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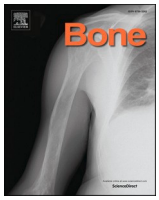

Full Length Article

Clinical value of RANKL, OPG, IL-6 and sclerostin as biomarkers for fibrous dysplasia/McCune-Albright syndrome

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ABSTRACT

Background: Fibrous dysplasia/McCune-Albright syndrome (FD/MAS) is a rare genetic bone disease caused by a somatic mutation in the *GNAS* gene. Currently used bone turnover markers (BTMs) do not correlate with the clinical picture and are not useful to predict or monitor therapy success. This study assessed the correlation of RANKL, OPG, RANKL/OPG ratio, IL-6 and sclerostin with the classic BTMs alkaline phosphatase (ALP), procollagen type 1 propeptide (P1NP) and beta crosslaps (CTX), with pain, skeletal burden score (SBS) and response to bisphosphonate or denosumab treatment.

Methods: Ninety-six serum samples of adult patients >18 years of age with any subtype of FD/MAS were included from the biobank facility of the Leiden University Medical Center, Center for Bone Quality between 2015 and 2021. Standard laboratory assessments were assessed as part of usual care. The concentrations of potential biomarkers RANKL, OPG, sclerostin, IL-6 were analyzed. Data on FD/MAS subtype, age, pain, treatment history and treatment response were retrieved from the electronic patient files. Baseline characteristics were summarized by descriptive statistics. Correlations of the concentrations of the potential biomarkers with classic bone turnover markers, SBS and pain scores were cross-sectionally assessed by Spearman rank order correlation. Correction for multiple testing was performed by Benjamini and Hochberg False Discovery Rate. A sensitivity analyses was performed by excluding patients with SBS below 15 and patients using antiresorptive medication at the time of blood withdrawal or within the wash-out period. In patients treated with bisphosphonates or denosumab after blood withdrawal, pre-treatment concentrations were compared in patients with and without therapy response by Mann Whitney *U* test.

Results: The median age of the patients was 41.2 (Q1-Q3 25.9–52.2) years, 62.5 % was female. Median SBS was 2.5 (Q1-Q3 0.5–7.8). RANKL level correlated weakly with ALP (Spearman rho 0.309, $p = 0.004$, $n = 84$), but not with P1NP or CTX. The RANKL/OPG ratio, OPG, IL-6 and sclerostin did not correlate with ALP, P1NP or CTX. None of the potential biomarkers correlated with SBS or pain. Results of the sensitivity analyses were comparable. Pre-treatment biomarker levels were similar in patients with and without improvement in pain scores following bisphosphonate therapy. Pre-treatment RANKL and sclerostin were comparable between patients with and without improvement in pain scores after denosumab therapy. Pre-treatment IL-6 level and the RANKL/OPG ratio seemed to be higher in patients with response to denosumab (IL-6: median 0.64 (Q1-Q3 0.53–0.74) pg/mL, $n = 6$, RANKL/OPG: median 0.062 (Q1-Q3 0.016–0.331), $n = 5$) compared to patients without response (IL-6: median 0.35 (0.20–0.54) pg/mL, n = 5, RANKL/OPG: 0.027 (0.024–0.046), $n = 4$). Pre-treatment IL-6 correlated with the improvement in maximum pain scores (rho 0.962, $p < 0.001$, $n = 9$) and average pain scores (rho 0.895, $p = 0.001$, n = 9) reported during denosumab therapy.

Conclusion: Increased concentrations of RANKL, IL-6, sclerostin and of the RANKL/OPG ratio do not indicate severity of FD/MAS, as no correlation was observed of these potential biomarkers with the classic BTMs and SBS. Biomarker levels did not correlate with pain and had no value in predicting bisphosphonate treatment response. These biomarkers are not superior over the currently used methods of assessing ALP, P1NP and CTX or evaluating SBS to establish disease extent or activity and provide no reliable results. Yet, possibly pre-treatment IL-6

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and the RANKL/OPG ratio may have some predictive value for clinical response to denosumab. Therefore, studies investigating disease activity and treatment response should include lesional imaging and patient-reported outcome measures.

