RESEARCH PROTOCOL

ACROMEGALY: BALANCE, FALLS AND FRACTURE RISK

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GENERAL INFORMATION

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I: TITLE

Acromegaly: balance, falls and fracture risk.

List of abbreviations:

GH: Growth Hormone

IGF-1: Insulin-like Growth Factor 1

SSA: Somatostatin Analog

DA: Dopamine Agonist

HR-pQCT: High Resolution peripheral Quantitative Computed Tomography

HGS: Hand Grip Strength

TUG: Timed Up-and-Go

DXA: Dual X-ray Absorptiometry

BMSi: Bone Material Strength Index

vBMD: Volumetric Bone Mineral Density

BMI: Body Mass Index

VFA: Vertebral Fracture Assessment

mSv: Milli-Sievert

CoP: Center of Pressure

WOMAC: Western Ontario and McMaster universities osteoarthritis Index

FES-I: Falls Efficacy Scale International

P1NP: procollagen-1 N-terminal propeptide a marker of bone formation) and

CTX: cross-linked C telopeptide of type I collagen

PTH: Parathyroid Hormone

II: BACKGROUND, AIMS AND HYPOTHESES

A. BACKGROUND

Acromegaly is a rare systemic disease characterized by hypersecretion of growth hormone (GH) from a pituitary adenoma, and consequently pathologically high levels of insulin-like growth factor I (IGF-I). Symptoms develop insidiously and include acral growth, coarse facial features and organ enlargement. The incidence of acromegaly is most often reported to be approximately 3-5 cases per 10^6 per year[1–5] whereas the reported prevalence varies considerably between studies from 20 - 85 cases per 10^6 [6].

Acromegaly is associated with several comorbidities including diabetes mellitus, hypertension, heart failure, thromboembolic events, ischemic heart disease, increased cancer risk, arthropathy and osteopathy, and untreated it is associated with a significant reduction in quality of life and life expectancy[2,7].

The preferred treatment, when feasible, is transsphenoidal surgical removal of the pituitary adenoma, which is performed in 60-80% of patients in Denmark. Medical treatment includes somatostatin analogs (SSAs), the GH receptor antagonist Pegvisomant and dopamine agonists (DAs), while radiotherapy is rarely used[2]. With optimal treatment and normalization of GH and IGF-I, acromegalic patients have a mortality rate close to that of the general population[1]. If left untreated, however, acromegaly is associated with an increased mortality, mainly due to cardiovascular complications[8].

B. ACROMEGALY AND MUSCULOSKELETAL DISORDERS

Of the acromegaly-related comorbidities, arthropathy is associated with a markedly reduced quality of life due to pain, reduced mobility, impaired activities of daily living as well as reduced work productivity and increased risk of early retirement[9]. Arthropathy is one of the most frequent complications of acromegaly, affecting more than 70% of all patients[10,11]. Acromegalic arthropathy is characterized initially by cartilage hypertrophy, joint space widening and osteophytosis, whereas in later stages it assumes features similar to primary osteoarthritis, such as joint space narrowing, subchondral cysts and bone edema[11,12]. In early stages of acromegalic arthropathy, progression of the condition may be halted or even reversed by achieving biochemical control/remission, but in later stages the arthropathy becomes irreversible and persistent even despite successful treatment of the underlying condition. However, acromegalic arthropathy has even been found to slowly progress despite achievement of disease control[2]. No effective treatment, except for surgical arthroplasty and analgesics, exists for this debilitating condition[11]. Novel imaging techniques such as HR-pQCT have not yet been applied in the field of acromegalic arthropathy, and may further our understanding of the condition, potentially leading to new treatment options. HR-pQCT imaging of joints is common in research regarding inflammatory joint diseases[13], but thus far, no studies exist regarding HR-pQCT joint imaging in acromegalic patients.

