ORIGINAL ARTICLE

Serum microRNAs Catalog Asthma Patients by Phenotype

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Abstract

Background: Asthma is a chronic inflammatory condition of the airways with a complex pathophysiology. Stratification of asthma subtypes into phenotypes and endotypes should move the field forward, making treatment more effective and personalized. Eosinophils are the key inflammatory cells involved in severe eosinophilic asthma. Given the health threat posed by eosinophilic asthma, there is a need for reliable biomarkers to identify affected patients and treat them properly with novel biologics. microRNAs (miRNAs) are a promising diagnostic tool.
Objective: The aim of this study was to identify serum miRNAs that can phenotype asthma patients.
Methods: Serum miRNAs of patients with eosinophilic asthma (N=40) and patients with noneosinophilic asthma (N=36) were evaluated using next-generation sequencing, specifically miRNAs-seq, and selected miRNAs were validated using RT-qPCR. Pathway enrichment analysis of deregulated miRNAs was performed.
Results: Next-generation sequencing revealed 15 miRNAs that were expressed differentially between eosinophilic and noneosinophilic asthma patients, although no differences were observed in the miRNome between atopic and nonatopic asthma patients. Of the 15 miRNAs expressed differentially between eosinophilic and noneosinophilic asthma patients, hsa-miR-26a-1-3p and hsa-miR-376a-3p were validated by RT-qPCR. Expression levels of these 2 miRNAs were higher in eosinophilic than in noneosinophilic asthma patients. Furthermore, expression values of hsa-miR-26a-1-3p correlated inversely with peripheral blood eosinophil count, and hsa-miR-376a-3p expression values correlated with FeNO values and the number of exacerbations. Additionally, in silico pathway enrichment analysis revealed that these 2 miRNAs regulate signaling pathways associated with the pathogenesis of asthma.
Conclusion: hsa-miR-26a-1-3p and hsa-miR-376a-3p could be used to differentiate between eosinophilic and noneosinophilic asthma.

Key words: Asthma patients. Eosinophilic asthma. microRNA-seq. Phenotypes/endotypes. Serum microRNAs.

Resumen

Antecedentes: El asma es una enfermedad inflamatoria crónica de las vías respiratorias con una fisiopatología compleja. La estratificación de los subtipos de asma en fenotipos y en endotipos debería hacer avanzar el campo, haciendo que el tratamiento sea más eficaz y personalizado. Los eosinófilos son las células inflamatorias clave implicadas en el asma eosinofílica grave. Debido a la amenaza para la salud que representa el asma eosinofílica, existe la necesidad de biomarcadores confiables para identificar a los pacientes y tratarlos adecuadamente con nuevos biológicos. Una herramienta prometedora para el diagnóstico son los microARNs (miARNs).
Objetivo: El objetivo de este estudio fue encontrar miRNAs séricos que puedan fenotipar a los pacientes asmáticos.
Métodos: Los miARNs séricos de individuos asmáticos eosinofílicos (N=40) y no eosinofílicos (N=36) fueron evaluados mediante secuenciación de próxima generación (NGS), específicamente miARN-seq, y los miARNs seleccionados fueron validados por RT-qPCR. Se realizó un análisis de enriquecimiento de rutas de miARNs desregulados.
Resultados: El análisis NGS reveló 15 miARNs expresados diferencialmente entre pacientes asmáticos eosinofílicos y no eosinofílicos, mientras que no mostró diferencias en el miRNoma entre individuos asmáticos atópicos y no atópicos. De los 15 miARNs expresados diferencialmente entre asmáticos eosinofílicos y no eosinofílicos, hsa-miR-26a-1-3p y hsa-miR-376a-3p fueron validados por RT-qPCR. Los niveles de expresión de estos dos miARNs fueron más altos en los asmáticos eosinofílicos que en los no eosinofílicos. Además, los valores de expresión de hsa-miR-26a-1-3p correlacionaron inversamente con el recuento de eosinófilos en sangre periférica y los valores

de expresión de hsa-miR-376a-3p con los valores de FeNO y el número de exacerbaciones. Además, el análisis de enriquecimiento de la vía *in silico* reveló que estos dos miARNs regulan vías de señalización relacionadas con la patogénesis del asma.
Conclusión: Hsa-miR-26a-1-3p y hsa-miR-376a-3p podrían usarse para distinguir pacientes asmáticos eosinofílicos y no eosinofílicos.
Palabras clave: Pacientes asmáticos. Asma eosinofílico. microARN-seq. Fenotipos/endotipos. microARNs séricos.

