Akershus Cardiac Examination (ACE) 4 Study: Pragmatic randomized controlled trial of early biomarker measurements and structured feedback in unselected patients with tachypnea

Protocol Identification Number: ACE 4 Study

ClinicalTrials.gov Identifier: NCT05699564

SPONSOR:

Akershus University Hospital
Division of Medicine
1478 Lørenskog, Norway
Tel : +47 02900

PRINCIPAL INVESTIGATOR:

Associate Professor Magnus Lyngbakken, MD PhD
Department of Cardiology, Division of Medicine
Akershus University Hospital
Sykehusveien 25
1478 Lørenskog, Norway
Tel: +47 93 40 88 37
E-mail: magnus.lyngbakken@medisin.uio.no

PROTOCOL VERSION NO. 3

CONTACT DETAILS

Sponsor: **Akershus University Hospital**
Division of Medicine
1478 Lørenskog, Norway
Tel: +47 67960000

Core Study Group

Principal Investigator: **Associate Professor Magnus Lyngbakken, MD PhD**
Division of Medicine
Akershus University Hospital
Sykehusveien 25
1478 Lørenskog, Norway
Tel: +47 93 40 88 37
E-mail: magnus.lyngbakken@medisin.uio.no

Co-Principal Investigator: **Professor Helge Røsjø, MD PhD**
Division of Research and Innovation
Akershus University Hospital
Sykehusveien 25
1478 Lørenskog, Norway
Tel:+47 91 54 58 64
E-mail: helge.rosjo@medisin.uio.no

Investigator: **Rahul Bhatnagar, MD**
Department of Cardiology, Division of Medicine
Akershus University Hospital
Sykehusveien 25
1478 Lørenskog, Norway
Tel: +47 41 21 83 49
E-mail: rahul.bhatnagar@studmed.uio.no

Investigator: **Kristian Berge, MD**
Department of Cardiology, Division of Medicine
Akershus University Hospital
Sykehusveien 25
1478 Lørenskog, Norway
Tel: +47 41 28 31 28
E-mail: kristian.berge@gmail.com

Investigator: **Professor Henrik Schirmer, MD PhD**
Department of Cardiology, Division of Medicine
Akershus University Hospital
Sykehusveien 25
1478 Lørenskog, Norway
Tel: +47 992 64 338
E-mail: henrik.schirmer@medisin.uio.no

Investigator: **Torbjørn Wisløff, MSc PhD**
Division of Research and Innovation,
Akershus University Hospital
Sykehusveien 25
1478 Lørenskog, Norway
Tel: +47 928 67 976
E-mail: torbjorn.wisloff@ahus.no

Investigators

Investigator: **Professor Torbjørn Omland, MD PhD**
Department of Cardiology, Division of Medicine
Akershus University Hospital
Sykehusveien 25
1478 Lørenskog, Norway
Tel: +47 401 07 050
E-mail: torbjorn.omland@medisin.uio.no

Investigator: **Lars Gunnar Klæbo, MD, PhD**
Department of Cardiology, Division of Medicine
Akershus University Hospital
Sykehusveien 25
1478 Lørenskog, Norway
Tel: +47 975 43 045
E-mail: lars.gunnar.klabo@ahus.uio.no

Investigator: **Associate Professor Gunnar Einvik MD, PhD**
Department of Pulmonary Medicine, Division of Medicine
Akershus University Hospital
Sykehusveien 25
1478 Lørenskog, Norway
Tel: +47 411 04 542
E-mail: gunnar.einvik@medisin.uio.no

Investigator: **Professor Stefan James, MD, PhD**
Uppsala Clinical Research Center,
Uppsala, Sweden
Tel: +46 70 594 44 04
E-mail: stefan.james@ucr.uu.se

SPONSOR SIGNATURE PAGE

Title:	Akershus Cardiac Examination (ACE) 4 Study: Pragmatic randomized controlled trial of early biomarker measurements and structured feedback in unselected patients with tachypnea
Protocol ID no:	ACE 4 Study
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Sponsor signatory approval

Irene Grundvold
Chief of Cardiology
Akershus University Hospital

Signature *Date*

I hereby declare that this Protocol has been developed in compliance with ICH GCP and the applicable regulatory requirements:

Magnus Lyngbakken
PI
MD PhD
Akershus University Hospital

Signature *Date*

PI signatory approval

I hereby declare that I will conduct the study in compliance with the Protocol, ICH GCP, and the applicable regulatory requirements:

Name:
Title:

PI signature *Date*

PROTOCOL SYNOPSIS

Intervention	Early biomarker measurements and structured feedback in patient's electronic medical records
Protocol no.	ACE 4 Study
Study phase	4
Study title	Akershus Cardiac Examination (ACE) 4 Study: Pragmatic randomized controlled trial of early biomarker measurements and structured feedback in unselected patients with tachypnea
Sponsor	Department of Cardiology, Division of Medicine, Akershus University Hospital, Norway
Responsible contact persons	Associate Professor Magnus Lyngbakken, MD PhD Division of Medicine Akershus University Hospital Sykehusveien 25 1478 Lørenskog, Norway Tel: +47 93 40 88 37 E-mail: magnus.lyngbakken@medisin.uio.no and Professor Helge Røsjø Division of Research and Innovation Akershus University Hospital Sykehusveien 25 1478 Lørenskog, Norway Tel: +47 91 54 58 64 E-mail: helge.rosjo@medisin.uio.no
Funding source(s)	Akershus University Hospital, University of Oslo, South-Eastern Norway Regional Health Authority
Study center	Akershus University Hospital, Lørenskog
Planned number of patients	Maximum 600 patients
Timelines	Estimated study start (first patient first visit [FPFV]): Feb 1, 2023 Estimated recruitment end (last patient first visit [LPFV]): June 28, 2024 Follow-up period end date (last patient [LP] off study): Dec 31, 2038 End of study: Sep 1, 2050
Background and rationale	Patients hospitalized with tachypnea, defined as respiratory rate ≥20/ min, have substantial mortality and may suffer from different conditions, including acute heart failure (HF). Symptoms of HF can be difficult to identify and ~15% of patients with HF will not be correctly diagnosed by the treating physician in the Emergency Department (ED). Biomarkers like B-type natriuretic peptides (BNPs) and cardiac troponins improve diagnostic accuracy and risk stratification. Whether early, structured biomarker assessment and structured feedback in the patient's electronic health records (EHR) improve management and outcomes among unselected patients with tachypnea have previously not been explored in a randomized controlled trial.

Study objectives	<u>Primary endpoint</u> To determine whether early structured biomarker assessment in unselected patients with tachypnea extends the time to the first event for either *(1)* all-cause readmission or *(2)* all-cause mortality; i.e. time to the combined endpoint, compared to the current strategy/ standard care <u>Secondary endpoints</u> • To estimate the difference in hospital length of stay during the index hospitalization for strategy with early structured biomarker assessment compared to current strategy/ standard care in unselected patients with tachypnea • To estimate the difference in length of stay in Intensive Care Unit/Medical Intensive Care Unit/ Cardiac Intensive Care Unit during the index hospitalization for strategy with early structured biomarker assessment compared to current strategy/ standard care in unselected patients with tachypnea • To estimate the difference in 30-day all-cause readmission for strategy with early structured biomarker assessment compared to current strategy/ standard care in unselected patients with tachypnea • To compare time to first all-cause readmission during follow-up for strategy with early structured biomarker assessment compared to current strategy/ standard care in unselected patients with tachypnea • To compare total number of all-cause readmissions during follow-up for strategy with early structured biomarker assessment compared to current strategy/ standard care in unselected patients with tachypnea • To estimate the difference in all-cause mortality during follow-up for strategy with early structured biomarker assessment compared to current strategy/ standard care in unselected patients with tachypnea • To estimate the difference in total cost for strategy with early structured biomarker assessment compared to current strategy/ standard care in unselected patients with tachypnea • To estimate the difference in all-cause mortality during the index hospitalization for strategy with early structured biomarker assessment compared to current strategy/ standard care in unselected patients with tachypnea • To estimate the difference in the cardiac troponin T and/or I and B-type natriuretic peptide and/or N-terminal pro-B-type natriuretic peptide concentrations from hospital admission to discharge for strategy with early structured biomarker assessment compared to current strategy/ standard care in unselected patients with tachypnea • To estimate the difference in guideline-defined medical therapy for HF, as defined by international guidelines, at discharge for strategy with early structured biomarker assessment compared to current strategy/ standard care in patients adjudicated with HF diagnosis and categorized into HF with reduced or mildly reduced ejection fraction (HFrEF/ HFmrEF) and HF with preserved ejection fraction (HFpEF) • To estimate cost-utility for the strategy with early structured biomarker assessment compared to current strategy/ standard care in unselected patients with tachypnea Total follow-up is permitted until 2038. We will assess the primary endpoint after 12 month follow-up from randomization; i.e. patients are followed-up either for 12 months or to the time of death (if this happens before 12 months from randomization). We will also report data for extended follow-up after 3 years, 5 years, and 10 years for the primary outcome.