Studies concerning balance and falls among acromegalic patients remain scarce. Thus far, it has been suggested that acromegalic patients have impaired balance and postural control, due to factors such as alterations in body composition, enthesopathy, myopathy, arthropathy or visual field disturbances[14–16]. Indeed, altered body composition has been demonstrated in acromegalic patients using whole-body DXA scans[17,18] and reduced peripheral muscle strength has been found using hand grip strength (HGS) as a measure[19]. Moreover, an increased fear of falling has been observed in patients with acromegaly, although the frequency of falling seems to be similar to healthy controls[16]. However, fall risk, as evaluated via the timed up-and-go (TUG) test, has been shown to be increased in acromegalic patients[20].
An improvement in several dynamic balance parameters, as evaluated by stabilometry, has been demonstrated among acromegalic patients after a relatively short training program, indicating a potential benefit of focused physical therapy for this patient group[14]. Given that acromegalic patients have impaired bone quality, preventing falls may be of considerable clinical relevance. However, this subject calls for further investigations[14–16]. To our knowledge, no studies have directly compared body composition and dynamic muscle parameters such as hand grip strength, leg extension strength, timed up-and-go and stabilometry in acromegalic subjects.

Acromegaly-related osteopathy has been a subject of interest in recent years, as it has been established that patients with acromegaly have increased bone turnover, reduced bone quality and an increased risk of fractures despite normal bone mineral density as assessed by DXA-scans[21]. Secondary hypogonadism due to tumor mass effect or pituitary surgery plays a significant role in the diminished bone quality of acromegalic patients, even after disease control is achieved[21].
Acromegalic subjects have been found to have reduced quality of the trabecular bone compartment, increasing the risk of vertebral fractures, which have indeed been found to be prevalent among acromegalic patients[22–25]. Using High-Resolution peripheral Quantitative Computed Tomography (HR-pQCT), significantly reduced measurements of both trabecular and cortical volumetric bone mineral density (vBMD) have been reported in acromegalic patients[18,26], even during biochemical disease control[26]. Disease duration and presence of hypogonadism appear to be the most influential risk factors for this impaired bone quality, although even eugonadal acromegalic patients have reduced trabecular bone quality[18,26,27]. HR-pQCT-derived bone parameters can be used to calculate physical properties such as bone stiffness and failure load, which has previously been performed in acromegalic patients, revealing no difference between acromegalic patients and healthy controls in this regard[18]. However, compromised bone quality has been confirmed *ex vivo* by means of iliac crest bone biopsies in a study of acromegalic patients, reporting both lower trabecular thickness and increased trabecular separation in acromegalic patients with vertebral fractures but normal BMD, as assessed by DXA scan[28].

Although normal or even increased cortical bone thickness has been reported using iliac crest bone biopsies[28], hip structure analysis[29] and high-resolution cone beam tomography[30], other cortical bone parameters such as bone material strength index (BMSi) have been shown to be reduced, even in well-controlled acromegalic patients[31], suggesting compromised bone fracture resistance in acromegalic patients. Decreased BMSi, as measured by micro-indentation using an OsteoProbe, has been shown to be

correlated with fragility fractures in postmenopausal women and in individuals with diabetes[31,32]. Only one study[31] has measured BMSi on acromegalic subjects and found significantly lower values compared to healthy controls, regardless of biochemical control, hypogonadism or presence of vertebral fractures. If and how BMSi values correlate to reduced bone quality, as assessed by HR-pQCT, remains to be examined in patients with acromegaly. Thus far, no studies exist regarding the integration of HR-pQCT-derived bone parameters, BMSi and the presence of vertebral fractures. By combining these diagnostic modalities, we aim to perform an integrated assessment of bone quality in acromegalic patients, examining both the cortical and trabecular bone compartments to further our understanding of acromegalic osteopathy. This may provide more insight into which diagnostic modality is optimal as a measure of fracture risk in acromegalic patients, since standard DXA scans have proved less useful in this regard[21].

C. OBJECTIVES

Aim:

A. To perform a comprehensive, integrated assessment of balance, bone and joint quality, body composition, muscle strength, fear and risk of falling and fracture risk of acromegalic patients.

Hypotheses:

A. Acromegalic patients have impaired bone quality (as assessed by HR-pQCT and BMSi), impaired balance, and increased risk of falling. Combining multiple diagnostic modalities will improve our understanding of the pathophysiological mechanisms behind acromegalic osteopathy.

III: METHODS

A. STUDY DESIGN

The study will be performed as a cross-sectional study examining patients with acromegaly and healthy controls. Participants will be informed about the study both orally and in writing and will be recruited from the pituitary endocrinology outpatient clinic at Aalborg University Hospital.

