INNOVATIVE THERAPIES IN BONE BIOLOGY: WHAT CAN BE LEARNED FROM RARE BONE DISEASES?

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PUBLISHED IN: Frontiers in Endocrinology

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88976-498-3
DOI 10.3389/978-2-88976-498-3

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Citation: Eekhoff, E. M. W., De Vries, T. J., Van Hul, W., Sakkers, R., eds. (2022). Innovative Therapies in Bone Biology: What can be Learned From Rare Bone Diseases? Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88976-498-3

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frontiers | Frontiers in Endocrinology

EDITORIAL
published: 09 June 2022
doi: 10.3389/fendo.2022.928667

Editorial: Innovative Therapies in Bone Biology: What Can Be Learned From Rare Bone Diseases?

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Keywords: rare bone disease, innovative, innovative, therapies, monitoring, collaboration

Editorial on the Research Topic

Innovative Therapies in Bone Biology: What can be Learned from Rare Bone Diseases?

OPEN ACCESS

Edited and reviewed by:
Jonathan H. Tobias,
University of Bristol, United Kingdom

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Specialty section:
This article was submitted to Bone Research, a section of the journal Frontiers in Endocrinology

Received: *26 April 2022*
Accepted: *27 April 2022*
Published: *09 June 2022*

Citation:
Eekhoff EMW, de Vries TJ, Sakkers RJB and Van Hul W (2022) Editorial: Innovative Therapies in Bone Biology: What Can Be Learned From Rare Bone Diseases? Front. Endocrinol. 13:928667. doi: 10.3389/fendo.2022.928667

INTRODUCTION

Due to their rarity and heterogeneity, rare bone diseases have been overlooked in recent decades, but the tide is turning. New developments in research models [see also the related, simultaneously hosted Research Topic "Innovative Models in Bone Biology: What can be Learned from Rare Bone Diseases?" (1)] and genetics help to gain more insight into the pathogenesis. But there is still a long way to go before effective treatments will be found for the various rare bone diseases.

In this Research Topic, 13 articles describe innovative and up-to-date options to improve the present treatment of rare bone diseases, including organization of care, setting up partnerships, innovative treatment monitoring, reuse of existing therapies and the developmental route to new therapies for various rare bone diseases.

HOW TO ORGANIZE PATIENT CARE AND ENTER INTO PARTNERSHIPS TO ENABLE OPTIMAL CARE AND SUPPORT FOR NEW TREATMENT OPTIONS?

Due to the often extreme manifestation of rare bone diseases, collaboration of dedicated specialists and researchers with specialized expertise targeting this small number of patients is necessary to improve good clinical care. An organizational form that can achieve such shared goals through a

committed, valued mentality of the employees is the organizational collaboration model, a good working example of which is described in a stimulating way in the article "Collaboration on rare bone diseases leads to the Unique Organizational Drive of the Amsterdam Bone Center" (Eekhoff et al.).

INNOVATIVE MONITORING OPTIONS OF NEW TREATMENTS

Change in bone properties during a new therapy can be monitored *in vitro* by analyzing sequential bone biopsies. (Treurniet et al.) provide an update on new options to characterize and explore these biopsy features. A summary of the current numerous techniques is elegantly described. Interestingly, it is now also possible to directly evaluate some properties of bone that contribute to bone strength, *in vivo*. This is possible with impact micro-indentation (IMI). Schoeb et al. describe its current developments and applicability.

Unambiguous international methods and cut-off points/ definitions for recording clinical measurements is of great importance. This becomes clear in an interesting overview of the current knowledge and clinical implications of hyperkyphosis on health by Koele et al. They show that when not using a fixed standard definition, the power of pooling many research results is complicated and thus hinders interpretation of the various studies. When not using the same standard definition, it is hard to compare (intervention) studies on hyperkyphosis.

NEW TREATMENT OPTIONS FOR RARE BONE DISEASES BY REUSE OR BY INNOVATIVE APPROACHES

Following a previously described concept, in a bone graft-implant study, commonly used medication was successfully and innovatively re-applied by Bhadada et al. for pain relief in a patient with fibrous dysplasia. In this study, intralesional administration of bisphosphonates was found to be more effective than intravenous administration. Likewise, bisphosphonates had a positive treatment effect in patients with sternocostoclavicular hyperostosis. Here, intravenous pamidronate was effective in reducing pain and improving shoulder function and also led to decreased bone turnover on skeletal scintigraphy Leerling et al.

While great progress can be made in understanding the pathogenesis of a disease, developing effective therapy can still be lagging behind, which has been elegantly described by Ralston and Gaston. They wrote a comprehensive review of current knowledge and future treatment options for Osteogenesis Imperfecta (OI). While explaining the shortcomings that remain in the current treatment, possible new treatment options were put into perspective, including the ongoing "reuse" studies and the status of new drug development.

Kloen et al. describe a combined surgical approach to address a non-union fracture along with a novel patellar fracture in a patient with OI. These fractures are among the rare injuries associated with a disrupted quadriceps extension mechanism in patients with OI. They put the result of this operation in the context of a literature review, which encourages to consider new combined approaches in OI surgery, if needed.

By monitoring treatments given for other concomitant diseases such as cancer, there is also a lot to learn about its effect on the underlying rare bone disease itself, as is the case with radiotherapy in fibrodysplasia ossificans progressiva (FOP). In this way, based on a case study and literature, possible future radiotherapy options for FOP lesions have been put into perspective by Botman et al.

In addition, Botman et al. showed in a second article that in emergency situations, surgery can be mandatory for an FOP patient. Such decisions require stringent innovative collaboration between various experts.

NEW CHALLENGING SOLUTIONS FOR RARE BONE DISEASES

An example of a new bold procedure to solve a clinical problem in a patient with Gorham-Stout disease is presented in the article by de Keyser et al. In this article the authors give an interesting review on the current knowledge of this very rare bone disease for which still no real or proven treatment options exist.

INNOVATIVE RESEARCH AND COLLABORATIVE APPROACHES TO FIND A CURE FOR RARE BONE DISEASES:

At present, the importance of different types of generated mouse models, human cell models and mutual collaboration in research is of great importance to find a good and effective therapy for various complex rare bone diseases. This also concerns fibrous dysplasia and McCune Albright syndrome, which are caused by a postzygotic mutation in the *GNAS* gene, leading to mosaic expression. The background of this rare bone disease, the current state of research and the difficulty and importance of finding a better therapy is explained and summarized by Lung et al.

A 1-week international scientific workshop on FOP provided by and according to the concept of the Lorentz Center has led to a concise overview of current knowledge and scientific gaps, described by de Ruiter et al. Such workshops are of vital importance in rare bone disease research, since bringing all researchers on a rare bone disease together provides an effective platform to update each other on the latest research. It further contributes to a clear roadmap for future research, joint efforts and

the scientific steps to be taken to find a cure for FOP. This workshop is an example of how international cooperation can be promoted.

CONCLUSION

The 13 contributions to this Research Topic on innovative therapies in rare bone diseases have highlighted the progress and developments of potentially new treatment options, treatment monitoring, collaboration and improvement of care in rare bone diseases.

It is clear that much research remains to be done. Only through increased knowledge of the underlying cellular processes, we can initiate new research models. These will lead to new drugs or to reuse of existing drugs. Above all, through collaboration, this can lead to novel treatment options to the benefit of the patient. The current topic provides a comprehensive overview of the status of some important rare bone diseases, with special emphasis on novel treatments. These described novelties may inspire others and can serve as an example or guideline for new developments in research, collaboration and care pathways in other rare bone diseases.

AUTHOR CONTRIBUTIONS

EE designed, wrote, and submitted the editorial. TV, RS and WH contributed to the design, writing and editing of the editorial. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

We thank Gudrun Stenbeck, Guillaume Mabilleau, Elaine Dennison, editors of Frontiers in Endocrinology, for managing five manuscripts that had a conflict of interest with the editors of this topic.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CASE REPORT
published: 19 November 2019
doi: 10.3389/fendo.2019.00803

Co-administration of Systemic and Intralesional Zoledronic Acid in a Case of Fibrous Dysplasia: A Potentially Novel Therapy

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Fibrous dysplasia (FD) is a benign bone lesion characterized by replacement of normal bone with abnormal fibrous tissue, clinically manifesting as deformities, bone pains, and pathological fractures. The standard medical management for FD includes systemic bisphosphonate therapy. The efficacy of systemic bisphosphonate is however limited with minimal functional improvement and pain relief. Keeping the above lacunae in mind, we have made a solitary attempt at treating FD with locally administered zoledronic acid. A 25-year-old gentleman had presented to our institute with swelling and pain involving the left thigh and left lower leg. He was diagnosed as having polyostotic FD, confirmed on bone histopathology. He was administered 4 mg of zoledronic acid intravenously while 1 mg of the drug was injected locally into the femoral lesion under ultrasound and fluoroscopy guidance. There were no peri-procedural complications. At 6 months follow-up, there was marked improvement in pain scores at the left thigh, while that at the left leg remained unchanged. In addition, repeat bone scintigraphy showed a 20.8% and 25.3% reduction in anterior and posterior uptake values, respectively, at the left femur while that at the left tibia remained unaltered.

Keywords: fibrous dysplasia (FD), bisphosphonate, zoledronate, local bisphosphonate, implant fixation

OPEN ACCESS

Edited by:
Elisabeth Marelise W. Eekhoff, VU University Medical Center, Netherlands

Reviewed by:
Panagiotis Anagnostis, Aristotle University of Thessaloniki, Greece
Stefano Pagano, University of Perugia, Italy

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Specialty section:
This article was submitted to Bone Research, a section of the journal Frontiers in Endocrinology

Received: *09 July 2019*
Accepted: *04 November 2019*
Published: *19 November 2019*

Citation:
Bhadada SK, Pal R, Sood A, Dhiman V and Saini UC (2019) Co-administration of Systemic and Intralesional Zoledronic Acid in a Case of Fibrous Dysplasia: A Potentially Novel Therapy. Front. Endocrinol. 10:803. doi: 10.3389/fendo.2019.00803

BACKGROUND

Fibrous dysplasia (FD) is a benign bone lesion characterized by replacement of normal bone by an excessive proliferation of cellular fibrous connective tissue intermixed with irregular trabecula (1). Long bones are most commonly affected. Three percent of all the cases occur in association with café-au-lait macules and/or hyper-functioning endocrinopathy (most common being precocious puberty), an entity referred to as McCune-Albright syndrome (MAS) (2, 3). FD has three clinical patterns, namely monostotic, polyostotic, and craniofacial forms (1). Clinically, patients with monostotic FD are usually asymptomatic and have a limited tendency to progress; presentation with pain, limp, or radiological evidence of microfracture predicts disease progression (4). Instead, polyostotic FD usually present with bone pains, fragility fractures, deformities, and facial asymmetries (2). MAS patients have the most extensive bone disease and regularly experience multiple fractures requiring recurrent surgical interventions (5). Diagnosis is usually based on plain radiographs that show an expansile radiolucent "ground-glass" lesion. Isotope bone scintigraphy

delineates the entire extent of the disease. Histopathology shows irregular trabeculae of woven bones (giving a "Chinese letter" pattern) without an osteoblastic rim (6). The lesions are often lined by an unusually large number of osteoclasts (7). FD results from activating mutations in *GNAS1* gene (most common being R201H substitution) that codes for $G_S\alpha$ protein. Constitutive activation of $G_S\alpha$ leads to overproduction of cAMP in bone marrow stromal cells (BMSCs) causing osteoblast maturation arrest and unrestricted proliferation of unorganized masses of fibro-osseous tissues (8). cAMP increases IL-6 production by BMSCs that activates osteoclasts with consequent bone resorption and expansion of FD lesions. Rarely, malignant transformation of FD can occur with reported prevalence ranging from 0.4 to 4.0% (6).

Medical treatment of FD involves use of bisphosphonates, either administered orally or intravenously. Bisphosphonates inhibit osteoclast-mediated further bone resorption, preserve cortical bone mass and thereby reduce fracture risk (9). In addition, oral alendronate therapy and intravenously administered zoledronate/pamidronate have been shown to reduce bone turnover and partially suppress disease activity in polyostotic FD with no significant effect on pain or functional parameters (10, 11). Keeping these limitations of currently available treatment modalities in mind, we went ahead with the combination of systemic and intralesional administration of zoledronic acid in a patient with polyostotic FD, an endeavor that has hitherto never been undertaken. The concept of intralesional administration of this drug stemmed from prior studies wherein local application of bisphosphonates have been used as a means of counteracting secondary bone resorption following bone grafting and promoting early implant fixation (12–19). Bisphosphonates, either incorporated into implants or surface coated onto implants prevent bone resorption and actively promote bone regrowth into endoprosthesis porosities, thereby extending the durability of implants (18). In a double-blinded randomized control trial of 50 patients, application of 1 ml of ibandronate to the tibial bone surface led to improved prosthesis fixation following knee replacement (16).

CASE PRESENTATION

A 25-year-old gentleman presented to us with pain and swelling in the left thigh and shin. He had noticed the swelling at the age of 10 years and had been increasing ever since. Bony pain at the left thigh and shin was of recent onset. He denied any history of fractures or proximal muscle weakness. He was not on any medications other than over-the-counter analgesics for symptomatic pain relief. Physical examination revealed bony hard swellings involving the whole of left thigh and anterior part of the left mid-shin (**Figures 1A,B**). Pain was assessed using the subjective 11-point Numeric Pain Rating Scale that has been widely used for assessing severity of pain in FD (20, 21). The pain score at left thigh was 8/10 and at left leg was 7/10. There were no similar swellings in other parts of the body. He did not have any café-au-lait macules. Radiograph of the affected part showed a deformed left femur and left ischium with multiple expansile lytic areas (**Figure 1C**). Radiograph of the left leg showed a similar lesion involving the upper and mid-third of tibia. Bone scintigraphy showed increased tracer uptake involving the left ischium, femur and part of tibia (**Figures 2A,C**, anterior and posterior views, respectively). There was no evidence of increased tracer uptake in other parts of the skeleton. Bone biopsy from the femoral lesion showed irregular trabeculae of woven bones suggestive of FD. Biochemical panel revealed normocalcemia and normophosphatemia. He was vitamin D sufficient. Bone turnover markers [P1NP = 143 ng/ml (age-specific range: 38.5–86 ng/ml) and CTX = 920 pg/ml (age-specific range: 112–584 pg/ml)] were elevated. His thyroid function test, insulin-like growth factor 1 (IGF-1) and testosterone levels were all normal, ruling out any endocrinopathy and MAS.

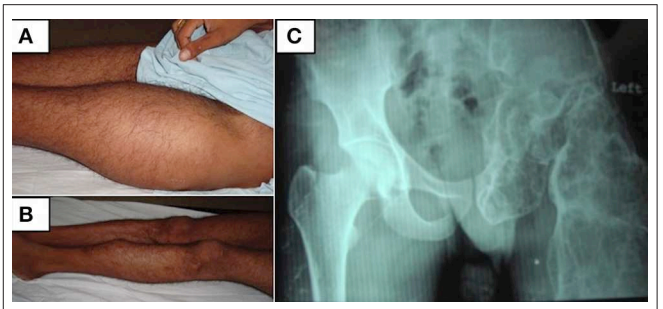

FIGURE 1 | (A,B) Clinical photographs of the patient showing swellings involving the whole of left thigh and anterior part of the left mid-shin. **(C)** Radiograph of the pelvis showing a grossly deformed left femur and left ischium with multiple expansile lytic areas.

After taking informed consent from the patient, 4 mg of zoledronic acid was administered intravenously. Simultaneously, 3 ml of zoledronic acid, containing 1 mg of the drug was injected within the dysplastic lesion involving the left femur. Under ultrasound guidance, a Jamshidi needle was inserted into the shaft of the left femur along the lateral aspect at a junction of the upper and middle third. The position of the tip of the needle was confirmed using C-arm fluoroscopy. The trocar was removed and subsequently the required amount of the drug was slowly injected. There were no intra-procedural complications. The following day he developed high-grade fever that lasted for 2 days, likely attributable to systemically administered zoledronate.

At 6 months follow-up, there was no noticeable change in the swelling of the left thigh or left shin. However, pain scores at left thigh was reduced to 4/10 while that in left shin remained unchanged at 7/10. He had not developed any fractures in this interim period. Complete biochemical panel was unremarkable. Bone turnover markers were slightly reduced from baseline values (P1NP = 105.3 ng/ml, CTX = 710 pg/ml). Bone scintigraphy was repeated; when compared with the baseline scan, there was a reduction in uptake at left thigh and no change at left shin. The anterior uptake values at left thigh and left tibia at baseline were 3.07 and 3.55, respectively (**Figure 2A**); at 6 months, the corresponding values were 2.43 and 3.60, respectively (**Figure 2B**). Similarly, the posterior uptake values at left thigh and left shin at baseline were 2.25 and 1.46, respectively (**Figure 2C**); the corresponding values at 6 months follow-up were 1.68 and 1.48, respectively (**Figure 2D**) (summarized in **Table 1**).

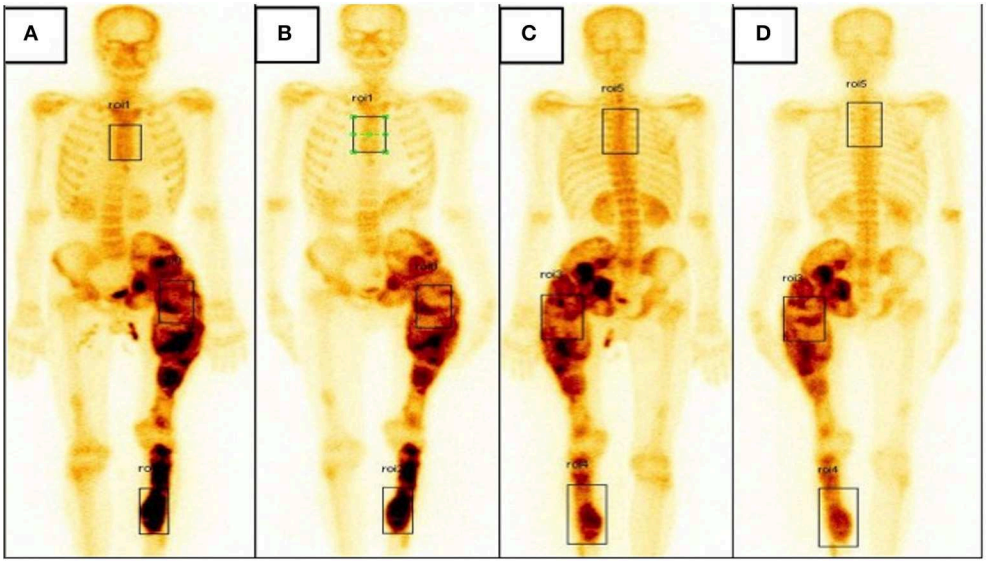

FIGURE 2 | (A,B) Bone scintigraphy image, anterior view showing increased tracer uptake involving the left ischium, femur and part of tibia at baseline **(A)** and slightly reduced tracer uptake at the left femur when followed up at 6 months **(B)**. **(C,D)** Bone scintigraphy image, posterior view showing increased tracer uptake involving the left ischium, femur, and part of tibia at baseline **(C)** and slightly reduced tracer uptake at the left femur when followed up at 6 months **(D)**.

TABLE 1 | Table showing anterior and posterior uptake values on bone scintigraphy at the left thigh and left tibia pre and 6 months post intravenous and intralesional zoledronate therapy.

	Left thigh		Left shin	
	Anterior uptake value	**Posterior uptake value**	**Anterior uptake value**	**Posterior uptake value**
Pre-zoledronate	3.07	2.25	3.55	1.46
Post-zoledronate	2.43	1.68	3.60	1.48
Percentage change (%)	−20.8	−25.3	+1.4	+1.3

DISCUSSION

We have demonstrated efficacy of intralesional zoledronate over and above systemic bisphosphonate in the treatment of FD. Our index patient was administered systemic zoledronate at a recommended dose of 4 mg followed by intralesional bisphosphonate at the femoral lesion with the tibial lesion acting as an auto-control. At 6 months follow-up, there was marked improvement in the pain scores at left thigh with no change at left shin. Moreover, bone scintigraphy showed reduction in uptake at left femur and no change at left tibia, adding testimony to the fact that locally administered bisphosphonate is effective and perhaps acts synergistically with the systemically administered drug in markedly reducing osteoclast activity.

Management of FD is challenging with systemic bisphosphonates being the treatment of choice. Systemic bisphosphonates do reduce bone turnover markers, however, improvement in pain and functional parameters are debatable (10, 11). There is a dire need for new treatment modalities for the management of FD. Keeping this in mind, we came up with the innovative idea of intralesional injection of bisphosphonate in addition to the conventional administration of the drug by intravenous route. The idea stemmed from observations that locally administered bisphosphonates is an effective means of enhancing bone-implant fixation. Bisphosphonates, acting locally has been shown to suppress osteoclast function at the bone-implant interface; at the same time it has been shown to activate osteoblast activity, promoting bone-implant integration (18). Systemic bisphosphonates administered in a patient with FD are concentrated at the sites of the lesions, however, only 50% of the intravenously administered drug is available for incorporation in the bone matrix (22), hence, the concentration can be expected to be much lower compared to the locally injected drug. At such low concentrations, local bone turnover at the FD lesions are minimally suppressed, as was evident at the left tibia of our index patient. When administered locally over and above the systemically administered one, the two perhaps acts synergistically leading to more profound suppression of local bone turnover, resulting in reduction of uptake values and pain scores. In addition, the osteo-anabolic activity of local bisphosphonate coupled with increased osteoclast inhibition might have contributed to transformation of immature woven to more mature lamellar bone and subsequently reduced tracer uptake on scintigraphy. A repeat bone biopsy at follow-up would have been required to prove or disprove the aforementioned hypothesis, however, the patient did not consent for the same. Moreover the higher concentrations of the drug achieved locally with intralesional administration might allow for smaller and less frequent dosing. The efficacy of locally administered bisphosphonate can further be augmented by the use of drug-coated scaffolds or carriers that would increase the biological permanence of the drug at the desired site of action

(23). In addition, this would further reduce the systemic side effects of bisphosphonate (that includes infusion-related reactions, myalgias, cutaneous reactions, osteonecrosis of jaw, atrial fibrillation, uveitis, and nephrotoxicity) which is otherwise negligible with local application of the drug (24, 25).

In conclusion, we have demonstrated a novel approach to effectively treat fibrous dysplasia. How practical will be this treatment modality in polyostotic FD is certainly debatable, however, it can certainly be considered as an option in patients with monostotic FD. A phase 3 trial on the efficacy of local bisphosphonate in reducing recurrence rates in extremity giant cell tumor of bone is currently underway (NCT 03295981). Similar, large-scale randomized-controlled trials need to be undertaken comparing the efficacy of intralesional bisphosphonate over and above the systemic drug in FD.

DATA AVAILABILITY STATEMENT

All datasets for this study are included in the article/supplementary material.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institute Ethics Committee PGIMER, Chandigarh. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

SB had conceived the idea of intralesional zoledronate therapy. RP had prepared the manuscript. AS had provided the bone scintigraphy images. VD had edited and revised the manuscript. US had performed bone biopsy and helped us in administering zoledronate intralesionally. All the four authors had approved the final revised version of the manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

MINI REVIEW
published: 24 January 2020
doi: 10.3389/fendo.2020.00005

The Clinical Relevance of Hyperkyphosis: A Narrative Review

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The kyphosis angle of the thoracic spine tends to increase with aging. Hyperkyphosis is a kyphosis angle, exceeding the normal range. This narrative literature review aims to provide an overview of the current literature concerning kyphosis measurement methods, the etiology and adverse health effects of hyperkyphosis. As of yet, a well-defined threshold for hyperkyphosis is lacking. To attain more generalizability and to be able to compare study results in older adults, we propose to define age-related hyperkyphosis as a Cobb angle of 50° or more in standing position. Hyperkyphosis may be a potentially modifiable risk factor for adverse health outcomes, like fall risk and fractures. Additionally, hyperkyphosis may indicate the presence of osteoporosis, which is treatable. Prospective and intervention studies, using a Cobb angle of 50° as a clear and uniform definition of hyperkyphosis, are warranted to investigate the clinical relevance of hyperkyphosis.

Keywords: hyperkyphosis, kyphosis, older adults, fracture, fall, measurement, review

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Bone Research, a section of the journal Frontiers in Endocrinology

Received: *02 September 2019*
Accepted: *07 January 2020*
Published: *24 January 2020*

Citation:
Koelé MC, Lems WF and Willems HC (2020) The Clinical Relevance of Hyperkyphosis: A Narrative Review. Front. Endocrinol. 11:5. doi: 10.3389/fendo.2020.00005

INTRODUCTION

Kyphosis is the curvature of the thoracic spine, formed by the shape of the vertebrae and the intervertebral discs and–in standing position–paraspinal muscle strength. Hyperkyphosis is present when the kyphosis angle exceeds the normal ranges. Apart from the consequences of normal aging, like decreasing muscle strength (1) and degenerative changes of the spine (2), other factors contribute to the increase of the kyphosis angle. Vertebral fractures are present in no less than 40% of the persons with hyperkyphosis (3), and with each vertebral fracture the kyphosis angle increases with 3.8° (4). There is growing evidence showing an association between hyperkyphosis and negative health effects, like a decreased physical performance and a doubled fall risk (4–15).

Currently, numerous kyphosis measurement methods have been used in literature and a clear definition of hyperkyphosis is lacking. If we had a uniform definition of hyperkyphosis, the association with adverse health effects and prognostic value of hyperkyphosis as well as the effectiveness of interventions could be investigated better. This review aims to provide an updated overview of the current studies and to conclude whether hyperkyphosis is relevant for clinical practice. We will discuss the etiology and adverse health effects of hyperkyphosis, and will focus on kyphosis measurement methods. Based on the literature described, we will propose to define hyperkyphosis as a Cobb angle of 50° or more in standing position.

METHODS

We conducted a literature search of PubMed and Embase from 1947 up to now, using the following search terms and derivatives: kyphosis, hyperkyphosis and thoracic spine. We screened the

abstracts (9238) and included 74 studies assessing kyphosis measurement methods, the pathogenesis of hyperkyphosis or the association with clinically relevant outcomes. We excluded non-English studies, duplicate or overlapping articles intervention studies assessing the effect of surgical procedures and studies in children or in participants with hyperkyphosis caused by disease and scoliosis.

Kyphosis Measurement Methods

The Cobb angle is considered to be the current gold standard method to measure kyphosis (16). Initially, the Cobb angle was developed to assess scoliosis angles. By modifying the direction of radiographic imaging from frontal to sagittal projection, the Cobb angle became useful to assess kyphosis angles (17). The vertebrae superior to the fourth thoracic vertebra (T4) are often less well visible due to over projection of other structures. Therefore, commonly the angle between T4 and T12 is used. The Cobb angle is measured by drawing a line through the superior endplate of T4 and a second line through the inferior endplate of T12. At the intersection of these two lines, the Cobb angle can be measured (**Figure 1**).

In addition to the Cobb angle, several clinimetric kyphosis measurement methods have been developed. A protractor is used to measure the kyphosis angle with the Debrunner kyphometer (18), goniometer (19), arcometer (20), and inclinometer (21). The upper arm of the protractor is placed on C7 or T1, and the lower arm on T12. Two other devices—the flexicurve ruler (22) and the spinal mouse (23)—document the contour of the spine. The flexicurve ruler is molded to the spine from C7 in caudal direction. The kyphosis index is the width divided by the length of the thoracic curve. The spinal mouse is a device with accelerometers, detecting distance and changes of inclination while rolled over the spine. Finally, the occiput-to-wall distance (OWD) and the blocks method are used to quantify kyphosis (**Figure 2**).

Every measurement method has its own characteristics, advantages and disadvantages. While the Cobb angle has the advantage of providing information on the anatomy of the vertebrae and spinal alignment, radiation exposure is inevitable. High interrater and intrarater reliability have been described in studies with well-trained examiners to score the Cobb angles. The correlation coefficients range from 0.80 to 1.00 (4, 7, 24, 25), which may be expected to be lower when performed in clinical practice by less experienced examiners. The clinimetric measurement methods make radiation exposure redundant. Some clinimetric measurement methods are easy to use in clinical practice and the result instantly available.

