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Original Research

Short term exacerbation risk and exhaled nitric oxide in COPD

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ABSTRACT

Introduction: Exacerbations of chronic obstructive pulmonary disease (COPD) significantly impact morbidity and healthcare utilization. Identifying biomarkers predictive of exacerbation risk can optimize management strategies. We evaluated the role of baseline fractional exhaled nitric oxide (F_ENO) as a predictor of moderate and severe exacerbations over 90 days.

Methods: A prospective cohort study included COPD patients attending pulmonology clinics. Patients were stratified based on baseline F_ENO levels: F_ENO <20 ppb and *FENO* ≥20 ppb. The primary outcome was time-to-first exacerbation, analysed using Kaplan-Meier survival curves and Cox proportional hazards models. Secondary outcomes included differences in baseline characteristics and hazard ratios (HR) for severe exacerbations.

Results: A total of 322 patients were included (220 with F_ENO <20 ppb, 102 with F_ENO ≥20 ppb). Kaplan-Meier analysis showed significantly shorter survival time without moderate/severe exacerbations in those with high F_ENO. Cox regression demonstrated a 3.01-fold increased risk of moderate/severe exacerbations in high F_ENO (HR: 3.01, 95 % CI: 1.83–4.93; $p < 0.001$). For severe exacerbations, those patients exhibited a non-significant trend toward increased risk (HR: 2.49, 95 % CI: 0.91–6.86; $p = 0.058$).

Conclusion: Elevated F_ENO (≥20 ppb) is associated with increased short-term risk of moderate and severe COPD exacerbations. These findings highlight F_ENO as a potential biomarker for early risk stratification and tailored interventions in COPD patients.

Take- home message

Elevated F_ENO (≥20 ppb) predicts a higher short-term risk of moderate COPD exacerbations. This non-invasive biomarker enables early risk stratification, supporting personalized treatment strategies to improve outcomes and optimize COPD management.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is one of the leading causes of morbidity and mortality worldwide, significantly affecting patients' quality of life and increasing healthcare resource utilization [1]. Characterized by symptom deterioration, COPD exacerbations are particularly detrimental, accelerating disease progression, impairing lung function, and increasing the risk of hospitalization and death. Exacerbations can be classified as mild, moderate, or severe, depending on the degree of symptom worsening and the required intervention, with severe exacerbations resulting in hospitalization and elevated mortality rates [2]. Predicting which patients are at greater risk of short-term exacerbations remains a clinical challenge [3].

Inflammation plays a significant role in COPD pathophysiology, with both neutrophilic and eosinophilic pathways contributing to disease manifestations[4–6]. Fractional exhaled nitric oxide (F_ENO) is a non-invasive biomarker of airway inflammation that reflects eosinophilic activity [7]. While F_ENO has long been established as a tool for asthma management, its utility in COPD is increasingly being recognized [8–10]. Elevated F_ENO levels (specially ≥20 ppb) [7,11] have been associated with eosinophilic inflammation, a phenotype linked to

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exacerbations and corticosteroid responsiveness in COPD patients [12] and an increased probability of response to biologics [13]. However, the evidence on F_ENO 's ability to predict short-term exacerbations in COPD remains limited, particularly in patients managed in real-world clinical settings.

Given the burden of COPD exacerbations, identifying reliable biomarkers for early risk stratification is crucial. F_ENO measurements could offer a simple, non-invasive method for identifying patients at increased risk, allowing for timely interventions to reduce exacerbation frequency and improve outcomes[14–16]. The present study aimed to evaluate whether baseline F_ENO levels ($\geq$20 ppb) are associated with an increased short-term risk (90 days) of moderate and severe exacerbations in COPD patients.

2. Methods

2.1. Study design

This prospective cohort study was conducted at three pulmonology outpatient clinics between Feb 2016 and Dec 2019. A consecutive sampling method was employed to ensure representative recruitment of patients attending routine COPD follow-up visits.

