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Original Article

Biomarkers related to respiratory symptoms and lung function in adults with asthma

Lucia Calciano¹, Laura Portas¹, Angelo Guido Corsico², Mario Olivieri³, Paolo Degan⁴, Marcello Ferrari⁵, Alessandro Fois⁶, Anna Maria Fratta Pasini⁵, Andrea Pasini⁵, Maria Elisabetta Zanolini¹, Roberto de Marco^{1*†} and Simone Accordini^{1*}

¹Unit of Epidemiology and Medical Statistics, Department of Diagnostics and Public Health, University of Verona, Verona, Italy

²Division of Respiratory Diseases, IRCCS ‘San Matteo’ Hospital Foundation, University of Pavia, Pavia, Italy

³Occupational Medicine, Department of Medicine and Public Health, University Hospital of Verona, Verona, Italy

⁴Mutagenesis Unit, Ospedale Policlinico San Martino, Genova, Italy;

⁵Department of Medicine, University of Verona, Verona, Italy

⁶Lung Disease Unit, Department of Clinical and Experimental Medicine, University of Sassari, Sassari, Italy

*Equal senior authors

† Deceased.

21 **Corresponding author**

22 Lucia Calciano, PhD

23 Unit of Epidemiology and Medical Statistics

24 Department of Diagnostics and Public Health

25 University of Verona

26 8 Strada le Grazie

27 Verona, Italy

28 E-mail: lucia.calciano@univr.it

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Abstract

Background. There is a need for easily measurable biomarkers that are able to identify different levels of asthma severity.

Aim. To assess the association between peripheral blood cell counts, fractional nitric oxide in exhaled air (FeNO), urinary biomarkers of oxidative stress (8-hydroxy-2'-deoxyguanosine and 8-isoprostane), and asthma severity in adult patients from the general population.

Methods. In the Gene Environment Interactions in Respiratory Diseases study, 287 subjects with asthma (aged 20-64) were identified from the general population in Verona (Italy) (2008-2010). Self-reported asthma attacks, asthma-like symptoms and the use of hospital services in the past year were synthesised in a score of respiratory symptoms (SRS). The association of biomarkers with SRS and lung function measures (pre-bronchodilator FEV1% predicted and FEV1/FVC) was assessed using the quasi-Poisson and Gaussian regression models, respectively.

Results. Eosinophils (Ratio of Expected Scores: RES[95%CI]=1.19[1.09,1.30]), basophils (RES[95%CI]=1.24[1.10,1.40]), lymphocytes (RES[95%CI]=1.27[1.12,1.45]) and FeNO (RES[95%CI]=1.18[1.02,1.37]) were positively associated with SRS. However, only eosinophils (RES[95%CI]=1.15[1.02,1.30]) and lymphocytes (RES[95%CI]=1.25[1.06,1.47]) showed an independent association. Furthermore, eosinophils (Change in the Expected Outcome for 1-SD increase: CEO[95%CI]=-1.18[-2.09,-0.27]%), basophils (CEO[95%CI]=-1.24[-2.16,-0.33]%) and lymphocytes (CEO[95%CI]=-1.07[-1.99,-0.14]%) were individually, but not independently, associated with FEV1/FVC. Finally, neutrophils were negatively associated with FEV1% predicted (CEO[95%CI]=-2.22[-4.00,-0.44]%).

Conclusions. We identified a pattern of association between a set of biomarkers and asthma endotypes in adult patients from the general population, which could improve understanding of the heterogeneity and severity of the disease and could be useful in defining targeted therapeutic approaches.

Key words

Asthma severity, biomarkers, lung function, score of respiratory symptoms, peripheral blood cell counts, oxidative stress, FeNO, GEIRD

Abbreviations used

8-OHdG: 8-hydroxy-2'-deoxyguanosine

95%CI: 95% confidence interval

ACT: Asthma control test

CEO: Change in the Expected Outcome for 1-SD increase in the biomarker

COPD: Chronic obstructive pulmonary disease

EBC: Exhaled breath condensate

FeNO: Fractional exhaled Nitric Oxide

FEV1: Forced expiratory volume in 1 second

FVC: Forced vital capacity

GEIRD: Gene Environment Interactions in Respiratory Diseases

GINA: Global Initiative for Asthma

OR: Odds Ratio

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74 RES: Ratio of Expected Scores
75 SD: Standard Deviation
76 SoB: Shortness of breath
77 SRS: Score of Respiratory Symptoms
78 WBC: White blood cell
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Accepted Manuscript

