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Interleukin-26 is associated with the level of systemic inflammation and lung functions in obese and non-obese moderate-to-severe asthmatic patients

Abstract

Introduction: Obese asthma is a complex syndrome, which includes different phenotypes of disease. At present, these phenotypes only have started to acquire a sufficient understanding. It was suggested that IL-26 is a potential biomarker of disease severity in asthma without signs of Th2-mediated inflammation. In this study, we investigated the serum and exhaled levels of IL-26 and its associations with the level of systemic inflammation, lung functions, and body weight in obese and non-obese moderate-to-severe asthmatic patients

Material and methods: The study included 10 healthy subjects, 10 obese subjects without lung pathologies, 10 non-obese asthmatics (NOA) (BMI 18.5–24.9 kg/m^2), and 40 obese asthmatics (OA) (BMI 25.0–49.9 kg/m^2). During the visit, patients' examination and spirometry with the bronchodilator reversibility test were conducted, the exhaled breath condensate (EBC) was obtained, and the blood samples were collected. The level of IL-26, interleukin-1β (IL-1β), interleukin-4 (IL-4), interleukin-6 (IL-6), TNF-α, interleukin-10 (IL-10), total and specific immunoglobulin E (IgE), and high sensitive C reactive protein (hs-CRP) were measured using the ELISA kits. Statistical comparison between 2 groups was analyzed using the Mann–Whitney rank-sum test. Chi-square with Yates' correction was used to compare frequencies. Spearman's rank test was used for correlating nonparametric variables. The Receiver Operating Characteristic (ROC) curve and the area under ROC curve (AUC) were used for evaluating the diagnostic power of IL-26 as a possible biomarker.

Results: NOA had a reversible airway obstruction with reduced FEV_1, FEV_1/FVC, FVC 25/75, and positive post-bronchodilator test (PBT), significantly increased serum levels of IL-10, IL-4, and slightly increased IL-26. NOA had significantly increased exhaled IL-26 in comparison with healthy subjects. The obese subjects had a normal ventilatory pattern without airway obstruction, and differences in serum IL-26, IL-10, and IL-4 concentrations in comparison with healthy subjects. Obese subjects had a significant escalation of hs-CRP and no differences in the levels of exhaled IL-26, IL-10, and hs-CRP as compared with healthy subjects. OA had reduced FEV_1, FEV_1/FVC, and FEV25–75 in comparison with non-obese asthmatics. OA had elevated IL-26, IL-10, IL-4, and hs-CRP concentrations as compared with healthy subjects. These patients had a partial similarity with both non-obese asthmatics (elevated IL-26, IL-10, and IL-4) and obese subjects (elevated, IL-1β, IL-6, TNF-α, hs-CRP). OA had a reduced concentration of exhaled IL-26 in comparison with NOA and elevated exhaled IL-10 in comparison with obese subjects. Furthermore, OA had an increased concentration of IL-1β and TNF-α in comparison with healthy individuals and NOA. Exhaled IL-26 concentration distinguished non-obese asthmatics from healthy subjects, asthmatic patients from non-asthmatics (healthy and obese subjects), all asthmatic patients from non-asthmatics (healthy and obese subjects).

Conclusions: Exhaled IL-26 elevated in obese and non-obese moderate-to-severe asthmatic patients. Exhaled IL-26 might be a perspective biomarker in non-obese and obese asthmatics. The obese asthmatic phenotype comprised the combined systemic and local airway inflammation.

Key words: interleukin-26, systemic inflammation, obesity, asthma

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Introduction

Both asthma and obesity are common conditions, leading to a substantial public health burden. Obese asthma is characterized by poor asthma control, impaired lung function, and decreased efficacy of inhaled treatment [1]. These patients experienced more hospitalizations and

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used more rescue medications than normal-weight asthmatics [2]. Obese asthma is a complex syndrome, which includes different phenotypes of disease. At present, these phenotypes only have started to acquire a sufficient understanding [3].

Obesity-associated asthma can be classified as non-type2-driven asthma [4] with a specific biomarker profile: increased adipose tissue inflammation with high serum level of leptin, interleukin-6, and low adiponectin; elevated oxidative stress, and decreased exhaled NO [3].

M1/M2 macrophages orchestrated cytokine network in asthma and obesity since M1 macrophages of obese adipose tissue produced pro-inflammatory cytokines such as IL-6, TNF-α, IL-1β, IFN-γ, CCL2, CCL5, CCL8, etc. This cytokine set activated innate lymphoid cells 3 (ILC3) to produce IL-17 in lung tissue with further lung impairment [5].

Recently, the role of interleukin-17 in obese asthma inflammation was proposed [6, 7]. The novel data have shown that Th17 cell generation can be supported by IL-26 produced in asthmatic sputum. IL-26 induced pro-inflammatory cytokine secretion by monocyte/macrophage locally in the airways [8]. It was suggested that IL-26 is a potential biomarker of disease severity in asthma without signs of Th2-mediated inflammation [9]. These data go in parallel with the correlation of IL-26 with the level of systemic inflammation, lung functions, and body weight in COPD patients [10].

In this study, we investigated the serum and the exhaled levels of IL-26, and their associations with the level of systemic inflammation, lung functions, and body weight in moderate-to-severe obese asthmatic patients.

Material and methods

Subjects

The study included 10 healthy subjects, 10 obese subjects without lung pathologies, 10 non-obese asthmatics, and 40 obese asthmatics during 2019.

Inclusion criteria: male or female adults aged 40–70 years, diagnosed with asthma at least 12 months before the screening visit. The diagnosis of asthma relied on the GINA 2017 criteria, which included post-bronchodilator spirometry to confirm reversible airflow obstruction. Asthma severity was estimated by Asthma Control Test (ACT), Asthma Control Questionnaire (ACQ) and Asthma Quality of Life Questionnaire Score (AQLQ). The included patients were in stable condition and had neither exacerbations nor viral infections for at least 1 month prior to the study.

