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Sputum TWEAK Expression Correlates with Severity and Degree of Control in Noneosinophilic Childhood Asthma

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ABSTRACT

Background: Tumor necrosis factor-like weak inducer of apoptosis (TWEAK) is known to play a role in the pathogenesis of various inflammatory diseases. However, no study has been performed on childhood asthma.

Methods: Ninety-five children with asthma and 78 controls aged 5–18 years were included. Sputum induction, pulmonary function test (PFT), and methacholine challenge test were performed. The subjects were divided into the eosinophilic airway (EA) and non-EA (NEA) groups based on sputum analysis and into the high and low TWEAK groups according to the TWEAK cutoff level (263.0 pg/mL). TWEAK in induced sputum supernatant was measured through enzyme-linked immunosorbent assay.

Results: Children with asthma had higher TWEAK levels than healthy controls [493.0 (157.1–904.3) vs. 118.2 (67.5–345.5) pg/mL, $P < 0.001$]. Sputum TWEAK levels were significantly correlated with PFT parameters reflecting airway obstruction. This association was particularly prominent in subjects with NEA inflammation. Significant differences in FEF_{25–75} (maximum midexpiratory flow, % predicted; $P = 0.017$), AX (reactance area; $P < 0.001$), R5-R20 (difference between resistance at 5 and 20 Hz; $P = 0.025$), and X5

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(reactance at 5 Hz, % predicted; $P < 0.001$) were noted between the high and low TWEAK groups within the NEA group. Sputum TWEAK level also showed significant positive correlations with asthma severity ($r = 0.358$, $P = 0.001$) and control status ($r = 0.470$, $P < 0.001$), distinctively in subjects with NEA inflammation.

Conclusions: Airway TWEAK may play a role in small airway inflammation especially in children with noneosinophilic asthma.

Key words: airway inflammation, asthma, children, tumor necrosis factor-like weak inducer of apoptosis (TWEAK)

INTRODUCTION

Although asthma was traditionally believed to result from inflammation driven by T-helper type 2 (Th2) responses (1, 2), recent studies have focused on the highly heterogeneous features of this disease, including multiple phenotypes and endotypes (3, 4). However, airway inflammation itself has been rarely measured in clinics, and the inflammatory process remains inconsistently identified (5). Recently, biomarkers such as fractional exhaled nitric oxide (FeNO) (6), sputum eosinophil count (7, 8), and serum periostin (9) for the determination and treatment of specific asthma subtypes are being emphasized (10, 11).

Human tumor necrosis factor-like weak inducer of apoptosis (TWEAK), a type II-transmembrane protein that can be cleaved to function as a soluble cytokine (12), is expressed in various cell types, including inflammatory cells, such as monocytes, macrophages, dendritic cells, T cells, and NK cells (13). TWEAK acts through its highly inducible receptor, fibroblast growth factor-inducible 14 (Fn14), and plays a multifunctional role by regulating diverse cellular responses, including pro-inflammatory activity, cell growth, angiogenesis, modulation of progenitor cell biology and even cell death (13). It is significantly elevated in inflammatory diseases (14–16) and cancer (17),

which are associated with infiltration of inflammatory cells or activation of resident innate immune cells.

A few studies (18–20) have investigated the effect of TWEAK/Fn14 signaling on human bronchial epithelial cells, and suggested its possible contribution to the development of airway inflammation. However, there has been no report on the expression of TWEAK in patients with respiratory diseases. Thus, we planned to evaluate the airway TWEAK levels in children with asthma, investigate the associations with sputum analysis and pulmonary function test (PFT) parameters, and potentially explain the specific subtypes of asthma.

