SUPPLEMENTARY MATERIAL

High-sensitivity troponin I and all-cause mortality in patients with stable COPD: An analysis of the COSYCONET study

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Methods

Study design and patients

This report describes a longitudinal observation analysis that involved 2085 patients with stable COPD from the ongoing COSYCONET (COPD and Systemic Consequences–Comorbidities Network) cohort study. The overall aims and methods of COSYCONET have been described previously.(1) In brief, the central purpose of COSYCONET is to analyze the prevalence and severity of extrapulmonary disorders and to quantify the impact of extrapulmonary organ manifestations on morbidity and mortality of patients with COPD.(1) The baseline visit took place between 2010 and 2013 in 31 German study centers. Patients were recruited primarily following referral from respiratory specialists and primary care practitioners. Inclusion criteria were broad (age ≥40 years; diagnosis of COPD or chronic bronchitis, and availability for repeated study visits for at least 18 months), with minimal exclusion criteria applied (i.e., major lung surgery, lung tumor and relevant physical or cognitive impairment) in order to cover a wide range of patterns of the disease.(1) All patients had to have stable COPD, which we defined as not having experienced a moderate or severe exacerbation during the four weeks preceding study entry. A total of 2741 patients were included and underwent a broad panel of assessments, guided by standard operating procedures on the basis of established guidelines, with priority given to the assessment of pulmonary function and cardiovascular comorbidities. Follow-up visits were scheduled at 6, 18, 36, and 54 months after the baseline visit. For the present report, we included patients with a confirmed GOLD stage from 1 to 4 (i.e., ratio of post-bronchodilator forced expiratory volume [FEV_1] to forced vital capacity [FVC] <0.7). A detailed patient flow chart is given in the online data supplement (Figure E1).

COSYCONET was approved by the ethical committees of all study centers and all patients gave written informed consent. The cohort study is registered on clinicaltrials.gov (NCT01245933).

COPD assessments

Lung function was measured using standardized procedures as described previously.(1, 2) Dyspnea was assessed using the modified Medical Research Council (mMRC) dyspnea scale. Exercise capacity was evaluated via the 6-minute walk distance (6MWD). Both variables were used as continuous variables and dichotomized using the cut-off of ≥2 for mMRC and <350 m for 6MWD.(3, 4) The BODE index (body-mass index [BMI], airway obstruction, dyspnea, and exercise capacity), which is the most frequently used prognostic assessment in COPD, was calculated, with a cut-off of >4 used to dichotomize the index.(4) Exacerbations during the 12 months prior to recruitment were recorded from a structured interview. Mild exacerbations were defined as those with symptomatic deterioration requiring any action, moderate exacerbations were defined as those with symptomatic deterioration requiring treatment with antibiotics or oral corticosteroids, whereas severe exacerbations were those with symptomatic deterioration resulting in hospital admission.(3) The patients were categorized into GOLD groups A to D based on the mMRC scale.(5) The exacerbation risk for the ABCD grouping was based on the 12-month history of exacerbations of all severities, with high risk indicated by a history of two or more non-hospitalized exacerbations or one or more exacerbation leading to hospital admission.(6) On the basis of blood gas analyses, hypoxemia was defined as PaO_2 <55 mmHg or that required oxygen therapy, with hypercapnia defined as $PaCO_2$ >50 mmHg.(7)

Assessment and definition of cardiovascular risk factors and cardiovascular diseases

History of myocardial infarction and history of stroke were systematically recorded in a structured interview.(1) A combination of self-reported comorbidities and disease-specific medication was the basis for the definition of arterial hypertension, diabetes mellitus, hyperlipidemia, and coronary artery disease, with objective measurements also taken into consideration for the detection of arterial hypertension (systolic blood pressure ≥140 mmHg), diabetes mellitus (glycated hemoglobin level ≥48 mmol per mole [≥6.5%]), and hyperlipidemia (total cholesterol / high-density lipoprotein [HDL] cholesterol ratio ≥5).(8, 9) All other cardiovascular risk factors, cardiovascular diseases, and cardiac dysfunctions were defined solely on the basis of objective measurements. Thus, chronic kidney disease more than mild was defined as estimated glomerular filtration rate (eGFR) <60 mL/min/1.73m^2.(9, 10) The ankle-brachial index (ABI) was measured as previously described, with a value ≤0.9 being both an established measure of peripheral artery disease and an indicator of asymptomatic atherosclerotic disease.(9, 11, 12). High-sensitivity C-reactive protein (hs-CRP) is the most frequently used marker of systemic inflammation and was used as an additional cardiovascular risk factor.(9) NT-proBNP was used as a marker for heart failure, given that an increased level is a main diagnostic criterion.(13) Echocardiography was performed using standard methodology as previously described with left ventricular ejection fraction (LVEF) and tricuspid annular plane systolic excursion (TAPSE) used to detect left and right heart dysfunction, respectively.(14, 15) Systolic left ventricular dysfunction was defined as LVEF <50% according to European Society of Cardiology (ESC) criteria, whereas right heart dysfunction was primarily defined as TAPSE <17mm as per American Society of Echocardiography (ASE) recommendations.(13, 15) In the case of missing values for

