



Adult but not childhood onset asthma is associated with the metabolic syndrome, independent from body mass index

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ABSTRACT

Introduction: Adult-onset asthma (AOA) is usually more severe compared to childhood onset asthma (CoA). Given the increasing evidence that AoA is associated with obesity, we investigated the relationship of other related metabolic comorbid conditions with AoA compared to CoA.

Study design and methods: This cross-sectional study compared the metabolic syndrome and lipid derived inflammatory markers in patients with AoA, CoA and age- and sex-matched control subjects without asthma. Participants were asthma patients visiting the outpatient clinic of two teaching hospitals in Rotterdam, The Netherlands. All participants underwent lung function tests, blood tests and physical activity tracking. AoA was defined as asthma age of onset after the age of 18 years. Metabolic syndrome was defined according to the international joint interim statement criteria.

Results: Eighty-one participants were included (27 AoA, 25 CoA, 29 controls). AoA was associated with the metabolic syndrome (Odds Ratio = 3.64 95% CI (1.16–11.42) $p = 0.03$, Nagelkerke $R^2 = 0.26$), adjusted for age, sex, body mass index and smoking habits. AoA patients had higher median serum IL-6 and leptin-adiponectin (LA) ratio compared to controls (IL-6 (pg/mL): 3.10 [1.11–4.30] vs. 1.13 [0.72–1.58], $p = 0.002$ and LA ratio (pg/mL): 6.21 [2.45–14.11] vs. 2.24 [0.67–4.71], $p = 0.0390$). This was not observed in CoA and controls.

Conclusion: AoA was associated with the metabolic syndrome and its related pro-inflammatory endocrine and cytokine status. This may suggest adipose tissue derived inflammatory markers play a role in the pathophysiology of AoA.

1. Introduction

In the past decade, adulthood onset asthma (AoA) and childhood onset asthma (CoA) have been distinguished as different phenotypes [1]. With the ageing global population, the prevalence of AoA will increase [2]. Disease burden of AoA is higher compared to CoA, and treatment seems more difficult; often high doses of ICS (inhaled corticosteroids) are used to attain control [3,4].

While CoA is more often of the allergic phenotype and related to viral respiratory tract infections in childhood, little is known about the risk factors and nature of the underlying pathology of AoA [3]. Studies suggest AoA can be incited by triggers such as respiratory tract infections and inhaled pollutants, while alterations in lung structure and lung function are also related to AoA [5].

AoA is often non-atopic, characterised by corticosteroid resistance and uncontrolled asthma [6,7]. AoA without T2 inflammation is

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correlated with obesity and corticosteroid resistance [6,7], whereas duration since asthma diagnosis is known to be associated with an increase of BMI [8].

Abdominal obesity is known to be a risk factor for asthma development [9]. Several studies advocate a causal relationship between obesity and asthma with a role for the metabolic syndrome (MetS) in the pathophysiology of AoA [10–12]. MetS can be defined as the presence of any three of five of the following factors: 1) Abdominal obesity: waist circumference ≥ 88 cm in women and ≥ 102 cm in men; 2) Hypertension: systolic blood pressure ≥ 130 mm Hg/diastolic blood pressure ≥ 85 mm Hg and/or use of antihypertensive medication in patients with known hypertension; 3) Hypertriglyceridemia: triglycerides ≥ 1.7 mmol/L/use of lipid-modifying drugs; 4) Low HDL-cholesterol (HDL-C): HDL-C < 1.3 mmol/L in women or < 1.0 mmol/L in men/use of lipid-modifying drugs; 5) Diabetes mellitus type 2 [13]. Several studies show these factors are associated with the development of asthma, whereas those associations were all dependent on BMI. In a prospective cohort study a causal relationship between MetS and AoA has been suggested, as patients with MetS more often developed AoA during follow-up compared to patients without MetS [14]. On the contrary, a recent meta-analysis reported that there is no association between AoA and MetS [10].

MetS is known to induce a low-grade systemic inflammation with elevated levels of serum IL-6 and leptin [15,16]. Serum IL-6 is elevated in asthma and negatively correlated with FEV1% [17,18]. Leptin and adiponectin are hormones produced in adipose tissue, and have immunomodulatory effects. Leptin mediates effects on monocyte proliferation and differentiation, and on the production of IL-6, IL-12 and TNF α [19]. Adiponectin has opposite immunomodulatory effects, with e.g., stimulation of anti-inflammatory cytokines and inhibition of TNF α and phagocytic activity, and is decreased in persons with obesity. It has been shown that in persons with obesity and/or MetS the ratio between leptin and adiponectin is elevated, contributing to a pro-inflammatory state.

Combined, MetS could be an important player in worsened asthma control in AoA and could be seen as a treatable trait [20]. Whether MetS itself is a risk factor for asthma, or if the risk is only one of its components, remains unclear [21].

In this study, we aimed to investigate the co-existence of MetS and AoA, compared to CoA and controls.