Introduction

Asthma is a prevalent chronic inflammatory disease of the airways with major public health consequences [1] in which the clinical and pathological characteristics are highly heterogeneous and diverse, thus complicating control. As a result, there is substantial room for improvement in the diagnostic and therapeutic tools used [2]. Asthma encompasses numerous disease variants [3], and phenotyping and endotyping of asthma can facilitate response to treatment, pinpoint the pathogenic mechanisms involved, and anticipate risks. Based on the predominant inflammatory cell, asthma phenotypes are as follows: eosinophilic, neutrophilic, mixed granulocytic, and paucigranulocytic. Taking an endotype-based approach, asthma is divided into type 2 asthma, non–type 2 asthma, and mixed complex endotypes [4]. Eosinophils emerged as the hallmark of eosinophilic asthma, which mainly involves T_H2, but also type 2 innate lymphoid cells [5].

microRNAs (miRNAs) are small, single-stranded, noncoding RNAs that are 21 to 25 nucleotides in length [6] and control gene expression by targeting specific mRNAs for degradation or translational repression. They are involved in multiple biological processes and simultaneously regulate various pathological processes [7]. Serum miRNAs are very stable and resistant to blood RNases. Some are differentially expressed in a range of diseases [8] and function as noninvasive, sensitive, and specific biomarkers [9].

The aim of this study was to determine whether miRNAs could serve as biomarkers to classify asthma patients into distinct phenotypes and endotypes using next-generation sequencing (NGS) and thus facilitate the choice of treatment.

Methods

Selection of Patients

Patients diagnosed with asthma were recruited from the allergy and pulmonology units of Fundación Jiménez Díaz Hospital (FJDH), Complejo Hospitalario de Navarra (CHN), and La Paz Hospital (LPH). Eighteen patients were selected for microRNA-sequencing (miRNA-seq) and distributed into 4 different groups: atopic asthma, nonatopic asthma, eosinophilic asthma, and noneosinophilic asthma. miRNAs were validated using semiquantitative real-time polymerase chain reaction (qPCR) in 67 additional patients, ie, 36 with eosinophilic asthma and 31 with noneosinophilic asthma. Descriptive data for the demographic, inflammatory, functional, and clinical characteristics of the study participants were compiled.

All patients (N=85) took part in the MEGA project, which is based on a cohort of asthma patients of varying grades of severity [10]. The inclusion criteria were as follows: (*1*) agreement to participate, with signed informed consent; (*2*) diagnosis of asthma following the 2019 GINA criteria [11]; (*3*) age between 18 and 75 years. The definitions of atopic and nonatopic disease were established based on a positive or negative prick test and/or specific IgE to at least 1 allergen. Asthma was classified as eosinophilic or noneosinophilic according to the peripheral blood eosinophil count (≥500/μL and <150/μL, respectively).

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the local ethics committees.

Sample Collection

Serum was obtained by blood clotting in anticoagulant-free tubes, centrifuged at 3000 rpm for 10 minutes at 4ºC, and stored at –80ºC until use.

Isolation of miRNA

RNA (including miRNAs) was obtained from 200 μL of serum using the miRNeasy Serum/Plasma Advanced Kit (Qiagen) according to the manufacturer's instructions. Three synthetic miRNA spike-ins (2, 4, and 5) were added to calibrate optimal RNA extraction (miRCURY LNA RNA Spike-in kit, Qiagen). The RNA enriched in miRNAs was eluted with 20 μL of RNase-free water.

miRNA-seq: Library Preparation and Sequencing

miRNA-enriched RNA isolated from the serum samples of 18 asthma patients was used for miRNA-seq.

Small RNA samples were converted to Illumina sequencing libraries using the NEXTFLEX Small RNA-Seq Kit v3 (Bioo Scientific Corporation), strictly adhering to the manufacturer's user guide. The size profile of the individual libraries was quantified using D1000 DNA High Sensitivity Screen Tape on a 4200 TapeStation System (both Agilent). Quantified libraries were sequenced on an Illumina MiniSeq 550 platform (Illumina) using a MiniSeq 500/550 75-cycle High Output Kit.

