SUPPLEMENTARY MATERIALS

Methods

Inclusion and exclusion criteria

Severity of asthma was categorized according to the Global Initiative for Asthma (GINA) (1) guidelines."Mild" asthma was defined as mild disease, treated with low daily dose of inhaled corticosteroids (ICS) (<250 μg of fluticasone propionate [FP] [dry powder inhaler] or equivalent) and/or short acting β_2-agonists on demand. "Moderate" asthma was defined as mild persistent disease treated with low (combined with long-acting β_2-agonists) or medium dose of ICS (250-500 μg of FP or equivalent). "Severe" asthma was defined as asthma that requires high dose of ICS (>500 μg of FP or equivalent) with long-acting β_2-agonist to prevent it from becoming uncontrolled or asthma that remains poorly controlled despite that treatment.

Arterial hypertension was defined based on a history of hypertension (blood pressure >140/90 mmHg), or administration of antihypertensive treatment. Hypercholesterolemia was defined as the previously diagnosed and treated with statins, or serum total cholesterol >5.2 mmol/l. Diabetes mellitus was defined as the current use of insulin, or oral hypoglycemic medications, or fasting serum glucose >7.0 mmol/l. Congestive heart failure was defined as the left ventricular ejection fraction below 40%. Coronary heart disease was defined as a clinical manifestation of angina pectoris or myocardial infarct in the past. Liver injury was defined as the liver enzymes above the upper limit of the reference range.

Ultrasound examinations

Flow-mediated dilatation (FMD) of the right brachial artery was assessed using Celermayer' method (2), as described previously (3,4). A baseline sagittal diameter (D1) of a

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distal part of the brachial artery was measured in the M-presentation by using a 10 MHz linear array ultrasonic transducer placed 2-3 cm proximal to the arterial bifurcation. A sphygmomanometer cuff was then placed on the forearm below the elbow and inflated to a pressure of 200 mmHg for 5 minutes and released. One minute after releasing the cuff, the brachial artery diameter was measured again (D2) at the same point. FMD was defined as the increase of brachial artery diameter after deflation of the cuff and was expressed as a percentage of the baseline diameter (FMD %= [(D2-D1)/D1] x 100%).

The intima-media thickness (IMT) of the common carotid artery was measured by ultrasound with a 10 MHz linear transducer. The thickness of the anterior and posterior walls were assessed bilaterally in the longitudinal projection immediately before they bifurcate. The mean value of the both sidesmeasurements was used in further analysis (3,4).

Postbronchodilator spirometry

Postbronchodilator spirometry (after 400 μg of albuterol) was made according to the American Thoracic Society standards (5), using a Jaeger MasterLabspirometer (Jaeger-Toennies GmbH, Hochberg, Germany).

Lung computed tomography (CT)

Lung CT was performed 1 hour after administration of inhaled albuterol (400 μg), using 64-raw multidetector computed tomography (Aquilion TSX-101A, Toshiba Medical Systems Corporation, Otawara, Japan), without administration of intravenous contrast medium, in a helical scanning mode, with CT parameters: 64 x 0.5 mm collimation, helical pitch of 53 and 0.5 second per rotation with standard radiation dose [150±50 mAs and 120 kVp]). Patients were scanned in the supine position at maximal inspiration with the arms held over the head.

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Results

Levels of interleukin (IL)-4 and IL-5 were below the detection point in almost allsubjects, while IL-6 was detectable in the majority of analyzed samples. Concentrations of IL-10 were measurable in about 14% of asthmatics and 27% of controls, while IL-12(p70), IL-17A, IL-23, and interferon gamma (INFγ) were above the detection threshold in about 60% and 35% of asthma and control individuals, respectively.

In asthmatics activity of von Willebrand factor (vWF) remained in positive associations with inflammatory markers, including C-reactive protein (CRP) (β=0.35 [95%CI: 0.03-0.23]), IL-6 (β=0.1 [95%CI: 0.01-0.19]), IL-10 (β=0.27 [95%CI: 0.17-0.37]), IL-17A (β=0.13 [95%CI: 0.03-0.23]), and ADAM-33 (β=0.22 [95%CI: 0.12-0.32]). In patient group vWF was higher in those with diabetes (119.3 [111.2-125], n=18 vs. 103.2 [89.9-120.3], n=74, p=0.007), hypercholesterolemia (117.4 [99.9-124.8], n=28 vs. 101.2 [86.7-115], n=64, p=0.003), deep vein thrombosis in medical history (127 [111.1- 134.8], n=9 vs. 104.6 [90.4-118.7], n=83, p=0.003), and treated with oral corticosteroids (124.3 [105.3-133.2], n=23 vs. 103.3 [89.9-119.7], n=69, p=0.004).

In controls, similarly to asthma patients, FMD% was inversely related to the age (β=-0.4 [95%CI: -0.53 to -0.27]) and body mass index (BMI) (β=-0.23 [95%CI: -0.38 to -0.08]). Among laboratory variables the onlypositive association was demonstrated with circulating pentraxin-3 (β= 0.23 [95%CI: 0.03-0.43]) and the only negative with vWF activity (β=-0.24 [95%CI: -0.37 to -0.11]). In turn, IMT in this group of subjects was positively related to the age only (β=0.26 [95%CI: 0.12-0.4]). Moreover, in controls IMT remained in relationships with selected echocardiographic parameters, including interventricular septum thickness (β=0.2 [95%CI: 0.02-0.38]), posterior wall thickness (β=0.19 [95%CI: 0.01-0.37]), right ventricle diameter (β=0.37 [95%CI: 0.17-0.57]), and ejection fraction (β=-0.2 [95%CI: -0.36

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to -0.04]). Among laboratory variables in controls IMT correlated with triglycerides (β=0.17 [95%CI: 0.01-0.33]) and high-density lipoproteins (β=-0.35 [95%CI: -0.49 to -0.21]).

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Table 1S. Selected basic laboratory tests results and lung computer tomography indices of airway remodeling in asthmatics.

	Patients n=92	Controls n=62	p-value
Basic laboratory tests			
Hemoglobin, g/dl	13.5 (12.9-14.4)	13.7 (12.6-15)	0.4
Red blood cells, 10^6/μl	4.62 (4.32-4.9)	4.51 (4.18-4.85)	0.14
Blood eosinophilia, 10^3/μl	210 (110-400)	140 (110-200)	0.1
Glucose, mmol/l	5.00 (4.70-5.40)	4.98 (4.76-5.20)	0.24
Alanine transaminase, IU/l	26 (21-37.5)	21.5 (15-29)	0.006
Aspartate transaminase, IU/l	23 (21-37.5)	21.5 (17-25)	0.04
Lung computed tomography airway remodeling indices			
Right lower lobe basal posterior bronchus wall thickness, mm	1.8 (1.6-2.1)	-	-
Right lower lobe basal posterior bronchus wall area ratio	72.15 (67.9-76.2)	-	-
Right lower lobe basal posterior bronchus wall thickness ratio	23.5 (21.4-25.6)	-	-
Left lower lobe basal posterior bronchus wall thickness, mm	1.9 (1.7-2.2)	-	-
Left lower lobe basal posterior bronchus wall area ratio	72.3 (68.1-75.4)	-	-
Left lower lobe basal posterior bronchus wall thickness ratio	23.6 (21.8-25)	-	-

Data is presented as median and interquartile range.

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