We excluded patients with COPD, conditions that were associated with inflammation, such as infection (by conducting complete blood count, and biochemical blood analysis). The study also excluded patients with cancer and severe cardiovascular conditions, such as unstable coronary heart disease, class III/IV according to New York Heart Association, acute myocardial infarction, uncontrolled endocrine disease, severe hepatic, renal, gastrointestinal impairments, osteoarthritis, current tobacco smoking, as well as patients taking systemic corticosteroids or biologics.

All subjects underwent treatment according to the GINA recommendation (inhalation of budesonide/formoterol or fluticasone propionate/salmeterol, salbutamol as a rescue medication) for at least 1 month prior to the study. The doses of inhaled corticosteroids were estimated as high, medium and low according to the guidelines [11].

According to BMI of asthmatic patients, they were divided into 2 groups: obese (n = 40) (BMI 25.0–49.9 kg/m^2) and non-obese (n = 10) (BMI 18.5–24.9 kg/m^2). 10 subjects with normal lung function and BMI formed the group of healthy subjects.

Ethics statement

This study is a part of the research project No. 0120U101166 "The study of the pathogenetic role of the circadian molecular clock in the development of metabolic diseases and systemic inflammation and the development of treatment methods aimed at these processes", which was approved by the Ethics Commission of Ukrainian Medical Stomatological Academy (Approval No. 177a as of 27.11.2019) and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013. All individuals signed written informed consent forms before enrollment in the study.

Assessments and study procedures

During the visit on the 17th ± 3 days after the screening, we conducted patients' examination and spirometry with bronchodilator reversibility test. We also obtained the exhaled breath condensate (EBC) and collected the blood samples.

Patients' examination included general clinical methods and measurement of anthropometric indices (BMI). BMI was calculated as weight (kg)/height (m)^2.

Spirometry

All subjects underwent spirometry (using Vitalograph, Clare, Ireland) in compliance with the American Thoracic Society and the European Respiratory Society guidelines. The bronchodilator reversibility test was conducted with subsequent evaluation of FEV_1, FEV_1/FVC, FEV 25–75% [12, 13].

Exhaled breath condensate

For EBC collection, we used a U-shape glass tube embedded in the water/ice bath. Patients exhaled air for 15 min at the normal resting breathing pattern. When a satisfactory sample of EBC was obtained, we discontinued the procedure. EBC samples were concentrated (up to 4 times) by centrifugation using Amicon® Ultra 4 mL Centrifugal Filters (3.000 MWCO) at 4.000 × g maximum for approximately 30–40 minutes. Concentrated EBC samples were centrifuged and stored at −70°C until analysis. This procedure was standardized according to the ATS/ERS Task Force [14].

Cytokine and IgE assay

The level of IL-26 protein (sensitivity 18.75 pg/mL) (Elabscience, USA), interleukin-4 (sensitivity 0.4 pg/mL), interleukin-10 (sensitivity 1.0 pg/mL), IL-1β (sensitivity 1.0 pg/mL), IL-6 (sensitivity 0.5 pg/mL), TNF-α (sensitivity 1.0 pg/mL) and CRP (sensitivity 0.05 IU/mL) concentrations (Vector-Best-Ukraine Ltd) were measured using the enzyme-linked immunosorbent assay kits according to the manufacturers' instructions. The levels of total IgE were measured using the enzyme-linked immunosorbent assay kit (NovaTec Immunodiagnostica GmbH) according to the manufacturers' instructions. The level of specific IgE to E1 (cat epithelium), Gx (grass mixture — timothy, dactylis, fescue, bluegrass, meadow foxtail, wheat grass, rye), Tx (spring trees — birch, alder, hazel), E2 (dog epithelium), D1 (Dermatophagoides spp.), W56 (wormwood) were measured using the enzyme-linked immunosorbent assay kit (CHEMA Ltd, Ukraine) according to the manufacturers' instructions. Specific IgE concentrations were classified as Class 0: up to 0.35 IU/mL, Class 1: 0.35–0.70 IU/mL, Class 2: 0.70–3.5 IU/mL, Class 3: 3.5–17.5 IU/mL, and Class 4: more than17.5 IU/mL.

Statistics

Data are expressed as M (25–75) where M is the median in the sample; (25–75) — 25th and 75th percentiles. Statistical comparison between 2 groups was analyzed using the Mann-Whitney rank-sum test. Chi-square with Yates' correction was used to compare frequencies. Spearman's test was used for correlating nonparametric variables. The Receiver Operating Characteristic (ROC) curve and the area under the ROC curve (AUC) were used for evaluating the diagnostic power of IL-26 as a possible biomarker. We used GraphPad Prism version 5.00 (GraphPad Software, Inc., San Diego, CA, USA). Probability of $P < 0.05$ was considered statistically significant.

Results

Characteristics of patients

According to the aim of the study, we distinguished four groups — healthy subjects, non-obese asthmatics, obese subjects without lung pathologies, and obese asthmatics. Patients' demographic information is summarized in Table 1.

The groups of patients were comparable in terms of age and gender with the predominance of female ratio. Obese subjects (52.50 (44.75–67.00), p = 0.0035), and obese asthmatics (59.00 (51.25–67.00), p = 0.0003) were significantly older than healthy subjects (35.00 (21.75–38.00)).

Obese subjects and obese asthmatics had a significantly higher body weight, BMI as well as waist and thigh circumferences than healthy subjects and non-obese asthmatics, respectively. There was a significant difference in BMI in obese subjects (41.00 (37.75–44.25)) and obese asthmatics (32.00 (29.00–35.00); p = 0.0005).

Eosinophil percentage was higher in non-obese (4.5 (2.975–5.625); p = 0.0002) and obese (3.55 (2.00–6.75); p = 0.0003) asthmatics than in healthy (1.00 (0.00–2.00)) and obese (1.00 (0.75–1.25)) subjects, respectively.

There were significant difference in total IgE concentrations between non-obese/obese asthmatics (132.8 (34.28–397.7), p = 0.0039; 97.6 (39.3–282.9), p = 0.0101, respectively) and healthy/obese subjects (16.6 (12.9–71.23); 22.95 (9.475–52.38), respectively).