81 INTRODUCTION

82 Asthma is a heterogeneous condition with different phenotypes that may be recognized by
83 means of different biomarkers. During recent years there has been an increasing interest in the
84 investigation of easily-obtained biomarkers for the assessment of airway inflammation,[1] which
85 is a common feature of asthma.[2] The induced sputum cell count is the most specific, valid and
86 discriminative method for evaluating airway inflammation in asthmatic patients[3], but this
87 method is difficult to obtain in routine practice. Other markers are currently being investigated,
88 including the measurement of FeNO and peripheral blood cell counts. These biomarkers are a
89 valid means for recognizing distinct inflammatory patterns, which are likely to correspond to
90 different phenotypes of asthma.[4,5] In fact, it has been shown that higher levels of FeNO are
91 associated with bronchial hyperresponsiveness[6] and asthma exacerbations.[7] FeNO levels
92 make it possible to distinguish eosinophilic and non-eosinophilic airway inflammation in severe
93 asthma.[8] Blood eosinophil cell counts are directly associated with the severity of asthmatic
94 symptoms, the frequency of asthma attacks and bronchial hyperresponsiveness, and they are
95 indirectly associated with the level of pulmonary function.[9,10] Furthermore, lymphocytes are
96 associated with clinical indicators in asthma,[11] and neutrophils (alone or in combination with
97 eosinophils) typically modifies the inflammatory profile.[5] Moreover, an oxidant-antioxidant
98 imbalance in the lung cells, which is measurable by means of urinary 8-hydroxy-2'-
99 deoxyguanosine (8-OHdG) and 8-isoprostane, has been shown to be a non-specific feature of the
100 inflammatory process involved in the severity of the disease.[12]

101 In order to improve the management of patients with asthma, the 2002 and 2004 Global Initiative
102 for Asthma (GINA) guidelines recommended combining symptom frequency, respiratory function

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and the daily treatment intensity into a single measure of disease severity[13]. In epidemiological studies on the risk factors of asthma severity, combining the frequency of asthma symptoms and lung function in a single measure does not make it possible to distinguish between their shared and specific determinants.[14] Furthermore, it has been shown that a quantitative score based on asthma symptoms,[15] rather than categorical definitions, reduces misclassification of the disease condition.

The present study is aimed at assessing the association between quantitative measures of asthma severity and the level of FeNO, peripheral blood cells and oxidative stress in adults with asthma. The analysis was carried out by using the data from the GEIRD study.[16]

METHODS

Design of the GEIRD study

GEIRD is an ongoing population-based, multicentre, (multi)case-control study on respiratory health in Italy. The design of the study has been fully described elsewhere.[16] In brief, adult cases and controls have been identified through a two-stage screening process in pre-existing cohorts [the European Community Respiratory Health Survey (ECRHS; 1991-1993)[17] and the Italian Study on Asthma in Young Adults (ISAYA; 1998-2000)[18] or in new random samples from the general population in Italy (2007-2010)[19]. In the GEIRD-stage 1 (2007-2010), a screening questionnaire was mailed to 7,413 subjects aged 20–64 (response rate: 70.7%) in the Verona centre. In the GEIRD-stage 2 (2008-2010), all the responders to the screening questionnaire with symptoms suggestive of asthma, chronic obstructive pulmonary disease (COPD) or chronic bronchitis, and a random sample of those with symptoms suggestive of rhinitis or without respiratory symptoms, were invited to undergo a detailed clinical interview, lung function tests, and laboratory tests for accurate phenotyping. By December 2013, 1,174 of these subjects (45%) attended the clinical stage in Verona and 293 individuals were classified as cases of asthma (see

Fig 1).

Ethics approval was obtained from the appropriate ethics committee (“Comitato Etico per la Sperimentazione dell'Azienda Ospedaliera Istituti Ospitalieri di Verona”). All participants were fully informed about all the aspects of the research project and they gave written informed consent.

Cases of asthma

A subject was defined as having asthma if he/she fulfilled at least one of the following criteria:

- 1) having reported physician-diagnosed asthma;
- 2) having reported asthma-like symptoms [wheezing, nocturnal tightness in the chest, shortness of breath (SoB) following strenuous activity, SoB at rest, SoB at night time] or anti-asthmatic treatment in the past 12 months at the clinical interview, and having at least one of the following conditions:
 - a. positive methacholine test ($PD_{20} \leq 1$ mg);
 - b. pre-bronchodilator airflow obstruction ($FEV_1/FVC < 70\%$ or $<$ Lower Limit of Normal, according to Quanjer[20]) and a positive reversibility test (increase in $FEV_1 > 12\%$ and > 200 ml with respect to pre-bronchodilator FEV_1 after 400 mcg of salbutamol);
 - c. pre- but not post-bronchodilator airflow obstruction, and post-bronchodilator $FEV_1 \geq 80\%$ predicted[20].

Biomarker measurement protocols

Peripheral blood cell counts. Venous blood was taken from each subject who had consented to the blood test. Differential white blood cell (WBC) counts were estimated in all samples by using a cell analyzer (Technicon H6000; Technicon; basophilsingstoke, UK). Total WBCs and differential eosinophils, neutrophils, basophils, lymphocytes and monocytes were counted optically on the peroxidase channel, hydrodynamically focused in a sheath stream flow cell. Granulocyte cell counts per cubic millimeter (cells/mm³) were obtained by multiplying the relative percentage by

the total leukocyte count. A blood eosinophil threshold ≥ 400 cells/mm³ was used to define eosinophilic asthma[21].

