Novel Biomarkes in Acute Respiratory Failure and Chronic Obstructive Pulmonary Disease.

Jónsdóttir, Brynja

2020

Document Version:
Publisher's PDF, also known as Version of record

Novel Biomarkers in Acute Respiratory Failure and Chronic Obstructive Pulmonary Disease

BRYNJA JÓNSDÓTTIR
DEPARTMENT OF CLINICAL SCIENCES, MALMÖ | LUND UNIVERSITY

Novel Biomarkers in Acute Respiratory Failure and Chronic Obstructive Pulmonary Disease

Novel Biomarkers in Acute Respiratory Failure and Chronic Obstructive Pulmonary Disease

Brynja Jónsdóttir

MD

LUND
UNIVERSITY

DOCTORAL DISSERTATION

by due permission of the Faculty of Medicine, Lund University, Sweden.
To be defended at Agardhsalen, CRC, Skåne University Hospital, Malmö.
Date 30th of May 2020 at 09.00.

Faculty opponent
Josefin Sundh, Associate Professor, School of Medical Sciences, Faculty of Medicine and Health, Örebro University. Senior consultant, Department of Respiratory Medicine, University Hospital, Örebro

Organization LUND UNIVERSITY Department of Clinical Sciences Malmö Faculty of Medicine	**Document name** DOCTORAL DISSERTATION **Date of issue** 30th of May 2020
Author Brynja Jónsdóttir, MD	Sponsoring organization None

Title and subtitle
Novel Biomarkers in Acute Respiratory Failure and Chronic Obstructive Pulmonary Disease

ABSTRACT

BACKGROUND: Dyspnea is a common manifestation of a range of conditions and diseases, sometimes with multiple contributing factors. A subgroup of these patients present with acute hypecapnic respiratory failure (AHRF) and are treated with non-invasive positive pressure ventilation (NPPV). Despite the biomarkers and clinical signs available to predict outcome in patients with respiratory failure of differing etiologies, further knowledge is needed to stratify patients according to risk of treatment failure or mortality at early stages of disease before the source of the deterioration may be apparent. Chronic obstructive pulmonary disease (COPD) is a common cause of dyspnea and, in its advanced stages, of respiratory failure. COPD is to some extent preventable, e.g. with smoking avoidance or cessation. Identification of predictors of COPD development in the general population is essential to recognise individuals at high risk of developing COPD and initiating preventive measures and early treatment.

AIMS: The aim of Study I was to evaluate whether the biomarkers interleukin-8 (IL-8) and growth differentiation factor 15 (GDF-15) are predictive of 28-day mortality in patients with AHRF of different underlying causes receiving NPPV treatment. In Study II, suppression of tumorigenicity 2 (ST2) was evaluated as predictive of 28-day and 18-month mortality, in the same cohort as in Study I. A secondary goal was to test the hypothesis that a decrease in ST2 concentration during the first 12 hours of NPPV treatment could indicate treatment efficacy. Study III evaluated the prognostic value of ST2 with respect to all-cause mortality in patients suffering from acute dyspnea, with stratification of the study cohort according to the suggested ST2 cut-off points for risk stratification in cardiac disease, 35 ng/mL and 70 ng/mL. Study IV assessed the value of IL-8, ST2, and GDF-15 in predicting diagnosis of COPD in secondary care and of all-cause mortality in a large population-based prospective cohort study.
SUBJECTS: Studies I and II were based on a small cohort (n=46) of patients with AHRF treated with NPPV in the Intermediate Emergency Care Department of Skåne University Hospital, Malmö, Sweden. Study III comprised a population presenting to the emergency department (ED) at Skåne University Hospital, Malmö, Sweden with acute dyspnea of differing etiologies (n=1251). Study IV was based on the cardiovascular cohort of the population-based Malmö Diet and Cancer Study (n=4292).

METHODS: Biomarker analysis was conducted with the Proseek biomarker panel (Proximity extension assay) and with the Presage ST2 assay for ST2. Statistical analysis was conducted with SPSS. In Studies I and II, patients were analysed in subgroups according to primary discharge diagnosis. The Study III subgroup analysis was based on hospital admission status, and Study IV according to COPD diagnosis in secondary care over a mean follow-up period 21 years. Cox proportional hazard models were used to determine the prognostic value of the biomarker concentrations for mortality and COPD diagnosis. Data regarding mortality was confirmed by the Swedish National Cause of Death Registry.

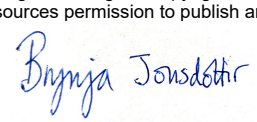
RESULTS: In Study I and II, the small cohort of 46 patients with AHRF treated with NPPV included three subgroups categorized by primary diagnosis: acute exacerbation of COPD (AECOPD, n=34), acute heart failure (AHF, n=8), and acute exacerbation in obesity hypoventilation syndrome (AEOHS, n=4). Plasma concentrations of IL-8 and ST2 were predictive of 28-day mortality independent of CRP concentration. This association was maintained when the subgroup with AECOPD was analyzed. In Study II, plasma ST2 was an independent predictor of 18-month mortality, although these findings may have been driven by deaths within the first 28 days. Decrease in ST2 values during the first 12 hours was not indicative of concurrent clinical improvement.In Study III, ST2 concentrations in patients seeking the ED with acute onset dyspnea (n=1251) were predictive of 3-month and 12-month mortality independent of NT-proBNP levels. Stratification of the Study III cohort according to the ST2 cut-off levels of 35 and 70 ng/mL was shown practicable in identifying high risk patients. In Study IV, comprising a large general population cohort (n=4292), GDF-15 was found positively associated with COPD diagnosis in secondary care during a mean follow-up period of 21 years, adjusted for smoking and other confounding factors. GDF-15, ST2, and IL-8 demonstrated a significant association with all-cause mortality during a mean follow-up period of 21.5 years, with GDF-15 showing the strongest association.

CONCLUSIONS: IL-8 and ST2 concentrations show association with acute clinical deterioration in respiratory failure and present targets for further exploration of prognostic value. The ST2 cut-off points of 35 ng/mL and 70 ng/mL used for risk stratification in acute cardiac disease can adequately identify high risk patients with acute dyspnea. Plasma GDF-15 concentration shows an association with long-term prognosis and mortality in respiratory failure, and, in the general population, can identify which individuals might develop COPD.

Key words
Chronic Obstructive Pulmonary Disease, dyspnea, respiratory failure, biomarkers, ST2, IL-8, GDF-15

Classification system and/or index terms (if any)

Supplementary bibliographical information	**Language:** English
ISSN and key title 1652-8220 Lund University, Faculty of Medicine Doctoral Dissertation Series 2020:69	**ISBN:** 978-91-7619-930-5
Recipient's notes / **Number of pages 94**	Price
Security classification	

Signature  Date 2020-04-20

Novel Biomarkers in Acute Respiratory Failure and Chronic Obstructive Pulmonary Disease

Brynja Jónsdóttir

MD

LUND
UNIVERSITY

Department of Clinical Sciences in Malmö,
Faculty of Medicine
Lund University, Sweden

Cover photo by the author

Lund University, Faculty of Medicine Doctoral Dissertation Series 2020:69
Department of Clinical Sciences Malmö
ISBN 978-91-7619-930-5
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2020

To my family, for giving me the strength

to believe that anything is possible

Table of Contents

Abbreviations

ADYS	Acute dyspnea study
AECOPD	Acute exacerbation of chronic obstructive pulmonary disease
AEOHS	Acute exacerbation of obesity hypoventilation syndrome
AHF	Acute heart failure
AHRF	Acute hypercapnic respiratory failure
ARDS	Acute respiratory distress syndrome
AUC	Area under the receiver operating characteristic curve
BE	Base excess
BMI	Body mass index
BNP	Brain natriuretic peptide
BP	Blood pressure
BPM	Breaths per minute
CI	Confidence interval
COPD	Chronic obstructive pulmonary disease
CPAP	Continuous positive airway pressure
CRP	C-reactive protein
DNR	Do not resuscitate
ED	Emergency department
ELISA	Enzyme-linked immunosorbent assay
EPAP	Expiratory positive airway pressure
FEV1	Forced expiratory volume in one second
GDF-15	Growth differentiation factor 15
HR	Hazard ratio
ICD	International classification of disease
IL-8	Interleukin 8
IL-33	Interleukin 33
IPAP	Inspiratory positive airway pressure
IQR	Interquartile range

LDL	Low density lipoprotein
LVEF	Left ventricle ejection fraction
METTS-A	Medical emergency triage and treatment system – adult score
MDC-CC	Malmö Diet and Cancer Cardiovascular Cohort
MDCS	Malmö Diet and Cancer Study
NPPV	Non-invasive positive pressure ventilation
NPs	Natriuretic peptides
NPX	Normalized protein expression
NT-proBNP	N-terminal prohormone of brain natriuretic peptide
OHS	Obesity hypoventilation syndrome
OR	Odds ratio
$PaCO_2$	Partial pressure of carbon dioxide in arterial blood
PEA	Proximity extension assay
PaO_2	Partial pressure of oxygen in arterial blood
RR	Respiratory rate
SD	Standard deviation
ST2	Suppression of tumorigenicity 2
ST2L	Suppression of tumorigenicity 2 ligand
TNF-α	Tumor necrosis factor α

List of papers

The thesis is based on four studies presented in two published papers and two manuscripts, referred to in text by corresponding Roman numerals. Permission for reprinting has been granted by the publishers.

I. **Jónsdóttir B**, Jaworowski Å, San Miguel C, Melander O. *IL-8 predicts early mortality in patients with acute hypercapnic respiratory failure treated with non-invasive positive pressure ventilation.* BMC Pulm Med, 2017. 17(1): p. 35.

II. **Jónsdóttir B**, Ziebell Severinsen M, Von Wowern F, San Miguel C, Goetze JP, Melander O. *ST2 Predicts Mortality in Patients with Acute Hypercapnic Respiratory Failure Treated with Non-invasive Positive Pressure Ventilation.* Int J Chron Obstruct Pulmon Dis, 2019. 14: pp. 2385–2393.

III. **Jónsdóttir B**, Ziebell Severinsen M, Von Wowern F, Lazkani H, Saleh M, Wiklund K, Goetze JP, Melander O. *ST2 predicts mortality in patients with acute dyspnea.* In manuscript.

IV. **Jónsdóttir B**, Engström G, Von Wowern F, Nilsson J, Orho-Melander M, Melander O. *Growth differentiation factor 15 measured in the general population predicts chronic obstructive pulmonary disease.* In manuscript.

Abstract

Background: Dyspnea is a common manifestation of a range of conditions and diseases, sometimes with multiple contributing factors. A subgroup of these patients present with acute hypecapnic respiratory failure (AHRF) and are treated with non-invasive positive pressure ventilation (NPPV). Despite the biomarkers and clinical signs available to predict outcome in patients with respiratory failure of differing etiologies, further knowledge is needed to stratify patients according to risk of treatment failure or mortality at early stages of disease before the source of the deterioration may be apparent. Chronic obstructive pulmonary disease (COPD) is a common cause of dyspnea and, in its advanced stages, of respiratory failure. COPD is to some extent preventable, e.g. with smoking avoidance or cessation. Identification of predictors of COPD development in the general population is essential to recognise individuals at high risk of developing COPD and initiating preventive measures and early treatment.

Aims: The aim of Study I was to evaluate whether the biomarkers interleukin-8 (IL-8) and growth differentiation factor 15 (GDF-15) are predictive of 28-day mortality in patients with AHRF of different underlying causes receiving NPPV treatment. In Study II, suppression of tumorigenicity 2 (ST2) was evaluated as predictive of 28-day and 18-month mortality, in the same cohort as in Study I. A secondary goal was to test the hypothesis that a decrease in ST2 concentration during the first 12 hours of NPPV treatment could indicate treatment efficacy. Study III evaluated the prognostic value of ST2 with respect to all-cause mortality in patients suffering from acute dyspnea, with stratification of the study cohort according to the suggested ST2 cut-off points for risk stratification in cardiac disease, 35 ng/mL and 70 ng/mL. Study IV assessed the value of IL-8, ST2, and GDF-15 in predicting diagnosis of COPD in secondary care and of all-cause mortality in a large population-based prospective cohort study.

Subjects: Studies I and II were based on a small cohort (n=46) of patients with AHRF treated with NPPV in the Intermediate Emergency Care Department of Skåne University Hospital, Malmö, Sweden. Study III comprised a population presenting to the emergency department (ED) at Skåne University Hospital, Malmö, Sweden with acute dyspnea of differing etiologies (n=1251). Study IV was based on the cardiovascular cohort of the population-based Malmö Diet and Cancer Study (n=4292).

Methods: Biomarker analysis was conducted with the Proseek biomarker panel (Proximity extension assay) and with the Presage ST2 assay for ST2. Statistical analysis was conducted with SPSS. In Studies I and II, patients were analysed in subgroups according to primary discharge diagnosis. The Study III subgroup analysis was based on hospital admission status, and Study IV according to COPD diagnosis in secondary care over a mean follow-up period 21 years. Cox proportional hazard models were used to determine the prognostic value of the

biomarker concentrations for mortality and COPD diagnosis. Data regarding mortality was confirmed by the Swedish National Cause of Death Registry.

Results: In Study I and II, the small cohort of 46 patients with AHRF treated with NPPV included three subgroups categorized by primary diagnosis: acute exacerbation of COPD (AECOPD, n=34), acute heart failure (AHF, n=8), and acute exacerbation in obesity hypoventilation syndrome (AEOHS, n=4). Plasma concentrations of IL-8 and ST2 were predictive of 28-day mortality independent of CRP concentration. This association was maintained when the subgroup with AECOPD was analyzed. In Study II, plasma ST2 was an independent predictor of 18-month mortality, although these findings may have been driven by deaths within the first 28 days. Decrease in ST2 values during the first 12 hours was not indicative of concurrent clinical improvement. In Study III, ST2 concentrations in patients seeking the ED with acute onset dyspnea (n=1251) were predictive of 3-month and 12-month mortality independent of NT-proBNP levels. Stratification of the Study III cohort according to the ST2 cut-off levels of 35 and 70 ng/mL was shown practicable in identifying high risk patients. In Study IV, comprising a large general population cohort (n=4292), GDF-15 was found positively associated with COPD diagnosis in secondary care during a mean follow-up period of 21 years, adjusted for smoking and other confounding factors. GDF-15, ST2, and IL-8 demonstrated a significant association with all-cause mortality during a mean follow-up period of 21.5 years, with GDF-15 showing the strongest association.

Conclusions: IL-8 and ST2 concentrations show association with acute clinical deterioration in respiratory failure and present targets for further exploration of prognostic value. The ST2 cut-off points of 35 ng/mL and 70 ng/mL used for risk stratification in acute cardiac disease can adequately identify high risk patients with acute dyspnea. Plasma GDF-15 concentration shows an association with long-term prognosis and mortality in respiratory failure, and, in the general population, can identify which individuals might develop COPD.

Svensk sammanfattning

Andfåddhet är ett vanligt förekommande symtom med flera olika bakomliggande orsaker. Ofta föreligger många anledningar till att en patient känner sig andfådd. En del av patienter som söker vård för andfåddhet har andningssvikt, vilket betyder att de har svårt att syresätta sig. Vissa patienter med andningssvikt har dessutom dålig förmåga för att vädra ut koldioxid, vilket leder till att koldioxidhalten i blodet stiger och orsakar en såkallad hyperkapnisk andningssvikt. Vid akut påkommen andningssvikt behandlas dessa patienter ofta med bifasisk noninvasiv ventilation (noninvasive positive pressure ventilation = NPPV). För att identifiera patienter i denna grupp som har hög risk för behandlingssvikt och död, har forskning påvisat vissa förutsägande faktorer. Trots det kan det vara svårt att förutsäga utfallet, särskild i början av förloppet när underliggande orsak inte alltid är tydlig. Därför är det viktigt att hitta nya faktorer som kan förutsäga utfall och död.

Kronisk obstruktiv lungsjukdom (KOL) är en vanligt förekommande sjukdom som kan orsaka andfåddhet och andningssvikt. Patienter med hyperkapnisk andningssvikt har dessutom ofta underliggande svår KOL. Sjukdomen är delvis möjlig att föhindra, t.ex. med rökstopp. För att kunna lägga vikt på sådana förebyggande åtgärder i tidigt skede är det viktigt att hitta faktorer som kan förutsäga vilka inidivider har högst risk för att utveckla sjukdomen.

Målet med denna avhandling var att hitta mätbara markörer som kan förutsäga utfall hos patienter med hyperkapnisk andningssvikt som behandlas med NPPV. De markörer som hittades i de första två studierna, har sedan utvärderats som förutsägande faktorer för dödlighet hos patienter med akut andfåddhet (tredje studien) och som förutsägande för utveckling av KOL och dödlighet i en populationsstudie (fjärde studien).

För studie I och II inkluderades 46 patienter med hyperkapnisk andningssvikt som behandlades med NPPV på Akutvårdsavdelningen, Skånes Universitetssjukhus i Malmö i januari – juni 2014. Patienternas tillstånd övervakades noggrant och regelbundna kontroller mättes. Information angående tidigare sjukdomar, det aktuella vårdtilfället och kliniska parametrar samlades ihop i en databas. Blodprover togs innan behandlingen påbörjades och regelbundet under vårdtiden.

I studie I utvärderades två inflammatoriska biomarkörer, Interleukin-8 (IL-8) och Growth Differentiation Factor 15 (GDF-15). Vi studerade om halterna av dessa biomarkörer i blod kunde förutsäga korttidsdödlighet (28 dagar). Analyserna visade att höga halter av IL-8 kunde förutsäga korttidsdödligheten oberoende av C-reactive protein (CRP), en av dem mest kända inflammatoriska biomarkörerna. Däremot kunde vi inte utesluta att GDF-15 var oberoende av IL-8 i sammanhanget. Resultaten verkar ha störst signifikans i den största undergruppen, patienter med akut försämring av KOL (34 patienter).

I studie II utvärderadas den inflammatoriska biomarkören Suppression of Tumorigenicity 2 (ST2) på samma patientgrupp som i studie I. Dessutom studerade vi om ST2 halter kunde förutsäga långtidsdödlighet (18 månader). I denna studie mättes ST2 med två olika metoder där resultaten samvarierade starkt med varandra. ST2 halter kunde förutsäga båda korttids- och långtidsdödligheten i patientgruppen, även om vi drar slutsatsen att sambandet är starkast mellan ST2 halter och korttidsdödligheten i undergruppen av patienter med försämring i KOL. Det andra målet med studie II var att utvärdera om förändringar i halter av ST2 under de första 12 timmarna av behandlingen kunde indikera utfallet av behandlingen. Våra resultat kunde inte bekräfta våran teori, att sjunkande halter av ST2 kunde tyda på ett samtidigt positivt behandlingssvar.

I studie III utvärderades om ST2 kunde förutsäga 3 månaders och 12 månaders dödlighet hos 1251 patienter som sökte akutmottagningen i Malmö med andfåddhet 2013-2019. I denna grupp kunde ST2 halter förutsäga dödlighet efter 3 och 12 månader. Resultaten var oberoende av rökning och NT-proBNP, en känd biomarkör relaterad till hjärtsvikt. Patientgruppen delades även upp utifrån två ST2 gränsvärden, 35 ng/mL och 70 ng/mL, kända som användbara för att hitta patienter med hjärtsjukdomar som har ökad risk för dåligt utfall. Även i denna grupp patienter med olika bakomliggande orsaker för andfåddhet var gränsvärderna informativa för riskstratifiering.

I studie IV utvärderades om biomarkörerna ST2, IL-8 och GDF-15 kunde förutsäga utveckling av KOL och dödlighet i en populationsstudie, Malmö Kost Cancer studien. 4292 individer följdes upp i ungefär 21 år. Av de tre biomarkörerna var det endast GDF-15 som kunde oberoende förutsäga KOL utveckling i kohorten. GDF-15 kunde även på mest övertygande sätt kopplas till dödlighet under uppföljningstiden. Detta samband var oberoende av rökning och andra möjliga störande faktorer.

Sammanfattningsvis visar resultaten av studierna i avhandlingen att biomarkörerna IL-8 och ST2 verkar ha starkt samband med akut andningssvikt av olika orsaker. I framtiden är det lämpligt att utvärdera deras roll i riskstratifiering och att förutsäga dödlighet. GDF-15 verkar mer relaterad till långtidsprognos i andningssvikt och kan möjligen vara till hjälp att hitta patienter som har risk för att utveckla KOL. Om dessa patienter kan hittas i tidigt skede kan förebyggande åtgärder och tidig behandling inledas.

Íslensk samantekt

Mæði er algengt einkenni ýmissa sjúkdóma. Margir þættir geta haft áhrif á upplifun sjúklings með mæði. Hluti sjúklinga sem leita hjálpar vegna mæði þjást af öndunarbilun, þar sem röskun verður á loftskiptum og sjúklingar eiga erfitt með að halda uppi súrefnismettun. Sumir sjúklingar með öndunarbilun eiga þar að auki erfitt með að skilja út koltvísýring, sem getur þá orðið of hár í blóði. Þetta ástand kallast koltvísýringsbilun. Í bráðri öndunarbilun með háum styrk koltvísýrings í blóði eru sjúklingar oft meðhöndlaðir með ytri öndunarvél sem hefur tveggja þrepa jákvæðan innöndunarþrýsting (e. noninvasive positive pressure ventilation, NPPV). Ýmsar rannsóknir hafa verið gerðar til að leita að forspárþáttum fyrir horfur þessa sjúklingahóps. Þrátt fyrir það getur verið erfitt að áætla hvort meðferðin muni hjálpa sjúklingunum, einkum í byrjun meðferðarinnar þegar ekki er vitað hvaða ástand eða sjúkdómur liggur að baki hinni bráðu versnun. Af þessum sökum er mikilvægt að skilgreina fleiri forspárþætti sem geta orðið til aðstoðar við meðferðarval.

Langvinn lungateppa (LLT) er algengur sjúkdómur sem getur valdið mæði og öndunarbilun, í sumum tilfellum koltvísýringsbilun. Reykingar eru einn af helstu áhættuþáttum LLT. Hægt er að hafa áhrif á þróun LLT með því að hvetja sjúklinga til að hætta að reykja. Til þess að geta hafið slíkar fyrirbyggjandi aðgerðir sem fyrst eftir að sjúkdómurinn hefur byrjað að þróast er mikilvægt að skilgreina þætti sem spá fyrir um hvaða einstaklingar eru í sérstakri hættu á að fá LLT og vinna að forvörnum í þeim hópi.

Í þessari doktorsritgerð eru fjórar rannsóknir kynntar. Markmiðið var að finna mælanlega lífmarka (e.biomarker) sem gætu hjálpað við að spá fyrir um útkomu sjúklinga með koltvísýringsbilun sem eru meðhöndlaðir með NPPV. Þeir lífmarkar sem fundust í rannsókn I og II voru síðan metnir með tilliti til forspárgildis fyrir lifun í sjúklingum sem leituðu á bráðamóttöku vegna mæði (rannsókn III) og þróunar á LLT og lifun hjá frískum einstaklingum (rannsókn IV).

Þýðið í rannsókn I og II samanstóð af 46 sjúklingum með bráða koltvísýringsbilun sem voru meðhöndlaðir með NPPV á hágæsludeild á Háskólasjúkrahúsinu í Malmö, Svíþjóð, í janúar – júní 2014. Fylgst var náið með ástandi sjúklinganna samtímis því sem lífsmörk og blóðprufur voru teknar reglulega. Upplýsingar um fyrri sjúkdóma, félagslega þætti, innlögnina sjálfa, niðustöður úr blóðprufur og mælingum sem voru gerðar, var safnað í gagnagrunn.

