

Inflammatory Biomarkers and Exacerbations in Chronic Obstructive Pulmonary Disease

Mette Thomsen, MD

Truls Sylvan Ingebrigtsen, MD

Jacob Louis Marott, MSc

Morten Dahl, DMSc

Peter Lange, DMSc

Jørgen Vestbo, DMSc

Børge G. Nordestgaard, DMSc

EXACERBATIONS OF RESPIRATORY symptoms in chronic obstructive pulmonary disease (COPD) are of major importance because of their profound and long-lasting adverse effects on patients.^{1,2} Frequent episodes accelerate loss of lung function,³ affect the quality of life of the patients,^{4,5} and are associated with poor survival.⁶⁻⁸ In general, exacerbations become more frequent with increasing disease severity, but the single best predictor of exacerbations in all grades of COPD is a previous exacerbation, suggesting the existence of a phenotype susceptible to exacerbations independent of degree of airflow limitation.⁹ However, when predicting risk of future exacerbations based on previous events, the positive predictive value remains low,⁹ indicating that additional determinants of exacerbation susceptibility remain to be identified.

Exacerbations are often caused by respiratory tract infections,¹⁰ and during the acute episode, levels of circulating acute phase proteins and inflammatory cells are elevated.^{11,12} However, some patients with COPD also have evidence of low-grade systemic inflammation with increased levels of such inflammatory biomarkers

For editorial comment see p 2390.

Importance Exacerbations of respiratory symptoms in chronic obstructive pulmonary disease (COPD) have profound and long-lasting adverse effects on patients.

Objective To test the hypothesis that elevated levels of inflammatory biomarkers in individuals with stable COPD are associated with an increased risk of having exacerbations.

Design, Setting, and Participants Prospective cohort study examining 61 650 participants with spirometry measurements from the Copenhagen City Heart Study (2001-2003) and the Copenhagen General Population Study (2003-2008). Of these, 6574 had COPD, defined as a ratio between forced expiratory volume in 1 second (FEV₁) and forced vital capacity below 0.7.

Main Outcomes and Measures Baseline levels of C-reactive protein (CRP) and fibrinogen and leukocyte count were measured in participants at a time when they were not experiencing symptoms of exacerbations. Exacerbations were recorded and defined as short-course treatment with oral corticosteroids alone or in combination with an antibiotic or as a hospital admission due to COPD. Levels of CRP and fibrinogen and leukocyte count were defined as high or low according to cut points of 3 mg/L, 14 μmol/L, and 9 × 10⁹/L, respectively.

Results During follow-up, 3083 exacerbations were recorded (mean, 0.5/participant). In the first year of follow-up, multivariable-adjusted odds ratios for having frequent exacerbations were 1.2 (95% CI, 0.7-2.2; 17 events/1000 person-years) for individuals with 1 high biomarker, 1.7 (95% CI, 0.9-3.2; 32 events/1000 person-years) for individuals with 2 high biomarkers, and 3.7 (95% CI, 1.9-7.4; 81 events/1000 person-years) for individuals with 3 high biomarkers compared with individuals who had no elevated biomarkers (9 events/1000 person-years; trend: $P=2 \times 10^{-5}$). Corresponding hazard ratios using maximum follow-up time were 1.4 (95% CI, 1.1-1.8), 1.6 (95% CI, 1.3-2.2), and 2.5 (95% CI, 1.8-3.4), respectively (trend: $P=1 \times 10^{-8}$). The addition of inflammatory biomarkers to a basic model including age, sex, FEV₁ percent predicted, smoking, use of any inhaled medication, body mass index, history of previous exacerbations, and time since most recent prior exacerbation improved the C statistics from 0.71 to 0.73 (comparison: $P=9 \times 10^{-5}$). Relative risks were consistent in those with milder COPD, in those with no history of frequent exacerbations, and in the 2 studies separately. The highest 5-year absolute risks of having frequent exacerbations in those with 3 high biomarkers (vs no high biomarkers) were 62% (vs 24%) for those with Global Initiative for Chronic Obstructive Lung Disease (GOLD) grades C-D (n=558), 98% (vs 64%) in those with a history of frequent exacerbations (n=127), and 52% (vs 15%) for those with GOLD grades 3-4 (n=465).

Conclusions and Relevance Simultaneously elevated levels of CRP and fibrinogen and leukocyte count in individuals with COPD were associated with increased risk of having exacerbations, even in those with milder COPD and in those without previous exacerbations. Further investigation is needed to determine the clinical value of these biomarkers for risk stratification.

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during stable conditions,¹³ and previous studies have found that elevated levels of inflammatory biomarkers like C-reactive protein (CRP), fibrinogen, and

Author Affiliations are listed at the end of this article. **Corresponding Author:** Børge G. Nordestgaard, DMSc, Department of Clinical Biochemistry, Herlev Hospital, Copenhagen University Hospital, Herlev Ringvej 75, DK-2730 Herlev, Denmark (boerge.nordestgaard@regionh.dk).

leukocytes during stable COPD are associated with poor outcomes.¹⁴⁻¹⁷ Thus, these biomarkers may also be associated with an increased risk of having exacerbations. In this study, we tested the hypothesis that elevated levels of inflammatory biomarkers in individuals with stable COPD are associated with an increased risk of having exacerbations.

