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Periostin as an important biomarker of inflammatory phenotype T2 in Brazilian asthma patients

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ABSTRACT

Objective: The aim of this study was to assess the laboratory performance of periostin associated with a panel of biomarkers to identify the inflammatory phenotype of Brazilian asthma patients. **Methods:** We evaluated 103 Brazilian individuals, including 37 asthmatics and 66 nonasthmatic controls. Both groups underwent analyses for serum periostin, eosinophil levels in the peripheral blood, the fraction of exhaled nitric oxide (FeNO), total serum IgE, urinary leukotriene E_4, and serum cytokines. **Results:** Higher levels of periostin ($p = 0.005$), blood eosinophils ($p = 0.012$), FeNO ($p = 0.001$), total IgE ($p < 0.001$), and IL-6 ($p \leq 0.001$) were found in the asthmatic patients than the controls. Biomarker analyses by the ROC curve showed an AUC greater than 65%. Periostin (OR: 12,550; 95% CI: 2,498–63,063) and IL-6 (OR: 7,249; 95% CI: 1,737–30,262) revealed to be suitable asthma inflammation biomarkers. Blood eosinophils, FeNO, total IgE, IL-6, TNF, and IFN-γ showed correlations with clinical severity characteristics in asthmatic patients. Periostin showed higher values in T2 asthma ($p = 0.006$) and TNF in non-T2 asthma ($p = 0.029$). **Conclusion:** The panel of biomarkers proposed for the identification of the inflammatory phenotype of asthmatic patients demonstrated good performance. Periostin proved to be an important biomarker for the identification of T2 asthma.

Keywords: Asthma; Biomarkers; Eosinophilia; Phenotype.

INTRODUCTION

Asthma is a chronic inflammatory disease characterized by hyperresponsiveness of the lower airways and variable airflow limitations that are reversible spontaneously or with treatment. Considered one of the most prevalent chronic diseases in the world, asthma is directly and indirectly responsible for various medical and productive losses,[1,2] with Brazil being one of the countries with the highest prevalence.[3,4]

The diagnosis of asthma is clinically based on the presence of compatible symptoms associated with variable airflow limitations and demonstrations of airway hyperresponsiveness.[5,6] Among asthma patients, there is a subgroup of individuals who have difficult-to-treat asthma. Although these patients use high doses of medication to control their symptoms, they exhibit attributes that hinder control, such as behavioral factors, low adherence, incorrect inhaler techniques, environmental or occupational exposure, and the presence of comorbidities.[7] A smaller subgroup of these patients have severe asthma. These individuals lack adequate symptom control, even when alternative diagnoses are excluded, comorbidities are treated, triggers are removed, and treatment adherence is satisfactory; they represent around 1 to 4% of the general population of asthmatics.[8,9]

Several immunobiological options are currently available for the treatment of severe asthma. For a target-specific approach among the existing options, the identification of the inflammatory phenotype is fundamental.[10] Among the inflammatory phenotypes, the most frequent is type 2 (T2) inflammation, which is characterized by the presence of type 2 T-helper cells (Th2) or innate immune cells (ILC-2) and manifests as airway and/or systemic eosinophilia.[11]

All immunobiological agents approved for use are directed to the treatment of T2 asthma, which can be identified through available biomarkers such as eosinophils in the peripheral blood (blood eosinophils) and total serum immunoglobulin E (total IgE), eosinophils in induced sputum (sputum eosinophils), and the fraction of exhaled nitric oxide (FeNO).[12] New biomarkers for the differentiation of T2 asthma have been proposed, such as serum periostin and urinary leukotriene E_4 (LTE_4).[13,14] There are also studies investigating the use of cytokines involved in the inflammatory pathways of Th1 and Th17 cells, such as IL-6, TNF, and IFN-γ, as biomarkers for non-T2 asthma.[15-17]

The 2021 Global Initiative for Asthma (GINA) guidelines recommend the identification of at least one of the following findings for the establishment of T2 asthma: (1) blood eosinophils ≥ 150 cells/μL and/or (2) FeNO ≥ 20 ppb and/or (3) sputum eosinophils ≥ 2% and/or (4) allergy-induced asthma and/or (5) the need for maintenance therapy with oral corticosteroids (OC). If necessary, the values of eosinophils in the blood and

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FeNO should be measured at least 3 times under the lowest possible dose of oral corticosteroids.[5]

Currently, the routine services of clinical laboratories are widely available for the quantification of total serum IgE and blood eosinophils, but lack methodologically-directed validation in the clinical context of asthma. There is also no service that provides quantifications of FeNO, periostin, and LTE_4 for use in clinical practice. The quantification of sputum eosinophils is an ideal test for asthma phenotyping; however, it is a complex and difficult-to-apply technique in the clinical routine.[18] The availability and accessibility of other biomarkers are essential, not only aiming at the replacement of less useful biomarkers but also for the complementation of information on the inflammatory phenotype of patients. In this sense, a more complete panel of biomarkers may be useful.[12,19,20] To this end, it is crucial that clinical laboratories validate these trials in the clinical context of asthma to determine the analytical performance of tests for this purpose.

In view of the above, the aim of the present study was to evaluate the analytical performance of a panel of biomarkers to identify the inflammatory phenotype in a group of asthmatic patients in Brazil.

METHODS

This cross-sectional, observational, analytical study was duly registered in Plataforma Brasil under CAAE No. 73640917.8.0000.5479 and approved by the Research Ethics Committees of the Irmandade da Santa Casa de Misericórdia de São Paulo and Fleury Group. All participants signed a free and informed consent form.