However, the correlation with the Cobb angle ranges extensively from low (0.28) to high (up to 0.92) (7, 19, 24, 26–28). These large differences may be explained by the variety in kyphosis measurement methods regarding the position of the person during measurement and which part of the spine is measured. In supine position, the back is passively stretched and the influence of muscle strength may be diminished when compared to a standing position. Most of the measurement methods only measure the curve of the thoracic spine. Yet the blocks method also takes the cervical spine into account, and the OWD is influenced by the posture of the patient when standing.

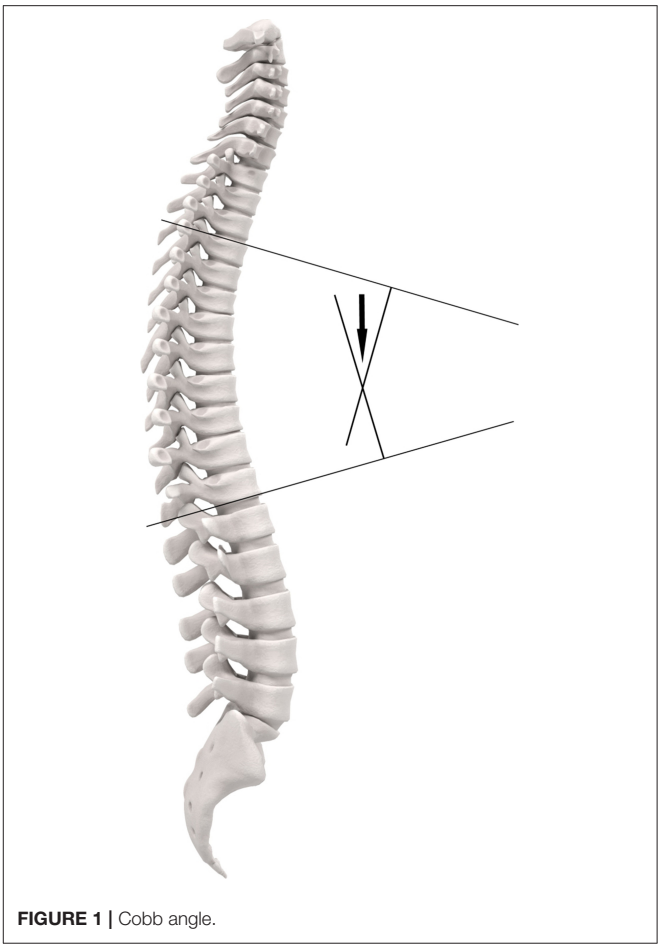

FIGURE 1 | Cobb angle.

Thus, in addition to the gold standard kyphosis measurement–the Cobb angle–various kyphosis measurement methods have been used in literature. Correlation among the measurement methods ranges extensively, possibly reflecting the large differences between methods.

Definition

With aging, kyphosis tends to increase. In younger adults, the Cobb angle averages from 20 to 29° (17). After the fourth decade, the kyphosis angle increases (17). In two cohort studies among older women, the Cobb angle increased with 2.6° in 3 years and 7° in 15 years (5), and 3.9 in 4 years (29). The mean kyphosis angle ranges from 35 to 38° in adults, aged 65 years and older (30, 31). In a cohort of older End Stage Renal Disease patients, the mean Cobb angle was 41° (32). In another cohort with women aged 65 years and older, the mean kyphosis angle increased with age from 47 to 52° (33). However, these values were measured in a cohort with underlying osteoporosis, with potentially more vertebral fractures and thereby a higher mean Cobb angle than the general population.

Currently, a well-defined threshold, differentiating between normal kyphosis and hyperkyphosis, is lacking. In some studies, the 95th percentile of the Cobb angle in younger adults is

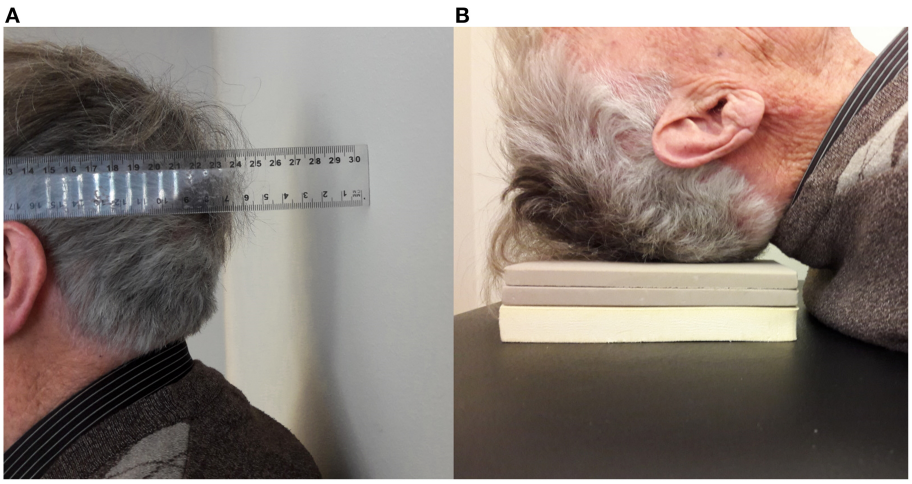

FIGURE 2 | (A) Occiput-to-wall distance and **(B)** Blocks method.

used as threshold (17). As the mean angle in older adults ranges from 35 to 52°, the prevalence of hyperkyphosis may be overestimated in these studies. In other studies, a higher threshold value of 50° is used (6, 14, 29, 34). When using these threshold values, hyperkyphosis is present in 20–40% of the older adults (7, 27, 35, 36). Even higher prevalences of up to 55% are reported in a geriatric population (14). The abundance of kyphosis measurement methods makes a large number of other definitions of hyperkyphosis necessary. Yet for some measurement methods no threshold value could be found in literature, and for some kyphosis measurement methods the threshold value differs between studies. McDaniels-Davidson et al. defined hyperkyphosis as 54°, measured with the Debrunner kyphometer and 17, measured with the Flexicurve ruler (6). Hyperkyphosis, measured with the OWD, is defined in literature as 4 cm (15, 37) or 5 cm (14, 38). Different threshold values to define hyperkyphosis are used for the blocks method: ≥1 block (39), ≥2 blocks (36, 40), and ≥4 blocks (6).

In conclusion, a large number of kyphosis measurement methods with various threshold values for hyperkyphosis are used in addition to the gold standard method, the Cobb angle Generalizability and comparison of study results is therefore limited. Though preferable in order to pursue more uniformity in hyperkyphosis research, differentiating between normal and abnormal kyphosis angles remains difficult.

PATHOGENESIS

Vertebral Fractures and Degenerative Disc Disease

The pathogenesis of hyperkyphosis has not yet been completely elucidated. Anterior wedging of the vertebrae and asymmetrical compression of the intervertebral discs may result in an increase of the kyphosis angle (41). In adults with vertebral fractures, hyperkyphosis is more prevalent (3, 33, 42). Kado et al. showed that with each compression fracture, the kyphosis angle increased with 3.8° (4). Yet, only 40% of the patients with hyperkyphosis has vertebral fractures (3), which suggests that other risk factors may play a role. With aging, the intervertebral discs desiccate. This process is referred to as degenerative disc disease. Manns et al. showed a significant correlation between anterior disc height and kyphosis angle ($r = -0.34$, $p < 0.001$) and a negative correlation to age ($r = -0.30$, $p = 0.01$), potentially indicating that disc degeneration is not a disease, but merely part of normal aging (43).

As most studies are cross-sectional, it is unclear whether degenerative disc disease is a cause or consequence of hyperkyphosis. Only Kado et al. has reported an association between degenerative disc disease and hyperkyphosis–and not kyphosis progression–in a longitudinal study. However, due to the retrospective design, no conclusions could be drawn on causality of the two phenomena (4). Another possibility is that–rather than being cause or consequence–degenerative disc disease and hyperkyphosis enhance each other. Anterior compression of the intervertebral discs may increase the kyphosis angle, and this in turn may enhance further compression of the discs.

Muscle Strength

Besides the vertebrae and intervertebral discs, paraspinal muscle strength may influence kyphosis. Back extensor muscle strength has been shown to be inversely correlated to kyphosis (44, 45). Hyperkyphosis may be an indicator of frailty, as grip strength is one of the Fried criteria. However, the association between kyphosis angle and grip strength remains controversial, as some cohort studies report a positive association (9, 40), and others a negative association (5, 46).

Genetic Predisposition

In some heritable diseases like Scheuermann's disease, hyperkyphosis is seen at an early age. Kado et al. reported that independent of vertebral fractures and bone mineral density

(BMD), women with 1–2 parents with hyperkyphosis had on average 2.6° worse kyphosis angle compared to women with parents without hyperkyphosis (4). A twin study among 241 twins found a heritability estimate of 61% (95%CI 46–72) (47). In the Framingham study, the heritability estimate was reported to be 54% (95%CI 43–64%) (48). Mouse knock-out and transgenic models show that hyperkyphosis may be enhanced by mutations in the genes involved in DNA repair and delaying senescence (49, 50).

ADVERSE HEALTH EFFECTS OF HYPERKYPHOSIS

Physical Performance

A large number of cohort studies has investigated the association of the kyphosis angle and physical performance (4, 5, 7–11, 51). In all studies, except the study of Demarteau et al., multivariate analyses were performed to adjust for age and comorbidity including vertebral fractures or BMD. Only Katzman et al. investigated this association prospectively in a large cohort of women (mean age 68 years) (5). Performance time on the Timed Up and Go test (TUG) increased with increasing kyphosis angle. Although statistically significant, the effect size of this difference is very small. Similar to this study, all studies consistently report a statistical significant lower physical performance in hyperkyphotic participants, potentially indicating publication bias. Yet, various kyphosis measurement methods and physical performance tests have been used, and reported differences are small. Therefore, the clinical relevance of this association between hyperkyphosis and physical performance is questionable.

Falls

The majority of studies, including two studies with a prospective design, show that hyperkyphosis is associated with falls (6, 12, 14, 15, 39). One relatively small study ($n = 73$) may have overestimated the association, as age was not added in the multivariate analyses (12). One prospective and two cross-sectional studies found no association between hyperkyphosis and falls (13, 32, 52).

The underlying cause of the increased fall risk in older adults with an increased kyphosis angle may be balance disruption due to a forward shift of the center of gravity of the body (53). Indeed, older adults with hyperkyphosis have an increased postural sway, wider stance and reduced gait speed (38, 53). However, conflicting results have been reported on the association between balance and hyperkyphosis. Some studies report a positive correlation (13, 34, 54), while others found no correlation between hyperkyphosis and impaired balance (7, 55). This difference may partly be explained by the balance test used, as clinical tests like used in one of the two negative studies (7), may be less sensitive to detect balance problems than post-urography, which is used in the studies reporting a positive association. Significant methodological limitations of the before mentioned studies may be a second reason for the conflicting results of the studies (7, 34, 54, 55). Only the study of Ishikawa et al. adjusted for potential confounders (13).

In conclusion, the majority of studies shows that in adjusted analyses, hyperkyphosis is associated to falls. Whether impaired balance is the underlying mechanism of the increased fall risk in persons with hyperkyphosis, is currently unknown.

Fractures

Hyperkyphosis increases pressure on the anterior part of the vertebrae. Consequent vertebral fractures may therefore be expected. Huang et al. indeed reported an increased risk of vertebral fractures in women (mean age 71 years) with hyperkyphosis (adjusted OR 1.7, 95%CI 1.0–3.0) (25). This is confirmed in another longitudinal study of Kado et al. in women (mean age 69 years) with hyperkyphosis (HR 1.50, 95%CI 1.10–2.06, model adjusted for age and BMD). Change of the Cobb angle was also independently associated to fracture risk (HR 1.28, 95%CI 1.06–1.55) (29). Opposite to these results, one large cohort study among older women (mean age 68 years) with low BMD or prevalent vertebral fracture reported no association (IRR 1.08, 95%CI 0.96–1.22, model adjusted for age and BMD). Change of the kyphosis angle was not associated with fracture risk (42).

Thus, conflicting results regarding the association between hyperkyphosis and future fractures have been reported in women with low BMD (25, 29, 42). These differences may be explained by the difference in regression models applied in the studies. The studies reporting a positive association have applied logistic regression and Cox regression, while the study reporting no association used Poisson regression. The chance of a future vertebral fracture is dependent on previous fractures. Therefore, the standard error is smaller and the confidence interval too narrow, which makes the test statistic too high and the estimated effect of the predictor on the outcome too high. Poisson regression corrects for the type I error caused by the correlation between a first fracture and next fractures. Therefore, the studies of Huang et al. and Kado et al. may have overestimated the effect of hyperkyphosis on fracture incidence.

Pulmonary Function

Literature on the association of hyperkyphosis with pulmonary function is scarce. Increased thoracic kyphosis may cause mechanical restriction of pulmonary function, as reported in all four articles included in the systematic review of Harrison et al. (56). Older adults with hyperkyphosis have more often dyspnoea and decreased vital capacity (57, 58) and forced expiratory volume (58, 59). Lombardi et al. was the only study, in which correlations were unadjusted (58). The retrospective study of Lee et al., found no association with acute respiratory failure in 51 hyperkophotic participants (unadjusted HR 3.20, 95%CI 0.86–12.14) (60).

Thus, consistent results on the association between hyperkyphosis and pulmonary function have been reported, though internal and external validity of the studies is limited. Whether hyperkyphosis leads to a higher incidence of diseases like pneumonia or COPD, is yet unknown.

Mortality

Four large cohort studies report that hyperkyphosis is associated with a higher all-cause mortality (32, 36, 61, 62). In the Rancho-Bernardo cohort, the odds ratio was 1.40 (95%CI 1.07–1.82) in the multivariable model adjusted for age, gender, smoking, physical activity and BMD (36). Goto et al. reported an association between hyperkyphosis and mortality in end stage renal disease patients, yet they may have overestimated the association as they did not adjust for potential confounders (32). Mortality rates increase with increasing kyphosis angle in older women with osteoporosis in the for age and comorbidity adjusted model (61), possibly reflecting the number of osteoporotic fractures and thus the severity of osteoporosis.

Pain

Remarkably, only in a few studies the association between hyperkyphosis and pain has been investigated (33, 63–65). Three out of four studies adjusted for age (33, 63, 64). All studies except Ettinger et al. (63) report a positive correlation or association with pain.

Quality of Life

As mentioned above, several negative health conditions, like pain and lower physical performance, have been linked to hyperkyphosis. Lower quality of life may therefore be a logical consequence. Less satisfaction with life in participants with a larger kyphosis angle has been described (66–68). However, results are difficult to interpret due to significant methodological limitations. Only Martin et al. adjusted for potential confounders, i.e., age and BMD (66).

DISCUSSION AND CONCLUSION

Hyperkyphosis is common in older adults. This review reveals several shortcomings in the literature concerning the clinical relevance of hyperkyphosis.

First of all, a well-defined threshold for hyperkyphosis is lacking. Yet, in order to attain more uniformity in research, applying one clear definition of hyperkyphosis is essential. As the Cobb angle is the gold standard kyphosis measurement method, a definition of hyperkyphosis based on the Cobb is preferable. The mean kyphosis angle has been reported to range from 35 to 42° in adults aged 65 years and older (30–32, 69), with a larger mean angle of 47–52° in older women with osteoporosis. We need to take the measurement error into account, as the interrater and intrarater variability ranges from 3 to 5° (70, 71). Defining hyperkyphosis based on means and interrater and intrarater variability may be preferable, as a definition based on the association with adverse health outcomes would only be applicable in similar populations. Based on the range of the mean kyphosis angle in older adults and interrater and intrarater variability, we propose to define hyperkyphosis as a Cobb angle of 50° or more in standing position. Additionally, identifying a pre-stage of hyperkyphosis–a Cobb angle ranging from 40 to 50°–may facilitate early recognition and potential intervention.

Secondly, many cohort studies report an association between hyperkyphosis and adverse health effects. However, most studies have a cross-sectional design and some outcome measures have been scantly investigated. Moreover, most studies have been performed in a population with osteoporosis. In order to gain knowledge on the consequences of hyperkyphosis, more prospective studies are warranted in other populations. While literature concerning the consequences of hyperkyphosis may be limited, osteoporotic vertebral fractures have consistently been identified as one of the causes of hyperkyphosis. Therefore, hyperkyphosis may be a clear clinical sign of the presence of osteoporosis. As osteoporosis is treatable, early recognition is highly important to prevent future fractures and the accompanying health-related problems. Finally, a few small intervention studies have shown that hyperkyphosis in itself is treatable through targeted training of back extensor muscles or yoga (72–75).

In conclusion, hyperkyphosis is a clinical sign of the presence of osteoporosis, and a potentially modifiable risk factor for adverse health outcomes. Prospective and intervention studies, using a Cobb angle of 50° as a clear and uniform definition of hyperkyphosis, are warranted to investigate the clinical relevance of hyperkyphosis.

AUTHOR CONTRIBUTIONS

MK performed the literature search and wrote the draft. MK and HW read the articles. WL and HW reviewed the draft of the article and provided expertise for revisions. All authors approved the submitted version of the manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer JN declared a shared affiliation, with no collaboration, with one of the authors, WL, to the handling editor at the time of the review.

frontiers in Endocrinology

REVIEW
published: 24 January 2020
doi: 10.3389/fendo.2019.00925

Advances in Models of Fibrous Dysplasia/McCune-Albright Syndrome

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The G_S G-protein coupled receptor pathway is a critical regulator of normal bone formation and function. The G_S pathway increases intracellular cAMP levels by ultimately acting on adenylate cyclase. McCune-Albright Syndrome (MAS) and fibrous dysplasia (FD) of the bone are two proto-typical conditions that result from increased cellular G_S signaling activity. Both are caused by somatic activating mutations in the *GNAS* gene that encodes for the $G_S\alpha$ subunit. FD bone lesions are particularly difficult to treat because of their variability and because of the lack of effective medical therapies. In this review, we briefly discuss the key clinical presentations of FD/MAS. We also review the current status of mouse models that target the G_S GPCR signaling pathway and human cellular models for FD/MAS. These powerful tools and our improving clinical knowledge will allow further elucidation of the roles of GPCR signaling in FD/MS pathogenesis, and facilitate the development of novel therapies for these medically significant conditions.

Keywords: GPCR (G protein-coupled receptors), McCune-Albright syndrome (MAS), fibrous dysplasia (FD), GNAS (guanine nucleotide-binding protein/[alpha]-subunit, $G_s\alpha$, cAMP, mouse models, human cell models

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Bone Research, a section of the journal Frontiers in Endocrinology

Received: *05 November 2019*
Accepted: *18 December 2019*
Published: *24 January 2020*

Citation:
Lung H, Hsiao EC and Wentworth KL (2020) Advances in Models of Fibrous Dysplasia/McCune-Albright Syndrome. Front. Endocrinol. 10:925. doi: 10.3389/fendo.2019.00925

INTRODUCTION

Musculoskeletal disorders such as skeletal dysplasias are a significant health problem affecting both children and adults. A variety of cellular pathways, including G-protein coupled receptors (GPCRs) and their ligands, have been identified as key regulators of osteoblast formation and function—two critical steps in normal bone formation and homeostasis. The human GPCR family includes over 340 non-olfactory and 400 olfactory receptors (1, 2), making it the largest class of receptors in the human genome. GPCRs mediate a wide variety of biological processes and are activated by multiple types of extracellular signals, ranging from photons and ions to small molecules to peptides. The diversity of GPCRs and their responses to small molecules has made them major targets for over 40% of modern pharmaceuticals (3).

GPCRs signal through a select number of canonical pathways (4): among these, the G_s and G_i pathways increase or decrease intracellular cAMP levels, respectively, by acting on adenylate cyclase, while the G_q pathway increases intracellular calcium by activating phospholipase C.

McCune-Albright Syndrome (MAS) is a proto-typical disease caused by activating mutations in the *GNAS* locus, encoding the $G_s\alpha$ protein (5). MAS is a mosaic genetic disease characterized by the classic triad of polyostotic fibrous dysplasia (FD) of the bone, café-au-lait skin hyperpigmentation, and peripheral precocious puberty. Patients with MAS may have other endocrinopathies, including

Cushing's syndrome, hyperthyroidism, acromegaly, and solid organ malignancies of the breast, thyroid, and pancreas. FD/MAS is caused by an acquired somatic mutation in *GNAS*, the gene that encodes the alpha subunit of the stimulatory G-protein ($G_s\alpha$), leading to constitutive activation of G_s signaling in affected cells. This mutation occurs post-zygotically, resulting in tissue mosaicism, and is not inherited through the germline. As a result of this mosaicism, the clinical disease spectrum of FD ranges from single bone involvement to multi-organ involvement. The most common cause is a missense mutation at either position c.602G>A (p.R201H) or c.601C>T (p.R201C). This mutation results in an amino acid substitution in the GTP hydrolase domain of the $G_s\alpha$ protein, inhibiting the intrinsic GTPase activity, and leading to persistently elevated intracellular cAMP levels (**Figure 1**).

Clinical Presentation of Fibrous Dysplasia

A major clinical feature of MAS is FD of the bone, where expansile bone lesions cause fragility, malformations, and pain (**Figure 2**). FD also occurs without MAS, and is a common congenital skeletal dysplasia that can affect one bone (monoostotic) or multiple bones (polyostotic) (5). FD is arguably the most significant medical complication of MAS, since no effective treatments are available to manage the bone complications. In addition, the broad clinical spectrum of FD/MAS and the mosaic nature of the disease leads to variability in the radiographic presentation, making FD challenging to accurately diagnose.

In 2019, the FD/MAS International Consortium put forth a position statement to help guide clinicians in the diagnosis and management of FD/MAS (6). The first step in diagnosing FD/MAS is to perform a complete skeletal and extra-skeletal evaluation to determine the extent of the disease. MAS can be diagnosed if a patient has FD and at least one extraskeletal manifestation (café-au-lait skin hyperpigmentation, peripheral precocious puberty, thyroid lesions consistent with FD/MAS, growth hormone excess, neonatal hypercortisolism) or the absence of skeletal involvement but 2 extra-skeletal manifestations (6). An accurate diagnosis of FD/MAS can usually be made after a complete physical examination, combined with biochemical, hormonal, and radiologic evaluation of the skeletal, dermatologic, and endocrine systems. Biopsy of FD lesions is needed only if there is uncertainty about the diagnosis (i.e., atypical radiologic features) or concern for underlying malignancy (6). In these situations, affected tissue can be tested for the presence of a *GNAS* activating mutation, with the understanding that false negatives may occur if the sample has a low mutational burden. Peripheral blood is usually not sufficient for diagnosis due to the mosaicism of the disease. Next-generation sequencing is associated with a lower false-positive rate (6).

Treatment and Monitoring of FD/MAS

Comprehensive guidelines regarding the management of the skeletal and extra-skeletal manifestations of FD/MAS were recently published, and should be considered when caring for patients along this clinical disease spectrum (6). The mainstay of therapy in FD/MAS remains adequate pain control, optimization of phosphate and vitamin D status, treatment of IGF-1 excess if present, and judicious consideration of surgical resection of FD lesions once they have stabilized. Unfortunately, there are no effective medical treatments available for FD/MAS. Bisphosphonate therapy in IV formulation has been reported to provide some benefits for pain control in patients with persistent moderate-to-severe pain from FD lesions, but why this helps in only some patients remains unclear (7–9). In addition, there is no evidence to suggest that bisphosphonates decrease the progression of FD lesions, and may not adequately control pain in some patients (7, 10).

Presently, there is minimal evidence for the use of denosumab and other anti-resorptive agents in FD, although there are case reports suggesting potential clinical benefits (8, 11–16). However, there are major concerns about rebound fractures and FD lesion progression after drug cessation (17–19). Ongoing clinical trials to address the utility of denosumab in FD are underway (NCT03571191). In addition, the TOCIDYS trial is evaluating the efficacy of IL-6 inhibition in patients with FD who did not have improvement in pain with prior bisphosphonate treatment (NCT01791842). These exciting trials hold promise for identifying potential medical strategies for mitigating the complications from FD.

MOUSE MODELS FOR UNDERSTANDING FD

One contributor to the dearth of effective treatments for FD/MAS is the complexity of the *GNAS* locus. This complexity has made it challenging to develop robust mouse and human models to dissect the mechanisms of FD/MAS. During the past several years, novel strategies for uncovering the roles of G_s-GPCR signaling in bone have been developed. These models provide critical insights into the pathogenesis and potential therapeutic approaches for FD.

One of the earliest models utilized the PTH/PTH-related protein (PTH/PTHrP) receptor (PPR), a GPCR, to study Jansen's metaphyseal chondrodysplasia (JMC). JMC is a rare form of short-limbed dwarfism caused by activating mutations of the PPR, leading to constitutive receptor activation and ligand-independent intracellular cAMP accumulation. Calvi et al. generated a mouse (Col1-caPPR) that expressed the human mutant PPR HKrk-H223R (caPPR), one of the causative mutations associated with JMC, in osteoblastic lineage cells in mice using a Col1 (2.3 kb) promoter (20). At 1 week of age, these mice showed increased osteoblast number and function in both the trabecular bone and the endosteal surface of cortical bone in the long bones. However, periosteal osteoblast activity was inhibited. This resulted in an increase in trabecular bone volume and a decrease in cortical bone thickness in the long bones. Calvarial thickness remained unchanged but there was increased porosity and bone remodeling on the endosteal surface of the skull. There was also an increased number of mature osteoclasts in these mice, which led to increased porosity of the cortical bone. At 2 weeks of age, excess bone formed in the

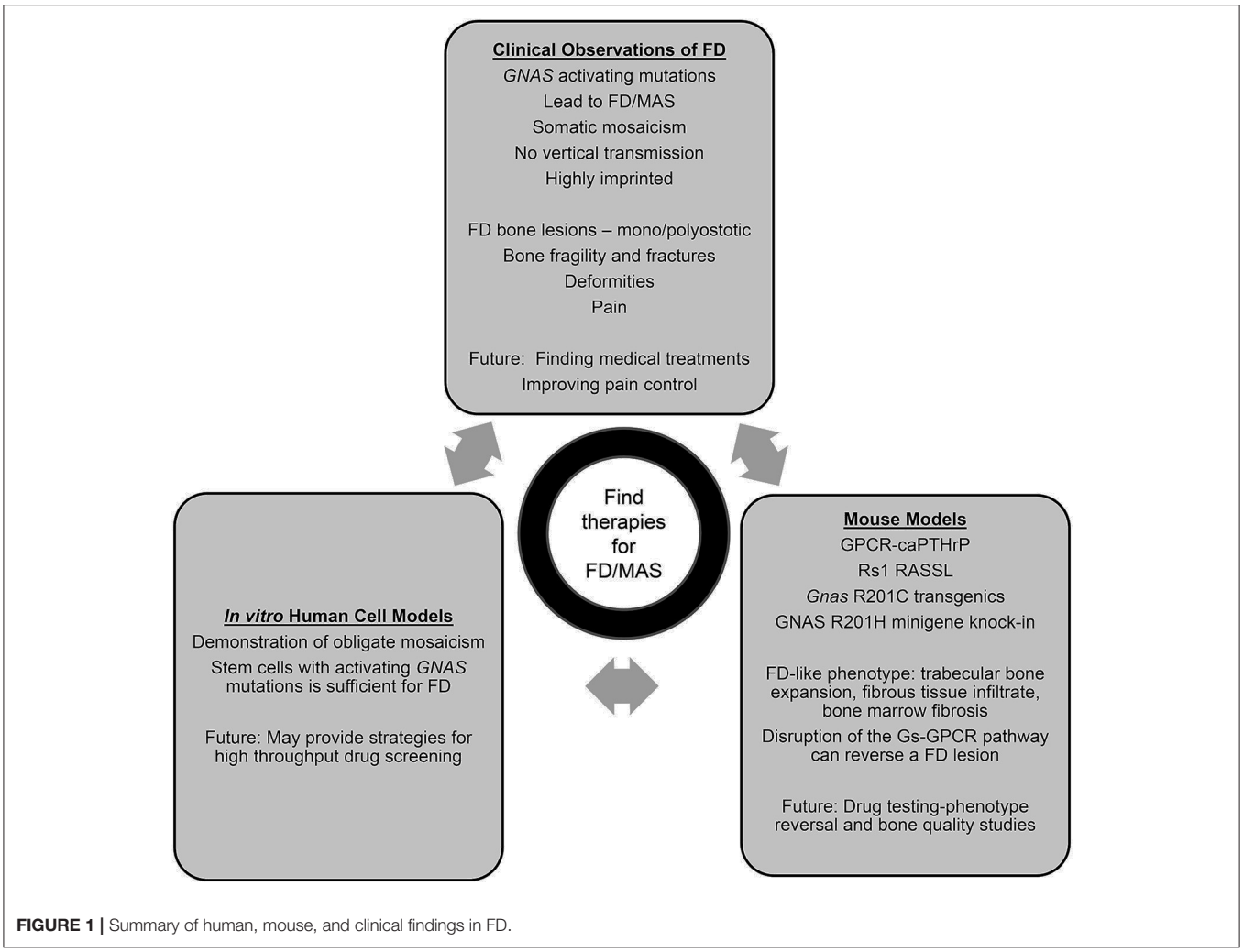

FIGURE 1 | Summary of human, mouse, and clinical findings in FD.

bone marrow space (21). The area between the trabeculae was occupied by fibrous cells, blood vessels, and osteoclasts. There was delayed formation of bone marrow cavities, adipocytes, and hematopoietic cells. Surprisingly, these dysplastic bone and fibrous tissue phenotypes gradually resolved over time, and were limited to the metaphyseal area at 4 months. These studies showed that when the constitutively active PPR was expressed in osteoblastic lineage cells, the receptor could mediate both the anabolic and resorptive effects of PTH, and that PPR is involved in the regulation of both bone marrow and stromal tissues.

