Mortality risk prediction in COPD by a prognostic biomarker panel

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ABSTRACT Chronic obstructive pulmonary disease (COPD) is a complex disease with various phenotypes. The simultaneous determination of multiple biomarkers reflecting different pathobiological pathways could be useful in identifying individuals with an increased risk of death.

We derived and validated a combination of three biomarkers (adrenomedullin, arginine vasopressin and atrial natriuretic peptide), assessed in plasma samples of 385 patients, to estimate mortality risk in stable COPD. Biomarkers were analysed in combination and defined as high or low.

In the derivation cohort (n=142), there were 73 deaths during the 5-year follow-up. Crude hazard ratios for mortality were 3.0 (95% CI 1.8–5.1) for one high biomarker, 4.8 (95% CI 2.4–9.5) for two biomarkers and 9.6 (95% CI 3.3–28.3) for three high biomarkers compared with no elevated biomarkers. In the validation cohort (n=243), 87 individuals died. Corresponding hazard ratios were 1.9 (95% CI 1.1–3.3), 3.1 (95% CI 1.8–5.4) and 5.4 (95% CI 2.5–11.4). Multivariable adjustment for clinical variables as well as the BODE (body mass index, airflow obstruction, dyspnoea, exercise capacity) index and stratification by the Global Initiative for Chronic Obstructive Lung Disease stages provided consistent results. The addition of the panel of three biomarkers to the BODE index generated a net reclassification improvement of 57.9% (95% CI 21.7–92.4%) and 45.9% (95% CI 13.9–75.7%) at 3 and 5 years, respectively.

Simultaneously elevated levels of adrenomedullin, arginine vasopressin and atrial natriuretic peptide are associated with increased risk of death in patients with stable COPD.

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Introduction

Chronic obstructive pulmonary disease (COPD) is associated with significant morbidity and mortality. It is currently estimated to be the fourth highest cause of death in the world, accounting for 3 million deaths worldwide annually [1]. Accurate prediction of mortality is important because it helps identify patients in whom the implementation of therapeutic measures can improve outcomes [2]. A recent approach explored the association of clinical characteristics with specific biomarkers of disease activity and/or progression [2, 3]. Several serum biomarkers have been independently associated with increased risk of death [4–8] and a few have been shown to further contribute to mortality prediction when added to known clinical variables such as the BODE (body mass index (BMI), airflow obstruction, dyspnoea, exercise capacity) index [2, 9, 10]. However, because COPD is a complex and heterogeneous disease with various phenotypes, it is likely that the goal of finding one single biomarker of COPD may not ever be realised [11]. Moreover, biomarkers may not necessarily have to come from the lung in order to be useful in predicting prognosis in COPD as these patients have other serious comorbidities that could contribute to mortality [12]. Therefore, a panel of prognostic biomarkers needs to be used reflecting the associated comorbidities and/or different pathobiological pathways that may be altered in this disease [3].

Adrenomedullin (ADM) is a 50/52 amino acid peptide expressed in bronchoalveolar epithelial cells, alveolar macrophages and pulmonary endothelium [13]. ADM is induced in response to bacterial exposure and hypoxia [14, 15]. In COPD airway inflammation, ADM may act primarily to promote tissue repair and maintain microvascular function [16, 17]. Arginine vasopressin (AVP) is a potent vasoconstrictor that promotes water retention and plays a role in the body's stress response by stimulating adrenocorticotrophic hormone secretion [18]. AVP also seems to reduce bronchial obstruction, shunt fraction and oedema formation, thereby improving gas exchange, reversing myocardial dysfunction, reducing plasma levels of nitrogen oxides and limiting pulmonary tissue concentrations of reactive nitrogen species [19]. Atrial natriuretic peptide (ANP), a 28 amino acid peptide hormone synthesised by the cardiac atria, has a wide range of cardioprotective effects, including inhibition of sympathetic nervous system activity and the renin–angiotensin–aldosterone system [20]. ANP also attenuates activation of inflammatory signalling by lipopolysaccharide and tumour necrosis factor-α in human pulmonary endothelial cells, and protects against bacterium-induced lung injury and pulmonary endothelial barrier dysfunction [21]. These mechanisms support its protective role against cardiopulmonary complications in patients with COPD undergoing lung cancer surgery [22]. Pro-ADM, pro-AVP and pro-ANP are stable, biologically inactive regional fragments of the respective prohormones and, as such, are surrogates for the mature proteins [23–25]. Circulating pro-ADM, pro-AVP and pro-ANP are predictors of mortality in COPD patients in the stable state as well as during exacerbation [4, 5, 9, 10].

As these three biomarkers are thought to mirror distinct aspects accounting for the complexity of COPD, we envisioned that their simultaneous measurement could be useful in identifying individuals with an increased risk of death. We also proposed that the accuracy achieved by the concomitant estimation of ADM, AVP and ANP would contribute to mortality prediction independent of clinical parameters. These hypotheses were derived and validated using data prospectively collected in two long-term studies that included well-phenotyped, stable patients with COPD and specifically aimed to identify predictors of outcome using systemic markers in COPD.

Methods

Study designs and ethics

Derivation cohort study subjects (n=142) were participants in the Pro-ProCOLD (procalcitonin-guided antibiotic therapy in acute exacerbations of COPD: a randomised trial) study, a single-centre, long-term, observational, nested-cohort study at University Hospital Basel, Basel, Switzerland. Derivation cohort study subjects were ProCOLD study participants with a history of severe acute exacerbation of COPD in the previous year. For the current analysis, clinical, laboratory and lung function data were collected in the stable state, 6 months after a severe exacerbation of COPD. The original ProCOLD publication describes all protocol details [26].

Validation cohort study subjects (n=243) were participants in the Predicting Outcome using Systemic Markers in Severe Exacerbations of COPD (PROMISE-COPD) study, which evaluated variables potentially identifying poor outcomes in patients with moderate-to-very severe COPD. Moderate-to-very severe disease was defined as post-bronchodilator forced expiratory volume in 1 s (FEV_1)/forced vital capacity ratio <70% and FEV_1 <80% predicted, *i.e.* Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage II–IV airway obstruction [27]. COPD exacerbation was defined as an acute change from baseline in one or more of dyspnoea, cough and sputum, beyond normal day-to-day variation and possibly warranting medication change. This nested analysis included 243 patients at University Hospital Basel and the

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anticipated follow-up period was 5 years. The PROMISE-COPD study was specifically designed to identify predictors of outcome using systemic markers in COPD. Participants had an initial baseline examination and were then followed for at least 2 years in scheduled semi-annual visits. In addition, patients made outpatient visits or were hospitalised for treatment of acute exacerbation of COPD, as necessary, and follow-up visits were specified for 4 weeks after exacerbation onset. Patients were treated as warranted by their clinical condition, without restriction, throughout the study period.