2.2. Study population

The study population comprised adult COPD patients, current or former smokers with at least 10 pack-years of tobacco consumption, attended at outpatient clinics from 3 tertiary hospitals. The inclusion criteria were diagnosis of COPD (as defined by post-bronchodilator FEV1/FVC ratio <0.70), age $\geq$40 years, stable clinical condition (no exacerbations or significant change in treatment within 4 weeks prior to enrolment), and baseline *F*ENO measurement available. The exclusion criteria were a diagnosis of concomitant asthma (based on GINA criteria or the current Asthma- COPD overlap criteria proposed by GesEPOC) or other active airway diseases, recent respiratory tract infections (<4 weeks), use of oral corticosteroids within the past 4 weeks, current participation in clinical trials, pulmonary rehabilitation program or another research study, and severe comorbid conditions that could limit follow up, such as active malignancy, severe renal impairment, or uncontrolled systemic diseases.

2.3. FENO measurement

Baseline F_ENO levels were measured using a standardized electrochemical analyser (NIOX VERO®, Aerocrine AB, Sweden) following established American Thoracic Society (ATS) and European Respiratory Society (ERS) guidelines [17]. The procedure was performed under the supervision of trained pulmonary function technicians. Patients were instructed to avoid food, caffeine, alcohol, and smoking for at least 2 h prior to the test, maintain a steady exhalation rate (50 mL/s) during F_ENO measurement, as specified by ATS/ERS.

Each participant performed three valid *F*ENO maneuvers, with the final F_ENO value taken as the mean of these measurements. *F*ENO levels were categorized into two groups: those with F_ENO <20 ppb and those with F_ENO $\geq$20 ppb (indicative of eosinophilic inflammation).

2.4. Clinical and functional assessments

Demographic and clinical data were collected at baseline, including age, sex, body mass index (BMI), smoking history (quantified as pack-years), and comorbidities (ischemic heart disease, diabetes, depression, etc.). COPD symptoms were assessed using the COPD Assessment Test (CAT) and the modified Medical Research Council (mMRC) scale for dyspnoea. Spirometry was performed according to ATS/ERS guidelines using standardized equipment to measure post-bronchodilator FEV1, FVC, and FEV1/FVC ratio [18]. Multidimensional indexes (COTE, BODE) were calculated according to the original authors [19,20].

Blood eosinophil counts were measured from peripheral blood samples obtained at baseline. Exacerbation history in the preceding 12 months was documented, including the frequency and severity of events.

3. Outcomes

The primary outcome was time-to-first moderate or severe COPD exacerbation within 90 days of baseline F_ENO measurement. Exacerbations were defined as moderate exacerbations (worsening respiratory symptoms requiring systemic corticosteroids and/or antibiotics without hospitalization) and severe exacerbations (worsening respiratory symptoms leading to hospitalization or emergency department visit for more than 24 h). Exacerbation events were recorded through structured follow-up visits and telephone interviews conducted at 30, 60, and 90 days. Hospitalization records and prescription data were cross-checked for validation.

Secondary outcomes included hazard ratios (HR) for severe exacerbations, baseline clinical and functional differences between F_ENO groups, and the association between F_ENO and peripheral eosinophil counts.

3.1. Ethical considerations

The study was conducted in accordance with the provisions of the 1975 Declaration of Helsinki and was approved by the Ethics Committee of Clinical Research at our Institution (*Comité de Ética de la Investigación Biomédica de la Provincia de Gran*ada, and added the approval date: *March 10, 2016 reference num*ber 1987-N-16). All participants gave signed informed consent.

3.2. Statistical analysis

Descriptive statistics were used to summarize baseline characteristics. Continuous variables were reported as mean $\pm$ standard deviation (SD) or median (interquartile range), as appropriate. Categorical variables were presented as counts and percentages. Comparisons between F_ENO groups were conducted using the Student's t-test or Mann-Whitney *U* test for continuous variables and chi-square tests for categorical variables.

Time-to-event analysis was performed using Kaplan-Meier survival curves, and differences in survival probability were assessed using the log-rank test. Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95 % confidence intervals (CIs) for exacerbation risk associated with elevated F_ENO levels. The multivariable Cox model adjusted for potential confounders, including age, sex, BMI, smoking status, FEV1, GOLD grade, ICS prescriptions at baseline and blood eosinophil counts. The proportional hazards assumption was verified using Schoenfeld residuals.

A p-value <0.05 was considered statistically significant. All statistical analyses were performed using Jamovi software (version 2.6). Sensitivity analyses were conducted to evaluate the robustness of results, including subgroup analyses based on eosinophil levels and exacerbation history.

