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.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-042786
Article Type:	Protocol
Date Submitted by the Author:	14-Jul-2020

Complete List of Authors:	Kamper, Rikke; Geriatric Research Unit, Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg; CopenAge; Copenhagen Center for Clinical Age Research, University of Copenhagen Schultz, Martin; Geriatric Research Unit, Department of Medicine, Copenhagen University Hospital, Herlev and Gentofte; CopenAge; Copenhagen Center for Clinical Age Research, University of Copenhagen Hansen, Sofie; Geriatric Research Unit, Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg; CopenAge; Copenhagen Center for Clinical Age Research, University of Copenhagen Andersen, Helle; Department of Occupational and Physiotherapy, Copenhagen University Hospital, Bispebjerg and Frederiksberg; CopenAge; Copenhagen Center for Clinical Age Research, University of Copenhagen Ekmann, Anette; Geriatric Research Unit, Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg; CopenAge; Copenhagen Center for Clinical Age Research, University of Copenhagen Nygaard, Hanne; Department of Emergency Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg; CopenAge; Copenhagen Center for Clinical Age Research, University of Copenhagen Wejse, Miriam; Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg Pressel, Eckart; Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg Rahbek, Camilla; Geriatric Research Unit, Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg Noerst, Tim; Geriatric Research Unit, Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg Helland, Fredrik; Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg Nielsen, Finn; Department of Emergency Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg; Copenhagen Center for Translational Research, Copenhagen University Hospital, Bispebjerg and Frederiksberg

	Suetta, Charlotte; Geriatric Research Unit, Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg; Geriatric Research Unit, Department of Medicine, Copenhagen University Hospital, Herlev and Gentofte
Keywords:	Risk management < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, STATISTICS & RESEARCH METHODS, ACCIDENT & EMERGENCY MEDICINE, GERIATRIC MEDICINE

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

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.

[1,3]Rikke S. Kamper, [4,3]Martin Schultz, [1,3]Sofie K. Hansen,[5,3]Helle Andersen, [1,3]Anette Ekmann,[3,6]Hanne Nygaard, [2]Miriam R. Wejse, [2]Eckart Pressel, [1]Camilla B. Rahbek, [1]Tim Noerst, [2]Fredrik Helland, [6,7]Finn E. Nielsen, [1,4]Charlotte Suetta

[1]Geriatric Research Unit, Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg, Copenhagen, Denmark.

[2]Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg, Copenhagen, Denmark.

[3]CopenAge; Copenhagen Center for Clinical Age Research, University of Copenhagen, Copenhagen, Denmark.

[4]Geriatric Research Unit, Department of Medicine, Copenhagen University Hospital, Herlev and Gentofte, Herlev, Denmark.

[5]Department of Occupational and Physiotherapy, Copenhagen University Hospital, Bispebjerg and Frederiksberg, Copenhagen, Denmark.

[6]Department of Emergency Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg, Copenhagen Denmark.

[7]Copenhagen Center for Translational Research, Copenhagen University Hospital, Bispebjerg and Frederiksberg, Copenhagen, Denmark.

Keywords: Risk stratification, Acute patients, Elderly, Biomarkers, Sarcopenia

Word count: 3466

Corresponding author

Rikke Stefan Kamper

Geriatric Research Unit, Department of Geriatric and Palliative Medicine,

Bispebjerg and Frederiksberg University Hospital,

Ebba Lunds vej 44,

2400 Copenhagen,

Denmark

Email: rikke.stefan.kamper.01@regionh.dk

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

Clinicaltrails.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

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] Furthermore, physical inactivity, chronic diseases, immobilization, and hospitalization are known to play a part in the development of sarcopenia.[6,11-13]

In 2018, approximately 45% of all hospital admissions in Denmark concerned patients aged 65 or older who had a mean length of stay of 3.5 days.[14] Older patients are often inactive during hospitalization spending 71%-83% of their time lying down,[15,16] and at least 35% of older patients lose independence in one basic Activity of Daily Living (ADL) as an unintended consequence of a medical illness and hospitalization.[17] Sarcopenia may aggravate this functional decline, as patients with sarcopenia have an attenuated recovery of their functional levels 3 months following discharge.[18] From a clinical perspective, sarcopenia is associated with infectious complications, readmissions, increased need for

rehabilitation following discharge, reduced quality of life, increased mortality, and longer hospitalization.[4,19]

Early mobilization protocols have proven effective in reducing hospital-acquired disability and hospital length of stay. However, frequently reported barriers for implementation of early mobilization include lack of staff and time to enable mobilization of the patient.[20] With an increasing aging population and the heterogeneousness of older individuals, the systematic identification of older individuals at risk of prolonged hospitalization and deconditioning during hospitalization are of outmost importance. As such, we need to develop risk stratification tools to identify older patients at risk of these adverse outcomes.

STUDY OBJECTIVES AND HYPOTHESES

Primary objectives and hypothesis

We aim to examine whether circulating biomarkers at admission are associated with length of hospital stay in older (≥ 65 years) acutely admitted medical patients and whether the combination of clinical and functional measures with these biomarkers can identify patients at risk of having a prolonged hospital stay (>96 hours). In addition, we aim to establish

circulating biomarkers associated with changes in muscle mass, muscle strength, and function in geriatric patients during hospitalization. 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 prolonged (≥ 96 hours) hospital stays and physical deconditioning during hospitalization.

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 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

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

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

Outcomes

The primary outcome in the PROTECT cohort is the length of hospital stay. As successive events of hospitalisation have been suggested to contribute to the development of sarcopenia, and even short periods (4-5 days) of skeletal muscle disuse are known to induce muscle atrophy,[21,22] we have defined a prolonged hospital length of stay as an admission lasting >96 hours. In 2018, the mean length of hospital stay in Denmark was 84 hours in patients aged 65 years or over.[14]

The primary outcomes in the Geriatric cohort are the relative changes in muscle mass, muscle strength, and muscle function during hospitalization. Primary and secondary outcomes for both cohorts are listed in table 2.

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

The research personnel might be unable to assess all patients, as the number of eligible patients (i.e. fulfilling inclusion criteria with the absence of exclusion criteria) varies daily. Thus, to avoid selection bias, all eligible patients on the day in question are randomized using a computer-generated randomization sequence to establish a randomized visitation sequence. Patients who wish to participate sign an informed consent and baseline measurements are performed within the first 24 hours of admission.

All included patients have blood samples drawn to determine concentrations of tumor necrosis factor (TNF)-α, interferon gamma (IFN-γ), interleukin (IL)-6, IL-10, IL-13, transforming growth factor (TGF)-β1, follistatin, insulin-like growth factor (IGF)-1, growth differentiation factor (GDF)-11, GDF-15, and soluble urokinase-type plasminogen activator receptor (suPAR).

Handgrip strength is assessed using a digital hand-held dynamometer (Model SH1001; SAEHAN Corporation, Yangdeok-Dong, Masan, South Korea). Patients able to leave the bed sit on a chair with the elbow flexed at 90° and the wrist in a neutral position, while bedridden patients are assessed in the hospital bed with the backrest elevated. The highest value of three attempts with the dominant hand is used for analyses. Should the

third trial elicit the highest value, the patient continues until a lower value is achieved.

Muscle function is assessed in the 30-second sit-to-stand test, where patients are asked to stand up from a standardized chair as many times as possible with their arms folded across the chest. Only full standing positions are counted.

Patients included in the Geriatric cohort also have their habitual gait-speed assessed. The gait-speed assessment is measured over a course of 4 meters and includes walking aids if they are used by the patient. Patients stand behind a starting line and are asked to start walking towards a visual goal at their habitual pace. The visual goal is placed after 5.5 m. to reduce the effect of deceleration. The fastest of the two attempts will be used for analyses and quantified as m/s.

The assessment of handgrip strength (kg) and habitual gait-speed have previously shown to be feasible and reliable measures in acutely older medical patients. However, the feasibility and reliability of the 30-second sit-to-stand test was moderate, as only half of the patients were able to perform the test as instructed.[23] Thus, we have included an additional nominal variable to categorize the sit-to-stand ability as either i) able to perform

the test as instructed, ii) ability to rise using the armrest, and iii) inability to rise independently from a chair.

Bodyweight (kg) is assessed using chair scales and height (cm) is estimated with a segmometer using the knee-height measurement and age with the equations from Chumlea et al.[24] Body composition is assessed using Direct-Segmental Multi-frequency Bioelectrical Impedance Analyses (DSM-BIA) (InBody S10®; Biospace Co., Ltd., Seoul, Korea). Self-reported current smoking is reported as a dichotomous variable. Patients included in the Geriatric cohort are also assessed at discharge to evaluate circulating biomarker concentrations as well as changes in body composition, muscle strength, and functional performance. Tests of strength, physical function and body composition measurements are performed by trained research personnel.

The presence of frailty is assessed by trained nurses associated with the study using the Canadian Study of Health and Aging Clinical Frailty Scale (CFS).[25] Patients are screened for sarcopenia using the SARC-F questionnaire,[26] while cognitive status is evaluated by the short Orientation-Memory-Concentration test (OMC).[27] The risk of malnutrition is assessed and validated using the Short Nutritional Assessment

Questionnaire (SNAQ).[28] A flowchart showing the timeline and assessments in the two cohorts can be seen in Figure 1.

INSERT FIGURE 1 AROUND HERE

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)[29] and obtained by evaluating the type and number of ICD-10 discharge diagnosis during the last 5 years of the index admission. 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 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

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

Patient and public involvement

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

Power calculation and statistics

To evaluate the prognostic abilities of circulating biomarkers (individually, in combination and combined with clinical and functional measures) we will use the area under the curve for receiver operating characteristics (AUROC) statistics. A reference group of 2058

patients over the age of 65 from Bispebjerg-Frederiksberg University Hospital and Herlev-Gentofte Hospital had a mean age of 78.3 years and a mean length of stay of 5.8 days during hospitalization. In these patients, 817 (39.7%) had a prolonged length of stay, defined as a hospitalization lasting more than 96 hours. With a sample size of 1700 and the assumption that approximately 40% of older medical patients have a prolonged hospital stay, an AUROC of 82 will have a power of 0.9 with a significance level of 0.05.

A table of summary statistics will be presented with baseline variables. Continuous variables will be summarized with: n (non-missing sample size), mean, standard deviation, median, interquartile range, and number of missing values. Categorical variables will be reported as frequency and percentages (based on non-missing sample size), and number of missing values. Data missing at random will be imputed using multiple imputation.

To evaluate whether clinical, functional, and circulating biomarkers are associated with length of stay we will perform multivariate logistic regression. Patients will be grouped in either normal (< 96 hours) or extended length of stay (≥ 96 hours) and Cox regression analysis will be used to compare differences in non-elective readmission and all-cause mortality. Patients will be followed from the date of discharge from the index admission

until the end of the follow-up period, emigration, readmission, or death as appropriate. To assess the discriminative ability of biomarkers with regards to an extended length of stay and all-cause mortality, we will use the area under the curve (AUC) for receiver operating characteristics (ROC) curves. AUCs for different ROC curves will be compared using the DeLong test. The association of circulating biomarkers with changes in muscle mass, muscle strength, and function in the Geriatric cohort will be assessed using a multivariate linear model adjusted for the relative length of stay.

Study organization

The study is a researcher initiated clinical study. The protocol was written by the steering committee composed of experts in geriatric medicine and acute medicine and a PhD student in clinical medicine. The committee is responsible for the design of the study, supervision of research personnel, data acquisition, communication and publication of results, approval of sub-studies and ensuring that future studies comply with the regulations regarding data management.

At present (July 2020), the study has included 377 patients, of which 62 are part of the Geriatric cohort. Inclusion was temporarily paused due to the impact of the Covid-19 pandemic.

ETHICS AND DISSEMINATION

All procedures are being conducted according to “Good Clinical Practice” standards, regarding initiation, monitoring, and reporting. The study protocol has been approved by the local ethics committee (H-19039214) and the Danish Data Protection Agency (P-2019-239), and the project complies with the regulations of the General Data Protection Regulation (GDPR) and the Data Protection Act.

All eligible patients receive oral and written information. In the case of severe dementia or delirium, some patients might be unable to provide participant consent. In these cases, we seek participant consent from a close relative or guardian, should the guardianship include access to sign participant consent for research purposes. Independent medical doctors who have knowledge of the project but are not associated with the project and are independent of the interests of the principal investigator evaluate whether these subjects can participate in the study.

The project entails minimal discomfort and no permanent side effects; thus, it is considered ethically sound. Findings from the project, regardless of the outcome, will be published in relevant peer-reviewed scientific journals ensuring the anonymity of the patients. The study is registered at clinicaltrials.gov (NCT04151108) where positive, negative, or inconclusive results will be published.

DISCUSSION

The importance of sarcopenia has recently been underlined by its inclusion as a reportable disease in the Centers for Disease Control and Prevention (ICD-10-CM code M62.84) in October 2016.[32] Even though the serious consequences of sarcopenia are widely recognized, the diagnose has yet to be implemented in clinical practice in Denmark. Lack of knowledge regarding the basic biological mechanisms driving sarcopenia in conjunction with other age-related diseases and lack of systematic assessment hinders the identification and treatment of sarcopenia and can lead to physical deconditioning during hospitalization. As such, the knowledge surrounding the prevalence and determinants of sarcopenia in older medical patients is scarce, and it is unknown whether circulating biomarkers, individually or in combination, can predict physical deconditioning during hospitalization.

Mechanisms that regulate skeletal muscle mass are central to the understanding of sarcopenia. Myostatin, also referred to as GDF-8, is a part of the TGF-β family and predominantly expressed in skeletal muscle. Myostatin and TGF-β are inducers of catabolic processes, inhibiting muscle growth, and inducing muscle protein breakdown via activation of the Small Mothers Against Decapentaplegic (SMAD)2 and SMAD3 transcription factors.[33,34] Myostatin is reportedly increased with aging,[35] and following prolonged bed rest,[36] and treatment with myostatin antibodies attenuates the loss of muscle mass and function induced by immobilization in mice.[37]

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

GDF-15, another member of the TGF-β family, is present in low levels under healthy conditions but can increase during disease or injury and contribute to muscle wasting by

suppressing appetite, which may result in anorexia and drastic weight loss.[40] In older patients, unintentional weight loss has been associated with an increased in-hospital morbidity and increased overall mortality.[41] GDF-15 may be induced in response to cellular stress signals or dysfunctions, and it has been suggested that circulating levels of GDF-15 could be biomarker of mitochondrial dysfunction.[42] Nonetheless, several studies demonstrate that GDF-15 levels are predictors of all-cause mortality.[43,44]

Follistatin acts as an antagonist to TGF-β family ligands including myostatin, TGF-β and GDF11.[34] Measurements of TGF-β ligands as well as their antagonist follistatin could represent biomarkers of muscle breakdown, physical function or mortality. However, the translation of these findings into clinical utility needs further validation in a larger cohort.

