STATISTICAL ANALYSIS PLAN for ACE 4 Study

Administrative information:

Sponsor name	Akershus University Hospital
Sponsor address	Division of Medicine 1478 Lørenskog, Norway
EudraCT number /REC no	REC 2015/1273
Trial title	Akershus Cardiac Examination (ACE) 4 Study: Pragmatic randomized controlled trial of early biomarker measurements and structured feedback in unselected patients with tachypnea
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SIGNATURE PAGE

PRINCIPAL/COORDINATING INVESTIGATOR:

Professor Magnus Lyngbakken, MD PhD
Department of Infectious Diseases, Division of Medicine, Akershus University Hospital

Signature

09/FEB/2026
Date (dd/mmm/yyyy)

TRIAL STATISTICIAN:

Torbjørn Wisløff, MSc, PhD
Health Services Research Unit
Akershus University Hospital

Torbjørn Wisløff
Signature

10.02.2026
Date (dd/mmm/yyyy)

DMC STATISTICIAN [if applicable]:

Maria Lie Selle, MSc, PhD
Health Services Research Unit
Akershus University Hospital

Maria Lie Selle
Signature

10/FEB/2026
Date (dd/mmm/yyyy)

ABBREVIATIONS

AE	Adverse Event
AUC	Area under the curve
CICU	Cardiac Intensive care unit
CI	Confidence Interval
DMC	Data Monitoring Committee
DSMB	Data safety monitoring board
ED	Emergency department
EHR	Electronic health record
HF	Heart failure
HFmrEF	HF with mildly reduced ejection fraction
HFpEF	HF with preserved ejection fraction
HFrEF	HF with reduced ejection fraction
ICU	Intensive care unit
KUHR	Control and payment of health reimbursements database
LVEF	Left ventricular ejection fraction
MICU	Medical intensive care unit
NEWS2	National Early Warning score 2
NPR	Norwegian Patient Registry
QALY	Quality-adjusted life-year
SAE	Serious Adverse Event
SD	Standard Deviation
SSB	Statistics Norway
TP	Time point
TSD	Services for sensitive data (University of Oslo)
TTE	Transthoracic echocardiography

TABLE OF CONTENTS

1 Introduction

1.1 Background and Rationale

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. In the ACE 4 Study we will test the strategy whether early biomarker assessment and structured feedback in the patient' EHR reduces clinical endpoints in unselected patients with tachypnea.

1.2 Intervention(s)

1.2.1 Brief description of the study intervention

Early structured biomarker assessment in unselected patients with tachypnea

1.2.2 Control

Current strategy/standard care

1.3 Trial Objectives

1.3.1 Primary Objective

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]

1.3.2 Secondary Objectives

The secondary objectives of this study are:

- 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

1.3.3 Exploratory Objectives (if applicable)

The exploratory objectives of this study is:

- 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 H2FPEF and HFA-PEFF scores
- The ACE 4 Study will phenotype participants with short-term Holter recordings (≤24 h), transthoracic echocardiography (TTE), and biospecimens collection for later molecular characterization in all participants and we will compare phenotypic traits with adjudicated diagnosis and clinical outcomes during follow-up

2 Trial Methods

2.1 Trial 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.

2.2 Randomisation

Randomization will be performed by a pre-determined computer algorithm that is set up by an independent statistician (Owen Thomas).

2.3 Statistical Framework

2.3.1 Hypothesis Test

This trial is designed 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

2.3.2 Confidence Intervals and p-values

All efficacy estimates will be presented with two-sided 95% confidence intervals. The primary outcome will be presented with confidence interval and two-sided p-value. As there is only one primary objective in this trial, there will be no adjustments for multiplicity.

2.4 Timing of Outcome Assessments

For analysis and tabulation purposes, we define study time points as:

Time Point Label	Definition (Day window)
TP1. Baseline	Information on admission
TP2. Discharge	Hospital stay

TP3. 30 days	TP1 + 30 days
TP4. 1 year	TP1 + 365 days
TP5. 3 years	TP1 + 3 years
TP6. 5 years	TP1 + 5 years
TP7. 10 years	TP1 + 10 years

If more than one visit fall into the same time point interval, information on all visits will be used in the analyses.

2.5 Statistical Interim Analyses and Stopping Guidance

The DSMB statistician has had access to data after 100, 200, and 400 included patients and the DSMB recommended continuation of the study at all DSMB meetings.

2.6 Timing of Main Analysis

The main analysis is planned when all patients have concluded 12 months of follow-up, all data up to 12 months have been entered, verified and validated and the primary database has been locked.

3 Trial Population

3.1 Screening Data, Eligibility and Recruitment

The total number of screened patients and reasons for not entering the trial will be summarised and tabulated.

