



IL-17 protein levels in both induced sputum and plasma are increased in stable but not acute asthma individuals with obesity



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ABSTRACT

Background: Obesity worsens asthma control partly through enhanced airway neutrophilia, altered lung mechanics and comorbidities, including obstructive sleep apnea syndrome, gastroesophageal reflux disease and depression. Although controversial, obesity may also cause poorer outcomes in acute asthma. IL-17 is associated with neutrophilic inflammation, steroid resistance and severe asthma, but its importance in the association between asthma and obesity is unknown.

Objective: To investigate the role of IL-17 in obese asthma in both acute and stable settings.

Methods: Both stable ($n = 177$) and acute ($n = 78$) asthmatics were recruited and categorized into lean ($n = 77$ and 39 respectively), overweight ($n = 41$ and 17 respectively) and obese ($n = 59$ and 22 respectively) groups and compared for clinical characteristics, including sputum and plasma IL-17 protein concentrations, sputum cellularity, spirometry and comorbidities. Correlations of IL-17 expression with other measures were explored.

Results: In stable subjects, airway neutrophilia and IL-17 concentrations were most prominent in the obese, and correlated positively with each other. Significant increase in plasma IL-17 levels was also noted and associated with elevated depressive symptoms in obesity. In acute asthma, IL-17 expression, like most other clinical measures, was similar among lean, overweight and obese groups, but was higher in acute versus stable asthma subjects, with sputum IL-17 correlating positively with sputum neutrophils and negatively with FEV₁ and plasma IL-17 showing a positive connection to airway eosinophilia during exacerbation.

Conclusions: IL-17 contributes to worse disease control in obese asthma through enhancing airway neutrophilia and depression, and may implicate in asthma exacerbations. Effects of adiposity on acute asthma remain uncertain.

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Abbreviations: ACQ-5, five-question Asthma Control Questionnaire; BDI, Beck Depression Inventory; BMI, body mass index; FEV₁, forced expiratory volume in 1 s; FVC, forced vital Capacity; GER, gastroesophageal reflux disease; ICS, inhaled corticosteroid; IL, interleukin; OSAS, obstructive sleep apnea syndrome; SaO₂, arterial oxygen saturation.

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1. Introduction

Recent years have witnessed a dramatic increase in prevalence of both obesity and asthma [1]. Higher body mass index (BMI) leads to steroid-resistance and worse asthma control [1,2]. In addition, although controversial, at least two studies [3,4], among the limited literature [3–6], reported poorer outcomes in overweight/obese versus lean acute asthma patients. Mechanisms underlying stable obese asthma have been widely explored, and may involve changes in lung mechanics [7,8] and comorbidities [9–11], including obstructive sleep apnea syndrome (OSAS), gastroesophageal reflux

disease (GER) and depression. Of note, there has been an emerging idea that adiposity is associated with enhanced airway neutrophilia in asthma [12,13], a phenotype, well known for its potential to cause steroid-resistant and severe asthma [14,15], while the typical Th2 cytokine-driven, eosinophil-dominated airway inflammation either unaffected or diminished with increasing of BMI in most reports [16,17], except one [18], which depicted increased sputum interleukin (IL)-5 and submucosal eosinophils in obese severe asthma. On the contrary, much less effort has been made to understand the association between obesity and acute asthma, and whether it also involves altered airway cellular inflammation is unknown.

IL-17 family cytokines, including IL-17A (hereinafter abbreviated as IL-17), IL-17E and IL-17F, play critical roles in asthma [19], with IL-17 and IL-17F definitely involved in severe asthma [20–22]. IL-17 has also been considered closely related to steroid-resistant type of asthma [23,24] and neutrophilic inflammation [25,26]. It is therefore interesting to find that IL-17-mediated immune response mimics some features of obesity-related asthma. We then suppose IL-17 may play a part in this context.

Accordingly, this paper is mainly aimed to explore the role of IL-17 in the pathogenesis of obese asthma in both stable and acute settings, focusing on its importance in airway inflammation.

2. Material and methods

2.1. Subjects

Both stable and acute asthma patients were recruited and categorized into lean ($18.5 \text{ kg/m}^2 \leq \text{BMI} < 25 \text{ kg/m}^2$), overweight ($25 \text{ kg/m}^2 \leq \text{BMI} < 30 \text{ kg/m}^2$) and obese ($\text{BMI} \geq 30 \text{ kg/m}^2$) groups [11,27,28]. The protocol was approved by the Ethics Committees of Huai'an Second People's Hospital and Xiangya Hospital of Central South University. Written informed consent was obtained from each participant. Acute asthmatics were recruited from outpatients and inpatients admitted to the Second People's Hospital of Huai'an and Xiangya Hospital of Central South University with an exacerbation during a period from September 2012 to August 2016. For stable subjects, the above-mentioned patients were recruited by telephone calls at least three months after their release from hospital, with additional subjects recruited by poster advertisement in the same period. Asthma was diagnosed according to Global Initiative for Asthma guidelines (2012), with exacerbations defined as episodes of a progressive increase in shortness of breath, cough, wheezing, or chest tightness, or a combination of these symptoms, requiring a change in treatment. All acute asthma subjects should finally show airway reversibility (FEV_1 improvement $> 12\%$ and 200 ml increase). All stable asthmatics should present with a documented history of episodic dyspnea and airway responsibility and had no asthma exacerbation, respiratory tract infection or systemic corticosteroid use in the preceding 4 weeks, parenchymal lung disease apparent on chest radiography, diabetes mellitus, cardiovascular disease, pregnancy, autoimmune diseases and tumors. Patients, in whom sputum induction could not be achieved, were excluded in this study, including 15 lean, 7 overweight and 9 obese subjects with stable asthma and 13 lean, 8 overweight and 8 obese subjects with acute asthma. All subjects were assessed for demographics, sputum and plasma IL-17 protein levels, airway cellular inflammation and spirometry data. Stable asthma patients were additionally assessed for the occurrence of OSAS, GER and depression. Among asthmatics with an exacerbation, respiratory rate, pulse, diaphoresis, wheezing, accessory muscle use, arterial oxygen saturation (SaO_2), medication management, cause and duration of exacerbation were recorded.

2.2. Lung function parameters

Spirometry was performed according to the American Thoracic Society and European Respiratory Society guidelines [29]. Forced expiratory volume in 1 s (FEV_1) and Forced vital Capacity (FVC) were recorded and expressed as percent of predicted value.

2.3. Evaluation of asthma control

Stable subjects were evaluated for asthma control levels by fulfilling an Asthma Control Questionnaire, 5-item version (ACQ5) [30], with an ACQ score of 0.0–0.75 classified as well-controlled asthma, 0.75–1.5 as partly-controlled asthma and > 1.5 as poorly-controlled asthma.

