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ABSTRACT

Denosumab (Dmab) treatment can benefit patients with fibrous dysplasia/McCune-Albright syndrome (FD/MAS) by suppressing the receptor activator of nuclear factor κB ligand (RANKL)-mediated increased bone resorption. However, limited data of two pediatric cases indicate that a rebound phenomenon may occur after withdrawal. Therefore we studied the safety of Dmab discontinuation in FD/MAS. Thirty-seven patients using Dmab, mostly after unsuccessful bisphosphonate (BP) treatment, were included. Health records were screened for pain scores, side effects, and bone turnover markers (BTMs) (calcium, alkaline phosphatase [ALP], procollagen 1 N-terminal propeptide [P1NP], and β-crosslaps [B-CTX, also termed β–C-terminal telopeptide]) during treatment, and for BTMs and clinical rebound effects after withdrawal. BTM levels after withdrawal were compared to pretreatment values. Data were calculated as median (interquartile range [IQR]). BTMs normalized in two-thirds of patients and pain scores decreased significantly during treatment ($p = 0.002$). One patient (2.7%) developed osteonecrosis of the jaw. Sixteen patients discontinued Dmab treatment after a median of 1.6 years (IQR 1.0 years) because of insufficient effect on pain ($n = 10$, 63%), side effects ($n = 4$, 25%), or other reasons ($n = 4$, 25%). Follow-up posttreatment was 3.2 (2.8) years, wherein no fractures, pain flares, or lesion progression occurred. Calcium remained normal in all but one patient, who had a mild asymptomatic hypercalcemia (2.73 mmol/L) 5 months after discontinuation. ALP passed pretreatment levels in five of 11 patients (46%), increased most after 6 months by 18 (43) U/L, and returned to baseline levels thereafter. P1NP exceeded pretreatment levels in four of nine patients (44%), CTX in eight of nine patients (89%). P1NP rose most after 3 months and stabilized thereafter. CTX showed the highest relative elevation. Patients with high pretreatment levels responding well to Dmab seemed to have the highest rebound. These results suggest beneficial effects of Dmab on pain and BTMs, and show a biochemical but asymptomatic rebound phenomenon after withdrawal in adults with FD/MAS, mainly in case of high pretreatment levels, good response, and multiple injections. Further studies on the safety of Dmab and withdrawal are needed and ongoing. © 2021 The Authors. *Journal of Bone and Mineral Research* published by Wiley Periodicals LLC on behalf of American Society for Bone and Mineral Research (ASBMR).

KEY WORDS: FIBROUS DYSPLASIA; McCUNE-ALBRIGHT SYNDROME; DENOSUMAB; WITHDRAWAL; REBOUND

Introduction

Fibrous dysplasia (FD) is a rare genetic disorder characterized by the presence of fibrous skeletal lesions in one or multiple bones (monostotic FD [MFD] vs. polyostotic FD [PFD]), sometimes accompanied by intramuscular myxomas in Mazabraud syndrome (MZB) or hyperfunctioning endocrinopathies in McCune-Albright syndrome (MAS).[1-3] FD/MAS is induced by a postzygotic mutation in the *GNAS* gene, encoding the α-subunit

of the stimulatory G-protein (Gsα). Altering various downstream cascades, the mutation disturbs bone remodeling and endocrine functions in MAS.[4] In bone, the mutation occurs in osteoprogenitor cells (OPCs) and hampers their differentiation into mature osteoblasts. In addition, the mutated OPCs stimulate osteoclast survival and activity through increased levels of cytokines, such as interleukin 6 (IL-6) and receptor activator of nuclear factor κ-B ligand (RANKL). RANKL is a member of the tumor necrosis factor family and has been detected excessively

in both FD bone tissue and serum of patients. RANKL also has been shown to correlate with skeletal burden in FD/MAS.[5-8] The above processes lead to an increase in both bone formation and bone resorption and to the development of weak, immature, woven bone lesions with disturbed adipogenesis and hematopoiesis. These lesions can induce a wide spectrum of symptoms including deformities, pain, and fractures.[9,10] For symptomatic patients, operative or medical treatment may be necessary and patients may require pain medication, surgery or antiresorptive drugs, mainly bisphosphonates (BPs).[10,11] However data on the effect of BPs are scattered with oral preparations not being effective in treating bone pain,[12] and intravenous BPs showing reductions in pain and markers of bone turnover in many patients, but not all.[13-17] The underlying overproduction of RANKL has led to the use of denosumab (Dmab), a humanized monoclonal antibody, mimicking the function of the decoy receptor osteoprotegerin (OPG) by binding to RANKL and preventing the connection to its receptor RANK on the membrane of osteoclasts.[18] As a result, the formation and stimulation of these osteoclasts is inhibited, leading to a suppression of bone resorption and an elevation in bone mass.[19,20] Dmab is mainly used for the treatment of osteoporosis[19,21,22] and skeletal metastases,[23,24] and recently for giant cell tumors of bone.[25,26] In an FD/MAS mouse model, Dmab halted the progression of lesions, induced the formation of mineralized bone within lesions, and prevented the development of new FD lesions.[27] In humans successful treatment has been observed in BP refractory patients, leading to diminished growth rate[28,29] or even regression of lesions,[30,31] to pain reduction and to decreased bone turnover.[32-35] Nevertheless, data on the safety of long-term Dmab use and especially on Dmab withdrawal in FD/MAS have not been reported. Withdrawal from Dmab in osteoporosis patients can provoke rebound increases in markers of bone turnover above pretreatment levels and enhances the risk for multiple vertebral fractures.[36-38] To prevent this, alternative antiresorptive posttreatment should be considered because it might prevent the rebound in bone resorption markers, although its ability to prevent bone loss is still under debate.[39,40] Besides bone loss in FD/MAS there is another concern because in the mouse model the lesions reoccurred after therapy cessation.[27] This was also observed in a case report of a young boy, resulting in such a severe rebound that besides rapid growth a life-threatening hypercalcemia occurred.[28] Also another child demonstrated a severe hypercalcemia after discontinuation from Dmab and lesional growth, although at a slower rate. Because we recently reported the successful treatment of symptomatic FD/MAS with Dmab,[35] we now set out to evaluate the safety of long-term use of Dmab in FD/MAS and to assess the effect of discontinuation on clinical parameters such as pain and fractures and on biochemical parameters such as serum calcium and markers of bone turnover.