METHODS

Study design

A total of 230 children aged 5–18 years, who visited Severance Children's Hospital for work-up or treatment of asthma or for a health check-up between June 2012 and June 2016, were enrolled. Subjects with asthma were confirmed on the basis of consistent respiratory symptoms verified by physicians, the presence of either a bronchodilator (BD) response of $\geq 12\%$ increase in the forced expiratory volume in 1 s (FEV_1), or bronchial hyperresponsiveness (BHR) defined as a decrease in FEV_1 of $\geq 20\%$ with inhalation of < 16 mg/mL methacholine. The asthma severity and level of control were assessed using the 2015 Global Initiative for Asthma guidelines. Healthy control subjects consisted of children who had visited the hospital for a general health check-up or vaccination, and had normal lung function without BHR and never had a physician diagnosis of asthma. Children with concurrent diagnosis of other respiratory diseases or a parenchymal lung disease evident on chest radiography performed within 4 weeks were excluded. After clinical assessment, PFT with BD response, sputum induction, and blood sampling were performed on the same day, followed by a methacholine challenge test (MCT).

The study was approved by the institutional review board (IRB) of Severance Hospital (Seoul, Korea; IRB no. 4-2004-0036). Written informed consent was obtained from the participants and their parents.

Spirometry with impulse oscillometry (IOS) and MCT

Spirometry and MCT were executed with a Jaeger MasterScreen PFT system (Jaeger Co., Würzburg, Germany) by using standard techniques in compliance with the American Thoracic Society/European Respiratory Society guidelines (21). For MCT, participants inhaled increasing doses of methacholine (0.075, 0.15, 0.31, 0.62, 1.25, 2.5, 5, 10, 25, and 50 mg/mL), nebulized by the dosimeter (MB3; Mefar, Brescia, Italy) until FEV₁ was reduced by 20% from a postnebulized saline solution value. The provocative concentration of methacholine that caused a 20% decrease in FEV₁ (PC₂₀) was determined.

IOS was performed with a Jaeger MasterScreen IOS system (Jaeger Co.) in accordance with standard recommendations (22). IOS parameters were recorded including the mean respiratory resistance at 5 Hz (R5) and 10 Hz (R10), difference between respiratory resistance at 5 and 20 Hz (R5-R20), reactance value at 5 Hz (X5), and reactance area (AX). As R5 and R20 are considered to reflect the total and central airway resistance, respectively, the difference between them (R5-R20) is considered a parameter of small airway obstruction (23). X5 and AX also have a relationship with peripheral airway obstruction (23).

FeNO measurement

FeNO was measured using a CLD 88 analyzer (Eco Medics, Duernten, Switzerland) at a constant expiratory flow rate of 50 mL/s. Measurements were made according to standard guidelines (21). All children refrained from eating nitrate-rich foods for 24 h before FeNO measurement because ingestion of nitrate-rich foods can affect the levels

of FeNO. The mean value of the three consecutive measurements was calculated and considered the actual value.

Sputum induction, analysis, and assessment of TWEAK levels

Sputum induction and processing were performed according to the protocol by Gibson et al. (24). All children were instructed to rinse their mouths thoroughly with water to clear debris and squamous epithelial cells, after which they inhaled a 3% saline solution nebulized in an ultrasonic nebulizer (NE-U12; Omron Co., Tokyo, Japan) at maximum output at room temperature. Then, they were encouraged to cough deeply at 3-min intervals. Spirometry was repeated thereafter, and when the FEV₁ had decreased to $\leq 80\%$ of the post-BD baseline value, the child was required to wait until it returned to baseline values. Induced samples were kept at 4°C for ≤ 2 h before further processing. Selected portions of the samples were dispersed with dithiothreitol (WACO Pure Chemical Industries Ltd., Osaka, Japan), and a total cell count was performed. We determined sputum viability with the trypan blue exclusion method to ensure adequate viability. Total cell counts were carried out using a hemocytometer, and slides were prepared with cytopsin (Cytospin3; Shandon, Tokyo, Japan) and stained with May-Grünwald-Giemsa solution for differential cell counts. An adequate specimen was defined as one producing countable cytopsin slides for estimation for differential cell count, with minimal squamous contamination ($< 50\%$) (24). At least 400 inflammatory cells were counted for each specimen by two individuals who were blinded to the clinical details. Sputum inflammatory subtypes were categorized, according to the percentages of sputum eosinophils and neutrophils, as eosinophilic (sputum eosinophils $\geq 3\%$ and sputum neutrophils $< 61\%$) and noneosinophilic (sputum neutrophils $\geq 61\%$ or sputum eosinophils $< 3\%$) inflammation (25). The supernatant was then stored at -70°C for subsequent TWEAK assay performed with enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN, USA).

