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MicroRNA expression profile in peripheral blood mononuclear cells of asthmatic patients and healthy individuals: The effect of age and *ex vivo* rhinovirus exposure

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Key Message

Age had a significant influence on the miRNA expression in PBMCs

Changes in miRNA expression with age may be associated with asthma status

Ex vivo rhinovirus exposure had no effect on miRNA expression in PBMCs

Ageing of the immune system (or “immunosenescence”) is associated with higher mortality due to infectious diseases and reduced response to vaccines. Dysregulation of the cellular response may be associated with an increased incidence of neoplasms, while an elevated level of proinflammatory cytokines, produced mostly by innate cells, contributes to age-related inflammatory diseases including osteoporosis, type 2 diabetes, atherosclerosis or Alzheimer's disease.¹ Immunosenescence, along with age-related decline in the lung function, may cause different manifestations of asthma in elderly patients.² Age-related

alterations in the immune response can be linked to epigenetic factors, for example miRNA changes, since they might affect the expression of multiple inflammatory genes. Altered expression of several miRNAs was associated with asthma control, severity, disease phenotype or treatment.³ Human rhinoviruses (RV) are considered to be the main contributors of asthma exacerbations and they may be involved in asthma pathogenesis. It has been documented that the miRNA profile could be altered upon infection with different respiratory viruses.^{4,5} Therefore, the purpose of the study was to assess the effect of rhinovirus on miRNA expression in peripheral blood mononuclear cells (PBMCs) isolated from asthma patients and healthy controls and to evaluate how age may contribute to this process.

To achieve these objectives, we performed the study in two consecutive steps. First, the profiling study was conducted. It included three elderly asthmatics (EA, aged over 65 years), three non-elderly asthmatics (nEA, aged 30–50 years), three elderly controls (EC, aged over 65 years) and three non-elderly controls (nEC, aged 30–50 years old). Next, validation was performed in 48 subjects, including 12 EA (aged over 65 years), 12 nEA (aged 30–50 years old), 12 EC (aged over 65 years) and 12 nEC (aged 30–50 years old). In both parts of the study, blood samples were collected from the subjects and PBMCs were isolated. Subsequently, cells were cultured in the presence of RV1b and with the medium only, and, after 48 h, miRNA was isolated from the PBMCs. miRNA profile and miRNA expression in the validation study were determined at single point, after 48 h of PBMCs culture. The detailed methods and clinical characteristics of the study subjects have been described in the Supplementary material.

The first part of the study, miRNA profiling, was performed by Exiqon (Vedbaek, Denmark). Out of 2097 miRNAs on the microarray, the profiling of PBMC samples detected the expression of 433 of them. For validation of the profiling results, only miRNAs showing more than a two-fold up- or down- regulation (adjusted p value < 0.05) and with signal intensity well above the array background (7.5–14.5) were selected for further analysis, which allowed to reduce the number of relevant miRNAs to 47 (Table 1). Since the study mainly focused on the influence of age and rhinovirus exposure on the expression pattern of miRNAs in PBMCs, we further analysed miRNAs upon infection that were differently expressed in EA than in age-matched controls. As regards EA, the following miRNAs were found to be downregulated: miRNA-19b, -22, -106a, -142, -146a, -451a, -491 and -3182, while miRNA -4286 was upregulated in EA as compared with EC. Seven of these miRNAs, miRNA-19b, -22, -142, -451a, -491, -3182 and -4286, were also differently expressed in EA and EC in noninfected PBMCs, indicating that the differences in the expression rather reflected age-related changes. Based on the profiling study, we observed a limited effect of RV1b exposure on miRNA expression in PBMCs as only one miRNA (miRNA-4732) was induced upon infection with RV1b (comparison between non-infected and infected cells), and the result was observed in nEC only.

TABLE 1. Results of miRNA profiling

Compared groups	Number of miRNAs	List of miRNAs
Medium exposed PBMCs		
All asthma vs. all controls	14	miRNA-19b-5p, -27a-3p, -29a-3p, -29b-3p, -30e-5p, 142-5p, -223, -1260a, -1260b, -4286, -4532, -4695, -4876, -5581
EA vs. EC	20	miRNA-15b, -19b-5p, -21, -22, -27a, -29b, -29c, -101, -106b, -142-5p, -342, -351a, -391, -451a, -491, -1260a, -3182, -4286, -4532, -4695, -5581
nEA vs. nEC	3	miRNA-30e, -1260b, -4532
RV1b exposed PBMCs		
EA vs. EC	9	miRNA-19b, -22, -106a, -142, -146a, -451a, -491, -3182, -4286
RV1b vs. medium exposed PBMCs		
nEC (medium) vs. nEC (RV1b)	1	miRNA-4732

Note

List of miRNAs with significant changes in the expression pattern (on microarrays). miRNAs showing a significant level of regulation were chosen based on fold change, significance level (adjusted p value <0.05) and array signal intensity.