A CONSORT flow diagram (appendix A) will be used to summarise the number of patients who were:

- assessed for eligibility at screening
- eligible at screening
- ineligible at screening*
- eligible and randomised
- eligible but not randomised*
- received the randomised allocation
- did not receive the randomised allocation*
- lost to follow-up*
- discontinued the intervention*
- randomised and included in the primary analysis
- randomised and excluded from the primary analysis*

*reasons will be provided.

3.2 Baseline Patient Characteristics

The patient demographics and baseline characteristics to be summarised separately for treatment and control groups include age, sex, comorbidities (coronary artery disease, heart failure, myocardial infarction, diabetes mellitus, chronic obstructive pulmonary disease, chronic kidney failure, peripheral artery disease), Charlson Comorbidity Index, and study-adjudicated cause for hospitalization for the index admission. Categorical data will be presented as absolute frequencies and percentages. For continuous data, N, mean±SD or median (25th and 75th percentiles).

3.3 Withdrawal/Follow-up

The status of eligible and randomised patients at trial end will be tabulated by treatment group according to

- completed intervention and assessments
- completed assessments but not intervention
- withdrew consent
- lost to follow-up

Outcomes with long-term follow-up (1 year) will be assessed based on data in the EHR. Data from registries will be merged to get update on whether persons are alive (SSB), still in the country (SSB), and all other potential contacts with the health services (NPR & KUHR).

Time to event variables and Kaplan-Meier product-limit estimates will be presented stratified by intervention group. Risk of events in time-to-event models will be assessed by adjusted Cox regression. The analyses will be conducted on all patient data one year after the time of inclusion of the last patient.

3.4 Adherence and Protocol Deviations

3.4.1 Adherence to Allocated Treatment

Patients will be analyzed based on the group they were randomized to, i.e. intention to treat. The number and % of participants receiving the intervention early structured biomarker assessment will be presented in a table grouped by randomization status.

3.4.2 Protocol Deviations

Patients will be analyzed based on the group they were randomized to, i.e. intention to treat. The number and % of participants receiving the intervention early structured biomarker assessment will be presented in a table grouped by randomization status.

3.5 Analysis Populations

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.

Over a period from February 1, 2023 until March 20, 2024, the ACE 4 Study enrolled a total of 575 patients hospitalized at Akershus University Hospital with tachypnea. At Akershus University Hospital, all patients are scored according to National Early Warning score 2 (NEWS2) in the triage section of the ED. 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 will 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 will check in the EHR of patients with tachypnea whether the patients fulfil 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 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 establish 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. 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.

The Intention To Treat Analysis Set (ITTAS) will be defined as all patients randomly assigned to a treatment group after randomisation.

The Per Protocol Analysis Set (PPAS) will include all randomised according to the treatment they received.

3.5.1 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 part of clinical routine in 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

3.5.2 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

4 Outcome Definitions

4.1 General Definitions and Derived Variables

4.1.1 ICU/MICU/CICU

- ICU: Intensive Care Unit
- MICU: Medical Intensive Care Unit
- CCU: Cardiac Care Unit

4.1.2 HFrEF, HFmrEF and HFpEF

- HFrEF (LVEF≤50%)
- HFmrEF (LVEF 40-50%)
- HFpEF (LVEF>50%)

4.2 Primary Outcome Definition

The primary outcome is defined as time to the first event for either (1) all-cause readmission or (2) all-cause mortality; i.e. time to the combined endpoint during the first 12 months after randomization. All-cause readmission include all emergency readmissions, but not including elective admissions (including but not limited to elective surgery or other elective admission to medicine) or transfers between departments or health trusts related to the index admission (including but not limited to transfers between departments for procedures or transfer to intensive care). We will also report data for extended follow-up after 3 years, 5 years, and 10 years for the primary outcome.

4.3 Secondary Outcomes Definitions

4.3.1 Hospital length of stay

Length of stay in hospital during index stay, as reported in EHR.

4.3.2 ICU/MICU/CICU length of stay

Length of stay in ICU/MICU/CCU, as reported in EHR.

4.3.3 All-cause readmission

All-cause readmission will be analysed in three different ways;

- as dichotomous variable during 30 days
- as time-to-event variable during 12 months (and later 3, 5 and 10 years)
- as continuous count variable counting number of admissions after the index stay

Readmission is defined as stay in hospital, i.e. not outpatient visit or other visit during daytime. They include all emergency readmissions but not including elective admissions (including but not limited to elective surgery or other elective admission to medicine) or transfers between departments or health trusts related to the index admission (including but not limited to transfers between departments for procedures or transfer to intensive care). In the time-to-event variable, time is only counted until the first admission. For the count variable, we also gather data on timing of each readmission to allow for possible analysis using multiple failure time models.