Bioinformatics Analysis of miRNA

Quality control, preprocessing, and statistical analysis of small RNA-seq data were carried out by the Bioinformatics Unit of IIS-Fundación Jiménez Díaz.

Adaptors were removed, and raw reads were trimmed using Cutadapt [12] by following the NEXTflex small RNA instructions. Adaptor-trimmed reads between 17 and 25 nt were retained and aligned to the reference genome (GRCh38 assembly) using Bowtie2 as an aligner [13]. Known miRNAs

were mapped with HTSeq-count2 [14] using mature miRNA annotation retrieved from the miRBase database (miRBase v22). Raw miRNA counts across samples were normalized by sequencing depth and RNA composition using the TMM function of the NOISeq Bioconductor R package [15]. Subsequent principal component analysis based on normalized and scaled values was applied using the prcomp R function from the R package [16]. Normalized expression levels across groups were compared following 2 alternative methods for testing differential expression in sequencing data: NOISeq [15] and DESeq2 [17]. Fold change and adjusted *P* values by false discovery rate were calculated and used to identify significant differentially expressed miRNAs.

miRNAs were considered biologically relevant if they were differentially expressed (adjusted $P<.05$) and presented a log2 fold change ≥1.5 between groups.

miRNA-seq Validation of Differentially Expressed miRNAs by RT-qPCR

For the validation of results obtained by NGS, serum miRNAs from 36 patients with eosinophilic asthma and 31 patients with noneosinophilic asthma were retrotranscribed to cDNA using the miRCURY LNA RT Kit (Qiagen), as described by the manufacturer. Briefly, 4 µL of total RNA was mixed with reverse transcription enzyme and with the synthetic miRNAs SP6 and cel-miR-39-3p, which were used to ensure correct retrotranscription to cDNA. The final volume was 10 µL. The reaction was performed in a Veriti 96 well Thermal Cycler (Applied Biosystems) for 60 minutes at 42ºC, then 5 minutes at 95ºC, and indefinitely at 4ºC; cDNA was stored at –20°C until use.

Subsequently, miRNA expression was evaluated using qPCR (miRCURY LNA SYBR Green PCR Kit, Qiagen) according to the manufacturer's protocol. Based on the miRNA-seq results, the probes were used were as follows: hsa-miR-206, hsa-miR-32-5p, hsa-miR-6511a-3p, hsa-miR-202-5p, hsa-miR-26a-1-3p, hsa-miR-200a-3p, hsa-miR-941, hsa-miR-195-5p, hsa-miR-376a-3p, hsa-miR-210-3p, and hsa-miR-450a-5p (Qiagen). Additionally, hsa-miR-103a-3p, hsa-miR-191-5p, SP6, and cel-miR-39-3p were selected as endogenous controls, and hsa-miR-23a-3p and hsa-miR-451a were used as hemolysis controls (Qiagen). All samples were run in triplicate, and reactions were generated in a Light Cycler 96 thermocycler (Roche). Cycle threshold (Ct) values were analyzed using LightCycler 96 SW 1.1 (Roche).

The values of the RT-qPCR analysis were normalized to the endogenous miRNA controls using the $2^{-\Delta Ct}$ method [18], where $\Delta Ct = Ct_{miRNA} - \Delta Ct\ (\overline{X}\ Ct_{hsa\text{-}miR\text{-}103a\text{-}5p} + Ct_{hsa\text{-}miR\text{-}191\text{-}5p})$. hsa-miR-103a-3p and hsa-miR-191-5p, which are known to be stably expressed in whole serum (normal and asthmatic patients), were used as internal controls, as no significant differences in expression were observed between the 2 groups, that is, eosinophilic and noneosinophilic asthma patients. Relative quantification of differences in expression ($RQ = 2^{-\Delta\Delta Ct}$; where $\Delta\Delta Ct = \Delta Ct_{eosinophilics} - \Delta Ct_{non\text{-}eosinophilics}$) was carried out using the ΔΔCt method [18].

In Silico Pathway Enrichment Analysis

Pathway enrichment analysis of dysregulated miRNAs was performed using the DIANA-miRPath v3.0 bioinformatic tool [19].

Statistical Analysis

The statistical analysis was performed using GraphPad Prism v6.0 (GraphPad Software Inc).

