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## Nasal IL-17F is related to bronchial IL-17F/neutrophilia and exacerbations in stable atopic severe asthma

Sorbello V, Ciprandi G, Di Stefano A, Massaglia GM, Favatà G, Conticello S, Malerba M, Folkerts G, Profita M, Rolla G, Ricciardolo FL.

### Abstract

Severe asthma (SA) is associated with neutrophil recruitment and T helper (T<sub>H</sub>)17 chemokine overexpression in bronchial biopsies. We aimed to evaluate IL-17A and IL-17F expression in nasal/bronchial lamina propria of atopic mild-to-severe asthmatics and controls in relation to neutrophilia and asthma exacerbations. Cryostat sections of nasal/bronchial biopsies obtained from 14 SA and 14 mild asthma (MA) stable atopic patients with rhinitis, and seven healthy controls were analyzed by immunohistochemistry for neutrophils, IL-17A and IL-17F expression. Atopic SA showed an increase in asthma exacerbations number, IL-17F and IL-17A expression in nasal/bronchial lamina propria compared to MA and controls, and a higher expression of bronchial neutrophils in SA compared to MA and controls. In all asthmatics, significant relationships were found between bronchial IL-17F and neutrophils/FEV<sub>1</sub>, nasal IL-17F and bronchial neutrophil/IL-17 markers and between the latter and exacerbations, suggesting that nasal IL-17F might be informative on bronchial IL17-driven neutrophilia in atopic SA.

Th17 cells seem to play a relevant role in severe asthma (SA) [1-3]. These cells are characterized by interleukin (IL)-17A and IL-17F production whose expression is supposed to be in relation to neutrophilic inflammation [4]. IL-17A and IL-17F act on several cell types, which respond with upregulation of pro-inflammatory cytokines and chemokines [4]. SA, characterized by steroid dependence/resistance and poor control [5], affects only the 10% of all asthmatics but represents a relevant economic burden for healthcare resources [6]. Among risk factors for asthma severity, there are upper airways disorders [7] and exacerbations [8]. It is still unclear the impact of upper on lower airways inflammatory processes in SA.

Because of the close relationship between upper and lower airways in asthma and between bronchial Th17-related cytokines and SA, we aimed to evaluate the expression of the Th17-related cytokines IL-17A and IL-17F in nasal and bronchial biopsies obtained from atopic mild-to-severe asthmatics and controls in relation to neutrophilia and exacerbations.

### Materials and methods

We analyzed bronchial biopsies from 14 stable SA, 14 stable mild asthma (MA), and from seven healthy controls (Table 1). Asthma diagnosis and severity were defined according to GINA and ERS/ATS guidelines [5, 9]. Atopy was assessed by skin prick tests and all subjects performed lung function test as previously described [10]. The study was approved by the Local Ethics Committee (San Luigi Hospital: protocol 1759, 2008) and conformed to the Declaration of Helsinki; written informed consent was obtained from each subject.

Table 1 Subjects' characteristics

	Sex	Smoke	Atopy	Rhinitis	Sinusitis		FEV <sub>1</sub> preb2	FEV <sub>1</sub> postb2	Asthma onset	Exacerbations	BECL0 daily
Age (years)	M/F	yes/no	yes/no	yes/no	yes/no	FeNO (ppb)	(%pred)	change (l)	(early/late)	frequency (N)	dose (lg)
Controls (N = 7)	48 ± 6 3/4	0/7	0/7	0/7	–	12 ± 2	120 ± 12	–	–	–	–
Mild (N = 14)	43.6 ± 8 7/7	0/14	14/0	14/0	7/7	32 ± 14**	98 ± 14**	+308 ± 166	7/7	0.7 ± 1	179 ± 79†

Severe (N = 14)	49 ± 10	7/7	0/14	14/0	14/0	9/5	29 ± 20**	59 ± 11**	+238 ± 47	7/7	2.7 ± 1.9§§	757 ± 285§§†
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FEV<sub>1</sub> preb2: baseline FEV<sub>1</sub>; FEV<sub>1</sub> postb2 change: FEV<sub>1</sub> postb2 change from baseline. Beclomethasone (BECLO) equivalent daily dose represents the dose of daily inhaled corticosteroids transformed in an equivalent dose of beclomethasone. Asthma onset: early ≤ 14 years; late ≥ 15 years.