The present study included Brazilian individuals aged above 18 years old from two different centers: patients diagnosed with severe asthma according to the international criteria described in the ATS/ERS guidelines,[21] who were followed up at the severe asthma outpatient clinic of the Irmandade da Santa Casa de Misericórdia de São Paulo, and patients with nonsevere asthma and control individuals, employees of the Fleury Group company, who volunteered to participate through an internal communication of the research project. The sample size was estimated following the CLSI (Clinical & Laboratory Standards Institute) EP09-A3 guideline, which determines a minimum requirement of 40 samples for user-conducted verification.

Patients and controls were recruited from February 2019 to May 2021. The patients were instructed not to discontinue ongoing treatment since the assessment was not interventional and did not address a specific treatment. For inclusion in the nonsevere asthma group, patients previously diagnosed by a specialist, who had asthma attacks during adulthood, and who did not meet the criteria for severe asthma by clinical history and the medications used for symptom control were accepted. The control group was determined by sex pairing with the asthma group.

All study participants (patients and controls) were evaluated regarding obesity (BMI calculation), allergy to inhalant antigens (detection of specific IgE against house dust, mites, grass pollen, animal epithelium and proteins, and/or fungi), and whether they had any of the following comorbidities: sleep apnea, bronchiectasis, atopic dermatitis, chronic urticaria, nasal polyposis, chronic rhinosinusitis, vocal cord dysfunction, or autoimmune diseases.

Asthmatic patients were evaluated concerning the history of the disease (late-onset asthma was defined as the onset of symptoms in adulthood), the drugs in use for categorization of the treatment stage according to GINA, asthma control through the Asthma Control Test (ACT), and pulmonary function by analyzing the values of forced vital capacity (FVC) and forced expiratory volume in the first second (FEV_1) of predicted (prior to bronchodilator use) spirometry (Koko® PFT Spirometer, nSpire Health Inc, Longmont, CO, USA) results, performed according to the quality and reproducibility criteria of ATS/ERS.[22] T2 asthma was defined when patients presented with both biomarkers: blood eosinophil values ≥ 150 cells/μL and FeNO ≥ 20 ppb.

Quantifications of total and specific IgE were conducted using automated immunofluorimetry and electrochemiluminescence platforms, and the results were expressed as kilo units per liter (kU/L). Blood eosinophil levels, expressed as cells per microliter (cells/μL), were determined by fluorescent flow cytometry and impedance, with confirmation of counts and morphological analysis performed by microscopy when applicable.

The quantification of serum periostin was carried out by enzyme-linked immunosorbent assay (ELISA) using the Human Periostin/OSF-2 kit (R&D Systems Inc, Minneapolis, MI, USA), and the results were expressed as nanograms per milliliter (ng/mL). FeNO quantification tests were performed according to the ATS/ERS recommendations[23] using a NIOX-MINO® device (Circassia AB, Uppsala, Sweden) and expressed as parts per billion (ppb). LTE4 quantification was conducted by competitive ELISA with the Leukotriene E4 ELISA Kit (Cayman Chemical, Ann Arbor, MI, USA). The LTE4 results were normalized by the serum creatinine concentration in the sample and expressed as picograms per milligram of creatinine (pg/mg Cr). As for the inflammatory cytokines IL-6, TNF, and IFN-γ, they were quantified using the BD™ Cytometric Array Human methodology (CBA) (BD Biosciences, San Diego, CA, USA) and expressed as picograms per milliliter (pg/mL). IL-6 was evaluated in the asthmatic patients and controls, and TNF and IFN-γ were analyzed only in asthmatic patients.

The distributions of the numerical variables were assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. In cases of nonparametric data distribution, the Mann-Whitney test was used for two-group comparisons, and the Kruskal-Wallis test was used for three-group comparisons. For categorical variables, frequency interactions were analyzed by Fisher's chi-square or exact test. The ROC curve was used to measure test performance as a discriminator of asthmatics and controls. The inference of the

predictor variables of the inflammatory phenotype was performed by bivariate log regression using the linear regression method.

The analyses were conducted using the IBM SPSS Statistics software (version 20.0), and the graphs were generated using the GraphPad PRISM software (version 5.01).

It was not possible to quantify all variables for all samples due to volume limitations or unavailability; however, this loss did not significantly impact any variable. The final sampling in each analysis is detailed in the Tables and Figures below.

RESULTS

Data collection was performed in 103 individuals, 37 (36%) of whom were asthmatic patients and 66 (64%), nonasthmatic controls. Of the 37 asthmatic patients, 15 (40.5%) were severe asthmatics who were followed up at the Severe Asthma Outpatient Clinic of the Irmandade da Santa Casa de Misericórdia de São Paulo, while 22 (59.5%) were patients with nonsevere asthma who were employees of the Fleury Group. The demographic data of the patients are shown in Table 1.

Comparative analysis of biomarkers in the asthma patients and control subjects

In order to evaluate the analytical methods used for biomarker quantification, the values obtained from the asthmatic patients were compared to those of the controls. Figure 1 illustrates the results obtained in this analysis, as well as the statistical significance found in each comparison. Higher values of blood eosinophils ($p = 0.012$), FeNO ($p \leq 0.001$), IgE ($p \leq 0.001$), periostin ($p = 0.005$), and IL-6 ($p \leq 0.001$) were observed in the asthmatic patients compared to the controls; however, there was no difference in LTE_4 values ($p = 0.353$).

Verification of the analytical performance of the biomarkers

In order to establish the cutoff points of optimal analytical performance regarding the methods evaluated in the context of asthma, biomarkers that presented significantly higher levels in asthmatic patients were evaluated by ROC curve analysis to assess sensitivity and specificity. Samples with missing data were excluded from the analyses. The results showed a statistically significant curve for all biomarkers analyzed, with an area under the curve (AUC) greater than 65%. The obtained curves are represented in Figure 2.

