Low levels of miR-143-3p are associated with severe chronic rhinosinusitis with nasal polyps*

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Dear Editor:

microRNAs (miRNAs) are small, single-stranded, non-coding RNA molecules that regulate post-transcriptional gene expression. Accumulating evidence suggests their involvement in regulating various biological and pathological processes, including inflammation.

Studies have revealed distinct expression patterns of miRNAs in Chronic Rhinosinusitis with (CRSwNP) and without (CRSsNP) nasal polyps [(1)]. Specifically, miR-155 and miR-21 have been observed to be upregulated in CRSwNP, increasing and attenuating the expression of pro-inflammatory cytokines, respectively [(2,3)]. Conversely, the downregulation of miR-34, miR-449, and members of the miR-200 family has been associated with impaired ciliogenesis and the regulation of epithelial-mesenchymal transition, respectively [(4,5)]. Nonetheless, the direct role of miRNAs in CRSwNP is still being investigated.

miR-143-3p has been extensively studied in cancer research, where it has been found to participate in cell proliferation, migration, and invasion. Teng et al. demonstrated that miR-143-3p had an anti-inflammatory function in nasal epithelial cells of patients with allergic rhinitis [(6)]. Based on these findings, we aimed to examine the expression of miR-143-3p in upper airway tissues obtained from severe CRSwNP patients and healthy controls, speculating on its regulatory effects on inflammation.

For this purpose, we conducted a study in which we retrospectively collected tissue samples from nasal polyps (NP, n=39) from severe CRSwNP patients and nasal mucosa (NM, n=26) from control individuals (Table 1, Supplementary data). In this study "severe" CRSwNP patients were considered those who underwent Endoscopic sinus surgery (ESS). The study was approved by the Ethics Committee of our Institution and all participant patients and subjects signed an informed consent. Blood eosinophil counts (BEC, cells/µL, and %) were determined while immunohistochemical staining using an anti-BMK13 antibody was performed to quantify nasal tissue eosinophils (NTE). Total RNA was extracted, and miR-143-3p expression levels were assessed using quantitative real-time-PCR employing the fold change (FC) = $2^{(-\Delta CT)}$ method. Additionally, the tissue location of miR-143-3p was determined using RNAscope technology.

miR-143-3p expression was significantly lower in the NP group compared to the NM group. In CRSwNP patients, NP with asthma showed however a higher miR-143-3p expression than NP without asthma ($p = 0.0236$, t-test). Non-significant differences were observed between patients with and without tolerance to aspirin/nonsteroidal anti-inflammatory drugs (NSAIDs) (Figure 1A). NTE were significantly higher in the NP group, regardless of the presence of comorbidities, vs. the NM group (Figure 1B). A significant negative correlation was found between NTE and miR-143-3p expression ($p = 0.0023$, t-test; $r = -0.469$, Spearman coefficient) (Figure 1C). Furthermore, we observed specific staining of miR-143-3p in the submucosal area, but not in the epithelium, in both NM and NP tissues (Figure 1D). In further studies, we aim to integrate and combine the RNAscope and the immunohistochemistry staining on the same tissue sample to achieve a simultaneous visualization of miR-143-3p and the protein marker of the cell location.

To gain a deeper understanding of the mechanism and specific functions of miR-143-3p, further in vitro studies will be needed. Identifying the targets of miR-143-3p will provide insights into the specific gene expression pathways involved. Promising outcomes in modulating inflammation such as reducing nasal polyp size and improving clinical outcomes could be achieved through preclinical studies utilizing miR-143-3p mimics.