Í rannsókn I voru tveir lífmarkar tengdir bólguviðbrögðum valdir til athugunar, Interleukin-8 (IL-8) og Growth Differentiation Factor 15 (GDF-15). Athugað var hvort þéttni þessara lífmarka gæti haft forspárgildi varðandi skammtímalifun sjúklinganna (skilgreind sem 28 dagar). Rannsóknin sýndi að þéttni IL-8 gat spáð fyrir um 28 daga lifun í sjúklingahópnum, óháð CRP sem er algengur lífmarki tengdur bráðri bólgu. GDF-15 hafði einnig forspárgildi fyrir skammtímalifun, en

niðurstöðurnar gátu ekki útilokað að GDF-15 væri háð IL-8 í þessu samhengi. Niðurstöðurnar hafa mest vægi í stærsta undirflokki rannsóknarþýðisins sem þjáðist af versnun á LLT (34 sjúklingar).

Í rannsókn II var lífmarkinn Suppression of Tumorigenicity 2 (ST2) sem er einnig tengdur bólguviðbrögðum, valinn til athugunar í sama sjúklingahóp og í rannsókn 1. Til viðbótar við skammtímalifun var einnig kannað hvort ST2 hefði forspárgildi hvað varðar langtímalifun (skilgreind sem 18 mánuðir). Í þessari rannsókn var ST2 mælt með tveimur mismunandi mæliaðferðum þar sem samsvörun reyndist góð. Þéttni ST2 hafði forspárgildi hvað varðar bæði skammtíma- og langtímalifun. Ályktun rannsóknarhópsins er þó sú að sambandið sé sterkara hvað varðar skammtímalifun og sterkast í undirflokki sjúklinga með versnun á LLT. Annað markmið þessarar rannsóknar var að kanna þá kenningu að endurteknar mælingar á ST2 á fyrstu klukkustundum meðferðar gæti spáð fyrir um útkomu meðferðarinnar. Niðurstöðurnar styrktu ekki þá kenningu að minnkandi þéttni ST2 á fyrstu 12 klukkustundunum eftir að meðferð er hafin sé tengd bætingu í ástandi sjúklinganna.

Í rannsókn III var kannað hvort ST2 hefði forspárgildi fyrir 3ja og 12 mánaða lifun hjá 1251 sjúklingum sem leituðu á bráðamóttöku á Háskólasjúkrahúsinu í Malmö vegna mæði á árunum 2013-2019. Í þessum hópi hafði ST2 forspárgildi fyrir lifun, óháð NT-proBNP, lífmarka sem tengist hjartabilun. Sjúklingahópnum var einnig skipt upp í hópa út frá tveimur viðmiðunargildum ST2 (35 og 70 ng/mL), sem eru þekkt fyrir að vera til aðstoðar við að spá fyrir um lifun og líðan sjúklinga með hjartasjúkdóma. Í þessum hópi sjúklinga með mæði af mismunandi orsökum höfðu þessi viðmiðunargildi hlutverk við að finna sjúklinga í mikilli áhættu á lélegri útkomu.

Í rannsókn IV voru allir þrír lífmarkarnir IL-8, ST2 og GDF-15 metnir með tilliti til forspárgildi fyrir þróun á LLT og langtímalifun, hjá 4292 einstaklingum tilheyrandi frísku þýði (Malmö Diet and Cancer Cohort) með eftirfylgd í u.þ.b. 21 ár. Af þessum þremur lífmörkum var það eingöngu GDF-15 sem hafði sjálfstætt forspárgildi varðandi þróun á LLT. Einnig var hægt að tengja GDF-15 þéttni við langtímalifun í þýðinu. Þetta samband var óháð reykingum og öðrum mögulegum flækjuþáttum.

Niðurstöður rannsóknanna í doktorsritgerðinni sýna þannig að lífmarkarnir IL-8 og ST2 virðast hafa mögulegt forspárgildi í bráðri öndunarbilun af ýmsum orsökum. Í framtíðinni er mögulegt að meta hlutverk þeirra við að finna sjúklinga með aukna áhættu á slæmri útkomu. Lífmarkinn GDF-15 virðist frekar tengdur langtímahorfum sjúklinga með öndunarbilun og getur mögulega haft hlutverki að gegna í að finna einstaklinga sem eru í mestri áhættu á að þróa LLT. Ef þessir einstaklingar finnast snemma í sjúkdómsferlinu væri hægt að leggja áherslu á fyrirbyggjandi aðgerðir svo sem reykingastopp, og hefja meðferð sjúkdómsins snemma.

Introduction

Dyspnea

Definition and presentation

Dyspnea, or breathlessness, is defined by the American Thoracic Society as a subjective experience of breathing discomfort that consists of qualitatively distinct sensations that vary in intensity [1]. It is a common symptom of both acute and chronic clinical conditions, frequently leading to visits to emergency departments and hospital admission. Approximately 5% of patients seeking the ED exhibit dyspnea as a primary symptom, and many experience dyspnea as a secondary symptom co-existing with other major disease manifestations [2,3]. A wide range of underlying conditions and diseases can cause dyspnea, including pulmonary, cardiovascular, neuromuscular, traumatic, psychiatric, toxic, and metabolic disorders [3,4]. The presence of dyspnea is prognostic of worse outcome in pulmonary and cardiac disease, and effective relief remains a challenge in these patients [1,4,5]. The subjective experience of dyspnea varies widely among individuals and with underlying condition, with severity ranging from mild discomfort to a sensation of imminent threat to life. The determination of which patients to prioritize for immediate assessment and treatment is therefore a clinical challenge [1-3].

Initial evaluation of acute dyspnea

The key factors in identifying the underlying disorder or disorders responsible for acute onset dyspnea or worsening of chronic dyspnea are review of medical records and history of the current illness along with physical examination, including respiratory rate and oxygen saturation [3]. Although the causal conditions can often be diagnosed and successfully treated after initial assessment, biomarkers and imaging methods are helpful in planning the most appropriate second-line treatment and predicting outcome [1,3]. When preventive actions such as modifications of medical treatment or life-style factors are being considered, biomarkers prognostic of short- and long-term outcome are especially important [4,6].

Assessment of the natriuretic peptides (NP), brain natriuretic peptide (BNP) and N-terminal prohormone of BNP (NT-proBNP), is used in diagnosis of acute heart failure (AHF) as a primary cause of acute dyspnea [4,6]. Other biomarkers, including cardiac troponins, have also been studied in the setting of acute dyspnea, although, in a recent review, Suzuki *et al.* point out that more research is necessary, particularly regarding dyspnea associated with pulmonary disease [6]. They stress that, even when the primary cause of dyspnea is diagnosed, there is often a complex pathophysiology involving both the heart and the lungs [6]. Thus, it remains difficult to differentiate clinically between cardiac and pulmonary causes, which often co-exist and show synergistic effects.

Respiratory Failure

Respiratory failure is caused by inadequate alveolar gas exchange resulting in hypoxia with or without hypercapnia. Common causes of hypoxic respiratory failure are pneumonia, acute respiratory distress syndrome (ARDS), and pulmonary oedema in AHF. Hypercapnic respiratory failure is more frequently seen in patients with acute exacerbations of chronic obstructive pulmonary disease (COPD) and is a signal of advanced disease [3,7]. Hypercapnic respiratory failure may also be a result of neuromuscular disorders and obesity hypoventilation syndrome (OHS) [7].

Analysis of arterial blood gases is essential in initial evaluation of respiratory failure. Hypoxia (PaO_2 <10.5 kPa) is frequently a component of stable chronic respiratory disease and can become more pronounced in acute deterioration. In the presence of hypercapnia ($PaCO_2$ >6.0 kPa) during acute respiratory failure, pH is reduced, resulting in respiratory acidosis. If hypercapnia is present in chronic respiratory failure, metabolic compensation can improve or rectify the acidosis by exchanging H+ for HCO3-, resulting in increased base excess (normal range -3 to +3) [8].

Respiratory failure can be acute or chronic as well as a manifestation of an acute exacerbation of a chronic condition, for example COPD. In the case of acute dyspnea, multiple clinical conditions may contribute to respiratory failure, presenting a challenge to diagnosis and treatment, as well as to evaluating risk of adverse outcome or mortality [3]. In this thesis, the causes of respiratory failure discussed are COPD, heart failure, and OHS.

Chronic Obstructive Pulmonary Disease

Pathophysiology

Chronic obstructive pulmonary disease is a heterogeneous disease characterized by irreversible airway obstruction and persistent respiratory symptoms such as

dyspnea, wheezing, and cough [9]. It is a systemic inflammatory disease with slowly progressing symptoms related to airway pathology, including fibrosis [10]. Remodelling of both small and large airways involves structural alterations such as increased smooth muscle mass, subepithelial fibrosis, glandular hypertrophy, and neovascularization, eventually leading to narrowing of airways and obstruction [11]. The development of therapies that target specific immune pathways is challenging, since the pathophysiology of COPD is not fully understood [12].

The airways are constantly exposed to airborne pathogens, and inflammatory cells such as macrophages, neutrophils, lymphocytes, and eosinophils are important components of first-line defence mechanism [13]. Recent studies suggest that the respiratory epithelium is the main contributor to the development of inflammatory airway disorders via its response to environmental stimuli with secretion of antimicrobial peptides, chemokines, and cytokines, thereby attracting and activating inflammatory cells [13]. Epithelial dysfunction seems to be one of the major factors contributing to altered mucosal integrity and disrupted barrier functions [13].

Epidemiology and diagnosis

The major risk factors for COPD development are smoking and second-hand smoke exposure, indoor and outdoor air pollutants, and industrial agents [14]. Since COPD is, to some extent, a preventable disease, it is important to diagnose patients early and to raise universal awareness regarding risk factors [14]. Chronic obstructive pulmonary disease is thought to affect more than 300 million people globally and is one of the most common causes of death worldwide [15,16]. The diagnosis of COPD is based on pulmonary function tests. Airway obstruction that is not fully reversible is diagnostic, combined with the presence of related symptoms evaluated by the COPD Assessment Test questionnaire [9,17]. The simplicity of this diagnostic tool makes it convenient, although the information provided is not sufficient to differentiate between COPD phenotypes [18-20]. Despite the availability of this simple and inexpensive diagnostic method, COPD is considered under-diagnosed [14]. Increasing the accuracy of COPD diagnosis would be beneficial, especially during earlier stages of the disease, as would development of phenotyping methods, since therapy approaches and optimal treatment strategies might be phenotype-specific [19,21].

Treatment and prognosis

Avoidance of smoking and other relevant exposures is important for preventing COPD development. There is currently no known therapy that can be initiated in the pre-clinical state that will prevent COPD progression to symptomatic disease, and medical treatment aims to relieve symptoms and prevent exacerbations [17,19]. The first line of treatment recommended by the Global Initiative for Chronic Obstructive Lung Disease is bronchodilators, including long-acting ß adrenergic receptor (ß2) agonists and long-acting muscarinic antagonists, with the addition of

inhaled corticosteroids (ICS) in the case of severe symptoms or frequent exacerbations [9].

Chronic obstructive pulmonary disease is a progressive condition leading to hospitalizations, increased morbidity, and mortality, mainly as a result of acute exacerbations (AECOPD) [22]. Exacerbations are defined as increased dyspnea, increased sputum production, and/or discoloration of sputum triggered by bacterial or viral respiratory infections, environmental pollutants, or unknown factors [21,23]. Acute exacerbations of COPD are commonly diagnosed on the basis of symptoms. Only about half of hospital admissions are thought to be associated with infection [19].

Research of biomarkers in COPD

The heterogeneity of COPD presents a challenge to identification of biomarkers useful in predicting disease progression or manifestations such as acute exacerbations, as well as for therapeutic guidance [12,19,20]. Biomarker research is nonetheless critical to revealing the pathogenesis of different disease phenotypes. Radiological and physiological biomarkers in lung tissue, blood, sputum, exhaled breath condensate, and the lung microbiome have been investigated in this context [10,18,20]. Associated endpoints include lung function decline, symptoms, exacerbation rate, hospitalization, and mortality [18].

In a recent meta-analysis of studies of patients with stable COPD, Fermont *et al*. found a positive association of mortality and shorter distance on 6-minute walk distance test and higher resting respiratory rate, C-reactive protein (CRP), fibrinogen, and white blood cell count [18]. In recent years, eosinophil count has become one the most commonly researched biomarkers for therapy guidance in COPD, as patients with high eosinophil levels are more likely so respond to treatment with ICS [10,12]. The top three biomarkers that have been associated with AECOPD are CRP, interleukin-6, and tumour necrosis factor-α (TNF-α) [10].

Potential approaches to the development of novel medical treatments include targeting the imbalance of airway proteases, i.e. enzymes that exert tissue-damaging effects in the airway and are implicated to have a role in COPD [12]. In order to establish novel and target-specific therapies, a better understanding of pro- and anti-inflammatory cytokines is needed [13].

Heart Failure

Heart failure is a clinical syndrome caused by a structural and/or functional cardiac abnormality resulting in reduced cardiac output and/or elevated intracardiac pressure that can lead to decreased peripheral perfusion and pulmonary oedema. The chief symptoms are dyspnea, orthopnoea, and lower limb oedema. Heart failure affects 1–2% of the population in the western world and its prevalence increases with age [24,25]. The most common aetiology is ischemic coronary artery disease,

but causes may be inflammatory, toxic, valvular failure, extra-cardial, or unknown [24,26]. Patients with heart failure often exhibit co-morbidities such as atrial fibrillation, valvular disease, and diabetes. Approximately 20–30% have concomitant COPD [27].

Patients with heart failure are differentiated based on echocardiographic measurements of left ventricular ejection fraction (LVEF) ranging from preserved (>50%), mid-range (40-49%), or reduced LVEF (<40%) [26]. Plasma concentrations of NPs can be used initially to establish a working diagnosis. An abnormal electrocardiogram is associated with increased likelihood of established heart failure, although echocardiography is the most useful widely available test to confirm the diagnosis [26].

Preventive measures such as hypertension management and life-style modification are important in the pre-clinical phase of heart failure. The goals of treatment are to improve clinical status, functional capacity, and quality of life as well as to prevent hospital admission and reduce mortality [26]. Beta-blockers, angiotensin converting enzyme inhibitors/angiotensin receptor blockers, and mineralocorticoid receptor antagonists are first-line treatment, with additional therapies individualised according to clinical status and co-morbidities [26].

Acute heart failure (AHF) is defined as a rapid onset of new or worsening signs and symptoms of heart failure. A new presentation is designated as *de novo* AHF, although the majority of patients have pre-existing cardiomyopathy with acute decompensated heart failure [28]. Acute heart failure is a common cause of ED visits and hospitalization, often presenting with acute-onset dyspnea widely varying in severity [3,29]. Older guidelines classified AHF into six groups based on the underlying condition. Given that the patients often present with a range of co-morbidities, the source of decompensation might be multifactorial, and it is not always possible to assign a patient to a single category. Revised methods of stratifying AHF patients according to results of a comprehensive evaluation based on severity of presentation and clinical signs are under development, in order to individualize intervention [24,26].

Obesity Hypoventilation Syndrome

Obesity hypoventilation syndrome (OHS) is defined by the association of obesity (BMI ≥ 30 kg/m2), daytime hypercapnia ($PaCO_2$ > 6.0 kPa), and sleep-disordered breathing, after ruling out other disorders that may cause alveolar hypoventilation. Obesity hypoventilation syndrome is thought to be the cause of sleep-disordered breathing and daytime hypersomnia in 8–20% of obese patients referred to sleep centres for evaluation [30]. In up to 40% of patients, OHS is initially diagnosed during acute-on-chronic hypercapnic respiratory failure leading to ED visit or hospitalization and is often misdiagnosed as acute exacerbation of COPD [30-32]. Since patients with OHS are more severely affected by cardiovascular disease than

are eucapnic obese patients, it is important to raise awareness of the condition, diagnose accurately, and initiate appropriate therapy. A comprehensive management program including weight loss and treatment with non-invasive ventilation is recommended [32].

Non-invasive positive pressure ventilation in acute respiratory failure

Non-invasive positive pressure ventilation (NPPV) is a form of ventilatory support applied via a mask connected to a ventilator that applies positive pressure during both inspiration and expiration [33]. The nomenclature of non-invasive ventilation is somewhat confusing, with use of different definitions and terminology. In this thesis, NPPV refers to non-invasive bilevel positive pressure ventilation, with higher inspiratory, compared with expiratory, pressure, as opposed to continuous positive airway pressure (CPAP), in which consistent positive pressure is applied during the entire respiratory cycle [34].

NPPV is widely used for treating acute hypoxic or hypercapnic respiratory failure, to which multiple underlying causes can contribute simultaneously [7,34-37]. NPPV can be pressure-controlled with consistent inspiratory and expiratory positive airway pressure (IPAP and EPAP) or volume-controlled with a target tidal volume, allowing adjustments to maintain a target mean ventilation over several respiratory cycles. The difference between IPAP and EPAP is the pressure support (**Figure 1**). Inspiratory positive airway pressure improves respiratory physiological parameters, allows respiratory muscle rest, and counterbalances the effects of dynamic hyperinflation in the case of COPD. Expiratory positive airway pressure improves gas exchange and, hence, oxygenation. Most often the patient triggers a switch from EPAP to IPAP by initiating an inspiration, although the ventilator can initiate a new respiratory cycle, and does so automatically if the patient's respiratory rate drops below a previously defined back-up rate [34,36].

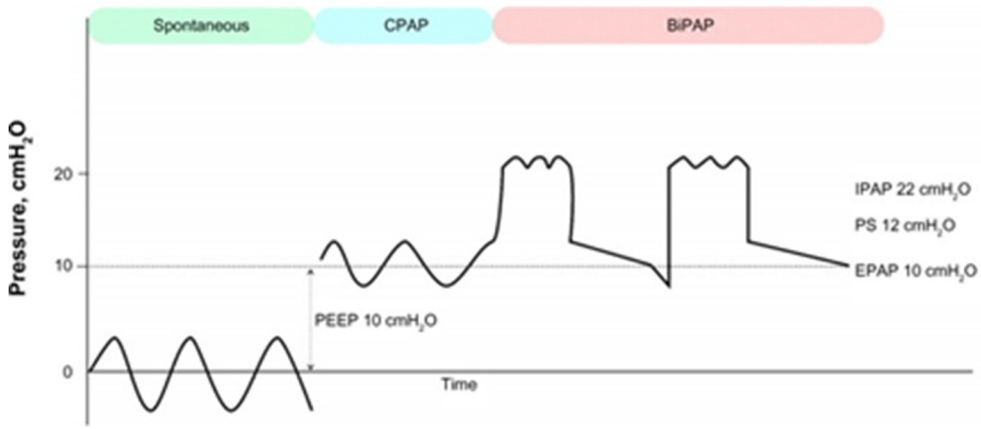


Figure 1: Pressure-time curves showing inhalation and exhalation pressure during spontaneous breathing and with CPAP and BiPAP (bilevel positive airway pressure, same as NPPV). From Mas *et al*. [36] Reprinted with permission of the: International Journal of COPD, 2014, 9 837–85.

Abbreviations: BiPAP, bilevel positive airway pressure; CPAP, continuous positive airway pressure; EPAP, expiratory positive airway pressure; IPAP, inspiratory positive airway pressure; NPPV, non-invasive positive pressure ventilation; PEEP, positive end-expiratory pressure; PS, pressure support.

NPPV in exacerbations of COPD

Non-invasive positive pressure ventilation is used to treat acute respiratory failure in AECOPD, especially if hypercapnia is present. In patients with AECOPD and hypercapnia unresponsive to appropriate medical therapy, NPPV reduces the need for endotracheal intubation, the length of hospital stay, and the in-hospital mortality rate [38-43]. The treatment is most effective during the early stages of respiratory acidosis, and selecting patients that will respond satisfactorily can be challenging [44-47]. In AECOPD, factors that predict treatment failure resulting in endotracheal intubation or death include severe acidosis (pH <7.25), low Glasgow Coma Scale scores, high respiratory rate, and high APACHE-II scores [48].

In early published randomized trials evaluating the use of NPPV in AECOPD, the patient groups were highly selected, and endotracheal intubation was frequently used when NPPV treatment failed [38-43]. Some studies have assessed the clinical use of NPPV in AECOPD and compared results with the original treatment guidelines based on the early randomized trials. Mortality rates seem to be higher in clinical practice, with in-hospital mortalities mortality of 9–10% in the early randomized trials compared with 20–30% in clinical practice [42,49-52]. Hedsund *et al.* conducted a sub-analysis comprising patients that fulfilled the exclusion criteria used by Plant *et al.* in 2000, and found that, when this group was treated with NPPV, the in-hospital and 1-year mortality was higher than in patients that would not have been excluded from the Plant *et al.* study [42,51].

It has been reported that, in clinical practice, severely acidotic patients are treated similarly with NPPV regardless of contributing factors and without an alternative plan in case of treatment failure [50,51]. There is need for a better defined standard of optimal care for the entire range of acidotic patients and for implementation of international guidelines, despite the challenges involved, since severely ill patients are rarely eligible for randomized controlled trials, although they are common in everyday clinical practice [50,51]. Nonetheless NPPV treatment has proven beneficial in patients receiving therapy as a component of palliative care because of old age or critical clinical condition and a do-not-intubate status [33,51].

NPPV in pulmonary oedema associated with acute heart failure

Non-invasive positive pressure ventilation is effective and commonly used in treatment of acute cardiogenic pulmonary oedema associated with AHF [26,35,36,53]. In this setting, CPAP is the first treatment option, although NPPV is recommended if there is evidence of hypercapnia or if the patient remains in distress despite CPAP treatment [54]. Some research has shown greater relief of respiratory distress, lower intubation rate, and decreased mortality with NPPV use in AHF compared to CPAP [54].

NPPV in exacerbations of obesity hypoventilation syndrome

Both NPPV and CPAP are used to treat exacerbations of OHS, which is defined as acute decompensated hypercapnic respiratory failure in OHS [31,55]. A comparison of NPPV treatment in patients with AHRF caused by OHS with AECOPD showed similar effectiveness with respect to survival, in-hospital mortality, and length of hospital stay [55]. NPPV treatment of AHRF in OHS is considered safe and effective, even though acidosis generally persists longer than in patients with AECOPD, and randomized clinical trials are lacking [56].

NPPV in AHRF of differing and multiple causes

Non-invasive positive pressure ventilation is also employed in acute respiratory failure from causes such as pneumonia in COPD patients and infections in immunocompromised patients [35,36,57,58]. In an unselected group of subjects with AHRF, a 60-day survival benefit was shown for those treated with NPPV for acute-on-chronic respiratory failure [59]. Lack of improvement within the first hour following initiation of NPPV is prognostic of worse outcome regardless of the underlying cause of AHRF [60]. In palliative settings, NPPV is used for respiratory failure of various aetiologies to relieve the sensation of dyspnea [61].

Role of biomarkers as prognostic factors of development and outcome in cardiovascular and pulmonary disease

Challenges of biomarker studies

A biomarker is defined as a characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or response to an exposure or intervention. This broad definition refers to biomarkers of molecular, histological, radiographic, or physiological derivation [62]. The identification of biomarkers that can potentially be an early indication of a worsening clinical state can expedite initiating preventive measures or intensifying/modifying treatment. The ideal biomarker should also be independent of other factors [63,64]. To be of use in clinical practice, procedures to assess biomarker levels must be safe, accurate, inexpensive, and efficient, with a simple validated methodology [64].

Biomarkers may play multiple roles: *Diagnostic* biomarkers detect the presence of a disease or condition or a subtype of disease. *Monitoring* biomarkers are those measured repeatedly to assess disease status or the effect of treatment. *Susceptibility/risk* biomarkers are involved in the transition from healthy state to disease. [62,64]. *Prognostic* biomarkers can predict and quantify likelihood of a disease-related outcome in a patient with a disease or condition and should be differentiated from *predictive* biomarkers, which can predict the likelihood of an individual in a defined cohort (with or without disease) to respond to a specific prevention intervention, over a specified time interval [62,64,65].

