Osteoprotegerin: A new biomarker for impaired bone metabolism in complex regional pain syndrome?

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Abstract

Osteoprotegerin (OPG) is important for bone remodeling and may contribute to complex regional pain syndrome (CRPS) pathophysiology. We aimed to assess the value of OPG as a biomarker for CRPS and a possible correlation with radiotracer uptake in 3-phase bone scintigraphy (TPBS). OPG levels were analyzed in 23 CRPS patients (17 women; mean age 50 ± 9.0 years; disease duration: 12 weeks [IQR 8–24]), 10 controls (6 women; mean age 58 ± 9.6 years) and 21 patients after uncomplicated fractures (12 women; mean age: 43 ± 15 years; time after fracture: 15 weeks [IQR: 6–22]). The CRPS and control patients also underwent TPBS. OPG in CRPS patients was significantly increased by comparison with both control groups (P = 0.001; Kruskal-Wallis test; CRPS patients: 74.1 pg/mL [IQR: 47.1–100.7]; controls: 46.7 pg/mL [IQR: 35.5–55.0]; P = 0.004; fracture patients: 45.9 pg/mL [IQR: 37.5–56.7]; P = 0.001). As a diagnostic test for CRPS, OPG had a sensitivity of 0.74, specificity of 0.80, positive predictive value of 68% and negative predictive value of 84%. Receiver operating characteristic curve analysis showed an area under the curve of 0.80 (CI: 0.68–0.91). For the CRPS-affected hand, a significant correlation between OPG and TPBS region of interest analysis in phase III was detected (carpal bones; r = 0.391; P = 0.03). The persistent OPG increase in CRPS indicates enhanced osteoblastic activity shown by increased radiotracer uptake in TPBS phase III. A contribution of bone turnover to CRPS pathophysiology is likely. OPG might be useful as a biomarker for CRPS.

1. Introduction

Limb trauma leads to localized and transient (usually days to weeks) signs of inflammation and pain. In about 2–5% of patients, inflammatory symptoms do not subside but rather intensify with time. In these cases, complex regional pain syndrome (CRPS) may have developed [3]. Diagnostic components included in the Budapest criteria [13] comprise sensory, vasomotor, sudomotor/edema, and motor/trophic changes.

Despite ongoing research, the symptoms arising from the deep somatic tissues (e.g., joint pain, limited range of motion) and CRPS-associated osteoporosis of periarticular bone are poorly understood [16,31,38]. Bone scintigraphy [38] in CRPS indicates a high turnover osteoporosis with increased osteocyte activity [4], which is the rationale for treatment with bisphosphonates [28,35].

The discovery of the receptor activator of nuclear factor (NF)-κB (RANK)/RANK ligand (RANKL)/osteoprotegerin (OPG) pathway might be a key to understanding bone symptoms in CRPS. This pathway contributes to both bone resorption and formation [15]. OPG, also known as osteoclastogenesis inhibitory factor, is produced by osteoblasts. OPG prevents the activation of the receptor activator of NF-κB (RANK) and thereby not only hinders osteoclast formation but also affects the immune system. OPG obviously contributes to fracture healing; following a fracture, OPG is immediately upregulated [11] but subsequently normalizes within a few weeks [20].

OPG elevation is not bone specific, since serum OPG might also be increased in coronary heart disease [27], irritable bowel syndrome [17], and other disorders. OPG might be of particular
interest in CRPS since it belongs to the tumor necrosis factor (TNF) receptor superfamily. TNF-α upregulation has consistently been shown to be central to the inflammatory pathophysiology of CRPS [18,34].

The standard method of assessing bone metabolism in CRPS is 3-phase bone scintigraphy (TPBS) with technetium-labeled bisphosphonates [38]. TPBS demonstrates significant lateral differences between CRPS-affected and nonaffected limbs. It is analyzed either qualitatively or quantitatively by region of interest (ROI) analysis [38]. The sensitivity and specificity of TPBS in CRPS is fair. The major disadvantage of TPBS is the use of radioactively labeled compounds. Performing TPBS in young patients is therefore inherently difficult and repetitive assessment, for example during the course of CRPS, is inappropriate.