Data are expressed as mean ± SD. For functional data, the comparisons between groups were performed by the Student's *t*-test. For other parameters, the Mann–Whitney *U*-test was applied.

†Only one mild asthmatic was not on inhaled corticosteroid therapy.

‡Only one severe asthmatic was on oral corticosteroid (prednisone 5 mg/day).

\**P* < 0.05, \*\**P* < 0.01 significantly different from controls; §*P* < 0.05, §§*P* < 0.01 significantly different from mild asthma.

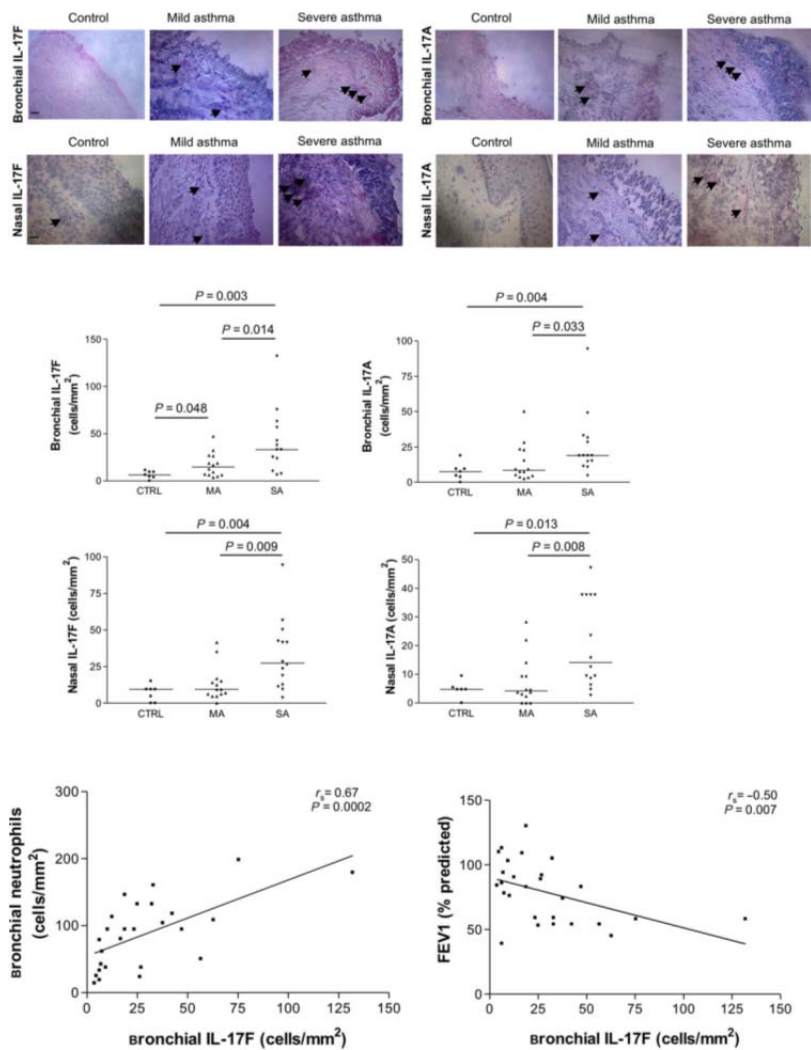
Bronchoscopy and rhinoscopy (1 week later) were performed in all subjects using a flexible fiber-optic bronchoscope (Pentax FB-18P; Asahi Optical Co. Ltd, Tokyo, Japan) and a flexible fiber-optic nasal endoscope (ENF Type P4; Olympus, Tokyo, Japan), respectively, after the appropriate premedication [for bronchoscopy: as previously described [10]; for nasal endoscopy: 0.05% oxymetazoline moistened nasal cottonoids with Xylocaine and 1 : 100 000 adrenaline solution for 10 min before procedure]. Bronchial (segmental/lobar airways) and nasal (inferior turbinate) biopsies were gently extracted from size 19 cupped [10] and 45° upward angle Blakesley forceps, respectively, and processed for light microscopy (40× magnification) as previously described [10]. Nasal and bronchial cryostat sections were immunostained using goat anti-IL-17A and IL-17F (R&D Systems, Minneapolis, MN, USA), and mouse antineutrophil elastase (DAKO Cytomation, Milan, Italy); immunostained cells were quantified as previously described [10].