Several studies have investigated the association of inflammatory biomarkers with muscle mass, muscle strength, and muscle function in healthy older subjects. Most commonly, studies have focused on a few biomarkers, such as TNF-α, IL-6 or C-Reactive Protein (CRP).[9,45-47] A recent study has demonstrated an inverse relationship between a composite of pro- and anti-inflammatory markers and muscle mass, strength and function in healthy older subjects.[48] However, results are inconsistent and lack clear evidence as to

whether these inflammatory biomarkers are associated with sarcopenia. Nonetheless, circulating levels of CRP are predictive of both the length of hospital stay and readmissions.[49,50] Indeed, geriatric patients with inflammation, evaluated by CRP levels at admission, stayed on average 3 days longer than patients without inflammation.[51]

The anabolic growth factor, IGF-1, and the IGF-1/phosphatidylinositol 3-kinase(PI3K)/Akt pathway is involved in skeletal muscle hypertrophy and atrophy.[52,53] Circulating IGF-1 levels decrease with aging, while inflammatory markers such as TNF-α and IL-6 can interfere with the IGF-1 signaling pathway.[54,55] As such, changes in IGF-1/PI3K/Akt signaling during aging may be a result of decreased IGF-1 expression as well as IGF-1 inhibition. Notably, no difference in circulating IGF-1 concentrations were found between older sarcopenic and non-sarcopenic women.[56]

Recently, suPAR was established as a biomarker of inflammation and immune activation, and elevated levels of suPAR are believed to reflect a state of chronic inflammation.[57] SuPAR correlates with other inflammatory markers, such as TNF-α, and patients with the highest levels of suPAR generally have the worst prognosis.[58] In one study, suPAR was associated with low muscle mass, while IL-6 was associated with low muscle mass and

increased fat mass in both patients and healthy controls.[59] Thus, there seem to be distinct inflammatory processes occurring simultaneously with different effects on muscle mass and fat mass, respectively.

Distinct patient populations with co-existing pathophysiological processes might exhibit different biomarker profiles. Further validation needs to be conducted in different patient populations to utilize the possible prognostic value of these biomarkers, either individually, or in combination with functional and clinical measures. Systematic identification of patients at risk of prolonged hospitalization and deconditioning should occur to enable early individualized interventions to counteract the adverse outcomes of prolonged bed rest.

CONCLUSIONS AND CLINICAL IMPLICATIONS

If the hypotheses from the Copenhagen PROTECT study are confirmed, the results can be helpful in the identification of older patients at risk of prolonged hospitalization. Additionally, circulating biomarker assays able to predict physical deconditioning during hospitalization will help in the early detection of geriatric patients at risk of deconditioning during hospitalization. This knowledge can then be tested in a future interventional study.

The Copenhagen PROTECT study is considered feasible, ethically sound, and with potential extensive implications for future identification and treatment of sarcopenia in older medical patients.

Acknowledgements

The authors would like to thank the doctors, nurses, secretaries and physiotherapists at the Department of Emergency Medicine and the Department of Geriatric and Palliative Medicine for their cooperation and patience during patient recruitment and assessment. Furthermore, they would like to thank Rasmus Roennemoes Iversen for his help in the recruitment of patients.

Contributors

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

Funding

The work is supported by funding from the Novo Nordisk Foundation; grant number NNF18OC0052826.

Disclaimer

The funders take no part in the design of the study; in the collection, analysis or interpretation of data; in the writing of manuscripts or decisions regarding publication.

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 (H-19039214) and the Danish Data Protection Agency (P-2019-239)

REFERENCES

1. Rosenberg IH. Sarcopenia: origins and clinical relevance. *Clin Geriatr Med*. 2011;27(3):337-339. doi:10.1016/j.cger.2011.03.003

2. Roubenoff R. Sarcopenia: a major modifiable cause of frailty in the elderly. *J Nutr Health Aging*. 2000;4(3):140-142.

3. Rantanen T, Avlund K, Suominen H, et al. Muscle strength as a predictor of onset of ADL dependence in people aged 75 years. *Aging Clin Exp Res*. 2002;14(3 Suppl):10-15.

4. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age Ageing*. 2010;39(4):412-423. doi:10.1093/ageing/afq034

5. von Haehling S, Morley JE, Anker SD. An overview of sarcopenia: facts and numbers on prevalence and clinical impact. *J Cachexia Sarcopenia Muscle*. 2010;1(2):129-133. doi:10.1007/s13539-010-0014-2

6. Roubenoff R, Heymsfield SB, Kehayias JJ, et al. Standardization of nomenclature of body composition in weight loss. *Am J Clin Nutr*. 1997;66(1):192-196. doi:10.1093/ajcn/66.1.192

7. Aagaard P, Suetta C, Caserotti P, et al. Role of the nervous system in sarcopenia and muscle atrophy with aging: strength training as a countermeasure. *Scand J Med Sci Sports*. 2010;20(1):49-64. doi:10.1111/j.1600-0838.2009.01084.x

8. La Colla A, Pronsato L, Milanesi L, et al. 17β-Estradiol and testosterone in sarcopenia: Role of satellite cells. *Ageing Res Rev*. 2015;24(Pt B):166-177. doi:10.1016/j.arr.2015.07.011

9. Bano G, Trevisan C, Carraro S, et al. Inflammation and sarcopenia: A systematic review and meta-analysis. *Maturitas*. 2017;96:10-15. doi:10.1016/j.maturitas.2016.11.006

10. Cruz-Jentoft AJ, Kiesswetter E, Drey M, et al. Nutrition, frailty, and sarcopenia. *Aging Clin Exp Res*. 2017;29(1):43-48. doi:10.1007/s40520-016-0709-0

11. Bautmans I, Van Puyvelde K, Mets T. Sarcopenia and functional decline: pathophysiology, prevention and therapy. *Acta Clin Belg*. 2009;64(4):303-316. doi:10.1179/acb.2009.048

12. Suetta C, Hvid LG, Justesen L, et al. Effects of aging on human skeletal muscle after immobilization and retraining. *J Appl Physiol (1985)*. 2009;107(4):1172-1180. doi:10.1152/japplphysiol.00290.2009

13. Gill TM, Allore HG, Holford TR, et al. Hospitalization, restricted activity, and the development of disability among older persons. *JAMA*. 2004;292(17):2115-2124. doi:10.1001/jama.292.17.2115

14. Statistics Denmark. https://www.statistikbanken.dk/IND05 (accessed July 2020)

15. Brown CJ, Redden DT, Flood KL, et al. The underrecognized epidemic of low mobility during hospitalization of older adults. *J Am Geriatr Soc*. 2009;57(9):1660-1665. doi:10.1111/j.1532-5415.2009.02393.x

16. Pedersen MM, Bodilsen AC, Petersen J, et al. Twenty-four-hour mobility during acute hospitalization in older medical patients. *J Gerontol A Biol Sci Med Sci*. 2013;68(3):331-337. doi:10.1093/gerona/gls165

17. Covinsky KE, Palmer RM, Fortinsky RH, et al. Loss of independence in activities of daily living in older adults hospitalized with medical illnesses: increased vulnerability with age. *J Am Geriatr Soc*. 2003;51(4):451-458. doi:10.1046/j.1532-5415.2003.51152.x

18. Sánchez-Rodríguez D, Marco E, Miralles R, et al. Sarcopenia, physical rehabilitation and functional outcomes of patients in a subacute geriatric care unit. *Arch Gerontol Geriatr*. 2014;59(1):39-43. doi:10.1016/j.archger.2014.02.009

19. Peterson SJ, Braunschweig CA. Prevalence of Sarcopenia and Associated Outcomes in the Clinical Setting. *Nutr Clin Pract*. 2016;31(1):40-48. doi:10.1177/0884533615622537

20. Surkan MJ, Gibson W. Interventions to Mobilize Elderly Patients and Reduce Length of Hospital Stay. *Can J Cardiol*. 2018;34(7):881-888. doi:10.1016/j.cjca.2018.04.033

21. Kouw IWK, Groen BBL, Smeets JSJ, et al. One Week of Hospitalization Following Elective Hip Surgery Induces Substantial Muscle Atrophy in Older Patients. *J Am Med Dir Assoc*. 2019;20(1):35-42. doi:10.1016/j.jamda.2018.06.018

22. Suetta C, Frandsen U, Jensen L, et al. Aging affects the transcriptional regulation of human skeletal muscle disuse atrophy. *PLoS One*. 2012;7(12):e51238. doi:10.1371/journal.pone.0051238

23. Bodilsen AC, Juul-Larsen HG, Petersen J, et al. Feasibility and inter-rater reliability of physical performance measures in acutely admitted older medical patients. *PLoS One*. 2015;10(2):e0118248. Published 2015 Feb 23. doi:10.1371/journal.pone.0118248

24. Chumlea WC, Roche AF, Steinbaugh ML. Estimating stature from knee height for persons 60 to 90 years of age. *J Am Geriatr Soc*. 1985;33(2):116-120. doi:10.1111/j.1532-5415.1985.tb02276.x

25. Rockwood K, Song X, MacKnight C, et al. A global clinical measure of fitness and frailty in elderly people. *CMAJ*. 2005;173(5):489-495. doi:10.1503/cmaj.050051

26. Malmstrom TK, Miller DK, Simonsick EM, et al. SARC-F: a symptom score to predict persons with sarcopenia at risk for poor functional outcomes. *J Cachexia Sarcopenia Muscle*. 2016;7(1):28-36. doi:10.1002/jcsm.12048

27. Katzman R, Brown T, Fuld P, et al. Validation of a short Orientation-Memory-Concentration Test of cognitive impairment. *Am J Psychiatry*. 1983;140(6):734-739. doi:10.1176/ajp.140.6.734

28. Kruizenga HM, Seidell JC, de Vet HC, et al. Development and validation of a hospital screening tool for malnutrition: the short nutritional assessment questionnaire (SNAQ). *Clin Nutr*. 2005;24(1):75-82. doi:10.1016/j.clnu.2004.07.015

29. Charlson ME, Pompei P, Ales KL, et al. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis*. 1987;40(5):373-383. doi:10.1016/0021-9681(87)90171-8

30. Harris PA, Taylor R, Thielke R, et al. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42(2):377-381. doi:10.1016/j.jbi.2008.08.010

31. Harris PA, Taylor R, Minor BL, et al. The REDCap consortium: Building an international community of software platform partners. *J Biomed Inform*. 2019;95:103208. doi:10.1016/j.jbi.2019.103208

32. World Health Organization. International statistical classification of diseases and related health problems. - 10th revision, Fifth edition, 2016; https://icd.who.int/browse10/2016/en#/M60-M63 (accessed July 2020)

33. Lee SJ, Reed LA, Davies MV, et al. Regulation of muscle growth by multiple ligands signaling through activin type II receptors. *Proc Natl Acad Sci U S A*. 2005;102(50):18117-18122. doi:10.1073/pnas.0505996102

34. Han HQ, Zhou X, Mitch WE, et al. Myostatin/activin pathway antagonism: molecular basis and therapeutic potential. *Int J Biochem Cell Biol*. 2013;45(10):2333-2347. doi:10.1016/j.biocel.2013.05.019

35. Yarasheski KE, Bhasin S, Sinha-Hikim I, et al. Serum myostatin-immunoreactive protein is increased in 60-92 year old women and men with muscle wasting. *J Nutr Health Aging*. 2002;6(5):343-348.

36. Reardon KA, Davis J, Kapsa RM, et al. Myostatin, insulin-like growth factor-1, and leukemia inhibitory factor mRNAs are upregulated in chronic human disuse muscle atrophy. *Muscle Nerve*. 2001;24(7):893-899. doi:10.1002/mus.1086

37. Murphy KT, Cobani V, Ryall JG, et al. Acute antibody-directed myostatin inhibition attenuates disuse muscle atrophy and weakness in mice. *J Appl Physiol (1985)*. 2011;110(4):1065-1072. doi:10.1152/japplphysiol.01183.2010

38. Nakashima M, Toyono T, Akamine A, et al. Expression of growth/differentiation factor 11, a new member of the BMP/TGFbeta superfamily during mouse embryogenesis. *Mech Dev*. 1999;80(2):185-189. doi:10.1016/s0925-4773(98)00205-6

39. Schafer MJ, Atkinson EJ, Vanderboom PM, et al. Quantification of GDF11 and Myostatin in Human Aging and Cardiovascular Disease. *Cell Metab*. 2016;23(6):1207-1215. doi:10.1016/j.cmet.2016.05.023

40. Tsai VW, Husaini Y, Manandhar R, et al. Anorexia/cachexia of chronic diseases: a role for the TGF-β family cytokine MIC-1/GDF15. *J Cachexia Sarcopenia Muscle*. 2012;3(4):239-243. doi:10.1007/s13539-012-0082-6

41. Gaddey HL, Holder K. Unintentional weight loss in older adults. *Am Fam Physician*. 2014;89(9):718-722.

42. Fujita Y, Taniguchi Y, Shinkai S, et al. Secreted growth differentiation factor 15 as a potential biomarker for mitochondrial dysfunctions in aging and age-related disorders. *Geriatr Gerontol Int*. 2016;16 Suppl 1:17-29. doi:10.1111/ggi.12724

43. Eggers KM, Kempf T, Wallentin L, et al. Change in growth differentiation factor 15 concentrations over time independently predicts mortality in community-dwelling elderly individuals. *Clin Chem*. 2013;59(7):1091-1098. doi:10.1373/clinchem.2012.201210

44. Wiklund FE, Bennet AM, Magnusson PK, et al. Macrophage inhibitory cytokine-1 (MIC-1/GDF15): a new marker of all-cause mortality. *Aging Cell*. 2010;9(6):1057-1064. doi:10.1111/j.1474-9726.2010.00629.