Specific IgE concentrations were measured to determine asthmatic patients' sensibilization to common aeroallergens. Healthy and obese subjects had no specific IgE. Non-obese asthmatics had an elevated level of specific IgE in 50% and obese asthmatics — 35% (p = 0.6101), class 1: 10% and 7.5% (p = 0.5675), class 2: 10% and 7.5% (p = 0.5675), class 3: 20% and 10% (p = 0.9295), class 4: 10% and 10% (p = 0.8275), respectively.

Table 1. Characteristics of the studied population (M (25–75))

Characteristics	Healthy subjects (n = 10)	Non-obese asthmatics (n = 10)	Obese subjects (n = 10)	Obese asthmatics (n = 40)
Male/Female (n)	4/6	6/4	4/6	10/30
Age [years]	35.00 (21.75–38.00)	43.00 (25.00–61.25) $P_1 = 0.0883$	52.50 (44.75–67.00) **$P_1 = 0.0035$** $P_2 = 0.1981$	59.00 (51.25–67.00) **$P_1 = 0.0003$** **$P_2 = 0.0341$** $P_2 = 0.3254$
Height [cm]	174.00 (164.30–181.30)	173.50 (162.80–179.80 $P_1 = 0.8795$	167.50 (164.30–172.30) $P_1 = 0.3247$ $P_2 = 0.2892$	165.00 (159.30–172.00) $P_1 = 0.0783$ $P_2 = 0.0988$ $P_3 = 0.3689$
Weight [kg]	71.00 (64.50–76.25)	69.50 (62.50–74.25) **$P_1 = 0.3838$**	116.50 (107.00–124.50) **$P_1 < 0.0001$** **$P_2 = 0.0002$**	89.5 0 (79.50–97.75) **$P_1 < 0.0001$** **$P_2 < 0.0001$** **$P_3 = 0.0005$**
Waist circumference [cm]	76.00 (66.50–79.25)	70.00 (64.25–78.00) $P_1 = 0.471$	120.50 (111.30–129.80) **$P_1 = 0.0002$** **$P_2 = 0.0002$**	101.50 (89–114.50) **$P_1 < 0.0001$** **$P_2 < 0.0001$** **$P_3 = 0.0007$**
Thigh circumference [cm]	56.50 (52.75–62.50)	51.50 (48.75–56.50) $P_1 = 0.1399$	82.00 (77.75–102.80) $P_1 = 0.0028$ $P_2 = 0.001$	68.50 (60.00–74.00) $P_1 = 0.0005$ $P_2 = 0.0003$ $P_3 = 0.0027$
BMI [kg/m^2]	24.00 (22.75–24.25)	24.00 (22.00–25.00) $P_1 = 0.3266$	41.00 (37.75–44.25) $P_1 = 0.0002$ $P_2 = 0.0002$	32.00 (29.00–35.00) $P_1 < 0.0001$ $P_2 < 0.0001$ $P_3 = 0.0005$
Eosinophils [%]	1.00 (0.00–2.00)	4.5 (2.975–5.625) $P_1 = 0.0002$	1.00 (0.75–1.25) $P_1 = 0.9672$ **$P_2 = 0.0002$**	3.55 (2.00–6.75) **$P_1 = 0.0003$** $P_2 = 0.4151$ **$P_3 = 0.0002$**
IgE, IU	16.6 (12.9–71.23)	132.8 (34.28–397.7) $P_1 = 0.0039$	22.95 (9.475–52.38) $P_1 = 0.9118$ **$P_2 = 0.0089$**	97.6 (39.3–282.9) **$P_1 = 0.0101$** $P_2 = 0.4278$ **$P_3 = 0.0074$**
ACT, score	NA	16 (11–17.25)	NA	13 (11–15) $P_2 = 0.1766$
ACQ, score	NA	1.66 (1.24–2.83)	NA	2.5 (2.16–2.95) **$P_2 = 0.0424$**
AQLQ, score	NA	4.67 (4.29–5.64)	NA	4.25 (3.78–4.69) **$P_2 = 0.0186$**
Doses of inhaled glucocorticoids				
Budesonide/Fluticasone propionate (high-dose), n [%]	NA	2 (20)	NA	21 (52.5) $P_2 = 0.066$
Budesonide/Fluticasone propionate (medium-dose), n [%]	NA	4 (40)	NA	13 (32.5) $P_2 = 0.370$
Budesonide/Fluticasone propionate (low-dose), n [%]	NA	4 (40)	NA	7 (17.5) $P_2 = 0.053$

ACT — Asthma Control Test; ACQ — Asthma Control Questionnaire; AQLQ — Asthma Quality of Life Questionnaire; BMI — body mass index; IgE — immunoglobulin E; NA — not available

Table 2. Spirometry data from the studied population (M (25–75))

Characteristics	Healthy subjects (n = 10)	Non-obese asthmatics (n = 10)	Obese subjects (n = 10)	Obese asthmatics (n = 40)
FEV_1 [%]	93 (85.75–99.25)	73.5 (57.25–87)	104.0 (90.5–115.3)	62 (51.25–78.25)
		$P_1 = 0.0051$	$P_1 = 0.121$ **$P_2 = 0.0015$**	**$P_1 = 0.0001$** $P_2 = 0.1742$ **$P_3 = 0.0001$**
FVC [%]	102.5 (96.25–111)	94 (76.25–110.5)	104.5 (93.25–112)	77 (69.25–96)
		$P_1 = 0.3845$	$P_1 = 1$ $P_2 = 0.4961$	$P_1 = 0.2431$ $P_2 = 0.0872$ **$P_3 = 0.0024$**
FVC 25/75 [%]	104.5 (73.75–111)	45 (20.75–55.75)	104.5 (73.75–111)	33.5 (24.25–47.5)
		$P_1 = 0.0172$	$P_1 = 0.0126$ $P_2 = 0.0001$	$P_1 = 0.0001$ $P_2 = 0.4163$ **$P_3 = 0.0001$**
FEV_1/FVC [%]	70.5 (50.75–88)	77 (73.75–86)	99.5 (92.75–100.5)	76.5 (69–84.5)
		$P_1 = 0.0483$	$P_1 = 0.0137$ $P_2 = 0.0064$	**$P_1 = 0.0001$** $P_2 = 0.4891$ **$P_3 = 0.0098$**
Post-bronchodilator test [mL]	31 (16.25–76.25)	320 (257.5–420)	2.5 (0–39.25)	298 (220–450)
		$P_1 = 0.0004$	$P_1 = 0.066$ **$P_2 = 0.0003$**	**$P_1 = 0.0001$** $P_2 = 0.9516$ **$P_3 = 0.0001$**