METHODS

We studied age-stratified, randomly selected white individuals from 2 similar general population studies, essentially conducted by the same investigators and using identical methods: the 2001-2003 examination of the Copenhagen City Heart Study and the 2003-2008 examination of the Copenhagen General Population Study.¹⁸⁻²⁰ Number and age distributions of individuals invited to participate, all participants, and participating individuals with COPD in the 2 studies are shown in eFigure 1 (available at <http://www.jama.com>). Random selection was conducted at invitation based on the Danish Central Person Register number given to all persons living in Denmark, that is, prior to ascertainment of COPD diagnosis. Both studies were approved by Herlev Hospital and a Danish ethics committee and were conducted according to the Declaration of Helsinki. Written informed consent was obtained from all participants. There was no overlap of individuals between the 2 studies. In both studies, all participants filled out a questionnaire reviewed by an examiner at attendance, had spirometry performed, and had blood samples drawn. The current study selected a subgroup of individuals with COPD for further studies. The participants with COPD in the 2 studies were analyzed as 1 collective cohort to obtain maximal statistical power.

Spirometry

Forced expiratory volume in 1 second (FEV₁) and forced vital capacity (FVC) were determined without inhalation of a bronchodilator using a dry wedge spirometer (Vitalograph; Maids More-

ton) in the Copenhagen City Heart Study and in the first 14 624 participants of the Copenhagen General Population Study. For the remaining participants of the Copenhagen General Population Study, an EasyOne Spirometer (ndd Medizintechnik) was used. Reference values for FEV₁ were internally derived for men and women separately in a subsample of healthy never-smokers using linear regression with age and height as covariates. In stratified analyses, participants were grouped according to Global Initiative for Chronic Obstructive Lung Disease (GOLD) grades 1 through 4 for airflow limitation and the recent GOLD grades A through D for assessing both symptoms and risk.² Breathlessness was assessed using the Medical Research Council questionnaire, and history of frequent exacerbations was defined as having had 2 or more exacerbations in the year before examination. Classification criteria for GOLD grades are shown in eTable 1. Distributions of FEV₁ percent predicted according to GOLD grades are shown in eFigure 2. In the analyses presented, GOLD grades A-B, C-D, 1-2, and 3-4 were combined to obtain maximum statistical power; however, if analyzed separately, the results were largely similar but attenuated in higher GOLD grades.

Inflammatory Biomarkers

Plasma levels of high-sensitivity CRP and fibrinogen and whole blood leukocyte count in both studies were measured using standard hospital assays at a central laboratory at Herlev Hospital. We chose these 3 biomarkers because they are widely available and commonly used to monitor disease in patients with COPD. As in our previous study,¹⁷ biomarkers were analyzed in combination defined as high or low according to cut points. Although such categorization may lead to loss of information compared with using the biomarkers as continuous variables, we used cut points because they are simple and clinically useful. Levels of CRP were categorized using the cut point 3 mg/L, a cut point that

previously has been used by us and others in cardiovascular/pulmonary medicine.^{15,20,21} We next defined equivalent cut points for fibrinogen and leukocyte count, 14 μmol/L for fibrinogen and 9 × 10⁹/L for leukocyte count, so that the numbers in each of the low and high groups for each biomarker were roughly the same. If each biomarker was analyzed separately or in any combination with one other biomarker, results were attenuated but largely similar to those presented.

Exacerbations

An exacerbation of COPD was defined as a short-course treatment with oral corticosteroids alone or in combination with an antibiotic or as a hospital admission due to COPD. This information was collected for each individual by linking our database to 2 national registries: the Danish Registry of Medicinal Product Statistics, which contains information on all prescriptions dispensed in all Danish pharmacies, and the Danish National Patient Registry covering all hospital contacts in Denmark. We identified treatment with oral corticosteroids (H02AB) and antibiotics (J01) using the Anatomic Therapeutic Chemical code²² and diagnoses of COPD (DJ41-44) using the World Health Organization International Classification of Diseases code.²³ This ascertainment seems valid because short-course treatments of oral corticosteroids are not used for other conditions in patients with COPD and because administrative data on hospital admissions in Denmark have shown a high validity and adequate completeness.²⁴ Treatments with oral corticosteroids or oral corticosteroids in combination with antibiotics and hospital admissions had to be a minimum 4 weeks apart to be considered separate exacerbations. Frequent exacerbations were defined as 2 or more exacerbations less than 1 year apart. A history of frequent exacerbations was defined as 2 or more exacerbations in the year before examination. For each participant, exacerbations were recorded 1 year prior to the examina-

tion date and forward until the end of follow-up in August 2010. Deaths due to respiratory failure without prior hospitalization were not captured in the Danish National Patient Registry; however, we expect these to be rare in Denmark where hospitalization is free of charge.

Covariates

Body mass index was calculated as weight in kilograms divided by height in meters squared. Participants were categorized as current smokers, former smokers, or never smokers. Any inhaled medication was any prescription of inhaled short- and long-lasting β_2 -agonists, anticholinergics, corticosteroids, or combination products in the year prior to examination. (eTable 2 lists the number of participants using each type of inhaled medication and corresponding Anatomic Therapeutic Chemical codes.)