	For secondary endpoints we will report data after the index hospitalization, after 30 days follow-up, after 1 year, 3 years, 5 years, and 10 years. Cost-utility will be evaluated based on data reported up to and including 12 months, and updated after 5 years. Explorative endpoints: • Assessing primary and secondary outcomes in the subgroup of patients classified as hospitalized due to HF, as assessed by the adjudication committee • Assessing primary and secondary outcomes in HF patients, classified by the adjudication committee, with patients stratified by LVEF 50%; e.g. HFrEF/ HFmrEF vs. HFpEF • Assessing primary and secondary outcomes with patients stratified by biomarker concentrations measured on the first study visit (study inclusion), including B-type natriuretic peptides, cardiac troponins, and additional biomarkers • Assessing accuracy for clinical and biochemical diagnostic scores for diagnosing HFpEF; e.g. the H_2FPEF and HFA-PEFF scores • The ACE 4 Study will phenotype participants with short-term Holter recordings (≤24 h), transthoracic echocardiography (TTE), and biospecimens collection on admission, discharge, and after 6 months for later molecular characterization in all participants and we will compare phenotypic traits with adjudicated diagnosis and clinical outcomes during follow-up
Study design	Single-center, pragmatic randomized controlled trial of a non-pharmacological intervention Screening phase: <24 h of admission to ED. Study phase: All patients will be randomized <24 h of admission to ED Total follow-up is permitted until 2038. We will assess the primary endpoint after 12 month follow-up from randomization; i.e. patients are followed-up either for 12 months or to the time of death (if this happens before 12 months from randomization). We will also report data for extended follow-up after 3 years, 5 years, and 10 years for the primary outcome. For secondary endpoints we will report data after the index hospitalization, after 30 days follow-up, after 1 year, 3 years, 5 years, and 10 years.
Patient population	Inclusion • Patients ≥18 years old • Tachypnea defined as respiratory rate ≥20/min, as noted in the National Early Warning Score (NEWS) 2 classification performed on admission in the Emergency Department and reported in the patient' EHR, which is performed as clinical routine for all admission at Akershus University Hospital • Admission to Departments under the Division of Medicine at Akershus University Hospital, except the Department of Neurology • <24 h from hospital admittance to inclusion in the study • Signed written informed consent during the initial phase of the hospitalization Exclusion • Previously included into the study (in case of patients presenting with a second hospitalization during the study period) • Known or suspected cancer outside of local control, documented in medical

	records, at the time of patient inclusion or diagnosed in relation to the index hospitalization • Neurological condition with short life expectancy; e.g. ALS, documented in medical records during screening prior to study entry • Other non-cardiac disease with life expectancy below 1 year, documented in medical records during screening prior to study entry • Obvious non-cardiac cause for tachypnea based on medical records and clinical findings during screening prior to study entry; e.g. anaphylaxis in young patient with known allergy, dyspnea after direct chest trauma, or young patient with fever and positive Covid-19 test on admission. • Patient assessed as non-Internal Medicine patient; e.g. surgical patient • Patients unwilling or unable to comply with the protocol, including Glasgow Coma Scale <13 on the time of study inclusion • Patients that are intubated for invasive ventilatory therapy before or shortly after hospital admission • History of non-compliance to medical management and patients who are considered potentially unreliable, based on documentation in medical records, during screening prior to study entry • History or evidence of alcohol or drug abuse with the last 12 months, based on medical records and clinical findings during screening prior to study entry, that will influence study participation • Any surgical or medical condition, based on medical records and clinical findings during screening prior to study entry, that will impair the ability of the patient to participate in the study
Visit schedule and assessments	Early biomarker assessment consists of the following examinations: the cardiac biomarkers N-terminal pro-B-type natriuretic peptide (NT-proBNP) and high-sensitivity cardiac troponin T (hs-cTnT), which will be recorded in all patients on hospital admission as part of routine care. In the group randomized to early biomarker assessment, we will use *a priori*-decided cutoffs and a specific biomarker algorithm to characterize patients as rule out, undetermined ("grey zone"), and rule in for HF. In the control group, biomarker concentrations will be provided in the electronic laboratory report without a specific note in the text-based section of the patient' EHR, which is the current standard for reporting laboratory data today at Akershus University Hospital. The patient will need to have signed a written informed consent prior to any study-specific note being included in the patient' EHR. In the group randomized to early biomarker assessment and structured feedback, biomarker-based assessment concerning probability for HF will be entered into the EHR of the individual patient. We will not provide any recommendations relating to diagnostic work up for individual patients. The study participants will receive all examinations considered appropriate by the treating physician, which includes TTE in the control group. Hence, there will be no restrictions to the diagnostic testing of our study participants, regardless of randomization status. If requested by the treating physician, a full TTE will be performed by trained study personnel in both groups. The TTE protocol will assess atrial and ventricular dimensions, systolic and diastolic properties of the left ventricle, right ventricular systolic function, the cardiac valves, pericardial effusion, and estimate arterial pulmonary pressure + look for other signs indicative of pulmonary hypertension. The conclusion of the TTE will be

	recorded in the patient' EHR, according to standard procedure. The decision of the patient to participate or to decline participation into the study will not influence the treatment that is offered to the patient at Akershus University Hospital and all patients, regardless of study participation, will receive current strategy/ standard care treatment. We will not provide any treatment recommendation for individual patients. However, we will include a short paragraph related to information for pharmacological treatment of patients with HFrEF (LVEF≤50%), HFmrEF (LVEF 40-50%), and HF with recovered LVEF according to the European Society of Cardiology (ESC) Heart Failure Guidelines 2021, which is the most recent recommendations for HF patients from the ESC. For such patients we will inform the physician that β-blocker, SGLT2-inhibitor, inhibition of renin-angiotensin with ACE inhibitors or angiotensin-neprilysin inhibition, and aldosterone inhibition are recommended. We will also inform that loop diuretics are recommended for volume control/ decongestion in all patients with HF according to the ESC guidelines. The recent ESC guidelines have no clear pharmacological recommendations for patients with LVEF≥50% (HFpEF), but based on emerging data from randomized clinical trials demonstrating effects from SGLT2-inhibitors across the spectrum of LVEF in HF patients (e.g. DELIVER trial) we will inform the treating physician about SGLT-2-inhibitiors in HFpEF patients. We will also inform the treating physician about aldosterone inhibition, as aldosterone inhibition reduced HF hospitalizations in the TOPCAT trial (secondary endpoint) and reduced the primary endpoint of TOPCAT in post-hoc analysis. Given that treatment of HFpEF seems to be developing with more documentation related to effect by pharmacological therapy on HF hospitalizations and mortality, we will update the information to the treating physicians and include new, ESC-reported recommendations if released during the study inclusion period. The recent US (AHA/ACC/HFSA) guidelines for heart failure (2022) supports this strategy. We will also inform that, according to several ESC guidelines, patients with suspected acute coronary syndromes are recommended to be clinically assessed for coronary artery disease and treated according to current guidelines. We will also, on a general basis, inform the treating physician that patients with atrial fibrillation should receive guideline-recommended treatment, including anti-coagulation and frequency control. We apply for permission to establish a real-time electronic surveillance of all patients hospitalized at the Division of Medicine, Akershus University Hospital that have tachypnea as defined by NEWS2 scoring, which is part of clinical routine. Only dedicated study personnel will have access to this information, which will be presented to the study team as a list that is stored on secure servers. We also apply for permission to check in the EHR of patients with tachypnea whether the patients fulfill inclusion criteria and do not meet exclusion criteria of the ACE 4 Study. After the study team has identified eligible patients, the study team will obtain written, informed consent from the patient. After this, trained study personnel will collect additional information on current and prior medical history by the strategy previously successfully used in the ACE 2 Study. This information will be checked against the EHR of the patient. All participants of the study, regardless of randomization status, will be subjected to blood sampling for biobank (admission and discharge), short-term ECG monitoring (≤24 h), as well as TTE if this has not yet been performed, after clinical stabilization and prior to hospital discharge. The results of short-term ECG monitoring is for research-use only purposes and can be evaluated also after patient discharge. Hence, the results for the short-term ECG monitoring will not be

	communicated back to the patient or the treating physician. Analogously; the discharge echocardiography is for research-use only purposes and will not be communicated back to the treating physician or patient. This discharge echocardiography will be performed only in the situation that no echocardiography was ordered by the treating physician during the index hospitalization. Still, if research personnel identify unrecognized, gross pathology on online analysis while performing the examination, like left ventricular ejection fraction<40%, right ventricular strain indicative of possible pulmonary embolus, or pericardial effusion with risk of cardiac tamponade, this information will be communicated directly to the treating physician and also noted in the patient' EHR. All echocardiographic examinations will later also be examined by off line-analysis (after the patient has been discharged), and these examinations are for research-use only and results will not be communicated back to the patient. To ensure that the selected population for the ACE 4 Study is representative to all patients hospitalized with tachypnea during the same period, we ask permission to collected basic, anonymized information related to the patients eligible for inclusion, but not included into the study (e.g. not enough resources to include all patients on a given day). For these patients we will only collect the basic information age, sex, and hospital length of stay for the index hospitalization, and we will anonymize the data before storage (i.e. not possible to correlate data to individual patients). The index hospitalization for all included patients will be classified by an endpoint committee of two expert physicians working independently, as (1) HF, (2) non-HF, but with myocardial dysfunction, and (3) non-HF and normal LV function. This process and these categories are analogous to the system previously used in the ACE 2 Study and international studies for adjudication committees in such studies. We will invite included patients back for a 6-month outpatient visit to register medication and to collect biospecimen for biobank storage. The rationale for biospecimen collection also after 6 months is to assess serial concentration of biomarker over time, and to correlate changes in biomarker concentration with medication. This 6-month visit is detailed in the signed informed consent for this study, where patients are informed that we can "*contact the patient after 1, 3, and 6 months, 1 year, 2 years, 4 years, 6 years, 8 years, 10 years, 12 years, and 15 years, and also other time points*".
Data management and statistical analysis	This is a single-center, pragmatic randomized controlled trial of early structured biomarker assessment in patients hospitalized to Internal Medicine with tachypnea, defined as respiratory rate ≥20/ min, as noted in the NEWS 2 classification performed on admission in the Emergency Department and reported in the patient' EHR. The data will be summarized with respect to demographic and baseline characteristics, efficacy observations and measurements, and safety observations and measurements. Categorical data will be presented as absolute frequencies and percentages. For continuous data, N, mean±SD or median (quartile 1-3) will be presented. Time to event variables and Kaplan-Meier product-limit estimates will be presented stratified by intervention group. The primary safety and efficacy analyses will be conducted on all patient data at the time of inclusion of the last patient. The final diagnosis of the hospitalization will be established by an adjudication committee with two senior physicians reviewing all information available on the patients, including information on the clinical

	outcome of the patient within 90 days after discharge from the index hospitalization. Financial resource utilization will be performed by collecting information on hospital length of stay and type of hospital ward (ICU/CCU vs. standard), by collecting information on all supplementary tests performed during or in conjunction with the index hospitalization, and data from local, regional and national registries. The primary end-point is whether early structured biomarker assessment in unselected patients with tachypnea extends the time to the first event for either *(1)* all-cause readmission or *(2)* all-cause mortality; i.e. time to the combined endpoint, compared to the current strategy/ standard care. We will assess the primary endpoint after 12 month follow-up from randomization; i.e. patients are followed-up either for 12 months or to the time of death (if this happens before 12 months from randomization). We will also report data for extended follow-up after 3 years, 5 years, and 10 years for the primary outcome. Secondary endpoints relate to all-cause mortality, hospital length of stay, need for ward in ICU/CCU, total number of re-admissions, resource utilization, medical therapy, and changes in biomarker concentrations. For secondary endpoints we will report data after the index hospitalization, after 30 days follow-up, after 1 year, 3 years, 5 years, and 10 years. We will include a Data Safety and Monitoring Board (DSMB), which will meet after 100, 200, and 400 included patients. The DSMB will assess key data like primary and some secondary endpoints in the two groups. The DSMB may then recommend that the study continues or stops based on safety or futility. The ACE 4 Study will have a large prospective epidemiological section related to various types of biomarkers in the total cohort or different subgroups and also to additional molecular biological characterizations. The planned sub-studies relate to the performance of novel cardiovascular biomarkers for diagnostic and prognostic accuracy compared to clinical assessment, established biomarkers, and individual echocardiographic indices. We will also examine ECG variables and echocardiographic parameters and link these variables to diagnosis during the index hospitalization and clinical outcomes. Patient follow-up and adjudication of clinical events during follow-up will be performed by either linking our data to national registries or by reviewing patient medical records or records from other health care providers. We will contact the patients for clinical information (e.g. events) during follow-up and for a physical visit after 6 months. With biospecimen collection also after 6 months, we plan to assess serial changes in biomarker concentration and examine correlation between biomarker concentration and medication. We will also investigate associations between biomarker concentration after 6 months, and between change in biomarker concentration from inclusion to 6 month, and clinical outcomes during follow-up. In studies of biomarkers and prognosis, the patients may also be stratified into subgroups based on the adjudicated diagnosis of the index hospitalization (e.g. acute heart failure, acute exacerbation of chronic obstructive pulmonary disease, bacterial pulmonary infection, etc.) and we will use multivariable statistical models to assess the individual performance of biomarkers/other tests. A health economic evaluation will be performed after completion of the primary endpoint analyses at 12 months. The health economic evaluation will be evaluating costs per quality-adjusted life years, i.e. a cost-utility analysis.

