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Studying small molecules that control how different eosinophil types behave in asthma

Submission date
26/03/2026

Recruitment status
No longer recruiting

Registration date
07/04/2026

Overall study status
Completed

Last Edited
07/04/2026

Condition category
Respiratory

[_] Prospectively registered
[_] Protocol
[_] Statistical analysis plan
[_] Results
[_] Individual participant data
[X] Record updated in last year

Plain English summary of protocol
Background and study aims
Asthma is a long-term condition where the airways become inflamed and narrow, making it harder to breathe. A type of white blood cell called an eosinophil plays a major role in this inflammation. Recent research shows that there are two types of eosinophils in the lungs: resident eosinophils, which help maintain normal lung function, and inflammatory eosinophils, which increase during allergic reactions.
This study aims to look at very small molecules called microRNAs found inside these eosinophils. MicroRNAs help control how genes behave, and changes in them may give clues about how asthma develops. The study compares microRNAs in people with allergic asthma and in healthy volunteers. It also looks at microRNAs found in blood and in tiny particles released by cells, called exosomes. The aim is to understand whether these microRNAs could help diagnose asthma or show how severe it is, and to understand more about what eosinophils do in asthma.

Who can participate?
Adults aged 18 to 70 years can take part. The study includes people with allergic asthma caused by house dust mites as well as healthy volunteers with no allergic or long-term breathing problems. Everyone must give written informed consent before taking part.

What does the study involve?
Participants provide a blood sample. Researchers isolate eosinophils from the blood and separate them into the two types of eosinophils. They then study the microRNAs inside the cells, in the blood, and in exosomes using laboratory methods. No medicines or treatments are given as part of this study. All procedures follow Good Clinical Practice guidelines.

What are the possible benefits and risks of participating?
There is no direct benefit to participants. However, the information collected may help improve future diagnosis and treatment of asthma. The risks are small and mainly relate to giving a blood sample, such as brief discomfort or bruising.

Where is the study run from?
The study is carried out at the Department of Pulmonology at the Hospital of the Lithuanian University of Health Sciences Kaunas Clinics in Lithuania.

When is the study starting and how long is it expected to run for?
The first participant was enrolled on 15 January 2021. The final enrolment was on 30 June 2025. The study is now completed.

Who is funding the study?
GlaxoSmithKline.

Who is the main contact?
Professor Kestutis Malakauskas, malakauskas@lsmu.lt

Contact information

Type(s)
Principal investigator, Scientific, Public

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Study information

Scientific Title
Non-coding RNAs analysis of eosinophil subtypes in asthma

Acronym
NCRA

Study objectives
1. To investigate the microRNA (miRNA) expression profiles of resident eosinophils (rEOS) and inflammatory eosinophils (iEOS) in the peripheral blood of healthy subjects and patients with asthma
2. To determine the expression of selected miRNAs in blood plasma and evaluate their potential diagnostic value as biomarkers of asthma
3. To analyze miRNAs contained in eosinophil-derived exosomes and assess their possible role in airway remodeling, particularly through miRNA-mediated effects of eosinophil subtypes

Ethics approval required

Ethics approval required

Ethics approval(s)
approved 09/01/2020, Kaunas Region Biomedical Research Ethics Committee (A. Mickeviciaus str. 9, Kaunas, LT-44307, Lithuania; +370 37326889; aunorbtek@lsmu.lt), ref: BE-2-58

Primary study design
Observational

Secondary study design
Cross sectional study

Study type(s)

Health condition(s) or problem(s) studied
Allergic asthma patients with sensitization to house dust mites (D. pteronyssinus) allergen

