Endothelial dysfunction and pentraxin-3 in clinically stable adult asthma patients

Short running title: **Endothelial dysfunction in asthmatics**

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.18176/jiaci.0563

Abstract

Background: Asthma is associated with low-grade systemic inflammation, prothrombotic state, and premature atherosclerosis.

Objective: To evaluate the relationships between asthma, inflammatory biomarkers, and parameters of endothelial dysfunction.

Patients and methods: In 92 adult, clinically stable asthmatics and 62 well-matched controls we analyzed flow-mediated dilatation (FMD) of the brachial artery and intima-media thickness (IMT) of the common carotid artery using ultrasonography. We also measured blood levels of selected inflammatory and asthma specific biomarkers, including interleukin (IL)-4, IL-5, IL-6, IL-10, IL-12(p70), IL-17A, IL-23, interferon γ, as well as a disintegrin and metalloproteinase domain-containing protein 33 (ADAM-33), together with endothelial damage laboratory markers: circulating pentraxin-3 and plasma activity of von Willebrand factor (vWF). We analyzed relationships of studied variables with asthma severity, lung function abnormalities, lung computer tomography (CT) indices of airway remodeling, and transthoracic echocardiography parameters.

Results: Asthmatics had higher IL-6, IL-10, and ADAM-33. They were also characterized by 23% lower FMD% and 15% thicker IMT, as compared to controls ($p<0.001$, both). In asthma vWF was related to age (β=0.28 [95%CI: 0.15 to 0.41]) and remained in an inverse relationship with FEV_1 (β=-0.2 [95%CI: -0.05 to -0.35]). Surprisingly, a negative correlation was revealed between vWF and pentraxin-3 (β=-0.17 [95%CI: -0.3 to -0.04]). Pentraxin-3 remained in positive associations with CT airway remodeling indices.

Conclusions: Asthma is characterized by endothelial dysfunction that was related to the airway obstruction. The biological role of pentraxin-3 is unknown, although our data suggests its protective role against endothelial damage and atherosclerosis.

Key words: Asthma, Endothelium, Flow-mediated dilatation, Intima-media thickness, Pentraxin-3.

doi: 10.18176/jiaci.0563

Resumen

Antecedentes: el asma se asocia con inflamación sistémica de bajo grado, con un estado protrombótico y la existencia de aterosclerosis prematura.

Objetivo: evaluar las relaciones entre asma, biomarcadores inflamatorios y parámetros de disfunción endotelial.

Pacientes y métodos: Se ha analizado la dilatación mediada por flujo (DMF) de la arteria braquial y el grosor íntima-media (GIM) de la arteria carótida común mediante ecografía, en 92 adultos asmáticos clínicamente estables y 62 controles. También se midieron los niveles sanguíneos de determinados biomarcadores inflamatorios específicos de asma, incluyendo interleucina (IL) -4, IL-5, IL-6, IL-10, IL-12 (p70), IL-17A, IL-23, interferón γ , así como desintegrina y la metaloproteinasa que contiene el dominio proteína 33 (ADAM-33), junto con marcadores de laboratorio de daño endotelial: pentraxina-3 circulante y actividad plasmática del factor von Willebrand (vWF). Analizamos las relaciones de las variables estudiadas con la gravedad del asma, las anomalías de la función pulmonar, los índices de tomografía computarizada (TC) pulmonar de remodelación de las vías respiratorias y los parámetros de ecocardiografía transtorácica.

Resultados: los asmáticos tuvieron mayores niveles de IL-6, IL-10 y ADAM-33. También se caracterizaron por tener un 23% menos de DMF y un 15% más grueso el GIM, en comparación con los controles (p <0,001, ambos). En el asma, vWF se relacionó con la edad (β = 0.28 [IC 95%: 0.15 a 0.41]) y se mantuvo en una relación inversa con FEV1 (β = -0.2 [IC 95%: -0.05 a -0.35]). Sorprendentemente, se observó una correlación negativa entre vWF y pentraxina-3 (β = -0.17 [IC 95%: -0.3 a -0.04]). La pentraxin-3 se asoció positivamente con los índices CT de remodelación de la vía aérea.

Conclusiones: el asma se caracteriza por una disfunción endotelial relacionada con la obstrucción de las vías respiratorias. Se desconoce el papel biológico de la pentraxina-3, aunque nuestros datos sugieren su papel protector contra el daño endotelial y la aterosclerosis.

Palabras clave: Asma, Endotelio, Dilatación mediada por flujo, Espesor íntima-media, Pentraxina-3.

doi: 10.18176/jiaci.0563

Introduction

Asthma is a chronic inflammatory disease of the airways [1]. A growing body of evidence supports its association with low-grade systemic inflammation, prothrombotic state [2,3], and premature development of atherosclerosis [4], accompanied by an increased risk of cardiovascular events, including cardiac death [5–8]. Atherosclerosis affects arterial wall, but is also related to the inflammation [9]. Premature atherosclerosis has been demonstrated in patients with various inflammatory diseases, such as lupus erythematosus [10], systemic sclerosis [11,12], or vasculitis [13,14]. In addition to inflammation, other well-established cardiovascular risk factors might play a proatherogenic role in asthmatics, including obesity, smoking, lack of physical activity, airway obstruction resulting in hypoxia, and corticosteroid therapy [6]. There are only a few studies that document premature development of atherosclerosis in asthmatics, also in pediatric population [15–19]. However, the exact mechanisms and pathogenesis of these unfavorable blood vessel alterations remain unknown and require further investigations.

Endothelial cell dysfunction is an important causal factor of atherosclerotic cardiovascular diseases. It is related to the various non-adaptive alterations in arterial endothelium function, which might have important implications for the regulation of blood-vessel hemostasis, local thrombosis, vascular tone maintenance and inflammatory response within the arterial wall [20]. Impaired endothelial function, together with atherosclerotic plaque rupture and subsequent thrombosis, is the main contributor of acute or chronic myocardial ischemia [21,22]. However, it might be reversed by the proper therapy or even lifestyle changes of affected individuals. Endothelial function may be evaluated by measuring the changes of arterial diameter in response to vasoactive agents, such as nitric oxide injected directly into the studied artery [23]. However, that approach is invasive and not routinely recommended. In turn, measurements of flow-mediated dilatation (FMD) and vascular

doi: 10.18176/jiaci.0563

reactive hyperemia index of the brachial artery are noninvasive and simple alternatives, thus might be used in clinic [24]. They measure endothelium-dependent brachial arteries dilatation in response to reactive hyperemia (shear stress) with subsequent endogenic nitric oxide release.

