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Biomarkers for length of hospital stay, changes in muscle mass, strength and physical function in older medical patients: rationale and methodology of the Copenhagen PROTECT study. A prospective cohort study.

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9 strength and physical function in older medical patients:
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12 rationale and methodology of the Copenhagen PROTECT
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17 study. A prospective cohort study.
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ABSTRACT

Introduction

Sarcopenia is generally used to describe the age-related loss of muscle mass and strength believed to play a major role in the pathogenesis of physical frailty and functional impairment that may occur with old age.

The knowledge surrounding the prevalence and determinants of sarcopenia in older medical patients is scarce, and it is unknown whether specific biomarkers can predict physical deconditioning during hospitalization. We hypothesize that a combination of clinical, functional, and circulating biomarkers can serve as a risk stratification tool and can i) identify older acutely ill medical patients at risk of prolonged hospital stays and ii) predict changes in muscle mass, muscle strength, and function during hospitalization.

Method and analysis

The Copenhagen PROTECT study is a prospective cohort study consisting of acutely ill older medical patients admitted to the acute medical ward at Copenhagen University Hospital, Bispebjerg and Frederiksberg, Denmark. Assessments are performed within 24 hours of admission and include blood samples, body composition, muscle strength, physical function, and questionnaires. A subgroup of patients transferred to the Geriatric Department are included in a smaller geriatric cohort and have additional assessments at discharge to evaluate the relative change in circulating biomarker concentrations, body composition, muscle strength, and physical function during hospitalization. Enrollment commenced November 4th, 2019, and proceeds until May 3rd, 2021.

Ethics and dissemination

The study protocol has been approved by the local ethics committee (H-19039214) and the Danish Data Protection Agency (P-2019-239). Findings from the project, regardless of the outcome, will be published in relevant peer-reviewed scientific journals and at www.clinicaltrials.gov.

Trial registration number

Clinicaltrials.gov, ID: NCT04151108

ARTICLE SUMMARY

Strengths and limitations of this study

- A strength of the study is the large heterogeneous population, which brings generalizability to the study results.
- The assessments of physical function applied in the study have previously been evaluated in acutely admitted older medical patients.
- Bio-electrical impedance analysis (BIA) may be affected by the hydration status of the patients.
- There are no direct measurements of the physical activity levels of the patients during admission.
- The study estimates stature by knee-height measurements, as many patients are unable to stand for height measurements.

BACKGROUND

It is well established that human skeletal muscle function declines with aging, and sarcopenia is generally used to describe the age-related skeletal muscle atrophy and loss of muscle strength believed to play a major role in the pathogenesis of physical frailty, loss of independence, and functional impairment that may occur with old age.[1-3] Clinical sarcopenia has been defined in statistical terms assuming a lower normal limit of two

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4 standard deviations below a mean relative appendicular muscle mass in young healthy
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8 adults.[4] The prevalence of sarcopenia is estimated at 5%-13% in 60-70 year-olds and
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11 11%-50% in individuals aged 80 years or older.[5]
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15 The etiology of sarcopenia is complex and involves neuronal, hormonal, immunological, and
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18 nutritional mechanisms.[6-10] Furthermore, physical inactivity, chronic diseases,
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21 immobilization, and hospitalization are known to play a part in the development of
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24 sarcopenia.[6,11-13]
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30 In 2018, approximately 45% of all hospital admissions in Denmark concerned patients aged
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33 65 or older who had a mean length of stay of 3.5 days.[14] Older patients are often inactive
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36 during hospitalization spending 71%-83% of their time lying down,[15,16] and at least 35%
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38
39 of older patients lose independence in one basic Activity of Daily Living (ADL) as an
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41
42 unintended consequence of a medical illness and hospitalization.[17] Sarcopenia may
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45 aggravate this functional decline, as patients with sarcopenia have an attenuated recovery
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48 of their functional levels 3 months following discharge.[18] From a clinical perspective,
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51 sarcopenia is associated with infectious complications, readmissions, increased need for
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4 rehabilitation following discharge, reduced quality of life, increased mortality, and longer
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8 hospitalization.[4,19]
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12 Early mobilization protocols have proven effective in reducing hospital-acquired disability
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15 and hospital length of stay. However, frequently reported barriers for implementation of early
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18 mobilization include lack of staff and time to enable mobilization of the patient.[20] With an
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21 increasing aging population and the heterogeneousness of older individuals, the systematic
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24 identification of older individuals at risk of prolonged hospitalization and deconditioning
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27 during hospitalization are of outmost importance. As such, we need to develop risk
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30 stratification tools to identify older patients at risk of these adverse outcomes.
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37 **STUDY OBJECTIVES AND HYPOTHESES**

41 **Primary objectives and hypothesis**

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45 We aim to examine whether circulating biomarkers at admission are associated with length
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48 of hospital stay in older (≥ 65 years) acutely admitted medical patients and whether the
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51 combination of clinical and functional measures with these biomarkers can identify patients
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54 at risk of having a prolonged hospital stay (>96 hours). In addition, we aim to establish
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4 circulating biomarkers associated with changes in muscle mass, muscle strength, and
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8 function in geriatric patients during hospitalization. We hypothesize that a combination of
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11 clinical and functional measures with circulating biomarkers has the potential to identify
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15 older (≥ 65 years) acutely admitted medical patients at risk of prolonged (≥ 96 hours)
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18 hospital stays and physical deconditioning during hospitalization.
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20 21 22 **Secondary objectives and hypothesis**

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27 The secondary objectives are to determine whether circulating biomarkers are associated
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30 with readmissions within 90 days of discharge, frailty, discharge to a higher level of care,
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33 and all-cause mortality within 90 days of the index admission and whether the combination
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37 of clinical and functional measures with these biomarkers can identify patients at risk of
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40 readmissions, discharge to a higher level of care, and all-cause mortality. We hypothesize
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44 that a combination of clinical and functional measures with circulating biomarkers has the
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47 potential to identify older (≥ 65 years) acutely admitted medical patients at risk of non-
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51 elective readmissions within 90 days of discharge, discharge to a higher level of care, and
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54 all-cause mortality within 90 days of the index admission.
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METHODS AND ANALYSIS

Setting and intervention

The Copenhagen PROTECT study is a prospective cohort study consisting of acutely ill older medical patients admitted to the acute medical ward at Copenhagen University Hospital, Bispebjerg and Frederiksberg, Denmark. A subgroup of these patients, subsequently transferred to the Geriatric Department, are also included in a smaller geriatric cohort. Enrollment commenced November 4th, 2019 and will proceed until May 3rd, 2021.

Eligible patients

The current study is recruiting participants during a 1.5-year period to avoid any seasonal differences in the patient population and to take into account the temporary pause in recruitment due to the Covid-19 pandemic. We aim to include a total of 1700 patients representing the PROTECT cohort, of which approximately 400 patients subsequently will be transferred to the Geriatric Department and constitute the Geriatric cohort. All patients admitted at the acute medical ward at Copenhagen University Hospital, Bispebjerg and Frederiksberg who fulfill the inclusion criteria and do not meet any exclusion criteria are

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4 eligible for the study (Table 1). The hospital admission during which the patient is recruited
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8 represents the index admission. Any subsequent non-elective admissions of included
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11 patients during the study inclusion period will be interpreted as readmissions. Included
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15 patients will be followed for 90 days following discharge from index admission to
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18 investigate future readmissions and mortality.
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27 *Table 1. Inclusion and exclusion criteria*

Inclusion criteria
Equal to or over the age of 65 years
Acutely admitted with a medical diagnosis (i.e. non-surgical)
Exclusion criteria
Admitted for more than 24 hours prior to baseline assessment
Terminal illness (expected life span of less than 6 months)
Temporary civil registration number
Droplet or airborne infections requiring isolation
Does not speak or read Danish
Patients judged medically contraindicated by health personnel
Inability to provide informed consent for participation

54 Outcomes

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4 The primary outcome in the PROTECT cohort is the length of hospital stay. As successive
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8 events of hospitalisation have been suggested to contribute to the development of
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11 sarcopenia, and even short periods (4-5 days) of skeletal muscle disuse are known to
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15 induce muscle atrophy,[21,22] we have defined a prolonged hospital length of stay as an
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18 admission lasting >96 hours. In 2018, the mean length of hospital stay in Denmark was 84
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22 hours in patients aged 65 years or over.[14]
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26 The primary outcomes in the Geriatric cohort are the relative changes in muscle mass,
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29 muscle strength, and muscle function during hospitalization. Primary and secondary
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33 outcomes for both cohorts are listed in table 2.
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40 *Table 2. Primary and secondary outcomes*

The PROTECT cohort	The Geriatric cohort
Primary outcome	Primary outcomes
Length of hospital stay	Changes in muscle mass during hospitalization
	Changes in muscle strength during hospitalization
	Changes in muscle function during hospitalization
Secondary outcomes	Secondary outcomes
Non-elective readmissions within 90 days of discharge	Length of hospital stay

All-cause mortality within 90 days of index admission	Non-elective readmissions within 90 days of discharge
In-hospital mortality	All-cause mortality within 90 days of index admission
Muscle mass at admission	In-hospital mortality
Muscle strength at admission	Discharge to an increased level of care
Muscle function at admission	Frailty
Frailty	

We have defined geriatric patients discharged to an increased level of care as i) patients receiving increased relief in terms of walking aids or patients with an increased need for caregiver assistance or home care, ii) patients referred to rehabilitation or 24-hour care, or iii) patients moving to a nursing home following discharge. Data on readmissions will be limited to non-elective readmissions in Region Zealand and the Capital Region of Denmark. A geriatrician will evaluate whether the readmission is related to the index admission; i.e. newly emerged acute illness following the index admission, acute aggravation of disease treated during the index admission, or complication to treatment during the index admission.

Assessment and randomization

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5 The research personnel might be unable to assess all patients, as the number of eligible
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8 patients (i.e. fulfilling inclusion criteria with the absence of exclusion criteria) varies daily.
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11 Thus, to avoid selection bias, all eligible patients on the day in question are randomized
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14 using a computer-generated randomization sequence to establish a randomized visitation
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18 sequence. Patients who wish to participate sign an informed consent and baseline
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22 measurements are performed within the first 24 hours of admission.
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26 All included patients have blood samples drawn to determine concentrations of tumor
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29 necrosis factor (TNF)- α , interferon gamma (IFN- γ), interleukin (IL)-6, IL-10, IL-13,
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33 transforming growth factor (TGF)- β 1, follistatin, insulin-like growth factor (IGF)-1, growth
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37 differentiation factor (GDF)-11, GDF-15, and soluble urokinase-type plasminogen activator
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40 receptor (suPAR).
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44 Handgrip strength is assessed using a digital hand-held dynamometer (Model SH1001;
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47 SAEHAN Corporation, Yangdeok-Dong, Masan, South Korea). Patients able to leave the
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51 bed sit on a chair with the elbow flexed at 90° and the wrist in a neutral position, while
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55 bedridden patients are assessed in the hospital bed with the backrest elevated. The
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59 highest value of three attempts with the dominant hand is used for analyses. Should the
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5 third trial elicit the highest value, the patient continues until a lower value is achieved.
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8 Muscle function is assessed in the 30-second sit-to-stand test, where patients are asked to
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11 stand up from a standardized chair as many times as possible with their arms folded
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15 across the chest. Only full standing positions are counted.
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19 Patients included in the Geriatric cohort also have their habitual gait-speed assessed. The
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22 gait-speed assessment is measured over a course of 4 meters and includes walking aids if
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25 they are used by the patient. Patients stand behind a starting line and are asked to start
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28 walking towards a visual goal at their habitual pace. The visual goal is placed after 5.5 m.
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32 to reduce the effect of deceleration. The fastest of the two attempts will be used for
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35 analyses and quantified as m/s.
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40 The assessment of handgrip strength (kg) and habitual gait-speed have previously shown
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43 to be feasible and reliable measures in acutely older medical patients. However, the
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47 feasibility and reliability of the 30-second sit-to-stand test was moderate, as only half of the
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50 patients were able to perform the test as instructed.[23] Thus, we have included an
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54 additional nominal variable to categorize the sit-to-stand ability as either i) able to perform
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4 the test as instructed, ii) ability to rise using the armrest, and iii) inability to rise
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8 independently from a chair.
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12 Bodyweight (kg) is assessed using chair scales and height (cm) is estimated with a
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15 segmometer using the knee-height measurement and age with the equations from

