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Serum Amyloid A Is a Biomarker of Acute Exacerbations of Chronic Obstructive Pulmonary Disease

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Rationale: Much of the total disease burden and cost of chronic obstructive pulmonary disease (COPD) is associated with acute exacerbations of COPD (AECOPD). Serum amyloid A (SAA) is a novel candidate exacerbation biomarker identified by proteomic screening.

Objectives: To assess SAA as a biomarker of AECOPD.

Methods: Biomarkers were assessed (1) cross-sectionally (stable vs. AECOPD; 62 individuals) and (2) longitudinally with repeated measures (baseline vs. AECOPD vs. convalescence; 78 episodes in 37 individuals). Event severity was graded (I, ambulatory; II, hospitalized; III, respiratory failure) based on consensus guidelines.

Measurements and Main Results: Presumptively newly acquired pathogens were associated with onset of symptomatic AECOPD. In the cross-sectional study, both SAA and C-reactive protein (CRP) were elevated at AECOPD onset compared with stable disease (SAA median, 7.7 vs. 57.6 mg/L; P < 0.01; CRP median, 4.6 vs. 12.5 mg/L; P < 0.01). Receiver operator characteristics analysis was used to generate area-under-curve values for event severity. SAA discriminated level II/III events (SAA, 0.88; 95% confidence interval, 0.80–0.94 vs. CRP, 0.80; 95% confidence interval, 0.70–0.87; P = 0.05). Combining SAA or CRP with major symptoms (Anthonisen criteria, dyspnea) did not further improve the prediction model for severe episodes. IL-6 and procalcitonin were not informative.

Conclusions: SAA is a novel blood biomarker of AECOPD that is more sensitive than CRP alone or in combination with dyspnea. SAA may offer new insights into the pathogenesis of AECOPD.

Keywords: chronic obstructive pulmonary disease; exacerbation; biomarker; inflammation

Chronic obstructive pulmonary disease (COPD) is now one of the leading causes of mortality and morbidity worldwide (1). Patients with moderate to severe COPD are particularly susceptible to recurrent acute exacerbations of COPD (AECOPD), which account for much of the total disease burden. AECOPD have been associated with accelerated decline in lung function (2), increased morbidity, decreased quality of life (3), and increased mortality, often from later cardiovascular system events (4). In most health care systems, AECOPD are also the major cost component of the disease, particularly if they lead to subsequent hospitalization (5, 6). Despite their importance, AECOPD and their severity remain difficult to define objectively. AECOPD are therefore usually defined operationally as “a sustained worsening of the patient’s condition that is acute in nature and necessitates change in regular medication” (7) and/or by increased consumption of health care resources (8). In this study, we used the American Thoracic Society/European Respiratory Society (ATS/ERS) consensus definition (“an acute change in a patient’s baseline dyspnea, cough, and/or sputum beyond day-to-day variability sufficient to warrant a change in therapy”) to define exacerbations, and the ATS/ERS exacerbation severity grades: level I, ambulatory; level II, hospitalization; and level III, respiratory failure (9).

The majority of AECOPD are known to be associated with acute respiratory tract infection, although some may also be triggered by pollutants, cold weather, or irritants (10). Respiratory viral infection and the acquisition of a new bacterial strain have both been characterized in AECOPD (11, 12). AECOPD caused by pathogens are associated with increased lung and systemic inflammation (13, 14). This increase in inflammation has opened the possibility of finding systemic biomarkers of disease induced by inflammatory mediators. Recently, considerable attention has focused on C-reactive protein (CRP), a hepatic, acute-phase protein induced by inflammatory mediators, such as IL-6, IL-1β, and tumor necrosis factor (TNF)-α, and the level of which, consequently, reflects inflammation. The potential utility of biomarkers is reflected in the observations that basal CRP levels are related to lung function decline (15). CRP may also be a marker of comorbid cardiovascular disease and mortality risk; in addition, CRP levels rise in AECOPD (16, 17).

On the basis of evidence that AECOPD are associated with systemic inflammation, forerunner experiments to this study, we used a highly sensitive, but nonquantitative, surface-enhanced laser desorption ionization time-of-flight (SELDI-ToF) proteomics method to screen for novel candidate biomarkers in an AECOPD serum collection. We found an 11,699-D protein mass peak corresponding to serum amyloid A (SAA) in the sera of patients with elevated CRP who had been hospitalized for severe AECOPD (see the online supplement). Because the association...
of SAA with AECOPD or AECOPD severity has not previously been reported, SAA was selected for prospective validation studies.