PATIENTS AND METHODS

Patient selection

All patients with FD/MAS were included who were treated with Dmab injections and attending the outpatient clinics of the Center for Bone Quality of the Leiden University Medical Center (LUMC) and of the Department of Endocrinology of the Radboud University Medical Center (RUMC) (n = 37). For the management of FD/MAS, a protocolled care trajectory is followed including regular assessments of pain, markers of bone turnover and radiological evaluation. Medical treatment involves supplementation of calcium and vitamin D3 or active D metabolites when necessary; symptomatic patients are, when a mechanic component is excluded, treated with analgesics and intravenous BPs. When these therapies are not tolerated or not (sufficiently) effective, which is for BPs defined as insufficient pain relief with biochemical progressive disease despite adequate treatment according to the guidelines,[18] Dmab (Prolia®; Amgen, Inc., Thousand Oaks, CA, USA) is offered after multidisciplinary consultation as last resort. Oral informed consent for the off-label use of Dmab is obtained from all patients. The present study is part of the PROFID study, a multicenter observational cohort study for FD/MAS, in which data on clinical, biochemical, radiological, and histological parameters are retrieved, as well as treatment effects and questionnaires on pain and quality of life. All patients have given informed consent prior to inclusion in the study. Approval from the Medical Ethics Committee of the LUMC was obtained.

Treatment protocol

A selection of the currently included patients has been described.[35] Currently, at our center Dmab is prescribed in a dose of 60 mg every 3 months.[35] Nine patients started on a -6-monthly schedule, one patient on a 4-monthly schedule, and 21 patients on 3-monthly schedule. Follow-up with clinical and biochemical evaluation including registration of adverse effects was scheduled every 3 months. Radiological examination is performed on indication. According to clinical and biochemical treatment response and side effects, the dosage or interval of Dmab treatment can be adjusted at the discretion of the treating physician, which was done in 12 patients. In nine patients treatment was intensified because of insufficient effect on pain or bone turnover markers and in four patients tapered off because of satisfactory outcomes. After withdrawal, appointments are also scheduled every 3 months.

Data collection

Patient data were collected in an online Castor database. Baseline characteristics were registered such as sex, type of FD, and skeletal burden score (SBS).[41] Clinical data concerning Dmab treatment were retrieved, such as age at start of treatment, treatment dosage and schedule, and adverse effects. Pain was examined during treatment by the Brief Pain Inventory (BPI), assessing scores for maximum, minimum, mean, and current pain on a scale from 0 to 10 as well as pain locations and the use of analgesics. In patients who discontinued treatment, clinical signs of rebound effects such as pain flares, fractures, or radiographic lesion progression were assessed at each visit during the entire follow-up period after withdrawal. All serum laboratory measurements were collected of albumin-corrected calcium and the bone turnover markers alkaline phosphatase (ALP), procollagen type 1 N-terminal propeptide (P1NP), and collagen type 1 C-terminal telopeptide (β-crosslaps [B-CTX]) performed for clinical purposes prior to starting Dmab, during treatment, and after withdrawal in a nonfasting state. Levels of albumin-corrected calcium (calcium reference range, 2.15–2.55 mmol/L) and phosphate (0.90–1.50 mmol/L) were measured by semiautomated techniques. ALP was determined by an automated P800 modulator system (Roche BV, Woerden, The Netherlands), the upper limit of normal (ULN) ALP <98 IU/L for women and <115 for men. P1NP, ULN <59 ng/ml for premenopausal women and

men, and <76 ng/ml for postmenopausal women, and B-CTX, ULN <0.573 ng/ml for premenopausal and <1.008 ng/ml for postmenopausal women, <0.704 ng/ml for men until 70 years and 0.854 ng/ml for men >70 years old, were measured by the E-170 system (Roche BV). The C-terminal of fibroblast growth factor 23 (FGF-23) (Immutopics, San Clemente, CA, USA), ULN <125 RU/ml, was measured by the BioTek ELx50 Washer (BioTek, Bad Friedrichshall, Germany). All analyses were performed according to the manufacturers' protocol. To evaluate treatment response, the values of calcium and the bone turnover markers (BTMs) closest to 12 months after the start of treatment (median 12; interquartile range [IQR] 11–13; range, 10–20 months) were compared with baseline pretreatment levels. Similarly the values of calcium and BTMs after discontinuation were grouped, whereas all measurements performed between two time points were regarded as measured at the first time point, at 3 months (median 5; range, 4–5 months), 6 months (median 7.5; range, 6–9 months) and 12 months (median 13; range, 12–21 months) after the last injection. These values were compared to the last available measurement before or within 3 months after the last injection.