In summary, we endotyped eosinophilic inflammation in the blood and nasal tissues from severe CRSwNP patients and healthy controls. Eosinophil levels, a marker of type 2 inflammation,

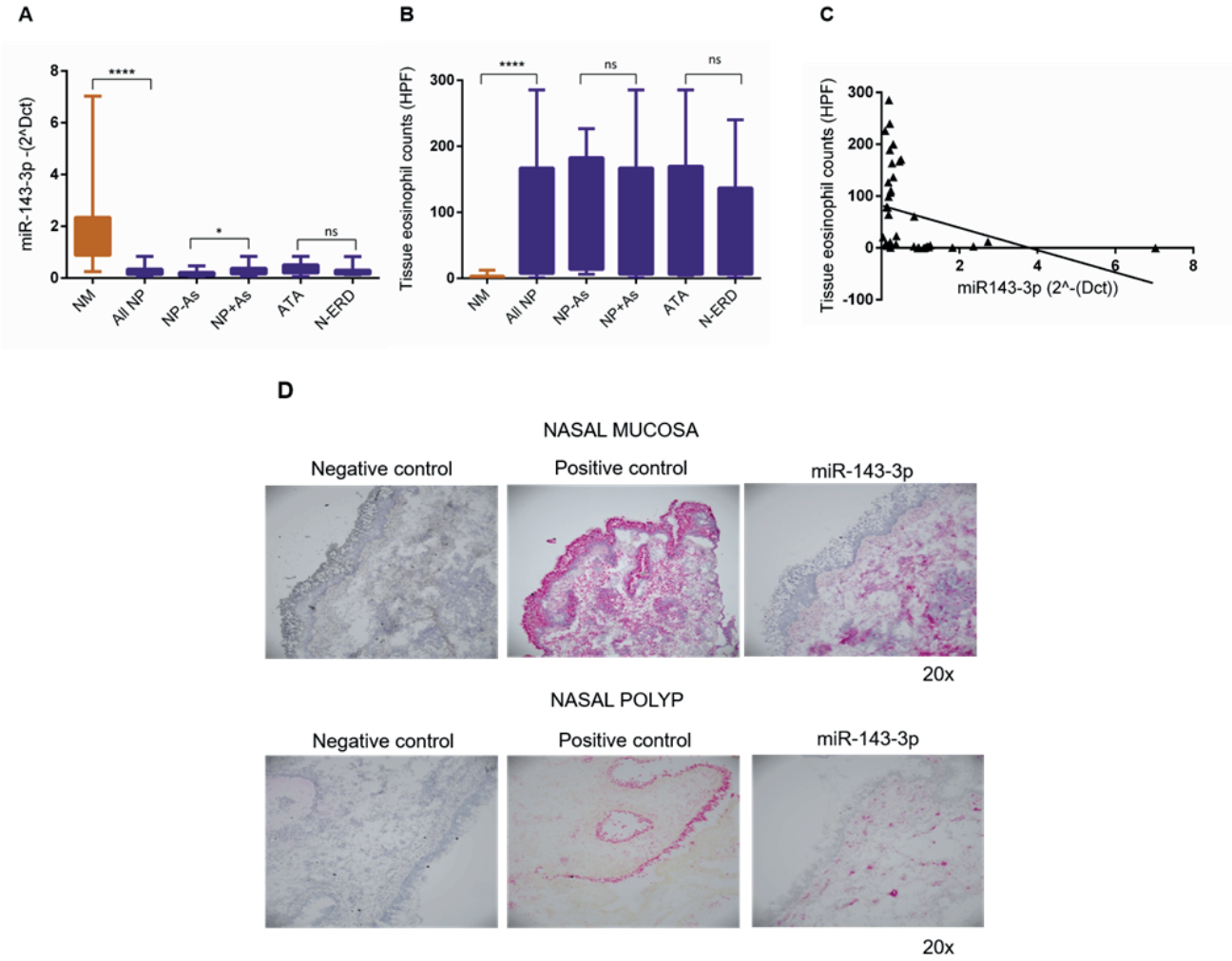

Figure 1. A) miR-143-3p expression (2^-(Dct)) was significantly lower in all nasal polyp (All NP) group compared to the nasal mucosa (NM) group ($p<0.0001$). miR-143-3p expression was also higher in patients with (NP+As) vs. without (NP-As) asthma ($p<0.0001$). B) Tissue eosinophil counts (at x 400 magnification) were significantly higher in the All NP compared to the NM group ($p < 0.0001$). C) A significant inverse correlation ($p = 0.0023$) was found between the tissue eosinophil counts and miR-143-3p expression ($r = -0.4687$, Spearman coefficient). D) Tissue location of miR-143-3p was analyzed by miRNAscope™. Scramble-SI Negative Contral Probe (left microphotographs) and Hs-RNU6-SI Positive Contral Probe (middle) were tested in both NM and NP tissues. Specific detection of miR-143-3p was observed in the submucosal area using has-miR-143-3p-SI probe (right); 20X magnification). As, asthma; ATA, aspirin tolerant asthma, HPF, high-power field, N-ERD, NSAID-Exacerbated Respiratory Disease. Mann-Whitney test was used for statistical group comparisons while Spearman test for correlations.

were significantly elevated in both NP and blood samples from CRSwNP patients. However, we observed a lack of correlation between blood eosinophils and nasal eosinophil infiltration, suggesting a novel perspective in evaluating eosinophilia in CRSwNP patients. Further studies are required to elucidate the discrepancy in eosinophil levels between these two different sources.

Furthermore, our study revealed a significant downregulation of miR-143-3p in NP compared to NM tissues, highlighting its potential anti-inflammatory role in CRSwNP pathogenesis. Moreover, because of the inverse correlation between NTE counts and miR-143-3p expression, we hypothesize a possible role of miR-143-3p as a low expression biomarker associated to the CRSwNP severity. This finding may help to advance in patients' care and outcomes under the umbrella of personalized and precision medicine.

List of abbreviations

