

Clinical Communications

High levels of plasma fibrinogen could predict frequent asthma exacerbations

Vitina Carriero, PhD^{a,*}, Francesca Bertolini, PhD^{a,*},
Andrea Elio Sprio, PhD^a, Michela Bullone, DVM^b,
Giorgio Ciprandi, MD^c, and
Fabio Luigi Massimo Ricciardolo, MD, PhD, FERS^a

Clinical Implications

- It is essential to find biomarkers that could predict asthma exacerbation and allow the development of precise interventions. Our data suggest that the measurements of plasma fibrinogen could be a predictor of exacerbation frequency.

Asthma exacerbation (AE) is an episodic worsening of asthmatic symptoms and lung function, occasionally leading to fatal events. The impaired quality of life and the high health care system costs caused by AEs make it fundamental to discover biomarkers to optimize their management.

AE frequency relates to different inflammatory patterns including high counts of sputum/blood eosinophils and neutrophils,¹ and activation of blood monocytes.² Patients with stable asthma have altered blood coagulation, thus explaining the increased risk of thromboembolic events in asthma.³ Fibrinogen, a coagulation mediator and systemic inflammation biomarker linked to uncontrolled asthma,⁴ indirectly contributes to triggering the type 2 immune response in the airways.⁵ On this basis, we can infer the involvement of the coagulation pathway in AE, but the association of fibrinogen with AE frequency still remains unraveled. Thus, we aimed to investigate the role of plasma fibrinogen and other hematologic markers (including blood cell count) in conjunction with the expression of T2 biomarkers in the bronchial wall of frequent exacerbators with asthma.

We stratified 70 patients with asthma on the basis of frequency (≥ 2) of their severe AE⁶: 44 nonfrequent exacerbators (nFEs) and 26 frequent exacerbators (FEs). Written informed consent was obtained from each subject. The study was approved by the local ethics committees (San Luigi Gonzaga University Hospital: protocols 1759/2008 and 14871/2009).

Their clinical, functional, and blood/bronchial inflammatory characteristics are listed in Table E1 in this article's Online Repository at www.jaci-inpractice.org. At the time of the study, all patients were in stable condition either for asthma or for other disorders (see Table E2 in this article's Online Repository at www.jaci-inpractice.org). We found increased levels of blood neutrophils ($P < .05$), monocytes ($P < .05$), and fibrinogen ($P < .001$) in FEs than in nFEs (Figure 1, A); the other hematological measures were similar (see Figure E1 in this article's Online Repository at www.jaci-inpractice.org). To assess the fibrinogen involvement in the T2 response, we evaluated the

expression of 2 T2 biomarkers (eotaxin-3 and IL-5) in bronchial biopsies of a study subject subset (see this article's Online Repository at www.jaci-inpractice.org). Cell counts in the lamina propria of bronchial sections by means of immunohistochemistry showed a higher number of eotaxin-3⁺ and IL-5⁺ cells ($P < .0001$) in the FE group compared with the nFE group (Figure 1, B and C).

In all our patients with asthma, monocytes, neutrophils, and fibrinogen positively correlated with each other (Figure 2, A; see Tables E3 and E4 in this article's Online Repository at www.jaci-inpractice.org), ICS dose, and the number of exacerbations per year (see Figure E2 in this article's Online Repository at www.jaci-inpractice.org).

Forced vital capacity %predicted, FEV₁ %predicted, and Tiffeneau Index (TI) (%) negatively correlated with fibrinogen (Figure 2, B). Monocytes correlated negatively with FVC % predicted, and TI%, and positively with residual volume % predicted (see Table E5 in this article's Online Repository at www.jaci-inpractice.org). Receiver-operating characteristic (ROC) curve analyses (Figure 2, C) provided possible thresholds to detect the probability of higher AE frequency. Patients with counts of monocytes greater than or equal to 705 cell/ μ L had almost 10-fold higher probability to belong to the FE group, but the ROC curve was not significant. Neutrophil counts of greater than 3570 cell/ μ L and fibrinogen values of greater than 361 mg/dL were thresholds of higher probability (3-fold and 8-fold, respectively) to have 2 or more AEs per year.

Here, FE and nFE displayed similar levels of blood eosinophils, contrary to previous findings¹ but in line with the Denlinger et al⁷ study. The latter study suggested that exacerbation frequency was not strictly associated with T2 inflammation, but divergent pathways may be involved. In this study, we found higher blood levels of IgE, neutrophils, and monocytes in FEs than in nFEs, consistent with our previous studies suggesting that both type 2 and non-type 2 inflammation could be involved in severe asthma and FEs.^{6,8} However, in this context a role of corticosteroids should be considered. By influencing eosinophil apoptosis and prolonging neutrophil cell survival, inhaled corticosteroids could modulate in our patients a switch to a neutrophilic asthma phenotype.⁸ Our research group demonstrated previously augmented peripheral neutrophils, TNF- α , and IL-8 in those with severe asthma compared with those with mild asthma.⁹

Neutrophils have a pivotal role in AE. Epithelial injury caused by allergens, pathogens, smoking, and environmental pollutants induces fibrin deposition, resulting in adhesion molecule expression in endothelium and monocyte recruitment. Monocytes secrete TNF- α and metalloproteases that release fibrinogen degradation products responsible for recruitment and activation of neutrophils, which release a plethora of mediators against pathogens and pollutants. Neutrophils induce further monocyte recruitment and fibrinogen extravasation, thus likely contributing to worsening of the bronchial/systemic inflammatory status and inducing airway hyperresponsiveness.