Statistical analyses

Statistical analyses were performed in SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY, USA) after data extraction from Castor. Results are described as number and percentage for categorical data and as median (IQR) for numerical data unless stated otherwise. The courses of scores for maximum and average pain during treatment were analyzed in a linear mixed model. Laboratory marker values were described as absolute values, absolute change, and relative change. Laboratory marker values after 1 year of treatment were compared with pretreatment values in a Wilcoxon signed rank test in case of symmetrical distribution of differences and in a sign test in the remainder of the comparisons. For laboratory marker values after withdrawal the number of patients was calculated in whom laboratory markers after withdrawal exceeded pretreatment levels and exceeded the individual upper reference limit at any time during the first 18 months after the last injection. Significance level was set at $p < 0.05$. Spaghetti graphs were computed to depict the percentage change in calcium and BTMs after withdrawal. Graphs were designed in GraphPad Prism, version 8.4.2 (GraphPad Software, La Jolla, CA, USA).

RESULTS

Patients' characteristics

Thirty-seven patients (Table 1) were treated with Dmab (24 females (64.9%), of whom eight were postmenopausal). Seven patients had MFD (18.9%), but the majority of patients had PFD ($n = 21$, 56.8%). Nine patients were diagnosed with MAS (24.3%). The median SBS was 11.8 (IQR 23.6). A total of 34 patients (91.9%) were previously treated with BP therapy, with a median cumulative dose of 713 mg (range, 0–6336 mg) over 6.0 years (range, 0–22.1 years). This treatment was in all cases unsatisfactory. Three patients had not used BPs. One of them was diagnosed with osteoporosis, for which Dmab treatment was initiated elsewhere, and two patients had FD in the thoracic region, one with MFD and one with MAS, and preferred Dmab over BP therapy because of fear of side effects. Six patients had received a single administration whereas 31 patients were

Table 1. Baseline characteristics

Characteristic	Value
Gender, n (%)	
Female	24 (64.9)
Male	13 (35.1)
Age at start of denosumab therapy (years), median (IQR)	42 (19)
Type of FD, n (%)	
Monostotic	7 (18.9)
Polyostotic	21 (56.8)
MAS	9 (24.3)
Skeletal burden score, median (IQR)	11.8 (23.6)
Bone turnover markers prior to denosumab therapy, median (IQR)	
Corrected calcium (mmol/L) ($n = 37$; normal 2.15–2.55 mmol/L)	2.31 (0.13)
Phosphate (mmol/L) ($n = 36$; normal 0.90–1.50 mmol/L)	0.96 (0.25)
ALP (U/L) ($n = 37$; normal: women <98 U/L, men <115 U/L)	103 (127)
P1NP (ng/ml) ($n = 33$; normal: men and premenopausal women <59 ng/ml; postmenopausal women <76 ng/ml)	85 (259)
B-CTX (ng/ml) ($n = 33$; normal: women, premenopausal <0.573, postmenopausal <1.008 ng/ml; men ≤70 years <0.704 ng/ml, >70 years <0.854 ng/mL)	0.306 (0.245)
FGF-23 (RU/ml) ($n = 25$, normal <125 RU/ml)	132 (84)
BP therapy	
BP use prior to denosumab, n (%)	34 (91.9)
Duration (years), median (IQR)	5.3 (0–22.1)

Abbreviations: ALP, alkaline phosphatase; B-CTX, β-crosslaps; BP, bisphosphonate; FD, fibrous dysplasia; FGF-23, fibroblast growth factor 23; IQR, interquartile range; MAS, McClune-Albright syndrome; P1NP, procollagen 1 N-terminal propeptide; RU, relative units.

treated with multiple injections over a median duration of 3.5 years (IQR 3.0; range, 0.5–5.3 years), with a median of 11 injections (IQR 13; range, 2–20 injections) in a median cumulative dose of 660 mg (IQR 900; range, 120–1860 mg). Reasons for withdrawal are discussed below in Outcomes of Withdrawal from Dmab.

Treatment outcomes

Laboratory markers

Median ALP at baseline was 103 U/L (IQR 127 U/L) and was elevated in 20 of 37 patients (54.1%). ALP normalized in 14 of 20 patients (70%) and decreased to 83 U/L (IQR 52 U/L) after 1 year of treatment (sign test $p = 0.001$) (Table 2, Fig. 1). P1NP and B-CTX were available at baseline in 33 patients and in 29 patients also during treatment. At baseline, P1NP was elevated in 20 of 33 patients (60.1%), and normal values were reached in 14 of them (70%) while on treatment. After 1 year of treatment P1NP diminished from 85 ng/mL (IQR 259 ng/mL) to 36 ng/mL (153 ng/mL) (sign test $p = 0.005$). B-CTX before treatment exceeded the ULN in four of 33 patients (11.4%) and normalized in three of them (75%) during treatment.

Table 2. Course of bone turnover markers before treatment, during treatment, and after withdrawal (n = 11)

BTM	Last before treatment (value)	After 1 year of treatment		6 months after withdrawal		12 months after withdrawal	
		Value	Change[a]	Value	Change[b]	Value	Change[b]
Ca (mmol/L)	2.31 (0.13)	2.37 (0.17)	−0.01 (0.22)	2.36 (0.17)	+0.2 (0.43)	2.33 (0.12)	+0.01 (0.20)
ALP (U/L)	103 (127)	83 (52)	−35 (83)	94 (170)	+18 (43)	86 (127)	+10 (66)
P1NP (ng/ml)	85 (259)	36 (153)	−45 (85)	62 (401)	+40 (132)	81 (56)	+35 (50)
B-CTX (ng/ml)	0.31 (0.25)	0.13 (0.25)	−0.12 (0.37)	0.34 (0.86)	+0.17 (0.42)	0.34 (0.40)	+0.27 (0.41)

Notes: Data are median (IQR). Normal ranges: Ca 2.15–2.55 mmol/L; ALP women <98 U/L, men <115 U/L; P1NP men and premenopausal women <59 ng/ml, postmenopausal women <76 ng/ml; B-CTX premenopausal women <0.573 ng/ml, postmenopausal women <1.008 ng/ml, men ≤70 years <0.704 ng/ml, men >70 years <0.854 ng/ml.