Both the Pro-ProCOLD and the PROMISE-COPD study were investigator-initiated and -driven studies that complied with the Helsinki Declaration and Good Clinical Practice Guidelines, and were approved by the participating centres' ethics committees. The PROMISE-COPD study was registered at www.controlled-trials.com under the identifier ISRCTN99586989. Patients provided prior written, informed consent for all study assessments.

Patients

Both the derivation and validation cohorts consisted of patients consecutively recruited and followed at University Hospital Basel. To be eligible for the study, patients had to meet the following inclusion criteria at baseline: 1) clinically stable moderate-to-very severe COPD based on anamnesis, physical examination and spirometry performed $\geqslant$4 weeks after resolution of the latest exacerbation; 2) age $\geqslant$40 years; and 3) smoking history $\geqslant$10 pack-years. Exclusion criteria were: 1) a non-COPD condition, for example bronchiectasis, asthma or pulmonary fibrosis, as the main respiratory disease; and 2) immunosuppression, including AIDS or a history of organ transplantation, or current chronic steroid use (>20 mg prednisolone-equivalent per day). Further exclusion criteria applied to the PROMISE-COPD study included rapid fatal disease and a musculoskeletal disorder preventing walking.

Baseline and scheduled visits assessment

For each patient, we performed a physical examination, registered vital signs, and obtained a detailed medical history including demographics, smoking status, current treatment, duration of disease, number and severity of exacerbations in the previous year, and comorbidities. Using the comorbidity data, an age-adjusted Charlson comorbidity index score was calculated for patients included in the validation cohort. We also obtained plasma samples for measurement of biomarkers and spontaneous sputum samples for quantitative bacterial culture. Spirometry and, for patients included in the validation cohort, 6-min walking distance (6MWD) were administered by trained technicians according to American Thoracic Society guidelines [28, 29]. Patients in the validation cohort were also assessed using the modified Medical Research Council (mMRC) dyspnoea scale, the St George's Respiratory Questionnaire (SGRQ) COPD version and the 36-item Short Form Health Survey (SF-36) health-related quality-of-life questionnaire. Except for 6MWD, which was performed annually, all other evaluations took place at each scheduled visit, *i.e.* on a semi-annual basis.

Outcome

Patients in the derivation and validation cohort were followed up for a median (interquartile (IQR)) duration of 1703 (615–3141) days and 1749 (1195–1943) days, respectively. Patients who survived throughout follow-up were categorised as survivors, whereas patients who died within the follow-up period were categorised as nonsurvivors. For the validation cohort, the cause of death was adjudicated based on the review of medical records (University Hospital Basel, neighbourhood institutions, nursing homes, daycare centres, emergency medical services and family physicians), and personal interviews with attending physicians and family physicians. Review of the medical records was performed by two independent, board certified pulmonary specialists. Discrepancies were settled by consensus. Vital status was additionally confirmed by family physicians and/or health insurance companies.

Pro-ADM, pro-AVP and pro-ANP

Blood samples for biomarker measurement were collected at visits into Vacutainer tubes, which were centrifuged at 3000 × *g* for 10–15 min to obtain plasma. Samples were stored at -80°C until analysis. For all three analytes, quantification was performed in duplicate within one run in a central, accredited laboratory by technicians unaware of the patients' clinical data, using automated sandwich immunoassays based on time-resolved amplified cryptate emission technology (KRYPTOR; Thermo Scientific Biomarkers, Hennigsdorf, Germany). The KRYPTOR MR-ProADM assay has a measurement range of 0.05–100 nmol·L^{-1} and a functional sensitivity of 0.25 nmol·L^{-1}. The Pro-AVP assay has a lower limit of detection of 0.4 pmol·L^{-1}and a functional sensitivity of <1 pmol·L^{-1} [12]. The Pro-ANP assay has a lower limit of detection of 4.3 pmol·L^{-1} and a functional sensitivity of 11.0 pmol·L^{-1}, with an interassay coefficient of variation <20% [8]. For each studied plasma biomarker, measurements below the limit of quantitation were imputed to that value.

Biomarkers were analysed in combination and defined as high or low according to cut-off points. Levels of pro-ADM, pro-AVP and pro-ANP were categorised using the cut-off points of 0.75 nmol·L^{-1}, 40 pmol·L^{-1} and 184 pmol·L^{-1}, respectively; all cut-off points had been previously, individually described during exacerbations of COPD in the derivation cohort [4, 5, 9, 10].

Statistics

Continuous variables are expressed as the mean±SD or median (IQR), and discrete variables as % (n). Comparisons of clinical characteristics at baseline between survivors and nonsurvivors were made with the Chi-squared test, Mann–Whitney U-test or the t-test, as appropriate.

First, we analysed the risk of death during the first 3 years of follow-up using logistic regression. For the derivation cohort, the calculation was based on Bayesian estimation for logistic regression [30]. It relies on the fact that very large odds ratios are nearly impossible. It has the advantage of providing reliable estimates if there are zero counts in cross-tables. Biomarker levels were log-transformed for analyses. Models were multivariable, adjusted for: age and comorbidities, including arterial hypertension, cardiopathy, malignancy, diabetes mellitus and renal failure (derivation cohort), or age-adjusted Charlson score (validation cohort); sex; FEV_1 % pred; and smoking status. For the confirmation cohort, a further model included all four domains of the BODE index [31]. Assessment of model fit was by Hosmer–Lemeshow test.

Secondly, we analysed the risk of death 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 based on the Schoenfeld residuals [32]. Follow-up time for each participant began at study entry and ended at study discontinuation, death (n=73 and n=87 in the derivation and validation cohorts, respectively) or December 2013, whichever came first. Multivariable models were adjusted by age and comorbidities (derivation cohort) or age-adjusted Charlson score (validation cohort), sex, FEV_1 % pred and smoking status or the BODE index (validation cohort).

