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Serum cytokine profiles as predictors of asthma control in adults from the EGEA study

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ABSTRACT

Background: To which extent serum cytokines may predict asthma control in adults remains understudied.
Objectives: We investigated cross-sectional and longitudinal associations between cytokine profiles and asthma outcomes.
Methods: Serum interleukin (IL)-1Ra, IL-5, IL-7, IL-8, IL-10, IL-13 and TNF-α levels were determined in 283 adults with current asthma from the 2nd survey of the Epidemiological Study on the Genetics and Environment of Asthma (EGEA2). Participants were followed-up seven years later. Asthma symptom control was assessed according to GINA 2015 guidelines. Cytokine profiles were identified by principal component (PC) analyses, and expressed as above/below the median.
Results: The first two PCs captured 82.5% of the variability. While all seven cytokines scored high on PC1, only IL-1Ra and IL-10 scored high on PC2. At EGEA2, neither PC1 nor PC2 were related to exacerbations, asthma attacks, asthma symptom control, lung function, or allergic diseases. High level of PC1 (above the median) was associated with higher blood neutrophil counts (P = 0.02), while high level of PC2 was associated with lower IgE levels (P = 0.04). High level of PC2 at EGEA2 was associated with lower bronchial hyperresponsiveness (adjusted(a) OR[95%CI] = 0.46[0.23; 0.91]) and with subsequent lower risk of worsening asthma control and attacks (aOR[95%CI] = 0.24[0.09; 0.60]; 0.31[0.11; 0.85] respectively).
Conclusions: Serum cytokine profiles with high levels of IL-1Ra and IL-10 were associated with lower subsequent risks of worsening asthma control and attacks in adults. This study adds new findings for the role of serum cytokine profiles to help identifying adults with subsequent risk of asthma burden that could be targeted for specific therapies.

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1. Introduction

Asthma is a chronic disorder of the airways, in which cytokines are likely to play a key role in the persistent inflammation and in the pathogenesis of the disease [1]. Recently, our understanding of the underlying mechanisms of asthma pathophysiology has evolved, opening new possibilities for disease management such as the development of target-directed therapies with anti-Immunoglobulin (Ig)E or anti-interleukin (IL)-5 and anti-IL-13 antibodies, but these therapies may be ineffective in some patients [2,3]. Although cytokines are at the core of recently developed asthma therapies [4,5], little is known about the association between serum cytokine levels and asthma control outcomes.

To better understand the inflammatory mechanisms in relation with asthma control outcomes, cytokine levels have been often measured after direct and invasive sampling procedures i.e. bronchoalveolar lavage (BAL) or induced sputum [6–9]. The measurement of cytokine levels in serum may be a valuable alternative, as standardized collection of peripheral blood is practical among participants of all ages for large epidemiological studies and for routine clinical practice [10,11]. Furthermore, multidimensional approaches for data analysis may allow the identification of pools of cytokines with diagnostic performance superior to single cytokines in terms of sensitivity, specificity and robustness [12]. To our knowledge, only two studies, a case-control study [8] and a cross-sectional study [13], have evaluated the associations between cytokines profiles in sputum or in serum and asthma control outcomes, lung function, blood granulocyte counts and IgE. However, no study has focused on associations between serum cytokine profile and evolution of asthma control outcomes in adults.

In this paper, serum levels of cytokine panel were determined in adults with current asthma from the longitudinal Epidemiological study on the Genetics and Environment of Asthma (EGEA). The cytokine panel included IL-5, IL-7 and IL-13, inflammatory cytokines implicated in eosinophilic asthma triggered by the TH2 pathway, IL-8, crucial in neutrophil recruitment [14–16], and Tumor Necrosis Factor-alpha (TNF-α) that has a central role in systemic inflammation [10]. Additionally, this panel included two cytokines that are less often investigated in asthma [12]: Interleukin (IL)-1 receptor antagonist (IL-1Ra), an antagonist of IL-1 with anti-inflammatory activity [17], and IL-10 able to inhibit pro-inflammatory cytokine synthesis [18,19].

The aim of this study was to build a cytokine signature and evaluate its association with asthma control outcomes in a cross-sectional and longitudinal setting.

2. Methods

2.1. Study design and population

EGEA study (https://egeanet.vjf.inserm.fr/) is a French cohort study based on an initial group of asthma cases and their first-degree relatives, and controls (first survey EGEA1). The protocol and descriptive characteristics have been described previously [20,21], and in the supplementary data.