4. Results

From March 2016 to December 2019, 412 COPD patients from 3 tertiary hospitals in Spain were assessed consecutively for eligibility, 325 were recruited and finally 322 patients were available for study. Fig. S1 presents the STROBE diagram of the study.

The participants' baseline characteristics are detailed in Table 1. In summary, 89 % were male and aged in their seventies, 28 % were current smokers and had a relatively high level of tobacco consumption (46 pack-years). Most presented moderate airflow limitation (FEV_1 56 %),

Table 1
Baseline characteristics of study participants. Data are expressed in mean (SD) or n (%).

	Overall (N = 322)
Sex, male (n, %)	287 (89.1 %)
Age, years	70.7 (10.2)
BMI, kg/m^2	29.9 (5.2)
Smoking history	
Current smokers, (n,%)	90 (28.0 %)
Pack- years	46.0 (17.1)
COTE index ≥4 (n,%)	69 (21.4 %)
Lung function	
FEV1, L	1.4 (0.6)
FEV1, % predicted	56.4 (17.0)
Severe to very severe AFL (n,%)	116 (36 %)
GOLD 2024 (n,%)	
GOLD A	29 (9.0 %)
GOLD B	133 (41.3 %)
GOLD E	160 (49.7 %)
Symptom burden	
CAT	19.1 (8.0)
mMRC ≥2 (n,%)	267 (82.9 %)
Mod & severe exacerbation, prev year	1.7 (1.4)
Baseline treatment	
ICS	190 (59.0 %)
LAMA	247 (76.7 %)
LABA	294 (91.3 %)
T2 inflammatory biomarkers	
Blood eosinophil count,/mm^3	0.2 (0.2)
F_ENO, ppb (median, IQR)	16.8 (11.4)

BMI: body mass index; COTE: COPD comorbidity index; FEV1: forced expiratory volume in the 1st second; AFL: airflow limitation; CAT: COPD assessment test; mMRC: modified Medical Research Council; ICS: inhaled corticosteroids; LAMA: long acting muscarinic antagonist; LABA: long acting beta-agonist; T2: type 2; FENO: exhaled nitric oxide; ppb: parts per billion. IQR: interquartile range.

had high symptom burden (mean CAT 19.1) and were classified as GOLD E group.

At baseline, 102 patients (31.6 % of the entire population) had increased F_ENO levels (≥20 ppb). This group of patients had similar disease characteristics than the group of patients with F_ENO <20 ppb except for an increased tobacco consumption burden and a reduced burden of comorbidities (COTE index). Results are showed in Table 2.

Table 2
Baseline differences between those patients with high (≥20 ppb) or low (<20 ppb) F_ENO.

	F_ENO <20 ppb (n = 220)	F_ENO ≥20 ppb (n = 102)	p value
Sex, male (n, %)	196 (89.1 %)	91 (89.2 %)	0.973
Age, years	71.06 (10.73)	69.97 (8.88)	0.139
BMI, kg/m^2	30.10 (5.48)	29.44 (4.52)	0.330
Smoking history			
Current smokers, (n,%)	62 (28.2 %)	28 (27.5 %)	0.892
Pack-years	44.41 (17.24)	49.37 (16.52)	0.016
COTE index ≥4 (n,%)	41 (18.6 %)	21 (20.5 %)	0.025
Lung function			
FEV1, L	1.40 (0.66)	1.36 (0.59)	0.534
FEV1, % predicted	57.44 (16.33)	54.23 (18.33)	0.170
GOLD 2024 (n,%)			0.191
GOLD A	24 (10.9 %)	5 (4.9 %)	
GOLD B	87 (39.5 %)	46 (45.1 %)	
GOLD E	109 (49.5 %)	51 (50.0 %)	
CAT	18.69 (7.8)	19.95 (8.33)	0.190
Mod & severe exacerbation, prev year	1.80 (1.38)	1.99 (1.78)	0.730
Blood eosinophil count,/mm^3	0.23 (0.14)	0.24 (0.18)	0.574
F_ENO, ppb (median, IQR)	13.3 (6.7)	25.3 (4.9)	<0.001
Baseline treatment			
ICS/LABA/LAMA	93 (42.3 %)	37 (36.3 %)	0.307
LAMA/LAMA	69 (31.4 %)	24 (23.5 %)	0.149
LABA/ICS	42 (19.1 %)	13 (12.7 %)	0.159

Regarding the main outcome of the study, patients with high F_ENO at baseline showed an increased risk for short-term moderate and severe exacerbations. Kaplan-Meier curves demonstrated a significantly higher incidence of moderate/severe exacerbations in those participants ($p < 0.001$, Fig. 1).