	Statistical power calculation is based on data from the ACE 2 Study, which included 314 patients with similar clinical characteristics as expected for the ACE 4 Study cohort. The power calculation is based on the assumption that approximately 45% of patients hospitalized with tachypnea will be diagnosed with acute HF and that a substantial proportion of the patients with non-HF related dyspnea will exhibit evidence of myocardial dysfunction; in total 65% of the ACE 2 cohort was classified as hospitalized with myocardial dysfunction. The 1-year readmission/all-cause mortality rate in the ACE 2 Study was approximately 40%, and we expect a rate reduction to 25% in the group with early structured biomarker assessment among patients adjudicated to have myocardial dysfunction. Based on these numbers, we will need to randomize 152 patients to early structured biomarker assessment and 152 patients to the control group to have 80% probability to detect a significant difference ($p<0.05$) between the two groups. Based on data from the ACE 2 Study, 65% of the population will suffer from myocardial dysfunction, thus we will need to randomize 234 patients to early cardiological assessment. With a 1:1 randomization strategy taking into account possible patient dropouts, we will aim to include at least 500 patients in the ACE 4 Study. However, we ask permission to include up to 600 patients if additional pilot experiments prior to study commencement indicate a need for a larger sample size.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or special term	Explanation
ACE 2 Study	Akershus Cardiac Examination 2 Study
ACE 4 Study	Akershus Cardiac Examination 4 Study
ACS	Acute coronary syndrome
AECOPD	Acute exacerbation of chronic obstructive pulmonary disease
AF	Atrial fibrillation
ALS	Amyotrophic lateral sclerosis
AMI	Acute Myocardial Infarction
AUC	Area under the curve
BNP/NT-proBNP	B-type natriuretic peptide/N-terminal pro-B-type natriuretic peptide
CRF	Case Report Forms
CT	Computer tomography
CV/CVD	Cardiovascular/ cardiovascular disease
DSMB	Data Safety and Monitoring Board
ECG	Electrocardiogram
EMR	Electronic medical records
ED	Emergency Department
FEV_1	Forced expiratory volume in 1 sec
FVC	Forced vital capacity
GOLD	Global initiative of chronic Obstructive Lung Disease
HF	Heart failure
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
HR	Heart rate
hs-cTnT	High-sensitivity cardiac troponin T
JVP	Jugular venous pulse
LoS	Hospital length of stay
LV	Left ventricular

LVEDD	Left ventricular end-diastolic dimension
NEWS2	National Early Warning Score 2
NYHA	New York Heart Association
PE	Pulmonary embolus
PI	Principal Investigator
RCT	Randomized controlled trial
TTE	Transthoracic echocardiogram

1 INTRODUCTION

1.1 Background – Diagnostic Challenges in Patients with Acute Dyspnea

Patients with dyspnea constitute a heterogeneous group of patients with substantial short- and long-term mortality. Major patient groups admitted to the Emergency department (ED) with dyspnea are patients with acute heart failure (HF), acute exacerbations of chronic obstructive pulmonary disease (AECOPD), and pulmonary infections. Other conditions that can also lead to dyspnea are pulmonary embolus (PE) and asthma. These are all potential life-threatening conditions and delayed diagnosis are associated with increased morbidity and mortality. In addition, missed diagnosis in the ED causes a delay in the initiation of appropriate therapy, which will increase hospital length of stay (LoS) and expenditure without improving patient care. An important clinical sign in patients with dyspnea are increased breathing rate (tachypnea) and 192 of 314 patients (61%) hospitalized with acute dyspnea and included into the Akershus Cardiac Examination 2 (ACE 2) Study, conducted at Akershus University Hospital during 2009-2010, demonstrated respiratory rate >20/ min on ED admission, which is characterized as tachypnea.

Unfortunately, making the correct diagnosis in the ED can be challenging as symptoms overlap between the different conditions and clinical signs may not accurately reflect the underlying pathology. Even patients with severe HF, as documented by invasive measurements of pulmonary artery pressures, can present without signs considered sensitive for acute HF; e.g. increased jugular venous pulse (JVP), pulmonary crackles, and peripheral edemas. Previous reports from multicenter trials have found physicians working in the ED to diagnose acute HF with 85% accuracy, as assessed by receiver operating statistics (area under the curve [AUC≈0.85]). In line with this, we recently found AUC=0.86 for physicians working in the ED at Akershus University Hospital to diagnose acute HF in the ACE 2 Study. Hence, diagnostic accuracy for HF among unselected patients with acute dyspnea and tachypnea can be improved.

1.2 Background –Testing in Patients with Acute Dyspnea and Tachypnea

Acute HF is a syndrome that is caused by different underlying pathophysiology (**Figure 1**). Hence, early cardiological assessment should cover all processes that potentially could cause acute HF. Current guidelines recommend the use of supplementary tests in the ED in patients with suspected HF, including electrocardiogram (ECG), measurements of cardiac biomarkers, chest X-ray and early transthoracic echocardiography (TTE). To the best of our knowledge, no study has previously tested whether a strategy combining (1) early structured biomarker assessment (a priori-based cutoffs) and (2) subsequent standardized reporting in the patient's electronic health records (EHR) will improve clinical outcomes compared to biomarker testing and assessment performed by attending physicians (standard care). We will base cardiological assessment on a few robust parameters that can be ascertained in all patients and evaluated also by junior physicians.

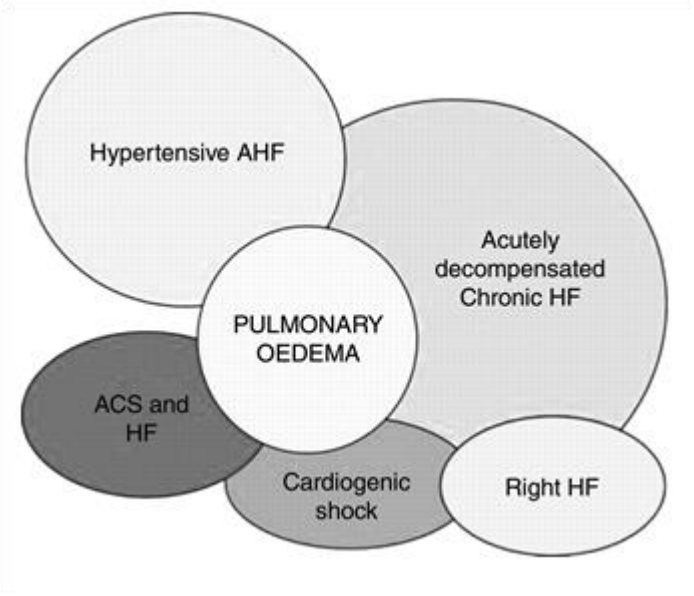

Fig.1. Acute HF may be caused by hypertensive heart disease, heart failure with preserved ejection fraction (HFpEF), acute coronary syndrome, de novo acute HF, or acute-on-chronic HF. The severity of acute HF may differ between New York Heart Association (NYHA) functional class II-IV with the most severe cases being classified as pulmonary edema (as diagnosed by chest x-rays). From McDonagh TA, et al. Eur J Heart Fail 2011;13:1253-60

ECG is an easily available supplementary test that is helpful to diagnose myocardial ischemia and supraventricular and ventricular arrhythmias. ECG assessment may also enable the physician to suspect prior myocardial injury or structural heart disease, which then should be further explored with additional tests, including TTE. The importance of obtaining information from the ECG can also be appreciated by the association between atrial fibrillation (AF) and acute HF. AF and other supraventricular tachycardias are especially associated with acute HF in patients with HFpEF, as these patients have impaired left ventricular (LV) relaxation. Hence, HFpEF patients are especially vulnerable to tachycardia, which will increase left atrial filling pressures, increase pulmonary artery pressures, and induce pulmonary congestion. Unfortunately, high quality echocardiographic images can be difficult to obtain in patients with rapid AF and subsequent tachypnea, which underlines the need to base early cardiological assessment on supplementary tests besides TTE alone. ECG is also helpful to diagnose acute coronary syndrome in the subgroup of patients with tachypnea due to acute coronary artery disease. However, as moderate/minimal ST-T segment abnormalities can be caused by tachycardia and tachypnea directly, and as physician experience influences ECG interpretation, we have not included ECG in the structured cardiological assessment to be tested in the Akershus Cardiac Examination 4 (ACE 4) Study. Moreover, as high-sensitivity cardiac troponin assays will detect even minute myocardial injury, cardiac biomarker testing will duplicate and add to the information that are provided by ECG readings. Still, one could argue that detection of AF could be included into early cardiology assessment, but given the large overlap in AF prevalence between patients with acute HF and non-HF related dyspnea, reflected in a modest AUC=0.65 for AF in the ACE 2 Study (vs. AUC=0.85 for NT-proBNP), ECG variables seem to have limited additional value to cardiac biomarkers when assessing risk of HF.