Pentraxin-3 is an acute phase glycoprotein, similar in structure to the C-reactive protein (CRP). It has been suggested that CRP reflects systemic inflammation, whereas pentraxin-3, which is synthesized locally by the endothelial cell, refers to the vascular system abnormalities [25,26]. It has been demonstrated that circulating pentraxin-3 is increased in patients with cardiovascular diseases [27], obstructive sleep apnea [28], but also in autoimmunity, including rheumatoid arthritis, systemic sclerosis, and vasculitis [25].

Given available data about potential links between asthma, inflammation, thrombosis, and atherosclerosis we sought to evaluate endothelial function in adult clinically stable asthma patients by measuring of FMD, together with assessment of intima-media thickness of the common carotid artery (IMT), as a marker of atherosclerosis. We also analyzed selected inflammatory and asthma specific parameters, as well as laboratory biomarkers of endothelial damage, including circulating von Willebrand factor (vWF) and pentraxin-3 [29,30]. We studied interrelationships between analyzed variables, asthma severity score, lung function abnormalities, lung computer tomography (CT) indices of airway remodeling, and transthoracic echocardiography parameters. Such studies have not yet been conducted.

doi: 10.18176/jiaci.0563

Methods

Our study has a retrospective format and received approval from the Bioethics Committee of the Jagiellonian University Medical College. The participation in that research was voluntary, and all the individuals were informed about methodology and potential side-effects. Written consents were recorded from patients and controls.

Participants

We enrolled 92 adult, white patients, 66 women and 26 men, with clinically stable asthma. They were recruited in the period from 2012 to 2016 at the Outpatient Clinic of the Department of Allergy and Clinical Immunology, University Hospital, Krakow, Poland. All patients were diagnosed with asthma on the basis of medical history (wheezing, shortness of breath, dyspnea, and cough) and postbronchodilator increase in forced expiratory volume in 1 second (FEV_1) of at least 12% and 200 ml documented currently or in the past. All asthma medications, with the exception of biological treatment, were permitted. Oral corticosteroids were allowed at a daily dose equivalent to ≤10 mg of prednisolone, unless the doses were unchanged during the preceding 3 months. Asthma patients could not be exacerbated during the preceding 6 months.

The control group consisted of 62 individuals, 39 women and 23 men, matched to the asthma group according to the race, gender, age, body mass index (BMI) and internal medicine comorbidities, including arterial hypertension, hypercholesterolemia, and diabetes mellitus. Controls were enrolled from the hospital's personnel and their relatives. They had no history of allergy or bronchial obstruction. Each control was individually matched with two patients considering the closest values of the matching criteria.

Representatives of both studied groups were 18-70 years old. The exclusion criteria for all participants were: congestive heart failure, coronary heart disease, stroke, liver injury,

doi: 10.18176/jiaci.0563

chronic kidney disease (stage 3 or more), and ongoing cancer treatment. Definitions of all comorbidities have been provided in an online Supplement.

Spirometry and lung computed tomography (CT)

Spirometry and lung CT were performed only in asthma subjects.

The airway cross-sectional geometry was quantified automatically by AW Server Software (General Electric Healthcare, Wauwatosa, WI, USA) in two peripheral bronchi: the right and the left lower lobe basal posterior bronchi (RB10 and LB10, respectively). Previously, we have demonstrated that both of them correlated best in CT-metrics with spirometry variables and histological features of airway remodeling, thus our current research was focused on their analysis [32]. In both peripheral airways we assessed average lumen and airway diameter, average wall thickness, lumen and wall area, wall area ratio (WAR), and wall thickness ratio (WTR), as it has been previously described [32]. Wall thickness was calculated based on average outer and inner bronchial diameters. The WAR was defined as an average difference between outer wall area and inner wall area divided by the outer wall area. The WTR was calculated as the average value of the ratio of the wall thickness and the airway diameter. CT-based indices were measured two times by an independent radiologist (J.Z.) on different days. The mean value was further analyzed. More detail on CT and spirometry investigations has been provided in an online Supplement.

Ultrasound examinations

Ultrasound studies were performed in all participants in a darkened, quiet room with subjects resting in a supine position for 10 minutes prior. Examinations were conducted by two independent ultrasound experts using the Siemens Acuson Sequoia 512 with a 10 MHz linear array ultrasonic transducer (MountainView, Ca, USA). Both experts made three consecutive measurements of each parameter (FMD and IMT). The mean of the 6

doi: 10.18176/jiaci.0563

measurements was presented as the final result. In addition, a transthoracic echocardiogram (TTE) was performed for every participant with measurements of the left ventricular ejection fraction (EF) and the systolic pulmonary artery pressure, using standard procedures (33). Detailed description of all ultrasound procedures has been provided in an online Supplement.

Laboratory analysis

Fasting blood samples were drawn into the serum tube and tube containing 0.109 mol/l sodium citrate (vol/vol,9:1) in the morning from the antecubital vein with minimal stasis and centrifuged at 2,000 x g for 10 minutes at room temperature within 2 hours from collection. Routine laboratory techniques were used to perform basic laboratory tests. C-reactive protein was measured by the Johnson & Johnson VITROS 250.

Commercially available high sensitivity immunoenzymatic assays (ELISAs) were applied to analyze levels of interleukin (IL)-4, IL-5, IL-6, IL-10, IL-12(p70), IL-17A, IL-23, and interferon (INF) γ (eBiosciencea, Vienna, Austria, all). A disintegrin and metalloproteinase domain-containing protein 33 (ADAM-33) in serum was assessed by the standard ELISA (Cloud-Clone Corp., Katy, TX, USA), similarly to pentraxin-3 (Elabscience, Houston, TX, USA). Blood concentrations of pentraxin-3 were measured calorimetrically according to the manufacturer's instruction, with the detection threshold of 0.252 ng/ml. Activity of vWF was analyzed in plasma by immunoenzymatic assay (Asserachrom, DiagnosticaStago, Asnieres, France).

