Clinical, functional and biological characterization of neutrophilic asthma phenotype and endotypes

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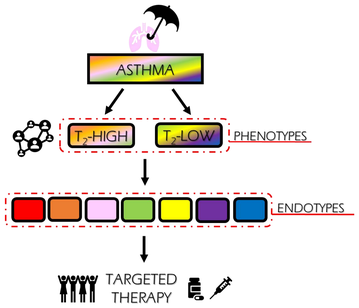
INTRODUCTION

Asthma is a complex and heterogeneous condition, characterized by chronic airway inflammation and remodeling. It can be seen as an "umbrella-like" diagnosis embracing several atopic/non-atopic diseases with different ages of onset, defined by a history of respiratory symptoms, variable and reversible airflow limitation and bronchial hyperresponsiveness.

Two major asthma phenotypes have been defined, according to the inflammatory status of the subjects: **eosinophilic** (type 2 (T2) high) – characterized by *T2 inflammation* and high blood/sputum eosinophilia, mainly mediated by T2 cytokines: **non-eosinophilic** (T2 low) – characterized by the *absence of T2 inflammatory signature*. It is associated with neutrophilic, pauci-granulocytic or mixed inflammatory infiltrate. Knowledge of T2 low asthma is poor and patients have an unmet clinical need.

Neutrophilic asthma, in particular, is characterized by severe refractory disease and it is thought to be associated with altered innate immune response and activation of T_H1/T_H17 pathways.



AIM & METHODS

AIM

The study aims to characterize the molecular pathways associated with neutrophilic asthma.

METHODS

We selected 46 bronchial biopsies obtained from mild-to-severe asthmatics and divided them into: **neutrophilic** (NEU) (n=23; NEU counts ≥47.17 cells/mm^2) and **eosinophilic** (EOS) (n=23; EOS counts ≥12.45 cells/mm^2 and NEU counts <47.17 cells/mm^2).

Through immunohistochemistry we assessed the expression of T_H1/T_H17-related molecules in the bronchial submucosa of the patients. We, then, collected clinical and functional data.

Cell counts were performed on immune-stained tissues at 40X magnification, by the same operator, who was blinded to subjects ID and group. All the positive cells in the submucosa have been quantified in the 100 um area beneath the epithelial basement membrane in several non-overlapping fields. The final result, expressed as cells per mm^2, was calculated as the average of all counts performed in each bronchial biopsies.

RESULTS

NEU did not differ from EOS for age, asthma severity, pre-bronchodilator FEV_1, and incidence of atopy, OCS use and exacerbation rate, while ICS dose was higher in NEU [TABLE 1].

	NEU (N=23)	EOS (N=23)
SEVERE/MILD	13/10	6/17
AGE, years	49.8	51.0
ALLERGY	17/23 (73.9%)	14/23 (60.9%)
FEV_1 pre (% pred.)	79.3±24.6	91.4±15.8
ICS/day (µg fluticasone HFA eq.)	596.6±430.6	317.9±251.1*
OCS (≥6 months/year)	3/23 (13.0%)	1/23 (4.3%)
FREQUENT EXACERBATOR (≥2exa/year)	6/23 (26.1%)	6/23 (26.1%)

TABLE 1 – The table shows **clinical and demographic data**.
Continuous variables are presented as mean ± SD. Incidences are reported as occurence/exposed cases and percentage. * p < 0.05
Abbreviations: Exa = exacerbations; HFA = hydrofluoroalkane; ICS = inhaled corticosteroids; OCS = oral corticosteroids

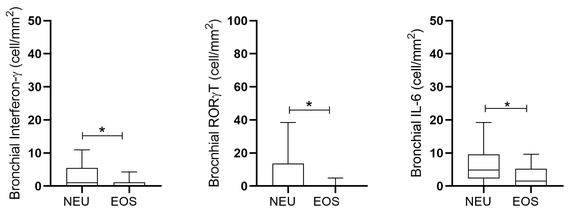


FIG. 1 – The panel shows the relative boxplots of the average counts of **RORγT, Interferon-γ** and **IL-6** in bronchial biopses.
* p < 0.05

The number of RORγT$^+$, IFNγ$^+$, and IL-6$^+$ cells was higher in NEU than EOS (p<0.05) *[FIG.1]*.

No statistical difference was observed in the expression of inflammasome pathway molecules (IL-18, IL-1β, NLRP3, cas1) or other NEU-related markers (STAT1, IL-8, IL-6R, YKL40) *(data not shown)*.

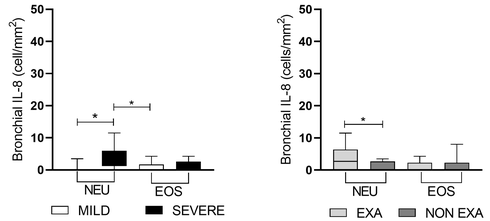


FIG. 2 – The panel shows the relative boxplots of the average counts of **IL-8** in bronchial biopses, divided into **mild** vs **severe** (on the left) or **exacerbators** (EXA) vs **non-exacerbators** (NON EXA) (on the right).
* p < 0.05

We, then, stratified the two groups in **mild** vs. **severe** and **exacerbators** (EXA) (≥2/years) vs. **non-exacerbators** (NON-EXA).

Both NEU-severe and NEU-EXA had higher IL-8$^+$ cells than NEU-mild and NEU-NON-EXA, respectively (p<0.05) *[FIG.2]*.

Conclusions

Our results suggest that the pathways associated with **T_H1/T_H17 responses are predominant in neutrophilic asthma**. This is in line with our previous observation of an higher expression of IL-17F in the bronchial submucosa of patients with neutrophilic asthma.

Conflict of Interests: the study has been funded by Chiesi Farmaceutici spa.