The present analysis included 283 asthma cases and their first-degree relatives with current asthma who were adult at the second survey (EGEA2, $\geq$16 years old) and were followed-up at EGEA3, about 7 years later. Only participants with available data for serum cytokines measurements were considered (supplementary Figure E1). Participants who reported other respiratory or cardio-respiratory diseases, cancer, diabetes, other chronic inflammatory diseases, tuberculosis (as significantly associated with cytokine levels) or who had smoked one hour prior to blood sampling were excluded. Participants selected for the analysis were younger, more often non-smokers, had lower Body Mass Index (BMI) and a better lung function than those not selected (n = 145, supplementary Table E1).

Ethical approval was obtained from the relevant institutional review board committees (Cochin Port-Royal Hospital and Necker-Enfants Malades Hospital, Paris). All participants signed a written informed consent.

2.2. Respiratory phenotypes

Inclusion criteria used to define asthma cases were based on self-reported positive responses to four questions from the validated and standardized British Medical Research Council, European Coal and Steel Community, American Thoracic Society (ATS) and European Community Respiratory Health Survey questionnaires: "*Have you ever had attacks of breathlessness at rest with wheezing?*", "*Have you ever had asthma attacks?*", "*Was this diagnosis confirmed by a physician?*" and "*Have you had an asthma attack in the last 12 months?*", or a positive response to at least two questions and a positive review of the medical records. Asthma in first-degree relatives of asthma cases was defined as a positive answer to at least one of the first two questions [22,23].

Among participants with asthma (*ever asthma*), "*current asthma*" was defined by a report of respiratory symptoms (wheeze, nocturnal chest tightness, attacks of breathlessness following strenuous activity, at rest or at night time, and asthma attacks) or use of inhaled and/or oral medicines because of breathing problems in the past twelve months.

Asthma exacerbation was defined by hospital or emergency admission because of respiratory problems or the use of oral corticosteroids for breathing difficulties in the last year. Exacerbations not requiring hospital or emergency admissions or use of oral corticosteroids was defined as asthma attack (considered as mild exacerbations).

Asthma symptom control at EGEA2 and EGEA3 has been assessed in 3 classes, over a 3-month period, using responses to survey questions to approximate as closely as possible the GINA 2015 definition, and as previously used [24]. Participants were defined with controlled, partly-controlled and uncontrolled asthma if they had none, 1–2 or 3–4 of the following criteria, respectively: frequent daytime symptoms (defined by at least 1 asthma attack or 1 or more trouble breathing per week in the past 3 months), any night-time symptoms (defined by woken up because of asthma or by an attack of shortness of breath in the last 3 months), frequent use of reliever medication (defined by on average twice a week in the past 3 months), and any activity limitation (defined by the following answer "totally limited", "extremely limited", "very limited", "moderate limitation", "some limitation" to the question "*Overall, in all the activities that you have done during the last two weeks, how limited have you been by your asthma?* ").

Asthma symptom control evolution between EGEA2 and EGEA3 was categorized as "worsening" if participants moved from controlled to partially controlled/uncontrolled asthma or from partially controlled to uncontrolled asthma, "stable" if there is no change in asthma symptom control status, and "improved" if they progressed from uncontrolled to partially controlled/controlled asthma or from partially controlled to controlled asthma. Similarly, asthma attack evolution was categorized as "worsening" (switch from no asthma attacks at EGEA2 to having asthma attacks at EGEA3), "stable" (no change), and "improved" (from having asthma attacks to no asthma attacks). Changes in asthma exacerbations between EGEA2 and EGEA3 were not computed due to the small sample size of participants in subgroups.

Allergic Rhinitis was defined by a positive answer to *"Have you ever had allergic rhinitis?"* or *"Have you ever had hay fever?"*, and a

positive answer to nasal symptoms: *"Have you had a problem with sneezing or runny or blocked nose when you did not have a cold or the flu in the last twelve months?"*.

Eczema ever was defined as a positive answer to *"Have you ever had eczema"*.

A lung function test with spirometry and methacholine challenge was performed at EGEA2 using a standardized protocol with similar equipment across centers according to the ATS/European Respiratory Society guidelines [25]. More details are given in supplemental data.

2.3. Medication

Information on use of controller medication for asthma is available in 274 of the participants (96.8%): 131 participants (47.8%) did not take any medication in the last twelve months. Among the 143 participants who reported medication use in the last twelve months, 67.8% (n = 97) reported inhaled corticosteroids (ICS) use only, and 28% (n = 40) reported ICS use in combination with long acting B2-agonists (LABA) and/or antileukotriene. Only 5 participants reported antileukotriene use only, and one participant reported LABA use only.