2. Methods

2.1. Study design and setting

This cross-sectional study included patients with asthma, and age- and sex-matched controls with no history of pulmonary disease, visiting two large teaching hospitals in Rotterdam, The Netherlands. The study was approved by the TWOR (NL60437.101.17), a nationally acknowledged medical research ethics committee and our institutional review board and registered at clinicaltrials.gov (NCT03278561). All participants provided written informed consent.

Two study visits were planned a week apart between June 2017 and June 2019 for each participant. During the first study visit, pulmonary function was measured and clinical examination. The second visit was scheduled a week apart for non-fasting blood collection. Study visits were postponed in cases of an upper or lower respiratory tract infection 4 weeks prior to the visit.

2.2. Study population

Asthma was diagnosed based on the presence of typical clinical symptoms and reversible airway obstruction ($>12\%$ and 200 mL improvement of FEV1% after bronchodilator) and/or bronchial hyper-reactivity ($PD_{20} \leq 1.76$ mg/ml) and/or a FeNO >50 parts per billion (ppb) [22]. All patients were on GINA step 3–4 medication. Controls were relatives, partners or close contacts of included asthma patients to rule out bias of social background. All subjects were between 18 and 80

years of age. Exclusion criteria for all participants were: 1) smoking history >10 pack years (PY); 2) disease which could alter pulmonary function and/or the immune system such as a current malignancy, rheumatic diseases and known immune deficiencies; 3) non-comprehension of the Dutch language; 4) not being able to perform pulmonary function tests or sputum induction; 5) sufficient information on age of onset was not available. Controls were excluded from study participation if lung function revealed the presence of asthma.

2.3. Variables and outcome measures

To define the age of onset, the patient's medical file was reviewed and the general practitioner was contacted. AoA was defined as asthma started after the age of 18, without a medical history of childhood asthma or infant wheezing. CoA was defined as asthma started before the age of 18. Allergies were verified by ImmunoCAP blood tests and patient interviews. T2 inflammation was defined according to GINA 2020 guidelines: blood eosinophils $\geq 150/\mu\text{l}$ and/or FeNO ≥ 20 ppb, and/or clinically allergen driven asthma [23]. Level of education was based on the highest completed level, classified according to Verhage, whereas 0–3 points stand equal to only completed primary education; 4–5 points to completed secondary education, and 6–7 points to completed university [24]. Atopy was defined as atopic sensitization, an increased specific IgE >0.7 kU/L. Clinical allergy was defined as allergic symptoms in combination with the presence of atopic sensitization. Categorization of body mass index (BMI) was performed according to the World Health Organization recommendations [25]. MetS was defined as demonstrated in the international joint interim statement criteria: 1) Abdominal obesity: waist circumference ≥ 88 cm in women and ≥ 102 cm in men; 2) Hypertension: systolic blood pressure ≥ 130 mm Hg/diastolic blood pressure ≥ 85 mm Hg and/or use of antihypertensive medication in patients with known hypertension; 3) Hypertriglyceridemia: triglycerides ≥ 1.7 mmol/L/use of lipid-modifying drugs; 4) Low HDL-cholesterol (HDL-C): HDL-C < 1.3 mmol/L in women or < 1.0 mmol/L in men/use of lipid-modifying drugs; 5) Diabetes mellitus type 2 [13].

Lung function tests were performed by a lung function technician with the Vmax Sensor Medics Viasys, type 6200 Encore. Values obtained were related to height, weight, age and sex and expressed as percentage of their predicted value [26]. FeNO measurement was performed with the Niox-Flex (Aerocrine AB, Sweden) [27].

Physical activity was monitored with a movement monitor (3D accelerometer, McRoberts). This monitor was carried for seven consecutive days for at least 22 h per day. This monitor measures physical activity levels and other physical activity measurements such as total numbers of steps per 24 h and active minutes sitting, lying and walking [28].

Laboratory measurements were carried out at the Department of Clinical Chemistry, Franciscus Gasthuis & Vlietland (Rotterdam, the Netherlands) according to standard procedures.

Serum IL-6, leptin and adiponectin concentrations were determined using commercially available ELISA kits (R&D Systems, Minneapolis, Minnesota). Measurements higher than the upper limit of detection (ULD) were set at detection limit (D) for analysis.

Asthma control was assessed with the asthma control questionnaire (ACQ-6) combined with FEV1% [29]. The asthma quality of life questionnaire (AQLQ) was used to assess asthma related quality of life; a lower score reflects increased functional problems [30].

2.4. Covariates and bias

Participants were stratified based on age, sex and age of asthma onset. To minimize confounding, we included potential covariates based on literature, statistical significance and since the groups were not completely matched: age, sex, BMI and pack years.