SAA is an acute-phase protein, induced, like CRP, by inflammatory mediators, including IL-6, IL-1β, and TNF-α, that rise acutely in AECOPD (18). SAA is secreted from the liver as the predominant apolipoprotein associated with plasma high-density lipoprotein cholesterol. The human SAA gene family is comprised of four members: SAA1 and SAA2 are induced during inflammation or in response to tissue injury; SAA3 is a pseudogene; and SAA4 is constitutively expressed.

Because SELDI-ToF is semiquantitative and poorly suited to repeat-measurement studies, we have used quantitative measurements of SAA by an ELISA assay, which detects induced SAA1 and SAA2 to compare the sensitivity and specificity of SAA to CRP, IL-6, and procollagen as biomarkers of AECOPD, both cross-sectionally and longitudinally. Furthermore, we analyzed whether SAA was better associated with event severity than was CRP and other putative biomarkers. Our data suggest that SAA is a systemic biomarker of AECOPD that contrasts favorably with CRP, whether measured alone or used in concert with major inflammatory mediators, including IL-6, IL-1β, and TNF-α.

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METHODS

Patients were recruited from the Melbourne Longitudinal COPD Cohort (MLCC), an open cohort of participants with moderate to severe COPD (GOLD [Global Initiative for Chronic Obstructive Lung Disease] stages II–IV). The MLCC is comprised of patients who have previously presented with acute severe AECOPD to the Royal Melbourne Hospital (RMH) in Melbourne, Australia. After discharge, patients in the MLCC are routinely managed in the community by practice nurses in consultation with chest physicians based at RMH and local general physicians. Additional subjects for cross-sectional analysis were recruited from patients presenting with acute AECOPD at RMH or at general practice. In addition, baseline SAA and CRP values were also obtained in nine age-matched, healthy volunteers and nine patients with obstructive sleep apnea recruited from the RMH sleep clinic.

Study Design

The primary objective of the study was to test the hypothesis that SAA is a blood biomarker, the level of which rises during infectious AECOPD. The secondary objective of the study was to relate changes in SAA to event severity in comparison with CRP, other putative biomarkers, and standard clinical assessment. We report a cross-sectional analysis at two time points (coinciding with stable COPD and AECOPD) in 62 patients for 62 events. We also report a more detailed longitudinal study of 37 patients for 78 events (measured at three time points: stable COPD, AECOPD, and recovery) with virological and microbiological assessment, including respiratory viruses (multiplex polymerase chain reaction [PCR], atypical pneumonia serology, sputum [if available] for bacterial culture, and serum collection). A patient flow chart is shown in Figure 1, and their characteristics and regular treatment are reported in Table 1. Inclusion criteria were stable, moderate to severe COPD (GOLD criteria). Baseline data from patients with clinically stable disease included demographics, spirometry, carbon monoxide diffusing capacity, exercise tolerance, and symptom severity. Exclusion criteria were living more than 50 kilometers from the hospital, lung cancer, chronic systemic inflammatory conditions, renal failure requiring dialysis, and the need for palliative care. The Human Research Ethics Committee of Melbourne Health (Royal Melbourne Hospital) approved the study, and written informed consent was obtained from all subjects.
nasocongestion, sore throat, myalgia or headaches, subjective fever, chills, or rigors (20–22). Patients were referred to hospital for additional assessments (chest X-ray and arterial blood gases) if their oxygen saturation was less than 88% on room air or usual domiciliary oxygen therapy, or there was clinical evidence of pneumonia, severe respiratory distress, or altered mental status.

**Acute Effect of Oral Steroids**

We directly tested whether oral steroids altered SAA or CRP in a separate analysis in a group of eight patients admitted for AECOPD in order to formally assess this potentially confounding variable. Blood was collected at four time points: (1) when stable; (2) on admission before steroid administration (100 mg i.v. hydrocortisone three times a day or 25–50 mg p.o. prednisolone); (3) 24 hours after steroids administration; and (4), when available, a recovery sample was collected.

