Z, Xu K et al. Next generation sequencing for miRNA profile of spleen CD4⁺ T cells in the murine model of acute asthma. *Epigenomics*. [Epub ahead of Print](2018).
12. Woodruff PG. Subtypes of asthma defined by epithelial cell expression of messenger RNA and microRNA. *Ann Am Thorac Soc*. 10 Suppl, S186-189 (2013).
13. Maes T, Cobos FA, Schleich F et al. Asthma inflammatory phenotypes show differential microRNA expression in sputum. *J Allergy Clin Immunol*. 137(5), 1433-1446 (2016).
14. Sin B, Togias A. Pathophysiology of allergic and nonallergic rhinitis. *Proc Am Thorac Soc*. 8(1), 106-114 (2011).
15. Patterson K, Strek ME. Allergic bronchopulmonary aspergillosis. *Proc Am Thorac Soc*. 7(3), 237-244 (2010).
16. Lötval J, Akdis C A, Bacharier L B, et al. Asthma endotypes: a new approach to classification of disease entities within the asthma syndrome . *J Allergy Clin Immunol*. 127(2), 355-360 (2011).
17. Milger K, Gotschke J, Krause L et al. Identification of a plasma miRNA

- biomarker signature for allergic asthma: A translational approach. *Allergy*. 72(12), 1962-1971 (2017).
18. Wu XB, Wang MY, Zhu HY, Tang SQ, You YD, Xie YQ. Overexpression of microRNA-21 and microRNA-126 in the patients of bronchial asthma. *Int J Clin Exp Med*. 7(5), 1307-1312 (2014).
19. Tu YL, Chang SW, Tsai HJ et al. Total serum IgE in a population-based study of Asian children in Taiwan: reference value and significance in the diagnosis of allergy. *PLoS One*. 8(11), e80996 (2013).
20. Chiappori A, De Ferrari L, Folli C, Mauri P, Riccio AM, Canonica GW. Biomarkers and severe asthma: a critical appraisal. *Clin Mol Allergy*. 13, 20 (2015).
21. Hoffmeyer F, Sucker K, Monsé C et al. Different Patterns in Changes of Exhaled Breath Condensate pH and Exhaled Nitric Oxide After Ozone Exposure. *Adv Exp Med Biol*. 834, 39-47 (2015).
22. Ahmad Al Obaidi AH, Mohamed Al Samarai AG, Yahya Al Samarai AK, Al Janabi JM. The Predictive Value of IgE as Biomarker in Asthma. *J Asthma*. 45(8), 654-663 (2008).
23. American Thoracic Society, European Respiratory Society. ATS/ERS Recommendations for Standardized Procedures for the Online and Offline Measurement of Exhaled Lower Respiratory Nitric Oxide and Nasal Nitric Oxide. *Am J Respir Crit Care Med*. 171, 912-930 (2005).
24. Haccuria A, Michils A, Michiels S, et al. Exhaled nitric oxide: a biomarker

integrating both lung function and airway inflammation changes.[J]. J Allergy Clin Immunol, 134(3):554-559(2014).

25. Parks C G, Biagini R E, Cooper G S, et al. Total serum IgE levels in systemic lupus erythematosus and associations with childhood onset allergies.[J]. Lupus, 19(14):1614-1622(2010).

Figure legends

Figure 1. Expression levels of serum miR-1165-3p. A) MiR-1165-3p expression levels in asthma patients (n = 53) and healthy controls (n = 22). B) Correlation analysis between serum miR-1165-3p levels and asthma ('0' stand for control and '1' stand for asthma).

Figure 2. MiR-1165-3p is shown to contribute to asthma severity. Serum miR-1165-3p levels in A) patients with differing levels of asthma severity, B) patients at different clinical stages, C) patients at different control levels, D) patients taking and not taking ICS, and E) patients with or without AR.