Statistical analysis

The results in asthma and control groups were compared using Statistica 13.3, TIBCO Software Inc. The continuous variables were all non-normally distributed according to the Shapiro-Wilk test. They were reported here as median and interquartile range and compared using the Mann-Whitney U-test. Categorical variables were reported as percentages and

doi: 10.18176/jiaci.0563

compared by $\chi 2$ test. To adjust for potential confounders, the results of a relative increase of the FMD (FMD%), IMT, pentraxin-3, and vWF activity were Box-Cox normalized and a one way covariance analysis (ANCOVA) was performed with an adjustment for age, sex, and BMI. The univariate linear regression models adjusted for the same confounders were used to analyze associations between two selected parameters. Independent determinants of FMD% and IMT were established in multiple linear regression models, built by a forward stepwise selection procedure, verified by Snedecor's F-distribution. The R^2 was assessed as a measure of the variance. Unconditional multivariate logistic regression and a one way variance analysis (ANOVA) were used to analyze independent impact of comorbidities, including hypertension, diabetes mellitus, hypercholesterolemia, and statin therapy on FMD% and IMT, respectively. To calculate odds ratio (OR) with 95% confidence interval (CI), the cut-off point of FMD% was calculated based on receiver operating characteristic (ROC) curves. Results were considered statistically significant, when the p value was less than 0.05.

doi: 10.18176/jiaci.0563

Results

Patients and controls

In the Table 1 we have demonstrated demographic and clinical characteristics of asthma patients and control subjects. As it has been shown, both groups were well matched according to the demographic variables, including age, sex, BMI, and smoking habit, as well as prevalence of internal medicine comorbidities, such as hypertension, diabetes mellitus, and hypercholesterolemia.

Basic laboratory tests, inflammatory and asthma specific biomarkers

Results of basic laboratory tests have been shown in the Table 2 of the main Manuscript and in the Table 1S of an online Supplement. Asthmatics had higher serum triglycerides, as well as raised inflammatory markers, including white blood cell count, CRP, circulating IL-6, and IL-10. They were also characterized by two times higher blood level of ADAM-33.

Plasma activity of vWF was similar in both studied groups (Table 2). In asthmatics, however, it remained in inverse relationships with FEV_1 (β=-0.2 [95%CI: -0.05to -0.35]) and FEV_1/VC index (β=-0.24 [95%CI: -0.35 to -0.13]). Interestingly, in both studied groups we documented positive relationships between vWF plasma activity and renal parameters (β=0.24 [95%CI: 0.06-0.42] and β=0.3 [95%CI: 0.15-0.45]; β=0.24 [95%CI: 0.06-0.42] and (β=0.21 [95%CI: 0.04-0.38], for serum creatinine and urea, in asthma and control, respectively).

Although simple comparison between asthma and control individuals has shown no difference in circulating pentraxin-3 (Table 2), we have documented that in controls much more subjects had its level below the ELISA detection threshold (n=16 [26%] vs. n=6 [5%], p=0.01). On the other hand, surprisingly, an analysis limited to those with detectable

doi: 10.18176/jiaci.0563

pentraxin-3 revealed its lower levels in asthmatics (1.2 [0.87-1.84], n=84 vs.1.67 [1.18-2.5], n=42, p=0.01 after adjustment for potential confounders). In asthma, pentraxin-3 correlated positively with IL-6 (β=0.2 [95%CI: 0.11-0.29]) and IL-10 (β=0.5 [95%CI: 0.42-0.58]), but inversely with white blood cell count (β= -0.19 [95%CI:-0.31 to -0.07]) and vWF (β= -0.17 [95%CI: -0.3 to -0.04]). Interestingly, positive association was revealed between pentraxin-3 and airway remodeling indices, including WAR and WTR of RB10 (β=0.2 [95%CI: 0.1-0.3] and β=0.2 [95%CI: 0.1-0.3], respectively) and LB10 (β=0.18 [95%CI: 0.05-0.31] and β=0.23 [95%CI: 0.1-0.36], respectively).

More details on laboratory test results, including their relationships with vWF activity and circulating pentraxin-3, as well as FMD% and IMT in controls have been provided in an online Supplement.

Ultrasonographic parameters

Asthma individuals had slightly lower ejection fraction and increased right ventricular diameter (Table 2). Interestingly, in both studied groups, we demonstrated positive associations between circulating pentraxin-3 and left ventricle diastolic and systolic diameters (β=0.37 [95%CI: 0.21-0.53], β=0.19 [95%CI: 0.05-0.33] and β=0.37 [95%CI: 0.14-0.5], β=0.28 [95%CI: 0.1-0.46], asthma and controls, respectively).

Asthmatics were characterized by 23% lower FMD%, together with 15% thicker IMT, as compared to control individuals ($p<0.001$ both, also after adjustment for potential confounders) (Table 2). Moreover, presented differences were demonstrated for all asthma severity subtypes (Fig. 1A and 1B). Asthmatics had markedly higher risk of having a lower FMD%, defined as values below the cut-off point of 8.33% (OR 3.65 [95%CI: 2.06-6.48], $p<0.0001$), compared with the control group.

The main relationships of FMD% with demographic, echocardiographic, spirometry and laboratory variables in asthma patients have been presented in Fig 1C. As it has been

doi: 10.18176/jiaci.0563

shown FMD% was unfavorable related to the older age, higher BMI, severity of bronchial obstruction, increased heart left ventricle posterior wall thickness and right ventricular diameter, as well as higher circulating ADAM-33 and plasma vWF activity. In turn, increased ejection fraction, as well as raised circulating pentraxin-3 and IL-12(p70) had favorable impact on this parameter (Fig.1C). Surprisingly, higher FMD% was revealed in patients on montelukast (9.38 [6.38-11.43] n=11 vs. 6.12 [4.65-8.82] n= 61, p=0.04). In turn, its lower values were independently demonstrated in those with hypertension (6 [5.76-10] n=53 vs. 9.38 [8.16-10.63] n=39, p=0.001) and hypercholesterolemia (6.45 [4.76-11.29] n=28 vs. 9.28 [7.89-11.11] n=64, p=0.02).