Fraction of exhaled Nitric Oxide. FeNO was measured before spirometry by using a chemiluminescence analyser (CLD88, Ecomedics, Switzerland) at an expiratory flow rate of 50 mL/s. Absolute FeNO values were expressed in part per billion (ppb).

Urinary Concentration of 8-OHdG and 8-isoprostane. The urine samples were stored at -80°C, and 8-OHdG and 8-isoprostane concentrations were measured by the ELISA assay (Cosmo Bio LTD and Cayman Chem Co, respectively). The values of creatinine-corrected 8-OHdG and 8-isoprostane concentrations (ng/mg) were used in the data analyses.

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166 **Measures of asthma severity**

Three measures of asthma severity were considered in the present analysis: a Score of Respiratory Symptoms (SRS); pre-bronchodilator FEV₁% predicted and pre-bronchodilator FEV₁/FVC.

SRS was computed as the sum of the answers to the following clinical items: (i) frequency of asthma attacks [none (0), 1-12 (1), ≥ 12 (2)] in the past 12 months; (ii) frequency of wheezing [never (0), sometimes (1), at least once a week (2)] in the past 12 months; (iii) tightness in the chest [no (0), yes (1)] in the past 12 months; (iv) SoB at rest [no (0), yes (1)] in the past 12 months; (v) SoB following strenuous activities [no (0), yes (1)] in the past 12 months; (vi) SoB at night time [no (0), yes (1)] in the past 12 months; (vii) chronic bronchitis (i.e. cough or phlegm on most days for a minimum of three months a year and for at least two successive years) [no (0), yes (1)]; (viii) episodes when symptoms were a lot worse than usual or having reported at least one emergency

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department visit/hospital admission for respiratory problems [no (0), yes (1)] in the past 12 months. SRS ranges from 0 to 10 (see **Fig E1**).

Statistical analysis

The statistical analysis was carried out on the 287 cases of asthma (out of the 293 patients identified in Verona) with complete information on the three measures of asthma severity. The correlation between log-transformed biomarker values was assessed by means of the Pearson's coefficient. The association of single biomarkers with SRS and the lung function measurements was evaluated by using generalized linear models with a quasi-Poisson (with a log link) and a Gaussian (with an identity link) distribution,[22] respectively. In the quasi-Poisson model, the scale parameter was estimated by dividing the Pearson's chi-squared by the residual degrees of freedom. The strength of the associations was measured through the Ratio of Expected Scores (RES) in the quasi-Poisson models, and the regression coefficient in the Gaussian models, which represents the Change in the Expected Outcome (CEO) for one standard deviation increase in the biomarker. The same analysis was repeated by evaluating all the biomarkers together as potential determinants. Moreover, the association of single biomarkers with each symptom included in the SRS was evaluated by using logistic regression models. All the regression models were adjusted for age, gender, smoking habits during lifetime and the use of controller medications in the past 3 months.

All the statistical analyses were conducted by using STATA 14 software (StataCorp, College Station, Tex).

199 RESULTS

201 Main characteristics of the subjects with asthma

202 The 287 cases of asthma (49% females) included in the present analysis were on average 42.5
203 years old (standard deviation, SD: 9.8 years). The percentage of atopic patients, current smokers
204 and patients with well-controlled asthma (ACT>19)[22] was 76%, 24% and 62%, respectively.
205 Sixty-four percent of the subjects reported at least one of the following conditions: asthma
206 attacks, asthma-like symptoms, worsening of symptoms, use of hospital services or use of anti-
207 asthmatic treatment in the past 12 months. In particular, 40% of these individuals reported to
208 have used controller medications in the past 12 months and 27% of them to have used controller
209 medications in the past 3 months. Finally, the mean pre-bronchodilator FEV1% predicted and
210 FEV1/FVC was 102.1% (SD: 14.6) and 77.6% (SD: 7.9), respectively. (**Tab 1**)

211 All the biomarkers had a highly skewed distribution (skewness coefficient>0. 5) (**Tab 2**). The
212 correlation between log-transformed biomarkers is presented in **Fig E2**. A weak but statistically
213 significant correlation was found between FeNO and eosinophils (0.256, $p<0.001$), FeNO and
214 basophils (0.141, $p=0.047$) and between all types of WBC (Persons' correlation coefficients >0.26,
215 $p<0.001$), except between eosinophils and neutrophils (0.075, $p=0.23$). Furthermore, a very
216 strong correlation between urinary 8-OHdG and 8-isoprostane (0.780, $p<0.001$) was found.

218 Association of biomarkers with SRS and each respiratory symptom

219 The eosinophil (Ratio of Expected Scores: RES=1.19, $p<0.001$), basophil (RES=1.24, $p<0.001$) and
220 lymphocyte counts (RES=1.27, $p<0.001$), and FeNO (RES=1.18, $p=0.025$) were positively

221 associated with SRS (**Tab 3**). When these biomarkers were included in the same model, only
222 eosinophil (RES=1.15, $p=0.020$) and lymphocyte (RES=1.25, $p=0.009$) counts showed independent
223 and statistically significant associations (**Tab E1**).

