COREUMAPT_OP: LINKING WNT PATHWAY WITH BONE STRUCTURE AND FRACTURE RISK IN OSTEOPOROSIS

Osteoporosis (OP) is a metabolic skeletal disease characterized by low bone mass and microarchitecture deterioration [1]. Its clinical consequence is the occurrence of fragility fractures, which represents a public health problem and results in increased mortality, morbidity and disability and also in a major and growing economic burden on health-care systems [2, 3].The current available tools for identifying patients at high risk of fracture are not satisfactory as almost half of the fractures occur in individuals that were not classified as being at high risk by dual X-ray absorptiometry [4]. The clinical challenge that we are facing today is to accurately select the individuals with high risk of fracture and with indication for treatment in order to minimize individual and societal costs [1, 4]. Recently, some studies pointed to the importance of Wnt signaling, a crucial pathway for osteoblast differentiation and a master bone mass regulator. In fact, serum levels of dickkopf 1 (DKK1) and sclerostin (SOST) [5, 6], negative regulators of Wnt signaling, increase with age and are associated with bone mass loss. Our previous work showed that in fragility fracture patients osteoblast terminal differentiation is impaired, which is associated with bone mechanical fragility [7]. Moreover we also showed that in hip fragility fracture patients there was deterioration of trabecular stiffness, the mechanical parameter directly associated with tissue mineralization[8]. We hypothesize that serum Wnt regulators are associated to bone fragility and with fractures and can constitute new markers for osteoporosis treatment decision.

AIMS OF THE PROPOSED STUDY

AIM 1: We will conduct a pilot work to explore if disturbances in the Wnt pathway are associated to mineralization derangement and bone fragility.

AIM 2: Conduct a prospective 2 year cohort study with 1200 woman with more than 65 years old in order to determine the association between baseline serum levels of Wnt pathway proteins and changes in vertebral stiffness assessed by computed tomography based structural rigidity analysis (CTRA), which is a validated tool to assess the load bearing capacity of bones based on the principles of structural mechanics, and with incident vertebral fractures assessed by spine radiographs.

DETAILED DESCRIPTION

1- Hip fragility fracture patients- pilot study

In this cross-sectional project we will study a cohort of 150 hip fragility fracture patients and compare it with osteoarthritis patients submitted to total hip arthroplasty in the Orthopedic Department of Hospital de Santa Maria, in Lisbon, for a period of 12 months, with the following specific objectives:

Our primary aim will be to assess the association between bone gene expression, protein expression and serum levels of Wnt pathway regulators (DKK1, DKK2, SOST, WIF-1 and sFRP) and bone structure at several hierarchical levels- bone mineralization density distribution, microstructure parameters assessed by micro-CT analysis and bone stiffness assessed by ex-vivo bone mechanical compressive tests, among hip fragility fracture patients.

Our secondary aim is to identify differences between bone gene expression, protein expression and serum levels of Wnt pathway regulators among fragility fracture and osteoarthritis patients.

Population

Hip fragility fracture and osteoarthritis patients will be post-menopausal women or men over 50 years and able to give clinical information and written informed consent. Patients under anti-osteoporotic drugs, bone metastasis, primary tumors and osteomyelitis will be excluded.

After surgery, femoral epiphysis will be collected and two bone cylinders will be drilled with a diamond saw from the femoral epiphysis. From one cylinder, we will obtain three small bone specimens with trabecular and cortical aspects and prepared for μCT imaging, qBEI and immunohistochemistry. With the other cylinder we will perform ex-vivo- mechanical tests. A small sample of trabecular bone will also be extracted to perform RNA and studies.

Outcome definition and assessment

Our outcomes will be bone structure at several hierarchical levels: bone mineralization density distribution (BMDD) parameters of cortical and trabecular bone samples reflecting bone matrix mineralization assessed by qBEI; micro-structural parameters such as cortical and trabecular bone volume fraction (BV/TV), trabecular structure model index (SMI), trabecular number (Tb.N), trabecular thickness (Tb.Th) evaluated by μ-CT; and ex-vivo mechanical trabecular compression tests (strength and stiffness).

Covariates of interest definition and assessment

Our covariates of interest will be Wnt pathway proteins (DKK1, DKK2, SOST, WIF-1 and sFRP) and we will determine levels of bone gene expression (accessed by reverse Transcriptase- Polymerase Chain Reaction (RT-PCR)), protein expression (accessed by immunohistochemistry) and serum levels (accessed by ELISA).

Covariates definition and assessment

A clinical questionnaire [9] will be applied in order to collect clinical risk factors (CRF)s – age, gender, body mass index (BMI), prior fragility fracture (other than the reason for surgery), parental history of hip fracture, long term use of oral glucocorticoids (≥ 3 months), rheumatoid arthritis, current smoking, alcohol intake (≥3 units/day) and other secondary causes of osteoporosis. These variables will be assessed as in FRAX United Kingdom study [10]. Ten year probability of fracture will be calculated with CRFs and DXA information using the FRAX tool available online [11].

Fasting blood samples will be collected to assess serum calcium and phosphorus, alkaline phosphatase, bone specific alkaline phosphatase, procollagen type 1 N-terminal propeptide, collagen type 1 beta C-terminal telopeptide, tartrate-resistant acid phosphatase), cathepsin K, PTH, total osteocalcin, undercarboxylated osteocalcin (ucOCL), vitamin D and IGF-1, using ELISA commercial kits.

Femoral neck aBMD of the contralateral hip will be measured by DXA scan at the Rheumatology and Bone Metabolic Diseases Department of Hospital de Santa Maria.

