Study protocol containing statistical analysis plan

The Copenhagen PROTECT study: Biomarkers for length of hospital stay and loss of muscle mass and function in old medical patients

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Protocol

As humans age, there is a gradual loss of skeletal muscle mass and strength, termed sarcopenia (1,2). The underlying causes of sarcopenia are yet not fully elucidated but are thought to be multifactorial and include increased levels of systemic pro-inflammatory cytokines, a decrease in anabolic hormones and changes in the neuromuscular system (3,4). Furthermore, physical inactivity, chronic diseases, immobilisation and hospitalisation are known to play an important part in the development of sarcopenia (4). Notably, muscle strength declines more rapidly than muscle mass with aging (3) and muscle weakness in the elderly is prevalent and closely linked to frailty, functional decline, immobility, falls, and injuries in this population (5). As such, measures of muscle strength and functional performance have shown to be sensitive markers of frailty in combination with appendicular muscle mass (5,6).

The prevalence of sarcopenia ranges from 20-30% (aged >70yrs) within the general community (2,7). However, the prevalence of sarcopenia in geriatric patients after an acute hospital admission is substantially higher, estimated at ≈50% (8). Furthermore, successive events of hospitalisation have been suggested to contribute to the development of sarcopenia, as even short periods (4-5 days) of skeletal muscle disuse are known to induce muscle atrophy (9,10). Mean length of hospital stay in geriatric wards due to acute illness or hip-fracture is typically 7 to 11 days during which the level of physical activity is strongly reduced (11, 12) leading to an accelerated loss of muscle mass that many older patients never recover from.

Notably, a substantial part of the deterioration in functional capacity could be avoided *just* by counteracting loss of muscle mass during hospitalization. As such, we need to identify sensitive biological, clinical and functional biomarkers for loss of muscle mass and function during hospitalization to identify patients at risk of developing sarcopenia and investigate the association of these biomarkers with hospital length of stay, readmissions, mortality, and discharge to a higher level of care.

Purpose of the study

The overall aim of the present study is to identify clinical, functional and biological biomarkers associated with length of hospital stay in older acutely ill patients. In addition,

we will examine sensitive biomarker assays to investigate whether biomarkers assays in combination with clinical and functional measures have the ability to predict loss of muscle mass and function in patients hospitalized in a geriatric ward. Collectively, these initiatives will have the potential to identify patients at risk of developing sarcopenia and thus develop individualized treatments that counteract physical deconditioning and improve independent life expectancy and quality of life in older people, and lead to substantial health-care savings.

The specific aims of the present project are therefore to:

I. Examine whether the level of a variety of biomarkers at admission are associated with length of hospital stay, time to first readmission within 90 days, and time to mortality within 90 days in old patients, and whether the combination of clinical and functional measures with these biomarkers can identify patients at risk of prolonged hospital stay, readmissions and mortality. Primary end-point is length of stay. Secondary end-points are time to first readmission, time to mortality, muscle mass, muscle strength and frailty score (WP1).

II. Establish biomarker assays for predicting muscle loss and loss of function in old patients hospitalized at a geriatric ward, assessing whether levels of a variety of biomarkers combined with clinical and functional measures are associated with length of hospital stay, readmissions, mortality and whether patients are discharged to a higher level of care. Primary end-points are the relative change in muscle mass, muscle strength and muscle function. Secondary end-points are length of stay, time to first readmission, time to mortality, frailty score and discharge to a higher level of care (WP2)

Background of the project

Physiological adaptations to ageing

It is well established that human skeletal muscle function decays with aging, and sarcopenia is now generally used to describe the age-related loss of muscle mass and strength, which is believed to play a major role in the pathogenesis of frailty and functional impairment that may occur with old age (13-19). The exact mechanism behind the loss of muscle strength observed with aging and development of sarcopenia is not yet elucidated

(20). However, increased levels of systemic pro-inflammatory mediators such as TNF-α, IL-6 and CRP, a decline in anabolic hormones and changes in the neuromuscular system have been identified as some of the contributing factors (3,4, 21, 22, 23). Furthermore, physical inactivity, chronic diseases, immobilization and hospitalization are known to play a part in the development of sarcopenia (4). In addition, it has been demonstrated that the age-related muscle atrophy is followed by an increased infiltration of non-contractile components within the muscle tissue, such as connective tissue and intramuscular fat (24;25). Considering these morphological changes it is not surprising that maximal muscle strength is reduced as a result of aging by approximately 1.5 % per year from the sixth decade and forth (26).