4.3.4 All-cause mortality

Mortality will be gathered from either the hospital system or SSB and will be recorded as a time variable, which then can be used in further defining dichotomous variables within the given time intervals.

4.3.5 Total cost

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.

4.3.6 Troponin T

Will be measured both at admission and in the stable phase prior to discharge. We will calculate relative change in each group by dividing the absolute change in troponin T concentration from admission to discharge (Δ troponin T) with the admission troponin T concentration.

4.3.7 Troponin I

Will be measured both at admission and in the stable phase prior to discharge. We will calculate relative change in each group by dividing the absolute change in troponin I concentration from admission to discharge (Δ troponin I) with the admission troponin I concentration.

4.3.8 B-type natriuretic peptide

Will be measured both at admission and in the stable phase prior to discharge. We will calculate relative change in each group by dividing the absolute change in BNP concentration from admission to discharge (Δ BNP) with the admission BNP concentration.

4.3.9 N-terminal pro-B-type natriuretic peptide

Will be measured both at admission and in the stable phase prior to discharge. We will calculate relative change in each group by dividing the absolute change in NT-proBNP concentration from admission to discharge (Δ NT-proBNP) with the admission NT-proBNP concentration.

4.3.10 Guideline-defined medical therapy for HF if categorized into HF

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).

We will assess these medication classes for HFrEF/HFmrEF (any dose):

- B-blockers
- ACEI/ARNI (ARB)
- Aldosterone inhibition
- SGLT2-receptor inhibitor

We will assess these medication classes for HFpEF (any dose):

- Aldosterone inhibition
- SGLT2-receptor inhibitor

4.3.11 Cost-utility

Cost-utility will be calculated by dividing difference in total cost by difference in total quality-adjusted life years (QALYs). QALYs in each arm will be calculated as quality-adjusted survival, i.e. quality of life measured by EQ-5D at different time points and assuming the value 0 after death.

4.4 Overview of Outcomes

The table includes both primary and secondary outcomes. Explorative outcomes not included.

Level	Outcome	Timeframe	Type
Primary	Time to readmission or death	12 months	Time to event
Secondary	Hospital length of stay	Index stay	Time
	ICU/MICU/CICU length of stay	Index stay	Time
	All-cause readmission	30-day	Dichotomous
	All-cause readmission	12 months	Time-to-event
	All-cause readmission	12 months	Count
	All-cause mortality	12 months	Time-to-event
	Total cost	12 months	Cost (continuous)
	All-cause mortality	Index stay	Time-to-event
	Troponin T	Index stay	Continuous
	Troponin I	Index stay	Continuous
	B-type natriuretic peptide	Index stay	Continuous
	N-terminal pro-B-type natriuretic peptide	Index stay	Continuous
	Guideline-defined medical therapy for HF if categorized into HF	Index stay	Dichotomous
	Cost-utility	12 months	Cost per QALY

5 Analysis Methods

5.1 Methods for Primary Outcome

5.1.1 Descriptive Statistics

Descriptive statistics will include number and percentage by treatment group. Descriptive statistics will be based on non-imputed data, thus the number of evaluable outcome measurements at the time of primary interest (12 months) will also be presented.

5.1.2 Primary Inferential Analysis

Time to the combined endpoint readmission or death will be analysed using a Cox regression model with treatment as dichotomous covariate, adjusted for covariates with assumed impact on outcome to reduce uncertainty. Patients still in hospital at the end of follow-up will be censored at maximum follow-up. Time-to-event outcomes will also be presented with a Kaplan-Meier curve with a separate curve for each intervention and with differences between groups assessed by the log-rank test. Analyses will be performed on the ITTAS.

5.1.3 Effect Estimates

The primary effect estimate will be the adjusted hazard ratio, computed from the Cox regression. The hazard ratio will also be reported together with the 95% confidence interval.

5.1.4 Assumption Checks and Alternative Analyses

For the primary analysis we will perform a test of the assumption of proportional hazards based on Schoenfeld residuals. If proportionality assumption is not fulfilled, the primary analysis will be performed as restricted mean survival time

5.1.5 Missing Data

For the primary outcome, missing data are likely not possible. If the event is not recorded as occurred during the follow-up, it is assumed censored.