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19 Chumlea et al.[24] Body composition is assessed using Direct-Segmental Multi-frequency

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23 Bioelectrical Impedance Analyses (DSM-BIA) (InBody S10®; Biospace Co., Ltd., Seoul,

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26 Korea). Self-reported current smoking is reported as a dichotomous variable. Patients

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29 included in the Geriatric cohort are also assessed at discharge to evaluate circulating

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33 biomarker concentrations as well as changes in body composition, muscle strength, and

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37 functional performance. Tests of strength, physical function and body composition

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39
40 measurements are performed by trained research personnel.
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44 The presence of frailty is assessed by trained nurses associated with the study using the

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46
47 Canadian Study of Health and Aging Clinical Frailty Scale (CFS).[25] Patients are

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50 screened for sarcopenia using the SARC-F questionnaire,[26] while cognitive status is

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54 evaluated by the short Orientation-Memory-Concentration test (OMC).[27] The risk of

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58 malnutrition is assessed and validated using the Short Nutritional Assessment
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4 Questionnaire (SNAQ).[28] A flowchart showing the timeline and assessments in the two
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8 cohorts can be seen in Figure 1.
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12 *INSERT FIGURE 1 AROUND HERE*
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15 Information on medical treatment is evaluated by counting all prescribed medications,
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18 including unscheduled medications, except for the following:
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- 20
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22
23 ▪ Eyedrops
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25 ▪ Eardrops
26
27 ▪ Lotions and ointments
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29 ▪ Antibiotic treatment of limited duration
30
31 ▪ Multivitamins
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33 ▪ Supplementary nutrition or tube feeding
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39 Medications listed twice containing the same substance are only counted once.
40

41
42 Comorbidity is evaluated by the Charlson Comorbidity Index (CCI)[29] and obtained by
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45
46 evaluating the type and number of ICD-10 discharge diagnosis during the last 5 years of
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48
49 the index admission. Data on emigration and all-cause mortality within 90 days of index
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53 admission is extracted from the Danish Civil Registration System. A summary of variables
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assessed by research personnel and extracted from the electronic patient system (EPIC)

or the Danish Civil Registration System are listed in table 3.

Table 3. Variables assessed by research personnel and extracted from EPIC or The Danish Civil Registration System.

	Assessed by research personnel	Extracted from EPIC or the Danish Civil Registration System
Descriptive information		
Age		x
Gender		x
Smoking	x	
Emigration		x
Clinical information		
Hospital length of stay		x
Main diagnosis (index admission)		x
Non-elective readmissions within 90 days of discharge		x
Main diagnosis (readmission)		x
In-hospital mortality		x
All-cause mortality within 90 days of index admission		x
Prescribed medications upon admission		x
ICD-10 discharge diagnoses 5 years prior to index admission		x
Number of hospitalizations (acute and elective) one year prior to the index admission		x
Vital values (saturation, respiratory rate, heart rate, blood pressure, core temperature, Glasgow coma scale) upon admission		x
Early Warning Score (EWS) upon admission		x
Electrocardiographic (ECG) abnormalities upon admission		x
Admission to the intensive care unit (ICU) (admission date, discharge date, treatment with vasopressors, dialysis and mechanical ventilation)		x

Sepsis during the index admission†	x
Braden Score	x
Anthropometry and physical function	
Bodyweight (kg)	x
Height (cm)	x
Body composition (DSM-BIA)	x
Handgrip strength (kg)	x
Sit-to-stand ability	x
Sit-to-stand ability, categorical	x
Habitual gait-speed* (m/s)	x
Canadian Study of Health and Aging Clinical Frailty Scale (CFS)	x
SARC-F score	x
Use of walking aids at index admission	x
Discharge to an increased level of care*	x
Barthel Index at admission*	x
Barthel Index at discharge*	x
Cumulated Ambulation Score (CAS)*	x
New Mobility Score (NMS)*	x
De Morton Mobility Index (DEMMI) score*	x
Cognition	
Dementia diagnosis	x
Orientation-Memory-Concentration test (OMC)	x
Nutrition	
Short Nutritional Assessment Questionnaire (SNAQ)	x
Blood	
Results of routine blood tests upon admission (C-reactive Protein (CRP), albumin, urea, creatinine, hemoglobin, white blood cells, platelets, potassium, sodium, glomerular filtration rate (GFR), liver biochemistry, glucose, calcium, magnesium, lactate, and other routine blood samples)	x
Results of PROTECT blood tests (TNF- α , IL-6, IL-10, IL-13, IFN- γ , TGF- β , GDF-11, GDF-15, follistatin, IGF-1, and suPAR)	x

* denotes variables included in the geriatric cohort only.

† Sepsis is defined in accordance with the Sepsis-3 criteria.[60]

Data management

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5 Following data acquisition, all physical documents are stored in accordance with the
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8 guidelines for data management from the Danish Data Protection Agency. Electronic data
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10
11 is managed and stored using Research Electronic Data Capture (REDCap)[30,31], a web-
12
13
14 based secure software platform hosted at Bispebjerg-Frederiksberg University Hospital. To
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16
17 ensure data quality, the REDCap database was built to ensure data integrity including real-
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20 time data validation, integrity checks, and range checks for data values.
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26 **Patient and public involvement**

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30 Upon request, patients with measures of muscle mass, strength or function can gain
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33 insight into their values and receive advice to improve from either an exercise physiologist
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36 or a physiotherapist. Patients are not involved in the study design, recruitment, or other
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40 aspects of the study.
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45 **Power calculation and statistics**

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49 To evaluate the prognostic abilities of circulating biomarkers (individually, in combination
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52 and combined with clinical and functional measures) we will use the area under the curve
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54
55 for receiver operating characteristics (AUROC) statistics. A reference group of 2058
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4 patients over the age of 65 from Bispebjerg-Frederiksberg University Hospital and Herlev-
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8 Gentofte Hospital had a mean age of 78.3 years and a mean length of stay of 5.8 days
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10
11 during hospitalization. In these patients, 817 (39.7%) had a prolonged length of stay,
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14 defined as a hospitalization lasting more than 96 hours. With a sample size of 1700 and
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16
17 the assumption that approximately 40% of older medical patients have a prolonged
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19 hospital stay, an AUROC of 82 will have a power of 0.9 with a significance level of 0.05.
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26 A table of summary statistics will be presented with baseline variables. Continuous
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28 variables will be summarized with: n (non-missing sample size), mean, standard deviation,
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30 median, interquartile range, and number of missing values. Categorical variables will be
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32 reported as frequency and percentages (based on non-missing sample size), and number
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34 of missing values. Data missing at random will be imputed using multiple imputation.
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44 To evaluate whether clinical, functional, and circulating biomarkers are associated with
45
46 length of stay we will perform multivariate logistic regression. Patients will be grouped in
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48 either normal (< 96 hours) or extended length of stay (\geq 96 hours) and Cox regression
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50 analysis will be used to compare differences in non-elective readmission and all-cause
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52 mortality. Patients will be followed from the date of discharge from the index admission
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4 until the end of the follow-up period, emigration, readmission, or death as appropriate. To
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8 assess the discriminative ability of biomarkers with regards to an extended length of stay
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11 and all-cause mortality, we will use the area under the curve (AUC) for receiver operating
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13
14 characteristics (ROC) curves. AUCs for different ROC curves will be compared using the
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17 DeLong test. The association of circulating biomarkers with changes in muscle mass,
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22 muscle strength, and function in the Geriatric cohort will be assessed using a multivariate
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26 linear model adjusted for the relative length of stay.
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29 **Study organization**

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34 The study is a researcher initiated clinical study. The protocol was written by the steering
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36
37 committee composed of experts in geriatric medicine and acute medicine and a PhD
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39
40 student in clinical medicine. The committee is responsible for the design of the study,
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44 supervision of research personnel, data acquisition, communication and publication of
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48 results, approval of sub-studies and ensuring that future studies comply with the
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51 regulations regarding data management.
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4 At present (July 2020), the study has included 377 patients, of which 62 are part of the
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8 Geriatric cohort. Inclusion was temporarily paused due to the impact of the Covid-19
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11 pandemic.
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14 15 16 **ETHICS AND DISSEMINATION**

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18 All procedures are being conducted according to “Good Clinical Practice” standards,
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21 regarding initiation, monitoring, and reporting. The study protocol has been approved by
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24 the local ethics committee (H-19039214) and the Danish Data Protection Agency (P-2019-
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27 239), and the project complies with the regulations of the General Data Protection
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30 Regulation (GDPR) and the Data Protection Act.
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36 All eligible patients receive oral and written information. In the case of severe dementia or
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39 delirium, some patients might be unable to provide participant consent. In these cases, we
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41
42 seek participant consent from a close relative or guardian, should the guardianship include
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45 access to sign participant consent for research purposes. Independent medical doctors
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48 who have knowledge of the project but are not associated with the project and are
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51 independent of the interests of the principal investigator evaluate whether these subjects
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54 can participate in the study.
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4 The project entails minimal discomfort and no permanent side effects; thus, it is
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8 considered ethically sound. Findings from the project, regardless of the outcome, will be
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11 published in relevant peer-reviewed scientific journals ensuring the anonymity of the
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14 patients. The study is registered at clinicaltrials.gov (NCT04151108) where positive,
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18 negative, or inconclusive results will be published.
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22 **DISCUSSION**

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27 The importance of sarcopenia has recently been underlined by its inclusion as a reportable
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30 disease in the Centers for Disease Control and Prevention (ICD-10-CM code M62.84) in
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33 October 2016.[32] Even though the serious consequences of sarcopenia are widely
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35
36 recognized, the diagnose has yet to be implemented in clinical practice in Denmark. Lack of
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39 knowledge regarding the basic biological mechanisms driving sarcopenia in conjunction with
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42 other age-related diseases and lack of systematic assessment hinders the identification and
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45 treatment of sarcopenia and can lead to physical deconditioning during hospitalization. As
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48 such, the knowledge surrounding the prevalence and determinants of sarcopenia in older
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51 medical patients is scarce, and it is unknown whether circulating biomarkers, individually or
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55 in combination, can predict physical deconditioning during hospitalization.
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4 Mechanisms that regulate skeletal muscle mass are central to the understanding of
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8 sarcopenia. Myostatin, also referred to as GDF-8, is a part of the TGF- β family and
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11 predominantly expressed in skeletal muscle. Myostatin and TGF- β are inducers of catabolic
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14 processes, inhibiting muscle growth, and inducing muscle protein breakdown via activation
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16
17 of the Small Mothers Against Decapentaplegic (SMAD)2 and SMAD3 transcription
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19 factors.[33,34] Myostatin is reportedly increased with aging,[35] and following prolonged bed
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22 rest,[36] and treatment with myostatin antibodies attenuates the loss of muscle mass and
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25 function induced by immobilization in mice.[37]

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33 Recently, GDF-11, a TGF- β family ligand,[38] has been measured in human blood
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36 samples.[39] High circulating GDF-11 levels have been related to increased disease burden
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39 and elevated risk of post-operative complications and mortality in older adults undergoing
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41
42 heart surgery. Notably, patients categorized as physically frail based on low handgrip
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45 strength and gait speed as well as self-reported activity measures had significantly higher
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47
48 GDF-11 levels compared to non-frail controls.[39]