Sarcopenia

Skeletal muscle tissue accounts for almost half of the human body mass and, in addition to its power-generating role; it is a crucial factor in maintaining homeostasis of glucose metabolism and as an energy reservoir in catabolic conditions. Given its central part in human mobility and metabolic function, any deterioration in the contractile, material, and metabolic properties of skeletal muscle has an extremely important effect on human health.

Sarcopenia specifically refers to the skeletal muscle atrophy and loss of muscle strength and function often observed with aging and is a major musculoskeletal cause of the loss of mobility, independence, and frailty in older adults. It typically appears as a decrease in muscle strength concurrent with a decrease in muscle mass. Clinical sarcopenia has been defined in statistical terms assuming a lower normal limit of two standard deviations below a mean relative appendicular muscle mass referred to findings in young healthy adults (27). The prevalence of sarcopenia ranges from 20-30% (age > 70years) within the general community (2,7), however, the prevalence of sarcopenia in geriatric patients after an acute hospital admission is substantially higher estimated at ≈50% (8). The etiology of sarcopenia is complex and involves neuronal, hormonal, immunological and nutritional mechanisms. Furthermore, physical inactivity and immobilization is known to play an important part in the development of sarcopenia (28;29). Yet, muscle mass in old age is not only lost due to reduced anabolism and increased catabolism, but also due to a reduced capacity of muscle

regeneration as muscle stem cell activation and proliferation becomes impaired in old age (30).

The knowledge surrounding the prevalence and determinants of sarcopenia in geriatric rehabilitation care is scarce, and it is unknown whether specific biomarkers can predict physical deconditioning during hospitalization. As such, the development of novel interventions for this group of patients is hampered by the lack of understanding of the basic biological mechanisms driving sarcopenia in conjunction with other age-related diseases in humans. The importance of sarcopenia has recently been underlined by its inclusion as a reportable disease in the Centers for Diseases Control and Prevention (ICD-10-CM code M62.84) in October 2016 (31). However, despite the serious consequences of sarcopenia being widely recognized, the diagnose has yet to be implemented in clinical practice in Denmark. Lack of knowledge and systematic assessment hinders diagnosis and treatment of sarcopenia and leads to physical deconditioning during hospitalization. Consequently, more than 50% of geriatric patients report a decline in functional capacity after discharge, often leading to a need for prolonged and expensive rehabilitation interventions (8).

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 (9,10), we have defined a prolonged hospital length of stay as an admission lasting >96 hours. In addition, we have defined patients discharged to a higher level of care as patients receiving increased relief, patients with increased need for a caregiver, patients referred to rehabilitation or 24-hour care, or patients moving to a nursing home following discharge. Readmission data will be limited to readmissions in the region of Zealand and the Capital Region of Denmark.

Biomarkers

Mechanisms that regulate skeletal muscle mass are central to the understanding of sarcopenia and related geriatric syndromes. Recently, GDF11, a close family member to myostatin (33), has been measured in human blood samples (34). When GDF11 is added to muscle and animals, identical signaling patterns to myostatin are seen with consequent myotube and muscle atrophy and inhibition of muscle differentiation (32,35). High circulating GDF11 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 frail based on low grip strength and gait speed as well as self-reported activity measures, had significantly higher GDF11 levels compared to non-frail controls (34). The data indicate that measurements of circulating GDF11 could be a biomarker that reflect frailty and related to geriatric syndromes. However, translation of these new findings into clinical utility needs further validation in a larger cohort.

In addition to the atrophic effects of GDF11, it is also able to induce expression of GDF15 (36). GDF15 is present in low levels under healthy conditions but can increase during disease or injury and contribute to muscle wasting by suppressing appetite, resulting in anorexia and drastic weight loss (37). In elderly, unintentional weight loss has been associated with increased in hospital morbidity and increased overall mortality (38). In an animal study, the suppression of appetite but not the loss of skeletal muscle could be reversed with a GDF15-neutralizing antibody (36). As such, the combined effects of GDF11 and GDF15 in muscle wasting during hospitalization might affect the length of hospital stay, readmissions and mortality.

