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

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Introduction

Osteoarthritis (OA) is the most frequent chronic musculoskeletal disease affecting approximately 40% of adults aged 70 years and over. 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.

One of the most important metabolic pathways regulating bone and cartilage homeostasis in adults is the Wnt-β-catenin signalling pathway. 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. We have speculated that the measurement of stimulators of the Wnt-β-signalling 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 fracture pathogenesis 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 but also in the cartilage matrix by the chondrocytes. It is an important mediator of the effects of mechanical factors and PTH on BMD, bone strength and fracture pathogenesis. Because POSTN is a secreted soluble factor, a specific immunoassay has been recently developed to measure its concentration in serum and plasma.

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.

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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.

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. 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 posteroanterior weight-bearing knee radiographs with fixed flexion using the SynaFlex X-ray Positioning Frame (Synarc, San Francisco, CA), as previously described. Radiographs were obtained in a single radiography unit by the same staff of 2 technicians using a previously described standardized protocol. The severity of OA was performed and graded according to the Kellgren & Lawrence (KL) at baseline and 4 years later. 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.

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. 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 to 1. All spine radiographs were scored by a single trained rheumatologist (ES-R).

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.

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.

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
“treatments and diseases” was assessed by the non parametric Spearman test because the variables were not normally distributed.

We estimated the association between serum POSTN or urine CTX-II, as continuous or categorized (per quartile) variables, and KL progression using the “logistic regression” function in the STATA software. Serum POSTN or urinary CTX-II values were tested as independent variables and age, BMI, prevalent knee OA, prevalent hand OA and prevalent lumbar spine OA as confounding variables. As a proportion of participants had missing information for one of the confounders [lumbar spine OA evaluated for 421 women (71%)] we created a category for patients with missing values in order to avoid ignoring these individuals in the multivariable analysis.

To distinguish progressors and non progressors, we developed receiver operating characteristic (ROC) curve by the method of cross validation. With this method, the entire dataset was used both for the development and the validation models. The data were randomly divided into 10 mutually exclusive subsets of equal size and ratio between progressors and non progressors. The model was refitted 10 times, omitting in turn one of the ten subsets. By this way, the predicted values were calculated for the entire dataset. In a final step, the cross-validation estimate of the area under the ROC curve was calculated using these fitted values in a logistic regression model including age and POSTN.

All statistical analyses were performed using Stata 12 (StataCorp LP, College Station, Texas, USA).

Results

Baseline characteristics of women (Table I) and associations of serum POSTN with other variables

Table I shows the baseline characteristics of the 594 women included in this analysis who had data available for baseline POSTN and CTX-II. Among them, 477 women (80.3%) were post menopausal. POSTN and age were significantly associated only in premenopausal women (r = 0.31, P < 0.001). Serum POSTN was not significantly associated with body mass index (BMI) (P = 0.99). We found also no significant association (P = 0.27) between POSTN levels and the variable grouping “treatments and diseases” including treatments known to affect bone metabolism [hormone replacement therapy (HRT, n = 138), bisphosphonates (n = 33), Selective Estrogen Receptor Modulators (SERMs, n = 8), corticosteroids (n = 1)] and diseases known to affect POSTN metabolism [breast cancer (n = 13) and hyperparathyroidism (n = 1)].

Table I

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total population (n = 594)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>62.7 ± 11.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>62.1 ± 10.2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>159.3 ± 6.1</td>
</tr>
<tr>
<td>BMI</td>
<td>24.5 ± 3.9</td>
</tr>
<tr>
<td>KL – 0 (n, %)</td>
<td>397 (66.8%)</td>
</tr>
<tr>
<td>KL – 1 (n, %)</td>
<td>114 (19.2%)</td>
</tr>
<tr>
<td>KL – 2 (n, %)</td>
<td>59 (9.9%)</td>
</tr>
<tr>
<td>KL – 3 (n, %)</td>
<td>16 (2.7%)</td>
</tr>
<tr>
<td>KL – 4 (n, %)</td>
<td>8 (1.3%)</td>
</tr>
<tr>
<td>Incident knee OA (n, %)</td>
<td>62 (10.4%)</td>
</tr>
<tr>
<td>Progressors (KL score) (n, %)</td>
<td>82 (13.8%)</td>
</tr>
<tr>
<td>Prevalent lumbar spine OA (n, %)</td>
<td>351 (59.1%)</td>
</tr>
<tr>
<td>Prevalent hand OA (n, %)</td>
<td>188 (31.6%)</td>
</tr>
<tr>
<td>Prevalent hip OA (n, %)</td>
<td>57 (9.6%)</td>
</tr>
<tr>
<td>s-POSTN ng/ml</td>
<td>11700 ± 295.6</td>
</tr>
<tr>
<td>u-CTX-II ng/mmol creat.</td>
<td>1844 ± 114.9</td>
</tr>
</tbody>
</table>

