Biomarkers of endothelial damage in patients with COPD.

A substudy of the CORTICO-COP Randomized Controlled Trial

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Background

Chronic obstructive pulmonary disease (COPD) is affecting approx. 210 mio. people worldwide, and being the cause of nearly 1.9 mio deaths in 2019.[1] Cardiovascular diseases (CVD) are common amongst COPD patients, with a high prevalence of unspecified cardiovascular disease, congestive heart failure, coronary heart disease, peripheral vascular disease, arrhythmia and stroke.[2] The CVDs are associated with an increased rate of hospitalization and mortality within COPD patients.[3,4]

It is known that COPD patients have an increased risk of cardiovascular disease (CVD) and venous thromboembolism (VTE), including pulmonary embolism (PE).[2,5,6] The CVDs are associated with an increased rate of both hospitalization and mortality in COPD patients.[3,4] It is now recognized that COPD is a heterogenic disease and often associated with a persistent systemic inflammation[7]. Systemic inflammation induces an increased stress on the endothelium, leading to varying degrees of endothelial damage and dysfunction.[8] Damage of endothelium leads to a healing process, where Von Willebrand Factor (vWF) is an initiator via exposure to extracellular matrix proteins through the damaged endothelium.[9] Initially, the healing process is centered around clotting, which is a complex interplay between clot formation and clot resolution, with both processes happening simultaneously. As part of the ongoing clot resolution, cross-linked fibrin is degraded, releasing bi-products, including X-fib, which is a neo-epitope of plasmin-mediated degradation of cross-linked fibrin.[10]

Thus, both vWF and products of fibrin degradation may help describe an ongoing process of endovascular damage and clot resolution in patients with COPD.

Regarding vWF, there is a significant correlation between vWF-levels and the CHA2DS2-VASc score, which is the validated score for risk of stroke in patients with atrial fibrillation.[11] In 2013, a review and meta-analysis concluding on 6556 cases showed a modest but significant increase in risk of coronary heart disease increasing with levels of vWF (odds ratio 1.1 (95% CI 1.10-1.22) per 1 standard deviation higher vWF).[12] Similar was found in a study of patients with carotic stenosis, where patients in the upper quartile of vWF-antigen was shown to have higher incidense of cardiac events.[13] In patients with COPD, vWF levels and relative serum activity is increased.[14] Another method of investigating vWF is by its processing products, allowing analyses of both VWF formed (as VWF-n) and VWF activated (as VWF-a),[15] a method which recently has been applied to samples from the ECLIPSE trial; revealing an association of vWF-n with the chronic condition of emphysema, and vWF-a with prior exacerbations.[16] Additionally, in a dichotomized form, higher values of both vWF products were associated with an increase in all-cause mortality.[16] X-fib has previously been

shown to predict mortality in stable COPD patients.[10] D-dimer, another marker of fibrin degradation has previously been shown to predict death and future VTE in healthy persons.[17,18] Additionally, in COPD patients with an acute exacerbation of COPD, D-dimer has been demostrated to predict in-hospital as well as 1-year mortality.[19]

Hypotheses

1) A high blood level* of activated von Willebrand Factor (vWF-a) predicts major cardio-vascular events** within 36 months among patients with severe chronic obstructive pulmonary disease***

2) A high blood level* of von Willebrand Factor formation (vWF-n) predicts major cardio-vascular events** within 36 months among patients with severe chronic obstructive pulmonary disease***

3) A high blood level* of X-fib predicts major cardio-vascular events** within 36 months among patients with severe chronic obstructive pulmonary disease***

*upper quartile. ** MACE is defined as the composite of all-cause mortality, myocardial infarction, coronary revascularization, ischemic stroke (including transient ischemic attack), and heart failure hospitalization. *** COPD patients admitted for COPD in exacerbation (i.e. GOLD groups C/D)

Aim

To evaluate the predictional properties of the three biomarkers vWF-a (marker of vWF activation), vWF-n (a marker of vWF formation) and X-fib (a marker of fibrin degradation) for the risk of future MACE within 36 months. The biomarkers will primarily be evaluated at two timepoints:

a) the acute phase

b) 30 days following the acute phase

and furthermore evaluated as delta values by:

c) Comparing the levels of the biomarkers at the acute phase and in stable phase (after 30 days), and taking this change into account.

This strategy will be applied to vWF-n and X-fib.