Laboratory and allergy screening tests

Complete blood count, including eosinophil count, and total and specific immunoglobulin E (IgE) levels were measured with the ADVIA 2120i hematology system with autoslide (Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA) and Pharmacia CAP assay (Uppsala, Sweden), respectively. A specific IgE test was done with six common allergens in Korea (two types of dust mites, egg white, cow's milk, German cockroach, and *Alternaria alternata*). We defined atopy as the presence of at least one positive allergen-specific IgE test result (sIgE $\geq$ 0.7 KUa/L).

Statistical analysis

Continuous data were reported as the mean ($\pm$ standard deviations) or median (interquartile range). Comparisons of two groups were performed with Student's *t*-test or Mann-Whitney *U*-test for continuous variables, and the chi-square test or Fisher's exact test was used to analyze categorical variables. For comparisons among the three groups of severity and control status, Kruskal-Wallis *H*-test was used, and multiple comparisons were conducted with Dunn's method. Correlations between TWEAK levels and PFT parameters were analyzed through Spearman's rank correlation test. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic accuracy and calculate the optimal cutoff level.

All statistical analyses were conducted with SPSS Statistics (version 22.0; IBM Corp., Armonk, NY, USA) and R (version 3.3.2; R Foundation for Statistical Computing, Vienna, Austria). *P* < 0.05 was considered statistically significant.

RESULTS

Subjects' characteristics

Of the 230 children enrolled, successful sputum induction was carried out in 196 subjects (85.2%). Among them, 35 subjects had inadequate samples of induced sputum, and the remaining 173 subjects were finally analyzed. They consisted of 95 children with asthma and 78 healthy controls, and their demographic characteristics and clinical indices are summarized in Table 1. The groups did not differ in sex, age, and body mass index. Atopic tendency was significantly higher in asthma patients. Blood eosinophil count, total IgE, and FeNO significantly differed between the groups. Compared with healthy controls, children with asthma showed a lower percentage of FEV₁/forced vital capacity (FVC) ratio, % predicted FEV₁, % predicted maximum midexpiratory flow (FEF₂₅₋₇₅), and peak expiratory flow (PEF). IOS parameters including AX, R5-R20, R5, R10, and X5 were also significantly elevated in children with asthma (Table 1).

Sputum TWEAK levels in children with asthma and healthy controls

Sputum TWEAK level was significantly elevated in children with asthma than in healthy controls [493.0 (157.1–904.3) vs. 118.2 (67.5–345.5) pg/mL, $P < 0.001$; Fig. 1A]. It was significantly higher in patients with severe asthma than in those with mild to moderate asthma [1212.6 (896.9–1418.5) vs. 447.0 (109.5–779.3) pg/mL, $P = 0.001$; 1212.6 (896.9–1418.5) vs. 408.4 (170.2–716.5) pg/mL, $P = 0.001$; Fig. 1B]. Similarly, TWEAK expression was significantly elevated in patients with poorly controlled asthma compared with those with well- or partly controlled asthma [1152.7 (315.3–1553.3) vs. 142.9 (46.4–585.2), $P < 0.001$; 1152.7 (315.3–1553.3) vs. 495.1 (172.3–753.9) pg/mL, $P = 0.014$; Fig. 1C). In correlation analysis, sputum TWEAK level showed significant positive correlations with asthma severity ($r = 0.250$, $P = 0.015$) and level of control ($r = 0.389$, $P < 0.001$). There were no significant associations between sputum TWEAK levels and sex or age (data not shown).