224 The association between the peripheral blood inflammatory cells and the risk of having each
225 respiratory symptom is reported in **Tab 4**. Remarkably, the eosinophil, basophil and lymphocyte
226 values were positively associated with an increased risk of having most of the respiratory
227 symptoms. The strongest association was observed between lymphocytes and SoB at rest, with
228 an expected 99% increase in the risk ($p=0.002$) for 1-SD increase in the lymphocyte value. The
229 neutrophil count was associated with SoB following strenuous activities (Odds ratio, OR=1.43,
230 $p=0.015$).

231 An increase in FeNO levels increased the risk of wheezing (OR= 1.65, $p=0.001$) and the risk of the
232 worsening of symptoms and/or use of hospital services (OR= 1.53, $p=0.038$).

233 Urinary biomarkers of oxidative stress showed a statistically significant association with the risk
234 of having chronic bronchitis (8-OHdG: OR=1.38, $p=0.036$) and the risk of the worsening of
235 symptoms and/or use of hospital services (8-OHdG: OR=1.51, $p=0.005$; 8-isoprostane: OR=1.53,
236 $p=0.025$) (**Tab 4**). However, these biomarkers were not associated with SRS (**Tab 3**).

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238 **Association between biomarkers and lung function**

239 Eosinophils (Change in the Expected Outcome for 1-SD increase in the biomarker: CEO=-1.18%,
240 $p=0.011$), basophils (CEO=-1.24%, $p=0.007$) and lymphocytes (CEO=-1.07%, $p=0.024$) were
241 negatively associated with pre-bronchodilator FEV1/FVC (**Tab 3**). However, when these three
242 biomarkers were included in the same regression model, no statistically significant independent

243 association with pre-bronchodilator FEV1/FVC was found (**Tab E2**). Furthermore, the neutrophil
244 count was negatively associated with pre-bronchodilator FEV1% predicted (CEO=-2.22%,
245 p=0.014) (**Tab 3**).

246 None of the other biomarkers was significantly associated with lung function.

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DISCUSSION

The assessment of non- or minimally invasive biomarkers may be a feasible approach for evaluating airway inflammation, the disease severity and the chance of exacerbations in patients with asthma, and for monitoring the effectiveness of anti-inflammatory treatment regimens. In order to evaluate the prognostic role of easily measurable biomarkers in asthma severity, we investigated their association with asthma symptoms and lung function impairment in adult subjects with asthma, who were identified from the general population in Italy.

The main results of the present analysis are the following:

- 1) Eosinophils and lymphocytes are independently associated with a continuous measure of symptom severity (SRS). Basophils and FeNO are also associated with SRS, even if this association does not reach statistical significance after adjusting for the eosinophil and lymphocyte counts.
- 2) The majority of the analyzed biomarkers are associated with the main asthma symptoms.
- 3) Eosinophils, basophils, lymphocytes and neutrophils are individually associated with a decrease in lung function.

Association between biomarkers and SRS

In our study, the positive statistically significant associations of eosinophils, basophils and lymphocytes with SRS support the well-known relationship between these inflammatory biomarkers and asthma severity.[5,23] In fact, it is well-known that patients with higher eosinophil levels are more likely to have a higher frequency of symptoms,[5,24] and that lymphocytes have a central role as a conductor of the immune orchestra that contributes to

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3 270 asthma. The number of activated lymphocytes has been shown to be related to the degree of
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8 272 exacerbations of the disease. Type 2 helper T-cells (Th2) secrete IL-4, IL-5 and IL-13, which induce
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10 273 B-cells to produce IgE and which can activate other inflammatory cells to release cytokines
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12 274 stimulating eosinophils and mast cells degranulation. In addition, a positive association has been
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14 275 found for FeNO confirming its role as a biomarker of airway inflammation in mild to moderate
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16 276 asthma.[4] However, when all the biomarkers that were individually associated with SRS were
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18 277 included in the same model, only a weak borderline association was observed for FeNO, whereas
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20 278 basophils were no longer associated with symptom severity. Eosinophils maturation,
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22 279 mobilization and recruitment to areas of inflammation are controlled by the Type 2 cytokines IL-
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24 280 5 and IL-13 and FeNO is produced by nitric oxide synthase, the expression of which is driven by
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26 281 IL-13 and IL-4. Therefore, a certain degree of overlap is expected between these biomarkers, as
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28 282 Type 2 cytokines are expected to be secreted in an interrelated way rather than in a singular
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30 283 mode.[25] These considerations can explain the independent association of FeNO with symptom
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32 284 severity and its weaker effect than that of eosinophils, when considered together with the others
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34 285 biomarkers. Despite the fact that our findings reiterate the central role of eosinophilic
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36 286 inflammation in asthma, they also suggest that both local and systemic Th2 cytokine-drive
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38 287 mechanisms represent two different inflammatory pathways with separate trigger mechanisms
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40 288 supporting the view that a combination of two markers might be a cost-effective way to
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42 289 characterize phenotyping and individualized treatment in asthmatic patients.[26] On the other
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44 290 hand, the results observed for basophils can be explained by the fact that they have a much
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46 291 shorter lifespan and that their number in the peripheral blood is lower with respect to the blood
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counts of other biomarkers. Therefore, it is plausible that their role as predictors of symptoms can be masked by the predominant effect of eosinophils and lymphocytes.