Abbreviations: ALP, alkaline phosphatase; B-CTX, β-crosslaps; IQR, interquartile range; P1NP, procollagen 1 N-terminal propeptide.

[a]Compared to last before treatment.
[b]Compared to last before withdrawal.

B-CTX declined from 0.31 (IQR 0.27) ng/mL at baseline to 0.13 (0.25) ng/mL after 1 year (Wilcoxon signed rank test $p = 0.044$) (Table 2, Fig. 1).

Pain and use of analgesics

Pain scores fluctuated in many patients during treatment. Scores during the first 2 years of treatment were included in the linear mixed model analysis because of a diminishing number of measurements thereafter. During this time frame scores for maximum pain decreased significantly from a mean ± standard deviation (SD) of 6.0 ± 2.7 out of 10 at baseline by $\beta = 0.09$ per month (95% confidence interval [CI], 0.03–0.15; $p = 0.002$). In other words, after 11 months of treatment maximum pain scores declined on average by 1 point (95% CI, 0.4–1.6), and after 2 years on average by 2.7 points (95% CI, 0.8–3.6). Scores for average pain decreased significantly from a mean ± SD of 4.4 ± 2.4 at baseline by $\beta = 0.05$ per month (95% CI, 0.005–0.099; $p = 0.48$), indicating a decline of 1.2 points (95% CI, 0.01–2.2) after 2 years of treatment. Analgesics were used by 18 patients (48.6%) at the start of Dmab therapy. Fourteen patients (37.8%) did not use any analgesics and data were missing in five patients (13.5%). Of those 18 patients, six were taking opioids. Four of them continued the use of opiates during Dmab therapy (66.7%), one could decrease the dosage of the opioids (16.7%), and one patient (16.7%) could withdraw completely from all painkillers during Dmab therapy. Of the remaining 12 patients using acetaminophen and/or nonsteroidal anti-inflammatory drugs (NSAIDs) prior to Dmab, three (25%) could reduce the use of pain killers, seven (58.3%) continued the medication, and two patients (16.7%) used analgesics infrequently. Of the 14 patients not using analgesics before the first Dmab injection, four (28.6%) used acetaminophen or NSAIDs infrequently and 10 (71.4%) used no analgesics during Dmab treatment. Four patients specifically reported that Dmab therapy had increased their quality of life despite unchanged pain levels in three of them. This occurred because these patients felt less tired and more active or because more (physical) activity was possible.

Side effects

Twenty patients (51.4%) reported in total 37 adverse effects. Side effects were severe in two patients: one patient (2.7%) reported oral blisters and one patient (2.7%) developed osteonecrosis of the jaw (ONJ). The latter was a 35-year-old male who was diagnosed with MAS with an SBS of 31.40 and had received 360 mg of Dmab in 1.5 years. He had also been treated with BPs for 12 years, but this was stopped 8 years before the start of Dmab treatment. The ONJ developed after a dental procedure without antibiotics prophylaxis nor primary suture of the lesion. In both patients Dmab therapy was discontinued because of these side effects. The majority of the side effects were mild to moderate, specifically malaise (n = 7, 18.9%), skin problems, mainly rash or dryness (n = 6, 16.2%), muscle pain of stiffness (n = 5, 13.5%), flare in FD-related pain (n = 5, 13.5%), which was in all cases self-limiting, diarrhea and flatulence (n = 3, 8.1%), fatigue (n = 2, 5.4%), tooth sensitivity (n = 2, 5.4%), nausea and vomiting (n = 1, 2.7%), headache (n = 1, 2.7%), brittle nails (n = 1, 2.7%), recurrent respiratory tract infections (n = 1, 2.7%), and the sensation of bladder pressure (n = 1, 2.7%) (Table 2).

Outcomes of withdrawal from Dmab

Clinical parameters

In 16 of 37 patients (43%) the use of Dmab therapy was ceased (Table 3). Two patients had MAS (14.3%), four had MFD (28.6%), and eight had PFD (57.2%). Six patients received a single injection and 10 patients were treated with multiple injections (median 4; IQR 6; range, 3–11) in a median cumulative dose of 240 mg (IQR 390; range, 120–780 mg) during a median duration of 1.6 years (IQR 1.0; range, 1.0–4.9 years). Reasons for withdrawal were (a combination of) insufficient relief of pain (n = 10, 62.5%) or side effects (n = 4, 25%) being osteonecrosis of the jaw (n = 1, 6.3%), oral blisters (n = 1), rash (n = 1), or both muscle ache and respiratory tract infections (n = 1). Other reasons for withdrawal were family planning (n = 1), complete pain alleviation after surgery (n = 1), treated osteoporosis (n = 1), and long-term use (n = 1). The median clinical follow up after the last injection was 3.2 years (IQR 2.8 years). No patients experienced a flare in FD-related pain after withdrawal. Moreover, no (atypical) fractures nor lesional growth were observed.