2.5. Statistical analysis

Statistical analysis was conducted with SPSS version 26.0 (IBM SPSS Statistics, New York, USA) and Prism version 8.0 (Graphpad, version 8.0, USA). Continuous and categorical variables between three groups were compared using the one-way ANOVA test, Kruskal Wallis test and Chi-square test, in accordance with the variable characteristics. Post-hoc testing between two groups was performed using the Student's t-test/Mann-Whitney-U test and Chi-square test, in accordance with the variable characteristics. Correlations within numerical data were analysed by Pearson's *r*, with covariates age, sex, BMI and pack years. Multinomial logistic regression was performed to describe possible correlations for MetS between patients with AoA and patients without AoA (response variable: MetS; covariates: age, sex, BMI and pack years). Binary logistic regression was used to determine a correlation between MetS and age of asthma onset (response variable MetS; covariates: age, sex, BMI and pack years). A multiple linear regression was calculated to predict the presence of MetS based on age of asthma onset (response variable: MetS; covariate: age of onset; confounders: age, sex, BMI and packyears). Missing data were deleted pairwise. Skewed data was log-transformed before regression analyses were performed. Results were considered statistically significant if p-values were (two-tailed) <0.05. Data is shown in mean \pm SD and median [25th-75th] or absolute (%) counts.

3. Results

Out of 310 screened asthma patients, 122 met the inclusion criteria, while only 52 were willing to participate and therefore included in this study (25 CoA and 27 AoA). Patients that refused to participate did not differ from patients that were included in this study with respect to clinical parameters such as asthma severity and BMI. The control group consisted of 28 subjects that were age and sex matched to the asthma patients included in this study.

3.1. Subject characteristics

Asthma patients and controls were comparable in terms of patient demographics. AoA patients were older compared to CoA patients (57.00 [45.00–64.00] vs. 42.00 [25.50–59.50], $p = 0.02$). Asthma

severity based on ACQ, exacerbation frequency, and GINA treatment level, did not differ between AoA and CoA patients (Table 1).

3.2. Comorbidities and metabolic syndrome

BMI of asthma patients was higher compared to controls (28.60 ± 5.48 vs. 25.63 ± 4.29 , $p = 0.02$). Also, asthma patients tended to have more comorbidities (cardiovascular, gastro-intestinal, endocrine, musculoskeletal, neurological and traumatic) compared to controls (67.30% vs. 46.40%, $p = 0.07$). Levels of HbA1c, LDL-C, HDL-C, TG or GGT were comparable between asthma patients and controls. MetS was more often present in asthma patients compared to controls (53.80% vs. 25.00%, $p = 0.01$), of which abdominal obesity and hypertension were mostly present in asthma patients (Table 2).

No differences were seen for non-MetS comorbidities between CoA and AoA. However, AoA patients met the criteria for MetS more often than CoA patients (CoA 36.00% vs. AoA 70.37%, $p = 0.01$), (Fig. 1).

In a regression model investigating risk for MetS, adult (versus childhood) onset asthma was associated with an increased risk of MetS (OR = 3.64 95% CI (1.16–11.42) $p = 0.03$, Nagelkerke $R^2 = 0.26$), adjusted for age, sex, BMI and pack years (Table 3A). In a multiple logistic regression model investigating the risk for MetS, age of asthma onset showed a trend for an increased risk of MetS at higher age of asthma onset (OR = 1.04, 95% CI (1.00–1.07), $p = 0.05$, Omnibus $\chi^2 = 14.51$), adjusted for age, sex, BMI and pack years (Table 3B).

Physical activity levels between asthma patients and controls did not differ (asthma: 1.51 ± 0.13 vs. controls: 1.53 ± 0.12 , $p = 0.10$), nor between CoA and AoA. No correlation between MetS and physical activity level was seen.

Asthma control level in terms of ACQ did not differ between AoA and CoA. ACQ was correlated with asthma related quality of life (AQLQ) for all asthma patients. ACQ and AQLQ were not correlated to MetS, adjusted for age, sex, BMI and pack years.

3.3. Immunological findings

FeNO, absolute blood eosinophil count and serum IgE – but not any of the other serum Ig isotypes – were significantly higher in asthma patients compared to controls (Table 4).

Absolute blood eosinophil counts showed a negative correlation to

Table 1
Participant demographics.