Table 2 describes the results of the ROC curve, as well as the cutoff points of best analytical performance for each biomarker, including sensitivity when specificity was above 90%.

Binary logistic regression analysis was performed to determine whether the association of biomarkers would contribute to better sensitivity in the clinical context of asthma. To that end, the biomarkers were categorized according to the cutoff points established as positive (above the cutoff point) and negative (below the cutoff point). Periostin (OR: 12,550; CI 95%: 2,498-63,063) and IL-6 (OR: 7,249; 95% CI: 1,737-30,262) were significant asthma predictors, whereas the other biomarkers were not.

Analysis of the interference of comorbidities on biomarker values

In order to analyze the interference of comorbidities on the biomarker values, the control group was stratified regarding the presence of allergies, obesity, and the presence of one or more comorbidities associated with worse prognoses in asthma (sleep apnea, bronchiectasis, atopic dermatitis, chronic urticaria, nasal polyposis, chronic rhinosinusitis, allergic rhinitis, or vocal cord dysfunction). None of the comorbidities was associated with high biomarker values (Table S1)

Comparative analysis of biomarkers in asthmatic patients according to clinical characteristics and phenotypes

In order to assess the association between the biomarker values and the patients' clinical data, the asthma group was analyzed separately regarding the clinical variables studied.

Table 1. Demographic data stratified by group.

Variables	Asthmatics (n = 37)	Controls (n = 66)
Sex (% women)[a]	27 (73%)	49 (74%)
Age (years)[b]	39 (33.5 - 54.0)	33 (27.8 - 38.3)
BMI (kg/m^2)[b]	28 (23 - 33)	25 (23 - 29)
Allergy (specific IgE)[a]	30 (81%)	26 (39%)
Other comorbidities[a]	19 (51%)	23 (35%)
Late-onset (adult asthma)[a]	15 (41%)	-
ACT[b]	20 (16 - 23)	-
Exacerbations (previous year)[a]	9 (24%)	-
FVC (% of predicted)[b]	90 (73 - 100)	-
FEV_1 (% of predicted)[b]	80 (55 - 92)	-
Steps 4 and 5 GINA treatment[a]	17 (46%)	-
Use of OC[a]	5 (14%)	-

BMI: Body mass index (kilograms per square meter); IgE: Immunoglobulin E; ACT: Asthma control test; FVC: Forced vital capacity; FEV_1: Forced expiratory volume in the first second; GINA: Global Initiative for Asthma; OC: Oral corticosteroids. [a]Number of cases (%); [b]Median (Interquartile range 25 - 75).

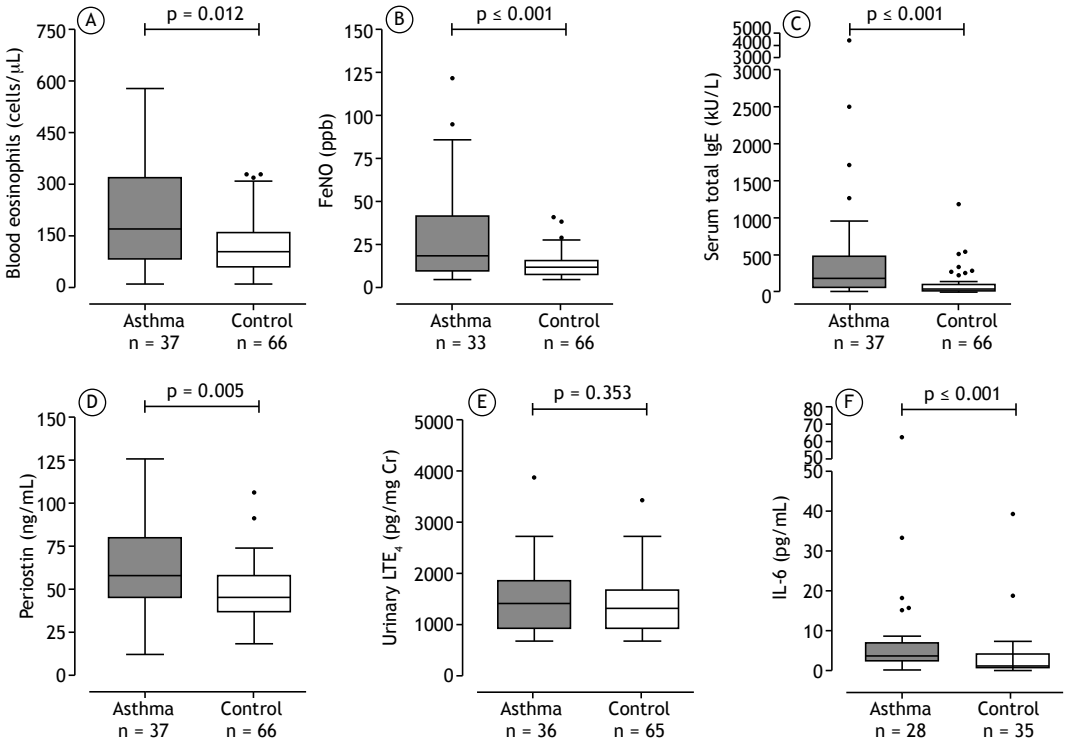

Figure 1. Biomarkers in asthmatic and control patients. Vertical boxes represent the interquartile intervals 25 - 75. The centerlines represent the medians and the stems indicate the lower and upper limits. Dots represent outliers. FeNO: Fraction of exhaled nitric oxide; LTE_4: Urinary leukotriene E_4; IL: Interleukin; IgE: Immunoglobulin E.