1. Introduction

Fibrous dysplasia is caused by a mutation in the *GNAS* gene. Due to the somatic mosaicism there is a broad clinical spectrum [1]. Hallmark of the disease is the development of fibrous, dysplastic bone lesions with abnormal bone turnover and inadequate mineralization, which may induce pain, nerve compression, fractures or malformations [2,3]. These lesions may affect one bone in monostotic fibrous dysplasia (MFD) or multiple sites in polyostotic disease (PFD). When accompanied by at least 2 extraskeletal manifestations like hyperpigmented skin macules, hormone overproduction or benign tumors, it is termed as McCune-Albright syndrome (MAS) [4]. To date predicting disease activity and progression has been difficult and disease burden, as measured by skeletal burden score [5], does not seem to be predictive [6]. Theoretically the downstream factors from the *GNAS* mutation could serve as a marker for disease activity. The GNAS mutation is located on chromosome 20q13.3 and encoding the stimulatory alpha subunit of the G-protein (Gsα), alters hydrolysis of GTP to GDP [1,7] and induces elevated levels of cyclic adenosine monophosphate (cAMP) and protein kinase A phosphorylation [1]. Physiologically, the increase in cAMP lowers sclerostin levels and leads to overexpression of factors of the Wnt signaling pathway and β-catenin by osteoblast progenitors. As a result, in FD/MAS this causes increased bone turnover, which may be reflected by increased markers of bone formation (alkaline phosphatase (ALP), osteocalcin, procollagen type 1 propeptide (P1NP)) and of bone resorption (beta crosslaps (CTX)). Together with insufficient mineralization, this leads to immature, woven bone [3,8]. In addition, mutated osteoblasts overexpress receptor activator of nuclear factor κ-B ligand (RANKL) [9] and interleukin-6 (IL-6) [10,11]. Currently disease activity is biochemically assessed by measuring the bone turnover markers (BTMs) ALP, osteocalcin, P1NP and beta crosslaps (CTX) [4]. However, these markers have several limitations in the management of FD: they do not correlate with pain [12] and correlate with skeletal burden score (SBS) in some studies but not all [5,13,14]. Moreover, a recent longitudinal study suggested an age-related decline in BTMs, regardless of antiresorptive treatment [12]. These limitations underline the need for biomarkers with the potential to predict and monitor treatment success and to accelerate the development of new therapeutic approaches. A previous study showed that RANKL and its decoy receptor osteoprotegerin (OPG) were systemically increased in patients with FD/MAS when compared to controls [15]. RANKL and OPG levels and the RANKL/OPG ratio correlated with SBS in a combined cohort of patients and healthy controls [15]. RANKL-inhibition has provided promising results, but the predictive value of RANKL levels for therapy success has not been investigated. Likewise treatment targeting IL-6 has been conducted, although systemic levels of IL-6 have not been evaluated in FD/MAS. Therefore, this study aimed to explore systemic levels of RANKL, OPG, and IL-6 and to assess the association with disease activity and extent, pain and treatment response. Moreover, this study focused on sclerostin, a key bone regulator, which inhibits bone formation, suppresses mineralization and stimulates RANKL expression and bone resorption [16]. Research on the sclerostin/Wnt pathway in FD/MAS is scarce. Some patients are described to exhibit gene upregulation of components of the Wnt signaling pathway [17,18] and in a mice model of FD, diminished expression of sclerostin was observed by immunohistochemistry, although mRNA expression was comparable to controls [19]. In summary, this study investigated the role of RANKL, OPG, IL-6 and sclerostin as potential biomarkers. Their clinical significance is evaluated by exploring the association between serum concentrations and SBS, classic BTMs, pain and response to antiresorptive therapy.

2. Methods

2.1. Subjects

In this cohort study 96 serum samples were collected from a IRB approved biobank [20]. All samples were retrieved from patients ≥18 years of age who visited the outpatient clinic of the Center for Bone Quality, Department of Endocrinology of the Leiden University Medical Center in the Netherlands between 2015 and 2021 and retrieved a definitive diagnosis of any subtype of FD/MAS. All samples were taken before the afternoon with a maximum processing time of 4 hours [20]. This study was approved by the Medical Ethics Committee and scientific biobank committee [20]. A subset of patients was included in previous studies [14,21–24], data for the present study were generated originally. At the time of serum withdrawal, 65 patients (67.7 %) were therapy naïve, 17 patients (17.7 %) had previously received bone-modulating treatment but underwent a minimum of 6 months wash-out, 14 patients (14.6 %) were under active treatment.