45. Schaap LA, Pluijm SM, Deeg DJ, et al. Inflammatory markers and loss of muscle mass (sarcopenia) and strength. *Am J Med*. 2006;119(6):526.e9-526.e5.26E17. doi:10.1016/j.amjmed.2005.10.049

46. Visser M, Pahor M, Taaffe DR, et al. Relationship of interleukin-6 and tumor necrosis factor-alpha with muscle mass and muscle strength in elderly men and women: the Health ABC Study. *J Gerontol A Biol Sci Med Sci*. 2002;57(5):M326-M332. doi:10.1093/gerona/57.5.m326

47. Cesari M, Penninx BW, Pahor M, et al. Inflammatory markers and physical performance in older persons: the InCHIANTI study. *J Gerontol A Biol Sci Med Sci*. 2004;59(3):242-248. doi:10.1093/gerona/59.3.m242

48. Calvani R, Marini F, Cesari M, et al. Systemic inflammation, body composition, and physical performance in old community-dwellers. *J Cachexia Sarcopenia Muscle*. 2017;8(1):69-77. doi:10.1002/jcsm.12134

49. Brown SH, Flint K, Storey A, et al. Routinely assessed biochemical markers tested on admission as predictors of adverse outcomes in hospitalized elderly patients. *Hosp Pract (1995)*. 2012;40(1):193-201. doi:10.3810/hp.2012.02.960

50. Hogarth MB, Gallimore R, Savage P, et al. Acute phase proteins, C-reactive protein and serum amyloid A protein, as prognostic markers in the elderly inpatient. *Age Ageing*. 1997;26(2):153-158. doi:10.1093/ageing/26.2.153

51. Bautmans I, Njemini R, Lambert M, et al. Circulating acute phase mediators and skeletal muscle performance in hospitalized geriatric patients. *J Gerontol A Biol Sci Med Sci*. 2005;60(3):361-367. doi:10.1093/gerona/60.3.361

52. Bodine SC, Stitt TN, Gonzalez M, et al. Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nat Cell Biol*. 2001;3(11):1014-1019. doi:10.1038/ncb1101-1014

53. Glass DJ. Skeletal muscle hypertrophy and atrophy signaling pathways. *Int J Biochem Cell Biol*. 2005;37(10):1974-1984. doi:10.1016/j.biocel.2005.04.018

54. Barbieri M, Ferrucci L, Ragno E, et al. Chronic inflammation and the effect of IGF-I on muscle strength and power in older persons. *Am J Physiol Endocrinol Metab*. 2003;284(3):E481-E487. doi:10.1152/ajpendo.00319.2002

55. Broussard SR, Zhou JH, Venters HD, et al. At the interface of environment-immune interactions: Cytokine and growth-factor receptors, *Journal of Animal Science*, Volume 79, Issue suppl_E, 2001, Pages E268–E284, https://doi.org/10.2527/jas2001.79E-SupplE268x

56. Hofmann M, Halper B, Oesen S, et al. Serum concentrations of insulin-like growth factor-1, members of the TGF-beta superfamily and follistatin do not reflect different stages of dynapenia and sarcopenia in elderly women. *Exp Gerontol*. 2015;64:35-45. doi:10.1016/j.exger.2015.02.008

57. Haupt TH, Kallemose T, Ladelund S, et al. Risk factors associated with serum levels of the inflammatory biomarker soluble urokinase plasminogen activator receptor in a general population. *Biomark Insights*. 2014;9:91-100. Published 2014 Dec 16. doi:10.4137/BMI.S19876

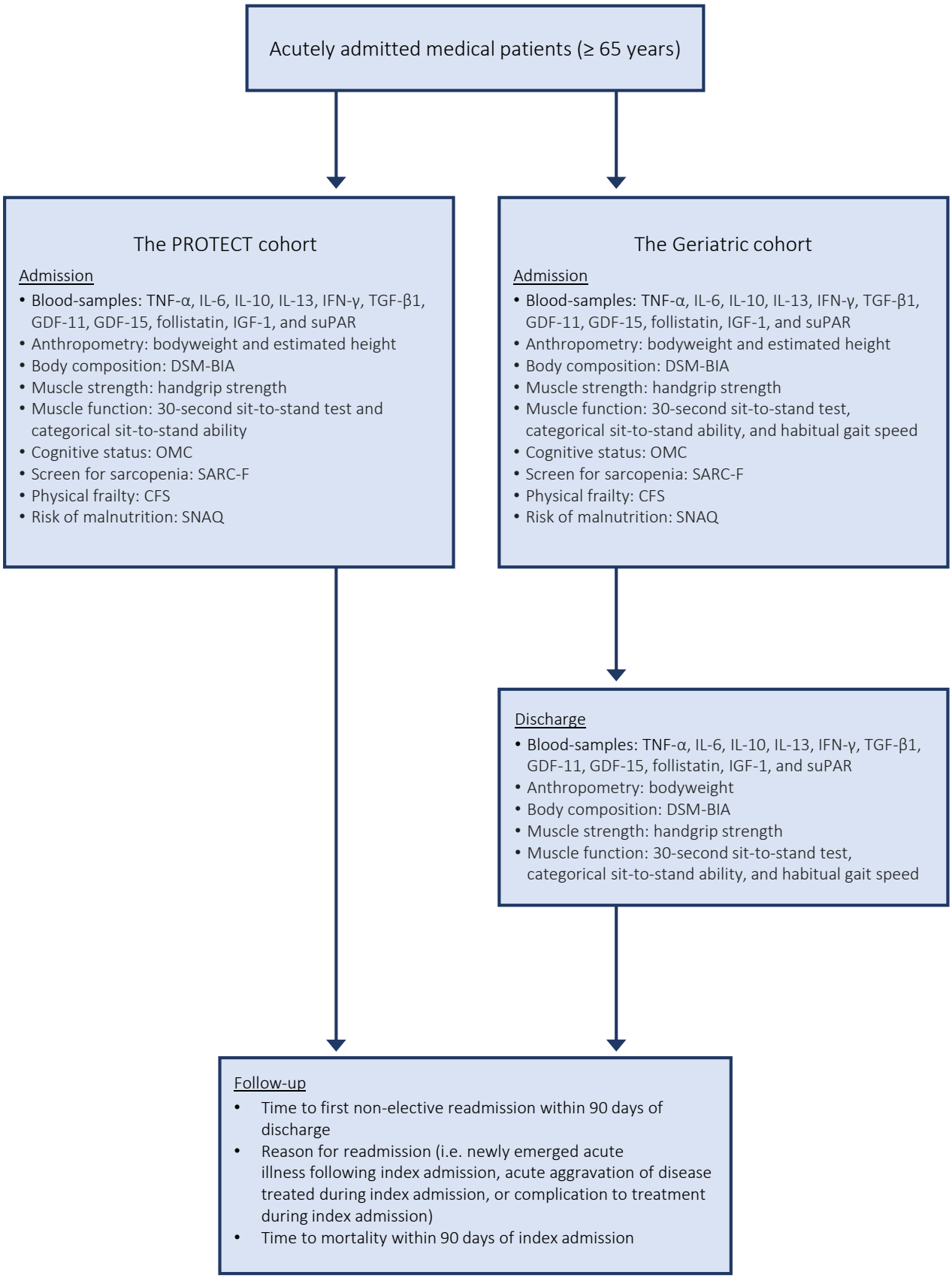
58. Thunø M, Macho B, Eugen-Olsen J. suPAR: the molecular crystal ball. *Dis Markers*. 2009;27(3):157-172. doi:10.3233/DMA-2009-0657

59. Langkilde A, Petersen J, Henriksen JH, et al. Leptin, IL-6, and suPAR reflect distinct inflammatory changes associated with adiposity, lipodystrophy and low muscle mass in HIV-infected patients and controls. *Immun Ageing*. 2015;12:9. Published 2015 Aug 4. doi:10.1186/s12979-015-0036-x

60. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA*. 2016;315(8):801-810. doi:10.1001/jama.2016.0287

Figure legends

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


Acutely admitted medical patients (≥ 65 years)
The PROTECT cohort
Admission
• Blood-samples: TNF-α, IL-6, IL-10, IL-13, IFN-γ, TGF-β1, GDF-11, GDF-15, follistatin, IGF-1, and suPAR
• Anthropometry: bodyweight and estimated height
• Body composition: DSM-BIA
• Muscle strength: handgrip strength
• Muscle function: 30-second sit-to-stand test and categorical sit-to-stand ability
• Cognitive status: OMC
• Screen for sarcopenia: SARC-F
• Physical frailty: CFS
• Risk of malnutrition: SNAQ
The Geriatric cohort
Admission
• Blood-samples: TNF-α, IL-6, IL-10, IL-13, IFN-γ, TGF-β1, GDF-11, GDF-15, follistatin, IGF-1, and suPAR
• Anthropometry: bodyweight and estimated height
• Body composition: DSM-BIA
• Muscle strength: handgrip strength
• Muscle function: 30-second sit-to-stand test, categorical sit-to-stand ability, and habitual gait speed
• Cognitive status: OMC
• Screen for sarcopenia: SARC-F
• Physical frailty: CFS
• Risk of malnutrition: SNAQ
Discharge
• Blood-samples: TNF-α, IL-6, IL-10, IL-13, IFN-γ, TGF-β1, GDF-11, GDF-15, follistatin, IGF-1, and suPAR
• Anthropometry: bodyweight
• Body composition: DSM-BIA
• Muscle strength: handgrip strength
• Muscle function: 30-second sit-to-stand test, categorical sit-to-stand ability, and habitual gait speed
Follow-up
• Time to first non-elective readmission within 90 days of discharge
• Reason for readmission (i.e. newly emerged acute illness following index admission, acute aggravation of disease treated during index admission, or complication to treatment during index admission)
• Time to mortality within 90 days of index admission

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.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-042786.R1
Article Type:	Protocol
Date Submitted by the Author:	17-Nov-2020
Complete List of Authors:	Kamper, Rikke; Geriatric Research Unit, Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg; CopenAge; Copenhagen Center for Clinical Age Research, University of Copenhagen Schultz, Martin; Geriatric Research Unit, Department of Medicine, Copenhagen University Hospital, Herlev and Gentofte; CopenAge; Copenhagen Center for Clinical Age Research, University of Copenhagen Hansen, Sofie; Geriatric Research Unit, Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg; CopenAge; Copenhagen Center for Clinical Age Research, University of Copenhagen Andersen, Helle; Department of Occupational and Physiotherapy, Copenhagen University Hospital, Bispebjerg and Frederiksberg; CopenAge; Copenhagen Center for Clinical Age Research, University of Copenhagen Ekmann, Anette; Geriatric Research Unit, Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg; CopenAge; Copenhagen Center for Clinical Age Research, University of Copenhagen Nygaard, Hanne; Department of Emergency Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg; CopenAge; Copenhagen Center for Clinical Age Research, University of Copenhagen Helland, Fredrik; Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg Wejse, Miriam; Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg Rahbek, Camilla; Geriatric Research Unit, Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg Noerst, Tim; Geriatric Research Unit, Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg Pressel, Eckart; Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg Nielsen, Finn; Department of Emergency Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg; Copenhagen Center for Translational Research, Copenhagen University Hospital, Bispebjerg and Frederiksberg

	Suetta, Charlotte; Geriatric Research Unit, Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg; Geriatric Research Unit, Department of Medicine, Copenhagen University Hospital, Herlev and Gentofte
Primary Subject Heading:	Research methods
Secondary Subject Heading:	Geriatric medicine, Rehabilitation medicine
Keywords:	Risk management < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, STATISTICS & RESEARCH METHODS, GERIATRIC MEDICINE

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

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.

[1,3]Rikke S. Kamper, [4,3]Martin Schultz, [1,3]Sofie K. Hansen,[5,3]Helle Andersen, [1,3]Anette Ekmann,[3,6]Hanne Nygaard, [2]Fredrik Helland, [2]Miriam R. Wejse, [1]Camilla B. Rahbek, [1]Tim Noerst, [2]Eckart Pressel, [6,7]Finn E. Nielsen, [1,4]Charlotte Suetta

[1]Geriatric Research Unit, Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg, Copenhagen, Denmark.

[2]Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg, Copenhagen, Denmark.

[3]CopenAge; Copenhagen Center for Clinical Age Research, University of Copenhagen, Copenhagen, Denmark.

[4]Geriatric Research Unit, Department of Medicine, Copenhagen University Hospital, Herlev and Gentofte, Herlev, Denmark.

[5]Department of Occupational and Physiotherapy, Copenhagen University Hospital, Bispebjerg and Frederiksberg, Copenhagen, Denmark.

[6]Department of Emergency Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg, Copenhagen Denmark.

[7]Copenhagen Center for Translational Research, Copenhagen University Hospital, Bispebjerg and Frederiksberg, Copenhagen, Denmark.

Keywords: Risk management, Statistics and research methods, Geriatric medicine

Word count: 3546

Corresponding author

Rikke Stefan Kamper

Geriatric Research Unit, Department of Geriatric and Palliative Medicine,

Bispebjerg and Frederiksberg University Hospital,

Ebba Lunds vej 44,

2400 Copenhagen,

Denmark

Email: rikke.stefan.kamper.01@regionh.dk

Telephone: +4520446333

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 of Copenhagen and Frederiksberg (H-19039214) and the Danish Data Protection Agency (P-2019-239) and all experimental procedures were performed in accordance with the Declaration of Helsinki. 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

Clinicaltrails.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 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]

Furthermore, physical inactivity, chronic diseases, immobilization, and hospitalization are known to play a part in the development of sarcopenia.[6,11-13]

In 2018, approximately 45% of all hospital admissions in Denmark concerned patients aged 65 or older who had a mean length of stay of 3.5 days.[14] Older patients are often inactive during hospitalization spending 71%-83% of their time lying down,[15,16] and at least 35% of older patients lose independence in one basic Activity of Daily Living (ADL) as an unintended consequence of a medical illness and hospitalization.[17] Sarcopenia may aggravate this functional decline, as patients with sarcopenia have an attenuated recovery of their functional levels 3 months following discharge.[18] From a clinical perspective, sarcopenia is associated with infectious complications, readmissions, increased need for rehabilitation following discharge, reduced quality of life, increased mortality, and longer hospitalization.[4,19]

Early mobilization protocols have proven effective in reducing hospital-acquired disability and hospital length of stay. However, frequently reported barriers for implementation of early mobilization include lack of staff and time to enable mobilization of the patient.[20] With an increasing aging population and the heterogeneousness of older individuals, the systematic

identification of older individuals at risk of prolonged hospitalization and deconditioning during hospitalization are of outmost importance. As such, the combination of clinical, functional and circulating biomarkers may serve as risk stratification tools to identify older patients at risk of these adverse outcomes.

STUDY OBJECTIVES AND HYPOTHESES

Primary objectives and hypothesis

We aim to examine whether circulating biomarkers at admission are associated with length of hospital stay in older (≥ 65 years) acutely admitted medical patients and whether the combination of clinical and functional measures with these biomarkers can identify patients at risk of having a prolonged hospital stay (>96 hours). In addition, we aim to establish circulating biomarkers associated with changes in muscle mass, muscle strength, and function in geriatric patients during hospitalization. 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 prolonged (≥ 96 hours) hospital stays and physical deconditioning during hospitalization.