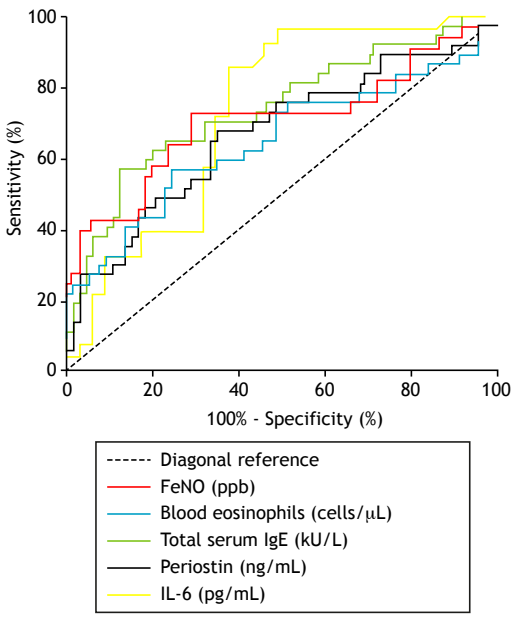

Figure 2. Receiver operator characteristic (ROC) curve based on the values of the biomarkers in the clinical context of asthma. Each biomarker has its own estimated curve.

Patients with more severe asthma (in higher levels of treatment according to GINA) presented higher values of IL-6 (p ≤ 0.001) and IFN-γ (p = 0.007) and lower levels of blood eosinophils (p = 0.030) than the nonsevere asthma patients. In the categorical assessment regarding asthma control, no differences were found in biomarker levels in uncontrolled (ACT < 20) and controlled asthma (ACT ≥ 20) or in asthmatics using oral corticosteroids. As for the variables concerning airflow limitations, lower percentages (≤ 80%) of FVC were associated with higher values of IL-6 (p = 0.002) and IFN-γ (p < 0.001). Similarly, lower percentages (≤ 80%) of FEV_1 also correlated with higher values of IFN-γ (p = 0.045).

Allergic asthma, defined as the presence of specific IgE, presented significantly higher values of FeNO (p = 0.008) and lower values of IFN-γ (p = 0.044). As for late-onset asthma, significantly lower FeNO values were found (p = 0.038). The stratification of patients according to the obesity phenotype (BMI ≥ 30) revealed significantly higher total IgE values in obese patients (p = 0.033) (Figure 3).

In the classification of asthma patients using the parameters FeNO ≥ 20 ppb and blood eosinophils ≥ 150 cells/μL for the establishment of T2 asthma,[5] periostin presented significantly higher levels in T2 asthma (p = 0.006), but not LTE_4 (p = 0.893) or IL-6 (p = 0.593). TNF showed lower values in T2 asthma (p = 0.029) (Figure 4).

DISCUSSION

The primary objective of the present study was to validate the use of a panel of biomarkers in clinical practice and to determine the analytical performance of

already used and new tests to identify the inflammatory phenotype of asthma. This is of paramount importance to physicians and patients, especially in the context of severe asthma, in which the identification of the inflammatory phenotype is fundamental for the implementation of target-specific therapy.[6] In view of this objective, the analytical performance (sensitivity and specificity) of the isolated trials was verified, as well as the benefits of offering a test panel with more information on the pathophysiological characteristics of patients.

Table 2. Results of ROC curve analysis and analytical performance of biomarkers considering cutoff points with specificity above 90%.

Variables	AUC	Standard Error	p-value	95% confidence interval		Cutoff point	Analytical performance	
				Lower limit	Upper limit		Sensitivity	Specificity
FeNO (ppb)	0.707	0.062	≤0.001	0.586	0.828	>27	42.4%	93.9%
Blood eosinophils (cells/μL)	0.649	0.061	0.012	0.530	0.769	>275	29.7%	92.4%
Total IgE (kU/L)	0.741	0.053	≤0.001	0.637	0.845	>265	40.5%	90.9%
Periostin (ng/mL)	0.669	0.058	0.005	0.555	0.782	>75	27.0%	97.0%
IL-6 (ng/mL)	0.731	0.064	0.001	0.605	0.857	>6	32.1%	91.4%

FeNO: Fraction of exhaled nitric oxide; IgE: Immunoglobulin E; IL: Interleukin.