A decrease in hormone concentrations appears to be associated with the development of sarcopenia. Especially in men, the decline in testosterone levels seems to be associated with a decline in muscle mass and muscle strength (39). Indeed, testosterone can increase protein synthesis in muscle and promote muscle regeneration by activating satellite cells (40). Estrogen may also promote muscle repair by activation and proliferation of satellite cells via the estrogen receptor α and β (41, 42). In addition, a murine study showed that ovariectomized rats (OVX) was unable to fully regain their muscle mass following a period of hindlimb unloading with a subsequent period of reloading compared to OVX rats with estrogen supplementation (43). The protective effect of estrogen on muscle mass during hindlimb unloading and reloading was also confirmed in a study of male rats (44) As such, estrogen might attenuate the degree of disuse atrophy.

Studies have demonstrated that ageing is accompanied by modifications of the immune system, specifically immunosenescence, leading to cytokine dysregulation and a chronic low-grade inflammation (45, 46). This has potential detrimental consequences, as research has shown that biomarkers of inflammation are associated with the pathology of the most common chronic age-related diseases including atherosclerosis (47), chronic heart failure (48), osteoporosis (49), type II diabetes mellitus (50), neurodegenerative disorders (51,52)

and sarcopenia (53, 54, 55). Several studies have investigated the association of inflammatory biomarkers with muscle mass, muscle strength and muscle function. In vitro, exposure of myoblasts to TNF-α inhibits muscle differentiation and causes a decrease in protein synthesis (56). In addition, TNF-α binding to its receptor TNFR activates the NF-κB pathway causing proteolysis (57). However, results regarding the effect of inflammatory biomarkers on muscle mass are inconsistent and lack clear evidence as to whether these inflammatory biomarkers are associated with sarcopenia.

Many studies have assessed low-grade inflammation by measuring a limited number of inflammatory proteins, and one study showed that elderly individuals exhibiting high levels of pro-inflammatory cytokines also tended to show high levels of anti-inflammatory cytokines (58). Thus, we aim to include a variety of both pro- and anti-inflammatory markers.

Furthermore, distinct patient populations with co-existing pathophysiological processes might exhibit different biomarker profiles. A recent study found a negative association between systemic levels of CRP and changes in muscle mass in geriatric patients in response to resistance training during hospitalization, indicating that patients with inflammation during their hospital stay may have an increased risk of deconditioning during hospitalization (59).

Regardless, systemic levels of the inflammatory marker C-reactive protein (CRP), has been found to be predictive of both the length of hospital stay and readmissions (60, 61). Indeed, geriatric patients with inflammation at admission stayed on average 3 days longer than patients without inflammation, evaluated by CRP levels at admission (62). Despite CRP being a nonspecific marker of inflammation, it has also been recognized as a predictive marker of in-hospital mortality in elderly patients admitted to the acute ward (63).

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 (64). suPAR correlates with other inflammatory markers, such as TNF-α, and patients with the highest levels of suPAR generally have the worst prognosis (65). Elevated levels of suPAR have been associated with increased risk of cardiovascular disease, cancer, type II diabetes and premature mortality in the general population, independent of CRP levels (66). However, a recent study found that the prognostic capability of suPAR did not affect all-cause mortality in patients admitted to the emergency department (67).

In a study of HIV-infected patients, suPAR was associated with low muscle mass, while IL-6 was associated with both low muscle mass and increased fat mass in both patients and healthy controls (68). Thus, there seem to be distinct inflammatory processes occurring simultaneously with different effects on muscle mass and fat mass, respectively.

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.

RESEARCH PLAN

This project includes 2 work packages (WP), of which WP1 is a prospective cohort study of acutely ill older patients (65+ years) referred to the hospital and will investigate the association of biomarkers combined with clinical and functional measures with length of hospital stay, time to first non-elective readmission, muscle mass, muscle strength, frailty, and time to mortality. WP2 is a prospective cohort study of older patients (+65 years) admitted to a geriatric ward for treatment of the acute disease and rehabilitation care and will investigate the possible association of biomarkers with the relative change in muscle mass and function, frailty score, length of hospital stay, time to first non-elective readmission, time to mortality and discharge to a higher level of care. Readmission data will be limited to readmissions in the region of Zealand and the Capital Region of Denmark.