As shown in Table 3 a multiple regression model has demonstrated that favorable higher FMD% in asthmatics was independently determined by younger age, less severe bronchial obstruction, thinner left ventricle heart posterior wall, and surprisingly by higher blood levels of IL-12(p70) and pentraxin-3. All those variables explained 46% of FMD% variability in asthmatics.

Fig.1D depicts main associations of IMT with demographic, echocardiographic, and laboratory variables in asthmatics. As it has been demonstrated, IMT was unfavorable associated with older age, higher BMI, increased heart left ventricle systolic diameter, hypercholesterolemia, and raised circulating IL-12(p70). Surprisingly, among laboratory biomarkers the only inverse, thus favorable relationship was demonstrated for pentraxin-3 (Fig.1D).

Higher IMTs were independently documented in asthma individuals with hypertension (0.72 [0.6-0.8], n=53 vs. 0.7 [0.6-0.75], n=39, p=0.04), diabetes mellitus (0.75 [0.7-0.83], n=18 vs. 0.7 [0.6-0.8], n=74, p=0.02), and on statins (0.8 [0.75-0.8], n=22 vs. 0.7 [0.6-0.8], n=70, p=0.03).

doi: 10.18176/jiaci.0563

A multiple regression model built for IMT in asthmatics has demonstrated that higher IMT was independently determined by older age, raised serum glucose, thicker heart left ventricle posterior wall, but lower serum pentraxin-3 (Table 3). As shown in that Table these parameters explained 48% of the IMT variability.

doi: 10.18176/jiaci.0563

Discussion

In the present study we have shown that asthma is characterized by endothelial dysfunction demonstrated by decreased FMD%, as well as raised risk of accelerated atherosclerosis, reflected by a thicker IMT complex of the common carotid artery. As expected, unfavorable lower FMD% was documented in patients with hypercholesterolemia and hypertension, as well as in those who were older and had higher BMI. Impaired endothelial function of brachial artery was related to the higher circulating ADAM-33, asthma specific biomarker, but also to the plasma activity of vWF, which in turn was positively associated with renal parameters, even if they were in normal range. That interesting finding indicates likely parallel alterations in kidney vessels and renal function, reflected by slightly higher concentrations of urea and creatinine in blood. It has been previously shown that chronic kidney disease and cardiovascular disease share similar risk factors, as well as endothelial function is significantly impaired in end-stage renal disease [34]. Our results stay in line with that observation, but also once again demonstrate that major cardiovascular risk factors, such as arterial hypertension, hypercholesterolemia, obesity, and diabetes mellitus might aggravate atherosclerosis partly by impairing the vessel endothelial cell function [14,35].

To date, only scarce papers have addressed endothelial dysfunction in asthmatics, however, the results of these studies are consistent with our findings [15–19]. It has been shown that children with asthma present with increased IMT complex [15,16] and diminished FMD% of the brachial artery [15], comparing to matched healthy individuals. Also studies performed in adult asthma population have demonstrated increased brachial-ankle pulse wave velocity [18], thickening of IMT [8], presence of atherosclerotic plaques in carotid arteries [19] and decreased FMD% [36]. However, aforementioned studies were performed in small groups of individuals. In turn, we analyzed more than 90 asthmatics with different asthma

doi: 10.18176/jiaci.0563

severity score and according to our knowledge, this is the largest asthma group described in the literature in that aspect. Particularly interesting in our study is an inverse correlation between FMD% and the severity of bronchial obstruction. Such relationship is revealed for the first time in asthmatics and might indicate that more severe asthma, reflected by the worse lung function, is related to the impaired endothelial function, thus raised risk of atherosclerosis. A similar conclusion may be also drawn from a documented by us a positive association between lung function and vWF plasma activity, which is a well-established marker of endothelial cell activation [37]. Our results in that context mirror epidemiological studies that provide further evidence for relationship between impaired lung function and cardiovascular outcomes, including heart failure, stroke, and coronary artery disease [38–41]. Moreover, demonstrated by us correlations between higher FMD%, thinner heart walls and better ejection fraction support the thesis, that healthier endothelium improves the whole cardiovascular system and protects against heart failure [42].

According to our knowledge pentraxin-3 in asthma subjects has not been comprehensively studied yet. In our study that inflammatory protein was more frequently above the ELISA detection point in asthmatics. However, the analysis limited to those with detectable circulating pentraxin-3 revealed, surprisingly that it was higher in control. This is an interesting finding, which requires a comment. Pentraxin-3 has become a novel and sensitive marker of endothelial activation, associated with peripheral artery disease, myocardial infarct, unstable angina pectoris, atherosclerosis and heart failure [27]. In our study, indeed circulating pentraxin-3 correlated with selected inflammatory biomarkers in asthmatics, but was also related to the better endothelial function in patients and controls, thinner IMT and lower plasma vWF activity in asthma, suggesting its protective function on vessel endothelium. Also animal models provide increasing evidence for immunomodulatory role of pentraxin-3 in cardiovascular outcomes and atherosclerosis. It has been shown, that

doi: 10.18176/jiaci.0563

lack of pentraxin-3 in double-knockout mice makes them susceptible to atherosclerosis via greater accumulation of macrophages and higher expression of adhesion molecules in the vascular wall [43]. In turn, Salio et al. [44] have found that pentraxin-3-deficient mice demonstrated increased heart damage and greater inflammatory response after ligation of the coronary artery. Pentraxin-3 also played a protective role in arterial thrombosis by inhibiting the prothrombotic effects of fibrinogen and collage [45]. In conclusion, pentraxin-3 is consistently related to the cardiovascular diseases, however its exact function continues to be virtually not understood.

Interestingly, in our study pentraxin-3 was unfavorable related to the lung CT airway remodeling parameters. That is also a novel and worth discussing finding. It has been demonstrated that pentraxin-3 might be raised in sputum of children with asthma and these increases correlate well with bronchial obstruction [46]. Moreover, Zhang et al. [47] have reported higher expression of pentraxin-3 in airway smooth muscle cells in asthma, hypothesizing its impact on airway remodeling. Our data stays in line with that observation, indicating possible contribution of pentraxin-3 to asthma remodeling. In this context interesting is also inverse relationship between FMD% and ADAM-33, protein overexpressed in epithelium and airway smooth muscle cells of asthma subjects, promoting angiogenesis and cell proliferation, thus likely airway remodelling [48]. However, the presented above relationship might also suggest that ADAM-33 is involved in endothelial cell function, directly by its complex structure and function, or by local lung release of other endothelial damage contributors [49].