Our group used a different approach, creating an engineered GPCR RASSL (receptor activated solely by a synthetic ligand) to regulate GPCR signaling (22). The G_s-coupled receptor, Rs1, was created by inserting a D100A mutation into the wild-type human 5HT4 serotonin receptor (23). This engineered receptor has a high basal level of constitutively active $G_s\alpha$ activity and is not responsive to the endogenous serotonin ligand. The Col1(2.3)/Rs1 mouse model limits Rs1 expression spatially to osteoblastic lineage cells by using a Col1a1(2.3 kb) promotor, and temporally using the tet-off system, which allows for controlled expression of Rs1 in the absence of doxycycline. The FD-like phenotype of mice born off doxycycline was first apparent at 6 days (24). These mice showed age-dependent, increased trabecular bone formation with loss of marrow space and thinned cortical bone. The histologic and radiographic features

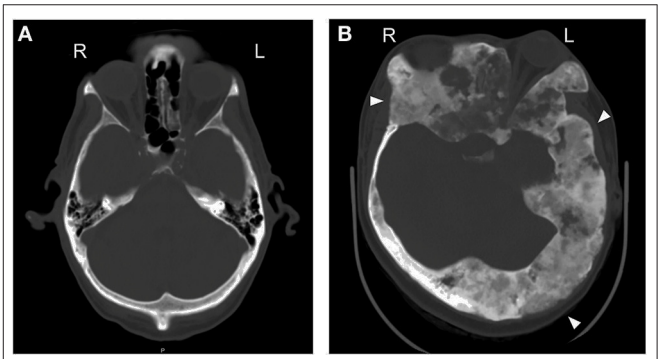

FIGURE 2 | Fibrous dysplasia commonly affects the skull and is mosaic. Axial CT across the orbit. **(A)** A normal CT scan of the craniofacial bones, from a skeletally normal 61 y.o. male. **(B)** A 29 y.o. female with craniofacial FD. Note the asymmetry in the skull [ground glass expanded lesions (arrowheads) and displaced left globe].

strongly resemble human FD of the bone. There was also a dramatic increase in the number of immature osteoblasts present in the FD lesions, deposition of immature bone tissue, and reduced mineralization seen in analyses by FTIR spectroscopy and synchrotron radiation micro-computed tomography (25). The mice also showed lower numbers of hematopoietic stem cells (26). Col1(2.3)/Rs1 mouse bones showed dramatically reduced mature adipocyte differentiation, and higher osteoblastic glucose utilization than control mice (27). RNA analysis on whole bone samples showed increased Wnt signaling, suggesting that Wnt proteins may be a major driver of this effect (27). Importantly, blocking G_s signaling by using doxycycline at 4 weeks gradually reversed the bone phenotype (24), providing a proof-of-concept that therapies inhibiting G_s signaling may be effective for reversing fibrous dysplastic bone lesions. This model provides a powerful tool for understanding the effects of G_s-GPCR signaling on dynamic bone growth and remodeling.

In contrast to strategies that did not directly manipulate *GNAS*, Saggio et al. generated a transgenic mouse model of human FD that constitutively expressed *GNAS*-R201C (28). The transgene expression did not show any appreciable effect on embryonic skeletal formation and unlike human FD, could be vertically transmitted. FD lesions were detected radiographically at 6 months of age. The lesions first appeared in the tail and later progressed to the femurs and the skull. The onset and progression of lesions were defined in three stages: an early phase (<2 months), defined by abnormal trabecular bone formation, endosteal thickening, and ectopic cortical lesions; an intermediate phase (2–6 months), with narrowed marrow cavity, expanded cortical bone and increased osteoclastic activity; and a late phase (>10 months) described as the fibrous dysplastic phase, with abnormal bone trabeculae and matrix fibrous tissue. At 1 year of age, the skeletal features resembled human FD.

When *GNAS*-R201C expression was limited to maturing osteoblasts using the Col1a1 (2.3 kb) promoter, changes in bone structure were detected on radiograph at 3 weeks in the tail, with excess bone mass (29). At 3 months, increased bone density was noted in all bones. The transgenic mice showed excess bone formation and remodeling of the bone marrow space. However, the expression in Col1a1(2.3 kb) cells failed to reproduce other features of human FD, such as bone marrow space fibrosis, and the loss of adipocytes and hematopoietic cells seen in other models (23, 28).

Palmisano et al. tested the effect of RANKL (receptor activator of nuclear factor kappa-B ligand) inhibition by treating the mice with an anti-RANKL antibody (30). They found that new and highly mineralized bone replaced the FD-like lesions in 2-month old mice, and the bone density increased. The treatment also stopped the growth of pre-existing FD lesions. In addition, there was a higher maximum load and stiffness of the bones in the treated group. However, the FD-like lesions progressed after RANKL inhibitor cessation.

More recently, Zhao et al. developed a mouse model expressing *GNAS*-R201C in the skeletal stem cell lineage using a tetracycline-inducible Cre-mediated Prrx1 driver (31). The FD bone phenotype was observed in embryos and adult mice <2 weeks after doxycycline administration (to activate the transgene) at E4.5. The FD lesions in the long bones showed reduced endochondral ossification, and in craniofacial bones showed decreased mineralization in the calvarial sutures. Poorly mineralized trabeculae and a dense fibrous matrix were present, and the bone marrow spaces were also decreased. When doxycycline was administered to 3-week old mice for 2 weeks (to induce expression), all limbs developed FD-like lesions with expansile bone deformities, fractures in limb bones, and defects in calvarial sutures. Doxycycline withdrawal resulted in reversal of the bone lesions. Hematopoietic cells and adipocytes appeared in the bone marrow of former FD lesions. The skulls of the reversed *GNAS*-R201C mice also showed normal morphology with healed sutures.

Finally, Khan et al. generated a new conditional knock-in mouse model [*GNAS*f(R201H)] using a minigene cassette to express *GNAS*-R201H, a human FD mutation, at the endogenous mouse *GNAS* locus (32). They found that expression of *GNAS*-R201H in the germ-line using Sox2-Cre resulted in embryonic lethality. Using Prrx1-Cre, they limited the expression of *GNAS*-R201H to osteochondral progenitor cells and early limb bud mesenchyme cells. At P0, control mice had well-formed cortical bone, cartilaginous epiphyses, and a normal marrow cavity. In contrast, Prrx1-Cre; *GNAS*f(R201H)/+ showed severely deficient long bone formation. At P6, Prrx1-Cre; *GNAS*f(R201H)/+ mice showed bone formation, but the marrow space was replaced by fibrous tissue and trabecular bone. In mutant long bones, higher levels of Wnt/β-catenin were also noted. At 3 weeks of age, there was more FD-like bone formation in the Prrx1-Cre; *GNAS*f(R201H)/+ mice; expansion of woven trabecular bone; lack of cortical bone; and increased osteoclasts. Furthermore, in this mouse model, Xu et al. demonstrated that $G_s\alpha$ signaling mediated intramembranous ossification of cranial bones by regulating both Hh and Wnt/β-catenin signaling (33).

Together, these mouse models demonstrate that FD bone lesions can develop from activating mutations at the G_s-GPCR level as well as from transgenic expression or knock-in expression of a *GNAS* allele carrying the R201C or R201H activating mutation. In addition, data from several mouse models suggest that blocking G_s pathway hyperactivity can lead to reversal of the FD bone lesions and may be a viable treatment strategy for human FD.

HUMAN CELLULAR MODELS OF G_S-SIGNALING

Human cellular models have contributed significantly to our understanding of FD/MAS pathophysiology, and patient-derived tissue samples have been an invaluable source. In 1998, Bianco et al. performed one of the earliest studies using FD/MAS patient bone marrow samples and showed that bone marrow stromal cell (BMSC) progenitors isolated from fibrous dysplastic lesions were either heterozygous for the $G_s\alpha$ activating mutant allele, or homozygous wildtype (34). This discovery demonstrated that the skeletal progenitor cell population in FD lesions is mosaic, with a resident population of bone marrow stromal cells harboring the mutation and another population that is unaffected (34).

When these cells were transplanted into immunocompromised mice, the wildtype colonies developed normal ossicles, but the colonies containing only the *GNAS* mutant allele did not survive and did not form ossicles. However, when a mixture of wildtype and mutant cell colonies was transplanted, an abnormal ossicle formed, with histopathologic features resembling FD. These experiments provided strong evidence that both wildtype and mutant cells were necessary to form a FD lesion, and that the mosaicism inherent to FD/MAS can be recapitulated within an FD lesion (34).

This novel concept of somatic mosaicism within an FD lesion was explored further by Kuznetsov et al. (35). In their study, they isolated colony forming unit-fibroblasts (CFU-Fs) from FD lesions of patients with FD/MAS and calculated the frequency of mutation-bearing CFU-Fs vs. normal CFU-Fs. They noted an inverse relationship between patient age and the number of mutated skeletal stem cell colonies present within an FD lesion, and concluded that the number of mutated stem cells must undergo apoptosis as patients age. Similarly, the bone histology in older patients (32–52 years of age) with FD/MAS was less severe than that of younger patients, and was more likely to be associated with a lower *GNAS* mutational burden. They hypothesized that as skeletal stem cells aged, there may be preferential apoptosis of mutated cells, resulting in loss of these cells and self-renewal of non-mutated cell populations. This could account for the clinically observed decreased incidence of new FD lesions and the relative stability of existing FD lesions as patients age. Kuznetsov et al. also transplanted cell colonies containing CFU-Fs into immunocompromised mice and showed that FD ossicles formed, whereas non-mutant CFU-Fs or mutation-positive strains without mutant skeletal stem cell populations did not form FD ossicles. Thus, they concluded that *GNAS* mutations within the skeletal stem cell population was sufficient to induce formation of FD lesions (35).

Piersanti et al. developed a model in which human skeletal progenitor cells were engineered to stably over-express the *GNAS*-R201C mutation using a lentiviral vector (36). These cells demonstrated elevated cAMP production, consistent with over-expression of $G_s\alpha$. When cultured *in vitro,* they did not exhibit mineralization and had lower osteocalcin levels than controls. Levels of the osteogenic markers alkaline phosphatase and bone sialoprotein were elevated compared to controls, and the cells exhibited robust RANKL expression, consistent with the profound osteoclastogenesis seen in most human FD lesions. Additionally, genes in the phosphodiesterase pathway were upregulated in these cells, suggesting an adaptive response to $G_s\alpha$ over-expression. When these cells were transplanted into immunocompromised mice, the stably-transduced *GNAS*-R201C cells formed ossicles but were unable to differentiate into adipocytes or hematopoietic components. Finally, silencing of the *GNAS*-R201C allele with lentiviral vectors containing short hairpin interfering RNA sequences caused these cells to revert to their normal state and no longer exhibit a mutant phenotype.

In 2019, de Castro et al. used human FD-derived BMSCs from a well-characterized cohort of FD patients at the NIH to show that RANKL expression in FD skeletal lesions may directly contribute to osteoclast induction in FD lesions (37). They showed that serum levels of RANKL were 16-fold higher in FD patients compared with healthy controls, and the serum RANKL/OPG ratio was 12-fold higher. The magnitude of increase in RANKL and RANKL/OPG was positively correlated with total body skeletal disease burden score, a well-validated scoring system used to determine the severity of FD.

de Castro et al. also isolated BMSCs from these FD patients and healthy volunteers and showed that RANKL levels were higher in the conditioned media of FD BMSCs compared with BMSCs derived from healthy volunteers when stimulated with prostaglandin E2 (PGE2) and 1,25 vitamin D3 (37). These cells also released OPG, but to a much lower degree than healthy volunteer controls. Additionally, when FD BMSCs were co-cultured in osteogenic media with peripheral monocytes from healthy volunteers, the monocytes differentiated into TRAP+ osteoclasts; however, when cultured in non-osteogenic media, they did not induce osteoclastogenesis. They subsequently showed that osteoclastogenesis could be inhibited when the cell co-cultures were treated with denosumab. This study provided strong evidence that RANKL is expressed in human FD lesions and is correlated with disease burden, thus implicating RANKL over-expression as an important contributor to FD pathogenesis and informing the design of ongoing studies testing denosumab in FD (NCT03571191).

CONCLUSIONS

The past 15 years have shown major advances in our understanding of FD/MAS. There are striking consistencies among the mouse models of FD, and a number of features of the human disease are replicated in the mouse genetic models, suggesting that direct targeting of the G_s pathway has strong potential as a therapeutic strategy for FD. In addition, the human cell models of FD are providing new tools for understanding FD pathogenesis and cell-type specific effects of the *GNAS* activating mutations. Finally, international collaborations among clinicians and researchers with strong experience in FD/MAS are yielding best-practice recommendations and treatment guidelines for optimal management of FD/MAS.

AUTHOR CONTRIBUTIONS

EH, KW, and HL conceived of this manuscript, wrote the manuscript, and edited the manuscript.

FUNDING

This work was supported by the University of California Department of Medicine (to EH), a Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine Doctoral Training Fellowship (to HL), and National Institutes of Health Career Development Grant K08 DE028946 (to KW).

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Conflict of Interest: EH and KW receive clinical trial research support through their institution from Clementia Pharmaceuticals for clinical trials unrelated to this work. EH is also a volunteer on the editorial board for the Journal of Bone and Mineral Research. EH serves in an unpaid capacity on the International FOP Association Medical Registry Advisory Board, on the International Clinical Council on FOP, and on the Fibrous Dysplasia Foundation Medical Advisory Board. EH has also received clinical trial research support through his institution from Regeneron Pharmaceuticals.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

CASE REPORT
published: 05 February 2020
doi: 10.3389/fendo.2020.00037

A Large Skull Defect Due to Gorham-Stout Disease: Case Report and Literature Review on Pathogenesis, Diagnosis, and Treatment

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A 24-year old man was referred to the Erasmus MC Bone Center because of an asymptomatic increasing skull defect of the left parietal bone. The defect was first noticed at the age of six, and gradually increased over the years. His medical history was unremarkable, without any known trauma and a negative family history for bone diseases. Laboratory tests showed a low vitamin D level without other abnormalities. Particularly, there was no increase in markers of inflammation or bone turnover. CT-scans of the skull showed an osteolytic region of the parietal skull bone, with a two-centimeter increase in diameter over 9 years. Contrast enhanced MRI showed lymphangiogenic invasion, which was compatible with our suspicion of Gorham-Stout disease. The patient was referred to the neurosurgeon for treatment with a bone graft while considering additional drug treatment. Gorham-Stout or vanishing bone disease is a rare entity characterized by progressive osteolysis with lymphangiogenic bone invasion. Although already reported in 1838, currently the diagnosis and treatment of Gorham-Stout disease is still challenging. The underlying pathophysiology is not clarified yet and several theories exist. The disease usually affects persons younger than 40 years and the majority present with bone disease of the maxillofacial region, the upper extremities or the torso. The clinical presentation includes most frequently pain, swelling, and functional impairment of the affected region, but the disease can also be asymptomatic. Laboratory investigations are usually normal, and diagnosis is based upon imaging and sometimes pathology examination of affected bone tissue. Treatment is experimental and there is no general consensus about the best option due to lack of randomized controlled trials. Case reports showed patients treated with bisphosphonates, interferon-alpha, anti-VEGF therapy, mTOR inhibitors, and radiotherapy. There are some reports of surgery with prosthetic or bone grafts but no long-term follow-up data exist. This paper describes a unique case of Gorham-Stout disease of the parietal skull bone and discusses the current state of knowledge about this rare bone disease.

Keywords: Gorham-Stout, osteolysis, rare bone disease, parietal bone, bone graft

OPEN ACCESS

Edited by:
Wim Van Hul,
University of Antwerp, Belgium

Reviewed by:
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Specialty section:
This article was submitted to Bone Research, a section of the journal Frontiers in Endocrinology

Received: *28 October 2019*
Accepted: *20 January 2020*
Published: *05 February 2020*

Citation:
de Keyser CE, Saltzherr MS, Bos EM and Zillikens MC (2020) A Large Skull Defect Due to Gorham-Stout Disease: Case Report and Literature Review on Pathogenesis, Diagnosis, and Treatment. Front. Endocrinol. 11:37. doi: 10.3389/fendo.2020.00037

CASE PRESENTATION

A 24-year old man was referred to the Erasmus MC Bone Center in Rotterdam, the Netherlands, because of a growing skull defect of the left parietal bone. He had been analyzed in the referring hospital because the defect became larger over time, but no treatment was initiated. The defect was first noticed when he was 6 years old, and he nor his parents could remember any traumatic incident. His medical history mentioned no relevant diseases and he did not use any medication. He reached his target height with no other skeletal deformities, had no other complaints and was in good clinical condition. Family history was negative for bone diseases.

Laboratory tests showed a low 25-hydroxy vitamin D level (21 nmol/L, reference values 50–120 nmol/L), no increase in inflammation markers [C-reactive protein (CRP), erythrocyte sedimentation rate (ESR)], and normal bone turnover markers in the form of alkaline phosphatase, procollagen type 1 N propeptide (P.1.N.P.), and beta isomer of C-terminal telopeptide of type 1 collagen (beta-CTX) with only slightly increased bone alkaline phosphatase (30.0 μg/L, reference value <20.1 μg/L). Also, serum levels of cytokines that may be involved in the pathogenesis [interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), interleukin-1-beta (IL-1β)], were normal. The results of the most relevant laboratory tests are shown in **Table 1**. Laboratory test were performed according to standard procedures. CT-scans of the referring hospital showed a region of osteolysis of the diploë and outer table of the parietal bone, with an intact inner table. The region size of the osteolytic region slowly increased in size over the years. The first CT-scan was performed at the age of 15 and showed a defect with a maximum diameter of 38 mm. One year later the defect had increased to 41 mm. CT-scans at the age of 22 and 24 showed an increase of the defect to a maximum diameter of 57 and 60 mm, respectively. **Figure 1** shows the most recently performed CT-scan from the referring hospital with the defect of 60 mm. 3D-CT reconstructions were made to visualize the extensiveness of the defect (**Figure 2**). Based on the clinical manifestation and radiological findings the diagnosis Gorham-Stout disease was suspected. To confirm this diagnosis, we performed a contrast enhanced MRI-scan which showed an enhancing zone of diploic vascularity at the edge of the osteolytic region (**Figure 3**), characteristic of Gorham-Stout. We therefore concluded that our patient suffered from Gorham-Stout disease of the parietal bone. Additional bone scintigraphy showed no other lesions. We were challenged by the decision to cover the defect with or without removal of affected bone and whether or not to start additional medical treatment. Due to the rarity of the disease, lack of literature with respect to the underlying pathophysiological mechanism and a standardized treatment guideline, we performed a literature search to choose the best clinical approach and also consulted experts in the field.

TABLE 1 | Results of the laboratory tests from the patient.

Laboratory test	Serum value (reference values)	Laboratory test	Serum value (reference values)
Calcium	2.51 (2.20–2.65 mmol/L)	CRP	<0.3 (<10 mg/L)
Phosphate	1.04 (0.80–1.40 mmol/L)	ESR	2 (0–15 mm/h)
Albumin	53 (35–50 g/L)	ALP	84 (<115 U/L)
25-OH-D	21 (50–120 mmol/L)	Gamma-GT	23 (<55 U/L)
IL-6	<10 (<10 pg/mL)	P.1.N.P.	93 (19.4–95.5 μg/L)
TNF-α	<15 (<15 pg/mL)	Beta-CTX	0.27 μg/L
IL-1β	<10 (<10 pg/mL)	Bone AF	30.0 (<20.1 μg/L)

IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha; IL-1β, interleukin-1-beta; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; ALP, alkaline phosphatase; Gamma-GT, gamma glutamyltransferase; P.1.N.P., beta isomer of C-terminal telopeptide of type 1 collagen; beta-CTX, beta isomer of C-terminal telopeptide of type 1 collagen.

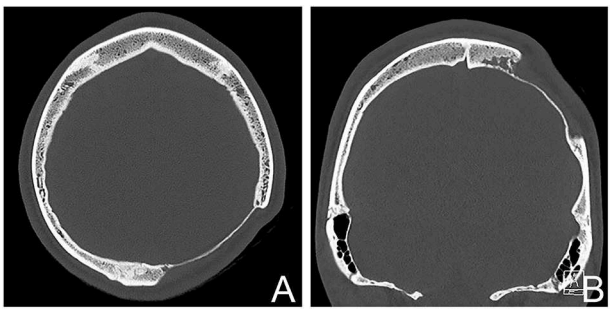

FIGURE 1 | CT-scan of the patient showing the osteolytic skull defect of 60 mm. **(A)** Axial plane, **(B)** coronal reconstruction showing irregular lobular vessel shaped osteolysis at the medial side.

OVERVIEW OF THE LITERATURE ON GORHAM-STOUT DISEASE

Gorham-Stout disease, also named vanishing bone disease, phantom bone disease, or idiopathic massive osteolysis, is a rare bone disorder characterized by progressive osteolysis with lymphatic and vascular proliferation (1). The incidence is very rare, with only a few hundred case reports described in literature (1, 2). The disorder does not seem to exhibit a preference for race, geographic area, or sex, although the disease might be slightly more prevalent in males. No definite pattern of genetic inheritance has been described, currently no cases with a family history of the disease have been reported. It can be diagnosed at every age, although most of the cases that have been described occurred under the age of 40 years, with an average age at diagnosis of 25 years (1, 3, 4).

Clinical Picture

Gorham-Stout disease most frequently involves the upper part of the body. Most cases present with affected bones of the maxillofacial region, vertebrae, ribs and the pelvic girdle, but it can affect any part of the skeleton. In the majority of the cases the disease is monostotic, involvement of multiple bones has been described (3). There is no clear trigger in clinical practice that causes the disease to occur. Sometimes the disease can occur after a traumatic incident. After a trauma, the disease is confined to a single location (5).

The clinical presentation and patient's complaints are dependent on the bone that is affected. Patients most frequently present with local pain. This can be accompanied by functional

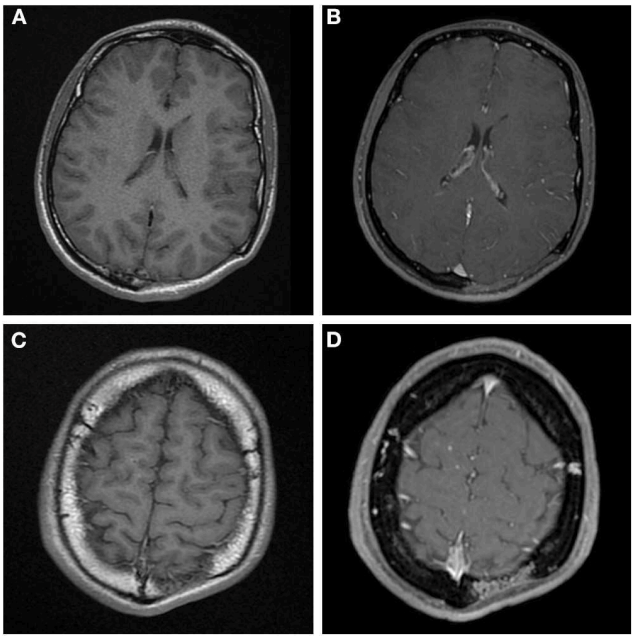

FIGURE 2 | (A,C) Axial T1 MR images before contrast administration, **(B,D)** post contrast T1 weighted MR images at the same level as **(A,C)**. The main area of osteolysis **(A,B)** is filled with non-enhancing soft-tissue. But there is vascular shaped contrast enhancement in the diploë in **(D)** at the edge of the osteolysis, this is the same area as mentioned in **Figure 1B**.

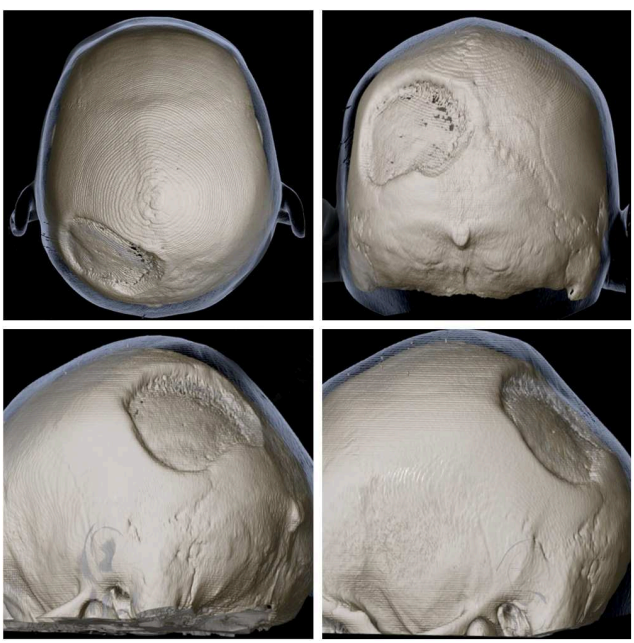

FIGURE 3 | 3D reconstruction of skull CT from multiple angles showing the extensiveness and location of the defect of the left parietal bone.

impairment, muscle weakness or local edema. The disease can also be completely asymptomatic, or may have a first presentation with a spontaneous or traumatic fracture. Dyspnea can occur due to chylothorax, a frequently seen complication in Gorham-Stout disease of the bones in the thorax with a prevalence up to 25%. Pleural effusion and chylothorax may develop when the lymphoangiogenic invasion extends to the pleural cavity or the thoracic duct (6).

The prognosis of Gorham-Stout disease is unpredictable and varying, based on the extent, severity, and localization of the disease. It is considered a benign disease and its natural progression is characterized by spontaneous resolution. When the disease has stabilized, the resorbed bone is not replaced by new bone. Severe complications have been described and can be life threatening. Besides the above described chylothorax due to pleural involvement, rarely described complications include hemangiomatous lesions of the skin, pericardial effusion, osteomyelitis with septic shock, increased intracranial pressure, meningitis, cerebrospinal fluid leakage, spinal cord involvement and paraplegia by vertebral involvement (4, 7–9). In general, visceral and spinal involvement are associated with a poor prognosis (4).