Area under the receiver operating characteristic curve (AUC), a numeric representation of the likelihood of a test to accurately predict a clinical outcome of interest, is the most widely used measure of biomarker performance [19,62]. An AUC of 1.0 signifies 100% sensitivity and 100% specificity, while an AUC of 0.5 represents a biomarker with performance no better than random chance. Tests showing an AUC > 0.85 are considered to have a clinical potential, while an AUC 0.70 – 0.85 may represent tests with clinical usefulness in selected settings [19]. The AUC should typically be calculated before clinical implementation of a new biomarker, however, at the discovery stage, before findings have been replicated and externally validated, it has limited value.

Although there has been considerable research into the role of biomarkers as prognostic of development and outcome of disease, significant barriers to clinical application of biomarker data remain. Many studies have comprised a small number of samples, and the results are poorly replicated in independent external cohorts. As a consequence, many novel biomarkers have not proven useful in a clinical setting [19].

As shown in **Figure 2**, biomarker research begins in response to a clinical need, with the initial step being to identify its intended use (feasibility phase). Content

development includes collection of biological samples from well-defined patient cohorts and application of these samples to a technology platform for biomarker discovery (content development phase). These platforms commonly generate large quantities of complex data that needs to be appropriately analysed and the results interpreted. When a promising biomarker is found, testing in an external, independent subject cohort is necessary to demonstrate replicability. This is followed by a transfer of the biosignature to a clinical platform with proper analytic validation of the assay (validation and qualification phase). Lastly, the clinical assay needs to be tested in a cohort of suitable patients in order to evaluate the biomarker performance for the management of the disease in question.

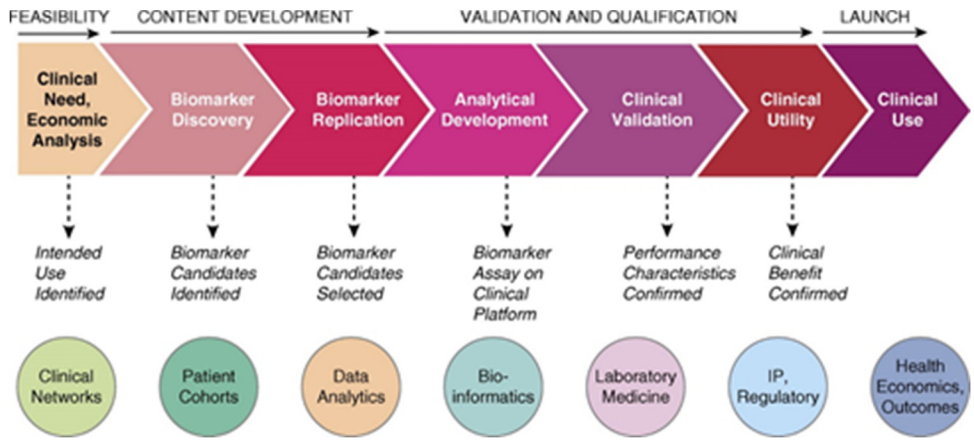


Figure 2: Biomarker development pipeline. IP = intellectual property. Reprinted with permission of the American Thoracic Society. Copyright © 2020 American Thoracic Society. All rights reserved. Sin, D.D., *et al.*/2015/Biomarker Development for Chronic Obstructive Pulmonary Disease. From Discovery to Clinical Implementation/Am J Respir Crit Care Med./192(10)/p. 1162-70 [19]. The American Journal of Respiratory and Critical Care Medicine is an official journal of the American Thoracic Society.

Biomarker studies have provided valuable information regarding the pathological processes leading to clinical presentation of a disease or condition [64]. In this thesis, the biomarkers discussed are suppression of tumorigenicity 2, interleukin 8, and growth differentiation factor 15.

Suppression of tumorigenicity 2

Suppression of tumorigenicity 2 (ST2) is a biomarker most widely known as prognostic of outcome and mortality in patients with acute or chronic cardiovascular disease [66]. Two forms of ST2 exist, one a membrane bound receptor of cytokine IL-33 (ST2L) and a second that is soluble and measurable in plasma (sST2) (**Figure 3**). The IL-33/ST2L complex activates the immune response by signalling presence of tissue damage to inflammatory cells [67]. Soluble ST2 is thought to act as a decoy receptor for IL-33 as well as playing a more complex immunomodulatory role in

binding to other receptors [4,63,68]. For purposes of simplification, sST2 will be henceforth referred to as ST2, as is most common in the literature.

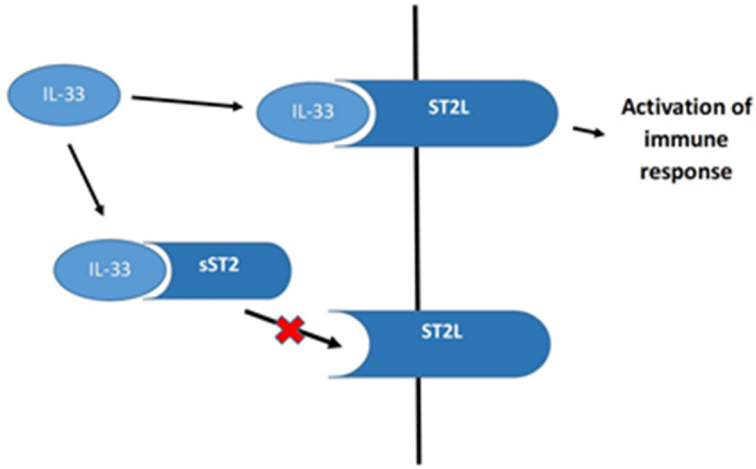


Figure 3: IL-33/ST2 pathway connections. Adapted from Mueller *et al.* [67]

ST2 plasma concentration is generally higher in healthy males than in females, and is not affected by age, body mass index (BMI), or renal function [68,69]. Pulmonary and vascular endothelial cells are known to secrete ST2, but the major source of its production in healthy individuals has not been established [67,68,70].

Prognostic value of ST2 in disease development and mortality in the general population

ST2 has been evaluated as a biomarker to predict cardiovascular as well as non-cardiac disease in the general population and is assumed to be a promising biomarker to identify patients at high risk. However, further study is needed to establish its value in screening. In addition, ST2 lacks specificity for disease type [71-74].

The Framingham and Dallas Heart studies found plasma ST2 predictive of all-cause mortality in the general population [70,71]. Chen *et al.* found, in the Dallas Heart Study, that this association was strongest in African Americans, and that the significance of the results diminished when adjusted for GDF-15, troponin T, NT-proBNP, and high sensitivity CRP [70]. Currently, it is assumed that ST2 might reflect the prognosis of mortality in the general population, although most likely in combination with other markers of cardiovascular stress [70-73].

Prognostic value of ST2 for outcome in chronic disease

ST2 is known to be elevated in patients with chronic cardiovascular or respiratory disorders, as well as in chronic conditions such as liver and renal disease, cancer, inflammatory disease, and diabetes [63,67,74-76]. In cardiac stress, ST2 is upregulated in cardiac myocytes and decreases the cardioprotective effects of IL-33 by binding to it as a decoy receptor. The outcome is thought to be interference with remodelling and increase in fibrosis [66,77].

In studies of patients with COPD, ST2 and IL-33 have been shown elevated in peripheral blood and in lung tissue compared to levels in control subjects without COPD [78]. Other studies have strengthened the suggestion that the IL-33/ST2 pathway might contribute to the pathogenesis and progression of COPD, in which airway epithelial cells are considered a major source of ST2 production, as well as of asthma and other respiratory inflammatory diseases [76,79].

Prognostic value of ST2 in acute exacerbations of chronic disease

Determination of plasma ST2 concentration can be valuable in predicting mortality and morbidity in patients with acute chest pain of differing causes, acute heart failure, and acute myocardial infarct [4,63,69,74,80-83]. Monitoring of ST2 levels over the course of treatment for heart failure has been shown to provide prognostic information, suggesting that serial measurements may play an important role in evaluating therapy of other health conditions in the future [69,84].

Several studies have found ST2 to be a strong and independent predictor of 1-year all-cause mortality in cohorts of patients presenting to emergency departments with acute dyspnea, regardless of the underlying condition [85-87]. This includes the Pro-Brain Natriuretic Peptide Investigation of Dyspnea in the Emergency Department (PRIDE) study, in which mean ST2 concentration was significantly higher at baseline in the segment of subjects with AHF (n = 208 of 599) compared to non-AHF subjects [87]. Martinez-Rumayor *et al.* looked specifically at a subgroup in the PRIDE study that suffered from dyspnea and pulmonary disease without AHF (n = 231) and found that, even in this group, ST2 was predictive of 1-year mortality [88]. The ST2 concentration was further found predictive of 4-year mortality in the PRIDE study [89].

In a study of patients with acute dyspnea, Socrates *et al.* found a powerful association of ST2 with 30-day mortality in those with AHF, and reported that the combination of ST2 and BNP/NT-proBNP was accurate in identifying those dyspnea patients with high risk of mortality [86].

ST2 cut-off levels to stratify patients according to prognosis have been proposed. In patients with AHF, a threshold level of 35 ng/mL provides a reasonable reference limit above which risk of adverse outcome rises linearly with the degree of elevation [90]. However, many patients with AHF exhibit substantially higher levels, and an ST2 concentration of 70 ng/mL might represent a practical cut-off level for patients

with AHF at extremely high risk of adverse outcome. High ST2 concentration is thought to be associated with activation of both neurohormone and pro-fibrotic pathways, increasing risk of adverse heart remodelling [69,84]. The same cut-off levels are suggested for risk stratification of patients with acute coronary syndrome [69].

The potential role of ST2 as a useful biomarker in acute clinical worsening of allergic and non-allergic pulmonary disease has been evaluated in recent years [91]. Plasma ST2 concentrations have been shown to be increased in patients with acute exacerbations of idiopathic pulmonary fibrosis, ARDS, and sepsis [91-93].

Interleukin 8

Interleukin 8 (IL-8) is a pro-inflammatory cytokine produced by several cell types of the inflammatory pathway. Upon release, IL-8 activates the immune system and contributes to the acute inflammatory response by recruiting neutrophils [13,94]. Among other actions, it induces the migration of neutrophils to the airways and promotes their degranulation [10,54,95]. In addition, IL-8 exerts angiogenic effects through binding to the IL8RB (CXCR2) receptor on endothelial cells [96].

Prognostic value of IL-8 in disease development and mortality in the general population

IL-8 is thought to play a role in the development of COPD, cardiovascular disease, and cancer [97-100]. Only a few studies have addressed the predictive value of IL-8 on all-cause mortality. In the largest of these, Velásquez *et al.* found that elevated IL8 levels were associated with increased risk of mortality regardless of the underlying cause [94].

Prognostic value of IL-8 for outcome in chronic disease

Studies have reported a positive association of IL-8 levels with the development of diastolic heart dysfunction [101]. However, the value of IL-8 in monitoring progression and establishing a prognosis in chronic heart failure is unclear, and it has not proven useful [102].

IL-8 is thought to be a key contributor to the development and progression of COPD in chronically inflamed airways by attracting and activating neutrophils [13,100,103]. Sputum and plasma IL-8 has been a target of research investigating COPD-related mortality and exacerbations [13,103,104]. Bronchial epithelial cells in patients with COPD exhibit higher baseline expression of IL-8, and this production is stimulated by exposure to cigarette smoke, TNF-α, and possibly by ST2L/IL-33 activation [13,105]. Agusti *et al.* identified six inflammatory biomarkers, including IL-8, related to systemic inflammation. COPD patients with elevated plasma levels of the studied biomarkers showed increased exacerbation frequency and all-cause mortality during a 3-year follow-up period [106].

Prognostic value of IL-8 in acute exacerbations of chronic disease

Elevated plasma concentration of IL-8 are associated with increased long-term risk of mortality in patients with acute coronary syndrome, and increased levels of circulating IL-8 have been shown to predict development of heart failure in patients with acute myocardial infarction. Hypoxia and reperfusion injury involve IL-8 and recruitment of neutrophils [107]. However, the precise role of IL-8 in cardiac damage is still not fully understood, and further studies are needed [99].

High concentrations of IL-8 have been found in patients with acute exacerbation of COPD [13,103]. A review by Koutsokera *et al.* concluded that IL-8 levels in spontaneous sputum of patients with AECOPD is indicative of clinical severity, recovery from exacerbation, and presence of bacterial infection and its eradication [104]. Shafiek *et al.* showed that, in patients with AECOPD, concentrations of IL-8 were higher in non-responders to NPPV compared with responders, with failure defined as decision to terminate NPPV trial and initiate invasive mechanical ventilation. In this study, however, IL-8 was not found to be related to the presence of bacterial infection [108].

Growth differentiation factor 15

Growth differentiation factor 15 (GDF-15) is a member of the transforming growth factor-ß cytokine superfamily [109]. In response to oxidative stress, inflammation, and hypoxia, among other stimuli, its expression is upregulated in cardiac myocytes, vascular smooth muscle cells, and the endothelial cells of various tissues. GDF-15 is thought to exert a broad range of homeostatic actions [81,110,111]. It is also expressed in activated macrophages and adipocytes [112,113]. It plays both positive and negative roles in regulation of inflammation, cell proliferation, and migration in multiple organ systems, depending on the state of the cells and their environment [111,114,115]. The exact nature of GDF-15 action and consequent activation of downstream mediators is not entirely understood, and its practical significance as a marker of disease development and progression is far from clear [81,111,115].

Prognostic value of GDF-15 in disease development and mortality in the general population

Numerous studies have assessed the value of GDF-15 in prediction of disease development in the general population, as well as the association of its level with underlying disease. Ho *et al.* found a strong positive association of GDF-15 concentration with age and reported that genetic factors influence concentrations in the general population. In that study, no sex difference was observed, but there was a significant link of high GDF-15 with cardiometabolic risk factors, including diabetes, hypertension, and smoking [116]. In the general population, high GDF-15 concentration has been shown to accompany underlying cardiovascular disease and subclinical atherosclerosis, and can predict the development of diabetes and chronic kidney disease [110,114,116-122].

Studies have found a positive association of GDF-15 with all-cause mortality, suggesting that GDF-15 plays a role in biological processes related to aging, although no risk stratification or therapeutic decision making tool is available [81,109,111,112,115,121]. In the Framingham Heart Study, elevated plasma concentration of GDF-15 was found strongly associated with development of heart failure and death [71].

Prognostic value of GDF-15 for outcome in chronic diseases

Concentrations of GDF-15 are elevated in patients with chronic heart failure, and correlate with disease severity [71]. Compared to healthy controls, patients with chronic pulmonary vascular disease, including pulmonary emboli and pulmonary hypertension, also exhibit elevated concentrations of GDF-15 [74,111].

The association of GDF-15 and COPD development has been studied [111]. In patients with established COPD, higher GDF-15 concentrations are linked to an increased annual rate of exacerbations, higher mortality, and an accelerated decline of lung function [123].

Prognostic value of GDF-15 in acute exacerbations of chronic disease

Elevated GDF-15 levels may indicate poorer prognosis in clinical deterioration of diseases such as acute myocardial infarct, acute or chronic heart failure, cancer, critical illness, infections, and inflammatory disease [71,81,111,115]. When compared to patients with stable COPD and to control subjects, patients with acute exacerbations of COPD showed higher GDF-15 concentrations, and elevated levels upon hospital admission were reported associated with adverse outcomes [124,125].

The findings of GDF-15 studies with respect to disease outcome and mortality support its value in identifying high-risk patients, but no current clinical guidelines have incorporated GDF-15 for this purpose [111].

Aims

The overall aim of the research reported in this thesis was to identify biomarkers and other clinical factors that could predict mortality and outcome in patients with acute hypercapnic respiratory failure (AHRF) treated with non-invasive positive pressure ventilation (NPPV). Based on the results of Studies I and II, the biomarkers investigated therein were evaluated as predictors of mortality in acute dyspnea (Study III) and as predictors of COPD diagnosis in a cohort from the general population (Study VI).

The aims of the individual studies were as follows:

Study I

To determine whether the biomarkers interleukin-8 (IL-8) and growth differentiation factor 15 (GDF-15) can predict 28-day mortality in patients with AHRF differing in underlying cause receiving NPPV treatment.

Study II

To determine whether the biomarker suppression of tumorigenicity 2 (ST2) can predict mortality in patients with AHRF differing in underlying cause receiving NPPV treatment. A secondary goal was to test the hypothesis that a decrease in ST2 concentration during the first 12 hours of NPPV treatment could indicate treatment efficacy.

Study III

To determine whether ST2 can predict all-cause mortality in patients suffering from acute dyspnea, with emphasis on differences between discharged and admitted patients and stratification of the studied cohort according to the ST2 risk cut-off points of 35 and 70 ng/mL, suggested to add prognostic value in cardiac disease. A secondary goal was to assess whether results of Study II regarding the predictive value of ST2 for mortality in patients with AHRF treated with NPPV could be replicated in a larger cohort.

Study IV

To assess whether IL-8, ST2, and GDF-15 can predict a diagnosis of COPD in secondary care and all-cause mortality in a large population-based prospective study comprising the cohort of the Malmö Diet and Cancer Study (MDCS).

Materials and Methods

Study populations and design

The acute hypercapnic respiratory failure cohort (Studies I and II)

In Studies I and II, the population consisted of patients admitted to the Intermediate Emergency Care Department of Skåne University Hospital in Malmö, Sweden that were treated with NPPV for AHRF differing in underlying cause. From January through June 2014, patients >18 years-old with AHRF that were treated with NPPV according to local clinical guidelines were enrolled. The study was observational and prospective. The treatment was controlled by the on-call physicians, and the study implementation did not intervene with the treatment. Written informed consent was obtained from all patients or from their next of kin. The patients consented to access of their medical history and current medication through medical records. Information regarding smoking habits and socioeconomic factors was obtained through patient interview when possible. The study was approved by the Regional Ethics Board of Lund, Sweden and followed the precepts established by the Declaration of Helsinki.

Fifty-one subjects were enrolled during the study period, with five excluded because of withdrawal of consent (n = 3), and neurological disease (n = 1) or sepsis (n = 1) as the major cause of respiratory failure, leaving forty-six patients in the final analysis.

Prior to the final analysis of data, medical records were examined by an internist to determine the primary discharge diagnosis, as well as a secondary diagnosis if present. Additional information obtained included records of previous and present disease, medications, smoking history, clinical presentation, blood test results, and radiographic examination. This assessment produced three groups with respect to primary diagnosis: acute exacerbation of COPD (AECOPD), acute heart failure (AHF), and acute exacerbation of OHS (AEOHS).

Vital signs were measured before and during NPPV treatment at 0, 1, 4, and 12 h after treatment initiation. Venous blood samples and arterial samples for evaluation of blood gases were taken simultaneously when possible. The arterial blood gases

were analysed immediately with an ABL800 Flex blood gas analyser (Radiometer, Copenhagen, Denmark), while the venous blood samples for biomarker determination were centrifuged and plasma was stored at -80 °C until analysis.

Study of patients with acute dyspnea (ADYS) (Study III)

The study of patients with acute dyspnea (ADYS) was conducted at the emergency department of Skåne University Hospital, Malmö Sweden. The ED receives 75 000–80 000 visits per year and serves approximately 400 000 inhabitants (official statistics from County of Skåne). Patients were enrolled by research nurses from 6 March 2013 to 31 January 2019. The study was observational and prospective, and all patients >18 years seeking the ED for acute dyspnea during clinic hours (6.45–16.30) were asked to participate. Written informed consent for inclusion and review of medical records was obtained from all subjects or from their next of kin. The study was approved by the Regional Ethics Board of Lund, Sweden and followed the precepts established by the Declaration of Helsinki.

Patients lacking a Swedish civil registration number and those not able to provide informed consent were excluded. Patients with pneumothorax or trauma as the underlying cause of dyspnea were excluded following review of medical records and discharge diagnoses.

Information regarding social factors and medical history, current medication, and clinical parameters such as vital signs, standard blood test results, and the medical emergency triage and treatment system-adult score (METTS-A) was documented [126]. Blood samples for analysis of ST2 and other biomarkers were secured within an hour of presentation at the ED, centrifuged, and plasma was stored at -80 °C until analysis. The research nurses manually reviewed the medical records to retrieve relevant patient data.

A total of 1573 subjects were enrolled in Study III. Of these, 322 were later excluded because of missing values of one of the possible confounding factors included in the study, leaving 1251 patients in the final analysis.

Malmö Diet and Cancer cardiovascular cohort (MDC-CC) (Study IV)

The Malmö Diet and Cancer study (MDCS) was designed as a prospective case-control study with two initial purposes: The first was to evaluate the association of a western diet and other life-style factors with certain forms of cancer. A second was to determine whether oxidative stress and DNA-repair systems could influence the impact of diet on cancer development. The collected data has subsequently been used as a resource for testing many other hypotheses [127].

Data was collected during the recruitment phase from 1 January 1991 to 25 September 1996. Invitation letters were initially sent to all persons living in Malmö born from 1926 through 1945, a total of 53 325 individuals. In 1995, the recruitment

was extended to females born 1923–1950 and males born 1923–1945. Notices were also published in local newspapers and posted in public spaces, resulting in recruitment of additional participants who had not received an invitation letter. Exclusion criteria were cognitive delay and inability to communicate in Swedish. When recruitment was concluded in 1996, 28 449 participants had contributed blood samples, among other measurements, and completed questionnaires providing information of medical history, current medication, education, occupation, smoking history, and social factors [127]. All participants signed written informed consent for inclusion and a review of medical records. The study was approved by the Regional Ethics Board of Lund, Sweden and followed the precepts established by the Declaration of Helsinki.

To investigate potential selection bias, characteristics of the participants in the MDCS study were later compared to those of the general population with respect to cancer incidence and mortality. The groups did not differ in sociodemographic structure, and smoking status was similar. However, non-participants had higher mortality during the recruitment period as well as during the follow-up [128].

The MDCS participants were invited to take part in a cardiovascular sub-study, the Malmö Diet and Cancer Cardiovascular Cohort (MDC-CC). The included subjects (n = 6103) underwent an ultrasound examination of the right carotid artery as part of the baseline examination and were asked to provide additional fasting blood samples [129]. The final number of subjects analysed was 4292, with exclusion mainly due to missing covariate data.

End points and Registries

The primary endpoint of Study I was 28-day all-cause mortality.

In Study II, all-cause mortality at 18 months was added as a primary endpoint, thus analyses were made regarding both short- and long-term mortality. Secondary endpoints were clinical factors related to improvement of AHRF (changes in pH and $PaCO_2$) observed during the hospital stay over the course of 12 hours.

In Study III, primary endpoints were all-cause mortality at three and twelve months post-ED-visit, and for patients with AHRF treated with NPPV also at 28 days. Secondary endpoints were final discharge diagnosis from the ED or the hospitalization, retrieved from medical records.

The primary endpoints of Study IV were COPD diagnosis in hospital-based secondary care units and all-cause mortality during a follow-up time of over 20 years.

In all studies, deaths and date of death were confirmed by linking the subject 10-digit civil registration number to the Swedish National Cause of Death Registry, which contains information from death records of all deceased registered residents of Sweden since 1952 [130].

In Study IV, information regarding the diagnosis of COPD according to ICD-8 (490-492), ICD-9 (491–492, 496), and ICD-10 (J41–J44) was retrieved through linkage of the 10-digit civil registration number with the Swedish National Inpatient Registry. This registry has previously been confirmed to have acceptable validity with respect to COPD diagnosis for purposes of epidemiological research, with less than 10% classified as having an uncertain or incorrect COPD diagnosis [131]. It includes diagnoses from in- and out-patient secondary care but not information from primary care.