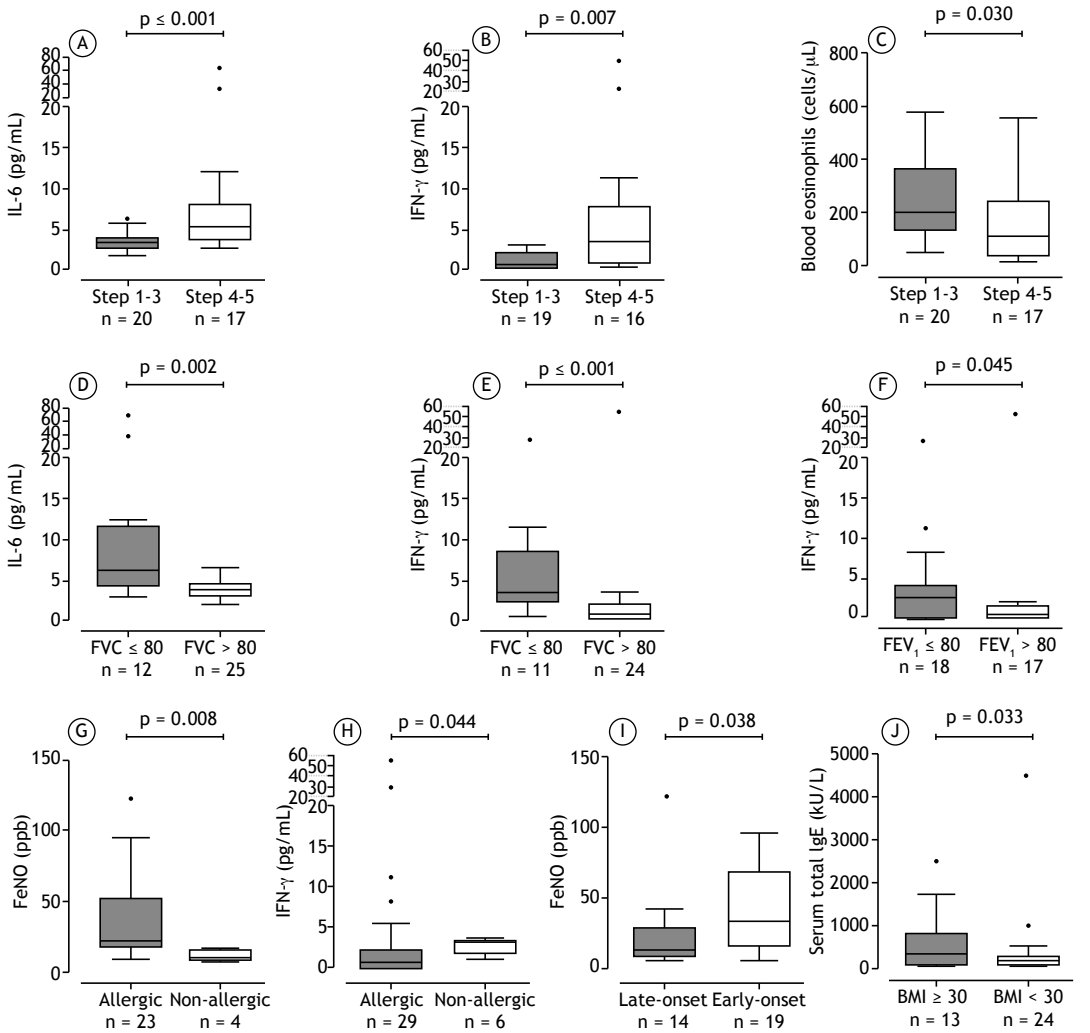

Figure 3. Biomarkers in asthmatic patients according to their clinical characteristics. Vertical boxes represent the interquartile intervals 25 - 75. The centerlines represent the medians and the stems indicate the lower and upper limits. Dots represent outliers. FeNO: Fraction of exhaled nitric oxide; IL: Interleukin; IFN-γ: Interferon-gamma; FVC: Forced vital capacity; FEV1: Forced expiratory volume in 1 second; BMI: Body mass index. (a - c) Treatment steps according to GINA.