Current guidelines recommend early testing of concentrations of B-type natriuretic peptide/N-terminal pro-B-type natriuretic peptide (BNP or NT-proBNP) in patients with suspected HF as they provide excellent discrimination between HF and non-HF dyspnea and improve physician accuracy for diagnosing HF. Still, BNP/NT-proBNP measurements will fail to identify HF in 10-15% of patients admitted with acute dyspnea. Moreover, cutoffs for BNP/NT-proBNP are different in patients with HFrEF and HFpEF. Thus, although useful for diagnosing HF, BNP/NT-proBNP concentrations should be interpreted in the context of HFrEF/HFpEF. The need to incorporate biomarker concentrations with additional knowledge could explain why some studies have found early natriuretic peptide testing to improve patient management, while results have not been as clear in other studies. Based on the literature, and also including information from our own ACE 2 Study, we will establish a combined NT-proBNP and high-sensitivity cardiac troponin T (hs-cTnT)-algorithm that should provide sensitive and specific for HF across the spectrum of HF. The definition of acute myocardial infarction also incorporates cardiac troponin testing as an essential criterion for the diagnosis and our algorithm will be accurate also to diagnose acute myocardial infarction in unselected patients with tachypnea. We have demonstrated that high-sensitivity cardiac troponin testing complements BNP/ NT-proBNP measurements for HFpEF diagnosis in the

ACE 2 Study, and therefore will combine hs-cTnT and BNP/ NT-proBNP to diagnose HF in unselected patients with tachypnea in the ACE 4 Study.

TTE is a non-invasive method to determine myocardial structure and function and is recommended as early as possible in patients with suspected acute HF. Although easy to perform by trained personnel, TTE is greatly influenced by the experience of the examiner and inter- and intrarater variability can be profound. In patients with tachypnea image quality will be suboptimal as the patient cannot lie down and there are rapid, high-frequency movements of the chest during respiratory work. In theory, performing early echocardiography in all patients with tachypnea could therefore impair patient safety and increase the time to appropriate therapy, as physicians will be preoccupied with performing echocardiography and potentially also could focus too strongly on HF as the cause of tachypnea. In this scenario, early TTE may divert the attention from other important etiologies that also can cause tachypnea, including AECOPD, PE, and pulmonary infections. Hence, we believe a standardized protocol based on the easily available cardiac biomarkers BNP/ NT-proBNP and high-sensitivity cardiac troponin, followed by structured feedback in the patient's EMR, will provide a balanced strategy to increase diagnostic accuracy for HF among unselected patients with tachypnea. Still, elevated biomarker concentrations will need additional work up, including TTE, and therefore we expect that biomarker profiles indicative of HF and structured feedback in the patient' EMR will result in referral by the treating physician for early TTE in the majority of cases.

1.3 Summary of Previous Clinical Studies and Current Strategies

1.3.1 Diagnostic Accuracy in Patients with Acute Dyspnea and Tachypnea

In general, physician AUCs for diagnosing HF in patients hospitalized with acute dyspnea (and thereby also tachypnoea) have been reported to be in the range of 0.76-0.90, and the heterogenous result could relate both to differences in the population assessed and the quality of diagnostic work up at the different centers. Previous multicenter trials have reported AUCs of 0.85 and 0.86 and we have found AUC=0.86 for physicians working in the ED of Akershus University Hospital to diagnose HF in patients with acute dyspnea (ACE 2 Study). Accordingly, novel strategies are needed to improve diagnostic accuracy for HF in patients hospitalized with dyspnea and tachypnea.

1.3.2 Current Strategies in Patients with Acute Dyspnea and Tachypnea

The current strategy for attending physicians in patients with acute dyspnea and suspected HF is to obtain patient history and perform clinical examination before ordering supplementary tests like ECG recordings, BNP/NT-proBNP measurements, chest X-ray and high-sensitivity cardiac troponin testing in the majority of cases. Normally, TTE is performed in the acute/subacute phase.

1.4 Rationale for the Study

Acute HF constitutes approximately 40% of patients hospitalized with acute dyspnea (and therefore tachypnea). In addition, approximately 1/3 of patients hospitalized with non-HF related dyspnea will exhibit evidence of myocardial dysfunction. Accordingly, the total proportion of patients hospitalized with acute dyspnea and tachypnea with evidence of myocardial injury or dysfunction will be 60-65%. Given that physician accuracy for diagnosing HF in the ED is AUC=0.85, strategies that increase diagnostic accuracy should improve patient care. Hence, in the ACE 4 Study we will test the strategy whether early biomarker assessment and structured feedback in the patient' EMR reduces clinical endpoints in unselected patients with tachypnea.

2 STUDY OBJECTIVES

The primary endpoint of the study will be whether early biomarker assessment and structured feedback in the patient' EHR assessment in unselected patients with tachypnea extends the time to the first event for either *(1)* all-cause readmission or *(2)* all-cause mortality; i.e. time to the combined endpoint, compared to the current strategy/ standard care. We hypothesize that the strategy of early biomarker assessment and structured feedback in unselected patients with tachypnea will also affect endpoints related to morbidity and health economics, and also that the strategy will help decipher underlying pathophysiology in acute HF, including conditions such as acute coronary syndromes, HFpEF, and HFrEF. We will also perform prespecified stratified analyses according to HF diagnosis and HF phenotype.

The study participants will be extensively characterized with echocardiography, ECG monitoring, and detailed molecular characterization and there will be explorative endpoints related to also these analyses.

2.1 Endpoints

Primary endpoint

To determine whether early structured biomarker assessment in unselected patients with tachypnea extends the time to the first event for either *(1)* all-cause readmission or *(2)* all-cause mortality; i.e. time to the combined endpoint, compared to the current strategy/ standard care

Secondary endpoints

- To estimate the difference in hospital length of stay during the index hospitalization for strategy with early structured biomarker assessment compared to current strategy/ standard care in unselected patients with tachypnea
- To estimate the difference in length of stay in Intensive Care Unit/Medical Intensive Care Unit/ Cardiac Intensive Care Unit during the index hospitalization for strategy with early structured biomarker assessment compared to current strategy/ standard care in unselected patients with tachypnea
- To estimate the difference in 30-day all-cause readmission for strategy with early structured biomarker assessment compared to current strategy/ standard care in unselected patients with tachypnea
- To compare time to first all-cause readmission during follow-up for strategy with early structured biomarker assessment compared to current strategy/ standard care in unselected patients with tachypnea
- To compare total number of all-cause readmissions during follow-up for strategy with early structured biomarker assessment compared to current strategy/ standard care in unselected patients with tachypnea
- To estimate the difference in all-cause mortality during follow-up for strategy with early structured biomarker assessment compared to current strategy/ standard care in unselected patients with tachypnea
- To estimate the difference in total cost for strategy with early structured biomarker assessment compared to current strategy/ standard care in unselected patients with tachypnea
- To estimate the difference in all-cause mortality during the index hospitalization for strategy with early structured biomarker assessment compared to current strategy/ standard care in unselected patients with tachypnea
- To estimate the difference in the cardiac troponin T and/or I and B-type natriuretic peptide and/or N-terminal pro-B-type natriuretic peptide concentrations from hospital admission to discharge for strategy with early structured biomarker assessment compared to current strategy/ standard care in unselected patients with tachypnea
- To estimate the difference in guideline-defined medical therapy for HF, as defined by international guidelines, at discharge for strategy with early structured biomarker assessment compared to current strategy/ standard care in patients adjudicated with HF diagnosis and categorized into HF with reduced or mildly reduced ejection fraction (HFrEF/ HFmrEF) and HF with preserved ejection fraction (HFpEF)
- To estimate cost-utility for the strategy with early structured biomarker assessment compared to current strategy/ standard care in unselected patients with tachypnea

Total follow-up is permitted until 2038. We will assess the primary endpoint after 12 month follow-up from randomization; i.e. patients are followed-up either for 12 months or to the time of death (if this happens before 12 months from randomization). We will also report data for extended follow-up after 3 years, 5 years, and 10 years for the primary outcome.

For secondary endpoints we will report data after the index hospitalization, after 30 days follow-up, after 1 year, 3 years, 5 years, and 10 years. Cost-utility will be evaluated based on data reported up to and including 12 months, and updated after 5 years

Explorative endpoints:

- Assessing primary and secondary outcomes in the subgroup of patients classified as hospitalized due to HF, as assessed by the adjudication committee
- Assessing primary and secondary outcomes in HF patients, classified by the adjudication committee, with patients stratified by LVEF 50%; e.g. HFrEF/ HFmrEF vs. HFpEF
- Assessing primary and secondary outcomes with patients stratified by biomarker concentrations measured on the first study visit (study inclusion), including B-type natriuretic peptides, cardiac troponins, and additional biomarkers
- Assessing accuracy for clinical and biochemical diagnostic scores for diagnosing HFpEF; e.g. the H_2FPEF and HFA-PEFF scores
- The ACE 4 Study will phenotype participants with short-term Holter recordings (≤24 h), transthoracic echocardiography (TTE), and biospecimens collection during the index hospitalization and after 6 months for later molecular characterization in all participants and we will compare phenotypic traits with adjudicated diagnosis and clinical outcomes during follow-up

3 STUDY POPULATION

3.1 Selection of Study Population

Over a period from February 1, 2023 until June 28, 2024, the ACE 4 Study will enroll a minimum of 400 patients and a maximum of 600 patients hospitalized at Akershus University Hospital with tachypnea. Hence, stopping criterion for the trial is either inclusion of 600 patients or June 18, 2024. At Akershus University Hospital, all patients are scored according to National Early Warning score 2 (NEWS2) in the triage section of the ED. Hence, scoring of NEWS2 is part of clinical routine at Akershus University Hospital and therefore not a study defined variable. For the ACE 4 Study, we will define tachypnea as respiratory rate ≥20/ min according to the NEWS2 scoring on admission in the ED. We apply for permission to establish a real-time electronic surveillance of all patients hospitalized at the Division of Medicine, Akershus University Hospital that have tachypnea as defined by NEWS2 scoring, which is part of clinical routine. Only dedicated study personnel will have access to this information, which will be presented to the study team as a list that is stored on secure servers. We also apply for permission to check in the EHR of patients with tachypnea whether the patients fulfill inclusion criteria and do not meet exclusion criteria of the ACE 4 Study. After the study team has identified eligible patients, the study team will obtain written, informed consent from the patient. Subsequently to consent, trained study personnel will collect additional information on current and prior medical history by the strategy previously successfully used in the ACE 2 Study. This information will be checked against the EHR of the patient. Participants must comply with all inclusion and exclusion criteria to be eligible for study participation.