WP1: Biomarkers in the acutely ill older patient

Hypothesis: We hypothesize that combining clinical and functional measures with systemic biomarkers has the potential to identify older acutely ill medical patients at risk of prolonged (>96 hours) hospital stays, non-elective readmissions, and mortality.

Overview of Actions: Acutely ill medical patients (age >65 years) referred to Bispebjerg-Frederiksberg Hospital will consecutively be enrolled in the study. We will recruit participants during a 1-year period.

Assessments: Blood tests, frailty (CSHA Clinical Frailty Scale), risk of pressure ulcers (Braden Score), sarcopenia (SARC-F), hand-grip strength, chair-rise test and body composition (BIA). Blood samples will be used to assess biomarkers of muscle atrophy and wasting (GDF-11 & GDF-15), inflammatory cytokines and acute-phase proteins, sex hormones and immune activation (suPAR). All assessments will be performed within the

first 24 hours of admission. Primary endpoint is length of hospital stay. Secondary endpoints are time to first non-elective readmission, time to mortality, muscle strength, muscle mass and frailty.

WP2: Biomarkers for changes in muscle mass and function in geriatric patients

Hypothesis: We hypothesize that combining clinical and functional measures with biomarkers will give us the opportunity to elucidate a composition of biomarkers associated with the change in muscle mass and function during hospitalization, thus having the potential to identify future geriatric patients at risk of physical deconditioning and prolonged (>96 hours) hospital stays, non-elective readmissions, mortality, and discharge to a higher level of care.

Overview of Actions: Geriatric patients (age >65 years) referred to geriatric rehabilitation care at Bispebjerg-Frederiksberg Hospitals will be enrolled in the study. We will recruit participants during a 1-year period.

Assessments: Included patients will be monitored by blood tests immediately following admission and at discharge. A geriatric assessment will be performed during admission (Barthel Index, the CSHA Clinical Frailty Scale, SARC-F, the Orientation-Memory-Concentration test (OMC) and De Morton Mobility Index (DEMMI). Measurements of muscle function (e.g. hand-grip strength, gait-speed and sit-to-stand tests) and body composition (BIA) will be performed shortly after admission and at discharge. Blood samples will be used to assess biomarkers of muscle atrophy and wasting (GDF-11 & GDF-15), inflammatory cytokines and acute-phase proteins, sex hormones and immune activation (suPAR). Preliminary assessments will be performed within the first 24 hours of admission. Primary end-points are relative change in muscle mass, muscle strength and muscle function. Secondary end-points are length of hospital stay, time to first non-elective readmission, time to mortality, frailty, and discharge to a higher level of care.

Project significance and future perspectives

The project includes; 1) Identifying biomarkers in acutely ill older patient groups associated with length of hospital stay, non-elective readmissions, muscle mass, muscle strength, frailty, and time to mortality within 90 days. 2) Combining biomarker measurements with clinical and functional measurements for predicting loss of muscle mass and function

during hospitalization in geriatric patients, and investigating the association with hospital length of stay, non-elective readmissions, time to mortality, frailty, and discharge to a higher level of care. The findings of the study will not only have implications for geriatric patients but has the potential to be translated across disciplines to any patient at risk of hospital induced muscle wasting.

Subjects and methods

Subjects

Acutely ill medical patients (age > 65 yrs) for WP1 (n=1100) will be enrolled ad hoc from Bispebjerg-Frederiksberg Hospital.

Geriatric patients (age > 65 yrs) for WP2 (n=200) will be enrolled ad hoc from Bispebjerg-Frederiksberg Hospital.