Statistical Analysis

We used Stata/SE version 12.0. A 2-sided *P* value less than .05 was considered significant. First, we analyzed risk of having frequent exacerbations during the first year of follow-up using logistic regression. Models were multivariable adjusted for age, sex, FEV₁ percent predicted, smoking, use of any inhaled medication, and body mass index. One model was in addition adjusted for history of frequent exacerbations and time since most recent prior exacerbation. Assessment of model fit was by Hosmer-Lemeshow test. Second, we analyzed risk of having at least 1 exacerbation and risk of having frequent exacerbations with maximum follow-up time using Cox proportional hazards regression models with delayed entry at examination (left truncation). The assumption of proportional hazards was tested with the Lin, Wei, and Ying score process test.²⁵ Misspecifications of the functional form of the covariates were tested by plotting the continuous covariates against the cumulative residuals and comparing them with random realizations under the model. Follow-up time for each par-

ticipant began at study entry and ended at event, death (*n*=744), or August 2010, whichever came first; we did not lose track of any individual.

Multivariable models were adjusted for age (as time scale), sex, FEV₁ percent predicted, smoking, use of any inhaled medication, body mass index, history of frequent exacerbations, and time since most recent prior exacerbation. The added discriminative power offered by the addition of inflammatory biomarkers to a basic model with clinical characteristics was analyzed using area under the curve, Harrell *C* index,²⁶ and the net reclassification index.²⁷ Positive and negative predictive values were calculated. For test for trend of risk estimates, groups based on increasing levels of CRP and fibrinogen and leukocyte count were coded 0, 1, 2, etc. Test of interaction in the Cox model was performed by introducing a 2-factor interaction term, and *P* values were by likelihood ratio test for comparing models with and without the interaction term. Finally, absolute 1-year, 3-year, and 5-year risk by groups of the 3 biomarkers was estimated using the regression coefficients from a Poisson regression model.²⁸ Because we only included individuals from the cohort with complete information on inflammatory biomarkers and covariates for adjustments, we had no individuals with missing values.

RESULTS

Among 5919 participants of the Copenhagen City Heart Study and 55 731 participants of the Copenhagen General Population Study, 8020 individuals had COPD, defined as a ratio between FEV₁ and FVC below 0.7. Aiming to avoid misclassification with asthma, we excluded individuals with self-reported asthma (*n*=1198) and individuals younger than 40 years of age (*n*=196). Also, individuals with missing values for covariates (*n*=3) or measurements of any of the 3 biomarkers (*n*=49) were excluded, leaving 6574 individuals available for analyses. Baseline characteristics of the 6574 participants with stable COPD stratified by GOLD grades and history of frequent

exacerbations are shown in the TABLE and eTables 3 through 5. During a median 4 years (interquartile range, 2.4-5.5) of follow-up time, 3083 exacerbations were recorded (mean, 0.5/participant). Among all 6574 participants, 244 individuals (4%) had 1 or more exacerbations in the year prior to examination, and 85 (1%) had their most recent exacerbation within 2 months of the examination date (eFigure 3).

Frequent Exacerbations During First Year of Follow-up

During the first year of follow-up time, 129 individuals had frequent exacerbations (≥ 2). The number of individuals with frequent exacerbations increased stepwise according to groups of inflammatory biomarkers (FIGURE 1). Numbers of events per 1000 person-years were 17 (95% CI, 12-25) for individuals with 1 high biomarker (*n*=1831), 32 (95% CI, 24-46) for individuals with 2 high biomarkers (*n*=1066), and 81 (95% CI, 59-120) for individuals with 3 high biomarkers (*n*=384), compared with 9 (95% CI, 6.6-13) for individuals with no elevated biomarkers (*n*=3293). Corresponding multivariable-adjusted odds ratios for having frequent exacerbations were 1.5 (95% CI, 0.9-2.6), 2.6 (95% CI, 1.6-4.3), and 6.4 (95% CI, 3.8-11) (trend: $P=3 \times 10^{-11}$). When including adjustment for history of frequent exacerbations and time since most recent prior exacerbation, corresponding odds ratios were 1.2 (95% CI, 0.7-2.2), 1.7 (95% CI, 0.9-3.2), and 3.7 (95% CI, 1.9-7.4), respectively (trend: $P=2 \times 10^{-5}$).

Exacerbations During Maximum Follow-up Time

During follow-up time, 931 individuals had at least 1 exacerbation. Among these individuals, 423 had frequent exacerbations (≥ 2 less than 1 year apart). Risk of having at least 1 exacerbation and risk of having frequent exacerbations increased stepwise from none through 3 high inflammatory biomarkers (FIGURE 2). Multivariable-adjusted hazard ratios for having at least 1 exacer-

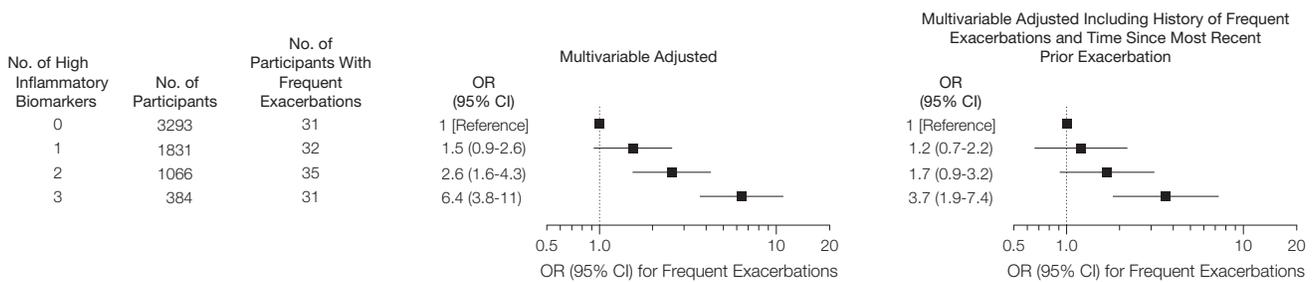
bation were 1.2 (95% CI, 1.0-1.4) for individuals with 1 high biomarker, 1.3 (95% CI, 1.1-1.6) for individuals with 2 high biomarkers, and 1.8 (95% CI, 1.4-2.2) for individuals with 3 high biomarkers, compared with individuals who had no elevated biomarkers (trend: $P=2 \times 10^{-7}$). Corresponding hazard ratios for having frequent exacerbations