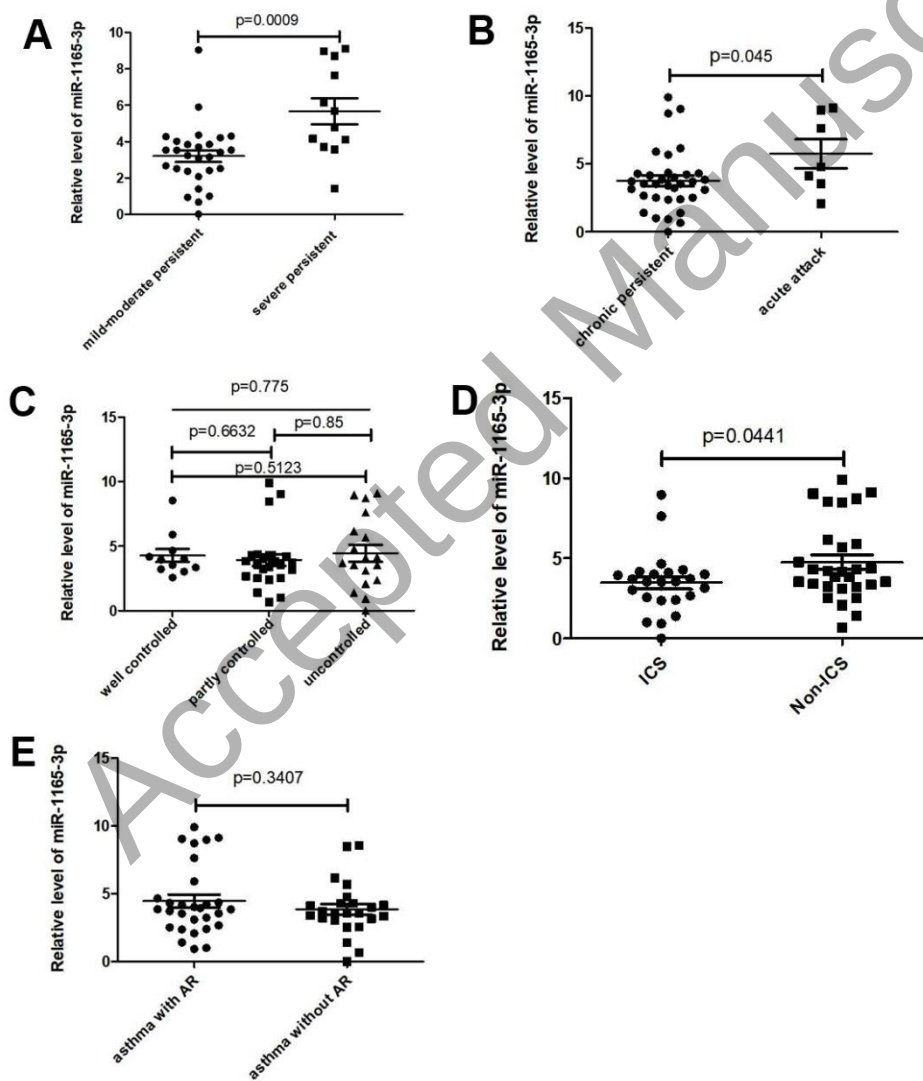
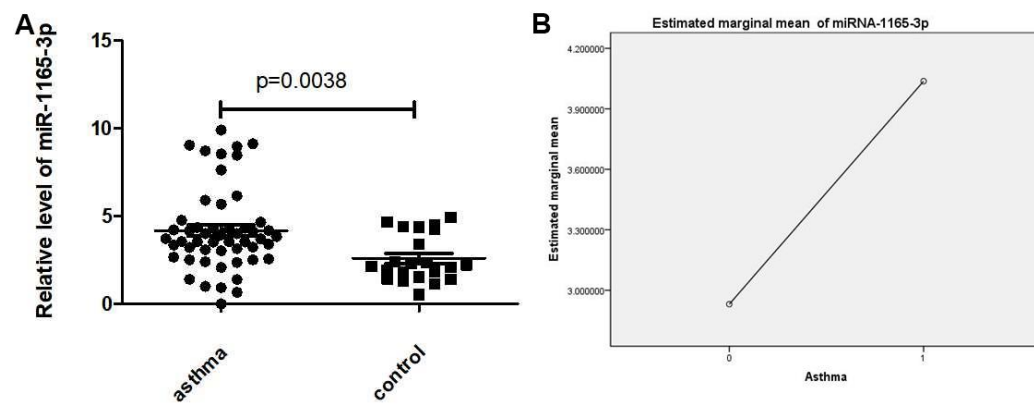
Figure 3. Correlation analysis between serum miR-1165-3p and FeNO level, and serum total IgE level and ACT score. Serum miR-1165-3p levels were not correlated with A) FeNO levels, B) serum total IgE levels, or C) ACT scores.