Data, expressed as mean ± SD for clinical/functional variables and as median (range) for morphological parameters, were analyzed using GraphPad Prism program (GraphPad 5.0 Software Inc., San Diego, CA, USA). We assumed a normal distribution for clinical/functional data and a non-normal distribution for morphological parameters applying, respectively, the analysis of variance (anova) or the nonparametric Kruskal–Wallis test for multiple comparisons and the Student's *t*-test or the Mann–Whitney *U*-test for comparisons between groups. Spearman's rank method was used for correlations. *P* < 0.05 was considered statistically significant.

## Results and discussion

We observed that the number of IL-17F<sup>+</sup> cells was significantly higher both in nasal and bronchial lamina propria of SA compared to MA (*P* = 0.009 and *P* = 0.014, respectively) and controls (*P* = 0.004 and *P* = 0.003, respectively) (Fig. 1A,B); IL-17F<sup>+</sup> cells were significantly higher in bronchial lamina propria of MA compared to controls (*P* = 0.048, Fig. 1A,B). Similarly, the number of IL-17A<sup>+</sup> cells was greater both in nasal and bronchial lamina propria of SA compared to MA (*P* = 0.008 and *P* = 0.033, respectively) and controls (*P* = 0.013 and *P* = 0.004, respectively) (Fig. 1A,B). The number of neutrophils was increased in bronchial, but not in nasal, lamina propria of SA (110 ± 46 cells/mm<sup>2</sup>) compared to MA (64 ± 45 cells/mm<sup>2</sup>, *P* = 0.017) and controls (29 ± 10, *P* = 0.0004).