Association between biomarkers and each respiratory symptom

In our study, we found a common pattern of association of eosinophils, basophils and lymphocytes with tightness in the chest, SOB at rest and SOB at night time. In addition, eosinophils and basophils showed a positive association with asthma attacks, a fact that underlines their central role as initiators of inflammation. Despite the fact that basophils represent less than 1% of peripheral blood leukocytes and their lifespan is much shorter than that of mast cells, their infiltration from the blood circulation to the airway lumen and their modulation of the immune responses in the surrounding tissues during asthma attacks has been reported.[27]

Basophils and eosinophils share many of their recruitment mechanisms and are regulated by exactly the same hemopoietic growth factors, namely IL-3, IL-5 and GM-CSF, which enhance all their functions or effects including chemotaxis and mediator release. IL-5 and GM-CSF are capable of activating both basophils and eosinophils at the same time, but low levels of IL-3 preferentially affect various aspects of cell biology in basophils, without affecting the function of eosinophils. Thus, eosinophil activation is always accompanied by basophil activation but not vice versa. In addition, basophils are the only cell type that constitutively expresses IL-4 and IL-13 mRNA and they play a key immunoregulatory role during both the initiation and amplification of the Th2-type immune response [27]. In fact, they secrete IL-4 and IL-13 upon FcεRI even cross-linking over IgE-independent activation (IL-3 plus C5a), contributing to the local symptoms of

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3 314 inflammation and airway hyper-responsiveness through degranulation and the release of
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6 315 histamines, eicosanoids and cytokines, and through antigen-presenting cell function.[28]
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8 316 Basophil-derived proteases generate chemotactic factors by acting on serum proteins, which can
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11 317 induce further basophil infiltration and the recruiting of other inflammatory cells, such as
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13 318 eosinophils and lymphocytes, thereby aggravating inflammation and symptoms. Our findings
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15 319 support this fact, highlighting a positive independent association of eosinophils with chronic
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18 320 bronchitis, as well as a positive independent association of basophils and lymphocytes with the
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20 321 worsening and/or use of hospital services.
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23 322 In the 1990s, the high level of FeNO in patients with asthma suggested that there was a
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25 323 relationship between the fraction of FeNO and the disease.[29] Further research has pointed out
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28 324 that FeNO is potentially a non-invasive marker of eosinophilic airway inflammation, which
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30 325 increases with increasing allergen exposure.[30] Other studies have shown that FeNO levels rise
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33 326 in asthmatic patients with worsening symptoms, suggesting that higher FeNO levels are a useful
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35 327 predictive marker of exacerbations and loss of control in subjects with asthma.[31] Our results
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38 328 on the positive association of high FeNO levels with wheezing and the worsening and/or use of
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40 329 hospital services, support this evidence.
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42 330 Interestingly, our study reveals a significant association of 8-OHdG with chronic bronchitis and
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44 331 highlights the key role of both 8-isoprostane and 8-OHdG in the worsening and/or use of hospital
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47 332 services. Since the level of oxidative stress may be used as an indirect marker of the severity of
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50 333 airway inflammation, these findings support the evidence from previous studies on the
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52 334 association between chronic bronchitis symptoms and a more severe or uncontrolled form of
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54 335 asthma.[32] Oxidative stress plays an important role in the pathophysiology of asthma, leading
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to the damage of the airway epithelium and the pulmonary smooth muscle. Neutrophils and eosinophils in asthmatic patients produce increased amounts of oxygen free radicals when stimulated in vitro, while antioxidant capacity is decreased in resting neutrophils from these subjects[12]. 8-isoprostane, which is the most common form of F2-isoprostanes (a group of stable PGF2 α isomers formed by the free radical peroxidation of arachidonic acid irrespective of the action of cyclooxygenase), and 8-OHdG are considered to be ideal markers for investigating the pathophysiology of oxidative injury[33]. Patients with asthma have been found to have a higher concentration of exhaled breath condensate (EBC) 8-isoprostane compared to healthy adults, and the concentration of EBC 8-isoprostane appears to be progressively elevated with asthma severity[12]. Overall, our findings suggest that the assessment of 8-isoprostane and 8-OHdG levels in the urine of patients with asthma could potentially reflect the inflammatory pattern involving oxidative stress, which seems to be present in severe/uncontrolled asthma with frequent exacerbations. However, we cannot exclude that the imbalance between oxidative stress and antioxidative capacity could be the expression of the overlap or transition to COPD. In fact, the importance of oxidative stress in COPD has been confirmed by several studies that identified the presence of markers of free radical damage.[34,35]