2.4. Cytokine measurements

An inflammation cytokine 9-plex assay was used. This panel included IL-1Ra, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, TNF-α and Leptin (Bio-Plex Pro human inflammation cytokine 9-plex assay, Bio-Rad Laboratories, Hercules, California, United States). Determination of serum cytokine levels (at EGEA2) was carried out at the IMIM (*Instituto Hospital del Mar de Investigaciones Médicas*, Barcelona, Spain) using the Luminex xMAG® technique in a BioPlex system. Analyses were restricted to IL-1Ra, IL-5, IL-7, IL-8, IL-10, IL-13 and TNF-α because, as expected, around 50% of the measurements of IL-6 were undetectable, and because leptin has multiple roles that are largely beyond inflammation [26]. No value above or below the limit of detection (LD) was observed for IL-7 and IL-13 measurements. Less than 3% of the values were above or below the LD for other cytokines. Values below the lower limit of detection (LLD) were assigned the LLD/2 and values above the upper limit of detection (ULD) were assigned the ULD+1. An aliquot of the same human serum pool was run by duplicate in each plate. In order to minimize inter-plate variability, each serum cytokine value was divided by the pool result obtained from the same plate and expressed as ratio. These ratios were included in the statistical analyses.

2.5. Statistical analyses

The Pearson's correlation coefficient was used to estimate correlations between cytokine levels. Principal Component Analysis (PCA) was performed by using the PRINCOMP procedure in the Statistical Analysis System (SAS) software to reduce the dimension of the cytokines data set (n = 7) to a few uncorrelated components that account for a meaningful amount of the variance contained in the original set of variables. Each PC produced from PCA corresponds to a different linear combination of the measured quantities [27]. To rigorously confirm the partitioning of the data set, the VARCLUS procedure was also performed (http://www.math.wpi.edu/saspdf/stat/chap68.pdf). The principal components (PC) obtained from PCA were expressed as above/below the median (high/low PC) as a result of their skewed distribution, and for clinical meaningfulness.

The associations between PCs and age, sex, BMI, smoking and medication use were explored. PCs were then studied in association with asthma control outcomes (exacerbation, attack, and asthma symptom control), lung function and methacholine challenge test, allergic diseases, blood IgE levels and neutrophil and eosinophils counts at EGEA2 (cross sectional analysis). The association between PCs and neutrophil counts was further studied in participants without respiratory infections in the past four weeks, which may affect blood neutrophil counts. PCs were also studied in association with asthma symptom control evolution and asthma attack evolution between EGEA2 and EGEA3 (longitudinal analyses).

In both cross-sectional and longitudinal analyses, multivariate analyses were conducted using generalized estimating equations (GEEs) to take into account the familial aggregation of the data except for polytomous logistic regression (used for asthma symptom control at EGEA2 and asthma symptom control evolution and asthma attack evolution between EGEA2 and EGEA3). In these multivariate analyses, asthma outcomes were the dependent variables and PCs the independent ones. Age (continuous), sex, smoking status (never, ex-, current smokers), BMI status (4 classes) at EGEA2 and center were included as covariates. Further adjustment on allergic diseases, allergic sensitization and IgE level were done. Moreover, further adjustment for controller medication use was performed for the associations with asthma symptom control evolution and asthma attacks evolution. Statistical analyses were performed using SAS software, version 9.4 (SAS Institute, Inc., Cary, North Carolina, USA).

3. Results

3.1. Participants' characteristics (Table 1)

At EGEA2, the mean age of the selected 283 adults with current asthma was 37 years, half of them were women, and 53% were non-smokers. Almost half of them reported an asthma attack in the last year, and 55% were classified as having controlled asthma. More than 80% were sensitized to at least one of the 12 aeroallergens tested, 63% had allergic rhinitis and 47% had eczema. The interrelationships between allergic rhinitis, eczema and allergic sensitization are summarized in a Venn diagram (supplementary Figure E2).

At EGEA3, 50% of them were classified as having controlled asthma. Asthma symptom control evolution between EGEA2 and EGEA3 showed that 23.4% (n = 43), 58.1% (n = 107) and 18.5% (n = 34) of the participants had worsening, stable and improved asthma symptom control, respectively. Regarding asthma attack evolution, 21.8% (n = 37), 58.2% (n = 99) and 20.0% (n = 34) of the participants had worsening, stable and improved asthma attacks, respectively.