All patients will be thoroughly informed about all aspects of the study and the study team will collect informed, written consent from all study participants. As a small subgroup of patients with tachypnea will have severely compromised clinical status on admission, including possibly hypercapnia that could influence cognitive status, the written informed

consent can also be obtained within the first days of the hospitalization after clinical stabilization. In case of severely compromised clinical status on admission, the patient will receive oral information on the study and we will limit testing to examinations that are indicated from a clinical perspective; e.g. ECG and blood sampling for standard cardiovascular (CV) biomarkers. An impartial witness should be present during the entire informed consent discussion, and this will be recorded in the patient study file. Informed written consent will be obtained in all subjects as soon as possible after stabilization of clinical status. Regardless of the time, at the request by the patient and not already included in a publication, all data collected in the study relating to the participant will be terminated. A short run-in phase in the ED will be permitted to assess whether the patient will meet the inclusion and exclusion criteria. Blood samples for biobank will require signed written informed consent. We will be establishing an eBiobank for our study, which is an electronic tracking system for research biobanks approved by the Regional Ethics Committee. It will allow us to keep track of the included participants and their respective blood samples in our research biobank. Registration of samples is standardized, we will get an overview where the samples are stored, with an overview of sample amount in the specimens. Sample search and withdrawals are logged. Sensitive information is encrypted, and the user is working with anonymized participant numbers. Each participant can have several samples linked to the eBiobank with unique sample numbers.

The study participants will receive all examinations as considered appropriate by the treating physician, which includes also early echocardiography in the control group. There will be no restrictions to the diagnostic testing of our study participants, regardless of randomization status in the study. Study personnel will not directly administrate management nor therapy of the patients, but the study team will provide structured biomarker-based risk of HF and general information related to guideline-based treatment of HF in the study intervention group. The decision of the patient to participate in the ACE 4 Study will not influence treatment that is offered to the patient at Akershus University Hospital and patients not participating in the study will receive current strategy/ standard care.

From an ethical perspective the delayed written informed consent can be justified by the argument that most severely compromised patients also would benefit most from early detection and treatment of myocardial dysfunction. Hence, although we clearly acknowledge the need for all research to adhere to Good Clinical Practice and consider written informed consent as foundation for all research, it is imperative that the most severely ill patients can be included into the ACE 4 Study. Of note, the non-invasive biomarker measurement is part of routine examinations in this patient group and do not in any way put the patient at risk. Accordingly, we consider a delayed written consent in a subgroup of patients to be acceptable. The study participants will also receive all additional examinations as considered appropriate by the treating physician and study personnel will not dictate diagnostic work up or the plans for the hospitalization or follow-up period. Delayed written consent will also only be performed in a small subgroup of patients as we plan to obtain written informed consent prior to study inclusion in the majority of our participants. The question regarding delayed informed written consent were addressed also by the independent Ethics Committee during formal approval of the study and we received support to pursue this strategy.

3.2 Inclusion Criteria

All the following conditions must apply to the prospective patient at screening prior to inclusion in the study:

- Patients ≥18 years old
- Tachypnea defined as respiratory rate ≥20/ min, as noted in the NEWS 2 classification performed on admission in the ED and reported in the patient' EHR, which is performed as routine in all admission at Akershus University Hospital (hence, part of clinical routine)
- Admission to Departments under the Division of Medicine at Akershus University Hospital, except the Department of Neurology
- <24 h from hospital admittance to inclusion in the study
- Signed written informed consent during the initial phase of the hospitalization

3.3 Exclusion Criteria

Patients with tachypnea will be excluded from the study if they meet any of the following criteria:

- Previously included into the study (in case of patients presenting with a second hospitalization during the study period)
- Known or suspected cancer outside of local control, documented in medical records, at the time of patient inclusion or diagnosed in relation to the index hospitalization
- Neurological condition with short life expectancy; e.g. ALS, documented in medical records during screening prior to study entry
- Other non-cardiac disease with life expectancy below 1 year, documented in medical records during screening prior to study entry
- Obvious non-cardiac cause for tachypnea based on medical records and clinical findings during screening prior to study entry; e.g. anaphylaxis in young patient with known allergy, dyspnea after direct chest trauma, or young patient with fever and positive Covid-19 test on admission.
- Patient assessed as non-Internal Medicine patient; e.g. surgical patient
- Patients unwilling or unable to comply with the protocol, including Glasgow Coma Scale <13 on the time of study inclusion
- Patients that are intubated for invasive ventilatory therapy before or shortly after hospital admission
- History of non-compliance to medical management and patients who are considered potentially unreliable, based on documentation in medical records, during screening prior to study entry
- History or evidence of alcohol or drug abuse with the last 12 months, based on medical records and clinical findings during screening prior to study entry, that will influence study participation
- Any surgical or medical condition, based on medical records and clinical findings during screening prior to study entry, that will impair the ability of the patient to participate in the study

3.4 Patient registration

Each patient in the study will be uniquely identified by a code starting with “ACE4-“ followed in numerical order from 1 up to the last patient that is included in the study. Once assigned to a patient, a patient number will not be re-used. If the patient fails to start on study for any reason, including patients that are randomized and later refuse study participation, the reason for not entering the study will be entered in the Screening Log.

To ensure that the selected population for the ACE 4 Study is representative to all patients hospitalized with tachypnea during the same period, we ask permission to collected basic, anonymized information related to the patients eligible for inclusion, but not included into the study (e.g. not enough resources to include all patients on a given day). For these patients we will only collect the basic information age, sex, and hospital length of stay for the index hospitalization, and we will anonymize the data before storage (i.e. not possible to correlate data to individual patients).

4 STUDY EXECUTION

4.1 Patient Screening, Inclusion, and Randomization

We will include only patients hospitalized with tachypnea at Akershus University Hospital, location Lørenskog. The identification of patients eligible for the study will be directly from automatic, real-time surveillance by the data warehouse of Akershus University Hospital of patients with tachypnea, as recorded by ED nurse during assessment of NEWS2 scoring and recorded in the patient' EHR (**Figure 2**).

Randomization will be performed by a pre-determined computer algorithm that is set up by a statistician. Clinical characteristics will be collected directly from the physician and from the medical records by a standardized protocol and plotted into the electronic case reports forms (eCRFs) of the study participants.

4.2 Early Biomarker-based Cardiological Assessment

The early cardiological assessment will be performed in the subgroup of patients randomized to this intervention (**Figure 2**). Using admission concentrations of NT-proBNP and hs-cTnT, we will use standardized criteria to assess risk of HF as the cause for hospitalization. Based on data from the ACE 2 Study and current recommendations for diagnosing acute HF, we will use the structured algorithm in **Figure 3**, which will only be distributed to the study team. The values for NT-proBNP are based on contemporary cutoffs for diagnosing HF in the ED setting, with NT-proBNP <300 ng/L largely excluding HF, and values above age-specific cutoffs increasing the likelihood of HF being the cause of respiratory distress.[1] For hs-cTnT, the cutoffs are based on the positive and negative predictive values from ACE 2 Study, with negative predictive values for HF >90% for hs-cTnT <10ng/L and positive predictive values for HF >90% for hs-cTnT ≥90ng/L for patients with intermediate concentrations of NT-proBNP.

In patients assessed to be in the "grey zone" after as determined by concentrations of NT-proBNP and hs-cTnT, where additional examinations are needed to assess risk of HF, and in the patients deemed to be high-risk patients for HF based on biomarker testing ("rule in"-group), we will recommend that the patient should perform TTE, if not recently performed. According to established guidelines, we will also recommend that an elevated cardiac troponin measurement should be followed by additional troponin testing (serial testing), which is required to make the diagnosis of acute myocardial infarction.

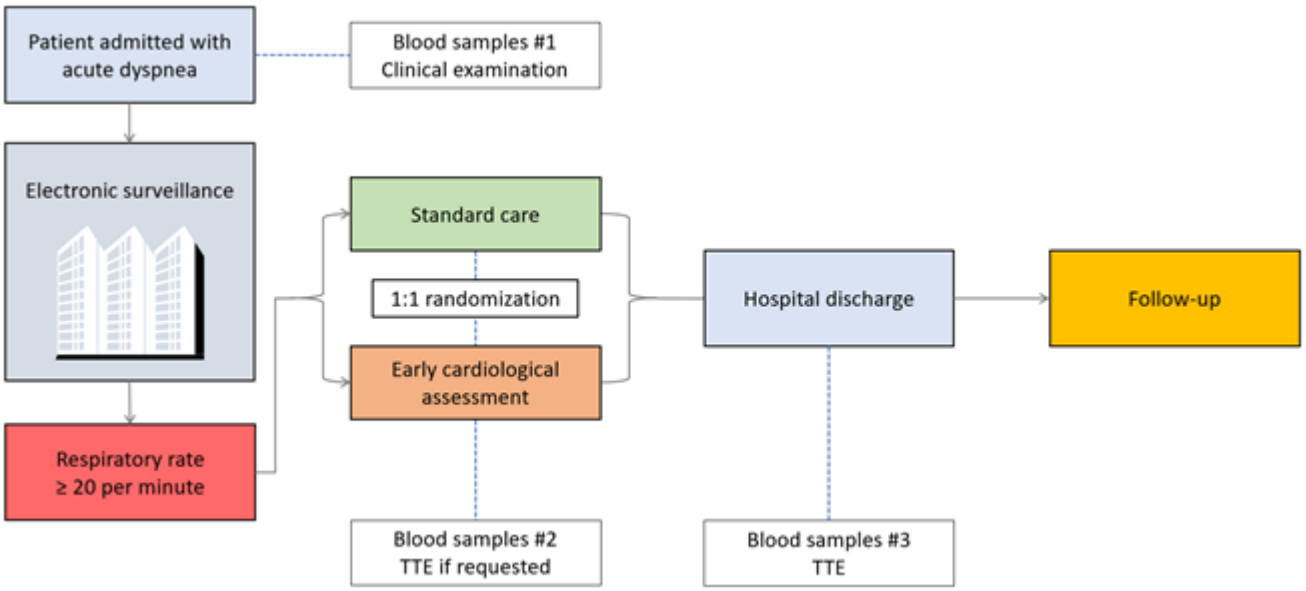

Figure 2. Flow chart of the ACE 4 Study. HF, heart failure. TTE, transthoracic echocardiogram.