Serum POSTN levels and prevalent knee OA (Table II)

Women with prevalent knee OA were older than women without OA (P < 0.0001) had a higher BMI (P < 0.0001) and most of them were postmenopausal (98%). After adjustment for age, BMI, treatments and diseases, prevalent hand OA and prevalent lumbar spine OA serum POSTN levels were moderately but significantly lower (−7%) in the group of women suffering from OA than in the 511 women without prevalent OA (P = 0.002). Urinary CTX-II levels were significantly increased in the OA group (+61%, P = 0.001 after adjusting for the same confounders).

When the analysis was focused on postmenopausal women, who represented the majority of the population, the serum POSTN levels were lower in women with prevalent OA than in women without OA (P = 0.002, after adjusting for the same confounders).

When the cut-off for prevalent OA was a KL score in 1, serum POSTN levels were always slightly but significantly lower (−4.2%) among the 197 women with prevalent OA compared to the women without OA (P = 0.013, after adjusting for the same confounders). Moreover, urinary CTX-II levels were also significantly increased in this OA group (+43.2%, P = 0.008, after adjusting for the same confounders).

Sensitivity analyses showed that serum POSTN was still significantly lower in women having prevalent OA compared to healthy women after eliminating treatments or diseases individually: bisphosphonates (P = 0.003), SERMs (P = 0.002), corticosteroids (P = 0.002), breast cancer (P = 0.002), and hyperparathyroidism (P = 0.002). After eliminating women taking HRT (n = 138), the significance was attenuated (P = 0.06). Similar results were obtained when the sensitivity analysis was restricted to postmenopausal women (P < 0.01 for all except for HRT: P = 0.08).

Serum POSTN levels and incidence/progression of knee OA (Table III and Figs. 1 and 2)

The women who had a progression of knee KL at 4 years (n = 82) were more likely to be older (P < 0.0001) had a higher BMI (P = 0.02) and most were postmenopausal (P < 0.0001). The progressors had more often prevalent lumbar spine OA (P = 0.003), prevalent knee OA (P = 0.004) and prevalent hand OA (P = 0.005) than non progressors. When considered as a continuous variable, serum POSTN levels in the group of progressors were not significantly decreased compared to non-progressors after adjustments for age, BMI, prevalent knee OA, prevalent hand OA and prevalent lumbar spine OA. Urinary CTX-II levels were significantly higher (+33%, P = 0.042) in knee OA women after the same adjustments. In the group of KL progressors, 76% (62 women) had a KL score at baseline strictly lower than 2, meaning that this group represents predominantly incident OA rather than progression of the disease in the strict sense (Table III). When considered alone, these 62 women with incident knee OA had lower serum POSTN levels that the other subjects although the difference did not reach statistical significance after adjustment for age (P = 0.089).

When baseline POSTN levels were categorized in quartiles, there was a significant association between increased POSTN levels and decreased incidence/progression [odds-ratio (95%CI): 0.79 (0.64–0.99, P = 0.043) for each quartile increase of POSTN after adjustment for age, BMI, prevalent knee OA, prevalent hand OA and prevalent lumbar spine OA (Fig. 1)].

We found a similar association when we excluded premenopausal women [odds-ratio (95%CI): 0.79 (0.64–0.99, P = 0.04)]. Moreover, we assessed the performance of the multivariable logistic regression model including age and POSTN levels to discriminate between progressors and non progressors. The ROC curve obtained by cross-validation showed an area under the curve...
of 0.66 (95% CI = 0.61–0.72) reflecting a modest predictive power ($P = 0.028$).