3.2. Identification of serum cytokine profiles

The mean, minimum and maximum serum levels of the seven studied cytokines, along with their ratio values in order to minimize the inter-plate variability are summarized in Table E2. As shown in Table E3, IL-1Ra was more strongly correlated with IL-10 ($r = 0.61$) than with other cytokines ($r = 0.29–0.50$), whereas IL-5, IL-7, IL-8, IL-13 and TNF-α were highly correlated together ($r = 0.65–0.90$). Patterns of correlations were similar when considering the raw cytokine levels (data not shown).

After PCA, we retained the first two PCs, which accounted for 82.5% of the variability. Even if all cytokines scored high on PC1, this PC was mainly explained by pro-inflammatory cytokines involved either in eosinophilic inflammation (IL-5, IL-7 and IL-13) or neutrophilic inflammation (IL-8 and TNF-α) in asthma (0.81–0.95) (Fig. 1), and was labeled the "*pro-inflammatory cytokine profile*". Only two cytokines scored high on PC2 which are the anti-

Table 1
Characteristics of the 283 adults with current asthma at baseline (EGEA2).

	At EGEA2	At EGEA3
Age, year, Mean ± SD	36.6 ± 15.4	43.5 ± 15.5
Sex, women, n (%)	140 [49.5]	140 [49.5]
Body mass index (BMI), kg/m^2, n (%)		
<20 kg/m^2	40 [14.1]	26 [11.7]
[20–25[kg/m^2	157 [55.5]	114 [51.1]
[25–30[kg/m^2	66 [23.3]	54 [24.2]
>=30 kg/m^2	20 [7.10]	29 [13.0]
Smoking, n (%)		*N = 280*
Non-smokers	151 [53.4]	136 [48.6]
Ex-smokers	60 [21.2]	86 [30.7]
Smokers	72 [25.4]	58 [20.7]
Age at asthma onset, n (%)	*N = 271*	*N = 271*
[0–4] years	93 [34.3]	93 [34.3]
[4–16] years	99 [36.5]	99 [36.5]
>16 years	79 [29.2]	79 [29.2]
Exacerbation, last 12 months, n (%)	*N = 261*	*N = 50*
	47 [18.0]	42 (84.0)
Asthma attack, last 12 months, n (%)	*N = 282*	*N = 261*
	132 [46.8]	128 [49.0]
Asthma symptom control, n (%)	*N = 283*	*N = 246*
Controlled	150 [54.9]	94 [49.7]
Partly controlled	96 [35.2]	73 [38.7]
Uncontrolled	27 [9.90]	22 [11.6]
FEV_1% predicted, mean ± SD	97.0 ± 16.8	NA
Methacholine test*, PD20 ≤ 4 mg, n (%)	*N = 189*	NA
	138 [73.0]	
Inhaled corticosteroids, last 12 months, n (%)	*N = 280*	*N = 223*
	142 [50.7]	125 [56.1]
SPT+, n (%)	*N = 271*	NA
	228 [84.1]	
Allergic rhinitis, n (%)	*N = 278*	NA
	175 [62.9]	
Eczema ever, n (%)	*N = 282*	NA
	132 (46.8)	
IgE, IU/mL, GM (Q1-Q3)	158 [69.4; 390]	NA
Eosinophils, cells/mm^3, GM (Q1-Q3)	215 [140; 320]	NA
Neutrophils, cells/mm^3, GM (Q1-Q3)	3745 [3000; 4690]	NA

Abbreviations: FEV1, Forced expiratory volume in 1 s; PD20, Dose of methacholine causing a 20% fall in FEV in 1 s from baseline; *Methacholine challenge test was not performed if baseline FEV_1 <80% predicted. SPT+: Skin Prick Test, a mean wheal diameter ≥3 mm than the negative control for at least one of 12 aeroallergens; GM = geometric mean, Q1-Q3 = first and third quartile, NA: not available.

inflammatory cytokines IL-1Ra and IL-10 (0.67), and PC2 was labeled the *"anti-inflammatory cytokine profile"*. Participants with high levels on one PC have therefore high levels for the cytokines that mainly explain the PC. The dendrogram obtained with the cluster analysis confirmed the partition in two clusters: cluster 1 {IL-5, IL-7, IL-8, IL-13 and TNF-α} and cluster 2 {IL-1Ra and IL-10} (supplementary Figure E3).

3.3. Associations between PCs and age, sex, BMI, smoking and medication use at EGEA2

At EGEA2, high level of the "*anti-inflammatory cytokine profile*" was associated with overweight or obesity (P = 0.004). No other associations were observed (data not shown).