Healthy controls will be recruited via social media and websites such as www.forsøgsperson.dk. An existing database where participants have given written consent (within the last year) to be contacted regarding research projects may also be used. This database is created and administrated by the North Denmark Region, and approved by its legal staff. Potential participants are required to use a secure access (NemID), and if they do not actively renew their consent within a year, it is automatically withdrawn.

B. STUDY POPULATION

We plan to include thirty male or female patients with acromegaly and thirty age- (+/- 5 years), BMI- (+/- 5 points), and sex-matched non-acromegalic control subjects.

Inclusion criteria for the acromegalic group:

1. Verified acromegaly diagnosis
2. Age ≥18 years
3. Ability to provide informed consent
4. Ability to stand on both legs for ≥5 minutes at a time and walk ≥6 meters
5. Patient is eugonadal, either naturally or by hormone replacement therapy

Exclusion criteria for the acromegalic group:

1. Established diagnosis of severe kidney or liver dysfunction, malabsorption, multiple myeloma or other diseases associated with reduced bone quality
2. Treatment with supraphysiological doses of glucocorticoids or other drugs that impair bone quality
3. Diagnosis of rheumatoid arthritis, psoriatic arthritis or other joint diseases unrelated to acromegaly
4. Active drug or alcohol abuse
5. Pregnancy
6. Known allergy/hypersensitivity to local anaesthetics or disinfectant
7. Other factors that render the subject unable to participate in the clinical study, based on the judgment of the investigator(s)

The control subjects will be recruited via social media, the aforementioned database and websites, and matched to the acromegalic study population.

Inclusion criteria for the control group:

1. Age ≥18 years
2. Ability to provide informed consent
3. Ability to stand on both legs for ≥5 minutes at a time and walk ≥6 meters

Exclusion criteria for the control group:

1. Established diagnosis of severe kidney or liver dysfunction, malabsorption, multiple myeloma, diabetes or other diseases associated with reduced bone quality
2. Treatment with supraphysiological doses of glucocorticoids or other drugs that impair bone quality
3. Diagnosis of rheumatoid arthritis, psoriatic arthritis or other joint diseases
4. Active drug or alcohol abuse
5. Pregnancy

6. Known allergy/hypersensitivity to local anaesthetics or disinfectant
7. Other factors that render the subject unable to participate in the clinical study, based on the judgment of the investigator(s)

C. ASSESSMENT OF RESOURCES

This study will be conducted in collaboration with the department of endocrinology of Aalborg University Hospital, wherefrom patients will be recruited and the physical locale for the procedures provided. The department has access to experienced personnel, who are trained in the use of both HR-pQCT and DXA scanners, and the Osteoprobe. Personnel and equipment for biochemical sampling are also accessible.

For assistance with the procedures regarding balance and postural control, we will collaborate with Rogerio P. Hirata under the group of Prof. Thomas Graven-Nielsen at Aalborg University, who is experienced in the use of the balance pad and interpretation of the results it yields.

D. STUDY PROCEDURES

Patients with an established diagnosis of acromegaly will be recruited from December 2022 to March 2025. Patients will be informed of the study during scheduled visits to the pituitary endocrinology outpatient clinic at Aalborg University Hospital by one of two specialists in pituitary endocrinology. Patients will be given information regarding the study, and if interested, they will be provided with a form to fill out with their contact information, so they can be contacted by the investigators or other staff affiliated with the study. Should the patient agree to be contacted via telephone or e-mail, they will be given an instruction regarding their rights as a participant in a scientific study (*"Dine rettigheder som forsøgsperson i et sundhedsvidenskabeligt forskningsprojekt"*) as well as written material specifically pertaining to the study (*"Deltagerinformation"*). Should the subject be deemed fit for the study, based on the in- and exclusion criteria, contact will be made via phone or e-mail and they will receive further information regarding the study, and offered a personal meeting with one of the investigators, where details of the study procedures will be outlined. The meeting will take place in a dedicated, undisturbed office at the disposition of the Department of Endocrinology, in order to ensure due privacy during the meeting. In advance, the subject will be informed of the opportunity to bring a relative or other representative to the meeting. At the meeting, the participant and any representative will be informed of the 24-hour deliberation period before providing informed consent. No study procedures will be performed until the informed consent form has been signed by both the participant and the investigator.