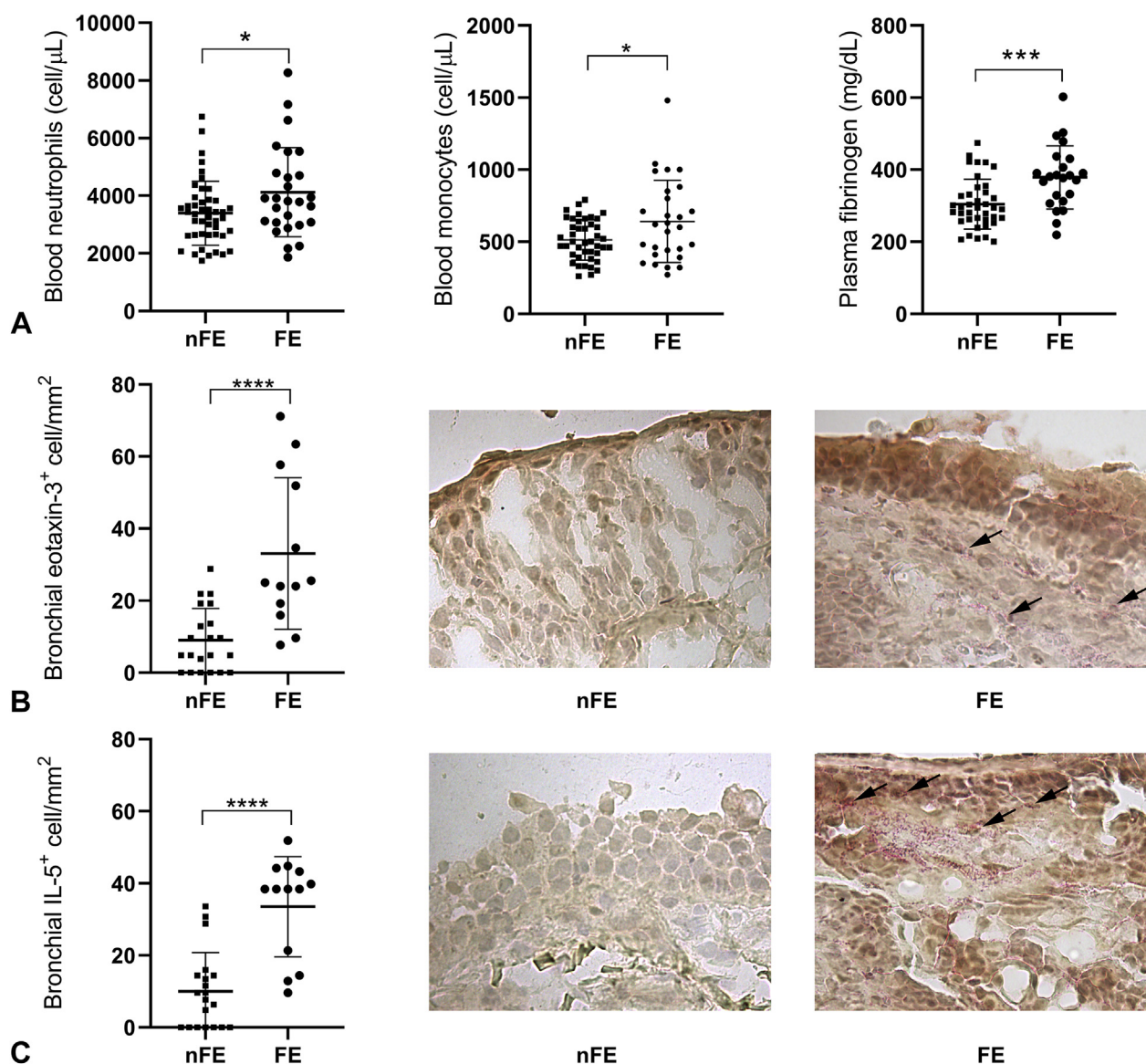


FIGURE 1. Blood neutrophils, monocytes, fibrinogen (A), and bronchial T2-high markers (B and C) in patients with asthma stratified by nFEs and FEs. Data are presented as individual data; each horizontal line indicates mean values. Representative photomicrographs showing cells staining positively for eotaxin-3 (B) and IL-5 (C) in bronchial biopsies of FE and nFE patients (40 $\times$ magnification). Arrows indicate positively stained cells. * $P < .05$. *** $P < .001$. **** $P < .0001$.

The literature connected asthma with thrombosis: in severe asthma and in frequent exacerbators with asthma, high blood neutrophilia is associated with increased coagulation markers.³ In contrast, FEs and nFEs had comparable platelet counts, international normalized ratio, and partial thromboplastin time, meaning that inflammatory stimuli did not alter coagulation in our cohort. However, FE patients had higher concentrations of fibrinogen. The explanation could reside in the dual role of fibrinogen as an inflammatory marker and coagulation factor, and in the above-discussed mechanisms of acute on chronic inflammation during AE, as neutrophils, monocytes, and fibrinogen concentrations positively correlated with each other. They could cooperate in promoting airway remodeling and

bronchial obstruction. Moreover, we assessed the possible fibrinogen involvement in T2 response in our patients with asthma. Similar to fibrinogen, we found higher levels of bronchial eotaxin-3, IL-5, and eosinophils in FEs than in nFEs. These findings at least in part support a fibrinogen contribution in T2 immune response, but we cannot rule out other pathways in FEs, such as non-type 2 immune response.