FEV1 — forced expiratory volume in one second; FVC — forced vital compacity

There was no significant difference in the ACT in non-obese (16 (11–17.25)) and obese asthmatics (13 (11–15); p = 0.1766). On the other hand, there were significant differences in ACQ in non-obese (1.66 (1.24–2.83)) and obese asthmatics (2.5 (2.16–2.95); p = 0.0424), in AQLQ in non-obese (4.67 (4.29–5.64)), and obese asthmatics (4.25 (3.78–4.69); p = 0.0186).

There were differences in doses of inhaled steroids between non-obese and obese asthmatics. Obese asthmatics had an increased proportion of high-doses inhaled steroids (52.5%) in comparison with normal weight asthmatics (20%) (p = 0.066).

Spirometry

Spirometry is a powerful tool that can be used to detect, follow, and manage patients with asthma. In the first step, we determined the validity of the test and determined an obstructive or restrictive ventilatory pattern in the studied population.

The healthy and obese subjects had a normal ventilatory pattern without airway obstruction. We found a reduced FEV_1 (73.5 (57.25–87); p = 0.0051) and absolute FEV_1/FVC ratio (77 (73.75–86); p = 0.0483) as well as FVC 25/75 (45 (20.75–55.75); p = 0.0483)) in non-obese asthmatics indicating an obstructive ventilatory pattern. After bronchodilator challenge testing in non-obese asthmatics, a reversible airway obstruction pattern (320 (257.5–420); p = 0.0004) was identified.

In obese asthmatics, there was a similar obstructive ventilatory pattern as in non-obese asthmatics. Obese asthmatics had a reduced FEV_1 (62 (51.25–78.25); p = 0.1742) and FEV_1/FVC (76.5 (69–84.5); p = 0.4891) confirmed by the decreased mid-expiratory flow rate (FEF 25–75%) (33.5 (24.25–47.5); p = 0.4163) and the post-bronchodilator test (298 (220–450); p = 0.9516) in comparison with non-obese asthmatics (Table 2).

Cytokine concentrations in the sera

The airway inflammation underlying asthma and obesity is regulated by a network of mutually interacting cytokines. The exact functional role of each individual cytokine in the pathogenesis of this comorbidity remains to be fully established.

We studied the IL-26, hs-CRP, T2 (IL-4, IL-10) and T1 (IL-1β, IL-6, TNF-α) cytokine levels in the sera of non-obese and obese asthmatics (Table 3).

Non-obese asthmatics had significantly increased serum levels of IL-10 (4.61 (3.2–7.798); p = 0.0001), IL-4 (0.543 (0.2673–0.9858); p = 0.0021), IL-1β (1.445 (1.145–1.636); p = 0.001),

Table 3. Biomarkers concentration in the sera from the studied populations

Characteristics	Healthy subjects (n = 10)	Non-obese asthmatics (n = 10)	Obese subjects (n = 10)	Obese asthmatics (n = 40)
IL-26 [pg/mL]	20.96 (4.4–41.54)	78.38 (21.67–321.7) $P_1 = 0.1508$	34.91 (27.37–259.3) $P_1 = 0.2222$ $P_2 = 0.6905$	76.04 (31.64–146.6) **$P_1 = 0.0317$** $P_2 = 0.6905$ $P_3 = 0.5476$
IL-10 [pg/mL]	1.99 (1.208–2.24)	4.61 (3.2–7.798) $P_1 = 0.0001$	2.455 (1.38–3.5) $P_1 = 0.2176$ **$P_2 = 0.0115$**	3.39 (2.64–4.41) **$P_1 = 0.0001$** $P_2 = 0.0653$ $P_3 = 0.0543$
IL-4 [pg/mL]	0.006 (0-0.164)	0.543 (0.2673–0.9858) $P_1 = 0.0021$	0 (0–0.5263) $P_1 = 0.9585$ $P_2 = 0.0536$	0.457 (0.1798–0.9655) **$P_1 = 0.0051$** $P_2 = 0.5935$ $P_3 = 0.0721$
IL-6 [pg/mL]	0.864 (0.7227–1.044)	2.219 (1.135–5.647) $P_1 = 0.0058$	2.069 (1.556–4.65) **$P_1 = 0.0040$** $P_2 = 0.9705$	3.534 (1.908–4.873) **$P_1 = 0.0001$** $P_2 = 0.3811$ $P_3 = 0.2429$
IL-1β [pg/mL]	0.97 (0.849–1.041)	1.445 (1.145–1.636) $P_1 = 0.0001$	1.154 (0.9993–1.422) **$P_1 = 0.0355$** $P_2 = 0.1903$	1.259 (1.161–1.603) **$P_1 = 0.0001$** $P_2 = 0.7127$ $P_3 = 0.1408$
TNF-α	1.664 (1.479–1.956)	2.262 (1.69–5.548) $P_1 = 0.0493$	2.6 (1.818–3.147) **$P_1 = 0.0185$** $P_2 = 0.7959$	2.444 (1.769–3.304) **$P_1 = 0.0047$** $P_2 = 0.9903$ $P_3 = 0.8084$
hs-CRP [IU]	1.305 (0.37–3.065)	2.06 (0.4475–12.39) $P_1 = 0.6305$	10.84 (4.313–12.51) **$P_1 = 0.014$** $P_2 = 0.0889$	5.525 (2.893–11.88) **$P_1 = 0.005$** $P_2 = 0.135$ $P_3 = 0.2636$

hs-CRP — high sensitive C reactive protein; IL-1β — interleukin-1β; IL-4 — interleukin-4; IL-6 — interleukin-6; IL-10 — interleukin-10; IL-26 — interleukin-10; TNF-α — tumor necrosis factor α

IL-6 (2.219 (1.135–5.647); p = 0.0058), TNF-α (2.262 (1.69–5.548); p = 0.0493) with slightly increased IL-26 (78.38 (21.67–321.7); p = 0.1508).