As mentioned above, healthy controls will be recruited via the aforementioned database, social media and websites such as www.forsoegsperson.dk. Potential participants who contact the principal investigator through these channels will be provided with written material pertaining to the study ("Deltagerinformation") and a description of their legal rights as a participant (*"Dine rettigheder som forsøgsperson i et sundhedsvidenskabeligt forskningsprojekt"*). Should the potential participant meet the in- and exclusion criteria, they will be invited to a personal meeting, where details of the study are explained,

having been informed of the opportunity to bring a representative or relative to the meeting in advance. Should they wish to participate, a 24-hour deliberation period will be given prior to obtaining written informed consent.

After providing informed consent, the participants will undergo a series of study procedures. Bone quality will be assessed by way of High-Resolution peripheral Quantitative Computed Tomography (HR-pQCT) images of the distal radius and tibia. Bone material strength index (BMSi) is assessed using an OsteoProbe. In addition to this, postural control will be assessed using a balance board to obtain a surrogate measure of fall risk. Body composition, bone mineral density and vertebral fracture assessment (VFA) will be analyzed via Dual X-ray Absorptiometry (DXA) scans and peripheral muscle strength will be assessed via hand grip strength and knee extension torque measurements, and visual acuity tested with standard methods. Data will be analyzed using relevant statistical analyses and presented as a cross-sectional study.

HIGH RESOLUTION PERIPHERAL QUANTITATIVE COMPUTED TOMOGRAPHY (HR-PQCT)

HR-pQCT is a 3-dimensional imaging modality that can be applied to bone and joints *in vivo*, in order to non-invasively assess the quantity and quality of trabecular and cortical bone compartments separately. HR-pQCT will be applied to peripheral skeletal sites, namely the distal radius and tibia, where image slices equivalent to 9 mm of bone will be acquired. From this, parameters such as volumetric bone mineral density (vBMD), cortical density, cortical porosity, trabecular density, trabecular thickness, trabecular spacing and others will be calculated using specialized software. In the same scanning session, images of the wrist and ankle joints will be produced and analyzed for features of arthrosis, such as erosions, osteophytosis and others.

The scanner's gantry is relatively narrow and shallow (rear physical stop) only allowing the distal peripheral skeleton to be accommodated. The limb being scanned is immobilized in a carbon fiber shell. A scout view, essentially a two-dimensional x-ray scan, is obtained so that the operator can identify a precise region for the three-dimensional measurement. Because HR-pQCT uses a polychromatic X-ray source it is subject to beam hardening as well as scatter artefacts, which can significantly impact geometric and densitometric measures. Once the images have been acquired, a default patient evaluation protocol is used to analyze the scans over the entire 9 mm three-dimensional region to assess a wide range of standard and optional structural and density parameters.

The effective radiation dose for one HR-pQCT scan at the distal tibia or radius is 0,003 – 0,005 mSv, which is considered a low radiation dose[33]. In comparison, a regular chest X-ray yields a dose of 0,01 – 0,1 mSv and dental X-ray yields 0,005 mSv. The worldwide average effective radiation dose from natural background radiation is 2,4 mSv per year. The average effective radiation dose in Denmark is 3 mSv per year.

Estimated time: 30 min.

WHOLE BODY DUAL X–RAY ABSORPTIOMETRY (DXA) SCAN AND VERTEBRAL FRACTURE ASSESSMENT

Through DXA scans, we will obtain information regarding body composition, hip and spine bone mineral density, as well as examine any previous vertebral fractures using vertebral fracture assessment (VFA). VFA is a function of DXA scanners which allows for visualization of thoracic and lumbar vertebrae (usually T4-L4) in order to detect vertebral fractures[34]. Vertebral fractures can then be classified as either mild, moderate or severe using the method described by Genant et al[35].

The effective radiation dose is between 0,001 and 0,01 mSv for one whole body DXA scan, between 0,003 and 0,03 mSv for one hip and lumbar spine DXA scan and 0,003 mSv for one VFA scan. As such, the total effective radiation dose for the DXA scans is between 0,007 and 0,043 mSv.

The whole body DXA scan, hip and lumbar spine DXA scan and VFA scan will be performed consecutively.