The incremental discriminative power offered by adding the biomarker panel to the clinical characteristics (comorbidities or age-adjusted Charlson score, sex, FEV_1 % pred and smoking status or the BODE index) was analysed using the net reclassification improvements (NRI) from combining these predictive modalities [33]. The NRI relies on reclassification tables generated separately for survivors and nonsurvivors, and quantifies the correct movement in categories, upwards for survivors and downwards for nonsurvivors. Positive and negative likelihood ratios (corrected for continuity) were calculated. To test for trend of risk estimates, groups based on increasing levels of pro-ADM, pro-AVP and pro-ANP were coded 0, 1, 2, *etc.* Absolute 1–5-year risk by groups of the biomarkers was estimated. All analyses utilised a two-sided p-value of 0.05 for significance and were performed using R version 2.5.1 (The R Project for Statistical Computing; www.r-project.org) or SPSS Statistics version 21.0 (IBM Corp., Armonk, NY, USA).

Results

The study design according to the STROBE (Strengthening the Reporting of Observational studies in Epidemiology) guidelines and patient disposition is summarised in figure 1. A total of 385 Swiss patients were included in the nested cohort analysis and completed the 5-year follow-up. For the validation cohort, the clinical characteristics of the patients excluded from the analysis because of incomplete biomarker data (n=14) were similar with regard to age, BMI, lung function, age-adjusted Charlson comorbidity score, BODE index, 6MWD and mMRC score (p-value nonsignificant for all). During a median (IQR) of 1703 (615–3141) days and 1749 (1195–1943) days of follow-up time, for the derivation and validation cohorts, respectively, 73 (51.4%) and 87 patients (35.8%) died. For the validation cohort, cause of death could be definitively ascertained in 48 cases (55.2%). The most common primary cause of death was cardiorespiratory failure (37.5%), followed by malignancy (11.4%) and others (6.8%).

Clinical characteristics of patients according to the number of increased biomarkers

Tables 1 and 2 compare the baseline clinical and physiological characteristics of subjects included in the derivation and validation cohorts according to number of increased biomarkers at inclusion. In both cohorts, there was a high prevalence of cardiovascular disease, including arterial hypertension, coronary arterial disease and congestive heart failure. For the derivation cohort, certain comorbidities (cardiopathy, diabetes mellitus and renal failure), the use of systemic glucocorticosteroids and lung function (forced vital capacity % predicted) differed significantly among patients characterised by the number of increased biomarkers. For the validation cohort, age, dyspnoea score, exercise capacity, the presence of certain comorbidities and their corresponding pharmacological treatment increased in parallel with the number of elevated biomarkers. By contrast, the BODE index, COPD pharmacological and nonpharmacological treatments, predicted lung function values as well as GOLD stages were generally similar among the four

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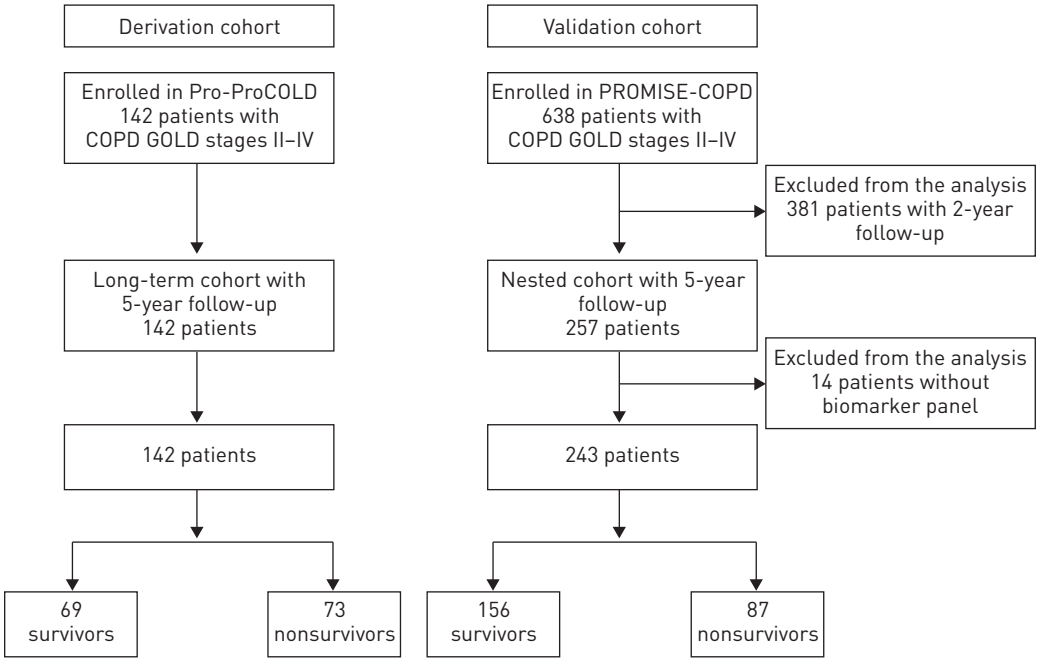

FIGURE 1 Study design for the present analysis according to the STROBE guidelines. Pro-ProCOLD: procalcitonin-guided antibiotic therapy in acute exacerbations of COPD: a randomised trial; PROMISE-COPD: Predicting Outcome using Systemic Markers in Severe Exacerbations of COPD; COPD: chronic obstructive pulmonary disease; GOLD; Global Initiative for Chronic Obstructive Lung Disease.

groups. For both cohorts, the absolute values of pro-ADM, pro-AVP and pro-ANP increased in parallel to the number of elevated biomarkers.

Risk of death during the first 3 years of follow-up

During the first 3 years of follow-up, 50 and 49 patients died in the derivation and validation cohorts, respectively. For both cohorts, the number of nonsurvivors increased stepwise according to the number of increased biomarkers (16 (19.8%) out of 81 and 15 (11.3%) out of 133 with zero, 22 (51.2%) out of 43 and 14 (24.6%) out of 57 with one, eight (57.1%) out of 14 and 13 (34.2%) out of 38 with two, and four (100%) out of four and seven (46.6%) out of 15 with three increased biomarkers in the derivation and validation cohorts, respectively; $p<0.001$ for both). Corresponding odds ratios for mortality were 4.3 (95% CI 1.9–9.6) for one increased biomarker, 2.4 (95% CI 1.2–5.1) for two increased biomarkers and 5.4 (95% CI 1.7–17.8) for three increased biomarkers *versus* no increased biomarkers in the derivation cohort, and were 2.4 (95% CI 1.2–5.1), 3.4 (95% CI 1.6–7.2) and 6.4 (95% CI 2.6–15.7) in the validation cohort. Adjusted odds ratios for the derivation cohort were 3.1 (95% CI 1.2–8.2), 4.6 (95% CI 1.0–20.2) and 6.9 (95% CI 0.3–180). For the validation cohort the multivariable-adjusted odds-ratio were 1.7 (95% CI 0.8–3.7), 2.5 (95% CI 1.1–5.5) and 4.4 (95% CI 1.7–11.4) (table 3). When adjusting for BODE index, the corresponding odds ratios were 1.6 (95% CI 0.7–3.7), 2.3 (95% CI 0.9–5.6) and 6.9 (95% CI 2.8–17.3).