In obese subjects, there were no differences in serum IL-26, IL-10, and IL-4 concentrations in comparison with healthy subjects. In our opinion, there were high variations of individual concentrations of these cytokines. Obese subjects had elevated levels of IL-1β (1.154 (0.999–1.422); p = 0.0355), IL-6 (2.069 (1.556–4.65); p = 0.0040), and TNF-α (2.6 (1.818–3.147); p = 0.0185)), with slightly increased IL-26 (78.38 (21.67–321.7); p = 0.1508). These patients had a significant escalation of hs-CRP (10.84 (4.313–12.51); p = 0.014).

Obese asthmatics had a specific cytokine profile with elevated IL-26 (76.04 (31.64–146.6); p = 0.0317), IL-10 (3.39 (2.64–4.41); p = 0.0001), IL-4 (0.457 (0.1798–0.9655); p = 0.0051), IL-1β (1.259 (1.161–1.603); p = 0.0001), IL-6 (3.534 (1.908–4.873); p = 0.0001), TNF-α (2.44 (1.769–3.304); p = 0.0047) and hs-CRP (5.525 (2.893–11.88); p = 0.005) concentrations as compared with healthy subjects. Moreover, these patients had a partial similarity with both non-obese asthmatics (elevated IL-26, IL-10, IL-4, IL-1β, IL-6, and TNF-α)) and obese subjects (elevated L-1β, IL-6, TNF-α, and hs-CRP).

Cytokine concentrations in the exhaled breath condensates

We studied exhaled IL-26, hs-CRP, T2 (IL-10), and T1 (IL-1β, IL-6, and TNF-α) cytokine levels in the exhaled breath condensate with healthy and obese subjects or non-obese and obese asthmatics (Table 4).

Non-obese asthmatics had a significantly increased exhaled IL-26 concentration (10.81 (3.245–23.63); p = 0.0001) and TNF-α (1.388 (0.4126–2.562); p = 0.0300) in comparison with healthy subjects.

Table 4. Biomarkers concentration in the exhaled breath condensates from the studied populations

Characteristics	Healthy subjects (n = 10)	Non-obese asthmatics (n =10)	Obese subjects (n = 10)	Obese asthmatics (n = 40)
IL-26 [pg/mL]	2.229 (1.201–3.809)	10.81 (3.245–23.63)	1.536 (1.071–2.911)	6.439 (5.113–8.028)
		P_1 = 0.0001	P_1 = 0.9118 **P_2 = 0.0005**	**P_1 = 0.0001** P_2 = 0.1490 **P_3 = 0.0002**
IL-10 [pg/mL]	0.9575 (0.798–1.303)	1.255 (0.955–1.38)	0.9111 (0.4121–1.199)	1.187 (0.7415–1.529)
		P_1 = 0.2799	P_1 = 0.4495 P_2 = 0.0892	P_1 = 0.2859 P_2 = 0.942 P_3 = 0.0538
IL-6 [pg/mL]	0.1292 (0.1076–0.2174)	0.1901 (0.1173–0.3163)	0.1093 (0.0655–0.1599)	0.1335 (0.1085–0.212)
		P_1 = 0.3527	P_1 = 0.3527 P_2 = 0.0524	P_1 = 0.9420 P_2 = 0.4958 P_3 = 0.2206
IL-1β [pg/mL]	0.9291 (0.7978–1.331)	1.262 (1.051–2.591)	1.097 (0.9117–2.159)	1.397 (1.032–2.861)
		P_1 = 0.0892	P_1 = 0.2475 P_2 = 0.4359	**P_1 = 0.0318** **P_2 = 0.0001** P_3 = 0.3142
TNF-α	0.0993 (0–1.09)	1.388 (0.4126–2.562)	0.8298 (0–1.685)	1.342 (0.5545–1.948)
		P_1 = 0.0300	P_1 = 0.2627 P_2 = 0.2551	**P_1 = 0.0030** P_2 = 0.8272 P_3 = 0.2345
hs-CRP [IU]	0.9758 (0.7584–1.261)	1.03 (0.845–1.443)	0.9036 (0.3418–1.025)	0.78 (0.31–0.9835)
		P_1 = 0.5288	P_1 = 0.1733 P_2 = 0.1207	P_1 = 0.058 **P_2 = 0.0277** P_3 = 0.8171

hs-CRP — high sensitive C reactive protein; IL-1β — interleukin-1β; IL-4 — interleukin-4; IL-6 — interleukin-6; IL-10 — interleukin-10; IL-26 — interleukin-10; TNF-α — tumor necrosis factor α

Obese subjects had no differences in the levels of exhaled IL-26, IL-10, IL-1β, IL-6, TNF-α, and hs-CRP in comparison with healthy subjects.

In OA in comparison with NOA the level of hsCRP in EBC was lower (p = 0.0277).

Obese asthmatics had elevated levels of IL-1β (1.397 (1.032-2.861); p = 0.0318) and TNF-α (1.342 (0.5545–1.948); p = 0.0030) in comparison with healthy subjects. Obese asthmatics had a reduced concentration of IL-26 (6.439 (5.113–8.028); p = 0.0001), elevated IL-1β (1.397(1.032–2.861); p = 0.0001) in comparison with non-obese asthmatics and elevated IL-10 (1.187 (0.7415–1.529); p = 0.0538) in comparison with obese subjects.