Abbreviations: EA, elderly asthmatics; EC, elderly controls; nEA, non-elderly asthmatics; nEC, non-elderly controls;

PBMCs, peripheral blood mononuclear cells; RV1b, Rhinovirus 1b.

In the second part, out of nine miRNAs differently expressed in RV1b exposed PBMCs isolated from EA as compared with EC in the profiling study, seven were selected for further analysis with RT-PCR: miRNA -19b, -22, -106a, -142, -146a, 451a and -4919. The remaining two miRNAs (miRNA-3182 and -4286) were not analysed since they had high consecutive numbers suggesting they were freshly added to the miRNA database, and, in fact, they could be the products of tRNA degradation, not real miRNAs. Additionally, we decided to include miRNA-126a into the analysis. It did not show any significant expression in the profiling, however, it had previously been reported as an important regulator of anti-inflammatory responses associated with asthmatic inflammation.^{3, 4} We also added miRNA-4732, which was induced in PBMCs upon exposure to RV1b in the nEC. Out the nine miRNAs selected in this way, three (miRNA-451a, -491 and -4732) were not amplified in our samples, therefore further analysis included the remaining six miRNAs.

In PBMCs exposed to the medium isolated from all the asthmatics, the expressions ($2^{-\Delta\text{Ct}}$) of miRNA -19b and miRNA -22 were higher than in the controls (Me (25–75%CI): 36.8 (21.8–65.7) vs. 16 (7.1–34); $p = 0.006$ and 7.4 (4.7–12.2) vs. 3.9 (2.7–6.3); $p = 0.015$). After stratification of the subjects by age, EA had a lower expression of miRNA-146a as compared with the age-matched controls, while nEA had a higher expression of miRNA -19b, -22, -106, -142 and -146 as compared with nEC. EA had a lower expression of miRNA -106a and -146a than nEA (Figure 1A). In PBMCs exposed to RV1b isolated from all the asthmatics, the expression of miRNA -22 ($2^{-\Delta\text{Ct}}$) was higher than in the controls (Me (25–75%CI): 6.7 (3.9–11.3), vs. 4.2 (2.3–5.5); $p = 0.03$). RV1bV exposed PBMCs from EA, as compared with EC, demonstrated a significantly lower expression of miRNA-146a. Whereas, nEA, as compared with the age-matched controls, had a higher expression of miRNA-19b, -106a and -146a in RV1b-infected cells. EC had a higher expression of miRNA- 19b and -146a in PBMCs exposed to RV1b than nEC (Figure 1B).

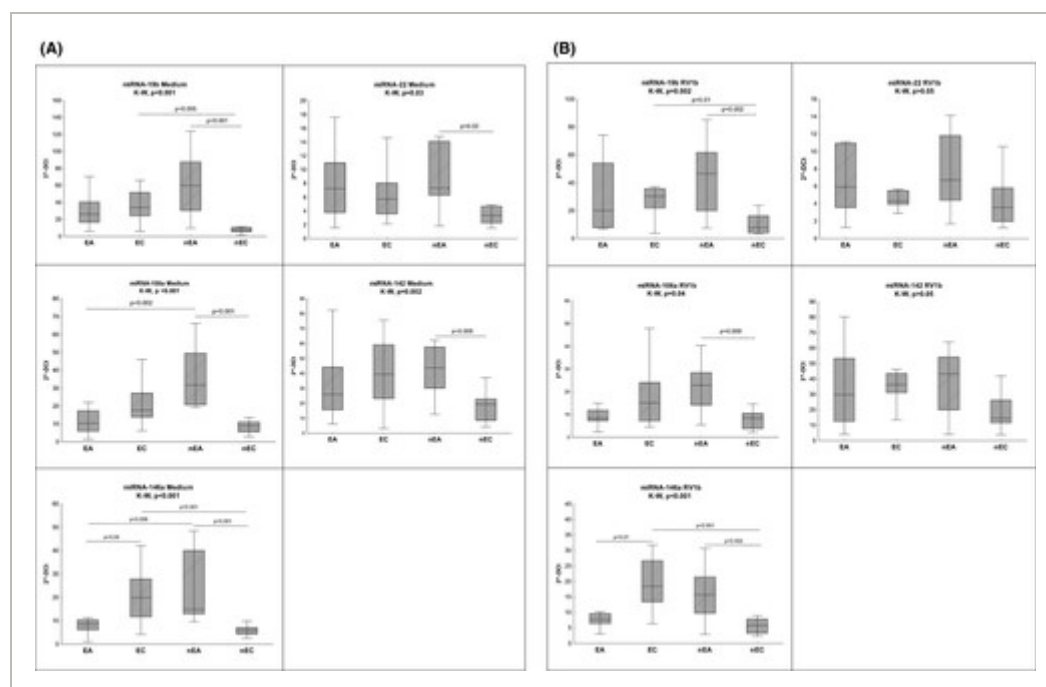


FIGURE 1

[Open in figure viewer](#) | [PowerPoint](#)