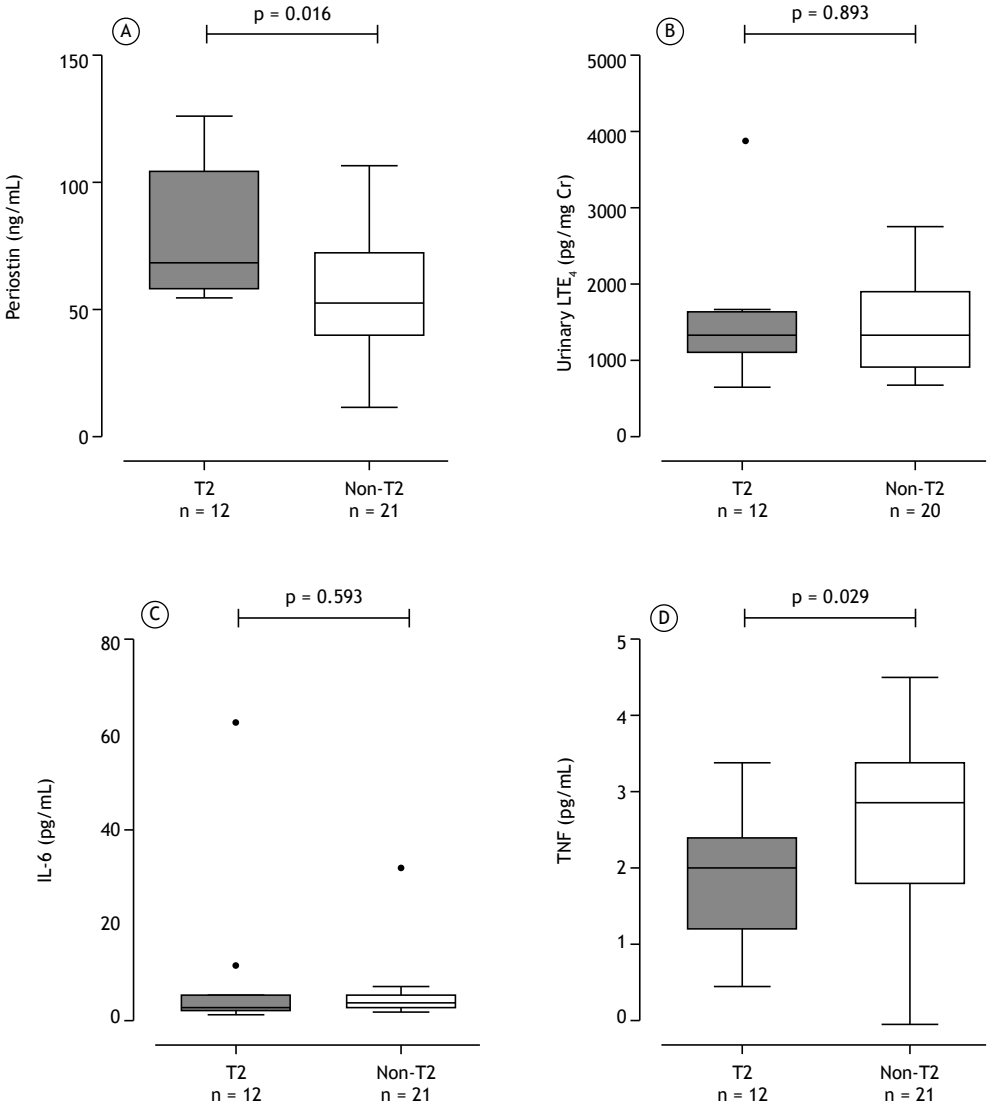

Figure 4. Biomarkers in asthmatic patients according to the inflammatory phenotype T2. Vertical boxes represent the interquartile intervals 25 - 75. The centerlines represent the medians and the stems indicate the lower and upper limits. Dots represent outliers. LTE_4: Urinary leukotriene E_4; IL: Interleukin; TNF: Tumor necrosis factor.

Corroborating some studies in the literature,[19,24] the values of blood eosinophils and total IgE were higher in asthmatic patients than in the controls. These biomarkers are currently widely used in clinical practice and assist in the identification of eosinophilic and allergic inflammatory phenotypes. However, targeted analytical validation is not performed in the clinical context of asthma by laboratories. Lower levels of blood eosinophils were found in asthmatic patients undergoing GINA treatment steps 4 and 5, a possible effect of high doses of inhaled corticosteroids and the use of systemic corticosteroids by these patients, resulting in eosinophilic suppression.[25] A previously described albeit poorly studied finding was higher total IgE levels in obese patients.[26]

FeNO is also an important biomarker not only for identifying the inflammatory phenotype but also as a predictor of the response to treatment with anti-IL-4/IL-13.[27] One of the ways of determining FeNO is by using a portable device that is easy to handle[28] in clinical practice. With the approval of this method by ANVISA, it became possible to use this biomarker during the management of asthma patients in Brazil. This study corroborated findings in the literature regarding the increased values of this biomarker in the clinical context of asthma and shed light on its analytical characteristics for national validation. The correlation between FeNO and allergic and early-onset asthma has also been previously described in the literature,[5,29] reinforcing the relationship between FeNO and T2 asthma.

Two biomarkers that are not currently used routinely in clinical practice were also evaluated: serum periostin and urinary LTE_4; both are related to T2 asthma,[14,30] although urinary LTE_4 often shows controversial results in the literature.[31] Periostin is a matricellular protein

involved in different biological functions[32] that has been identified in asthma in the process of airway tissue remodeling.[33] Its expression is induced by IL-13, a cytokine that plays a key role in T2 asthma response and is involved in airway hyperresponsiveness.[13] Periostin is used as a biomarker of T2 asthma, and its role in the identification of good responders to corticosteroids[34] and anti-IL-13[35] has already been described. This study not only corroborated the increase in periostin values in the clinical context of asthma but also reinforced its complementary role in the predictive value of the disease when compared to the controls and its high levels in patients with elevated blood eosinophils and FeNO (T2 asthma). Regarding urinary LTE_4, no differences were observed between the values obtained in the controls or regarding clinical characteristics.

Another investigated biomarker, IL-6, presented higher values in asthmatic patients than the controls and even higher values in non-T2 asthma patients. Similar to the other biomarkers, IL-6 may be elevated in different clinical conditions, such as COVID-19,[36] by its known participation in chronic inflammatory processes.[37] In this study, higher IL-6 values were also predictors of asthma and related to steps 4 and 5 of GINA treatment and lower percentages of FVC, which directly reflect a possible relationship with the severity of the disease. Higher values of IFN-γ were also observed in patients in higher stages of treatment, with lower FVC and FEV_1, and in nonallergic asthma, showing possible immunological activation mediated by Th1 cells.[16] The TNF cytokine also presented higher values in non-T2 asthma, a fact that reinforces the identification of other pathophysiological pathways in these patients.

The data obtained with the ROC curves revealed cutoff points with a better balance between specificity and sensitivity in asthma. These points are higher than those indicated by GINA[5] but closer to the cutoff, with solid clinical applications such as FeNO ≥ 25 ppb for indication of treatment with anti-IL-4/IL-13[27] and blood eosinophils ≥ 300 cell/μL with anti-IL-5/anti-IL-5-receptor.[38,39] The modest sensitivity found was expected since all the evaluated biomarkers, with the exception of IL-6, are increased in eosinophilic asthma, which represents most, but not all cases of the disease.[40] Regarding specificity, this cohort showed that the presence of comorbidities associated with difficulty controlling asthma did not significantly affect biomarker levels. However, the number of patients analyzed was a limitation; further studies are needed to support this finding.

Finally, an important limitation of this study was the absence of induced sputum collection, one of the most important tests for the identification of the inflammatory phenotype. With the COVID-19 pandemic, procedures capable of releasing aerosols from respiratory material, such as nebulization during the collection of induced sputum, have been prohibited outside emergency contexts. Thus, future studies with the addition of induced sputum tests may strengthen our findings. In addition, access to a larger sample of individuals with severe asthma and patients of other centers in Brazil is crucial to better evaluate the clinical applicability of this panel, as well as outcomes related to the response to treatment. It is possible that the lack of differences regarding the levels of biomarkers and uncontrolled patients was due to the small number of severe patients.

The use of a panel of biomarkers as a single test, with technical laboratory information regarding the validation of each examination in the clinical context of asthma, is a fundamental approach to assist clinicians and patients in individualized and effective treatment. The biomarkers already consolidated in clinical practice, together with new ones, offered jointly, could provide reliable and accurate information for the determination of the inflammatory phenotype of asthma, allowing for targeted interventions that could foresee severe symptoms and complications related to the disease.