TAPSE but available visual grading of the right ventricular function, we categorized subjects with a reduced RV function based on visual inspection as right ventricular dysfunction.

Outcome

The primary outcome of this report was all-cause mortality over three years of follow-up. After baseline, patients were invited for each follow-up visit via telephone and letters. If a patient missed a follow-up visit without formally withdrawing from the study, research assistants ascertained the survival status (and in case of death the date of death) by contacting partners, relatives, primary care practitioners and hospitals. Where an exact date of death was not available the date was imputed (assuming the 15th of the month if the month but not the date was known, the middle of the year if only the year was known, and on the basis of the date of the last contact if the date was totally unknown).

The risk of future COPD exacerbations was assessed by the history of exacerbations reported at the follow-up visits 2 to 4 over three years after the baseline visit. In case of one or two missing follow-up visits the information was used from the available follow-up visit (i.e. at least one).

Statistical analyses

For the descriptive analyses hs-TnI was categorized into quartiles. For the further analyses hs-TnI was both dichotomized according to the cut-off of 6 ng/L, and was used as a continuous variable (after log-transformation since hs-TnI was not normally distributed across the study patients). Linear trends in patient

demographics, cardiovascular markers and COPD assessments across the hs-TnI quartiles were analyzed using χ^2 tests for dichotomized variables and analysis of variance or Kruskal-Wallis test for continuous variables (depending on distribution and homogeneity of variances). The association between continuous log-transformed hs-TnI values and COPD assessments was also tested after adjustment for the traditional cardiovascular risk factors age, sex, smoking status, hypertension, and hyperlipidemia along with the presence of coronary artery disease and history of myocardial infarction.(9) Therefore, we used separate logistic regression analyses with each dichotomized COPD assessment as the dependent variable, i.e., severe or very severe COPD, mMRC score ≥2, 6MWD <350m, BODE score >4, ≥1 severe exacerbation in the prior year, hypoxemia, and hypercapnia, respectively.

To identify the best model of independent predictors for higher hs-TnI levels among cardiovascular markers and COPD assessments, we categorized hs-TnI according to the cut-off value of 6 ng/L for the identification of high-risk patients, which served as the dependent variable. We performed a logistic regression analysis with backward elimination including all variables which showed $p < 0.10$ across the hs-TnI quartiles on the univariate level.

The relation between hs-TnI quartiles and all-cause mortality was analyzed by Kaplan-Meier survival plots and log-rank tests. To calculate the relative risk of mortality associated with hs-TnI levels, we used a series of four Cox proportional hazard regression models, adjusting stepwise for established mortality predictors of COPD, cardiovascular risk factors, and prevalent cardiovascular diseases. The first model was unadjusted. The second was adjusted for established mortality predictors in COPD, specifically the components of the BODE score (BMI, FEV_1, mMRC, and 6MWD) plus age, hypoxemia, hypercapnia, presence of severe exacerbations in the

prior year, and a correction for alpha-1 antitrypsin deficiency. The third model added further adjustments for the cardiovascular risk factors sex, hypertension, diabetes, smoking status, hyperlipidemia, eGFR, and log hs-CRP. The final model also added adjustments for cardiovascular diseases, i.e., coronary artery disease, history of myocardial infarction, history of stroke, decreased ABI, and log NT-proBNP. All Cox analyses were performed twice, first including continuous log-transformed hs-TnI values and second with hs-TnI dichotomized with the cut-off of 6 ng/L.

Finally, we calculated a composite variable from BODE index and hs-TnI to visualize the effect of hs-TnI as an additional predictor of survival, and in particular the relative risk in patients with high hs-TnI and high BODE index compared to those with low hs-TnI and high BODE index. The added discriminative power offered by the addition of hs-TnI to FEV_1 and to the BODE score was analyzed using C statistics based on the beta coefficients derived from Cox regression models.(16, 17) Differences in the C statistic, i.e., hs-TnI plus FEV_1 versus FEV_1 alone and hs-TnI plus BODE score versus BODE score alone, were estimated using the method described by Antolini et al.(18)

Logistic regression analyses were used to analyze the predictive value of hs-TnI for future exacerbations and exacerbation related hospitalizations, separately.