In our asthmatic population, we found a progressive small airway obstruction resulting in higher air trapping (reduced forced vital capacity and augmented residual volume), related to both systemic monocytes and fibrinogen. Airflow limitation correlated only with increasing fibrinogen (reduced FEV₁), whereas monocytes and fibrinogen negatively correlated with TI

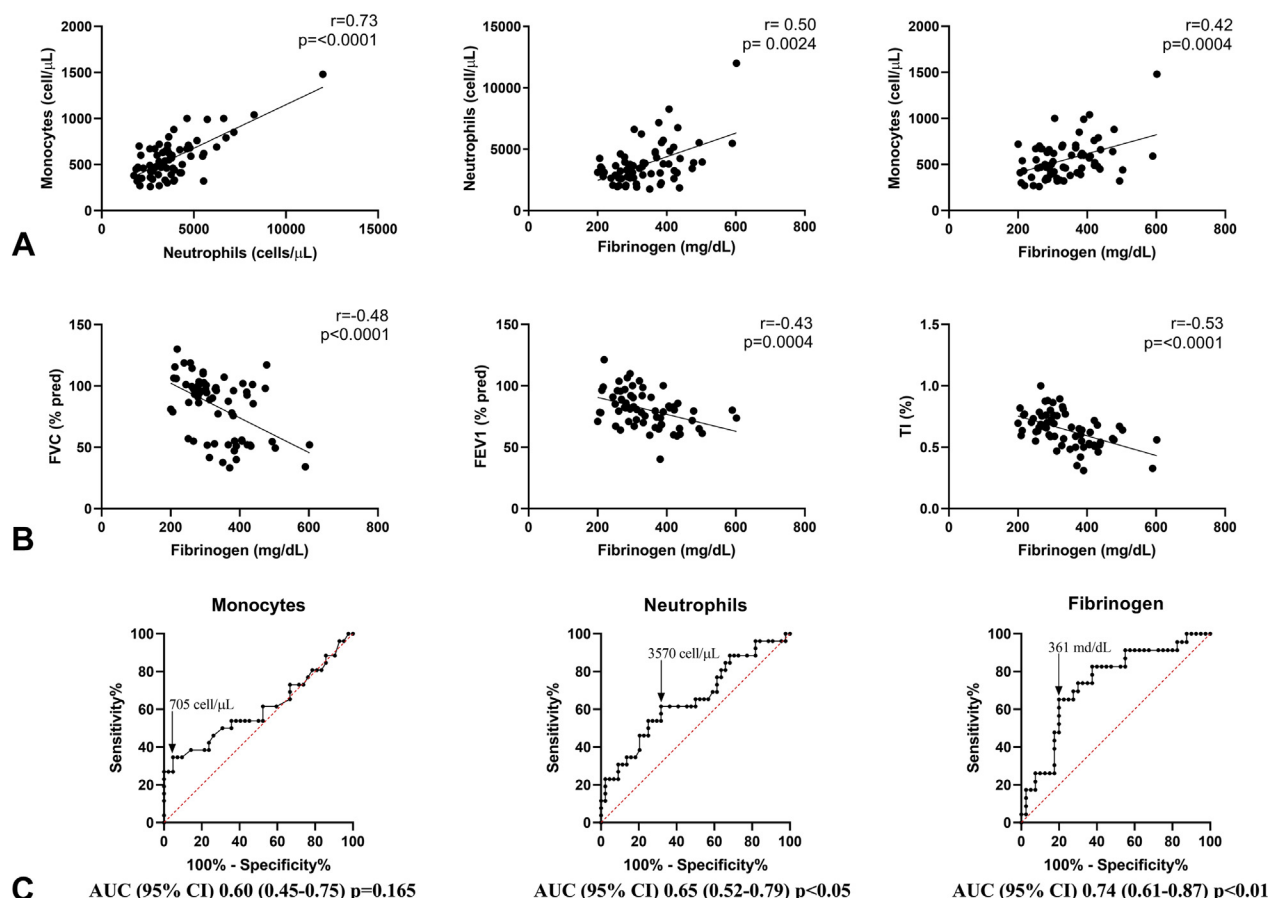


FIGURE 2. (A) Correlations among blood markers in all patients with asthma. (B) Correlation between lung function parameters and fibrinogen. Straight line represents the best-fitting line. r = Pearson correlation coefficient. (C) ROC curves for optimal cutoff points of monocytes, neutrophils, and fibrinogen. AUC, Area under the ROC curve; FVC, forced vital capacity. r_s = Spearman's rank correlation coefficient.

%, indicating a link between airway obstruction and both systemic inflammation markers associated with FE.

ROC analyses provided cutoff values potentially useful as "diagnostic tests." Blood neutrophils appeared to be a moderate FE phenotype predictor, whereas fibrinogen provided a better predictive result. The explanation could reside in neutrophils cutoff that was within the intermediate range of the general population, whereas that of fibrinogen was in the higher range (see Table E6 in this article's Online Repository at www.jaci-inpractice.org).

In conclusion, our study highlights the potential use of a simple routine hematological examination to detect the FE phenotype. Although inhaled corticosteroids play a confounding role in blood neutrophil count, fibrinogen could be a promising predictor of higher exacerbation frequency in patients with asthma, although further prospective studies are required in larger populations to confirm these findings.