Laboratory marker values

Laboratory markers after withdrawal were available in 11 patients (Table 2, Fig. 1), of whom two received a single injection and eight had multiple injections. In eight of 11 patients (72.7%) calcium levels exceeded pretreatment levels within 18 months after withdrawal. However, in all but one patient (90.9%) calcium remained below the upper limit of normal. In one patient a mild, asymptomatic hypercalcemia of 2.73 mmol/L was observed 5 months after the last injection (Fig. 1C,D, patient 3). The
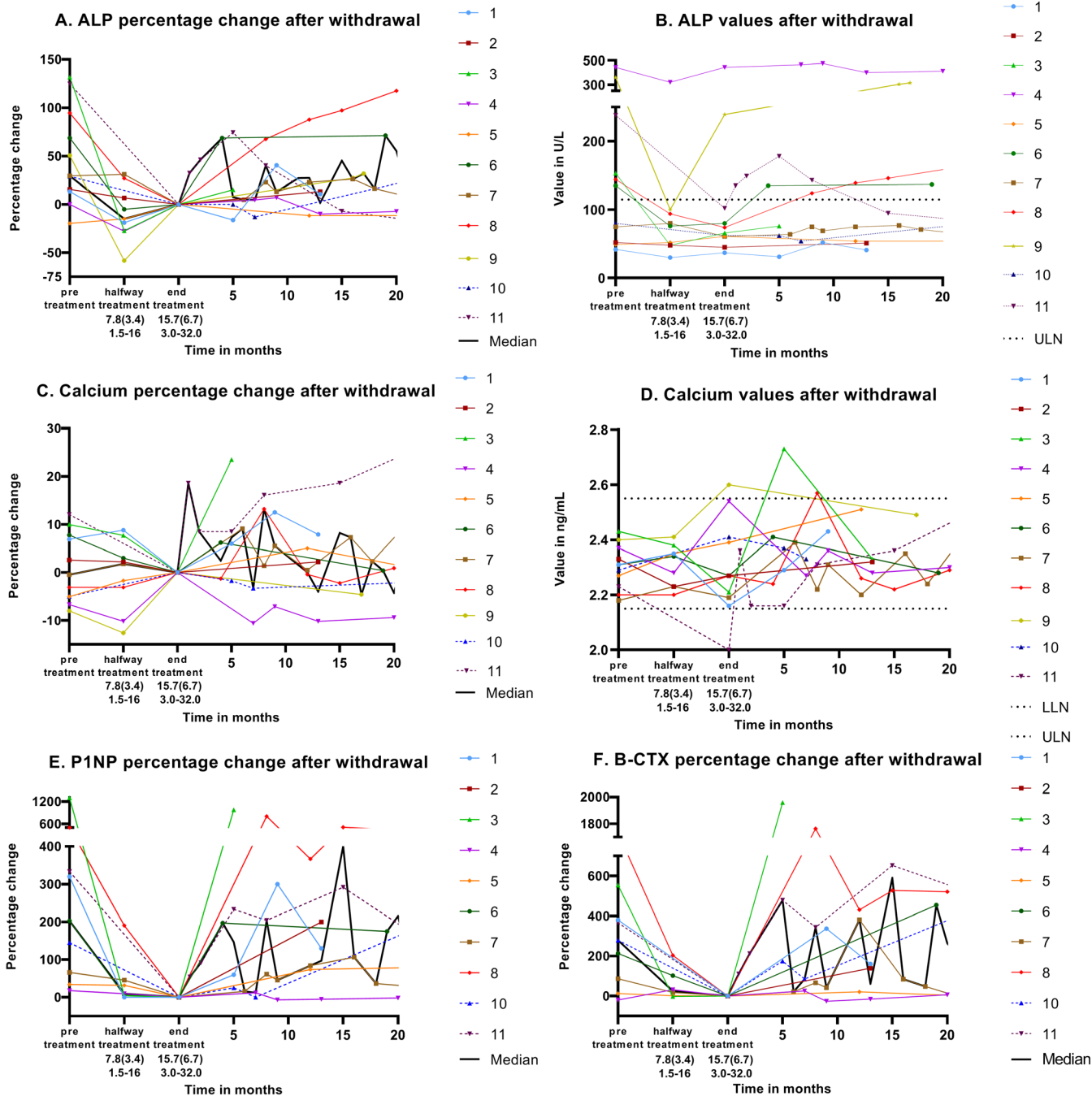
Fig. 1. (*A–F*) Levels of calcium and of markers of bone turnovers ALP, P1NP, and B-CTX during treatment and after withdrawal. Graphs demonstrating the values of albumin corrected calcium, ALP, B-CTX, and P1NP before treatment, halfway during treatment, at the end of denosumab treatment and after withdrawal. The numbers below halfway and end treatment correspond to the median (interquartile range) and range. The dotted lines of patient 10 and 11 represent the patients who received a single injection. Calcium values remain generally stable and below the ULN, except for one patient. ALP, P1NP, and B-CTX show a distinct increase after withdrawal in several patients, mainly in patients with high pretreatment levels. The steepest slope is observed in the first months after withdrawal. However in a subset of the patients the bone turnover markers remain quite stable. Abbreviations: ALP, alkaline phosphatase; B-CTX, β-crosslaps; LLN, lower limit of normal; P1NP, procollagen 1 N-terminal propeptide; ULN, upper limit of normal.

patient was diagnosed with MAS and had used Dmab for 2.1 years (Table 4, patient 3). Treatment to decrease the calcium was not necessary. After 6 months the calcium was 2.45 mmol/mL, but Dmab had already been restarted because of gradually increasing FD-related pain. The median calcium level of the entire cohort increased slightly in the first 6 months after withdrawal but did not exceed the upper limit of normal (Table 2).