**Definition and Severity Grading of Exacerbations**

Stable AECOPD was defined as no requirement for increased treatment above maintenance therapy, other than bronchodilators, for 30 days. AECOPD were defined by ATS/ERS consensus criteria (8), and also met the Anthonisen criteria; (i.e., increased dyspnea, increased sputum purulence or amount or increased dyspnea accompanied by signs of viral upper respiratory tract infection) (23). Resolution of an AECOPD was defined as: (1) completion of treatment with antibiotics and increased steroids; (2) return of symptoms to baseline levels for 48 hours; or (3), if symptoms had not fully resolved within 30 days of onset, that symptoms were stable for 48 hours and did not require further acute treatment.

**Grading of exacerbation severity.** Peak clinical severity reached between Days 1 and 14 of each exacerbation episode was used as the severity outcome. At resolution, the overall exacerbation severity was coded as the maximum severity of each episode. We used the ATS/ERS consensus operational criteria to grade severity as follows: ambulatory (level I); requiring hospitalization (level II); and acute respiratory failure (level III) (9). Chest X-ray was performed if there was clinical evidence of pneumonia.

**Virology and Microbiology**

Respiratory virus multiplex PCR and atypical serology were performed at the Victorian Infectious Disease Reference Laboratory (24), a World Health Organization reference laboratory. Nose and throat swabs for multiplex PCR were collected at onset of the episode (Days 1–5). The following viruses were screened: influenza A and B; picornavirus (rhinovirus); respiratory syncytial virus (RSV); parainfluenza; and adenovirus (24). Spontaneously expectorated sputum samples were obtained at AECOPD onset. In patients who were chronically colonized with potentially pathogenic bacteria, increased color and amount of sputum (25), or a change in pathogen detected on sputum culture, was used to indicate an AECOPD. Bacteria cultured from sputum with a bacterial load of at least $2 \times 10^6$ cfu were coded as bacteria positive. Sputum microscopy and culture were performed by the clinical diagnostic laboratory, Melbourne Pathology, using standard techniques.

Blood was obtained on Days 1 and 30 of each AECOPD and screened for antibodies for influenza A and B, Chlamydia pneumoniae, C. psittaci, Legionella pneumophila, and Mycoplasma pneumoniae (24). An AECOPD associated with an atypical pathogen was defined as at least a fourfold rise in serum antibody titer between the Day-1 and Day-30 specimens.

**Measurement of Inflammatory Serum Markers**

Measurements were performed blinded and independently from the clinical and microbiological assessment of exacerbations using standard clinical tests. CRP was first assessed by immunoturbidimetry (detection range, 5–300 mg/L; values > 300 mg/L were obtained by automated dilution; Olympus Diagnostica GmbH, Hamburg, Germany). CRP values below 5 mg/L were determined by high-sensitivity ELISA (detection limit, 0.35 ng/ml; Alpha Diagnostics, San Antonio, TX). ELISA was used to determine SAA (detection limit, 1.1 ng/ml; Anogen, Mississauga, ON, Canada) and IL-6 (OptEIA ELISA, detection limit, 4.7 pg/ml; Becton-Dickinson, San Diego, CA). Procalcitonin was determined by immunoluminometric assay (PCT LIA, detection limit, 0.3 ng/ml; BRAHMS, Henningsdorf, Germany). SAA is composed of four family members (SAA1–SAA4), with only SAA1 and SAA2 induced during the acute response (26). The assay we used identifies both SAA1 and SAA2.

**Identification of SAA by SELDI-ToF Proteomics**

See Figure 2 and the online supplement for details.

**Statistical Analyses**

The distributions of SAA and CRP values were log-normal, and data were natural logarithm transformed. Data are presented as median and interquartile range with 95% confidence interval (CI). Analyses of key findings are also reported for absolute values.