Both profiles were unrelated with medication use in the last 12 months expressed as: no medication (reference) – ICS use only - ICS use in combination with LABA, antileukotriene or both (supplementary Table E4).

3.4. Cross-sectional associations between serum cytokine profiles and asthma outcomes at EGEA2

Both profiles were unrelated to exacerbations, asthma attacks, asthma symptom control, lung function, and allergic diseases. Participants with high level of the "*anti-inflammatory cytokine profile*" had a lower risk of BHR (Table 2), and the association remained significant after further adjustment on allergic diseases (not shown). Participants with high level of the "*pro-inflammatory cytokine profile*" had significantly higher neutrophil counts, an association that remained significant after adjustment for respiratory infections in the last four weeks. Participants with high level of the "*anti-inflammatory cytokine profile*" had significantly lower total IgE levels, and lower neutrophil counts, an association of borderline significance. None on the profiles was associated with eosinophil counts (all *P*-values>0.2) (Table E5).

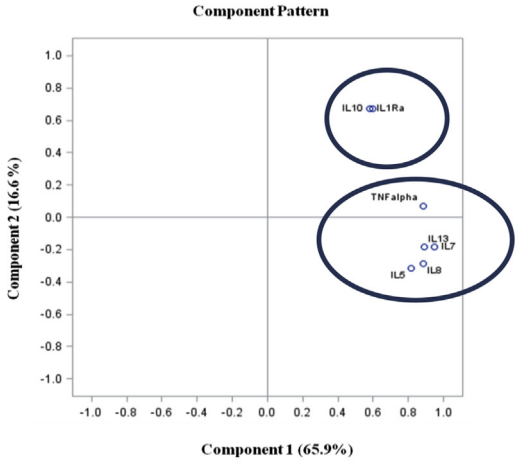
3.4.1. Associations between serum cytokine profiles at EGEA2 and asthma outcomes evolution between EGEA2 and EGEA3

Participants with high level of the "*anti-inflammatory cytokine profile*" at baseline (EGEA2) had significantly lower risks of worsening asthma symptom control (Fig. 2) and of asthma attacks (Fig. 3) between EGEA2 and EGEA3 than those with low level of PC2 (Table E6). No associations were observed with the "*pro-inflammatory cytokine profile*".

Further adjustment for allergic diseases (allergic rhinitis, eczema), allergic sensitization or IgE level or for controller medication use did not change the results (data not shown).

4. Discussion

Our study identified for the first time two serum cytokine profiles in adults with current asthma; the first profile (PC1) labeled



Marker	*"Pro-inflammatory cytokine profile"*	*"Anti-inflammatory cytokine profile"*
Ratio IL-7/ pool	**0.95**	-0.18
Ratio IL-13/ pool	**0.90**	-0.18
Ratio IL-8/ pool	**0.89**	-0.29
Ratio TNF-α/ pool	**0.88**	0.07
Ratio IL-5/ pool	**0.81**	-0.32
Ratio IL1-Ra/ pool	0.58	**0.67**
Ratio IL-10/ pool	0.60	**0.67**

Fig. 1. Graphical representation of the two profiles in the space obtained from the PCA. The "*pro-inflammatory cytokine profile*" (PC1) mainly included IL-5, IL-7, IL-8, IL-13, TNF-α, even though all seven cytokines score high on this profile. The "*anti-inflammatory cytokine profile*" (PC2) included IL-1Ra and IL-10. **Abbreviations:** PCA, Principal component analysis.

Table 2
Cross-sectional associations between cytokines profiles and asthma outcomes (EGEA2).