Table. Baseline Characteristics of Study Participants With Chronic Obstructive Pulmonary Disease

	All (n = 6574)	GOLD Grades A-B (n = 6016) ^a	GOLD Grades C-D (n = 558) ^a	Frequent Exacerbations		GOLD Grades 1-2 (n = 6109) ^a	GOLD Grades 3-4 (n = 465) ^a
				No History (n = 6447)	History (n = 127) ^b		
Age, median (IQR), y	67 (58-75)	66 (58-75)	72 (65-77)	67 (58-75)	75 (69-80)	67 (58-75)	70 (64-77)
Men, No. (%)	3120 (47)	2844 (47)	276 (49)	3073 (48)	47 (37)	2874 (47)	246 (53)
FEV ₁ percent predicted, median (IQR)	80 (67-92)	82 (70-93)	44 (39-48)	80 (67-92)	69 (48-85)	82 (70-93)	43 (37-47)
BMI, median (IQR) ^c	25 (23-28)	25 (23-28)	25 (22-28)	25 (23-28)	25 (23-28)	25 (23-28)	25 (22-28)
Current smokers, No. (%)	2566 (39)	2313 (38)	253 (45)	2530 (39)	36 (28)	2343 (38)	223 (48)
Former smokers, No. (%)	2573 (39)	2329 (39)	244 (44)	2502 (39)	71 (56)	2374 (39)	199 (43)
Use inhalants, No. (%)	660 (10)	456 (8)	204 (37)	609 (9)	51 (40)	480 (8)	180 (39)
History of exacerbations, No. (%)	127 (2)	0	127 (23)	0	127 (100)	93 (2)	34 (7)
C-reactive protein, median (IQR), mg/L	1.8 (1.2-3.5)	1.7 (1.2-3.3)	2.9 (1.6-6.4)	1.8 (1.2-3.4)	3.4 (1.9-8.1)	1.7 (1.2-3.3)	2.8 (1.5-6.1)
Fibrinogen, median (IQR), μmol/L	11.9 (10.3-14.1)	11.8 (10.2-13.9)	13.4 (11.4-15.6)	11.9 (10.2-14.0)	13.8 (11.7-16.5)	11.8 (10.2-13.9)	13.4 (11.3-15.4)
Leukocyte count, median (IQR), ×10 ⁹ /L	7.4 (6.3-8.7)	7.3 (6.3-8.6)	7.9 (6.6-9.3)	7.4 (6.3-8.7)	8.3 (6.9-10.4)	7.4 (6.3-8.7)	7.9 (6.5-9.2)

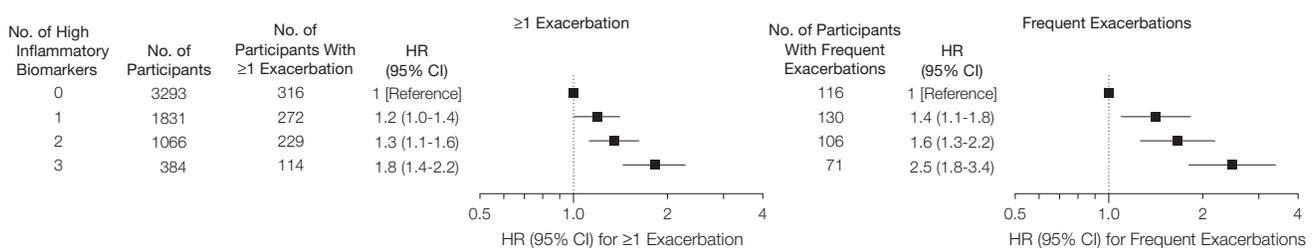
Abbreviations: BMI, body mass index; FEV₁, forced expiratory volume in 1 second; GOLD, Global Initiative for Chronic Obstructive Lung Disease; IQR, interquartile range.
^aStratification in GOLD grades 1-4 is based solely on levels of FEV₁, whereas GOLD grades A-D also include dyspnea and the history of exacerbations (eTable 1), which explains the observed differences.
^bHistory of frequent exacerbations was ≥2 exacerbations in the year before examination.
^cCalculated as weight in kilograms divided by height in meters squared.

Figure 1. Risk of Having Frequent Exacerbations (≥2) in the First Year of Follow-up According to Inflammatory Biomarkers in Individuals From the General Population With COPD



Plasma C-reactive protein and fibrinogen and blood leukocyte count were defined as high or low according to cut points of 3 mg/L, 14 μmol/L, and 9 ×10⁹/L, respectively. The first model was multivariable adjusted for age, sex, forced expiratory volume in 1 second percent predicted, smoking, use of any inhaled medication, and body mass index (trend $P=3 \times 10^{-11}$), while the second model also included adjustment for history of frequent exacerbations and time since most recent prior exacerbation (trend $P=2 \times 10^{-5}$). COPD indicates chronic obstructive pulmonary disease; OR, odds ratio.