Associations between sputum TWEAK levels and PFT parameters

Among the total population, FEV₁, FEV₁/FVC, PEF, FEF_{25–75}, and post-BD FEV₁/FVC had significant negative correlations with TWEAK levels. Of the IOS parameters, AX, R5, and X5 revealed significant positive correlations with TWEAK levels (Table 2). When the total subjects were further divided into the eosinophilic airway (EA) group (n = 44) and non-EA (NEA) group (n = 129), the NEA group demonstrated significant inverse correlation with FEF_{25–75}, and positive correlations with AX, R5, and X5. In the EA group, sputum TWEAK showed no significant associations with any PFT parameter (Table 2).

Sputum TWEAK levels in children with asthma and healthy controls according to types of airway inflammation

In the NEA group, TWEAK level was significantly elevated in children with asthma than in healthy controls [486.0 (140.9–1025.0) vs. 123.6 (72.8–415.3) pg/mL, $P < 0.001$; Fig. 2A]. Moreover, a positive progression of increasing sputum TWEAK levels with increasing asthma severity [208.9 (50.0–717.7), 455.7 (177.7–841.4), and 1269.6 (1057.9–1636.6) pg/mL, respectively; Fig. 2B] and poorer control status [66.9 (15.7–466.8), 489.5 (185.5–825.0), and 1233.4 (812.2–1658.5) pg/mL, respectively; Fig. 2C] was noted. However, in the EA group, TWEAK did not show any significant difference according to severity class or control status (data not shown). Consistently, sputum TWEAK level showed significant positive correlations with asthma severity ($r = 0.358$, $P = 0.001$) and level of control ($r = 0.470$, $P < 0.001$) only in the NEA group.

Associations between sputum TWEAK levels and PFT parameters according to types of airway inflammation

The demographic characteristics and clinical indices of the total subjects according to airway cellular phenotypes are summarized in supplementary Table E1. The subjects were subdivided into high and low TWEAK groups based on the optimal TWEAK cutoff level (263.0 pg/mL) obtained from ROC curve analysis (Supplementary Fig. 1). In the total

population, subjects with high TWEAK revealed significantly lower FEF₂₅₋₇₅ ($P = 0.001$) and higher AX ($P = 0.008$) and X5 ($P < 0.001$) than those with low TWEAK (Fig. 3A). In the NEA group, similar findings with lower FEF₂₅₋₇₅ ($P = 0.017$) and higher AX ($P < 0.001$), R5-R20 ($P = 0.025$), and X5 ($P < 0.001$) were found in subjects with high TWEAK (Fig. 3B), whereas no significant differences were noted between the high and low TWEAK groups in the above parameters in subjects with EA inflammation (data not shown).

Sputum TWEAK and FeNO

Among the total study population, sputum TWEAK showed no meaningful association with FeNO. In children with asthma, a significant negative correlation was noted ($r = -0.364$, $P = 0.007$) between sputum TWEAK level and FeNO, and when these subjects were further divided into the EA and NEA groups, only NEA group demonstrated a significant inverse correlation ($r = -0.377$, $P = 0.030$). In addition, when asthmatic children were categorized into the high and low FeNO groups according to the cutoff value of 20 ppb, the sputum TWEAK level was significantly lower in children in the high FeNO group than in those in the low FeNO group [176.0 (134.6–512.1) vs. 507.0 (253.7–838.2) pg/mL, $P = 0.018$].