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54 GDF-15, another member of the TGF- β family, is present in low levels under healthy
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57 conditions but can increase during disease or injury and contribute to muscle wasting by
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4 suppressing appetite, which may result in anorexia and drastic weight loss.[40] In older
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8 patients, unintentional weight loss has been associated with an increased in-hospital
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11 morbidity and increased overall mortality.[41] GDF-15 may be induced in response to
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14 cellular stress signals or dysfunctions, and it has been suggested that circulating levels of
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17 GDF-15 could be biomarker of mitochondrial dysfunction.[42] Nonetheless, several studies
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20 demonstrate that GDF-15 levels are predictors of all-cause mortality.[43,44]
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26 Follistatin acts as an antagonist to TGF- β family ligands including myostatin, TGF- β and
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29 GDF11.[34] Measurements of TGF- β ligands as well as their antagonist follistatin could
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32 represent biomarkers of muscle breakdown, physical function or mortality. However, the
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35 translation of these findings into clinical utility needs further validation in a larger cohort.
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41 Several studies have investigated the association of inflammatory biomarkers with muscle
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44 mass, muscle strength, and muscle function in healthy older subjects. Most commonly,
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47 studies have focused on a few biomarkers, such as TNF- α , IL-6 or C-Reactive Protein
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50 (CRP).[9,45-47] A recent study has demonstrated an inverse relationship between a
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53 composite of pro- and anti-inflammatory markers and muscle mass, strength and function in
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56 healthy older subjects.[48] However, results are inconsistent and lack clear evidence as to
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4 whether these inflammatory biomarkers are associated with sarcopenia. Nonetheless,
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8 circulating levels of CRP are predictive of both the length of hospital stay and
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11 readmissions.[49,50] Indeed, geriatric patients with inflammation, evaluated by CRP levels
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15 at admission, stayed on average 3 days longer than patients without inflammation.[51]
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19 The anabolic growth factor, IGF-1, and the IGF-1/phosphatidylinositol 3-kinase(PI3K)/Akt
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22 pathway is involved in skeletal muscle hypertrophy and atrophy.[52,53] Circulating IGF-1
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24
25 levels decrease with aging, while inflammatory markers such as TNF- α and IL-6 can
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29 interfere with the IGF-1 signaling pathway.[54,55] As such, changes in IGF-1/PI3K/Akt
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31
32 signaling during aging may be a result of decreased IGF-1 expression as well as IGF-1
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35 inhibition. Notably, no difference in circulating IGF-1 concentrations were found between
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40 older sarcopenic and non-sarcopenic women.[56]
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44 Recently, suPAR was established as a biomarker of inflammation and immune activation,
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47 and elevated levels of suPAR are believed to reflect a state of chronic inflammation.[57]
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51 SuPAR correlates with other inflammatory markers, such as TNF- α , and patients with the
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54 highest levels of suPAR generally have the worst prognosis.[58] In one study, suPAR was
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58 associated with low muscle mass, while IL-6 was associated with low muscle mass and
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4 increased fat mass in both patients and healthy controls.[59] Thus, there seem to be distinct
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8 inflammatory processes occurring simultaneously with different effects on muscle mass and
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11 fat mass, respectively.
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15 Distinct patient populations with co-existing pathophysiological processes might exhibit
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18 different biomarker profiles. Further validation needs to be conducted in different patient
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22 populations to utilize the possible prognostic value of these biomarkers, either individually,
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25 or in combination with functional and clinical measures. Systematic identification of
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28 patients at risk of prolonged hospitalization and deconditioning should occur to enable
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32 early individualized interventions to counteract the adverse outcomes of prolonged bed
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36 rest.
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38 39 40 **CONCLUSIONS AND CLINICAL IMPLICATIONS**

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43 If the hypotheses from the Copenhagen PROTECT study are confirmed, the results can be
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46 helpful in the identification of older patients at risk of prolonged hospitalization.
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50 Additionally, circulating biomarker assays able to predict physical deconditioning during
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53 hospitalization will help in the early detection of geriatric patients at risk of deconditioning
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56 during hospitalization. This knowledge can then be tested in a future interventional study.
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4 The Copenhagen PROTECT study is considered feasible, ethically sound, and with
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8 potential extensive implications for future identification and treatment of sarcopenia in
9
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11 older medical patients.
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14

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23 recruitment of patients.
24

25 **Contributors**

26 RSK, CS, MS, and FEN drafted the manuscript. CS, FEN, RSK, MS, AE, HN, HA, MRW, and EP designed the study.
27
28 SKH, HA, HN, AE, FH, MRW, EP, TN and CBR contributed to the discussion, edited and reviewed the manuscript. All
29
30 authors read and approved the final manuscript.
31

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37
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39
40 manuscripts or decisions regarding publication.
41

42 **Competing interests**

43
44 None
45

46 **Patient consent for publication**

47
48 Not required
49

50 **Data availability statement**

51
52 There is no data in this work
53
54

55 **Ethics approval**

56
57 The study protocol has been approved by the local ethics committee (H-19039214) and the Danish Data Protection
58
59 Agency (P-2019-239)
60

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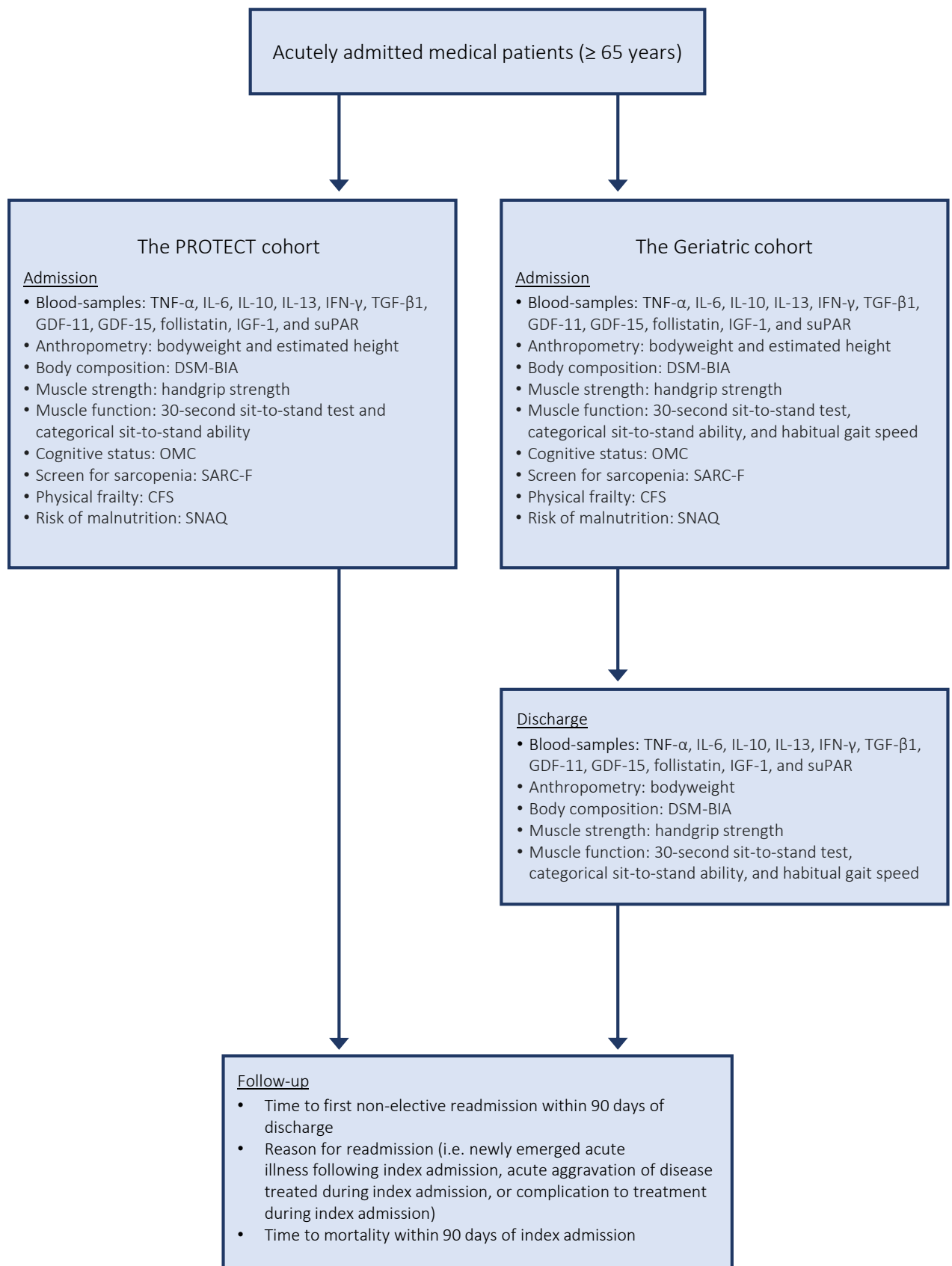
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Figure legends

Figure 1. Timeline and assessments in the PROTECT cohort and the Geriatric cohort

For peer review only



BMJ Open

Biomarkers for length of hospital stay, changes in muscle mass, strength and physical function in older medical patients: rationale and methodology of the Copenhagen PROTECT study - a prospective cohort study.

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5 Biomarkers for length of hospital stay, changes in muscle mass, strength and
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8 physical function in older medical patients: rationale and methodology of the
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11 Copenhagen PROTECT study - a prospective cohort study.
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22 **ABSTRACT**

23 **Introduction**

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28 Sarcopenia is generally used to describe the age-related loss of muscle mass and strength believed to play
29
30 a major role in the pathogenesis of physical frailty and functional impairment that may occur with old age.
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32
33 The knowledge surrounding the prevalence and determinants of sarcopenia in older medical patients is
34

35 scarce, and it is unknown whether specific biomarkers can predict physical deconditioning during
36
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38 hospitalization. We hypothesize that a combination of clinical, functional, and circulating biomarkers can
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40 serve as a risk stratification tool and can i) identify older acutely ill medical patients at risk of prolonged
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43 hospital stays and ii) predict changes in muscle mass, muscle strength, and function during hospitalization.
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49 **Method and analysis**

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52 The Copenhagen PROTECT study is a prospective cohort study consisting of acutely ill older medical
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54 patients admitted to the acute medical ward at Copenhagen University Hospital, Bispebjerg and
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57 Frederiksberg, Denmark. Assessments are performed within 24 hours of admission and include blood
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4 samples, body composition, muscle strength, physical function, and questionnaires. A subgroup of patients
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6
7 transferred to the Geriatric Department are included in a smaller geriatric cohort and have additional
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10 assessments at discharge to evaluate the relative change in circulating biomarker concentrations, body
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12
13 composition, muscle strength, and physical function during hospitalization. Enrollment commenced
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15
16 November 4th, 2019, and proceeds until May 3rd, 2021.

17 18 19 20 **Ethics and dissemination**

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22
23 The study protocol has been approved by the local ethics committee of Copenhagen and Frederiksberg (H-
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26 19039214) and the Danish Data Protection Agency (P-2019-239) and all experimental procedures were
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29 performed in accordance with the Declaration of Helsinki. Findings from the project, regardless of the
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32 outcome, will be published in relevant peer-reviewed scientific journals and at www.clinicaltrials.gov.

33 34 35 36 **Trial registration number**

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39 Clinicaltrials.gov, ID: NCT04151108
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47 **ARTICLE SUMMARY**

48 49 50 51 **Strengths and limitations of this study**

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55 • A strength of the study is the large heterogeneous population, which brings
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58 generalizability to the study results.
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- The assessments of physical function applied in the study have previously been evaluated in acutely admitted older medical patients.
- Bio-electrical impedance analysis (BIA) may be affected by the hydration status of the patients.
- There are no direct measurements of the physical activity levels of the patients during admission.
- The study estimates stature by knee-height measurements, as many patients are unable to stand for height measurements.