Cox regression revealed a 3.01-fold increased risk of moderate/severe exacerbations in those participants with high baseline F_ENO (HR: 3.01, 95 % CI: 1.83–4.93; $p < 0.001$). The risk was especially high during the first 6 weeks (Fig. 2). For severe exacerbations, the HR was 2.49 (95 % CI: 0.91–6.86; $p = 0.077$), suggesting a trend toward increased risk (Fig. 3).

In the multivariate analysis, prior ICS use at baseline and F_ENO levels correlated with increased exacerbation frequency. Other covariates, including FEV1, GOLD grades and eosinophil counts, showed no significant impact (Fig. 4).

5. Discussion

Our findings confirm that elevated F_ENO levels (≥20 ppb) are associated with a higher short-term risk of moderate COPD exacerbations, with a hazard ratio of 3.01. This reinforces the role of F_ENO as a potential biomarker for identifying patients at higher risk for acute deterioration. Importantly, these results are consistent with previous studies suggesting a link between eosinophilic inflammation and exacerbation risk, particularly in patients responsive to corticosteroid therapy [12].

The Kaplan-Meier survival curves demonstrated clear stratification between the two F_ENO groups, with significantly shorter exacerbation-free survival in patients with elevated F_ENO. This supports the hypothesis that F_ENO is a marker of active eosinophilic inflammation, contributing to airway instability and increased susceptibility to exacerbations [7,8,21]. This risk seemed to be increased specially in the first 60 days of follow up, allowing to a prompt intervention which could reduce eosinophilic inflammation with inhaled therapies as ICS [22]. As most of the patients were already on ICS, this could also allow to a prompt intervention with targeted therapies [23]. It is worth noting that while severe exacerbations exhibited a trend toward increased risk in the high F_ENO group, this did not reach statistical significance. This could be due to the relatively low number of severe events during the 90-day follow-up period, suggesting that larger studies with longer follow-up durations are needed to confirm this association.

Our results are in line with other studies that have suggested an increased risk for future exacerbations among patients with increased

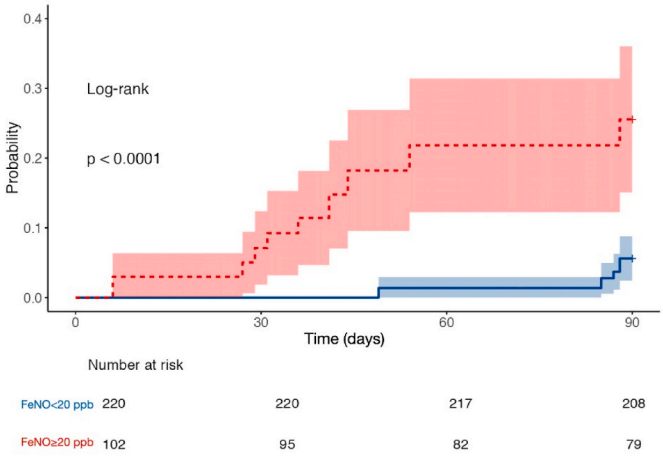

Fig. 1. Kaplan-Meier analysis of time (days) to moderate or severe COPD exacerbations, adjusted for age, gender, smoking, ICs and previous exacerbation history, and stratified by high or low F_ENO at baseline. Lines represent time to event. Shadows reflect 95 % confidence intervals. See text for further explanation.