2.4. Sputum induction and processing

All patients were pre-treated with two puffs of salbutamol (200 μg) and inhaled a nebulized solution of normal saline followed by increasing concentrations (3, 4, and 5%) of hypertonic saline using an ultrasonic nebulizer. Patients were asked to rinse their mouth and were then instructed to cough sputum into a sterilized sputum container. These procedures were repeated for up to a total of 5 min of inhalation sequentially with each concentration until the FEV_1 had fallen to $\geq 20\%$ (for stable asthma) or 10% (for acute asthma) or an adequate sputum sample ($\sim 1 \text{ ml}$) was obtained.

Sputum was processed according to the method of Fahy et al. with some modifications [31]. Briefly, sputum separated from saliva was dispersed with an equal volume of dithiothreitol 0.1%, and was then centrifuged at 450 g for 10 min at 4 °C. The cell pellet was resuspended in phosphate-buffered saline and cytopins were stained with May-Grunwald-Giemsa. No protease inhibitors were used in processing of induced sputum. Differential cell counts were counted from 400 nonsquamous cells. Samples with a contamination of $> 80\%$ squamous cells were excluded from analyses. Eosinophilic phenotype was defined as $> 3\%$ sputum eosinophil counts while neutrophilic phenotype consisted of $> 61\%$ sputum neutrophil counts [32].

2.5. Determine of IL-17 concentration

IL-17 concentrations in supernatant of sputum and plasma were quantified using an Enzyme-Linked Immunosorbent Assay kit (Abcam, Cambridge, United Kingdom) according to the manufacturer's protocol. Concentrations below the standard range of the assay (1.6 pg/ml) were set as zero.

2.6. Evaluation of obesity-related aggravating factors

OSAS was diagnosed by polysomnography, or by heavy snoring and frequent apnea periods of $> 10 \text{ s}$ reported by the partner in combination with a history of daytime sleepiness [17]. GER was diagnosed as mild heartburn or regurgitation ≥ 2 days per week, or moderate/severe heartburn or regurgitation ≥ 1 day a week [33]. The presence and severity of depressive symptoms were detected by the Chinese version of Beck Depression Inventory II (BDI-II; Chinese Behavioral Sciences Society, 2000) [34]. It is a 21-item questionnaire with scoring between 0 (symptom not present) and 3 (symptom strongly present) for each item. Subjects with a score of at least 14 were considered to have significant depressive symptoms.

2.7. Statistical analysis

Statistical analysis was carried out in SPSS version 20 (IBM

Corporation, Chicago, IL, USA). Group comparisons for continuous data were performed using either one-way analysis of variance followed by Student-Newman-Keuls post hoc test (normally distributed data) or Kruskal-Wallis test and Dunn-Bonferroni post hoc test (non-normally distributed data). Group comparisons of proportions were made using Pearson's Chi-squared test or Fisher's exact test with Bonferroni adjustment. Correlations were examined with Spearman rank test. Neutrophil percentages in sputum from stable asthma patients were analyzed by stepwise regression until the most parsimonious model was achieved. Univariate *P* values of ≤ 0.2 entered the multivariate model. *P* values of < 0.05 were considered statistically significant.

3. Results

3.1. Characteristics of subjects with stable asthma

A total of 177 (including 77 lean, 41 overweight and 59 obese) stable asthmatics were recruited. Their characteristics were presented in Table 1. Levels of sputum and plasma IL-17 were highest in obese group, with increase in sputum IL-17 compared with lean ($P = 0.010$) and plasma IL-17 compared with overweight ($P = 0.049$) individuals reaching statistical significance (Fig. 1A and B). A trend towards increasing sputum neutrophil percentages with increasing BMI was observed ($P = 0.095$), which was confirmed by a

Table 1
Characteristics of lean, overweight and obese subjects with stable asthma.

	Lean n = 77	Overweight n = 41	Obese n = 59	<i>P</i> value
Female (%)	40 (51.9)	24 (58.5)	33 (55.9)	0.773
Age (year)	40.9 (13.1)	41.29 (13.4)	44.1 (10.7)	0.302
BMI (kg/m ²)	22.5 (21.2, 23.5)	26.7 (25.8, 27.6) ^b	35.9 (32.2, 38.1) ^{b,c}	< 0.001
Smoking (%)	22 (28.6)	11 (26.8)	13 (22.0)	0.683
Sputum IL-17 (pg/ml)	3.7 (2.2, 6.1)	3.8 (2.6, 8.0)	5.5 (3.6, 9.0) ^b	0.013
Plasma IL-17 (pg/ml)	8.1 (5.0, 15.7)	7.8 (3.5, 13.5)	13.1 (6.7, 18.5) ^c	0.028
FEV ₁ % predicted	86.0 (16.4)	84.9 (16.2)	82.6 (14.9)	0.483
FVC% predicted	93.9 (14.1)	92.6 (13.6)	89.4 (14.3)	0.232
Duration of sputum induction (minute)	12.0 (10.0, 15.50)	12.0 (10.0, 15.0)	13.0 (9.0, 15.0)	0.922
Sputum neutrophils (%)	43.1 (18.3)	42.5 (17.5)	49.6 (21.4)	0.095
Neutrophilic asthma (%)	14 (18.2)	9 (22.0)	22 (37.3) ^b	0.034
Sputum eosinophils (%)	1.8 (0.3, 5.5)	1.5 (0.4, 4.1)	1.5 (0.3, 3.8)	0.817
Eosinophilic asthma (%)	26 (33.8)	11 (26.8)	17 (28.8)	0.695
OSAS (%)	8 (10.4)	6 (14.6)	12 (20.3)	0.267
GER (%)	16 (20.8)	11 (26.8)	23 (39.0)	0.063
BDI II score	8.0 (5.5, 13.0)	9.0 (6.5, 13.5)	10.0 (7.0, 17.0)	0.062
BDI II score ≥ 14 (%)	18 (23.4)	10 (24.4)	21 (35.6)	0.249
Regular ICS use (%)	12 (15.6)	7 (17.1)	12 (20.3)	0.767
ICS dose ^a (g/d)	800 (425, 1000)	400 (400, 1000)	900 (525, 1000)	0.487
ACQ-5 score	1.0 (0.6, 1.6)	1.0 (0.6, 1.5)	1.2 (0.8, 1.8)	0.052

Data are presented as mean (standard deviation) or median (interquartile range) or number (percentage). BMI: body mass index; FEV₁: forced expiratory volume in 1 s; FVC: forced vital capacity; OSAS, obstructive sleep apnea syndrome; GER, gastroesophageal reflux; BDI, Beck Depression Inventory; ICS: inhaled corticosteroid; ACQ: Asthma Control Questionnaire.