Figure 4. ROC curve analyses of serum miR-1165-3p and serum total IgE to

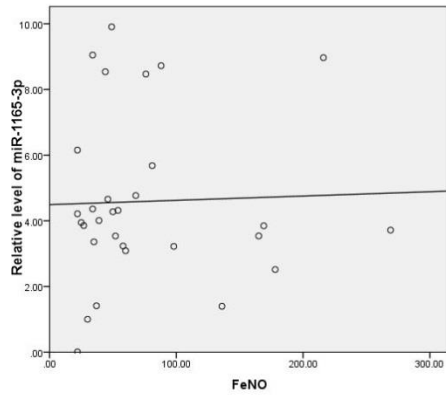
determine efficacy as an asthma diagnostic. ROC curve analyses for A) serum miR-1165-3p, B) serum total IgE, and C) a comparison of the serum miR-1165-3p and serum total IgE curves.

Figure 5. ROC curve analyses of serum miR-1165-3p in different asthma patient subgroup. ROC curve analyses of serum miR-1165-3p to A) diagnose severe asthma, B) determine clinical stage, and C) determine if ICS is administered.

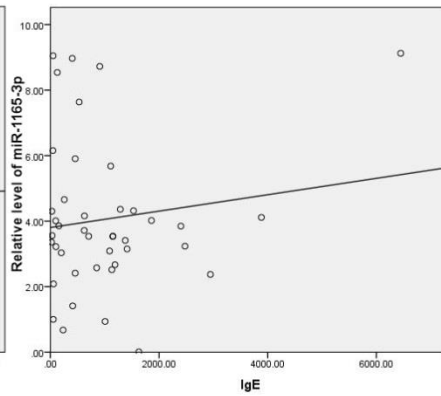
Figure 6. Serum miR-1165-3p levels in allergic rhinitis (AR) and allergic bronchopulmonary aspergillosis (ABPA) patients. Serum miR-1165-3p levels were significantly increased in both A) AR and B) ABPA patients relative to the healthy controls. ROC curve analyses of serum miR-1165-3p in C) AR and D) ABPA patients.



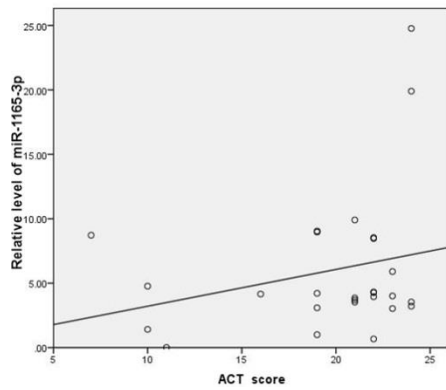
A The correlation between miR-1165-3p and FeNO