Results are expressed as median and interquartile range (IQR). Normality was analyzed using the Shapiro-Wilk test. For continuous variables, parametric data were compared between nonpaired groups using an unpaired *t* test (compared groups have equal SD values) and the Welch *t* test (assumption that the population may have different SD values), and nonparametric and nonpaired groups were compared using the Mann-Whitney test.

The Spearman correlation (nonparametric data) or Pearson correlation (parametric data) was applied for comparisons between miRNA expression levels (ΔCt) and some clinical parameters (quantitative variables). The Fisher exact test was performed on a 2×2 contingency table to test the null hypothesis of independence of the 2 groups and some clinical characteristics (qualitative variables).

The expression profile (ΔCt) of each differentially expressed miRNA was used to create receiver operator characteristic (ROC) curves. Logistic regression models were developed for diagnosis.

Statistical significance was set at $P<.05$.

Results

Clinical Parameters of the Study Patients

Descriptive data for demographic, inflammatory, functional, and clinical characteristics of 85 study patients are summarized in Supplementary Table 1S. The patients were distributed into 4 groups: 40 with eosinophilic asthma, 36 with noneosinophilic asthma, 5 with atopic asthma, and 4 with nonatopic asthma.

The groups were homogeneous in terms of demographic characteristics (ie, age, sex, body mass index [BMI], and smoking habit), with no significant differences. Women were predominant in all 4 groups (67.5%, 66.7%, 80%, and 75%, respectively). Patients from all the groups presented overweight (BMI>25), except in the atopic group (BMI=22.1). Smoking habit was similar across all the groups ($P>.05$). As expected, the peripheral blood count differed significantly between eosinophilic and noneosinophilic asthma patients (700 vs 100/µL, $P<.0001$). Regarding other inflammatory parameters, such as sputum eosinophils, atopy, and IgE levels, we did not observe significant differences, although sputum eosinophils (2.3% and 1%) and IgE levels (209 and 124 IU) were higher in eosinophilic than in noneosinophilic asthma patients, in contrast to atopic patients (60% and 77.8%), where values were slightly higher in noneosinophilic individuals.

Patients with eosinophilic asthma had significantly higher FeNO values than noneosinophilic asthma patients (50 vs 19 ppb, $P<.0001$). Moreover, higher values were recorded in patients with eosinophilic asthma for exacerbations in the previous year (22 vs 14) and severe or moderate asthma (22 vs 11 and 9 vs 5, respectively), although the difference was not statistically significant (Supplementary Table 1S). Similarly,

no significant differences were observed for the Asthma Control Test scores or other clinical traits, such as treatment with inhaled corticosteroids (ICS) and long-acting ß-agonists (LABAs) (Supplementary Table 1S).

Finally, when the atopic and nonatopic asthma groups were compared, significant differences were observed only for atopy ($P<.01$).

miRNA-seq: Differential Expression of miRNAs

Analysis of data obtained by NGS (miRNA-seq) revealed 15 differentially expressed miRNAs between patients with eosinophilic asthma and patients with noneosinophilic asthma (adjusted $P<.05$). Of these 15 miRNAs, 14 were upregulated and 1 was downregulated in patients with eosinophilic asthma. Moreover, both groups were clearly differentiated when principal component analysis was performed using miRNA expression values (Supplementary Figure 1S).

In a different population selected based on atopy, the analysis of miRNA-seq data did not show differentially expressed miRNAs between atopic and nonatopic patients (data not shown).

Validation by RT-qPCR of Differentially Expressed miRNAs Identified Using miRNA-seq: Eosinophilic and Noneosinophilic Asthma

After miRNA-seq, we validated miRNAs using RT-qPCR. Of the 15 miRNAs expressed differentially by miRNA-seq between eosinophilic and noneosinophilic asthma patients, 14 were checked (1 was not available). We found a significant increase in hsa-miR-26a-1-3p and hsa-miR-376a-3p expression levels in patients with eosinophilia (Figure 1A and B).

