STUDY TITLE:

BIOMARKERS EVALUATION AND EFFECT OF MEPOLIZUMAB ON LOWER AND UPPER AIRWAYS INFLAMMATION IN SEVERE REFRACTORY EOSINOPHILIC ASTHMA AND NASAL POLYPOSIS

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Partecipants:

PI: Mauro Maniscalco	**ICS Maugeri IRCCS Telese**
Dina Visca and Antonio Spanevello	**ICS Maugeri IRCCS Tradate**
Mauro Carone and Maria Aliani	**ICS Mageri IRCCS Bari**
Antonino di Stefano	**ICS Maugeri IRCCS Veruno**
Andrea Motta	**CNR Pozzuoli Napoli**

Scientific rationale

Patients with severe or difficult-to-treat asthma represent a small amount of total asthmatic patients, but weight on the national health system for the costs of disease management. Chronic rhinosinusitis with nasal polyposis (CRSwNP) [1], which the Italian severe/uncontrolled asthma registry reported with a prevalence of 30% [2], represents a comorbidity that significantly impact lung function and asthma control in severe asthma [3]. Recent evidence indicates that there is a consistent heterogeneity regarding mucosal alterations present in subjects with nasal polyposis involving different pathways: inflammatory cells, remodeling, T cell activation, local IgE production, alteration induced by interactions between microorganisms and epithelial cells.

Hypothesis

Nasal, bronchial and systemic inflammation can differ among patients with severe refractory eosinophilic asthma with or without CRSwNP. Treatment with Mepolizumab could modify nasal, bronchial and systemic inflammation of patients with severe refractory eosinophilic asthma and nasal polyposis acting on disease control.

Objectives (or Research questions)

The aim of the study is to evaluate:

- differences in specific inflammatory pattern and metabolites between severe refractory asthma patients with or without nasal polyposis
- nasal, bronchial and systemic inflammation and metabolomics profile in patients with severe refractory eosinophilic asthma before and after treatment with Mepolizumab.

Study population

We will enroll 10 patients with severe refractory eosinophilic asthma plus CRSwNP and 10 patients with severe refractory eosinophilic asthma and no CRSwNP, for whom treatment with mepolizumab is indicated, according to the AIFA recommendations.

Inclusion criteria

- age >18 years

- diagnosis of severe refractory asthma according to the ERS/ATS criteria ATS

- Blood eosinophils >150 cells/mcL at the screening visit and at least one value of 300 or more eosinophils/mcL during the previous year;

- at least two exacerbations despite maximal therapy with long-acting bronchodilators and high dose inhaled corticosteroids in the previous year or the need for continued therapy with oral corticosteroids in addition to maximal inhaled therapy for at least 6 months in the previous year

- written informed consent.

- For CRSwNP diagnosis, the EPOS 2020 guidelines will be considered as reference.

All the above-mentioned inclusion criteria must be met.

Exclusion criteria

- Pregnancy

- Eosinophilic Granulomatosis with Polyangiitis (EGPA);

- Serious life threatening cardiopulmonary disorders;

- Systemic immunologic disorder in the last 12 months;

- Positive history for malignant tumors ever in patient's life;

- Immunodeficiency.

Study design and methods

Duration of the study.

The study will last 2 years. The enrollment period will last 1 year. The follow-up period will last 1 year. Visits and collection of biological fluids will be performed at baseline and at 6 and 12 months after initiation of treatment with Mepolizumab.

The following parameters will be evaluated

- Clinical history (age of asthma onset, duration of rhinosinusitis symptoms, ASA intolerance, number of asthma exacerbations in the previous year, ACT, ACQ, Morisky Medication Adherence Scale MMAS - 8, Lund-Mackay scores, treatment, etc..);
- Snot 22;
- Nasal polyps score;
- Visual analogue scale (VAS) for main symptoms of CRSwNP
- Lung function;
- Nasal endoscopy evaluation.

Nasal inflammation. Nasal cytology: cytospin preparations for differential cell count of eosinophils, neutrophils, macrophages, T-cells. Nasal secretions by rhinowash: ELISA tests for identification and quantitation of IL-4, IL-5, IL-3, IL-13, IFγ, eotaxin (CCL11), TSLP GMCSF.

Bronchial inflammation. In induced sputum cytospin preparations: differential cell count of eosinophils, neutrophils, macrophages, T-cells.

In bronchial sputum supernatants by ELISA tests: IL-4, IL-5, IL-3, IL-13, IFγ, eotaxin (CCL11), TSLP GMCSF.

In cytospin preparations some selected receptors expressed by eosinophils, neutrophils or macrophages will be analyzed after evaluation of the prevalent secreted cytokines in the supernatants.

Systemic inflammation. Blood eosinophils and neutrophil count; serum specific IgE evaluation with microarray technology; ELISA tests for IL-4, IL-5, IL-3, IL-13, IFγ, eotaxin (CCL11), TSLP GMCSF.

Metabolomic analysis in exhaled breath condensate (EBC)

Pulmonary function tests. Flow rates will be determined using automated equipment (V Max 22 System Sensor Medics, Milan, Italy) according to ATS/ERS recommendations. Static lung volumes will be determined by the helium dilution method.