2.2. Clinical parameters

The electronic patient file was consulted to assess type FD/MAS, age, results of routine assessments of ALP, P1NP and CTX, presence of pain and pain score at the time of serum withdrawal. For pain assessment, the Brief Pain Inventory was used [25]. This questionnaire investigates pain severity, where patients score their maximum pain, least pain, pain on average and pain right now. The range is 0–10, higher scores reflect more pain. We considered the items maximum pain and average pain most relevant for our research and excluded other domains. Next, we classified pain scores into pain levels, where score 0 was categorized as zero pain, score 1–3 as mild pain, score 4–6 as moderate pain, and ≥ 7 as severe pain. Additionally, the medical history was reviewed for history of treatment with denosumab or bisphosphonates either before or after serum withdrawal, and for therapy response in terms of pain, bone turnover markers. Treatment status during blood withdrawal was established as on or off active treatment, with a 6-month wash-out period for bisphosphonates and 3-months wash-out for denosumab. Skeletal burden score [5] was assessed on bone scintigraphy or 18F-NaF PET/CT.

2.3. Laboratory analysis

From each patient, a serum sample of 450 uL was obtained from storage at −80 °C in the biobank. The concentrations of RANKL, OPG, sclerostin and IL-6 were assessed according to the manufacturer's instructions. RANKL was detected by MILLIPLEX MAP Human RANKL Magnetic Bead - Single Plex - Metabolism Assay of Merck Millipore with a Luminex Platform, intra assay variation <10 %, inter assay variation <15 % (reported by manufacturer). Sclerostin was assessed by SOST Human Sclerostin Kit Immunoassay of MSD, intra assay variation <15 %, inter assay variation <18 %. OPG was measured by Human Osteoprotegerin/TNFRSF11B DuoSet ELISA with DuoSet ELISA Ancillary Reagent Kit 2 and normal goat serum, all of R&D systems, intra and inter assay variation not reported by manufacturer. IL-6 was measured by V-plex Human IL-6 kit of MSD, intra-assay variation 4 %, inter assay variation 6.4 %. OPG was merely assessed in the first batch of patients in 2019 (n = 57) and not performed in the samples retrieved from the biobank in 2021, of newly included patients between 2019 and 2021 (n = 39), due to our preliminary results. Standard laboratory assessments were routinely performed directly after venipuncture, total ALP by

automated P800 modulator system (Roche BV, Woerden, The Netherlands), P1NP and B-CTX were measured by the E-170 system (Roche BV).

2.4. Statistical analysis

Baseline characteristics were summarized by descriptive statistics: number and percentage for categorical data and mean and standard deviation for normally distributed data, otherwise median with interquartile range. Results of each potential biomarker were screened for outliers by Grubb's outlier test with a significance level of <0.05. Outliers with a *Z*-score > 3 were indicated. Correlations were assessed by Spearman rank order correlation, as the presence of relevant outliers in all laboratory assessments precluded the use of Pearson's correlation. Correlations of the potential biomarkers RANKL, OPG, IL-6 and sclerostin with classic BTMs, SBS, and presence of pain at the time of serum withdrawal were cross-sectionally analyzed. A sensitivity analyses was performed by excluding patients with SBS below 15 (to investigate whether associations were affected by disease severity) and excluding patients using antiresorptive medication at the time of blood withdrawal or within the wash-out period (to assess whether associations were affected by use of antiresorptives). Serum concentrations of the potential biomarkers were compared in patients with and without therapy response after bisphosphonate or denosumab therapy by Mann Whitney *U* test. This analysis was performed in the subgroup of patients off therapy during blood withdrawal and starting therapy after withdrawal. Correction for multiple testing was performed for all statistical tests (correlations and analyses of group differences, $n = 26$) by Benjamini and Hochberg False Discovery Rate.

3. Results

3.1. Baseline characteristics

Ninety-six patients were included with median age 41.2 (Q1-Q3 25.9–52.2) years, 62.5 % female (Table 1). Monostotic disease was present in 53 patients (55.2 %), polyostotic involvement in 43 patients (44.8 %). An additional diagnosis of MAS was established in 14 patients (20.8 %). Median skeletal burden score was 2.5 (Q1-Q3 0.5–7.8). Median serum concentration of RANKL was 33.0 (Q1-Q3 19.5–62.5) pg/mL, of OPG 1093 (949–1478) pg/mL, of IL-6 0.342 (0.145–0.595) pg/mL, of sclerostin 0.185 (0.150–0.220) ng/mL. The median RANKL/OPG ratio was 0.024 (0.014–0.046). Grubb's outlier test was significant for all potential biomarkers, indicating at least one outlier. The number of outliers with *Z*-score > 3.0 was 2 for RANKL (Z-score 3.1 and 7.1), 1 for OPG (Z-score 4.9), 1 for IL-6 (Z-sore 8.6), 1 for sclerostin (Z-score 3.4), and 1 for the RANKL/OPG ratio (Z-score 5.7).