	High level of "*pro-inflammatory cytokine profile*"		High level of "*anti-inflammatory cytokine profile*"	
	Unadjusted *OR [95% CI]*	Adjusted* *OR [95% CI]*	Unadjusted *OR [95% CI]*	Adjusted* *OR [95% CI]*
Exacerbation (last 12 months)	0.79 [0.42; 1.49]	0.72 [0.33; 1.53]	0.68 [0.36; 1.28]	0.65 [0.29; 1.47]
Asthma attack (last 12 months)	1.17 [0.73; 1.85]	1.09 [0.65; 1.82]	0.74 [0.46; 1.18]	0.68 [0.40; 1.16]
Asthma symptom control				
Partly controlled vs. controlled	1.26 [0.76; 2.11]	1.23 [0.69; 2.19]	0.65 [0.39; 1.08]	0.67 [0.37; 1.20]
Uncontrolled vs. controlled	0.89 [0.39; 2.03]	0.86 [0.32; 2.32]	0.89 [0.39; 2.03]	0.79 [0.29; 2.19]
Methacholine test, PD20 ≤ 4 mg(N = 189)	1.13 [0.61; 2.11]	0.90 [0.43; 1.89]	**0.45 [0.24;0.84]**	**0.46 [0.23;0.91]**
SPT+	0.95 [0.51; 1.79]	0.82 [0.38; 1.78]	0.82 [0.42; 1.60]	0.68 [0.30; 1.58]
Allergic rhinitis	0.77 [0.48; 1.24]	0.75 [0.44; 1.29]	0.79 [0.48; 1.30]	0.74 [0.42; 1.29]
Eczema ever	0.79 [0.49; 1.30]	1.01 [0.58; 1.77]	1.34 [0.83; 2.15]	1.04 [0.61; 1.77]
	β [95% CI]	***β [95% CI]***	***β [95% CI]***	***β [95% CI]***
FEV_1% predicted	2.09 [-1.77; 5.95]	1.75 [-2.27; 5.77]	−1.34 [-5.20; 2.53]	−0.41 [-4.46; 3.63]

*β/OR [95% CI] were adjusted for age, sex, smoking status, BMI and center; GEE regression method was performed, except for asthma symptom control where a polytomous logistic regression was applied. **Bolded results** are statistically significant at P < 0.05.
Abbreviations: FEV1: Forced expiratory volume in 1 s; PD20: Dose of methacholine causing a 20% fall in FEV in 1 s from baseline; SPT+: Positive skin prick test, a mean wheal diameter ≥3 mm than the negative control for at least one of 12 aeroallergens; High level of "*pro-inflammatory cytokine profile*" = PC1 above the median; High level of "*anti-inflammatory cytokine profile*" = PC2 above the median; PC, Principal component.

the "*pro-inflammatory cytokine profile*" included mainly cytokines involved either in eosinophilic (IL-5, IL-7 and IL-13) or in neutrophilic inflammation (IL-8 and TNF-α) in asthma, and the second one (PC2), labeled the "*anti-inflammatory cytokine profile*" included two anti-inflammatory cytokines (IL-1Ra and IL-10). These profiles were differentially associated with asthma outcomes in adults with current asthma. The high "*pro-inflammatory cytokine profile*" was significantly associated with higher blood neutrophil count at baseline. The high "*anti-inflammatory cytokine profile*" was significantly associated with low total IgE level and lower BHR at baseline and with a lower risk of worsening asthma control and asthma attacks.

In the cross-sectional analysis, we found that participants with high (above the median) "*pro-inflammatory cytokine profile*" had higher blood neutrophil counts than those with low "*pro-inflammatory cytokine profile*". These results are consistent with the positive association between IL-8 levels and neutrophil counts found in sputum [28] and in blood [14]. However, we found no association between "*pro-inflammatory cytokine profile*" and blood eosinophil counts or total IgE level, in contrast with previous studies on IL-5 and IL-13 in sputum [18,29–31]. The "*pro-inflammatory cytokine profile*" was not correlated with asthma control outcomes, allergic diseases, and lung function. In contrast, numerous reports found associations between sputum cytokines levels and clinical outcomes of asthma. Norzilla et al. reported an association between sputum IL-5 and IL-8 levels and exacerbations [32], and IL-8 levels in BAL were found to be inversely correlated with FEV_1 in a clinical study [7]. It is to be noted that we found no association between the "*pro-inflammatory cytokine profile*" and asthma symptom control. A previous study on 217 asthmatic patients reported a threshold of 156 pg/mL of IL-13 in sputum to have good diagnostic accuracy for the detection of not-well-controlled asthma [9]. Likewise, IL-8 levels in BAL were found to be higher in participants with uncontrolled than in those with controlled asthma [7]. To our knowledge, only one case-control study has studied the association between sputum levels of a panel of 12 cytokines including IL-5, IL-10 and TNF-α and asthma control in 106 asthmatic patients [8]: uncontrolled asthma being more common among participants with the 'IL-5/IL-17A-high' profile than in those with the 'IL-5/IL-17A-low' profile. However, differences in study designs and protocols (profiles *versus* individual cytokines, biological compartments, asthma control assessment) prevent any direct between-study comparison. Interestingly, the recent review by Fatj and Wenzel [2] showed that therapies targeting the canonical type 2 cytokines IL-4, IL-5, and IL-