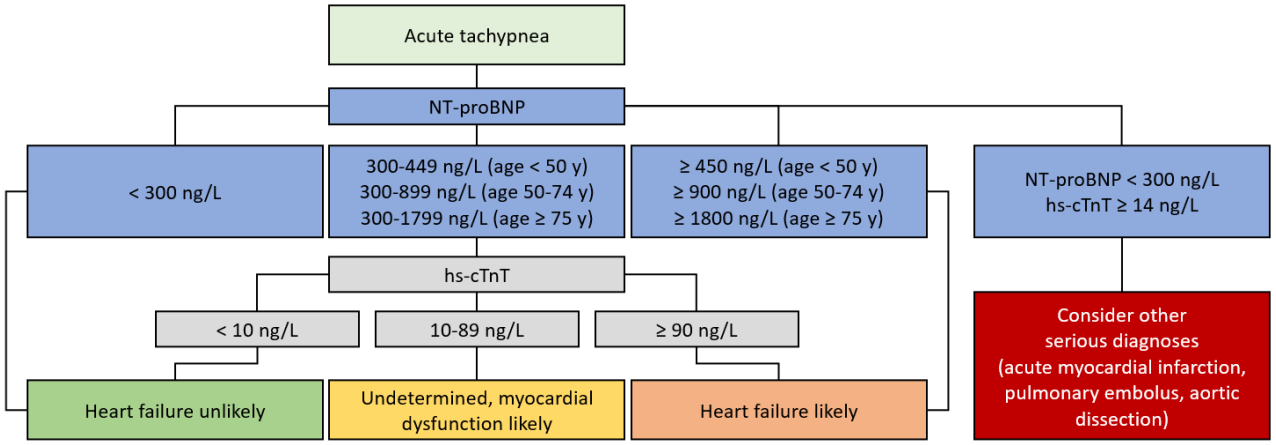

Figure 3. Early cardiological assessment in patients with acute tachypnea. Cutoffs for NT-proBNP are derived from Januzzi JL, et al, Journal of the American College of Cardiology 2018;71:1191-200, cutoffs for hs-cTnT in the grey zone are based on results from the ACE 2 Study, and the cutoff hs-cTnT ≥14 ng/L is the 99-percentile in the healthy population.

4.3 Patient Follow-Up During the Index Hospitalization and Data Storage

All patients that might prove eligible for inclusion in the ACE 4 Study will be identified through automatic, real-time surveillance from the data warehouse of Akershus University Hospital. This screening will provide a list of all subjects scored with respiratory rate ≥20/min, as noted in the NEWS2 classification performed on admission in the ED and reported in the patient EMR. We have identified that median 13 patients/ day (range 2-30 patients) fulfill this first inclusion criteria at Akershus University Hospital, location Lørenskog. Only dedicated study personnel will have access to this screening log and will then, based on EHR notes, make a first assessment whether the patient might be eligible for inclusion into the study. Study personnel will subsequently contact the patient to see whether the patient is compliant with the inclusion and exclusion criteria of the ACE 4 Study, provide information regarding the ACE 4 Study, and ask whether the patient will provide written informed consent. Study personnel will also consider whether delayed consent is more appropriate in patients who are considered to be in a severe clinical situation. Participants will be randomized with an electronic algorithm using block randomization and the randomization scheme will not be revealed to study personnel. Preferably, only patients with signed written consent will be randomized. Patients that are randomized but do not provide consent or later are deemed non-compliant with inclusion or exclusion criteria, will be marked as screening failures and not included into the ACE 4 Study.

We will perform cardiac biomarker testing with NT-proBNP (ProBNP assay, Roche Diagnostics, Basel, Switzerland) and hs-cTnT (Elecsys TnT hs stat, Roche Diagnostics) measurements on ED admission in all participants, regardless of randomization status. The results will be provided in the patient's EHR, regardless of randomization status in the ACE 4 Study. We will provide a note in all patient's EHR that the patient is participating in the ACE 4 Study, has signed a written informed consent, and we will provide an overview of the study protocol. In addition, **and only for patients randomized to the intervention group**, we will include assessment of probability that myocardial injury or dysfunction are the underlying pathophysiology responsible for tachypnea in the patient' EHR, as evaluated by the cardiac biomarker algorithm of the ACE 4 Study (**Fig. 3**). Furthermore, **in patients randomized to the intervention group**, we will also inform on general recommendations for work up and treatment, but no specific instructions for individual patients as the treating physician should make all clinical decisions.

The study participants will receive all examinations considered appropriate by the treating physician, including TTE in the control group if this is considered appropriate from a clinical perspective. Hence, there will be no restrictions to diagnostic testing of our study participants, regardless of randomization status. If requested by the treating physician, a full TTE will be performed by trained study personnel in both groups. The TTE protocol will assess atrial and ventricular dimensions, systolic and diastolic properties of the left ventricle, right ventricular systolic function, the cardiac valves, pericardial effusion, and estimate arterial pulmonary pressure + look for other signs indicative of pulmonary

hypertension. The conclusion of the TTE will be recorded in the patient' EMR, according to standard procedure. The decision of the patient to participate or to decline participation into the study will not influence the treatment that is offered to the patient at Akershus University Hospital and also patients declining participation will receive current strategy/ standard care.

After written, informed consent from the patient, trained study personnel will collect additional information on prior medical history by the strategy previously used in the ACE 2 Study. This information will be checked against the EHR of the patient. All participants of the study, regardless of randomization status, will be subjected to extensive CV phenotyping, including blood sampling for biobank (admission, discharge, and after 6 months), and short-term ECG monitoring (≤24 h). Furthermore, and only in patients not receiving TTE early after admission as part of clinical work-up, we will perform a TTE for research purposes prior to hospital discharge. Hence, all participants of the ACE 4 Study will be examined by TTE during the index hospitalization. The results of TTE performed for research purpose late during the hospitalization will not be communicated to the treating physician, unless we identify unrecognized, gross pathology like left ventricular ejection fraction<40%, right ventricular strain indicative of possible pulmonary embolus, severe valvular disease, or pericardial effusion with risk of cardiac tamponade.

We will not provide any treatment recommendation for individual patients. However, we will include a short paragraph related to information for pharmacological treatment of patients with HFrEF (LVEF≤50%), HFmrEF (LVEF 40-50%), and HF with recovered LVEF according to the ESC Heart Failure Guidelines 2021. For these patients we will inform the physician that β-blocker, SGLT2-inhibitor, inhibition of renin-angiotensin with ACE inhibitors or angiotensin-neprilysin inhibition, and aldosterone inhibition is recommended. We will also inform that loop diuretics are recommended for volume control/ decongestion in all patients with HF according to the ESC guidelines. The recent ESC guidelines have no clear pharmacological recommendations for patients with LVEF≥50% (HFpEF), but based on emerging data from randomized clinical trials demonstrating effects from SGLT2-inhibitors across the spectrum of LVEF in HF patients (e.g. DELIVER trial) we will inform the treating physician about SGLT-2-inhibitiors in HFpEF patients. We will also inform the treating physician about aldosterone inhibition, as aldosterone inhibition reduced HF hospitalizations in the TOPCAT trial (secondary endpoint) and reduced the primary endpoint of TOPCAT in post-hoc analysis. The recent US (AHA/ACC/HFSA) guidelines for heart failure (2022) supports this strategy.

We will also inform that, according to several ESC guidelines, patients with suspected acute coronary syndromes are recommended to be clinically assessed for coronary artery disease and treated according to current guidelines. We will also, on a general basis, inform the treating physician that patients with atrial fibrillation have guideline-recommendations for treatment, including anti-coagulation and frequency control.

Patients will be followed up during the index hospitalization for all endpoints related to the hospitalization. We will record the time in hospital, and the time spent in high-resource units (Intensive Care Unit/Medical Intensive Care Unit/Cardiac Intensive Care Unit). We will also record all additional diagnostic testing performed in the patients and calculate the total resource utilization for the individual patient. We will also collect data on 30-day all-cause readmission from the data warehouse of Akershus University Hospital and from national registries. We will use similar strategy to collect data also for secondary and exploratory endpoints.

4.4 Adjudication of Diagnosis and Clinical Events

The diagnosis of the index hospitalization will be adjudicated by two experts working independently and reviewing all data, including follow-up data, as previously also performed in the ACE 2 Study and other international studies. For the ACE 4 Study the experts will have access to the results of CV biomarkers measured during the hospitalization and to the results of TTEs, however, the experts will not have direct access to the conclusion of the early cardiological assessment. A diagnosis of HF as the cause for the hospitalization will be based on the criteria proposed by ESC

requiring typical signs and symptoms of HF and objective evidence of structural or functional myocardial abnormality, most recently updated with ESC Guidelines for Heart Failure from 2021. Discrepancy regarding the diagnosis between the two experts will be resolved by consensus and we will document the proportion of cases where the experts initially do not agree.

For follow-up we will collect all data and any hospitalization, regardless of illness and diagnosis (all-cause morbidity and all-cause mortality). A diagnosis of acute myocardial infarction will be based on the definition by the most recent WHO Universal Definition for myocardial infarction; i.e. (1) Either patient history suggestive of acute myocardial infarction (AMI) but sudden death before blood samples could be obtained OR (2) troponin rise and/or fall. In addition, one of the following criteria also needs to be present: (i) Symptoms suggestive of acute coronary syndrome (ACS), (ii) ECG signs of AMI, (iii) TTE signs of AMI, or (iiii) obstructive coronary artery disease on angiogram. Exacerbation of chronic obstructive pulmonary disease (AECOPD) will be defined by the Global initiative of chronic Obstructive Lung Disease (GOLD) criteria: "[…] a worsening of the patient's symptoms [dyspnea, cough and/or sputum production] that is beyond day-to-day variation and that leads to a change in medication." COPD will require pulmonary testing with FEV_1/FVC <0.7 and we will use the GOLD category 1-4 according to FEV_1. Pulmonary embolus (PE) will be diagnosed based on evidence of PE on CT scan (or other modality), or TTE evidence of TTE in the absence of other known cause for right ventricular strain/other indices possibly reflecting PE. Pulmonary infections will be diagnosed based on clinical symptoms of infections (e.g. sputum production, fever, etc), biomarkers reflective of inflammation during the hospitalization and/or chest X-ray/CT scan demonstrating pulmonary infection. Mortality during follow-up will be collected from the data warehouse of Akershus University Hospital and national registries.

4.5 Quality of Life and Resource Utilization

We will use the EQ-5D-5L questionnaire as basis for calculating health related quality of life (HRQoL) in our study participants. Information from EQ-5D-5L will be collected soon after admission and prior to discharge during the index hospitalization. Planned time points for QoL assessment are at discharge, after 1 month, 3 months, 6 months, 12 months, and yearly afterward for maximum 10 years.

