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Serum periostin is associated with prevalent knee osteoarthritis and disease incidence/progression in women: the OFELY study

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SUMMARY

Objective: Our aim was to investigate the relationships between serum periostin (POSTN) and both prevalence and incidence/progression of knee osteoarthritis (OA) in women.
Methods: We investigated 594 women (62.7 ± 11.2 yr) from the OFELY cohort. Knee radiographs were scored according to the Kellgren & Lawrence (KL) grading system at baseline and 4 years later. Spine, hip and hand OA were assessed at baseline. Prevalent knee OA was defined by a KL score higher or equal in 2. Progression of KL was defined as an increase of the KL score ≥1 during the 4 years follow-up. Serum POSTN was measured at baseline by ELISA.
Results: By non-parametric tests, POSTN was significantly lower in 83 women with a KL score ≥2 at baseline, compared to those with a KL score <2 ($n = 511$; 1101 ± 300 vs 1181 ± 294 ng/ml, $P = 0.002$) after adjustment for age, body mass index (BMI), treatments and diseases, prevalent hand OA and prevalent lumbar spine OA. By logistic regression analyses, the odds-ratio of knee OA incidence/progression was significantly reduced by 21% ($P = 0.043$) for each quartile increase in serum POSTN at baseline, after adjustment for age, BMI, prevalent knee OA, prevalent hand OA and prevalent lumbar spine OA.
Conclusions: We show for the first time that serum POSTN is associated with prevalence and the risk of development/progression of knee OA in women.

Introduction

Osteoarthritis (OA) is the most frequent chronic musculoskeletal disease affecting approximately 40% of adults aged 70 years and over[1]. It is considered as a slowly progressive disease deteriorating all tissues of the affected joint and appearing as a degradation of cartilage, the hallmark of OA, but also a mild-to-moderate synovial inflammation and an impairment of subchondral bone structure[2–5].

One of the most important metabolic pathways regulating bone and cartilage homeostasis in adults is the Wnt-β-catenin signalling pathway[6–8]. Several lines of evidence showed that Wnt-β-catenin is involved in the OA process although animal models and data on the association of circulating inhibitors of this pathway in human OA have generated conflicting results[9]. We have speculated that the measurement of stimulators of the Wnt-β-signaling pathway could bring valuable information concerning the implication of Wnt-β-catenin signalling in OA. Among them, periostin (POSTN) appears as a potential candidate. Indeed, two studies in rats and in human subjects[10–12] found that the POSTN gene is significantly upregulated in the subchondral bone during OA disease. POSTN is a matricellular protein expressed predominantly in the periosteum which covers a large majority of bones[13,14] but also in the cartilage matrix by the chondrocytes[15]. It is an important mediator of the effects of mechanical factors and PTH on BMD, bone strength and fracture pathogenesis[16]. Because POSTN is a secreted soluble factor, a specific immunoassay has been recently developed to measure its concentration in serum and plasma. Serum POSTN has been investigated mainly in the context of oncogenesis[17]. To our best knowledge, there is no published study that has investigated the relationships between serum POSTN and OA. Consequently, the main objective of this study was to investigate the relationship

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between the levels of serum POSTN measured by a new sensitive assay and prevalence and incidence/progression of knee OA in women. We also studied the relationship between POSTN and CTX-II, one of the most efficient prognostic marker of knee OA[18].

Materials and methods

Patients

The study group included French women belonging to a population-based cohort. These women were participants in a prospective investigation of the determinants of bone loss, the Os des FEmmes de LYon (OFELY) study. This cohort has previously been described in details elsewhere[19]. In the present analysis, we studied a group of 594 women, who had both serum POSTN and urinary CTX-II measurements, and an evaluation of OA disease by radiography for spine disc degeneration and knee OA, by clinical examination for the hand OA and by a questionnaire for the hip OA. These evaluations have been performed at the same visit, 8 years after the recruitment of the cohort (ninth follow-up visit). After baseline OA assessment, women were followed prospectively for 4 years.