Figure 2. Risks of Having at Least 1 Exacerbation and Having Frequent Exacerbations (≥2 Less Than 1 Year Apart) During Maximum Follow-up Time According to Inflammatory Biomarkers in Individuals From the General Population With COPD



Plasma C-reactive protein and fibrinogen and blood leukocyte count were defined as high or low according to cut points of 3 mg/L, 14 μmol/L, and 9 ×10⁹/L, respectively. Models were multivariable adjusted for age (as time scale), sex, forced expiratory volume in 1 second percent predicted, smoking, use of any inhaled medication, body mass index, history of frequent exacerbations, and time since most recent prior exacerbation. For having ≥1 exacerbation, trend $P=2 \times 10^{-7}$; for having frequent exacerbations, trend $P=1 \times 10^{-8}$. COPD indicates chronic obstructive pulmonary disease; HR, hazard ratio.

were 1.4 (95% CI, 1.1-1.8), 1.6 (95% CI, 1.3-2.2), and 2.5 (95% CI, 1.8-3.4), respectively (trend: $P=1 \times 10^{-8}$). All models included other covariates and also adjustment for history of frequent exacerbations and time since most recent prior exacerbation.

Model Accuracy and Predictive Values

Discrimination and predictive values of the 3 inflammatory biomarkers for risk of frequent exacerbations are shown in eTable 6. The addition of inflammatory biomarkers to a basic model including age, sex, FEV₁ percent predicted, smoking, use of any inhaled medication, body mass index, history of previous exacerbations, and time since most recent prior exacerbation improved the C statistics from 0.71 to 0.73 (comparison: $P=9 \times 10^{-5}$). Positive and negative predictive values for 3 high biomarkers vs none for frequent exacerbations were 8% and 99% during the first year of follow-up and 18% and 96% during maximum follow-up, respectively. For comparison, positive and negative predictive values for history vs no history of frequent exacerbations were 58% and 99% during the first year of follow-up and 72% and 95% during maximum follow-up time, respectively. For frequent exacerbations during the first year of follow-up, adding the inflammatory biomarkers to the basic model yielded a combined net reclassification index of 40% (95% CI, 22%-57%; $P=8 \times 10^{-6}$) (eFigure 4).

Sensitivity Analyses

When the models shown in Figure 2 were also adjusted for number of previous exacerbations on a continuous scale, or individuals with exacerbations in the first year of follow-up were excluded, the results were similar to those presented (eFigures 5 and 6). Also, in the analyses stratified according to GOLD grades A-D, history of frequent exacerbations, or GOLD grades 1-4, risks of having at least 1 exacerbation or risks of having frequent exacerbations were similar in all strata (FIGURE 3) (test of interaction: all $P > .06$). The wider con-

fidence intervals for risk estimates for those with GOLD C-D (n=558) vs A-B (n=6016), for those with a history of frequent exacerbations (n=127) vs no history (n=6447), and for those with GOLD 3-4 (n=465) vs GOLD 1-2 (n=6109) are explained by lower statistical power in the former vs latter strata. Also, in analyses stratified by smoking habits (never, former, current), results were similar in former and current smokers but attenuated in never smokers (eFigure 7). Furthermore, when the 2 studies were analyzed separately, results were similar when comparing individuals with 3 high biomarkers with individuals who had no elevated biomarkers.

Absolute Risk of Frequent Exacerbations

There was a stepwise increase in the absolute 1-year, 3-year, and 5-year risk of having frequent exacerbations from none through 3 high inflammatory biomarkers, in all strata of GOLD grades A-D, history of frequent exacerbations, and GOLD grades 1-4 (FIGURE 4). The highest 5-year absolute risks of having frequent exacerbations in those with 3 high biomarkers (vs no high biomarkers) were 62% (vs 24%) for those with GOLD grades C-D (n=558), 98% (vs 64%) in those with a history of frequent exacerbations (n=127), and 52% (vs 15%) for those with GOLD grades 3-4 (n=465).

DISCUSSION

The principal finding of this study is that simultaneously elevated levels of CRP, fibrinogen, and leukocytes were associated with increased risk of frequent exacerbations in individuals with stable COPD. Risk of having frequent exacerbations was increased approximately 4-fold in the first year of follow-up and 3-fold using maximum follow-up time in individuals with 3 high inflammatory biomarkers compared with individuals who had no elevated biomarkers. Importantly, relative risk estimates were consistent even in those with milder COPD and in those with no history of frequent exacerbations,

suggesting that these biomarkers provide additional information to the latest GOLD 2011 grading.² These findings may enable clinicians using absolute risk estimates to provide a more stratified management focusing on exacerbation prevention and are yet another step toward personalized medicine in COPD.

A subgroup of patients with COPD have increased levels of inflammatory biomarkers during stable conditions,¹³ and we found that these individuals seem particularly prone to developing future exacerbations. One explanation could be that high levels of inflammatory biomarkers reflect bacterial colonization or latent viral infections persisting in airway epithelial after a previous exacerbation,^{10,29} which is an important predictor for subsequent exacerbations.⁹ Also, the degree of lung inflammation and thus disease activity is another important factor.⁹ However, in this study, the association was independent of previous exacerbations and degree of airflow limitation, suggesting that low-grade systemic inflammation in itself may have a negative effect on the immunological response to airborne pathogens increasing exacerbation susceptibility. In human experimental models of virus-induced exacerbations, patients with COPD had impaired production of interferon- β by alveolar macrophages compared with healthy controls.³⁰ Also, the presence of systemic low-grade inflammation could hypothetically have a negative synergistic effect on innate cytokine response, leading to an insufficient immune response, but the exact biological mechanism behind the present observations remains unclear.