BACKGROUND

It is well established that human skeletal muscle function declines with aging, and sarcopenia is generally used to describe the age-related skeletal muscle atrophy and loss of muscle strength believed to play a major role in the pathogenesis of physical frailty, loss of independence, and functional impairment that may occur with old age.[1-3] Clinical sarcopenia has been defined in statistical terms assuming a lower normal limit of two standard deviations below a mean relative appendicular muscle mass in young healthy adults.[4] The prevalence of sarcopenia is estimated at 5%-13% in 60-70 year-olds and 11%-50% in individuals aged 80 years or older.[5] The etiology of sarcopenia is complex and involves neuronal, hormonal, immunological, and nutritional mechanisms.[6-10]

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4 Furthermore, physical inactivity, chronic diseases, immobilization, and hospitalization are
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8 known to play a part in the development of sarcopenia.[6,11-13]
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12 In 2018, approximately 45% of all hospital admissions in Denmark concerned patients aged
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15 65 or older who had a mean length of stay of 3.5 days.[14] Older patients are often inactive
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18 during hospitalization spending 71%-83% of their time lying down,[15,16] and at least 35%
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22 of older patients lose independence in one basic Activity of Daily Living (ADL) as an
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25 unintended consequence of a medical illness and hospitalization.[17] Sarcopenia may
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28 aggravate this functional decline, as patients with sarcopenia have an attenuated recovery
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32 of their functional levels 3 months following discharge.[18] From a clinical perspective,
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35 sarcopenia is associated with infectious complications, readmissions, increased need for
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38 rehabilitation following discharge, reduced quality of life, increased mortality, and longer
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42 hospitalization.[4,19]
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48 Early mobilization protocols have proven effective in reducing hospital-acquired disability
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51 and hospital length of stay. However, frequently reported barriers for implementation of early
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54 mobilization include lack of staff and time to enable mobilization of the patient.[20] With an
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58 increasing aging population and the heterogeneousness of older individuals, the systematic
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4 identification of older individuals at risk of prolonged hospitalization and deconditioning
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8 during hospitalization are of utmost importance. As such, the combination of clinical,
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11 functional and circulating biomarkers may serve as risk stratification tools to identify older
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15 patients at risk of these adverse outcomes.
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18 19 **STUDY OBJECTIVES AND HYPOTHESES**

20 21 22 23 **Primary objectives and hypothesis**

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27 We aim to examine whether circulating biomarkers at admission are associated with length
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30 of hospital stay in older (≥ 65 years) acutely admitted medical patients and whether the
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33 combination of clinical and functional measures with these biomarkers can identify patients
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36 at risk of having a prolonged hospital stay (>96 hours). In addition, we aim to establish
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39 circulating biomarkers associated with changes in muscle mass, muscle strength, and
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42 function in geriatric patients during hospitalization. We hypothesize that a combination of
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45 clinical and functional measures with circulating biomarkers has the potential to identify
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48 older (≥ 65 years) acutely admitted medical patients at risk of prolonged (≥ 96 hours)
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51 hospital stays and physical deconditioning during hospitalization.
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Secondary objectives and hypothesis

The secondary objectives are to determine whether circulating biomarkers are associated with readmissions within 90 days of discharge, frailty, discharge to a higher level of care, and all-cause mortality within 90 days of the index admission and whether the combination of clinical and functional measures with these biomarkers can identify patients at risk of readmissions, discharge to a higher level of care, and all-cause mortality. We hypothesize that a combination of clinical and functional measures with circulating biomarkers has the potential to identify older (≥ 65 years) acutely admitted medical patients at risk of non-elective readmissions within 90 days of discharge, discharge to a higher level of care, and all-cause mortality within 90 days of the index admission.

METHODS AND ANALYSIS

Setting and intervention

The Copenhagen PROTECT study is a prospective cohort study consisting of acutely ill older medical patients admitted to the acute medical ward at Copenhagen University Hospital, Bispebjerg and Frederiksberg, Denmark. A subgroup of these patients, subsequently transferred to the Geriatric Department, are also included in a smaller

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4 geriatric cohort. Enrollment commenced November 4th, 2019 and will proceed until May
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8 3rd, 2021.
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10 11 12 **Eligible patients** 13

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16 The current study is recruiting participants during a 1.5-year period to avoid any seasonal
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18 differences in the patient population and to take into account the temporary pause in
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20 recruitment due to the Covid-19 pandemic. We aim to include a total of 1700 patients
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22
23 representing the PROTECT cohort, of which approximately 400 patients subsequently will
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25
26 be transferred to the Geriatric Department and constitute the Geriatric cohort. All patients
27
28
29 admitted at the acute medical ward at Copenhagen University Hospital, Bispebjerg and
30
31 Frederiksberg who fulfill the inclusion criteria and do not meet any exclusion criteria are
32
33
34 eligible for the study (Table 1). The hospital admission during which the patient is recruited
35
36
37 represents the index admission. Any subsequent non-elective admissions of included
38
39
40 patients during the study inclusion period will be interpreted as readmissions. Included
41
42
43 patients will be followed for 90 days following discharge from index admission to
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54 investigate future readmissions and mortality.
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9 *Table 1. Inclusion and exclusion criteria*

10 **Inclusion criteria**

11 Equal to or over the age of 65 years

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14 Acutely admitted with a medical diagnosis (i.e. non-surgical)

15
16 **Exclusion criteria**

17 Admitted for more than 24 hours prior to baseline assessment

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19
20 Terminal illness (expected life span of less than 6 months)

21
22 Temporary civil registration number

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24 Droplet or airborne infections requiring isolation

25
26 Does not speak or read Danish

27
28 Patients judged medically contraindicated by health personnel

29
30 Inability to provide informed consent for participation

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36 **Outcomes**

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40 The primary outcome in the PROTECT cohort is the length of hospital stay. Successive

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42
43 events of hospitalisation have been suggested to contribute to the development of

44
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46 sarcopenia, and even short periods (4-5 days) of skeletal muscle disuse are known to

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49 induce muscle atrophy.[21,22] In 2018, the mean length of hospital stay in Denmark was

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55 84 hours in patients aged 65 years or over.[14] while the mean LOS in the two largest local

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4 hospitals was 96 hours. As such, we have defined a prolonged hospital length of stay as
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8 an admission lasting >96 hours.
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12 The primary outcomes in the Geriatric cohort are the relative changes in muscle mass,
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15 muscle strength, and muscle function during hospitalization. Primary and secondary
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18 outcomes for the PROTECT cohort and the Geriatric cohort are listed in table 2 and 3,
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22 respectively.
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29 *Table 2. Primary and secondary outcomes in the PROTECT cohort*

30
31 **Primary outcome**

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33 Length of hospital stay
34

35 **Secondary outcomes**

36
37 Non-elective readmissions within 90 days of discharge
38

39 All-cause mortality within 90 days of index admission
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41 In-hospital mortality
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43 Muscle mass at admission
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45 Muscle strength at admission
46

47 Muscle function at admission
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49 Frailty
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54 *Table 3. Primary and secondary outcomes in the Geriatric cohort*

55
56 **Primary outcomes**

57
58 Changes in muscle mass during hospitalization
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4 Changes in muscle strength during hospitalization
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6 Changes in muscle function during hospitalization
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8 **Secondary outcomes**
9

10 Length of hospital stay
11

12 Non-elective readmissions within 90 days of discharge
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14 All-cause mortality within 90 days of index admission
15

16 In-hospital mortality
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18 Discharge to an increased level of care
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20 Frailty
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26 We have defined geriatric patients discharged to an increased level of care as i) patients
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28 receiving increased relief in terms of walking aids or patients with an increased need for
29
30 caregiver assistance or home care, ii) patients referred to rehabilitation or 24-hour care, or
31
32 iii) patients moving to a nursing home following discharge. Data on readmissions will be
33
34 limited to non-elective readmissions in Region Zealand and the Capital Region of Denmark.
35
36 A geriatrician will evaluate whether the readmission is related to the index admission; i.e.
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38 newly emerged acute illness following the index admission, acute aggravation of disease
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40 treated during the index admission, or complication to treatment during the index admission.
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54 **Assessment and randomization**
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5 The research personnel might be unable to assess all patients, as the number of eligible
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8 patients (i.e. fulfilling inclusion criteria with the absence of exclusion criteria) varies daily.
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11 Thus, to avoid selection bias, all eligible patients on the day in question are randomized
12
13
14 using a computer-generated randomization sequence to establish a randomized visitation
15
16
17
18 sequence. Patients who wish to participate sign an informed consent and baseline
19
20
21
22 measurements are performed within the first 24 hours of admission. All included patients
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25
26 have blood samples drawn to determine concentrations of tumor necrosis factor (TNF)- α , ,
27
28
29 interleukin (IL)-6, IL-10, transforming growth factor (TGF)- β 1, follistatin, insulin-like growth
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32 factor (IGF)-1, growth differentiation factor (GDF)-11, GDF-15, and soluble urokinase-type
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36 plasminogen activator receptor (suPAR).
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40 Handgrip strength is assessed using a digital hand-held dynamometer (Model SH1001;
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43 SAEHAN Corporation, Yangdeok-Dong, Masan, South Korea). Patients able to leave the
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47 bed sit on a chair with the elbow flexed at 90° and the wrist in a neutral position, while
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51 bedridden patients are assessed in the hospital bed with the backrest elevated. The
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55 highest value of three attempts with the dominant hand is used for analyses. Should the
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59 third trial elicit the highest value, the patient continues until a lower value is achieved.
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5 Muscle function is assessed in the 30-second sit-to-stand test, where patients are asked to
6
7
8 stand up from a standardized chair as many times as possible with their arms folded
9
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11 across the chest. Only full standing positions are counted.[23, 24] Patients included in the
12
13
14 Geriatric cohort also have their habitual gait-speed assessed. The gait-speed assessment
15
16
17 is measured over a course of 4 meters and includes walking aids if they are used by the
18
19
20 patient. Patients stand behind a starting line and are asked to start walking towards a
21
22
23 visual goal at their habitual pace. The visual goal is placed after 5.5 m. to reduce the effect
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25
26 of deceleration. The fastest of the two attempts will be used for analyses and quantified as
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29 m/s.[23] The assessment of handgrip strength (kg) and habitual gait-speed have
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33 previously shown to be feasible and reliable measures in acutely older medical patients.
34
35
36 However, the feasibility and reliability of the 30-second sit-to-stand test was moderate, as
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38
39 only half of the patients were able to perform the test as instructed.[23] Thus, we have
40
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43 included an additional nominal variable to categorize the sit-to-stand ability as either i) able
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47 to perform the test as instructed, ii) ability to rise using the armrest, and iii) inability to rise
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53 independently from a chair.
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4 Bodyweight (kg) is assessed using chair scales and height (cm) is estimated with a
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8 segmometer using the knee-height measurement and age with the equations from
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11 Chumlea et al.[25] Body composition, including whole body phase angle, is assessed
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14 using Direct-Segmental Multi-frequency Bioelectrical Impedance Analyses (DSM-BIA)
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16
17 (InBody S10®; Biospace Co., Ltd., Seoul, Korea), which has previously been used in
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19 elderly acutely admitted patients with a mean LOS of 5 days.[26] Self-reported current
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21
22 smoking is reported as a dichotomous variable. Patients included in the Geriatric cohort
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24
25
26 are also assessed at discharge to evaluate circulating biomarker concentrations as well as
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29
30 changes in body composition, muscle strength, and functional performance. Tests of
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32
33 strength, physical function and body composition measurements are performed by trained
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35
36 research personnel. The presence of frailty is assessed by trained nurses associated with
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40 the study using the Canadian Study of Health and Aging Clinical Frailty Scale (CFS).[27]
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44 Patients are screened for sarcopenia using the SARC-F questionnaire,[28] while cognitive
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48 status is evaluated by the short Orientation-Memory-Concentration test (OMC).[29] The
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52 risk of malnutrition is assessed and validated using the Short Nutritional Assessment
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Questionnaire (SNAQ).[30] A flowchart showing the timeline and assessments in the two cohorts can be seen in Figure 1.

Information on medical treatment is evaluated by counting all prescribed medications, including unscheduled medications, except for the following:

- Eyedrops
- Eardrops
- Lotions and ointments
- Antibiotic treatment of limited duration
- Multivitamins
- Supplementary nutrition or tube feeding

Medications listed twice containing the same substance are only counted once.

Comorbidity is evaluated by the Charlson Comorbidity Index (CCI)[31] and obtained by evaluating the type and number of ICD-10 discharge diagnosis during the last 5 years of the index admission. Sepsis is defined in accordance with the Sepsis-3 criteria.[32] Data on emigration and all-cause mortality within 90 days of index admission is extracted from the Danish Civil Registration System. A summary of variables assessed by research

personnel and extracted from the electronic patient system (EPIC) or the Danish Civil

Registration System are listed in table 4.

Table 4. Variables assessed by research personnel and extracted from EPIC or The Danish Civil Registration System.