Table 1
Baseline characteristics.

	$N = 96$
Age years, median (Q1-Q3)	41.2 (25.9–52.2)
Sex, female (n, %)	60 (62.5 %)
Type of FD [a] (n, %)	
MFD (non-CFD)	53 (55.2 %)
PFD	43 (44.8 %)
MAS	14 (20.8 %)
Skeletal Burden Score median (Q1-Q3)	2.5 (0.5–7.8)
Use of bone modulating medication (lifetime)	
Bisphosphonates	64 (66.7 %)
Denosumab	13 (13.5 %)
Tocilizumab	1 (0.01 %)
At time of serum withdrawal	
Bisphosphonates	12 (12.5 %)
Denosumab	1 (0.01 %)
Tocilizumab	1 (0.01 %)
FD-pain present at baseline	50 (52.1)

MFD: monostotic fibrous dysplasia, PFD: polyostotic FD, MAS: McCune-Albright syndrome.

3.2. Correlation of biomarkers with classic bone turnover markers

RANKL correlated weakly with ALP levels (Spearman rho 0.309, $p = 0.004$, $n = 84$), but not with P1NP (rho: 0.149, $p = 0.173$, $n = 85$) or with CTX (rho: −0.077, $p = 0.483$, n = 85) (Table 2). OPG did not correlate with ALP, P1NP or CTX (rho: - 0.057, $p = 0.676$, rho: - 0.034, $p = 0.804$, rho: - 0.127, $p = 0.346$ respectively) (Table 2). Initially the RANKL/OPG ratio did correlate weakly with ALP (rho 0.365, $p = 0.014$, $n = 45$) but not after correction for multiple testing. RANKL/OPG ratio did not correlate with P1NP or CTX (rho: 0.162, $p = 0.282$, rho: −0.155, $p = 0.304$ resp.) (Table 2). Likewise initially IL-6 correlated with ALP (rho 0.245, $p = 0.019$, $n = 92$), but not after correction for multiple testing. IL-6 did not correlate with P1NP or CTX (Table 2). Sclerostin did not correlate with any of the BTMs (Table 2).

3.3. Correlation with skeletal burden score

None of the biomarkers correlated with SBS (Table 2). Of note, also none of the classic bone turnover markers (ALP, P1NP, CTX) correlated with SBS (data not shown).

3.4. Correlation with pain

RANKL, the RANKL/OPG ratio, IL-6 and sclerostin did not correlate with average pain or maximum pain score or level at the time of serum withdrawal. Table 3 shows the median serum concentrations of the biomarkers for each level of average pain. Results for maximum pain are not shown but comparable.

3.5. Sensitivity analyses

After excluding patients with SBS < 15, the more severely affected subgroup consisted of nineteen patients. In this group, results were similar to the total group regarding the correlation between investigated biomarkers and clinical endpoints (classic bone turnover markers, SBS or pain score). Fourteen patients used bone modulating therapy at the time of blood withdrawal (14.6 %) or were within 6 months prior to blood withdrawal. Exclusion of these patients from the analysis improved the correlations of RANKL with ALP and with P1NP, of RANKL/OPG ratio with ALP, and of IL-6 with ALP. However, this did not alter the (absence of) correlation between RANKL and RANKL/OPG ratio with SBS and of the (absence of) correlation between biomarkers and pain scores.

Table 2
Correlation biomarkers with standard markers of bone turnover and SBS.

		ALP	P1NP	CTX	SBS
RANKL	Coefficient	0.309	0.149	−0.077	0.116
	Significance	0.004*	0.173	0.483	0.294
	N	84	85	85	84
OPG	Coefficient	−0.057	−0.034	−0.127	−0.078
	Significance	0.676	0.804	0.346	0.564
	N	56	57	57	57
RANKL/OPG	Coefficient	0.365	0.162	−0.155	0.170
	Significance	0.014	0.282	0.304	0.259
	N	45	46	46	46
IL-6	Coefficient	0.245	−0.086	−0.139	0.133
	Significance	0.019	0.413	0.182	0.208
	N	92	93	93	92
Sclerostin	Coefficient	0.029	0.104	0.169	−0.141
	Significance	0.783	0.313	0.101	0.173
	N	95	96	96	95

P-values with * are statistically significant after correction for multiple testing.