**Figure 1.**

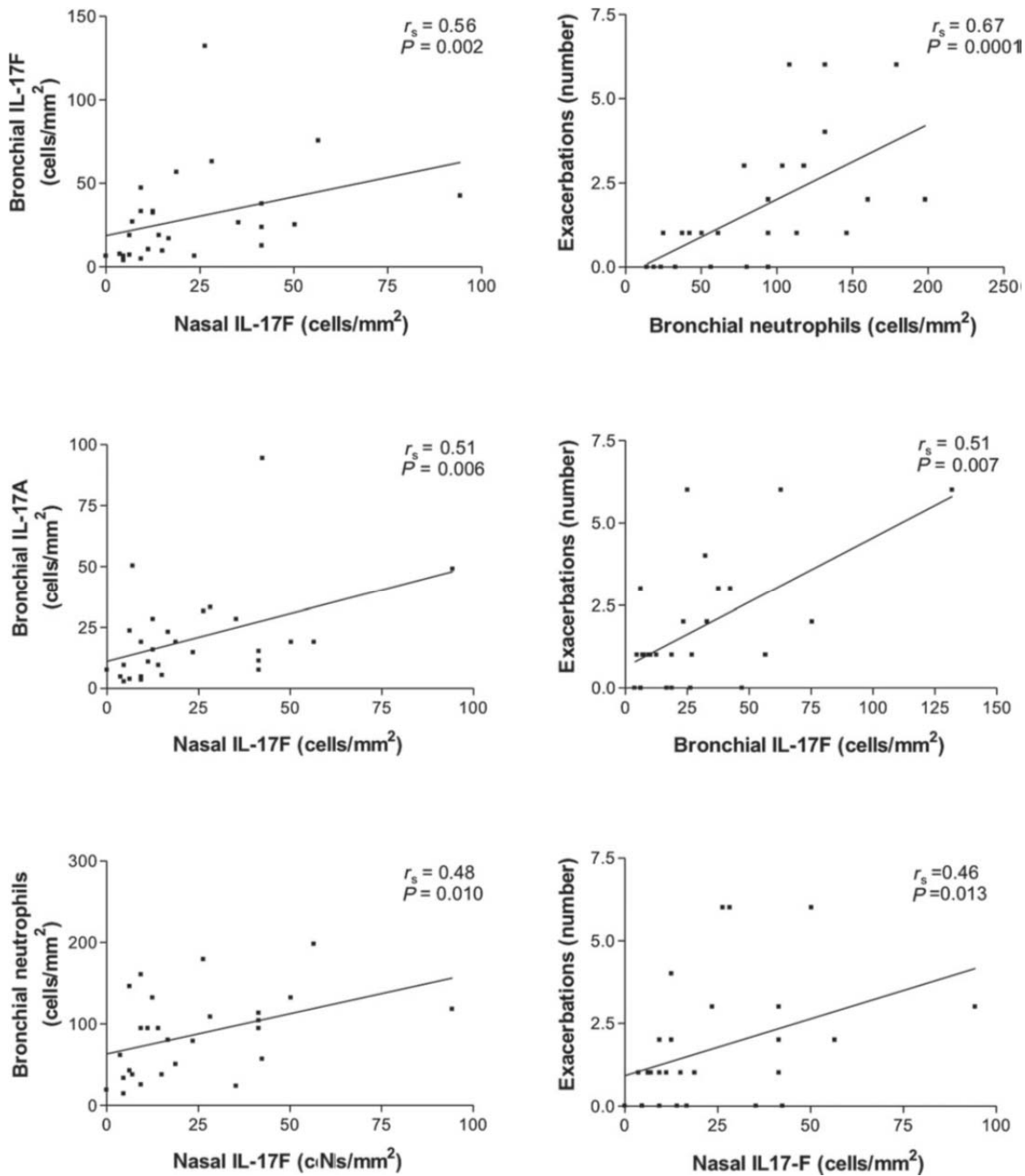


IL-17F and IL-17A expression in bronchial and nasal biopsy specimens obtained from severe asthma (SA) and mild asthma (MA) atopic patients and from controls, and correlations with inflammatory cells and clinical parameters. (A) Photomicrographs showing the immunostaining for IL-17F and IL-17A in the bronchial and nasal lamina propria of SA and MA and of controls. Original magnification 40 $\times$ . Internal scale: 25  $\mu$ m. Arrows indicate positive cells. (B) Number of IL-17F<sup>+</sup> and IL-17A<sup>+</sup> cells/mm<sup>2</sup> of bronchial and nasal lamina propria from SA and MA and from controls. Each bar indicates the median value in the respective groups. (C, D) Correlations between the number of bronchial IL-17F<sup>+</sup> cells and the number of bronchial neutrophils (C) or FEV<sub>1</sub> (%predicted) (D) in all asthmatics. The correlation coefficient was obtained using the Spearman rank method ( $r_s$ ).

The number of bronchial IL-17F<sup>+</sup> and IL-17A<sup>+</sup> cells correlated positively with bronchial neutrophils [IL-17F:  $r = 0.67$ ,  $P = 0.0002$  (Fig. 1C); IL-17A:  $r = 0.39$ ,  $P = 0.044$ ] and negatively with FEV<sub>1</sub>% predicted [IL-17F:  $r = -0.50$ ;  $P = 0.007$  (Fig. 1D); IL-17A:  $r = -0.37$ ,  $P = 0.049$ ].