	AoA N = 27	CoA N = 25	Controls N = 28	P-value ^S (CoA/AoA/ Controls)	P-value ^{SS} (CoA/ AoA)
Sex female, N	15 (55.56)	13 (52.00)	15 (53.57)	0.97	0.78
Age (years) [#]	57.00 [45.00–64.00]	42.00 [25.50–59.50]	41.00 [27.00–57.00]	0.01	0.02
Age of asthma onset (years) [#]	43.00 [26.00–56.00]	6.00 [6.00–7.00]	.	.	<0.01
Infant wheeze, N	0 (0.00%)	20 (80.00)	2 (0.07)	<0.01	<0.01
ACQ	0.83 [0.50–1.67]	1.00 [0.59–1.84]	.	.	0.96
Exacerbations <12months	1.00 [0.00–2.00]	1.00 [0.00–2.50]	.	.	0.73
GINA 2/3/4	0/6/21	1/2/22	.	.	0.23
Dose inhaled corticosteroids (mg/day)	640.00 [400.00; 800.00]	640.00 [400.00; 800.00]	.	.	1.00
FEV1% pred*	99.55 \pm 13.30	90.25 \pm 16.12	103.12 \pm 11.23	0.03	0.14
FEV1/FVC*	78.48 \pm 12.44	76.90 \pm 11.82	87.77 \pm 11.83	<0.01	1.00
T2 inflammation, N	21 (77.78)	19 (76.00)	.	.	0.88
FeNO \geq 20 ppb, N*	17 (62.96)	13 (52.00)	6 (21.43)	0.01	0.58
Blood eosinophils \geq 150/ μ l, N	14 (51.85)	18 (72)	11 (39.29)	0.12	0.51
Allergy, N*	19 (70.37)	18 (72)	5 (17.86)	<0.01	0.55
Duration of asthma (years) [#]	7.00 [2.00–24.00]	36.00 [18.50–55.00]	.	.	<0.01
Smoking, N yes/never/quit	1/18/8	0/19/6	1/23/4	0.41	0.54
Level of education, N low/medium/high	1/10/16	0/6/19	1/6/21	0.47	0.47

Data shown in mean \pm SD or median [25th-75th] or absolute (%) counts. ACQ = Asthma Control Questionnaire; GINA = Global Initiative for Asthma; FEV1 = Forced Expiratory Volume in 1 s, FVC = Forced Volume Capacity. ^S Numeric data analysis One-way ANOVA was used for normally distributed data and Kruskal Wallis for parametric data. Categorical data was analysed with a Chi-square test; ^{SS} Numeric data analysis post-hoc testing was performed with unpaired t-tests for normally distributed data and Mann-Whitney U test for parametric data. Categorical data post-hoc testing was performed with a Chi-square test. *significant difference between groups; [#] significant difference between CoA and AoA, according to post-hoc test.

Table 2
Comorbidities.

	AoA N = 27	CoA N = 25	Controls N = 28	P-value [§] (CoA/AoA/Controls)	P-value ^{§§} (CoA/AoA)
Body Mass Index*	28.58 ± 5.96	28.62 ± 5.02	25.63 ± 4.29	<0.01	0.98
Comorbidity	20 (74.07)	15 (60.00)	13 (46.43)	0.11	0.28
HbA1c (mmol/mol)	36.00 [33.50–40.00]	36.00 [33.00–37.00]	35.50 [32.25–37.75]	0.32	0.18
LDL-C (mmol/l)	1.61 [1.53–1.70]	1.71 [1.59–1.78]	1.74 [1.59–1.96]	0.11	0.10
HDL-C (mmol/l)	4.00 [3.20–4.70]	3.45 [3.13–4.10]	3.15 [2.40–4.38]	0.11	0.30
Triglycerides (mmol/l)	1.73 [1.29–2.26]	1.30 [1.00–1.80]	1.20 [0.84–1.79]	0.10	0.13
gamma-GT (U/l)	29.00 [24.50–40.50]	21.50 [12.50–43.50]	23.00 [16.00–29.75]	0.06	0.06
Statin use	3 (11.11)	0 (0.00)	3 (10.71)	0.23	0.09
Dose of statins (mg)*	2.59 ± 8.59	0.00 ± 0.00	2.50 ± 8.44	<0.01	0.14
Metabolic syndrome* [#]	19 (70.37)	9 (36.0)	7 (25.00)	<0.01	0.01
Abdominal obesity*	20 (74.07)	13 (52.00)	10 (35.71)	0.04	0.28
Hypertriglyceridemia* [#]	17 (62.96)	6 (24.00)	9 (32.14)	0.04	0.01
Hypertension, N (%) ^{*#}	22 (81.48)	11 (44.00)	10 (35.71)	<0.01	0.02
HDL-C <1.4 mmol/L	4 (14.81)	0 (0.00)	3 (10.71)	0.15	0.08
DM type 2	2 (7.41)	0 (0.00)	1 (3.57)	0.37	0.17

Data shown in mean ± SD or median [25th–75th] or absolute counts (%); [§] Numeric data analysis One-way ANOVA was used for normally distributed data and Kruskal Wallis for parametric data. Categorical data was analysed with a Chi-square test; ^{§§} Numeric data analysis post-hoc testing was performed with unpaired t-tests for normally distributed data and Mann-Whitney *U* test for parametric data. Categorical data post-hoc testing was performed with a Chi-square test. *significant difference between groups; [#] significant difference between CoA and AoA, according to post-hoc test. *significant difference between groups; [#] significant difference between CoA and AoA.