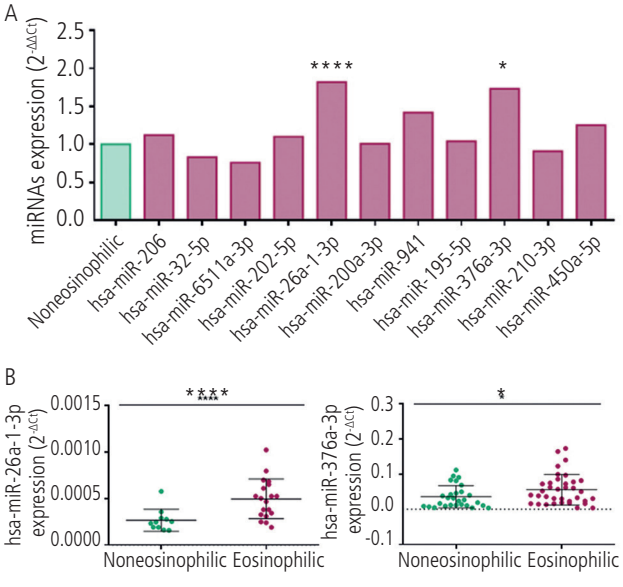

Figure 1. Serum miRNA deregulation in eosinophilic and noneosinophilic asthmatic patients A, Patients with eosinophilic asthma had higher expression levels of hsa-miR-26a-1-3p and hsa-miR-376a-3p than noneosinophilic asthma patients. B, ****, $P<.0001$; *, $P<.05$.

hsa-miR-6513, hsa-miR-1185-1, and hsa-miR-6503 were not detected, and no significant differences were observed for the remaining miRNAs evaluated (Figure 1A).

These results confirm that serum hsa-miR-26a-1-3p and hsa-miR-376a-3p are differentially expressed between eosinophilic and noneosinophilic asthma patients, suggesting that they could serve as diagnostic biomarkers.

Correlation of miRNAs With Clinical Characteristics

In order to establish an association between the expression levels of these miRNAs (ΔCt) and clinical parameters, we performed a correlation analysis depending on whether the variables were quantitative or qualitative. The laboratory data and clinical parameters were as follows: severe asthma, peripheral blood eosinophil count, sputum eosinophil percentage, atopy, total IgE, FEV_1/FVC percentage, FeNO, exacerbations during the previous year, number of exacerbations during the previous year, ICS and LABAs, and the Asthma Control Test result.

Given that higher ΔCt values imply lower miRNA expression levels, we emphasize that ΔCt values of hsa-miR-26a-1-3p were inversely correlated with the peripheral blood eosinophil count (Spearman r=–0.5736; $P<.001$) in all patients (eosinophilic and noneosinophilic; Figure 2A), that is, the peripheral blood eosinophil count increased with the hsa-miR-26a-1-3p expression level. Furthermore, a negative correlation was observed between ΔCt values of hsa-miR-376a-3p and FeNO levels (Spearman r=–0.2594; $P<.05$) in both groups together (Figure 2B) and the number of exacerbations in the previous year (Spearman r=–0.3391; $P<.05$) among eosinophilic asthma patients (Figure 2C) and in the total population (Spearman r=–0.2592; $P<.05$).

Differentially Expressed miRNAs: ROC Curves and Logistic Regression Models

ROC curves were generated and the area under the curve (AUC) was calculated. The AUC values for hsa-miR-26a-1-3p and hsa-miR-376a-3p were 0.76 and 0.68, respectively; therefore, only hsa-miR-26a-1-3p was acceptable as a univariate predictor (Figure 3).

Multivariate logistic regression models were constructed to better differentiate between eosinophilic and noneosinophilic asthma. These were based on the values in their original form (continuous predictors) and on the cut-off values of hsa-miR-26a-1-3p and hsa-miR-376a-3p expression defined by the Youden index as explanatory variables (categorical predictors) (Figure 3). Furthermore, the model combining hsa-miR-26a-3p and hsa-miR-376a-3p, which was created using the original values, yielded an AUC of 0.76, with a sensitivity of 0.79 and a specificity of 0.45 (Figure 3). The model based on categorical predictors combining both miRNAs, which was created using the cut-off values for miRNA expression, yielded an AUC of 0.79, with a sensitivity of 0.84 and a specificity of 0.91 (data not shown). However, despite the favorable AUC, sensitivity, and specificity of the latter model, it would not be acceptable owing to a value <0.1 in the Hosmer-Lemeshow test, which was used to assess calibration of the model.

According to these results, the hsa-miR-26a-1-3p univariate predictor model seems to be the best for discriminating between eosinophilic and noneosinophilic asthma patients, despite being similar to the multivariate regression model with hsa-miR-26a-1-3p and hsa-miR-376a-3p.