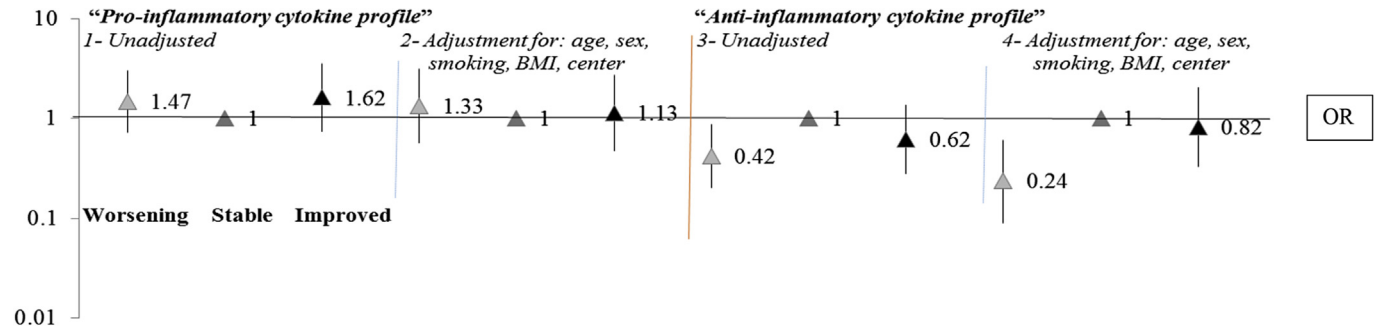


Fig. 2. Association between the "*pro-inflammatory cytokine profile*" and the "*anti-inflammatory cytokine profile*" and asthma symptom control evolution between EGEA2 and EGEA3. 1- Association between the "*pro-inflammatory cytokine profile*" and asthma symptom control evolution between EGEA2 and EGEA3 (unadjusted). 2- Association between the "*pro-inflammatory cytokine profile*" and asthma symptom control evolution between EGEA2 and EGEA3 further adjustment for age, sex, BMI, smoking and center. 3- Association between the "*anti-inflammatory cytokine profile*" and asthma symptom control evolution between EGEA2 and EGEA3 (unadjusted). 4- Association between the "*anti-inflammatory cytokine profile*" and asthma symptom control evolution between EGEA2 and EGEA3 further adjustment for age, sex, BMI, smoking and center. Stable is the reference value with OR = 1; Worsening and improved status are compared to stable. The triangle expresses the OR and the line the 95% CI. **Abbreviations:** BMI, Body mass index.