The identification of different biomarkers mirroring CRPS-induced bone changes is therefore essential. The role of OPG in bone turnover, as indicated earlier, and the ease of assessing OPG in serum samples, led to our investigation of a potential diagnostic role for OPG in CRPS. In order to gain further insight into the role of bone turnover in CRPS, we compared the results from CRPS patients with those from a control group as well as from patients after uncomplicated fractures. We were particularly interested in finding a relationship between OPG and TPBS, and assessing clinical symptoms that might be correlated to serum OPG levels. Finally, a possible relationship between the bone-related findings and the pain and sensory profiles of CRPS patients was investigated.

2. Materials and methods

2.1. Participants

Twenty-three patients with CRPS (17 women; mean age 50 ± 9.0 years) were recruited from the CRPS outpatient clinic of the Department of Neurology, University Medical Center, Mainz. The median disease duration was 12 weeks (interquartile range [IQR]: 8–24 weeks). To assess the influence of disease duration on different parameters, the CRPS patients were divided using a median split. In 14 patients, disease duration was >12 weeks. Since 4 of our patients had a disease duration of 12 weeks, the median split resulted in imbalanced numbers.

All patients fulfilled the current Budapest criteria for the diagnosis of CRPS [13] and suffered from an “initially warm” CRPS subtype [36] as a clinical hallmark for the presence of inflammation. Only patients with CRPS located in an upper extremity were recruited. All patients reported significant limb trauma (eg, fracture, surgery). Fifteen patients had a limb fracture as the inciting event, 7 patients underwent soft tissue surgery, and 1 patient had a sprain of the wrist. Comedication was assessed routinely and 19 of the 23 patients did not take sex hormones that could possibly affect bone turnover. In the other 4 cases, estrogen replacement therapy was not mentioned explicitly in the charts. CRPS patients were excluded from the study if they 1) did not fulfill the Budapest criteria; 2) had a history of cancer; 3) had bone disease; or 4) had CRPS located on the lower extremity.

In order to avoid radiation exposure to volunteers, 10 sex-matched patients undergoing TPBS necessary for cancer staging were included as a control group (6 women; mean age 58 ± 9.6 years). Tumor patients were slightly older than the CRPS patients (P = 0.027). In all patients, tumor diagnosis was incidental. Six patients had lung cancer, two had breast cancer, one had gastric cancer and one had a fibrous dysplasia of the skull. All these control patients were staged T1–2, N0, and M0 (post staging). TPBS was performed before any treatment commenced.

To further control for the effects of the fracture itself on OPG values, 21 patients not meeting CRPS criteria after limb trauma were investigated (12 women; mean age: 43 ± 15 years). The patients were age-matched to the CRPS group and the time between fracture and OPG analysis equaled the duration of the CRPS (median: 15 weeks; IQR: 6–22 weeks; not significant [ns]).

Written informed consent according to the Declaration of Helsinki was obtained from all participants. The study was approved by the ethics committee of the Rhineland-Palatinate Medical Association.

2.2. Quantitative sensory testing and McGill Pain Questionnaire

Quantitative sensory testing (QST) of pain parameters was performed in all CRPS patients one day before TPBS using the technique of the German Research Network on Neuropathic Pain (DFNS) [29]. We aimed to evaluate the sensory profile of CRPS patients in order to establish a possible relationship between positive and negative symptoms of neuropathic pain and features of bone metabolism. Control subjects as well as fracture patients were pain free. QST was not performed in these patients because multicenter German QST normal values have been assessed previously, including contributions from our laboratory [29].