The last issue, which deserves a commenting our results is a favorable higher FMD% observed in asthma patients treated with montelukast, antileukotriene medication. Leukotrienes are potent proinflammatory modulators important for asthma pathology. Emerging data indicates that they are also active in atherosclerotic plaques and contribute to

doi: 10.18176/jiaci.0563

the atherosclerosis and cardiovascular outcomes [50]. It cannot be excluded that those very well-tolerated medications, currently used in asthma therapy, might become a future drug in the atherosclerosis treatment, although large experimental and observational studies are needed to verify this hypothesis.

Conclusions

In summary, our study demonstrates that asthma is characterized by endothelial dysfunction that might be noninvasively monitored by FMD%, a simple ultrasonographic test. Impaired endothelial function was related to the airway obstruction and blood level of ADAM-33, an asthma specific biomarker. Although atherosclerosis is regulated by complex mechanistic pattern, our data suggests that classical cardiovascular risk factors, including hypercholesterolemia, arterial hypertension, diabetes, and obesity need to be more comprehensively controlled by life style changes and/or medications in asthmatics, particularly in those with more severe type of the disease. The biological role of pentraxin-3 is unknown, although our data suggests its protective role against endothelial dysfunction. Pentraxin-3 might be also involved in airway remodeling pathology.

Study limitations

The patient and control groups were relatively small. Our patients were relatively old and most of them were moderate to severe asthmatics. We measured each laboratory variable at a single time point, thus we cannot exclude their changes in time. Statistical associations reported here may not necessarily indicate cause-effect relationships. In vitro models are needed to elucidate the role of pentraxin-3 in asthma pathology, endothelial dysfunction and atherosclerosis development. Finally, the clinical relevance of demonstrated relationships particularly in terms of vascular outcomes and asthma remodeling remains to be established.

doi: 10.18176/jiaci.0563

Funding: This project was funded by the National Science Centre based on decision No: DEC-2013/09/B/NZ5/00758 (to S. B-S.) and research grant of Jagiellonian University Medical College No: K/ZDS/007900 (to S. B-S.).

The authors have no conflicts of interest to declare.

References

1. Becker AB, Abrams EM. Asthma guidelines: the Global Initiative for Asthma in relation to national guidelines.Curr Opin Allergy Clin Immunol. 2017;17(2):99-103.

2. Bazan-socha S, Mastalerz L, Cybulska A, Zareba L, Kremers R, Zabczyk M, et al. Prothrombotic State in Asthma Is Related to Increased Levels of Inflammatory Cytokines , IL-6 and TNF α , in Peripheral Blood. Inflammation. 2017;40(4):1225–35.

3. Bazan-socha S, Mastalerz L, Cybulska A, Zareba L, Kremers R, Zabczyk M, et al. Asthma is associated with enhanced thrombin formation and impaired fibrinolysis Experimental Allergy. Clin Exp Allergy. 2016;46:932–44.

4. Kuczia P, Mastalerz L, Potaczek DP, Cybulska A, Zareba L, Bazan-Socha S, et al. Increased activity of lipoprotein-associated phospholipase A2 in non-severe asthma. Allergol Int [Internet]. 2019;68(4):450–5.

5. Tattersall M, Guo M, Korcarz C. Asthma predicts cardiovascular disease events: the multi-ethnic study of atherosclerosis. Arter Thromb Vasc Biol. 2015;35(6):1520–5.

doi: 10.18176/jiaci.0563

6. Strand LB, Tsai MK, Wen CP, Chang S, Brumpton BM. Is having asthma associated with an increased risk of dying from cardiovascular disease ? A prospective cohort study of 446 346 Taiwanese adults. BMJ Open. 2018;8(5):e019992.

7. Xu M, Xu J, Yang X. Asthma and risk of cardiovascular disease or all-cause mortality: a meta-analysis. Ann Saudi Med. 2017;31(2):99–105.

8. Onufrak S, Abramson J, Vaccarino V. Atherosclerosis risk in communities (aric) study. Atherosclerosis. 2007;195(1):129–37.

9. Conglin L, Jingying Z, Guo-ping S. Interaction between allergic asthma and atherosclerosis. Transl Res. 2016;174:5–22.

10. Wang DG, Tang XW, Fan Y, Leng RX, Ni J, Deng SM, et al. Decreased Flow-Mediated Dilatation in Patients with Systemic Lupus Erythematosus: a Meta-analysis. Inflammation. 2014;37(6):2067–75.

11. Frech T, Walker AE, Barrett-O'Keefe Z, Hopkins PN, Richardson RS, Wray DW, et al. Systemic sclerosis induces pronounced peripheral vascular dysfunction characterized by blunted peripheral vasoreactivity and endothelial dysfunction. Clin Rheumatol. 2015;34(5):905–13.

12. Cypiene A, Laucevicius A, Venalis A, Dadoniene J, Ryliskyte L, Petrulioniene Z, et al. The impact of systemic sclerosis on arterial wall stiffness parameters and endothelial function. Clin Rheumatol. 2008;27(12):1517–22.

13. Pacholczak R, Bazan-Socha S, Iwaniec T, Zaręba L, Kielczewski S, Walocha JA, et al. Endothelial dysfunction in patients with granulomatosis with polyangiitis: a case–control study. Rheumatol Int. 2018;38(8):1521-30.

doi: 10.18176/jiaci.0563

14. Pacholczak R, Bazan-socha S, Iwaniec T, Zar L, Kielczewski S. Endothelial dysfunction in patients with eosinophilic granulomatosis with polyangiitis. Clin Rheumatol. 2019;38(2):417-424.

15. Butov AD, Makieieva N, Vasylchenko Y. Interrelationship of endothelial function parameters in children with bronchial asthma in exacerbation and remission. Adv Respir Med. 2019;87(1):7-13.