^aDepartment of Clinical and Biological Sciences, University of Turin, San Luigi University Hospital, Turin, Italy

^bDepartment of Veterinary Sciences, University of Turin, Grugliasco, Turin, Italy

^cAllergy Clinic, Casa di Cura Villa Montalegre, Genoa, Italy

*These authors contributed equally to this work.

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Corresponding author: Fabio Luigi Massimo Ricciardolo, MD, PhD, FERS, Department of Clinical and Biological Sciences, University of Turin, San Luigi University Hospital, Regione Gonzole 10, 10043 Orbassano, Turin, Italy. E-mail: fabioluigimassimo.ricciardolo@unito.it.

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ONLINE REPOSITORY

METHODS

Patients

For this observational cross-sectional study, 70 patients with asthma were evaluated. We enrolled at the San Luigi Gonzaga University Hospital (Orbassano, Torino, Italy) 70 patients with asthma. Written informed consent was obtained from each subject. The study conformed to the Declaration of Helsinki and was approved by the local ethics committees (San Luigi Gonzaga University Hospital: protocols 1759/2008 and 14871/2009). At the time of the study, all patients were in stable condition and none of them was treated with theophylline, antibiotics, antioxidants, mucolytics, and/or systemic glucocorticoids in the 6 weeks before bronchial biopsy. Asthma was defined according to Global Initiative for Asthma^{E1} and European Respiratory Society/American Thoracic Society Guidelines.^{E2} Clinical data and patient history were obtained at recruitment: patients who had at least 2 severe exacerbations per year have been defined as FEs (N = 26), whereas the others have been classified as nFEs (N = 44).^{E3} According to European Respiratory Society/American Thoracic Society Guidelines, a severe exacerbation was defined as 2 or more bursts of systemic corticosteroids (3 days each) in the previous year.^{E2}

Evaluation of clinical, functional, and blood parameters

Cell counts (white blood cells and platelets) and coagulation parameters (fibrinogen, international normalized ratio, and partial thromboplastin time) were collected from routinely performed hematological examinations.

White blood cell count and white blood cell differential count were performed on the basis of optical and impedance characteristics using a Cell-Dyn Sapphire (Abbott, Rome, Italy) automated hematology analyzer (data were expressed as an absolute number). Fibrinogen content in plasma was turbidimetrically measured using ACL-TOP CTS 700 (Werfen Italia, Milan, Italy) coagulation analyzer. International normalized ratio and partial thromboplastin time were evaluated using the same coagulation analyzer. Ranges of reference values are reported in Table E6.

Pulmonary function test was performed assessing spirometry and lung volumes before and 15 minutes after the administration of albuterol (400 µg) using body plethysmograph (Vmax Encore 62, Carefusion, Germany). Fractional exhaled nitric oxide was also measured as in the previous study.^{E3}

Skin prick tests were performed and serum total IgE levels were measured to identify patients with allergy according to validated criteria.^{E4} Patients sensitized to 2 or more allergens have been considered as polysensitized.^{E5}

Fiberoptic bronchoscopy collection

Bronchoscopy was performed in all subjects using a flexible fiberoptic bronchoscope (Pentax FB-18P; Asahi Optical Co. Ltd, Tokyo, Japan) as previously described.^{E6}

Immunohistochemistry

Bronchial biopsy samples were collected as previously described.^{E3} Immunostaining was performed on 6-µm-thick frozen sections using specific antibodies against eosinophil cationic protein (1:100, rabbit polyclonal IgG, cod. bs-8615R, Bioss,

Woburn, Mass) and neutrophil elastase (1:100, mouse monoclonal IgG, clone NP57, Agilent Dako, Santa Clara, Calif). We performed bronchial biopsies immunostaining on 34 randomly selected patients (14 FEs and 20 nFEs). We used antibody against human CCL26/eotaxin-3 (1:40, goat polyclonal IgG, cod. AF653, R&D System, Minneapolis, Minn) and IL-5 (1:250, rabbit polyclonal IgG, cod. 26677-1-AP, Proteintech, Rosemont, Ill). Appropriated biotinylated secondary antibodies (Vector Laboratories, Peterborough, UK) were then applied (1:200); avidin-biotin complex and alkaline phosphatase were used as detection systems (PK6100 and AK5000, Vector Laboratories) with Fast Red and DAB as substrates (Sigma Aldrich S.r.l., Milan, Italy).

Cell counts were performed at 40× magnification by the same operator, who was blinded to the patients group. Immunostained cells were quantified in the area 100 µm beneath the basement membrane (lamina propria) in several nonoverlapping hpfs until all the available area was covered. The final result, expressed as the number of positive cells/mm², was calculated as the average of all the cellular counts performed in all fields. We quantified the immunostained cells with at least a portion of the nucleus seen close to immunopositivity.

Statistical analysis

Data distributions were assessed by the D'Agostino-Pearson test to detect departure from normality. Unpaired *t* test (Welch-corrected, if different SD were detected) or Mann-Whitney test was used to compare differences between nFE and FE groups. Data were graphed reporting the mean values. Data reported in Tables E1 and E2 are represented by mean ± SD. χ^2 tests were used to compare frequencies. Correlations were determined using Pearson correlation or Spearman rank correlation coefficient: best-fitting line was graphed, whereas values of slope and *Y* intercept, as well as their SEs and 95% CI, are reported in Table E5. *P* values less than .05 were considered significant. For the purpose of this analysis, correlation coefficients were considered as follows: greater than or equal to 0.8 = very strong; 0.6 to 0.79 = strong; 0.4 to 0.59 = moderate; 0.2 to 0.39 = weak; and less than 0.2 = very weak.^{E7} Optimal cutoff values of possible exacerbation predictors were determined by ROC curves; area under the ROC curve (AUC) was used to measure the accuracy of each score. Odds ratios (ORs) were used to estimate probability of becoming FEs. Statistical evaluations were performed with GraphPad Prism 8.0 (GraphPad Software, San Diego, Calif).