Interventions
- Eosinophils isolation. Eosinophils from study subjects peripheral blood will be isolated by combined centrifugation at the high-density gradient and magnetic separation with commercial eosinophil isolation kits, according to the manufacturer's instruction. Eosinophils will be used if their viability and purity will be > 97 %;
- Eosinophils subtyping. Whole peripheral blood eosinophils will be separated to eosinophil subtypes – rEOS- and iEOS-like blood eosinophils with human antibodies against CD62L, conjugated with magnetic beads (rEOS is CD62L positive, iEOS- negative). Confirmation will be performed with a flow cytometer by measuring the expression of several surface proteins: iEOS - Siglec-8hiCD62L–CD101hi, rEOS Siglec-8intCD62L+CD101lo. All antibodies are human orthologs discriminating surface markers found in the mouse;
- mRNA isolation. Total mRNA from eosinophils will be extracted by using commercial mRNA isolation kits with special columns, according to manufacturers' instruction or Trizol reagent. The current procedure will be selected after the evaluation of mRNA quality after primary isolation. To avoid possible mRNA degradation we will use RNA stabilizing solutions before lysis of eosinophils, to avoid possible release of high active eosinophils RNases. Samples will be used in the experiments after appropriate washes steps;
- RNA sequencing. By small RNA the NGS with the designed appropriate libraries will be used to found a miRNA and piRNA (in one analysis) in eosinophils subtypes (Qiagen, Venlo, Netherlands). Moreover, as lncRNA is significantly longer compared with small RNAs, the NGS will be performed as a separate experiment (Qiagen, Venlo, Netherlands);
- Gene expression. The validation process and ncRNAs levels in blood plasma and exosomes will be performed by quantitative polymerase chain reaction (qPCR) with specific nucleotides primers against investigating ncRNAs. ncRNA which will show the sufficient amount for its detection with qPCR analysis will be considered as validated.
- Exosomes isolation. Eosinophils-derived exosomes will be collected from cells culture supernatants after appropriate activation with eosinophilopoietins in vitro and from blood plasma after series centrifugations and magnetic separation. Exosomes will be isolated by magnetic separation with positive selection using MicroBeads recognizing the tetraspanin proteins CD9, CD63, and CD81 on exosomes surface.

Intervention Type
Procedure/Surgery

Primary outcome(s)

1. Fold changes and statistical differences of miRNAs in HS iEos vs rEos measured using NGS data of miRNA expression in rEOS and iEOS in healthy subjects. Data presented as iEOS versus rEOS (positive value means that gene expression is higher in iEOS, negative value - gene expression is higher in rEOS). Statistical significance was evaluated using the Wilcoxon signed-rank test for log# fold change values, and p-values were adjusted for multiple comparisons using the Benjamini–Hochberg False Discovery Rate (FDR) method at 6 to 12 months

2. Fold changes and statistical differences of miRNAs in AA iEos vs rEos measured using NGS data of miRNA expression in rEOS and iEOS inAA patients. Data presented as iEOS versus rEOS (positive value means that gene expression is higher in iEOS, negative value - gene expression is higher in rEOS). Statistical significance was evaluated using the Wilcoxon signed-rank test for log# fold change values, and p-values were adjusted for multiple comparisons using the Benjamini–Hochberg False Discovery Rate (FDR) method at 6 to 12 months

3. Fold changes and statistical differences of miRNAs in AA iEos vs HS iEos measured using NGS data of miRNA expression in AA iEos vs HS iEos. Data presented as AA iEOS versus HS iEOS (positive value means that gene expression is higher in AA iEOS, negative value - gene expression is higher in HS iEOS). Statistical significance was evaluated using the Wilcoxon signed-rank test for log# fold change values, and p-values were adjusted for multiple comparisons using the Benjamini–Hochberg False Discovery Rate (FDR) method at 6 to 12 months

4. Fold changes and statistical differences of miRNAs in AA rEos vs HS rEos measured using NGS data of miRNA expression in AA rEos vs HS rEos. Data presented as AA rEOS versus HS rEOS (positive value means that gene expression is higher in AA rEOS, negative value - gene expression is higher in HS rEOS). Statistical significance was evaluated using the Wilcoxon signed-rank test for log# fold change values, and p-values were adjusted for multiple comparisons using the Benjamini–Hochberg False Discovery Rate (FDR) method at 6 to 12 months

Key secondary outcome(s))

1. Circulating plasma levels of miRNA in asthma patients measured using selected miRNA levels measurements in patients' blood plasma samples based on miRNA expression profiles in eosinophil subtypes. Data presented as log#(2#ΔΔCt) gene expression changes between AA group and HS group. Positive values indicate that gene expression is higher in the plasma of AA group, while negative values indicate that gene expression is higher in the HS group. Differences between the two independent groups were assessed using the Mann-Whitney U test. A p-value of < 0.05 was considered statistically significant at From 12 to 24 months