Previous studies on inflammatory biomarkers and COPD exacerbations are scarce, but the studies that have been conducted so far support our findings.^{9,16,31} A study from the ECLIPSE cohort found elevated levels of CRP and fibrinogen and leukocyte count to be associated with the occurrence of exacerbations in the first year of follow-up in univariate analyses.⁹ How-

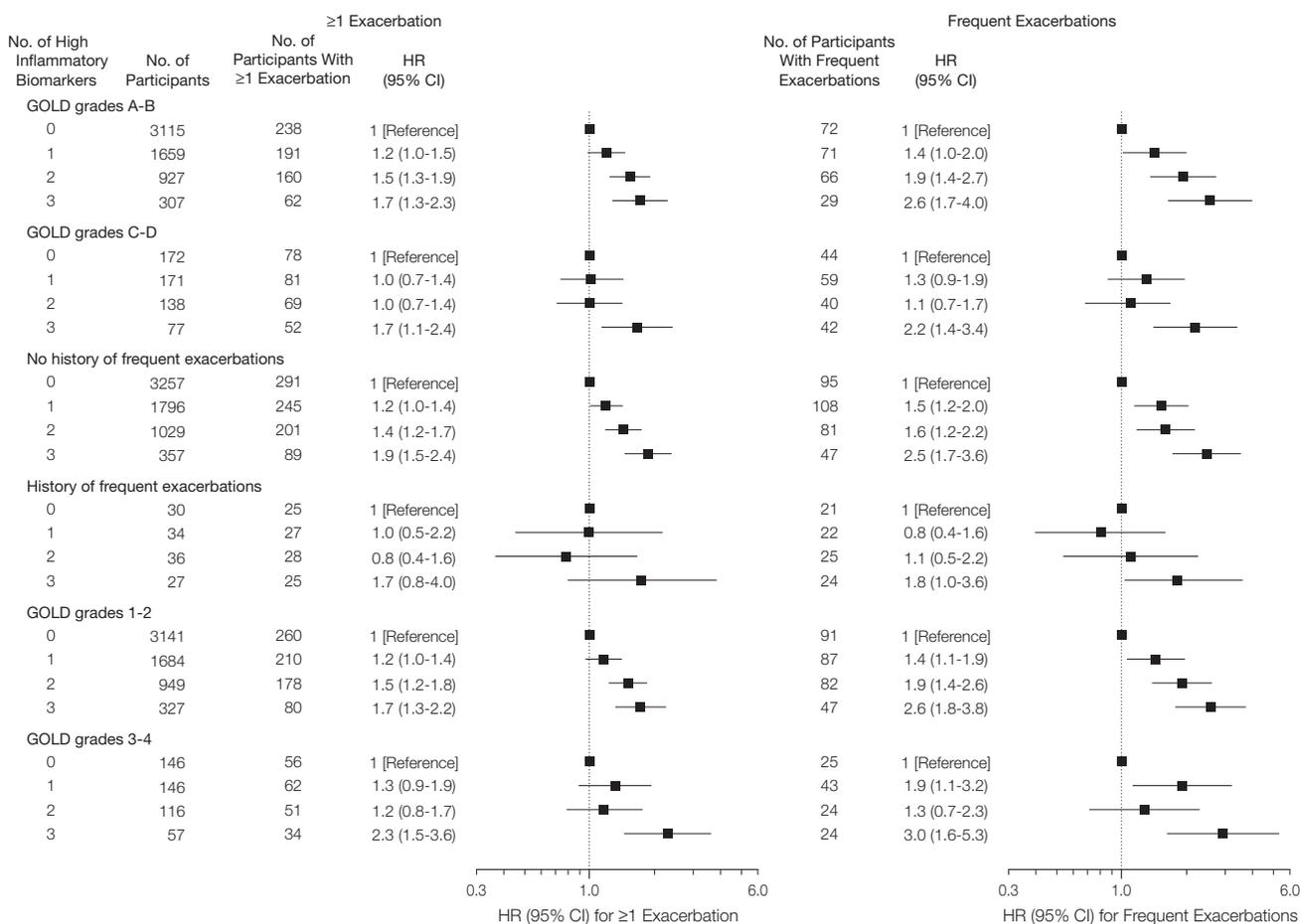
ever, after multivariable adjustment including previous exacerbations, only the association found for elevated leukocyte count remained statistically significant.

Another study found elevated levels of fibrinogen to be an independent risk factor for COPD exacerbation,³¹ but in that study, all participants had a history of previous exacerbations, which may have confounded results. It seems that by combining elevated levels of several inflammatory biomarkers, as done by us, the association with exacerbations becomes more robust.