Univariate analysis of variance adjusted for administered inhaled corticosteroid (ICS) dose was performed to evaluate the possible confounding role of ICSs in correlations between being FE and detected possible predictor factors. Analysis was performed with IBM SPSS Statistics for Mac, version 24.0 (IBM Corp, Armonk, NY).

RESULTS

Comparison of clinical, functional, and biological characteristics

Characteristics of the 70 patients (44 nFEs and 26 FEs) included in this study are reported in Table E1. Briefly, FEs and nFEs were comparable for demographic and physical parameters (ie, age, sex, body mass index) and smoker incidence. Although atopy frequency was also comparable, FEs had higher serum total

IgE levels ($P < .05$) and were more frequently sensitized to perennial allergens ($P < .05$) than nFEs. FEs had more compromised pulmonary function than nFEs: forced vital capacity %predicted, FEV₁ %predicted, and TI (%) were lower, whereas residual volume (RV) %predicted was higher. Furthermore, FEs received higher corticosteroid administration: the ICS dose (fluticasone hydrofluoroalkane equivalent) was more than double ($P < .001$) and 8 of them received oral corticosteroids for 6 months or more per year, whereas no patients in the nFE group received long-term oral corticosteroids ($P < .001$). Finally, both eosinophil and neutrophil bronchial counts were higher in FEs after evaluation of biopsy data.

Comorbidities

In Table E2, we reported the comorbidities at the time of bronchial biopsies. On comparing comorbidities between FEs and nFEs, we found that only osteoporosis was more present in the FE group ($P < .05$). No significant differences in time period from onset were found between FEs and nFEs.

Correlations among blood biomarkers of inflammation

We obtained significant positive correlations among monocytes, neutrophils, and fibrinogens both in FE and in nFE groups (Table E3).

Best-fitting lines of nFEs and FEs, for the 3 blood markers studied, were comparable in terms of both slope and Y intercept for all considered correlations (Table E5), meaning a similar behavior in the general population with asthma.

Lung function parameters and inflammatory biomarkers in blood

In Table E4, we show correlation data regarding patients subdivided into nFE and FE groups. In nFEs, monocytes positively correlated with RV %predicted but negatively with TI (%); neutrophils positively correlated with RV %predicted; fibrinogen negatively correlated with forced vital capacity %predicted and TI (%). In FEs, fibrinogen correlated with RV %predicted, but negatively correlated with FEV₁ %predicted.

Predictor of frequent exacerbations

ROC curve analyses (Figure 2, C) provided possible cutoff values to detect a higher probability of AE. Although the ROC analysis was not significant, patients with counts of monocytes greater than 705 cell/ μ L had almost 10 times the probability ($P < .01$) to belong to the FE group (AUC, 0.60; diagnostic OR, 10.6); sensitivity was 34.6%, specificity 95.2%, positive predictive value (PPV) 95.2%, and negative predictive value (NPV) 34.6%. Patients with value of neutrophils greater than 3570 cell/ μ L had a significant 3-fold greater probability ($P < .05$) to be FEs than patients with less than or equal to 3570 cell/ μ L (AUC, 0.65; diagnostic OR, 3.4); sensitivity was 61.5%, specificity 68.2%, PPV 68.2%, and NPV 61.5%. Patients with value of fibrinogen greater than 361 mg/dL were almost 8 times more likely ($P < .001$) to be FEs than patients with less than or equal to 361 mg/dL (AUC, 0.74; diagnostic OR, 7.5); sensitivity was 65.2%, specificity 80.0%, PPV 80.0%, and NPV 65.2%.

Univariate analysis of variance highlighted a confounding role of ICS administration in blood neutrophil count, but confirmed the consistency of fibrinogen correlation with the higher AE frequency ($P < .05$).