Results

Levels of hs-TnI according to the GOLD groups A to D are presented in Figure E2. There were significant differences of hs-TnI values across the groups ($p < 0.001$) with higher levels in group B and D and lower levels in group A and C (Figure E2).

Cardiovascular risk factors and prevalent cardiovascular diseases according to hs-TnI quartiles differentiated for women and men separately are presented in tables E1 and E2. Some markers only differed significantly across the hs-TnI quartiles in men, such as coronary artery disease and history of myocardial infarction.

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Table E1. Patients' demographics, cardiovascular risk factors and cardiovascular diseases according to quartiles of high-sensitivity troponin I in men.

	High-sensitivity troponin I quartiles				P for trend in men
	<2.49 ng/L	**2.50–3.79 ng/L**	**3.80–6.59 ng/L**	**≥6.60 ng/L**	
Age, years, mean (SD)	63.8 (8.4)	65.5 (8.1)	66.2 (8.2)	67.3 (7.9)	<0.001
BMI, kg/m^2, mean (SD)	26.5 (4.4)	27.1 (4.9)	27.2 (4.9)	27.8 (5.1)	0.001
Current smoker, n (%)	69 (27.1)	72 (23.3)	76 (23.0)	80 (21.3)	0.12
Hypertension, n (%)	184 (72.2)	219 (70.9)	244 (73.7)	307 (81.9)	0.002
Glycated hemoglobin, mmol/mol, mean (SD)	41.2 (7.3)	41.6 (8.2)	41.7 (8.0)	42.4 (8.6)	0.071
Diabetes mellitus, n (%)	46 (18.0)	57 (18.4)	74 (22.4)	91 (24.3)	0.026
Total cholesterol / HDL cholesterol ratio, median (IQR)	3.5 (2.8–4.5)	3.7 (2.9–4.6)	3.6 (2.9–4.6)	3.7 (2.9–4.6)	0.70
Hyperlipidemia, n (%)	127 (49.8)	153 (49.5)	186 (56.2)	216 (57.6)	0.016
eGFR, mL/min, median (IQR)	85 (71–99)	83 (69–96)	81 (70–94)	78 (66–95)	0.004
eGFR <60 mL/min, n (%)	24 (9.5)	33 (10.7)	32 (9.8)	60 (16.1)	0.014
hs-CRP, mg/L, median (IQR)	3.9 (1.8–6.3)	4.9 (2.0–7.1)	4.8 (2.2–7.3)	4.3 (2.1–9.0)	0.27
Coronary artery disease, n (%)	59 (23.1)	51 (16.5)	68 (20.5)	113 (30.1)	0.006
History of myocardial infarction, n (%)	23 (9.0)	21 (6.8)	35 (10.6)	66 (17.6)	<0.001
History of stroke, n (%)	9 (3.5)	15 (4.9)	12 (3.6)	20 (5.3)	0.42
Ankle-brachial index ≤0.9, n (%)	20 (7.9)	22 (7.3)	33 (10.1)	45 (12.4)	0.025
NT-proBNP, pg/mL, median (IQR)	184 (35–366)	187 (28–386)	175 (32–378)	235 (52–501)	0.024
Left ventricular ejection fraction <50%, n (%)	15 (6.6)	13 (5.1)	21 (7.6)	39 (12.7)	0.004
Right ventricular dysfunction, n (%)	8 (3.6)	14 (5.3)	14 (4.9)	34 (10.6)	0.001

Abbreviations: BMI = body mass index; eGFR = estimated glomerular filtration rate; HDL = high-density lipoprotein; hs-CRP = high-sensitivity C-reactive protein; NT-proBNP = N-terminal pro-brain natriuretic peptide.

Variables showing a skewed distribution are presented as median (IQR) and analyzed with Kruskal Wallis Test.

Table E2. Patients' demographics, cardiovascular risk factors and cardiovascular diseases according to quartiles of high-sensitivity troponin I in women.