Bone expression of Wnt signaling members (SOST, DKK1, DKK2, LRP5, LRP6, LRP4, Wnt3a, Wnt10b, SFRP, WIF-1), osteoblast genes (RUNX2, OSX, ALP, COL1A1, RANKL, OPG, M-CSF, ESR-alpha, ESR-beta, PTHR1 and VDR), osteoclast genes (RANK, cathepsin K, TRAP, integrin beta3, ATP6V0D2) will be accessed by reverse Transcriptase-Polymerase Chain Reaction (RT-PCR).

2 - Cohort of postmenopausal woman with more than 65 years old from CooReumaPT

We will follow for 2 years a Cohort of 768 Portuguese woman over 65 years old from the project EpiReumaPt. Our primary aim will be to assess the association between

markers of Wnt signaling pathway and computed tomography based structural rigidity analysis (CTRA). Our secondary aim will be to identify new serum markers of a fracture event in women over 65 years old.

Population

EpiReumaPt is a national Portuguese epidemiological study with the primary aim of studying rheumatic diseases prevalence. This cross-sectional study started on September 2011 and it is going to use a random sample of 10 000 Portuguese non-institutionalized adults [12]. We will invite to participate in our study women over 65 years-old and willing to participate and with the ability to give informed consent subjects. We will exclude patients under anti-osteoporotic drugs in the last year before inclusion; other bone metabolic diseases (osteomalacia, Paget's disease, osteogenesis imperfect); bone metastasis; primary bone tumors and osteomyelitis

Patients will be assessed at baseline and every 12 months by a trained rheumatologist. EpiReumaPt's diagnostic van fully equipped will be available to perform the following exams: X-ray and blood sample collection. Axial DXA and vertebral CT scan will be performed at baseline and 24 months at pre-specified centers in Braga, Porto, Viseu, Coimbra, Lisboa, Évora, Faro, Madeira and Azores.

Outcome Definition and assessment

Our primary outcome in this cohort study will be rigidity measures evaluated by Computed tomography based structural rigidity analysis (CTRA) at the end of the follow-up and rigidity changes between baseline and the end of the follow-up. We will use the EA, EI and GJ rigidities information.

CTRA that has been introduced by the Center for Advanced Orthopaedic Studies (CAOS), Beth Israel Deaconess Medical Center and Harvard Medical School in Boston, Massachusetts, USA is a validated tool to assess the load bearing capacity of bones based on the principles of structural mechanics. The accuracy of this approach, has been validated in a series of *ex vivo* [13] and *in vivo* [14] studies. This tool is now going to be validated in osteoporosis with this cohort in a study leathered by a rheumatologist from my research unit Helena Canhão, MD PhD. Patients will perform a vertebral CT scan in order to obtain the rigidity measures at baseline and 24 months.

Our secondary outcome will be a new fragility fracture which is considered any low-trauma fracture that occurs during the follow-up period confirmed clinically and radiologically. Fractures of the skull, fingers and toes as well as any traumatic fracture will be excluded. We will also perform a questionnaire of incident fractures, falls, hospitalization, living status and functional status. Self-reported medical history and incident fractures will be verified by a physician using medical records and imaging exams. The subjects will also perform spine X-ray at baseline, 12 and 24 months and we will determine incident vertebral fracture as described by Wustrack and colleagues [15].

In this nested case-control analysis, controls will be defined by the complete absence of fragility fractures, either in previous history or during the follow-up period.

Covariates of interest definition and assessment

Covariates of interest will be serum DKK1, DKK2, SOST, WIF-1 and sFRP assessed by ELISA.

Covariates assessment

Demographic, clinical data and biochemical assessment will be performed and the same variables will be obtained as in hip fragility fracture patients.

Patients will also perform density scans of the lumbar spine and hip acquired by dual-energy X-ray absorptiometry at baseline and 24 months.

Analysis Plan

Analysis will be performed with STATA. Continuous variables will be reported as mean and standard deviation (or in case of non-normal distribution as median and interquartile range). Categorical variables will be displayed as frequency or proportions.

In the hip fragility fracture pilot study, serum, protein and bone expression of Wnt pathway regulators will be compared across hip fragility patients and osteoarthritis using general linear model (GLM) analysis adjusted for differences in demographic, clinical and biochemical characteristics identified as possible confounders.

In hip fragility fracture patients we will explore the association between each of the Wnt pathway regulators and the outcomes: bone mineral matrix (BMDD parameters), microstructure (BV, SMI) and with ex- vivo bone mechanical properties (stiffness and strength) using generalized linear models adjusted to clinical characteristics and aBMD. Multivariate linear regression models will be performed for each outcome, having Wnt signaling regulators as the covariate of interest. Clinical characteristics, aBMD, bone gene expression and hormones will also be included in the model. The results of these analyses will be presented as a coefficient (β) with 95% confidence interval (CI). The candidate covariates will be first analyzed by univariate regression.

In the cohort study, the association of baseline levels of Wnt signaling with vertebral rigidity (EA, EI, and GJ rigidities) changes and with fragility fractures (new fragility fracture- 0/1) in women over 65 years old will be explored through general linear models adjusted for clinical characteristics. To further assess the independence of the association we will use multivariate logistic and linear (according to the outcome) regression analysis considering Wnt signaling regulators as predictors. We also include in this model, vertebral aBMD, clinical characteristics and hormones. The candidate covariates will be first analyzed by univariate regression. Covariates will enter in the multivariate models if their p-value<0.25 in univariate analysis or if they will considered clinically relevant in this setting. The selection of covariates will be stepwise by backward selection, according to the level of significance<0.05.

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