DISCUSSION

Sputum TWEAK levels were significantly elevated in children with asthma compared with healthy controls. TWEAK levels were notably increased in severe asthma and uncontrolled asthma. Furthermore, sputum TWEAK level was significantly correlated with PFT parameters reflecting airway obstruction, which was particularly prominent in subjects with NEA inflammation. Significant differences in FEF₂₅₋₇₅, AX, R5-R20, and X5 were noted between the high and low TWEAK groups within the NEA group, whereas no meaningful differences were found within the EA group. Correlation studies also showed significant associations of sputum TWEAK levels with PFT indices, asthma severity, and control status distinctively in the NEA group.

TWEAK signals through Fn14, which contains a TNF receptor-associated factor binding sequence, resulting in the activation of nuclear factor- κ B signaling pathways (26), which have been known to induce multiple cellular responses depending on the cell type and context (13). It contributes to a physiological process promoting tissue repair after acute injury, whereas its persistent upregulation leads to pathological tissue degeneration and remodeling in chronic inflammation (13). Since the first description from 1997 (12), there have been several animal and human studies that aimed to elucidate the role of the TWEAK/Fn14 pathway in inflammatory diseases, exemplified by rheumatoid arthritis (14), systemic lupus nephritis (15), multiple sclerosis (16), and tumors (17).

Accordingly, a few studies reported associations between TWEAK and airway inflammation. Xu et al. (18) reported that TWEAK stimulates human bronchial epithelial cells to produce proinflammatory IL-8 and granulocyte-macrophage colony-stimulating factor. They speculated that infiltrating monocytes/macrophages might express TWEAK upon inflammation, and subsequent signaling leads to the development of inflammatory airway and lung diseases. Itoigawa et al. (19) identified that TWEAK reduced the expression of E-cadherin and accelerated the transforming growth factor- β -induced features of epithelial-mesenchymal transition in bronchial epithelial cells, which may contribute to chronic airway changes and remodeling. A recent report (20) also demonstrated that dexamethasone can reduce airway inflammation through inhibition of TWEAK/Fn14 expression in asthmatic mice.

The present findings of sputum TWEAK levels, which were significantly elevated in children with asthma and higher with greater asthma severity and poorer control status, are consistent with the known molecular function of TWEAK in chronic inflammatory processes. Furthermore, our study revealed significant associations between sputum TWEAK levels and several PFT parameters. In the total population, forced expiratory indices including FEV₁, FEV₁/FVC, PEF, FEF₂₅₋₇₅, and post-BD FEV₁/FVC were negatively correlated with TWEAK. Among the IOS parameters, AX, R5-R20, R5, and X5 were positively correlated with TWEAK. These significant negative and positive

correlations also support the possible associations of TWEAK with airway obstruction and remodeling.

Interestingly, according to subgroup analysis of airway inflammatory types, significant correlations with PFT parameters were noted distinctively in the NEA group. In the NEA group, FEF_{25-75} was significantly lower in the high TWEAK group than in the low TWEAK group, and FEF_{25-75} had a statistically significant inverse correlation with sputum TWEAK. AX, R5-R20, and X5 were significantly higher in the high TWEAK group, and AX and X5 showed statistically significant positive correlations with TWEAK. As decreased FEF_{25-75} and increased AX, R5-R20, and X5 are all characteristics of small airway dysfunction (23), airway TWEAK level may reflect small airway inflammation in NEA.

Clinically, noneosinophilic asthma, which is mainly characterized by neutrophilic airway inflammation, is frequently associated with severe asthma and steroid resistance, indicating the need for alternative treatment strategies (5). However, much less is understood about the underlying phenotypes and molecular mechanisms compared with eosinophilic asthma. In the present study, the sputum TWEAK levels in children with asthma within the NEA group were higher with greater asthma severity and poorer control status, and had significant positive correlations with these parameters. In contrast, there were no significant differences in TWEAK levels across subjects with asthma stratified according to severity and control status in the EA group, and no statistically significant correlations were noted. Accordingly, TWEAK may have a greater association with NEA inflammation, potentially explaining the underlying molecular process of noneosinophilic asthma. The correlation assay demonstrated a significant negative association between the sputum TWEAK level and the measured FeNO in asthmatic children. This correlation was distinctively statistically significant in asthmatics with NEA. Considering that FeNO is a well-established marker associated with eosinophilic airway inflammation in asthma (6), the above findings further support the possible contribution of TWEAK in noneosinophilic childhood asthma. A previous report of Nakayama et al. (27), which showed TWEAK expression from monocytes upon stimulation with interferon- γ , and a recent report of Bilgiç et al. (28), which identified elevated serum levels of TWEAK in patients with psoriasis vulgaris, a T-cell-mediated