2. Validated fold changes of selected miRNA Expression in AA rEos vs HS rEos measured using quantitative polymerase chain reaction data of selected miRNA expression. Data presented as log# fold changes in gene expression between AA rEos vs HS rEos. Positive values indicate that gene expression is higher in the plasma of AA group, while negative values indicate that gene expression is higher in the HS group. Differences between the two independent groups were assessed using the Mann-Whitney U test. A p-value of < 0.05 was considered statistically significant. Only statistically significant data are presented at 6 to 12 months

3. Validated fold changes of selected miRNA Expression in AA iEos vs HS iEos measured using quantitative polymerase chain reaction data of selected miRNA expression. Data presented as log# fold changes in gene expression between AA iEos vs HS iEos. Positive values indicate that gene expression is higher in the plasma of AA group, while negative values indicate that gene expression is higher in the HS group. Differences between the two independent groups were

assessed using the Mann-Whitney U test. A p-value of < 0.05 was considered statistically significant. Only statistically significant data are presented at 6 to 12 months

4. Fold changes and statistical differences of miRNAs in AA rEos Exosomes vs HS rEos Exosomes measured using quantitative polymerase chain reaction data of selected miRNA expression in eosinophil-derived exosomes. Data presented as log# fold changes in gene expression between AA rEos vs HS rEos. Positive values indicate that gene expression is higher in the plasma of AA group, while negative values indicate that gene expression is higher in the HS group. Differences between the two independent groups were assessed using the Mann-Whitney U test. A p-value of < 0.05 was considered statistically significant. Only statistically significant data are presented at 6 to 12 months

5. Fold changes and statistical differences of miRNAs in AA iEos Exosomes vs HS iEos Exosomes measured using quantitative polymerase chain reaction data of selected miRNA expression in eosinophil-derived exosomes. Data presented as log# fold changes in gene expression between AA iEos vs HS iEos. Positive values indicate that gene expression is higher in the plasma of AA group, while negative values indicate that gene expression is higher in the HS group. Differences between the two independent groups were assessed using the Mann-Whitney U test. A p-value of < 0.05 was considered statistically significant. Only statistically significant data are presented at 6 to 12 months

Completion date
01/11/2025

Eligibility

Key inclusion criteria
1. Men and women between the ages of 18-70 years
2. Allergic asthma and sensitization to house dust mites (D. pteronyssinus) allergen, approved with
2.1. Medical history and symptoms more than one year
2.2. Skin prick test positive for D. pteronyssinus (positive wheals are those exceeding 3 mm in diameter greater than the negative control)
2.3. Positive bronchial challenge with methacholine or documented reversible bronchial obstruction
3. Premenopausal women if pregnancy test is negative
4. Healthy subjects without allergic and other chronic respiratory diseases (control group)
5. Participants who gave his/her informed written consent

Healthy volunteers allowed
Yes

Age group
Mixed

Lower age limit
18 years

Upper age limit
70 years

Sex
All

Total final enrolment
72

Key exclusion criteria
1. Asthma exacerbation 1 month prior to study
2. Clinically significant permanent allergy symptoms such as cat or dog dander induced allergy
3. Contraindications to perform an allergy skin test and or bronchial provocation test
3.1. Active airway infection 1 month prior to the study
3.2. Used medicaments
3.2.1. Inhaled glucocorticoids intake 1 month prior to the study
3.2.2. Antihistamines intake 7 days prior to the study
3.3. Short acting beta 2 agonists 12 hours prior to the study
3.4. Long acting beta 2 agonists 2 days prior to the study
3.5. Leukotriene receptor antagonists 14 days prior to the study
4. Contraindications for epinephrine
5. Other significant mental and or internal diseases and conditions which could be exclusion criteria in the opinion of the researcher
6. Alcohol or narcotic abuse
7. Pregnancy
8. Breast feeding

Date of first enrolment
15/01/2021

Date of final enrolment
30/06/2025

Locations

Countries of recruitment
Lithuania

Study participating centre
Department of Pulmonology at the Hospital of Lithuanian University Health Sciences Kaunas Clinics
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Sponsor information

Organisation
Lithuanian University of Health Sciences

ROR
https://ror.org/0069bkg23

Funder(s)

Funder type

Funder Name
GlaxoSmithKline

Alternative Name(s)
GlaxoSmithKline plc., GSK plc., GlaxoSmithKline plc, GSK

Funding Body Type
Government organisation

Funding Body Subtype
For-profit companies (industry)

Location
United Kingdom

Results and Publications

Individual participant data (IPD) sharing plan

IPD sharing plan summary
Not expected to be made available