^a : Beclomethasone equivalents, assessed only in subjects taking ICS.

^b : $P < 0.05$ versus lean group.

^c : $P < 0.05$ versus overweight group.

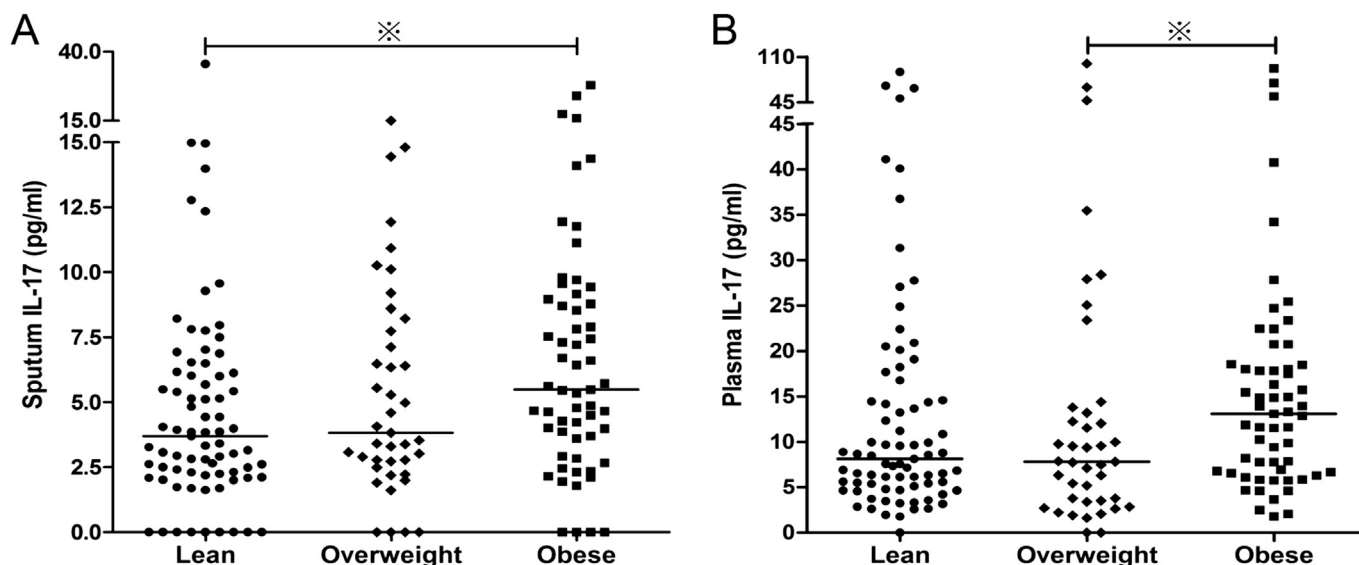


Fig. 1. Sputum IL-17 concentrations (A) and plasma IL-17 concentrations (B) in lean, overweight and obese subjects with stable asthma. Horizontal bars represent median concentrations. *: $P < 0.05$.

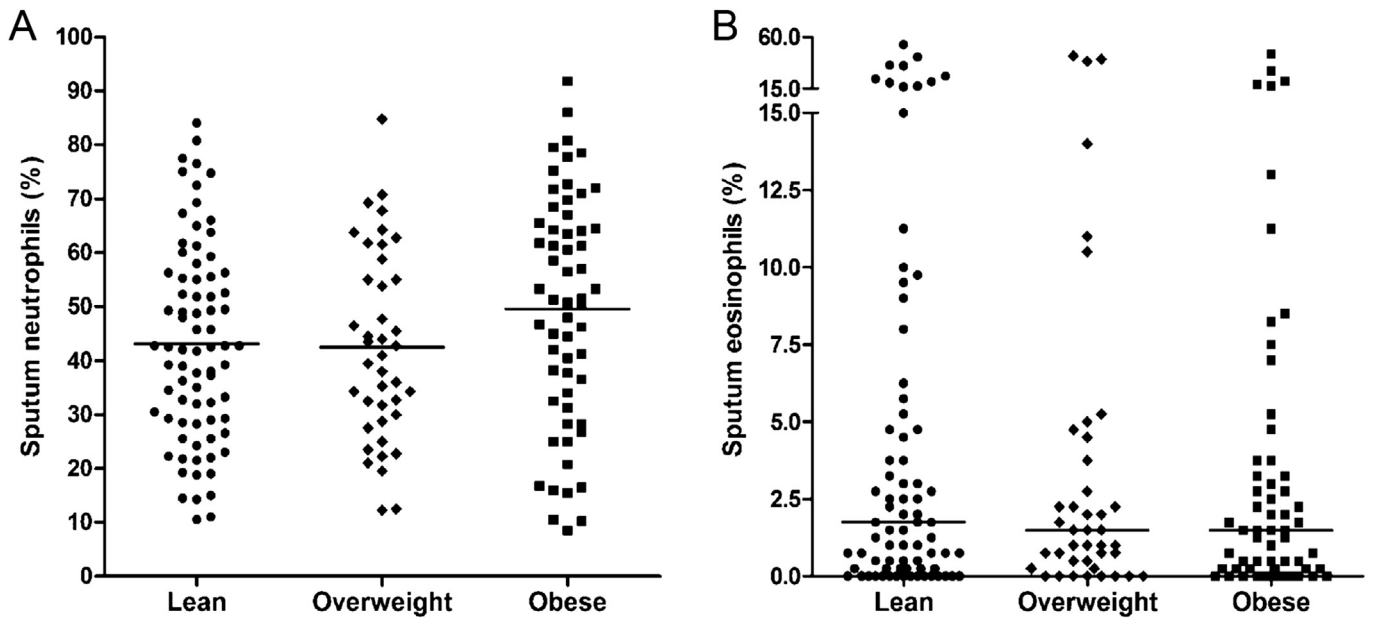


Fig. 2. Sputum neutrophil percentages (A) and sputum eosinophil percentages (B) in lean, overweight and obese subjects with stable asthma. Horizontal bars represent mean (A) and median (B) percentages, respectively.