In nine of 11 patients (81.8%) at least one BTM exceeded pretreatment values and in seven of them (77.8%), at least one of

these BTMs also passed the ULN. In two patients (18.2%) all BTMs exceeded pretreatment levels, although in one patient ALP and B-CTX remained below the ULN (Fig. 1).

ALP exceeded pretreatment levels after withdrawal in five of 11 patients (45.5%), but remained below the ULN in two. Median

ALP levels peaked at 94 U/L (IQR 170 U/L) at 6 months, due to a median increase of 18 U/L (IQR 43 U/L), and stabilized after 12 months. The highest increase in ALP was +25% compared to pretreatment values (Table 2, Fig. 1A,B).

P1NP and B-CTX levels after cessation were available in 10 patients, but in one patient pretreatment values were missing. In four of nine (44.4%) patients P1NP exceeded pretreatment values, in three of them above the ULN. The greatest increase in P1NP was 153% compared to pretreatment. Median P1NP was 24 ng/ml (IQR 22 ng/ml) at the end of treatment, rose to 80 ng/ml (157 ng/ml) 3 months after withdrawal and remained elevated around that level afterward (Table 2, Fig. 1E).

Of all laboratory markers, CTX showed the highest relative increase after Dmab discontinuation (Fig. 1). Median B-CTX during treatment was 0.067 ng/ml (IQR 0.081 ng/ml) and rose to 0.29 ng/ml (IQR 0.18 ng/ml) after 6 months of withdrawal. In eight of nine patients (88.9%), B-CTX levels exceeded pretreatment levels, in four of them (50%) above the ULN. The maximum rise in B-CTX was +209% compared to pretreatment values. Overall, of all BTMs exceeding pretreatment values ($n = 17$), median time to stabilization was 12.5 months (IQR 17 months) (Table 2, Fig. 1F).

The laboratory markers calcium, ALP, B-CTX, and P1NP seem to increase and decrease simultaneously, because a few patients account for the elevations in all four laboratory marker values, whereas some other patients appear to remain stable in all values, as is shown in Fig. 1. Mainly calcium, B-CTX, and P1NP

Table 3. Adverse effects of denosumab therapy

Adverse effects	n (%)
Malaise	7 (18.9)
Skin problems	6 (16.2)
Muscle pain or stiffness	5 (13.5)
Flare in FD-related pain	5 (13.5)
Diarrhea/flatulence	3 (8.1)
Fatigue	2 (5.4)
Tooth sensitivity	2 (5.4)
Nausea/vomiting	1 (2.7)
Headache	1 (2.7)
Brittle nails	1 (2.7)
Multiple respiratory tract infections	1 (2.7)
Sensation of bladder pressure	1 (2.7)
Vertigo	1 (2.7)
Transient hyperparathyroidism	1 (2.7)

Note: Number of patients with adverse effects: 20 (54.1% of total); number of adverse effects: 37.

Abbreviations: FD, fibrous dysplasia.

Table 4. Characteristics of patients withdrawn from denosumab therapy

Patient ID	Age at start denosumab (years), gender	Type of FD	Skeletal burden score	Duration of bisphosphonate use (years)/cumulative dose (mg)	Duration of denosumab use (years)/cumulative dose (mg)	Denosumab scheme	Follow-up after last injection (years)
1	26, F	MFD	0.5	6.6/691	1.2/240	60 mg sc/3 months	1.6
2	59, F	PFD	2.8	10.0/75	1.6/180	60 mg sc/6 months	1.6
3	43, F	MAS	44.2	22.0/2204	2.1/540	60 mg sc/3 months	1.3
4	33, F	PFD	25.1	11.8/2314	2.7/780	60 mg sc/6 months > 60 mg/4 months > 120 mg/3 months	3.2
5	54, F	MFD	0.8	4.9/521	1.6/240	60 mg sc/6 months	2.5
6	28, F	PFD	13.8	8.6/4740	1.3/120	60 mg sc/6 months	3.6
7	42, F	PFD	0.4	2.5/3160	1.0/180	60 mg sc/6 months	4.1
8	33, M	MAS	31.4	11.8/3305	1.5/360	60 mg sc/3 months	3.2
9	38, M	PFD	43.3	0.01/0.60	1.3/240	60 mg sc/6 months > 60 mg/3 months > 60 mg/6 months	1.6
10	63, F	MFD	0.3	0/0	0/60	Single injection	4.8
11	58, F	PFD	0.1	2.2/735	0/60	Single injection	2.8
12	34, F	PFD	8.5	1.9/7	4.9/660	60 mg sc/6 months > 60 mg/10 months > 60 mg/8 months > 60 mg/3 months	0.4
13	39, M	PFD	15.8	3.0/1607	0/60	Single injection	4.7
14	42, F	PFD	17.1	4.8/20	0/60	Single injection	4.4
15	39, M	PFD	16.5	1.5/157	0/60	Single injection	4.1
16	63, M	MFD	0.4	0/0	0/60	Single injection	4.8

Abbreviations: F, female; FD, fibrous dysplasia; M, male; MAS, McClune-Albright syndrome; MFD, monostotic FD; PFD, polyostotic FD; sc, subcutaneously.