Association between biomarkers and lung function

Our finding of a negative association between neutrophils and FEV1% predicted is unexpected in asthma, but it is not completely surprising, as previous studies on COPD and on alpha-1-antitrypsin deficiency have detected a relation between a high neutrophil count and higher levels of neutrophil elastase, with a faster decline in FEV1.[35,36] Moreover, we found a relationship

358 between neutrophils and SOB following strenuous activities, which may represent an early sign
359 of COPD. On the basis of our results, we can only suppose that asthma and COPD could share
360 mechanisms that are responsible for the FEV1 decline.

361 The association between blood eosinophils and pre-bronchodilator FEV1/FVC decrease is
362 biologically plausible. Eosinophils within the airway wall are associated with features of
363 remodeling, in particular the thickening of the basement membrane, and their inflammatory
364 factors can increase contractility and can reduce the relaxation of airway smooth muscle.[37] It
365 has been shown that targeting eosinophils in sputum with anti-IL-5 increases luminal patency
366 when compared to placebo.[38] Therefore, eosinophils might modulate lung function through
367 some mechanisms, which include airway wall remodeling, airway constriction and mucus
368 hypersecretion.

369 In addition, we found an association of basophils and lymphocytes with a decrease in pre-
370 bronchodilator FEV1/FVC. This finding is supported by the fact that increased numbers of mast
371 cells[39], basophils[40] and lymphocytes[41] have been found in the lung tissue of asthmatic
372 patients. Furthermore, a study on mice has demonstrated that lung-infiltrating basophils[42]
373 contribute to the induction of early-phase airway obstruction, whereas in vitro data have
374 suggested an influence of T lymphocytes on the late-phase tissue damaging process and the
375 structural change in the bronchi.[41]

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377 **Strengths and weaknesses of the study**

378 The main strength of the present analysis is that the cases of asthma were identified in the
379 general population, rather than in clinically selected groups, which should guarantee that our

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sample encompasses a wide spectrum of disease severity. In addition, these patients underwent an accurate phenotyping by means of an extensive clinical interview and lung function tests[17]. However, the relatively small number of cases and the fact that they were identified in a single centre should be taken into account when interpreting our results.

Conclusions

We identified a pattern of associations between a set of biomarkers and asthma endotypes in adult patients from the general population. Our results could advance the understanding of the heterogeneity and severity of the disease and could be useful in defining targeted therapeutic approaches.

COMPETING INTERESTS

None declared.

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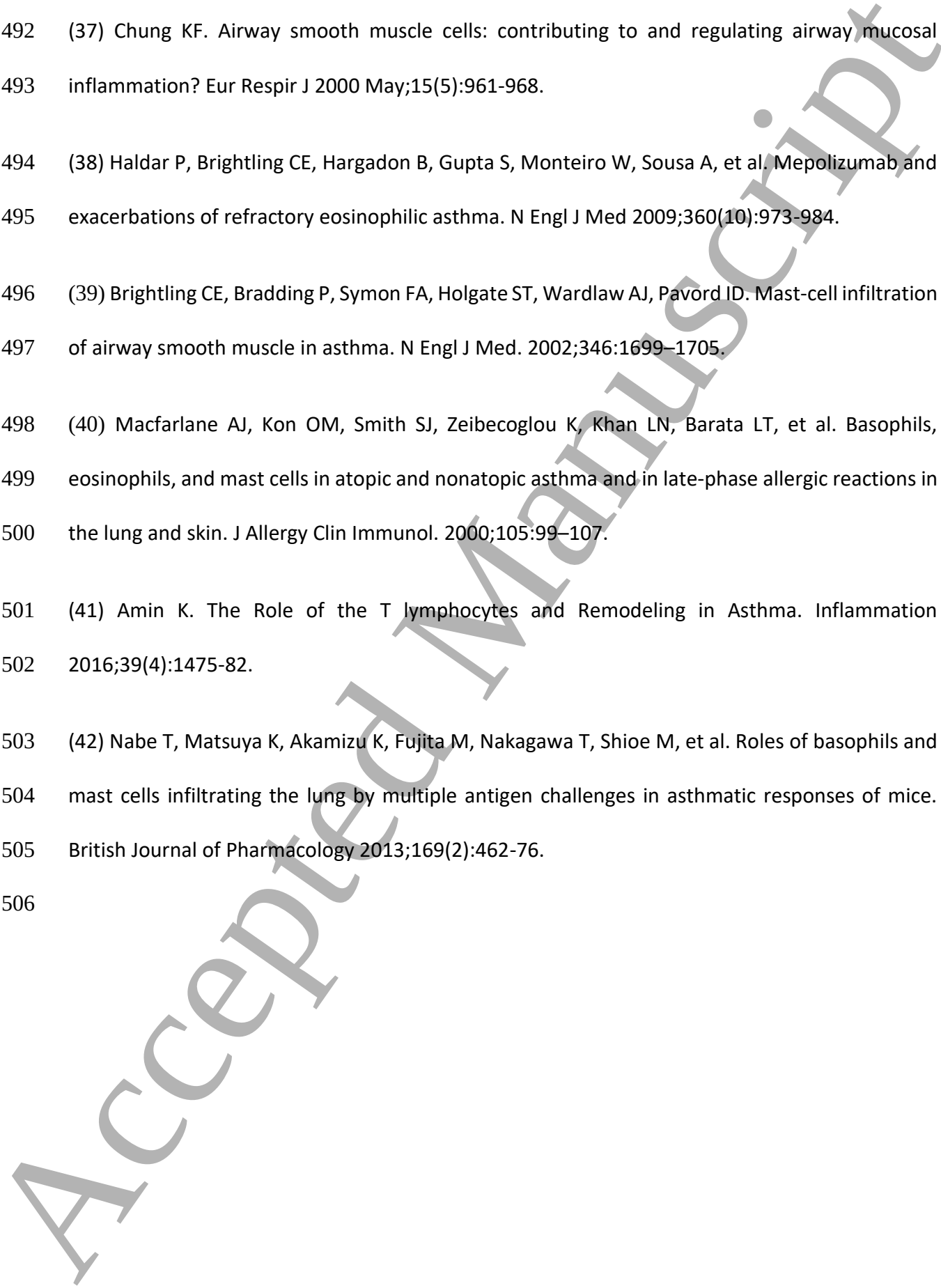
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507 **Tab 1.** Main characteristics of the asthmatic subjects identified in the GEIRD study (n=287)