Financial resource utilization will be performed by collecting information on hospital length of stay and type of hospital ward (ICU/CCU vs. standard) and by collecting information on all supplementary tests performed during or in conjunction with the index hospitalization. To calculate total costs during follow-up we will obtain information on hospital re-admissions during follow-up, medication use, sick leave from work, and nursing home care. We will obtain this information from hospital systems (re-admissions) and regional and national registries (medications [national medication registry], sick leave [NAV/SSB], and nursing home use [IPLOS]). We will also seek to obtain data on medical contacts with primary care physicians from national registries [KUHR/KPR] and travel costs for patients. In addition, we will also obtain information from other registries (e.g. FD-trygd and Norwegian Patient Registry) to inform resource use in the health economic evaluation.

4.6 Deep Phenotyping and Exploratory Endpoints

There is a need to understand more closely the pathophysiology and comorbidities of the major conditions causing hospitalization for acute dyspnea. Accordingly, in the ACE 4 Study we will also characterize all participants with TTE, ECG monitoring in the acute phase, and collection of biospecimens that later can permit quantification of protein and non-protein biomarkers. We will collect blood samples for study biobank from all participants within 24 h from hospital admission and before hospital discharge (**Figure 2**). We will invite included patients back for a 6-month outpatient visit to register medication and to collect biospecimen for biobank storage. The rationale for biospecimen collection also after 6 months is to assess serial concentration of biomarker over time, and to correlate changes in biomarker

concentration with medication. Blood sampling will be performed by standard venous access or from indwelling arterial cannula if already present due to clinical need. We will obtain serum and citrate, heparin, and EDTA plasma.

We will measure established and novel CV protein and non-protein biomarkers. Categories of biomarkers that will be measured relate to markers of hemodynamic stress, myocardial injury and function, neurohormones, cell necrosis, fibrosis, inflammation, renal disease, and pulmonary or other non-cardiac organ status. In addition, we ask permission to use -omics based methodology to perform unbiased analyses of RNA, protein, and metabolites. The revised protocol will be reviewed by the Regional Ethics Committee prior to the start of the ACE 4 Study. We ask permission to send samples to collaborating researchers outside of Akershus University Hospital and Norway. Possible countries that may receive samples are all Western European countries, but especially Sweden, Denmark, Finland, Germany, Great Britain, Switzerland, Italy, and Austria. We also ask permission to send samples outside of Europe, including to countries with different legislation compared to Norway for biological samples, and the main recipients are UK, USA, Australia, China, and India. If samples are sent out from Akershus University Hospital the collaborating center will only receive de-identified samples; i.e. no samples will be sent out with linked personal information so that the material can be linked back to individual patients of the ACE 4 Study.

Clinical phenotyping will be performed by obtaining short-term continuous ECG monitoring (≤24 h) during day 1 after patient inclusion into the study in all subjects, regardless of randomization status. This will be performed by the use of portable ECG monitors (Holter recordings) and the ECG recordings will be assessed by trained research personnel. Possible traits related to the ECG recordings are AF, ventricular ectopies, complex ventricular ectopies, non-sustained ventricular tachycardias, and sustained ventricular tachycardias. We will also obtain data on indices of heart rate variability. Also data from these recordings may be shared with national and international collaborators analogous to the countries listed above for biological analysis. Only de-identified data will be sent out from Akershus University Hospital.

We will perform a full TTE examination in all subjects during the hospitalization, regardless of initial randomization status. TTE will be performed by the Vivid E95 (GE VingMed, Horten, Norway) and the images will be digitally stored for offline analysis on custom software (EchoPac, GE VingMed). We will acquire 2D images and loops with a 2.5 MHz transducer and 3D images with a 4 V matrix-array transducer. Standard parasternal long axis and three apical views recordings will be done in the end-expiratory phase with the subjects in supine left lateral position. LV dimensions, septal and posterior wall thickness, and LV mass will be measured as recommended by American Society of Echocardiography. LV ejection fraction (EF) will be calculated using the modified Simpson's rule from biplane 4-chamber and long-axis views. LV diastolic function will be assessed by pulsed Doppler transmitral peak early (E), peak late (A) and E deceleration time. TVI-derived indices will be recorded at the base of the septal and lateral mitral annulus to determine peak systolic (S'), early diastolic (e') and late (a') diastolic velocities. Global and regional longitudinal strain will be analyzed by an offline semi-automated speckle tracking technique from the three apical views. The digital storage of echocardiographic images in Echopac will permit later analyses of novel imaging indices. The echocardiographic recordings are obtained by a limited number of researchers, and we will ensure standardization of all recordings. The results of the TTE considered standard in the art and available at the time of the examination, will be recorded in the medical records of the patient, but will not be communicated directly to the physician of the patient except in cases of gross pathology as outlined in section #4.3. Also data from these recordings may be shared with national and international collaborators analogous to the countries listed above for biological analysis. Only de-identified data will be sent out from Akershus University Hospital.

5 DATA MANAGEMENT AND STATISTICAL ANALYSIS

5.1 Electronic Case Report Forms (eCRFs)

Case report forms (CRF) will be provided for the recording of all data and we will use RedCap for data storage. Data will be recorded directly and legibly onto the eCRF and verified by the investigator. If any assessments are omitted, the reason for such omissions will be noted on the eCRFs. Corrections, with the reason for the corrections if applicable, should be dated and initialed. Data will be transferred to a secure area on TSD, University of Oslo. Study participants will only be identified by the study ID in the eCRF and the key to connect the eCRF data to the identity of the patients will be stored in a secure place with access control (only accessible to the PI and dedicated members of the study team).

5.2 Source Data

We will automatically monitor all admissions through the ED at Akershus University Hospital meeting the inclusion criteria of tachycardia, as documented in the respiratory variable of the NEWS2 score. Of note, NEWS2 scoring is performed routinely in all admissions at Akershus University Hospital for clinical purposes and therefore not a study specific action for the ACE 4 Study. Only study team members will have access to this screening list and these data will only be available on safe data systems connected to the data warehouse at Akershus University Hospital. Members of the study team will access the patient' EHR of patients that have been identified by the automatic surveillance to make initial assessment regarding study eligibility. We will record data on the number of patients detected by the screening log and prevalence of these patients that are approached for possible study inclusion, but no other information will be stored related to the initial, automatic surveillance for eligible patients. The study team also apply for permission to access the patient' EMR to determine whether the patient may be eligible for participation in the ACE 4 Study.

For patients that are included in the ACE 4 Study, we will review all patient' EHR and perform a structured interview of study participants, which is analogous to the strategy used in the ACE 2 Study. Data obtained during the hospitalization (**Figure 2**) will also be collected, de-identified, and stored in a secure web solution. Only de-identified data (marked with study code) will be shared with national and international collaborators as outlined in section #4.5. We also ask permission to collect some standard, demographic data on the patients that are found eligible for study inclusion, but decline to participate in the ACE 4 Study. We believe some key information on age, gender, and hospital length of stay are relevant to report for non-included patients to document that the final study population is representative for the total population that could have been included into the study.

We will ask permission to link our data to registries and the hospital records during follow-up. Morbidity and mortality data will be obtained from the data warehouse at Akershus University Hospital and from national registries.

5.3 Source Data Verification

The investigator will be visited on a regular basis by a Clinical Study Monitor, who will check and collect completed eCRFs, discuss the progress of the study, and perform source data verification.

When the responsible study monitor has checked and verified the CRFs, the data will be entered into a computer database at TSD, University of Oslo for further handling and statistical evaluation.

Sponsor's representatives (e.g. monitors, auditors) and/or regulatory authorities will be allowed access to source data for source data verification in which case a review of those parts of the hospital records relevant to the study may be required.

5.4 Storage of Study Documentation

The investigator shall arrange for the retention of the patient identification and the code list. Patient files shall be kept for the maximum period of time permitted by each hospital. The study documentation (eCRFs, Site File etc) shall be retained and stored during the study and for 5 years after study closure, which was reported as a requirement from the Regional Ethics Committee when granting permission for study start. All information concerning the study will be stored in a safe place inaccessible to unauthorized personnel.

5.5 Study Design

This is a single center, pragmatic randomized controlled trial of a non-pharmacological intervention.

The data will be summarized with respect to demographic and baseline characteristics and efficacy observations and measurements. Categorical data will be presented as absolute frequencies and percentages. For continuous data, N, median, and quartile 1-3 will be presented. Time to event variables and Kaplan-Meier product-limit estimates will be presented for the primary endpoint and other endpoints. The primary analyses will be conducted on all patient data at the time all patients have been included in the study and after hospital discharge of the last patient (regarding the index diagnosis). The following populations will be used for analysis:

- **Intention to Treat Analysis.** Contains all patients of the population that were included in the study and randomized to early structured biomarker assessment or standard care.
- **Per-protocol population.** Consists of all patients of the intent-to-treat population who show no major protocol violations, i.e. violations that may have an impact on the study outcome.

5.6 Sample Size & Statistical Considerations

Statistical power calculations are based on data from the ACE 2 Study, which included 314 patients with acute dyspnea using a similar strategy as employed for the ACE 4 Study. The power calculations are based on the assumptions that approximately 45% of patients hospitalized with dyspnea will be diagnosed with acute HF and that a substantial proportion of the patients with non-HF related dyspnea will also exhibit evidence of myocardial dysfunction; in total 65% of the ACE 2 cohort was classified as hospitalized with myocardial dysfunction. The 1-year readmission/all-cause mortality rate in the ACE 2 Study was approximately 40%, and we expect a rate reduction to 25% in the early cardiological intervention group among the patients with myocardial dysfunction. Based on these numbers and using a two-sided test, we will need to include 152 patients in the control group and 152 patients in the early cardiological assessment group to have 80% probability to detect a significant difference ($p<0.05$). Based on data from the ACE 2 Study, 65% of the population will suffer from myocardial dysfunction, thus we will need to randomize 234 patients to early cardiological assessment. With a 1:1 randomization strategy taking into account possible patient dropouts, we will aim to include at least 500 patients in the ACE 4 Study. However, we ask permission to include up to 600 patients if additional pilot experiments prior to study commencement indicate a need for a larger sample size.

The study will be analyzed on an intention-to-treat basis. That includes all patients enrolled in the study, irrespective if they have received the intervention or not. As a scenario analysis, we will also assess all outcomes by per-protocol analysis (i.e. including only the patients that actually received the intervention into the analysis).

5.7 Safety Analysis

The assessment of safety will be based mainly on the frequency of Adverse Events during the index hospitalization. Adverse Events will be summarized by presenting the number and percentage of patients having any adverse event by

body system and type of Adverse Event. Those Adverse Events that result in death or life-threatening event will be presented separately. We will include a Data Safety and Monitoring Board (DSMB) that will review the data for safety signals after inclusion of 100, 200, and 400 patients. The DSMB will assess key data like primary and some secondary endpoints in the two groups. The DSMB may then recommend that the study continues or stops based on safety or futility.