follow identical curves. Patients 3 and 8 exhibit the highest rebound (Fig. 1). These patients were affected with MAS, had high pretreatment levels of BTMs and an excellent biochemical response to Dmab. They had used 2204 and 3305 mg BPs over 22 and 12 years, respectively, and had used high doses of Dmab (540 and 360 mg) over 2.1 and 1.5 years, respectively. Patients with low rebound mainly had low levels before treatment; those patients had MFD or PFD, but not MAS, and had used variable amounts of BPs and of Dmab. Remarkably, the patient with the highest pretreatment levels, the longest duration of Dmab use, and the highest cumulative dose showed a low rebound phenomenon. In this patient the levels of the BTMs remained high throughout the entire treatment. BTMs hardly increased after withdrawal: ALP rose from 444 to 475 U/L, P1NP from 1050 to 1177 ng/ml, B-CTX from 0.745 to 0.936 ng/ml, and calcium decreased after discontinuation. The two patients receiving a single injection of Dmab showed mild rebound effects. In one patient, all four laboratory markers remained within normal limits, although calcium, P1NP, and B-CTX slightly exceeded pretreatment levels. The other patient had elevations in ALP and P1NP to above the upper normal limit, although not above pretreatment values, and in calcium and B-CTX over pretreatment values but not over the ULN.

Antiresorptive therapy after withdrawal

Dmab therapy had been restarted after withdrawal in two patients (12.5%). In both patients the most severe pain did not respond well to Dmab and was surgically treated. However, after a while the FD-related pain recurred in other lesions, for which Dmab was restarted successfully. BP therapy was never acutely indicated because of rebound phenomenon. One patient had received zoledronic acid for a slow incline in P1NP levels. Six patients were treated with BP therapy because FD bone pain increased gradually over time, one patient received zoledronic acid according to the treatment protocol for osteoporosis, one patient received zoledronic acid twice after 5 years of Dmab therapy according to the treatment protocol for long-term use, and in seven patients no further antiresorptive therapy was prescribed.

Discussion

The primary aim of this study was to evaluate the safety of withdrawal from Dmab treatment in patients with FD/MAS. Withdrawal from Dmab has not caused flares in FD-related pain in our cohort, neither were fractures observed, nor progression of the lesions, although patients were under surveillance for a median of 3.2 years. However, the main concern regarding the discontinuation of Dmab is the rise in serum BTMs and calcium, because two children have been reported who developed severe rebound effects in BTMs as well as hypercalcemia after discontinuation of therapy.[28] Hazardous hypercalcemia was not observed in our cohort; one patient developed a mild, asymptomatic hypercalcemia (serum calcium: 2.73 mmol/L) 5 months after the last injection. Next to hypercalcemia, this patient had a prominent increase in B-CTX, reflective of a very active bone resorption. An explanation for the discrepancy between our cohort and the two reported pediatric cases is that we only treated adults with matured skeletons of whom 92% were pretreated with BPs. Despite the absence of hypercalcemia, the majority of patients showed a relapse in BTMs to around pretreatment levels and some to above pretreatment levels. Values

after discontinuation became at maximum 25% higher than pretreatment for ALP, 143% for P1NP, and 203% for B-CTX. Stabilization was observed after 1 year. In osteoporosis patients, BTMs also exceeded the pretreatment levels or the upper normal limit after discontinuation of Dmab, and rebound increases in BTMs were observed 3 to 9 months after the last injection, although individual responses were variable just like in our study.[38,40] In our cohort patients with large rebound effects had high pretreatment levels of BTMs, had received high cumulative doses of Dmab, and had a good treatment response. On the contrary, patients with low pretreatment levels tended to lack rebound effects, and remarkably hardly any rebound was observed in a patient with high pretreatment levels, but poor response to Dmab, although treated with the longest duration and highest cumulative dose. These observations may cautiously indicate that the degree of bone turnover before treatment combined with the magnitude of the skeletal improvement on treatment are more important contributors to the rebound phenomenon than the duration or cumulative dose of Dmab. In patients with osteoporosis, the rebound after Dmab discontinuation appears to be larger in patients with a higher pretreatment bone turnover, after a longer treatment duration, or after higher-dose therapy, whereas pretreatment with BPs was found to be protective against a rebound, although consensus on this topic has not yet been reached.[39,42-48] A hypothesis for the mechanism behind the rebound phenomenon is that an excessive remodeling rate occurs after withdrawal from Dmab. In mice it is observed that OPG, the natural decoy receptor for RANKL mimicked by Dmab, induced the fission of active, multinucleated osteoclasts into smaller, less active daughter cells termed *osteomorphs* by McDonald et al.[49] Upon withdrawal from OPG, the osteomorphs quickly reassembled into bone resorbing osteoclasts and bone resorption rebounded. In humans after Dmab discontinuation a similar process may occur, leading to a decrease in trabecular bone volume more than in cortical bone volume, and to an elevated amount of unmineralized bone.[44,49-51] Such a mechanism could be aggravated by a long treatment duration of Dmab, a high cumulative dose, or by a good response to therapy. Our results support this hypothesis, because patients with a good response and supposedly a high number of inactivated osteomorphs had a higher rebound and vice versa, although in our data the effect of treatment duration remains questionable. Another hypothesis is that of a mechanostatic reset, where a homeostatic, intrinsic mechanism defines the setpoint of bone remodeling in a patient, and where the suppressed bone turnover naturally regresses to this setpoint after discontinuation of antiresorptive therapy.[52] This theory is supported by two studies with osteoporosis patients, in which a correlation was observed between the gain in bone mineral density after Dmab therapy and the loss after discontinuation, although it has to be noted that patients with more severe disease generally require a longer treatment exposure in order to reach treatment targets.[36,48] Specifically for FD/MAS this theory is of great interest, because both the activity and the extent of bone lesions are markedly variable between patients.[35,53] Our data provides preliminary support for this theory, because in most patients the BTMs returned to roughly around pretreatment levels, but further research is needed to confirm these findings and to provide further evidence for the mechanisms responsible for the relapse or rebound in BTMs in FD/MAS. Another feature of the two patients in our study with the largest rebound effect was the presence of MAS. Likewise further research is needed to clarify whether the hormonal abnormalities in MAS are contributing

to the rebound effect or were in our cohort merely a coexisting trait in these patients.