Death during the maximum follow-up time

During the maximum follow-up time, 73 and 87 individuals died in the derivation and validation cohorts, respectively. For both the derivation and validation cohorts risk of death increased stepwise from none through to three increased biomarkers. Crude hazard ratios for the derivation cohort were 3.0 (95% CI 1.8–5.1) for one increased biomarker, 4.8 (95% CI 2.4–9.5) for two increased biomarkers and 9.6 (95% CI 3.3–28.3) for three increased biomarkers *versus* no increased biomarkers. Figure 2 shows the crude cumulative survival for patients with zero, one, two or three increased biomarkers from the panel for the validation cohort. Corresponding hazard ratios were 1.9 (95% CI 1.1–3.3), 3.1 (95% CI 1.8–5.4) and 5.4 (95% CI 2.5–11.4) for one, two and three increased biomarkers, respectively. For the derivation cohort, multivariable adjusted hazard ratios for death were 2.4 (95% CI 1.3–4.4) for one increased biomarker, 3.2 (95% CI 1.4–7.3) for two increased biomarkers and 4.4 (95% CI 1.1–17.6) for three increased biomarkers *versus* no increased biomarkers. As shown in table 4, multivariable adjusted hazard ratios for death for the validation cohort were 1.4 (95% CI 0.8–2.4), 1.9 (95% CI 1.0–3.4) and 3.3 (95% CI 1.5–7.3). The cumulative survival of patients in the validation cohort according to the number of increased biomarkers

TABLE 1 Baseline characteristics of 142 patients with COPD (derivation cohort) according to the number of increased biomarkers at baseline

Characteristic	All	Number of increased biomarkers				p-value
		0	1	2	3	
Subjects n	142	81	43	14	4	
Age years	69.8±9.8	65.9±9.9	74.5±6.9	77.4±5.9	71.5±5.8	0.268
Males	55.6 (79)	49.4 (40)	62.7 (27)	71.4 (10)	50 (2)	0.303
Current smokers	45.7 (65)	48.1 (39)	46.5 (20)	35.7 (5)	25 (1)	0.680
Smoking history pack-years	46±29	49±30	44±27	43±30	29±10	0.201
Duration of COPD months	122±79	117±85	136±70	90±65	180±69	0.149
Pharmacological COPD treatment						
LABA	90.1 (128)	88.9 (72)	90.7 (39)	92.9 (13)	100 (4)	0.911
LAMA	59.9 (85)	59.3 (48)	60.5 (26)	57.1 (8)	75 (3)	0.931
ICS	83.8 (119)	85.2 (69)	79.1 (34)	85.7 (12)	100 (4)	0.485
Methylxanthines	7.0 (10)	9.9 (8)	2.3 (1)	0 (0)	25 (1)	0.103
Systemic glucocorticosteroid	18.3 (26)	13.6 (11)	23.3 (10)	14.3 (2)	75 (3)	0.044
Oxygen therapy	21.8 (31)	22.2 (18)	20.9 (9)	14.3 (2)	50 (2)	0.556
Comorbidities						
Arterial hypertension	26.1 (37)	22.2 (18)	30.2 (13)	42.9 (6)	0 (0)	0.215
Cardiopathy	43.7 (62)	30.9 (25)	51.2 (22)	78.6 (11)	100 (4)	<0.001
Pulmonary hypertension	16.9 (24)	18.5 (15)	11.6 (5)	21.4 (3)	25 (1)	0.431
Malignancy	10.6 (15)	6.2 (5)	16.3 (7)	7.1 (1)	50 (2)	0.063
Diabetes mellitus	12.7 (18)	9.9 (8)	14.0 (6)	7.1 (1)	75 (3)	0.026
Renal failure	4.9 (7)	0 (0)	2.3 (1)	28.6 (4)	50 (2)	<0.001
GOLD stage						
II	33.1 (47)	35.8 (29)	32.6 (14)	28.6 (4)	0 (0)	0.733
III	40.1 (57)	41.3 (33)	39.5 (17)	35.7 (5)	50 (2)	
IV	25.4 (36)	22.2 (18)	25.6 (11)	35.7 (5)	50 (2)	
Post-bronchodilator FVC % predicted	73.9±24.8	74.3±25.1	78.6±23.5	61.1±23.4	45.7±13.3	0.048
Post-bronchodilator FEV_1 % predicted	46.1±23.2	45.7±23.8	48.8±23.5	42.0±21.1	36.7±15.4	0.720
Post-bronchodilator FEV_1/FVC %	49.1±15.6	48.6±14.7	48.8±17.7	51.5±13.6	55.3±17.1	0.823
Pro-ADM nmol·L^{-1}	0.67 (0.48–0.95)	0.50 (0.44–0.62)	0.94 (0.83–1.21)	1.19 (1.01–1.53)	1.9 (1.88–)	<0.001
Pro-AVP pmol·L^{-1}	6.4 (4.0–11.3)	4.9 (3.4–8.2)	6.9 (5.2–16.2)	15.7 (6.9–18.3)	50.9 (40.7–)	<0.001
Pro-ANP pmol·L^{-1}	18.3 (53.5–116.3)	60.9 (40.5–82.4)	95.3 (77–136.5)	269.5 (193.3–406.8)	474 (366–)	<0.001

Data are presented as mean±SD, % (n) or median (interquartile range), unless otherwise stated. COPD: chronic obstructive pulmonary disease; LABA: long-acting β_2-agonist; LAMA: long-acting anti-muscarinic agents; ICS: inhaled corticosteroid; GOLD: Global Initiative for Chronic Obstructive Lung Disease; FVC: forced vital capacity; FEV_1: forced expiratory volume in 1 s; ADM: adrenomedullin; AVP: arginine vasopressin; ANP: atrial natriuretic peptide.