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

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

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

Outcomes

The primary outcome in the PROTECT cohort is the length of hospital stay. Successive events of hospitalisation have been suggested to contribute to the development of sarcopenia, and even short periods (4-5 days) of skeletal muscle disuse are known to induce muscle atrophy.[21,22] In 2018, the mean length of hospital stay in Denmark was 84 hours in patients aged 65 years or over.[14] while the mean LOS in the two largest local

hospitals was 96 hours. As such, we have defined a prolonged hospital length of stay as an admission lasting >96 hours.

The primary outcomes in the Geriatric cohort are the relative changes in muscle mass, muscle strength, and muscle function during hospitalization. Primary and secondary outcomes for the PROTECT cohort and the Geriatric cohort are listed in table 2 and 3, respectively.

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

Table 3. Primary and secondary outcomes in the Geriatric cohort

Primary outcomes
Changes in muscle mass during hospitalization

Changes in muscle strength during hospitalization
Changes in muscle function during hospitalization
Secondary outcomes
Length of hospital stay
Non-elective readmissions within 90 days of discharge
All-cause mortality within 90 days of index admission
In-hospital mortality
Discharge to an increased level of care
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

The research personnel might be unable to assess all patients, as the number of eligible patients (i.e. fulfilling inclusion criteria with the absence of exclusion criteria) varies daily. Thus, to avoid selection bias, all eligible patients on the day in question are randomized using a computer-generated randomization sequence to establish a randomized visitation sequence. Patients who wish to participate sign an informed consent and baseline measurements are performed within the first 24 hours of admission. All included patients have blood samples drawn to determine concentrations of tumor necrosis factor (TNF)-α, , interleukin (IL)-6, IL-10, transforming growth factor (TGF)-β1, follistatin, insulin-like growth factor (IGF)-1, growth differentiation factor (GDF)-11, GDF-15, and soluble urokinase-type plasminogen activator receptor (suPAR).

Handgrip strength is assessed using a digital hand-held dynamometer (Model SH1001; SAEHAN Corporation, Yangdeok-Dong, Masan, South Korea). Patients able to leave the bed sit on a chair with the elbow flexed at 90° and the wrist in a neutral position, while bedridden patients are assessed in the hospital bed with the backrest elevated. The highest value of three attempts with the dominant hand is used for analyses. Should the third trial elicit the highest value, the patient continues until a lower value is achieved.

Muscle function is assessed in the 30-second sit-to-stand test, where patients are asked to stand up from a standardized chair as many times as possible with their arms folded across the chest. Only full standing positions are counted.[23, 24] Patients included in the Geriatric cohort also have their habitual gait-speed assessed. The gait-speed assessment is measured over a course of 4 meters and includes walking aids if they are used by the patient. Patients stand behind a starting line and are asked to start walking towards a visual goal at their habitual pace. The visual goal is placed after 5.5 m. to reduce the effect of deceleration. The fastest of the two attempts will be used for analyses and quantified as m/s.[23] The assessment of handgrip strength (kg) and habitual gait-speed have previously shown to be feasible and reliable measures in acutely older medical patients. However, the feasibility and reliability of the 30-second sit-to-stand test was moderate, as only half of the patients were able to perform the test as instructed.[23] Thus, we have included an additional nominal variable to categorize the sit-to-stand ability as either i) able to perform the test as instructed, ii) ability to rise using the armrest, and iii) inability to rise independently from a chair.

Bodyweight (kg) is assessed using chair scales and height (cm) is estimated with a segmometer using the knee-height measurement and age with the equations from Chumlea et al.[25] Body composition, including whole body phase angle, is assessed using Direct-Segmental Multi-frequency Bioelectrical Impedance Analyses (DSM-BIA) (InBody S10®; Biospace Co., Ltd., Seoul, Korea), which has previously been used in elderly acutely admitted patients with a mean LOS of 5 days.[26] Self-reported current smoking is reported as a dichotomous variable. Patients included in the Geriatric cohort are also assessed at discharge to evaluate circulating biomarker concentrations as well as changes in body composition, muscle strength, and functional performance. Tests of strength, physical function and body composition measurements are performed by trained research personnel. The presence of frailty is assessed by trained nurses associated with the study using the Canadian Study of Health and Aging Clinical Frailty Scale (CFS).[27] Patients are screened for sarcopenia using the SARC-F questionnaire,[28] while cognitive status is evaluated by the short Orientation-Memory-Concentration test (OMC).[29] The risk of malnutrition is assessed and validated using the Short Nutritional Assessment

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

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

Patient and public involvement

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

Power calculation and statistics

To evaluate the prognostic abilities of circulating biomarkers (individually, in combination and combined with clinical and functional measures) we will use the area under the curve for receiver operating characteristics (AUROC) statistics. A reference group of 2058

patients over the age of 65 from Bispebjerg-Frederiksberg University Hospital and Herlev-Gentofte Hospital had a mean age of 78.3 years and a mean length of stay of 5.8 days during hospitalization. In these patients, 817 (39.7%) had a prolonged length of stay, defined as a hospitalization lasting more than 96 hours. With a sample size of 1700 and the assumption that approximately 40% of older medical patients have a prolonged hospital stay, an AUROC of 82 will have a power of 0.9 with a significance level of 0.05.

A table of summary statistics will be presented with baseline variables. Continuous variables will be summarized with the following: n (non-missing sample size), mean, standard deviation, median, interquartile range, and number of missing values. Categorical variables will be reported as frequency and percentages (based on non-missing sample size), and number of missing values. Data missing at random will be imputed using multiple imputation.

To evaluate whether clinical, functional, and circulating biomarkers are associated with length of stay we will perform multivariate logistic regression. Patients will be grouped in either normal (< 96 hours) or extended length of stay (≥ 96 hours) and Cox regression analysis will be used to compare differences in non-elective readmission and all-cause

mortality. Patients will be followed from the date of discharge from the index admission until the end of the follow-up period, emigration, readmission, or death as appropriate. To assess the discriminative ability of biomarkers with regards to an extended length of stay and all-cause mortality, we will use the area under the curve (AUC) for receiver operating characteristics (ROC) curves. AUCs for different ROC curves will be compared using the DeLong test. The association of circulating biomarkers with changes in muscle mass, muscle strength, and function in the Geriatric cohort will be assessed using a multivariate linear model adjusted for the relative length of stay.

Study organization

The study is a researcher initiated clinical study. The protocol was written by the steering committee composed of experts in geriatric medicine and acute medicine and a PhD student in basic and clinical research in musculoskeletal sciences. The committee is responsible for the design of the study, supervision of research personnel, data acquisition, communication and publication of results, approval of sub-studies and ensuring that future studies comply with the regulations regarding data management.

At present (July 2020), the study has included 377 patients, of which 62 are part of the Geriatric cohort. Inclusion was temporarily paused due to the impact of the Covid-19 pandemic.

ETHICS AND DISSEMINATION

All procedures are being conducted according to “Good Clinical Practice” standards, regarding initiation, monitoring, and reporting. 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) and all experimental procedures are performed in accordance with the Declaration of Helsinki. The project complies with the regulations of the General Data Protection Regulation (GDPR) and the Data Protection Act.

All eligible patients receive oral and written information. In the case of severe dementia or delirium, some patients might be unable to provide participant consent. In these cases, we seek participant consent from a close relative or guardian, should the guardianship include access to sign participant consent for research purposes. Independent medical doctors who have knowledge of the project but are not associated with the project and are

independent of the interests of the principal investigator evaluate whether these subjects can participate in the study.

The project entails minimal discomfort and no permanent side effects; thus, it is considered ethically sound. Findings from the project, regardless of the outcome, will be published in relevant peer-reviewed scientific journals ensuring the anonymity of the patients. The study is registered at clinicaltrials.gov (NCT04151108) where positive, negative, or inconclusive results will be published.

DISCUSSION

The importance of sarcopenia has recently been underlined by its inclusion as a reportable disease in the Centers for Disease Control and Prevention (ICD-10-CM code M62.84) in October 2016.[35] Even though the serious consequences of sarcopenia are widely recognized, the diagnose has yet to be implemented in clinical practice in Denmark. Lack of knowledge regarding the basic biological mechanisms driving sarcopenia in conjunction with other age-related diseases and lack of systematic assessment hinders the identification and treatment of sarcopenia and can lead to physical deconditioning during hospitalization. As

such, the knowledge surrounding the prevalence and determinants of sarcopenia in older medical patients is scarce, and it is unknown whether circulating biomarkers, individually or in combination, can predict physical deconditioning during hospitalization.

Mechanisms that regulate skeletal muscle mass are central to the understanding of sarcopenia. Myostatin, also referred to as GDF-8, is a part of the TGF-β family and predominantly expressed in skeletal muscle. Myostatin and TGF-β are inducers of catabolic processes, inhibiting muscle growth, and inducing muscle protein breakdown via activation of the Small Mothers Against Decapentaplegic (SMAD)2 and SMAD3 transcription factors.[36,37] Myostatin is reportedly increased with aging,[38] and following prolonged bed rest,[39] and treatment with myostatin antibodies attenuates the loss of muscle mass and function induced by immobilization in mice.[40]

Recently, GDF-11, a TGF-β family ligand,[41] has been measured in human blood samples.[42] High circulating GDF-11 levels have been related to increased disease burden and elevated risk of post-operative complications and mortality in older adults undergoing heart surgery. Notably, patients categorized as physically frail based on low handgrip

strength and gait speed as well as self-reported activity measures had significantly higher GDF-11 levels compared to non-frail controls.[42]

GDF-15, another member of the TGF-β family, is present in low levels under healthy conditions but can increase during disease or injury and contribute to muscle wasting by suppressing appetite, which may result in anorexia and drastic weight loss.[43] In older patients, unintentional weight loss has been associated with an increased in-hospital morbidity and increased overall mortality.[44] GDF-15 may be induced in response to cellular stress signals or dysfunctions, and it has been suggested that circulating levels of GDF-15 could be biomarker of mitochondrial dysfunction.[45] Nonetheless, several studies demonstrate that GDF-15 levels are predictors of all-cause mortality.[46,47]

Follistatin acts as an antagonist to TGF-β family ligands including myostatin, TGF-β and GDF11.[37] Measurements of TGF-β ligands as well as their antagonist follistatin could represent biomarkers of muscle breakdown, physical function or mortality. However, the translation of these findings into clinical utility needs further validation in a larger cohort.

Several studies have investigated the association of inflammatory biomarkers with muscle mass, muscle strength, and muscle function in healthy older subjects. Most commonly, studies have focused on a few biomarkers, such as TNF-α, IL-6 or C-Reactive Protein (CRP).[9,48-50] A recent study has demonstrated an inverse relationship between a composite of pro- and anti-inflammatory markers and muscle mass, strength and function in healthy older subjects.[51] However, results are inconsistent and lack clear evidence as to whether these inflammatory biomarkers are associated with sarcopenia. Nonetheless, circulating levels of CRP are predictive of both the length of hospital stay and readmissions.[52,53] Indeed, geriatric patients with inflammation, evaluated by CRP levels at admission, stayed on average 3 days longer than patients without inflammation.[54]

The anabolic growth factor, IGF-1, and the IGF-1/phosphatidylinositol 3-kinase(PI3K)/Akt pathway is involved in skeletal muscle hypertrophy and atrophy.[55,56] Circulating IGF-1 levels decrease with aging, while inflammatory markers such as TNF-α and IL-6 can interfere with the IGF-1 signaling pathway.[57,58] As such, changes in IGF-1/PI3K/Akt signaling during aging may be a result of decreased IGF-1 expression as well as IGF-1

inhibition. Notably, no difference in circulating IGF-1 concentrations were found between older sarcopenic and non-sarcopenic women.[59]

Recently, suPAR was established as a biomarker of inflammation and immune activation, and elevated levels of suPAR are believed to reflect a state of chronic inflammation.[60] SuPAR correlates with other inflammatory markers, such as TNF-α, and patients with the highest levels of suPAR generally have the worst prognosis.[61] In one study, suPAR was associated with low muscle mass, while IL-6 was associated with low muscle mass and increased fat mass in both patients and healthy controls.[62] Thus, there seem to be distinct inflammatory processes occurring simultaneously with different effects on muscle mass and fat mass, respectively.

Distinct patient populations with co-existing pathophysiological processes might exhibit different biomarker profiles. Further validation needs to be conducted in different patient populations to utilize the possible prognostic value of these biomarkers, either individually, or in combination with functional and clinical measures. Systematic identification of patients at risk of prolonged hospitalization and deconditioning should occur to enable

early individualized interventions to counteract the adverse outcomes of prolonged bed rest.

CONCLUSIONS AND CLINICAL IMPLICATIONS

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

Acknowledgements

The authors would like to thank the doctors, nurses, secretaries and physiotherapists at the acute ward and the Department of Geriatric and Palliative Medicine for their cooperation and patience during patient recruitment and assessment. Furthermore, they would like to thank Rasmus Rønnemoes Iversen for his help in the recruitment of patients.

Contributors

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

Funding

The work is supported by funding from the Novo Nordisk Foundation; grant number NNF18OC0052826.

Disclaimer

The funders take no part in the design of the study; in the collection, analysis or interpretation of data; in the writing of manuscripts or decisions regarding publication.

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)

REFERENCES

1. Rosenberg IH. Sarcopenia: origins and clinical relevance. *Clin Geriatr Med*. 2011;27(3):337-339. doi:10.1016/j.cger.2011.03.003

2. Roubenoff R. Sarcopenia: a major modifiable cause of frailty in the elderly. *J Nutr Health Aging*. 2000;4(3):140-142.

3. Rantanen T, Avlund K, Suominen H, et al. Muscle strength as a predictor of onset of ADL dependence in people aged 75 years. *Aging Clin Exp Res*. 2002;14(3 Suppl):10-15.

4. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age Ageing*. 2010;39(4):412-423. doi:10.1093/ageing/afq034

5. von Haehling S, Morley JE, Anker SD. An overview of sarcopenia: facts and numbers on prevalence and clinical impact. *J Cachexia Sarcopenia Muscle*. 2010;1(2):129-133. doi:10.1007/s13539-010-0014-2

6. Roubenoff R, Heymsfield SB, Kehayias JJ, et al. Standardization of nomenclature of body composition in weight loss. *Am J Clin Nutr*. 1997;66(1):192-196. doi:10.1093/ajcn/66.1.192

7. Aagaard P, Suetta C, Caserotti P, et al. Role of the nervous system in sarcopenia and muscle atrophy with aging: strength training as a countermeasure. *Scand J Med Sci Sports*. 2010;20(1):49-64. doi:10.1111/j.1600-0838.2009.01084.x

8. La Colla A, Pronsato L, Milanesi L, et al. 17β-Estradiol and testosterone in sarcopenia: Role of satellite cells. *Ageing Res Rev*. 2015;24(Pt B):166-177. doi:10.1016/j.arr.2015.07.011

9. Bano G, Trevisan C, Carraro S, et al. Inflammation and sarcopenia: A systematic review and meta-analysis. *Maturitas*. 2017;96:10-15. doi:10.1016/j.maturitas.2016.11.006

10. Cruz-Jentoft AJ, Kiesswetter E, Drey M, et al. Nutrition, frailty, and sarcopenia. *Aging Clin Exp Res*. 2017;29(1):43-48. doi:10.1007/s40520-016-0709-0

11. Bautmans I, Van Puyvelde K, Mets T. Sarcopenia and functional decline: pathophysiology, prevention and therapy. *Acta Clin Belg*. 2009;64(4):303-316. doi:10.1179/acb.2009.048

12. Suetta C, Hvid LG, Justesen L, et al. Effects of aging on human skeletal muscle after immobilization and retraining. *J Appl Physiol (1985)*. 2009;107(4):1172-1180. doi:10.1152/japplphysiol.00290.2009

13. Gill TM, Allore HG, Holford TR, et al. Hospitalization, restricted activity, and the development of disability among older persons. *JAMA*. 2004;292(17):2115-2124. doi:10.1001/jama.292.17.2115

14. Statistics Denmark. https://www.statistikbanken.dk/IND05 (accessed July 2020)

15. Brown CJ, Redden DT, Flood KL, et al. The underrecognized epidemic of low mobility during hospitalization of older adults. *J Am Geriatr Soc*. 2009;57(9):1660-1665. doi:10.1111/j.1532-5415.2009.02393.x

16. Pedersen MM, Bodilsen AC, Petersen J, et al. Twenty-four-hour mobility during acute hospitalization in older medical patients. *J Gerontol A Biol Sci Med Sci*. 2013;68(3):331-337. doi:10.1093/gerona/gls165

17. Covinsky KE, Palmer RM, Fortinsky RH, et al. Loss of independence in activities of daily living in older adults hospitalized with medical illnesses: increased vulnerability with age. *J Am Geriatr Soc*. 2003;51(4):451-458. doi:10.1046/j.1532-5415.2003.51152.x

18. Sánchez-Rodríguez D, Marco E, Miralles R, et al. Sarcopenia, physical rehabilitation and functional outcomes of patients in a subacute geriatric care unit. *Arch Gerontol Geriatr*. 2014;59(1):39-43. doi:10.1016/j.archger.2014.02.009

19. Peterson SJ, Braunschweig CA. Prevalence of Sarcopenia and Associated Outcomes in the Clinical Setting. *Nutr Clin Pract*. 2016;31(1):40-48. doi:10.1177/0884533615622537

20. Surkan MJ, Gibson W. Interventions to Mobilize Elderly Patients and Reduce Length of Hospital Stay. *Can J Cardiol*. 2018;34(7):881-888. doi:10.1016/j.cjca.2018.04.033

21. Kouw IWK, Groen BBL, Smeets JSJ, et al. One Week of Hospitalization Following Elective Hip Surgery Induces Substantial Muscle Atrophy in Older Patients. *J Am Med Dir Assoc*. 2019;20(1):35-42. doi:10.1016/j.jamda.2018.06.018

22. Suetta C, Frandsen U, Jensen L, et al. Aging affects the transcriptional regulation of human skeletal muscle disuse atrophy. *PLoS One*. 2012;7(12):e51238. doi:10.1371/journal.pone.0051238

23. Bodilsen AC, Juul-Larsen HG, Petersen J, et al. Feasibility and inter-rater reliability of physical performance measures in acutely admitted older medical patients. *PLoS One*. 2015;10(2):e0118248. Published 2015 Feb 23. doi:10.1371/journal.pone.0118248

24. Suetta C, Haddock B, Alcazar J, et al. The Copenhagen Sarcopenia Study: lean mass, strength, power, and physical function in a Danish cohort aged 20-93 years. *J Cachexia Sarcopenia Muscle*. 2019;10(6):1316-1329. doi:10.1002/jcsm.12477

25. Chumlea WC, Roche AF, Steinbaugh ML. Estimating stature from knee height for persons 60 to 90 years of age. *J Am Geriatr Soc*. 1985;33(2):116-120. doi:10.1111/j.1532-5415.1985.tb02276.x

26. Van Ancum JM, Scheerman K, Pierik VD, et al. Muscle Strength and Muscle Mass in Older Patients during Hospitalization: The EMPOWER Study. *Gerontology*. 2017;63(6):507-514. doi:10.1159/000478777

27. Rockwood K, Song X, MacKnight C, et al. A global clinical measure of fitness and frailty in elderly people. *CMAJ*. 2005;173(5):489-495. doi:10.1503/cmaj.050051

28. Malmstrom TK, Miller DK, Simonsick EM, et al. SARC-F: a symptom score to predict persons with sarcopenia at risk for poor functional outcomes. *J Cachexia Sarcopenia Muscle*. 2016;7(1):28-36. doi:10.1002/jcsm.12048

29. Katzman R, Brown T, Fuld P, et al. Validation of a short Orientation-Memory-Concentration Test of cognitive impairment. *Am J Psychiatry*. 1983;140(6):734-739. doi:10.1176/ajp.140.6.734

30. Kruizenga HM, Seidell JC, de Vet HC, et al. Development and validation of a hospital screening tool for malnutrition: the short nutritional assessment questionnaire (SNAQ). *Clin Nutr*. 2005;24(1):75-82. doi:10.1016/j.clnu.2004.07.015

31. Charlson ME, Pompei P, Ales KL, et al. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis*. 1987;40(5):373-383. doi:10.1016/0021-9681(87)90171-8

32. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA*. 2016;315(8):801-810. doi:10.1001/jama.2016.0287

33. Harris PA, Taylor R, Thielke R, et al. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42(2):377-381. doi:10.1016/j.jbi.2008.08.010

34. Harris PA, Taylor R, Minor BL, et al. The REDCap consortium: Building an international community of software platform partners. *J Biomed Inform*. 2019;95:103208. doi:10.1016/j.jbi.2019.103208

35. World Health Organization. International statistical classification of diseases and related health problems. - 10th revision, Fifth edition, 2016; https://icd.who.int/browse10/2016/en#/M60-M63 (accessed July 2020)

36. Lee SJ, Reed LA, Davies MV, et al. Regulation of muscle growth by multiple ligands signaling through activin type II receptors. *Proc Natl Acad Sci U S A*. 2005;102(50):18117-18122. doi:10.1073/pnas.0505996102

37. Han HQ, Zhou X, Mitch WE, et al. Myostatin/activin pathway antagonism: molecular basis and therapeutic potential. *Int J Biochem Cell Biol*. 2013;45(10):2333-2347. doi:10.1016/j.biocel.2013.05.019

38. Yarasheski KE, Bhasin S, Sinha-Hikim I, et al. Serum myostatin-immunoreactive protein is increased in 60-92 year old women and men with muscle wasting. *J Nutr Health Aging*. 2002;6(5):343-348.

39. Reardon KA, Davis J, Kapsa RM, et al. Myostatin, insulin-like growth factor-1, and leukemia inhibitory factor mRNAs are upregulated in chronic human disuse muscle atrophy. *Muscle Nerve*. 2001;24(7):893-899. doi:10.1002/mus.1086

40. Murphy KT, Cobani V, Ryall JG, et al. Acute antibody-directed myostatin inhibition attenuates disuse muscle atrophy and weakness in mice. *J Appl Physiol (1985)*. 2011;110(4):1065-1072. doi:10.1152/japplphysiol.01183.2010

41. Nakashima M, Toyono T, Akamine A, et al. Expression of growth/differentiation factor 11, a new member of the BMP/TGFbeta superfamily during mouse embryogenesis. *Mech Dev*. 1999;80(2):185-189. doi:10.1016/s0925-4773(98)00205-6

42. Schafer MJ, Atkinson EJ, Vanderboom PM, et al. Quantification of GDF11 and Myostatin in Human Aging and Cardiovascular Disease. *Cell Metab*. 2016;23(6):1207-1215. doi:10.1016/j.cmet.2016.05.023

43. Tsai VW, Husaini Y, Manandhar R, et al. Anorexia/cachexia of chronic diseases: a role for the TGF-β family cytokine MIC-1/GDF15. *J Cachexia Sarcopenia Muscle*. 2012;3(4):239-243. doi:10.1007/s13539-012-0082-6

44. Gaddey HL, Holder K. Unintentional weight loss in older adults. *Am Fam Physician*. 2014;89(9):718-722.

45. Fujita Y, Taniguchi Y, Shinkai S, et al. Secreted growth differentiation factor 15 as a potential biomarker for mitochondrial dysfunctions in aging and age-related disorders. *Geriatr Gerontol Int*. 2016;16 Suppl 1:17-29. doi:10.1111/ggi.12724

46. Eggers KM, Kempf T, Wallentin L, et al. Change in growth differentiation factor 15 concentrations over time independently predicts mortality in community-dwelling elderly individuals. *Clin Chem*. 2013;59(7):1091-1098. doi:10.1373/clinchem.2012.201210

47. Wiklund FE, Bennet AM, Magnusson PK, et al. Macrophage inhibitory cytokine-1 (MIC-1/GDF15): a new marker of all-cause mortality. *Aging Cell*. 2010;9(6):1057-1064. doi:10.1111/j.1474-9726.2010.00629.

48. Schaap LA, Pluijm SM, Deeg DJ, et al. Inflammatory markers and loss of muscle mass (sarcopenia) and strength. *Am J Med*. 2006;119(6):526.e9-526.e5.26E17. doi:10.1016/j.amjmed.2005.10.049

49. Visser M, Pahor M, Taaffe DR, et al. Relationship of interleukin-6 and tumor necrosis factor-alpha with muscle mass and muscle strength in elderly men and women: the Health ABC Study. *J Gerontol A Biol Sci Med Sci*. 2002;57(5):M326-M332. doi:10.1093/gerona/57.5.m326

50. Cesari M, Penninx BW, Pahor M, et al. Inflammatory markers and physical performance in older persons: the InCHIANTI study. *J Gerontol A Biol Sci Med Sci*. 2004;59(3):242-248. doi:10.1093/gerona/59.3.m242

51. Calvani R, Marini F, Cesari M, et al. Systemic inflammation, body composition, and physical performance in old community-dwellers. *J Cachexia Sarcopenia Muscle*. 2017;8(1):69-77. doi:10.1002/jcsm.12134

52. Brown SH, Flint K, Storey A, et al. Routinely assessed biochemical markers tested on admission as predictors of adverse outcomes in hospitalized elderly patients. *Hosp Pract (1995)*. 2012;40(1):193-201. doi:10.3810/hp.2012.02.960

53. Hogarth MB, Gallimore R, Savage P, et al. Acute phase proteins, C-reactive protein and serum amyloid A protein, as prognostic markers in the elderly inpatient. *Age Ageing*. 1997;26(2):153-158. doi:10.1093/ageing/26.2.153

54. Bautmans I, Njemini R, Lambert M, et al. Circulating acute phase mediators and skeletal muscle performance in hospitalized geriatric patients. *J Gerontol A Biol Sci Med Sci*. 2005;60(3):361-367. doi:10.1093/gerona/60.3.361

55. Bodine SC, Stitt TN, Gonzalez M, et al. Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nat Cell Biol*. 2001;3(11):1014-1019. doi:10.1038/ncb1101-1014

56. Glass DJ. Skeletal muscle hypertrophy and atrophy signaling pathways. *Int J Biochem Cell Biol*. 2005;37(10):1974-1984. doi:10.1016/j.biocel.2005.04.018

57. Barbieri M, Ferrucci L, Ragno E, et al. Chronic inflammation and the effect of IGF-I on muscle strength and power in older persons. *Am J Physiol Endocrinol Metab*. 2003;284(3):E481-E487. doi:10.1152/ajpendo.00319.2002

58. Broussard SR, Zhou JH, Venters HD, et al. At the interface of environment-immune interactions: Cytokine and growth-factor receptors, *Journal of Animal Science*, Volume 79, Issue suppl_E, 2001, Pages E268–E284, https://doi.org/10.2527/jas2001.79E-SupplE268x

59. Hofmann M, Halper B, Oesen S, et al. Serum concentrations of insulin-like growth factor-1, members of the TGF-beta superfamily and follistatin do not reflect different stages of dynapenia and sarcopenia in elderly women. *Exp Gerontol*. 2015;64:35-45. doi:10.1016/j.exger.2015.02.008

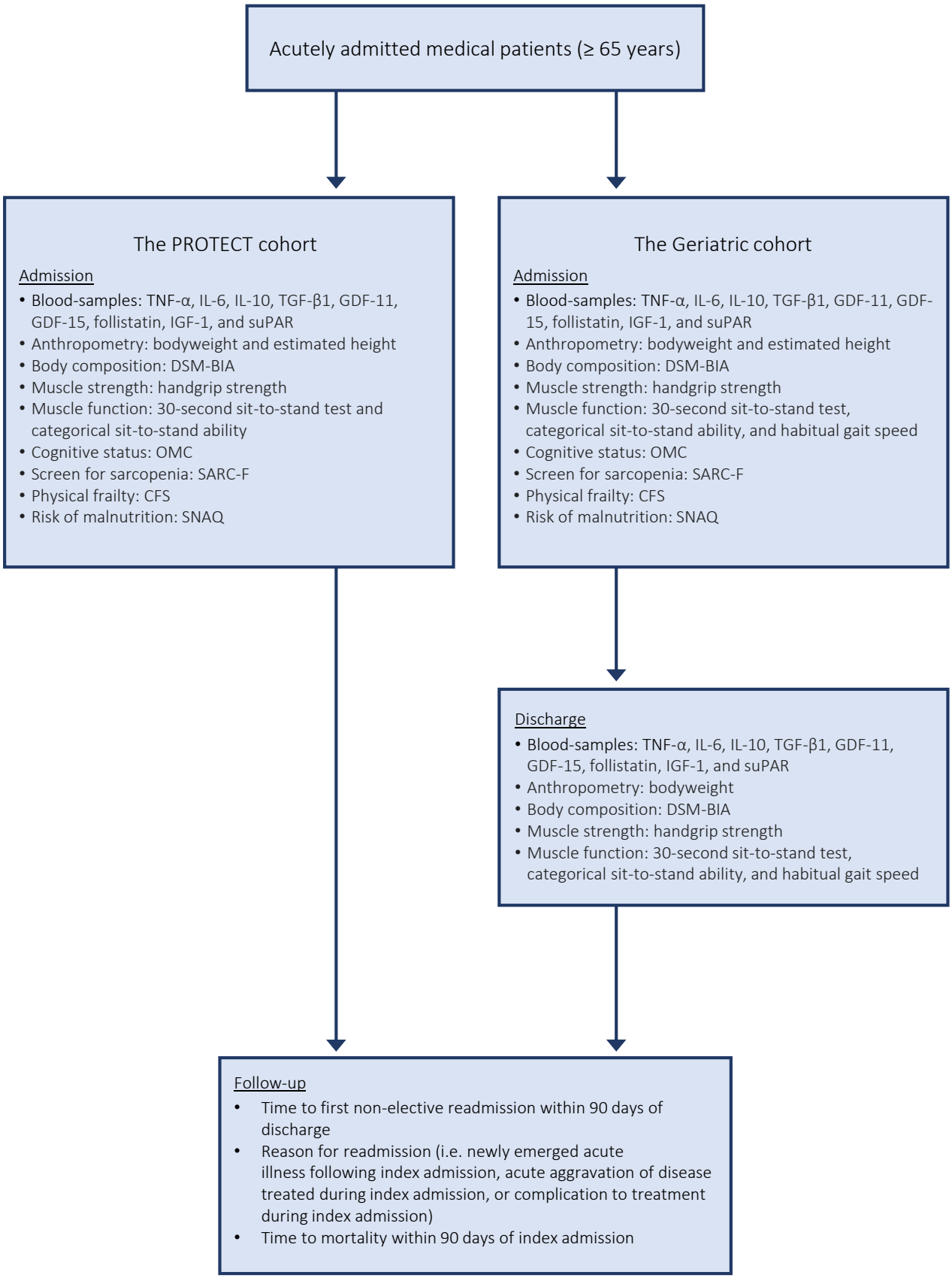
60. Haupt TH, Kallemose T, Ladelund S, et al. Risk factors associated with serum levels of the inflammatory biomarker soluble urokinase plasminogen activator receptor in a general population. *Biomark Insights*. 2014;9:91-100. Published 2014 Dec 16. doi:10.4137/BMI.S19876