For both WP1 and WP2 we aim to include patients with a dementia diagnosis and patients with delirium, as this group of patients represent a large portion of the elderly patients admitted to the hospital. Thus, the project aims to be representative of the general elderly patient population. Patients with dementia will be identified through patient journals. Depending on the severity of dementia and delirium, it might be possible that some patients are unable to sign participant consent, and in these cases, we will seek participant consent from a close relative or guardian. Independent medical doctors who have knowledge of the project but are not associated with the project and are independent to the interests of the principal investigator will evaluate whether these subjects are able to participate in the study. The medical doctors designated to perform this evaluation is chosen based on available independent doctors on duty in the department that day. Two large prospective cohort studies have previously reported that dementia is independently associated with frailty (69,70). Another study has shown that frail patients have an increased risk of developing delirium and that frail patients with delirium have a reduced survival rate following hospitalization compared to non-frail patients with delirium (71). This puts this specific patient population at increased risk of adverse outcomes and muscle wasting during hospitalization. As such, it is essential that we increase our knowledge of the underlying mechanisms and predictors of muscle wasting and the predictors of longer hospital stays and mortality in this specific patient population so that we might help future

patients with dementia and delirium. The project entails minimal discomfort for the subjects and has no permanent side effects. Should the patients with delirium regain their legal competency during their hospital stay, they will have to sign an independent participant consent.

All subjects will have blood tests performed to evaluate levels of GDF11 & GDF15, inflammatory cytokines and acute-phase proteins, sex hormones and immune activity (suPAR). In addition, all subjects will have their hand-grip strength, chair-rise ability and body composition (BIA) assessed. Their frailty score and risk of pressure ulcers will be assessed by the CHSA Clinical Frailty Scale and Braden score, respectively. The Charlson Comorbidity Index will be used to assess comorbidity, SARC-F will evaluate the presence of sarcopenia and the Orientation-Memory-Concentration test (OMC) will evaluate cognitive function in all subjects

Subjects included in WP2 will have an additional geriatric assessment including the Barthel Index and De Morton Mobility Index (DEMMI), which are already part of standard clinical practice. Functional status will additionally be evaluated by a gait-speed test.

Information from patient records and other data sources

Prior to obtaining participant consent we will need access to the summary in patient records when dementia is suspected. This is solely to confirm the dementia diagnosis as these patients will need to be evaluated by an independent physician to determine whether they are able to participate in the study.

After obtaining participant consent, direct access to information from patient records and/or the National Patient Registry (NPR) is necessary for project implementation and to control for confounders and covariates in analyses. Participants will be informed of this in the participant information sheet. This information is disclosed to the investigator and includes information regarding:

- age, height and weight
- smoking habits
- results of routine bloodwork
- admission to the intensive care unit
- Early Warning Score (EWS)

- disease history and the number of ICD-10 discharge diagnoses 5 years prior to admission, to assess comorbidity
- discharge to a higher level of care (WP2)
- hospital length of stay
- readmissions within 90 days in the region on Zealand and Capital region of Denmark
- number of hospitalizations (acute and elective) 1 year prior to admission
- number of medications prescribed at admission, to evaluate polypharmacy
- results of bloodwork associated with the project
- results from questionnaires (Barthel Index, OMC, SARC-F, DEMMI, Braden Score & CSFA Clinical Frailty Scale)

Information regarding patient mortality within 90 days of admission will be extracted from the Danish Civil Registry System.

The participant consent will give the investigators as well as possible supervisory authorities access to obtain information from patient records to view health information necessary for the implementation of the project, as well as required quality-control and monitoring.

Processing of personal information

All participant data are confidential, are subject to confidentiality and will be handled in compliance with the law on the processing of personal information. Results from participants will be published in scientific journals, securing the complete anonymity of participants. All procedures will be conducted according to “Good Clinical Practice” standards, regarding initiation, monitoring and reporting. All protocols will be submitted for approval by the local ethics committee and Danish Data Protection Agency before trial-initiation and the project will comply with the regulations of the General Data Protection Regulation (GDPR) and the Data Protection Act.

Recruitment and participant consent

All acutely ill old medical patients (>65 years) are included in the project. Initial contact to the patients will be made immediately after admission to Bispebjerg-Frederiksberg Hospitals, by research personnel directly associated with the project. Initial contact will entail informing the eligible participants, that a scientific study is being conducted and that they have the possibility to take part in it. Interested participants will be given the written participant information and we will set up a meeting immediately or within the following hours for the verbal information. Interested participants will be informed that they have the right to bring an assessor to the meeting.