	High-sensitivity troponin I quartiles				P for trend in women
	<2.49 ng/L	**2.50–3.79 ng/L**	**3.80–6.59 ng/L**	**≥6.60 ng/L**	
Age, years, mean (SD)	61.6 (8.7)	65.1 (8.1)	64.7 (8.0)	64.5 (8.2)	0.002
BMI, kg/m^2, mean (SD)	25.4 (5.1)	26.2 (5.7)	25.9 (5.8)	26.0 (5.8)	0.37
Current smoker, n (%)	87 (34.3)	55 (26.2)	42 (22.0)	39 (24.5)	0.009
Hypertension, n (%)	168 (66.1)	157 (74.8)	140 (73.3)	122 (76.3)	0.030
Glycated hemoglobin, mmol/mol, mean (SD)	39.2 (7.0)	39.8 (5.8)	40.3 (6.5)	39.7 (5.5)	0.326
Diabetes mellitus, n (%)	25 (9.8)	19 (9.0)	21 (11.0)	20 (12.5)	0.34
Total cholesterol / HDL cholesterol ratio, median (IQR)	3.0 (2.5–3.7)	3.2 (2.5–3.8)	3.0 (2.5–3.7)	3.1 (2.6–3.7)	0.81
Hyperlipidemia, n (%)	91 (35.8)	93 (44.3)	88 (46.1)	70 (43.8)	0.062
eGFR, mL/min, median (IQR)	83 (71–93)	77 (67–87)	75 (65–87)	79 (68–98)	0.005
eGFR <60 mL/min, n (%)	22 (8.8)	28 (13.5)	29 (15.5)	25 (15.8)	0.021
hs-CRP, mg/L, median (IQR)	3.3 (1.5–7.8)	4.6 (2.0–7.0)	4.0 (1.7–6.2)	4.0 (1.6–9.0)	0.76
Coronary artery disease, n (%)	19 (7.5)	19 (9.0)	13 (6.8)	21 (13.1)	0.14
History of myocardial infarction, n (%)	8 (3.1)	8 (3.8)	6 (3.1)	5 (3.1)	0.93
History of stroke, n (%)	9 (3.5)	6 (2.9)	11 (5.8)	3 (1.9)	0.85
Ankle-brachial index ≤0.9, n (%)	18 (7.1)	9 (4.4)	12 (6.5)	11 (7.1)	0.91
NT-proBNP, pg/mL, median (IQR)	130 (16–324)	154 (14–361)	180 (32–353)	178 (46–394)	0.056
Left ventricular ejection fraction <50%, n (%)	9 (4.1)	6 (3.2)	5 (3.0)	6 (4.4)	0.98
Right ventricular dysfunction, n (%)	15 (6.6)	12 (6.3)	10 (5.8)	13 (9.3)	0.46

Abbreviations: BMI = body mass index; eGFR = estimated glomerular filtration rate; HDL = high-density lipoprotein; hs-CRP = high-sensitivity C-reactive protein; NT-proBNP = N-terminal pro-brain natriuretic peptide.

Variables showing a skewed distribution or a heteroscedasticity are presented as median (IQR) and analyzed with Kruskal Wallis Test.