Medical emergency triage and treatment system - adult score

In Study III, all patients were evaluated with the medical emergency triage and treatment system-adult score (METTS-A), a 5-level rating tool based on a combination of symptoms and vital signs [126]. The levels of priority are: (1) red, life threatening; (2) orange, potentially life threatening; (3) yellow, not life threatening but in need of medical attention; (4) green, not life threatening and not in need of immediate care; (0) blue, not in need of emergency care and should be referred to primary care.

Analysis of biomarkers

Biomarkers were analysed in frozen plasma samples with a Proseek Multiplex Biomarker Panel CVD 1 (Olink Bioscience, Uppsala, Sweden). The method is a multiplex immunoassay based on the Proximity Extension Assay (**Figure 4**) [132]. The data are presented as the unit normalized protein expression (NPX) that provides relative quantification, and values can be compared only for the same protein across samples analysed, not as absolute values. Assay characteristics such as detection limits and measures of assay performance and validation are available from the manufacturer's website [133].

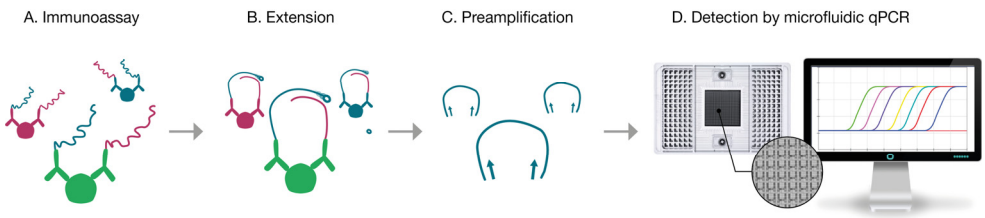


Figure 4: Overview of Proximity Extension Assay procedure for biomarker analysis. (A) 92 antibody pairs labelled with DNA oligonucleotides bind to target antigen in a solution. (B) Oligonucleotides that are brought into proximity with one another hybridize and are extended by DNA polymerase. (C) This creates a segment of DNA barcode, which is amplified by PCR. (D) The quantity of each DNA barcode is determined by microfluidic qPCR [133]. Image courtesy of Olink Proteomics

In Studies II and III, the ST2 concentration was analysed from frozen plasma samples with Presage ST2 Clinical Assay (Sopachem Diagnostics; Copenhagen, Denmark). This is an in vitro diagnostic assay that quantifies ST2 in serum or plasma by enzyme-linked immunosorbent assay (ELISA) in a microtitre plate format. Assay details are available from the manufacturer´s website [134].

NPPV treatment data

In Studies I and II, all patients in the AHRF cohort received NPPV treatment with Trilogy100 and an appropriate NPPV mask (Respironics; Murrysville, Pennsylvania, USA). Inspiratory positive airway pressure was automatically regulated with average volume assured pressure support, with pressure ranging from 10 to 25 cm H2O to obtain a goal tidal volume of 8 mL/kg. Expiratory positive airway pressure was maintained at a constant 5 cm H2O. Spontaneous/Timed mode was used, in which the patient's inspiration triggers initiation of IPAP, but, if the respiratory rate drops below a pre-set backup rate, the NPPV initiates a new respiratory cycle [135]. Backup respiratory rate was set at 10 breaths/minute. Oxygen was applied as needed based on a target peripheral capillary oxygen saturation of 88–90% as opposed to a pre-defined oxygen level.

All patients were monitored closely during NPPV treatment. The treatment was continuous with short breaks, for example during meals. The on-call physicians decided if and when the treatment was discontinued. Information of treatment length and device installations was obtained from the Directview Program (Respironics; Murrysville, Pennsylvania, USA).

Monitoring of respiration with a sleep monitor

In the AHRF cohort (Studies I and II), one of the study questions raised before initiation of data collections was whether monitoring of the patients respiration patterns using a sleep monitor could be of use in predicting outcome. Patients receiving NPPV were fitted with a Nox T3 sleep monitor (Nox Medical; Reykjavík, Iceland) during the first 24 hours of treatment to record respiration patterns with and without NPPV. This was accomplished technically in 32 patients.

When analysis began, it quickly became clear that data collected from the Nox T3 monitor would be difficult to interpret, mainly because of technical issues resulting in disturbances in the recordings. Despite efforts to overcome this problem, the data could not be evaluated, and the decision was made to discontinue this aspect of the study.

Databases

For Studies I and II, a database of variables of interest was developed in the web-based program BC Platforms (BC Platforms, Esbo, Finland). The variable values were entered into the database by the primary investigator and a research nurse. When data collection was complete, SPSS files were created for processing and analysis.

For Study III, a similar database was designed and developed in BC Platforms by the project supervisor, and data were compiled by research nurses. For Study IV, data were gathered by research nurses, and a database was created in the Oracle program (Oracle Corporations; Redwood Shores, California, USA) and subsequently transferred to SPSS files.

Statistics

SPSS versions 21–24 (SPSS Inc.; Chicago, Illinois, USA) were used for all analyses. Figure 10 in Study IV was produced in R v.3.3.0 (R Foundation; Vienna, Austria). All tests were two-sided, and a P-value <0.05 was considered significant unless otherwise indicated.

Studies I and II

Since NPPV treatment in AHRF has proven beneficial, it was not possible to include a control group deprived of the treatment in Studies I and II [36,40,43,55,59]. Hence, levels of the biomarkers were ranked and compared within the studied

cohort. No power calculation was performed prior to the initiation of the study, as no suggested effect size for the association of the evaluated biomarkers with short- or long-time mortality in AHRF patients receiving NPPV treatment exist.

The small cohort of 46 patients comprised three subgroups based on primary discharge diagnosis. Baseline characteristics were summarized using descriptive statistics for the entire cohort and the subgroups. Since the data did not show normal distribution, variables are expressed as median value and interquartile range (IQR). Differences among subgroups were assessed by the Kruskal-Wallis test to analyse continuous variables and by Fischer´s exact test for the categorical variables sex and smoking status. Logistic regression was used to evaluate correlation between radiological evidence of heart failure or consolidation and 28-day mortality, with results expressed as odds ratio (OR) with 95% confidence intervals (CI).

Cox proportional hazard models were used to relate baseline levels of the biomarkers IL-8, GDF-15 (Study I), and ST2 (Study II) to mortality during 28-day follow-up and, in Study II, during 18-month of follow-up. Since the biomarker concentrations were not normally distributed, they were transformed with the natural logarithm with the results expressed as hazard ratio (HR) with 95% CI, on a standardized scale of 1 standard deviation (SD) increment.

Cox proportional hazard multivariate models were as follows:

- Model 1: Adjusted for age and sex.
- Model 2 (backward elimination): Adjusted for age, sex, and CRP (non-parametric, transformed with a natural logarithm).
- Model 3 (backward elimination): As in model 2 with addition of the potential confounding factors BMI; respiratory rate at start of treatment; pH, PaO_2, $PaCO_2$, and lactate levels; and primary discharge diagnosis, with reported biomarker effect sizes adjusted for covariates retained at $P < 0.10$.

Patients with acute exacerbation of COPD were the largest subgroup in Studies I and II (n = 34), and biomarker levels were analysed separately with models 1 and 2. The three biomarkers were analysed both independently and in combination.

The biomarker levels were ranked and divided into tertiles with the lowest tertile as the reference group, and crude Kaplan-Meier plots were created.

In Study I, CRP alone was analysed in model 2. IL-8 and GDF-15 were analysed in combination using model 2 in order to assess their accuracy as independent prognostic factors.

In Study II, ST2 was measured by two methods, the Proximity Extension Assay and ELISA (Presage ST2 clinical assay). Pearson´s correlation coefficient was calculated to evaluate the correlation between methods.

In Study II, ST2 was repeatedly measured during the first 12 hours of treatment, in order to test the hypothesis that a decrease in ST2 concentration might indicate a positive effect of NPPV treatment. Changes in values representing the severity of respiratory failure (pH and $PaCO_2$) during the first 4 and 12 hours of treatment were used as indicators of respiratory acidosis. First, the Wilcoxon paired rank test was used to determine whether changes from baseline in ST2, pH and $PaCO_2$ during the first 4 or 12 hours of treatment were significant. Subsequently, a linear regression model was used to compare baseline ST2 levels and changes in ST2 in the first 4 and 12 hours to changes in pH and $PaCO_2$ over the same time frame. Both baseline levels and delta values (the difference between variables at start of treatment and after 4 and 12 hours) were transformed with the natural logarithm before being submitted to the linear regression analyses.

Study III

Baseline characteristics were summarized using descriptive statistics for the entire cohort and for the cohort categorized according to hospitalization status. Data were expressed as median values and interquartile range (IQR) for all variables except age and BMI, which were normally distributed and expressed as means and standard deviation (SD). Differences among hospitalization groups were tested using an independent *t*-test for age and BMI, the Mann-Whitney U test for non-normally distributed continuous variables, and chi-square for categorical variables. The Mann-Whitney U test was used analyse differences in plasma ST2 concentrations of survivors vs. non-survivors at 3 and 12 months, admitted vs. non-admitted, and admitted patients treated with NPPV vs. untreated.

Cox proportional hazard models 1 and 2 were used to relate baseline ST2 levels to mortality at 3 and 12 months. Levels of ST2, NT-proBNP, CRP, and creatinine were transformed with the natural logarithm due to non-parametric distribution, with results expressed as HR with 95% CI, on a standardized scale of 1 SD increments. The models were as follows:

- Model 1: Adjusted for age and sex.
- Model 2 (backward elimination): Adjusted for age, sex, BMI, smoking status (never smoked vs. current or former smoker), METTS-A score, CRP, creatinine, and underlying disease (asthma, COPD, restrictive lung disease, other lung disease, chronic heart failure, hypertension, atrial fibrillation, ischemic heart disease, and cancer), with a reported biomarker effect size adjusted for covariates retained at $P < 0.10$.

To evaluate the proportion of ST2 variance explained by NT-proBNP or CRP in a subset of 700 patients with available NT-proBNP data, a Pearson correlation test was performed, and the r-value squared (r^2) was calculated to express the proportion of variance explained. All three biomarkers were subsequently analysed in combination using the Cox proportional hazard model 1.

The ST2 concentrations were divided into quartiles with the lowest quartile as the reference group, and crude Kaplan-Meier plots were generated for ST2 quartiles. In a separate analysis, subject ST2 levels were separated into three groups according to the suggested cut-off levels of 35 and 70 ng/mL. The relationship of the groups with 12-month mortality was assessed with the Cox proportional hazard model 1, and crude Kaplan-Meier plots for the subgroups were created using the group with concentrations <35 ng/mL as reference.

An additional analysis was conducted using logistic regression to assess the relationship of ST2 concentration with the discharge diagnosis according to medical records, with the results expressed as OR and 95% CI on a standardized scale of 1 SD increments.

Study IV

The study cohort (n = 4292) was divided according to subjects presence or absence of a COPD diagnosis in secondary care during the mean follow-up time of 21.0 years. To analyse differences in baseline characteristics of COPD vs. non-COPD individuals, an independent *t*-test was used for continuous variables and chi-square test for categorical variables. Variables were presented as mean±SD.

Due to non-normal distribution, biomarker levels were transformed with the natural logarithm. Cox proportional hazard models were used to relate biomarker levels at baseline to risk of COPD diagnosis, all-cause mortality, or respiratory-related mortality, with a mean follow-up time of 21.5 years. Results are expressed as HR with 95% CI on a standardized scale of 1 SD increments. The models were as follows:

- Model 1: Adjusted for age and sex.
- Model 2 (backward elimination): Adjusted for age, sex, BMI, smoking (current, former, never), antihypertensive therapy, diabetes, systolic blood pressure, CRP, NT-proBNP, and plasma LDL with reported biomarker effect size adjusted for covariates retained at $P < 0.10$

Plasma GDF-15 levels were separated into quartiles, and crude Kaplan-Meier plots were generated with the lowest quartile as reference. To analyse whether the association between GDF-15 and mortality remained significant after adjustment for COPD diagnosis, logistic regression was used with outcome presented as OR with 95% CI.

Results

Study I

Interleukin-8 predicts early mortality in patients with acute hypercapnic respiratory failure treated with non-invasive positive pressure ventilation.

Clinical characteristics

Of the 51 subjects enrolled during the study period, five were excluded because of withdrawal of consent (n = 3), and presence of neurological disease (n = 1) or sepsis (n = 1), leaving 46 patients for the final analysis. Three subgroups based on primary diagnosis were AECOPD (n = 34), AHF (n = 8), and AEOHS (n = 4). Clinical characteristics and variables related to AHRF of the entire cohort and subgroups are presented in **Table 1**. Significant differences were observed among subgroups with respect to age, smoking status, and BMI. In the variables related to AHRF, a significant difference in base excess (BE) was observed among subgroups. 45 patients presented with $PaCO_2$ > 6.0 kPa.

Nine patients in the AECOPD subgroup had been receiving long-term oxygen therapy at home prior to hospitalization, and three received long-term NPPV therapy at home. No patient in the other subgroups had long-term oxygen therapy; a single patient in the AEOHS group received home NPPV.

Bedside chest radiographies were performed on 39 patients (85%), with two (4%) showing radiological evidence of consolidation. 21 patients exhibited radiological signs of possible pulmonary oedema (46%). Logistic regression analysis showed no correlation between evidence of consolidation or possible pulmonary oedema and 28-day mortality [OR 0.67 (95% CI 0.16 – 2.75, P = 0.57)] when adjusted for age and sex.

All patients were evaluated at admission according to applicable guidelines with respect to advanced care restrictions. In 33 patients (72%) the “Do Not Resuscitate” order was made and recorded in the medical records and, of those, 24 patients (52%) were considered ineligible for intensive care in case of worsening. No patient was intubated and treated with invasive ventilation during the hospital stay. Median length of hospitalization was seven days (IQR 4–11 days). 13 patients (28%) died within the 28 days following admission, 10 of whom (22%) died while hospitalized.

Table 1. Characteristics of the subjects of Study I, complete cohort and subgroups.

General characteristics	Complete cohort	AECOPD	AHF	AEOHS	P-value[b]
Number of subjects	46	34	8	4	
Age, years	77.1 (68.7–84.0)	76.9 (68.8–83.9)	82.3 (77.7–86.8)	65.4 (60.5–73.0)	0.035
BMI, kg/m2	23.4 (20.5–36.1)	24.0 (18.8–28.2)	27.7 (21.7–39.2)	46.6 (39.3–55.2)	0.004
Female, n (%)	30 (65)	22 (65)	5 (63)	3 (75)	0.054
Active or former smoker, n (%)	40 (87)	33 (97)	5 (63)	2 (50)	<0.001
FEV1%	31 (24–43)	29 (22–36)	47 [a]	43 [a]	0.058
Variables related to AHRF	**Complete cohort**	**AECOPD**	**AHF**	**AEOHS**	**P-value[b]**
pH	7.28 (7.24–7.36)	7.31 (7.24–7.37)	7.24 (7.10–7.31)	7.30 (7.25–7.35)	0.17
PaO_2 kPa	7.45 (6.33–8.73)	6.85 (6.10–8.48)	7.50 (5.60–9.58)	8.40 (8.23–10.68)	0.24
$PaCO_2$ kPa	8.75 (7.78–10.5)	8.90 (7.78–10.35)	8.05 (6.38–10.73)	10.05 (8.45–11.73)	0.30
Base excess, mEq/L	5.1 (1–10)	6.4 (4–10)	- 3 (-7.5 – 0)	11.3a	<0.001
Respiratory rate, bpm	26 (20–29)	26 (20–29)	24 (22–29)	25 (21–27)	0.89
CRP, mg/L	15.5 (8.3–76.5)	36.5 (9.7–93.0)	8.7 (6.4–11.0)	12.5 (7.5–37.0)	0.076
Lactate, mmol/L	1.40 (0.80–2.75)	1.10 (0.80–2.10)	3.80 (2.78–5.63)	1.20 (0.80–2.35)	0.09
NPPV use first 4 hours	3.57 (3.50–4.00)	3.67 (3.50–4.00)	3.50 (2.50–4.00)	3.75 (3.50–4.00)	0.67

Data presented with medians (IQR) unless otherwise stated.

Notes: [a]Insufficient numbers to analyse IQR. [b]Kruskal-Wallis test was used for all variables with the exception of sex and smoking status, where Fisher´s exact test was used.

Abbreviations: IQR, interquartile range; AECOPD, acute exacerbation of COPD; AHF, acute heart failure; AEOHS, acute exacerbation of obesity hypoventilation syndrome; BMI, body mass index; FEV1, forced expiratory volume in 1 sec; CRP, C-reactive protein; NPPV, non-invasive positive pressure ventilation.

IL-8 and GDF-15

Based on results of our earlier research assessing the role of inflammatory biomarkers in risk stratification of patients suffering from acute dyspnea, we chose IL-8 and GDF-15 for analysis in Study I [136]. Plasma concentrations were measured on admission, prior to initiation of treatment. Analysis with Cox proportional hazard model 1, adjusted for age and sex, and 2, adjusted for age, sex, and CRP, showed a significant predictive value of biomarker level with 28-day mortality. For IL-8, each 1 SD increment was associated with a nearly four-fold risk of 28-day mortality, and for GDF-15 the risk increase was approximately three-fold (**Table 2**). C-reactive protein was also analysed as an independent biomarker adjusted for age and sex, with resulting HR for 28-day mortality of 1.61 (95% CI 1.08–2.41, $P = 0.02$) for each 1 SD increment.

The possible confounding factors BMI; blood gas values pH, PaO_2, $PaCO_2$, and lactate; respiratory rate at treatment initiation; and primary discharge diagnosis were entered into model 3 (data missing in 13 subjects). In the analysis, both IL-8 ($P = 0.015$) and GDF-15 ($P = 0.008$) remained significantly associated with increased risk of 28-day mortality.

Table 2. Association of IL-8 and GDF-15 on admission and risk of 28-day mortality in patients with AHRF treated with NPPV.

IL-8	**Continuous IL-8 analysis (per SD increment)**	***P*-value**	**Tertile 1**	**Tertile 2**	**Tertile 3**	***P* for trend**
events[a]/n	13/46		1/15	3/16	9/15	
HR (95% CI) Model 1*	3.88 (1.86–8.06)	<0.001	1.0 (ref)	2.79 (0.29–26.89)	10.02 (1.24–80.77)	0.009
HR (95% CI) Model 2**	3.76 (2.02–7.03)	<0.001	1.0 (ref)	3.11 (0.32–29.93)	13.47 (1.70–106.91)	0.003
GDF-15	**Continuous GDF-15 analysis (per SD increment)**	***P*-value**	**Tertile 1**	**Tertile 2**	**Tertile 3**	***P* for trend**
events[a]/n	13/46		2/15	3/16	8/15	
HR (95% CI) Model 1*	2.76 (1.37–5.56)	0.004	1.0 (ref)	1.21 (0.19–7.77)	3.48 (0.54–22.34)	0.124
HR (95% CI) Model 2**	3.48 (1.78–6.80)	<0.001	1.0 (ref)	1.14 (0.16–8.21)	2.65 (0.36–19.41)	0.036

Notes: [a]All-cause mortality within 28 days of admission. *Adjusted for age and sex. **Backward elimination model; adjusted for age, sex and CRP.

Abbreviations: IL-8, interleukin 8; GDF-15, growth differentiation factor 15; SD, standard deviation; HR, hazard ratio; CI, confidence interval; CRP, C-reactive protein

Biomarker levels were ranked, separated into tertiles, and entered into model 1 and 2 adjusted Cox proportional hazard models. For IL-8, patients in the highest tertile showed a significant 10-fold (model 1) and 13-fold (model 2) risk of 28-day mortality, compared to the lowest tertile (**Table 2**). The event rate in IL-8 tertiles according to a Kaplan-Meier plot is shown in **Figure 5**. For GDF-15 tertiles, model 1 showed no significant association, while model 2 resulted in a significant 2.5-fold risk of mortality for the highest tertile as compared to the lowest (**Table 2**).

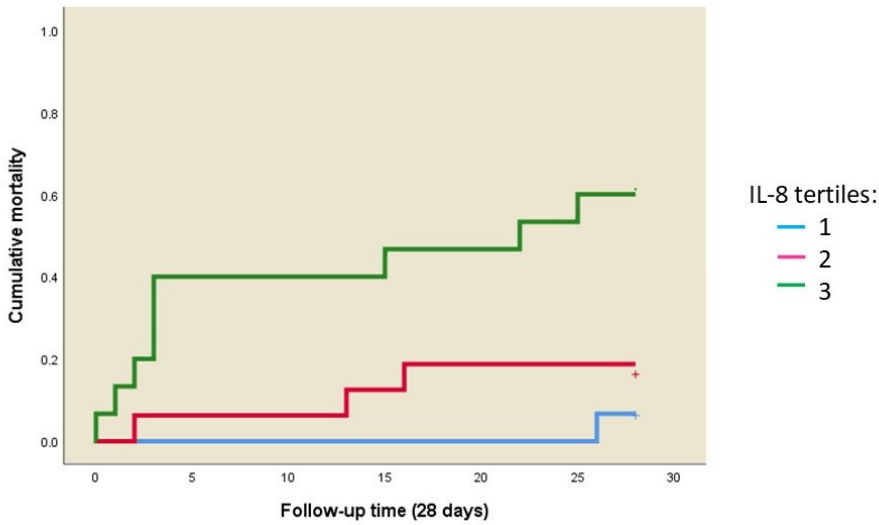


Figure 5. Kaplan-Meier plot showing cumulative mortality during 28 days of follow-up from hospital admission. Tertile 1 denotes the lowest levels of IL-8 and tertile 3 the highest levels.

To determine whether IL-8 and GDF-15 are independent risk factors, they were entered in combination as continuous variables into model 1. Only IL-8 remained significantly associated with 28-day mortality, with HR 3.38 (95% CI 1.35–8.43, *P* = 0.009) per SD increment.

The largest subgroup of patients, comprising those with AECOPD as a primary diagnosis (n = 34), was analysed separately. Concentrations of IL-8 and GDF-15 at admission showed significant prognostic value for 28-day mortality in both models 1 and 2 with approximately four-fold (IL-8) and three-fold (GDF-15) risk per 1 SD increment (**Supplementary Table 1**). When both were entered into model 1 to determine independence of effect, only IL-8 remained significantly associated with 28-day mortality [HR 4.31 (95% CI 1.57–11.84, *P* = 0.005)].

Study II

ST2 predicts mortality in patients with acute hypercapnic respiratory failure treated with NPPV.

Clinical characteristics

Studies I and II comprised the same cohort, with clinical characteristics and subgroups as described (**Table 1**). The biomarker analysed in Study II was ST2, previously proven to be of prognostic value in patients with cardiovascular disease [63,68,137]. Plasma ST2 concentrations showed no significant difference among subgroups as measured by the Kruskal-Wallis test ($P = 0.22$). Both short-term (28-day) and long-term (18-month) mortality were assessed, with 30 patients (65%) dying during the 18-month follow-up.

Correlation of ST2 analysis methods

Plasma ST2 was measured and analysed as a part of a biomarker panel (Proseek Multiplex CVD 1). To verify the results of the panel, baseline ST2 concentrations were also quantified with the ELISA Presage ST2 clinical assay. The correlation coefficient of results of the techniques was r = 0.95 (Pearson´s correlation coefficient test, P <0.001) (**Figure 6**). The Presage ST2 assay results were subsequently used for all further analyses, with the exception of changes in ST2 levels during the first 4 and 12 hours of NVVP treatment, since ST2 was measured only as a component of the biomarker panel at time points other than baseline.