	Assessed by research personnel	Extracted from EPIC or the Danish Civil Registration System
Descriptive information		
Age		x
Gender		x
Smoking	x	
Emigration		x
Clinical information		
Hospital length of stay		x
Main diagnosis (index admission)		x
Non-elective readmissions within 90 days of discharge		x
Main diagnosis (readmission)		x
In-hospital mortality		x
All-cause mortality within 90 days of index admission		x
Prescribed medications upon admission		x
ICD-10 discharge diagnoses 5 years prior to index admission		x
Number of hospitalizations (acute and elective) one year prior to the index admission		x
Vital values (saturation, respiratory rate, heart rate, blood pressure, core temperature, Glasgow coma scale) upon admission		x
Early Warning Score (EWS) upon admission		x
Electrocardiographic (ECG) abnormalities upon admission		x
Admission to the intensive care unit (ICU) (admission date, discharge date, treatment with vasopressors, dialysis and mechanical ventilation)		x

Sepsis during the index admission†		x
Braden Score		x
Anthropometry and physical function		
Bodyweight (kg)	x	
Height (cm)	x	
Body composition (DSM-BIA)	x	
Whole body phase angle (DSM-BIA)	x	
Handgrip strength (kg)	x	
Sit-to-stand ability	x	
Sit-to-stand ability, categorical	x	
Habitual gait-speed* (m/s)	x	
Canadian Study of Health and Aging Clinical Frailty Scale (CFS)	x	
SARC-F score	x	
Use of walking aids at index admission		x
Discharge to an increased level of care*		x
Barthel Index at admission*		x
Barthel Index at discharge*		x
Cumulated Ambulation Score (CAS)*		x
New Mobility Score (NMS)*		x
De Morton Mobility Index (DEMMI) score*		x
Cognition		
Dementia diagnosis		x
Orientation-Memory-Concentration test (OMC)	x	
Nutrition		
Short Nutritional Assessment Questionnaire (SNAQ)	x	
Blood		
Results of routine blood tests upon admission (C-reactive Protein (CRP), albumin, urea, creatinine, hemoglobin, white blood cells, platelets, potassium, sodium, glomerular filtration rate (GFR), liver biochemistry, glucose, calcium, magnesium, lactate, and other routine blood samples)		x
Results of PROTECT blood tests (TNF- α , IL-6, IL-10, TGF- β , GDF-11, GDF-15, follistatin, IGF-1, and suPAR)	x	

* denotes variables included in the geriatric cohort only.

Data management

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5 Following data acquisition, all physical documents are stored in accordance with the
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7
8 guidelines for data management from the Danish Data Protection Agency. Electronic data
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10
11 is managed and stored using Research Electronic Data Capture (REDCap)[33,34], a web-
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14 based secure software platform hosted at Bispebjerg-Frederiksberg University Hospital. To
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17 ensure data quality, the REDCap database was built to ensure data integrity including real-
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20 time data validation, integrity checks, and range checks for data values.
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26 **Patient and public involvement**

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30 Upon request, patients with measures of muscle mass, strength or function can gain
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33 insight into their values and receive advice to improve from either an exercise physiologist
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36 or a physiotherapist. Patients are not involved in the study design, recruitment, or other
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40 aspects of the study.
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45 **Power calculation and statistics**

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49 To evaluate the prognostic abilities of circulating biomarkers (individually, in combination
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51
52 and combined with clinical and functional measures) we will use the area under the curve
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54
55 for receiver operating characteristics (AUROC) statistics. A reference group of 2058
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4 patients over the age of 65 from Bispebjerg-Frederiksberg University Hospital and Herlev-
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8 Gentofte Hospital had a mean age of 78.3 years and a mean length of stay of 5.8 days
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11 during hospitalization. In these patients, 817 (39.7%) had a prolonged length of stay,
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13
14 defined as a hospitalization lasting more than 96 hours. With a sample size of 1700 and
15
16
17 the assumption that approximately 40% of older medical patients have a prolonged
18
19 hospital stay, an AUROC of 82 will have a power of 0.9 with a significance level of 0.05.
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26 A table of summary statistics will be presented with baseline variables. Continuous
27
28 variables will be summarized with the following: n (non-missing sample size), mean,
29
30 standard deviation, median, interquartile range, and number of missing values. Categorical
31
32 variables will be reported as frequency and percentages (based on non-missing sample
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34 size), and number of missing values. Data missing at random will be imputed using
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36 multiple imputation.
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48 To evaluate whether clinical, functional, and circulating biomarkers are associated with
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50 length of stay we will perform multivariate logistic regression. Patients will be grouped in
51
52 either normal (< 96 hours) or extended length of stay (\geq 96 hours) and Cox regression
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54 analysis will be used to compare differences in non-elective readmission and all-cause
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4 mortality. Patients will be followed from the date of discharge from the index admission
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8 until the end of the follow-up period, emigration, readmission, or death as appropriate. To
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11 assess the discriminative ability of biomarkers with regards to an extended length of stay
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13
14 and all-cause mortality, we will use the area under the curve (AUC) for receiver operating
15
16
17 characteristics (ROC) curves. AUCs for different ROC curves will be compared using the
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19 DeLong test. The association of circulating biomarkers with changes in muscle mass,
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21
22 muscle strength, and function in the Geriatric cohort will be assessed using a multivariate
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29 linear model adjusted for the relative length of stay.
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33 **Study organization**

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37 The study is a researcher initiated clinical study. The protocol was written by the steering
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39
40 committee composed of experts in geriatric medicine and acute medicine and a PhD
41
42
43 student in basic and clinical research in musculoskeletal sciences. The committee is
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46
47 responsible for the design of the study, supervision of research personnel, data
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51 acquisition, communication and publication of results, approval of sub-studies and
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55 ensuring that future studies comply with the regulations regarding data management.
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4 At present (July 2020), the study has included 377 patients, of which 62 are part of the
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8 Geriatric cohort. Inclusion was temporarily paused due to the impact of the Covid-19
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11 pandemic.
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14 15 **ETHICS AND DISSEMINATION**

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18 All procedures are being conducted according to “Good Clinical Practice” standards,
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20
21 regarding initiation, monitoring, and reporting. The study protocol has been approved by
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23
24 the local ethics committee of Copenhagen and Frederiksberg (H-19039214) and the
25
26
27 Danish Data Protection Agency (P-2019-239) and all experimental procedures are
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29
30 performed in accordance with the Declaration of Helsinki. The project complies with the
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32
33 regulations of the General Data Protection Regulation (GDPR) and the Data Protection
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35
36 Act.
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43 All eligible patients receive oral and written information. In the case of severe dementia or
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46 delirium, some patients might be unable to provide participant consent. In these cases, we
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48
49 seek participant consent from a close relative or guardian, should the guardianship include
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51
52 access to sign participant consent for research purposes. Independent medical doctors
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56
57 who have knowledge of the project but are not associated with the project and are
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4 independent of the interests of the principal investigator evaluate whether these subjects
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8 can participate in the study.
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11
12 The project entails minimal discomfort and no permanent side effects; thus, it is
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15 considered ethically sound. Findings from the project, regardless of the outcome, will be
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18 published in relevant peer-reviewed scientific journals ensuring the anonymity of the
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21 patients. The study is registered at clinicaltrials.gov (NCT04151108) where positive,
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24 negative, or inconclusive results will be published.
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30 DISCUSSION

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34 The importance of sarcopenia has recently been underlined by its inclusion as a reportable
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37 disease in the Centers for Disease Control and Prevention (ICD-10-CM code M62.84) in
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40 October 2016.[35] Even though the serious consequences of sarcopenia are widely
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43 recognized, the diagnose has yet to be implemented in clinical practice in Denmark. Lack of
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46 knowledge regarding the basic biological mechanisms driving sarcopenia in conjunction with
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49 other age-related diseases and lack of systematic assessment hinders the identification and
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52 treatment of sarcopenia and can lead to physical deconditioning during hospitalization. As
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4 such, the knowledge surrounding the prevalence and determinants of sarcopenia in older
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8 medical patients is scarce, and it is unknown whether circulating biomarkers, individually or
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11 in combination, can predict physical deconditioning during hospitalization.
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15 Mechanisms that regulate skeletal muscle mass are central to the understanding of
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18 sarcopenia. Myostatin, also referred to as GDF-8, is a part of the TGF- β family and
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22 predominantly expressed in skeletal muscle. Myostatin and TGF- β are inducers of catabolic
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26 processes, inhibiting muscle growth, and inducing muscle protein breakdown via activation
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28
29 of the Small Mothers Against Decapentaplegic (SMAD)2 and SMAD3 transcription
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32 factors.[36,37] Myostatin is reportedly increased with aging,[38] and following prolonged bed
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36 rest,[39] and treatment with myostatin antibodies attenuates the loss of muscle mass and
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40 function induced by immobilization in mice.[40]
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44 Recently, GDF-11, a TGF- β family ligand,[41] has been measured in human blood
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48 samples.[42] High circulating GDF-11 levels have been related to increased disease burden
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52 and elevated risk of post-operative complications and mortality in older adults undergoing
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55 heart surgery. Notably, patients categorized as physically frail based on low handgrip
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4 strength and gait speed as well as self-reported activity measures had significantly higher
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8 GDF-11 levels compared to non-frail controls.[42]
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12 GDF-15, another member of the TGF- β family, is present in low levels under healthy
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15 conditions but can increase during disease or injury and contribute to muscle wasting by
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17
18 suppressing appetite, which may result in anorexia and drastic weight loss.[43] In older
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21 patients, unintentional weight loss has been associated with an increased in-hospital
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24 morbidity and increased overall mortality.[44] GDF-15 may be induced in response to
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27 cellular stress signals or dysfunctions, and it has been suggested that circulating levels of
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30 GDF-15 could be biomarker of mitochondrial dysfunction.[45] Nonetheless, several studies
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33 demonstrate that GDF-15 levels are predictors of all-cause mortality.[46,47]
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41 Follistatin acts as an antagonist to TGF- β family ligands including myostatin, TGF- β and
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44 GDF11.[37] Measurements of TGF- β ligands as well as their antagonist follistatin could
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47 represent biomarkers of muscle breakdown, physical function or mortality. However, the
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50 translation of these findings into clinical utility needs further validation in a larger cohort.
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5 Several studies have investigated the association of inflammatory biomarkers with muscle
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8 mass, muscle strength, and muscle function in healthy older subjects. Most commonly,
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11 studies have focused on a few biomarkers, such as TNF- α , IL-6 or C-Reactive Protein
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14 (CRP).[9,48-50] A recent study has demonstrated an inverse relationship between a
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17 composite of pro- and anti-inflammatory markers and muscle mass, strength and function in
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20 healthy older subjects.[51] However, results are inconsistent and lack clear evidence as to
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23 whether these inflammatory biomarkers are associated with sarcopenia. Nonetheless,
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26 circulating levels of CRP are predictive of both the length of hospital stay and
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28
29 readmissions.[52,53] Indeed, geriatric patients with inflammation, evaluated by CRP levels
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32 at admission, stayed on average 3 days longer than patients without inflammation.[54]
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40 The anabolic growth factor, IGF-1, and the IGF-1/phosphatidylinositol 3-kinase(PI3K)/Akt
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43 pathway is involved in skeletal muscle hypertrophy and atrophy.[55,56] Circulating IGF-1
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45
46 levels decrease with aging, while inflammatory markers such as TNF- α and IL-6 can
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49 interfere with the IGF-1 signaling pathway.[57,58] As such, changes in IGF-1/PI3K/Akt
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52 signaling during aging may be a result of decreased IGF-1 expression as well as IGF-1
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4 inhibition. Notably, no difference in circulating IGF-1 concentrations were found between
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8 older sarcopenic and non-sarcopenic women.[59]
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12 Recently, suPAR was established as a biomarker of inflammation and immune activation,
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14
15 and elevated levels of suPAR are believed to reflect a state of chronic inflammation.[60]
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19 SuPAR correlates with other inflammatory markers, such as TNF- α , and patients with the
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21
22 highest levels of suPAR generally have the worst prognosis.[61] In one study, suPAR was
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25 associated with low muscle mass, while IL-6 was associated with low muscle mass and
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28 increased fat mass in both patients and healthy controls.[62] Thus, there seem to be distinct
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33 inflammatory processes occurring simultaneously with different effects on muscle mass and
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36 fat mass, respectively.
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40 Distinct patient populations with co-existing pathophysiological processes might exhibit
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44 different biomarker profiles. Further validation needs to be conducted in different patient
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48 populations to utilize the possible prognostic value of these biomarkers, either individually,
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52 or in combination with functional and clinical measures. Systematic identification of
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55 patients at risk of prolonged hospitalization and deconditioning should occur to enable
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4 early individualized interventions to counteract the adverse outcomes of prolonged bed
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8 rest.
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10 11 12 **CONCLUSIONS AND CLINICAL IMPLICATIONS**

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15 If the hypotheses from the Copenhagen PROTECT study are confirmed, the results can be
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18 helpful in the identification of older patients at risk of prolonged hospitalization.
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21 Additionally, circulating biomarker assays able to predict physical deconditioning during
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25 hospitalization will help in the early detection of geriatric patients at risk of deconditioning
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28 during hospitalization. This knowledge can then be tested in a future interventional study.
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32 The Copenhagen PROTECT study is considered feasible, ethically sound, and with
33
34
35 potential extensive implications for future identification and treatment of sarcopenia in
36
37
38
39 older medical patients.
40
41

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44
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46
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48
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50
51 patients.