In the case of severe dementia or delirium, it might be possible that some patients are unable to sign participant consent, and in these cases, we will seek participant consent from a close relative or guardian, should the guardianship include access to signing participant consent in research. Independent medical doctors who have knowledge of the project but are not associated with the project and are independent to the interests of the principal investigator will evaluate whether these subjects are able to participate in the study, and if so, they will also sign the participant consent. The medical doctors designated to perform this evaluation is chosen based on available independent doctors on duty in the department that day. Interested participants and their relatives or guardian will be given the written participant information and we will set up a meeting immediately or within the following hours for the verbal information. The verbal information will be adjusted to fit the patient's cognitive abilities.

The verbal information will be given by research personnel directly associated with the project. The meeting will take place in the hospital room to ensure that the conversation is as undisturbed as possible, and this will give eligible participants, relatives or guardians time to listen and ask questions. The verbal information will be understandable without the use of technical terms and will be based on the written participant information including aim, methods, possible risks and side-effects and disclosure of personal information. Possible participants, relatives or guardians will be given time to consider whether they would like to participate in the study before signing a participant consent. Should the patients with delirium regain their legal competency during their hospital stay, they will have to sign an independent participant consent.

The appendix “Forsøgspersoners rettigheder i et sundhedsvidenskabeligt Forskningsprojekt” will be enclosed with the written information and reviewed during the verbal information.

Following the written and verbal information, all participants will be informed of any newly discovered risks, side effects or any essential changes in study design. Participants will also be informed if the study is terminated and the reasons for termination.

Inclusion criteria

All acutely ill medical patients aged 65+ years admitted to Bispebjerg-Frederiksberg Hospital.

Exclusion criteria

Exclusion criteria includes being under the age of 65 years, terminal illness, droplet or airborne isolation, patients who do not understand Danish and patients with temporary civil person registration numbers (CPR).

Statistical considerations

The current study will recruit participants during a 1-year period to avoid any seasonal differences in the patient population. The acute medical unit receives approximately 10-20 patients a day over the age of 65, giving a total of roughly 5475 patients a year. We estimate a participation rate of 20% due to unwillingness to participate, inability to perform measurements or cognitive impairments or delirium affecting their ability to sign participant consent. We aim to include a total of roughly 1100 participants, of which 200 patients are admitted to the geriatric rehabilitation care unit.

Overall outcome analysis

To test whether clinical, functional and biological biomarkers are associated with length of stay we will perform multivariate logistic regression and to test whether clinical, functional and biological biomarkers are associated with loss of muscle mass and function we will perform multivariate linear regression. To evaluate the prognostic abilities of biomarkers (individual, in combination and combined with clinical and functional measures) we will use area under the curve for receiver operating characteristics (AUROC) statistics.

Table of summary statistics will be presented with a number of baseline variables (age, sex, functional measures, results of blood tests, comorbidities).
Continuous variables will be summarized with: n (non-missing sample size), mean, standard deviation, median, interquartile range, number of missing values. Categorical variables will be reported as frequency and percentages (based on non-missing sample size) and number of missing values.
Continuous and ordinal variables will be tested by Wilcoxon analysis for differences between groups. Categorical variables will be tested with chi-square. Missing data will be imputed.

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. 817 of these patients (39.7%) had a prolonged length of stay, defined as a hospitalization lasting more than 96 hours.

With a sample size of 1100 and the assumption that approximately 40% of old medical patients have a prolonged hospital stay, an AUROC of 82 will have a power of 0.9 and a significance level of 0.05. The primary outcome will be mean length of stay. Patients will be grouped in either normal or extended length of stay (<96 hours) and Cox regression analysis will be used to compare differences in readmission and mortality. To assess the discriminative ability of biomarkers with regards to extended length of stay, 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.

Methods

Blood tests are standard clinical procedures and will be performed by trained personnel. For the measurement of muscle strength and function, validated tests will be used and will be performed by trained physiotherapists associated with the study. Measurement of total and regional body composition are standard methods at Bispebjerg-Frederiksberg Hospital and will also be performed by trained physiotherapists associated with the study. Questionnaires are validated and will be carried out by healthcare professionals associated with the study.