Table 3
Serum concentations of biomarkers for levels of average pain.

		Zero pain	Mild pain	Moderate pain	Severe pain
RANKL	Median	34	20	41	32
	IQR (Q1-Q3)	20–67	15–57	24–74	24–56
	N	43	7	17	8
OPG	Median	1171	1260	1135	1064
	IQR (Q1-Q3)	916–1557	862–1570	1018–1677	851–1187
	N	27	5	10	9
RANKL/OPG	Median	0.024	0.018	0.024	0.042
	IQR (Q1-Q3)	0.011–0.057	0.011–0.053	0.012–0.033	0.026–0.057
	N	21	4	9	7
IL-6	Median	0.346	0.580	0.315	0.455
	IQR (Q1-Q3)	0.128–0.535	0.325–0.830	0.150–0.640	0.370–0.708
	N	48	7	17	10
Sclerostin	Median	0.196	0.180	0.165	0.175
	IQR (Q1-Q3)	0.150–0.244	0.166–0.200	0.144–0.255	0.140–0.210
	N	49	8	18	10

3.6. Pain response after bisphosphonate therapy

Sixty-four patients were treated with bisphosphonate therapy (66.7 %). Thirty-six patients (56.3 %) received their first dose after blood withdrawal, 17 patients (26.6 %) had received bisphosphonate therapy prior to the blood withdrawal but underwent >6 months wash-out, of which 10 had received only therapy before the study assessments and 7 both before and after. Lastly, 11 patients (17.2 %) were treated with bisphosphonates at the time of blood withdrawal. Of the 43 patients (44.8 %) with treatment after but not during blood withdrawal, 4 had no pain prior to treatment, and data regarding pain response was missing or inconclusive in 3 patients. Of the 36 remaining patients, 22 patients reported improvement in pain scores following bisphosphonate therapy and 14 experienced no improvement. Patients reporting improvement in pain had similar pretreatment RANKL levels compared to patients without improvement in pain (respectively median 24 (Q1-Q3 15–49) pg/mL, $n = 20$, versus median 26 (17–58), $n = 11$, Mann Whitney U: $p = 0.451$). Similarly for the pretreatment RANKL/OPG ratio no difference was observed between groups (group with improvement: median 0.014 (Q1-Q3 0.009–0.026), $n = 10$, versus group without improvement: median 0.019 (Q1-Q3 0.010–0.024), $n = 9$, MWU: $p = 0.740$, neither for IL-6 (group with improvement: 0.33 (Q1-Q3 0.13–0.65) pg/mL, n = 20, group without improvement: median 0.44 (0.18–0.70) pg/mL, $n = 14$, MWU $p = 0.545$), neither for sclerostin (group with improvement: median 0.20 (Q1-Q3 0.15–0.25) ng/mL, $n = 22$, group without improvement: 0.16 (Q1-Q3 0.15–0.22) ng/mL, n = 14, MWU $p = 0.240$).

3.7. Clinical response after denosumab therapy

Thirteen patients (13.5 %) were treated with denosumab. Of those, one received treatment during blood withdrawal, one had received therapy prior to but not after blood withdrawal, and 11 patients used denosumab after blood withdrawal but not during (10 denosumab naive patients and 1 patient who restarted therapy after a wash-out period). Of the latter group, six patients reported an improvement in pain scores while five did not. The concentrations of the biomarkers were evaluated but a statistical comparison between groups was not possible due to the low sample size. The pre-treatment RANKL levels were comparable between the group with and without clinical improvement following denosumab therapy (respectively median 57 (Q1-Q3 19–545) pg/mL, $n = 5$, and 33 (Q1-Q3 20–71) pg/mL, n = 5). The pretreatment RANKL/OPG ratio was slightly higher in patients with improvement in pain scores (median 0.062 (Q1-Q3 0.016–0.331), $n = 5$) than in patients with no improvement (0.027 (0.024–0.046), $n = 4$), although the spread of the data was high. Concentrations of IL-6 seemed to be higher in patients with clinical response (median 0.64 (Q1-Q3 0.53–0.74) pg/mL, $n = 6$) compared to patients without improvement in pain (median 0.35 (0.20–0.54) pg/mL, $n = 5$). Sclerostin levels were median 0.21 (Q1-Q3 0.16–0.28) ng/mL in the patients with improvement in pain scores (n = 6) and 0.17 (0.16–0.18) ng/mL in patients without improvement (n = 5). The correlation of IL-6 and pain was assessed because of the largest difference in groups for this biomarker. IL-6 level correlated with the improvement in maximum pain scores after denosumab therapy (Spearman's rho 0.962, $p < 0.001$, $n = 9$) and with the improvement in average pain scores (rho 0.895, $p = 0.001$, n = 9).