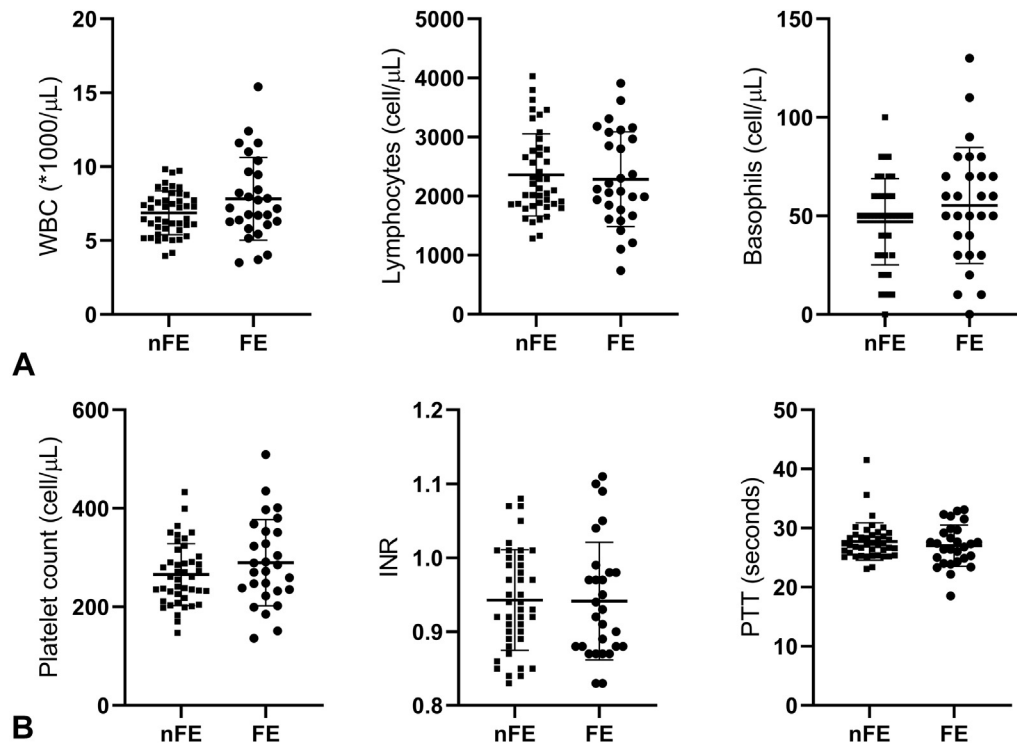


FIGURE E1. Blood (A) and coagulation (B) biomarkers in patients with asthma stratified by nFEs and FEs. Data are presented as individual data; each horizontal line indicates mean values. *INR*, International normalized ratio; *PTT*, partial thromboplastin time; *WBC*, white blood cell.

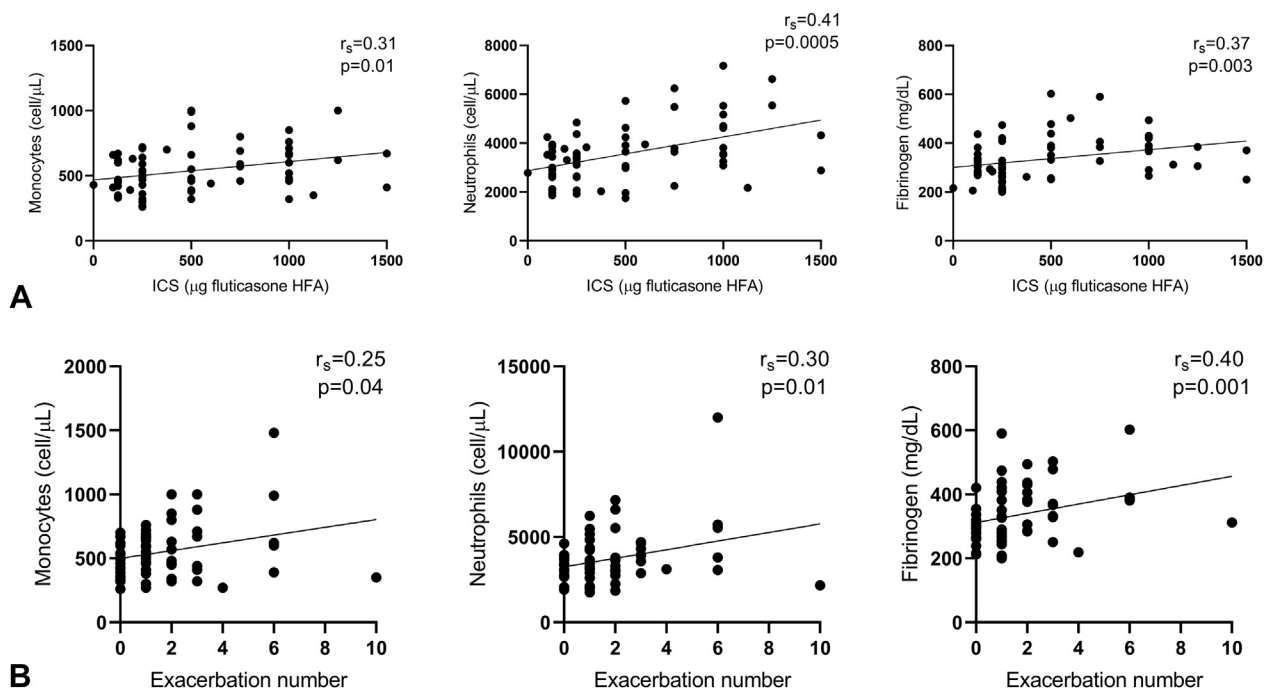


FIGURE E2. Correlation between ICS dose and inflammatory biomarkers in blood (A) and correlation between exacerbation number and inflammatory biomarkers (B). r_s = Spearman's rank correlation coefficient.