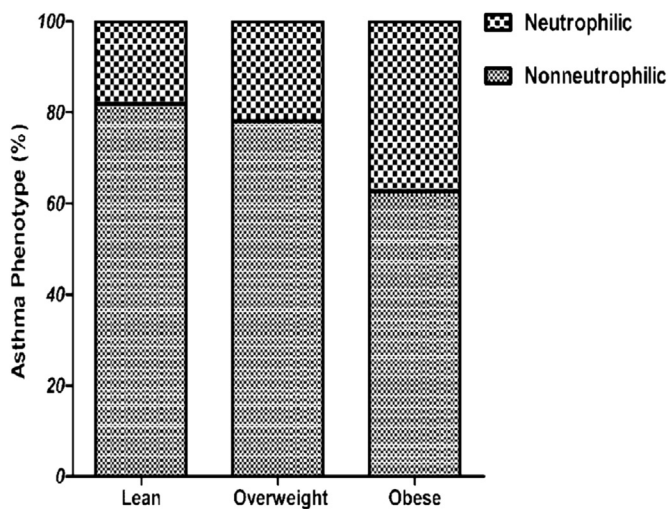


Fig. 3. Proportions of neutrophilic asthma (sputum neutrophils >61%) in lean, overweight and obese subjects with stable asthma.

significant elevation in neutrophilic asthma prevalence in obese versus lean individuals ($P = 0.012$) (Fig. 2A; Fig. 3). No differences were found for airway eosinophils (Fig. 2B). GER prevalence and BDI II score increased as BMI increased, although the differences were not significant ($P = 0.063$ and 0.062 , respectively). Finally and expectedly, obese individuals tended to suffer worse asthma control ($P = 0.052$).

3.2. Association of IL-17 in sputum and plasma with other clinical parameters in stable asthma patients

Sputum IL-17 correlated positively with sputum neutrophil percentages ($R_s = 0.337$, $P < 0.001$) (Fig. 4A). The association was also found when analyzed separately in lean ($R_s = 0.249$, $P = 0.029$), overweight ($R_s = 0.325$, $P = 0.038$) and obese ($R_s = 0.366$, $P = 0.004$) groups. The association between plasma IL-17 and sputum

neutrophils was marginal as a whole ($R_s = 0.148$, $P = 0.049$) (Fig. 4B) and insignificant in separate BMI groups (lean: $R_s = 0.171$, $P = 0.137$; overweight: $R_s = 0.058$, $P = 0.721$; obese: $R_s = 0.080$, $P = 0.546$). Further investigation using multiple regression analysis showed that sputum, but not plasma, IL-17 remained an independent factor of airway neutrophilia after adjusting for confounding factors (Tables 2 and 3). Neither sputum nor plasma IL-17 correlated with sputum eosinophils (Fig. 4C and D).

Sputum IL-17 showed a weak association with FEV₁ as a whole ($R_s = -0.136$, $P = 0.071$) (Fig. 4E), but not in separate BMI groups (lean: $R_s = -0.149$, $P = 0.196$; overweight: $R_s = -0.091$, $P = 0.570$; obese: $R_s = -0.109$, $P = 0.410$). No association could be detected between plasma IL-17 and FEV₁ (Fig. 4F).

BDI II score ≥ 14 correlated with IL-17 in plasma ($R_s = 0.279$, $P < 0.001$), but not in sputum ($R_s = 0.055$, $P = 0.470$), as a whole and separately, in overweight ($R_s = 0.317$, $P = 0.044$) and obese ($R_s = 0.302$, $P = 0.020$) groups, with a trend observed in lean ($R_s = 0.186$, $P = 0.105$) group. There was no association of sputum IL-17 with OSAS ($R_s = 0.029$, $P = 0.698$) and GER ($R_s = 0.090$, $P = 0.234$) and of plasma IL-17 with OSAS ($R_s = 0.105$, $P = 0.163$) and GER ($R_s = 0.057$, $P = 0.449$).

3.3. Details of current exacerbation in subjects with acute asthma

A total of 78 patients with acute asthma were enrolled in this study, including 39 lean, 17 overweight and 22 obese individuals. Most clinical features, including IL-17 concentrations and sputum neutrophil and eosinophil percentages, were similar among the three groups, except for age and BMI (Table 4; Fig. 5 and Fig. 6).

3.4. IL-17 concentrations in sputum and plasma in different asthma control levels

Stable asthma subjects were divided into well-controlled, partly-controlled and poorly-controlled asthma and compared with individuals with acute asthma for sputum and plasma IL-17 levels. Results indicated that both sputum and plasma IL-17 concentrations steadily increased as the level of asthma control