**Analysis of cross-sectional study.** In the cross-sectional study of 62 AECOPD (one AECOPD event per patient with COPD, obstructive sleep apnea, and normal reference groups), the difference in log$_2$(SAA) and log$_2$(CRP) between stable COPD, reference groups, and AECOPD was assessed using analysis of variance. These data are reported in Figure 3. To measure whether there was a significant difference in the magnitude of change in CRP and SAA associated with milder community-managed AECOPD (level I) and AECOPD requiring hospitalization (level II/III), the change in log$_2$(SAA) and log$_2$(CRP) was compared between groups using unpaired $t$-tests. To determine whether SAA and CRP differentiated between stable COPD and AECOPD onset, the area under the curve (AUC) receiver operating characteristics (ROC) for each marker was analyzed, first to differentiate level I AECOPD from stable COPD, and second to differentiate level II/III from stable COPD. Absolute SAA and CRP values were used in this analysis so that potential diagnostic cutoffs could be evaluated. These data are reported in Figure 4.

**Acute effect of oral glucocorticosteroids on biomarker levels over 24 hours.** The effect of steroids on biomarker levels was assessed by paired $t$ test. These data are reported in Figure 5.

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**Figure 2.** Surface-enhanced laser desorption ionization time-of-flight (SELDI-ToF) ProteinChip analysis. The top panel of each pair is a representative mass spectral serum profile from exacerbating samples (acute exacerbations of chronic obstructive pulmonary disease [AECOPD]) or stable samples (Stable) subjected to SELDI-ToF mass spectrometry, as detailed in Methods (n = 4). The bottom panel of each pair is a pseudogel image (pseudogel) of the spectral profiles showing relative abundance and identifying a cluster of proteins approximately 11.7 kDa in size (rectangle) induced during an AECOPD, which correspond to the molecular weight of serum amyloid A (SAA).
AECOPD, severity was subsequently defined as a binary categorical mine cutoff values for blood marker levels to potentially predict severe disease severity and susceptibility to more severe AECOPD. To deter-

developed to adjust for between-patient differences in the underlying logistic regression model that included FEV1 % predicted was then included in the same patient during the course of the study. A multivariable, ordinal regression models for repeated AECOPD episodes where they occurred were similar when the analysis incorporated multiple exacerbations on restricted to one exacerbation per subject, but the results and conclusions errors (27). The primary analysis of within-subject change was stringently applied. The statistical methodology used was a linear regression model based upon a generalized estimating equation (GEE) with an exchangeable correlation structure and robust standard correlation of repeated measures (both within-exacerbation and between-exacerbations) was applied. The statistical methodology used was a linear regression model based upon a generalized estimating equation (GEE) with an exchangeable correlation structure and robust standard errors (27). The primary analysis of within-subject change was stringently restricted to one exacerbation per subject, but the results and conclusions were similar when the analysis incorporated multiple exacerbations on individual subjects (allowing for correlation within subjects over repeated exacerbations). These data and further details of GEE analysis are presented in the online supplement.

**Analysis of longitudinal study.** First, in the longitudinal study of 78 AECOPD from 37 individuals, the change in SAA and CRP between stable COPD, AECOPD, and recovery was assessed by analysis of variance of log data, and is reported in Figure 6. As AECOPD severity increases with increasing disease severity, it is easily conceivable that repeated AECOPD in a subset of patients could skew the data; therefore, the primary analysis of these data was restricted to one AECOPD (first event) per patient. Second, to analyze repeated AECOPD episodes from the same patient, a further statistical model that allows for within-subject correlation of repeated measures (both within-exacerbation and between-exacerbations) was applied. The statistical methodology used was a linear regression model based upon a generalized estimating equation (GEE) with an exchangeable correlation structure and robust standard errors (27). The primary analysis of within-subject change was stringently restricted to one exacerbation per subject, but the results and conclusions were similar when the analysis incorporated multiple exacerbations on individual subjects (allowing for correlation within subjects over repeated exacerbations). These data and further details of GEE analysis are presented in the online supplement.

**Analysis of relationship between biomarkers and severity of AECOPD.** CRP is known to be elevated in stable COPD (28, 29). To control for raised blood marker levels in stable disease, a difference score was generated as the difference between log-transformed values at stable COPD, AECOPD, and recovery was assessed by analysis of variance. Note the broken axis.