We focused on the following QST parameters: heat pain thresholds, mechanical pain thresholds to pinprick stimuli, and pain thresholds to blunt pressure. Testing of the different pain thresholds was carried out on the dorsum of the affected hand. The corresponding area of the contralateral extremity served as the control site. In brief, heat pain thresholds were determined using a TSA 2001-II (MEDOC, Israel). The baseline temperature was 32°C, the contact area of the thermode was 9 cm², and the ramp rate was 1°C/second. The mean threshold temperature of 3 consecutive measurements was calculated. Mechanical pain thresholds were obtained by employing sets of calibrated pinpricks with a 0.25 mm flat-top cylindrical tip and a series of 7 forces (8-512 mN) geometrically spaced by a factor of 2 (The PinPrick, Department of Physiology, Mainz, Germany). Pressure pain thresholds were assessed at the thenar eminence using a handheld blunt pressure-gauge device (1 cm² contact area; FDN200, Wagner Instruments, USA).

In addition to pain thresholds, skin temperature of the affected and nonaffected extremities was measured using a contact thermometer (Fluke, Germany) in order to support the diagnosis of primarily warm CRPS subtype.

Patients were asked to rate their pain intensity on a verbal rating scale with the anchors 0 (no pain) and 10 (maximum pain imaginable). After completion of the QST, the German version of the McGill Pain Questionnaire was completed by all CRPS patients [33].

2.3. Blood samples

Blood samples for quantifying OPG were drawn before TPBS in the CRPS and control groups. In the fracture patients, the blood was drawn during a routine follow-up visit or during an explanatory discussion in preparation for surgical removal of osteosynthesis material. Blood samples were centrifuged at room temperature at 1600 rpm for 5 minutes. The supernatant serum was collected in 2 mL Eppendorf caps and stored at −80°C. OPG was quantified using a commercial OPG enzyme-linked immunospecific assay (Immundiagnostik, Bensheim) with a detection limit of 2.8 pg/mL (0.17 pmol/litre) and a coefficient of variation (CV) <5%. All analyses were carried out at the Dresden Technical University Medical Center.

2.4. TPBS

TPBS was performed using 99mTc-DPD. Images were acquired using a dual-headed E.CAM (Siemens, Erlangen, Germany) gamma camera equipped with low-energy high-resolution collimators. The fields of view contained both hands and forearms. The right hand was always positioned in front of the left hand in order to
prevent confusion. Dynamic flow images (128 × 128 matrix, 3 seconds per frame) were acquired for a total of 5 minutes, starting directly after injection of 10 MBq/kg $^{99m}$Tc-DPD (activity of 500–700 MBq per patient) using an intravenous cannula secured in the antecubital vein of the nonaffected arm. The dynamic scan for phase I (the blood-flow phase) was obtained directly after the intravenous bolus injection. Images for phase II (blood-pool phase) were subsequently obtained in dynamic sequence for 5 minutes. The mean time period between injection of the radiotracer and acquisition of the phase II scan was 3 minutes 26 seconds (±8.7 seconds). The static phase III (delayed phase) scan was taken 2–3 hours after injection of the radiotracer.

### 2.5. Image interpretation

The TPBS results were analyzed by an experienced nuclear medicine physician who was blinded to the status of the subject (M. S.). Pattern analysis was performed comparing radiotracer uptake between the CRPS-affected and contralateral extremities in CRPS patients as well as with the control group for each phase. Two ROIs were chosen for each scan: the carpal region (carpal bone; CB) and the metacarpal region (metacarpal bones including the fingers I–V; MCP). In order to quantitatively assess radiotracer accumulation, absolute values for counts per second per pixel, as well as the corresponding radiotracer uptake ratio counts per pixel per second for comparison of the left and right hand in each individual, were calculated for each ROI.