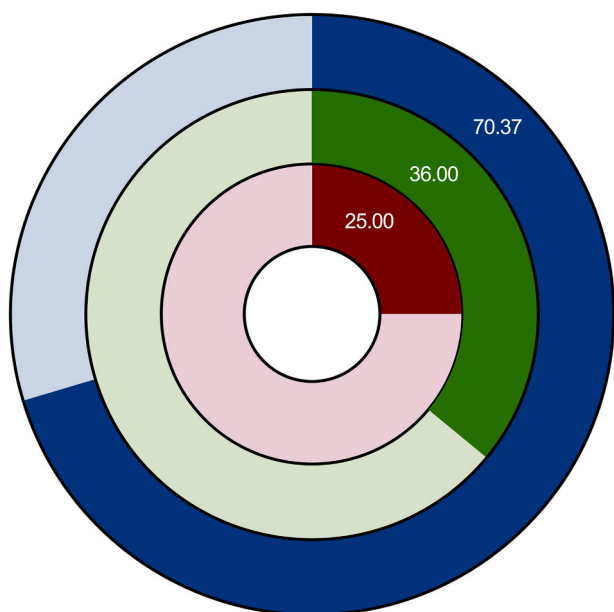


Fig. 1. Prevalence of metabolic syndrome in, darker colors show percentages of participants with metabolic syndrome, light colors show percentages of participants without metabolic syndrome: **Blue** Adult onset Asthma; **Green** Childhood onset Asthma and **Red** Controls. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

FEV1% in AoA and not in CoA ($R = -0.509$, $p = 0.013$), after adjustments for age, sex, BMI and pack years.

IL-6 was measured above the ULD in four samples (1 CoA, 3 control subjects); adiponectin was measured above the ULD in 19 samples (3 CoA, 7 AoA and 9 control subjects); leptin was not measured above the ULD in any sample. As shown in Fig. 2, IL-6 was higher in patients with asthma compared to control subjects (2.02 [1.06–3.65] vs. 1.13 [0.72–1.58], $p = 0.019$). Additionally, leptin-adiponectin (LA) ratio was higher in asthma compared to controls (0.005 [0.00–0.01] vs. 0.003 [0.00–0.01], $p = 0.045$). Both differences were also observed if AoA was compared to controls (Fig. 2).

Comparisons within patients with MetS were performed for explorative aims. As the groups are small, no firm conclusions can be drawn

Table 3
Regression models.

Factor	OR	95% CI	p-value
Age (years)	1.03	0.96–1.07	0.09
BMI	1.01	0.91–1.12	0.81
Sex	1.29	0.44–3.80	0.64
Pack years	1.04	0.94–1.16	0.45
Metabolic Syndrome	3.64	1.16–11.42	0.03

Factor	OR	95% CI	p-value
Age (years)	1.04	0.99–1.09	0.14
BMI	1.13	0.99–1.28	0.06
Sex	0.62	1.61–2.35	0.48
Pack years	0.97	0.86–1.10	0.64
Age of onset	1.04	1.00–1.07	0.05

A: Multinomial regression with presence of adult onset asthma = 1 and childhood onset asthma = 2; Reference category = Presence of MetS = 1; Reference category Sex: Female = 1. Nagelkerke $R^2 = 0.27$. B: Logistic regression with asthma age of onset in years; Predicted probability = membership of MetS; = 1; Reference category Sex: Female = 1. Omnibus $\chi^2 = 14.51$. OR = Odds Ratio; CI = Confidence interval.

from these data. No differences in terms of sex and BMI were seen between AoA, CoA and controls. Participants with MetS were slightly older compared to participants without MetS. FEV1% seems to decrease in MetS + patients with asthma. While BMI did not differ between MetS+ and MetS- asthma patients, BMI was higher in MetS + controls (Table 5). Increased waist circumference (corrected for age and sex) was correlated with increased leptin and LA ratios in asthma patients but not controls (leptin: asthma: $R = 0.54$, $p < 0.01$ vs. controls: $R = 0.34$, $p = 0.24$ and LA ratio asthma: $R = 0.51$, $p = 0.002$ vs. controls: $R = 0.30$, $p = 0.29$).

Type 2 inflammation was dominant in asthma patients with MetS and asthma patients without MetS. AoA patients with abdominal obesity and type 2 inflammation had a higher serum leptin-adiponectin ratio (10.75 [4.27–22.71] vs. 2.06 [0.66–3.22] ng/mL, $p = 0.03$), compared to AoA patients with type 2 inflammation without abdominal obesity. This was not seen in CoA patients with abdominal obesity.

4. Discussion

In this study we demonstrate that MetS is more prevalent in asthma compared to controls, and that specifically AoA is associated with MetS, even when adjusted for age, sex, BMI, and smoking history.

Table 4
FeNO and blood immunological parameters.