Methods

Patients

The study population originates from the CORTICO-COP study. This study is described in a previous publication.[20] Briefly, patients presenting with an exacerbation of COPD within 24 h of hospital admission during August 2016 and September 2018 were recruited in the trial. Blood samples (full blood and plasma) from 318 patients with severe or very severe COPD (age $\geq$ 40 and hospitalised

patients with exacerbation) were taken from three different Respiratory Departments in the Capital Region of Denmark (Herlev & Gentofte University Hospital, Bispebjerg University Hospital and Hvidovre University Hospital). All patients provided written informed consent. The study has been approved by the Ethics Committees of all participating sides (H-15012207) and the Danish Medicines Agency (EudraCT no: 2015-003441-26), and the Danish Data Protection Agency (HGH-2015-038 and I-Suite number 04014). The study was registered at clinicaltrials.gov (NCT02857842). A follow-up visit was performed at 30 and 90 days after the onset of an exacerbation. In the present study, only participants who had the biomarkers in question measured at baseline were included (299 participants).

Exposure

Three biomarkers (VWF-A, VWF-N and X-Fib) were assessed at two different time points (at admission and 30 days following admission).

Power

Assuming a yearly event-rate of the the primary outcome of 2% and an HR of 3 over three years of follow-up, corresponding to a MACE rate of 6% vs 18%, 326 participants are required.

Measurements

Venous blood samples were collected by venipuncture in vacuum EDTA tubes from 318 patiens during the recruitment, and at recovery at 30 and 90 days follow-up for all patients. Of these samples, 299 patients had blood drawn for biomarker analysis at baseline and 214 after 30 days. These samples were all used for the current study. Plasma was obtained by centrifugation of vacutainer tubes at 2000 g for 10–15 min. Plasma was stored at –80 °C until analysed. Plasma vWF-a, vWF-n, X-fib levels were measured as previously described elsewhere.[15,21]

Furthermore, lung function and the Medical Research Council (MRC) Dyspnoea Scale were performed and the patients completed the COPD Assessment (CAT) test during both visits.

Confounding factors:

Sex, age, smoking status (current, previous or never smoker), C-reactive protein, forced expiratory volume in one second, known inhaled corticosteroids use, ischemic heart disease, heart failure, atrial fibrillation / flutter, hypertension and diabetes mellitus.

Outcome

Registry-based follow-up on future MACE during 12-month follow-up. Hospital-related events and death is obtained by linking CORTICO-COP data to the Danish National Patient Registry and the Danish Civil Registration System, respectively.

Statistical analyses

Descriptive statistics will be performed on baseline data. The correlation between each biomarker at the index admission and after 30 days will be examined. Each of the three biomarkers will be dichotomized at the upper quartile, allowing comparison of the "high value groups" with the "low value groups" in a Cox proportional hazards regression, which will be adjusted for the previously mentioned confounding factors.

A stratified analysis will be applied for patients with known chronic ischemic heart disease or MACE prior to the baseline samples.

Four sub-group analyses for the primary outcome will be performed on each of the three biomarkers. Firstly, on participants who did not experience a recurrence of acute exacerbation of COPD resulting in either contact with a hospital or a need to intensify pharmalogical treatment. Secondly, the analyses will be performed with delta values (differences) combined with a high baseline value for the biomarker. Thirdly, a sub-group analysis for the primary outcome will be performed on the population, where imputation is performed on missing values of each of the three biomarkers. Finally, a comparison of biomarker levels will be done between the two treatment groups in the randomized controlled trial.

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Definition of primary outcome for registry follow-up

Diagnosis	**ICD-code**
Myocardial infarction	
Acute myocardial infarction	DI21
Complications following acute myocardial infarction	DI23
Other types of acute ischemic heart disease	DI24
Unstable angina pectoris	DI200
Coronary revascularization	
Coronary artery surgery	KFNA – KFNH (SKS)
Stroke	
Cerebral infarction	DI63
Stroke without information reg. bleed/infarct.	DI64
Transient ischemic attack	DG459
Heart failure hospitalization	
Heart failure	DI50
Hypertensive heart disease with incomp. Heart failure	DI110
Hypertensive heart- and kidney disease with heart failure	DI130
Hypertensive heart- and kidney disease with heart and kidney failure	DI132
Pulmonary edema	DJ81
Liver stasis	DK761

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