Females, %		49.1
Age (years), mean $\pm$ sd		42.5 $\pm$ 9.8
Smoking habits, %	Never smoker	49.1
	Past smoker	26.5
	Current smoker	24.4
Atopic asthma, %	Absent	20.2
	Present	76.3
	Missing	3.5
Pre-bronchodilator FEV ₁ % predicted, mean $\pm$ sd		102.1 $\pm$ 14.6
Pre-bronchodilator FVC % predicted, mean $\pm$ sd		110.7 $\pm$ 14.1
Pre-bronchodilator FEV ₁ /FVC (%), mean $\pm$ sd		77.6 $\pm$ 7.9
SRS, median (IQR)		1 (0-3)
Wheezing [†] , %	Never	60.6
	Sometimes	32.8
	At least once a week	6.6
Attacks of asthma [†] , %	0 (none)	77.7
	1 (1-12 attacks)	17.8
	3 ($\geq$ 12 attacks)	4.5
Tightness in the chest [†] , %		19.5
Shortness of breath at rest [†] , %		10.1
Shortness of breath following strenuous activities [†] , %		23.0
Shortness of breath at night-time [†] , %		13.6
Chronic bronchitis, %		12.2
Worsening of symptoms and/or use of hospital services [†] , %		13.6
Use of controller medications* [†] , %	Absent	73.9
	Present	25.4
	Missing	0.7

Use of controller medications* ^{††} , %	Absent	81.9
	Present	17.4
	Missing	0.7
ACT, %	5-19	9.8
	20-24	26.5
	25	35.2
	Missing	28.5

Detailed legend: IQR: interquartile range; ACT: Asthma Control Test; [†]In the past 12 months; ^{††}In the past 3 months; *Inhaled glucocorticosteroids (ICS) or Cromones or Oral Methylxanthines or Leukotriene modifiers or ICS & Long-acting β 2-agonists or ICS & Methylxanthines or ICS & Leukotriene modifiers with/without taking relievers in the past 12 months.

Tab 2. Distribution of the biomarkers of asthma severity.

Biomarkers	n	Median (IQR)	Skewness coefficient
Eosinophils (cells/mmc)	262	165.2 (99.5 – 261.0)	3.3
Neutrophils (cells/mmc)	262	4039.7 (3246.1- 5096.7)	1.3
Basophils (cells/mmc)	262	30.7 (20.6 - 43.7)	0.8
Lymphocytes (cells/mmc)	262	1880.7 (1557.3 - 2360.5)	0.5
Monocytes (cells/mmc)	262	356.1 (271.8 – 439.7)	0.8
FeNO(ppb)	216	21.5 (13.4 - 40.7)	1.5
8-OHdG (ng/mg)	276	4.1 (2.2 - 7.5)	5.2
8-isoprostane (ng/mg)	151	0.5 (0.2 – 1.1)	4.2

Tab 3. Association of each biomarker with the Score of Respiratory Symptoms (SRS), pre-bronchodilator FEV1/FVC and FEV1% predicted.