16. Cakmak A, Zeyrek D, Cece H, Erel O. The relationship between carotid intima media thickness and oxidative stress in asthmatic children. Asian Pacific J Allergy Immunol. 2010;28(4):256–61.

17. Ülger Z, Gülen F, Özyürek A. Abdominal aortic stiffness as a marker of atherosclerosis in childhood-onset asthma: a case–control study. Cardiovasc J Afr. 2015;26(1):8–12.

18. Sun WX, Jin D, Li Y, Wang RT. Increased arterial stiffness in stable and severe asthma. Respir Med [Internet]. 2014;108(1):57–62.

19. Tuleta I, Skowasch D, Aurich F, Eckstein N, Schueler R, Pizarro C. Asthma is associated with atherosclerotic artery changes. PLoS One [Internet]. 2017;12(10):e0186820.

20. Gimbrone M, García-Cardeña G. Endothelial Cell Dysfunction and the Pathobiology of Atherosclerosis. Physiol Behav. 2017;176(1):139–48.

21. Byoung-Joo C, Matsuo Y, Aoki T, Kwon T, Prasad A. Coronary endothelial dysfunction is associated with inflammation and vasa vasorum proliferation in patients with early atherosclerosis. Circ Res. 2016;118(4):620–36.

22. Godo S, Shimokawa H. Endothelial Functions. Arterioscler Thromb Vasc Biol.

doi: 10.18176/jiaci.0563

2017;37(9):e108–14.

23. Tousoulis D, Simopoulou C, Papageorgiou N, Oikonomo E, Hatzis G, Siasos G, et al. Endothelial dysfunction in conduit arteries and in microcirculation. Pharmacol Ther.; 2014;144(3):253-67.

24. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: A report of the international brachial artery reactivity task force. J Am Coll Cardiol [Internet]. 2002;39(2):257–65.

25. Inoue K, Kodama T, Daida H. Pentraxin 3: A novel biomarker for inflammatory cardiovascular disease. Int J Vasc Med. 2012;2012:657025.

26. Yasunaga T, Ikeda S, Koga S, Nakata T, Yoshida T, Masuda N, et al. Plasma pentraxin 3 is a more potent predictor of endothelial dysfunction than high-sensitive C-reactive protein. Int Heart J. 2014;55(2):160–4.

27. Bonacina F, Baragetti A, Catapano AL, Norata GD. Long pentraxin 3: Experimental and clinical relevance in cardiovascular diseases. Mediators Inflamm. 2013;2013:725102.

28. Kasai T, Inoue K, Kumagai T, Kato M, Kawana F, Sagara M, et al. Plasma pentraxin3 and arterial stiffness in men with obstructive sleep apnea. Am J Hypertens. 2011;24(4):401–7.

29. Gupta S, Hartley R, Khan UT, Singapuri A, Hargadon B, Monteiro W, et al. Quantitative computed tomography–derived clusters: Redefining airway remodeling in asthmatic patients. J Allergy Clin Immunol. 2014;133(3):729-738.e18.

doi: 10.18176/jiaci.0563

30. Choi S, Hoffman EA, Wenzel SE, Castro M, Fain S, Jarjour N, et al. Quantitative computed tomographic imaging-based clustering differentiates asthmatic subgroups with distinctive clinical phenotypes. J Allergy Clin Immunol. 2017;140(3):690-700.e8.

31. Culver BH, Graham BL, Coates AL, Wanger J, Berry CE, Clarke PK, et al. Recommendations for a Standardized Pulmonary Function Report. An Official American Thoracic Society Technical Statement. Am J Respir Crit Care Med. 2017;196(11):1463–72.

32. Kozlik P, Zuk J, Bartyzel S, Zarychta J, Okon K, Zareba L, et al. The relationship of airway structural changes to blood and bronchoalveolar lavage biomarkers, and lung function abnormalities in asthma. Clin Exp Allergy. 2019;(February):1–14.

33. Hillis GS, Bloomfield P. Basic transthoracic echocardiography. BMJ. 2005;330(7505):1432–6.

34. Chen J, Hamm LL, Mohler ER, Hudaihed A, Arora R, Chen CS, et al. Interrelationship of multiple endothelial dysfunction biomarkers with chronic kidney disease. PLoS One. 2015;10(7):1–10.

35. Pacholczak R, Bazan-Socha S, Iwaniec T, Zaręba L, Kielczewski S, Walocha JA, et al. Endothelial dysfunction in patients with granulomatosis with polyangiitis: a case–control study. Rheumatol Int. 2019;38(2):417-424.

36. Yildiz P, Oflaz H, Cine N, Genchallac H, Erginel-Unaltuna N, Yildiz A, et al. Endothelial dysfunction in patients with asthma the role of polymorphisms of ACE and endothelial NOS genes. J Asthma; 2004;41(2):159–66.

37. Widlansky ME, Gokce N, Keaney JF, Vita JA. The clinical implications of endothelial dysfunction. J Am Coll Cardiol. 2003;42(7):1149–60.

doi: 10.18176/jiaci.0563

38. Gulsvik AK, Gulsvik A, Skovlund E, Thelle DS, Mowé M, Humerfelt S, et al. The association between lung function and fatal stroke in a community followed for 4 decades. J Epidemiol Community Health. 2012;66(11):1030–6.

39. Engström G, Melander O, Hedblad B. Population-based study of lung function and incidence of heart failure hospitalisations. Thorax. 2010;65(7):633–8.

40. Agarwal SK, Heiss G, Barr RG, Chang PP, Loehr LR, Chambless LE, et al. Airflow obstruction, lung function, and risk of incident heart failure: The Atherosclerosis Risk in Communities (ARIC) study. Eur J Heart Fail. 2012;14(4):414–22.

41. Schroeder EB, Welch VL, Couper D, Nieto FJ, Liao D, Rosamond WD, et al. Lung Function and Incident Coronary Heart Disease: The Atherosclerosis Risk in Communities Study. Am J Epidemiol. 2003;158(12):1171–81.

42. Gevaert AB, Lemmens K, Vrints CJ, Van Craenenbroeck EM. Targeting Endothelial Function to Treat Heart Failure with Preserved Ejection Fraction: The Promise of Exercise Training. Oxid Med Cell Longev. 2017;2017(Cv).