4. Discussion

This study aimed to explore the value of the potential biomarkers RANKL, OPG, IL-6 and sclerostin for assessing disease activity and extent and for predicting and monitoring therapy success in patients with FD/MAS. The results suggested a weak correlation of the RANKL levels, the RANKL/OPG ratio and IL-6 levels with the ALP level, although highly variable data with multiple relevant outliers reduced the strength of this correlation. No correlations were observed between these potential biomarkers and P1NP or CTX, despite the correlations of the classic BTMs with each other. An explanation might be found in the large spread of the laboratory assessments, but nevertheless the absence of robustness of the results implies that RANKL, the RANKL/OPG ratio and IL-6 might not reflect disease activity. Similarly, OPG and sclerostin did not correlate with the BTMs. Selecting merely patients with SBS >15 did not change the results, so apparently the degree of bone remodeling assessed by the BTMs was not adequately captured by the potential biomarkers.

Correspondingly, no correlation was observed between the biomarkers and SBS, even after the exclusion of patients with SBS < 15 or under active FD-related treatment. These results are in contrast with the study of De Castro et al [15], which demonstrated a strong correlation between RANKL and SBS and a moderate correlation between the RANKL/OPG ratio and SBS. This difference may be explained by the considerably higher SBS and less outliers in the cohort of De Castro et al. Apart from SBS, their study provided no patient characteristics, hindering the comparison of patient and disease characteristics between both cohorts. The ELISA kit used for detecting RANKL was similar in both studies, but one major difference was the study population: healthy subjects were included in the study of De Castro, and analyzed with a SBS set at 0, and more severely affected patients with FD/MAS compared to our study. This methodological consideration could explain the differences in the observed correlations between RANKL or the RANKL/OPG ratio and SBS in both studies. Regardless the explanation, these differences confirm that validating previous results in a less severely affected cohort is important before drawing conclusion on the clinical utility of the data. Results of both studies combined suggest that increased concentrations of RANKL or the RANKL/OPG ratio may indicate presence but not severity of FD/MAS, and therefore provide no clinical usefulness over the currently used approach of assessing BTMs

with the SBS on nuclear imaging. In addition, in our study none of the assessed biomarkers correlated with average or maximum pain. Ideally, a biomarker would aid in discriminating FD-related pain from pain arising from other sources and consequently in allocating the most suitable treatment. The identification of such markers is hampered by the lack of a gold standard to distinguish between causes of pain in FD/MAS and the complex pain mechanisms with both nociceptive and neuropathic components involved [26]. Previous research postulated increased osteoclast activity as a possible cause for pain in FD/MAS, with IL-6 and RANKL as key osteoclastic factors [11,26,27], possibly combined with neuropathic changes induced by IL-6, proposed because of the role of IL-6 in bone cancer pain, neuropathic and inflammatory pain [28]. Although elucidating the complex pain mechanisms of FD/MAS in general is of utmost importance, the absence of any correlation between RANKL, IL-6 and pain in our cohort argues against further research on this topic in patients with mild to moderate disease severity. This is intensified by our observation that none of the biomarkers was associated with clinical response after bisphosphonate treatment, as the pre-treatment concentrations of the biomarkers were similar between patients with and without pain response after therapy and therefore not predictive for therapy success. A heterogenous clinical and biochemical response has been described in multiple studies on bisphosphonates, but the reasons for this remain unknown [14,29–33]. The limited treatment response in some patients, combined with emerged results on RANKL overexpression [9,15], has led to research on RANKL-inhibition in FD/MAS. Mice studies demonstrated halted lesion growth and improved mineralization within lesions after therapy [34,35] and in open-label, uncontrolled human studies with the RANKL-inhibitor Denosumab, registered for i.a. osteoporosis, a reduction in pain, BTMs, lesion size and lesion activity was observed [21,22,36]. Similarly, IL-6 inhibition has gained attention in recent years, resulting from studies which detected overexpression of IL-6 in vitro in FD, with a glucocorticoid-induced decline in IL-6 and bone resorption [10,11,37]. A clinical study into the IL-6 inhibitor tocilizumab for patients with FD/MAS did not show benefits for pain or BTMs after therapy in general, although a subset of patients reported a remarkable decrease in pain [38]. Collectively all therapeutic studies in patients with FD/MAS have shown a heterogenous clinical and biochemical response in humans, despite the clear role of RANKL and IL-6 in preclinical studies. None of the studies assessed pre-treatment RANKL and IL-6 levels, and this had led to our hypothesis that high concentrations of RANKL or IL-6 might be predictive for therapy response. Yet, neither RANKL, the RANKL/OPG ratio, IL-6 or sclerostin was associated with bisphosphonate treatment response. These observations dismiss any clinical value of these biomarkers in predicting success of therapy with bisphosphonates over classic BTMs, which are likewise not suitable for this purpose [14,21,22,29,30]. Possibly RANKL, the RANKL/OPG ratio and IL-6 might have some predictive value for clinical response of denosumab, as levels appeared to be higher in patients with adequate response, but the sample size of the subgroup of patients who had received denosumab is too limited to draw firm conclusions. Further research is needed to explore this association, and placebo-controlled studies on denosumab therapy will provide useful information on the effect on bone turnover markers and pain. Researchers may well consider adding assessments of RANKL and IL-6 in these future studies, to evaluate the predictive value of these biomarkers for treatment success.