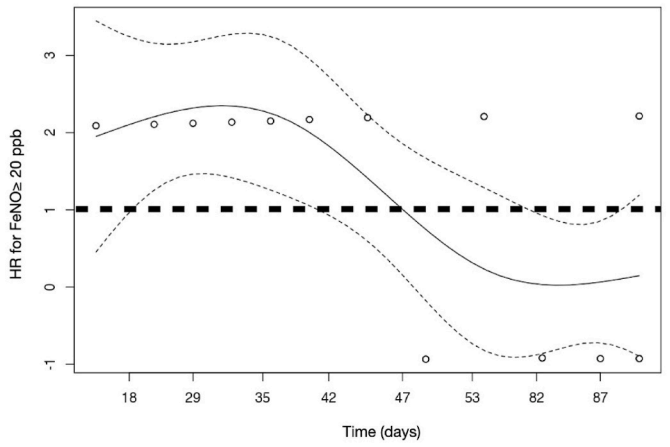

Fig. 2. Time-dependent hazard ratio for moderate and severe COPD exacerbations throughout the 90-day follow-up period, comparing patients with baseline $F_ENO \geq 20$ ppb to those with $F_ENO < 20$ ppb. The solid line represents the estimated hazard ratio, and the shaded area indicates the 95 % confidence interval. The horizontal dotted line corresponds to a hazard ratio of 1.0 (no difference in risk). Elevated hazard ratios in the early weeks suggest a higher short-term risk of exacerbation in the high F_ENO group.

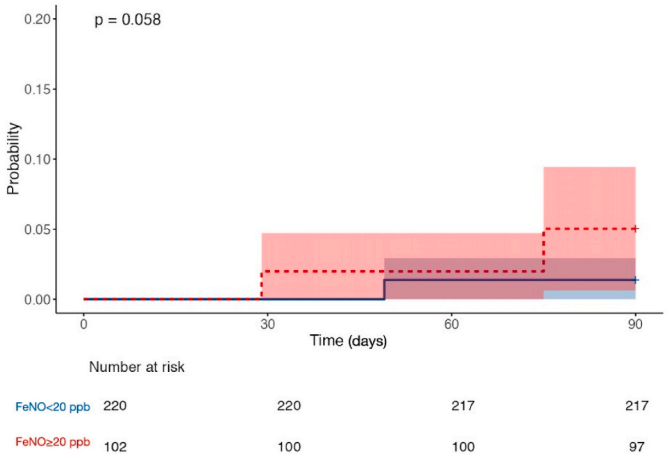

Fig. 3. Kaplan-Meier analysis of time (days) to severe COPD exacerbations, adjusted for age, gender, smoking, ICs and previous exacerbation history, and stratified by high or low F_ENO at baseline. Lines represent time to event. Shadows reflect 95 % confidence intervals.

F_ENO levels at stable state or during exacerbations [12,21,24]. However, other studies have found an increased risk not in the group of patients with high F_ENO levels but in those were there is variability during follow-up measurements [25].

Despite the strengths of our study, including a multicentric, well-defined outpatient cohort, several limitations should be acknowledged. First, the study was conducted in specialized pulmonology clinics, which may limit the generalizability of findings to primary care settings. Second, F_ENO was measured at baseline, but this measurement could have been influenced by previous inhaled therapy and by inherent variability between F_ENO measurements which has been observer in different studies [9,26]. Although we adjusted for ICS at baseline, we did not take into account the overall ICS dose and therefore the effects of different doses of ICS could not be detected. Additionally, while peripheral blood eosinophil counts were collected, the relationship between F_ENO and eosinophil levels warrants further exploration to refine patient phenotyping and could be explained by different citokines involved (IL-13 for airway inflammation and Il-5 for eosinophil recruitment from blood) or other factors that could influence F_ENO levels such as infections (bacterial, viral …). Finally, the exclusion of asthma- COPD overlap patients could limit generability of the results, especially when other T2 biomarkers such as IgE levels were not available in most of the study population.

The clinical implications of these findings are significant. F_ENO measurement is non-invasive, widely accessible, and relatively inexpensive compared to other biomarkers such as imaging or invasive sampling. Integrating F_ENO into routine COPD management could enable early identification of high-risk patients and facilitate personalized treatment strategies. For example, patients with elevated F_ENO may benefit from closer monitoring, early initiation of anti-inflammatory therapies, or non-pharmacological approaches including enhanced smoking cessation support or improving physical activity, all of which are strongly associated with reduced risk of future exacerbations.

In conclusion, elevated F_ENO levels ($\geq$20 ppb) are associated with an increased risk of moderate COPD exacerbations over a 90-day period. These findings highlight the utility of F_ENO as a practical biomarker for risk stratification in COPD. Further research is needed to validate these results in larger, more diverse populations and to investigate the potential role of F_ENO in guiding therapeutic interventions.

