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Effect of Biologicals on Alternative Functions of Eosinophils in Severe Asthma

EudraCT: NCT04520165

Scientific Background and goal

Refractory asthma still represents between 3 to 10% of asthmatic patients. These patients represent a high burden in terms of healthcare costs. Biotherapies have been developed within the last years to reduce the use of oral corticosteroids (OCS), which are responsible for long-term and costly side effects. Monoclonal antibodies directed against interleukin (IL)-5 or its receptor (IL-5R) are approved to treat severe eosinophilic asthma with tangible improvements of the patient conditions. But it appears that the patients do not respond the same way to biotherapies.

In addition to the "super-response", a new concept of asthma "remission" has recently emerged as a therapeutic target to achieve after 12 months of treatment and was defined according to results of randomized control trials (RCT) as obtaining asthma control, no exacerbation, no treatment with OCS while there is no consensus on the importance of improvement in lung function and reduction in type-2 (T2) inflammation in the current definition of remission.

Post-hoc analysis of RCT looked at the predictive factors of response to benralizumab or mepolizumab in vast cohorts of patients and observed that high blood eosinophil level was linked to a better improvement in terms of reduction of exacerbation rate. Even if blood eosinophils help to predict a general response to anti IL-5/ anti IL-5R, biomarkers reflecting local inflammation, such as those measured in induced sputum, have a better potential to predict the intensity of response to biologics as they reflect what really happens in the bronchi. So are there biomarkers of remission after therapy targeting IL-5 in the sputum of severe eosinophilic asthmatics? There are, to our knowledge, no studies analyzing inflammatory mediators as predictors of response after one year of anti-IL-5 treatment directly in the sputum itself in severe eosinophilic asthmatics. The goal of this paper is to analyze the role of mediators of the T2 cascade in the sputum as predictors of remission.

Study design and methods

Patients

Fifty-two severe asthmatics initiated with an anti-IL-5 (51 with Mepolizumab and one with Reslizumab) were recruited from our asthma clinic. This observational study was performed at the CHU of Liege, Belgium, between 2014 and 2021. Inclusion criteria included a diagnosis of asthma defined by the Global Initiative for Asthma (http://ginasthma.org/), severe asthma was defined according to European Respiratory Society (ERS)/ American Thoracic Society (ATS) criteria. The treatment was stable for all patients at the time of the sampling and the time between baseline and the next evaluation was in average one year. Remission was defined as patients combining after the instauration of the biotherapy: no oral corticosteroids, no exacerbation, ACQ lower than 1.5 and/or asthma control test (ACT) greater than 19 and, a FEV_1 ≥ 80% predicted and/or an improvement of FEV_1 ≥ 10% and a blood eosinophil count lower than 300 cells/µl.

Sub-analyses were also performed to assess the predictive values of sputum mediators in terms of response for some specific parameters alone, such as: an improvement of ACT greater than 19 post-treatment, a decrease of ACQ lower than 1.5 post-treatment, a decrease of sputum eosinophil under 3% or by 50%, stopping OCS post-treatment, no more exacerbations post-treatment and improvement of FEV_1 of at least 10% post-treatment.

A diagnosis of nasal polyps by an Ear-Nose-Throat specialist and a diagnosis of atopic dermatitis or urticaria done by a dermatologist were reported.

This study was approved by the Ethics committee of CHU Liege (2005/181) and all subjects gave written informed consent for participation. The study ClinicalTrials.gov Identifier is clintrial.org EudraCT: NCT04520165.

Study design

This study aims to investigate the airway expression of mediators of the T2 cascade as predictors of remission after anti-IL-5 treatment in a cohort of patients with severe eosinophilic asthma. To detect a difference between groups with a power of 70-80% and a significance level of 5%, the sample size was estimated as 10-13 patients per group (https://www.sealedenvelope.com/). These calculations were based on a significant difference of sputum IL-5 obtained as preliminary results. Usually, in the different studies, even if there is no consensus on the remission definition in the asthma context, there is mention of a percentage around 20% of patients exhibiting a good response to anti-IL-5. Consequently, with a cohort of 52 patients, we expected a sample size of at least 10 patients in the remission group.

Respiratory function

FeNO was measured using NiOX (Aerocrine, Solna, Sweden) at a flow rate of 50 mL/s. Spirometry was performed before and after bronchodilation according to the ATS/ERS standard criteria.

Blood samples

Blood samples of patients were analyzed by the routine laboratory of the University Hospital of Liege for leukocyte count, c reactive protein (CRP) and fibrinogen levels.

Sputum induction and processing.

The sputum was induced and processed as previously described and the sputum supernatant was collected before the addition of dithiothreitol (DTT), which could impact the measurements. Cell viability was determined by trypan blue exclusion and the differential cell count was performed by counting 500 non-squamous cells on cytospins stained with May-Grünwald-Giemsa.

Sputum inflammatory mediators measurement

Eosinophil peroxidase (EPX) was measured by classic ELISA (Mybiosource, CA, USA). Detection limit was 5.4 ng/ml. Immunoglobulin E (IgE) levels were measured with Human IgE ELISA kit from Abcam (Amsterdam, Netherlands). The detection limit was 30.5 pg/ml. Finally, interleukin (IL)-3, IL-4, IL-5, IL-13, IL-25, IL-33, granulocyte-macrophage colony-stimulating factor (GM-CSF), thymic stromal lymphopoietin (TSLP) and eotaxin-1 were measured by multiplex electrochemiluminescent assays (Meso Scale Discovery). This technic was already used with success in sputum supernatant in the past and spiking recovery were optimal.

Statistical analysis

Categorical variables are presented as numbers and percentages, continuous variables are presented as mean ± standard deviation or as median (interquartile range P25-p75) when appropriate.

The normality of continuous variables was assessed by Shapiro-Wilk test (normal distribution was found for age, BMI, ACQ, AQLQ, pulmonary function parameters, fibrinogen level, and sputum neutrophil percentage). To investigate baseline characteristics of patients remission group, Chi-square or Fisher exact test was used for categorical variables and Student t test or Mann-Whitey U test was performed for continuous variables when appropriate.

Univariate logistic regression was considered to model the association between patient remission status and demographic, clinical and biological variables. Results of logistic regression were presented using odds ratios and corresponding 95% confidence interval.

All tests were performed 2-sided and $p<0.05$ was considered significant. All statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC), except for ROC curve calculation, GraphPad Prism 7 (GraphPad Software San Diego, CA, USA) was used. The p-values associated with the ROC curves tested the null hypothesis that the area under the curve equals 0.50.