- *Blood tests:* Blood from a peripheral vein (50 ml) will be examined for inflammatory biomarkers, sex hormones, immune activation (suPAR) as well as biomarkers of muscle atrophy and muscle wasting (GDF11 and GDF15).
- *Maximal muscle strength:* Maximal hand grip strength will be measured by a hand-held dynamometer.
- *Functional tests:* Habitual and maximal gait speed and sit to stand test for 30 seconds.
- *Questionnaires:* A frailty score will be assessed by the CSHA Clinical Frailty Scale, the risk of pressure ulcers will be assessed by the Braden Scale, sarcopenia will be assessed by SARC-F, cognitive status by the Orientation-Memory-Concentration test (OMC) and the degree of multimorbidity will be assessed using the Charlson Comorbidity index. Participants included in WP2 will have an additional geriatric assessment as part of clinical practice, including the Barthel Index and the De Morton Mobility Index (DEMMI).
- *Anthropometry:* All subjects will have their body composition calculated by Bioelectrical Impedance Analyses (BIA) at admission. Patients from geriatric rehabilitation care will have an additional BIA measurement at discharge to evaluate the change in body composition during hospitalization. Weight will be assessed using chair scales. Height will be estimated with patients lying in a supine position by a segmometer using knee-height measurement and age using the equation from Chumlea et al (72).

Risks, side effects and disadvantages

The potential discomfort from having a blood test is very brief and rarely cause a small blood accumulation (bruise), which will disappear within a few days. The total amount of blood collected is 25-50ml, which is a quite modest amount compared to the amount collected as a blood donor (500 ml). There are no risks or discomfort associated with assessments of muscle strength or muscle function or by body composition analyses by BIA.

Ethical considerations and dissemination of results

All procedures will be conducted according to “Good Clinical Practice” standards, regarding initiation, monitoring and reporting. All protocols will be submitted for approval by the local ethics committee and Danish Data Protection Agency before trial-initiation and the project will comply with the regulations of the General Data Protection Regulation (GDPR) and the Data Protection Act. Findings from the project, regardless of the outcome, will be published in relevant peer-reviewed scientific journals and positive, negative or inconclusive results will be published on www.clinicaltrials.gov. Subjects will be anonymous.

The project is expected to provide insight into the biomarkers associated with changes in muscle mass, strength and function in acutely ill older patients and to elucidate the role of a variety of biomarkers on length of hospital stay, readmissions and mortality. Each subject will gain knowledge regarding their physical function and body composition, and patients admitted to geriatric rehabilitation will gain additional knowledge regarding their muscle function. Furthermore, the gained insight into biomarkers of changes in muscle mass and function during hospitalization and their association with length of hospital stay, readmissions and mortality, might prove beneficial for future geriatric patients. The project entails minimal discomfort and no permanent side effects; thus, it is considered ethically sound. All subjects are covered by Bispebjerg-Frederiksberg Hospital patient insurance.

The results will be available following the end of the project with a subsequent period of data processing. As such, individual participant results will not be available immediately following examinations. However, participants will be informed of individual preliminary results, to the desired extent.

Research biobank

It is necessary to create a research biobank for this study, as participants are recruited ad hoc from the hospital based on admission during a 1-year period. We will apply for approval from The Danish Privacy Act to create the biobank. As such, the participant consent will entail permission to create a biobank with biological material.

As per the Danish Privacy Act, excess biological material must generally be destroyed following the end of the project. However, we will apply for approval from the Danish Data Protection Agency to create a Biobank with excess material for future research. In addition,

we will seek permission from all subjects to store excess biological material for future research. The scientific ethics committee will be notified of any possible new projects. Excess biological material will be coded, frozen and stored at Bispebjerg hospital for the next 20-40 years, with anonymization of the subjects. It is important that the material is not destroyed as new biomarkers are constantly being discovered and further analyses into the complex association between various disease states and biomarkers of loss of muscle mass, strength and function, might increase our knowledge of the mechanisms behind atrophy and loss of function in elderly patient groups during hospitalization.

Financial conditions

The project is initiated by Professor Charlotte Suetta, who is also the principal investigator. Funding has been rewarded to the Geriatric Research Unit at Bispebjerg Hospital by the Novo Nordisk Foundation, and will be used for staff salaries, laboratory tests and other equipment in the current project. It is planned to seek additional funding in the future through both public and private sponsors. The principal investigator has no financial interests in the project and no financial association with the sponsor. No remuneration will be given to participants in the study, as they are all recruited directly from the hospital and therefore have no financial costs from participating (i.e. transportation costs).

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