CRediT authorship contribution statement

A. Romero- Linares: Writing – review & editing, Writing – original draft, Project administration, Methodology, Data curation. **L. Álvarez-Muro:** Writing – review & editing, Investigation. **A. Hammadi:** Writing – review & editing, Conceptualization. **C. Hoyas- Sánchez:** Writing – review & editing. **A. Jiménez- Antón:** Writing – review & editing. **A. Almansa- López:** Writing – review & editing. **L. Casares- Martin-Moreno:** Writing – review & editing. **E. Sánchez- Álvarez:** Writing – review & editing, Visualization. **A. Murillo- Rodríguez:** Writing – review & editing. **M. Gómez- Mora:** Writing – review & editing. **T. Gómez- Pontes Cabrera:** Writing – review & editing. **P.J. Romero-Palacios:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Conceptualization. **B. Alcázar- Navarrete:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Bernardino Alcazar-Navarrete reports equipment, drugs, or supplies was provided by Fundacion Neumosur. Bernardino Alcazar Navarrete reports a relationship with AstraZeneca Pharmaceuticals LP that includes: consulting or advisory, funding grants, non-financial support, and speaking and lecture fees. Bernardino Alcazar Navarrete reports a relationship with GSK that includes: consulting or advisory, funding grants, non-financial support, and speaking and lecture fees. Bernardino Alcazar Navarrete reports a relationship with Chiesi Pharmaceuticals Inc that includes: speaking and lecture fees and travel reimbursement. Bernardino Alcazar Navarrete reports a relationship with Menarini Laboratories that includes: travel reimbursement. Bernardino Alcazar Navarrete reports a relationship with Sanofi SA that includes: consulting or advisory, non-financial support, speaking and lecture fees, and travel reimbursement. Pedro J Romero Palacios reports a relationship with Menarini Laboratories that includes: funding grants, speaking and lecture fees, and travel reimbursement. Pedro J Romero Palacios reports a relationship with GSK that includes: speaking and lecture fees and travel reimbursement. Pedro J Romero Palacios reports a relationship with Chiesi Pharmaceuticals Inc that includes: speaking and lecture fees and

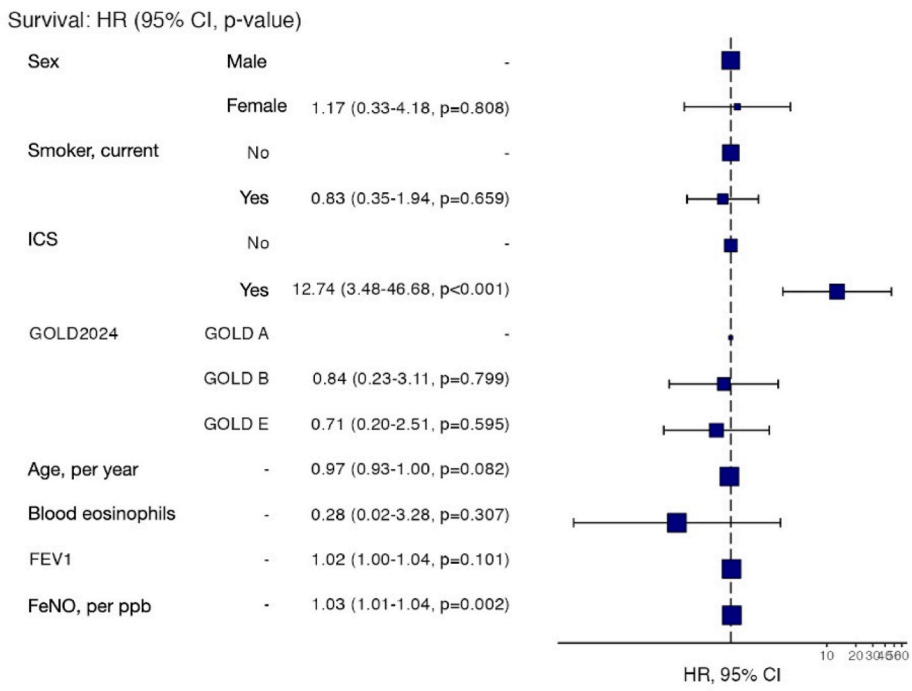

Fig. 4. Hazard regression plot showing hazard risk for moderate & severe exacerbations among participants according to multivariate analysis.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rmed.2025.108134.

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