In line with these findings, the addition of inflammatory biomarkers in our study increased the capacity to predict frequent exacerbations, but the increment in the C statistics was limited. However, obtaining increases in Harrell C index for a new model is very difficult when strong risk factors such as FEV₁ percent predicted and history of frequent exacerbations are already included in the basic model.³³ Thus, the magnitude of the increase in C statistics is difficult to translate into a clinical value, as is the increase in continuous net reclassification

tions becomes more robust. In line with this, another recent study measured 6 inflammatory biomarkers at baseline and found that COPD patients with elevated levels of 2 or more inflammatory biomarkers persisting at least 1 year after follow-up had increased all-cause mortality and exacerbation frequency, when compared with controls.¹⁶ Also, addition of several biomarkers to established clinical measures in COPD increased the capacity to predict mortality compared with adding a single biomarker.³²

Figure 3. Risks of Having at Least 1 Exacerbation and Having Frequent Exacerbations (≥2 Less Than 1 Year Apart) During Maximum Follow-up Time According to Inflammatory Biomarkers in Individuals From the General Population With COPD, by GOLD Grades and History of Frequent Exacerbations



Plasma C-reactive protein and fibrinogen and blood leukocyte count were defined as high or low according to cut points of 3 mg/L, 14 μmol/L, and 9 × 10⁹/L, respectively. Models were multivariable adjusted for age (as time scale), sex, forced expiratory volume in 1 second percent predicted, smoking, use of any inhaled medication, body mass index, history of frequent exacerbations, and time since most recent prior exacerbation. For ≥1 exacerbation and for frequent exacerbations, respectively, trend P values were as follows: GOLD grades A-B, P=2 × 10⁻⁶ for both; GOLD grades C-D, P=.04 and P=6 × 10⁻³; no history of frequent exacerbations, P=4 × 10⁻⁸ and P=1 × 10⁻⁶; history of frequent exacerbations, P=.32 and P=.07; GOLD grades 1-2, P=6 × 10⁻⁷ and P=4 × 10⁻⁸; GOLD grades 3-4, P=5 × 10⁻³ and P=8 × 10⁻³. COPD indicates chronic obstructive pulmonary disease; GOLD, Global Initiative for Chronic Obstructive Lung Disease; HR, hazard ratio.

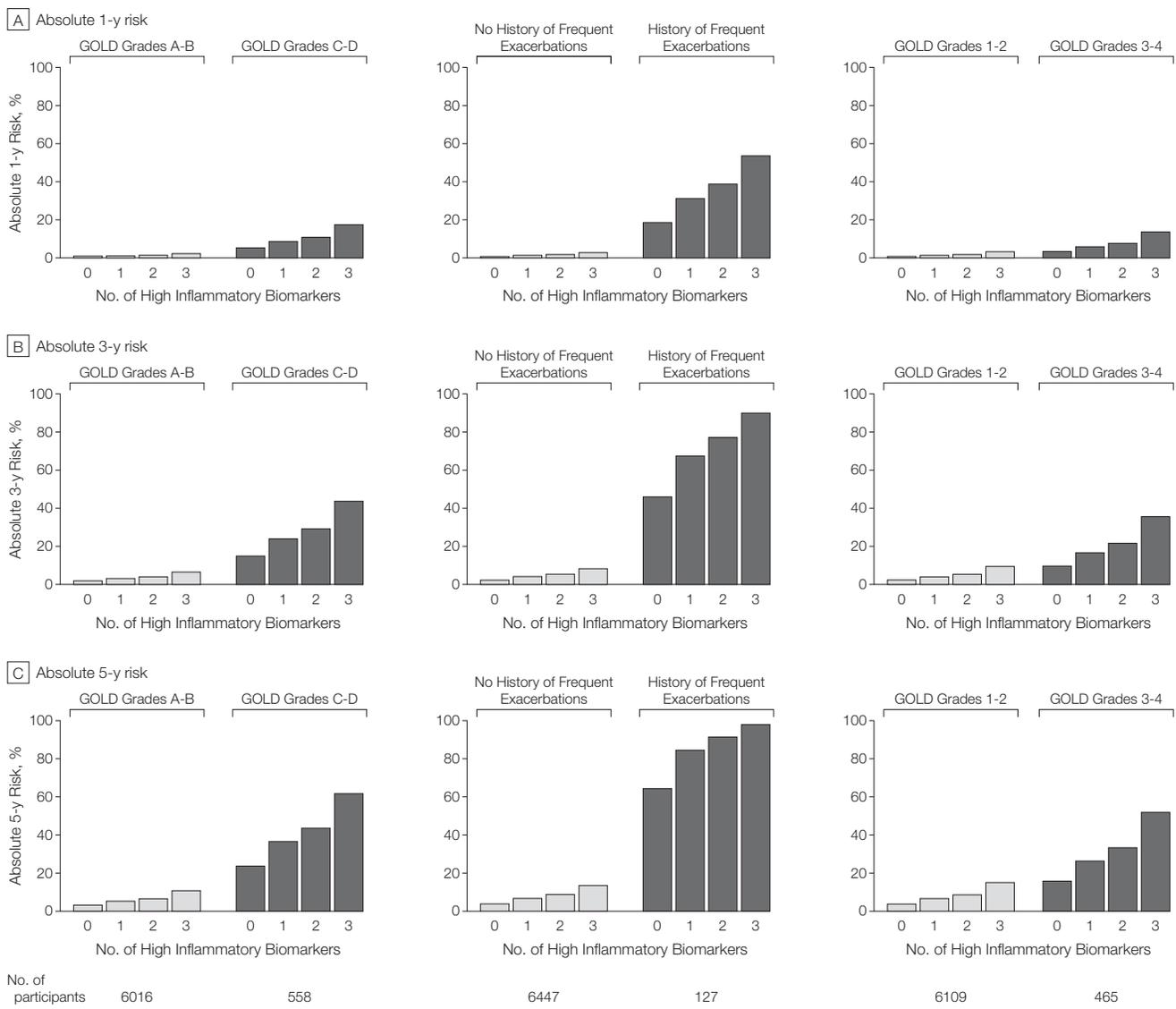
tion index, and the utility of the inflammatory biomarkers in a clinical setting need to be evaluated in future studies. Nevertheless, the current absolute 1-year, 3-year, and 5-year absolute risks of frequent exacerbations are directly applicable to clinical practice, as are the positive and negative predictive values. To our knowledge, our observational study is the first to show that elevated levels of 3 commonly measured inflam-

matory biomarkers combined were associated with increased risk of frequent exacerbations, even in patients without previous exacerbations.