chronic inflammatory disease with a mixed Th1/Th17 cytokine environment, also support this association with non-Th2 immunity. Another study (29) also revealed that TWEAK induced the production of IL-17 in rheumatoid arthritis, and blockade of Fn14 significantly suppressed Th17 differentiation. Together, these molecular findings and favorable result of anti-TWEAK treatment in the clinical improvement of rheumatoid arthritis (30) may also suggest its relevance to Th17 immunity. However, further molecular and clinical studies are needed to determine the explicit role of TWEAK in noneosinophilic asthma.

This study has limitations in that its cross-sectional nature does not allow the determination of cause-and-effect relationships. In addition, although there was a statistically meaningful correlation between sputum TWEAK levels and PFT parameters reflecting small airway obstruction, the strength of the association was modest at best. Such a modest correlation could have resulted from various reasons. First, noneosinophilic asthma is a heterogeneous entity that encompasses different subgroups of distinct inflammatory phenotypes such as neutrophilic, mixed granulocytic, and paucigranulocytic asthma. Although we could not find any significant associations between sputum TWEAK levels and asthma status, and different lung function parameters when these subclassifications were applied, this might be partly due to the small sample size in each inflammatory subtype. In addition, TWEAK expression could have been affected by diverse clinical conditions including the duration of illness, stable or exacerbating course, environmental exposures such as passive smoking and heavy pollutant exposure, other comorbid inflammatory or infectious diseases, and accompanying treatments. Despite these limitations, the present findings may provide directions for future research. Subsequent prospective research with a larger sample size is necessary to elucidate the specific inflammatory subtype in which TWEAK has a stronger correlation with disease activity, and to reveal the pathophysiologic roles of TWEAK on disease progression over an extended period of time.

In conclusion, sputum TWEAK levels were elevated in children with asthma, and significantly correlated with asthma severity, level of control, and indices of small airway obstruction, distinctively in children with NEA inflammation. We thus suggest that TWEAK may play a role in small airway inflammation in children with noneosinophilic asthma.

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TABLES

Table 1. Subjects' characteristics (N = 173)

	Control (n = 78)	Asthma (n = 95)	P-value
Age, years	9.7 (7.8–12.1)	8.5 (6.6–11.3)	0.053
Sex, M/F (%M)	50/28 (64.1)	61/34 (64.2)	>0.999
BMI, kg/m ²	18.6 (16.0–20.7)	17.5 (15.9–19.9)	0.195
Asthma severity, mild:moderate:severe (n)	N/A	32:53:10	
Blood eosinophil, /μL (n = 152)	220.0 (110.0–360.0)	415.0 (180.0–710.0)	<0.001
Sputum eosinophil, %	0 (0–1)	3 (0–12)	<0.001
Sputum neutrophil, %	62 (33–77)	53 (31–83)	0.902
Serum total IgE, IU/mL (n = 138)	109.0 (67.9–195.0)	340.0 (139.0–629.0)	<0.001
FeNO, ppb (n = 106)	10.9 (8.0–23.2)	18.6 (10.0–42.9)	0.008
Atopy, n (%)	15 (19.2)	35 (36.8)	0.004
Spirometry parameters			
FEV ₁ , % predicted	105.5 (98.6–113.9)	94.9 (83.0–103.7)	<0.001
FEV ₁ /FVC, %	88.5 (85.4–91.5)	81.8 (76.5–87.2)	<0.001
Change in FEV ₁ after BD, %	3.0 (0.2–5.6)	7.9 (3.8–13.7)	<0.001
FEF _{25–75} , % predicted	98.8 ± 21.1	67.3 ± 25.0	<0.001
Post-BD FEV ₁ /FVC, %	90.6 (88.0–93.5)	86.2 (81.2–89.7)	<0.001
IOS parameters			
AX, kPa/L	1.8 (1.0–2.5)	3.0 (1.8–3.8)	<0.001
R5-R20, kPa/(L/s)	0.5 ± 0.2	0.6 ± 0.2	<0.001
R5, % predicted	92.2 (79.7–104.8)	113.8 (94.4–128.1)	<0.001
R10, % predicted	81.8 (70.0–92.6)	90.1 (75.7–111.5)	0.002
X5, % predicted	101.8 (71.4–124.9)	141.0 (103.3–183.9)	<0.001