The number of nasal IL-17F<sup>+</sup> cells significantly correlated with the number of IL-17F<sup>+</sup> ( $r = 0.56$ ,  $P = 0.002$ ; Fig. 2A), IL-17A<sup>+</sup> ( $r = 0.51$ ,  $P = 0.006$ ; Fig. 2B) cells, and neutrophils ( $r = 0.48$ ,  $P = 0.01$ ; Fig. 2C) in bronchial biopsies. Bronchial neutrophils ( $r = 0.67$ ,  $P = 0.0001$ ; Fig. 2D), bronchial IL-17F<sup>+</sup> cells ( $r = 0.51$ ,  $P = 0.007$ ; Fig. 2E), and nasal IL-17F<sup>+</sup> cells ( $r = 0.46$ ,  $P = 0.013$ ; Fig. 2F) significantly correlated with exacerbations number.

**Figure 2.**



(A–C) Relationship between the number of nasal IL-17F<sup>+</sup> cells and the number of bronchial IL-17F<sup>+</sup> cells (A), bronchial IL-17A<sup>+</sup> cells (B), or bronchial neutrophils (C) in all asthmatics. (D–F) Relationship between the number of bronchial neutrophils (D), bronchial IL-17F<sup>+</sup> cells (E), or nasal IL-17F<sup>+</sup> cells (F) and the number of exacerbations in all asthmatics. The correlation coefficient was obtained using the Spearman rank method ( $r_s$ ).

These data indicate that atopic SA is featured by a distinct neutrophilic phenotype with increased expression of Th17-related cytokines (IL-17F and, to a lesser extent, IL-17A) in both bronchial and nasal lamina propria.

We demonstrated a positive correlation between IL-17F and neutrophils count in bronchial lamina propria, for the first time, and a negative correlation between bronchial IL-17F and FEV<sub>1</sub>, suggesting a direct role for IL-17 in neutrophils recruitment and in pulmonary function decline in asthma in agreement with Hamid's study [2] and partly in contradiction with Doe's study [11], all based on asthmatic populations unselected

for atopy. We evidenced a positive correlation between the number of exacerbations and of bronchial neutrophils or of bronchial and nasal IL-17F<sup>+</sup> cells indicating that IL-17 can be related to exacerbations which are a strong risk factor for lung function decline [8, 12] and near-fatal event in SA [6].

We found that only bronchial IL-17F was slightly increased in MA compared to controls in line with previous studies in sputum and BAL [13] and in bronchial biopsies [11] suggesting that a minimal persistent IL-17-driven inflammation is a weak feature of stable mild atopic asthma.

We also showed that SA is characterized by a more intense Th17-polarized nasal inflammation than MA highlighting the close pathogenic association between nose and bronchi and suggesting an interaction between upper and lower airway inflammation [14]. The augmented expression of nasal IL-17F in atopic SA and the relationship between nasal IL-17F and bronchial IL-17F, IL-17A, and neutrophils suggest that IL-17-related nasal inflammation reflects the inflammatory pattern observed in SA bronchi. Thus, in SA, the inflammation assessment could be addressed, at least as first line, at nasal level. A former study provided clear evidence that nasal cytology closely correlates with bronchial inflammation evaluated by biopsy, so the nose may be considered the window of bronchi in asthma [15]. To our knowledge, this is the first study comparing bronchial and nasal mucosa in relation to IL-17 pattern in asthma; we found a report in nasal polyps of chronic rhinosinusitis with asthma, with unknown atopic status, showing a higher expression of IL-17A compared to normal sinus mucosa in controls and a correlation between IL-17A<sup>+</sup> cells and eosinophils in sinus mucosa suggesting that IL-17A plays a role in eosinophilic inflammation of nasal polyps of chronic rhinosinusitis with asthma [16].

In conclusion, in this study we demonstrated that the atopic SA phenotype is characterized by IL-17-driven neutrophil infiltration, both in nasal and bronchial lamina propria, also confirmed by the strong relation between nasal IL-17F expression and bronchial neutrophil/IL-17 markers, suggesting that nasal IL-17F inflammation might be informative of bronchial IL-17 inflammatory pattern associated with bronchial neutrophilia in SA prone to asthma exacerbations. We may speculate that early recognition of nasal IL-17F-related inflammation in atopic SA could be clinically relevant in selecting the 'frequent exacerbator' and steroid-resistant patient potentially responding to anti-IL-17 biologic therapy.

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