Estimated time: 50 min.

MICROINDENTATION, BONE MATERIAL STRENGTH INDEX (BMSI) AND THE OSTEOPROBE®

Using the OsteoProbe®, we will by microindentation measure the bone material strength index (BMSi), an *in vivo* surrogate measure of the fracture resistance of the cortical bone in the tibia. The participant is placed in a supine position with the examined leg rotated slightly outward. After identifying the site of interest, located midway between the medial tibial plateau and the medial malleolus, disinfectant is applied to the skin of the tibia being examined. Finally, under local anesthesia and fully sterile conditions, a test probe is inserted through the skin and onto the midshaft of the tibia, and the fracture resistance of the bone tissue is measured. Without removing the probe from the skin, this process is repeated a minimum of eight (maximally 18) times to provide a sufficient number of measurements. By means of a synthetic polymer calibration phantom, the BMSi is calculated via specialized software provided by the manufacturer. After the procedure, a sterile bandage will be applied to the puncture site and all sharp and/or biohazardous materials will be disposed of in an appropriate manner.

Estimated time: 20 min.

HAND GRIP STRENGTH (HGS) AND LEG EXTENSION STRENGTH

Hand grip strength will be assessed using a digital hand dynamometer as a measure of peripheral muscle strength. Leg extensor strength will be examined using the peak torque measured by an isometric dynamometer mounted on a fixed chair. 3 attempts will be given for each method, and the maximum value recorded.

Estimated time: 10 min.

TIMED UP-AND-GO (TUG)

The TUG test is a functional test frequently used to assess balance in older individuals and is performed by measuring the time it takes for an individual to go from sitting to standing position, walk 3 meters, turn around and return to sitting position. A TUG score of >13,5 seconds is associated with an increased risk of falls.

Estimated time: 10 min.

STABILOMETRY

Postural control will be assessed by means of a force platform that registers Center of Pressure (CoP) as a measure of postural stability. Measurements are performed under a variety of conditions, including eyes open/closed and on different surfaces. From the CoP measurements, parameters such as CoP range and CoP velocity in both antero-posterior and medial-lateral directions will be calculated using specialized software.

Estimated time: 20 min.

NERVE CONDUCTION TEST

To fully examine all components of balance, nerve conduction will be tested using a handheld device called NC-stat DPN-Check in order to assess any peripheral neuropathy.

Estimated time: 10 min.

VISUAL ACUITY TEST

Visual acuity will be tested using an automated refractometer (KR-800S Auto-kerato-refractometer, Topcon Healthcare, The Netherlands).

Estimated time: 10 min.

Our laboratory has ample experience and know-how regarding the above-mentioned methods and their application in different patient groups.

QUESTIONNAIRES

Through questionnaires we will obtain information regarding previous falls and fractures, overall quality of life (Acro-QoL), joint-related symptoms (Western Ontario and McMaster universities osteoarthritis Index, WOMAC), fear of falling (Falls Efficacy Scale International, FES-I) and balance (Berg Balance Scale). Medicine lists will be collected from all participants.

BIOCHEMICAL BONE MARKERS AND BLOOD SAMPLES

To assess biochemical markers of bone metabolism, we will measure fasting serum levels of procollagen-1 N-terminal propeptide (P1NP, a marker of bone formation) and cross-linked C telopeptide of type I collagen (CTX, a marker of bone resorption) as well as sclerostin (an inhibitor of bone formation). To assess other hormones with influence on bone quality, we will measure levels of vitamin D and parathyroid hormone (PTH). In total, a volume of approximately 16mL of blood (4x 4mL vials) will be collected from each participant, both acromegalic and controls. For practical reasons, these blood samples are frozen after collection, stored in a research biobank, and analyzed in one session upon all participants' completion of the study procedures. Before analysis, Danish Tissue Utilization Register (Vævsanvendelsesregistret) will be consulted; should the participant be listed herein, the blood sample will not be analyzed. After analysis, any remaining material will be destroyed.

