Short communication

Combining biomarkers of clot resolution and alveolar basement membrane destruction predicts mortality in the ECLIPSE COPD cohort

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ABSTRACT

Background: Chronic obstructive pulmonary disease (COPD) is characterized by abnormal epithelial repair resulting in a hypercoagulable state with intra-alveolar accumulation of fibrin and alveolar basement membrane destruction. This study aimed to investigate if the combination of two serological biomarkers evaluating these pathological processes could improve the prediction of mortality risk compared to single biomarkers.

Methods: Matrix metalloproteinase-mediated degradation of the type IV collagen α3 chain (C4Ma3), located in the alveolar basement membrane, and plasmin-mediated degradation of crosslinked fibrin (X-FIB), an end-product of fibrinogen, were assessed serologically in a subset of the ECLIPSE cohort (n = 982). Biomarker data were dichotomized into high versus low at the median. Cox regression and Kaplan-Meier curves were used to analyze the predictive value of having one or two high biomarkers for all-cause mortality over two years.

Results: COPD participants with high levels of two biomarkers were at significantly higher risk of all-cause mortality with a hazard ratio of 7.66 (95% CI 1.75–33.48; p = 0.007) while participants with one high biomarker were not at significantly higher risk (HR 3.79 [95% CI 0.85–16.94]; p = 0.08).

Conclusions: A combination of serological biomarkers of alveolar basement membrane destruction and clot resolution was predictive of all-cause mortality in COPD. The combination of two different pathological aspects may strengthen prognostic accuracy and could be used in conjunction with clinical assessment to guide treatment decisions.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is the third leading cause of death worldwide and it is essential to identify the patients at increased risk of mortality [1]. COPD is characterized by abnormal epithelial repair, resulting in a hypercoagulable state, aberrant wound healing and alveolar basement membrane (BM) destruction [2–4]. It is likely that the combination of biomarkers reflecting different aspects of pathology may be a tool to assess mortality risk in COPD.

The BM of the lungs contains the tissue specific networking type IV collagen isoform α3α4α5(IV) that is found in the alveoli and allows gas diffusion [5–7]. Consequently, the turnover of α3(IV) may be of particular importance in COPD, where the BM is replaced by fibrillar collagens [8]. This confers a shift in the functionality of the tissue, to a more rigid and dense structure which is impermeable for gasses. The biomarker C4Ma3 assesses a neo-epitope of α3(IV) generated by matrix metalloproteinase (MMP) cleavage and released into the circulation, allowing for quantification of alveolar BM destruction.

Fibrinogen is a coagulation factor essential for the blood clotting process. Plasma fibrinogen was the first blood-based biomarker to be qualified by the U.S. Food and Drug Administration (FDA) in 2015 for the enrichment of subjects with COPD at higher risk of mortality [9,10]. The persistent injuries that occur in COPD increase the total fibrinogen pool. Fibrinogen is converted to fibrin and deposited into active wound

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healing sites, resulting in a fibrin clot that is further strengthened by crosslinks. In COPD, this clot is more dense and resistant to lysis [3]. In the resolution phase, proteases degrade the fibrin clot and release D-dimer, a crosslinked fragment of fibrin. The biomarker X-FIB assesses the plasmin-generated neo-epitope of D-dimer and thus quantifies clot resolution and completed wound healing.

We previously showed that high levels of C4Ma3 and X-FIB individually were independently associated with increased risk of mortality in COPD [11,12]. We hypothesized that having high levels of two biomarkers that reflect different aspects of pathology, rather than one, would increase prognostic accuracy.

2. Materials and methods

The study design of Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points (ECLIPSE) (clinicaltrials.gov identifier NCT00292552; GSK study code SCO104960) has previously been described [13]. C4Ma3 [14] and X-FIB [15] plasma levels were assessed at the year 1 time point in a subpopulation of 982 COPD participants out of the total of 2164, as previously described [11,12]. Only patients with both C4Ma3 and X-FIB data were included in the current analyses. Data were dichotomized into high versus low based on the median. All-cause mortality at the year 3 time point was compared by Cox regression analysis and Kaplan-Meier survival curves for patients with no, one or two high biomarkers using the Statistical Software MedCalc version 14.8.1 (MedCalc Software bvba, Ostend, Belgium).