Diagnosis

It can be a challenge to diagnose patients with Gorham-Stout disease on the clinical, histological, and radiological features. The disease can be misdiagnosed since it resembles other clinical diseases that also present with pain of the musculoskeletal tract, including rheumatoid arthritis, osteoarthritis, or osteomyelitis, and other causes of osteolysis such as intraosseous malignancies, granulomatous diseases, or central nervous system diseases such as a meningioma or syringomyelia. In clinical practice, it is often a diagnosis by exclusion, after ruling out inflammatory, infectious, metabolic and neoplastic diseases (10). There is no standardized guideline for diagnosis. Heffez et al. proposed an algorithm with eight diagnostic criteria for Gorham-Stout disease. These are based on clinical, radiological and histopathological findings and are the following: (1) angiomatous tissue present in biopsy tissue, (2) absence of cellular atypia, (3) minimal or no osteoblastic response and absence of dystrophic calcifications, (4) evidence of local progressive osseous resorption, (5) non-expansive, non-ulcerative lesion, (6) absence of visceral involvement, (7) osteolytic radiographic pattern, (8) negative hereditary, metabolic, neoplastic, immunologic, and infectious etiology (11).

Laboratory tests are generally not helpful in the diagnosis of Gorham-Stout disease since they are usually normal. There is currently no specific biomarker that can be used to assess the disease severity or that can be used in the follow-up to monitor the response to therapy. Alkaline phosphatase may be increased but is frequently normal (4, 10). Authors from a recent case report on Gorham-Stout disease with vanishing of three ribs and an osteolytic lesion of the left humerus bone suggest an initial set of laboratory tests to use in the diagnostic phase, including bone turnover markers. Since in this case, the bone turnover markers P.1.N.P. and bone-specific alkaline phosphatase were increased, the authors propose to use the bone turnover markers also for follow-up (12). In reported studies, several factors were measured in the blood of patients with Gorham-Stout disease (e.g., vascular endothelial growth factors, IL-6, sRANKL, and osteoprotegerin). IL-6 and VEGF-A were elevated in the blood of patients with

Gorham-Stout disease (3), and in a recent study also the levels of pyridinoline cross-linked carboxyterminal telopeptide of type I (ICTP) and sclerostin (13). However, they can also be normal.

Conventional X-rays are not useful for early diagnosis of the disease. Radiology findings at an early stage resemble patchy osteoporosis and show radiolucent foci at intramedullary and subcortical zones. If the disease progresses, bone deformities develop with bone mass loss, atrophy, fracture, fragmentation, and finally the image of vanishing bone (10, 14). Bone scintigraphy mostly shows increased uptake in the blood pool phase at the region of increased lymphatic and vascular proliferation, and a region of decreased uptake at the osteolytic region of vanished bone (4, 10, 15, 16). CT-scan is useful to investigate the location and extent of the osteolytic bone, and can sometimes show vessel shaped defects at the edge of osteolysis, as in our case, but is mostly not characteristic for diagnosis. MR imaging also shows the osteolysis and can clearly show the vascular and/or lymphatic vessels within the bone which enhance after contrast administration at the region of active osteolysis (17). Finally, the diagnosis can be confirmed by histology from a biopsy of the affected bone. In our patient, no biopsy of the affected region was taken, as the MR characteristics of an osteolytic region filled with non-enhancing soft-tissue and vascular shaped contrast enhancement in the diploë at the edge of the osteolysis, were very characteristic for the disease, and there was no clinical or laboratory suspicion for an alternative inflammatory, neoplastic, or infectious cause of the osteolysis. Recently, a multidisciplinary guideline for the initial evaluation of complicated lymphatic anomalies including Gorham-Stout disease was published (18). Although based on expert opinion consensus, authors present a diagnostic approach including laboratory, radiological, and histological evaluation. They state that the need for a histological biopsy should be discussed in a multidisciplinary expert team after the clinical and radiological evaluation. A possible complication of a biopsy is the oozing of lymphatic fluid from the biopsy site, which may need lymph drainage or can induce a chylothorax in the case of a biopsy of an affected rib (18).

Pathogenesis

The first case of Gorham-Stout disease was already reported in 1838 and described a patient with a vanishing of the complete humerus bone over 11 years (19). After more than a century, in 1955, Gorham and Stout investigated the characteristic histopathological findings of the massive osteolysis based on 8 cases. They discovered that the massive osteolysis was accompanied by angiogenesis and lymphangiomatosis, with proliferation of benign vascular structures and destruction of osseous matrix. The lymphangiomatosis resulted in a zone of hyperemia, which disrupts normal bone metabolism and shifts the bone metabolism to favor osteoclastic activity. The destructed bone tissue is replaced by fibrous tissue and no new bone formation occurs (20). Further research with the development of immunohistochemical markers of lymphatic endothelial cells revealed that in Gorham-Stout disease lymphatic anomalies are present in cortical and medullary parts of the bone, and that these lymphatic vessels are normally not present in bone tissue (3, 21). Although these findings were discovered decades ago, currently the exact pathophysiologic mechanism is not clarified. The proliferation of lymphatic vessels plays a central role in the pathogenesis of the disease and several mechanisms appear to be involved in the process of osteolysis.

An important role is attributed to the activation of osteoclasts through the immune system. In Gorham-Stout disease, an imbalance exists between osteoblasts and osteoclasts, favoring osteoclast activity leading to bone resorption over bone formation. Studies showed that *in vitro*, the osteoclast differentiation is increased, while the function of the osteoblasts is impaired with decreased bone mineralization ability (13). Osteoclasts are activated by different cytokines which are secreted by cells from the immune system. T-lymphocytes produce cytokines such as interferon-gamma, tumor necrosis factor alpha, prostaglandin E2, and interleukin 17, which induce the cytokine receptor activator of nuclear factor kappa-B ligand (RANKL). RANKL binds to the RANK receptor expressed on osteoclast precursors, leading to differentiation and activation of the osteoclasts (3). Furthermore, T-lymphocytes, but also leukocytes, osteoblasts, and dendritic cells, produce IL-6 and consequently activate mesenchymal stem cells in the bone marrow, stimulating RANKL production (22, 23). As described above, in case reports of Gorham-Stout disease the serum levels of IL-6 are sometimes elevated (3, 13, 24). Whether there is a systemic immune response, reflected by increased levels of cytokines in the serum of some patients, or whether these cytokines act locally at the defective bone, is currently not known.

Another role in the pathophysiological mechanism of the disease is attributed to macrophages, that can induce osteoclast activity and stimulate lymphangiogenesis through the production of VEGF-A, -C, and -D (13, 22, 25). Furthermore, they can inhibit osteoblast function by the production of TNF-α. The growth factors VEGF-A and VEGF-C, but also platelet-derived growth factor-BB, play a role in the invasion of lymphatic vessels (26). These growth factors signal through the same pathway which ends in the mammalian target of rapamycin (mTor) (27–29). mTor is an important kinase in the progression of the cell cycle and a key regulator of immune responses.

Furthermore, a role may be played by calcitonin, a hormone which is produced by the parafollicular (C-cells) of the thyroid gland and which has anti-osteoclastic properties. Interestingly, a patient who has been reported with agenesis of these C-Cells developed Gorham-Stout disease (30). Calcitonin binds to the calcitonin receptor on osteoclasts, thereby inhibiting their activity and thus inhibit osteoclastic bone resorption. This way, lack of calcitonin can contribute to the osteolysis in Gorham-Stout disease.

In the occurrence of Gorham-Stout disease after a trauma or fracture, it is hypothesized that the pro-inflammatory response after injury with cytokine secretion plays a role in the development of the disease. For example, secreted interleukin-1 (IL-1) induces angiogenesis and enhances the formation of cartilaginous callus, which is also a histological finding in Gorham-Stout disease.

However, to reveal the exact molecular triggers and subsequent mechanisms that cause Gorham-Stout disease,

more research is needed. It would be of most interest to reveal the trigger or the origin of the lymphangiomatosis, finally leading to osteolysis. Elucidating this may lead to a possible target for therapy, which might allow early cessation of the cascade and preventing bone destruction and loss.

Treatment

Since the underlying pathogenesis has not been clarified, a targeted treatment strategy is lacking. Furthermore, no prospective randomized controlled trials on treatment of Gorham-Stout disease have been performed due to the rarity of the disease. Several therapeutic options have been suggested to be beneficial but results appear to be variable. Generally, treatment consists of conservative therapy with medication, radiotherapy, or invasive therapy with surgery. The choice for a certain treatment is currently based on case reports from literature and expert opinion.

The pharmacological treatment options target inhibition of osteoclast activity, inhibition of angiogenesis or suppression of the immune system. Pharmacological agents that have been reported are bisphosphonates, interferon-a 2b, mTor inhibitors, vitamin D, calcium, calcitonin, anti-VEGF antibodies, bevacizumab, bleomycin, thalidomide, somatostatin, androgens, propranolol, low-molecular-weight heparin, and glucocorticosteroids (3). Patients have been treated with monotherapy, but frequently a combination of drugs has been used, such as bisphosphonates with sirolimus (27) or bisphosphonates with interferon-a-2b (31). Pharmacological treatment is also frequently combined with surgery. Bisphosphonates are thought to be beneficial through inhibition of osteoclast-mediated bone resorption, and therefore counteract the osteolysis in attempt to stabilize the disease process. Clinically, improvement of local pain and inhibition of further osteolysis has been described (31, 32). In the reported cases, bisphosphonates were mostly supplied intravenously. Interferon-a 2b and thalidomide may be beneficial through immunomodulatory and antiangiogenic effects. M-TOR inhibitors such as sirolimus and everolimus inhibit the activation of T-lymphocytes by inhibition of the intracellular signal transduction. mTOR is an important kinase in the progression of the cell cycle. The net result is immune suppression by inhibition of lymphocytes and decreased lymphangiomatous invasion by inhibition of lymphangiogenic growth factors (33, 34). The beta-blocking agent propranolol has also been reported as therapeutic option for Gorham-Stout disease, possibly through lowering of VEGF-A levels (35). In their description of 186 reported cases from literature with Gorham-Stout disease Dellinger et al. present the clinical features including affected bones and applied treatments in an overview table in the supplementary material (3).

Radiation therapy may prevent progression of bone osteolysis by inhibition of endothelial cell proliferation. It may prevent disease progression or induce disease regression in 77–80% of the Gorham-Stout disease cases. These percentages are based on a literature analysis on 44 cases with Gorham-Stout disease in which radiation therapy was used, and of results of 10 cases with Gorham-Stout disease obtained from structured questionnaires taken in radiation therapy settings, respectively. The treatment seemed safe, with a mild early and late toxicity after a dose of 30–45 Gy in total (36).

Surgical treatment is applied if patients complain about severe pain and impaired function (7, 37, 38), if there is a fracture or a high risk of fracture, or if complications occur such as an increased intracranial pressure (39) or chylothorax (40) that need surgical intervention. Surgery can consist of removal of the affected bone tissue, with subsequent reconstruction by the use of bone grafts, prostheses, or both.

Regarding the treatment of our case, lesions of the skull have rarely been described in the parietal bone. More frequently reported are skull-base defects, associated with cerebro-spinal fluid leak and meningitis (3, 41). Furthermore, involvement of the mandibulum is a frequently described location in the skull (3). There are only two other reported case reports of Gorham-Stout disease of the parietal bone. Amirjamshidi et al. described a 62-year old man with a painful and tender lesion of the right skull region, caused by a few centimeters large lytic lesion of the parietal bone with MRI characteristics of Gorham-Stout disease. Treatment with pharmacological therapy including bisphosphonates and interferon-alpha−2b did not result in pain relief. Subsequently, he underwent craniotomy with removal of all affected bone and replacement by a titanium plate. After this surgery, he was free of complaints and follow-up after 1 year showed no recurrent disease (42). Parihar et al. described a 35-year old female patient with Gorham-Stout disease of the left parietal bone of the skull, at the same location that was also painless. The defect had a diameter of about 40 mm. Treatment only consisted of surgery with replacement of the affected bone using cranioplasty with bone cement. Unfortunately, no follow-up data is available (43).

CASE FOLLOW-UP

After the MRI-scan of the skull, we concluded that our patient suffered from Gorham-Stout disease of the parietal bone. Since his disease was progressive over the years, we attempt to determine a proper treatment strategy with the goal to protect the brain from mechanic injury locally at the defective zone, and to prevent further osteolysis. But determining the most adequate treatment approach is challenging since literature is not conclusive on the precise treatment approach, reports only a few patients with Gorham-Stout disease of the parietal bone, and currently no guideline or widely accepted treatment approach exists. We referred the patient to the neurosurgeon for surgical treatment of the defect. Since only the inner table is still present over a large area, there is an indication for cranioplasty to protect the brain against mechanical injury. Based on experience and literature from comparable bone defects of the skull with other underlying causes, there are two possible surgical approaches. First, to cover the defect with a custom made 3D printed polyetheretherketone (PEEK) graft without removing all of the affected bone. A possible complication of this treatment is progression of the osteolysis around the

graft and release of the graft with the potential necessity for repeat surgery in the future. This approach is less invasive and carries less risk than the second approach, which would consist of the removal of all the affected remaining bone including a margin of surrounding healthy bone tissue and replacement with a custom made PEEK graft. This included the skull over the superior sagittal sinus. Complications of this operation are the risk of major bleeding from the venous sinuses, venous sinus thrombosis causing cerebral infarction, and -although unlikely- death. From literature, it cannot be concluded whether removal of the affected bone with a margin will prevent recurrence of disease and whether a more invasive and riskier operation should be considered to cure the patient. Importantly, no long-term follow-up data of either procedure are available.

We decided to treat our patient with the first above mentioned surgical approach in combination with an intravenously supplied bisphosphonate. Currently, a custom made PEEK-bone graft is being developed to cover the defect. During surgery we will collect tissue for histological confirmation of the diagnosis. After the surgery, we will supply zoledronic acid five milligrams intravenously with the intent to stop further osteolysis. Follow-up visits and CT and MRI-scans on a regular basis will be used to determine the duration of bisphosphonate treatment and will explain whether the allocated therapy brings the osteolysis to a halt.

CONCLUSION

We describe the case of a young man presenting with a 60 mm defect in the parietal bone of the skull due to Gorham-Stout disease and discuss the challenging task to determine an appropriate treatment strategy to prevent further osteolysis and protect his brain from mechanical damage.

Gorham-Stout disease is a rare bone disease characterized by progressive osteolysis with lymphatic and vascular proliferation. Physicians should be aware of the existence of this disease and once the diagnosis is suspected, referral to a medical center dedicated to rare bone diseases is recommended. Future research is needed since the exact underlying mechanism is not revealed yet. Research should focus on the triggers that initiate and maintain the underlying pathophysiological mechanisms, to get more insight directing toward the most appropriate treatment strategy.

Our case is unique, since it is only the third patient reported in literature with Gorham-Stout disease of the parietal bone of the skull with the largest defect. Future follow-up visits are needed to show whether further osteolysis occurs.

ETHICS STATEMENT

The patient provided written informed consent for publication of the case report.

AUTHOR CONTRIBUTIONS

CK wrote the first draft of the manuscript. EB, MS, and MZ contributed to the design and writing. All authors made critical revisions of the manuscript. EB provided the 3D reconstructions of the skull. MS provided the other figures. All authors read and approved the submitted and revised version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SYSTEMATIC REVIEW
published: 07 February 2020
doi: 10.3389/fendo.2020.00015

Added Value of Impact Microindentation in the Evaluation of Bone Fragility: A Systematic Review of the Literature

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The current gold standard for the diagnosis of osteoporosis and the prediction of fracture risk is the measurement of bone mineral density (BMD) using dual energy x-ray absorptiometry (DXA). A low BMD is clearly associated with increased fracture risk, but BMD is not the only determinant of bone strength, particularly in secondary osteoporosis and metabolic bone disorders in which components other than BMD are affected and DXA often underestimates true fracture risk. Material properties of bone which significantly contribute to bone strength have become evaluable *in vivo* with the impact microindentation (IMI) technique using the OsteoProbe® device. The question arises whether this new tool is of added value in the evaluation of bone fragility. To this effect, we conducted a systematic review of all clinical studies using IMI *in vivo* in humans also addressing practical aspects of the technique and differences in study design, which may impact outcome. Search data generated 38 studies showing that IMI can identify patients with primary osteoporosis and fractures, patients with secondary osteoporosis due to various underlying systemic disorders, and scarce longitudinal data also show that this tool can detect changes in bone material strength index (BMSi), following bone-modifying therapy including use of corticosteroids. However, this main outcome parameter was not always concordant between studies. This systematic review also identified a number of factors that impact on BMSi outcome. These include subject- and disease-related factors such as the relationship between BMSi and age, geographical region and the presence of fractures, and technique- and operator-related factors. Taken together, findings from this systematic review confirm the added value of IMI for the evaluation and follow-up of elements of bone fragility, particularly in secondary osteoporosis. Notwithstanding, the high variability of BMSi outcome between studies calls for age-dependent reference values, and for the harmonization of study protocols. Prospective multicenter trials using standard operating procedures are required to establish the value of IMI in the prediction of future fracture risk, before this technique is introduced in routine clinical practice.

Keywords: fracture risk, osteoporosis primary and secondary, rare bone diseases, bone quality, bone material strength index (BMSi), osteoprobe, bone mineral density, dual energy x-ray absorptiometry (DXA)

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Bone Research, a section of the journal Frontiers in Endocrinology

Received: *28 August 2019*
Accepted: *09 January 2020*
Published: *07 February 2020*

Citation:
Schoeb M, Hamdy NAT, Malgo F, Winter EM and Appelman-Dijkstra NM (2020) Added Value of Impact Microindentation in the Evaluation of Bone Fragility: A Systematic Review of the Literature. Front. Endocrinol. 11:15. doi: 10.3389/fendo.2020.00015

INTRODUCTION

Bone fragility is complex and its evaluation represents a significant challenge in clinical practice. The tools used to assess bone strength and thus fracture risk have so far included the measurement of bone mineral density using dual energy x-ray absorptiometry (DXA) and the evaluation of clinical risk factors for increased bone fragility using the FRAX algorithm. BMD measurements have been routinely performed in the clinic for over three decades and experience with their use is substantial. A low BMD has been clearly associated with increased fracture risk, but evidence has been accumulating over the past decade for factors contributing to bone strength other than BMD, as only one third of an individual's fracture risk is being explained by BMD values (1). The strength of bone thus not only depends on bone mineral density but also on its architecture at the macro-, micro- and nanolevel and on its material composition (2). Available tools for the evaluation of these various components of bone strength have so far included primarily histological evaluation of bone biopsies to assess bone histomorphometry parameters and nanoindentation to assess material properties of bone. Other tools more recently included high resolution peripheral quantitative computed tomography (HR-pQCT) to assess bone structure, and finite element analysis (3, 4). However, these methods are either invasive and time-consuming in their analysis, or associated with high radiation exposure. Material properties of bone, which significantly contribute to bone strength could until recently only be assessed *ex vivo* on a transiliac bone biopsy specimen. Since the introduction of Reference Point Indentation, the possibility has emerged for directly evaluating tissue-level properties of bone in humans *in vivo* (5, 6).

Impact microindentation (IMI) using the handheld OsteoProbe® device has been developed for use in the clinic as an adaption of the original Reference Point Indentation technique (7). Experience has been accumulating with the use of this technique and data have been collected from an increasing number of patients mainly with primary or secondary osteoporosis. A standard operating procedure for IMI has also been recently published to harmonize collection of data (8). Although results have been so far promising, outcomes have not always been concordant between studies or centers so that the added value of this technique in the evaluation of bone fragility still remains to be established. To address this issue, we conducted a systematic review of the literature of all clinical studies in which impact microindentation was performed *in vivo* in humans using the OsteoProbe® device including those published as meeting abstracts. Our objective was to assess the potential added value of impact microindentation in the evaluation of fracture risk in clinical practice. In this process, we also reviewed available literature on practical aspects of the technique and on differences in study design, which may explain differences in outcome between studies, adding new data to those studies published in the last review on the topic in 2017 (9).

The Reference Point Indentation Technique

Reference Point Indentation (RPI) is a technique which enables the assessment of material properties of bone by indenting the bone surface of the tibia *in vivo* in humans. The principle of RPI is based on the hypothesis that indentation of the bone surface results in separation of mineralized collagen microfibers, resulting in microcracks (10). The observation that RPI induced microcracks similar to those observed in fractured cadaveric human bone samples led to the development of the technique *in vivo* in humans in 2006 with a view to assessing the ability of bone to resist fractures. Two Reference Point Indentation devices have so far been used. The original Biodent™ device has been used in the laboratory in animal studies, on human cadaveric bone, and also in early studies *in vivo* in humans (10, 11). The handheld device OsteoProbe® was adapted from the Biodent™ device for *in vivo* use in large animals and in the clinic (7). To avoid confusion in the interpretation of data, a different nomenclature has been proposed for the two devices in 2016 using the term cyclic reference point microindentation (CMI) for the Biodent™ device, and impact microindentation (IMI) for the OsteoProbe® device (8). Since the OsteoProbe® is currently the only tool used in the clinic, and there have been no new publications using the Biodent™ *in vivo* in humans since 2013, with all prior publications included in the last review (9), this systematic review of the impact microindentation technique covers published literature of studies only using the OsteoProbe®.

Using the OsteoProbe®, measurements are performed at a localization at the mid-shaft of the tibia after applying local anesthetic with the patient in the supine position (**Figure 1A**). The probe is gently inserted in the skin at the point of interest until the bone surface is reached, following which the cortical bone of the tibia is indented by an impact delivered by the OsteoProbe®. The resistance of bone tissue to this applied mechanical challenge is expressed as the measured distance covered by the test probe after impact of the probe into bone: the Indentation Distance Increase (IDI). Poorly performed measurements, usually due either to slipping of the test probe on the surface of the bone or to unintentional moving of the subject's leg, are discarded.

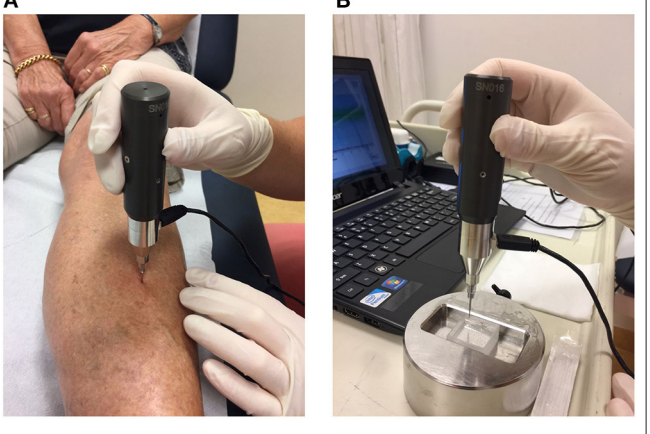

FIGURE 1 | (A) Method of use of the OsteoProbe® on the midshaft of the tibia after the application of a local anesthetic, **(B)** measurement performed on the polymethylmethacrylate (PMMA) reference phantom.

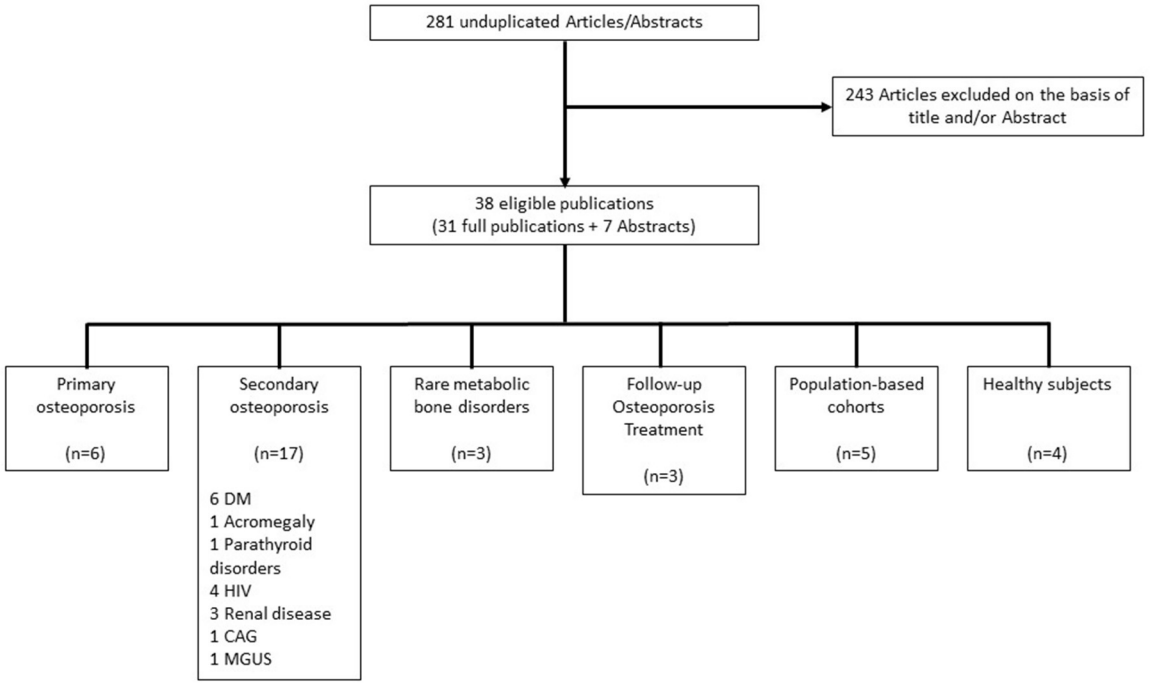

FIGURE 2 | Flowchart of study selection. DM, Diabetes mellitus; HIV, Human immunodeficiency virus; CAG, Chronic atrophic gastritis; MGUS, Monoclonal gammopathy of undetermined significance.

After 10 adequate measurements are obtained, five further measurements are performed on a polymethylmethacrylate (PMMA) reference phantom (**Figure 1B**). The software then calculates the outcome parameter of IMI: the Bone Material Strength index (BMSi). BMSi is defined as 100 times the harmonic mean of IDI from impact into the PMMA phantom divided by the average IDI from impact into bone tissue (7). The lower bone strength is, the deeper the probe indents the bone surface (high IDI) and the lower is the outcome parameter BMSi.

METHODS

Search Strategy and Eligibility Criteria

A search strategy was designed with the help of an experienced librarian for the systematic review of the literature on impact microindentation. The search, which was conducted in PubMed, EMBASE and Web of Science, included all original published articles and meeting abstracts in the English language, updated up to August 2019. Relevant keywords were used, including free text words (see **Supplementary Material** for details of complete search). Studies using the cyclic RPI technique with the pre-clinical device Biodent™, and studies using early OsteoProbe I™ and II™ devices were excluded from analyses. All articles identified by the search were assessed by two independent investigators.

Search Data Extraction

Studies were independently reviewed by the two independent investigators and the following data were extracted: (1) Demographic data on patient and control populations (clinical characteristics, population size); (2) outcome data (BMSi and BMD at the femoral neck (FN)); (3) Factors potentially influencing BMSi outcome (age, gender, body mass index (BMI), geographical region, prevalent fractures, BMD, number of operators, intra- and interobserver coefficient of variation, number of indentations per BMSi obtained); (4) potential complications arising from IMI. Extracted data were reported as presented in the articles, as mean ± SD, mean (SEM), or median (IQR).

Quality Assessment

The quality of full publications was independently assessed using the Newcastle-Ottawa (NO) Scale, which includes three domains: selection, comparability and exposure/outcome and was adapted for this review (see **Supplementary Table**). Based on the total quality score, articles were scored out of a maximum score of ten as unsatisfactory (0–4), satisfactory (5–7), or good (8–10).

RESULTS

Study Selection

The search yielded 456 articles and 102 meeting abstracts. Two hundred and seventy-seven duplicate articles in ≥2 electronic data bases were excluded, and 281 unique studies were further assessed; (**Figure 2**). Two hundred and forty-three of these were excluded on the basis of title and abstract: 218 studies were not using the OsteoProbe®, eight were using the OsteoProbe® either in animal studies (12–14) or in *ex vivo* human bone (15–19), two described primarily technical

TABLE 1 | Impact microindentation studies in subjects with osteoporosis/fractures.