5.1.6 Sensitivity Analyses

- A separate analysis of the primary outcome will be performed based on the PPAS.
- If there are patients without either discharge date or date of death at 12 months, we will perform a separate analysis where patients without discharge date will be given the maximum number of hospital days among the population

5.1.7 Subgroup Analyses

Predefined subgroup analysis will be done by including a treatment-diagnosis interaction term in the Cox regression model, and the resulting treatment effect by diagnosis will be presented using a forest plot. Planned subgroups include:

- Patients classified as hospitalized due to HF, as assessed by the adjudication committee (vs. patients hospitalized due to other causes)
- Patients stratified by LVEF: HFrEF vs. HFmrEF vs. HFpEF
- Patients stratified by biomarker concentrations measured on the first study visit (study inclusion), including:
    - B-type natriuretic peptides
    - Cardiac troponins
    - Additional biomarkers (such but not limited to secretoneurin)
- Patients admitted to cardiac wards vs. patients admitted to non-cardiac wards

5.2 Methods for Dichotomous Secondary Outcomes

5.2.1 Primary Inferential Analysis

All dichotomous outcomes will be analysed with logistic regression adjusting for age, sex, comorbidities, and cause of index admission.

5.2.2 Effect Estimates

Results will be presented as odds ratios with 95% confidence intervals.

5.2.3 Assumption Checks and Alternative Analyses

5.2.4 Missing Data

Missing data for the outcomes will not be imputed. Missing data for explanatory factors may be imputed if more than 5%.

5.3 Methods for Continuous Secondary Outcomes

5.3.1 Primary Inferential Analysis

All continuous outcomes will be analysed with linear regression adjusting for age, sex, comorbidities, and cause of index admission.

5.3.2 Effect Estimates

Effect estimates will be reported as estimated with 95% confidence intervals.

5.3.3 Assumption Checks and Alternative Analyses

Assumptions will be checked using QQ plots. If QQ plots indicate diversion from assumption of normal distribution of residuals, different link functions and distributional families will be tested to optimize best fit.

5.3.4 Missing Data

Missing data for the outcomes will not be imputed. Missing data for explanatory factors may be imputed if more than 5%.

5.4 Methods for Time to Event Secondary Outcomes

Time-to-event secondary outcomes will be analysed as the primary endpoint, except for no sensitivity and subgroup analyses.

5.5 Methods for Count Secondary Outcomes

5.5.1 Primary Inferential Analysis

Count endpoints will be analysed using a negative binomial regression model with treatment as dichotomous covariate, adjusted for covariates with assumed impact on outcome.

5.5.2 Effect Estimates

Results will be presented as incidence rate ratios with 95% confidence intervals.

5.5.3 Assumption Checks and Alternative Analyses

Alternative analyses will be performed using Poisson regression. If the Poisson regression indicates better fit than the negative binomial, analyses will be presented using Poisson instead of negative binomial regression.

5.5.4 Missing Data

Since all data are gathered from the EHR at Ahus, we assume that all counts without a value indicates no events. Missing data for the outcomes will therefore not be imputed. Missing data for explanatory factors may be imputed if more than 5%.

5.6 Methods for Cost Secondary Outcomes

5.6.1 Primary Inferential Analysis

All cost data will be analysed with generalised linear regression with gamma family and log link adjusting for age, sex, comorbidities, and cause of index admission.

5.6.2 Effect Estimates

5.6.3 Assumption Checks and Alternative Analyses

5.6.4 Missing Data

5.6.5 Sensitivity Analyses

5.6.6 Subgroup Analyses

5.7 Methods for Cost-utility analysis

5.7.1 Primary Inferential Analysis

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.

All cost data will be analysed with generalised linear regression with gamma family and log link adjusting for age, sex, comorbidities, and cause of index admission. EQ-5D will be analysed using beta regression.

5.7.2 Effect Estimates

5.7.3 Assumption Checks and Alternative Analyses

5.7.4 Missing Data

5.7.5 Sensitivity Analyses

5.7.6 Subgroup Analyses

5.8 Additional Analyses

- Assessing accuracy for clinical and biochemical diagnostic scores for diagnosing HFpEF; e.g. the H2FPEF and HFA-PEFF scores
- The ACE 4 Study will phenotype participants with short-term Holter recordings (≤24 h), transthoracic echocardiography (TTE), and biospecimens collection for later molecular characterization in all participants and we will compare phenotypic traits with adjudicated diagnosis and clinical outcomes during follow-up

5.9 Sample size

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).

6 Safety Analyses

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.

At each interim analysis, data on the following four variables will be extracted and analysed by the DSMB statistician:

- Hospital length of stay
- (30-day) readmission
- All-cause mortality

No formal stopping rule for safety will be applied, as this will be up to the data safety monitoring committee.

6.1 Adverse Events

Death is the only adverse event registered in the trial. For safety analyses, see previous paragraph

7 Statistical Software

All statistical analyses will be done in R using Rstudio in the version provided by TSD.

8 References

1. Guidelines for the Content of Statistical Analysis Plans in Clinical Trials. 2017;318(23):2337-2343. doi:10.1001/jama.2017.18556.