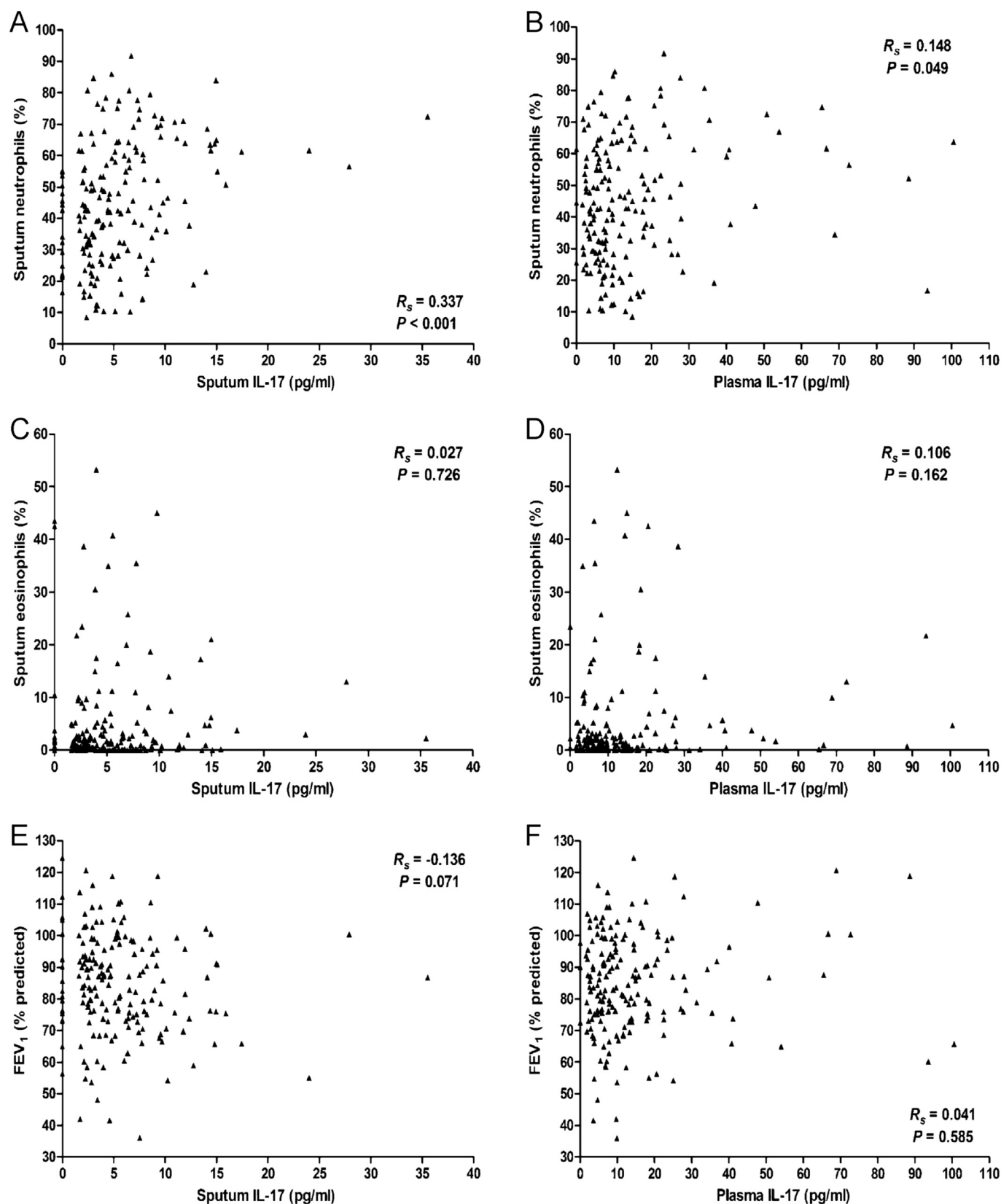


Fig. 4. Association of sputum IL-17 concentrations with sputum neutrophil percentages (A), sputum eosinophil percentages (C) and FEV₁ (E) and association of plasma IL-17 concentrations with sputum neutrophil percentages (B), sputum eosinophil percentages (D) and FEV₁ (F) in subjects with stable asthma.

Table 2

Multiple linear regression model for predicting sputum neutrophil percentages using sputum IL-17, sex, age, BMI, smoking status, ICS dose and duration of sputum induction as covariates in subjects with stable asthma.

	Unadjusted model			Adjusted model		
	β coefficient	Standard error	P value	β coefficient	Standard error	P value
Female (yes/no)	−2.109	3.364	0.532			
Age (year)	0.147	0.116	0.209			
BMI (kg/m ²)	0.296	0.203	0.147			
Smoking (yes/no)	1.888	3.865	0.626			
ICS dose (g/d)	0.000	0.004	0.904			
Duration of sputum induction (minute)	−0.737	0.340	0.032	−0.753	0.330	0.024
Sputum IL-17 (pg/ml)	1.120	0.285	<0.001	1.216	0.277	<0.001

BMI: body mass index; ICS: inhaled corticosteroid.

Table 3

Multiple linear regression model for predicting sputum neutrophil percentages using plasma IL-17, sex, age, BMI, smoking status, ICS dose and duration of sputum induction as covariates in subjects with stable asthma.

	Unadjusted model			Adjusted model		
	β coefficient	Standard error	P value	β coefficient	Standard error	P value
Female (yes/no)	−1.243	3.486	0.722			
Age (year)	0.216	0.119	0.071	0.267	0.114	0.021
BMI (kg/m ²)	0.321	0.210	0.128			
Smoking (yes/no)	1.709	4.006	0.670			
ICS dose (g/d)	0.001	0.004	0.744			
Duration of sputum induction (minute)	−0.790	0.351	0.026	−0.800	0.342	0.021
Plasma IL-17 (pg/ml)	0.157	0.087	0.071			

BMI: body mass index; ICS: inhaled corticosteroid.

Table 4

Characteristics of lean, overweight and obese subjects with acute asthma.

	Lean (n = 39)	Overweight (n = 17)	Obese (n = 22)	P value
Female (%)	21 (53.8)	11 (64.7)	12 (54.5)	0.737
Age (year)	35.4 (9.7)	42.9 (12.7) ^a	40.8 (11.6)	0.037
BMI (kg/m ²)	22.2 (21.3, 23.4)	26.9 (25.9, 27.7) ^a	36.0 (33.1, 41.3) ^{a,b}	<0.001
Smoking (%)	9 (23.1)	5 (29.4)	8 (36.4)	0.537
Sputum IL-17 (pg/ml)	10.2 (4.7, 17.0)	11.4 (5.9, 17.2)	11.9 (8.1, 24.9)	0.364
Plasma IL-17 (pg/ml)	22.5 (10.6, 38.8)	23.0 (10.5, 77.6)	16.0 (8.5, 50.5)	0.820
Duration of Sputum induction (minute)	10.0 (8.0, 15.0)	10.0 (7.0, 15.0)	9.0 (7.5, 14.3)	0.672
Sputum eosinophils (%)	8.0 (4.5, 19.3)	6.8 (4.0, 20.4)	8.8 (4.3, 26.8)	0.796
Sputum neutrophils (%)	55.6 (19.9)	53.3 (23.0)	52.5 (16.7)	0.822
Pulse (beat/min)	100.0 (92.0, 114.0)	99.0 (91, 107.5)	97.0 (91.5, 113.3)	0.881
Respiratory rate (breaths/min)	23.0 (19.0, 28.0)	25.0 (22.0, 27.5)	23.0 (20.8, 25.3)	0.535
Accessory muscle use (%)	12 (30.8)	5 (29.4)	6 (27.3)	0.959
Wheezing (%)	36 (92.3)	15 (88.2)	20 (90.9)	0.887
Diaphoresis (%)	4 (10.3)	3 (17.6)	2 (9.1)	0.724
Initial FEV ₁ % predicted	47.9 (41.3, 64.6)	54.4 (41.1, 64.2)	52.7 (46.5, 63.9)	0.784
SaO ₂ (%)	95.0 (94.0, 96.0)	95 (93.5, 96)	95.0 (94.0, 96.3)	0.580
Causes of exacerbation				0.655
Upper respiratory infections (%)	20 (51.3)	11 (64.7)	11 (50.0)	
Exposure to allergens (%)	13 (33.3)	4 (23.5)	4 (18.2)	
Therapy interruption (%)	2 (5.1)	1 (5.9)	3 (13.6)	
Others (%)	4 (10.3)	1 (5.9)	4 (18.2)	
Treatment measures				
Inhaled β -agonists (%)	39 (100)	17 (100)	22 (100.0)	1.000
Inhaled anticholinergics (%)	9 (23.1)	2 (11.8)	4 (18.2)	0.653
Systemic corticosteroids (%)	37 (94.9)	17 (100.0)	22 (100.0)	0.714
Intravenous magnesium (%)	0 (0)	0 (0)	2 (9.1)	0.122
Duration of exacerbation				0.887
Less than 1 day (%)	23 (59.0)	9 (52.9)	11 (50.0)	
1 - 2 days (%)	13 (33.3)	7 (41.2)	8 (36.4)	
More than 2 days (%)	3 (7.7)	1 (5.9)	3 (13.6)	

Data are presented as mean (standard deviation) or median (interquartile range) or number (percentage). BMI: body mass index; FEV₁: forced expiratory volume in 1 s; SaO₂: arterial oxygen saturation.