AUTHOR CONTRIBUTIONS

Study conception and design: DCB and RS; acquisition of data: DCB and JGR; data analysis and interpretation: DCB and RS; writing the manuscript: DCB; critical revision of the manuscript: DCB, JGR, MMMP, JEDC, AD, and RS; final approval of the manuscript: RS.

REFERENCES

1. Peters SP, Ferguson G, Deniz Y, Reisner C. Uncontrolled asthma: a review of the prevalence, disease burden and options for treatment. Respir Med. 2006;100(7):1139-51. https://doi.org/10.1016/j.rmed.2006.03.031.
2. To T, Stanojevic S, Moores G, Gershon AS, Bateman ED, Cruz AA, et al. Global asthma prevalence in adults: findings from the cross-sectional world health survey. BMC Public Health. 2012;12:204. https://doi.org/10.1186/1471-2458-12-204.
3. Pearce N, Aït-Khaled N, Beasley R, Mallol J, Keil U, Mitchell E, et al. Worldwide trends in the prevalence of asthma symptoms: phase III of the International Study of Asthma and Allergies in Childhood (ISAAC). Thorax. 2007;62(9):758-66. https://doi.org/10.1136/thx.2006.070169.
4. Menezes AM, Wehrmeister FC, Horta B, Szwarcwald CL, Vieira ML, Malta DC. Prevalence of asthma medical diagnosis among Brazilian adults: National Health Survey, 2013. Rev Bras Epidemiol. 2015;18 Suppl 2:204-13. https://doi.org/10.1590/1980-5497201500060018.
5. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention. 2021.
6. Pizzichini MMM, Carvalho-Pinto RM, Cançado JED, Rubin AS, Cerci Neto A, Cardoso AP, et al. 2020 Brazilian Thoracic Association recommendations for the management of asthma. J Bras Pneumol. 2020;46(1):e20190307. https://doi.org/10.1590/1806-3713/e20190307.
7. Carvalho-Pinto RM, Cançado JED, Pizzichini MMM, Fiterman J, Rubin AS, Cerci Neto A, et al. 2021 Brazilian Thoracic Association recommendations for the management of severe asthma. J Bras Pneumol. 2021;47(6):e20210273. https://doi.org/10.36416/1806-3756/e20210273.
8. Hekking PW, Wener RR, Amelink M, Zwinderman AH, Bouvy ML, Bel EH. The prevalence of severe refractory asthma. J Allergy Clin Immunol. 2015;135(4):896-902. https://doi.org/10.1016/j.jaci.2014.08.042.