### Discussion

We observed that circulating levels of POSTN are lower in women with prevalent knee OA and in OA progressors than in controls. We also found a positive association between POSTN and age in the group of pre-menopausal women only. Before the menopause, the periostal apposition compensated partially the endocortical bone loss. With advancing age, the cortices become thinner whereas the overall bone mass is unchanged because the same amount of bone is distributed around a larger perimeter and thus bone diameter increases. This increase of bone diameter is associated with an increase of the periostea surface. Consequently, we can hypothesize that the quantity of POSTN synthesized may also increase with age in premenopausal women.

POSTN is a secreted protein synthesized by osteoblasts and chondrocytes and in this way it could participate to the crosstalk between subchondral bone and cartilage facilitated by alteration of the tidemark in OA disease. Moreover, recent independent studies in rats and in human showed that the POSTN gene is up-regulated in the subchondral bone from OA cartilage. Furthermore, we found that POSTN protein concentrations in serum were significantly decreased in women with prevalent OA and in progressors in the next four years. The reasons for the discrepancy between gene expression at the joint tissue level and serum concentration of POSTN are unclear but could be related to the following factors.

The clearance of POSTN from the articular tissues to the serum is unknown. It could undergo a degradation process leading to an underestimation of its serum concentration. Similarly the structure of the circulating forms of POSTN — particularly the level of gamma-carboxylation that could interfere with the antibodies recognition—is also not determined. It was recently showed that the tertiary structure of the FAS-I domains could be modified by the binding of a ligand or post-translational modification suggesting that the epitope recognized by antibodies of the POSTN assay could be hidden in some situations. Finally, although POSTN can exist in different isoforms, the POSTN immunoassay has been developed to recognize all of them.

Secondly, Ma et al. showed recently that the Wnt-β-catenin signaling pathway has an anti-catabolic role in human chondrocytes. The decreased serum levels of POSTN could lead to an inhibition or an absence of stimulation of the Wnt-β-catenin pathway, by the direct action of POSTN on this pathway or indirectly via the inhibition of POSTN on the SOST expression. Consequently, the Wnt-β-catenin pathway may be not enough stimulated to exert its anti-catabolic role. This hypothesis is supported by the significant and concomitant increase of collagen type II degradation — assessed with CTX-II measurement—in the group of women suffering from OA at baseline suggesting that the cartilage degradation increases when levels of serum POSTN decrease.

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**Table II**

Baseline characteristics of women with and without prevalent OA (BMI — body mass index). Baseline serum levels of POSTN and CTX-II in both groups (mean ± SD, s — serum, u — urine). Prevalent knee OA was defined by a KL score higher or equal to 2. Treatments known to affect bone metabolism [hormone replacement therapy (n = 138), bisphosphonates (n = 33), SERMs (n = 8) and corticosteroids (n = 1)] and diseases known to affect POSTN metabolism [breast cancer (n = 13) and hyperparathyroidism (n = 1)].

<table>
<thead>
<tr>
<th>Variables</th>
<th>No prevalent knee OA n = 511</th>
<th>Prevalent knee OA n = 82 (KL ≥ 2)</th>
<th>$P$</th>
<th>$P^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>60.4 ± 10</td>
<td>70.1 ± 9</td>
<td>&lt;0.0001</td>
<td>—</td>
</tr>
<tr>
<td>Menopausal women (n, %)</td>
<td>399 (78%)</td>
<td>81 (98%)</td>
<td>0.001</td>
<td>—</td>
</tr>
<tr>
<td>BMI (kg. m$^{-2}$)</td>
<td>24.2 ± 3.8</td>
<td>25.6 ± 4.4</td>
<td>&lt;0.0001</td>
<td>—</td>
</tr>
<tr>
<td>Prevalent lumbar spine (n, %) excluding missing values</td>
<td>280 (54.8%)</td>
<td>71 (85.5%)</td>
<td>0.021</td>
<td>—</td>
</tr>
<tr>
<td>Missing values (n, % of the total)</td>
<td>167 (32.7%)</td>
<td>6 (7.2%)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Prevalent hip OA (n, %)</td>
<td>45 (8.8%)</td>
<td>12 (14.3%)</td>
<td>0.105</td>
<td>—</td>
</tr>
<tr>
<td>Prevalent hand OA (n, %)</td>
<td>141 (27.6%)</td>
<td>47 (56.6%)</td>
<td>&lt;0.0001</td>
<td>—</td>
</tr>
<tr>
<td>Treatments and diseases (n, %)</td>
<td>188 (36.8%)</td>
<td>16 (19.3%)</td>
<td>0.002</td>
<td>—</td>
</tr>
<tr>
<td>s-POSTN (ng/ml)</td>
<td>1181 ± 294</td>
<td>1101 ± 300</td>
<td>0.023</td>
<td>0.002</td>
</tr>
<tr>
<td>u-CTX-II (ng/mmol creat.)</td>
<td>173 ± 100</td>
<td>278 ± 154</td>
<td>&lt;0.0001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* adjusted for age, BMI, treatments and diseases, prevalent hand OA and prevalent lumbar spine OA.