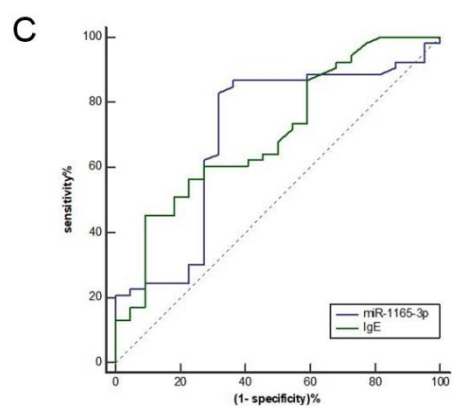
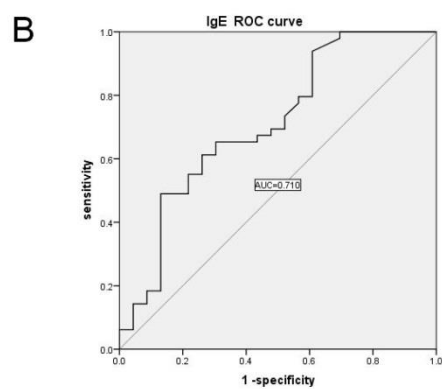
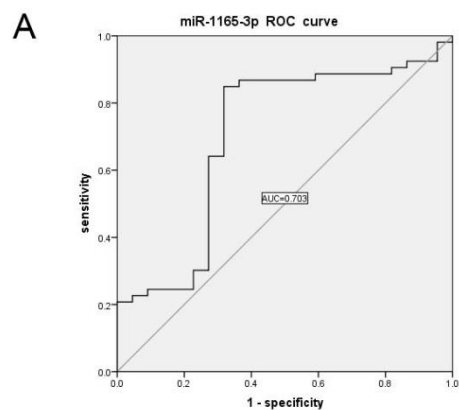
B The correlation between miR-1165-3p and serum IgE



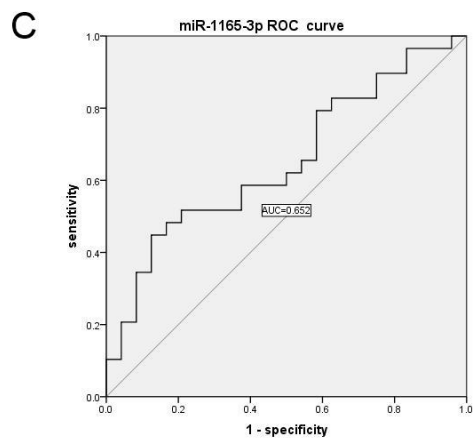
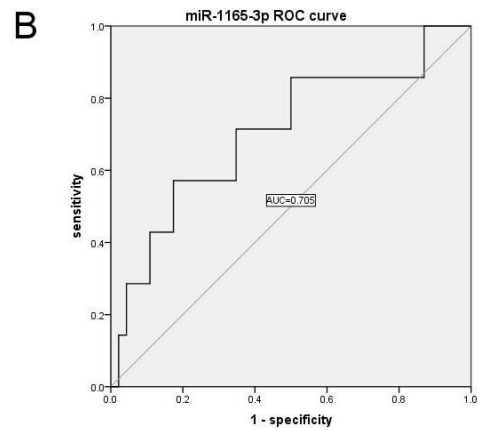
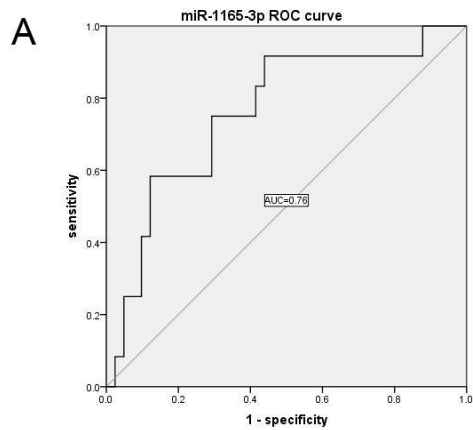
C The correlation between miR-1165-3p and ACT score