9. Kerkhof M, Tran TN, Soriano JB, Golam S, Gibson D, Hillyer EV, et al. Healthcare resource use and costs of severe, uncontrolled eosinophilic asthma in the UK general population. Thorax. 2018;73(2):116-24. https://doi.org/10.1136/thoraxjnl-2017-210531.
10. Campo P, Rodríguez F, Sánchez-García S, Barranco P, Quirce S, Pérez-Francés C, et al. Phenotypes and endotypes of uncontrolled severe asthma: new treatments. J Investig Allergol Clin Immunol. 2013;23(2):76-88. PMID: 23654073.
11. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. Nat Med. 2012;18(5):716-25. https://doi.org/10.1038/nm.2678.
12. Wagener AH, de Nijs SB, Lutter R, Sousa AR, Weersink EJ, Bel EH, et al. External validation of blood eosinophils, FE(NO) and serum periostin as surrogates for sputum eosinophils in asthma. Thorax. 2015;70(2):115-20. https://doi.org/10.1136/thoraxjnl-2014-205634.
13. Izuhara K, Ohta S, Ono J. Using Periostin as a Biomarker in the Treatment of Asthma. Allergy Asthma Immunol Res. 2016;8(6):491-8. https://doi.org/10.4168/aair.2016.8.6.491.
14. Kolmert J, Gómez C, Balgoma D, Sjödin M, Bood J, Konradsen JR, et al. Urinary Leukotriene E_4 and Prostaglandin D_2 Metabolites Increase in Adult and Childhood Severe Asthma Characterized by Type 2 Inflammation. A Clinical Observational Study. Am J Respir Crit Care Med. 2021;203(1):37-53. https://doi.org/10.1164/rccm.201909-1869OC.
15. Liang Z, Liu L, Zhao H, Xia Y, Zhang W, Ye Y, et al. A Systemic Inflammatory Endotype of Asthma With More Severe Disease Identified by Unbiased Clustering of the Serum Cytokine Profile. Medicine. 2016;95(25):e3774. https://doi.org/10.1097/MD.0000000000003774.
16. Zhu M, Liang Z, Wang T, Chen R, Wang G, Ji Y. Th1/Th2/Th17 cells imbalance in patients with asthma with and without psychological symptoms. Allergy Asthma Proc. 2016;37(2):148-56. https://doi.org/10.2500/aap.2016.37.3928.
17. Israel E, Reddel HK. Severe and Difficult-to-Treat Asthma in Adults. N Engl J Med. 2017;377(10):965-76. https://doi.org/10.1056/NEJMra1608969.
18. Vijverberg SJ, Hilvering B, Raaijmakers JA, Lammers JW, Maitland-van der Zee AH, Koenderman L. Clinical utility of asthma biomarkers: from bench to bedside. Biologics. 2013;7:199-210. https://doi.org/10.2147/BTT.S29976.
19. Buhl R, Korn S, Menzies-Gow A, Aubier M, Chapman KR, Canonica GW, et al. Prospective, Single-Arm, Longitudinal Study of Biomarkers in Real-World Patients with Severe Asthma. J Allergy Clin Immunol Pract. 2020;8(8):2630-9.e6. https://doi.org/10.1016/j.jaip.2020.03.038.
20. Holguin F, Cardet JC, Chung KF, Diver S, Ferreira DS, Fitzpatrick A, et al. Management of severe asthma: a European Respiratory Society/American Thoracic Society guideline. Eur Respir J. 2020;55(1):1900588. https://doi.org/10.1183/13993003.00588-2019.
21. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. Eur Respir J. 2014;43(2):343-73. https://doi.org/10.1183/09031936.00202013.
22. Standardization of Spirometry, 1994 Update. American Thoracic Society. Am J Respir Crit Care Med. 1995;152(3):1107-36. https://doi.org/10.1164/ajrccm.152.3.7663792.
23. American Thoracic Society; European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med. 2005;171(8):912-30. https://doi.org/10.1164/rccm.200406-710ST.
24. Pavlidis S, Takahashi K, Ng Kee Kwong F, Xie J, Hoda U, Sun K, et al. "T2-high" in severe asthma related to blood eosinophil, exhaled nitric oxide and serum periostin. Eur Respir J. 2019;53(1):1800938. https://doi.org/10.1183/13993003.00938-2018.
25. Lommatzsch M, Klein M, Stoll P, Virchow JC. Impact of an increase in the inhaled corticosteroid dose on blood eosinophils in asthma. Thorax. 2019;74(4):417-8. https://doi.org/10.1136/thoraxjnl-2018-212233.
26. Carballo I, Alonso-Sampedro M, Gonzalez-Conde E, Sanchez-Castro J, Vidal C, Gude F, et al. Factors Influencing Total Serum IgE in Adults: The Role of Obesity and Related Metabolic Disorders. Int Arch Allergy Immunol. 2021;182(3):220-8. https://doi.org/10.1159/000510789.
27. Castro M, Corren J, Pavord ID, Maspero J, Wenzel S, Rabe KF, et al. Dupilumab Efficacy and Safety in Moderate-to-Severe Uncontrolled Asthma. N Engl J Med. 2018;378(26):2486-96. https://doi.org/10.1056/NEJMoa1804092.
28. Hemmingsson T, Linnarsson D, Gambert R. Novel hand-held device for exhaled nitric oxide-analysis in research and clinical applications. J Clin Monit Comput. 2004;18(5-6):379-87. https://doi.org/10.1007/s10877-005-1158-z.
29. Akar-Ghibril N, Casale T, Custovic A, Phipatanakul W. Allergic Endotypes and Phenotypes of Asthma. J Allergy Clin Immunol Pract. 2020;8(2):429-40. https://doi.org/10.1016/j.jaip.2019.11.008.
30. Johansson MW, Evans MD, Crisafi GM, Holweg CTJ, Matthews JG, Jarjour NN. Serum periostin is associated with type 2 immunity in severe asthma. J Allergy Clin Immunol. 2016;137(6):1904-7.e2. https://doi.org/10.1016/j.jaci.2015.12.1346.
31. Lueke AJ, Meeusen JW, Donato LJ, Gray AV, Butterfield JH, Saenger AK. Analytical and clinical validation of an LC-MS/MS method for urine leukotriene E_4: A marker of systemic mastocytosis. Clin Biochem. 2016;49(13-14):979-82. https://doi.org/10.1016/j.clinbiochem.2016.02.007.
32. Izuhara K, Nunomura S, Nanri Y, Ogawa M, Ono J, Mitamura Y, et al. Periostin in inflammation and allergy. Cell Mol Life Sci. 2017;74(23):4293-303. https://doi.org/10.1007/s00018-017-2648-0.
33. Conway SJ, Izuhara K, Kudo Y, Litvin J, Markwald R, Ouyang G, et al. The role of periostin in tissue remodeling across health and disease. Cell Mol Life Sci. 2014;71(7):1279-88. https://doi.org/10.1007/s00018-013-1494-y.
34. Woodruff PG, Boushey HA, Dolganov GM, Barker CS, Yang YH, Donnelly S, et al. Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. Proc Natl Acad Sci U S A. 2007;104(40):15858-63. https://doi.org/10.1073/pnas.0707413104.
35. Corren J, Lemanske RF, Hanania NA, Korenblat PE, Parsey MV, Arron JR, et al. Lebrikizumab treatment in adults with asthma. N Engl J Med. 2011;365(12):1088-98. https://doi.org/10.1056/NEJMoa1106469.
36. Coomes EA, Haghbayan H. Interleukin-6 in Covid-19: A systematic review and meta-analysis. Rev Med Virol. 2020;30(6):1-9. https://doi.org/10.1002/rmv.2141.
37. López-Mejías R, González-Gay MA. IL-6: linking chronic inflammation and vascular calcification. Nat Rev Rheumatol. 2019;15(8):457-9. https://doi.org/10.1038/s41584-019-0259-x.
38. Bleecker ER, Wechsler ME, FitzGerald JM, Menzies-Gow A, Wu Y, Hirsch I, et al. Baseline patient factors impact on the clinical efficacy of benralizumab for severe asthma. Eur Respir J. 2018;52(4):1800936. https://doi.org/10.1183/13993003.00936-2018.
39. Harrison T, Canonica GW, Chupp G, Lee J, Schleich F, Welte T, et al. Real-world mepolizumab in the prospective severe asthma REALITI-A study: initial analysis. Eur Respir J. 2020;56(4):2000151. https://doi.org/10.1183/13993003.00151-2020.
40. Hudey SN, Ledford DK, Cardet JC. Mechanisms of non-type 2 asthma. Curr Opin Immunol. 2020;66:123-8. https://doi.org/10.1016/j.coi.2020.10.002.