Biomarkers (1-SD increase)	n	SRS Ratio of expected scores [95%CI]	FEV1/FVC Change in the expected outcome (%) [95%CI]	FEV1% predicted Change in the expected outcome (%) [95%CI]
Eosinophils (cells/mmc)	262	1.19*** [1.09,1.30]	-1.18* [-2.09,-0.27]	-1.18 [-3.01,0.65]
Neutrophils (cells/mmc)	262	1.09 [0.96,1.27]	-0.40 [-1.31,0.50]	-2.22* [-4.00,-0.44]
Basophils (cells/mmc)	262	1.24*** [1.10,1.40]	-1.24** [-2.16,-0.33]	-0.95 [-2.78,0.88]
Lymphocytes (cells/mmc)	262	1.27*** [1.12,1.45]	-1.07* [-1.99,-0.14]	-1.58 [-3.42,0.27]
Monocytes (cells/mmc)	262	1.06 [0.92,1.22]	-0.55 [-1.50,0.40]	-1.58 [-3.45,0.30]
FeNO(ppb)	216	1.18* [1.02,1.37]	-0.93 [-1.92,0.06]	-0.32 [-2.33,1.70]
8-OHdG (ng/mg)	276	1.07 [0.96,1.19]	-0.42 [-1.30,0.47]	0.06 [-1.67,1.80]
8-isoprostane (ng/mg)	151	1.02 [0.86,1.21]	-0.50 [-1.70,0.71]	0.28 [-2.09,2.65]

Detailed legend: All the estimates were obtained after adjusting for age, gender, smoking habits and recent use of controller medications in the past 3 months. * p<0.05, ** p<0.01, *** p<0.001.

Tab 4. Association of each biomarker with asthma attacks, asthma-like symptoms, worsening of symptoms and/or use of hospital services in the past 12 months.

Biomarkers (1-SD increase)	n	Asthma attacks	Wheezing	Tightness in the chest	Shortness of breath (SoB) at rest	SoB following strenuous activities	SoB at night time	Chronic bronchitis	Worsening and/or use of hospital services
		OR [95%CI]	OR [95%CI]	OR [95%CI]	OR [95%CI]	OR [95%CI]	OR [95%CI]	OR [95%CI]	OR [95%CI]
Eosinophils (cells/mmc) [¶]	262	1.42* [1.02,1.96]	1.36 [1.00,1.85]	1.54* [1.11,2.15]	1.84** [1.24,2.72]	1.21 [0.91,1.61]	1.93*** [1.34,2.78]	1.48* [1.04,2.10]	1.32 [0.97,1.80]
Neutrophils (cells/mmc) [¶]	262	1.07 [0.78,1.45]	1.11 [0.85,1.45]	0.91 [0.66,1.27]	1.07 [0.69,1.67]	1.43* [1.07,1.90]	1.09 [0.76,1.56]	0.93 [0.63,1.36]	0.99 [0.68,1.45]
Basophils (cells/mmc) [¶]	262	1.39* [1.02,1.89]	1.21 [0.925,1.59]	1.45* [1.05,1.99]	1.70** [1.14,2.54]	1.12 [0.84,1.50]	1.89*** [1.32,2.71]	1.20 [0.83,1.73]	1.47* [1.04,2.08]
Lymphocytes (cells/mmc) [¶]	262	1.37 [1.0,1.89]	1.23 [0.94,1.62]	1.63** [1.16,2.27]	1.99** [1.28,3.10]	1.28 [0.95,1.73]	1.83** [1.25,2.69]	1.27 [0.86,1.87]	1.58* [1.09,2.27]
Monocytes (cells/mmc) [¶]	262	0.91 [0.65,1.27]	1.12 [0.85,1.47]	1.12 [0.80,1.56]	1.38 [0.89,2.14]	1.10 [0.81,1.50]	0.98 [0.66,1.45]	1.03 [0.69,1.53]	0.91 [0.62,1.35]
FeNO(ppb) [¶]	216	1.15 [0.83,1.60]	1.65** [1.22,2.24]	1.34 [0.95,1.89]	0.92 [0.55,1.56]	0.99 [0.71,1.38]	1.32 [0.92,1.90]	1.10 [0.71,1.69]	1.53* [1.02,2.29]
8-OHdG (ng/mg) [¶]	276	0.97 [0.70,1.33]	1.20 [0.91,1.60]	0.99 [0.75,1.32]	1.00 [0.66,1.52]	1.02 [0.78,1.34]	0.89 [0.58,1.37]	1.38* [1.02,1.86]	1.51** [1.13,2.02]
8-isoprostane (ng/mg) [¶]	151	0.68 [0.33,1.37]	1.05 [0.75,1.47]	0.95 [0.64,1.41]	0.77 [0.35,1.70]	0.96 [0.65,1.41]	0.68 [0.27,1.71]	1.39 [0.95,2.04]	1.53* [1.06,2.22]

Detailed legend: All the estimates were obtained after adjusting for age, gender, smoking habits and recent use of controller medications in the past 3 months. * p<0.05, ** p<0.01, *** p<0.001.

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FIGURE LEGENDS

Fig 1. Flow chart of the GEIRD study in the Verona centre and the selection of the asthma cases.

Fig 1.