Nasal cytology. Nasal cytology will be assessed with nasal brushing. Nasal blown secretions will be collected by rhino-wash and processed as previously reported and supernatants stored at -80°C for soluble mediator evaluations [4].

Induced sputum. Sputum induction will be performed using a modification of the method suggested by prof. Howarth. The supernatant will be stored at -80°C for soluble markers determination by high sensitive tests ELISA

Exhaled NO. Exhaled will be measured by an electrochemical analyzer (Hypair FeNO medisoft Exp'air, 2010) according to ATS-ERS recommendations for online measurement of FeNO in adults [5] and as previously shown [6].

Inflammatory markers. Blood will be withdrawn, percentage of blood eosinophils will be determined and serum and plasma stored at -20°C for soluble biomarkers determinations. IL-4, IL-5, IL-3, IL-13, IFγ, eotaxin (CCL11), TSLP GMCSF, will be evaluated in serum or plasma, induced sputum and nasal secretion supernatants with commercially available ELISA [7] .

Furhermore the Humanitas Laboratory under direction of prof. Heffler will assess the following biological markers: Innate Lymphoid Cells 2 (ILC2) in sputum, Migration Stimulating Factor (MSF) Pentatraxin 3 (PTX3) Galectin 3 (GAL3) in plasma and sputum. Interleukina-5 (IL-5) in EBC.

EBC sampling. EBC will be collected with a TURBO-DECCS condenser set at -5.0 ± 1.0 °C (Medivac, Pilastrello, Parma, Italy, Medivac.it). Briefly, all subjects will be asked to breathe at tidal volume through the mouthpiece for 15 min while sitting comfortably and wearing a nose-clip. We will obtain, on average, 2.0 ± 0.3 ml (mean ± SD) of EBC from each subject. EBC samples will be immediately sealed in polypropylene tubes and stored first in dry ice and then at -80°C until NMR acquisition [8].

The salivary contamination of the samples will be tested by measuring their α-amylase activity.

NMR-based metabolomics. Metabolomic analysis will be performed by NMR spectroscopy. 1D and 2D NMR spectra will be recorded at 27°C in a randomized sequential order on a 600-MHz Bruker Avance-III spectrometer equipped with a CryoProbe. Metabolites will be identified by resorting to 2D experiments and comparison with metabolite data banks.

Study Endpoints

The primary outcomes will be:

Evaluation of the differences in specific inflammatory pattern and metabolites between severe refractory asthma patients with or without nasal polyposis and correlation with clinical data.

Variation of specific inflammatory pattern and metabolites between severe asthmatic patients with or without nasal polyposis metabolites after treatment with Mepolizumab.

Statistical Plan

We will use multivariate analysis to discriminate signals/metabolites to identify hidden phenomena and trends in ensembles of NMR spectra. Subregions (buckets) of 0.02-ppm width of the spectra will be integrated using the AMIX 3.9.15 software (Bruker Biospin GmbH, Rheinstetten, Germany). Each bucket will be normalized to the total spectrum area, and the data matrix analyzed with multivariate statistical regressions. Principal Component Analysis (PCA) and Orthogonal Projection to Latent Structures Discriminant Analysis (OPLS-DA) will be carried out with the SIMCA P+14 package (Umetrics, Umeå, Sweden). PCA will first be applied to detect EBC metabolites trends and clusterings in an unsupervised (*i.e.*, no prior group knowledge is used in the calculation) manner, while OPLS-DA will be used to correlate the metabolic variations to pathophysiological changes.

We will also apply pathway topology and biomarker analysis on selected and more representative metabolites in class separation by resorting to MetaboAnalyst 4.0 software. Discriminating metabolites will be selected by evaluating both Variable Importance in Projection values greater than 1 in class discrimination and correlation values $|\rho|$ greater than 0.6.

Data will be reported as mean ± standard deviation or median with interquartile range. Between-group comparisons will be performed by using Analysis of Variance (cross-sectional evaluation) and Analysis of Covariance (longitudinal study) with baseline values as covariates. Depending on the distribution of variable, the use of transformations or non parametric tests will be evaluated. Categorical variables will be reported as percentage and their associations will be analysed by chi-squared or Fisher exact test as appropriate. Because of the exploratory nature of the study, sample size is not formally computed but decided a priori. A p value of <0.05 will be considered statistically significant. The statistical analyses will be carried out using professional statistical software.

Pharmacovigilance

All adverse reactions encountered during the study for both the biological drug and the concomitant drugs will be reported to local pharmacovigilance officers, as required.

Limitations

Patients will be asked to return to the clinic with a frequency not much different to that of routine disease control to reduce possible drop out.

A possible problem could be the unsuitable sputum sample or patient with too compromised lung functions to undergo sputum induction function (FEV1 < 1.5L). We will collect spontaneous sputum when available, while if the patient cannot expectorate or the induced sputum collected is not suitable (sample mainly constituted by saliva), FeNO measurement will be the surrogate evaluation for eosinophilic airway inflammation.

At the co-investigator unit, a specific asthma school is present in order to properly train severe asthmatic patients to manage the disease and to reduce non adherence. This approach will be useful to avoid drop-out and loss of adherence to the protocol.

References

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