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TABLE 2 Baseline characteristics of 243 patients with COPD (validation cohort) according to the number of increased biomarkers at baseline

Characteristic	All	Number of increased biomarkers				p-value
		0	1	2	3	
Subjects n	243	133	57	38	15	
Age years	66.1±10.5	63.6±9.3	67.8±12.6	73.6±6.4	73.7±6.5	<0.001
Males	70.8 (172)	70.7 (94)	66.7 (38)	73.7 (28)	80 (12)	0.744
Caucasians	98.8 (240)	97.7 (130)	100 (57)	100 (38)	100 (15)	0.867
Current smokers	36.6 (89)	37.6 (50)	38.6 (22)	31.6 (12)	33.3 (5)	0.888
Smoking history pack-years	52±25	53±28	51±25	54±29	50±22	0.701
Duration of COPD symptoms months	77±76	82±63	109±98	99±67	100±98	0.313
Time elapsed since diagnosis months	92±77	70±63	92±98	82±75	92±100	0.428
BMI kg·m^{-2}	26.2±6.4	25.5±5.3	28.5±9.1	24.8±4.5	25.4±5.1	0.976
mMRC dyspnoea scale grade	1.5 (1–2)	1 (1–2)	2 (2–3)	2 (1.5–3)	2 (1–3)	<0.001
6MWD m	365±106	388±98	351±105	323±107	305±124	0.004
BODE index	3 (1–4)	3 (1–4)	3 (1–5)	4 (1–6)	4 (1.75–5.5)	0.184
Pharmacological COPD treatment						
Combination therapy						
SABA/SAMA	10.7 (26)	8.3 (11)	15.8 (9)	15.8 (6)	0 (0)	0.158
LABA/ICS	80.6 (196)	79.7 (106)	84.2 (48)	76.3 (29)	80.3 (12)	0.832
Single drug inhaler						
SABA	36.2 (88)	42.1 (56)	38.6 (22)	18.4 (7)	20 (3)	0.029
LAMA	69.5 (169)	67 (89)	71.9 (41)	71.1 (27)	80 (12)	0.839
ICS	4.5 (11)	3.8 (5)	5.3 (3)	7.9 (3)	0 (0)	0.580
Methylxanthines	5.3 (13)	4.6 (6)	7.1 (4)	5.1 (2)	6.7 (1)	0.959
Systemic glucocorticosteroid	8.6 (21)	9.8 (13)	7.0 (4)	7.9 (3)	6.7 (1)	0.917
Mucolytics/antioxidants	9.5 (23)	4.5 (6)	17.5 (10)	13.2 (5)	13.3 (2)	0.029
Non-pharmacological COPD treatment						
Supervised rehabilitation	27.6 (67)	30.1 (40)	24.6 (14)	26.3 (10)	20 (2)	0.768
Oxygen therapy	21.8 (53)	18.8 (25)	22.8 (13)	31.6 (12)	20 (3)	0.408
Noninvasive ventilation	5.3 (13)	4.5 (6)	8.8 (5)	5.3 (2)	0 (0)	0.503
Volume reduction therapy	7.4 (18)	9 (12)	7 (4)	2.6 (1)	6.7 (1)	0.616
Comorbidities						
Arterial hypertension	55.1 (134)	44.4 (59)	64.9 (37)	71.1 (27)	73.3 (11)	0.002
Coronary arterial disease	32.1 (78)	27.1 (36)	42.1 (24)	31.6 (12)	40 (6)	0.204
Congestive heart failure	24.7 (60)	15 (20)	22.8 (13)	42.1 (16)	73.3 (11)	<0.001
Myocardial infarction	9.9 (24)	8.3 (11)	5.3 (3)	18.4 (7)	20 (3)	0.086
Pulmonary hypertension	18.9 (46)	15.8 (21)	24.6 (14)	21.1 (8)	20 (3)	0.541
Malignancy	8.2 (20)	3.8 (5)	8.8 (5)	15.8 (6)	26.7 (4)	0.004
Diabetes mellitus	12.7 (31)	8.3 (11)	22.9 (13)	10.5 (4)	20 (3)	0.037
Renal failure	12.7 (31)	6.8 (9)	8.8 (5)	23.7 (9)	53.3 (8)	<0.001

TABLE 2 continued

Characteristic	All	Number of increased biomarkers				p-value
		0	1	2	3	
Age-adjusted Charlson comorbidity score	4 (3–5)	4 (3–5)	5 (3–6)	5 (5–7)	7 (4–9)	<0.001
Comorbidities treatment						
Aspirin/clopidogrel	44.9 (109)	33.1 (44)	50.9 (29)	60.5 (23)	86.7 (13)	<0.001
Diuretics	34.6 (84)	22.6 (30)	43.9 (25)	47.4 (18)	73.3 (11)	<0.001
Statins	26.3 (64)	21.8 (29)	26.3 (15)	36.8 (14)	40 (6)	0.171
ACE inhibitor/AT-II antagonists	39.1 (95)	32 (42)	37 (21)	55.3 (21)	73.3 (11)	0.002
Calcium channel antagonists	16 (39)	10.5 (14)	15.8 (9)	31.6 (12)	26.7 (4)	0.011
β-blockers	26.7 (65)	13.5 (18)	31.6 (18)	47.4 (18)	73.3 (11)	<0.001
Antidepressives	12.3 (30)	11.3 (15)	8.8 (5)	23.9 (9)	6.7 (1)	0.123
Oral antidiabetics	7.8 (19)	6 (8)	10.5 (6)	7.9 (3)	13.3 (2)	0.612
Insulin	2.9 (7)	2.3 (3)	5.3 (3)	2.6 (1)	0 (0)	0.616
GOLD stage						
II	42.7 (104)	43.6 (58)	40.4 (23)	40.5 (15)	53.3 (8)	0.894
III	38.3 (93)	36.8 (49)	42.1 (24)	37.8 (14)	10 (6)	
IV	18.5 (45)	19.5 (26)	17.5 (10)	21.6 (8)	6.7 (1)	
Post-bronchodilator FVC % predicted	77.0±19.8	78.1±20.5	75.8±20.5	76.4±18.9	72.1±15.1	0.271
Post-bronchodilator FEV_1 % predicted	47.0±16.6	47.3±17.5	46.1±15.4	46.6±17.7	48.9±12.3	0.745
Post-bronchodilator FEV_1/FVC %	46.5±14.0	46.7±14.9	46.9±13.5	44.5±12.0	49.4±13.9	0.497
Pro-ADM nmol·L^{-1}	0.7 (0.52–1.0)	0.54 (0.47–0.61)	0.90 (0.79–1.11)	1.16 (0.99–1.67)	1.59 (1.42–3.21)	<0.001
Pro-AVP pmol·L^{-1}	9.5 (5.1–18.6)	6.9 (2.4–10.8)	11.1 (7.5–18.7)	19.6 (9.5–30.7)	61.2 (52.1–93.2)	<0.001
Pro-ANP pmol·L^{-1}	96.1 (62–181)	63.9 (46.6–87.9)	121.1 (90.4–160.8)	279 (221.2–321.8)	410.4 (277.9–714.7)	<0.001

Data are presented as mean±SD, % (n) or median (interquartile range), unless otherwise stated. COPD: chronic obstructive pulmonary disease; BMI: body mass index; mMRC: modified Medical Research Council; 6MWD: 6-min walking distance; BODE: BMI, airflow obstruction, dyspnoea, exercise capacity; SABA: short-acting β_2-agonist; SAMA: short-acting anti-muscarinic agents; LABA: long-acting β_2-agonist; ICS: inhaled corticosteroid; LAMA: long-acting anti-muscarinic agents; ACE: angiotensin-converting enzyme; AT-II: angiotensin II receptor; GOLD: Global Initiative for Chronic Obstructive Lung Disease; FVC: forced vital capacity; FEV_1: forced expiratory volume in 1 s; ADM: adrenomedullin; AVP: arginine vasopressin; ANP: atrial natriuretic peptide.