Data are given as number (%), mean (± standard deviation), or median (interquartile range), as appropriate.

Abbreviations: BMI, body mass index; IgE, immunoglobulin E; FeNO, fraction of nitric oxide; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; BD, bronchodilator; FEF_{25–75}, maximum midexpiratory flow; PC₂₀, provocative concentration of methacholine inducing a 20% decrease in FEV₁; IOS, impulse oscillometry; AX, reactance area; R5-R20, difference between resistance at 5 and 20 Hz; R5, resistance at 5 Hz; R10, resistance at 10 Hz; X5, reactance at 5 Hz; N/A, not applicable.

Table 2. Correlations between sputum TWEAK levels and PFT parameters

	Total population (N = 173)		Noneosinophilic airway (n = 129)		Eosinophilic airway (n = 44)	
	<i>r</i>	<i>P</i> -value	<i>r</i>	<i>P</i> -value	<i>r</i>	<i>P</i> -value
Spirometry parameters						
FVC, % predicted	-0.120	0.115	-0.065	0.463	-0.202	0.189
FEV ₁ , % predicted	-0.171	0.024 [†]	-0.116	0.191	-0.207	0.178
FEV ₁ /FVC, %	-0.168	0.027 [†]	-0.127	0.152	-0.117	0.450
PEF, % predicted	-0.211	0.005 [‡]	-0.166	0.060	-0.247	0.105
FEF ₂₅₋₇₅ , % predicted	-0.230	0.002 [‡]	-0.183	0.038 [‡]	-0.165	0.285
Post BD FEV ₁ /FVC, %	-0.184	0.016 [†]	-0.139	0.119	-0.241	0.115
IOS parameters						
AX, kPa/L	0.248	0.001 [‡]	0.317	<0.001 [¶]	-0.052	0.736
R5-R20, kPa/(L/s)	0.126	0.101	0.168	0.058	-0.013	0.931
R5, % predicted	0.169	0.028 [†]	0.203	0.022 [†]	0.046	0.765
R10, % predicted	0.037	0.630	0.048	0.590	0.011	0.945
X5, % predicted	0.389	<0.001 [¶]	0.407	<0.001 [¶]	0.239	0.118

r, Spearman's correlation coefficient.

Abbreviations: TWEAK, tumor necrosis factor-like weak inducer of apoptosis; PFT, pulmonary function test; FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s; PEF, peak expiratory flow; FEF₂₅₋₇₅, maximum midexpiratory flow; BD, bronchodilator; IOS, impulse oscillometry; AX, reactance area; R5-R20, difference between resistance at 5 and 20 Hz; R5, resistance at 5 Hz; R10, resistance at 10 Hz; X5, reactance at 5 Hz.

[†]*P* < 0.05, [‡]*P* < 0.01, [¶]*P* < 0.001 in correlation with TWEAK by Spearman's rank correlation test.