In addition, our results show that Dmab therapy had beneficial effects on bone turnover in the majority of patients in our cohort. In two-thirds of patients with elevated levels of ALP, P1NP or B-CTX normalization was observed during treatment. Moreover, all markers showed a significant and clinically relevant decrease after 1 year of treatment. The results also suggest a beneficial effect on bone pain, because maximum pain scores declined significantly by 2.7 points (95% CI, 0.8–3.6) in the first 2 years of treatment, a patient-determined clinically relevant response.[54] However, in future studies it should be investigated whether this finding resulted from a treatment effect or whether concepts such as regression to the main or the placebo effect interfered with our results. Nevertheless, it is important to remember that pain scores alone are not sufficient to reflect the benefits of Dmab therapy. This is illustrated by three patients who felt more energetic despite unchanged pain levels. Moreover, one in four patients were able to reduce the dosage of or withdraw from analgesics.

Concerning adverse effects, in our cohort one patient (2.7%) developed ONJ after a dental procedure without antibiotics prophylaxis nor primary sutures of the wound. The risk for ONJ in FD/MAS on Dmab has never been studied but it is postulated that patients with a low SBS (<10) have a higher risk for developing ONJ, because the large amount of non-FD bone is particularly at risk for ONJ.[55] Our results do not support this hypothesis, because the patient with ONJ had an SBS of 31.4. The risk for ONJ in our cohort is comparable to the incidence in patients receiving Dmab for solid tumors (1.7%)[56] and for osteoporosis (0–3.0%),[22,57] although these risks are hard to compare due to variable patient characteristics, treatment schedules, and research methods. Supposedly the risk is not higher than the risk for ONJ in FD/MAS patients treated with BPs where incidences from 0% to 5.4% have been reported.[17,58]

Our study is the largest on the treatment with Dmab in FD/MAS and the first to assess the safety of Dmab discontinuation. A limitation of our study is that BPIs and laboratory marker values were assessed at normal follow-up before, during, and after treatment and that time points may vary. Especially after withdrawal some patients have merely one or a few available measurements, which complicates the interpretation of the direction of the rebound and whether it is resolving or exacerbating. In addition, in some patients the laboratory measurements were first evaluated several months after withdrawal, when it cannot be ruled out that an rebound occurred and resolved rapidly and unnoticed. Future studies with standardized laboratory measurements should address this issue, which are currently ongoing (https://clinicaltrials.gov/ct2/show/NCT03571191). Nevertheless, even when rebounding BTMs would be present but missed, we still did not observe clinically relevant consequences of high calcium levels, such as vomiting, or of an excessive bone remodeling, such as fractures. Another limitation is the lack of standardized treatment schemes in our patients. Patients were treated with either 60 or 120 mg of Dmab in intervals of 2, 3, 4, or 6 months according to their clinical and biochemical response. Currently, an initial treatment scheme of 60 mg Dmab every 3 months is recommended based our previous cohort study, which analyzed a subset of the patients included in this present study.[35] On the other hand, this heterogeneity in treatment schemes as well as in patient characteristics makes this study generalizable to the clinical situation. Last, the amount of patients who ceased Dmab treatment was rather small, 14 patients, although this is still the largest case series until now. Small populations are a common problem in research on rare diseases. The absence of fractures in this cohort after discontinuation of Dmab is reassuring, albeit a much larger cohort is needed to assess a risk similar to the risk for multiple vertebral fractures after withdrawal in case of osteoporosis.[36,40]

Notwithstanding these limitations, our results suggest a beneficial effect of Dmab treatment on pain, on the use of analgesics, as well as on BTMs. Future research should determine whether Dmab is indeed more effective than placebo in relieving pain and whether Dmab could prevent the development or growth of FD lesions. Similarly, the incidence of ONJ needs further attention. In addition, the currently recommended treatment scheme should be investigated in a larger randomized controlled trial and the safety and efficacy of Dmab in children requires more research.[55]

Concerning withdrawal, the results show that cessation of Dmab induces a biochemical rebound in BTMs and possibly in calcium, but without significant morbidity, acute deterioration, or hospital admittance. We cautiously suggest that withdrawal can be considered when Dmab treatment is no longer indicated. Nevertheless, Dmab should initially only be applied when BPs are not tolerated or not effective and only in a tertiary referral center with ample experience in the management of FD/MAS. After discontinuation, close biochemical and clinical surveillance is recommended and additional antiresorptive therapy should be considered. More research is needed into the extent of the observed rebound phenomenon, into risk factors as well as into the histologic response to cessation. Last, treatment regimens to prevent or treat discontinuation rebound should be explored.

Disclosures

Author Contributions

Stance N. Clerkx: data curation; formal analysis; investigation; resources; writing; review; editing. Elizabeth M. Winter: conceptualization; resources; writing; review; editing. Alberto M. Pereira: resources; writing; review; editing. Annenienke C. van de Ven: data curation; investigation; resources; writing; review; editing. Michiel AJ van de Sande: funding acquisition; supervision; writing; review; editing. Natasha M. Appelman-Dijkstra: conceptualization; data curation; funding acquisition; methodology; resources; supervision; validation; writing; review; editing.

Peer Review

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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