**Table III**

Baseline characteristics of progressors and non-progressors (mean ± SD, BMI — body mass index). Progressors are women with a KL score at follow up 9. POSTN and CTX-II levels at baseline in the progressor and non-progressor groups (mean ± SD, s — serum, u — urine). Treatments known to affect bone metabolism [hormone replacement therapy (n = 138), bisphosphonates (n = 33), SERMs (n = 8) and corticosteroids (n = 1)] and diseases known to affect POSTN metabolism [breast cancer (n = 13) and hyperparathyroidism (n = 1)].

<table>
<thead>
<tr>
<th>Variables</th>
<th>NON progressors n = 512</th>
<th>Progressors n = 82</th>
<th>$P$</th>
<th>$P^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>61 ± 10</td>
<td>67 ± 8</td>
<td>&lt;0.0001</td>
<td>—</td>
</tr>
<tr>
<td>Menopausal women (n, %)</td>
<td>397 (78%)</td>
<td>80 (98%)</td>
<td>&lt;0.0001</td>
<td>—</td>
</tr>
<tr>
<td>BMI (kg. m$^{-2}$)</td>
<td>24.3 ± 3.9</td>
<td>25.4 ± 3.9</td>
<td>0.02</td>
<td>—</td>
</tr>
<tr>
<td>Prevalent lumbar spine OA (n, %)</td>
<td>279 (81%)</td>
<td>72 (55%)</td>
<td>0.003</td>
<td>—</td>
</tr>
<tr>
<td>excluding missing values</td>
<td></td>
<td></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Missing values (n, % of the total)</td>
<td>176 (34.4%)</td>
<td>6 (7.3%)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Prevalent knee OA (n, %)</td>
<td>63 (12%)</td>
<td>20 (24%)</td>
<td>0.004</td>
<td>—</td>
</tr>
<tr>
<td>Prevalent hip OA (n, %)</td>
<td>48 (9%)</td>
<td>9 (11%)</td>
<td>0.39</td>
<td>—</td>
</tr>
<tr>
<td>Prevalent hand OA (n, %)</td>
<td>151 (30%)</td>
<td>37 (43%)</td>
<td>0.005</td>
<td>—</td>
</tr>
<tr>
<td>Treatments and diseases (n, %)</td>
<td>182 (35.5%)</td>
<td>22 (26.8%)</td>
<td>0.12</td>
<td>—</td>
</tr>
<tr>
<td>s-POSTN (ng/ml)</td>
<td>1179 ± 295</td>
<td>1112 ± 293</td>
<td>0.059</td>
<td>0.073</td>
</tr>
<tr>
<td>u-CTX-II (ng/mmol creat.)</td>
<td>179 ± 109</td>
<td>238 ± 131</td>
<td>&lt;0.0001</td>
<td>0.042</td>
</tr>
</tbody>
</table>

* adjusted for age, BMI, prevalent knee OA, prevalent hand OA and prevalent lumbar spine OA.
The POSTN synthesis may also be inhibited by the abnormal mechanical force due to the cartilage and bone alterations\(^{11}\).

Finally, it is also possible that the difference between gene expression and protein levels could be due to alterations in transcriptional, translation or post-translational processes.

Longitudinal studies investigating for a long period of time -4 years - the value of molecular markers in predicting incidence/progression of joint damage are scarce\(^{18}\). Here, we found that for each quartile increase in the levels of serum POSTN at baseline, the odds-ratio of incidence/progression was significantly increased by 21% after adjustment for age, BMI, prevalent knee OA, prevalent hand OA and prevalent lumbar spine OA. Simultaneously, urinary CTX-II levels were significantly increased among the progressors compared to the non-progressors (\( P = 0.042 \) same adjustments).