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TABLE 3 Multivariable logistic model for death prediction during the first 3 years of follow-up according to the number of increased biomarkers for the validation cohort

Characteristic	Odds ratio (95% CI)#	p-value
1 increased biomarker *versus* 0	1.7 (0.8–3.7)	0.014
2 increased biomarkers *versus* 0	2.5 (1.1–5.5)	0.025
3 increased biomarkers *versus* 0	4.4 (1.7–11.4)	0.002
Smoking (current *versus* former)	1.8 (1.0–3.2)	0.044
Sex (female *versus* male)	0.8 (0.4–1.6)	0.586
Age-adjusted Charlson comorbidity score	1.2 (1.1–1.3)	0.003
Post-bronchodilator FEV1 % predicted	0.970 (0.91–0.99)	0.004

FEV1: forced expiratory volume in 1 s. #: per interquartile range increase.

from the panel and adjusted by the BODE index is shown in figure 3. The corresponding hazard ratios were 1.5 (95% CI 0.8–2.7), 2.4 (95% CI 1.2–4.5) and 6.4 (95% CI 3.0–13.8) for one, two and three increased biomarkers, respectively. There was a stepwise increase in the absolute 1–5-year risk of death from none to through three increased biomarkers (fig. 4).

Model accuracy and predictive values

The addition of the biomarker panel to the clinical basic model including either age and comorbidities, or age-adjusted Charlson comorbidity score, sex, FEV1 % pred and smoking status improved the C statistics in both the derivation and validation cohort (table 5). For the validation cohort, there was a corresponding improvement for a model including the BODE index. As compared with the BODE index, the combination of three high biomarkers *versus* none generated lower negative likelihood ratios and higher positive likelihood ratios.

The addition of the panel to the clinical model for both cohorts and to the BODE index for the validation cohort generated a substantial combined NRI for the first 3 years of follow-up and for the maximum follow-up time. Risk of death in the analyses stratified by GOLD stages II–IV were similar to those presented in all strata (table 6).

Discussion

The principal finding of this study is that simultaneously elevated levels of three circulating biomarkers, *i.e.* ADM, AVP and ANP, are associated with an increased risk of death in patients with stable COPD. The crude relative risk of death increased two-, three- and five-fold for one, two or three elevated biomarkers, respectively. Multivariable adjustment for clinical variables as well as the BODE index and stratification by

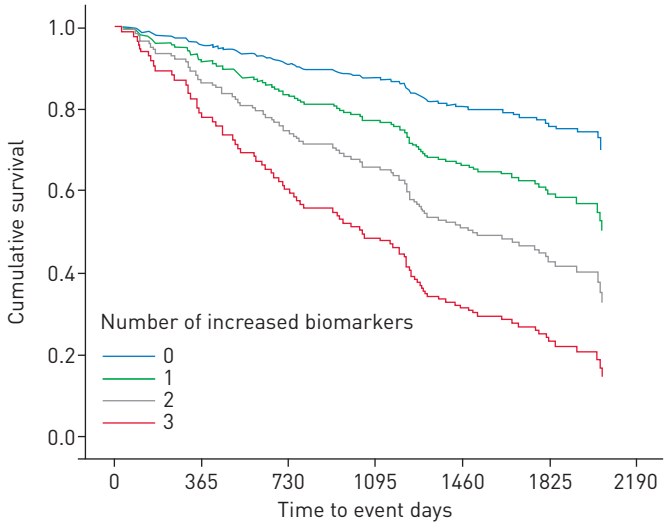

FIGURE 2 Crude cumulative survival in patients according to the number of increased biomarkers for the validation cohort. The corresponding hazard ratios were 1.9 (95% CI 1.1–3.3), 3.1 (95% CI 1.8–5.4) and 5.4 (95% CI 2.5–11.4) for one, two and three increased biomarkers, respectively.

TABLE 4 Multivariable Cox regression model for death prediction during the maximum follow-up time according to the number of increased biomarkers for the validation cohort

Characteristic	Hazard ratio (95% CI)#	p-value
1 increased biomarker *versus* 0	1.4 (0.8–2.4)	0.238
2 increased biomarkers *versus* 0	1.9 (1.01–3.4)	0.046
3 increased biomarkers *versus* 0	3.3 (1.5–7.3)	0.003
Smoking (current *versus* former)	1.4 (0.9–2.1)	0.184
Sex (female *versus* male)	0.98 (0.6–1.6)	0.923
Age-adjusted Charlson comorbidity score	1.2 (1.1–1.3)	<0.001
Post-bronchodilator FEV1 % predicted	0.970 (0.96–0.99)	<0.001

FEV1: forced expiratory volume in 1 s. #: per interquartile range increase.

the GOLD stage provided largely consistent results. The addition of all three biomarkers to the BODE index produced a NRI of approximately 50%. Thus, the biomarker panel appears to offer additional information to clinical variables and to the BODE index, which, to date, has been considered the gold standard for mortality risk prediction in COPD [31]. In addition, the biomarker panel generated a higher positive likelihood ratio (>5) than the BODE index, suggesting its clinical applicability. In this context, this biomarker panel might allow timely monitoring of the severity, the progression or hopefully the regression, if effective interventions follow, of disease processes in COPD.