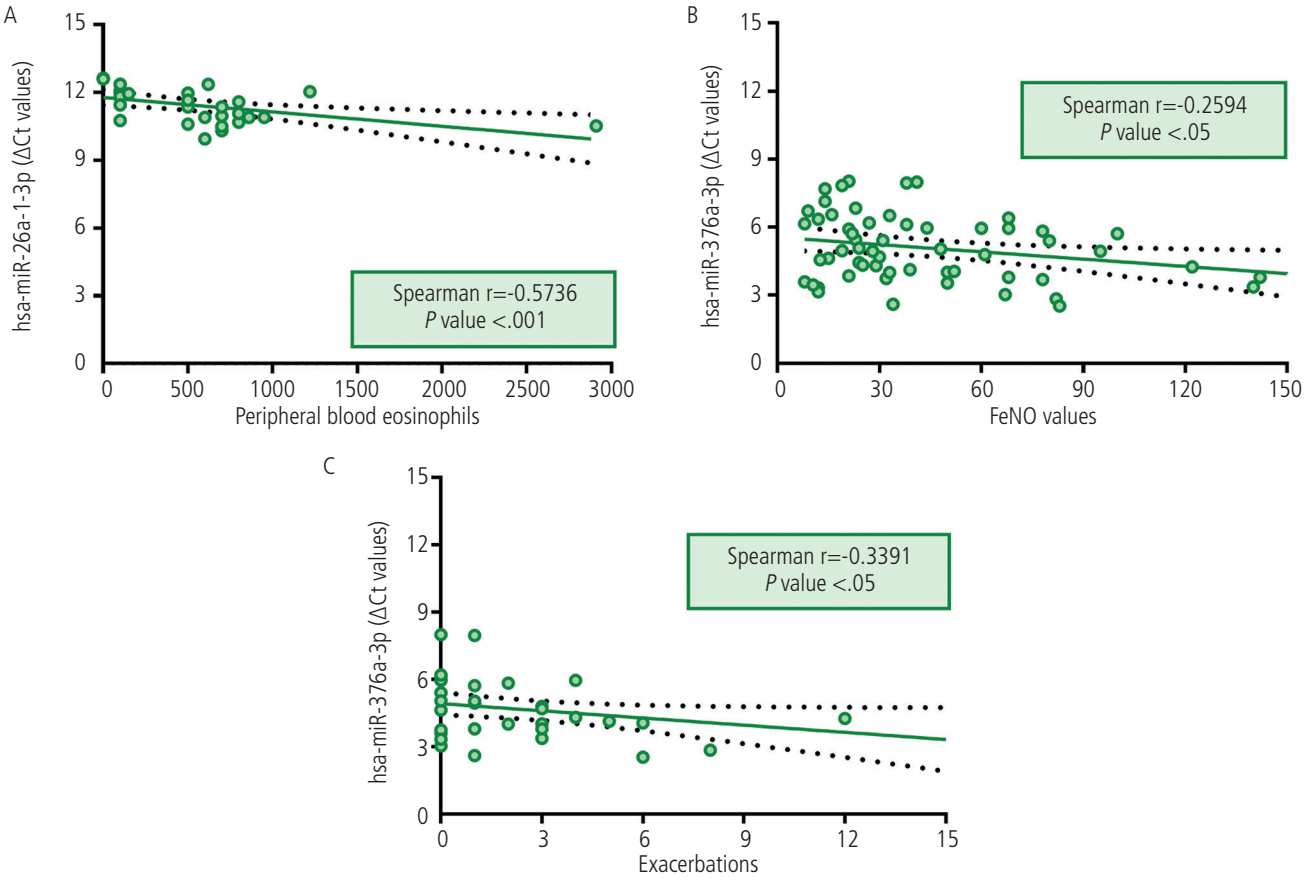

Figure 2. hsa-miR-26a-1-3p and hsa-miR-376a-3p correlate with clinical parameters. A negative correlation was observed in all patients between ΔCt values of hsa-miR-26a-1-3p and peripheral blood eosinophil count. A, ΔCt values of hsa-miR-376a-3p also were inversely correlated with FeNO values in both groups together (B) and the number of exacerbations in eosinophilic asthmatics (C).