	AoA N = 27	CoA N = 25	Controls N = 28	P-value [§] (CoA/AoA/Controls)	P-value ^{§§} (CoA/AoA)
FeNO (ppb)*	23.00 [18.00–41.00]	20.00 [15.50–29.50]	12.50 [10.00–18.50]	<0.01	0.26
Blood leukocytes (10 ⁹ /l)	5.60 [5.00–6.50]	6.10 [5.20–7.20]	5.85 [5.33–6.83]	0.72	0.47
Blood neutrophils (10 ⁹ /l)*	3.74 ± 1.63	3.82 ± 1.53	3.86 ± 1.83	<0.01	0.86
Blood eosinophils (10 ⁹ /l)*	0.26 ± 0.21	0.22 ± 0.19	0.14 ± 0.09	<0.01	0.51
Total IgE (kU/l)*	63.00 [26.00–220.00]	135.00 [36.00–287.00]	20.0 [9.25–50.75]	<0.01	0.31
IgA (g/l)	2.24 [1.60–2.97]	1.85 [1.32–2.78]	1.94 [1.16–2.99]	0.61	0.30
IgG (g/l)	9.50 [8.10–11.40]	9.70 [8.58–11.80]	10.65 [8.94–12.18]	0.46	0.52
IgM (g/l)	0.83 [0.67–1.13]	0.99 [0.81–1.33]	1.02 [0.60–1.33]	0.58	0.25
Adiponectin (mcg/mL)	3.64 [2.17–5.13]	4.52 [2.87–5.52]	3.07 [1.83–5.38]	0.45	0.24
Leptin (pg/mL)	14.90 [9.07–35.44]	15.35 [6.28–52.45]	8.95 [2.41–15.87]	0.24	0.49
L/A Ratio	0.006 [0.00–0.01]	0.004 [0.00–0.01]	0.003 [0.00–0.01]	0.11	0.46
IL-6 (pg/mL)*	3.10 [1.11–4.30]	1.65 [0.94–3.44]	1.13 [0.72–1.58]	<0.01	0.13

Data shown in mean ± SD or median [25th–75th] or absolute counts (%); *significant difference between groups. FeNO = Fraction exhaled Nitrogen Oxide; ppb = parts per billion; Ig = Immunoglobulin. [§] Numeric data analysis One-way ANOVA was used for normally distributed data and Kruskal Wallis for parametric data. Categorical data was analysed with a Chi-square test; ^{§§} Numeric data analysis post-hoc testing was performed with unpaired t-tests for normally distributed data and Mann-Whitney *U* test for parametric data. Categorical data post-hoc testing was performed with a Chi-square test. *significant difference between groups; #significant difference between CoA and AoA, according to post-hoc test. *significant difference between groups; #significant difference between CoA and AoA.

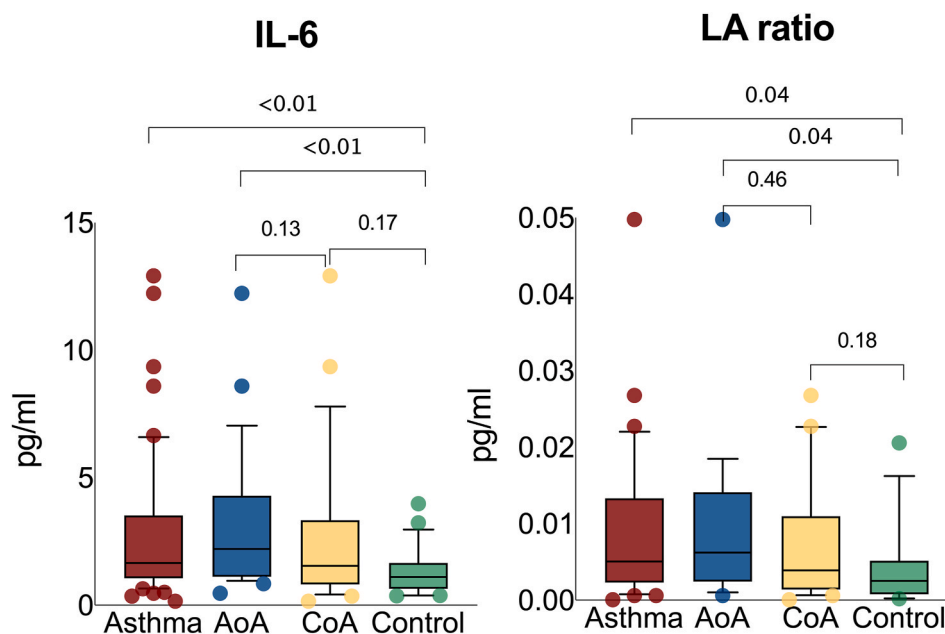


Fig. 2. A) IL-6 and B) Leptin-Adiponectin ratio are presented as median, IQR and upper and lower 90% values.

MetS is more common in adults and associated with low-grade systemic inflammation, dyspnea and asthma [10,11,14]. Our study shows a trend towards an association with age of asthma onset and MetS. Causality between asthma and MetS is difficult to disentangle because of the immense number of interdependent factors. In accordance with the HUNT trial, which showed adults with MetS were more prone to develop asthma, we found MetS to be more prevalent in AoA. More importantly, even after adjustment for BMI we found AoA was associated with MetS.