61. Thunø M, Macho B, Eugen-Olsen J. suPAR: the molecular crystal ball. *Dis Markers*. 2009;27(3):157-172. doi:10.3233/DMA-2009-0657

62. Langkilde A, Petersen J, Henriksen JH, et al. Leptin, IL-6, and suPAR reflect distinct inflammatory changes associated with adiposity, lipodystrophy and low muscle mass in HIV-infected patients and controls. *Immun Ageing*. 2015;12:9. Published 2015 Aug 4. doi:10.1186/s12979-015-0036-x

Figure legends

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


Acutely admitted medical patients (≥ 65 years)
The PROTECT cohort
Admission
• Blood-samples: TNF-α, IL-6, IL-10, TGF-β1, GDF-11, GDF-15, follistatin, IGF-1, and suPAR
• Anthropometry: bodyweight and estimated height
• Body composition: DSM-BIA
• Muscle strength: handgrip strength
• Muscle function: 30-second sit-to-stand test and categorical sit-to-stand ability
• Cognitive status: OMC
• Screen for sarcopenia: SARC-F
• Physical frailty: CFS
• Risk of malnutrition: SNAQ
The Geriatric cohort
Admission
• Blood-samples: TNF-α, IL-6, IL-10, TGF-β1, GDF-11, GDF-15, follistatin, IGF-1, and suPAR
• Anthropometry: bodyweight and estimated height
• Body composition: DSM-BIA
• Muscle strength: handgrip strength
• Muscle function: 30-second sit-to-stand test, categorical sit-to-stand ability, and habitual gait speed
• Cognitive status: OMC
• Screen for sarcopenia: SARC-F
• Physical frailty: CFS
• Risk of malnutrition: SNAQ
Discharge
• Blood-samples: TNF-α, IL-6, IL-10, TGF-β1, GDF-11, GDF-15, follistatin, IGF-1, and suPAR
• Anthropometry: bodyweight
• Body composition: DSM-BIA
• Muscle strength: handgrip strength
• Muscle function: 30-second sit-to-stand test, categorical sit-to-stand ability, and habitual gait speed
Follow-up
• Time to first non-elective readmission within 90 days of discharge
• Reason for readmission (i.e. newly emerged acute illness following index admission, acute aggravation of disease treated during index admission, or complication to treatment during index admission)
• Time to mortality within 90 days of index admission

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.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-042786.R2
Article Type:	Protocol
Date Submitted by the Author:	08-Dec-2020
Complete List of Authors:	Kamper, Rikke; Geriatric Research Unit, Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg; CopenAge; Copenhagen Center for Clinical Age Research, University of Copenhagen Schultz, Martin; Geriatric Research Unit, Department of Medicine, Copenhagen University Hospital, Herlev and Gentofte; CopenAge; Copenhagen Center for Clinical Age Research, University of Copenhagen Hansen, Sofie; Geriatric Research Unit, Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg; CopenAge; Copenhagen Center for Clinical Age Research, University of Copenhagen Andersen, Helle; Department of Occupational and Physiotherapy, Copenhagen University Hospital, Bispebjerg and Frederiksberg; CopenAge; Copenhagen Center for Clinical Age Research, University of Copenhagen Ekmann, Anette; Geriatric Research Unit, Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg; CopenAge; Copenhagen Center for Clinical Age Research, University of Copenhagen Nygaard, Hanne; Department of Emergency Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg; CopenAge; Copenhagen Center for Clinical Age Research, University of Copenhagen Helland, Fredrik; Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg Wejse, Miriam; Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg Rahbek, Camilla; Geriatric Research Unit, Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg Noerst, Tim; Geriatric Research Unit, Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg Pressel, Eckart; Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg Nielsen, Finn; Department of Emergency Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg; Copenhagen Center for Translational Research, Copenhagen University Hospital, Bispebjerg and Frederiksberg

	Suetta, Charlotte; Geriatric Research Unit, Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg; Geriatric Research Unit, Department of Medicine, Copenhagen University Hospital, Herlev and Gentofte
Primary Subject Heading:	Research methods
Secondary Subject Heading:	Geriatric medicine, Rehabilitation medicine
Keywords:	Risk management < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, STATISTICS & RESEARCH METHODS, GERIATRIC MEDICINE

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

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.

[1,3]Rikke S. Kamper, [4,3]Martin Schultz, [1,3]Sofie K. Hansen,[5,3]Helle Andersen, [1,3]Anette Ekmann,[3,6]Hanne Nygaard, [2]Fredrik Helland, [2]Miriam R. Wejse, [1]Camilla B. Rahbek, [1]Tim Noerst, [2]Eckart Pressel, [6,7]Finn E. Nielsen, [1,4]Charlotte Suetta

[1]Geriatric Research Unit, Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg, Copenhagen, Denmark.

[2]Department of Geriatric and Palliative Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg, Copenhagen, Denmark.

[3]CopenAge; Copenhagen Center for Clinical Age Research, University of Copenhagen, Copenhagen, Denmark.

[4]Geriatric Research Unit, Department of Medicine, Copenhagen University Hospital, Herlev and Gentofte, Herlev, Denmark.

[5]Department of Occupational and Physiotherapy, Copenhagen University Hospital, Bispebjerg and Frederiksberg, Copenhagen, Denmark.

[6]Department of Emergency Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg, Copenhagen Denmark.

[7]Copenhagen Center for Translational Research, Copenhagen University Hospital, Bispebjerg and Frederiksberg, Copenhagen, Denmark.

Keywords: Risk management, Statistics and research methods, Geriatric medicine

Word count: 3537

Corresponding author

Rikke Stefan Kamper

Geriatric Research Unit, Department of Geriatric and Palliative Medicine,

Bispebjerg and Frederiksberg University Hospital,

Ebba Lunds vej 44,

2400 Copenhagen,

Denmark

Email: rikke.stefan.kamper.01@regionh.dk

Telephone: +4520446333

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 of Copenhagen and Frederiksberg (H-19039214) and the Danish Data Protection Agency (P-2019-239) and all experimental procedures were performed in accordance with the Declaration of Helsinki. 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

Clinicaltrails.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.

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]

Furthermore, physical inactivity, chronic diseases, immobilization, and hospitalization are known to play a part in the development of sarcopenia.[6,11-13]

In 2018, approximately 45% of all hospital admissions in Denmark concerned patients aged 65 or older who had a mean length of stay of 3.5 days.[14] Older patients are often inactive during hospitalization spending 71%-83% of their time lying down,[15,16] and at least 35% of older patients lose independence in one basic Activity of Daily Living (ADL) as an unintended consequence of a medical illness and hospitalization.[17] Sarcopenia may aggravate this functional decline, as patients with sarcopenia have an attenuated recovery of their functional levels 3 months following discharge.[18] From a clinical perspective, sarcopenia is associated with infectious complications, readmissions, increased need for rehabilitation following discharge, reduced quality of life, increased mortality, and longer hospitalization.[4,19]

Early mobilization protocols have proven effective in reducing hospital-acquired disability and hospital length of stay. However, frequently reported barriers for implementation of early mobilization include lack of staff and time to enable mobilization of the patient.[20] With an increasing aging population and the heterogeneousness of older individuals, the systematic

identification of older individuals at risk of prolonged hospitalization and deconditioning during hospitalization are of outmost importance. As such, the combination of clinical, functional and circulating biomarkers may serve as risk stratification tools to identify older patients at risk of these adverse outcomes.

STUDY OBJECTIVES AND HYPOTHESES

Primary objectives and hypothesis

We aim to examine whether circulating biomarkers at admission are associated with length of hospital stay in older (≥ 65 years) acutely admitted medical patients and whether the combination of clinical and functional measures with these biomarkers can identify patients at risk of having a prolonged hospital stay (>96 hours). In addition, we aim to establish circulating biomarkers associated with changes in muscle mass, muscle strength, and function in geriatric patients during hospitalization. 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 prolonged (≥ 96 hours) hospital stays and physical deconditioning during hospitalization.

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

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

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

Outcomes

The primary outcome in the PROTECT cohort is the length of hospital stay. Successive events of hospitalisation have been suggested to contribute to the development of sarcopenia, and even short periods (4-5 days) of skeletal muscle disuse are known to induce muscle atrophy.[21,22] In 2018, the mean length of hospital stay in Denmark was 84 hours in patients aged 65 years or over.[14] while the mean LOS in the two largest local

hospitals was 96 hours. As such, we have defined a prolonged hospital length of stay as an admission lasting >96 hours.

The primary outcomes in the Geriatric cohort are the relative changes in muscle mass, muscle strength, and muscle function during hospitalization. Primary and secondary outcomes for the PROTECT cohort and the Geriatric cohort are listed in table 2 and 3, respectively.

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

Table 3. Primary and secondary outcomes in the Geriatric cohort

Primary outcomes
Changes in muscle mass during hospitalization

Changes in muscle strength during hospitalization
Changes in muscle function during hospitalization
Secondary outcomes
Length of hospital stay
Non-elective readmissions within 90 days of discharge
All-cause mortality within 90 days of index admission
In-hospital mortality
Discharge to an increased level of care
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

The research personnel might be unable to assess all patients, as the number of eligible patients (i.e. fulfilling inclusion criteria with the absence of exclusion criteria) varies daily. Thus, to avoid selection bias, all eligible patients on the day in question are randomized using a computer-generated randomization sequence to establish a randomized visitation sequence. Patients who wish to participate sign an informed consent and baseline measurements are performed within the first 24 hours of admission. All included patients have blood samples drawn to determine concentrations of tumor necrosis factor (TNF)-α, , interleukin (IL)-6, IL-10, transforming growth factor (TGF)-β1, follistatin, insulin-like growth factor (IGF)-1, growth differentiation factor (GDF)-11, GDF-15, and soluble urokinase-type plasminogen activator receptor (suPAR).

Handgrip strength is assessed using a digital hand-held dynamometer (Model SH1001; SAEHAN Corporation, Yangdeok-Dong, Masan, South Korea). Patients able to leave the bed sit on a chair with the elbow flexed at 90° and the wrist in a neutral position, while bedridden patients are assessed in the hospital bed with the backrest elevated. The highest value of three attempts with the dominant hand is used for analyses. Should the third trial elicit the highest value, the patient continues until a lower value is achieved.

Muscle function is assessed in the 30-second sit-to-stand test, where patients are asked to stand up from a standardized chair as many times as possible with their arms folded across the chest. Only full standing positions are counted.[23, 24] Patients included in the Geriatric cohort also have their habitual gait-speed assessed. The gait-speed assessment is measured over a course of 4 meters and includes walking aids if they are used by the patient. Patients stand behind a starting line and are asked to start walking towards a visual goal at their habitual pace. The visual goal is placed after 5.5 m. to reduce the effect of deceleration. The fastest of the two attempts will be used for analyses and quantified as m/s.[23] The assessment of handgrip strength (kg) and habitual gait-speed have previously shown to be feasible and reliable measures in acutely older medical patients. However, the feasibility and reliability of the 30-second sit-to-stand test was moderate, as only half of the patients were able to perform the test as instructed.[23] Thus, we have included an additional nominal variable to categorize the sit-to-stand ability as either i) able to perform the test as instructed, ii) ability to rise using the armrest, and iii) inability to rise independently from a chair.

Bodyweight (kg) is assessed using chair scales and height (cm) is estimated with a segmometer using the knee-height measurement and age with the equations from Chumlea et al.[25] Body composition, including whole body phase angle, is assessed using Direct-Segmental Multi-frequency Bioelectrical Impedance Analyses (DSM-BIA) (InBody S10®; Biospace Co., Ltd., Seoul, Korea), which has previously been used in elderly acutely admitted patients with a mean LOS of 5 days.[26] Self-reported current smoking is reported as a dichotomous variable. Patients included in the Geriatric cohort are also assessed at discharge to evaluate circulating biomarker concentrations as well as changes in body composition, muscle strength, and functional performance. Tests of strength, physical function and body composition measurements are performed by trained research personnel. The presence of frailty is assessed by trained nurses associated with the study using the Canadian Study of Health and Aging Clinical Frailty Scale (CFS).[27] Patients are screened for sarcopenia using the SARC-F questionnaire,[28] while cognitive status is evaluated by the short Orientation-Memory-Concentration test (OMC).[29] The risk of malnutrition is assessed and validated using the Short Nutritional Assessment

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

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

Patient and public involvement

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

Power calculation and statistics

To evaluate the prognostic abilities of circulating biomarkers (individually, in combination and combined with clinical and functional measures) we will use the area under the curve for receiver operating characteristics (AUROC) statistics. A reference group of 2058

patients over the age of 65 from Bispebjerg-Frederiksberg University Hospital and Herlev-Gentofte Hospital had a mean age of 78.3 years and a mean length of stay of 5.8 days during hospitalization. In these patients, 817 (39.7%) had a prolonged length of stay, defined as a hospitalization lasting more than 96 hours. With a sample size of 1700 and the assumption that approximately 40% of older medical patients have a prolonged hospital stay, an AUROC of 82 will have a power of 0.9 with a significance level of 0.05.