Two extra vials of blood (10mL total) will be extracted and stored in a biobank for future research, to enable analysis of any future bone markers or other biomarkers of interest for this project. An application will be submitted to the Danish Data Protection Agency in this regard. Blood samples will be frozen and stored in an encrypted fashion and in compliance with the Danish Data Protection Act for 15 years after completion of the study, or until their destruction is requested by the participant. Blood samples will be collected from both acromegalic subjects and healthy controls, in order to determine differences in biochemical markers between acromegalic subjects and control subjects. Participants will be asked to sign a separate consent form regarding storage of their biological material (blood sample) in a biobank for future research.

All blood samples are stored in a freezer at -80 degrees Celsius. The freezer is located on the premises of Aalborg University Hospital (Mølleparkvej 4, room 20.01.130) and is only accessible by authorized personnel. Sample vials are marked only with a participant number, and the key to link each participant number to a specific participant will be stored on a secure server only accessible by personnel affiliated with the study.

IV: DATA COLLECTION

Following the participants' informed consent, data regarding age, sex, medicine use and clinical information regarding acromegaly (duration, treatment, comorbidities, disease status and more) will be collected from the participants' Electronic Patient Journal (EPJ). For both groups, anthropometric data such as height and weight will be gathered in relation to the DXA scans. The aforementioned data will be entered in a REDCap database on a secure server owned by the North Denmark Region. The information gathered is both for use in the research project itself and for mandatory quality assurance and monitoring, as per the Committee Act, §3, subsection 3. By providing informed consent, the participants acknowledge that the investigators, sponsors and relevant control authorities may access their Electronic Patient Journal with this intent.

V: DATA ANALYSIS

A. STATISTICAL METHODS

SAMPLE SIZE CALCULATION

Based on another study using HR-pQCT on acromegalic subjects[27], using the HR-pQCT-derived parameter "trabecular density", mean values of 180 (SD: 40) and 150 (SD: 40) were found for controls and acromegalic patients, respectively. With a significance level of 5% and using 28 acromegalic subjects and 28 controls, the power of this study will be 80% to demonstrate a difference in trabecular density of 30 units.

STATISTICAL METHODS

Statistical testing will be performed using STATA® version 17 for Mac (StataCorp LLC, Texas, USA).

Data will be summarized using descriptive statistics such as frequency, percentage, mean and standard deviation. Statistical testing will be applied to compare acromegalic subjects to healthy controls, including t-tests, chi-squared tests and Mann-Whitney U tests, where applicable.

STATISTICAL SIGNIFICANCE

We use a statistical significance level of $\alpha < 5\%$.

VI: DATA SAFETY

The project will be registered with the North Denmark Region and all sensitive data will be stored on the secure servers of Aalborg University Hospital or in a REDCap database administrated by this institution and in accordance with the Data Protection Act (*databeskyttelsesloven*). All stored data will be anonymized after termination of the project. No data will be given over to a third party.

VII: STUDY LIMITATIONS

Due to the study's single-center nature, our findings may not be applicable to the entire population of acromegalic patients.

VIII: ETHICAL CONSIDERATIONS

A. INFORMED CONSENT

The initial inquiry about participation of patients with acromegaly will be made by one of two specialists in pituitary endocrinology. Potential participants will receive both oral and written information

(*"Deltagerinformation"* and *"Før du beslutter dig"*). Further information will later be given, by the principal investigator or another member of the research team, and will include details about the tests involved:

- This is a scientific project designed to investigate the optimal method for diagnosing acromegalic osteopathy, as well as testing balance and strength
- Information regarding the included examinations, including the risk of minimal to moderate pain associated with the BMSi measurement and the minimal exposure to ionizing radiation associated with HR-pQCT and DXA scans
- Information and separate consent regarding the creation of a biobank consisting of participants' blood samples, which will be stored for future analysis, but no more than 15 years after completion of the study. A renewed application will be submitted to the regional ethics committee in case the research team should wish to analyze the blood samples in the future. Prior to analysis, the Danish Tissue Utilization Register (Vævsanvendelsesregistret) will be consulted; should the participant be listed herein, the blood sample will not be analyzed.
- That withdrawal of consent, and as such withdrawal from participation, is possible at any time and with no given notice or explanation
- That collected data will be published in anonymized form with no possibility of being traced to the participant
- That collected data will be securely stored in accordance with the rules in place
- That at least 24 hours are given to deliberate before providing informed consent
- That potential participants have the right to bring a relative or other representative to the informational meeting

Potential participants are given at least 24 hours to deliberate, before any consent form is signed.