References	Subjects	(m/f)	Age (years)	FN BMD (g/cm^2)	BMSi	Relationship of BMSi with			Study quality scale (0–10)
						Age	BMD	Fx	
Malgo et al. (33)	Fx	63 (24/39)	62.6 ± 9.6 (40–85)	0.67 ± 0.09	79.9 (0.6) (78.7–81.1)	Neg	No	Pos	8
	No Fx	27 (13/14)	57.1 ± 9.5 (40–85)	0.69 ± 0.08	82.4 (1.0) (80.3–84.5)				
Malgo et al. (34)	NVF only	53 (14/39)	62.8 ± 8.3 (40–85)	0.65 ± 0.07	78.9 (0.7) (77.5–80.3)	Neg	No	Pos	9
	VF + NVF	34 (14/20)	62.8 ± 9.9 (40–85)	0.69 ± 0.09	78.3 (0.9) (76.5–80.1)				
	VF only	14 (8/6)	64.7 ± 9.3 (40–85)	0.70 ± 0.09	78.4 (1.4) (75.4–81.4)				
	No Fx	31 (11/20)	57.5 ± 9.9 (40–85)	0.68 ± 0.07	82.5 (0.9) (80.7–84.3)				
Duarte Sosa and Fink Eriksen (38)	Stress Fx	30 (0/30)	39.0 ± 13.9 (19–64)	0.92 ± 0.25	70.5 ± 8.7 (67.4–73.6)	No	No	Pos	8
	No Fx	30 (0/30)	42.3 ± 9.8 (23–66)	1.05 ± 0.11	77.1 ± 7.2 (74.5–79.7)				
Sosa and Eriksen (35)	NH/NVF	17 (0/17)	66.2 ± 9.1 (50–85)	0.77 ± 0.07	73.1 ± 6.5 (69.8–76.4)	No	No	Pos	10
	HF	25 (0/25)	68.2 ± 10.0 (50–85)	0.76 ± 0.11	72.0 ± 6.5 (69.3–74.7)				
	VF	24 (0/24)	67.8 ± 10.1 (50–85)	0.71 ± 0.10	70.1 ± 7.1 (67.2–73.0)				
	No Fx	66 (0/66)	66.5 ± 7.9 (50–85)	1.02 ± 0.13	76.4 ± 6.2 (74.9–77.9)				
Rozental et al. (50)	DRF	57 (0/57)	64.2 ± 10.5 (>50)	0.68 ± 0.11	74.4 ± 8.8 (72.1–76.7)	Neg	Pos	DRF Pos HF No	6
	HF	42 (0/42)	75.7 ± 10.9 (>50)	0.61 ± 0.12	74.6 ± 8.5 (72.0–77.2)				
	No Fx	93 (0/93)	67.3 ± 7.6 (>50)	0.72 ± 0.10	77.4 ± 8.8 (75.6–79.2)				
Popp et al. (55)	AFF	15 (0/15)	71.8 ± 10.8	0.69 ± 0.03	76.5 ± 10.9 (70.5–82.5)	No	No	No	5
	HF	20 (0/20)	74.6 ± 8.3	0.62 ± 0.03	78.3 ± 9.3 (73.9–82.7)				
	BP >5 years	30 (0/30)	71.9 ± 9.1	0.60 ± 0.19	76.6 ± 10.5 (72.8–80.4)				
	BP–naive	88 (0/88)	65.9 ± 5.6	0.71 ± 0.01	80.1 ± 8.3 (78.4–81.8)				

Age is presented as mean ± SD (range, if reported), BMD is presented as mean ± SD and BMSi is presented as mean ± SD (95% CI) or mean (SE) (95% CI). 95% CI calculated from data provided in the publication. AFF, Atypical femoral fracture; BMD, Bone mineral density; BMSi, Bone material strength index; BP, Bisphosphonate; DRF, Distal radius fracture; FN, Femoral neck; Fx, Fracture; HF, Hip fracture; NH/NVF, Non-hip non-vertebral fracture; NVF, Non-vertebral fracture; VF, Vertebralfracture.

aspects of RPI devices (8, 20) and 15 were review articles, eight describing different techniques for the assessment of bone quality including RPI (21–28), and seven specifically on RPI (7, 9, 20, 29–32). Included in the final evaluation were 31 original articles and seven studies published only as meeting abstracts using the OsteoProbe® microindentation device *in vivo* in humans.

Quality Assessment

Based on the adapted Newcastle-Ottawa Scale (adapted NO Scale: **Supplementary Table**), methodological quality of studies was good in eight studies (33–40), satisfactory in 15 studies (41–55), and unsatisfactory in six studies (56–61) (**Tables 1**–**6**). The NO Scale could not be used for the evaluation of two fully published studies (67, 69) and for all meeting abstracts (62–66, 68, 70) because of incomplete data. Of the 31 full articles, 8 (25.8%) were thus of unsatisfactory methodological quality or unevaluable. Notwithstanding, for the purpose of completeness of this systematic review of the literature we report on all full publications as well as meeting abstracts yielded by our search strategy reporting the use of IMI *in vivo* in humans.

Characteristics of Studies Generated by the Search

Of the 38 reported studies using the OsteoProbe®, 17 included patient groups with a known increased risk for fragility fractures on the basis of secondary osteoporosis, although this was not fully reflected by BMD values (36, 41, 45–49, 52, 53, 56, 58, 59, 62–66). The majority of these studies included patients with endocrine disorders such as diabetes mellitus and acromegaly ($n = 8$) (36, 41, 48, 49, 62–65). Six studies focused on patients having sustained fragility or stress fractures (33–35, 38, 50, 55), three studies evaluated BMSi outcomes in patients receiving osteoporosis treatment (37, 44, 68) and another three studies reported on patients with a rare metabolic bone disorder: Type 1 Gaucher Disease, Paget's and Camurati-Engelmann disease (57, 60, 67). Seven studies, two of which included patients with diabetes mellitus (41, 63), were performed in two population-based cohorts from Sweden and Australia, both designed to assess epidemiological data in osteoporosis (39, 43, 51, 54, 69). Four other studies included healthy individuals (40, 42, 61, 70) (**Figure 2**).

The number of patients in whom BMSi was measured varied greatly between studies, with the smallest study including only seven subjects (68) and the largest 489 subjects (41). All studies were performed in adults with ages ranging from a median of 33.9 years (27.6–53.8 years) (46) to a mean of 78.3 ± 1.1 years (51). Fourteen studies included only women (35, 37–43, 48–51, 55, 68), and four studies included only men (54, 63, 64, 69). Although not always explicitly stated, 17 studies appear to have overlapping patient or control cohorts (33, 36, 39, 41, 43, 45, 51–54, 57–60, 63, 67, 69).

TABLE 2 | Impact microindentation studies in subjects with secondary osteoporosis.

(a) Endocrine disorders

References	Subjects	(m/f)	Age (years)	FN BMD (g/cm^2)	Fx prevalence (%)	BMSi	Relationship of BMSi with			Study quality scale (0–10)
							Age	BMD	Disease	
Farr et al. (48)	Type 2 DM	30 (0/30)	65.4 (1.4)	0.93 (0.03)	10.0	77.2 (1.6) (73.9–80.6)	NA	NA	Lower in DM	5
	Controls	30 (0/30)	65.7 (1.6)	0.94 (0.03)	10.0	85.7 (1.6) (82.4–89.0)				
Nilsson et al. (41)	Type 2 DM	99 (0/99)	77.6 ± 1.5 (75–80)	0.69 ± 0.10	55.0	74.6 ± 7.6* (72.5–76.7)	NA	NA	Lower in DM	7
	Controls	954 (0/954)	77.7 ± 1.5 (75–80)	0.66 ± 0.10*	52.0	78.2 ± 7.5* (77.5–78.9)				
Furst et al. (49)	Type 2 DM	16 (0/16)	65.4 ± 2.4	0.77 ± 0.03	19.0	63.7 (1.9) (59.7 –67.8)	NA	No	Lower in DM	7
	Controls	19 (0/19)	65.6 ± 1.2	0.69 ± 0.01	11.0	70.1 (1.9) (66.1–74.1)				
Barnouin et al. (62) (Ab)	Type 2 DM	27 (NA)	NA (65–85)	NA	NA	70.5 ± 6.5 (67.9–73.1)	NA	NA	NA	NA
Holloway et al. (63) (Ab)	DM	34 (34/0)	NA (33–92)	0.97 (0.92–1.01)	NA	80.6 (78.9–82.9)	NA	NA	Lower in DM, but not in IFG	NA
	IFG	37 (37/0)		0.95 (0.91–0.99)		83.6 (81.7–85.6)				
	Controls	140 (140/0)		0.96 (0.94–0.98)		83.4 (82.4–84.4)				
Syversen et al. (64) (Ab)	Type 1 DM	33 (33/0)	42.7 ± 12.1	NA	NA	NA	NA	NA	Lower in DM	NA
	Controls	28 (28/0)	41.8 ± 12.0							
Malgo et al. (36)	Acromegaly	48 (26/22)	60.2 ± 11.0	0.84 ± 0.16	58.0	79.4 (0.7) (78.0–80.8)	Pos	No	Lower in Acromegaly	8
	Controls	44 (22/22)	60.5 ± 8.5	0.80 ± 0.09	16.0	83.2 (0.7) (81.8–84.6)	Neg	No		
Starr et al. (65) (Ab)	PHPT	13 (4/9)	59.3 ± 15.0	NA	NA	67.8 ± 9.0 (62.3–73.2)	NA	NA	Lower in PHPT + HypoPT	NA
	HypoPT	15 (4/11)	44.3 ± 12.5			68.4 ± 10.0 (62.9–73.9)				
	Controls	22 (5/17)	49.2 ± 17.0			77.2 ± 8.0 (73.7–80.7)				

(b) HIV, Chronic kidney disease, CAG, MGUS

CROSS-SECTIONAL DESIGN										
Guerri-Fernandez et al. (46)	HIV	50 (35/15)	36.7 [31.7–46.2]	0.81 [0.77–0.88]	4.0	84.5 [83.0–87.0]	NA	NA	Lower in HIV	7
	Controls	35 (24/11)	33.9 [27.6–53.8]	0.79 [0.73–0.96]	0	90.0 [88.5–93.0]				
Guerri-Fernandez et al. (47)	HIV >5 years TDF/FTC	36 (27/9)	56.4 ± 6.3	0.72 [0.2]	5.5	81.0 [0.8]	No	No	Lower in TDF/FTC	7
	HIV >5 years ABC/3TC	27 (20/7)	63.0 ± 9.8	0.74 [0.2]	3.7	82.7 [1.3]				

(Continued)

TABLE 2 | Continued

References	Subjects	(m/f)	Age (years)	FN BMD (g/cm^2)	Fx prevalence (%)	BMSi	Relationship of BMSi with			Study quality scale (0–10)
							Age	BMD	Disease	
Perez-Saez et al. (45)	ESRD before KT	53 (25/28)	55.8 ± 12.1	0.73 ± 0.15	26.4	79* [71.8–84.2]	NA	NA	Lower in ESRD	5
	Controls	94 (20/74)	50.2 ± 16.0	0.78 ± 0.12	0	82.6 [77.5–88.9]				
Perez-Saez et al. (58)	KT recipients >10 years after KT	40 (17/23)	63.8 ± 11.1	0.67 ± 0.13*	32.5	79.1 ± 7.7* (76.7–81.5)	NA	No	No	4
	Controls	94 (20/74)	50.2 ± 16.0	0.78 ± 0.12*	0	82.9 ± 7.8* (81.3–84.5)				
Aasarod et al. (56)	CAG	17 (9/8)	54.1 ± 12.6 (20–70)	0.79 ± 0.14	41.1	82.0 ± 9.6* (76.5–87.5)	NA	NA	No	1
	Controls	41 (20/21)	53.2 ± 11.4 (20–70)	0.80 ± 0.16	44.0	80.0 ± 7.0* (76.5–83.5)				
Gonzalez et al. (66) (Ab)	MGUS	22 (NA)	NA	0.73	NA	68.3 ± 5.0 (66.1–70.5)	NA	NA	Lower in MGUS	NA
	Controls	NA		0.78		83.0 ± 4.0				

References	Intervention	Subjects	(m/f)	Age (years)	FN BMD (g/cm^2)	Fx prevalence (%)	BMSi	Relationship of BMSi with			Study quality scale (0–10)
								Age	BMD	Intervention	
LONGITUDINAL DESIGN											
Guerri-Fernandez et al. (52)	ART with TDF/FTC, FU 24 (not shown) + 48 weeks	HIV	40 (33/7)	38 ± 9	**BL:** 0.84 ± 0.12 **FU:** 0.81 ± 0.11	5.0	**BL:** 86.1 ± 6.1 (84.2–88.0) **FU:** 89.0 ± 4.2 (87.7–90.3)	Neg	Pos	Increase with ART	7
Lerma-Chippirraz et al. (59)	ART with TDF/FTC, FU 48 weeks (HIV only)	HIV	20 (16/4)	37 [31–43]	**BL:** 0.84 [0.79–1.02] **FU:** 0.82 [0.73–0.96]	0	**BL:** 86 [83–90] **FU:** 90 [88–93]	NA	NA	Increase with ART	4
		Controls	20 (15/5)	38 [35–42]	**BL:** 0.83 [0.75–0.98]	0	**BL:** 89 [88–93]				
Perez-Saez et al. (53)	Low-dose GC after KT, FU 3 (not shown) + 12 months	ESRD receiving KT	36 (19/17)	54.9 ± 11.6	**BL:** 0.75 ± 0.15 **FU:** 0.73 ± 0.14	16.7	**BL:** 79.2* [73.2–85.4] **FU:** 80.1* [73.0–85.4]	NA	NA	No	6

*Age is presented as mean ± SD (range, if reported), mean (SE) or median [IQR], BMD is presented as mean ± SD, mean (95% CI), median [IQR] or median (SE) and BMSi is presented as mean ± SD (95% CI), mean (SE) (95% CI) or median [IQR]. 95% CI calculated from data provided in the publication. Ab, published only in Abstract form; ABC/3TC, Abacavir/lamivudine; ART, Antiretroviral therapy; BL, Baseline; BMD, Bone mineral density; BMSi, Bone material strength index; CAG, Chronic atrophic gastritis; ESRD, End-stage renal disease; FN, Femoral neck; FU, Follow-up; Fx, Fracture; IFG, Impaired fasting glucose; HypoPT, Hypoparathyroidism; MGUS, Monoclonal gammopathy of undetermined significance; PHPT, Primary hyperparathyroidism; DM, Diabetes mellitus; TDF/FTC, Tenofovir/emtricitabine. *Measured in a subgroup of subjects.*

TABLE 3 | Impact microindentation studies in subjects with rare metabolic bone disorders.

References	Subjects	(m/f)	Age (years)	FN BMD (g/cm^2)	Fx prevalence (%)	BMSi	Relationship of BMSi with			Study quality scale (0–10)
							Age	BMD	Disease	
Herrera et al. (57)	Gaucher's disease	16 (7/9)	51.3 ± 14.4 (21–69)	NA	0.0	72.7 ± 10.0 (67.4–78.0)	NA	NA	Lower in Gaucher's disease	4
	Controls	29 (5/24)	48.7 ± 15.8 (20–69)		0.0	81.8 ± 1.4 (81.3–82.3)				
Malgo et al. (67)	Unilateral Paget's disease of the tibia	9 (4/5)	69.5 (55–87)	NA	NA	Pagetic Tibia: 74.7 (1.7) (70.8–78.6) Non-pagetic Tibia: 78.7 (1.3) (75.7–81.7)	NA	NA	NA	NA
	Controls	11 (7/4)	61.9 (51–72)	NA	72.7	Dominant Tibia: 82.1 (1.3) (79.2–85.0) Non-dominant Tibia: 81.4 (1.3) (78.5–84.3)				
Herrera et al. (60)	Camurati-Engelmann	3 (1/2)	44.0 [43–47]	NA	0.0	76.9	NA	NA	Lower in Camurati-Engelmann	2
	Controls	29 (5/24)	56.0 (NA)		0.0	81.4			(ns)	

Age is presented as mean ± SD (range), mean (range) or median [range] and BMSi is presented as mean ± SD (95% CI), mean (SE) (95% CI) or median. 95% CI calculated from data provided in the publication. BMD, Bone mineral density; BMSi, Bone material strength index; FN, Femoral neck; Fx, fracture.

Impact Microindentation Studies in Patients with Fractures (Table 1)

Initial studies using IMI aimed at establishing the value of the then novel technique in the evaluation of bone fragility in patients who had sustained a fracture or were at high risk of sustaining one. Six of these studies evaluated the association between BMSi and prevalent fractures (33–35, 38, 50, 55).

Confirming data initially obtained by the Spanish group using the earlier device Biodent™ (10, 11), our group demonstrated that in the presence of comparable BMD values, BMSi was lower in 63 patients who had sustained a fragility fracture compared to 27 patients who had not [respectively, 79.9 (SE 0.6) vs. 82.4 (SE 1.0), $p = 0.032$]. BMSi was also comparable in patients with fragility fractures irrespective of whether they had osteopenia or osteoporosis (33). A subsequent study conducted in 132 patients, including data from the 90 patients from the original publication, confirmed that BMSi was lower in patients with fragility fractures ($n = 101$) compared to those who never sustained a fracture ($n = 31$) [respectively, 79.0 (SE 0.5) vs. 82.5 (SE 0.9), $p = 0.001$], independently of BMD measurements. Interestingly we also observed that measured BMSi values were comparable in fracture patients, regardless of type of fracture: non-vertebral ($n = 53$) [BMSi 78.9 (SE 0.7)], vertebral ($n = 14$) [BMSi 78.4 (SE 1.4)] or combined non-vertebral and vertebral fractures ($n = 34$) [BMSi 78.3 (SE 0.9)] (34).

Duarte Sosa et al. confirmed these findings by demonstrating that 66 postmenopausal women with osteoporosis and fragility fractures had significantly lower BMSi than age-matched postmenopausal women who had normal BMD values and no fractures (respectively, 71.5 ± 7.3 vs. 76.4 ± 6.2, $p = 0.008$) (35). Also in keeping with results from our group (34) there was no difference in BMSi whether patients had vertebral fractures ($n = 24$), hip fractures ($n = 25$) or non-hip non-vertebral fractures ($n = 17$), with BMSi 70.1 ± 7.1 vs. 72.0 ± 6.5 vs. 73.1 ± 6.5, respectively (35). Findings from this study also demonstrated that BMSi was inversely related to severity of vertebral fractures as evaluated by Genant's grading score in the 24 patients with vertebral fractures ($r^2 = 0.19$, $p < 0.05$), although these results could not be reproduced by our group or by that of Rudäng et al. (34, 43). Another study from the Norwegian group excluding patients with osteoporosis or previous low-energy fractures, but including women with stress fractures of the lower extremities or pelvis, demonstrated significantly lower BMSi in 30 patients with stress fractures (BMSi 70.5 ± 8.7), than in controls without fractures (BMSi 77.1 ± 7.2, $p = 0.01$) (38).

In a cohort of 192 postmenopausal women, Rozental et al. showed that BMSi was lower in addition to lower BMD at the FN and LS in 57 patients with distal radius fractures than in 93 fracture-free controls (BMSi, respectively, 74.4 ± 8.8 vs. 77.4 ± 8.8, $p = 0.04$) (50). This was also the case for the 42 patients with hip fractures although this did not reach statistical significance compared to controls (BMSi 74.6 ± 8.5, $p = 0.09$).

In a study addressing whether cortical tissue properties of bone are altered in atypical femoral fractures (AFF), BMSi was measured in 15 postmenopausal women with this rare fracture. In these patients, BMSi was lower, albeit not significantly, compared to that in 20 patients with hip fractures, 30 long-term bisphosphonate users, and 88 patients never treated with antiresorptives (BMSi 76.5 ± 10.9 vs. 78.3 ± 9.3 vs. 76.6 ± 10.5 vs. 80.1 ± 8.3, respectively) (55).

In summary, BMSi was found to be lower in all patients with fragility fractures compared to non-fracture controls

TABLE 4 | Impact microindentation studies in subjects receiving osteoporosis treatment.

References	Subjects	(m/f)	Age (years)	FN BMD (g/cm^2)	BMSi	Relationship of BMSi with			Study quality scale (0–10)
						Age	BMD	Fx	
CROSS-SECTIONAL DESIGN									
Nogues et al. (37)	BP >4 years Fx	21 (0/21)	69.5 ± 5.9	0.62 ± 0.08	73.8 ± 6.5 (70.8–76.8)	NA	No	Pos	8
	BP > 4 years No Fx	19 (0/18)	71.5 ± 6.8	0.66 ± 0.09	81.6 ± 6.3 (78.5–84.7)				

References	Intervention	OP treatment	(m/f)	Age (years)	FN BMD (g/cm^2)	Fx prevalence (%)	BMSi	Relationship of BMSi with			Study quality scale (0–10)
								Age	BMD	Intervention	
LONGITUDINAL DESIGN											
Mellibovsky et al. (44)	GC and OP prophylaxis, FU 7 and 20 weeks (not shown)	Ca/Vit D	19 (11/8)	55.3 ± 17.9	0.83 ± 0.13 FU NA	0.0	**BL:** 81.6 (74.3–86.9) **FU**: 71.9 (65.4–77.1)	NA	NA	Decrease in Ca/Vit D Increase in TPTD + Dmab	7
		BP	14 (10/4)	66.1 ± 17.0	0.75 ± 0.14 FU NA	7.1	**BL:** 81.1 (75.6–89.6) **FU:** 83.4 (76.6–93.0)				
		TPTD	5 (1/4)	69.8 ± 8.0	0.62 ± 0.12 FU NA	60.0	**BL:** 70.0 (64.0–72.6) **FU:** 81.8 (73.3–88.9)				
		DMAb	14 (5/9)	58.9 ± 12.8	0.72 ± 0.15 FU NA	14.3	**BL:** 76.2 (72.0–84.9) **FU:** 84.0 (79.2–90.0)				
Tsai et al. (68) (Ab)	TPTD treatment, FU 3 months	TPTD 20 mg	33 (0/33)	NA (52–83)	NA	NA	**BL:** 82.1 ± 8.3* (61.5–102.7) **FU**: −4.8%	NA	NA	Decrease in TPTD 20 + 40 mg	NA
		TPTD 40 mg	29 (0/29)				**BL:** 83.2 ± 10.1* (67.1–99.3) **FU:** - 7.0%				

*Age is presented as mean ± SD (range, if reported), BMD is presented as mean ± SD and BMSi is presented as mean ± SD (95% CI) or median (IQR). 95% CI calculated from data provided in the publication. Ab, published only in Abstract form; BL, Baseline; BMD, Bone mineral density; BMSi, Bone material strength index; BP, Bisphosphonate; Ca, Calcium; DMAb, Denosumab; FN, Femoral neck; FU, Follow-up; Fx, Fracture; GC, Glucocorticoid; OP, Osteoporosis; TPTD, Teriparatide. *Measured in a subgroup of subjects.*

TABLE 5 | Impact microindentation studies in population-based cohorts.

References	Subjects	(m/f)	Age (years)	FN BMD (g/cm^2)	Fx prevalence (%)	BMSi	Relationship of BMSi with			Study quality scale (0–10)
							Age	BMD	Fx	
Rudang et al. (51)	Elderly women	211 (0/211)	78.3 ± 1.1 (75–80)	0.65 ± 0.10	55.5	75.6 ± 7.6 (74.6–76.6)	No	Pos	No	6
Sundh et al. (40)	Elderly women	202 (0/202)	78.2 ± 1.1 (75–80)	NA	NA	75.6 ± 7.6 (74.6–76.6)	NA	NA	No	8
Johansson et al. (43)	Elderly women VF	277 (0/277)	77.8 ± 1.4 (75–80)	0.64 ± 0.09*	100	76.9 ± 7.3* (75.7–78.1)	NA	NA	No	6
	Elderly women No VF	750 (0/750)	77.7 ± 1.6 (75–80)	0.67 ± 0.10*	0	77.9 ± 7.4* (77.1–78.7)				
Rufus-Membere et al. (69)	Men	252 (252/0)	63.2 ± 12.6 (33–96)	NA	NA	83.0 ± 6.4 (82.2–83.8)	No	NA	NA	NA
Rufus-Membere et al. (54)	Men	357 (357/0)	63.2 ± 13.8 (33–96)	0.96 ± 0.13	11.9	Fx 80.2 ± 6.9 (78.0–82.4) No Fx 82.8 ± 6.1 (82.1–83.5)	Neg	No	Pos	5

*Age is presented as mean ± SD (range), BMD is presented as mean ± SD and BMSi is presented as mean ± SD (95% CI). 95% CI calculated from data provided in the publication. BMD, Bone mineral density; BMSi, Bone material strength index; FN, Femoral neck; Fx, Fracture; VF, Vertebral fracture. *Measured in a subgroup of subjects.*

independently of site of fracture and generally independently of BMD values, suggesting that tissue material properties of bone are altered in fragility fracture patients and that BMSi measured at the tibia is associated with increased bone fragility at all relevant skeletal sites.

Impact Microindentation Studies in Secondary Osteoporosis (Table 2)

Any systemic disorder may affect the skeleton and alter the material properties of bone thus increasing fracture risk. Our literature search yielded 17 studies addressing the value of IMI in assessing fracture risk in secondary osteoporosis: eight in patients with a variety of endocrine diseases (36, 41, 48, 49, 62–65), four in patients infected with the Human Immunodeficiency Virus (HIV) (46, 47, 52, 59), three in patients with chronic kidney disease (45, 53, 58), one in patients with chronic atrophic gastritis (56) and one in patients with monoclonal gammopathy of undetermined significance (MGUS) (66).

Endocrine Disorders

Secondary osteoporosis is a common co-morbidity of endocrine disorders, resulting from direct and indirect effects of hormonal excess or deficiency on the bone remodeling cycle, bone mineral content and bone matrix composition (71). The eight published studies in patients with endocrine disorders (four in abstract form) included a total of 304 patients in whom IMI was performed in addition to standard evaluation of fracture risk using DXA. Six of these studies were performed in patients with Diabetes mellitus (one in type 1, five in type 2) (41, 48, 49, 62–64), one in Acromegaly (36), and one in parathyroid disorders (primary hyperparathyroidism and hypoparathyroidism) (65).

Diabetes mellitus (DM)

The effect of DM on the skeleton is multifactorial. The main cause of osteoporosis in DM is low bone formation with the two main contributing factors for this being a shift from osteoblastogenesis to adipogenesis, and the toxic effects of the accumulation of advanced glycation end products (AGE) on osteoblasts, both leading to the characteristic low bone turnover and increased fracture risk particularly observed in type 1 DM (T1DM). Fracture risk is however increased in both T1DM and type 2 DM (T2DM) with BMD measurements shown to underestimate fracture risk (72). This suggests that tissue material properties of bone are likely to be impaired in patients with DM and that the evaluation of BMSi may provide information on bone strength not captured by BMD. Five of the six studies in DM patients (T1DM $n = 33$, T2DM $n = 131$), also including patients with impaired fasting glucose concentrations (IFG, $n = 37$), compared BMSi in DM and prediabetes patients with that of non-diabetic controls ($n = 655$). Findings from these studies show significantly lower BMSi values in all DM patients (type 1 and 2), but not in prediabetes, compared to controls, with values ranging from 63.7 (SE 1.9) (49) to 80.6 (63) in DM patients and from 70.1 (SE 1.9) (49) to 85.7 (SE 1.6) (48) in controls. Data from a US group also show an inverse relationship between BMSi and mean glycated hemoglobin level (HbA1c) over 10 years prior to the study ($r = -0.41$; $p = 0.026$) (48), suggesting a direct negative effect

TABLE 6 | Impact microindentation studies in healthy subjects.