The most common clinical variable associated with mortality in COPD is the severity of airflow obstruction, as assessed by the FEV1 % pred [34]. However, COPD has important extrapulmonary manifestations and other physiological variables not related to lung function itself, *e.g.* BMI, degree of breathlessness, hypoxaemia, hypercapnia and decreased exercise capacity, have been identified as predictors of mortality in stable COPD [9, 35–38]. This lead to evolution of the concept that the ability to predict outcome could be improved if the different variables were combined into a multidimensional index that captured the complexity of COPD, such as the BODE or ADO (age, dyspnoea and FEV1) [31, 39]. We now introduce the concept that the multidimensional evaluation of COPD by means of blood biomarkers might be more accurate in predicting mortality in COPD than predictors based on any single biomarker or clinical variables alone. This approach has been pursued in a few previous studies; however, these studies exclusively focused on inflammatory markers and, thus, explored the "inflammatory COPD phenotype" [11]. For instance, an earlier report evaluated a combination of two biomarkers (C-reactive protein and fibronectin) as a composite unit to score mortality risk in COPD [40]. Neither serum levels of fibronectin nor the circulating fibronectin/C-reactive protein ratio (stratified into quintiles) were significantly related to

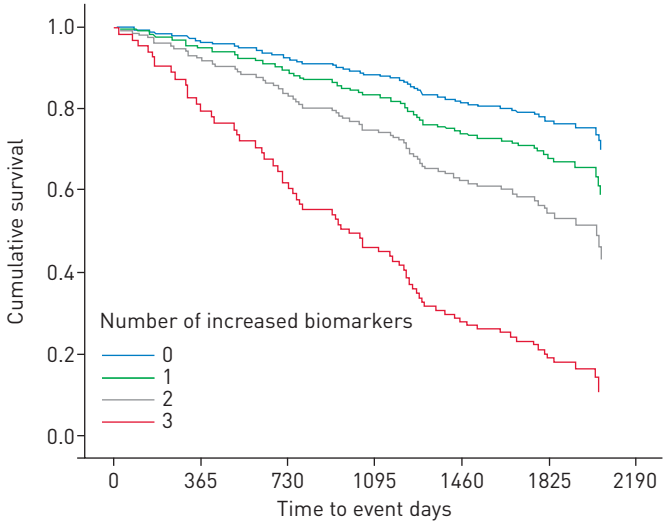

FIGURE 3 Cumulative survival in patients according to the number of increased biomarkers from the biomarker panel and adjusted by the BODE (body mass index, airflow obstruction, dyspnoea, exercise capacity) index for the validation cohort. The corresponding hazard ratios were 1.5 (95% CI 0.8–2.7), 2.4 (95% CI 1.2–4.5) and 6.4 (95% CI 3.0–13.8) for one, two and three increased biomarkers, respectively.

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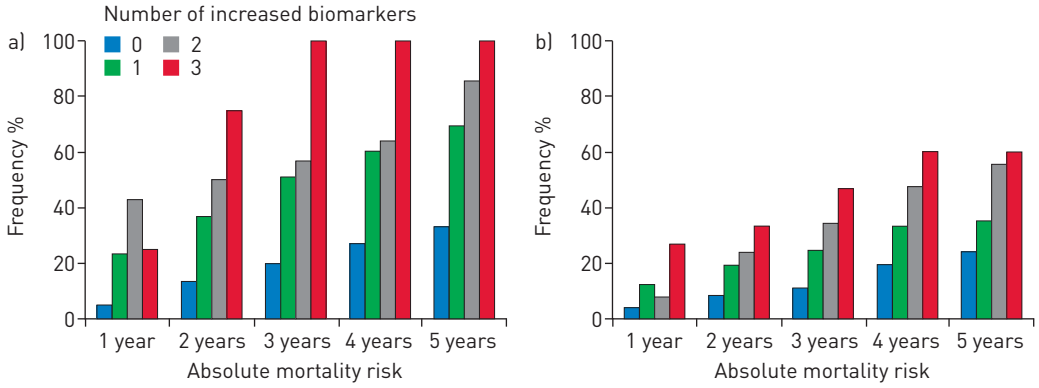

FIGURE 4 Absolute 1–5-year mortality risk by the number of increased biomarkers at baseline for a) the derivation and b) the validation cohort.

all-cause mortality of COPD patients. Moreover, the relationship between fibronectin/C-reactive protein ratio and mortality appeared to be L-shaped (and not linear), hampering the interpretation of its association with pulmonary repair and inflammation in COPD. Another study evaluated white blood cell count, interleukin (IL)-6, fibrinogen, pulmonary and activation-regulated chemokine, C-reactive protein, IL-8 and surfactant protein-D, both individually and in combination with each other, and clinical parameters in a large COPD cohort [2]. All tested biomarkers were independently associated with mortality over 3 years after adjustment for clinical variables. However, with the use of C-statistics, only IL-6 was shown to have independently added predictive power to the clinical model. A combination of two or more biomarkers improved the model only marginally. More recently, attention has been focused on fibrinogen as an inflammatory marker of mortality in COPD. A large meta-analysis of prospective studies demonstrated a correlation between plasma fibrinogen and death from COPD [41]. Correspondingly, data from the Atherosclerosis Risk in Communities/Cardiovascular Health Study and National Health and Nutrition Examination Survey III general population cohorts showed increased all-cause mortality in individuals with higher circulating fibrinogen [42, 43]. Although fibrinogen seems to be related to survival,

TABLE 5 ROC AUC, negative and positive likelihood ratios, and net reclassification improvement for death prediction at 3 years and maximum follow-up time according to the number of increased biomarkers in the derivation and validation cohort

	Derivation cohort	Validation cohort
Subjects n	142	243
ROC AUC		
Clinical basic model[#]	0.711	0.715
Clinical basic model + 3 biomarkers	0.744	0.729
BODE		0.616
BODE + 3 biomarkers		0.669
Likelihood ratio at 3 years negative/positive		
3 biomarkers *versus* none	0.89 (0.80–1.00)/3.06 (1.08–8.71)	0.73 (0.55–0.97)/5.01 (2.02–12.42)
BODE 7–10 vs 1–6		0.87 (0.73–1.02)/2.53 (1.15–5.55)
Likelihood ratio at 5 years negative/positive		
3 biomarkers *versus* none	0.95 (0.87–1.05)/1.71 (0.60–4.89)	0.84 (0.71–0.98)/3.66 (1.39–9.66)
BODE 7–10 *versus* 1–6		0.88 (0.78–0.99)/2.74 (1.26–5.96)
Net reclassification improvement at 3 years %		
Clinical basic model + 3 biomarkers *versus* clinical basic model	89.4 (53.0–100)	40.9 (9.4–71.5)
BODE + 3 biomarkers *versus* BODE		57.9 (21.7–92.4)
Net reclassification improvement at 5 years %		
Clinical basic model + 3 biomarkers *versus* clinical basic model	89.4 (51.7–100)	38.7 (10.7–64.7)
BODE + 3 biomarkers *versus* BODE		45.9 (13.9–75.7)

Data are presented as likelihood ratio (95% CI), unless otherwise stated. ROC: receiver operating characteristic; AUC: area under the curve; BODE: body mass index, airflow obstruction, dyspnoea, exercise capacity. [#]: the clinical basic model included age and comorbidities, sex, forced expiratory volume in 1 s (FEV_1) % predicted and smoking status (derivation cohort) or age-adjusted Charlson comorbidity score, sex, FEV_1 % pred and smoking status (validation cohort).