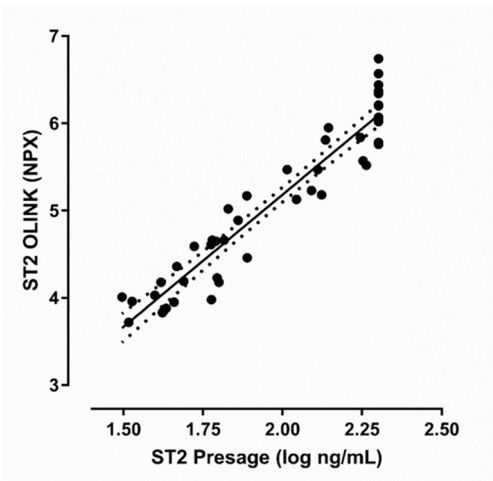


Figure 6. A scatterplot showing correlation between ST2 concentrations in patients with AHRF treated with NPPV as measured by the Olink biomarker panel and Presage ST2 clinical assay.

ST2 as predictor of short- and long-term mortality

Baseline ST2 concentrations prior to treatment initiation were transformed with the natural logarithm. Analysis with Cox proportional hazard model 1 (adjusted for age and sex) and 2 (adjusted for age, sex, and CRP) showed a significant prognostic value of biomarker level for mortality. Each 1 SD increment of ST2 was associated with an eleven- to twelve-fold risk of 28-day mortality. The association remained significant with 18-month mortality, at an approximately doubled risk (**Table 3**). As in Study I, ST2 concentrations were analysed in model 3 and were found prognostic of mortality independent of blood gas values, BMI, respiratory rate, and primary discharge diagnosis (data not shown).

The ST2 levels were ranked, separated into tertiles, and entered into model 1 and model 2. Patients in the highest tertile showed a significant more than five-fold risk of 18-month mortality, compared to the lowest tertile (**Table 3**). The event rate in ST2 tertiles according to a Kaplan-Meier plot is shown in **Figure 7**.

Table 3. Relationship of baseline plasma ST2 concentration and risk of 28-day and 18-month mortality in patients with AHRF treated with NPPV.

ST2 concentration on admission vs. 28-day mortality						
Continuous ST2 analysis (per SD increment)		*P*-value	Tertile 1	Tertile 2	Tertile 3	*P* for trend
events[a]/n	13/46		0/15	2/16	11/15	
HR (95% CI) Model 1*	11.00 (1.8–67.2)	0.009	n/a[b]	n/a[b]	n/a[b]	0.002
HR (95% CI) Model 2**	12.55 (2.0–77.2)	0.006	n/a[b]	n/a[b]	n/a[b]	0.001
ST2 concentration on admission vs. 18-month mortality						
Continuous ST2 analysis (per SD increment)		*P*-value	Tertile 1	Tertile 2	Tertile 3	*P* for trend
events[a]/n	30/46		7/15	10/15	13/16	
HR (95% CI) Model 1*	2.11 (1.4–3.2)	0.001	1.0 (ref)	1.54 (0.6–4.2)	5.50 (2.1–14.4)	0.001
HR (95% CI) Model 2**	2.47 (1.6 –3.9)	<0.001	1.0 (ref)	1.74 (0.7–4.6)	5.71 (2.2–4.7)	<0.001

Notes: [a]All-cause mortality within the follow-up time. [b]Analysis not possible due to zero number of events in Tertile 1. *Adjusted for age and sex. **Backward elimination model; adjusted for age, sex and CRP.

Abbreviations: AHRF, acute hypercapnic respiratory failure; NPPV, non-invasive positive pressure ventilation; ST2, suppression of tumorigenicity 2; SD, standard deviation; HR, hazard ratio; CI, confidence interval; CRP, C-reactive protein

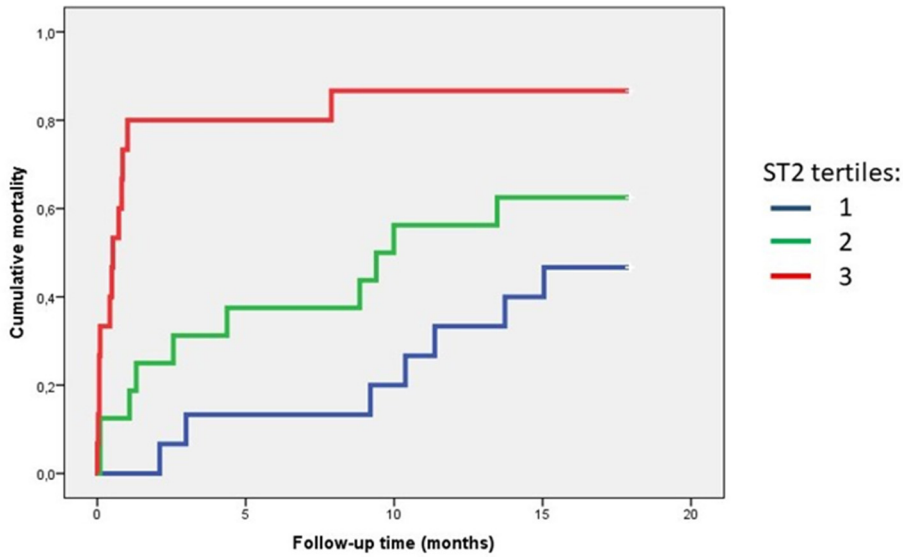


Figure 7. Kaplan-Meier plot showing cumulative mortality during 18-month follow-up period in patients with AHRF relative to baseline plasma ST2. Tertile 1 denotes the lowest levels of ST2 and tertile 3 the highest levels.

As in Study I, the subgroup of patients with AECOPD as a primary diagnosis was analysed separately. Each 1 SD increment of ST2 was associated with a greater than seven-fold risk of 28-day mortality [HR 7.11 (95% CI 1.3–38.1, $P = 0.022$)]. This association remained significant during long-term follow-up with a two-fold risk of 18-month mortality [HR 2.07 (95% CI 1.3–3.3, $P = 0.003$)].

Serial ST2 measurements and correlation with NPPV treatment response

Plasma ST2 concentrations measured in the biomarker panel prior to initiation of NPPV treatment and at 4 and 12 hours were compared with concurrent pH and $PaCO_2$ levels, factors that signify respiratory acidosis and in which change can indicate response to treatment (**Table 4**).

Table 4. ST2, $PaCO_2$, and pH levels at hospital admission and after 4 and 12 hours of NPPV treatment in patients with AHRF

	Prior to treatment initiation	4 hours post- treatment-initiation	12 hours post-treatment-initiation
ST2, NPX, median (IQR)	5.15 (5.19–5.87)	5.69 (4.92–6.20)*	5.99 (5.21–6.35)*
$PaCO_2$, kPa, median (IQR)	8.75 (7.78–10.50)	8.10 (6.55–8.70)*	7.40 (6.40–8.70)*
pH median (IQR)	7.28 (7.24–7.36)	7.37 (7.34–7.40)*	7.39 (7.34–7.43)*

Notes: *Significant difference between levels prior to initiation of treatment and after 4 and 12 hours of NVVP treatment. Wilcoxon parade rank test.

Abbreviations: ST2, suppression of tumorigenicity 2; NPPV, non-invasive positive pressure ventilation; AHRF, acute Hypercapnic respiratory failure; IQR, interquartile range; NPX, normalized protein expression.

Linear regression analysis did not reveal a correlation of ST2 levels prior to treatment initiation and subsequent changes in pH and $PaCO_2$ (data not shown). The ST2 levels had increased at the 12-hour follow-up (**Table 4**). The level of increase was positively correlated to change in pH from baseline to 4 hours post-treatment-initiation [ß 0.21 (95% CI 0.07–0.34, *P* = 0.004)] and from baseline to 12 hours post-treatment-initiation [ß 0.23 (95% CI 0.12–0.34, *P* <0.001)], analysed with linear regression. On the other hand, the change in ST2 levels from baseline to 4 hours post-treatment-initiation was negatively related to change of $PaCO_2$ [ß 0.21 (95% CI -0.36 to -0.06, *P* = 0.008)] in the same time period. A similar result was found from baseline to 12 hours post-treatment-initiation [ß 0.29 (95% CI -0.46 to -0.14), *P* <0.001)]. There were missing values in the ST2 measurements, at admission n = 46, after 4 hours n = 41, after 12 hours n = 36.

Additional analyses

Since we concluded that both IL-8 and ST2 showed a prognostic value with respect to mortality in the same model and the same cohort (Studies I and II), we chose to analyse if the two biomarkers were independent of each other. They were both entered as continuous variables into model 1 along with GDF-15, which was also analysed in Study I, and both remained significantly associated with 28-day mortality; HR for each SD increment of IL-8 was 2.67 (95% CI 1.39–5.15, *P* = 0.003) and for ST2 it was 8.60 (95% CI 1.65–44.90, *P* = 0.011). When ST2 and GDF-15 were analysed in combination, both remained significantly predictive.

When the same analysis was made regarding the association between the three biomarkers and 18-month mortality, ST2 and GDF-15 remained significantly associated with mortality, but IL-8 did not. The hazard ratio for each SD increment in ST2 was 2.09 (95% CI 1.38–3.16, *P* = 0.001) and for GDF-15 was 1.81 (95% CI 1.19–2.74, *P* = 0.005).

Study III

ST2 predicts mortality in patients with acute dyspnea.

Clinical characteristics

In study III, ST2 was measured in a cohort of patients suffering from acute dyspnea, using the Presage ST2 assay method. A flow-chart showing the number of patients enrolled and excluded is shown in **Figure 8**. Twelve patients were excluded because of pneumothorax and 322 because of missing values or information about confounding factors.

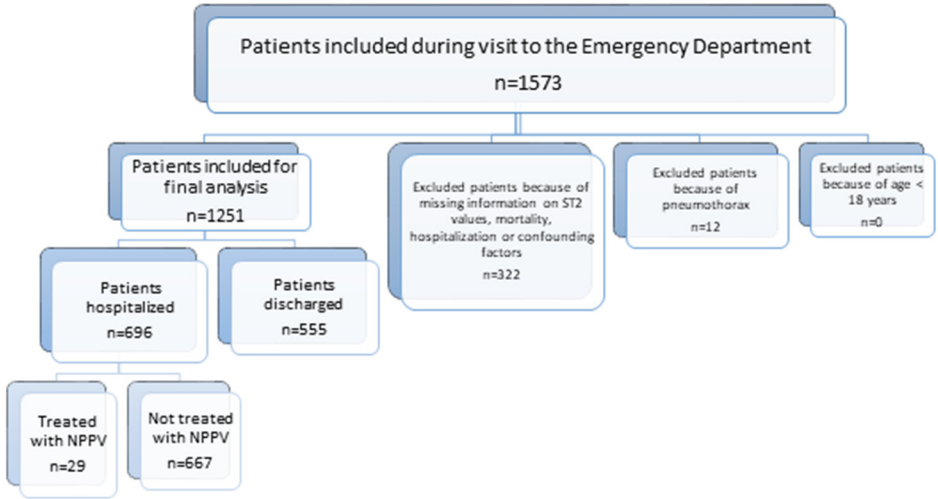


Figure 8. Acute dyspnea patients included and excluded in Study III.

We compared baseline characteristics in patients discharged from the ED (n = 555) with those hospitalized (n = 696) (**Table 5**). Significant differences were observed between groups with respect to all included characteristics except BMI. The patients admitted were more likely to be older and male and to have a history of smoking; higher respiratory rate; lower oxygen saturation; higher concentrations of CRP, creatinine, and ST2; and a higher METTS-A score.

Table 5: Baseline characteristics of the study population with acute dyspnea , divided by hospitalization status

	All	Discharged	Hospitalized	*P* value[a]
Number of individuals	1251	555	696	
Age, years	74.1 (61.4–83.6)	66.0 (48.3–77.5)	79.3 (69.4–86.5)	<0.001
Female, n (%)	692 (55)	334 (60)	358 (51)	0.002
BMI, kg/m^2, mean±SD	26.6±6.1	26.6±5.8	26.5±6.4	0.74
Current or former smokers, n (%)	870 (70)	375 (68)	495 (71)	0.007
Respiratory rate, BPM	22 (20–28)	20 (18–24)	24 (20–30)	<0.001
Oxygen saturation %	95 (91–98)	97 (95–99)	93 (88–96)	<0.001
CRP, mg/dL	8.8 (3.2–32.0)	5.1 (1.8–13.0)	16.0 (5.4–45.0)	<0.001
Creatinine, mmol/L	79.0 (64.0–104.0)	73.0 (62.0–89.0)	88.0 (69.0– 120.8)	<0.001
METTS-A red/orange[b], n (%)	520 (42)	113 (20)	407 (58)	<0.001
ST2, ng/L	41.0 (27.1–62.1)	31.0 (22.1–44.1)	51.6 (35.4–83.4)	<0.001

Data presented as median values and interquartile range (IQR) unless otherwise stated.

Notes: [a]Independent t-test for continuous variables and chi-square test for categorical variables. [b]METTS-A: red, life-threatening, orange, potentially life threatening

Abbreviations: BMI, Body Mass Index; BPM, breaths per minute; CRP, C-reactive protein; METTS-A, medical emergency triage and treatment system–adult score; ST2, Suppression of Tumorigenicity 2.

ST2 as predictor of mortality

Baseline plasma ST2 concentrations with respect to mortality rates at 3 and 12 months are shown in **Table 6**.

Table 6. Baseline ST2 concentrations and cumulative mortality in acute dyspnea patients after 3 and 12 months of follow-up.

Follow-up time	Cumulative mortality	ST2 ng/mL (median, IQR) survivors	ST2 ng/mL (median, IQR) non-survivors	*P* value*
3 months	140/1251 (11%)	38.4 (25.7–57.3)	72.6 (50.2–117.4)	<0.001
12 months	283/1251 (23%)	36.6 (24.1–54.7)	57.8 (39.6–99.2)	<0.001

Notes: *Mann-Whitney U test used to analyse differences between median values in survivors vs. non-survivors.

Abbreviations: ST2, Suppression of tumorigenicity 2; IQR, interquartile range.

Plasma ST2 concentrations were analysed with regard to mortality after 3 and 12 months with adjusted Cox proportional hazard models 1 and 2 (**Table 7**). In model 1, we adjusted for age and sex, and in model 2 for the potential confounding factors age, sex, BMI, smoking, CRP, creatinine, METTS-A score, and underlying disease including asthma, COPD, restrictive lung disease, other lung disease, chronic heart failure, hypertension, atrial fibrillation, ischemic heart disease, and cancer. The ST2 level was predictive of mortality in both models. ST2 concentrations were separated into quartiles and related to mortality risk (**Table 7**).

Table 7: Relationship between plasma ST2 concentrations and mortality in patients with acute dyspnea, presented as continuous base levels and divided into quartiles.

3-month mortality	**Continuous ST2 analysis (per SD increment)**		***P*-value**	**Quartile 1**	**Quartile 2**	**Quartile 3**	**Quartile 4**	***P* for trend**
	Events[a]/n	140/1251		6/312	14/313	38/313	82/313	
	HR (95% CI) Model 1 *	2.08 (1.76 - 2.46)	<0.001	1.0 (ref)	1.94 (0.74–5.01)	4.54 (1.90–10.81)	9.81 (4.23 - 22.78)	<0.001
	HR (95% CI) Model 2 **	1.93 (1.61 - 2.32)	<0.001	1.0 (ref)	1.87 (0.71 - 4.94)	3.66 (1.50 - 8.93)	7.18 (2.99 - 17.26)	<0.001
12-month mortality	**Continuous ST2 analysis (per SD increment)**		***P*-value**	**Quartile 1**	**Quartile 2**	**Quartile 3**	**Quartile 4**	***P* for trend**
	Events[b]/n	283/1251		22/312	54/313	77/313	130/313	
	HR (95% CI) Model 1 *	1.59 (1.42 - 1.79)	<0.001	1.0 (ref)	2.04 (1.24 - 3.35)	2.53 (1.57 - 4.09)	4.56 (2.87 - 7.24)	<0.001
	HR (95% CI) Model 2 **	1.41 (1.23 - 1.61)	<0.001	1.0 (ref)	1.82 (1.10 - 3.00)	2.01 (1.23 - 3.27)	3.13 (1.93 - 5.09)	<0.001

Notes: [a]All-cause mortality within 3 months. [b]All-cause mortality within 12 months. *Adjusted for age and sex. ** Backward elimination model, adjusted for age, sex, BMI, smoking, CRP, creatinine, METTS-A score and underlying diseases asthma, COPD, restrictive lung disease, other lung disease, chronic heart failure, hypertension, atrial fibrillation, ischaemic heart disease, and cancer.

Abbreviations: ST2, Suppression of tumorigenicity 2; SD, standard deviation; HR, hazard ratio; CI, confidence interval; BMI, body mass index; CRP, C-reactive protein; METTS-A, medical emergengy triage and treatment system–adult score; COPD, chronic obstructive pulmonary disease.

NT-proBNP values were available for the first 700 participants (of 1251) of Study III. Data from these patients was analysed separately. ST2 was predictive of 3-month (89/700) and 12-month mortality (168/700) using Cox proportional hazard model 1. For each 1 SD increment of ST2, the HR for 3-month mortality was 2.04 (95% CI 1.66–2.51, *P* <0.001) and for 12-month mortality was 1.63 (95% CI 1.40–1.90, *P* <0.001).

A Pearson correlation test was used to evaluate the correlation among ST2, NT-proBNP, and CRP transformed with the natural logarithm. NT-proBNP explained 14% of the variance of ST2 ($r^2 = 0.14$), and CRP explained 15% of the variance of ST2 ($r^2 = 0.15$). When ST2, NT-proBNP, and CRP were included in combination as continuous variables with Cox regression in model 1, using backward elimination, only ST2 remained significantly associated with mortality. In this analysis, the HR per SD increment in ST2 was 2.05 (95% CI 1.68–2.51, *P* <0.001) for 3-month mortality and 1.58 (95% CI 1.35– 1.85, *P* <0.001) for 12-month mortality.

ST2 cut-off points of 35 ng/mL and 70 ng/mL

The cohort was separated into subgroups according to the suggested ST2 risk stratification cut-off points of 35 ng/mL and 70 ng/mL [69,84]. **Table 8** shows the relationship of ST2 concentration in the subgroups and risk of mortality analysed with Cox regression model 1. The group with ST2 concentrations >70 ng/mL showed the highest risk of both 3-month and 12-month mortality. **Figure 9** shows a Kaplan Meier plot illustrating cumulative mortality of the groups.

Table 8: Relationship of baseline plasma ST2 concentration according to cut-off levels with 3-month and 12-month mortality in 1251 patients with acute dyspnea.

	ST2 <35 ng/mL	ST2 35–70 ng/mL	ST2 >70 ng/mL	*P* for trend
Number of patients (%)	505 (40%)	517 (41%)	229 (18%)	
events[a]/n	14/505	53/491	73/255	
HR (95% CI) for 3-month mortality*	1.0 (ref)	3.02 (1.66–5.48)	7.92 (4.40–14.27)	<0.001
Events[b]/n	54/505	116/491	113/255	
HR (95% CI) for 12-month mortality*	1.0 (ref)	1.73 (1.25–2.41)	3.39 (2.42–4.76)	<0.001

Notes: [a]All-cause mortality within 3 months. [b]All-cause mortality within 12 months. * Model 1, adjusted for age and sex.

Abbreviations: ST2, Suppression of tumorigenicity 2; HR, hazard ratio; CI, confidence interval.

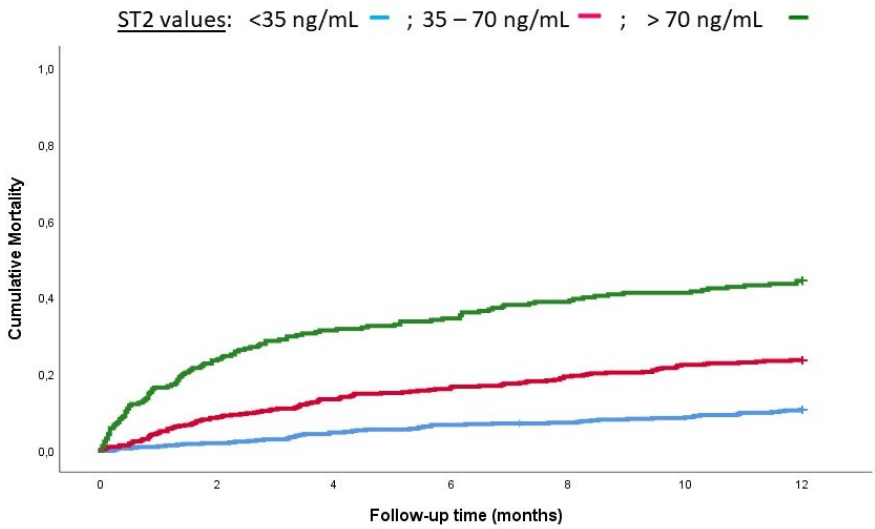


Figure 9. Kaplan-Meier plot showing cumulative mortality during a follow-up period of 12 months in patients presenting with acute dyspnea. Groups comprise patients with baseline plasma ST2 concentrations <35 ng/mL, 35–70 ng/mL and >70 ng/mL.

ST2 and treatment with NPPV

Twenty-nine of 696 patients presenting to the ED with acute dyspnea received treatment with NPPV for respiratory failure. These patients showed significantly higher median ST2 concentrations (63.5 ng/mL, IQR 47.4–99.8) than the group not treated with NPPV (50.1 ng/mL, IQR 35.3–80.5), when compared by the Mann-Whitney U test (P = 0.026). Cox regression analysis showed that, in the NPPV group, ST2 concentrations were significantly prognostic of 1-year mortality with an HR per SD increment of 2.76 (95% CI 1.13–6.70, P = 0.025). The association with 28-day and 3-month mortality was not significant. Three of the 29 patients presented ST2 concentrations <35 ng/mL at baseline, all of whom survived the follow-up period of 12 months. 14 of the 29 patients exhibited ST2 concentrations in the ≥35 to 70 ng/mL range, three of whom died within 12 months (21%). Concentrations >70 ng/mL ST2 were measured in 12 of the 29 patients, with eight of these patients dying within one year (67%). When stratified into groups, patients with the highest ST2 concentrations showed a significant increased risk of 1-year mortality compared with the group with the lowest concentrations (P for trend 0.029). Results were not significant for 28-day or 3-month mortality.

Additional analyses

The relationship between the most common final discharge diagnoses [acute exacerbation of obstructive lung disease (n = 205), acute heart failure (n = 234), acute coronary syndrome (n = 22), thromboembolic disease (n = 37), pneumonia or

severe infection (n = 142)] and plasma ST2 levels was evaluated using logistic regression. We found a positive correlation of ST2 concentration with acute heart failure [OR per SD increment 1.34 (95% CI 1.14–1.57, *P* <0.001)], thromboembolic disease [OR 1.41 (95% CI 1.01–1.97, *P* = 0.046)] and acute pneumonia or severe infection [OR 1.50 (95% CI 1.25–1.79, *P* <0.001)], but not with acute exacerbation of obstructive lung disease.

IL-8 and GDF-15 measurements were available for the first 700 participants (of 1251) in Study III. As the research reported in this thesis includes assessment of ST2, IL-8, and GDF-15, additional analyses were conducted to evaluate whether these three biomarkers were independently prognostic of 3- and 12-month mortality in the ADYS cohort. All three biomarkers were independently prognostic of 3- and 12-month mortality after adjustment for age and sex (**Table 9).**

Table 9. Relationship between baseline biomarker levels and all-cause mortality in 700 patients with acute dyspnea during follow-up time of 3 and 12 months

		3-month mortality		**12-month mortality**	
Biomarker		Continuous biomarker analysis (per SD increment)	*P*-value	Continuous biomarker analysis (per SD increment)	*P*-value
	events[a]/n	89/700		168/700	
ST2	HR (95% CI)*	1.57 (1.25–1.97)	<0.001	1.29 (1.09–1.52)	0.003
IL-8	HR (95% CI)*	1.52 (1.20–1.94)	0.001	1.60 (1.34–1.90)	<0.001
GDF-15	HR (95% CI)*	1.44 (1.04–1.98)	0.029	1.35 (1.08–1.70)	0.01

Notes: [a]All-cause mortality during the follow-up period. *Adjusted for age and sex, all biomarkers in the same model with backward elimination.