Cytokines concentrations and correlations with BMI and some spirometric indices

Given the importance of spirometry parameters (FEV_1, FEV_1/FVC), we conducted a correlation analysis between these indicators and cytokine concentrations to determine the severity of inflammation in normal-weight asthmatics. We found a negative correlation between FEV_1/FVC and serum hsCRP (R = –0.65; p = 0.0438). The results of the study show a positive correlation between serum IL-4 with eosinophil count (R = 0.70; p = 0.0306), and positive correlation between exhaled IL-10 and hsCRP (R = 0.67; p = 0.0390).

Due to the secondary aims of our study, we conducted a correlation analysis between spirometry indicators, body weight, and cytokine levels in obese subjects. We found negative correlations of the post-bronchodilator test with FEV_1 and FVC 25/75 (R = –0.77; p = 0.0088; R = –0.69; p = 0.0306, respectively). These data went in parallel with observed correlations between serum IL-26 with thigh circumference (R = 0.68; p = 0.0347), serum IL-4 with thigh circumference (R = 0.64; p = 0.0490), serum hs-CRP with BMI (R = 0.62; p = 0.0560), and eosinophil count and weight (R = 0.66; p = 0.0438). There were positive correlations between serum and exhaled cytokines levels: eosinophil count with serum IL-26 (R = 0.65; p = 0.0490) and IL-4 (R = 0.65;

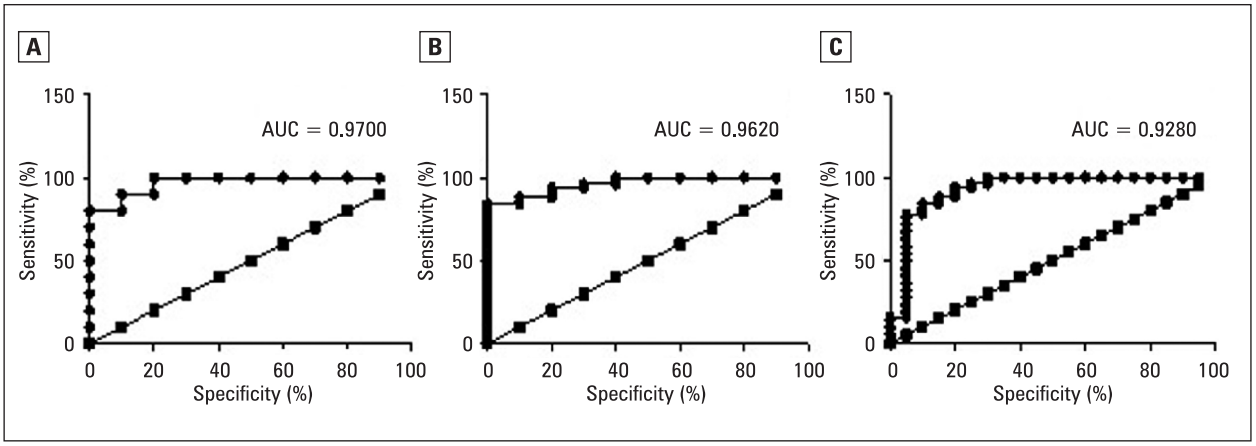

Figure 1. Receiver operating characteristic (ROC) analysis evaluating the diagnostic accuracy of exhaled IL-26 in asthmatic patients. **A.** ROC of healthy subjects and non-obese asthmatics; **B.** ROC of healthy subjects and asthmatics (non-obese and obese); **C.** ROC on including all cases of non-asthmatics and asthmatics

p = 0.0412), and serum IL-4 and serum IL-26 (R = 0.64; p = 0.0456), exhaled hs-CRP with exhaled IL-10 (R = 0.74; p = 0.0174).

We conducted a correlation analysis between spirometry indicators, body weight, and cytokine levels in obese asthmatics.

We found significant negative correlation between FEV_1/FVC and eosinophil count (R = –0.47; p = 0.0024). Serum hsCRP correlated positively with weight and BMI (R = 0.63; p = 0.0001; and R = 0.63; p = 0.0001, respectively). Exhaled IL-10 correlated with serum IL-10 (R = –0.43; p = 0.0063), as well as with exhaled hs-CRP (R = 0.46; p = 0.0027).

ROC analysis of diagnostic value for exhaled IL-26 in asthmatic patients

The ROC curves were plotted based on exhaled levels of IL-26 in study populations. We estimated the ROC curve displayed that non-obese asthmatics could be distinguished from healthy subjects according to their levels of exhaled IL-26 with an AUC value of 0.9700 (99% CI: 0.9071–1.033; p = 0.00038) (Figure 1A). The cut-off values for exhaled IL-26 diagnosing asthma in non-obese persons were 3.334 pg/mL with a sensitivity of 100% and a specificity of 80%, 3.676 pg/mL with a sensitivity of 90% and a specificity of 80%, 3.686 pg/mL with a sensitivity of 90% and a specificity of 90%, 3.938 pg/mL with a sensitivity of 80% and a specificity of 90% (Table 5A).

The ROC curve showed that all asthmatic patients could be distinguished from healthy subjects according to their levels of exhaled IL-26 with an AUC value of 0.9620 (95% CI: 0.9162–1.008; p = 0.0001) (Figure 1B). The cut-off values for exhaled IL-26 diagnosing asthma in non-obese persons were 3.302 pg/mL with a sensitivity of 94% and a specificity of 80%, 3.631 pg/mL with a sensitivity of 92% and a specificity of 80%, 3.663 pg/mL with a sensitivity of 90% and a specificity of 80%, 3.676 pg/mL with a sensitivity of 88% and a specificity of 80% (Table 5B).

The ROC curve showed that all asthmatic patients could be distinguished from non-asthmatics (healthy and obese subjects) according to their levels of exhaled IL-26 with an AUC value of 0.9280 (95% CI: 0.8166–1.039; p = 0.0001) (Figure 1C). The cut-off values for exhaled IL-26 diagnosing asthma in non-obese persons were 3.302 pg/mL with a sensitivity of 94% and a specificity of 80%, 3.631 pg/mL with a sensitivity of 92% and a specificity of 80%, 3.663 pg/mL with a sensitivity of 90% and a specificity of 80%, 3.676 pg/mL with a sensitivity of 88% and a specificity of 80% (Table 5C).