^a : $P < 0.05$ versus lean group.

^b : $P < 0.05$ versus overweight group.

decreased, with highest levels observed in acute asthma (Fig. 7A and B). The medians (interquartile range) in the four groups were

4.0 (2.1, 6.3), 3.8 (2.5, 7.2), 6.2 (2.8, 9.6) and 11.0 (6.2, 17.6) pg/ml respectively for sputum IL-17 and 7.6 (4.8, 15.5), 7.8 (4.6, 13.7), 14.0

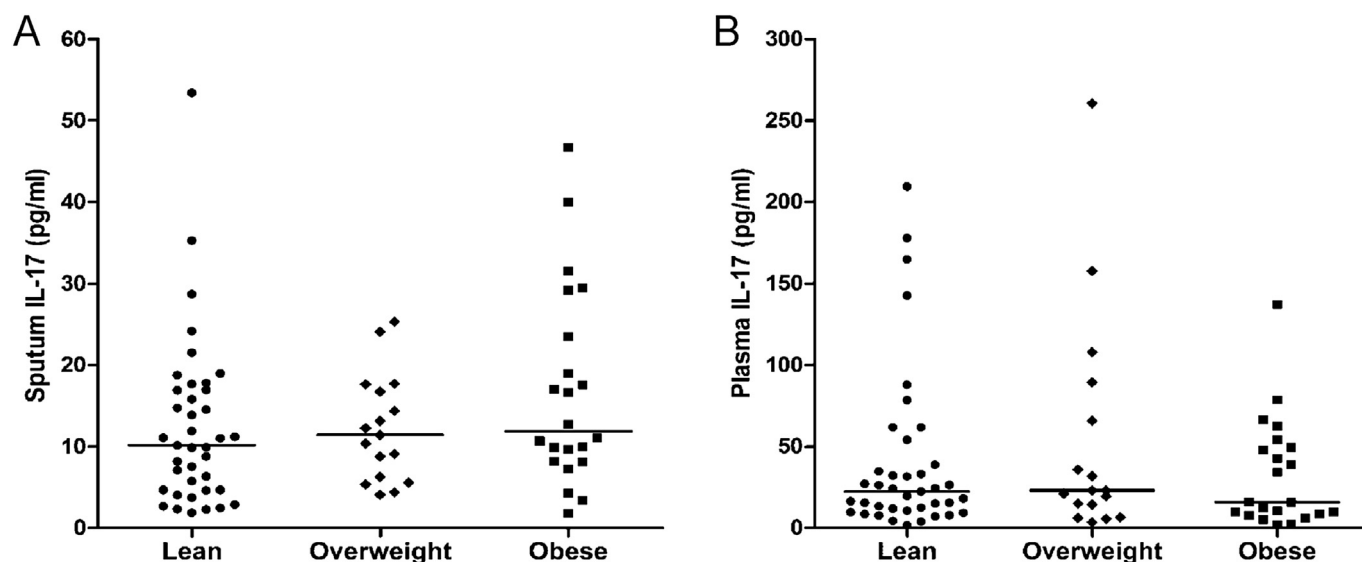


Fig. 5. Sputum IL-17 concentrations (A) and plasma IL-17 concentrations (B) in lean, overweight and obese subjects with acute asthma. Horizontal bars represent median concentrations.

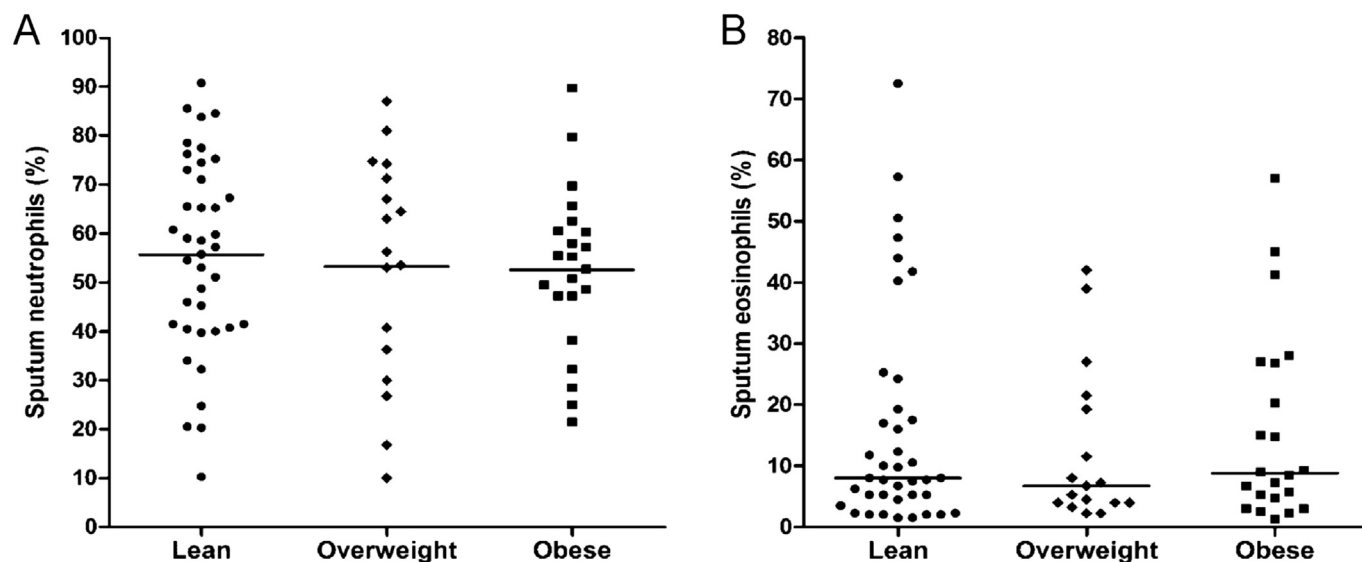


Fig. 6. Sputum neutrophil percentages (A) and sputum eosinophil percentages (B) in lean, overweight and obese subjects with acute asthma. Horizontal bars represent mean (A) and median (B) percentages, respectively.

(7.9, 22.5) and 21.8 (9.7, 50.5) pg/ml respectively for plasma IL-17.