**RESULTS**

**SAA Proteomics**

SAA was initially identified as a candidate biomarker by SELDI-ToF proteomics (see Figure E2 in the online suppl-
Cross-sectional Study: Patient and AECOPD Characterization

The first AECOPD event occurring following recruitment in a total of 62 patients was included in this analysis. Participants’ baseline characteristics and regular treatment are shown in Table 1. Spirometry confirmed that all patients had moderate to severe COPD (GOLD II–IV); mean FEV₁ was 35% predicted (range, 20–56%), and reversibility was less than 15%. A total of 26% of patients were on long-term home oxygen. Mean pack-years of smoking was 52 (range, 10–160 pack-years), and 26% was current smokers. For comparison, inflammatory marker levels were also obtained in 18 age-matched control subjects without COPD, 9 healthy volunteers, and 9 patients attending the outpatient sleep disorders clinic (Figure 3). A total of 62 AECOPD was included in this analysis; 31 (50%) events were level I, and were therefore managed in the community with oral antibiotics and corticosteroids, and 31 (50%) required hospitalization (level II/III).

Serum CRP and SAA Levels during an AECOPD

The first AECOPD per patient (62 patients and 62 AECOPD first events) was included in the primary analysis. Mean time from AECOPD onset to obtaining serum for measurement of inflammatory markers was 6.2 days. SAA and CRP were elevated at exacerbation onset compared with stable disease (SAA median: stable COPD, 7.7 mg/L vs. AECOPD, 57.6 mg/L [P < 0.01]; CRP median: stable COPD, 4.6 mg/L vs. AECOPD, 12.5 mg/L [P < 0.01]) (Figure 3).

AECOPD severity and change in SAA and CRP at event onset. Only SAA was significantly elevated in level II/III events vs. level I (Figure 4A). Median change in SAA level II/III events was 2.12 mg/L (95% CI, 1.80–3.21 mg/L) versus level I events (1.23 mg/L; 95% CI, 0.56–2.05 mg/L) (P < 0.05). No significant difference in CRP level I events (0.63 mg/L; 95% CI, 0.30–1.47) versus level II/III events (1.49 mg/L; 95% CI, 0.81–2.0) was observed (Figure 4A). ROC curves are useful to assess possible diagnostic cut-off point(s) for biomarker levels. Both markers modestly distinguished level I AECOPD from stable COPD, as ROC analysis generated AUC values of 0.71 (95% CI, 0.61–0.80) for SAA and 0.66 (95% CI, 0.56–0.76) for CRP. SAA was significantly better at differentiating level II/III AECOPD from stable COPD with an AUC value of 0.88 (95% CI, 0.80–0.94) versus CRP with an AUC value of 0.80 (95% CI, 0.70–0.87) (P = 0.05; see Figure E4B and the online supplement). A biomarker cut-off of 12.5 mg/L was 87% sensitive for severe AECOPD with a negative predictive value of 92% for SAA and 54% sensitive with a negative predictive value of 79% for CRP.

Effect of oral glucocorticosteroids. Oral glucocorticosteroids neither acutely enhanced nor suppressed biomarker levels significantly (Figure 5).

Longitudinal Community-based Cohort

A total of 78 AECOPD from 37 patients were assessed, with 63% of patients exacerbating more than once during the study period. Participants’ baseline characteristics and regular treatment are shown in Table E1. Spirometry confirmed that all patients had moderate to severe COPD (GOLD II–IV); mean FEV₁ was 41% predicted (range, 15–69%), and mean FEV₁/FVC ratio was 45% (24–69%). Reversibility was less than 15%. A total of 24% were on long-term home oxygen, mean pack-years
of smoking was 45 (range, 10–115 pack-years), and 16% were current smokers.

**Exacerbation characteristics.** The mean time of 2.4 days from symptom onset to obtaining serum for measurement of inflammatory markers was shorter than that for the cross-sectional study. Figure 6 includes single first events only, and shows that both SAA and CRP rose significantly during AECOPD and then resolved: SAA stable COPD, 6.81 mg/L (95% CI, 7.1–19.3); AECOPD, 68.00 mg/L (95% CI, 50.9–601.4); recovery, 4.41 mg/L (95% CI, 0.5–58.0); CRP stable COPD, 6.0 mg/L (95% CI, 4.6–13.1); AECOPD, 13.0 mg/L (95% CI, 11.9–52.0); recovery, 4.0 mg/L (95% CI, 2.2–28.7). Figure 7 presents a descriptive account of four randomly selected patients experiencing multiple AECOPD. Although the severity of underlying COPD is a known predictor of AECOPD severity, it is noteworthy that biomarker changes are reflected in individual AECOPD severity events, and that CRP and SAA are not always concordant.