As, Asthma; ATA, Aspirin tolerant asthma; BEC, Blood Eosinophil Counts; CRSsNP, Chronic rhinosinusitis without nasal polyp; CRSwNP, Chronic rhinosinusitis with nasal polyp; ESS, Endoscopic sinus surgery; HPF, High-power field; N-ERD, NSAID-Exacerbated Respiratory Disease; NM, Nasal mucosa; NP, Nasal polyp; NSAIDs, Nonsteroidal anti-inflammatory drugs; NTE, Nasal Tissue Eosinophils.

Authorship contribution

VT: Conception and design, data curation, methodology, acquisition of data, data analysis, interpretation of data, writing – review & editing. MF: data curation, methodology, acquisition of data. BC: Conception and design, data analysis, interpretation of data, writing – review & editing. MB: Conception and design, data analysis, interpretation of data, writing – review & editing. CM: Conception and design, data analysis, interpretation of data, writing – review & editing. IA: Conception and design, interpretation of data, writing – review & editing, project administration, funding acquisition. JB: Conception and design, interpretation of data, writing – review & editing, project administration, funding acquisition. AV: Conception and design, interpretation of data, writing – review & editing, project administration, funding acquisition. JRF: Conception and design, data analysis, interpretation of data, writing – review & editing. JM: Conception and design, data analysis, interpretation of data, writing – review & editing, project administration, funding acquisition.

Conflict of interest

JM is or has been member of national and international scientific advisory boards, consulting, received fees for lectures, and grants for research projects or clinical trials from AstraZeneca, Genentech-Roche, GSK, LETI, Menarini, MSD, Mitsubishi-Tanabe, NOUCOR/Uriach Group, Novartis, OPTINOSE, Proctor & Gamble, Regeneron Pharmaceuticals Inc., Sanofi-Genzyme, UCB Pharma, and Viatris/MEDA Pharma. All other authors on this manuscript have no relevant financial or other relationships to disclose. There are no patents, products in development or marketed products to declare.

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This manuscript contains online supplementary material

SUPPLEMENTARY MATERIAL

Table 1. Demographic, clinical, and inflammatory (eosinophilia) characteristics of the study population, controls and severe CRSwNP before ESS.