A. Differences in the expression of miRNAs [$2^{-\Delta\text{Ct}}$] in the asthmatics and controls in specific age groups in medium exposed peripheral blood mononuclear cells (PBMCs). 1.B. Differences in the expression of miRNAs in the asthmatics and controls in specific age groups in RV1b-infected PBMCs. The groups were compared with the use of the Kruskal–Wallis test followed by the Dunn correction. The boxes present 25th–75th percentiles, the bars- median value, the whiskers- non-outlier range. EA-elderly asthmatics, nEA non-elderly asthmatics, EC- elderly controls, nEC- non-elderly controls, RV1b- Rhinovirus 1b

We did not observe any changes in the miRNA expression in PBMCs exposed to RV1b as compared with the medium.

In the first phase of the study, the miRNAome obtained by microarray profiling was found to be significantly different, depending on age. There was a higher number of miRNAs differentiating the asthmatic and healthy subjects in PBMCs collected from elderly individuals than in younger study participants. Moreover, there was only one the same miRNA expressed in medium exposed cells in both age groups. In the validation study, we observed that the expression of miRNA-22 was higher in RV1b-infected PBMCs isolated from the asthmatics as compared with the controls, however, after stratification according to age, most of the miRNAs (miRNA-19b, -22, -106a, -142, -146a) were upregulated in younger patients as compared with age-matched controls in infected or non-infected cells, while in elderly group asthmatics had a lower expression of miRNA-146a than healthy subjects. In earlier studies, associations between miRNA expression and asthma have been well documented and certain miRNAs could be related to asthma traits or exacerbation.³ However, most of the studies were conducted in younger populations and they did not take patients' age into account, despite the fact that the ageing process is an important factor modifying asthma clinical phenotypes and pathophysiology. In our previous report, expression of selected circulating miRNAs (miRNA-19b, -106a, -126a and -146a) measured in the serum of 59 EA and nEA and 59 age-matched controls was associated with the age of the subjects. Additionally, only in younger asthmatics miRNAs (miRNA-106a, -126a and -146a) were correlated with clinical features of asthma.⁶ It was proven that age-related changes occur in miRNA expression in PBMCs⁷ and other types of cells or serum,⁶ which suggests that epigenetic changes are a key player in the process of senescence.

Surprisingly, we did not observe any significant effect of rhinovirus exposure on the expression of miRNAs in PBMCs. According to the miRNA profiling results, only one of the miRNAs was changed in PBMCs exposed as compared with those not exposed to RV1b (in younger controls, though), unfortunately, miRNA-4732 was not amplified in a larger group of subjects. In the second part of the study (validation stage), we did not observe any change of miRNA expression in PBMCs exposed to rhinovirus. Such results may be related to the fact that PBMCs are not a primary target of HRV, however it has been shown that rhinoviruses are able to infect these cells.⁸ In previous studies on epithelial cell infection with RV, RSV and Influenza virus caused dysregulation of several miRNAs.^{4, 5, 9} Only few studies described expression of miRNA isolated from PBMCs inoculated with RSV or Influenza,⁴ and to the best of our knowledge, this is the first report analysing the effect of RV1b exposure on the miRNA profile in PBMCs.

Our study has several limitations, mainly those resulting from a small number of individuals participating in the profiling study and the subsequent validation. It is therefore required that the results be confirmed in a larger group of subjects, preferably in a different population. Moreover, the model adopted in this study, evaluation of miRNA expression at only one time point (at 48 h) as well as use of unpurified cells- PBMCs, is not perfect. It is highly possible that the miRNA profile may differ depending on the type of cells. Additionally, factors, such as viral exposure as well as age or clinical characteristics, may

exert a different effect depending on a subpopulation of PBMCs. Finally, when comparing both parts of the study, we found discrepancies between the profiling results and validation. In both parts of the study there was a significant difference in the expression of miRNA-19b in non-stimulated PBMCs from the asthmatics as compared with the controls and in miRNA-146a in RV1b-infected cells in the elderly individuals (EA vs. EC), however, the remaining results have not been confirmed.

In conclusion, we established that the miRNA profile in PBMCs isolated from asthmatics and controls varies depending on the age of the subjects, while ex vivo rhinovirus exposure had only a limited effect on the expression of miRNA in PBMCs.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: M.L.K., A.W. and J.S.M.; laboratory analysis: M.P. and J.R.; patient recruitment: A.W. and J.J.B.; data analysis and writing – preparation of the original draft: A.W., M.C. and M.L.K. All the authors have read and agreed to the published version of the manuscript.

INSTITUTIONAL REVIEW BOARD STATEMENT

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Bioethical Committee at the Medical University of Lodz (Approval no. RNN/166/14/14/KE).

INFORMED CONSENT STATEMENT

Informed consent was obtained from all the subjects participating in the study.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. Additional information about study methods are available in the following repository: <https://doi-org.bibliosan.clas.cineca.it/10.5281/zenodo.5577974>.

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