**Inflammatory markers and AECOPD etiology.** The likelihood of detecting a pathogen increased with AECOPD severity. Presumptively newly acquired pathogens at AECOPD onset were detected (Figure 8A); respiratory viruses were identified by multiplex PCR in 26% of exacerbations (picornavirus, n = 14; parainfluenza virus, n = 3; influenza A, n = 1; RSV, n = 1; adenovirus, n = 1). Mean viral symptom score was 3.4 in PCR-negative and 4.4 in PCR-positive AECOPD; 23% of patients with AECOPD had a viral symptom score of greater than or equal to 4 in the absence of a positive PCR, and were therefore considered to have a probable viral infection. Bacteria were identified on sputum culture in 38% of exacerbations; *H. influenzae* (n = 9), *P. aeruginosa* (n = 7), and *S. pneumoniae* (n = 5) were prominent. Coinfection with both virus and bacteria identified on pathology samples occurred in 15% of AECOPD. Both CRP and SAA levels were elevated in bacteria-associated AECOPD versus nonbacterial AECOPD, although only SAA levels in coinfection events was significantly different from noninfective episodes (Figures 8B–8C). We also assessed the value of acute changes in CRP and SAA to predict bacterial infection using logistic regression. Specifically, the odds of a bacterial AECOPD, irrespective of the cut-point used, increased 57% per 1-unit increase in loge CRP (odds ratio, 1.57; 95% CI, 1.03–2.39; *P* = 0.036), and increased 39% per 1-unit increase in loge SAA (odds ratio, 1.39; 95% CI, 1.04–1.84; *P* = 0.022).

**Serum CRP, SAA, and IL-6 Levels during an AECOPD**

A table comparing first events to all events (including patients with multiple exacerbations) is presented in the online supplement.
It is noteworthy that the inclusion of patients with recurrent exacerbations would not have biased this result. When the analysis was restricted to one exacerbation per subject, the data showed strong evidence of within-subject increase in both SAA and CRP levels from “stable” to “acute.” Specifically, SAA levels increased by a ratio of 3.70 (95% CI, 1.87–7.32; P < 0.001), whereas CRP levels increased by a ratio of 2.60 (95% CI, 1.00–6.88; P = 0.05). The absolute median value for stable COPD was 3.6 (95% CI, 4.6–8.0) versus 5.6 for AECOPD (95% CI, 3.1–38.2; P < 0.05), but there was no significant increase in procalcitonin, which rose in only one patient with confirmed pneumonia. The results and conclusions were similar when we assessed data on all exacerbations (including multiple exacerbations in some individuals) using a linear regression model based upon a GEE that corrects for within-subject correlation of repeated measures within and between exacerbations (see online supplement).

### Predicting severe AECOPD using the ratio of AECOPD onset to stable baseline values for each inflammatory marker.

For IL-6, the AUC value for the ratio of AECOPD onset to stable IL-6 was 0.59 (95% CI, 0.41–0.78), and was not informative. The SAA AUC of 0.84 (95% CI, 0.74–0.94) was significantly different from the CRP AUC of 0.71 (95% CI, 0.56–0.87; P < 0.05). At a cutpoint of a twofold change in inflammatory marker levels, CRP was 80% sensitive and 54% specific, with a positive likelihood ratio of 1.73 and a negative likelihood ratio of 0.37. A twofold change in SAA was 100% sensitive and 44% specific, with a positive likelihood ratio of 1.80 and negative likelihood ratio of 0.00. These data indicate that, if a twofold change in SAA is not observed at the onset of AECOPD, a severe AECOPD (level III; respiratory failure) can be excluded. In contrast, a twofold change in CRP was both less sensitive and less specific, and alternative cut-off points did not improve its diagnostic precision. If the cutoff was increased to a 4.6-fold change in SAA, the specificity increased from 44 to 68%, whereas the sensitivity remained high (93%). At the same 4.6-fold change cut-point, CRP was 60% sensitive and 74% specific.