TABLE 6 Cox regression model for death prediction during the maximum follow-up time according to the number of increased biomarkers stratified by GOLD stages for the validation cohort

Characteristic	Hazard ratio (95% CI)	p-value
GOLD II		
1 increased biomarker *versus* 0	2.1 (0.9–5.4)	0.106
2 increased biomarkers *versus* 0	4.4 (1.7–11.0)	0.002
3 increased biomarkers *versus* 0	7.3 (2.5–21.5)	<0.001
GOLD III		
1 increased biomarker *versus* 0	1.5 (0.5–4.1)	0.482
2 increased biomarkers *versus* 0	3.5 (1.3–9.4)	0.014
3 increased biomarkers *versus* 0	5.8 (1.5–21.7)	0.009
GOLD IV		
1 increased biomarker *versus* 0	2.8 (1.2–6.7)	0.020
2 increased biomarkers *versus* 0	1.6 (0.6–4.6)	0.364
3 increased biomarkers *versus* 0	12.5 (1.4–112.6)	0.024

GOLD: Global Initiative for Chronic Obstructive Lung Disease.

due to the strong relationship between fibrinogen and cardiovascular mortality and the exclusion of individuals with cardiovascular disease from many studies evaluating fibrinogen as a surrogate marker in COPD, the usefulness of fibrinogen in clinical practice warrants further investigation [44].

It does not seem surprising that inflammatory biomarkers alone may not be capable of reflecting the whole picture of the pathophysiological process underlying COPD. Several other disease manifestations, such as tissue remodelling, hypoxia, pulmonary hypertension, an abnormal redox state and cardiovascular comorbidity, among others, are major contributors to morbidity and mortality in COPD. Most of these factors cannot be captured by inflammatory markers alone. Indeed, biomarkers targeting common comorbidities such as congestive heart failure would add to the usefulness of biomarkers in COPD. In this study, we have chosen an integrative approach by combining three serum biomarkers. These were expected to capture not only inflammation but also other systemic repercussions of the disease as well as associated comorbidities. While ADM is associated with inflammation, tissue remodelling and hypoxia, AVP modulates fluid homeostasis and oxidative stress, and ANP is related to cardiac dysfunction [18, 19, 45]. Hypoxia leads to ADM upregulation through the hypoxia inducible factor-1 pathway, which interacts with nuclear factor-κB to promote the expression of inflammatory genes [15, 46–48]. Systemic inflammation promotes cardiovascular disease, drives atherosclerosis and contributes to the development of skeletal muscle dysfunction, osteopenia and depression in COPD [49, 50]. ADM is also linked to vascular remodelling and related to the development of pulmonary hypertension in COPD [51, 52]. Midregional pro-ADM significantly predicted all-cause death, major adverse cardiac events and especially heart failure even beyond the predictive ability of N-terminal pro-brain natriuretic peptide [53]. ADM predicts death and rehospitalisation in patients with exacerbated COPD, refines the BODE index (“BODE-A” index) and replaces the more cumbersome 6MWD (“BOD-A” index) without sacrificing the predictive properties of the original composite score [9]. AVP decisively controls fluid homeostasis in COPD [54]. AVP seems to be inappropriately stimulated due to a reduction in effective circulating volume, but also due to non-osmotic stimuli, leading to a continuous expansion of extracellular volume and associated oedema formation in COPD [54]. AVP levels are also elevated in proportion to severity of heart failure and are associated with higher cardiovascular mortality in ambulatory patients [55]. ANP helps to relax the pulmonary vasculature and lower pulmonary artery pressures in COPD [45, 56]. Additionally, ANP exerts its cardioprotective function not only as a circulating hormone but also as a local autocrine and/or paracrine factor [20]. In this context, ANP can attenuate the effects of noradrenaline on the growth of cardiac myocytes and fibroblasts *via* a cyclic GMP-mediated inhibition of noradrenaline-stimulated Ca^{2+} influx, which raises the possibility that endogenous ANP suppressively regulates the development of cardiac myocyte hypertrophy and fibrosis [57, 58]. ANP has also been linked to major cardiovascular events and mortality in stable cardiovascular disease [59]. Hence, taken together, the broad pluripotential stimuli, including cardiovascular disease, leading to increased circulating levels of the panel biomarkers could serve as an “early” surrogate of disease repercussions, progression and/or activity that may signal an increased risk of poor outcome and assist in an attempts to prevent that outcome.

Our study has some limitations. First, we examined a modest size well-characterised and adequately treated monocentric cohort of COPD patients in GOLD stages II–IV seeking care in the respiratory division of a

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tertiary care academic institution. Thus, our results may not be generalisable to milder disease, asymptomatic or untreated patients, or to those individuals included in population-based studies. Secondly, although cause of death has been ascertained, isolated autopsies and uncertainty about the cause of death in a large proportion of cases (mainly due to the presence of relevant cardiovascular comorbidities) prevented the analysis of the predictive power of the panel with regard to individual causes of death. In this sense, it is unknown whether the panel specifically relates to COPD mortality. However, difficulties in evaluating the cause of death in COPD are well described and an affirmative categorisation in this setting is challenging. Finally, although we have shown an association between the biomarker panel and mortality, we cannot infer its pathobiological role, differentiate between the rates of progression of each of the associated conditions or infer its reversibility under proactive interventions. Thus, specific mechanistic research in lung tissue and intervention studies evaluating the benefit of therapeutic approaches stratified by biomarker levels are urgently warranted to clarify the usefulness of this panel in clinical practice. It should be noted that, in the current resource-limited environment, the establishment of priorities based on quality-adjusted life-years gained could advantageously refine indication for interventions such as lung transplantation, lung volume reduction procedures, intensified physiotherapy and/or noninvasive ventilation.

In conclusion, the simultaneous elevation of levels of three prognostic biomarkers, *i.e.* ADM, AVP and ANP, is associated with an increased risk of mortality in patients with stable COPD, independently of clinical parameters. Thus, this biomarker panel may enable the determination of the severity and course of disease progression or its response to therapy.

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