### 2.6. Statistics

Data were analyzed using the SPSS Statistics (IBM, Version 19.0 for Windows) software package. QST data were transformed into standard normal distributions corrected for body region, sex, and age (Z values). Z transformation allows the comparison of values independent of their physical dimensions. Increased sensitivity results in positive z scores, whereas decreased sensitivity results in negative z scores. Comparison of QST data transformed into z scores was performed using paired t tests. Owing to non-normal distribution, comparison of skin temperature between the CRPS-affected and nonaffected extremities was carried out using Wilcoxon signed rank tests. For analysis of the TPBS results, Mann-Whitney tests were carried out to identify differences between CRPS patients and controls, and Wilcoxon signed rank tests in order to allocate differences between the CRPS-affected and CRPS-nonaffected extremities. To investigate the impact of disease duration (≥12 weeks vs <12 weeks) Mann-Whitney tests were performed. To assess interrelations between CRPS variables, Spearman correlations were carried out. In the case of dichotomous variables (CRPS symptoms), point-biserial correlations were calculated. In order to evaluate differences in OPG serum levels between the different groups of patients (CRPS, fracture, and control patients), a Kruskal-Wallis test was done. Posthoc tests were carried out using Mann-Whitney tests between CRPS, control, and fracture patients, and corrected for multiple testing. To compare our OPG results with a larger normative sample that has been published previously [19], a two-tailed t test was carried out employing GraphPad software (http://www.graphpad.com/quickcalcs). The sensitivity and positive and negative predictive value of OPG serum levels for CRPS diagnosis was calculated based on cross-tabulation of the results obtained from our CRPS patients with the pooled control groups (owing to a lack of statistical differences between these groups). OPG values were classified as pathological if they exceeded the 90% CI calculated from our pooled control groups. The true positive rate (sensitivity) as a function of the false positive rate (1-specificity) was plotted to a receiver operating characteristic (ROC) curve. To estimate the discriminatory accuracy of OPG values, the area under the ROC curve (AUC) was calculated. All values are given as medians and IQR in the case of a non-normal distribution and as means ± SD in the case of a normal distribution. Values were considered significant if P < 0.05.

### 3. Results

#### 3.1. Patient characteristics

In CRPS patients, skin temperature was higher on the affected extremity (temperature median: affected hand: 32.6°C [IQR: 30.7–33.1]; nonaffected hand: 31.5°C [IQR: 30.7–32.3]; P = 0.02). CRPS patients had a median pain intensity of 5.0 (IQR: 3.0–8.0) on a verbal rating scale ranging from 0 to 10. The median McGill Pain Rating Index was 18 points (IQR: 10–24). OPG revealed heat hyperalgesia (heat pain threshold: CRPS-affected hand: z score 1.1 ± 1.7; nonaffected hand: 0.5 ± 1.3; P = 0.031) and pressure pain hyperalgesia (pressure pain threshold: CRPS-affected hand: z score 1.7 ± 2.8; nonaffected hand: −0.9 ± 1.8; P < 0.001) on the CRPS-affected hand. There was no evidence of pinprick hyperalgesia on the affected limb (mechanical pain threshold: CRPS-affected hand: z score 1.1 ± 1.5; nonaffected hand: 1.0 ± 1.3; ns).

#### 3.2. OPG serum levels

OPG in CRPS patients was significantly increased by comparison with both control groups (P = 0.001; Kruskal-Wallis test; CRPS patients: median: 74.1 pg/mL [IQR: 47.1–100.7]; control patients: median: 46.7 pg/mL [IQR: 35.5–55.0]; P = 0.004; fracture patients: median: 45.9 pg/mL [IQR: 37.5–56.7]; P = 0.001; Mann-Whitney test). No difference could be detected between the fracture and control groups.

For validation of the results, we also compared (2-tailed t test) the OPG values with a published normative data sample from a comparable age group (51–60 years) obtained with the same enzyme-linked immunospecific assay. Since July of our 23 CRPS patients were women, and the normative values for women are higher than for men, we chose to compare our results with the published OPG results for women (median OPG in women aged 51–60 years: 35.5 pg/mL [range 160], n = 150; median OPG in men aged 51–60: 34 pg/mL [range 134], n = 176; mean OPG ± SD in women aged 51–60 years: 39.5 ± 22.3; mean OPG ± SD in men aged 51–60 years: 36.9 ± 20.2 [19]). We also found that in this confirmation test, the CRPS patients had significantly increased OPG serum values (P < 0.001) while our controls and fracture patients did not.