Systemic inflammation in MetS is characterized by increased levels of circulating leptin, IL-6 and IL-8 [15,16,31]. Leptin mediates effects on monocyte proliferation and differentiation, and on the production of IL-6, IL-12 and TNF α [19]. Several studies have shown IL-6 is associated with neutrophilic airway inflammation and stimulation of neutrophil recruitment in the airways. Thus IL-6 might be of importance in non-eosinophilic asthma. This would be in line with Haldar et al., who characterized the obese asthma cluster as the non-eosinophilic type [16, 20]. Nevertheless, IL-6 is also seems to stimulate T helper differentiation into Th2 and Th17 cells [9]. In our study, possibly due to small sample size, IL-6 was not associated to MetS, nor total number of neutrophils.

Also, leptin was not associated. We did however, in accordance to Dixon et al., see increased serum IL-6 in patients with asthma compared to controls [32]. Nevertheless, in our data no relations between blood neutrophils and MetS, BMI or waist circumference were seen. It is suggested that metabolic inflammation activates neutrophils and induces bronchial hyperreactivity in patients with increased waist circumference and obesity [33]. Our study supports this hypothesis and suggests this is only seen in AoA. Thus the combination of MetS and AoA could be a distinctive asthma phenotype, making MetS a possible meaningful treatable trait in clinical practice of AoA.

We found increased waist circumference was associated with the prevalence of MetS in all asthma patients and not controls, adjusted for age, sex and smoking habits. In our study, serum leptin was also positively correlated to waist circumference within asthma patients and not in controls. In accordance with previous studies, we did find a correlation between abdominal obesity and increased serum leptin, IL-6 and L/A ratio in asthma patients [15,16,31]. More specifically, we found higher serum leptin-adiponectin ratios in AoA patients with abdominal obesity and type 2 inflammation, compared to AoA patients with type 2

Table 5
Patient characteristics of participants with and without MetS.

	AoA MetS+ N = 19	AoA MetS-N = 8	P	CoA MetS+ N = 9	CoA MetS-N = 16	P	Controls MetS+ N = 7	Controls MetS- N = 21	P
Sex female, N	10 (52.63)	5 (62.50)	0.64	4 (44.44)	9 (56.25)	0.57	4 (57.14)	11 (52.38)	0.83
Age (years)	62.00 [50.00–67.00]	49.00 [38.75–57.25]	0.31	57.00 [33.25–59.00]	38.00 [24.00–60.00]	0.15	63.00 [55.50–73.50]	42.00 [26.00–61.00]	<0.01
Age of onset (years)	53.00 [36.50–58.00]	36.50 [23.75–54.50]	0.18	6.00 [4.50–6.75]	7.00 [6.00–7.00]	0.23	.	.	.
ACQ	0.67 [0.26–1.17]	2.25 [1.12–2.99]	0.24	0.75 [0.18–2.33]	1.00 [0.67–1.67]	0.59	.	.	.
GINA 2/3/4, N	0/6/13	0/0/8	0.07	0/0/9	1/2/13	0.38	.	.	.
Dose ICS	400 [360.00–720.00]	720 [475.00–850.00]	0.06	724.50 [535.00–800.00]	640.00 [400.00–800.00]	0.23	.	.	.
Years of asthma	8.00 [2.00–13.00]	4.50 [1.00–22.75]	0.70	49.50 [28.75–53.75]	34.00 [17.00–56.00]	0.12	.	.	.
FEV1% pred	88.83 ± 19.75	94.17 ± 9.77	0.25	77.13 ± 10.99	96.36 ± 15.63	0.12	100.83 ± 12.23	104.30 ± 11.44	0.54
FEV1/FVC	79.85 ± 14.16	81.33 ± 7.12	0.56	67.63 ± 11.04	81.51 ± 10.04	<0.01	97.28 ± 13.42	83.69 ± 9.27	<0.01
T2 ⁺ , N	15 (78.95)	6 (75.00)	0.82	7 (77.78)	12 (75.00)	0.88	.	.	.
BMI	28.26 ± 4.22	27.71 ± 6.32	0.43	29.97 ± 2.97	27.90 ± 6.51	0.16	28.45 ± 2.78	24.68 ± 4.33	0.04
Eosinophils	0.32 ± 0.26	0.17 ± 0.11	0.44	0.21 ± 0.13	0.26 ± 0.26	0.54	0.10 ± 0.05	0.15 ± 0.09	0.19
Neutrophils	3.78 ± 1.65	3.64 ± 1.68	0.84	4.16 ± 1.72	3.63 ± 1.42	0.42	4.46 ± 2.27	3.67 ± 1.69	0.33
IL-6	3.44 [1.18–3.99]	1.94 [0.93–5.18]	0.89	1.74 [1.05–7.89]	1.27 [0.64–3.33]	0.17	1.23 [0.96–3.12]	1.01 [0.64–1.57]	0.18
Leptin	12.07 [8.56–31.04]	20.31 [11.95–47.36]	0.94	15.44 [10.22–42.49]	14.99 [3.31–77.87]	0.88	18.76 [11.43–41.16]	3.66 [1.63–10.74]	0.18
Adiponectin (mcg/L)	2.76 [2.07–4.44]	5.08 [3.32–5.52]	0.09	3.67 [2.08–4.93]	5.33 [3.43–5.95]	0.14	2.97 [2.34–5.51]	3.49 [2.69–5.35]	0.75
LA ratio	0.01 [0.00–0.01]	0.01 [0.00–0.02]	0.89	0.01 [0.00–0.01]	0.00 [0.00–0.01]	0.38	0.01 [0.00–0.02]	0.01 [0.00–0.01]	0.02
Abdominal obesity	15 (78.95)	4 (50.00)	0.13	8 (88.89)	6 (37.50)	0.01	5 (71.43)	5 (23.81)	0.02
Hypertriglyceridemia	16 (84.21)	0 (0.00)	<0.01	4 (44.44)	3 (18.75)	0.17	6 (85.71)	3 (14.29)	<0.01
Hypertension	19 (100.00)	3 (37.50)	<0.01	9 (100.00)	2 (12.50)	<0.01	7 (100.00)	3 (14.29)	<0.01
HDL-C <1.4 mmol/L	4 (21.05)	0 (0.00)	0.16	0 (0.00)	0 (0.00)	n/a	3 (42.86)	0 (0.00)	<0.01
DM type 2	2 (10.53)	0 (0.00)	0.34	0 (0.00)	0 (0.00)	n/a	0 (0.00)	1 (4.76)	0.56
Level of education, N low/medium/ high	1/9/9	0/1/7	0.15	0/3/6	0/3/13	0.41	0/2/5	1/3/17	0.63