A limitation of this study is the heterogenous patient population. Serum samples were withdrawn upon hospital intake in most patients, but a subset of patients ($n = 14$) had received previous antiresorptive treatment or was using antiresorptive therapy during the study procedures. This limitation was partially addressed by the sensitivity analysis of excluding patients under active treatment at time of biomarker assessment, which yielded similar results as in the entire population, as well as by assessing the predictive value of the potential biomarkers only in patients who received bisphosphonates or denosumab after but not during blood withdrawal. Lastly, OPG levels were measured merely in 56 patients, constituting the first batch of samples enquired from the biobank. The analyses for RANKL, IL-6 and sclerostin were also performed in the second set of patients, the newly included patients in the biobank between 2019 and 2021, which were retrieved in 2021 to increase sample size, due to preliminary results combined with limited resources, OPG measurements were not performed in this subgroup. Strengths of this study include the overall large sample size for a disorder of this rarity and the exploratory nature of the study. Additionally, this study is the first to assess systemic IL-6 and sclerostin values in patients with FD/MAS. Another important aspect of our study is the validation of results of previous studies on RANKL and OPG, as this demonstrated that results may lack generalizability when conducted in small cohorts subjected to selection bias.

So far, it seems that trying to extrapolate disease activity from systemic markers is not the correct approach and we must conclude that methods that investigate lesion activity like 18F-NaF PET/CT scan are so far the only methods showing a correlation between pain, bone markers and treatment response. Therefore, studies investigating treatment responses should include lesional imaging in order to discuss disease activity, as markers of bone turnover or cytokines related to bone turnover do not provide reliable results. Altogether, this study investigated the clinical usefulness of potential biomarkers: RANKL, OPG, their ratio, IL-6 and sclerostin in an exploratory setting. The assessed biomarkers do not have superior discriminative value compared to the currently used methods in assessing disease extent or activity. In addition, these biomarkers did not correlate with pain and had no value in predicting treatment response of bisphosphonate therapy. Future research may address the role of RANKL and IL-6 in predicting or monitoring treatment success of denosumab, and may shine further light on the complex pathophysiology of FD/MAS to identify possible new treatment targets.

CRediT authorship contribution statement

M.E. Meier: Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **M. Hagelstein-Rotman:** Investigation, Resources, Writing – review & editing. **T.C.M. Streefland:** Investigation, Resources, Validation, Writing – review & editing. **E.M. Winter:** Resources, Writing – review & editing, Project administration. **N. Bravenboer:** Conceptualization, Methodology, Validation, Writing – review & editing, Visualization, Supervision. **N.M. Appelman-Dijkstra:** Conceptualization, Methodology, Formal analysis, Resources, Writing – review & editing, Visualization, Supervision, Funding acquisition, Project administration.

Declaration of competing interest

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Data availability

The authors do not have permission to share data.

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