In 17 of our 23 CRPS patients, OPG serum levels exceeded the CI of our pooled fracture data as well as the control data, representing a sensitivity of 0.74. There were 8 of 25 false positive OPG values, representing a positive predictive value of 68%. The specificity was calculated to be 0.8 (false positives out of 39) and the negative predictive value was 84% (31 true negatives out of 37). For the diagnostic value of OPG in CRPS, a ROC analysis was conducted and the AUC was 0.80; [CI: 0.68–0.91]. The AUC indicates good performance of OPG as a biomarker in CRPS diagnosis. For details see Fig. 1.

Eight of our CRPS patients had no evident bone damage prior to the development of CRPS (soft tissue surgery: n = 7, distortion of the wrist: n = 1). No difference regarding OPG levels could be found between CRPS patients with and without bone affection (ns).

#### 3.3. TPBS

During phase I (blood-flow phase), ROI analysis of CB and MCP revealed no significant differences (counts per pixel per second) between the affected and nonaffected hands in the CRPS patients or control group. During phase II (blood-pool phase), the radiotracer
uptake ratio was significantly higher in the CRPS-affected hand for both ROIs compared with the contralateral arm (CB: \( P < 0.001 \); MCP: \( P = 0.002 \); Wilcoxon test) as well as with the control group (CB: \( P = 0.013 \); MCP: 0.003; Mann-Whitney test). In phase III (delayed phase) the uptake ratio was again significantly higher in the CRPS-affected hand for both ROIs compared with the contralateral arm (CB: \( P < 0.001 \); MCP: \( P = 0.001 \)) as well as with the control group (CB: \( P < 0.001 \); MCP: \( P < 0.001 \); Mann-Whitney test). Differences between the CRPS-nonaffected limb and the control patients could not be established. For details see Table 1.

### 3.4. Correlation between OPG levels and CRPS symptoms

OPG serum levels were not different between patients with a CRPS duration of <12 weeks and those with a duration of \( \geq 12 \) weeks. There were no significant correlations between OPG concentration and the presence or absence of CRPS symptoms (point-biserial correlations).

### 3.5. Correlation between serum OPG and TPBS

In CRPS patients, a significant correlation between serum OPG and ROI values for the carpal bone in phase III of the TPBS of the affected hand was detected (ROI values: CB: CRPS-affected side: \( 0.32 \pm 0.04; r = 0.391; P = 0.03 \)). OPG was not related to other TPBS parameters and ratios. For details see Fig. 2.

### 3.6. Correlation between TPBS parameters and evoked pain responses in CRPS patients

A significant negative correlation between radiotracer enhancement (ROI values) in phase II for the carpal bones and the pressure pain threshold for the CRPS-affected hand was detected (CB: \( r = -0.344; P = 0.037 \)). A trend towards a negative correlation between pressure pain threshold and the uptake ratio was detected for MCP during phase III (\( r = -0.312; P = 0.06; \) ns).

In CRPS patients with a disease duration <12 weeks, compared with patients with a longer disease duration, a significantly higher uptake ratio was detected for the MCP in phase III (\( P = 0.023 \)) and marginally in phase II (\( P = 0.062; \) Mann-Whitney test). No further correlations between TPBS parameters and either the presence or absence of CRPS symptoms was found.

### 4. Discussion

This is the first study in which elevated OPG serum levels in CRPS patients have been detected several months after the initial trauma. Our results demonstrate a moderate connection between osteoblast-derived systemic OPG and localized technetium uptake in phase II of TPBS. Therefore, increased osteoblast activity is present for a long time after trauma in CRPS, possibly contributing to the phenotype of post-traumatic CRPS. The results are in line with current theories concerning the inflammatory mechanisms of CRPS and open an avenue for future CRPS research. According to our findings, serum OPG has good specificity and sensitivity with respect to clinical CRPS diagnosis, and it seems possible that after further studies with appropriate control groups OPG may turn out to be a confirmative marker in ambiguous post-traumatic CRPS cases or for monitoring outcomes of CRPS treatment.