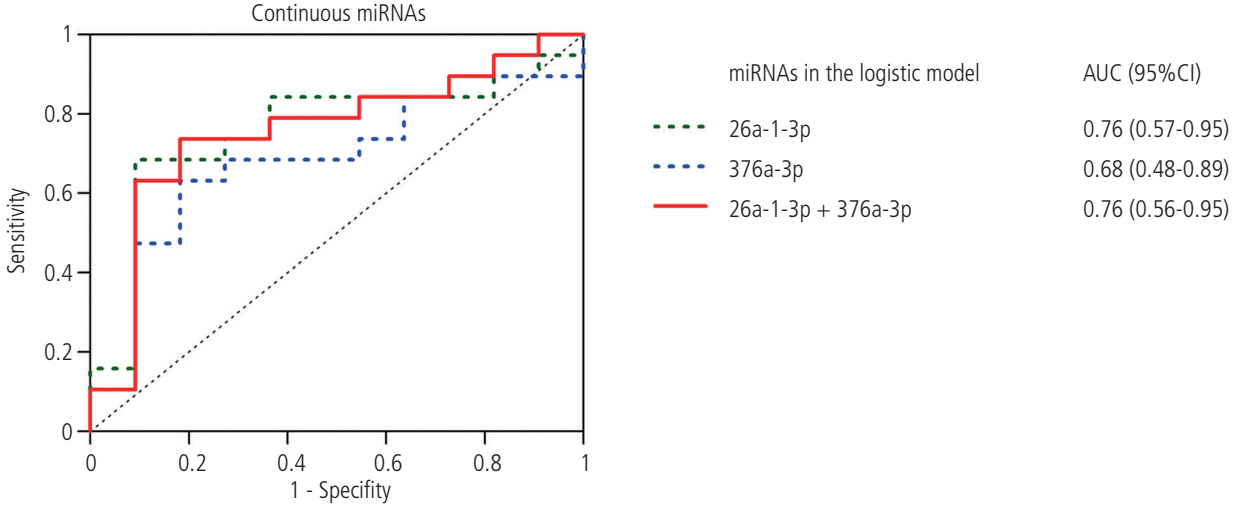

Figure 3. Receiver operator characteristic curves of differentially expressed miRNAs and multivariate logistic regression models created.

Signaling Pathways Involving Dysregulated miRNAs

An in silico analysis was carried out with the 2 differently expressed miRNAs to determine their involvement in biological processes.

As Supplementary Figure 2S shows, hsa-miR-26a-1-3p and hsa-miR-376a-3p are involved in the regulation of several crucial pathways: extracellular matrix (ECM)–receptor interaction, non–small cell lung cancer, and the p53 signaling pathway. hsa-miR-26a-1-3p modulates the non–small cell lung cancer and p53 signaling pathways, and hsa-miR-376a-3p modulates the ECM–receptor interaction. While the p53 and non–small cell lung cancer signaling pathways are more involved in the inflammatory response, the ECM–receptor interaction plays a major role in airway remodeling. Therefore, we can infer that the signaling pathways regulated by these miRNAs play a role in the pathogenesis of asthma.

Discussion

Ours is the first report to show altered expression of serum hsa-miR-26a-1-3p and hsa-miR-376a-3p in eosinophilic and noneosinophilic asthma patients. This profile could be used as a phenotypic biomarker to classify asthma patients into 2 groups in order to choose the appropriate treatment for each individual.

Asthma is a heterogeneous disease with varying severity [20]. Therefore, the identification of biomarkers to recognize endotypes and guide therapy has recently become a priority [21]. These biomarkers include sputum eosinophil percentage, peripheral blood eosinophil count, FeNO, and serum IgE levels [22], with eosinophil count in induced sputum proving to be the most sensitive for phenotyping patients with severe eosinophilic asthma [23]. Blood eosinophil counts are a potential surrogate biomarker of eosinophilic inflammation in asthma [24], although this relationship may differ according to the population and study. In fact, a systematic review and meta-analysis revealed that FeNO, blood eosinophils, and serum IgE have moderate diagnostic accuracy, suggesting the need for new techniques to improve this accuracy [25]. Furthermore, there is no consensus regarding eosinophil cut-off levels. The cut-off used in clinical trials to define high blood eosinophil counts ranges between 150 and 300/mL [26], and a blood eosinophil count of 0.22-0.27×10^9/L differentiates between eosinophilic and noneosinophilic asthma with 78%-86% sensitivity [27]. In our study, the cut-off point for eosinophilia was 0.5×10^9/L or higher.