The project will be performed in compliance with the ICH GCP and the Helsinki Declaration for biomedical research involving human test subjects. The study will be subject to continuous internal quality monitoring, in accordance with GCP guidelines.

The project will be prospectively registered with www.clinicaltrials.gov.

B. RISKS AND SIDE EFFECTS

All methods used have been tested in previous studies with no side effects being reported. The risks associated with the above-mentioned procedures are limited as only low-dose radiation is used, and all measures are taken to ensure sterility when relevant.

No changes in the participants' regular medication will be made, and no new medication will be administered, except for local anaesthetics given during the BMSi measurement.

The main side effect that may be anticipated is pain in relation to the BMSi measurement, however relevant measures will be taken to counter this, and the pain is transient. Aside from this, there is a minimal amount of ionizing radiation from HR-pQCT and DXA scans, as mentioned above.

These are the anticipated side effects related to each examination included in the protocol:

- *Blood samples*: minimal risk of infection; disinfectant will be applied before skin puncture. Risk of small hematoma around the puncture site.
- *HR-pQCT scan*: low dose of ionizing radiation (0,003 – 0,005 mSv). Mild discomfort from sitting still during the scan.
- *Whole body, hip and spine DXA and VFA*: low dose of ionizing radiation (0,007 - 0,043 mSv).
    - Theoretically, the radiation doses associated with the HR-pQCT and DXA scans may increase the life-time risk of cancer from approximately 25% to less than 25,000003 %.
- *Microindentation (BMSi measurement)*: mild to moderate pain/discomfort related to administration of local anaesthesia. Mild to moderate pain when contact is made between the probe and the periosteum; local anaesthesia will be administered to alleviate this. Minimal risk of infection after skin puncture; the procedure will be performed under sterile conditions to minimize this risk.
- *TUG, stabilometry, hand grip strength, leg extensions, nerve conduction test, visual examination*: no side effects or risks expected.

As all the procedures are associated with a low risk of lasting side effects or complications, and the results of the study are expected to contribute to improved diagnosis and treatment of debilitating conditions in the future, the investigators consider the study procedures ethically responsible.

C. BENEFITS TO SUBJECTS

The participants will not benefit directly from participation in this study. However, as we aim to examine the optimal means of diagnosing acromegalic osteopathy, our findings may contribute to earlier detection of this condition. This is of potential future benefit for patients with acromegaly, as early diagnosis and treatment may prevent fractures, and thus save patients considerable morbidity. Identifying patients at risk for falling will enable treating physicians to implement relevant measures to prevent falls, and thus, potentially prevent fractures. Should HR-pQCT prove valuable in the diagnosis of acromegalic arthropathy, this may also be beneficial to patients in the future.

As such, the potential future benefits to patients with acromegaly are deemed sufficient justification for performing the study.

D. COSTS TO SUBJECTS

Apart from transportation to the hospital to participate, no other costs will be incurred to participants.

E. COMPENSATION TO SUBJECTS

No economical compensation will be paid to participants.

In case of unforeseen side effects or other adverse events related to the examinations, affected participants will be covered by the Danish Health Act and "*patienterstatningsordningen*".

F. PLAN FOR DISSEMINATION OF FINDINGS

The results of the project are planned to take the form of a research article, to be published in an internationally acknowledged scientific journal such as JCEM or EJE. All data will be published in anonymized form. Results will be published regardless of submission acceptance or denial, and both positive, negative and inconclusive results will be published.

G. ECONOMY

The initiative for this project has been made solely by the main supervisor, Jakob Dal and PhD student, Christian Rosendal.

Funding has been provided by research grants from Ipsen (€40.000, approx. 300.000 kr.) and Pfizer (500.000 kr.) as well as in-house research funds. The aforementioned pharmaceutical companies do not stand to gain financially from any specific result this project may yield. The grants are transferred to the endocrinology research department and cover the salary for the PhD student for three years, while expenses related to the practical aspects of the study (materials, analyses etc.) are covered by in-house funds . None of the investigators have any economical connection to, and are not employed by, the aforementioned pharmaceutical companies. The described funds sufficiently cover the completion of the study.

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