#### 4.1. Bone remodeling in CRPS pathophysiology

Recent studies of the biological effects of the OPG/RANK/RANKL system have generated insights into the molecular and cellular basis of bone metabolism [24]. In brief, bone remodeling is initiated (initiation phase, lasting days to 3 weeks) by trauma, nonuse, and inflammatory cytokines (TNF-\( \alpha \), interleukin 18) or chemokines (monocyte chemotactic protein 1 [MCP-1], chemokine [C-C motif] ligand 2 [CCL2]), all of which induce RANKL expression on osteoblast precursors. All these factors contribute to CRPS pathophysiology [23]. Subsequently, osteoclast precursors bind via RANK to RANKL, become activated, and proliferate. In parallel, inflammatory cytokines and prostaglandins directly induce osteoclast activation via their specific receptors. In the transition phase, osteoclasts undergo apoptosis by, for example, estrogen-dependent pathways and activate via humoral (eg, TGF-\( \beta \)) or direct signaling, osteoblast proliferation and maturation. Interestingly,
The maintenance of normal bone mass has been shown to be at greater risk of developing CRPS after fracture [7,30]. However, no association between CRPS and the use of hormonal replacement therapy or oral contraceptives has been determined [8]. In the termination phase, lasting 3 months, osteoblasts secrete OPG, which in turn inhibits RANK on osteoclasts and terminates bone turnover [24]. In summary, OPG creates osteoblast activity, usually acting locally in the tissue. In mice, exposure to recombinant OPG led to increased bone density owing to a reduction of osteoclast numbers [32], and mice deficient in OPG developed osteopenia at an early age owing to increased osteoclast activity, highlighting a physiological role for OPG in the maintenance of normal bone mass [5].

The role of serum OPG as a biomarker for bone turnover in humans is under investigation. A negative correlation between serum OPG and bone mineral density at the trochanter major has been described [6], as has decreased OPG (and increased RANKL) in patients with rheumatoid arthritis-induced osteoporosis [39]. The contribution of the OPG/RANKL system to the pathogenesis of osteoporosis in CRPS remains unknown. We describe for the first time increased OPG in CRPS patients. This fits with recent findings that indicate an inflammatory component of CRPS. The correspondingly increased inflammatory cytokines [18,34] and the chemokine MCP-1 [14] in CRPS are possibly “overactivating” the osteoblast precursor/osteoclast pathway and the reduced TGF-β [34] possibly contributes to relative “underactivation” of osteoblasts, resulting in negative bone turnover. Our finding of increased OPG in CRPS indicates that bone remodeling by osteoblasts is still not complete even months after trauma. Moreover, no obvious direct bone injury is needed in order to induce an elevation of OPG since we also found increased OPG levels in CRPS resulting from soft tissue injury. This underscores the likely role of locally acting inflammatory tissue molecules in CRPS pathophysiology. Bisphosphonates [28,35], which inhibit osteoclasts, successfully treat CRPS, indicating that the ongoing bone turnover might be critical for the generation of CRPS symptoms. OPG has been found to be increased immediately as well as 10 weeks after an uncomplicated fracture [11]. OPG subsequently normalizes within a few weeks [20], as happened in our fracture group. Therefore, no significant difference between the fracture and control groups regarding OPG concentration could be found. However, one could speculate that our results can be partly explained by the initial fracture. We found OPG significantly increased in CRPS independently of disease duration and bone damage. Therefore, the conclusion that OPG is playing a role in CRPS pathophysiology appears justified.