3.5. Association of IL-17 in sputum and plasma with airway inflammation and FEV₁ in acute asthma patients

As shown in Fig. 8, sputum IL-17 correlated positively with sputum neutrophil percentages ($R_s = 0.353$, $P = 0.002$) in the whole asthma group and in overweight ($R_s = 0.517$, $P = 0.034$) and obese ($R_s = 0.509$, $P = 0.016$) groups separately, with a trend ($R_s = 0.270$, $P = 0.096$) found in lean group. Similar findings were recorded for sputum IL-17 and FEV₁ ($R_s = -0.470$, $P < 0.001$ as a whole and $R_s = -0.431$, $P = 0.006$ in lean, $R_s = -0.500$, $P = 0.041$ in overweight and $R_s = -0.688$, $P = 0.001$ in obese group). Additionally, plasma IL-17 was positively associated with sputum eosinophils ($R_s = 0.268$, $P = 0.018$) as a whole. However, the association may only exist in obese ($R_s = 0.463$, $P = 0.030$), but not in lean ($R_s = 0.180$, $P = 0.273$)

and overweight ($R_s = 0.365$, $P = 0.150$) groups.

4. Discussion

Consistent with a recently published correspondence by Gudrun S. Marijsse [35], both sputum and plasma IL-17 concentrations, accompanied by airway neutrophilia, increased with increasing BMI among subjects with chronic asthma. By using Spearman rank test and multiple regression analysis, our research showed sputum, but not plasma, IL-17 was closely related to sputum neutrophil percentages, even after adjusting for multiple confounding factors, including BMI. Therefore, airway IL-17-driven neutrophilic inflammation represents a potential pathway, whereby obesity adversely affects asthma control. Though not a strong predictor of airway neutrophilia, increase in plasma IL-17 should remain of clinical relevance in the pathogenesis of obese asthma considering

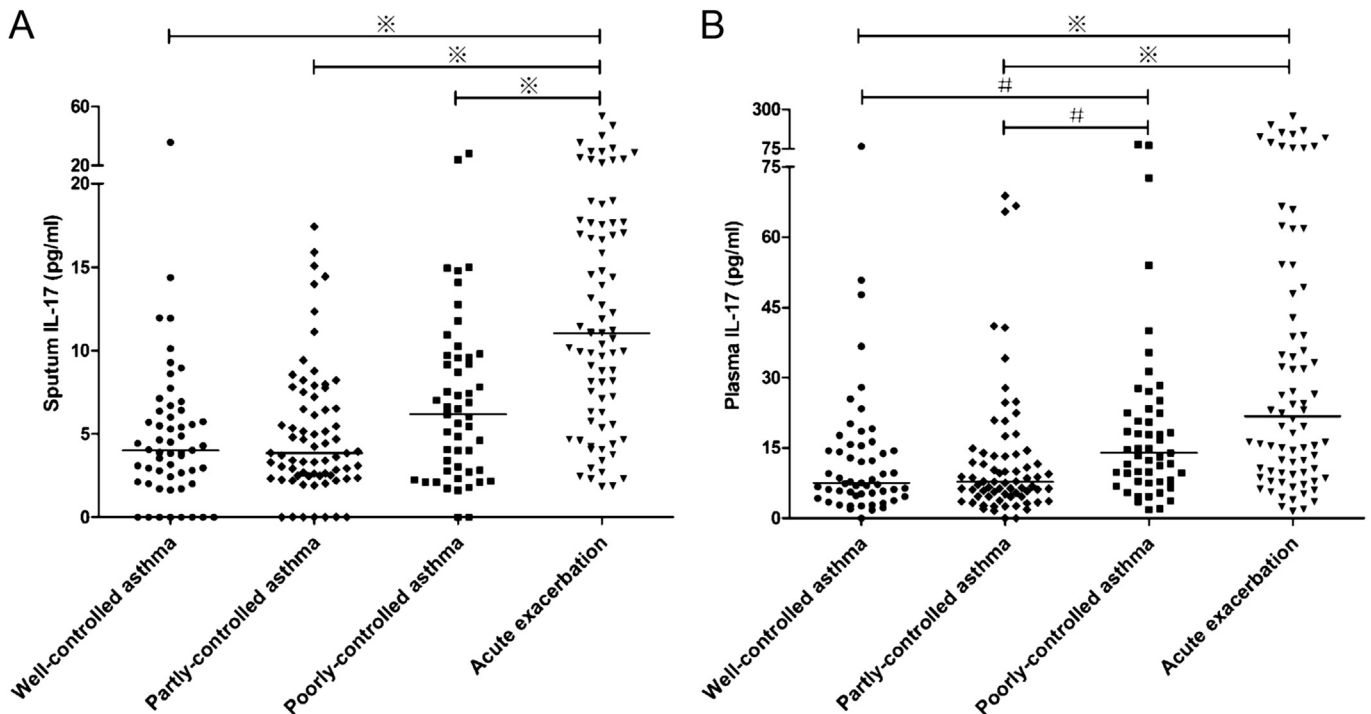


Fig. 7. Sputum IL-17 concentrations (A) and plasma IL-17 concentrations (B) in stable (well controlled, partly controlled and well controlled) and acute asthma subjects. Horizontal bars represent median percentages. *: $P < 0.001$; #: $P < 0.05$.

the concurrent finding, in this research, that there was an association between plasma IL-17 and depression especially in overweight and obese individuals, which may be a causal one, as indicated in an experimental study of established mouse model for depression [36], and the fact that depression may lead to damaged cognitive function, poor health behaviors, increased exposure to asthma triggers and poor adherence to ICS therapy and has been found to be more prevalent in obese asthma and completely mediate the association between BMI and worse asthma control [10].

The sources of increased IL-17 are a problem. Th17 cells should be first considered. Since Th17 cells increased in spleen [37] and adipose tissue [38] in obese mice. And activation of Th17/IL-17 axis participated in the development of several obesity-related inflammatory disease models [37,39]. Furthermore, adipose-derived stem cells from obese but not lean humans enhanced IL-17 secretion by Th17 cells [40]. However, we were puzzled by a recent study [41], where obese asthmatic patients showed a paradoxical increase in lymphocytes in bronchoalveolar lavage and cytokine production, including IL-17, from activated peripheral blood CD4⁺ T cells after bariatric surgery. However, we note subjects in the study were severely obese with a mean BMI of 51.37 kg/m². It is most likely that these subjects consumed extremely large amounts of high-fat diet, which has been shown to inhibit pro-inflammatory cytokine response of lymphocytes [42]. Other potential producers of IL-17, including CD4⁺ lineage-negative type 3 innate lymphoid cells [43] and IL-17⁺ $\gamma\delta$ T cells [44], have also been reported. Their importance in obese asthma deserves our attention and further discussion.

Meanwhile, clinical manifestations and treatment responses, including IL-17 expression and airway cellular inflammation were similar across BMI groups among acute asthmatics. Of course, these negative findings could naturally be attributed to a relatively small sample size. An older average age in overweight group could also confound the results. However, these findings agreed with data

from two previous larger studies [5,6], which respectively recruited 180 and 572 subjects. Therefore, our study tended to support the notion that BMI does not influence acute asthma.