References	Subjects	(m/f)	Age (years)	FN BMD (g/cm^2)	Fx prevalence (%)	BMSi	Relationship of BMSi with			Study quality scale (0–10)
							Age	BMD	Fx	
CROSS-SECTIONAL DESIGN										
Duarte Sosa et al. (42)	Women from Norway	42 (0/42)	46.3 ± 13.6	1.03 ± 0.10	0	77.0 ± 7.1 (74.9–79.2)	No	No	NA	5
	Women from Spain	46 (0/46)	46.7 ± 15.4	0.83 ± 0.12	0	80.7 ± 7.8 (78.4–83.0)				
Taymouri et al. (61)	Healthy volunteers	88 (19/69)	Men: 34 (24–98) Women: 49 (30–81)	NA	NA	88.0 ± 7.6 (84.3–91.7) 82.0 ± 7.4 (80.3–83.8)	No	NA	NA	3
Guerri et al. (70) (Ab)	Subjects prior to knee replacement	10 (5/5)	72 ± 5 (59–83)	0.66 ± 0.08	NA	75.8 ± 6.0 (71.5–80.1)	NA	No	NA	NA
References	**Intervention**	**Subjects**	**(m/f)**	**Age (years)**	**FN BMD (g/cm^2)**	**Fx prevalence (%)**	**BMSi**	**Relationship of BMSi with**		**Study quality scale (0–10)**
								Age	**BMD**	**Fx**
LONGITUDINAL DESIGN										
Sundh et al. (40)	Exercise of one leg, FU after 3 months	Inactive women	20 (0/20)	55.5 ± 2.3 (51–59)	**BL:** 0.72 ± 0.08 **FU:** NA	NA	**BL:** 73.4 ± 5.8 (70.7–76.1) **FU:** 76.8 ± 9.0 (72.6–81.0)	NA	NA	NA	8

Age is presented as mean ± SD (range, if reported) or median (range), BMD is presented as mean ± SD and BMSi is presented as mean ± SD (95% CI). 95% CI calculated from data provided in the publication. Ab, published only in Abstract form; BL, Baseline; BMD, Bone mineral density; BMSi, Bone material strength index; FN, Femoral neck; FU, Follow-up; Fx, Fracture.

of prolonged hyperglycemia on bone material properties. This finding was also supported by a significant inverse relationship between BMSi and the duration of T2DM ($r = -0.68$, $p < 0.05$) observed in another US study (49). Data further show that BMSi is significantly inversely correlated with AGE data obtained from skin analysis as detected by autofluorescence ($r = -0.65$, $p < 0.05$) (49). In all these studies but one, LS and FN BMD were comparable between T2DM patients and controls, which was not the case in T1DM where a larger number of patients also had low bone mass. Data on fracture risk were not reported in any of the studies in patients with DM. Literature findings in DM therefore suggest that bone material properties are impaired in both T1DM and T2DM, independently of BMD. In the last of the six studies conducted in DM patients, BMSi was significantly higher in T2DM patients with higher ergonometrically measured fitness although this was only published in abstract form (62).

Acromegaly

Skeletal changes in acromegaly are due to GH excess and are characterized by high bone turnover in favor of increased bone formation. Although BMD is generally normal or increased, the disorder is associated with an increased risk for vertebral fractures (71). Our group showed that BMSi was significantly lower in 48 patients with acromegaly despite long-term remission: 16.1 years (range 0.5–37.8 years), compared with BMSi measurements in 44 age-matched controls, 79.4 (SE 0.7) vs. 83.2 (SE 0.7), $p < 0.001$, although LS and FN BMD were comparable between groups (36). This finding suggests that tissue material properties of bone are likely to be irreversibly altered in patients with acromegaly leading to persistent increased fracture risk despite long-term adequate control of GH excess (73). Intriguingly, we could not demonstrate a difference in BMSi between patients with ($n = 28$) or without ($n = 20$) vertebral fractures (36).

Parathyroid disorders

BMSi was measured in patients with parathyroid disorders in a single study only published in abstract form (65). Parathyroid hormone (PTH) plays an important role in the maintenance of bone mass and integrity, and both excess or decrease of the circulating hormone may potentially affect fracture risk. Whereas bone turnover is low in hypoparathyroidism, it is high in hyperparathyroidism in favor of bone resorption, resulting in bone loss particularly at cortical sites. Although both non-vertebral and vertebral fracture risk have been shown to be increased in hyperparathyroidism (74, 75), data in hypoparathyroidism are scarce and conflicting (76). Starr et al. found that BMSi was significantly lower in 13 patients with primary hyperparathyroidism than in 22 age- and sex-matched controls with normal PTH values (respectively, 67.8 ± 9.0 vs. 77.2 ± 8.0, $p < 0.05$). Interestingly, BMSi was also found to be lower in 15 patients with hypoparathyroidism compared to controls (respectively, 68.4 ± 10.0 vs. 77.2 ± 8.0, $p < 0.05$). As expected, BMD T-scores were higher in hypoparathyroidism than in hyperparathyroidism patients at all sites except at the LS. No data on fracture risk were provided (65).

Patients Infected With HIV

BMSi was measured in a total of 153 patients with HIV in four studies conducted by the Spanish group (46, 47, 52, 59). Patients infected with HIV are at increased fracture risk possibly due to the viral infection itself or its treatment, particularly when using the antiretroviral drug tenofovir disoproxil fumarate (TDF), although the exact mechanism by which this drug increases bone fragility remains elusive. In an initial study by Güerri-Fernández et al. BMSi was found to be significantly lower in 50 untreated HIV patients compared to 35 healthy HIV-negative controls [84.5 (83.0–87.0) vs. 90.0 (88.5–93.0), respectively, $p < 0.001$], in the presence of similar BMD values (46). Findings from two further studies showed a significant increase in BMSi from 86.1 ± 6.1 to 89.0 ± 4.2 ($p < 0.001$), reaching comparative values to those of healthy controls, 12 months after starting antiretroviral therapy with TDF, while BMD values decreased on treatment (52, 59). No data are provided on the effect of these findings on fracture risk. BMSi values were not found to be different in patients on long-term treatment with TDF compared to patients using the different antiretroviral agent abacavir, BMSi 81.0 (0.8) vs. 82.7 (1.3), $p = 0.27$, respectively (47).

Chronic Kidney Disease (CKD)

Data on BMSi in CKD were retrieved from three studies conducted by the Spanish group (45, 53, 58). BMSi values were significantly lower in 35 CKD patients on dialysis compared to 94 healthy non-CKD controls [respectively, 79.0 (71.8–84.2) vs. 82.6 (77.5–88.9), $p < 0.05$] (45). In a second study, BMSi was measured cross-sectionally in 38 kidney transplant recipients more than 10 years after kidney transplantation. BMSi was found to be low in long-term transplant recipients compared to 93 younger healthy controls, although the difference was no longer observed after adjusting for age, sex, and BMI. This suggests that bone material properties may improve in kidney transplant recipients in the long-term (58). The most recent data were from a longitudinal study conducted in 14 patients undergoing kidney transplantation on a low glucocorticoid dosing protocol with follow-up BMSi performed 3 and 12 months post-transplantation. These data showed no significant change in BMSi compared to baseline values both at 3 months and at 12 months post-transplant [respectively, 80.1 (73.0–85.4) vs. 79.2 (73.2–85.4), no *p*-value provided] despite a significant transient decrease of FN BMD at month 3 and a significant decrease of LS BMD at month 12 (53).

Chronic Atrophic Gastritis (CAG)

A small study in 14 Norwegian patients with CAG and 18 age- and sex-matched healthy controls reported no difference in BMSi between patients and controls (respectively, 82.0 ± 9.6 vs. 80.0 ± 7.0, no *p*-value provided) in the presence of similar LS and FN BMD values. No fracture data were provided in this study, and the only suggested potential contributory factor to fracture risk in this condition was the chronic gastric hypoacidity (56).

Monoclonal Gammopathy of Undetermined Significance (MGUS)

BMSi was measured in a single study only published in abstract form comparing data between 22 patients with MGUS and age-matched controls, the number of whom was not provided. Despite normal and comparable BMD values between MGUS patients and controls, a significantly lower BMSi was observed in MGUS patients compared to controls (respectively, 68.3 ± 5.0 vs. 83.0 ± 4.0, $p < 0.001$) (66). These findings suggest that impaired material properties of bone contribute to the increased fracture risk reported in MGUS (77).

Impact Microindentation Studies in Rare Metabolic Bone Disorders (Table 3)

Three studies were performed in a total of 28 patients with rare metabolic bone disorders: One in Type 1 Gaucher Disease ($n = 16$) (57), one in Paget's disease of bone ($n = 9$) (67) and one in Camurati-Engelmann disease ($n = 3$) (60).

Type 1 Gaucher Disease

Type 1 Gaucher Disease is a rare lysosomal lipid storage disorder due to beta-glucocerebrosidase deficiency leading to the accumulation of glucocerebroside in cells of the macrophage lineage. In this disorder the mechanism of bone fragility is multifactorial including mechanical replacement of the bone marrow by direct infiltration by Gaucher cells and the increased production of inflammatory factors such as cytokines leading to an imbalance in bone turnover in favor of bone resorption (78). The lower BMSi values measured in 16 patients with type 1 Gaucher Disease compared to those of 29 age- and sex-matched healthy volunteers (respectively, 72.7 ± 10.0 vs. 81.8 ± 1.4, $p < 0.05$) are in keeping with the changes observed at predominantly cortical skeletal sites, although LS BMD was also decreased in these patients compared to controls. The main marker of disease activity, chitotriosidase, was also found to be inversely correlated with BMSi ($R^2 = 0.516$, $p < 0.05$), suggesting that in Gaucher disease material properties of bone are more severely altered the more severe the disease is (57).

Paget's Disease of Bone

In a study performed by our group, BMSi values were measured in 9 patients with unilateral Paget's disease of the tibia, in remission after treatment with bisphosphonates. Data were compared with BMSi values of the contralateral non-pathologic tibia. We observed no significant difference in BMSi between affected and non-affected tibia, 74.7 (SE 1.7) vs. 78.7 (SE 1.3), $p = 0.12$. However, we did observe a significant difference in serial indentations of pathologic and normal tibia. The variation of consecutive single indentation values was significantly greater in the affected tibia compared to the contralateral healthy tibia, suggesting heterogenous tissue material properties of the pagetic bone. This high variability in measurements may represent a potential sign of altered material bone properties in Paget's disease of bone (67).

Camurati-Engelmann Disease

In a series of three patients with Camurati-Engelmann disease, a rare bone disease characterized by progressive hyperostosis mainly of the diaphysis of long bones, BMSi values were lower compared to BMSi values in 29 healthy controls, albeit non-significantly (76.9 vs. 81.4, $p = 0.17$), despite the characteristic cortical hyperostosis. This finding suggests that

bone material properties can also be altered despite increased cortical volume (60).

Impact Microindentation Studies in Patients Receiving Osteoporosis Treatment (Table 4)

BMSi was measured in three studies in patients receiving anti-osteoporotic therapy ($n = 117$) in order to evaluate whether IMI had the potential to be used in evaluating treatment-induced changes in bone fragility in osteoporosis (37, 44, 68).

In the first study, BMSi was measured in 52 patients with various underlying diseases requiring glucocorticoid therapy before starting treatment with prophylactic anti-osteoporosis therapy. There was a significant decrease in BMSi after 7 weeks of treatment in the Calcium/Vitamin D3-treated group ($n = 19$), from 81.6 (74.3–86.9) to 71.9 (65.4–77.1) $p < 0.05$, compared to no significant changes in the Calcium/Vitamin D3 and additional oral bisphosphonate-treated group ($n = 14$), 81.1 (75.6–89.6) vs. 83.4 (76.6–93.0), $p = 0.83$. In contrast, a significant increase in BMSi [76.2 (72.0–84.9) to 84.0 (79.2–90.0), $p < 0.05$] was observed in the denosumab-treated group ($n = 14$), with the largest increase observed in a teriparatide-treated group ($n = 5$) [BMSi 70.0 (64.0–72.6) to 81.8 (73.3–88.9), $p < 0.05$]. It is of note that a significant increase of BMSi to 87.7 (78.7–96.5) was eventually also demonstrated in the oral bisphosphonate-treated group but only at the 20 weeks measurement timepoint compared to baseline ($p = 0.043$). This study was the first to show a change in BMSi in response to medical treatment (44).

In a second study published as a meeting abstract, BMSi was measured in seven patients with osteoporosis before and after 3 months of treatment with daily subcutaneous teriparatide 20 mcg ($n = 3$) or 40 mcg ($n = 4$). In this study a significant decrease in BMSi from baseline ($-4.8 \pm 10.7\%$, $p = 0.011$) was observed in the three patients receiving the lower dose of 20 mcg and the decrease was larger in the four patients receiving 40 mcg a day, $-7.0 \pm 15.5\%$, $p = 0.011$ (68). The Abstract format did not allow the authors to formulate a hypothesis for this discrepant finding. A full paper has not been published.

In the third study, BMSi was measured cross-sectionally in 39 patients with osteoporosis but without fractures before receiving bisphosphonate treatment for 4–14 years. BMSi values were found to be significantly lower in 21 patients with incident fractures under treatment with bisphosphonates, compared to 18 patients who did not sustain fractures during treatment (respectively, 73.8 ± 6.5 vs. 81.6 ± 6.3, $p < 0.05$). BMD at the LS was also lower in the 21 patients who had sustained incident fractures (0.66 ± 0.1 vs. 0.82 ± 0.1, $p < 0.05$) (37).

Impact Microindentation Studies in Population-Based Cohorts (Table 5)

BMSi was measured in 489 individuals from a Swedish population-based cohort and in 357 subjects from an Australian cohort. The objective of the Swedish cohort, initiated in 2013, was to identify factors contributing to fracture risk in older women aged 75–80 years (39, 41, 43, 51). The Australian cohort was designed to investigate the epidemiology of osteoporosis in men across ages 33–96 years from the Geelong Osteoporosis study (54, 63, 69).

In the Swedish cohort, there was no difference in BMSi between fracture ($n = 117$) and non-fracture patients ($n = 63$), 76.1 ± 7.4 vs. 75.7 ± 7.9 ($p = 0.4$), also after stratification for low bone mass (51). BMSi did not also differ between women with vertebral fractures ($n = 141$) and those without ($n = 331$), 76.9 ± 7.3 vs. 77.9 ± 7.4, $p = 0.15$, nor was there an association between BMSi and number and/or severity of vertebral fractures (43). An inverse relationship was found between BMSi and the amount of subcutaneous fat at the tibia, whole body fat mass and BMI, suggesting a possible negative influence of adipose tissue on bone strength. BMSi was also found to be associated with cortical porosity as measured by HR-pQCT and cortical volumetric BMD at the distal tibia (39).

In a recent publication of data from the Australian cohort, analysis of the association between BMSi and FRAX clinical risk factors showed that BMSi was significantly lower in men with a prior fracture ($n = 38$), compared to those without ($n = 319$) (respectively, 80.2 ± 6.9 vs. 82.8 ± 6.1, $p = 0.024$), and in men with a history of parental hip fracture ($n = 34$) compared to those without ($n = 323$) (respectively, 80.1 ± 6.1 vs. 82.8 ± 6.9, $p = 0.029$). Data also showed that BMSi tended to be lower in the presence of T2DM ($n = 44$) and alcohol consumption ($n = 60$), albeit non-significantly, but not in the presence of smoking ($n = 21$) or secondary osteoporosis ($n = 44$) (54). A further study conducted in the Australian cohort addressed feasibility and tolerability of BMSi measurements in 252 consecutive individuals from the cohort. Data showed that the procedure was well accepted suggesting the potential promising use of this technique in the clinic as well as in research settings (69).

Impact Microindentation Studies in Healthy Subjects (Table 6)

BMSi measurements were performed in healthy non-osteoporotic individuals ($n = 206$) in four studies. The first study focused on ethnical differences in BMSi measurements and was performed in 42 Norwegian women, aged 46.3 ± 13.6 years, and in 46 age-matched Spanish women, none of whom had ever sustained a fracture. BMSi was found to be significantly lower in Norwegian women compared to Spanish women (respectively, 77.0 ± 7.1 vs. 80.7 ± 7.8, $p < 0.001$), while total hip BMD was significantly higher in Norwegian than Spanish women (42). Differences in tissue-level material properties of bone might therefore partially explain geographically observed differences in fracture risk.

BMSi was sequentially measured in 20 healthy non-osteoporotic postmenopausal women, aged 51–60 years, before and 3 months after starting a unilateral high impact exercise program. Findings from this study showed an increase in BMSi of 7% from 73.4 ± 5.8 to 76.8 ± 9.0 ($p = 0.03$) in the exercised leg, without concomitant changes in volumetric BMD or bone microarchitecture. The authors concluded that sequential IMI may detect improvement in bone material properties within 3

months of high-impact loading before changes in bone mass or architecture can be detected (40).

Two further studies performed in non-osteoporotic individuals specifically investigated the relationship between BMSi and age in 69 women with a median age of 49 years (range 30–81 years), and 19 men with a median age of 34 years (range 24–98 years) (61), and the relationship between BMSi and BMD in 5 men and 5 women, aged 72 ± 5 years (59–83 years) (70). Data from these studies show that neither age nor BMD were associated with BMSi in non-osteoporotic individuals.

Factors Influencing BMSi

The systematic review of the literature on IMI performed for any indication revealed variable outcomes with the use of this tool depending not only on subject- or disease-related factors such as age, gender, BMI, geographical region or prevalent fractures and BMD, but also depending on technique-related factors such as experience and number of operators per study, number of indentations obtained per BMSi measured, and strategies used to assess inadequate quality of single indentations.

Subject-Related Factors

Age

Published data on the relationship between BMSi and age are conflicting. Whereas, eight studies failed to show a significant relationship between these two parameters (35, 38, 42, 47, 51, 55, 61, 69), six studies did demonstrate an inverse relationship between the two (33, 34, 36, 50, 52, 54) and only one study performed in 48 patients with acromegaly, aged 60.2 ± 11.0 years, showed a significant positive relationship between BMSi and age ($r = 0.291$, $p = 0.045$) (36).

Four of the eight studies showing no significant correlation between BMSi and age included subjects with very low BMSi values (35, 38, 42, 51) or a narrow age range (75–80 years) (51). A fifth and a sixth study included heterogenous groups of postmenopausal women with atypical femoral fractures ($n = 20$), hip fractures ($n = 15$), long-term bisphosphonate use ($n = 30$), and treatment-naive controls ($n = 88$) (55), or two groups of HIV-infected patients on long-term treatment with different types of antiretroviral agents (47). A relationship between BMSi and age could not be observed in a study designed to address this issue conducted in 88 patients: 69 women aged 49 years (range 30–81 years), and 19 men aged 34 years (range 24–98 years) (61). The last study that failed to show a relationship between BMSi and age was performed in the Australian cohort, which included 252 men with a wide age range: 33–96 years (69). However, after increasing the sample size of the cohort to 357 subjects, an inverse relationship between BMSi and age did become apparent ($r = -0.13$, $p = 0.014$) (54).

Among the five other studies showing an inverse relationship between BMSi and age, three were from our group and included a total of 164 subjects, aged 61.8 ± 9.4 years (range 40–85 years; $r = -0.457$, $p = 0.002$), all of whom were evaluated for increased fracture risk (33, 34, 36). The two other studies reported similar results in 191 postmenopausal women older than 50 years of age ($r = -0.15$, $p = 0.03$) (50), albeit non-significant in 40 HIV-positive subjects, aged 38 ± 9 years ($r = -0.28$, $p = 0.07$) (52).

Gender

Six studies have directly compared BMSi values between men ($n = 208$) and women ($n = 186$) (33, 34, 36, 46, 47, 56), one of which was conducted in HIV-infected patients. A significantly higher BMSi was observed in men ($n = 35$) compared to women ($n = 15$), 85.0 [83–87] vs. 80.0 (77–83), $p < 0.001$. In the same study there was no difference observed in 24 HIV-negative men and 11 HIV-negative women, BMSi 92.0 [88–96] vs. 89.0 [86–93], $p = 0.07$ (46). There was also no gender difference in the other five studies comparing BMSi values in men and women (33, 34, 36, 47, 56).

BMI

Data from three studies show BMSi to be significantly inversely correlated with BMI ($n = 559$) (39, 54, 69), although this association could not be confirmed in four other studies ($n = 365$) (33, 34, 36, 55).

Geographical variation

One study specifically addressed geographical variation in BMSi and significant differences were observed between different countries, with healthy Norwegian women having lower BMSi than healthy Spanish women (42). No other study addressed geographical variation in BMSi.

Fracture-Related Factors

BMD and fractures

Six studies reported significantly lower BMD values at one or more sites in patients with fractures compared to those without (35, 37, 38, 43, 50, 51). One study found a relationship between BMD and hip fractures but not atypical femoral fractures (55), while five studies found no significant difference in BMD between patients with or without fractures (33, 34, 45, 54, 58). Whereas the majority of reported studies ($n = 14$) did not elicit a significant association between BMSi values and BMD measurements (33–38, 42, 45, 47, 49, 54, 55, 58, 70), three did observe a weak correlation between the two measurements (50–52).

BMSi and fractures

Six studies found significantly lower BMSi values in patients with low bone mass and fragility or stress fractures, compared to non-fracture controls (33–35, 37, 38, 54). One study reported a significant relationship between BMSi and distal radius fractures, but not hip fractures (50). However, seven other studies did not observe a significant relationship between BMSi and fractures. These were three studies in patients with a diagnosis other than osteoporosis (36, 45, 58), three epidemiological studies from the Swedish cohort including patients with fractures irrespective of trauma type (39, 43, 51), and a study including 15 patients with the rare atypical femoral fractures and 20 patients with hip fractures (55).

Technique-Related Factors

As mentioned above, variability of data collected might also be due to technique-related factors such as experience of the operator, number of operators performing the technique per study, number of indentations obtained per BMSi evaluation and strategies used to assess false single indentations.

Operator-related factors

Literature data about operator training and experience are scarce and only provided in eight of the 38 publications yielded by our search (33, 43, 48, 50, 52, 54, 61, 69). Data on intra-observer coefficient of variation (CV) provided in 16 of the 38 publications (33–36, 38–43, 46, 48, 49, 51, 52, 67) report variations ranging from 1.65% (48) to 9.1% (42). Nineteen studies provided information about the number of operators performing the IMI measurements, ranging from one to nine different operators per study (33–35, 37–40, 42–44, 49–52, 54, 55, 61, 67, 69). Data on interobserver variability were however scarce, only reported in five of the 19 studies (39, 40, 43, 51, 61), with four of the five studies being from the same group reporting an interobserver CV of 5.2% for which data were adjusted before analysis (39, 40, 43, 51). The fifth study reported an interobserver CV of "<5%" (61).

Measurement-related factors

BMSi, the outcome parameter of IMI, is a dimensionless value calculated by the OsteoProbe® software from the mean of repeated indentations on the tibia, normalized to the mean of indentations performed on the phantom PMMA material. Although 13 studies (37, 45, 47, 50, 52–55, 57, 60, 61, 67, 69) stated that they performed the IMI measurements according to the standard operating procedure published in 2016 (8), the number of indentations obtained on the tibia, which was provided by all 31 full publications, varied widely from 5 up to 25 indentations. In contrast, the number of indentations performed on PMMA material provided by the majority of studies ($n = 28$) was consistently five as per standard protocol (33–35, 37–41, 43–55, 57–61, 67, 69). Since the older software does not automatically flag inadequate indentations, the evaluation of the adequacy of an indentation was entirely left to the judgement of the operator. Data on how inadequate indentations were identified, and methods used to delete these indentations, were not available for seven of the full publications (39, 42, 43, 48, 56, 58) and when reported, differed between studies (33–38, 40, 41, 44–47, 49–55, 57, 59–61, 67, 69).

Tolerability and Safety

Fourteen publications reported that the microindentation investigation using the OsteoProbe® was well tolerated and not associated with any major complications (35, 37, 38, 42, 44, 46–48, 51, 52, 54, 57, 60, 69). Only two minor complications were reported, a mild skin infection and a mild allergic reaction to the local anesthetic, both of which readily responded to treatment.

DISCUSSION

Identifying the patient at increased risk for a fragility fracture may be challenging, particularly in patients with secondary factors for osteoporosis, as bone mineral density measurements using DXA have been shown to underestimate true fracture risk in these patients (71, 72, 79–81). Over the past decade, evidence has been accumulating about the value of impact microindentation in the *in vivo* assessment of tissue-level material properties of bone, an important contributor to bone strength in addition to that of BMD. The number of studies addressing the value of bone material strength index (BMSi) measurements in the evaluation of fracture risk has been steadily increasing, but outcomes are not always concordant. The main aim of this systematic review of the literature was to examine the added value of impact microindentation in the evaluation of fracture risk in clinical practice.

Our search yielded 38 studies that were published over the past 5 years including 19 studies published in the first 3 years and reported in a previous review (9). Data from these 38 studies highlight the ability of IMI in identifying patients with increased bone fragility, be it patients with primary osteoporosis and fractures, or patients with or at risk for secondary osteoporosis due to a variety of underlying systemic disorders including endocrine disorders such as diabetes mellitus or acromegaly. Data on the value of IMI in the follow-up of patients with increased fracture risk remain scarce but do suggest that the technique is also able to detect changes in BMSi following bone-modifying therapy in both in primary and secondary osteoporosis. The scarce data published in patients with rare metabolic bone disorders such as Type 1 Gaucher Disease or Camurati-Engelmann disease also provide valuable insights into the relationship between tissue-level material properties of bone and fracture risk.

The evaluation of BMSi is of particular interest in patients who have sustained a fragility fracture in the presence of osteopenia or normal BMD values, where DXA BMD measurements underestimate fracture risk. More than 50% of fragility fractures have been found to occur in patients with osteopenia (1), the majority of whom are currently not being offered treatment with bone-modifying agents according to most nationwide adopted treatment protocols. Studies in primary osteoporosis show that IMI could identify patients with fragility fractures, also in the subgroup of those with osteopenia (33, 38), suggesting that tissue material properties of bone are altered in patients who have sustained a fragility fracture and that IMI might therefore help in identifying patients with primary osteoporosis at increased fracture risk where DXA fails to do so. Although BMSi is measured at a cortical site, most studies are concordant in showing that low BMSi is associated with increased bone fragility at all relevant skeletal sites, vertebral, non-vertebral and hip sites (34, 35, 50). Studies that did not elicit a relationship between BMSi and fractures were either performed in small subgroups of patients with secondary osteoporosis (36, 45, 58), or in studies including patients with the lowest reported BMD values among all studies (43, 51). These findings suggest that BMSi may not be of added value in evaluating bone fragility in the presence of severely decreased bone mass which is highly predictive of high fracture risk. On the other hand, findings also suggest that in patients with very low BMSi values and high fracture risk due to impaired tissue material properties of bone, bone mass may be of less important contribution to bone fragility, as observed in studies investigating BMSi and BMD in type 2 diabetes mellitus patients (41, 48, 63). In keeping with this hypothesis, BMSi was indeed found to be low in almost all studies including patient groups with a variety of underlying systemic disorders associated with secondary osteoporosis, where bone quality rather than

bone quantity is likely to have the most impact on fracture risk. It is of note that BMSi was also found to be inversely correlated with markers of underlying disease activity in secondary osteoporosis such as AGE accumulation in type 2 diabetes mellitus (49), and serum chitotriosidase levels in type 1 Gaucher Disease (57), suggesting that material properties of bone are more severely altered the more severely uncontrolled the disease is. Since BMSi values have been found to be independent of BMD values in almost all studies, findings from this systematic review strongly suggest that IMI captures elements of bone fragility not captured by BMD measurements. However, although IMI measurements have been shown to identify patients at increased risk for fracture, it has not fully been elucidated which specific mechanical properties of bone are captured by IMI. A recent study from our group addresses this issue for the first time by simultaneously evaluating tissue material properties of bone by measuring BMSi and bone composition of trans-iliac bone biopsies in humans *in vivo* in 12 patients with a variety of metabolic bone disorders and variable fracture risk (five patients with osteopenia and fractures, four patients with secondary osteoporosis, and three patients with rare bone diseases). The demonstration of both a negative correlation between BMSi and cortical porosity at the micro- and nanolevel and of a positive correlation of BMSi with the bone organic matrix parameters glycosaminoglycan and pyridinoline, and with mineral parameters, suggests that BMSi is affected by the composition of bone organic matrix and by bone mineral properties (82). The IMI technique has therefore the potential to be used as an additional tool to DXA in the evaluation, and also probably follow-up, of bone fragility in the clinic since sequential BMSi measurements have been shown to increase in situations associated with a decrease in fracture risk (44, 52, 59), and to decline where fracture risk increases, such as observed in patients starting glucocorticoids (44).