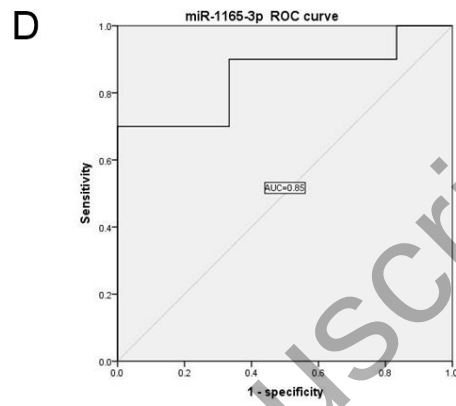
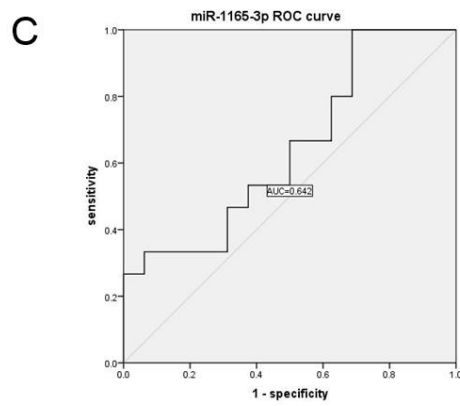
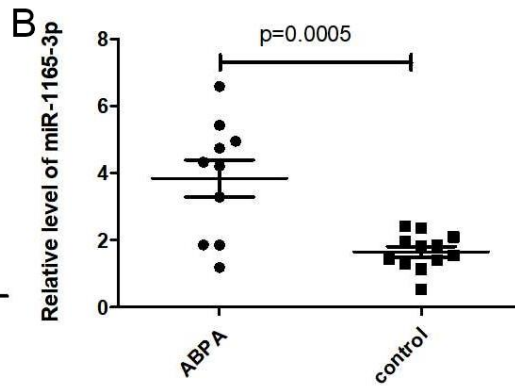
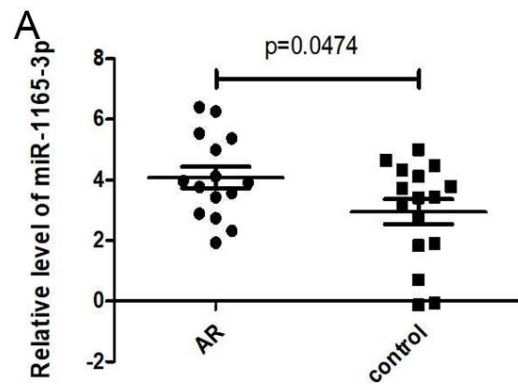
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Table 1 Descriptive statistics of characteristics of different groups

		Total	Asthma	AR	ABPA	Control
Sex	N	100	53	15	10	22
	Male	46	20	11	5	10
	Female	54	33	4	5	12
Age (years)		37.7±19.4	47.7±18.3	21.9±14.2	45.4±10.0	20.7±1.7
Smoking status	Ever	9	6	0	1	2
	Never	91	47	15	9	20
Medication use	ICS	35	24	7	4	0
	Non-ICS	65	29	8	6	22

Table 2 Different asthma subgroups based on severity, clinical stage and control level of asthma

	Asthma subgroup	Cases
Severity of asthma	Intermittent attack	13
	Mild persistent	15
	Moderate persistent	13
	Severe persistent	12
	Clinical remission	12
Clinical stage	Chronic persistent	34
	Acute attack	7
	Well controlled	11
Control level	Partly controlled	24
	Uncontrolled	18

Table 3 Covariance analysis of basic characteristics and the expression level of serum miR-1165-3p

Characteristics	III	quadratic	df	mean square	F	Sig.
	sum					
Correction model	53.202		5	10.64	2.42	0.044
Nodal increment	222.049		1	222.049	50.506	0.000
Sex	0.216		1	0.216	0.049	0.825
Age	1.588		1	1.588	0.361	0.55
Smoking status	7.881		1	7.881	1.793	0.185
With/without AR	4.371		1	4.371	0.994	0.322
Asthma/non-asthma	18.586		1	18.586	4.227	0.044

Sig: p value df: degree of freedom