The differences in serum POSTN between progressors and non-progressors are modest. However, we recently showed that even such a small change of POSTN serum levels, comparable to those highlighted in this study, can have a significant impact on fracture risk\(^{34}\). Moreover, the relationships between serum POSTN and the risk of disease incidence/progression appear to be in an opposite direction in osteoporosis and OA. Indeed, higher levels of serum POSTN were associated with an increased risk of fracture\(^{34}\) but a lower risk of radiological incidence/progression in OA. It means that a small difference in POSTN level, as observed in our study for prevalent OA and progressors, may have a sizeable effect on the metabolism of structural proteins, because we observed a parallel increase of CTX-II levels. Moreover, POSTN is involved in regulating the Wnt signaling pathway which is an important regulator of both bone and joint metabolism\(^{16,32}\). We also showed recently that POSTN is not only expressed in osteoblasts but also in osteoclasts from mouse long bones differentiated in vitro\(^{35}\).

The prevalence of OA increases dramatically in women around the age of 50 coinciding with the onset of menopause and suggesting a link between OA and ovarian function with a protective effect of estrogen. However when the analysis was restricted to postmenopausal women who accounted for 80% of the population, the relationship between POSTN and OA was not modified. When each bone disease or treatment was individually excluded, the results were also virtually unaltered, except for HRT probably for lack of statistical power.

Additional studies are needed to better understand the complex role of POSTN in the maintenance of bone strength considering its preferential localisation in the periostium which is highly sensitive to mechanical stimuli. Also, this protein being expressed both in osteoblasts and osteoclasts, its respective roles in cartilage and bone remain elusive. Nevertheless, the large overlap in serum POSTN levels between progressors and non-progressors and the modest AUC (\( < 0.7 \)) of the ROC curve suggests that its clinical value as a prognostic test is limited in the present stage of the assay technique.

Our study has strengths and some limitations. We investigated for the first time the association between serum POSTN and prevalence and incidence/progression of OA in a large and well characterized population of women followed prospectively over a long period of time. We verified that the loss of POSTN during long term storage is very limited\(^{15}\). Because all samples were stored for an average of 10 years, if a loss of immunoreactivity has occurred this would affect similarly samples from OA cases and controls. Therefore, the potential long-term storage effect has probably limited impact on the investigated association. Prevalence of lumbar spine OA in our cohort was high, but consistent with previously published data\(^{36,37}\). Moreover, using Nevitt criteria\(^{38}\), we observed that 73% of women had symptomatic lumbar spine OA. Finally, a single rheumatologist scored the radiographs for knee OA. Consequently, it is difficult to judge how intra-observer variability might influence the data analysis.

The number of progressors was limited (\( n = 82 \)) and our results need to be confirmed by additional larger studies.

The assay we used is believed to detect all isoforms of circulating POSTN. However, we do not know which isoform(s) is/are the most relevant to assess cartilage metabolism and if POSTN isoform(s) expressed by cartilage is/are the same than POSTN isoform(s) expressed by bone. Finally we investigated only predominantly healthy women. Thus, these findings may not apply to men or patients with a more severe OA.

In conclusion, lower serum POSTN levels are associated with increased prevalence and incidence/progression of OA in women. The molecular mechanisms relating serum POSTN to OA prevalence and incidence/progression remain to be further explored.

**Contributors**

This work was funded by the Institut National de la Santé et de la Recherche Medicale (INSERM). JCR contributed to the coordination of the study and wrote the manuscript. CB performed all the laboratory work. ESR and JCR performed the statistical data analysis. PC, RC and ESR participated in interpretation of data and contributed to the preparation of the final manuscript. All authors read and approved the final manuscript.

![Fig. 1. Number (percentage) of KL progressors at 4 years according to quartiles of serum POSTN at baseline. The odds-ratio per quartile is 0.79 (95% CI – 0.64–0.99; \( P = 0.043 \)) after adjustment for age, BMI, prevalent knee OA, prevalent hand OA and prevalent lumbar spine OA.](image1)

![Fig. 2. Receiver operating characteristic (ROC) curve of serum POSTN and age for predicting OA incidence/progression over 4 years. Using a cross validation model, we conducted a ROC analysis to evaluate the discriminating value of serum POSTN levels and age to predict an increase of at least 1 KL grade over 4 years. The area under the curve was 0.66 (95% CI – 0.61–0.72, \( P = 0.028 \)).](image2)
Competing interests
None.

Supplementary data
Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.joca.2015.05.015.

References