	Control NM (n = 26)	All NP (n = 39)	NP - Asthma (n = 13)	NP + Asthma (n=26)	ATA (n = 12)	N-ERD (n = 14)
Age, years, mean (SD)	42.9 (15.1)	56.1 (14.3)	61 (12.5)	53.7 (14.7)	54.2 (17.8)	50.8 (14.2)
Female, N (%)	11 (42)	17 (44)	2 (15)	16 (58)	5 (42)	10 (71)
Asthma, N (%)	0 (0)	26 (67)	0 (0)	26 (100)	12 (100)	14 (100)
N-ERD, N (%)	0 (0)	14 (36)	0 (0)	14 (54)	0 (0)	14 (100)
≥ 1 previous ESS, N (%)	0 (0)	11 (28)	2 (15)	9 (35)	4 (33)	5 (36)
Pre-ESS treatment, N (%)	0 (0)	11 (28)	2 (15)	9 (35)	4 (33)	5 (36)
INCs	0 (0)	11 (28)	2 (15)	9 (35)	3 (25)	6 (43)
OCs	0 (0)	2 (5)	0 (0)	2 (8)	2 (17)	0 (0)
Biologics	0 (0)	1 (3)	0 (0)	1 (4)	0 (0)	1 (7)
BEC, cells/μL, mean (SD)	195.6 (151.6)	397.5 (264.0)	280.0 (210.2)	454.1 (271.8)	575.0 (336.1)	357.3 (160.9)
BEC, % , mean (SD)	2.5 (1.7)	5.6 (3.0)	4.1 (2.3)	6.0 (3.1)	7.6 (3.4)	4.7 (2.2)
NTE, cells/HPF, mean (SD)	2.5 (3.6)	96.1 (85.6)	101.2 (86.9)	94.0 (87.4)	101.2 (101.4)	88.8 (80.4)

ATA, aspirin tolerant asthma; BEC, blood eosinophil count; Eos, eosinophils; ESS, endoscopic sinus surgery; INCs, intranasal corticosteroids; N-ERD, NSAID-exacerbated respiratory disease; NM, nasal mucosa; NP, nasal polyps; NTE, nasal tissue eosinophilia; OCs, oral corticosteroids; SD, standard deviation.

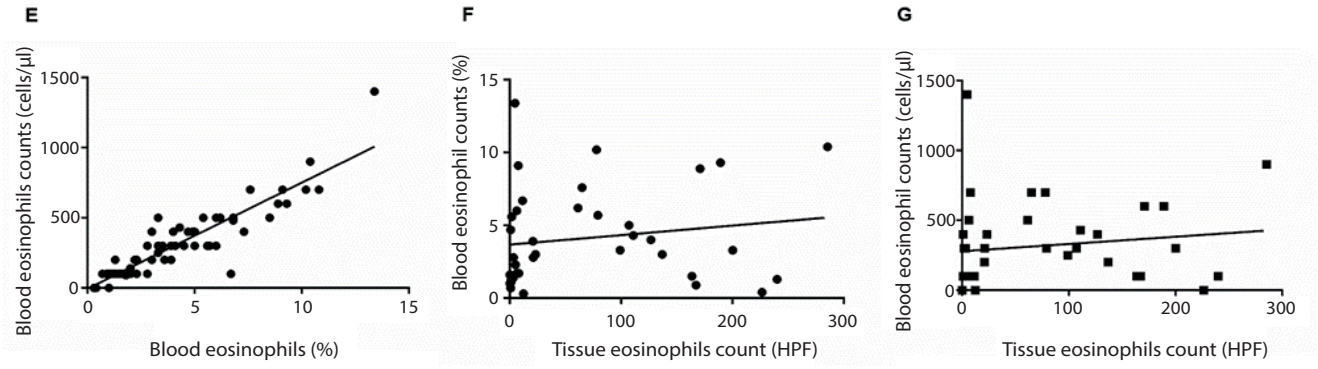

Figure S1. Correlations between blood eosinophil counts. (E) Only the correlation between blood eosinophil counts (cells/μL) and blood eosinophil percentage was statistically significant ($p < 0.0001$, Spearman $r = 0.8897$). (F) Non-significant correlations were found between blood eosinophil counts, either in percentage (p = ns; Spearman $r = 0.2797$) or cells/μL (G) (p = ns; Spearman $r = 0.0852$), with tissue eosinophil counts (HPF). Mann Whitney test was used for statistical group comparisons while Spearman test for correlations.