Data shown in mean ± SD or median [25th–75th] or absolute (%) counts. ACQ = Asthma Control Questionnaire; GINA = Global Initiative for Asthma; FEV1 = Forced Expiratory Volume in 1 s; IL=Interleukin; LA = leptin/adiponectin ratio; HDL-C = high density lipoprotein-cholesterol; DM = Diabetes Mellitus. Differences were calculated with unpaired-t tests, Mann-Whitney *U* test and Chi square test, according to the variable.

inflammation without abdominal obesity. Therefore, we hypothesize that an increase in visceral fat tissue in asthma patients is responsible for the increase of serum leptin, thereby promoting Th2 inflammation in AoA patients with abdominal obesity. Earlier studies suggest that adiponectin decreases in obese patients. Our study suggests that decreased adiponectin, adjusted for BMI, is associated with MetS. Although numbers in our study are relatively low, we did not see a difference in the occurrence of type 2 inflammation in asthma patients with MetS.

Previous studies show worse respiratory symptoms in multimorbid asthma patients. We show patients with MetS tend to experience worsened FEV1%, while asthma control and GINA classification tends to be comparable between MetS+ and MetS- patients [34]. No correlations were seen between adipose tissue derived inflammatory markers and asthma control or FEV1%.

There was no difference in numbers of blood eosinophils and neutrophils between AoA and CoA. However, all patients used medium to high dose inhaled corticosteroids (ICS) (according to GINA 2020), which may have suppressed systemic inflammation. Also, not all patients were obese meaning the results are not specific for obese asthma only.

Our study has several limitations; first, the sample size was powered for immunological differences and not comorbidity solely, meaning results are at risk to be underpowered for patient related outcomes. This is especially true for analysis performed in the MetS group. Therefore, no associations could be made within this small group of patients. Second, only 42.62% of eligible patients were willing to participate in this study. This could lead to selection bias, making this small sample less representative. However, a wide range of patients in terms of educational level, BMI and asthma control were included. Third, AoA patients were older compared to CoA and controls, therefore, age was added as a

covariate in the regression analysis. To minimize the effect of selection bias, we included asthma patients regardless of the level of control. Last, in our study cohort, CoA patients did not differ from AoA patients in terms of allergen driven asthma, sex and FeNO. Most cohorts show CoA to be more often allergen driven, with higher FeNO and more female compared to AoA. Patients in this study were selected in a tertiary asthma center. It is possible that this makes our cohort a specific cohort of more severe asthmatic patients. Hence, the results of this study should be regarded as an important signal and can be helpful in the design of future studies in AoA.

In conclusion, we show that MetS, independent of BMI, may be associated with AoA. We suggest that adipose tissue derived inflammatory markers, produced in adipose tissue, are contributing to the immunopathology of AoA. MetS and not only obesity, should be regarded as an important treatable trait in AoA.

Author statement

GJB had the original idea for this study. GMB designed the study protocol in collaboration with GJB, HiV, EFCR and GTS. GMB carried out the clinical part of the study, with assistance of LH and CMZ under supervision of GJB, GTS and GV. NP and RWH assisted GMB with immunological analysis. GMB performed statistical analysis under close supervision of BBK. GMB wrote the manuscript, with special assistance on metabolic topics from EFCR and asthma topics from GJB. All authors reviewed the final manuscript and approved it before submission.

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