Discussion

Obesity induces the development of asthma with a difficult-to-control phenotype [15]. These difficulties are associated with the direct mechanical influence of obesity on the lungs function, as well as the indirect one by inflammatory cytokine network.

Overweight and obesity directly led to lung compression, FRC and OFV1/FVC reduction, attenuation of tethering force between the airway and parenchyma, substantial narrowing and collapse of central airways, alveolar derecruitment and collapse of small airways [3, 16].

Table 5. Values of exhaled IL-26 cut-off with a range of sensitivity and specificity for asthma diagnosis in the study population

Exhaled IL-26 cut-off value [pg/mL]	Sensitivity, [%] (95%CI)	Specificity, [%] (95%CI)
A. Results on including healthy and none-obese asthmatics		
> 3.334	100	80
> 3.676	90	80
> 3.686	90	90
> 3.938	80	90
B. Results on including healthy subjects and asthmatics		
> 3.302	94	80
> 3.631	92	80
> 3.663	90	80
> 3.676	88	80
C. Results on including all cases of non-asthmatics and asthmatics		
> 3.302	94	80
> 3.631	92	80
> 3.663	90	80
> 3.676	88	80

IL-26 — interleukin 26

Obesity affects both cellular and humoral immune factors. Cellular immune factors include T-cells, ILC2, and ILC3, etc. Humoral factors comprise TNF-α, IFN-γ, IL-1β, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-13, IL-17, IL-25, IL-33. There are many transcriptional factors, pathways, receptors involved in obese asthma pathogenesis, such as NF-κB, MAPK, NOS2, TLRs, NLRP3, RORγτ, TSLP, GATA-3 [17].

In this study, we investigated the serum and exhaled levels of IL-26 and its associations with the level of systemic inflammation, lung functions, and body weight in obese moderate-to-severe asthmatic patients.

According to the study design, we formed 4 groups of healthy subjects, non-obese asthmatics, obese subjects, and obese asthmatics.

Asthma severity in all groups was the same per ACT score, obese asthmatics had less controlled asthma per ACQ with lower quality of life per AQLQ, but these differences were not clinically significant. Obese asthmatics had an increased proportion of high-doses inhaled steroids (52.5%) in comparison with normal-weight asthmatics (20%). Recently it was shown that moderate-to-severe asthma remains poorly treated despite therapy with inhaled steroids and long-acting beta-agonists: the disease is not adequately controlled in 10 to 20% of patients [18]. Our data went in parallel with observations that obese asthmatics had worse asthma control and quality of life and needed high-doses inhaled steroids [19].

According to our data, non-obese asthmatics had a significant increase in total IgE concentration in comparison with healthy subjects. Obese asthmatics had less increased levels of serum total IgE than the non-obese ones. At the same time, there were no significant differences in frequencies of specific IgE positive levels between non-obese and obese asthmatics. These results supported the position for the identification of clinically relevant T1 and T2 phenotype overlap [20].

Non-obese asthmatics had a reversible airway obstruction with a reduced FEV_1, FEV_1/FVC, FVC 25/75, and positive bronchodilator test. It was shown that high airway reversibility is more often associated with an elevation in T2 biomarkers [21]. Besides, these patients had significantly increased serum levels of IL-10, IL-4, and slightly increased IL-26. These data confirmed the concept of Th2 polarized immune response in asthmatics [22] as well as an increased level of serum IL-26 [23].

In our study, we investigated the level of cytokines in exhaled breath condensate (EBC) to examine the local airway inflammation. Recently, the procedure for EBC collection has been well standardized and validated for investigations of airway inflammation [23]. The correlations between EBC and sputum concentrations of

different substances, including cytokines, were proved [24]. Furthermore, EBC collection had additional benefits in asthmatic patients due to low sputum production.

We received new data that non-obese and obese asthmatics had significantly increased exhaled IL-26 in comparison with healthy subjects. Our results showed that obese asthmatics had lower exhaled IL-26 than non-obese asthmatics. We suggest that this difference might depend on the increased proportion of high-doses inhaled steroids (52.5%) in comparison with normal-weight asthmatics (20%). These data confirmed previous results of increased sputum IL-26 in different asthmatic patients [23]. IL-26 local production has been demonstrated by bronchial and lung biopsies with immunostaining of bronchial epithelial cells and macrophages/monocytes [25].

We observed a significant negative correlation between FEV_1/FVC and serum hsCRP. The results of the study show a positive correlation between serum IL-4 with eosinophil count and a positive correlation between exhaled IL-10 and hsCRP.

These data were of importance because IL-26 local production also reflected asthma control level in adults [25].

According to the data, tobacco smoking increased sputum IL-26, and thus interfered with results [26]. Fortunately, we did not involve tobacco smokers in the study.

As the next step, we characterized the level of systemic inflammation, lung functions, and body weight in obese subjects. The obese subjects had a normal ventilatory pattern without airway obstruction. In these subjects, there were no differences in serum IL-26, IL-10, and IL-4 concentrations in comparison with healthy subjects. In our opinion, there were high variations of individual concentrations of these cytokines. Obese subjects had a significant escalation of hs-CRP, IL-1β, IL-6, and TNF-α, reflecting an increased level of systemic inflammation. Obese subjects had no differences in the levels of exhaled IL-26, IL-10, and hs-CRP in comparison with healthy subjects. Nevertheless, we observed negative correlations of the post-bronchodilator test with FEV_1 and FVC 25/75. These data went in parallel with observed correlations between serum IL-26 with thigh circumference, serum IL-4 with thigh circumference, serum hs-CRP with BMI, and eosinophil count and weight. There were positive correlations between serum and exhaled cytokines levels: eosinophil count with serum IL-26 and IL-4, and serum IL-4 and serum IL-26, exhaled hs-CRP with exhaled IL-10.