### Combining SAA or CRP with clinical symptoms to predict severe AECOPD.

We analyzed whether change in a major symptom (dyspnea severity; sputum purulence or volume) (23, 31) at onset of AECOPD was predictive of severe AECOPD. CRP was no better then dyspnea (P = 0.51) or Anthonisen criteria (P = 0.60) in predicting severe episodes, whereas SAA proved to be significantly better than dyspnea (P = 0.01) and Anthonisen (P = 0.04). Using logistic regression to combine CRP or SAA with a major symptom did not improve the prediction model for severe AECOPD over SAA alone. This indicates that SAA is a highly sensitive and specific marker of severe AECOPD that is discriminative even in the absence of additional clinical information.

### DISCUSSION

In this article, we have identified SAA as a novel blood biomarker of AECOPD. Our data show that change in SAA is associated with event severity, and SAA was found to have greater sensitivity and specificity in describing AECOPD than did CRP when assessed by ROC. Our data indicate that a fourfold or greater increase above basal in SAA alone was associated with severe AECOPD, and this discriminative value was not further improved by combining SAA with major clinical signs (dyspnea and sputum). SAA levels may therefore be a useful adjunct in the objective early identification and management of AECOPD, and may also help to identify those patients at greatest risk of respiratory failure in most need of hospitalization. The inflammatory markers, IL-6 and procalcitonin, which have been used to guide antibiotic treatment in pneumonia (32), were uninformative.

The association of change in SAA with severity is of considerable interest, as objectively grading the severity of AECOPD is difficult. Current approaches use a combination of functional criteria, clinical symptoms, and measurements (9). Objective surrogates of severity have proven difficult to identify. In a meta-analysis of 268 studies involving 142,407 patients, Franciosi and colleagues (30) found that only arterial CO₂ and respiratory rate were potential surrogates for severity. Pinto-Plata and colleagues have recently reported the clinical association of serum IL-6, TNF, and IL-8 with clinical indices of severity (33, 34). CRP is known to rise acutely during infective AECOPD (35), and its levels may also represent a useful predictor of exacerbations when combined with clinical signs, notably dyspnea (31). Consistent with Hurst and colleagues (31) we found that CRP rise was not significantly associated with event severity. Further prospective research will be necessary to determine the role of SAA as a predictor of event severity.
We found that SAA was associated with infection. The present study is consistent with the recent findings of Papi and colleagues, who showed that AECOPD severity tracks with increased airway inflammation (13). Almost all these exacerbations were associated with proven infection or signs of infection (13). Like CRP, SAA is induced in the liver by IL-1β and IL-6, the levels of which are known to rise in AECOPD (14, 36). Accordingly, increases in SAA have previously been reported in a number of inflammatory diseases (37, 38) and in viral lung infections, including severe acute respiratory syndrome (39). We found that SAA was modestly elevated at baseline. To our knowledge, there has been no previous systematic study of SAA in COPD or AECOPD. Nel and colleagues reported acute-phase changes in bronchiectasis and bronchial carcinoma (40); Gao and colleagues reported twofold elevations of SAA in lung cancer (protein array), but not in COPD (41); Parnham and colleagues reported SAA in the normal range (<5 mg/L) in stable disease (42); Falsey and colleagues reported three patients with elevated SAA 4 weeks after influenza infection (43). Elevated SAA was also found in patients with cardiovascular disease where COPD was a comorbidity (44). About half of all deaths from COPD are associated with cardiovascular events, and the incidence of such events increases markedly after AECOPD (4, 45). Because SAA level is a good predictor of coronary artery disease and a strong predictor of future cardiovascular events in women (44), we also speculate that blocking SAA or its actions may be of therapeutic benefit in COPD, AECOPD, and, possibly, comorbid cardiovascular disease.

As both CRP and SAA are induced in the liver by the same inflammatory cytokines, their differential behavior in our study is interesting. The main difference between SAA and CRP production is that SAA can also be produced extracellularly in inflamed tissue (26, 46, 47). This raises the possibility that SAA production in the inflamed lung, as well as hepatic SAA, may have contributed to the SAA that we measured in this study. Consistent with this possibility, SAA, but not CRP, has recently been detected in the lungs of smoke-exposed, endotoxin-challenged mice, and SAA has been identified, but not quantified, in bronchoalveolar lavage fluid of smokers with COPD using high-sensitivity proteomics (48, 49). It is currently not possible to reliably detect SAA in sputum, and we are developing methods to test this possibility during AECOPD. This would also allow direct comparison to be made with sputum inflammatory indices and sputum virology and bacteriology.