Despite so, our study suggested a key role for IL-17 during an asthma episode by showing a gradual rise in both sputum and plasma IL-17 levels with increasing asthma severity. Furthermore, we again found a significant correlation between sputum IL-17 and neutrophils. To our knowledge, the role of IL-17 in neutrophilic asthmatic airway inflammation is supported, mainly from mouse models of asthma and the important pathologic effects of IL-17 in neutrophilic inflammation in other diseases, such as psoriasis [21], while much less from asthma patients [25,26]. Therefore, our findings not only add to the clinical literature, but also extend their relationship to acute asthma. And for the first time, we reported a moderate association between plasma IL-17 and airway eosinophils, which could not be detected in stable asthma, leading to the assumption that IL-17 is essential in the induction of an exacerbation, but not maintenance of eosinophilic airway inflammation. It is intriguing that a similar phenomenon was also observed for sputum IL-17 and FEV₁. In fact, the role of IL-17 in asthma is complex and may have different functions, depending on the cellular sources, timing, surrounding environment such as cytokines and other mediators, as well as the concentration of IL-17 [45].

Several issues and limitations should be mentioned here. Firstly, increase in airway neutrophilia in obesity occurred only in females as suggested in previous reports [12,13], and thus does not completely consist with our data, where such increases were not significance in both genders. And neither did IL-17 (data not shown). Therefore, whether sex modifies the association of BMI with airway neutrophils in Chinese asthma population remains to be resolved. Secondly, sputum cellularity is definitely affected by the total duration of sputum induction [46,47], which was not kept similar for all individuals in our study. However, we don't think it greatly influenced our results because mean sputum induction time was comparable among BMI groups and has been adjusted in

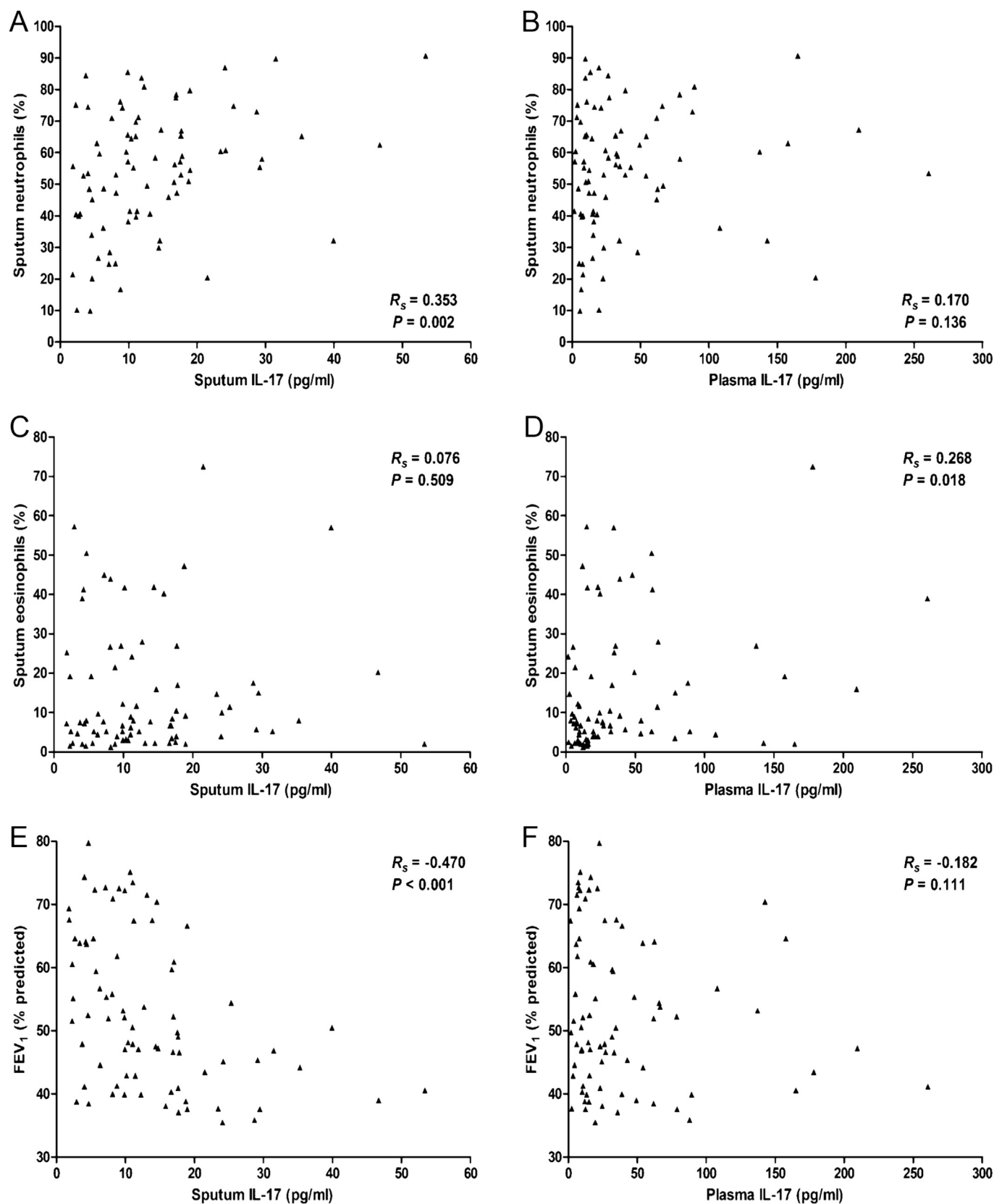


Fig. 8. Association of sputum IL-17 concentrations with sputum neutrophil percentages (A), sputum eosinophil percentages (C) and FEV₁ (E) and association of plasma IL-17 concentrations with sputum neutrophil percentages (B), sputum eosinophil percentages (D) and FEV₁ (F) in subjects with acute asthma.

multiple regression analysis for predictors of sputum neutrophil percentages. Finally, since the obese showed only a mild increase and no difference in airway neutrophil infiltration in stable and acute asthma individuals, respectively, addition of the analysis of IL-8 and myeloperoxidase is warranted to fully depict neutrophilic inflammation in obese asthma.

5. Conclusion

Our study shows that obesity promotes IL-17 expression in stable asthma patients. While sputum IL-17 is responsible for the enhanced airway neutrophilia in obese patients, plasma IL-17 explains their predisposition to depressive symptoms. Therefore, this paper justifies an intervention targeting IL-17 in therapy of this phenotype of asthma. Though our research does not support obesity as an aggravating factor for acute asthma, IL-17 may still play a role in this context.

Conflicts of interest

None.

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