Abbreviations: SD, standard deviation; ST2, suppression of tumorigenicity 2; IL-8, interleukin 8; GDF-15, growth differentiation factor 15; HR, hazard ration; CI, confidence interval.

Study IV

Growth differentiation factor 15 levels in the general population predict chronic obstructive pulmonary disease.

Clinical characteristics

Fifty-three the 6103 individuals in the MDC-CC presenting with a history of COPD or heart failure were excluded from analysis in Study IV. Confounding factors selected based on comorbidity of COPD and heart failure (smoking status; BMI; plasma LDL, CRP, and NT-proBNP; systolic blood pressure, antihypertension therapy; and diabetes) were adjusted for in the analysis [138]. Data of one or more

of these covariates were missing in 1758 subjects, and these individuals were excluded, leaving 4292 in the final analysis.

Biomarkers as predictors of COPD development

Baseline characteristics of the subjects are shown in **Table 10** as the complete cohort and stratified according to COPD diagnosis in secondary care during the follow-up period of 21.0±5.3 years (n = 402). Subjects that developed COPD were older at the time of inclusion, had higher CRP concentrations, and were more likely to have a history of smoking.

Table 10: Baseline characteristics of the study population relative to COPD diagnosis in secondary care at follow-up end (mean 21.0 years).

	All	No COPD	COPD	*P* value[a]
Number of individuals	4292	3890	402	
Age, years	57.5 (5.9)	57.3 (6.0)	58.8 (5.4)	<0.001
Female n (%)	2618 (61)	2387 (61)	231 (57)	0.13
BMI, kg/m^2	25.7 (3.9)	25.7 (3.8)	25.6 (4.4)	0.70
Current or former smoker, n (%)	2531 (59)	2178 (56)	353 (88)	<0.001
Diabetes, n (%)	318 (7.4)	280 (7.2)	38 (9.5)	0.10
Antihypertension therapy, n (%)	711 (16.6)	638 (16.4)	73 (18.2)	0.37
Systolic BP, mmHg	141 (19)	140 (19)	141 (19)	0.51
CRP, mg/L	0.25 (0.43)	0.24 (0.40)	0.36 (0.62)	<0.001
NT-proBNP, ng/L	98.3 (148)	97.8 (152)	102.4 (110)	0.56
LDL, mmol/L	4.16 (1.0)	4.16 (1.0)	4.18 (0.9)	0.61
IL-8, NPX	4.69 (0.6)	4.68 (0.6)	4.79 (0.6)	0.002
ST2, NPX	2.67 (0.6)	2.68 (0.6)	2.66 (0.5)	0.66
GDF-15, NPX	8.75 (0.6)	8.73 (0.6)	8.99 (0.6)	<0.001

Data presented as mean±SD unless otherwise stated.
Notes: [a]Independent t-test for continuous variables and chi-square test for categorical variables, comparing COPD group with no COPD group

Abbreviations: COPD, chronic obstructive pulmonary disease; BMI, body mass index; BP, blood pressure; CRP, C-reactive protein; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; LDL, low density lipoprotein; IL-8, Interleukin-8; ST2, suppression of tumorigenicity 2; GDF-15, growth differentiation factor 15; NPX, normalized protein expression.

Levels of ST2, IL-8, and GDF-15 were prognostic of COPD diagnosis during the follow-up period using adjusted Cox proportional hazard models 1 (adjusted for age and sex) and 2 (adjusted for additional confounding factors) (**Table 11**). Baseline ST2 levels showed no significant association with development of COPD. IL-8 was significantly prognostic of COPD diagnosis in model 1, but not in model 2. The baseline concentration of GDF-15 was significantly prognostic of COPD diagnosis

in both model 1 [HR 1.65 per SD increment (95% CI 1.49–1.83, *P* <0.001)] and model 2 [HR 1.29 per SD increment (95% CI 1.15–1.44, *P* <0.001)].

Table 11: Relationship between measured baseline biomarkers in the general population and COPD diagnosis in secondary care during a mean follow-up period of 21.0 years.

Biomarker	Risk of COPD diagnosis per SD increment		*P*-value
	N / n events[a]	4292/402	
ST2	HR (95% CI) Model 1*	0.97 (0.88 - 1.08)	0.60
	HR (95% CI) Model 2**	1.03 (0.93 - 1.15)	0.54
IL-8	HR (95% CI) Model 1*	1.17 (1.07 - 1.29)	0.001
	HR (95% CI) Model 2**	1.05 (0.95 - 1.16)	0.35
GDF-15	HR (95% CI) Model 1*	1.65 (1.49 - 1.83)	<0.001
	HR (95% CI) Model 2**	1.29 (1.15 - 1.44)	<0.001

Notes: [a] New COPD diagnosis during the follow-up period. *Adjusted for age and sex. **Backward elimination model, adjusted for age, sex, BMI, smoking status, antihypertensive therapy, diabetes, systolic blood pressure, CRP, NT-proBNP and LDL.

Abbreviations: COPD, chronic obstructive pulmonary disease; BMI, body mass index; CRP, C-reactive protein; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; LDL, low density lipoprotein; IL-8, Interleukin-8; ST2, suppression of tumorigenicity 2; GDF-15, growth differentiation factor 15; SD, standard deviation; HR, hazard ratio; CI, confidence interval.

Plasma GDF-15 levels were separated into quartiles and analysed relative to eventual COPD diagnosis. Cox proportional models showed HR of the highest versus the lowest quartile in model 1 of 5.18 (95% CI 3.64–7.36, *P* for trend <0.001) and, with model 2, 2.26 (95% CI 1.60–3.20, *P* for trend <0.001) **(Supplementary Table 2**). The relationship of GDF-15 quartiles with COPD diagnosis is shown in **Figure 10** with a Kaplan-Meier curve.

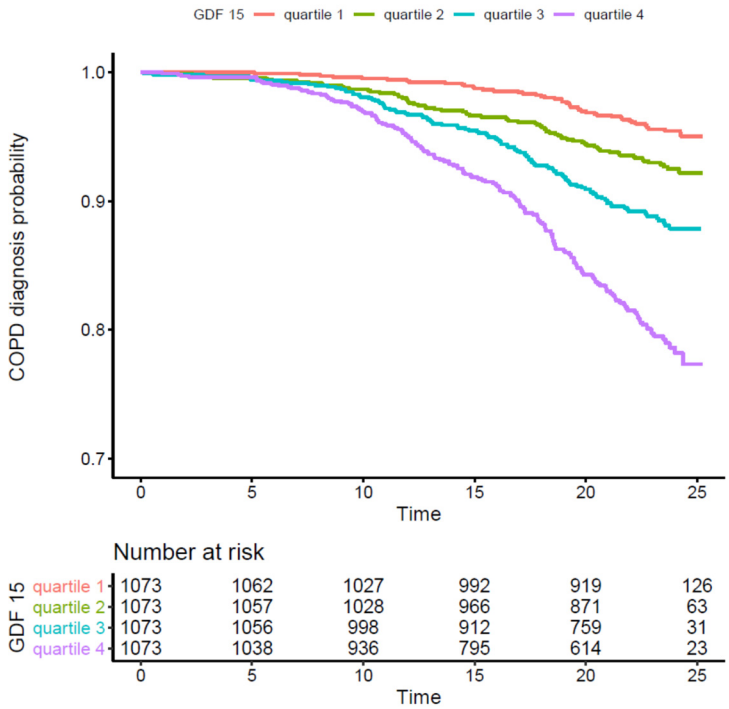


Figure 10. Kaplan-Meier plot showing cumulative COPD diagnosis in secondary care during follow-up time (21.0±5.3 years) in the general population. Quartile 1 denotes the lowest levels of GDF-15 and Quartile 4 the highest levels.

Additional analyses were conducted of current/former smokers versus those who had never smoked. The predictive value of GDF-15 for COPD diagnosis did not remain significant in the never-smoked group [HR per SD increment 1.29 (95% CI 0.95–1.76, $P = 0.10$)] in model 1. In the smoking subgroup the results remained significant. When former smokers were analysed separately from current smokers, in model 1, HR per SD increment of GDF-15 in former smokers was 1.29 (95% CI 1.05–1.5, $P = 0.015$) and 1.36 (95% CI 1.18–1.56, $P < 0.001$) in current smokers.

Biomarkers as predictors of mortality

The predictive value of ST2, IL-8, and GDF-15 for all-cause mortality was evaluated during the follow-up period of 21.5±4.9 years. All biomarkers exhibited a significant predictive value with respect to mortality in models 1 and 2, with GDF-15 showing the strongest association (**Table 12**). In order to evaluate whether the biomarkers showed independent prognostic value, we entered all three as continuous variables into model 2. Only GDF-15 remained significantly prognostic of all-cause mortality.

Table 12: Relationship between baseline biomarker concentration and all-cause mortality during a mean follow-up time of 21.5 years.

Biomarker	Risk of mortality per SD increment		*P*-value
	N / n events[a]	4292/1379	
ST2	HR (95% CI) Model 1*	1.10 (1.04 - 1.16)	0.002
	HR (95% CI) Model 2**	1.10 (1.03 - 1.17)	0.002
IL-8	HR (95% CI) Model 1*	1.15 (1.10 - 1.21)	<0.001
	HR (95% CI) Model 2**	1.10 (1.05 - 1.17)	<0.001
GDF-15	HR (95% CI) Model 1*	1.47 (1.39 - 1.56)	<0.001
	HR (95% CI) Model 2**	1.32 (1.24 - 1.40)	<0.001

Notes: [a]Mortality during the follow-up period. *Adjusted for age and sex. **Backward elimination model, adjusted for age, sex, BMI, smoking status, antihypertensive therapy, diabetes, systolic blood pressure, CRP, NT-proBNP and LDL.

Abbreviations: COPD, chronic obstructive pulmonary disease; BMI, body mass index; CRP, C-reactive protein; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; LDL, low density lipoprotein; IL-8, Interleukin-8; ST2, suppression of tumorigenicity 2; GDF-15, growth differentiation factor 15; SD, standard deviation; HR, hazard ratio; CI, confidence interval.

When GDF-15 levels were divided into quartiles analysed with respect to to all-cause mortality, *P* for trend was <0.001 in both models. With the first quartile defined as reference, the HR in the highest quartile was 2.26 (95% CI 1.89–2.68, *P* for trend <0.001) for model 1 and 1.74 (95% CI 1.45–2.09, *P* for trend <0.001) for model 2 (**Supplementary Table 3**).

GDF-15, COPD diagnosis, and all-cause mortality

Our results indicate that GDF-15 levels can predict both development of COPD and all-cause mortality. In a logistic regression analysis, the association between GDF-15 and all-cause mortality remained significant after adjustment for COPD diagnosis. Both GDF-15 and COPD diagnosis were independently significantly associated with mortality [OR 1.52 (95%CI 1.40–1.65, *P* <0.001) and 2.81 (95% CI 2.24 - 3.54, *P* <0.001), respectively].

Additional analyses

When the Study IV cohort was stratified according to COPD diagnosis, GDF-15 was significantly associated with mortality irrespective of COPD development, with HR for non-COPD of 1.46 (95% CI 1.36–1.55, *P* <0.001) per SD increment of GDF-15 and 1.35 (95% CI 1.17–1.52, *P* <0.001) for COPD. The association between GDF-15 and COPD incidence measured with Cox regression analysis showed similar effect size and was significant in both survivors and those who died during follow-up [HR for survivors 1.56 (95% CI 1.33–1.82, *P* <0.001) per SD increment of GDF-15 and 1.42 (95% CI 1.23–1.63, *P* <0.001) for non-survivors].

Adjustment for education level did not alter results regarding GDF-15 and diagnosis of COPD. Cox regression analysis showed HR of 1.64 per SD increment of GDF-

15 (95% CI 1.48–1.81, P <0.001) adjusted for age, sex, and education level. When the same adjustment was made for the association of GDF-15 with all-cause mortality, the analysis produced a HR of 1.46 per SD increment (95% CI 1.38–1.55, P <0.001).

A separate analysis was conducted for GDF-15 and mortality due to respiratory disease according to the Swedish National Cause of Death Registry (n = 78). GDF-15 remained significantly predictive of mortality, with HR 2.04 (95% CI 1.62–2.56, P <0.001) per SD increment in this subgroup.

Discussion

Main findings and significance

Studies I and II

In an unselected group of patients with AHRF differing in cause treated with NPPV, plasma IL-8 and ST2 were independently predictive of 28-day mortality and represent targets to explore further for establishing prognosis. The findings were driven by the largest subgroup of patients, those with acute exacerbation of COPD, and were independent of the traditional inflammatory biomarker CRP. Concentration of GDF-15 was also predictive of 28-day mortality, but our results cannot confirm it as an independent predictive biomarker. On the contrary, the findings suggested that GDF-15 association with mortality may be dependent on IL-8 levels.

Plasma ST2 was predictive of 18-month mortality, but the results seem to primarily reflect the strong association with 28-day mortality, making the long-term prognostic value of ST2 in this patient group uncertain. Our conclusion is that ST2 concentration is most informative for short-term mortality in AHRF and likely depends more on the acute illness as opposed to the underlying chronic disease.

Serial assessments of ST2 have been suggested to aid in assessment of need for, and effect of, therapeutic intervention, with post-treatment levels in acute or chronic settings adding substantially to the prognostic information gathered [69,90,139,140]. Our initial hypothesis that decreasing ST2 levels during the first 12 hours of NPPV treatment may be indicative of concurrent clinical improvement was not supported by the study results. Missing ST2 data in the repeat measurements might have been due to patient death in the initial hours after admission or instances in which blood samples were not retrievable for other reasons. This is a possible source of bias, as the missing values might represent patients with worsening clinical state. Although repeated ST2 measurements have been of value in assessing treatment response, most available studies report results of ST2 measurements ≥48 hours post-presentation, calling into question the validity of comparison with our results [84]. Due to the small size and heterogeneity of the patient cohort, no conclusions can be reached at this point concerning this matter.

NPPV is a well-known treatment for patients with acute respiratory failure as an adjunct to standard medical therapy, chiefly in hypercapnic respiratory failure, but also in some forms of hypoxic respiratory failure in selected patient groups [7,34,39,40,54]. Patients with acute exacerbation of COPD and AHRF have the most well-established treatment indication, and NPPV reduces the necessity of intubation as well as shortening the hospital stay and decreasing in-hospital mortality [42,52].

Although NPPV is widely used for a range of conditions, the specific cause of illness is not always obvious at an ED visit. Moreover, patients may have multiple conditions or diseases contributing to the clinical deterioration [3,4,7]. NPPV treatment is costly with regard to surveillance and technology, and not all patients tolerate or benefit from, the procedure [35,141]. Despite knowledge of factors that predict outcome, existing selection tools are not always helpful in treatment decisions, and more are needed in order to choose the most appropriate treatment for the individual patient [45,48]. While expanded access to such information might lead to an earlier and more aggressive line of treatment with intensive care and intubation, we suggest that conversion to a greater focus on palliative care might be the most reasonable approach when the prognosis is poor.

To the best of our knowledge, no prior study has been published regarding IL-8 or ST2 as predictors of short-term mortality in patients with AHRF treated with NPPV. Potential clinical use of the studied biomarkers might be valuable in primary patient evaluation and influential in treatment decisions. Since many forms of acute or chronic illness are characterized by enhanced inflammation, it is likely that high baseline levels of IL-8 and ST2 reflect not only severity of an acute condition but also more serious underlying chronic illness. However, our results suggest that the utility of these inflammatory biomarkers in predicting mortality seems to be optimal over the short-term. Although no definite conclusions can be currently drawn with respect to clinical utility of the studied biomarkers, it seems probable that biomarker levels are more directly related to acute illness as opposed to underlying chronic disease. The findings of Studies I and II represent the discovery stage and need to be replicated in larger studies, with the performance value of the biomarkers determined, for example with AUC calculations.

Study III

The biomarker ST2 was predictive of 3- and 12-month mortality in an unselected group of patients suffering from dyspnea and seeking the ED. The ST2 cut-off levels of 35 ng/mL and 70 ng/mL, previously suggested for risk-stratification in cardiac conditions [69,90], can also be applied in the setting of acute dyspnea. The observed ST2 prognostic value with regard to mortality was independent of NT-proBNP levels.

Several studies have evaluated the accuracy of ST2 in predicting mortality in patients with acute dyspnea [85-89]. Taken together, these studies have shown a predictive association of ST2 concentrations with 1- and 4-year mortality, especially in patients with AHF, and also in those with pulmonary disease. The combined evaluation of ST2 and BNP/NT-proBNP has been reported optimal in detecting high risk dyspnea patients, although, importantly, patients with high ST2 concentrations and low levels of BNP and NT-proBNP were also found to be at high risk [86]. Our study comprised one of the largest cohorts of published studies, and the results support the predictive accuracy of ST2 for mortality, independent of NT-proBNP, and highlights its value from an ED perspective, i.e. with closer relationship to the acute event.

In a 2015 review, Januzzi *et al.* proposed a cut-off level of 35 ng/mL of plasma ST2 in patients with AHF, based on the premise that this threshold provides a reasonable reference limit above which risk of adverse outcome rises linearly with elevation [90]. However, many patients with AHF show substantially higher levels, and Aleksova *et al.* have argued that an ST2 concentration of 70 ng/mL might represent a potential cut-off level for patients with AHF at very high risk of adverse outcome. The mechanism of the ST2 effect is thought to be associated with activation of neurohormonal and fibrotic pathways and increased risk of adverse heart remodelling [69,84]. The same cut-off levels are suggested for evaluating patients with acute coronary syndrome [69]. Our results based on a large group of patients seeking the ED with acute dyspnea imply that categorizing patients according to the cut-off levels of 35 ng/mL and 70 ng/mL might be valuable for risk stratification and identification of patients with acute dyspnea that have poor prognosis.

A secondary goal of Study III was to confirm that our previous results (Study II) regarding the predictive value of ST2 on mortality in patients with AHRF treated with NPPV could be replicated in a larger group. Compared with 46 participants in the AHRF cohort (Studies I and II), only 29 patients received NPPV treatment for AHRF in the ADYS cohort, despite the much larger number of patients included (n = 1251). The source of the lower than expected number might be the inclusion in the ADYS cohort of only those seen during office hours or the inability to include patients with such severe illness at the ED, while all patients with AHRF treated with NPPV at the Intermediate Emergency Care Department were included in Studies I and II. Within this subgroup, ST2 concentrations were significantly predictive of 1-year mortality, although no definitive conclusions can be drawn as to the value of these findings because of the small number of samples.

Despite the lack of specificity for disease type, we conclude that high ST2 concentrations in patients with dyspnea may be indicative underlying life-threatening disease. This information might be useful in the decision of whether to admit patients to intensive therapy or possibly consider a more palliative line of treatment.

Study IV

The main finding of Study IV was that plasma GDF-15 concentration in the general population was independently predictive of COPD diagnosis in secondary care during a follow-up period of approximately 21 years. Our results do not support the use of ST2 or IL-8 as predictors of COPD development over the long-term.

Although multiple roles of GDF-15 in regulation of inflammation and cell proliferation and migration have been defined, the significance of GDF-15 as a biomarker for disease development and progression is far from clear, since the exact function of GDF-15 is not well understood [81,114,115]. Ho *et al.* observed a strong positive association of GDF-15 concentration with age, diabetes, hypertension, and smoking as well as a link between genetic factors and GDF-15 concentration. No sex difference was seen, but there was a strong association between higher GDF-15 levels and cardiometabolic risk factors, including diabetes, hypertension, and smoking [116]. Other studies in the general population have demonstrated that high GDF-15 concentrations indicate underlying cardiovascular disease or subclinical atherosclerosis and can predict the development of diabetes and chronic kidney disease [114,116-122]. Elevated GDF-15 levels have also been associated with adverse prognosis in various states of acute or chronic deterioration of diseases such as heart failure, myocardial infarction, cancer, and inflammatory disease [81,115].

In patients with established COPD, high GDF-15 levels are associated with a greater rate of exacerbations, higher mortality, and increased rate of decline in lung function [123]. No previous study has, to our knowledge, evaluated the association of plasma GDF-15 concentration in the general population with later COPD diagnosis. Considering the broad spectrum of disease and conditions associated with high GDF-15 levels, we suggest that it is unlikely that GDF-15 levels represent a specific pathophysiological pathway of relevance for COPD. Rather, GDF-15 levels might increase in a range of subclinical conditions associated with inflammation, including airway inflammation.

Interestingly, participants in the study who later developed COPD also showed higher baseline CRP levels than those who remained free of COPD. We conclude that elevated GDF-15 might be indicative of a separate pathway of inflammation, since its association with COPD diagnosis was independent of CRP.

GDF-15 was associated with a diagnosis of COPD independent of smoking, the strongest and most well-established risk factor for COPD. Hence, our results add new information for COPD risk assessment to established risk factors (i.e. age and smoking habits), regardless of the underlying molecular source of the relationship between GDF-15 level and later COPD diagnosis. In those who have never smoked, we cannot conclude that GDF-15 predicts COPD, although this component of the analysis had less power because of a lower COPD incidence rate in the never-smoked group. With this taken into account, we also cannot rule out such a relationship.

Our results support the previously reported association between GDF-15 and all-cause mortality [81,112,115,121]. Since ST2 and IL-8 were not similarly independently associated with increased mortality, we conclude that GDF-15 is a valid biomarker for predicting all-cause mortality, while ST2 and IL-8 seem to be primarily associated with prognosis in acute conditions.

A sub-analysis of individuals reported to have died of respiratory disease according to the Swedish National Cause of Death Registry (n = 78) showed a significant association between plasma GDF-15 and respiratory-related mortality. However, in a study of the validity of this registry showing high (77%) overall agreement between the cause of death listed on death certificates and the expected cause of death based on case summaries, the agreement for COPD and death from other respiratory causes was only 49% [142]. Our results should therefore be interpreted with caution.

Strengths and limitations

The main limitation of Studies I and II is the small sample size, which results in wide confidence intervals. Another limitation is the heterogeneity of the AHRF group. The classification of patients into subgroups according to primary diagnosis was made by the same internist in all cases, and a wrong diagnosis seems unlikely because of the large quantity of available clinical information pertaining to admission and prior history, although some data regarding prior disease, such as spirometry and echocardiography results, were missing. Subgroup evaluation by two independent internists would have strengthened the results.

Subgroup analyses did not seem to influence results, although the subgroups of patients with AHF and acute exacerbation of OHS were too small for results to be considered reliable. In our opinion, this reflects the emergency medicine setting, in which the primary cause of deterioration might be multifactorial and difficult to determine, and the results can be interpreted in that context.

The cohort of Study I and II might be considered to exhibit more severe illness than those of previous comparable studies. The majority of patients in the cohort (72%) had a Do Not Resuscitate (DNR) order in place, which is higher than the population of a comparable clinical audit from Roberts et al. in 2008, with 40% under a documented DNR order [50]. The in-hospital mortality was similar between these studies (25%) [50]. A possible reason for the higher percentage of patients having a DNR order, might be the application of local guidelines, in which the admitting physician is required to make a resuscitation judgement before beginning NPPV treatment. In spite of the small number of patients, our results contribute important information. Larger studies with evaluation of biomarker performance could be valuable for physicians in making clinical decisions regarding this group of severely

ill patients, in which clinical research is extremely challenging despite its importance.

In Study II, ST2 levels were measured with two methods (Proximity Extension Assay and ELISA), the results of which correlated highly. Previous studies have shown that levels of plasma GDF-15 measured by the Proximity Extension Assay used in Studies I and IV correlated closely with those measured by an electrochemiluminescence immunoassay [122]. The high correlation between measurement methods strengthens the reliability of our results.