TABLE E1. Clinical, functional, and biological parameters in the asthmatic population

Clinical parameters	All patients with asthma (n = 70)	nFEs (n = 44)	FEs (n = 26)
Age (y)	51.1 ± 12.5 (20-72)	49.8 ± 11.6 (26-69)	53.3 ± 13.8 (20-72)
Sex (M/F)	34/36	23/21	11/15
Current smokers	4 of 70 (5.7%)	3 of 44 (6.8%)	1 of 26 (3.8%)
Past smokers	22 of 70 (31.4%)	16 of 44 (36.4%)	6 of 26 (23.1%)
BMI (kg/m ²)	26.2 ± 4.7	26.5 ± 4.7	25.6 ± 4.7
Atopy*	44 of 70 (62.9%)	26 of 44 (59.0%)	18 of 26 (69.2%)
Polysensitized	38 of 70 (54.3%)	23 of 44 (52.3%)	15 of 26 (57.7%)
Perennial allergen sensitization	28 of 70 (40.0%)	16 of 44 (36.4%)	10 of 26 (38.5%)
Serum IgE (KUI/L)	241.0 ± 409.0	182.4 ± 338.9	337.9 ± 496.3*
Serum IgE (>100 KUI/L)	38 of 70 (54.3%)	21 of 44 (47.7%)	17 of 26 (65.4%)
FENO (ppb)	30.8 ± 23.8	31.7 ± 26.5	29.2 ± 18.8
FENO (>30 ppb)	29 of 70 (41.4%)	19 of 44 (43.2%)	10 of 26 (38.5%)
Blood eosinophils (cell/μL)	314.0 ± 273.2	260.2 ± 163.7	347.2 ± 248.7
Blood eosinophils (>300 cell/μL)	28 of 70 (40%)	14 of 44 (31.8%)	14 of 26 (53.8%)
FVC pre (% predicted)	84.6 ± 25.1	92.8 ± 19.7	70.9 ± 27.5†
FEV ₁ pre (% predicted)	80.6 ± 16.9	83.1 ± 16.8	76.3 ± 16.2*
TI % (FEV ₁ /FVC)	0.64 ± 0.13	0.68±0.12	0.58 ± 0.13†
RV (% predicted)	126.1 ± 41.2	117.5 ± 26.8	139.2 ± 52.4*
Asthma onset, mean ± SD	26.1 ± 17.4	25.5 ± 16.4	27.0 ± 19.2
Asthma onset (<18 y)	26 of 70 (37.1%)	17 of 44 (38.6%)	9 of 26 (34.6%)
Asthma duration (y)	25.0 ± 16.8	24.2 ± 15.4	26.3 ± 19.2
ICS/d (μg fluticasone HFA equivalent)	483.8 ± 382.3	339.5 ± 285.8	727.9 ± 405.4‡
OCS (≥6 mo/y)	8 of 70 (11.4%)	0 of 44 (0.0%)	8 of 26 (30.8%)†
Omalizumab	5 of 70 (7.1%)	2 of 45 (4.5%)	3 of 28 (11.5%)
Bronchial eosinophils (cell/mm ²)	36.7 ± 25.2	30.9 ± 18.5	46.5 ± 31.6*
Bronchial neutrophils (cell/mm ²)	64.9 ± 46.8	51.5 ± 33.3	87.8 ± 57.3§

F, Female; FENO, fractional exhaled nitric oxide; FVC, forced vital capacity; HFA, hydrofluoroalkane; M, male; OCS, oral corticosteroid.

Continuous variables are presented as mean ± SD. For the age variable, the minimum to maximum range is also provided. Incidences are reported as occurrence/exposed cases and as percentage.

**P* < 0.05 compared with nFE.

†*P* < .001 compared with nFE.

‡*P* < .0001 compared with nFE.

§*P* < .01 compared with nFE.

TABLE E2. Comorbidities at the time of bronchoscopy

	nFEs (n = 44)		FEs (n = 26)	
	Prevalence	Years from onset, mean $\pm$ SD	Prevalence	Years from onset, mean $\pm$ SD
Rhinitis	28 of 44 (63.6%)	23.6 $\pm$ 12.3	11 of 26 (42.3%)	26.2 $\pm$ 15.6
Nasal polyposis	5 of 44 (11.4%)	1.4 $\pm$ 0.5	9 of 26 (34.6%)	8.6 $\pm$ 13.5
Sinusitis	10 of 44 (22.7%)	1.8 $\pm$ 2.0	11 of 26 (42.3%)	3.5 $\pm$ 7.1
Bronchiectasis	3 of 44 (6.8%)	2.3 $\pm$ 2.1	0 of 26 (0.0%)	0.0 $\pm$ 0.0
Pneumonia	6 of 44 (13.6%)	14.2 $\pm$ 16.7	3 of 26 (11.5%)	29.3 $\pm$ 22.0
Obstructive sleep apnea syndrome	3 of 44 (6.8%)	2.4 $\pm$ 3.2	1 of 26 (3.8%)	4.0 $\pm$ 0.0
Gastroesophageal reflux disease	7 of 44 (15.9%)	1.2 $\pm$ 1.6	2 of 26 (7.7%)	4.0 $\pm$ 1.4
Obesity	6 of 44 (13.6%)	2.8 $\pm$ 4.2	2 of 26 (7.7%)	1.5 $\pm$ 0.7
Diabetes	0 of 44 (0.0%)	1.0 $\pm$ 0.0	1 of 26 (3.8%)	3.0 $\pm$ 0.0
Hypertension	9 of 44 (20.5%)	1.8 $\pm$ 1.3	8 of 26 (30.8%)	9.0 $\pm$ 11.7
Heart failure	0 of 44 (0.0%)	0.0 $\pm$ 0.0	1 of 26 (3.8%)	9.0 $\pm$ 0.0
Acute myocardial infarction	0 of 44 (0.0%)	0.0 $\pm$ 0.0	0 of 26 (0.0%)	0.0 $\pm$ 0.0
Arrhythmia	0 of 44 (0.0%)	0.0 $\pm$ 0.0	1 of 26 (3.8%)	1.0 $\pm$ 0
Anxiety depression syndrome	0 of 44 (0.0%)	0.0 $\pm$ 0.0	0 of 26 (0.0%)	0.0 $\pm$ 0.0
Osteoporosis	1 of 44 (2.3%)	2.0 $\pm$ 0.0	5 of 26 (19.2%)*	1.0 $\pm$ 1.7
Arthropathy	3 of 44 (6.8%)	2.3 $\pm$ 2.5	0 of 26 (0.0%)	0.0 $\pm$ 0.0
Chronic pain	0 of 44 (0.0%)	0.0 $\pm$ 0.0	0 of 26 (0.0%)	0.0 $\pm$ 0.0

Prevalence rates are reported as occurrence cases and as percentage.