Important observation that obese asthmatics had significantly lower level of hsCRP in EBC in comparison with non-obese asthmatics ($p = 0.0277$). This observation might be explained by higher dose of inhaled corticosteroids in obese asthmatics.

These data taken together suppose that moderate obesity (BMI 40.60 ± 4.95 kg/m^2) is accompanied by T1 systemic inflammation without clinically significant local airway inflammation.

The obtained data supported the idea that obesity alone influences airway function slightly, but obesity accompanied by systemic inflammation can induce airway impairment [3, 27].

Finally, we investigated the level of systemic inflammation, lung functions, and body weight in obese asthmatics.

In obese asthmatics, a similar obstructive ventilatory pattern as in non-obese asthmatics was observed but with a more expressed obstruction degree. Obese asthmatics had reduced FEV_1 and FEV_1/FVC, confirmed by the decreased mid-expiratory flow rate (FEF 25–75%) and the post-bronchodilator test in comparison with non-obese asthmatics.

Obese asthmatics had a specific serum cytokine profile with elevated IL-26, IL-10, IL-4 as compared with healthy subjects, and IL-1β, TNF-α, hs-CRP concentrations as compared with healthy subjects and non-obese asthmatics. Moreover, these patients had a partial similarity with both non-obese asthmatics (elevated IL-26, IL-10, and IL-4) and obese subjects (elevated hs-CRP). Obese asthmatics had a reduced concentration of exhaled IL-26 in comparison with non-obese asthmatics and elevated exhaled IL-10 in comparison with obese subjects. We found a significant negative correlation between FEV_1/FVC and eosinophil count. Serum hsCRP correlated positively with weight and BMI. Exhaled IL-10 correlated with serum IL-10, as well as with exhaled hs-CRP.

In our study the obese subjects and obese asthmatics were older than healthy and non-obese asthmatics that might possibly influence the hsCRP level. According to a literature healthy older peoples showed serum CRP level within the normal range, but the level were greater in participants ≥ 65 (2.62 ± 1.68 mg/L) than < 65 years of age [28]. Thus, the age influence was significantly lower than the obesity.

Taken together, these findings suggest that obese asthmatics had combined systemic and local airway inflammation with different pathways and mechanisms involved. In these patients, systemic inflammation involved hs-CRP, IL-1β, IL-6,

and TNF-α, as well as IL-26, IL-10, and IL-4. The local airway inflammation was characterized by relatively low exhaled IL-26 and high exhaled IL-10, IL-1β, and TNF-α. This data goes in parallel with the observation that sputum IL-1β and TNF-α might be associated with severe asthma [29, 30].

According to our results, we didn't find a significant correlation between BMI and exhaled IL-26 in obese asthmatics. This data is partially inconsistent with results of sputum IL-26 overexpression and its correlation with BMI in severe asthma [31]. We suppose that there are several reasons for this inconsistence: 1) different target populations (only women were included); 2) no data provided about pharmacological treatments for asthmatic patients, which might influence local IL-26 production.

We used ROC analysis to estimate exhaled IL-26 as a possible biomarker in asthmatic patients. The ROC curve is the most popular graphical tool for evaluating the diagnostic power of a biomarker. It provides an exhaustive look at the trend of sensitivity over all cut-offs, and thus presents information about the relationship between the sensitivity and specificity of a biomarker. The area under the ROC curve, which integrates the curve over all cut-offs, is proposed for an efficient summarization [32].

Exhaled IL-26 concentration distinguished non-obese asthmatics from healthy subjects, ROC curve analysis (area: 0.9700) showed 100% sensitivity and 80% specificity; asthmatic patients from non-asthmatics (healthy and obese subjects), ROC curve analysis (area: 0.9620) showed 94% sensitivity and 80% specificity; all asthmatic patients from non-asthmatics (healthy and obese subjects), ROC curve analysis (area: 0.9280) showed 94% sensitivity and 80% specificity. Thus, exhaled IL-26 can discriminate asthmatics from non-asthmatic subjects, but obesity influenced test specificity.

Sputum IL-26 was proposed as a possible biomarker in pediatric [9] and adult [25] asthma but elevated IL-26 was found in COPD patients [10] and tobacco smokers [26]. Thus, we suggest that lung-derived IL-26 (in sputum or condensate) as a biomarker reflected more the lung inflammation rather than the distinguished nosologies.

IL-26 is a member of the highly pleiotropic IL-10 superfamily, and the members of this superfamily mediate diverse activities, including immune suppression, enhanced antibacterial and antiviral immunity, antitumor activity, and promotion of self-tolerance in autoimmune diseases [33]. In obese asthmatics, we observed a shift in the exhaled IL-26/IL-10 ratio, which probably characterized the low production of IL-26 and high IL-10 in airways. At the same time, these patients had elevated systemic production of IL-26, similar to non-obese asthmatics and obese patients.

Our study had several limitations, such as the limited number of subjects in comparison groups and high individual variations in cytokine concentrations. The obese subjects and obese asthmatics were older than healthy and non-obese asthmatics that might possibly influenced hsCRP level due to high prevalence of the atherosclerosis in older peoples [34]. Unfortunately, there were no recent publications about the connection of atherosclerosis and IL-26 We will have though to expand our population to be able to explore more deeply the relationships between IL-26, cellular populations, clinical phenotypes, and inhaled treatment, as IL-26 production has been reported to be modulated in vitro by asthma medications [35].

Nowadays, IL-17/IL-26 cascade might be a target for the treatment of obese asthma [36]. The novel anti-IL-17 antibody was successfully used for the experimental treatment of allergic inflammation in an obesity-related asthma model in mice [37]. Recently, novel anti-IL-26 neutralizing monoclonal antibodies were reported for the treatment of inflammatory diseases [38], which might have a perspective for the treatment of obese asthma.

Conclusions

Exhaled IL-26 elevated in obese and non-obese moderate-to-severe asthmatic patients. Exhaled IL-26 might be a perspective biomarker in non-obese and obese asthmatics. The obese asthmatic phenotype comprised the combined systemic and local airway inflammation.

Conflict of interests

None declared.

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