Notwithstanding, this systematic review of the literature encountered a number of limitations in the interpretation of the generated data. One of these limitations was the high variability in the methodological quality of the published studies. Although on the whole study quality was judged to be satisfactory, quality scores ranged widely from 1 to 10 out of 10 possible points using the adapted Newcastle-Ottawa Scale. Most studies included small numbers of patients and were performed in selected patient groups, potentially resulting in a selection bias. It should also be emphasized that none of the published studies on IMI had longitudinal fracture data and the majority had a cross-sectional design except for six studies with sequential BMSi measurements under an intervention with either bone-modifying agents or exercise. Although low BMSi as measured by IMI has been clearly linked with the presence of any type of fracture, the limitations attached to a number of the studies addressing this issue do not permit, so far, to extrapolate that BMSi measurements may be predictive for increased future fracture risk. In addition, although tissue material properties of bone as measured by IMI have been shown to be altered in almost all studies investigating patient groups with secondary osteoporosis, only three of these studies compared BMSi values of fractured patients with those of non-fractured ones. No significant difference in BMSi values was observed between patients with and without fractures, although subgroups were small.

Another important limitation encountered in the interpretation of IMI data generated by our systematic literature review is the high variability of BMSi values observed in the control arm subjects between studies. Some studies reported BMSi values of 81 or higher in control subjects, whilst others reported lower BMSi values in the range of 70–78, corresponding to the range of BMSi values observed in patient groups with increased fracture risk; **Tables 1**–**6**.

Our systematic review has identified a number of potential subject-, disease- and technique-related factors for this discrepancy. Our and other groups consistently reported a significant relationship between BMSi and age, with a significant decrease in BMSi observed with increasing age. This indicates that tissue material properties of bone as measured with IMI do decline with age, which would also be expected given the increasing fracture risk associated with aging. The absence of an observed relationship between BMSi and age in some studies may possibly be due to a narrow age range (46, 51), a small number of patients included (61), or the inclusion of heterogenous groups of patients (55). In addition, studies that did not observe a relationship between BMSi and age report some of the lowest BMSi values measured (35, 38, 42, 51). Geographical differences might also influence BMSi values as observed in a study specifically designed to address this issue (42), and this is supported by the observation of this review that non-fractured elderly women from Sweden had BMSi values below 78 (39, 43, 51), which were remarkably lower than BMSi values of non-fractured controls from The Netherlands (33, 34) and Spain, who had BMSi values above 81 (37, 45, 46, 57, 58, 60, 61). Different BMSi values of subjects from different geographical regions could reflect their difference in fracture risk and this should be taken into account when comparing studies. The only currently available age-dependent reference values are those suggested by the manufacturer (Active Life Scientific) and these remain to be confirmed.

Another complicating issue in the interpretation of BMSi results is that currently used IMI protocols vary in a number of key points, which hampers pooling of data and general application of the technique and may partly explain the variation in BMSi outcome between studies. The OsteoProbe® is a relatively easy to use handheld device specifically designed for *in vivo* use in humans, but it is also prone to variability in its results, which is at least partly due to the lack of a standard operating procedure until the recently published technical recommendations by Diez-Perez et al. (8). However, studies still use different protocols even after this publication. Part of the identified technique-related aspects of IMI will be addressed by the introduction of an updated system in which the software will automatically discard measurements that are more than two standard deviations away from the subject's mean, although large deviations might reflect bone disease (67). Of additional importance is adequate operator training and limitation of the number of operators per study, to minimize intra- and interobserver variability. Only after these methodological inconsistencies are addressed and future measurements are strictly conducted according to the standard protocol can normative values be obtained, and data compared between studies and centers.

Taken together, findings from this systematic review of the literature show that impact microindentation is a promising technique enabling physicians to evaluate *in vivo* tissue-level material properties of bone in a minimally invasive, simple and safe manner. Data generated by impact microindentation have contributed to the better understanding of factors involved in the pathogenesis of bone fragility. Data have also been shown to be valuable not only in the evaluation of bone fragility, but possibly also in the follow-up, particularly of patients with potentially underestimated fracture risk. BMSi is not a measure of bone mass, and no clear relationship has been demonstrated between the two, implying that IMI could be used as an additional tool to DXA BMD in the assessment of bone health rather than as a replacement of the latter in the individual patient. However, the value of IMI in predicting future fractures and treatment outcomes has yet to be established in prospective multicenter trials using standard operating procedures before recommending the routine use of the technique in the clinic.

AUTHOR CONTRIBUTIONS

MS, NH, and NA-D: conception of the work. MS and NA-D: data collection, data analysis, and interpretation. MS, NH, FM, and NA-D: drafting the article. MS, NH, FM, EW, and NA-D: critical revision of the article and final approval of the version to be published.

FUNDING

MS was supported by a European Calcified Tissue Society (ECTS) Clinical Research Fellowship and a European Union of Medical Specialists (UEMS) Exchange in Endocrinology Expertise (EEE) Fellowship.

ACKNOWLEDGMENTS

The authors thank Johannes W. Schoones, medical librarian of the Leiden University Medical Center, for his assistance in performing the literature search.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2020.00015/full#supplementary-material

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Conflict of Interest: NA-D is an unpaid member of the Scientific Board of Active Life Scientific, manufacturer of the OsteoProbe®.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

REVIEW
published: 11 February 2020
doi: 10.3389/fendo.2019.00924

Management of Osteogenesis Imperfecta

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Osteogenesis imperfecta (OI) is the term used to describe a group of rare inherited skeletal disorders characterized by a greatly increased risk of fragility fractures (1). Mutations in several genes can cause OI but the condition is most commonly caused by mutations of *COLIA1* or *COL1A2* resulting in the production of collagen which is abnormal or present in reduced amounts. Fractures in OI are particularly common during childhood but the elevated fracture risk continues throughout life. Bone mineral density (BMD) can be reduced in OI but the magnitude of increase in fracture risk is far greater than can be accounted for by low BMD, highlighting that a key mechanism of bone fragility is reduced bone quality due to defects of bone matrix and mineralization. A multidisciplinary approach is needed to optimize management of OI, with input from physicians, orthopedic surgeons, physiotherapists, occupational therapists, and other allied health professionals. Orthopedic surgery plays a key role both in the fixation of fractures and in the correction of limb deformities. Bisphosphonates have been widely used in the treatment of children and adults with OI. Although there is good evidence that they increase BMD, it is uncertain to what extent they reduce fracture risk. Clinical trials of bone anabolic drugs such as teriparatide and inhibitors of sclerostin have also been studied; although they increase BMD, studies of these agents have not been powered to look at fracture endpoints. Various other treatment modalities including denosumab, and cell therapy have been explored but haven't gained acceptance in routine clinical practice. There have been huge advances in understanding the pathogenesis of OI but these have not been accompanied by advances in treatment. This signals need for well-designed clinical trials with fracture endpoints in OI, both with existing agents and with the newer therapeutic agents that are now starting to emerge.

Keywords: teriparatide (TPTD), clinical trials, fracture, bisphosphonates (BP), osteogenesis imperfecta (OI)

OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Bone Research, a section of the journal Frontiers in Endocrinology

Received: *23 October 2019*
Accepted: *18 December 2019*
Published: *11 February 2020*

Citation:
Ralston SH and Gaston MS (2020) Management of Osteogenesis Imperfecta. Front. Endocrinol. 10:924. doi: 10.3389/fendo.2019.00924

INTRODUCTION

Osteogenesis imperfecta is the term used to describe a group of inherited disorders characterized by multiple low trauma fractures, first presenting in infancy. Depending on the subtype, other features may be observed such as bone deformity, growth retardation, dental abnormalities, blue sclera, hearing loss, and ligament laxity.

The Sillence classification which was devised in 1979 (1), divided patients with OI into four subtypes based on clinical severity, ranging from mild to lethal. As new genes for osteogenesis imperfecta have been discovered a new classification system has been suggested

(2) which introduces new subtypes related to the underlying genetic abnormality while retaining the Sillence classification for defects associated with mutations in the type 1 collagen genes.

The genetics of OI have recently been the subject of two recent comprehensive reviews (2, 3) and is also discussed in detail in another article in this series. In view of this, the gene mutations responsible for OI will be referred to only briefly in this article (**Table 1**). According to the new classification system, group A subtypes of OI are caused by defects in collagen synthesis, structure, or processing. The vast majority of patients with OI fall into this category. It has been estimated that between 85 and 90% of individuals with group A OI carry a mutation in *COLIA1 and COLIA2* which are the genes that encode the alpha 1 and alpha 2 chains of type 1 collagen. These are dominant mutations that impair the ability of type collagen to assemble normally or reduce the amount of collagen produced due to null mutations that evoke nonsense mediated RNA decay. Although, it was traditionally considered that null alleles were the underlying genetic defect in most individuals with Type I OI, recent information suggests that up to one third have a missense mutation in collagen (4). Mutations in the *BMP1* gene also cause a recessive form of OI that falls within group A classification since abnormalities of collagen assembly occur due to the protease function of *BMP1*. Group B subtypes of OI are recessively inherited and are due to mutations in genes that are responsible for post-translational modification of collagen. These are severe disorders which present in early infancy Group C subtypes can be dominantly or recessively inherited and most result in a moderate to severe phenotype. They are caused by mutations in genes that play a role in collagen folding and crosslinking of collagen. Heterozygous mutations in *P4HB* deserve special mention since that can cause a mild OI phenotype (5) as well as the Cole-Carpenter syndrome (6) in which an OI phenotype is accompanied by other features such as craniosynostosis, proptosis, hydrocephalus, and other distinctive facial features. Group D subtypes of OI are characterized by abnormalities of mineralization. Mutations in *IFTM5* cause an autosomal dominant form of OI where there is increased mineralization, whereas mutations in *SERPINF1* lead to a recessive form of OI characterized by reduced mineralization. Finally, group E subtypes of OI are recessively inherited severe forms of the disease which are caused by mutations in genes that affect osteoblast differentiation including *SP7, WNT1,* and *CREB3L1.*

There have been huge advances in understanding the molecular basis of OI but unfortunately this has not been accompanied by similar progress in terms of treatment. Furthermore, there is very little information on the relationship between the subtype of OI and the response to treatment. Hopefully the greater understanding of the molecular basis of OI that we now have will provide an avenue for the development of more effective targeted therapies in the future.

TABLE 1 | Clinical and genetic subtypes of osteogenesis imperfect.

Group	Subtype	Genes	Inheritance	Mechanism
Group A	Type I	*COL1A1 or COL1A2*	AD	Defects in collagen synthesis, structure, or processing
	Type II	*COL1A1 or COL1A2*	AD	
	Type III	*COL1A1 or COL1A2*	AD	
	Type IV	*COL1A1 or COL1A2*	AD	
	Type XIII	*BMP1*	AD	
Group B	Type VII	*CRTAP*	AR	Post-translational modification of collagen
	Type VIII	*LEPRE1*	AR	
	Type IX	*PPB*	AR	
	Type XIV	*TMEM38B*	AR	
Group C	X	*SERPINH1*	AR	Collagen folding or cross-linking
	XI	*FKBP10*	AR	
	–	*PLOD2*	AR	
	–	*P4HB*§	AD	
Group D	V	*IFTM5*	AD	Defects in bone mineralization
	VI	*SERPINF1*	AR	
Group E	XII	*SP7*	AR	Defects in osteoblast differentiation
	XV	*WNT1*	AR	
	XVI	*CREB3L1*	AR	

§*Mutations in P4HB have been associated with a Cole-Carpenter syndrome in which an OI-like phenotype occurs as part of a wider syndrome but recent reports indicate that P4HB mutations can also result in a mild OI phenotype.*

PATHOPHYSIOLOGY OF BONE FRAGILITY IN OSTEOGENESIS IMPERFECTA

Bone fragility is greatly increased in osteogenesis imperfecta. Reduced bone mass (7) and abnormalities of cortical thickness and trabecular architecture play a role (8) but these abnormalities are compounded by defects in bone matrix, which profoundly affect bone quality. There is also evidence that rates of bone turnover are abnormally increased particularly in types III and IV OI (8, 9) which may also contribute to bone fragility. Increases in TGF signaling have been implicated in the pathogenesis of increased bone remodeling in animal models of OI (10, 11) but the role of this cytokine in human OI has not yet been investigated. In another study, serum concentrations of DKK1 and RANKL were found to be elevated in a series of 18 children with OI (both untreated and after treatment with neridronate) leading the authors to conclude that these cytokines may play a role in causing increased bone resorption and reduced one formation in OI (12).

A puzzling feature of OI that still remains poorly understood is increased mineralization of bone. This was first described by Boyde et al. (13), but subsequently had been confirmed in most types of OI by various investigators (14–18). This has relevance to the pathogenesis of fractures since bone that is highly mineralized is also more brittle.

OVERALL MANAGEMENT STRATEGY IN OSTEOGENESIS IMPERFECTA

This review will mainly focus on options for medical management, but it is important to point out that optimal management utilizes a multidisciplinary team (MDT) approach (19). There isn't a strong evidence base for this in the sense

that the efficacy of individual components hasn't been tested in randomized clinical trials but clinical experience suggests that the MDT approach is important for the management of OI. Typically, the MDT is co-ordinated by a physician who usually will make the initial diagnosis of OI, decide upon the need for medical therapy and referral to other members of the MDT.

Occupational Therapy

Occupational therapy plays a central role, particularly in individuals with more severe forms of OI to assess the patient and advise on devices and mobility aids to allow affected individuals to optimize function.

Physiotherapy

The physiotherapist provides a key role in the care of patients with OI from when they are first diagnosed. They can assist parents of young OI babies and children with safe handling and positioning of the baby followed by setting realistic goals at reaching developmental milestones, dependent on the severity of the individual patient's condition. Probably the most important of these milestones is for the child to gain independent mobility whether this is with walking aids or ultimately using a wheelchair. The long-term input of the physiotherapist is key to optimizing the musculoskeletal health of children with OI and to help lead them into an independent adulthood. There is evidence from observational studies that supervised exercise programmes can positively influence muscle strength and physical ability (20, 21). Anecdotal evidence suggests that improving muscle strength can also have beneficial effects in stabilizing joints in people with hypermobility which is a common feature of OI. There has been a tendency for people with OI to be discouraged from participating in sports but there are many examples of individuals with OI reaching a high level in sporting activities. This in turn can have beneficial effects on well-being and quality of life.

Orthotics

Orthotics may also have a role, particularly the ankle foot orthoses (AFO) which can control the position of the distal lower extremity and may have a role in preventing the worsening of foot deformity. Removable splints for the upper limb can be used to help ambulation for example in aiding the use of a self-propelled wheelchair. An individualized approach to the use of splints is required and an experienced orthotist can be invaluable for these patients.

Other Health Care Professionals

The input of other health care professionals including clinical psychologists, speech and language therapists, dieticians, and social workers all play an important role in the multidisciplinary team (19). The orthopedic surgeon also plays a critical role in OI management, not only in the management of fractures, but also in performing prophylactic surgery to both prevent and correct deformities. The principles of orthopedic management of OI are discussed in more detail later in this review.

PRINCIPLES OF MEDICAL MANAGEMENT OF OSTEOGENESIS IMPERFECTA

The medical management of osteogenesis imperfecta is currently based on giving drugs that are used to treat osteoporosis, working on the assumption that medications which increase bone density and reduce bone turnover might favorably influence clinical outcome and reduce fracture risk. Other strategies such as stem cell therapy have been investigated but remain within the realm of research rather than routine clinical practice.

BISPHOSPHONATES IN OSTEOGENESIS IMPERFECTA

Bisphosphonates are the most widely used agents for the treatment of osteogenesis imperfecta. In the present review, I will review the results that have been reported with individual bisphosphonates followed by an overall summary of the effects of bisphosphonates at the end of this section.

Pamidronate

Pamidronate became widely used in children with OI following the observational study of Glorieux et al. (22) who treated 30 children with severe OI with intermittent infusions of pamidronate over a period of between 1 and 5 years using a dosage regimen of 1.5–3.0 mg/kg on 3 consecutive days at 4–6 monthly intervals. Most of the individuals had a severe phenotype such that about one-third had type III, and the remainder type IV with a few individuals that had clinical features of type V disease.

Following treatment, the authors reported that the most striking difference was an improvement in bone pain which was associated with an improvement in mobility. Bone mineral density assessed by DEXA also increased and the fracture rate decreased from 3.2 fractures per patient per year to 0.6 fractures per patient per year with time – a reduction of about 75%. The authors also noted an increase in vertebral area following treatment. The most common adverse effects were fever and a transient increase in bone pain which are well-recognized to occur with pamidronate in other indications (23). Reductions in serum calcium were also noted but these were usually asymptomatic. Since this wasn't a placebo-controlled study, further research was conducted to try and determine to what extent the reduction in fracture risk and increase in bone density was due to the therapeutic intervention or to skeletal growth or other confounding factors. To try and address this, Rauch and colleagues compared the increases in BMD following pamidronate therapy in 56 children with OI as compared with 167 patients who had not been treated. Regression analysis showed that pamidronate treated individuals had a greater increase in BMD than untreated subjects and this was further supported by a subgroup analysis of 51 treated individuals who were matched with 51 untreated individuals of a similar age and OI type (24). Another study of similar design was conducted by Land and colleagues who compared changes in vertebral shape in pamidronate treated individuals compared

with controls matched for age and type of OI. This also showed that pamidronate seemed to preserve vertebral shape (25).

At the time of writing this review, a search of PubMed identified over 150 publications referring to the use of pamidronate in OI. These studies generally supported the findings noted by Glorieux in showing increased BMD, reduced fracture incidence and symptomatic improvement after treatment as compared with before but with one exception which compared pamidronate with alendronate (26) these were observational studies with no control group. While the data suggest that pamidronate increases BMD and may affect vertebral modeling it remains less clear to what extent the reduction in fracture rate is due to pamidronate or confounding factors such as the tendency for fracture rates to decrease spontaneously with skeletal growth in children with OI, even in the absence of treatment (27, 28).

Alendronate

There have been three randomized trials of alendronate in OI, two in children and one in adults. DiMeglio and Peacock conducted a small randomized open study of 18 children with OI who were treated with oral alendronate 1 mg/kg/day or intravenous pamidronate 4 mg/kg every 4 months over a 2-year period (26). Bone density increased at the spine and total body to a similar extent with both treatments, but with a better response in type I as compared with type III and IV irrespective of the treatment given. The authors noted a reduction in fracture incidence during treatment with both drugs as had been reported in uncontrolled studies with pamidronate. Ward and colleagues conducted a double-blind randomized study of oral alendronate (n-109) and placebo ($n = 30$) in children with OI (29). The dose of alendronate was 5 mg daily in those <40 kg and 10 mg daily in those more than 40 kg. There was a significantly greater increase in BMD at the spine in the alendronate group as compared with placebo and this was accompanied by a significantly greater reduction in urinary N-telopeptide of type I collagen (uNTX/Cr) than in the placebo group. There was no significant difference in the proportion of patients with new fractures between the placebo group (92%) and alendronate group (83%), although the authors commented that the study had been powered to look at changes in BMD and not fracture. No difference between the groups was observed in secondary outcomes such as pain, mobility and physical activity. The alendronate was well-tolerated with an adverse event profile similar to that of placebo. Chevrel et al. (30) conducted a randomized double-blind trial or oral alendronate 10 mg daily vs. a matching placebo in 64 adults with OI over a 3-year period. Their average age was about 36 years and most had type I OI. All individuals received calcium and vitamin D supplements. There was a significant increase in hip BMD and spine BMD in the alendronate treated patients and this was accompanied by a decrease in biochemical markers of bone turnover. The fracture rate was similar in both groups; 11/31 (35%) in the placebo group experienced a non-vertebral fracture compared with 10/31 (32%) in the alendronate group. Two vertebral fractures occurred in the placebo group compared with none in the alendronate group and overall, 17 fractures occurred in each group during the treatment period. With regard to adverse events, pain score increased from baseline in the alendronate group in the intention to treat analysis but was not different in the per-protocol analysis. Gastro-intestinal adverse events were more common in the alendronate group occurring in 18/31 (58%) individuals compared with 5/31 (16%) in the placebo group.

Risedronate

The effects of risedronate have been studied in three randomized trials of children with OI.

A dose-ranging study by Bishop et al. (31) compared the effects of risedronate in doses of 0.2, 1, and 2 mg/kg in 53 children with OI. The average age at entry to the study was 11 years and most of the participants had type I OI. The study was powered to show a 75% reduction in fracture rate, based on the pamidronate study of Glorieux et al. (22). No effect of either 1 or 2 mg/kg was observed on fracture incidence as compared with the 0.2 mg/kg dose but BMD increased significantly with the 2 mg/kg dose as compared with the lowest dose. No differences in functional outcomes such as pain and grip strength were found between the treatment groups. The authors referred to a dose-related effect of treatment on bowing deformities in the text of the paper but no data on bowing deformities were presented. A further study by Bishop randomized 147 children with OI to receive risedronate 2.5 or 5 mg daily (depending on body weight) or placebo for a period of 1 year, at which point all patients were switched to risedronate. The average at entry to the study was about 8 years and most participants had mild OI. The study was powered to detect a difference in BMD of 5% between the risedronate and placebo groups at 1 year. At the end of the placebo-controlled phase, BMD increased significantly in risedronate treated patients. As expected, urinary NTx and bone specific alkaline phosphatase values were reduced in the risedronate treated patients during the blinded phase of the study. The proportion of patients with new clinical fractures was lower in the risedronate group 29/94 (30.8%) vs. 24/49 (48.9%) of the placebo group. The difference between groups was significant ($p = 0.0446$). A time to event analysis also showed that the hazard ratio for fractures was 0.53 (95% CI, 0.31–0.92) for risedronate compared with placebo. There was a trend for fewer vertebral deformities in the risedronate group but the difference from placebo was not significant. A third study Rauch randomized 26 children with type I OI to receive risedronate or placebo (32). The dose of risedronate was 15 mg weekly for those with a body weight of <40 kg and 30 mg weekly for those >40 kg. Treatment was continued for 2 years. In the risedronate group, BMD increased significantly and serum NTX decreased by about 30%. The authors noted that the decrease in NTX was proportionately less than that observed in previous observational studies with pamidronate but the age of patients and severity of OI was different in these studies, making a direct comparison difficult (22). There was no significant difference between the risedronate and placebo groups in vertebral morphometry, grip strength or bone pain. Eleven fractures occurred in each group, a difference that was not significant. Adverse events were similar in the treatment groups.

Neridronate

Neridonate is a nitrogen containing bisphosphonate that is structurally similar to pamidronate and alendronate but with six carbon atoms in the side chain as opposed to three with pamidronate and four with alendronate. It is currently licensed in Italy for the treatment of bone disease. Intravenous neridronate has been studied in one randomized controlled trial of children with OI performed by Gatti et al. (33). The trial was of 3 years duration but only the first year was placebo controlled at which point all patients were switched to neridronate which was given in a dose of 2 mg/Kg body weight by intravenous infusion every 3 months. Vitamin D supplements were also given to both groups in those with a vitamin D level of <50 nmol/l. The average age of participants was about 9 years and the majority had type I OI. During the placebo-controlled phase, BMD increased at the spine and hip to a significantly greater degree in the neridronate group. Gain of height and bone area in the lumbar spine were also reported to be greater during the first year in the neridronate group. During the placebo-controlled phase, 10/22 (45%) of the placebo group and 12/42 (28%) of the neridronate group had fractures; a difference that was not significant (OR 0.6, 95% CI 0.21–1.59). The number of fractures were fewer in the neridronate group however (OR 0.36, 95% CI 0.15–0.87, $p < 0.05$) and a similar reduction in fracture numbers was reported when the data were adjusted for baseline BMD, type of OI, and gender. However, the authors did not adjust for the proportion of individuals who had experienced fractures in the 12 months before the study, and this was higher in the placebo group (82 vs. 57%). In the open (observational) phase of the study, fracture rate decreased in both groups as expected. Another open study (34) randomized 46 adults with OI to receive neridronate 100 mg intravenously every 4 months or no treatment in a 2:1 ratio. The average age of participants 35 years and most had type I OI. There was a greater increase in spine and hip BMD in the neridronate group as compared with the untreated controls. Biochemical markers of bone turnover including bone specific ALP, serum CTX and urinary deoxypyridinoline/creatinine fell in both groups although the reductions tended to be greater in the neridronate group. Reporting of fracture data in this study was incomplete. The authors reported that 2 clinical fractures occurred in the placebo group during year 1 compared with 1 fracture in the neridronate group. No information was provided on fractures that occurred during the second year in the two groups. Flu-like symptoms were observed in 13/31 (42%) patients treated with neridronate but no serious adverse events were reported.

Olpadronate

Olpadronate is a bisphosphonate which is structurally similar to pamidronate but with an additional methyl group on the nitrogen atom. There has been one randomized study with olpadronate in OI and that was performed by Sakkers and colleagues (35) who randomized 34 children with OI to receive olpadronate 10 mg daily ($n = 16$) or placebo ($n = 18$). The average age of participants at baseline was about 10 years. There was a mix of OI types in each group; about half of the individuals in the placebo group had type I OI compared with one third in the olpadronate group. Spine BMD increased in the olpadronate group as compared with placebo, but there was no difference between groups in grip strength, mobility and biochemical markers of bone turnover. Fractures were recorded in 12/18 (77%) of the olpadronate group compared with 8/16 (50%) of the placebo group; a difference that was not significant. The total number of fractures was greater in the placebo group however (50 vs. 18) and this was significant with a reported hazard ratio of 0.69 (95% CI 0.52–0.91). A subsequent analysis of quality of life in participants of the same study (36) revealed very few differences between groups with the exception of a marginal improvement in pain at one time point only.

Summary

Randomized controlled trials of bisphosphonates in OI have consistently shown that BMD is increased and biochemical markers of bone turnover are decreased as compared with no treatment or placebo. There haven't been clear benefits in quality of life and functional status in controlled studies suggesting that the improvements in these domains that were seen in observational studies may not have been fully been attributable to the bisphosphate therapy. The data on fracture are also conflicting. A Cochrane review and a meta-analysis of randomized trials have both concluded that the effects of bisphosphonates on fracture rate are uncertain (27, 37), while also acknowledging that the studies performed so far have not generally been powered to detect a reduction in fracture incidence. A possible reason for the slightly disappointing results with bisphosphonates in terms of fracture reduction is that they increase mineralization of bone (38, 39) which might cause the bone to be more brittle.

DENOSUMAB

Denosumab is a monoclonal antibody directed against Receptor Activator of Nuclear Factor Kappa B ligand (RANKL) a molecule that plays an essential role in osteoclast activation. Through this action, denosumab acts as a highly potent inhibitor of osteoclastic bone resorption. When given in a dose of 60 mg subcutaneously every 6 months, denosumab has been shown to significantly reduce the risk of fractures in postmenopausal osteoporosis (40).

There have been some reports of denosumab use in OI, but no randomized trials. The first published case series of denosumab treatment in OI was by Hoyer-Kuhn et al. (41) who administered denosumab in a dose of 1 mg/kg every 12 weeks 6 months to 10 children with OI over a 4-year period. All individuals had previously been treated with bisphosphonates but these were discontinued 6 months before entry to the study. Calcium and vitamin D supplements were given routinely. The average age of participants was 7 years and most had type 1 OI. Bone density increased during the study but there were no changes in spine morphometry or general mobility. Urinary Deoxypyridinoline/Creatinine values (a marker of bone resorption) fell significantly after the first administration of denosumab with a nadir at day 8 and the values rose once

again such that by 10 weeks, the values were approaching pre-treatment levels. Various adverse events were reported including one instance of hypocalcaemia.

Another study by the same group reported outcomes in a case series of four individuals with type VI OI treated with denosumab (42). Bone density and vertebral area increased with time in these individuals. Urinary DpD values decreased on day 14 after the injections and then tended to recover by day 28. In another study Trejo and colleagues reported outcomes in four individuals with type VI OI treated with denosumab. Somewhat surprisingly they reported the occurrence of hypercalcaemia and hypercalciuria following treatment. In addition, rapid bone loss at the lumbar spine was noted when the interval between injections was increased to 6 months (43). A systematic review of denosumab in children with OI by Li et al. (44) identified and evaluated the three studies above and concluded that further research was necessary to evaluate its role in treatment.