A table of summary statistics will be presented with baseline variables. Continuous variables will be summarized with the following: n (non-missing sample size), mean, standard deviation, median, interquartile range, and number of missing values. Categorical variables will be reported as frequency and percentages (based on non-missing sample size), and number of missing values. Data missing at random will be imputed using multiple imputation.

To evaluate whether clinical, functional, and circulating biomarkers are associated with length of stay we will perform multivariate logistic regression. Patients will be grouped in either normal (< 96 hours) or extended length of stay (≥ 96 hours) and Cox regression analysis will be used to compare differences in non-elective readmission and all-cause

mortality. Patients will be followed from the date of discharge from the index admission until the end of the follow-up period, emigration, readmission, or death as appropriate. To assess the discriminative ability of biomarkers with regards to an extended length of stay and all-cause mortality, we will use the area under the curve (AUC) for receiver operating characteristics (ROC) curves. AUCs for different ROC curves will be compared using the DeLong test. The association of circulating biomarkers with changes in muscle mass, muscle strength, and function in the Geriatric cohort will be assessed using a multivariate linear model adjusted for the relative length of stay.

Study organization

The study is a researcher initiated clinical study. The protocol was written by the steering committee composed of experts in geriatric medicine and acute medicine and a PhD student in basic and clinical research in musculoskeletal sciences. The committee is responsible for the design of the study, supervision of research personnel, data acquisition, communication and publication of results, approval of sub-studies and ensuring that future studies comply with the regulations regarding data management.

At present (July 2020), the study has included 377 patients, of which 62 are part of the Geriatric cohort. Inclusion was temporarily paused due to the impact of the Covid-19 pandemic.

ETHICS AND DISSEMINATION

All procedures are being conducted according to “Good Clinical Practice” standards, regarding initiation, monitoring, and reporting. 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) and all experimental procedures are performed in accordance with the Declaration of Helsinki. The project complies with the regulations of the General Data Protection Regulation (GDPR) and the Data Protection Act.

All eligible patients receive oral and written information. In the case of severe dementia or delirium, some patients might be unable to provide participant consent. In these cases, we seek participant consent from a close relative or guardian, should the guardianship include access to sign participant consent for research purposes. Independent medical doctors who have knowledge of the project but are not associated with the project and are

independent of the interests of the principal investigator evaluate whether these subjects can participate in the study.

The project entails minimal discomfort and no permanent side effects; thus, it is considered ethically sound. Findings from the project, regardless of the outcome, will be published in relevant peer-reviewed scientific journals ensuring the anonymity of the patients. The study is registered at clinicaltrials.gov (NCT04151108) where positive, negative, or inconclusive results will be published.

DISCUSSION

The importance of sarcopenia has recently been underlined by its inclusion as a reportable disease in the Centers for Disease Control and Prevention (ICD-10-CM code M62.84) in October 2016.[35] Even though the serious consequences of sarcopenia are widely recognized, the diagnose has yet to be implemented in clinical practice in Denmark. Lack of knowledge regarding the basic biological mechanisms driving sarcopenia in conjunction with other age-related diseases and lack of systematic assessment hinders the identification and treatment of sarcopenia and can lead to physical deconditioning during hospitalization. As

such, the knowledge surrounding the prevalence and determinants of sarcopenia in older medical patients is scarce, and it is unknown whether circulating biomarkers, individually or in combination, can predict physical deconditioning during hospitalization.

Mechanisms that regulate skeletal muscle mass are central to the understanding of sarcopenia. Myostatin, also referred to as GDF-8, is a part of the TGF-β family and predominantly expressed in skeletal muscle. Myostatin and TGF-β are inducers of catabolic processes, inhibiting muscle growth, and inducing muscle protein breakdown via activation of the Small Mothers Against Decapentaplegic (SMAD)2 and SMAD3 transcription factors.[36,37] Myostatin is reportedly increased with aging,[38] and following prolonged bed rest,[39] and treatment with myostatin antibodies attenuates the loss of muscle mass and function induced by immobilization in mice.[40]

Recently, GDF-11, a TGF-β family ligand,[41] has been measured in human blood samples.[42] High circulating GDF-11 levels have been related to increased disease burden and elevated risk of post-operative complications and mortality in older adults undergoing heart surgery. Notably, patients categorized as physically frail based on low handgrip

strength and gait speed as well as self-reported activity measures had significantly higher GDF-11 levels compared to non-frail controls.[42]

GDF-15, another member of the TGF-β family, is present in low levels under healthy conditions but can increase during disease or injury and contribute to muscle wasting by suppressing appetite, which may result in anorexia and drastic weight loss.[43] In older patients, unintentional weight loss has been associated with an increased in-hospital morbidity and increased overall mortality.[44] GDF-15 may be induced in response to cellular stress signals or dysfunctions, and it has been suggested that circulating levels of GDF-15 could be biomarker of mitochondrial dysfunction.[45] Nonetheless, several studies demonstrate that GDF-15 levels are predictors of all-cause mortality.[46,47]

Follistatin acts as an antagonist to TGF-β family ligands including myostatin, TGF-β and GDF11.[37] Measurements of TGF-β ligands as well as their antagonist follistatin could represent biomarkers of muscle breakdown, physical function or mortality. However, the translation of these findings into clinical utility needs further validation in a larger cohort.

Several studies have investigated the association of inflammatory biomarkers with muscle mass, muscle strength, and muscle function in healthy older subjects. Most commonly, studies have focused on a few biomarkers, such as TNF-α, IL-6 or C-Reactive Protein (CRP).[9,48-50] A recent study has demonstrated an inverse relationship between a composite of pro- and anti-inflammatory markers and muscle mass, strength and function in healthy older subjects.[51] However, results are inconsistent and lack clear evidence as to whether these inflammatory biomarkers are associated with sarcopenia. Nonetheless, circulating levels of CRP are predictive of both the length of hospital stay and readmissions.[52,53] Indeed, geriatric patients with inflammation, evaluated by CRP levels at admission, stayed on average 3 days longer than patients without inflammation.[54]

The anabolic growth factor, IGF-1, and the IGF-1/phosphatidylinositol 3-kinase(PI3K)/Akt pathway is involved in skeletal muscle hypertrophy and atrophy.[55,56] Circulating IGF-1 levels decrease with aging, while inflammatory markers such as TNF-α and IL-6 can interfere with the IGF-1 signaling pathway.[57,58] As such, changes in IGF-1/PI3K/Akt signaling during aging may be a result of decreased IGF-1 expression as well as IGF-1

inhibition. Notably, no difference in circulating IGF-1 concentrations were found between older sarcopenic and non-sarcopenic women.[59]

Recently, suPAR was established as a biomarker of inflammation and immune activation, and elevated levels of suPAR are believed to reflect a state of chronic inflammation.[60] SuPAR correlates with other inflammatory markers, such as TNF-α, and patients with the highest levels of suPAR generally have the worst prognosis.[61] In one study, suPAR was associated with low muscle mass, while IL-6 was associated with low muscle mass and increased fat mass in both patients and healthy controls.[62] Thus, there seem to be distinct inflammatory processes occurring simultaneously with different effects on muscle mass and fat mass, respectively.

Distinct patient populations with co-existing pathophysiological processes might exhibit different biomarker profiles. Further validation needs to be conducted in different patient populations to utilize the possible prognostic value of these biomarkers, either individually, or in combination with functional and clinical measures. Systematic identification of patients at risk of prolonged hospitalization and deconditioning should occur to enable

early individualized interventions to counteract the adverse outcomes of prolonged bed rest.

Results from the Copenhagen PROTECT study can be helpful in the identification of older patients at risk of prolonged hospitalization. Additionally, circulating biomarker assays able to predict physical deconditioning during hospitalization will help in the early detection of geriatric patients at risk of deconditioning during hospitalization. This knowledge can then be tested in a future interventional study. The Copenhagen PROTECT study is considered feasible, ethically sound, and with potential extensive implications for future identification and treatment of sarcopenia in older medical patients.

Acknowledgements

The authors would like to thank the doctors, nurses, secretaries and physiotherapists at the acute ward and the Department of Geriatric and Palliative Medicine for their cooperation and patience during patient recruitment and assessment. Furthermore, they would like to thank Rasmus Rønnemoes Iversen for his help in the recruitment of patients.

Contributors

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

Funding

The work is supported by funding from the Novo Nordisk Foundation; grant number NNF18OC0052826.

Disclaimer

The funders take no part in the design of the study; in the collection, analysis or interpretation of data; in the writing of manuscripts or decisions regarding publication.

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)

Open access

REFERENCES

1. Rosenberg IH. Sarcopenia: origins and clinical relevance. *Clin Geriatr Med*. 2011;27(3):337-339. doi:10.1016/j.cger.2011.03.003

2. Roubenoff R. Sarcopenia: a major modifiable cause of frailty in the elderly. *J Nutr Health Aging*. 2000;4(3):140-142.

3. Rantanen T, Avlund K, Suominen H, et al. Muscle strength as a predictor of onset of ADL dependence in people aged 75 years. *Aging Clin Exp Res*. 2002;14(3 Suppl):10-15.

4. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age Ageing*. 2010;39(4):412-423. doi:10.1093/ageing/afq034

5. von Haehling S, Morley JE, Anker SD. An overview of sarcopenia: facts and numbers on prevalence and clinical impact. *J Cachexia Sarcopenia Muscle*. 2010;1(2):129-133. doi:10.1007/s13539-010-0014-2

6. Roubenoff R, Heymsfield SB, Kehayias JJ, et al. Standardization of nomenclature of body composition in weight loss. *Am J Clin Nutr*. 1997;66(1):192-196. doi:10.1093/ajcn/66.1.192

7. Aagaard P, Suetta C, Caserotti P, et al. Role of the nervous system in sarcopenia and muscle atrophy with aging: strength training as a countermeasure. *Scand J Med Sci Sports*. 2010;20(1):49-64. doi:10.1111/j.1600-0838.2009.01084.x

8. La Colla A, Pronsato L, Milanesi L, et al. 17β-Estradiol and testosterone in sarcopenia: Role of satellite cells. *Ageing Res Rev*. 2015;24(Pt B):166-177. doi:10.1016/j.arr.2015.07.011

9. Bano G, Trevisan C, Carraro S, et al. Inflammation and sarcopenia: A systematic review and meta-analysis. *Maturitas*. 2017;96:10-15. doi:10.1016/j.maturitas.2016.11.006

10. Cruz-Jentoft AJ, Kiesswetter E, Drey M, et al. Nutrition, frailty, and sarcopenia. *Aging Clin Exp Res*. 2017;29(1):43-48. doi:10.1007/s40520-016-0709-0

11. Bautmans I, Van Puyvelde K, Mets T. Sarcopenia and functional decline: pathophysiology, prevention and therapy. *Acta Clin Belg*. 2009;64(4):303-316. doi:10.1179/acb.2009.048

12. Suetta C, Hvid LG, Justesen L, et al. Effects of aging on human skeletal muscle after immobilization and retraining. *J Appl Physiol (1985)*. 2009;107(4):1172-1180. doi:10.1152/japplphysiol.00290.2009

13. Gill TM, Allore HG, Holford TR, et al. Hospitalization, restricted activity, and the development of disability among older persons. *JAMA*. 2004;292(17):2115-2124. doi:10.1001/jama.292.17.2115

14. Statistics Denmark. https://www.statistikbanken.dk/IND05 (accessed July 2020)

15. Brown CJ, Redden DT, Flood KL, et al. The underrecognized epidemic of low mobility during hospitalization of older adults. *J Am Geriatr Soc*. 2009;57(9):1660-1665. doi:10.1111/j.1532-5415.2009.02393.x

16. Pedersen MM, Bodilsen AC, Petersen J, et al. Twenty-four-hour mobility during acute hospitalization in older medical patients. *J Gerontol A Biol Sci Med Sci*. 2013;68(3):331-337. doi:10.1093/gerona/gls165

17. Covinsky KE, Palmer RM, Fortinsky RH, et al. Loss of independence in activities of daily living in older adults hospitalized with medical illnesses: increased vulnerability with age. *J Am Geriatr Soc*. 2003;51(4):451-458. doi:10.1046/j.1532-5415.2003.51152.x

18. Sánchez-Rodríguez D, Marco E, Miralles R, et al. Sarcopenia, physical rehabilitation and functional outcomes of patients in a subacute geriatric care unit. *Arch Gerontol Geriatr*. 2014;59(1):39-43. doi:10.1016/j.archger.2014.02.009

19. Peterson SJ, Braunschweig CA. Prevalence of Sarcopenia and Associated Outcomes in the Clinical Setting. *Nutr Clin Pract*. 2016;31(1):40-48. doi:10.1177/0884533615622537

20. Surkan MJ, Gibson W. Interventions to Mobilize Elderly Patients and Reduce Length of Hospital Stay. *Can J Cardiol*. 2018;34(7):881-888. doi:10.1016/j.cjca.2018.04.033

21. Kouw IWK, Groen BBL, Smeets JSJ, et al. One Week of Hospitalization Following Elective Hip Surgery Induces Substantial Muscle Atrophy in Older Patients. *J Am Med Dir Assoc*. 2019;20(1):35-42. doi:10.1016/j.jamda.2018.06.018

22. Suetta C, Frandsen U, Jensen L, et al. Aging affects the transcriptional regulation of human skeletal muscle disuse atrophy. *PLoS One*. 2012;7(12):e51238. doi:10.1371/journal.pone.0051238

23. Bodilsen AC, Juul-Larsen HG, Petersen J, et al. Feasibility and inter-rater reliability of physical performance measures in acutely admitted older medical patients. *PLoS One*. 2015;10(2):e0118248. Published 2015 Feb 23. doi:10.1371/journal.pone.0118248

24. Suetta C, Haddock B, Alcazar J, et al. The Copenhagen Sarcopenia Study: lean mass, strength, power, and physical function in a Danish cohort aged 20-93 years. *J Cachexia Sarcopenia Muscle*. 2019;10(6):1316-1329. doi:10.1002/jcsm.12477

25. Chumlea WC, Roche AF, Steinbaugh ML. Estimating stature from knee height for persons 60 to 90 years of age. *J Am Geriatr Soc*. 1985;33(2):116-120. doi:10.1111/j.1532-5415.1985.tb02276.x