43. Norata GD, Marchesi P, Pulakazhi Venu VK, Pasqualini F, Anselmo A, Moalli F, et al. Deficiency of the long pentraxin ptx3 promotes vascular inflammation and atherosclerosis. Circulation. 2009;120(8):699–708.

44. Salio M, Chimenti S, Angelis N De, Molla F, Maina V, Nebuloni M, et al. Cardioprotective function of the long pentraxin PTX3 in acute myocardial infarction. Circulation. 2008;117(8):1055–64.

45. Bonacina F, Barbieri SS, Cutuli L, Amadio P, Doni A, Sironi M, et al. Vascular pentraxin 3 controls arterial thrombosis by targeting collagen and fibrinogen induced platelets aggregation. Biochim Biophys Acta - Mol Basis Dis [Internet].

doi: 10.18176/jiaci.0563

2016;1862(6):1182–90.

46. Kim MJ, Lee HS, Sol IS, Kim MN, Hong JY, Lee KE, et al. Sputum pentraxin 3 as a candidate to assess airway inflammation and remodeling in childhood asthma. Med. 2016;95(51):1–6.

47. Zhang J, Shan L, Koussih L, Redhu NS, Halayko AJ, Chakir J, et al. Pentraxin 3 (PTX3) Expression in Allergic Asthmatic Airways : Role in Airway Smooth Muscle Migration and Chemokine Production. 2012;7(4):1–9.

48. Tripathi P, Awasthi S, Gao P. ADAM metallopeptidase domain 33 (ADAM33): A promising target for asthma. Mediators Inflamm. 2014;2014:572025.

49. Dijkstra A, Postma DS, Noordhoek JA, Lodewijk ME, Kauffman HF, Ten Hacken NHT, et al. Expression of ADAMs ("a disintegrin and metalloprotease") in the human lung. Virchows Arch. 2009;454(4):441–9.

50. Aiello RJ, Bourassa PA, Lindsey S, Weng W, Freeman A, Showell HJ. Leukotriene B4 receptor antagonism reduces monocytic foam cells in mice. Arterioscler Thromb Vasc Biol. 2002;22(3):443–9.

doi: 10.18176/jiaci.0563

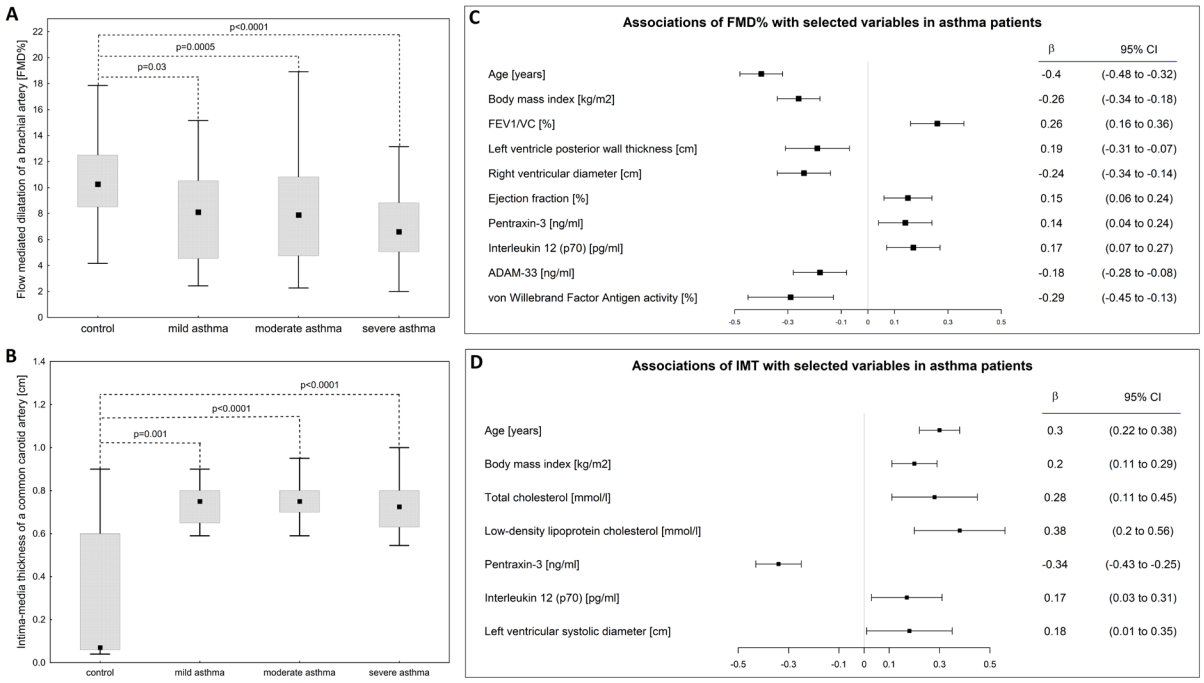
Figure description

Figure 1.

Left side: Relative increase of a flow mediated dilatation of the brachial artery (A) and intima-media thickness of the common carotid artery (B) in control and asthma subjects.

Data is presented as median, interquartile range, and maximum and minimum values. Numbers on the graph represent p-values in comparison to control.

Right side: Main associations of a relative increase of a flow mediated dilatation of the brachial artery (FMD%) (C) and intima-media thickness of a common carotid artery(IMT) (D) with demographic, echocardiographic, spirometry and laboratory parameters in asthma subjects.



Abbreviations:

ADAM-33 - a disintegrin and metalloproteinase domain-containing protein 33,

FMD% - a relative increase of a flow mediated dilatation of the brachial artery,

FEV_1/VC - forced expiratory volume in 1 second/vital capacity,

IMT- intima-media thickness of the common carotid artery

doi: 10.18176/jiaci.0563

Tables

Table 1. Characteristics of demographic factors and comorbidities in asthmatics and controls, as well as asthma severity parameters.