Figure E1. Patient flow diagram

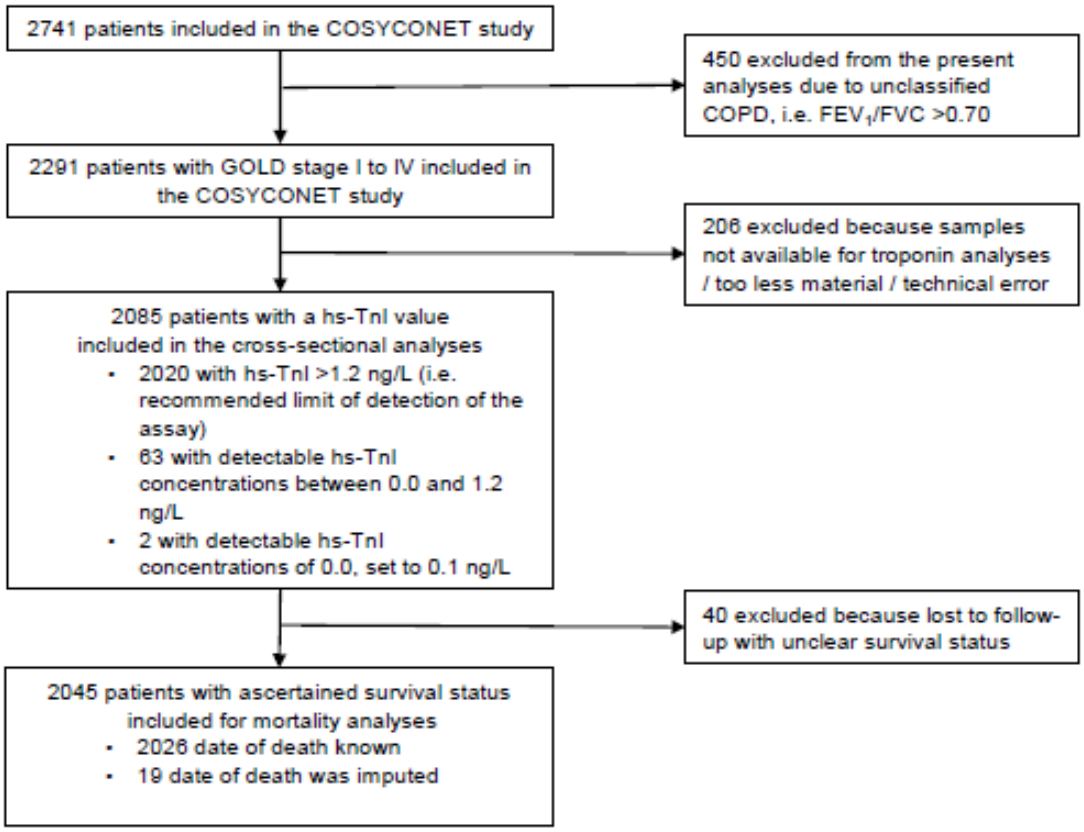
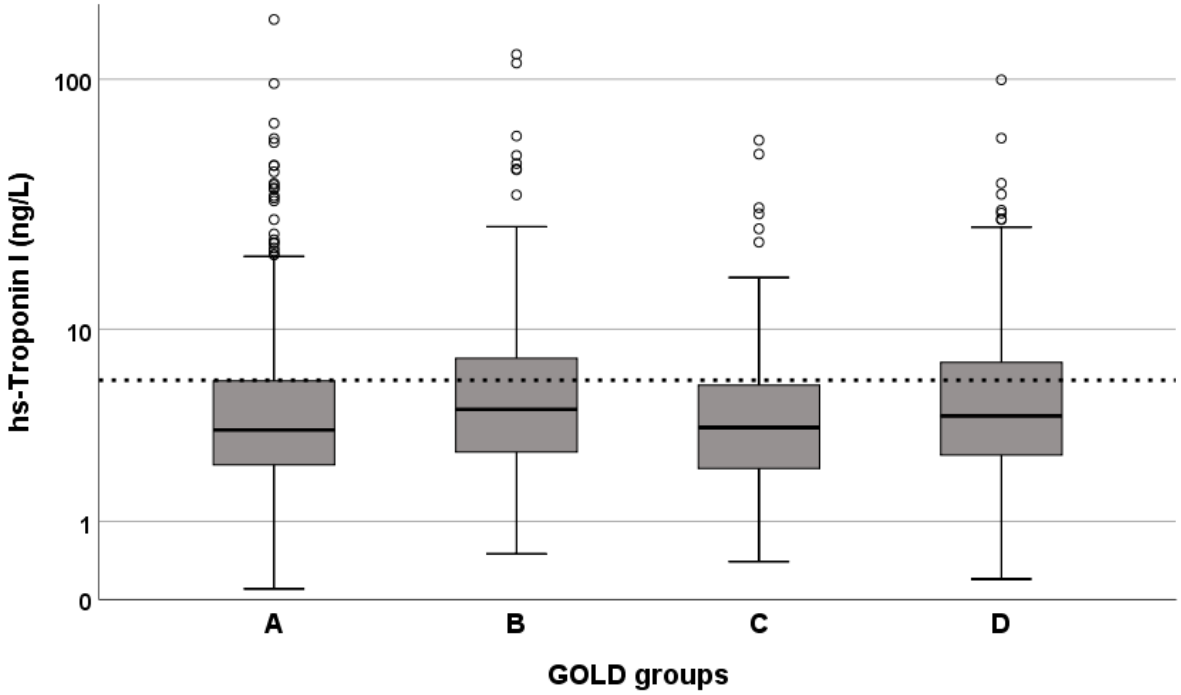


Figure E2. High-sensitivity troponin I levels according to GOLD groups



Legend Figure E2:

Hs-troponin I levels differed significantly across the GOLD groups ($p < 0.001$). The bars represent the interquartile range and the solid lines within the bars the median values. The dashed line represent the established hs-TnI cut-off of 6 ng/L, indicating an elevated cardiovascular risk.