5.8 Interim Analysis

We will not perform interim analysis.

6 ETHICAL AND REGULATORY REQUIREMENTS

6.1 Ethical Considerations

6.1.1 General Considerations

The responsible investigator will ensure that this study is conducted in agreement with the Declaration of Helsinki (Tokyo, Venice, Hong Kong, Somerset West and Edinburgh amendments) and the laws and regulations of the country where the trial is performed. The protocol has been written, and the study will be conducted according to the ICH Harmonized Tripartite Guideline for Good Clinical Practice (ref: http://www.ifpma.org/ethics/ifpma-code-of-practice/about-ifpma-code-of-practice.html). The study will be evaluated by the Regional Ethics Committee and other government agencies before initiation. The protocol will be registered in www.clinicaltrials.gov before inclusion of the first patient.

6.1.2 Informed Consent

All patients will be thoroughly informed about all aspects of the study, including the examinations during the index hospitalization, and the required evaluations for informed consent, which will be obtained during or just after hospital admission. The patients will also be informed that we will obtain information from national health registries and also information from other health care provides. As a small subgroup of patients with acute tachypnea have severely compromised clinical status on admission, including hypercapnia that may reduce cognitive status, the written informed consent can also be obtained within the first days of the hospitalization after stabilization. In case of severely compromised clinical status on admission, the patient will receive oral information on the study and we will limited testing to examinations that may also be indicated from a clinical perspective; e.g. ECG and blood sampling for standard CV biomarkers. An impartial witness should be present during the entire informed consent discussion and this will be recorded in the patient study file. Informed written consent will be obtained in all subjects as soon as possible after stabilization of clinical status. Regardless of the time, at the request by the patient and if data are not already part of a publication, all data collected in the study relating to the particular participant will be terminated. A short run in phase in the ED will be permitted to assess whether the patient meet the inclusion and exclusion criteria. Blood samples for biobank will require signed written informed consent. From an ethical perspective the delayed written informed consent can be justified by the argument that the most severely compromised patients also, potentially, will have the largest benefit of early detection of myocardial dysfunction. Hence, although we clearly acknowledge the need for all research to adhere to Good Clinical Practice and consider written informed consent as a foundation for all research, the non-invasive strategies implemented in our early structured biomarker assessment is part of routine examinations in this patient group and in no way put the patient at risk. Accordingly, we consider a delayed written consent in a subgroup of patients to be acceptable. Of note, we will obtain written informed consent in the large majority of patients prior to patient inclusion and randomization, thus, delayed written consent will only be performed in a small subgroup of patients. However, as this subgroup also will be the most severely ill patients, including them also in the

study will be important for internal and external validity of the study, and as this subgroup potentially may benefit the most from early structured biomarker assessment.

It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever he wants. This will not prejudice the patient's subsequent care and all patients, regardless of study participation, will receive current strategy/ standard care. Documented informed consent must be obtained for all patients included in the study before they are registered for the study. This must be done in accordance with the national and local regulatory requirements.

The written informed consent form should be signed and personally dated by the patient or by the patient's legally acceptable representative.

To ensure that the selected population for the ACE 4 Study is representative to all patients hospitalized with tachypnea during the same period, we ask permission to collected basic, anonymized information related to the patients eligible for inclusion, but not included into the study (e.g. not enough resources to include all patients on a given day). For these patients we will only collect the basic information age, sex, and hospital length of stay for the index hospitalization, and we will anonymize the data before storage (i.e. not possible to correlate data to individual patients).

6.2 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with the Declaration of Helsinki and Good Clinical Practice (ICH-GCP). The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all local and federal regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator is also responsible for assuring that all the required data will be collected and entered onto the CRFs. Periodic monitoring visits will be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all case report forms will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

6.3 Record Retention

Study documentation includes all CRFs, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms). Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that the study investigator must retain all study documentation pertaining to the conduct of a clinical trial. Study documents should be kept on file for 15 years after the completion and final study report.

6.4 Audits

To ensure the quality of data, study integrity, and compliance with the protocol and the various applicable regulations and guidelines, the sponsor may conduct site visits to institutions participating in this study. The investigator, by accepting to participate to this protocol, agrees to co-operate fully with any quality assurance visit undertaken by third parties, including representatives from the sponsor as well as to allow direct access to documentation pertaining to the

clinical trial (including CRFs, source documents and other study files) to these authorized individuals. The investigator must inform the sponsor immediately in case of a scheduled inspection by a regulatory authority.

6.5 Publication Policy

The findings of this study will be published independent of its outcome. All personnel who have contributed significantly with the planning or to perform the study (Vancouver convention 1988) may be included in the list of authors.

7 STUDY MANAGEMENT

7.1 Investigator Delegation Procedure

The Principal Investigator is responsible for making and updating a "delegation of tasks" listing all the involved co-workers and their role in the project. He will ensure that appropriate training relevant to the study is given to all of these staff, and that any new information of relevance to the performance of this study is forwarded to the staff involved.

7.2 Study Amendments

If it is necessary for the study protocol to be amended, the amendment and/or a new version of the study protocol (Amended Protocol) must be notified to and approved by the Competent Authority and the Ethics Committee according to EU and national regulations.

7.3 Audit and Inspections

Authorized representatives of a regulatory authority and Ethics Committee may visit the center to perform inspections, including source data verification. Likewise, the representatives from the sponsor may visit the center to perform an audit. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice (ICH GCP), and any applicable regulatory requirements. The Principal Investigator will ensure that the inspectors and auditors will be provided with access to source data/documents.

8 TRIAL SPONSORSHIP AND FINANCING

The study is sponsored by Akershus University Hospital with financial support from Helse Sør-Øst to a PhD position.

9 TRIAL INSURANCE

This study is covered by the general insurance of Akershus University Hospital.

10 ACKNOWLEDGEMENTS

A study protocol for a clinical trial of patients hospitalized with acute dyspnea as the inclusion criterion was initially developed in 2014-2015 by Prof Helge Røsjø in collaboration with Drs. Aagaard, Kvisvik, Caspersen, and Gravning.

The initial study protocol was never used for patient inclusion. Hence, Prof Røsjø in collaboration with Ass Prof Lyngbakken and R. Bhatnagar MD, Kristian Berge MD, and Professor Henrik Schirmer performed a revision of the protocol in 2021 and 2022 where inclusion criteria, screening strategy, intervention, and endpoints were revised. Hence, we consider version #2 to represent a revised *de novo* protocol and we will apply for renewed approvals from the Regional Ethics Committee and Data Protection Officer at Akershus University Hospital. Torbjørn Wisløff ,PhD, also contributed to the *de novo* revised protocol. The total study group, which is reported on the front page of the protocol, have also contributed to the new protocol by providing oral and written suggestions to the revised protocol (version #2).

11 APPENDIX

11.1 Echocardiographic protocol

ACE 4 – Akershus Cardiac Examination 4 Study

ECHOCARDIOGRAPHIC RECORDING PROTOCOL:

Required views for 2nd echo (approx. 30 min)

The number of loops and images to be recorded is minimum numbers (fifth column). All loops and images will be recorded in 50 mm/sec with the participants in supine and left lateral decubitus position at the end of expiration**.** The echo machine has to be set to "Cardiac mode", which is important for the 2D strain recordings. Also important for 2D strain analysis is to include all LV and LA endocardium (must be visible). Blood pressure in supine position between echo and linear recordings.

No	**Projection**	**Mode**	**Measurements to perform. Comments**	**Recordings**
1	PLAX	2D	Left atrium (LA), Left ventricle (LV) and LV outflow tract	4 loops
2	PLAX	M-mode	LV dimensions, LV mass	1 image with at least 5 HS
3	PSAX	2D	Presentation of the aortic valve, three or bi-leaflet	4 loops
4	PSAX	2D	At the papillary muscle level	4 loops
(4)	PSAX	M-mode	LV dimensions, LV mass. Recording only necessary if PLAX, M-mode (#2) not good	1 image with at least 5 HS
5	Apical 4-chamber (4CH)	2D	LV, RV, LA and RA. Measure: end systolic (es) and end diastolic (ed) volumes of LV (4C and 2C ad mode Simpson`s), es volume of VA (areal - length, biplane, 4C and 2C). 2-D strain systolic measurements of LV, RV and LA. (Important: endocardium must be visible and framerate per second between 40 – 90)	4 loops (Often 4 loops x 2, if RV and RA are not good enough recorded)
6	4CH	Pulsed Doppler (PW)	Transmitral early (E) and late (A) for the assessment of LV diastolic function. Peak velocity of E and A and deceleration time of E.	2 images with at least 5 HS
7	4CH	M-mode	TAPSE (tricuspid annular plane systolic excursion), which reflect RV systolic function.	1 image with at least 5 HS
8	4CH	Cont. wave Doppler (CW)	Pressure gradient between RV and RA	1 image with at least 5 HS

9	4CH	Tissue velocity imaging (TVI*)	Possibility to measure Systolic and diastolic TVI velocities in LA, LV, RA and RV: Frame rate >100/s. See point 4 regarding RA and RV	4 loops (often 4 loops x 2
10-11	4CH	TVI* + PW	Systolic S, diastolic E` an A` at septal and lateral mitral attachment.	2 images with at least 5 HS
12-13	4CH	TVI* + PW	Systolic S, diastolic E` and A` at lateral part of tricuspid valve and isovolumetric relaxation time.	2 images with at least 5 HS
15	Apical 2-Chamber (2CH)	2D	Volumes of VV and VA for the 2D strain analysis. 4 loops x 2 if VA not good recorded	4 loops
16	**2CH**	**TVI***	**TVI with frame rate >100/s**	**4 loops**
17	Apical long-axis (APLAX)	2D	Volumes of VV and VA for the 2D strain analysis.	4 loops
18	**APLAX**	**TVI***	**TVI with frame rate >100/s**	**4 loops**
19	4CH	3D	Volumes of VV and VA for the 3D strain. 4 loops extra if VA not good recorded	4 loops
20	2CH	3D	Volumes of VV and VA for the 3D strain. 4 loops extra if VA not good recorded.	4 loops
21	APLAX	3D	Volumes of VV and VA for the 3D strain. 4 loops extra if VA not good recorded	4 loops

***All TVI projections may be divided into 2 or more recordings if needed for frame rate >100/s.**

See separate description of additional echo recordings by pathological findings.

12 REFERENCES

1. Januzzi JL, et al. N-terminal pro–b-type natriuretic peptide in the emergency department. Journal of the American College of Cardiology 2018;71:1191-200.