52 53 **Contributors**

54 RSK, CS, MS, and FEN drafted the manuscript. CS, FEN, RSK, MS, AE, HN, HA, MRW, and EP designed the study.
55
56 SKH, HA, HN, AE, FH, MRW, EP, TN and CBR contributed to the discussion, edited and reviewed the manuscript. All
57
58 authors read and approved the final manuscript.
59
60

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Disclaimer

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Competing interests

None

Patient consent for publication

Not required

Data availability statement

There is no data in this work

Ethics approval

The study protocol has been approved by the local ethics committee of Copenhagen and Frederiksberg (H-19039214) and the Danish Data Protection Agency (P-2019-239)

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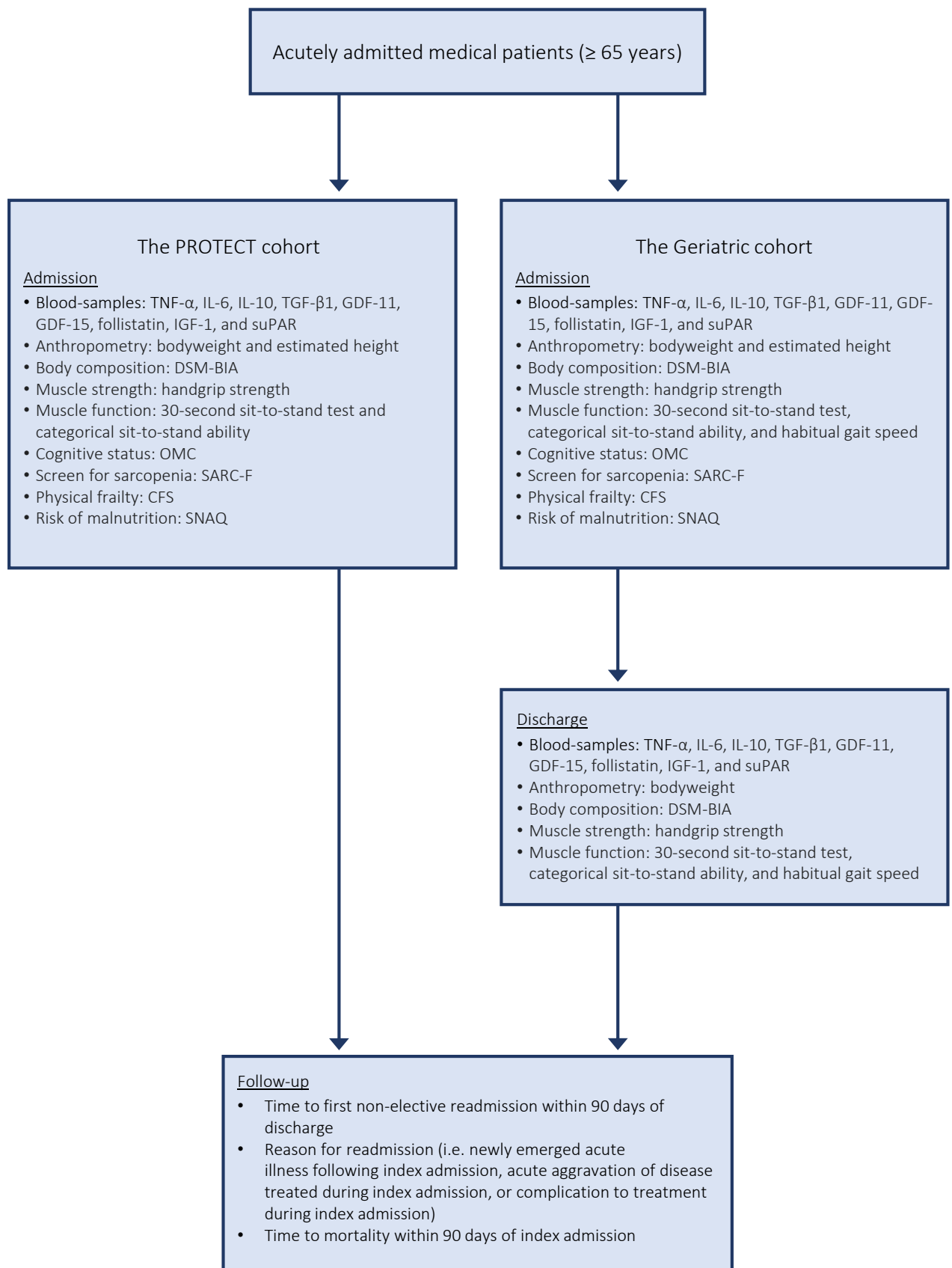
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Figure legends

Figure 1. Timeline and assessments in the PROTECT cohort and the Geriatric cohort

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BMJ Open

Biomarkers for length of hospital stay, changes in muscle mass, strength and physical function in older medical patients: protocol for the Copenhagen PROTECT study - a prospective cohort study.

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5 Biomarkers for length of hospital stay, changes in muscle mass, strength, and
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8 physical function in older medical patients: protocol for the Copenhagen PROTECT
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11 study - a prospective cohort study.
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22 **ABSTRACT**

23 **Introduction**

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28 Sarcopenia is generally used to describe the age-related loss of muscle mass and strength believed to play
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30 a major role in the pathogenesis of physical frailty and functional impairment that may occur with old age.
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34 The knowledge surrounding the prevalence and determinants of sarcopenia in older medical patients is
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36 scarce, and it is unknown whether specific biomarkers can predict physical deconditioning during
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38 hospitalization. We hypothesize that a combination of clinical, functional, and circulating biomarkers can
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40 serve as a risk stratification tool and can i) identify older acutely ill medical patients at risk of prolonged
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43 hospital stays and ii) predict changes in muscle mass, muscle strength, and function during hospitalization.
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49 **Method and analysis**

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53 The Copenhagen PROTECT study is a prospective cohort study consisting of acutely ill older medical
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55 patients admitted to the acute medical ward at Copenhagen University Hospital, Bispebjerg and
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58 Frederiksberg, Denmark. Assessments are performed within 24 hours of admission and include blood
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4 samples, body composition, muscle strength, physical function, and questionnaires. A subgroup of patients
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7 transferred to the Geriatric Department are included in a smaller geriatric cohort and have additional
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10 assessments at discharge to evaluate the relative change in circulating biomarker concentrations, body
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13 composition, muscle strength, and physical function during hospitalization. Enrollment commenced
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16 November 4th, 2019, and proceeds until May 3rd, 2021.

17 18 19 20 **Ethics and dissemination**

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23 The study protocol has been approved by the local ethics committee of Copenhagen and Frederiksberg (H-
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26 19039214) and the Danish Data Protection Agency (P-2019-239) and all experimental procedures were
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29 performed in accordance with the Declaration of Helsinki. Findings from the project, regardless of the
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32 outcome, will be published in relevant peer-reviewed scientific journals and at www.clinicaltrials.gov.

33 34 35 36 **Trial registration number**

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39 Clinicaltrials.gov, ID: NCT04151108

40 41 42 43 44 45 46 47 **ARTICLE SUMMARY**

48 49 50 51 **Strengths and limitations of this study**

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55 • A strength of the study is the large heterogeneous population, which brings
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58 generalizability to the study results.

- The assessments of physical function applied in the study have previously been evaluated in acutely admitted older medical patients.
- Bio-electrical impedance analysis (BIA) may be affected by the hydration status of the patients.
- There are no direct measurements of the physical activity levels of the patients during admission.
- The study estimates stature by knee-height measurements, as many patients are unable to stand for height measurements.

INTRODUCTION

It is well established that human skeletal muscle function declines with aging, and sarcopenia is generally used to describe the age-related skeletal muscle atrophy and loss of muscle strength believed to play a major role in the pathogenesis of physical frailty, loss of independence, and functional impairment that may occur with old age.[1-3] Clinical sarcopenia has been defined in statistical terms assuming a lower normal limit of two standard deviations below a mean relative appendicular muscle mass in young healthy adults.[4] The prevalence of sarcopenia is estimated at 5%-13% in 60-70 year-olds and 11%-50% in individuals aged 80 years or older.[5] The etiology of sarcopenia is complex and involves neuronal, hormonal, immunological, and nutritional mechanisms.[6-10]

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4 Furthermore, physical inactivity, chronic diseases, immobilization, and hospitalization are
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8 known to play a part in the development of sarcopenia.[6,11-13]
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12 In 2018, approximately 45% of all hospital admissions in Denmark concerned patients aged
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15 65 or older who had a mean length of stay of 3.5 days.[14] Older patients are often inactive
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18 during hospitalization spending 71%-83% of their time lying down,[15,16] and at least 35%
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22 of older patients lose independence in one basic Activity of Daily Living (ADL) as an
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25 unintended consequence of a medical illness and hospitalization.[17] Sarcopenia may
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28 aggravate this functional decline, as patients with sarcopenia have an attenuated recovery
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32 of their functional levels 3 months following discharge.[18] From a clinical perspective,
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35 sarcopenia is associated with infectious complications, readmissions, increased need for
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38 rehabilitation following discharge, reduced quality of life, increased mortality, and longer
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42 hospitalization.[4,19]
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48 Early mobilization protocols have proven effective in reducing hospital-acquired disability
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51 and hospital length of stay. However, frequently reported barriers for implementation of early
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54 mobilization include lack of staff and time to enable mobilization of the patient.[20] With an
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58 increasing aging population and the heterogeneousness of older individuals, the systematic
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4 identification of older individuals at risk of prolonged hospitalization and deconditioning
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8 during hospitalization are of utmost importance. As such, the combination of clinical,
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11 functional and circulating biomarkers may serve as risk stratification tools to identify older
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15 patients at risk of these adverse outcomes.
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18 19 **STUDY OBJECTIVES AND HYPOTHESES**

20 21 22 **Primary objectives and hypothesis**

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24 We aim to examine whether circulating biomarkers at admission are associated with length
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27 of hospital stay in older (≥ 65 years) acutely admitted medical patients and whether the
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30 combination of clinical and functional measures with these biomarkers can identify patients
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34 at risk of having a prolonged hospital stay (>96 hours). In addition, we aim to establish
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37 circulating biomarkers associated with changes in muscle mass, muscle strength, and
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40 function in geriatric patients during hospitalization. We hypothesize that a combination of
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43 clinical and functional measures with circulating biomarkers has the potential to identify
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47 older (≥ 65 years) acutely admitted medical patients at risk of prolonged (≥ 96 hours)
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50 hospital stays and physical deconditioning during hospitalization.
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Secondary objectives and hypothesis

The secondary objectives are to determine whether circulating biomarkers are associated with readmissions within 90 days of discharge, frailty, discharge to a higher level of care, and all-cause mortality within 90 days of the index admission and whether the combination of clinical and functional measures with these biomarkers can identify patients at risk of readmissions, discharge to a higher level of care, and all-cause mortality. We hypothesize that a combination of clinical and functional measures with circulating biomarkers has the potential to identify older (≥ 65 years) acutely admitted medical patients at risk of non-elective readmissions within 90 days of discharge, discharge to a higher level of care, and all-cause mortality within 90 days of the index admission.