3. Results

Baseline characteristics of the 982 COPD participants are listed in Table 1. Within the two years follow-up, 29 (3%) participants died. 272 (28%) participants had high levels of both C4Ma3 and X-FIB while 437 had high levels of one biomarker. Participants with two high biomarkers had significantly higher risk of all-cause mortality over two years as

Table 1
Baseline demographics.

	All	No high biomarkers	One high biomarker	Two high biomarkers	P-value
n	982	273	437	272	–
Male (n (%))*	626 (64%)	162 (59%)	288 (66%)	176 (65%)	0.194
Age, years	63.1 (7.2)	61.1 (7.2)	63.4 (7.1)	64.4 (7.1)	<0.001
BMI, kg/m^2	26.8 (5.9)	26.9 (5.7)	26.7 (5.8)	27.0 (6.2)	0.692
Current smokers (n (%))*	363 (37%)	109 (40%)	150 (34%)	104 (38%)	0.283
Smoking history, pack-years	47 (26)	46 (27)	47 (24)	49 (28)	0.293
FEV$_1$, L	1.41 (0.51)	1.42 (0.53)	1.41 (0.51)	1.41 (0.47)	0.924
FEV$_1$, % predicted	50.6 (15.2)	50.7 (16.0)	50.3 (15.2)	50.9 (14.3)	0.860
FVC, L	3.11 (0.90)	3.07 (0.90)	3.13 (0.92)	3.10 (0.87)	0.724
mMRC (median (IQR))*	1 (1–2)	1 (1–2)	2 (1–2)	2 (1–2)	0.126
SGRQ (median (IQR))	47 (33–61)	46 (30–59)	48 (35–61)	46 (33–61)	0.294
BODE (median (IQR))	3 (1–4)	2 (1–4)	3 (1–4)	3 (2–4)	0.151

Data are shown as mean (SD) unless stated otherwise. FEV$_1$, forced expiratory volume in 1 s.

compared to participants with no high biomarkers with a hazard ratio (HR) of 7.66 (95% CI 1.75–33.48, p = 0.007). Participants with only one high biomarker had a numerically higher risk of mortality (HR 3.79 [95% CI 0.848–16.936, p = 0.081]). When adjusting for age and smoking status, having two high biomarkers remained significantly associated with a higher risk of mortality with a HR of 5.78 (95% CI 1.31–25.49, p = 0.0205). Kaplan-Meier survival curves for groups with no, one and two high biomarkers were significantly different (p = 0.0043, Fig. 1).

(post-bronchodilator); FVC, forced vital capacity; mMRC, modified medical research council dyspnea scale; SGRQTOT, St. George's Respiratory Questionnaire total score; BODE, BMI, airflow obstruction, dyspnea and exercise index. Statistically significant difference between the no, one and two high biomarker groups was determined by one-way ANOVA or chi-squared test. *analysed by chi-squared test.

4. Discussion

We assessed two different pathological biomarkers of COPD, alveolar BM destruction (C4Ma3) and fibrin clot resolution (X-FIB), and showed that this combination improved prediction of mortality outcome compared to the individual biomarkers.

The rationale for this combination was that whereas the body may have wound healing in many places at the same time, represented by tissue nonspecific X-FIB, combining it with C4Ma3, a more tissue specific biomarker mainly localized in the lungs and kidney, could improve assessment of lung remodeling driven by wound healing. Elevated levels of C4Ma3 and X-FIB individually have previously been associated with increased mortality risk in COPD [11,12], and C4Ma3 levels were additionally elevated during acute exacerbations of COPD [14], indicating a role of wound healing and alveolar BM destruction in disease progression.