26. Van Ancum JM, Scheerman K, Pierik VD, et al. Muscle Strength and Muscle Mass in Older Patients during Hospitalization: The EMPOWER Study. *Gerontology*. 2017;63(6):507-514. doi:10.1159/000478777

27. Rockwood K, Song X, MacKnight C, et al. A global clinical measure of fitness and frailty in elderly people. *CMAJ*. 2005;173(5):489-495. doi:10.1503/cmaj.050051

28. Malmstrom TK, Miller DK, Simonsick EM, et al. SARC-F: a symptom score to predict persons with sarcopenia at risk for poor functional outcomes. *J Cachexia Sarcopenia Muscle*. 2016;7(1):28-36. doi:10.1002/jcsm.12048

29. Katzman R, Brown T, Fuld P, et al. Validation of a short Orientation-Memory-Concentration Test of cognitive impairment. *Am J Psychiatry*. 1983;140(6):734-739. doi:10.1176/ajp.140.6.734

30. Kruizenga HM, Seidell JC, de Vet HC, et al. Development and validation of a hospital screening tool for malnutrition: the short nutritional assessment questionnaire (SNAQ). *Clin Nutr*. 2005;24(1):75-82. doi:10.1016/j.clnu.2004.07.015

31. Charlson ME, Pompei P, Ales KL, et al. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis*. 1987;40(5):373-383. doi:10.1016/0021-9681(87)90171-8

32. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA*. 2016;315(8):801-810. doi:10.1001/jama.2016.0287

33. Harris PA, Taylor R, Thielke R, et al. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42(2):377-381. doi:10.1016/j.jbi.2008.08.010

34. Harris PA, Taylor R, Minor BL, et al. The REDCap consortium: Building an international community of software platform partners. *J Biomed Inform*. 2019;95:103208. doi:10.1016/j.jbi.2019.103208

35. World Health Organization. International statistical classification of diseases and related health problems. - 10th revision, Fifth edition, 2016; https://icd.who.int/browse10/2016/en#/M60-M63 (accessed July 2020)

36. Lee SJ, Reed LA, Davies MV, et al. Regulation of muscle growth by multiple ligands signaling through activin type II receptors. *Proc Natl Acad Sci U S A*. 2005;102(50):18117-18122. doi:10.1073/pnas.0505996102

37. Han HQ, Zhou X, Mitch WE, et al. Myostatin/activin pathway antagonism: molecular basis and therapeutic potential. *Int J Biochem Cell Biol*. 2013;45(10):2333-2347. doi:10.1016/j.biocel.2013.05.019

38. Yarasheski KE, Bhasin S, Sinha-Hikim I, et al. Serum myostatin-immunoreactive protein is increased in 60-92 year old women and men with muscle wasting. *J Nutr Health Aging*. 2002;6(5):343-348.

39. Reardon KA, Davis J, Kapsa RM, et al. Myostatin, insulin-like growth factor-1, and leukemia inhibitory factor mRNAs are upregulated in chronic human disuse muscle atrophy. *Muscle Nerve*. 2001;24(7):893-899. doi:10.1002/mus.1086

40. Murphy KT, Cobani V, Ryall JG, et al. Acute antibody-directed myostatin inhibition attenuates disuse muscle atrophy and weakness in mice. *J Appl Physiol (1985)*. 2011;110(4):1065-1072. doi:10.1152/japplphysiol.01183.2010

41. Nakashima M, Toyono T, Akamine A, et al. Expression of growth/differentiation factor 11, a new member of the BMP/TGFbeta superfamily during mouse embryogenesis. *Mech Dev*. 1999;80(2):185-189. doi:10.1016/s0925-4773(98)00205-6

42. Schafer MJ, Atkinson EJ, Vanderboom PM, et al. Quantification of GDF11 and Myostatin in Human Aging and Cardiovascular Disease. *Cell Metab*. 2016;23(6):1207-1215. doi:10.1016/j.cmet.2016.05.023

43. Tsai VW, Husaini Y, Manandhar R, et al. Anorexia/cachexia of chronic diseases: a role for the TGF-β family cytokine MIC-1/GDF15. *J Cachexia Sarcopenia Muscle*. 2012;3(4):239-243. doi:10.1007/s13539-012-0082-6

44. Gaddey HL, Holder K. Unintentional weight loss in older adults. *Am Fam Physician*. 2014;89(9):718-722.

45. Fujita Y, Taniguchi Y, Shinkai S, et al. Secreted growth differentiation factor 15 as a potential biomarker for mitochondrial dysfunctions in aging and age-related disorders. *Geriatr Gerontol Int*. 2016;16 Suppl 1:17-29. doi:10.1111/ggi.12724

46. Eggers KM, Kempf T, Wallentin L, et al. Change in growth differentiation factor 15 concentrations over time independently predicts mortality in community-dwelling elderly individuals. *Clin Chem*. 2013;59(7):1091-1098. doi:10.1373/clinchem.2012.201210

47. Wiklund FE, Bennet AM, Magnusson PK, et al. Macrophage inhibitory cytokine-1 (MIC-1/GDF15): a new marker of all-cause mortality. *Aging Cell*. 2010;9(6):1057-1064. doi:10.1111/j.1474-9726.2010.00629.

48. Schaap LA, Pluijm SM, Deeg DJ, et al. Inflammatory markers and loss of muscle mass (sarcopenia) and strength. *Am J Med*. 2006;119(6):526.e9-526.e5.26E17. doi:10.1016/j.amjmed.2005.10.049

49. Visser M, Pahor M, Taaffe DR, et al. Relationship of interleukin-6 and tumor necrosis factor-alpha with muscle mass and muscle strength in elderly men and women: the Health ABC Study. *J Gerontol A Biol Sci Med Sci*. 2002;57(5):M326-M332. doi:10.1093/gerona/57.5.m326

50. Cesari M, Penninx BW, Pahor M, et al. Inflammatory markers and physical performance in older persons: the InCHIANTI study. *J Gerontol A Biol Sci Med Sci*. 2004;59(3):242-248. doi:10.1093/gerona/59.3.m242

51. Calvani R, Marini F, Cesari M, et al. Systemic inflammation, body composition, and physical performance in old community-dwellers. *J Cachexia Sarcopenia Muscle*. 2017;8(1):69-77. doi:10.1002/jcsm.12134

52. Brown SH, Flint K, Storey A, et al. Routinely assessed biochemical markers tested on admission as predictors of adverse outcomes in hospitalized elderly patients. *Hosp Pract (1995)*. 2012;40(1):193-201. doi:10.3810/hp.2012.02.960

53. Hogarth MB, Gallimore R, Savage P, et al. Acute phase proteins, C-reactive protein and serum amyloid A protein, as prognostic markers in the elderly inpatient. *Age Ageing*. 1997;26(2):153-158. doi:10.1093/ageing/26.2.153

54. Bautmans I, Njemini R, Lambert M, et al. Circulating acute phase mediators and skeletal muscle performance in hospitalized geriatric patients. *J Gerontol A Biol Sci Med Sci*. 2005;60(3):361-367. doi:10.1093/gerona/60.3.361

55. Bodine SC, Stitt TN, Gonzalez M, et al. Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nat Cell Biol*. 2001;3(11):1014-1019. doi:10.1038/ncb1101-1014

56. Glass DJ. Skeletal muscle hypertrophy and atrophy signaling pathways. *Int J Biochem Cell Biol*. 2005;37(10):1974-1984. doi:10.1016/j.biocel.2005.04.018

57. Barbieri M, Ferrucci L, Ragno E, et al. Chronic inflammation and the effect of IGF-I on muscle strength and power in older persons. *Am J Physiol Endocrinol Metab*. 2003;284(3):E481-E487. doi:10.1152/ajpendo.00319.2002

58. Broussard SR, Zhou JH, Venters HD, et al. At the interface of environment-immune interactions: Cytokine and growth-factor receptors, *Journal of Animal Science*, Volume 79, Issue suppl_E, 2001, Pages E268–E284, https://doi.org/10.2527/jas2001.79E-SupplE268x

59. Hofmann M, Halper B, Oesen S, et al. Serum concentrations of insulin-like growth factor-1, members of the TGF-beta superfamily and follistatin do not reflect different stages of dynapenia and sarcopenia in elderly women. *Exp Gerontol*. 2015;64:35-45. doi:10.1016/j.exger.2015.02.008

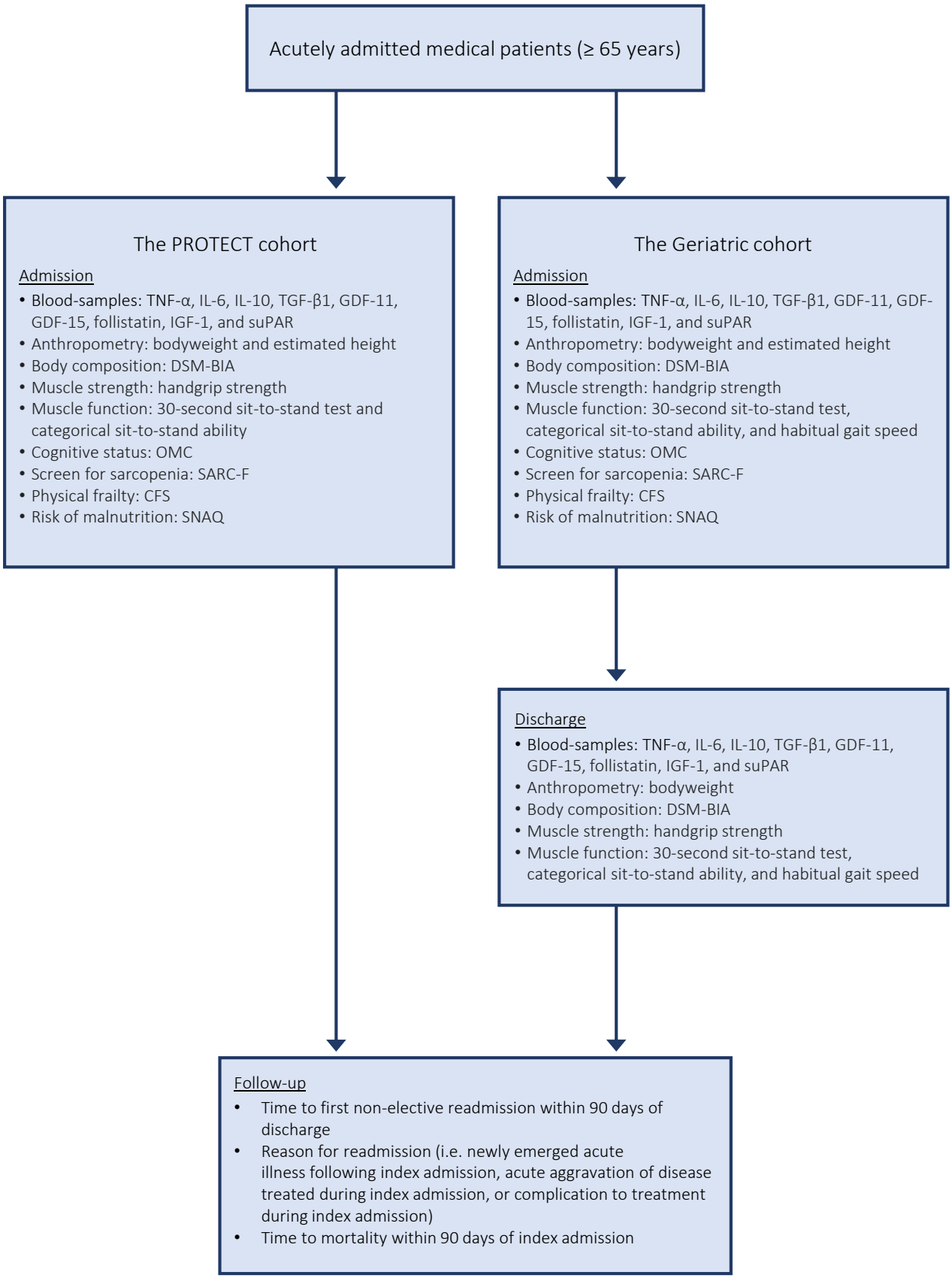
60. Haupt TH, Kallemose T, Ladelund S, et al. Risk factors associated with serum levels of the inflammatory biomarker soluble urokinase plasminogen activator receptor in a general population. *Biomark Insights*. 2014;9:91-100. Published 2014 Dec 16. doi:10.4137/BMI.S19876

61. Thunø M, Macho B, Eugen-Olsen J. suPAR: the molecular crystal ball. *Dis Markers*. 2009;27(3):157-172. doi:10.3233/DMA-2009-0657

62. Langkilde A, Petersen J, Henriksen JH, et al. Leptin, IL-6, and suPAR reflect distinct inflammatory changes associated with adiposity, lipodystrophy and low muscle mass in HIV-infected patients and controls. *Immun Ageing*. 2015;12:9. Published 2015 Aug 4. doi:10.1186/s12979-015-0036-x

Figure legends

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


Acutely admitted medical patients (≥ 65 years)
The PROTECT cohort
Admission
• Blood-samples: TNF-α, IL-6, IL-10, TGF-β1, GDF-11, GDF-15, follistatin, IGF-1, and suPAR
• Anthropometry: bodyweight and estimated height
• Body composition: DSM-BIA
• Muscle strength: handgrip strength
• Muscle function: 30-second sit-to-stand test and categorical sit-to-stand ability
• Cognitive status: OMC
• Screen for sarcopenia: SARC-F
• Physical frailty: CFS
• Risk of malnutrition: SNAQ
The Geriatric cohort
Admission
• Blood-samples: TNF-α, IL-6, IL-10, TGF-β1, GDF-11, GDF-15, follistatin, IGF-1, and suPAR
• Anthropometry: bodyweight and estimated height
• Body composition: DSM-BIA
• Muscle strength: handgrip strength
• Muscle function: 30-second sit-to-stand test, categorical sit-to-stand ability, and habitual gait speed
• Cognitive status: OMC
• Screen for sarcopenia: SARC-F
• Physical frailty: CFS
• Risk of malnutrition: SNAQ
Discharge
• Blood-samples: TNF-α, IL-6, IL-10, TGF-β1, GDF-11, GDF-15, follistatin, IGF-1, and suPAR
• Anthropometry: bodyweight
• Body composition: DSM-BIA
• Muscle strength: handgrip strength
• Muscle function: 30-second sit-to-stand test, categorical sit-to-stand ability, and habitual gait speed
Follow-up
• Time to first non-elective readmission within 90 days of discharge
• Reason for readmission (i.e. newly emerged acute illness following index admission, acute aggravation of disease treated during index admission, or complication to treatment during index admission)
• Time to mortality within 90 days of index admission