More useful biomarkers must be found to classify asthma patients into different phenotypes and endotypes. In this sense, NGS is a powerful technique that can analyze the whole genome profile, including mRNA and small RNA expression [28]. To date, this high-throughput profiling technology has revealed critical miRNAs in diseases such as asthma and has proven feasible because it can sequence and detect low-frequency variants [29,30]. Furthermore, in this study, we obtained higher AUC values than those shown by Hastie et al [31], who found that blood eosinophils lack sufficient accuracy for predicting sputum eosinophils in asthma.

This study is the first to reveal a specific profile for 2 miRNAs that could serve to differentiate between eosinophilic and noneosinophilic asthma. Dysregulation of hsa-miR-26a-1-3p and hsa-miR-376a-3p has previously been associated with various aspects of the pathogenesis of asthma and other respiratory diseases [32-34]. hsa-miR-26a-1-3p and hsa-miR-376a-3p could be relevant miRNAs with a phenotypic role in the identification of eosinophilic and noneosinophilic asthma patients, since they were significantly correlated with several clinical parameters. Thus, hsa-miR-26a-1-3p was associated with eosinophil counts and hsa-miR-376a-3p seems to be associated with FeNO values and the number of exacerbations in the previous year. When hsa-miR-376a-3p expression levels were high, the number of exacerbations increased in patients with eosinophilic asthma. It has been shown that blood eosinophil counts and other clinical variables can predict frequent asthma exacerbations and that an increased blood eosinophil count (>400/μL) indicates a greater likelihood of having 2 or more exacerbations per year [35].

Regarding the downstream effects, we found in silico that hsa-miR-26a-1-3p modulates 2 statistically significant pathways that are implicated mainly in inflammation and that hsa-miR-376a-3p significantly altered the ECM–receptor interaction signaling pathway. It is very interesting that the pathways regulated by these miRNAs play a key role in the development of airway inflammation and remodeling of asthma [36]. Asthma-associated airway inflammation is thought to be caused by eosinophilic inflammation, which is favored by airway epithelial barrier involvement and induced by the excessive release of T_H2 cytokines [37]. The deregulation of some miRNAs in noneosinophilic asthma could indicate less involvement of the pathways they regulate and more appropriate expression of the target genes, thus enabling better asthma control. However, the specific role of these miRNAs in distinguishing the phenotypes needs to be further studied.

Finally, in contrast with Bélanger et al [38], we found no differences in the expression of miRNAs between atopic and nonatopic asthma patients. This discrepancy in the results can be due to differences in patient selection: in the study by Bélanger et al, patients were selected from a family cohort and, most importantly, the miRNAs were obtained from purified eosinophils. Since no differentially expressed miRNAs were observed in atopic and nonatopic patients, we evaluated the possible statistical differences between the presence and absence of atopy in eosinophilic and noneosinophilic asthma and did not find statistically significant differences (Supplementary Table 1S). Moreover, the ΔCt values of the differentially expressed miRNAs obtained by validation did not correlate with the presence of atopic and nonatopic asthma. Therefore, in addition to the lack of miRNAs expressed differentially between atopic and nonatopic patients, the presence or absence of atopic asthma is not related to the presence or absence of eosinophilic asthma.

In summary, we describe significant differences in the expression of 2 miRNAs, hsa-miR-26a-1-3p and hsa-miR-376a-3p, which could be used as biomarkers to phenotype eosinophilic and noneosinophilic asthma patients in order to facilitate the choice of treatment.

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Conflicts of Interest

JS: having served as a consultant to Thermo Fisher, MEDA, Novartis, Sanofi, Leti, Faes Farma, Mundipharma, and GSK; having been paid lecture fees by Novartis, GSK, Stallergenes, Leti, and Faes Farma; having received grant support for research from Thermo Fisher, Sanofi, and ALK.

SQ: personal fees from AstraZeneca, personal fees from Novartis, personal fees from Sanofi, personal fees from Boehringer Ingelheim, personal fees from Teva, personal fees from ALK, personal fees from Mundipharma, personal fees from GSK, personal fees from Chiesi, personal fees from Leti, outside the submitted work.

JMO: grants from Sanofi during the conduct of the study; personal fees from AstraZeneca, personal fees from Mundipharma, outside the submitted work.

VdP: honoraria (advisory board, speaker) and/or institutional grant/research support from AstraZeneca and GSK.

The remaining authors declare that they have no conflicts of interest.

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