NT-proBNP data were only available for 56% of the cohort of Study III, a limitation considering the importance of comparing and combining the predictive values of the evaluated biomarkers. However, a sub-analysis of the group for which NT-proBNP levels were available did not alter the findings, and we conclude that the overall results strengthen the role of ST2 as a prognostic biomarker in the emergency department setting.

Study III was a single-centre study in which data were collected over an extended period of time by designated nurses. Although a multi-centre study with a larger number of subjects may have further strengthened results, the continuity of the personnel collecting the data throughout the study period ensures data quality. A limitation of the study is that the discharge diagnoses were retrieved through medical records without specific assessment from an external party, and that we did not have access to the cause of death in deceased patients.

The MDC-CC cohort in Study IV consisted primarily of individuals of northern European origin, and the associations revealed might differ from that in other populations. The participation rate was only slightly above 40% [127]. Data regarding lung function and possible confounding factors such as air pollution, COPD severity, and time of onset were not available. Data from primary care centres are not included, which might have led to fewer cases being identified. However, in Sweden only those patients suffering from a severe stage of COPD are treated in secondary care [143]. Thus, we conclude that, although the lack of demographic information from primary care may represent a limitation, the assumption can be made that individuals with a registered COPD diagnosis in secondary care are those most severely affected by the disease. Findings in these patients might provide information of relevance to treatment of those in the pre-clinical phase of COPD with respect to initiating preventive measures and improving prognosis. The long follow-up period, relatively large size of the cohort, and large quantity of demographic data are all strengths of the study.

Future aspects

Since our research of patients with AHRF treated with NPPV consisted of a very small cohort, further research is needed to validate the results and assess whether the findings can be generalized to groups other than patients with acute exacerbations of COPD. It would be of interest to include patients with AHRF in whom the level of care would be intensified, such as with intubation, when NPPV provides insufficient clinical improvement. Monitoring for a longer period of time in order to assess the association of changes in biomarker levels with clinical status may be of value, including for evaluation of outcome in chronic hypercapnic respiratory failure.

Further research is needed with respect to the prognostic value of ST2 in patients accessing the ED with acute dyspnea, especially in combination with other biomarkers such as NPs, and with serial measurements of ST2 in this patient group, irrespective of the underlying condition.

The evidence of elevated GDF-15 as a predictor of COPD diagnosis in the general population may be of clinical relevance. High GDF-15 may be a motivating factor in smoking cessation therapy. Other preventive actions triggered by high GDF-15 might include regular assessment of lung function, physical activity programs, and early medication. Randomized clinical trials of GDF-15-guided intervention in broad segments of the general population might clarify the value and practicality of such interventions.

In this thesis, the feasibility of using biomarkers that participate in the inflammatory pathway to predict outcome in acute respiratory failure and acute dyspnea was evaluated. In light of the current Covid-19 pandemic, the findings reported in this thesis might inspire studies to identify biomarkers associated with mortality in patients with Covid-19-related acute respiratory failure. Identification of such biomarkers might be helpful in decisions related to intensive care and, potentially, to the use of medications targeting specific biomarkers in order to inhibit the reported aggressive immunologic response [144].

Conclusions

Study I

Plasma IL-8 concentration can predict short-term mortality in patients with acute hypercapnic respiratory failure treated with non-invasive positive pressure ventilation.

Study II

Plasma ST2 concentration can predict short-term mortality in patients with acute hypercapnic respiratory failure treated with non-invasive positive pressure ventilation. The predictive value regarding long-term mortality is less certain.

Study III

Assessment of plasma ST2 concentration is valuable for risk stratification and as a prognostic marker of up to 12-month mortality in patients seeking the emergency care for acute dyspnea, irrespective of the underlying clinical condition and independent of NT-proBNP level. The suggested cut-off points of 35 ng/mL and 70 ng/mL appear useful in identifying high-risk patients in this group.

Study IV

Plasma GDF-15 measured in the general population can predict later COPD diagnosis in secondary care as well as all-cause mortality, even after adjustment for smoking status and other possible confounding factors.

Summary

Plasma levels of IL-8 and ST2 were shown to be related to acute clinical deteriotation in respiratory failure of differing etiologies and are worthwhile targets of exploration in predicting mortality. The ST2 cut-off points of 35 ng/mL and 70 ng/mL used for risk stratification of acute cardiac disease can adequately identify patients with acute dyspnea at high risk of adverse outcome and mortality. Plasma GDF-15 concentration shows an association with long-term prognosis and mortality in respiratory failure, and, in the general population, GDF-15 could predict individual risk of developing COPD.

Supplementary material

Supplementary table 1. Prognostic value of plasma IL-8/GDF-15 concentration at admission for 28-day mortality in patients with AECOPD treated with NPPV (n = 34).

	IL-8		**GDF-15**	
	Continuous IL-8 analysis (per SD increment)	P-value	Continuous IL-8 analysis (per SD increment)	*P*-value
events[a]/n	11/34		11/34	
HR (95% CI) Model 1*	4.16 (1.84–9.39)	0.001	3.10 (1.30–7.40)	0.011
HR (95% CI) Model 2**	4.34 (2.05–9.19)	<0.001	3.28 (1.56–6.88)	0.002

Notes: [a]All-cause mortality within 28 days of admission. *Adjusted for age and sex. **Backward elimination model; adjusted for age, sex and CRP.

Abbreviations: IL-8, interleukin 8; GDF-15, growth differentiation factor 15; AECOPD, acute exacerbation of COPD; SD, standard deviation; HR, hazard ratio; CI, confidence interval; CRP, C-reactive protein.

Supplementary Table 2: Increasing quartiles of GDF-15 concentration associated with risk of COPD diagnosis in secondary care during mean follow-up time of 21.0 years.

Biomarker	**Statistical model**	**Quartile 1**	**Quartile 2**	**Quartile 3**	**Quartile 4**	***P* for trend**
GDF-15	events[a]/n	46/1073	73/1073	109/1073	174/1073	
	HR (95% CI) Model 1*	1.0 (ref)	1.70 (1.17–2.47)	2.73 (1.91–3.90)	5.18 (3.64–7.36)	<0.001
	HR (95% CI) Model 2**	1.0 (ref)	1.27 (0.87–1.83)	1.54 (1.09–2.20)	2.26 (1.60–3.20)	<0.001

Notes: [a] New COPD diagnosis during the follow-up period. *Adjusted for age and sex. **Backward elimination model, adjusted for age, sex, BMI, smoking status, antihypertensive therapy, diabetes, systolic blood pressure, CRP, NT-proBNP and LDL.

Abbreviations: COPD, chronic obstructive pulmonary disease; BMI, body mass index; CRP, C-reactive protein; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; LDL, low density lipoprotein; GDF-15, growth differentiation factor 15; HR, hazard ratio; CI, confidence interval.

Supplementary Table 3: Increasing quartiles of plasma GDF-15 concentration associated with risk of all-cause mortality during a mean follow-up period of 21.5 years.

Biomarker	Statistical model	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* for trend
GDF-15	events[a]/n	184/1073	258/1073	390/1073	574/1073	
	HR (95% CI) Model 1*	1.0 (ref)	1.21 (1.00–1.46)	1.64 (1.37–1.96)	2.26 (1.89–2.68)	<0.001
	HR (95% CI) Model 2**	1.0 (ref)	1.13 (0.94–1.37)	1.40 (1.17–1.68)	1.74 (1.45–2.09)	<0.001

Notes: [a] All-cause mortality during the follow-up period. *Adjusted for age and sex. **Backward elimination model, adjusted for age, sex, BMI, smoking status, antihypertensive therapy, diabetes, systolic blood pressure, CRP, NT-proBNP and LDL.

Abbreviations: COPD, chronic obstructive pulmonary disease; BMI, body mass index; CRP, C-reactive protein; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; LDL, low density lipoprotein; GDF-15, growth differentiation factor 15; HR, hazard ratio; CI, confidence interval.

Erratum

In Table 2, 3 and 4 in Paper I, and Table 2 in Paper II, there is an error in the way events are expressed. Events are expressed with N/N^{a} events, with N being the total number of patients and N^{a} being the total number of events (for example, 28-day mortality 46/13). The way events are expressed in this thesis is: $Events^{a}/n$, where the number of events (^{a}) is expressed before the number of patients (n) in a cohort (for example, 28-mortality 13/46). The corrected tables in this thesis are Table 2 and 3, and Supplementary Table 1.

Acknowledgements

At the end of my journey to become a PhD in 2020 the world has suddenly changed and we all have to adjust the way we look at it. I am glad to have embarked on this research journey many years ago. Even if the final distance might not exactly be how I imagined, I am above all grateful for having gotten the opportunity to become a researcher and for being surrounded by amazing friends, colleagues and family who have supported me.

I want to begin by thanking all the people that participate in scientific studies – your input is invaluable for the following generations.

My supervisor **Olle Melander**, thank you so much for your endless patience and for answering every email right away. You are enormously hard-working and I sincerely believe that you have more hours each day than the rest of us. I still can't understand why you decided to listen to my crazy idea of a project and give me the opportunity to design and go forward with it. Whatever the reason, I will always be very grateful! I hope our collaboration will continue in the future and that I will continue to get the opportunity to learn from you (I still need to understand those outliers or why the sky is blue in Malmö…).

Thank you to all my co-authors for your input and support; **Fredrik von Wowern**, **Gunnar Engström**, **Jan Nilsson** and **Marju Orho-Melander**. Thank you to the Danish collaborators and co-authors **Jens Peter Goetze** and **Marie Ziebell Severinsen**, you have shared your knowledge in the most valuable way. And thank you **Gunilla Hughes-Wulkan** for always being able to help – I believe that you can truly fix anything!

Carmen San Miguel, thank you for becoming my research assistant during the gathering of information. Your thorough and disciplined work was amazing! The personnel at the Intermediate Care Department (AVA) in Malmö – thank you for your work during the study time. Thank you also to the study nurses that gathered data for the ADYS study at the Emergency Department in Malmö. And of course to all who contributed to gathering of data in the Malmö Diet and Cancer study – I don´t know you but I am very grateful.

Hassan Lazkani and **Munti Saleh**, you gathered information for my third study during your free time while working as assistants in the Emergency Department – I

will always be thankful for that. I am certain that you will become great doctors in the future with your selfless work ethics.

I have been so lucky to be surrounded by great role models ever since I decided to become a doctor and later a researcher. **Unnur Steina Björnsdóttir**, thank you for introducing me to, and including me in, the world of research as a student. I learned incredibly much from reading all those medical journals!

My future as a pulmonary physician was decided while working with research at the former lung hospital of Vífilsstaðir in Iceland and I would like to thank all the doctors there that welcomed me there and made me feel at home. Thank you **Þórarinn Gíslason** for being a mentor, helping me with ideas for my research and for always believing in me. Thank you **Ólafur Baldursson** and **Gunnar Guðmundsson** for introducing me to new aspects of pulmonary research.

While working in Sweden I have had the privilege to be surrounded by even more role models.

Thank you everyone at the **Internal Medicine Department in Malmö**, for making me feel welcomed right away and helping me grow as a doctor and as a researcher. I want to thank the head of department **Maria Ohlson Andersson**, for being such a great and down-to-earth boss. I am grateful for getting the chance to develope guidelines for NPPV use in the Intermediate Care Department, even if I was only a resident. That work laid ground for my choice of research field. Thank you everyone else I don't mention here, you really are the best gang!

To all the great colleagues at the **Pulmonary Department in Malmö and Lund –** thank you for helping me become the person I am today and always believing in me. I am grateful to have been able to learn from each and every one of you.

Johan Svahn, thank you for encouraging me as my head of department to continue with my research, and for many other life lessons you have taught me. I have felt greatly supported by you all these years and I value our collaboration deeply.

Eeva Piitulainen, you were one of the first people I got to know in the Pulmonary Department and it has been such an honour to work with you. You once told me that instead of being irritated over the shortcomings of clinical reality, it was easier to just do your best every day with what you have, and then go home and live your life. I have always tried to keep this in mind and tried following in your footsteps (even if I do take the elevator quite a lot…).

Åsa Jaworowski, thank you for our friendship and for all the research discussion we have had, you really helped me make a difficult decision when I was half-way through. Thank you **Behrouz Mostafavi** for always having my back and all the great research guidance, **Anthony Prakash** for always thanking me more than I deserve, and both of you for all the laughs. **Ruzica Mitrovic**, thank you for all the

guidance and warmth, you have taken such good care of me. Thank you **Ingela Nilsson** for encouraging me on the way.

Jónas Einarsson, thank you for becoming my clinical supervisor when I needed one, and for continuing to always be there when I needed to think out loud. You are one of the cleverest people I know and I value our friendship greatly. **Ulrika Lindberg**, I am so happy that we became side-by-side "schemaläggare" and friends – and for all you have taught me about life. Thank you **Maria Planck**, for all the laughs in our room at Ögon B and for being there for me when I really needed a friend.

A special thank you to **Arne Egesten** professor in pulmonary medicine, for always encouraging us clinical researchers and trying to find ways for us to go on, and for having so much faith in me. Not to mention how grateful I am for you volunteering to proofread this thesis and give me valuable input.

Through my work I have also gained so many great ST-friends. Thank you **Adriana**, for becoming my friend that I can always talk to and for always believing in me. **Zainab**, you are one of the most humble and hard-working people I know – thank you for your friendship and constant encouragement. **Marija**, it has been an honour growing up with you in the pulmonary world! Thank you for the great talks and warm friendship.

Guðrún Nína, how lucky I am that you decided to come to Lund and start working with me. We found each other directly and I am so glad for our friendship and your clever advice, you are always there for me!

I would not be where I am today without the help and support from my beloved **friends and family**!

Beta – one of if my oldest and most dear friends – I am happy that our friendship has lasted for over 30 years, even if we have lived in different countries we have always kept in touch. We are very different but have great respect for one another, I think that is why you are such an important advisor to me.

Thank you **Rannveig**, for doing this research journey side by side with me during the last years - I could not have done it without you! I am so grateful for our friendship, you help me see the world from antother perspective and inspire me with your achievements and energy.

Jórunn and **Kári**, what a lucky day when you moved to our street and we acquired the best neighbours ever! Even after you moved away, our friendship has grown during good times and bad and I know that we will always be there for each other. You have believed in me and made me feel that I actually really can do this!

Erna – my forever friend. We found each other during the first weeks in medical school and have been there for one another ever since. You are one of the most

clever and beautiful souls in the world. To have you as a friend that I can call anytime and talk about anything is invaluable. Thank you for listening and always being there!

Kristbjörg and **Lena**, I am so lucky to be able to call you both friends and family - what a break! Your positivity and humour lifts me up to touch the sky (in golden shoes). Thank you for making me believe that I will cross the finishing line even in this race.

In 2003 I was included into the family in Hjallabrekka. **Alda** and **Derek**, thank you for your tremendous support during the years. You inspire me with your curiosity and good listening skills, and have shown me that it is ok to change your path a few times and choose to do the things you love. I am grateful for all your encouragement and for being there for us! **Eva Hlín** – I am so lucky to have gained you as a big sister. You never give up and for you, every problem is a challenge that can be solved. I admire your strength. Thank you for appreciating and encouraging me, I look forward to more trips up and down hills together!

Amma – my grandmother, thank you for always being there for me. I love your sarcastic sense of humour and your story-telling skills. You believe in me even when I make mistakes. Your home has always been my home, I thank you and **Afi** for taking care of me when I needed it during the first years of medical school and right before I moved to Sweden.

Pabbi – my dad, thank your for enriching my life with all the stories and the songs, for giving me and my sisters and brothers your kindness and warmth. I claim that I have inherited getting crazy ideas from you, sometimes executing my ideas doesn´t work out but sometimes it does – as in the case of the beginning for this thesis of mine.

Mamma - my mom, you are the one who taught me to appreciate the little things in life. Your warm smile and embrace makes me feel strong enough to move mountains. You are caring and beautiful and I am so grateful for the support and faith you have in me.

Þórdís – my little sister and beam of light in difficult times. I love being your sister now when you are all grown up even more than I loved being your second mom when you were little. You are such a good listener and advisor, you teach me more and more every day and I am so proud of you.

And finally, my husband **Kristján** and our wonderful children **Þórhildur, Matthías** and **Vala**. You cheer me on and make me want to be a better person. Sometimes you drive me crazy, but always happy, always proud, always loved. You make everything worth fighting for. Takk!

References

1. Parshall MB, Schwartzstein RM, Adams L, et al. An official American Thoracic Society statement: update on the mechanisms, assessment, and management of dyspnea. *American journal of respiratory and critical care medicine.* 2012;185(4):435-452.
2. Johnson MJ, Hutchinson A. Breathlessness in the emergency care setting. *Current opinion in supportive and palliative care.* 2018;12(3):232-236.
3. DeVos E, Jacobson L. Approach to Adult Patients with Acute Dyspnea. *Emergency medicine clinics of North America.* 2016;34(1):129-149.
4. Stokes NR, Dietz BW, Liang JJ. Cardiopulmonary laboratory biomarkers in the evaluation of acute dyspnea. *Open access emergency medicine : OAEM.* 2016;8:35-45.
5. Laviolette L, Laveneziana P. Dyspnoea: a multidimensional and multidisciplinary approach. *The European respiratory journal.* 2014;43(6):1750-1762.
6. Suzuki T, Lyon A, Saggar R, et al. Editor's Choice-Biomarkers of acute cardiovascular and pulmonary diseases. *Eur Heart J Acute Cardiovasc Care.* 2016;5(5):416-433.
7. Davidson AC, Banham S, Elliott M, et al. BTS/ICS guideline for the ventilatory management of acute hypercapnic respiratory failure in adults. *Thorax.* 2016;71 Suppl 2:ii1-35.
8. Wagner PD. The physiological basis of pulmonary gas exchange: implications for clinical interpretation of arterial blood gases. *The European respiratory journal.* 2015;45(1):227-243.
9. Mirza S, Clay RD, Koslow MA, Scanlon PD. COPD Guidelines: A Review of the 2018 GOLD Report. *Mayo Clinic proceedings.* 2018;93(10):1488-1502.
10. Aggarwal T, Wadhwa R, Thapliyal N, Sharma K, Rani V, Maurya PK. Oxidative, inflammatory, genetic, and epigenetic biomarkers associated with chronic obstructive pulmonary disorder. *Journal of cellular physiology.* 2019;234(3):2067-2082.
11. Jindal SK. Remodeling in asthma and COPD-recent concepts. *Lung India : official organ of Indian Chest Society.* 2016;33(1):1-2.
12. Heaney LG, McGarvey LP. Personalised Medicine for Asthma and Chronic Obstructive Pulmonary Disease. *Respiration; international review of thoracic diseases.* 2017;93(3):153-161.

13. Garth J, Barnes JW, Krick S. Targeting Cytokines as Evolving Treatment Strategies in Chronic Inflammatory Airway Diseases. *International journal of molecular sciences.* 2018;19(11).
14. Bousquet J, Dahl R, Khaltaev N. Global Alliance against Chronic Respiratory Diseases. *The European respiratory journal.* 2007;29(2):233-239.
15. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet (London, England).* 2017;390(10100):1151-1210.
16. Lopez-Campos JL, Tan W, Soriano JB. Global burden of COPD. *Respirology (Carlton, Vic.).* 2016;21(1):14-23.
17. Singh D, Agusti A, Anzueto A, et al. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease: the GOLD science committee report 2019. *The European respiratory journal.* 2019;53(5).
18. Fermont JM, Masconi KL, Jensen MT, et al. Biomarkers and clinical outcomes in COPD: a systematic review and meta-analysis. *Thorax.* 2019;74(5):439-446.
19. Sin DD, Hollander Z, DeMarco ML, McManus BM, Ng RT. Biomarker Development for Chronic Obstructive Pulmonary Disease. From Discovery to Clinical Implementation. *American journal of respiratory and critical care medicine.* 2015;192(10):1162-1170.
20. Shaw JG, Vaughan A, Dent AG, et al. Biomarkers of progression of chronic obstructive pulmonary disease (COPD). *J Thorac Dis.* 2014;6(11):1532-1547.
21. Global strategy for the prevention, diagnosis and management of chronic obstructive lung disease (2020 Report). . *Global initiative for chronic obstructive lung disease (GOLD).* 2020; Available from: https://goldcopd.org./.
22. Soler-Cataluña JJ, Martínez-García MA, Román Sánchez P, Salcedo E, Navarro M, Ochando R. Severe acute exacerbations and mortality in patients with chronic obstructive pulmonary disease. *Thorax.* 2005;60(11):925-931.
23. Hurst JR, Skolnik N, Hansen GJ, et al. Understanding the impact of chronic obstructive pulmonary disease exacerbations on patient health and quality of life. *Eur J Intern Med.* 2020:S0953-6205(0919)30443-30441.
24. Kurmani S, Squire I. Acute Heart Failure: Definition, Classification and Epidemiology. *Curr Heart Fail Rep.* 2017;14(5):385-392.
25. Mosterd A, Hoes AW. Clinical epidemiology of heart failure. *Heart (British Cardiac Society).* 2007;93(9):1137-1146.
26. Ponikowski P, Voors AA, Anker SD, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *European heart journal.* 2016;37(27):2129-2200.
27. Triposkiadis F, Giamouzis G, Parissis J, et al. Reframing the association and significance of co-morbidities in heart failure. *European journal of heart failure.* 2016;18(7):744-758.

28. Gheorghiade M, Zannad F, Sopko G, et al. Acute heart failure syndromes: current state and framework for future research. *Circulation.* 2005;112(25):3958-3968.
29. Hunter BR, Martindale J, Abdel-Hafez O, Pang PS. Approach to Acute Heart Failure in the Emergency Department. *Prog Cardiovasc Dis.* 2017;60(2):178-186.
30. Masa JF, Pépin J-L, Borel J-C, Mokhlesi B, Murphy PB, Sánchez-Quiroga MÁ. Obesity hypoventilation syndrome. *Eur Respir Rev.* 2019;28(151):180097.
31. Jones SF, Brito V, Ghamande S. Obesity hypoventilation syndrome in the critically ill. *Critical care clinics.* 2015;31(3):419-434.
32. Piper AJ, BaHammam AS, Javaheri S. Obesity Hypoventilation Syndrome: Choosing the Appropriate Treatment of a Heterogeneous Disorder. *Sleep Med Clin.* 2017;12(4):587-596.
33. Scarpazza P, Incorvaia C, di Franco G, et al. Effect of noninvasive mechanical ventilation in elderly patients with hypercapnic acute-on-chronic respiratory failure and a do-not-intubate order. *International journal of chronic obstructive pulmonary disease.* 2008;3(4):797-801.
34. Bello G, De Pascale G, Antonelli M. Noninvasive Ventilation. *Clinics in chest medicine.* 2016;37(4):711-721.
35. Liesching T, Kwok H, Hill NS. Acute applications of noninvasive positive pressure ventilation. *Chest.* 2003;124(2):699-713.
36. Mas A, Masip J. Noninvasive ventilation in acute respiratory failure. *International journal of chronic obstructive pulmonary disease.* 2014;9:837-852.
37. Rochwerg B, Brochard L, Elliott MW, et al. Official ERS/ATS clinical practice guidelines: noninvasive ventilation for acute respiratory failure. *The European respiratory journal.* 2017;50(2):1602426.
38. Brochard L, Mancebo J, Wysocki M, et al. Noninvasive ventilation for acute exacerbations of chronic obstructive pulmonary disease. *The New England journal of medicine.* 1995;333(13):817-822.
39. Celikel T, Sungur M, Ceyhan B, Karakurt S. Comparison of noninvasive positive pressure ventilation with standard medical therapy in hypercapnic acute respiratory failure. *Chest.* 1998;114(6):1636-1642.
40. Roberts CM, Brown JL, Reinhardt AK, et al. Non-invasive ventilation in chronic obstructive pulmonary disease: management of acute type 2 respiratory failure. *Clinical medicine (London, England).* 2008;8(5):517-521.
41. McCurdy BR. Noninvasive positive pressure ventilation for acute respiratory failure patients with chronic obstructive pulmonary disease (COPD): an evidence-based analysis. *Ontario health technology assessment series.* 2012;12(8):1-102.
42. Plant PK, Owen JL, Elliott MW. Early use of non-invasive ventilation for acute exacerbations of chronic obstructive pulmonary disease on general respiratory wards: a multicentre randomised controlled trial. *Lancet (London, England).* 2000;355(9219):1931-1935.
43. Cabrini L, Landoni G, Oriani A, et al. Noninvasive ventilation and survival in acute care settings: a comprehensive systematic review and metaanalysis of randomized controlled trials. *Critical care medicine.* 2015;43(4):880-888.