Our study also raises the issue of whether SAA is a marker or a mediator of AECOPD. There is now extensive evidence that CRP is both a marker and a likely mediator of disease. SAA directly binds gram-negative bacteria and acts as an innate immune opsonin, leading to enhanced clearance of pathogens, which suggests a beneficial role (50). However, SAA has direct proinflammatory activities by inducing activation and chemotaxis of neutrophils and other inflammatory cells (51–53), promoting release of matrix metalloproteases (54), and inducing proinflammatory cytokines, including those in the IL-17/IL-23 axis now implicated in mediating neutrophil inflammation (55, 56). Surprisingly, SAA is also a G-protein–coupled receptor (FPLR1/LXA4R) agonist. The FPLR1/LXA4R receptor is unusual, because it is shared by two ligands, both the inflammatory bacterial formyl peptide and also the antiinflammatory eicosanoid, lipoxin A4 (57). Lipoxins have recently been recognized as important inflammation-resolving mediators. Furthermore, it has very recently been discovered that SAA promotes neutrophil survival via P2X7 purinergic receptors independently of FPLR1 (58). SAA could therefore both worsen inflammation and, we speculate, blunt resolution of inflammation by interfering with lipoxin signaling, collectively intensifying or prolonging AECOPD. This possibility remains to be tested. Although we found that systemic steroids had no acute effect on SAA, SAA levels measured longitudinally might also be a surrogate marker for the efficacy of other COPD treatments.

There are a number of caveats to this initial study. We analyzed both change in biomarker levels and absolute amount, and found identical statistical outcomes. Similarly, we found that stable SAA levels in this study (7.7 mg/L) were modestly higher than the normal range (1–5 mg/L), and we calculated absolute value “diagnostic cut-off” levels from ROC curves. However, until replicated independently and further validated independently, it would be injudicious to use the SAA absolute values reported here to guide practice. SAA performed best when change from basal levels were determined early in the course of an AECOPD, as the ROC values in the longitudinal substudy (2.3 d from onset of symptoms to collection of sample) were higher than the those in the cross-sectional study (6.7 d lag). In the future, SAA may emerge as a particularly well-suited biomarker for at-home, community-based, or early generalist point-of-care disease management approaches. SAA might also form part of a panel of markers analyzed in an algorithm, because the feasibility of protein miniaarray methods in AECOPD has now been demonstrated by Pinto-Plata and colleagues (33, 34). RSV rates were consistent with those that we have previously reported in this cohort and the community rate recorded during the study period (Infectious Disease Reference Laboratory), but are lower than those reported in the East London cohort (59). Although almost all of our patients were being treated with an inhaled, long-acting β2-adrenoceptor agonist/steroid combination, it is known that SAA induction is not suppressed by glucocorticosteroids in vitro or in vivo (60, 61). We also found no acute effect of oral prednisolone or intravenous hydrocortisone on SAA when we directly tested this possibility. Further validation will also be required in patient populations excluded from the present study, such as those with mild disease or comorbid, chronic, systemic inflammatory conditions. In the cross-sectional study, many of our patients with AECOPD developed fluid overload, and it would be of some interest to study whether combining SAA with β naturetic peptide, a marker of heart failure, might be useful to distinguish these distinct causes of breathlessness (62). 27% of our subjects had congestive heart failure. However, future studies would need to account for potentially confounding elevated β naturetic peptide secondary to right heart failure to pulmonary hypertension, which is common in COPD. Procalcitonin has also been suggested as a marker of severity, but we did not find it informative, perhaps because procalcitonin levels might better reflect pneumonia than AECOPD severity (32, 63, 64). Copeptin, the precursor of vasopressin, has also recently been described as a possible biomarker of type III AECOPD (63, 64). It would also be particularly useful to discover other biomarkers that can be combined with SAA to distinguish bacterial from viral infectious causes of AECOPD. IFN-γ–inducible 10-kD protein is one possibility that is an area of ongoing research in our group (65).

In conclusion, our data identify SAA as a novel biomarker of AECOPD, the levels of which are related to event severity. Accordingly, SAA may have utility in the clinical identification, classification, and management of AECOPD. We propose that the biology of SAA may also offer new insights into pathogenic mechanisms in COPD and AECOPD.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.
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