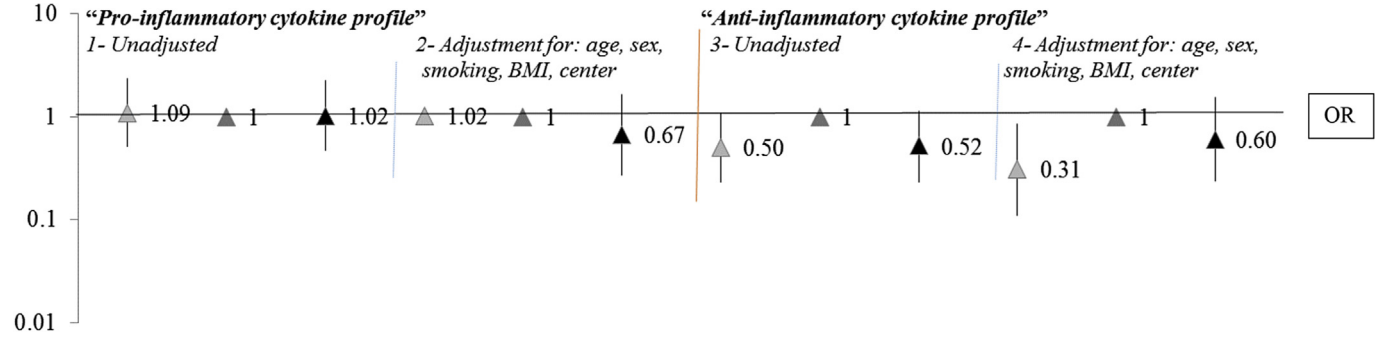


Fig. 3. Association the "*pro-inflammatory cytokine profile*" and the "*anti-inflammatory cytokine profile*" and asthma attacks evolution between EGEA2 and EGEA3. 1- Association between the "*pro-inflammatory cytokine profile*" and asthma attacks evolution between EGEA2 and EGEA3 (unadjusted). 2- Association between the "*pro-inflammatory cytokine profile*" and asthma attacks evolution between EGEA2 and EGEA3 further adjustment for age, sex, BMI, smoking and center. 3- Association between the "*anti-inflammatory cytokine profile*" and asthma attacks evolution between EGEA2 and EGEA3 (unadjusted). 4- Association between the "*anti-inflammatory cytokine profile*" and asthma attacks evolution between EGEA2 and EGEA3 further adjustment for age, sex, BMI, smoking and center. Stable is the reference value with OR = 1; Worsening and improved status are compared to stable. The triangle expresses the OR and the line the 95%CI. **Abbreviations:** BMI, Body mass index.

13 have shown consistent efficacy, especially in asthmatic patients with evidence of TH2 inflammation.

Interestingly, a high "*anti-inflammatory cytokine profile*" including IL-1Ra and IL-10 was significantly associated with low IgE levels. We also found that the "*anti-inflammatory cytokine profile*" was significantly associated with lower BHR at baseline, and lower risks of worsening asthma control. Further, the "*anti-inflammatory cytokine profile*" was associated with lower risk of asthma attack. Hakonarson et al. were the first to show that the treatment of antigen-sensitized animals with IL-1Ra inhibited airway hyperresponsiveness and inflammation [33]. Later, it was reported that sputum IL-1Ra levels were also significantly and positively correlated with lung function in patients with asthma [6,34].

Recently [35], the IL-1Ra/IL-1β ratio in sputum was compared between four sputum inflammatory profiles (paucigranulocytic, eosinophilic, neutrophilic, mixed) in 80 adults with asthma, and was significantly reduced in neutrophilic asthma, suggesting an imbalance of available anti-inflammatory mediators. Moreover, in the large-scale consortium-based Genomewide Association Study of Asthma (GABRIEL), association of genomewide significance was found between asthma and a genetic variant implicating *IL1R1/IL18R1* [36]. Previous studies have shown that IL-10 treatment reduced airway hyperresponsiveness [37,38] and that suppression of airway hyperresponsiveness by regulatory T cells is IL-10-dependent [39]. Overall, these results highlight the importance of including IL-1Ra and IL-10, and possibly other anti-inflammatory cytokines, in future studies of associations between cytokine profiles and asthma control outcomes with the possibility to monitor the evolution of the disease in uncontrolled patients.

To our knowledge, our study is the first to investigate the association between serum cytokine profiles and asthma control outcomes evolution such as asthma symptom control and asthma attacks in a prospective design. Most participants with asthma included in the analysis were recruited in chest clinics as asthma cases, with a careful procedure set up to include true asthmatics. Some were also recruited as relatives of asthmatic cases, based on answers to questions on asthma diagnosis. This leads to a group of asthmatics with a wide range of response to methacholine. Asthma symptom control was defined according to GINA 2015. Cytokines were measured in serum, a less invasive approach than sputum assays for further implementation in the context of epidemiological studies, or in clinical practice. We acknowledged that we have only explored a small part of the cytokines network, but we have included key inflammatory, anti-inflammatory and TH2 cytokines as well as a receptor antagonist, cytokines often neglected in previous studies [12]. Finally, since chronic inflammation in asthma is orchestrated by multiple cytokines through complex interactions [6], we performed a multiplex analysis, allowing the parallel analysis of multiple cytokines in one serum sample [40], and avoiding multiple testing. It is interesting to note that since IL-8 and TNF-α were clustered together with TH2 cytokines, suggesting that systemic neutrophil activation and inflammation may occur in asthma

patients regardless of the type of local inflammatory profile. Overall, we found significant associations between cytokine profiles and asthma control outcomes in a longitudinal but not in a cross-sectional way. We acknowledge that these results may be surprising and could be considered as possible spurious association. It is noteworthy that biological markers play a role either in the development, the activity or the evolution of the disease but our findings needs now to be replicated in other epidemiological studies on asthma.

In conclusion, the present study identified for the first time that serum cytokine profiles with high levels of IL-1Ra and IL-10 were associated with lower risks of worsening asthma control and asthma attacks in adults in a longitudinal design. More generally, this study suggests that assessing cytokine profiles when studying the associations with asthma control outcomes may be more efficient than assessing individual cytokine levels, and may help to target patients with uncontrolled asthma for therapy.

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Conflict of interest

VS has received speaker honorarium from TEVA, Novartis and AstraZeneca.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.rmed.2017.03.002.

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