Assessment of knee OA

Radiographs of both knees were obtained in all women. Radiologic evaluation of the knees consisted of bilateral postero-anterior weight-bearing knee radiographs with fixed flexion using the SynaFlex X-ray Positioning Frame (Synarc, San Francisco, CA), as previously described[20]. Radiographs were obtained in a single radiography unit by the same staff of 2 technicians using a previously described standardized protocol[21]. The severity of OA was performed and graded according to the Kellgren & Lawrence (KL) at baseline and 4 years later[22]. Prevalent knee OA was defined by a KL score higher or equal in 2 at baseline. Incident OA was defined by a KL score higher or equal in 2 at year 4 and a KL score <2 at baseline. Progressors were women with a KL score at year 4 strictly higher than the KL score at baseline. All knee radiographs were scored by a single trained rheumatologist (ES-R). Measurements were made paired but not blinded to sequence, which has been shown not to modify the sensitivity to change[23].

Assessment of lumbar spine OA

At baseline, lateral radiographs of the spine were available and interpretable in 421 women (age 50 years and older at the recruitment of the cohort). Spine films were graded with a standard atlas to document the severity of disc space narrowing (DSN) and osteophyte (OPH) formation, using the grading system described by Lane *et al.*[24] with a 4-point scale: normal 0, mild 1, moderate 2, and severe 3. Then, a grade was defined as 0 if both scores were normal, 1 if there was mild OPH or DSN, or 2 if there was moderate or severe OPH or DSN. Lumbar OA was assessed at 4 levels from L1–L2 to L4–L5. Lumbar OA was defined with the highest score of OPH and DSN and with the highest grade among the four levels. In our study, women were considered as having a spine OA when the final grade at baseline was higher or equal in 1. All spine radiographs were scored by a single trained rheumatologist (ES-R)[25].

Assessment of clinical hand OA

Clinical hand OA was assessed by a trained rheumatologist who systematically evaluated each hand in all subjects for Heberden's nodes of the distal interphalangeal joints, Bouchard's nodes of the proximal interphalangeal joints, and swelling of the first carpometacarpal joint. The presence of hand OA was defined according to the American College of Rheumatology criteria[26].

Assessment of hip OA

Hip OA was self-reported. Women from the OFELY cohort answered to the following question: “Has a doctor ever told you that you had hip osteoarthritis?” Subjects who answered “yes” to this question were considered as having a hip OA only if the general practitioner had detected the hip OA on radiographs of the affected hip.

Biochemistry

Blood samples were collected between 8:00 and 9:30 a.m. after an overnight fast at the ninth annual follow-up visit (baseline visit of the current analysis). Serum samples were stored frozen at −80°C until assayed.

POSTN assay

Serum POSTN was measured by a novel sandwich ELISA assay (USCN, China) using a polyclonal antibody raised against the fasciclin-1 like domain (amino acids 97–230) common to all isoforms. Briefly, microtiter plates were pre-coated with the polyclonal antibody as capture antibody. Serum samples or standards (recombinant fasciclin-1 like domain of POSTN) are incubated with the same polyclonal antibody but biotinylated for 2 h at 37°C. After washing, avidin conjugated to horseradish peroxidase (HRP) was added to each microplate well and incubated for 1 h at 37°C. After a washing step, the substrate TMB is added to the well which reacts with HRP and color is formed. After incubation, the reaction is stopped with the addition of a sulphuric acid solution and the plate is read at 450 nm.

In our laboratory, the intra- and interassay coefficients of variation were lower than 10% and 15% respectively. The linearity of the assay was assayed by testing four human serum samples with appropriate concentration of human POSTN and their serial dilution. The mean recovery was 83%. The detection limit defined as the concentration of POSTN corresponding to the OD value of standard 0 + 3 standard deviations was 29 ng/ml. The recovery of spiked standards which was tested by adding two different concentrations of standard into four different serum human samples presenting with various levels of endogenous POSTN ranged from 91 to 103% (see additional Figure 1 for antibody specificity).

Measurement of urinary CTX-II

For each woman, fasting first-void morning urine samples were collected and stored at −80°C until measurement of urinary CTX-II, a specific proteolytic fragment of type II collagen (CartiLaps; *IDS*). Intra and interassay coefficients of variation were less than 8% and 10%, respectively[27].

Statistical analysis

All data were reported as mean ± SD unless otherwise specified. Chi-square Wilcoxon tests and logistic regression were used to compare characteristics between women with prevalent OA or not before and after adjusting for confounding variables (age, treatments and diseases, prevalent hand OA and prevalent lumbar spine OA) which have been found significantly different between groups in the univariable analysis. Concerning the variable “treatments and diseases”, sensitivity analysis was performed to identify the effect of eliminating each of them individually upon the results. Correlation analysis between serum POSTN and age or the variable