METHODS AND ANALYSIS

Setting and intervention

The Copenhagen PROTECT study is a prospective cohort study consisting of acutely ill older medical patients admitted to the acute medical ward at Copenhagen University Hospital, Bispebjerg and Frederiksberg, Denmark. A subgroup of these patients, subsequently transferred to the Geriatric Department, are also included in a smaller

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4 geriatric cohort. Enrollment commenced November 4th, 2019 and will proceed until May
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8 3rd, 2021.
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10 11 12 **Eligible patients** 13

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16 The current study is recruiting participants during a 1.5-year period to avoid any seasonal
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18 differences in the patient population and to take into account the temporary pause in
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20 recruitment due to the Covid-19 pandemic. We aim to include a total of 1700 patients
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23 representing the PROTECT cohort, of which approximately 400 patients subsequently will
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26 be transferred to the Geriatric Department and constitute the Geriatric cohort. All patients
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29 admitted at the acute medical ward at Copenhagen University Hospital, Bispebjerg and
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31 Frederiksberg who fulfill the inclusion criteria and do not meet any exclusion criteria are
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34 eligible for the study (Table 1). The hospital admission during which the patient is recruited
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37 represents the index admission. Any subsequent non-elective admissions of included
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40 patients during the study inclusion period will be interpreted as readmissions. Included
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43 patients will be followed for 90 days following discharge from index admission to
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46 investigate future readmissions and mortality.
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9 *Table 1. Inclusion and exclusion criteria*

Inclusion criteria
Equal to or over the age of 65 years
Acutely admitted with a medical diagnosis (i.e. non-surgical)
Exclusion criteria
Admitted for more than 24 hours prior to baseline assessment
Terminal illness (expected life span of less than 6 months)
Temporary civil registration number
Droplet or airborne infections requiring isolation
Does not speak or read Danish
Patients judged medically contraindicated by health personnel
Inability to provide informed consent for participation

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36 **Outcomes**

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40 The primary outcome in the PROTECT cohort is the length of hospital stay. Successive
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44 events of hospitalisation have been suggested to contribute to the development of
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48 sarcopenia, and even short periods (4-5 days) of skeletal muscle disuse are known to
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51 induce muscle atrophy.[21,22] In 2018, the mean length of hospital stay in Denmark was
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55 84 hours in patients aged 65 years or over.[14] while the mean LOS in the two largest local
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4 hospitals was 96 hours. As such, we have defined a prolonged hospital length of stay as
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8 an admission lasting >96 hours.
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12 The primary outcomes in the Geriatric cohort are the relative changes in muscle mass,
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15 muscle strength, and muscle function during hospitalization. Primary and secondary
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18 outcomes for the PROTECT cohort and the Geriatric cohort are listed in table 2 and 3,
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22 respectively.
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29 *Table 2. Primary and secondary outcomes in the PROTECT cohort*

Primary outcome
Length of hospital stay
Secondary outcomes
Non-elective readmissions within 90 days of discharge
All-cause mortality within 90 days of index admission
In-hospital mortality
Muscle mass at admission
Muscle strength at admission
Muscle function at admission
Frailty

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54 *Table 3. Primary and secondary outcomes in the Geriatric cohort*

Primary outcomes
Changes in muscle mass during hospitalization

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4 Changes in muscle strength during hospitalization
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6 Changes in muscle function during hospitalization
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8 **Secondary outcomes**
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10 Length of hospital stay
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12 Non-elective readmissions within 90 days of discharge
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14 All-cause mortality within 90 days of index admission
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16 In-hospital mortality
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18 Discharge to an increased level of care
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20 Frailty
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26 We have defined geriatric patients discharged to an increased level of care as i) patients
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29 receiving increased relief in terms of walking aids or patients with an increased need for
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32 caregiver assistance or home care, ii) patients referred to rehabilitation or 24-hour care, or
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34
35 iii) patients moving to a nursing home following discharge. Data on readmissions will be
36
37
38 limited to non-elective readmissions in Region Zealand and the Capital Region of Denmark.
39
40
41
42
43 A geriatrician will evaluate whether the readmission is related to the index admission; i.e.
44
45
46 newly emerged acute illness following the index admission, acute aggravation of disease
47
48
49 treated during the index admission, or complication to treatment during the index admission.
50
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52
53

54 **Assessment and randomization**
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3
4 The research personnel might be unable to assess all patients, as the number of eligible
5
6
7
8 patients (i.e. fulfilling inclusion criteria with the absence of exclusion criteria) varies daily.
9
10
11 Thus, to avoid selection bias, all eligible patients on the day in question are randomized
12
13
14 using a computer-generated randomization sequence to establish a randomized visitation
15
16
17 sequence. Patients who wish to participate sign an informed consent and baseline
18
19
20 measurements are performed within the first 24 hours of admission. All included patients
21
22
23 have blood samples drawn to determine concentrations of tumor necrosis factor (TNF)- α , ,
24
25
26 interleukin (IL)-6, IL-10, transforming growth factor (TGF)- β 1, follistatin, insulin-like growth
27
28
29 factor (IGF)-1, growth differentiation factor (GDF)-11, GDF-15, and soluble urokinase-type
30
31
32 plasminogen activator receptor (suPAR).
33
34
35
36
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38
39

40 Handgrip strength is assessed using a digital hand-held dynamometer (Model SH1001;
41
42
43 SAEHAN Corporation, Yangdeok-Dong, Masan, South Korea). Patients able to leave the
44
45
46 bed sit on a chair with the elbow flexed at 90° and the wrist in a neutral position, while
47
48
49
50 bedridden patients are assessed in the hospital bed with the backrest elevated. The
51
52
53 highest value of three attempts with the dominant hand is used for analyses. Should the
54
55
56
57 third trial elicit the highest value, the patient continues until a lower value is achieved.
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5 Muscle function is assessed in the 30-second sit-to-stand test, where patients are asked to
6
7
8 stand up from a standardized chair as many times as possible with their arms folded
9
10
11 across the chest. Only full standing positions are counted.[23, 24] Patients included in the
12
13
14 Geriatric cohort also have their habitual gait-speed assessed. The gait-speed assessment
15
16
17 is measured over a course of 4 meters and includes walking aids if they are used by the
18
19
20 patient. Patients stand behind a starting line and are asked to start walking towards a
21
22
23 visual goal at their habitual pace. The visual goal is placed after 5.5 m. to reduce the effect
24
25
26 of deceleration. The fastest of the two attempts will be used for analyses and quantified as
27
28
29 m/s.[23] The assessment of handgrip strength (kg) and habitual gait-speed have
30
31
32
33 previously shown to be feasible and reliable measures in acutely older medical patients.
34
35
36
37 However, the feasibility and reliability of the 30-second sit-to-stand test was moderate, as
38
39
40 only half of the patients were able to perform the test as instructed.[23] Thus, we have
41
42
43
44 included an additional nominal variable to categorize the sit-to-stand ability as either i) able
45
46
47
48 to perform the test as instructed, ii) ability to rise using the armrest, and iii) inability to rise
49
50
51
52
53 independently from a chair.
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4 Bodyweight (kg) is assessed using chair scales and height (cm) is estimated with a
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6
7
8 segmometer using the knee-height measurement and age with the equations from
9
10
11 Chumlea et al.[25] Body composition, including whole body phase angle, is assessed
12
13
14
15 using Direct-Segmental Multi-frequency Bioelectrical Impedance Analyses (DSM-BIA)
16
17
18 (InBody S10®; Biospace Co., Ltd., Seoul, Korea), which has previously been used in
19
20
21
22 elderly acutely admitted patients with a mean LOS of 5 days.[26] Self-reported current
23
24
25
26 smoking is reported as a dichotomous variable. Patients included in the Geriatric cohort
27
28
29
30 are also assessed at discharge to evaluate circulating biomarker concentrations as well as
31
32
33
34 changes in body composition, muscle strength, and functional performance. Tests of
35
36
37
38 strength, physical function and body composition measurements are performed by trained
39
40
41
42 research personnel. The presence of frailty is assessed by trained nurses associated with
43
44
45
46 the study using the Canadian Study of Health and Aging Clinical Frailty Scale (CFS).[27]
47
48
49
50 Patients are screened for sarcopenia using the SARC-F questionnaire,[28] while cognitive
51
52
53
54 status is evaluated by the short Orientation-Memory-Concentration test (OMC).[29] The
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60 risk of malnutrition is assessed and validated using the Short Nutritional Assessment

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Questionnaire (SNAQ).[30] A flowchart showing the timeline and assessments in the two cohorts can be seen in Figure 1.

Information on medical treatment is evaluated by counting all prescribed medications, including unscheduled medications, except for the following:

- Eyedrops
- Eardrops
- Lotions and ointments
- Antibiotic treatment of limited duration
- Multivitamins
- Supplementary nutrition or tube feeding

Medications listed twice containing the same substance are only counted once.

Comorbidity is evaluated by the Charlson Comorbidity Index (CCI)[31] and obtained by evaluating the type and number of ICD-10 discharge diagnosis during the last 5 years of the index admission. Sepsis is defined in accordance with the Sepsis-3 criteria.[32] Data on emigration and all-cause mortality within 90 days of index admission is extracted from the Danish Civil Registration System. A summary of variables assessed by research

personnel and extracted from the electronic patient system (EPIC) or the Danish Civil

Registration System are listed in table 4.

Table 4. Variables assessed by research personnel and extracted from EPIC or The Danish Civil Registration System.

	Assessed by research personnel	Extracted from EPIC or the Danish Civil Registration System
Descriptive information		
Age		x
Gender		x
Smoking	x	
Emigration		x
Clinical information		
Hospital length of stay		x
Main diagnosis (index admission)		x
Non-elective readmissions within 90 days of discharge		x
Main diagnosis (readmission)		x
In-hospital mortality		x
All-cause mortality within 90 days of index admission		x
Prescribed medications upon admission		x
ICD-10 discharge diagnoses 5 years prior to index admission		x
Number of hospitalizations (acute and elective) one year prior to the index admission		x
Vital values (saturation, respiratory rate, heart rate, blood pressure, core temperature, Glasgow coma scale) upon admission		x
Early Warning Score (EWS) upon admission		x
Electrocardiographic (ECG) abnormalities upon admission		x
Admission to the intensive care unit (ICU) (admission date, discharge date, treatment with vasopressors, dialysis and mechanical ventilation)		x

Sepsis during the index admission†		x
Braden Score		x
Anthropometry and physical function		
Bodyweight (kg)	x	
Height (cm)	x	
Body composition (DSM-BIA)	x	
Whole body phase angle (DSM-BIA)	x	
Handgrip strength (kg)	x	
Sit-to-stand ability	x	
Sit-to-stand ability, categorical	x	
Habitual gait-speed* (m/s)	x	
Canadian Study of Health and Aging Clinical Frailty Scale (CFS)	x	
SARC-F score	x	
Use of walking aids at index admission		x
Discharge to an increased level of care*		x
Barthel Index at admission*		x
Barthel Index at discharge*		x
Cumulated Ambulation Score (CAS)*		x
New Mobility Score (NMS)*		x
De Morton Mobility Index (DEMMI) score*		x
Cognition		
Dementia diagnosis		x
Orientation-Memory-Concentration test (OMC)	x	
Nutrition		
Short Nutritional Assessment Questionnaire (SNAQ)	x	
Blood		
Results of routine blood tests upon admission (C-reactive Protein (CRP), albumin, urea, creatinine, hemoglobin, white blood cells, platelets, potassium, sodium, glomerular filtration rate (GFR), liver biochemistry, glucose, calcium, magnesium, lactate, and other routine blood samples)		x
Results of PROTECT blood tests (TNF- α , IL-6, IL-10, TGF- β , GDF-11, GDF-15, follistatin, IGF-1, and suPAR)	x	

* denotes variables included in the geriatric cohort only.