	Patients n=92	Controls n=62	p-value
Age, years	54 (45-62)	48 (41.5-60)	0.36
Male gender, n(%)	26 (28.3)	23 (37.1)	0.4
Body mass index, kg/m^2	27 (24.3-31.3)	26.8 (24.2-29.4)	0.27
Other cardiovascular risk factors			
Hypertension, n(%)	53 (47.3)	22 (39.3)	0.08
Diabetes mellitus, n(%)	18 (16.1)	5 (8.93)	0.11
Hypercholesterolemia, n(%)	28 (25)	18 (32)	0.76
Smoking currently, n(%)	8 (7.14)	3 (5.36)	0.64
Smoking, pack-years	0 (0-18)	0 (0-3)	0.1
Asthma severity (GINA)			
Mild, n(%)	14 (15)	-	-
Moderate, n(%)	37 (40)	-	-
Severe, n(%)	41 (45)	-	-
Spirometry results			
FEV_1, % of predicted value	93 (68.1-108.1)	-	-
FEV_1/VC, %	68 (59.7-75.5)	-	-

Categorical variables are presented as numbers (percentages), continuous variables as median and interquartile range.

Abbreviations: n – number

GINA - Global Initiative for Asthma

FEV_1- forced expiratory volume in 1 second

VC- vital capacity

Table 2. Results of selected laboratory and ultrasonographic parameters in both studied groups.

	Patients n=92	Controls n=62	p-value
Basic laboratory tests			
Total cholesterol, mmol/l	4.9 (4.2-5.7)	5 (4.2-5.3)	0.88
Low-density lipoprotein cholesterol, mmol/l	2.67 (2.25-3.14)	3.2 (2.49-3.64)	<0.001
High-density lipoprotein cholesterol, mmol/l	1.34 (1.09-1.6)	1.39 (1.18-1.8)	0.27
Triglycerides, mmol/l	1.5 (1.09-2.08)	1.11 (0.76-1.65)	<0.001
Creatinine, mmol/l	69.0 (62.0-78.5)	76.1 (68.8-89.2)	0.004
Urea, mmol/l	4.75 (4.0-5.65)	4.65 (3.93-5.5)	0.34
Inflammatory and asthma specific biomarkers			
White blood cell count, $10^3/\mu l$	6.56 (5.44-7.72)	6 (5.02-7.02)	0.02
C-reactive protein, mg/l	2.67 (0.58-8.0)	1.78 (0.89-2.29)	0.03
Interleukin 4, pg/ml	0.005 (0.005-0.005)	0.005 (0.005-0.005)	0.95
Interleukin 5, pg/ml	0.005 (0.005-0.005)	0.005 (0.005-0.005)	0.44
Interleukin 6, pg/ml	0.78 (0.45-1.72)	0.56 (0.26-0.84)	0.01
Interleukin 10, pg/ml	0.57 (0.26-0.99)	0.23 (0.005-0.54)	0.001
Interleukin 12 (p70), pg/ml	0.005 (0.005-1.25)	0.005 (0.005-0.89)	0.46
Interleukin 17A, pg/ml	0.005 (0.005-0.15)	0.005 (0.005-0.11)	0.7
Interleukin 23, pg/ml	0.005 (0.005-18.18)	0.005 (0.005-17.67)	0.79
Interferon γ, pg/ml	0.005 (0.005-0.31)	0.04 (0.005-0.19)	0.46
A disintegrin and metalloproteinase domain-containing protein 33, ng/ml	0.86 (0.23-1.91)	0.43 (0.21-1.05)	0.02
Cardiovascular parameters			
von Willebrand Factor plasma activity, %	108.5 (90.9- 123.4)	102.2 (91.4- 119.2)	0.42

Pentraxin-3, ng/ml	1.17 (0.7-1.7)	1.25 (0.25-2.24)	0.78
Echocardiographic parameters			
Left ventricular diastolic diameter, cm	4.7 (4.5-4.9)	4.7 (4.5-5.0)	0.49
Left ventricular systolic diameter, cm	3 (2.9-3.1)	3 (2.9-3.1)	0.6
Right ventricular diameter, cm	2.2 (2.1-2.5)	2.1 (1.9-2.3)	0.005
Left ventricle posterior wall thickness, cm	0.9 (0.9-1.0)	0.9 (0.8-1.0)	0.13
Ejection fraction, %	67 (65-68)	68 (68-68)	<0.001
Pulmonary artery pressure, mmHg	36 (34-38)	34 (32-40)	0.46
Ultrasound parameters of the endothelial dysfunction and atherosclerosis			
Relative increase of a flow mediated dilatation of the brachial artery, %	7.89 (4.76-10.53)	10.26 (8.51-12.5)	<0.001
Intima-media thickness of the common carotid artery, cm	0.75 (0.65-0.8)	0.65 (0.55-0.75)	<0.001

Categorical variables are presented as numbers (percentages), continuous variables as median and interquartile range.

Table 3.Multiple linear regression models for a relative increase of a flow-mediated dilatation of the brachial artery (FMD%) and intima-media thickness of the common carotid artery (IMT) in asthmatics. Presented variables have been documented as independent determinants of both studied parameters, explaining 46% and 48% of FMD and IMT variability, respectively.

	β (95% CI)	R^2
Relative increase of a flow-mediated dilatation of the brachial artery (%)		
Age, years	-0.17 (-0.28 to -0.07)	0.46
FEV_1/VC index	0.24 (0.15 to 0.34)	
Interleukin 12 (p70), pg/ml	0.3 (0.21 to 0.39)	
Posterior wall thickness, cm	-0.29 (-0.39 to -0.2)	
Pentraxin-3, ng/ml	0.17 (0.08 to 0.27)	
Adjustment statistics	F=11.4, p<0.001	
Intima-media thickness of the common carotid artery (cm)		
Age, years	0.46 (0.37 to 0.55)	0.48
Glucose, mmol/l	0.38 (0.29 to 0.47)	
Posterior wall thickness, cm	0.14 (0.05 to 0.23)	
Pentraxin-3, ng/ml	-0.1 (-0.19 to -0.02)	
Adjustment statistics	F=13.7, p<0.001	

The resulting standardized regression coefficient (β) with 95% confidence interval (95%CI) for a factor (independent variable) indicates the increase/decrease in standard deviations (SDs) of dependent variable (FMD% or IMT), when that particular factor increases with 1 SD and all other variables in the model remain unchanged.

For other abbreviations see Table 1

doi: 10.18176/jiaci.0563