* $P < .05$ compared with nFE.**TABLE E3.** Correlation among systemic biomarkers in FEs and nFEs

Blood cells	Fibrinogen		Monocytes	
	nFEs	FEs	nFEs	FEs
Monocytes	0.33 (.04)	0.40 (.056)	—	—
Neutrophils	0.33 (.04)	0.52 (.01)	0.43 (.005)	0.76 (<.0001)

Data are presented as r (P value).

TABLE E4. Tabular results of linear regressions among inflammatory biomarkers

Comparisons between biomarkers	All patients with asthma			nFEs			FEs			nFEs vs FEs
	Slope $\pm$ SE (95% CI)	Y intercept $\pm$ SE (95% CI)	<i>r</i> (<i>P</i>)	Slope $\pm$ SE (95% CI)	Y intercept $\pm$ SE (95% CI)	<i>r</i> (<i>P</i>)	Slope $\pm$ SE (95% CI)	Y intercept $\pm$ SE (95% CI)	<i>r</i> (<i>P</i>)	<i>P</i>
Monocyte count vs neutrophil count	0.095 $\pm$ 0.011 (0.073 to 0.116)	204.1 $\pm$ 44.6 (115.2 to 293.0)	0.73 (<.001)	0.062 $\pm$ 0.017 (0.028 to 0.095)	302.5 $\pm$ 59.4 (182.5 to 422.4)	0.50 (<.001)	0.105 $\pm$ 0.016 (0.071 to 0.128)	179.9 $\pm$ 79.7 (15.9 to 343.8)	0.78 (<.001)	.212
Monocyte count vs fibrinogen concentration	0.175 $\pm$ 0.470 (0.082 to 0.268)	242.0 $\pm$ 27.7 (186.6 to 297.4)	0.42 (<.001)	0.223 $\pm$ 0.087 (0.047 to 0.400)	200.5 $\pm$ 46.0 (107.3 to 292.6)	0.38 (.014)	0.115 $\pm$ 0.056 (0.000 to 0.231)	307.2 $\pm$ 38.4 (227.7 to 386.8)	0.40 (.051)	.541
Neutrophil count vs fibrinogen concentration	9.626 $\pm$ 2.101 (5.428 to 13.82)	537.5 $\pm$ 736.0 (−932.7 to 2008)	0.50 (<.001)	5.522 $\pm$ 2.062 (1.351 to 9.694)	1648 $\pm$ 669.8 (293.3 to 3003)	0.39 (.011)	13.45 $\pm$ 4.749 (3.627 to 23.27)	−620.5 $\pm$ 1844 (−4436 to 3195)	0.51 (.009)	.085

r = Pearson correlation coefficient.**TABLE E5.** Correlation between monocytes, neutrophils, and fibrinogen vs lung functional parameters in all populations, nFEs, and FEs

Systemic biomarkers	FVC (% predicted)			FEV1 (% predicted)			RV (% predicted)			TI (%)		
	nFEs	FEs	All	nFEs	FEs	All	nFEs	FEs	All	nFEs	FEs	All
Monocytes (cell/ μ L)	−0.23 (.13)	−0.25 (.20)	−0.29 (.02)	−0.07 (.63)	0.05 (.79)	−0.07 (.57)	0.47 (.004)	0.27 (.17)	0.32 (.01)	−0.36 (.02)	−0.20 (.29)	−0.30 (.01)
Neutrophils (cell/ μ L)	−0.14 (.36)	−0.29 (.13)	−0.10 (.41)	−0.14 (.36)	−0.10 (.61)	−0.04 (.74)	0.37 (.02)	−0.13 (.53)	−0.13 (.31)	−0.18 (.23)	−0.02 (.92)	0.03 (.79)
Fibrinogen (mg/dL)	−0.39 (.01)	−0.27 (.19)	*	−0.21 (.19)	−0.44 (.02)	*	0.24 (.17)	0.41 (.05)	0.34 (.01)	−0.41 (.009)	−0.21 (.30)	*

Data are presented as *r* (*P* value).

*Data are shown in Figure 2, B.

TABLE E6. Ranges of reference values

Blood markers	Reference values
WBC ($\times 1000$ cell/ μ L)	4.5-11.0
Eosinophils ($\times 1000$ cell/ μ L)	0.00-0.80
Neutrophils ($\times 1000$ cell/ μ L)	1.90-8.00
Lymphocytes ($\times 1000$ cell/ μ L)	0.90-5.20
Monocytes ($\times 1000$ cell/ μ L)	0.16-1.00
Basophils ($\times 1000$ cell/ μ L)	0.00-0.20
Platelets ($\times 1000$ cell/ μ L)	150-450
Fibrinogen (mg/dL)	150-450
INR	0.80-1.20
PTT (s)	22.0-34.0

INR, International normalized ratio; PTT, partial thromboplastin time; WBC, white blood cell.

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