Plasma fibrinogen was qualified by regulatory agencies (FDA and European Medicines Agency) as a biomarker for the enrichment of patients at high risk of all-cause mortality and hospitalized exacerbations mainly based on data from the ECLIPSE cohort [15]. However, fibrinogen reflects the total pool of "building blocks" available for the initial burst of the clotting process and does not fully reflect the wound healing process. Consequently, more refined biomarkers of the actual process, rather than the capacity for wound healing, may provide a more accurate assessment. D-dimer is released during the degradation of crosslinked fibrin clots and has previously been associated with mortality risk in COPD [16]. In this study, we utilized the biomarker X-FIB, a more accurate neo-epitope specific D-dimer assay. We have previously shown that in the current subset of ECLIPSE, high levels of plasma fibrinogen predicted mortality risk with an unadjusted hazard ratio of 3.3 [11]. Thus, the current data indicate that the combination of C4Ma3 and X-FIB may be an equally good, or potentially better, predictor of mortality. We
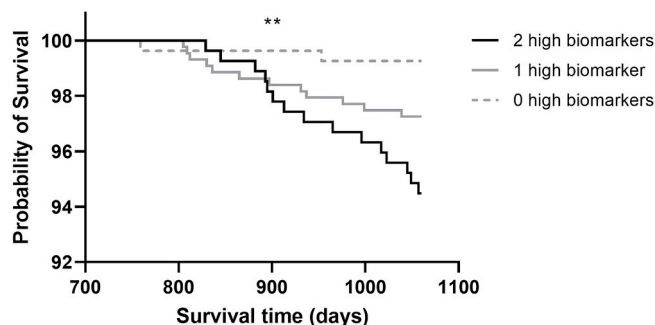
Fig. 1. The combination of alveolar basement membrane destruction and fibrin clot resolution strengthened the prediction of all-cause mortality. Kaplan-Meier survival curves for no, one and two high biomarkers as defined by the median. Data are unadjusted. **p = 0.0043.

identified patients with high levels of both C4Ma3 and X-FIB as having significantly elevated risk of mortality with an adjusted HR of 5.78. However, it must be noted that the confidence interval was wide due to the low number of deceased participants which is the major limitation of this study. Thus, the large hazard ratio needs to be validated in other populations.

These data support the hypothesis that alveolar epithelial cell damage in COPD results in activation of the coagulation cascade and destruction of the BM. Normally, the fibrin clot is resolved, and the BM is remodeled and re-epithelialized. In COPD, injury is repetitive, and the repair response is uncontrolled, resulting in continuous wound healing leading to excessive clot resolution and lung remodeling that may replace type IV collagen with fibrillar collagens and thus cause airflow limitations. This combination of biomarkers may be closer associated with COPD-related deaths as compared to individual biomarkers reflecting a single aspect of disease or even systemic wound healing or inflammation. The identification of patients at increased risk of mortality may be utilized in clinical practice to guide therapy decisions or for the enrichment of clinical trials. These results may also translate to other indications where aberrant wound healing and lung remodeling is crucial, such as pulmonary fibrosis or COVID-19.

5. Conclusions

Here we showed that combining biomarkers reflecting two different pathological aspects of COPD may improve prediction of outcome. The combination of alveolar BM destruction and clot resolution strengthened the association with all-cause mortality when compared to using individual biomarkers. High levels of C4Ma3 and X-FIB may reflect overactive repair processes and fibrosis, and, if replicated, these biomarkers may be used in conjunction with clinical assessment to guide treatment decisions.

CRediT authorship contribution statement

Jannie M.B. Sand: Conceptualization, Formal analysis, Writing - original draft. **Sarah R. Rønnow:** Formal analysis, Investigation, Writing - review & editing. **Lasse L. Langholm:** Formal analysis, Investigation, Writing - review & editing. **Morten A. Karsdal:** Conceptualization, Resources, Writing - review & editing. **Tina Manon-Jensen:** Conceptualization, Writing - review & editing. **Ruth Tal-Singer:** Conceptualization, Resources, Writing - review & editing. **Bruce E. Miller:** Conceptualization, Resources, Writing - review & editing. **Jørgen Vestbo:** Conceptualization, Resources, Writing - review & editing. **Diana J. Leeming:** Conceptualization, Supervision, Writing - review & editing.

Declaration of competing interest

JMBS, SRR, LLL, MAK, TMJ and DJL are employed by Nordic Bioscience, Biomarkers and Research. MAK, TMJ and DJL hold stocks in Nordic Bioscience. Nordic Bioscience is a privately owned small-medium sized enterprise, partly focused on the development of biomarkers for connective tissue disorders. None of the authors from Nordic Bioscience received any kind of financial benefits or other bonuses for the work described in this manuscript. RTS and BEM are employees and shareholders of GSK. JV has received honoraria for presenting and advising from Astra Zeneca, Boehringer-Ingelheim, Chiesi, GlaxoSmithKline and Novartis, outside the submitted work.

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