44. Lightowler JV, Wedzicha JA, Elliott MW, Ram FS. Non-invasive positive pressure ventilation to treat respiratory failure resulting from exacerbations of chronic obstructive pulmonary disease: Cochrane systematic review and meta-analysis. *BMJ (Clinical research ed.).* 2003;326(7382):185.
45. Plant PK, Owen JL, Elliott MW. Non-invasive ventilation in acute exacerbations of chronic obstructive pulmonary disease: long term survival and predictors of in-hospital outcome. *Thorax.* 2001;56(9):708-712.
46. Budweiser S, Jorres RA, Pfeifer M. Treatment of respiratory failure in COPD. *International journal of chronic obstructive pulmonary disease.* 2008;3(4):605-618.
47. Ambrosino N, Vagheggini G. Non-invasive ventilation in exacerbations of COPD. *International journal of chronic obstructive pulmonary disease.* 2007;2(4):471-476.
48. Confalonieri M, Garuti G, Cattaruzza MS, et al. A chart of failure risk for noninvasive ventilation in patients with COPD exacerbation. *The European respiratory journal.* 2005;25(2):348-355.
49. Titlestad IL, Lassen AT, Vestbo J. Long-term survival for COPD patients receiving noninvasive ventilation for acute respiratory failure. *International journal of chronic obstructive pulmonary disease.* 2013;8:215-219.
50. Roberts CM, Stone RA, Buckingham RJ, Pursey NA, Lowe D. Acidosis, non-invasive ventilation and mortality in hospitalised COPD exacerbations. *Thorax.* 2011;66(1):43-48.
51. Hedsund C, Ankjærgaard KL, Rasmussen DB, et al. NIV for acute respiratory failure in COPD: high in-hospital mortality is determined by patient selection. *Eur Clin Respir J.* 2019;6(1):1571332-1571332.
52. Osadnik CR, Tee VS, Carson-Chahhoud KV, Picot J, Wedzicha JA, Smith BJ. Non-invasive ventilation for the management of acute hypercapnic respiratory failure due to exacerbation of chronic obstructive pulmonary disease. *The Cochrane database of systematic reviews.* 2017;7:Cd004104.
53. Vital FM, Ladeira MT, Atallah AN. Non-invasive positive pressure ventilation (CPAP or bilevel NPPV) for cardiogenic pulmonary oedema. *The Cochrane database of systematic reviews.* 2013(5):Cd005351.
54. Berg KM, Clardy P, Donnino MW. Noninvasive ventilation for acute respiratory failure: a review of the literature and current guidelines. *Intern Emerg Med.* 2012;7(6):539-545.
55. Carrillo A, Ferrer M, Gonzalez-Diaz G, et al. Noninvasive ventilation in acute hypercapnic respiratory failure caused by obesity hypoventilation syndrome and chronic obstructive pulmonary disease. *American journal of respiratory and critical care medicine.* 2012;186(12):1279-1285.
56. Lemyze M, Taufour P, Duhamel A, et al. Determinants of noninvasive ventilation success or failure in morbidly obese patients in acute respiratory failure. *PloS one.* 2014;9(5):e97563.
57. Confalonieri M, Potena A, Carbone G, Porta RD, Tolley EA, Umberto Meduri G. Acute respiratory failure in patients with severe community-acquired pneumonia. A prospective randomized evaluation of noninvasive ventilation. *American journal of respiratory and critical care medicine.* 1999;160(5 Pt 1):1585-1591.

58. Bello G, De Pascale G, Antonelli M. Noninvasive ventilation for the immunocompromised patient: always appropriate? *Current opinion in critical care.* 2012;18(1):54-60.
59. Schnell D, Timsit JF, Darmon M, et al. Noninvasive mechanical ventilation in acute respiratory failure: trends in use and outcomes. *Intensive care medicine.* 2014;40(4):582-591.
60. Garpestad E, Brennan J, Hill NS. Noninvasive ventilation for critical care. *Chest.* 2007;132(2):711-720.
61. Gifford AH. Noninvasive ventilation as a palliative measure. *Current opinion in supportive and palliative care.* 2014;8(3):218-224.
62. Califf RM. Biomarker definitions and their applications. *Exp Biol Med (Maywood).* 2018;243(3):213-221.
63. Ciccone MM, Cortese F, Gesualdo M, et al. A novel cardiac bio-marker: ST2: a review. *Molecules (Basel, Switzerland).* 2013;18(12):15314-15328.
64. Hollander Z, DeMarco ML, Sadatsafavi M, McManus BM, Ng RT, Sin DD. Biomarker Development in COPD: Moving From P Values to Products to Impact Patient Care. *Chest.* 2017;151(2):455-467.
65. Burke HB. Predicting Clinical Outcomes Using Molecular Biomarkers. *Biomark Cancer.* 2016;8:89-99.
66. Ghashghaei R, Arbit B, Maisel AS. Current and novel biomarkers in heart failure: bench to bedside. *Current opinion in cardiology.* 2016;31(2):191-195.
67. Mueller T, Jaffe AS. Soluble ST2--analytical considerations. *The American journal of cardiology.* 2015;115(7 Suppl):8b-21b.
68. Mueller T, Dieplinger B. Soluble ST2 and Galectin-3: What We Know and Don't Know Analytically. *Ejifcc.* 2016;27(3):224-237.
69. Aleksova A, Paldino A, Beltrami AP, et al. Cardiac Biomarkers in the Emergency Department: The Role of Soluble ST2 (sST2) in Acute Heart Failure and Acute Coronary Syndrome-There is Meat on the Bone. *Journal of clinical medicine.* 2019;8(2).
70. Chen LQ, de Lemos JA, Das SR, Ayers CR, Rohatgi A. Soluble ST2 is associated with all-cause and cardiovascular mortality in a population-based cohort: the Dallas Heart Study. *Clinical chemistry.* 2013;59(3):536-546.
71. Wang TJ, Wollert KC, Larson MG, et al. Prognostic utility of novel biomarkers of cardiovascular stress: the Framingham Heart Study. *Circulation.* 2012;126(13):1596-1604.
72. Hughes MF, Appelbaum S, Havulinna AS, et al. ST2 may not be a useful predictor for incident cardiovascular events, heart failure and mortality. *Heart (British Cardiac Society).* 2014;100(21):1715-1721.
73. Ho JE, Sritara P, deFilippi CR, Wang TJ. Soluble ST2 testing in the general population. *The American journal of cardiology.* 2015;115(7 Suppl):22b-25b.
74. Mueller T, Leitner I, Egger M, Haltmayer M, Dieplinger B. Association of the biomarkers soluble ST2, galectin-3 and growth-differentiation factor-15 with heart

failure and other non-cardiac diseases. *Clinica chimica acta; international journal of clinical chemistry.* 2015;445:155-160.

75. Miller AM, Purves D, McConnachie A, et al. Soluble ST2 associates with diabetes but not established cardiovascular risk factors: a new inflammatory pathway of relevance to diabetes? *PloS one.* 2012;7(10):e47830.
76. Zhao J, Zhao Y. Interleukin-33 and its Receptor in Pulmonary Inflammatory Diseases. *Critical reviews in immunology.* 2015;35(6):451-461.
77. Sanada S, Hakuno D, Higgins LJ, Schreiter ER, McKenzie AN, Lee RT. IL-33 and ST2 comprise a critical biomechanically induced and cardioprotective signaling system. *The Journal of clinical investigation.* 2007;117(6):1538-1549.
78. Xia J, Zhao J, Shang J, et al. Increased IL-33 expression in chronic obstructive pulmonary disease. *American journal of physiology. Lung cellular and molecular physiology.* 2015;308(7):L619-627.
79. Kim SW, Rhee CK, Kim KU, et al. Factors associated with plasma IL-33 levels in patients with chronic obstructive pulmonary disease. *International journal of chronic obstructive pulmonary disease.* 2017;12:395-402.
80. Sabatine MS, Morrow DA, Higgins LJ, et al. Complementary roles for biomarkers of biomechanical strain ST2 and N-terminal prohormone B-type natriuretic peptide in patients with ST-elevation myocardial infarction. *Circulation.* 2008;117(15):1936-1944.
81. Wang J, Tan GJ, Han LN, Bai YY, He M, Liu HB. Novel biomarkers for cardiovascular risk prediction. *Journal of geriatric cardiology : JGC.* 2017;14(2):135-150.
82. Mallick A, Januzzi JL, Jr. Biomarkers in acute heart failure. *Revista espanola de cardiologia (English ed.).* 2015;68(6):514-525.
83. Marino R, Magrini L, Orsini F, et al. Comparison Between Soluble ST2 and High-Sensitivity Troponin I in Predicting Short-Term Mortality for Patients Presenting to the Emergency Department With Chest Pain. *Annals of laboratory medicine.* 2017;37(2):137-146.
84. Maisel AS, Richards AM, Pascual-Figal D, Mueller C. Serial ST2 testing in hospitalized patients with acute heart failure. *The American journal of cardiology.* 2015;115(7 Suppl):32B-37B.
85. Dieplinger B, Gegenhuber A, Kaar G, Poelz W, Haltmayer M, Mueller T. Prognostic value of established and novel biomarkers in patients with shortness of breath attending an emergency department. *Clinical biochemistry.* 2010;43(9):714-719.
86. Socrates T, deFilippi C, Reichlin T, et al. Interleukin family member ST2 and mortality in acute dyspnoea. *Journal of internal medicine.* 2010;268(5):493-500.
87. Januzzi JL, Jr., Peacock WF, Maisel AS, et al. Measurement of the interleukin family member ST2 in patients with acute dyspnea: results from the PRIDE (Pro-Brain Natriuretic Peptide Investigation of Dyspnea in the Emergency Department) study. *Journal of the American College of Cardiology.* 2007;50(7):607-613.
88. Martinez-Rumayor A, Camargo CA, Green SM, Baggish AL, O'Donoghue M, Januzzi JL. Soluble ST2 plasma concentrations predict 1-year mortality in acutely

dyspneic emergency department patients with pulmonary disease. *American journal of clinical pathology.* 2008;130(4):578-584.

89. Januzzi JL, Jr., Rehman S, Mueller T, van Kimmenade RR, Lloyd-Jones DM. Importance of biomarkers for long-term mortality prediction in acutely dyspneic patients. *Clinical chemistry.* 2010;56(12):1814-1821.
90. Januzzi JL, Mebazaa A, Di Somma S. ST2 and prognosis in acutely decompensated heart failure: the International ST2 Consensus Panel. *The American journal of cardiology.* 2015;115(7 Suppl):26B-31B.
91. Bajwa EK, Mebazaa A, Januzzi JL. ST2 in Pulmonary Disease. *The American journal of cardiology.* 2015;115(7 Suppl):44b-47b.
92. Tajima S, Oshikawa K, Tominaga S, Sugiyama Y. The increase in serum soluble ST2 protein upon acute exacerbation of idiopathic pulmonary fibrosis. *Chest.* 2003;124(4):1206-1214.
93. Hoogerwerf JJ, Tanck MW, van Zoelen MA, Wittebole X, Laterre PF, van der Poll T. Soluble ST2 plasma concentrations predict mortality in severe sepsis. *Intensive care medicine.* 2010;36(4):630-637.
94. Moreno Velasquez I, Gajulapuri A, Leander K, Berglund A, de Faire U, Gigante B. Serum IL8 is not associated with cardiovascular events but with all-cause mortality. *BMC cardiovascular disorders.* 2019;19(1):34.
95. Bhowmik A, Seemungal TA, Sapsford RJ, Wedzicha JA. Relation of sputum inflammatory markers to symptoms and lung function changes in COPD exacerbations. *Thorax.* 2000;55(2):114-120.
96. Heidemann J, Ogawa H, Dwinell MB, et al. Angiogenic effects of interleukin 8 (CXCL8) in human intestinal microvascular endothelial cells are mediated by CXCR2. *The Journal of biological chemistry.* 2003;278(10):8508-8515.
97. Moreno Velasquez I, Arnlov J, Leander K, Lind L, Gigante B, Carlsson AC. Interleukin-8 is associated with increased total mortality in women but not in men-findings from a community-based cohort of elderly. *Annals of medicine.* 2015;47(1):28-33.
98. Lippitz BE. Cytokine patterns in patients with cancer: a systematic review. *The Lancet. Oncology.* 2013;14(6):e218-228.
99. Bartekova M, Radosinska J, Jelemensky M, Dhalla NS. Role of cytokines and inflammation in heart function during health and disease. *Heart failure reviews.* 2018;23(5):733-758.
100. Su B, Liu T, Fan H, et al. Inflammatory Markers and the Risk of Chronic Obstructive Pulmonary Disease: A Systematic Review and Meta-Analysis. *PloS one.* 2016;11(4):e0150586.
101. Mocan M, Mocan Hognogi LD, Anton FP, et al. Biomarkers of Inflammation in Left Ventricular Diastolic Dysfunction. *Disease markers.* 2019;2019:7583690.
102. Nymo SH, Aukrust P, Kjekshus J, et al. Limited Added Value of Circulating Inflammatory Biomarkers in Chronic Heart Failure. *JACC. Heart failure.* 2017;5(4):256-264.

103. Zhang J, Bai C. The Significance of Serum Interleukin-8 in Acute Exacerbations of Chronic Obstructive Pulmonary Disease. *Tanaffos.* 2018;17(1):13-21.
104. Koutsokera A, Kostikas K, Nicod LP, Fitting JW. Pulmonary biomarkers in COPD exacerbations: a systematic review. *Respiratory research.* 2013;14:111.
105. Wu H, Yang S, Wu X, et al. Interleukin-33/ST2 signaling promotes production of interleukin-6 and interleukin-8 in systemic inflammation in cigarette smoke-induced chronic obstructive pulmonary disease mice. *Biochemical and biophysical research communications.* 2014;450(1):110-116.
106. Agustí A, Edwards LD, Rennard SI, et al. Persistent systemic inflammation is associated with poor clinical outcomes in COPD: a novel phenotype. *PloS one.* 2012;7(5):e37483-e37483.
107. Metinko AP, Kunkel SL, Standiford TJ, Strieter RM. Anoxia-hyperoxia induces monocyte-derived interleukin-8. *The Journal of clinical investigation.* 1992;90(3):791-798.
108. Shafiek HA, Abd-Elwahab NH, Baddour MM, El-Hoffy MM, Degady AA-E, Khalil YM. Assessment of some inflammatory biomarkers as predictors of outcome of acute respiratory failure on top of chronic obstructive pulmonary disease and evaluation of the role of bacteria. *ISRN Microbiol.* 2012;2012:240841-240841.
109. Bootcov MR, Bauskin AR, Valenzuela SM, et al. MIC-1, a novel macrophage inhibitory cytokine, is a divergent member of the TGF-beta superfamily. *Proceedings of the National Academy of Sciences of the United States of America.* 1997;94(21):11514-11519.
110. Wollert KC, Kempf T, Wallentin L. Growth Differentiation Factor 15 as a Biomarker in Cardiovascular Disease. *Clinical chemistry.* 2017;63(1):140-151.
111. Verhamme FM, Freeman CM, Brusselle GG, Bracke KR, Curtis JL. GDF-15 in Pulmonary and Critical Care Medicine. *American journal of respiratory cell and molecular biology.* 2019;60(6):621-628.
112. Wiklund FE, Bennet AM, Magnusson PK, et al. Macrophage inhibitory cytokine-1 (MIC-1/GDF15): a new marker of all-cause mortality. *Aging cell.* 2010;9(6):1057-1064.
113. Ding Q, Mracek T, Gonzalez-Muniesa P, et al. Identification of macrophage inhibitory cytokine-1 in adipose tissue and its secretion as an adipokine by human adipocytes. *Endocrinology.* 2009;150(4):1688-1696.
114. Adela R, Banerjee SK. GDF-15 as a Target and Biomarker for Diabetes and Cardiovascular Diseases: A Translational Prospective. *Journal of diabetes research.* 2015;2015:490842.
115. Corre J, Hebraud B, Bourin P. Concise review: growth differentiation factor 15 in pathology: a clinical role? *Stem cells translational medicine.* 2013;2(12):946-952.
116. Ho JE, Mahajan A, Chen MH, et al. Clinical and genetic correlates of growth differentiation factor 15 in the community. *Clinical chemistry.* 2012;58(11):1582-1591.
117. Gopal DM, Larson MG, Januzzi JL, et al. Biomarkers of cardiovascular stress and subclinical atherosclerosis in the community. *Clinical chemistry.* 2014;60(11):1402-1408.

118. Ho JE, Hwang SJ, Wollert KC, et al. Biomarkers of cardiovascular stress and incident chronic kidney disease. *Clinical chemistry.* 2013;59(11):1613-1620.
119. Xanthakis V, Larson MG, Wollert KC, et al. Association of novel biomarkers of cardiovascular stress with left ventricular hypertrophy and dysfunction: implications for screening. *Journal of the American Heart Association.* 2013;2(6):e000399.
120. Lind L, Wallentin L, Kempf T, et al. Growth-differentiation factor-15 is an independent marker of cardiovascular dysfunction and disease in the elderly: results from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) Study. *European heart journal.* 2009;30(19):2346-2353.
121. Rohatgi A, Patel P, Das SR, et al. Association of growth differentiation factor-15 with coronary atherosclerosis and mortality in a young, multiethnic population: observations from the Dallas Heart Study. *Clinical chemistry.* 2012;58(1):172-182.
122. Bao X, Borne Y, Muhammad IF, et al. Growth differentiation factor 15 is positively associated with incidence of diabetes mellitus: the Malmo Diet and Cancer-Cardiovascular Cohort. *Diabetologia.* 2019;62(1):78-86.
123. Husebo GR, Gronseth R, Lerner L, et al. Growth differentiation factor-15 is a predictor of important disease outcomes in patients with COPD. *The European respiratory journal.* 2017;49(3).
124. Mutlu LC, Altintas N, Aydin M, et al. Growth Differentiation Factor-15 Is a Novel Biomarker Predicting Acute Exacerbation of Chronic Obstructive Pulmonary Disease. *Inflammation.* 2015;38(5):1805-1813.
125. Kim M, Cha SI, Choi KJ, et al. Prognostic value of serum growth differentiation factor-15 in patients with chronic obstructive pulmonary disease exacerbation. *Tuberculosis and respiratory diseases.* 2014;77(6):243-250.
126. Widgren BR, Jourak M. Medical Emergency Triage and Treatment System (METTS): a new protocol in primary triage and secondary priority decision in emergency medicine. *The Journal of emergency medicine.* 2011;40(6):623-628.
127. Berglund G, Elmstahl S, Janzon L, Larsson SA. The Malmo Diet and Cancer Study. Design and feasibility. *Journal of internal medicine.* 1993;233(1):45-51.
128. Manjer J, Carlsson S, Elmståhl S, et al. The Malmö Diet and Cancer Study: representativity, cancer incidence and mortality in participants and non-participants. *Eur J Cancer Prev.* 2001;10(6):489-499.
129. Rosvall M, Janzon L, Berglund G, Engström G, Hedblad B. Incident coronary events and case fatality in relation to common carotid intima-media thickness. *Journal of internal medicine.* 2005;257(5):430-437.
130. Brooke HL, Talbäck M, Hörnblad J, et al. The Swedish cause of death register. *European journal of epidemiology.* 2017;32(9):765-773.
131. Inghammar M, Engstrom G, Lofdahl CG, Egesten A. Validation of a COPD diagnosis from the Swedish Inpatient Registry. *Scandinavian journal of public health.* 2012;40(8):773-776.
132. Lundberg M, Eriksson A, Tran B, Assarsson E, Fredriksson S. Homogeneous antibody-based proximity extension assays provide sensitive and specific detection of low-abundant proteins in human blood. *Nucleic acids research.* 2011;39(15):e102.

133. Proseek Multiplex: A Precision proteomics solution for targeted protein biomarker discovery. . [Webpage]. https://www.olink.com/data-you-can-trust/technology/. Accessed 18 april, 2018.
134. Presage® ST2 Assay. [Webpage]. 2018; http://www.sopachem.com/diagnostics/portfolio/presage-st2-assay/. Accessed 18 april, 2018.
135. Rabec C, Rodenstein D, Leger P, et al. Ventilator modes and settings during non-invasive ventilation: effects on respiratory events and implications for their identification. *Thorax.* 2011;66(2):170-178.
136. Wiklund K, Gransbo K, Lund N, et al. Inflammatory biomarkers predicting prognosis in patients with acute dyspnea. *The American journal of emergency medicine.* 2016;34(3):370-374.
137. Januzzi JL, Jr. ST2 as a cardiovascular risk biomarker: from the bench to the bedside. *Journal of cardiovascular translational research.* 2013;6(4):493-500.
138. Morgan AD, Zakeri R, Quint JK. Defining the relationship between COPD and CVD: what are the implications for clinical practice? *Ther Adv Respir Dis.* 2018;12:1753465817750524-1753465817750524.
139. Tang WH, Wu Y, Grodin JL, et al. Prognostic Value of Baseline and Changes in Circulating Soluble ST2 Levels and the Effects of Nesiritide in Acute Decompensated Heart Failure. *JACC. Heart failure.* 2016;4(1):68-77.
140. Januzzi JL, Pascual-Figal D, Daniels LB. ST2 testing for chronic heart failure therapy monitoring: the International ST2 Consensus Panel. *The American journal of cardiology.* 2015;115(7 Suppl):70B-75B.
141. Plant PK, Owen JL, Parrott S, Elliott MW. Cost effectiveness of ward based non-invasive ventilation for acute exacerbations of chronic obstructive pulmonary disease: economic analysis of randomised controlled trial. *BMJ (Clinical research ed.).* 2003;326(7396):956.
142. Johansson LA, Björkenstam C, Westerling R. Unexplained differences between hospital and mortality data indicated mistakes in death certification: an investigation of 1,094 deaths in Sweden during 1995. *J Clin Epidemiol.* 2009;62(11):1202-1209.
143. Sundh J, Janson C, Lisspers K, Montgomery S, Ställberg B. Clinical COPD Questionnaire score (CCQ) and mortality. *International journal of chronic obstructive pulmonary disease.* 2012;7:833-842.
144. Sarzi-Puttini P, Giorgi V, Sirotti S, et al. COVID-19, cytokines and immunosuppression: what can we learn from severe acute respiratory syndrome? *Clinical and experimental rheumatology.* 2020;38(2):337-342.

Printed by Media-Tryck, Lund 2020 NORDIC SWAN ECOLABEL 3041 0903

"I wish it need not have happened in my time," said Frodo. "So do I," said Gandalf, "and so do all who live to see such times. But that is not for them to decide. All we have to decide is what to do with the time that is given us."

J.R.R. Tolkien – The Fellowship of The Ring

FACULTY OF MEDICINE

Department of Clinical Sciences, Malmö

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2020:69
ISBN 978-91-7619-930-5
ISSN 1652-8220