4.2. Correlation between OPG, TPBS, and clinical CRPS symptoms

We only detected a positive correlation between serum OPG and ipsilateral technetium uptake in phase III in the carpal bone. However, high linear correlations between serum OPG and local findings were not expected since OPG acts locally in the tissue. Interestingly, OPG elevation was found to be independent of CRPS duration in our CRPS population, whereas TPBS changes were pronounced in patients with a short disease duration (<12 weeks), in agreement with previous studies [38]. Although in a recent retrospective study a weak association was made with an inflammatory phenotype, no direct correlation was demonstrated between clinical symptoms and TPBS parameters [1]. In our prospective study, radiotracer uptake in phase II was associated (borderline in phase III) with pressure pain hyperalgesia. The pain in acute CRPS mainly comes from deep tissues, including the bones [2], and pressure pain hyperalgesia is the most prevalent sensory sign [22]. The innervation of the bone is extensive [21]. The inflammatory mediators (including nerve growth factor) [24] that are responsible for bone remodeling (see earlier) are all able to activate and sensitize deep tissue nociceptors [12]. However, the impact of OPG itself in the development of pain needs to be further elucidated. OPG is not bone specific and is produced by various nonskeletal tissues, and is known to be involved in a complex cytokine network that regulates numerous functions in the immune system [25,37]. Thus, OPG could also be a surrogate for immune activation that itself may contribute to nociceptive sensitization.

4.3. The diagnostic value of serum OPG in CRPS

The diagnostic sensitivity and specificity of the delayed phase of TPBS for CRPS is moderate to fair [38]. This might be owing to the imperfect “gold standard” diagnostic criteria being exclusively based on clinical findings [13]. If uncritically used (ie, neglecting point 4: “There is no other diagnosis that better explains the signs and symptoms”), these criteria themselves might not be specific enough [10]. However, TPBS is often analyzed qualitatively and between-rater reliability represents a pitfall. Moreover, TPBS does not allow follow-up investigations because of radiation exposure. Therefore, other objective biomarkers for bone metabolism are desirable as part of CRPS pathophysiology. In the present study, we identified that OPG can fill this gap. In CRPS diagnosis, the emphasis should be on specificity. OPG levels in CRPS patients reached a specificity of 0.8 and the negative predictive value was found to be 84%—comparable to clinical diagnosis employing the Budapest criteria. The specificity of our OPG results resembles the respective value of the Budapest research criteria for CRPS vs non-CRPS neuropathic pain [13]. The negative predictive value in our patients even exceeded the negative predictive power of the Budapest research criteria, which was found to be 0.6 for an assumed 70% CRPS prevalence and 0.78 for an assumed 50% CRPS prevalence [13]. Moreover, the ROC analysis supported OPG being a useful biomarker for CRPS diagnosis. However, our sample size was rather small and the negative as well as the positive predictive value are very sensitive with respect to prevalence. Therefore, no definitive conclusions can be drawn. However, OPG might become useful for CRPS diagnosis when more studies have been done in this field.

4.4. Limitations

In this pilot study, we investigated patients with post-traumatic CRPS [13] with an initially warm phenotype indicating persistent
post-traumatic inflammation [9]. Our controls were patients with local nonmalignant tumors not affecting bones. We are confident that this did not affect the results because an extensive PubMed search retrieved exclusively reports on increased serum OPG that at most would reduce, but never inflate, the difference between our groups. The control group was significantly older than the CRPS group. This does not hamper but rather strengthens our results, since OPG serum levels are known to increase with age [9]. Since OPG serum levels are known to increase with age, the OPG difference between fracture and CRPS patients was remarkable, whereas no difference was to be found between the fracture and control groups.

4.5. Perspectives

We have provided evidence that elevated serum OPG reflects the occurrence of the pathophysiological processes of CRPS and might be a useful biomarker for CRPS. Longitudinal studies in different stages of CRPS must follow in order to substantiate our hypothesis that OPG is more prevalent in women. However, the sex differences in OPG serum levels were not so pronounced that they could explain our findings [19], and the OPG difference between fracture and CRPS patients was remarkable, whereas no difference was to be found between the fracture and control groups.

Conflicts of interest statement

There are no conflicts of interest.

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