Data management

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4
5 Following data acquisition, all physical documents are stored in accordance with the
6
7
8 guidelines for data management from the Danish Data Protection Agency. Electronic data
9
10
11 is managed and stored using Research Electronic Data Capture (REDCap)[33,34], a web-
12
13
14 based secure software platform hosted at Bispebjerg-Frederiksberg University Hospital. To
15
16
17 ensure data quality, the REDCap database was built to ensure data integrity including real-
18
19
20 time data validation, integrity checks, and range checks for data values.
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26 **Patient and public involvement**

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29
30 Upon request, patients with measures of muscle mass, strength or function can gain
31
32
33 insight into their values and receive advice to improve from either an exercise physiologist
34
35
36 or a physiotherapist. Patients are not involved in the study design, recruitment, or other
37
38
39
40 aspects of the study.
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43
44

45 **Power calculation and statistics**

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47
48
49 To evaluate the prognostic abilities of circulating biomarkers (individually, in combination
50
51
52 and combined with clinical and functional measures) we will use the area under the curve
53
54
55
56 for receiver operating characteristics (AUROC) statistics. A reference group of 2058
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4 patients over the age of 65 from Bispebjerg-Frederiksberg University Hospital and Herlev-
5
6
7
8 Gentofte Hospital had a mean age of 78.3 years and a mean length of stay of 5.8 days
9
10
11 during hospitalization. In these patients, 817 (39.7%) had a prolonged length of stay,
12
13
14 defined as a hospitalization lasting more than 96 hours. With a sample size of 1700 and
15
16
17 the assumption that approximately 40% of older medical patients have a prolonged
18
19 hospital stay, an AUROC of 82 will have a power of 0.9 with a significance level of 0.05.
20
21
22
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25

26 A table of summary statistics will be presented with baseline variables. Continuous
27
28 variables will be summarized with the following: n (non-missing sample size), mean,
29
30 standard deviation, median, interquartile range, and number of missing values. Categorical
31
32 variables will be reported as frequency and percentages (based on non-missing sample
33
34 size), and number of missing values. Data missing at random will be imputed using
35
36 multiple imputation.
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47 To evaluate whether clinical, functional, and circulating biomarkers are associated with
48
49 length of stay we will perform multivariate logistic regression. Patients will be grouped in
50
51 either normal (< 96 hours) or extended length of stay (\geq 96 hours) and Cox regression
52
53
54 analysis will be used to compare differences in non-elective readmission and all-cause
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4 mortality. Patients will be followed from the date of discharge from the index admission
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6
7
8 until the end of the follow-up period, emigration, readmission, or death as appropriate. To
9
10
11 assess the discriminative ability of biomarkers with regards to an extended length of stay
12
13
14 and all-cause mortality, we will use the area under the curve (AUC) for receiver operating
15
16
17 characteristics (ROC) curves. AUCs for different ROC curves will be compared using the
18
19 DeLong test. The association of circulating biomarkers with changes in muscle mass,
20
21
22 muscle strength, and function in the Geriatric cohort will be assessed using a multivariate
23
24
25
26
27
28
29 linear model adjusted for the relative length of stay.
30
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32

33 **Study organization**

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35
36
37 The study is a researcher initiated clinical study. The protocol was written by the steering
38
39
40 committee composed of experts in geriatric medicine and acute medicine and a PhD
41
42
43 student in basic and clinical research in musculoskeletal sciences. The committee is
44
45
46
47 responsible for the design of the study, supervision of research personnel, data
48
49
50
51 acquisition, communication and publication of results, approval of sub-studies and
52
53
54
55 ensuring that future studies comply with the regulations regarding data management.
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4 At present (July 2020), the study has included 377 patients, of which 62 are part of the
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6
7
8 Geriatric cohort. Inclusion was temporarily paused due to the impact of the Covid-19
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10
11 pandemic.
12

13 14 15 **ETHICS AND DISSEMINATION**

16
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18 All procedures are being conducted according to “Good Clinical Practice” standards,
19
20
21 regarding initiation, monitoring, and reporting. The study protocol has been approved by
22
23
24 the local ethics committee of Copenhagen and Frederiksberg (H-19039214) and the
25
26
27 Danish Data Protection Agency (P-2019-239) and all experimental procedures are
28
29
30 performed in accordance with the Declaration of Helsinki. The project complies with the
31
32
33 regulations of the General Data Protection Regulation (GDPR) and the Data Protection
34
35
36 Act.
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43 All eligible patients receive oral and written information. In the case of severe dementia or
44
45
46 delirium, some patients might be unable to provide participant consent. In these cases, we
47
48
49 seek participant consent from a close relative or guardian, should the guardianship include
50
51
52 access to sign participant consent for research purposes. Independent medical doctors
53
54
55 who have knowledge of the project but are not associated with the project and are
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4 independent of the interests of the principal investigator evaluate whether these subjects
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6
7
8 can participate in the study.
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10
11
12 The project entails minimal discomfort and no permanent side effects; thus, it is
13
14
15 considered ethically sound. Findings from the project, regardless of the outcome, will be
16
17
18 published in relevant peer-reviewed scientific journals ensuring the anonymity of the
19
20
21 patients. The study is registered at clinicaltrials.gov (NCT04151108) where positive,
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24 negative, or inconclusive results will be published.
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30 DISCUSSION

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34 The importance of sarcopenia has recently been underlined by its inclusion as a reportable
35
36
37 disease in the Centers for Disease Control and Prevention (ICD-10-CM code M62.84) in
38
39
40 October 2016.[35] Even though the serious consequences of sarcopenia are widely
41
42
43 recognized, the diagnose has yet to be implemented in clinical practice in Denmark. Lack of
44
45
46 knowledge regarding the basic biological mechanisms driving sarcopenia in conjunction with
47
48
49 other age-related diseases and lack of systematic assessment hinders the identification and
50
51
52 treatment of sarcopenia and can lead to physical deconditioning during hospitalization. As
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4 such, the knowledge surrounding the prevalence and determinants of sarcopenia in older
5
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8 medical patients is scarce, and it is unknown whether circulating biomarkers, individually or
9
10
11 in combination, can predict physical deconditioning during hospitalization.
12
13

14
15 Mechanisms that regulate skeletal muscle mass are central to the understanding of
16
17
18 sarcopenia. Myostatin, also referred to as GDF-8, is a part of the TGF- β family and
19
20
21 predominantly expressed in skeletal muscle. Myostatin and TGF- β are inducers of catabolic
22
23
24 processes, inhibiting muscle growth, and inducing muscle protein breakdown via activation
25
26
27 of the Small Mothers Against Decapentaplegic (SMAD)2 and SMAD3 transcription
28
29
30 factors.[36,37] Myostatin is reportedly increased with aging,[38] and following prolonged bed
31
32
33 rest,[39] and treatment with myostatin antibodies attenuates the loss of muscle mass and
34
35
36 function induced by immobilization in mice.[40]
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43
44 Recently, GDF-11, a TGF- β family ligand,[41] has been measured in human blood
45
46
47 samples.[42] High circulating GDF-11 levels have been related to increased disease burden
48
49
50 and elevated risk of post-operative complications and mortality in older adults undergoing
51
52
53 heart surgery. Notably, patients categorized as physically frail based on low handgrip
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4 strength and gait speed as well as self-reported activity measures had significantly higher
5
6
7
8 GDF-11 levels compared to non-frail controls.[42]
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11
12 GDF-15, another member of the TGF- β family, is present in low levels under healthy
13
14
15 conditions but can increase during disease or injury and contribute to muscle wasting by
16
17
18 suppressing appetite, which may result in anorexia and drastic weight loss.[43] In older
19
20
21 patients, unintentional weight loss has been associated with an increased in-hospital
22
23
24 morbidity and increased overall mortality.[44] GDF-15 may be induced in response to
25
26
27 cellular stress signals or dysfunctions, and it has been suggested that circulating levels of
28
29
30 GDF-15 could be biomarker of mitochondrial dysfunction.[45] Nonetheless, several studies
31
32
33 demonstrate that GDF-15 levels are predictors of all-cause mortality.[46,47]
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41 Follistatin acts as an antagonist to TGF- β family ligands including myostatin, TGF- β and
42
43
44 GDF11.[37] Measurements of TGF- β ligands as well as their antagonist follistatin could
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46
47 represent biomarkers of muscle breakdown, physical function or mortality. However, the
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50 translation of these findings into clinical utility needs further validation in a larger cohort.
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5 Several studies have investigated the association of inflammatory biomarkers with muscle
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7
8 mass, muscle strength, and muscle function in healthy older subjects. Most commonly,
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10
11 studies have focused on a few biomarkers, such as TNF- α , IL-6 or C-Reactive Protein
12
13
14 (CRP).[9,48-50] A recent study has demonstrated an inverse relationship between a
15
16
17 composite of pro- and anti-inflammatory markers and muscle mass, strength and function in
18
19
20 healthy older subjects.[51] However, results are inconsistent and lack clear evidence as to
21
22
23 whether these inflammatory biomarkers are associated with sarcopenia. Nonetheless,
24
25
26 circulating levels of CRP are predictive of both the length of hospital stay and
27
28
29 readmissions.[52,53] Indeed, geriatric patients with inflammation, evaluated by CRP levels
30
31
32 at admission, stayed on average 3 days longer than patients without inflammation.[54]
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40 The anabolic growth factor, IGF-1, and the IGF-1/phosphatidylinositol 3-kinase(PI3K)/Akt
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42
43 pathway is involved in skeletal muscle hypertrophy and atrophy.[55,56] Circulating IGF-1
44
45
46 levels decrease with aging, while inflammatory markers such as TNF- α and IL-6 can
47
48
49 interfere with the IGF-1 signaling pathway.[57,58] As such, changes in IGF-1/PI3K/Akt
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52 signaling during aging may be a result of decreased IGF-1 expression as well as IGF-1
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4 inhibition. Notably, no difference in circulating IGF-1 concentrations were found between
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6
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8 older sarcopenic and non-sarcopenic women.[59]
9

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11
12 Recently, suPAR was established as a biomarker of inflammation and immune activation,
13
14
15 and elevated levels of suPAR are believed to reflect a state of chronic inflammation.[60]
16

17
18
19 SuPAR correlates with other inflammatory markers, such as TNF- α , and patients with the
20
21
22 highest levels of suPAR generally have the worst prognosis.[61] In one study, suPAR was
23
24
25 associated with low muscle mass, while IL-6 was associated with low muscle mass and
26
27
28 increased fat mass in both patients and healthy controls.[62] Thus, there seem to be distinct
29
30
31 inflammatory processes occurring simultaneously with different effects on muscle mass and
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33
34 fat mass, respectively.
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41 Distinct patient populations with co-existing pathophysiological processes might exhibit
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43
44 different biomarker profiles. Further validation needs to be conducted in different patient
45
46
47 populations to utilize the possible prognostic value of these biomarkers, either individually,
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51 or in combination with functional and clinical measures. Systematic identification of
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54 patients at risk of prolonged hospitalization and deconditioning should occur to enable
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4 early individualized interventions to counteract the adverse outcomes of prolonged bed
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6
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8 rest.
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10
11 Results from the Copenhagen PROTECT study can be helpful in the identification of older
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13 patients at risk of prolonged hospitalization. Additionally, circulating biomarker assays able
14
15 to predict physical deconditioning during hospitalization will help in the early detection of
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17 geriatric patients at risk of deconditioning during hospitalization. This knowledge can then
18
19 be tested in a future interventional study. The Copenhagen PROTECT study is considered
20
21 feasible, ethically sound, and with potential extensive implications for future identification
22
23 and treatment of sarcopenia in older medical patients.
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38
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40
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42
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44
45 patients.
46

46 **Contributors**

47
48 RSK, CS, MS, and FEN drafted the manuscript. CS, FEN, RSK, MS, AE, HN, HA, MRW, and EP designed the study.
49
50 SKH, HA, HN, AE, FH, MRW, EP, TN and CBR contributed to the discussion, edited and reviewed the manuscript. All
51
52 authors read and approved the final manuscript.
53

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54
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Competing interests

None

Patient consent for publication

Not required

Data availability statement

There is no data in this work

Ethics approval

The study protocol has been approved by the local ethics committee of Copenhagen and Frederiksberg (H-19039214) and the Danish Data Protection Agency (P-2019-239)

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Figure legends

Figure 1. Timeline and assessments in the PROTECT cohort and the Geriatric cohort

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