A strength of this large study on inflammatory biomarkers and exacerbations in COPD is that we recruited participants from the general population. Our prospective design and registry-based definition of exacerbations with 100% follow-up avoiding investigator

bias is another major strength. Limitations include that we only had pre-bronchodilator measurements available and that we defined COPD based on airflow limitation alone. Also, as in other epidemiological studies, our findings are affected by selection bias due to possible overrepresentation of relatively healthy patients with COPD. Compared with studies recruiting in a hospital setting, there are fewer participants

Figure 4. Absolute 1-Year, 3-Year, and 5-Year Risk of Having Frequent Exacerbations, by Number of High Inflammatory Biomarkers, GOLD Grades of COPD, and History of Frequent Exacerbations



Plasma C-reactive protein and fibrinogen and blood leukocyte count were defined as high or low according to cut points of 3 mg/L, 14 μmol/L, and 9 × 10⁹/L, respectively. COPD indicates chronic obstructive pulmonary disease; GOLD, Global Initiative for Chronic Obstructive Lung Disease.

with severe COPD, fewer users of inhaled medication, and relatively low numbers of patients experiencing frequent exacerbations, all of which will reduce the statistical power to detect an association in those with severe COPD and previous exacerbations. However, this would tend to draw the results in a direction toward the null hypothesis and cannot explain our positive results.

Another potential limitation is the changes in treatment of COPD over the study period. Over time, there has been a gradual increase in the use of tiotropium and combination therapy with inhaled corticoids and long-acting β_2 -agonists in maintenance treatment. However, this is not likely to have influenced our results to a major extent, because tiotropium does not affect levels of inflammatory biomarkers³⁴ and because the use of inhaled corticosteroids is relatively rare in population-based cohorts. Finally, although we have previously published data on reasonable stability of CRP and fibrinogen levels over 10 years in individuals with COPD (Figure E1 in the online data supplement of Thomsen et al¹⁷), we do not have similar data for leukocyte count; however, a previous study found low variability in levels of fibrinogen and leukocytes in patients with COPD over time,³⁵ and this is likely to be similar in our cohort.

Until recently, the most important criterion for therapy guiding in COPD has been the degree of airflow limitation. The findings from the ECLIPSE study,⁹ among others, have led to a new classification of COPD,² enabling identification of more individuals at risk of frequent exacerbations and disease progression than the previous classification.³⁶ A recent study found pulmonary artery enlargement on chest computed tomography to be an independent predictor of exacerbations.³⁷ However, our study provides novel information that may lead to a simpler assessment using measurements of inflammatory biomarkers in individuals with stable COPD to further stratify preventive therapies based on absolute risk of frequent exacerbations. The potential ben-

efits of such stratification should be tested in future clinical trials that could include drugs of particular current interest, such as macrolides³⁸ or statins.³⁹

In conclusion, simultaneously elevated levels of CRP and fibrinogen and leukocyte count were associated with increased risk of exacerbations in stable COPD, even for individuals with milder COPD and for those without previous exacerbations. Further investigation is needed to determine the clinical value of these biomarkers for risk stratification.

Author Affiliations: Department of Clinical Biochemistry and the Copenhagen General Population Study, Herlev Hospital, Copenhagen University Hospital, Herlev, Denmark (Drs Thomsen and Nordestgaard); Copenhagen City Heart Study, Frederiksberg Hospital, Copenhagen University Hospital, Frederiksberg, Denmark (Drs Ingebrigtsen, Lange, and Nordestgaard and Mr Marott); Department of Clinical Biochemistry, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark (Dr Dahl); Respiratory Section, Hvidovre Hospital, Copenhagen University Hospital, Hvidovre, Denmark (Dr Lange); Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen (Drs Thomsen, Dahl, Lange, and Nordestgaard); Department of Public Health, Section of Social Medicine, University of Copenhagen, Copenhagen (Dr Lange); Department of Respiratory Medicine, Odense University Hospital, University of Southern Denmark, Odense, Denmark (Drs Ingebrigtsen and Vestbo); and Respiratory Research Group, Manchester Academic Health Sciences Centre, University Hospital of South Manchester NHS Foundation Trust, Manchester, United Kingdom (Dr Vestbo).

Author Contributions: Dr Nordestgaard had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Thomsen, Ingebrigtsen, Dahl, Lange, Vestbo, Nordestgaard.

Acquisition of data: Lange, Nordestgaard.

Analysis and interpretation of data: Thomsen, Ingebrigtsen, Marott, Dahl, Lange, Vestbo, Nordestgaard.

Drafting of the manuscript: Thomsen, Lange, Vestbo, Nordestgaard.

Critical revision of the manuscript for important intellectual content: Thomsen, Ingebrigtsen, Marott, Dahl, Lange, Vestbo, Nordestgaard.

Statistical analysis: Thomsen, Ingebrigtsen, Marott, Dahl, Nordestgaard.

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Study supervision: Dahl, Lange, Vestbo, Nordestgaard.

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