In summary there are limited data on the use of denosumab in OI and its effects on clinical outcome of the disease remains unclear.

BONE ANABOLIC AGENTS IN OSTEOGENESIS IMPERFECTA

Teriparatide

Teriparatide (TPTD) is the 1–34 fragment of parathyroid hormone. It has been successfully used for many years in the treatment of osteoporosis and has been found to be superior to bisphosphonates in the prevention of vertebral fractures in severe osteoporosis in observational studies (45). Moreover, TPTD was found to be superior to oral risedronate in preventing vertebral fractures in a double-blind randomized trial of individuals with severe osteoporosis (46). There has been one double blind randomized trial of Teriparatide in OI (47); in this study; Orwoll and colleagues randomized 79 adults with OI to receive TPTD 20 mcg daily or a matching placebo for an 18-month period. The average age of participants was 41 years and most had type I OI. There was a significantly greater increase in BMD in the TPTD group compared with placebo and biochemical markers of bone turnover (PINP and NTX) were both significantly elevated following TPTD therapy. The authors noted that changes in BMD and biochemical markers in response to TPTD were greater in those with type I OI as compared with those that had type III/IV. Fractures were observed in 11 (29%) of the TPTD group and 14 (36%) of the placebo group, a difference that was not significant (odds ratio 0.73, 95% CI 0.28–1.90). An observational study by Gatti and colleagues (48) treated 13 adults with type I OI with TPTD over an 18-month period. These individuals were selected for treatment on the basis that they had suffered fractures despite neridronate treatment. The study showed increased in BMD and biochemical markers with TPTD treatment. None of the patients experienced a fracture during TPTD therapy.

Sclerostin Inhibitors

Romosozumab, a monoclonal antibody which neutralizes sclerostin. Through these effects, romosozumab inhibits bone resorption, probably by increasing production of osteoprotegerin by osteoblasts (49) and stimulates bone formation by counteracting the inhibitory effects of sclerostin on osteoblast activity (50). Interestingly, the bone forming effect of romosozumab is transient and is lost after about 12 months, even with continued treatment. Despite this, romosozumab is a powerful anabolic agent which has been found to be effective at increasing BMD and reducing the risk of fractures in postmenopausal osteoporosis when compared with placebo (51). Prompted by these observations, and preclinical studies which have suggested that sclerostin antibodies can increase bone mass and bone strength in several mouse models of OI (52), sestrusumab (BPS-804) a monoclonal antibody which neutralizes sclerostin is now being investigated in the treatment of adults with OI.

In a randomized phase 2a trial, Glorieux conducted a dose ranging study with sestrusumab in nine adults with osteogenesis imperfecta in comparison with a reference group who were randomly allocated to receive no treatment. Participants were predominantly male, with an average age of 30 years and similar numbers of individuals with type I, III, and IV OI were included in the study. A sequential design was used in the active group such that participants received an initial intravenous infusion of sestrusumab 5 mg/kg followed by a further infusion of 10 mg/kg after an interval of 2 weeks later and a final infusion of 20 mg/kg after a further interval of 2 weeks with a final study visit follow-up 21 weeks after the initial dose. The control group were followed up without treatment for 21 weeks. The primary efficacy endpoints were changes in biochemical markers of bone turnover but bone density was also measured by DEXA. Biochemical markers of bone formation including PINP and BSAP increased significantly in the active group when compared with the control group. Lumbar spine BMD also increased in the active group but did not change significantly in the control group. The treatment was generally well-tolerated. The authors concluded that BPS-804 was effective at stimulating bone formation and bone density in OI. A further phase 2b study is now in progress with four different doses of sestrusumab in 100 adults with OI (Asteroid study, NCT03118570) The study has completed recruitment and is currently in follow-up.

TGF Beta Inhibition

There is evidence that TGFβ signaling is increased two mouse models of OI (10); one with targeted inactivation of CRTAP (*Crtap*$^{-/-}$) and another with a knock-in of the G610C mutation of *Col1a2* (*Col1a2*$^{tm1.1Mcbr}$). In both models, treatment with an antibody that neutralized the effect of TGFβ increased bone mass and bone structure and reduced bone turnover. Based on these data, fresolimumab, a monoclonal antibody which inhibits all three isoforms of TGFβ is currently being investigated in a phase I trial (NCT03064074) in adults with osteogenesis imperfecta.

CELL THERAPY

Bone marrow transplantation has been investigated as a possible means of treating severe OI. The first clinical study in this area was that of Horwitz and colleagues who performed bone

marrow transplantation in three individuals with type III severe deforming OI (53). Two were aged 13 months and one 32 months. The donor marrow was obtained from unaffected HLA identical or single antigen mismatched siblings and infused intravenously without modifications. The recipients also had preconditioning with cytotoxic therapy and in one case, total body irradiation. This was an exploratory study with no control group. In order to determine whether the procedure was successful in engrafting cells of the osteoblast lineage to the recipients, the authors were able to culture osteoblasts from bone marrow about 3 months after the procedure in two patients and estimated that between 1.5 and 2% were of donor origin. Some changes in bone mass, linear growth and bone histology were observed post-transplant. In two patients the procedure was well-tolerated but in the third individual it was complicated by sepsis, pulmonary compromise and a hygroma.

The same group of investigators performed a further study in five children of a similar age. All had type III OI and were treated using a similar approach (54). In two of these individuals the researchers were unable to document donor osteoblast engraftment after the procedure and they were excluded from analysis. A further two individuals with type III OI of a similar age acted as controls. The authors reported an increase in growth velocity following the procedure which was not observed in the controls but this subsequently slowed. The treated individuals showed a gain in total body mineral content following the procedure but data were not reported for the control subjects.

Subsequently, the same group of researchers investigated the effects of mesenchymal stem cells (MSC) on growth and bone mineral content in a further six patients with type III OI aged between 3 and 5 years (55). In this case bone marrow was obtained from donors and then cultured *in vitro* with the aim of selecting MSC by their ability to adhere to the plastic surface of culture dishes. The adherent MSC were then transfected with retrovirus encoding marker genes; one was a neomycin resistance gene which was expressed; the other was a cassette for a neomycin resistance gene and a beta-galactosidase gene which were not expressed. The purpose of transfecting the MSC with these sequences was simply to provide a means by which the transplanted cells could be detected. The study showed that engraftment with the donor cells in bone, bone marrow and skin was variable but that the proportion of engrafted cells (estimated by PCR of target tissues) never exceeded 1%. Most of the treated individuals showed an increased growth velocity in the 6 months after transplant but in four cases this was not accompanied by an increase in bone mineral content. Adverse events included an urticarial rash in one treated patient and the development of antibodies to bovine serum in another.

It is difficult to interpret whether or not the treated individuals derived clinical benefit from the transplant procedure due to limitations in study design and the short term follow up of participants. Based on the information available however it seems unlikely that bone marrow transplantation or MSC transplantation has durable clinical benefits in severe OI.

GENE BASED THERAPIES

Correcting the genetic defects that cause OI would represent an attractive approach to treatment that could potentially be curative. This has been explored in the studies of Chamberlain et al. (56, 57) who used an Adeno-Associated Virus to target exon 1 of the *COLIA1* gene with the aim of replacing a deleterious missense mutation with a non-functioning allele using homologous recombination *in vitro*. The authors were able to demonstrate that it was possible to target cultured mesenchymal stem cells from affected patients using this approach and correct the abnormalities in collagen processing, stability and structure. In order for this approach to be used clinically, the next step would be to perform an autograft with the targeted cells but this has not been attempted.

Further gene-based approaches that could be explored in severe OI but which have not yet been attempted are to try and correct the genetic defect in stem cells or mesenchymal cells using CRISPR-Cas technology which is being trialed in Thalassaemia (NCT03655678) or to use anti-sense RNA or oligonucleotides to suppress expression of damaging mutant transcripts (58).

ORTHOPEDIC MANAGEMENT OF OSTEOGENESIS IMPERFECTA

The aims of the orthopedic management are to maximize function and minimize pain for the patients affected. As mentioned previously, this is best done within a multidisciplinary team setting consisting of physiotherapists, orthotists, and rheumatologists or endocrinologists specializing in the medical management of OI. Orthopedic treatment begins in early life and remaining physically active is very important both for the strength of the bone and muscle as well as for the general well-being of the patient.

Treatment of Fractures

Children with Osteogenesis Imperfecta unfortunately become very well-known to their local Accident and Emergency and Orthopedic Services due to recurrent fractures. A conservative approach in the orthopedic management of fractures in patients with OI is usually taken and fixation is rarely performed in the acute fracture setting. Splinting and casting is normally used and after a number of fractures the parents and patients themselves become very adept at applying their own cast and indeed will often do this at home reducing the need for hospital visits. The local policy in NHS Lothian is to use "soft cast" which is a pliable material which is kind to the soft tissues but provides enough stability to the extremity to allow the fracture to heal. Early mobilization is encouraged and if the child is ambulant prior to a lower limb fracture then weight bearing as soon as tolerated is the main aim as repetitive periods of non-weight bearing lead to muscle wastage, fatigue and potentially the loss of ability to independently ambulate. The prevalence of fractures in OI drops in adulthood but they continue to occur such that 25% of lifetime fractures are reported to occur in adulthood. Fractures in adulthood can be even more challenging due to the presence

of deformity, previous surgery and/or metalwork and previous or ongoing bisphosphonate treatment (59).

Orthopedic Management of Deformity

The most challenging aspect that OI patient's provide to the orthopedic surgeon is the management of their sometimes severe and multiplanar deformities. As already noted, the patient should be managed within a multidisciplinary setting and the medical management of the bone quality be optimized where possible. Functional gains are the main indication for deformity correction surgery and this has greater implications in the ambulant patient. The use of multiple long bone osteotomies secured with intramedullary rod fixation was described by Sofield in the 1950's and this principle continues to be used to the current time (60). This corrects all of the deformities in single long bone in one surgical event and is fixed with an intramedullary nail. The intramedullary fixation device is much less likely to give rise to a peri-prosthetic fracture compared to other fixation devices and is thus seen as the fixation method of choice in these patients. In children, telescopic rods such as the Fassier-Duval and the Sheffield telescopic intramedullary rod system have gained popularity. These have been shown to correct deformity, prevent fractures, and enhance function (61, 62). However, re-operation and complication rates are high. Delayed and non-union is often encountered and repeat surgery incorporating the use of bone graft is often required (63). Over recent times a more proactive approach has been taken to the upper limb in correcting deformities of the humerus and forearm (64, 65). This is due to the functional importance of this in wheelchair operation and the performance of independent self-care. However, again these are complex surgeries and post-operative problems are common. High rates of revision surgery are reported from most centers (66).

Deformity of the spine including scoliosis, kyphosis, spondylolisthesis, and base of skull problems are also commonly seen in OI patients, particularly adolescents. The mechanisms of scoliosis are incompletely understood but it is associated with low BMD and low BMI (67) The scoliosis is thought to be due in part to vertebral fractures coupled with abnormalities of the vertebral growth plates (68). The influence of medical treatment on the spine in OI is uncertain. Bisphosphonate therapy was associated with slowing of the rate of progression of scoliosis in a retrospective observational study of 316 children with OI but only found in a subgroup of patients with type IV OI that were treated before the age of 6 years (69). A prospective randomized trial would be required to determine if this apparent benefit is due to the intervention or confounding factors.

The orthopedic management of scoliosis is challenging and reduced bone quality makes spinal fusion difficult. Fusion performed before the curve becomes too severe (Cobb angle 45 degrees) is preferred and is known to prevent respiratory complications in these patients (70). Cranio-cervical junction abnormalities such as basilar invagination may also observed in OI and can results in hydrocephalus and brainstem problems. The input of experienced neurosurgeons in regard to this and treatment with cranio-cervical fusion can be required and comes with the same challenges as noted in extremity and spinal orthopedic surgery (71). Spondylolisthesis at the L5 level is found in up to 10% of patients with OI; these patients often have an increased lumbar lordosis predisposing them to this condition and again careful consideration of this should be given when assessing an OI patient with any lower limb neurological symptoms (72).

The Osteogenesis Imperfecta Patient in the Operating Theater

Patients with OI who do need to come to theater provide additional challenges. Great care must be taken during the transfer of such patients on and off the operating table to prevent iatrogenic fracture. Careful pre-assessment by the anesthetic team should be carried out and precautions taken in airway management both due to the fact that the shape of the airway is often abnormal and the risk of cervical spine fracture during intubation. Lung function and bleeding should be taken into particular consideration during surgical correction of spinal deformity in OI (73).

AUTHOR CONTRIBUTIONS

The manuscript was jointly written by SR and MG.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

CASE REPORT
published: 12 February 2020
doi: 10.3389/fendo.2020.00006

Radiotherapy in Fibrodysplasia Ossificans Progressiva: A Case Report and Systematic Review of the Literature

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OPEN ACCESS

Edited by:
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Reviewed by:
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Melissa Orlandin Premaor, Federal University of Minas Gerais, Brazil

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Specialty section:
This article was submitted to Bone Research, a section of the journal Frontiers in Endocrinology

Received: *11 November 2019*
Accepted: *07 January 2020*
Published: *12 February 2020*

Citation:
Botman E, Netelenbos JC, Rustemeyer T, Schoonmade LJ, Nieuwenhuijzen JA, Teunissen BP, Visser M, Raijmakers P, Lammertsma AA, Dahele M and Eekhoff M (2020) Radiotherapy in Fibrodysplasia Ossificans Progressiva: A Case Report and Systematic Review of the Literature. Front. Endocrinol. 11:6. doi: 10.3389/fendo.2020.00006

Fibrodysplasia ossificans progressiva (FOP) is an autosomal dominant disease, characterized by the formation of heterotopic ossification (HO) in muscles, ligaments, and tendons. Flare-ups, an inflammatory process that often precedes the formation of HO, can occur spontaneously, but trauma is also a common trigger. It is not known whether radiotherapy, especially in higher doses, might cause sufficient trauma or inflammation to trigger a flare-up and subsequent HO in FOP patients. We report the case of a patient undergoing radiotherapy for the treatment of a 1-cm-wide basal cell carcinoma (BCC) of the lower lip. In addition, we present a systematic review of the available literature. Our patient received 54 Gy in 18 fractions with orthovoltage therapy, resulting in a clinical complete response of the tumor. Six months after treatment, there were no signs of HO either clinically or on [^{18}F]NaF PET/CT. The systematic review identified 11 publications describing either radiation treatment in FOP or radiation therapy as a cause of HO in non-FOP patients. Six case reports described the use of radiation in FOP patients for various reasons, including one with a high-dose treatment of a lip BCC using superficial X-ray therapy. The remaining five studies described the use of low-dose radiotherapy to prevent or treat either an FOP flare-up or HO formation. None of these cases showed worsening of disease that could be attributed to the use of radiation therapy. Radiation induced HO in non-FOP patients was rare and occurred in five studies. The largest of these studies suggested that HO was induced after treatment with high doses, resulting in more widespread evidence of tissue damage, potentially being the end result of this damage. In conclusion, available reports suggest no contraindication to radiotherapy in FOP patients; although the number of cases was small, systematic toxicity reports often were not available, and none of the reports described high-dose, high-energy radiation treatment at locations such as muscle and joint regions.

Keywords: fibrodysplasia ossificans progressiva (FOP), radiotherapy, heterotopic ossification (HO), [^{18}F]NaF PET/CT, ACVR1 gene mutation

INTRODUCTION

Fibrodysplasia ossificans progressiva (FOP) is an autosomal dominant disorder, which is characterized by heterotopic ossification (HO) in muscle, ligaments, and tendons (1, 2). First ossifications usually develop at the age of 6, often affecting the upper back or neck region. With aging, the formation of HO extends to appendicular regions (3). Often, HO formation is preceded by a flare-up, an inflammatory process of uncertain origin (2, 3). Flare-ups can be provoked by (minor) trauma and infections but can also occur spontaneously (3). Whether radiotherapy can cause sufficient trauma to trigger a flare-up, leading to HO, is unclear. Previously, we have demonstrated that [^{18}F]NaF PET can be used to detect activity of disease just prior to the formation or progression of HO (4–6). Intravenously administered labeled sodium fluoride ([^{18}F]NaF) binds to newly formed hydroxyapatite and, therefore, can be used to detect osteoblastic activity (7). We previously reported that increased [^{18}F]NaF uptake was observed within 1 month of surgery as the first sign of HO recurrence in an FOP patient, confirmed 6 months later with CT (6). If radiotherapy does indeed lead to HO formation, it should be detectable by either increased [^{18}F]NaF uptake on PET or the presence of HO at the irradiated site on a follow-up CT.

In this paper, we describe a 67-year-old male patient with FOP, who underwent radiation treatment for a basal cell carcinoma (BCC) of the lower left lip. To place results into context, we then performed a systematic review of the literature to address whether radiotherapy is safe in FOP patients.

CASE REPORT

A 67-year-old male patient with FOP presented with a 1-cm-wide, progressive lesion of the lower left lip. The patient has the classic variant (R206H) of FOP. The cumulative analog joint involvement scale (CAJIS) score was 25 (8). The patient had not had a flare-up for at least 5 years. However, disease activity was observed at multiple sites on [^{18}F]NaF PET/CT performed during annual follow-ups.

A skin biopsy, performed with caution to minimize damage to surrounding tissues, diagnosed an infiltrative BCC. It extended up to the deep biopsy margin (2 mm). Since surgery is known as a trigger for a flare-up, radiation treatment was preferred over surgical excision. Because the patient is wheelchair bound due to FOP, orthovoltage therapy was considered as the most practical method, as he could remain in his wheelchair during treatment. The patient underwent 18 sessions (fractions) of radiotherapy over a period of ∼4 weeks, with each fraction delivering a dose of 3 Gy for a total dose of 54 Gy. The BCC showed complete clinical remission after treatment. However, soon after treatment, the patient reported increased difficulty in eating because of decreased mobility of the lower lip. In combination with pre-existing jaw ankyloses, the loss of lip mobility increased the difficulty of eating and drinking. To assess whether these problems were caused by formation of HO in the irradiated area, [^{18}F]NaF PET/CT (Gemini TF-64; Philips Medical Systems, Best, Netherlands) was performed. This scan, performed 6 months after completion of radiation therapy, did not show any evidence of HO formation, i.e., no increased tracer uptake in the irradiated area, nor any CT evidence of HO in the treated region. In addition, the radiation therapy did not lead to a significant increase in overall activity of disease throughout the body. Almost 2 years after the irradiation, there was still no sign of HO formation at the irradiated site, confirmed by physical examination.

SYSTEMATIC REVIEW

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement (www.prisma-statement.org). A comprehensive search was performed in the bibliographic databases PubMed and Embase.com from inception to December 6th, 2018, in collaboration with a medical librarian (LS). Search terms included controlled terms (MesH in PubMed and Emtree in Embase) as well as free text terms. The following terms were used (including synonyms and closely related words) as index terms or free text words: "fibrodysplasia ossificans," "radiotherapy," "heterotopic ossification," and "myositis ossificans." The search was performed without date or language restrictions. Duplicate articles were excluded. The full search strategies for all databases can be found in the **Supplementary Material**.

Using this search strategy, 731 articles were identified. Articles describing radiotherapy in FOP patients or radiation therapy as a (probable) cause of HO were eligible for inclusion (**Figure 1**). The articles were systematically assessed by two independent reviewers (EB and JCN). Discrepancies were resolved by consensus. After screening titles, abstract, and articles, 11 publications were selected for this systematic review. Of these 11 articles, 6 articles addressed radiotherapy in FOP, and 5 the relationship between irradiation and the formation of HO.

Radiotherapy in FOP

Not including our own case, radiotherapy in FOP has been described in six other case reports (**Table 1**). One case reported the radical (high-dose) treatment of a lip BCC using superficial (90 Kv) X-ray therapy (12). The remaining five cases described the use of low-dose radiotherapy to prevent or treat FOP flare-ups or HO formation (9–11, 13, 14). In 4/5 of these cases, a beneficial effect on flare-up symptoms or HO formation was reported (9–11, 14). In 2/5 cases, one or two additional treatment modalities were also reported: a non-steroidal anti-inflammatory drug (NSAID) in both cases and a bisphosphonate in one of them (9, 11). None of the cases reported clinical deterioration or excessive toxicity as a result of radiotherapy (containing) treatment. All but one reported a relatively low dose of radiation (9, 11, 13, 14), consistent with the literature on HO prevention in non-FOP patients (15). Interestingly, Soldic et al. described clinical and radiological benefits after very low doses of fractionated radiotherapy (as low as 2 Gy in two fractions) (14). In the remaining case, which was very similar to ours, a patient received 35 Gy in five fractions on consecutive days for the treatment of a right upper lip BCC. There was a complete

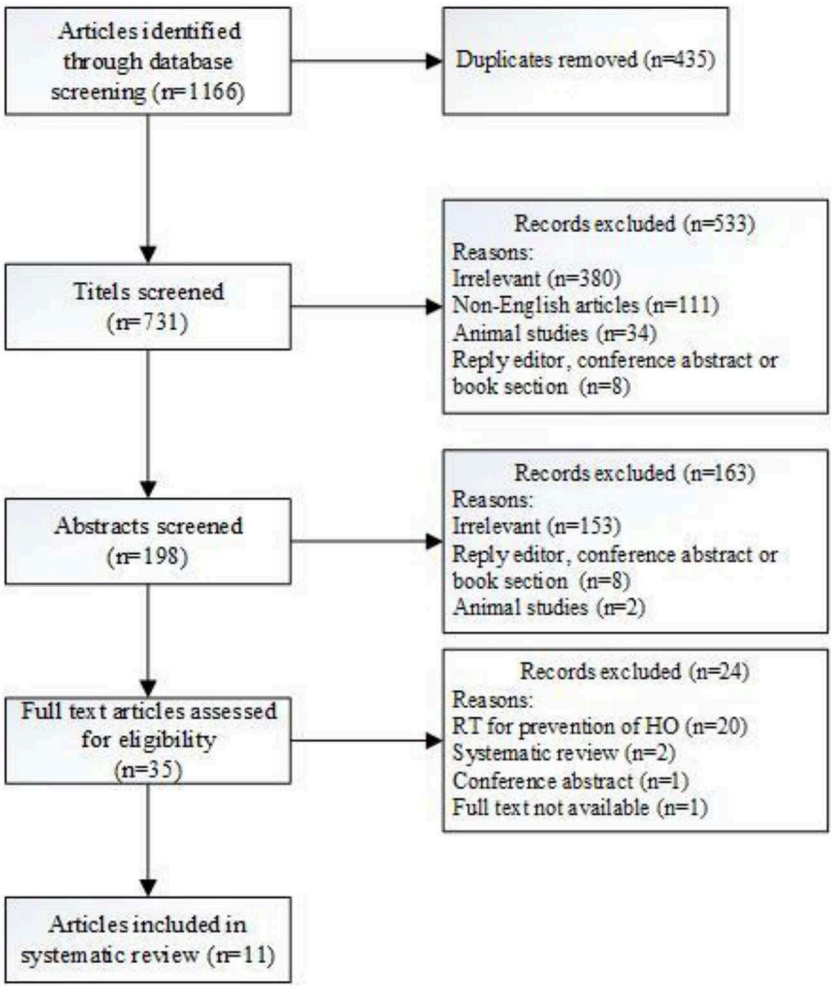

FIGURE 1 | Flowchart of the study selection process.

response with no evidence of HO at the irradiated site (12). Whether this was confirmed radiographically is not known. In addition, the time interval between radiation therapy and follow-up was not reported. In summary, based on a limited sample of seven patients with FOP (including ours), a range of radiotherapy doses appear to have been well-tolerated, with no reports of excessive or unexpected HO formation and no reports to suggest that the intended outcomes (primarily prevention, treatment of HO, and treatment of BCC) were any worse than expected. However, there was no systematic toxicity reporting, and none of the reports described high-dose, high-energy treatment at specific sites, including muscle and joint regions.

Development of HO in Non-FOP Patients Treated With Radiotherapy

Five studies were found suggesting that radiation received by non-FOP patients eventually led to HO at the irradiated site (**Table 2**) (16–20). The interval between actual treatment and formation of HO varied between 1 and 33 years. The largest patient series was from Carl et al. who reported on 15 cases with a range of primary tumors (breast, anal, endometrial, sarcoma, seminoma, bladder, and cervical) (16). Radiation types varied and include cobalt, neutrons, and brachytherapy. Biologically effective doses (for late normal tissue damage, with $\alpha/\beta = 3$) ranged from 67 to 214 Gy. However, potential overlap between fields means that local doses may have been higher. HO developed 2–31 years after radiotherapy. Importantly, all patients first developed other signs of tissue damage ranging from plexopathy to ulceration and necrosis as a result of radiation therapy, leading the authors to propose that HO in these patients was an end stage response to the tissue damage caused by radiotherapy. In the other four case reports, neither dose nor tissue damage as a result of treatment was specified (17–20). In three of these cases, no trigger other than radiotherapy for HO was present (18–20). In the remaining case, the authors stated that HO in the ankylosed mandible might have been caused by a combination of factors, including chemotherapy,

TABLE 1 | Articles describing radiotherapy in patients with fibrodysplasia ossificans progressiva.

	References	Age	Sex	Location	Dose (fractions)	Indication for RT	Follow-up interval after RT	Outcome	HO formed despite RT-containing treatment?
1	Benetos et al. (9)	18	♂	Hip	7 Gy (1)	Prevention of post-operative HO, combined with NSAID	1 year	Increased ROM	Yes[a]
2	Dharra et al. (10)	35	♂	Shoulder	10 Gy (5)	Treatment of flare-up	15 months	Relief of symptoms, increased ROM	Unknown
3	Druce et al. (11)	34	♀	Knee	10 Gy (1)	Treatment of flare-up, combined therapy with NSAID and bisphosphonate	2 months	Relief of symptoms.	Yes[b]
4	Frew and Kelly (12)	46	♂	Lip	35 Gy (5)	Basal cell carcinoma	Unknown	Complete response BCC	No
5	Jayasundara et al. (13)	47	♂	Thigh	26 Gy (13)	Prevention of post-operative recurrence of HO	Unknown	Outcome thigh lesion not described	Unknown
6	Soldić et al. (14)	35	♀	Various ($n = 9$) locations	2 (2)−10 Gy (5)[c]	Treatment of ossification after flare-ups	1–10 years	Relieve of symptoms within days, halted progression HO	No

[a] *Authors state "a small amount of heterotopic bone formed," suggests less HO than expected.*
[b] *Amount of HO not quantified, unclear if less than expected.*
[c] *Also 8 Gy in two fractions, 6 Gy in six fractions, 4 Gy in four fractions, and 3 Gy in three fractions.*
RT, radiotherapy; HO, heterotopic ossification; Gy, Gray; ROM, range of motion; BCC, basal cell carcinoma; NSAID, non-steroidal anti-inflammatory drug.

TABLE 2 | Articles describing the formation of heterotopic ossification in non-FOP patients as a late effect of radiotherapy.

	References	Design	Number of patients	Reason RT	Dosage	Time interval RT and HO (years)
1	Carl and Hartmann (16)	Case series	15	Various carcinomas	BED 67–214 Gy[a]	19 (range 2–31)
2	Kruse et al. (17)	Case report	1	Nasopharyngeal carcinoma	Unknown	3[b]
3	Park et al. (18)	Case report	1	Tonsil cancer	Unknown	14
4	Portha et al. (19)	Case report	1	Metastasized mamma carcinoma	Unknown	1
5	Harmon and Nielsen (20)	Case report	1	Testicular tumor	Unknown	33

[a] *Various kinds of radiotherapy given, potential for overlap could lead to underestimate of radiation dose.*
[b] *Additional factors: chemotherapy, intubation on intensive care, immobilization, critical illness neuromyopathy.*
BED, biological effective dose (with $\alpha/\beta = 3$ for late tissue effects).

radiation, prolonged intubation, immobilization, and critical illness neuromyopathy (17).

DISCUSSION

To the best of our knowledge, this is the first systematic review of literature relating to the use of radiotherapy in patients with FOP. Including our own case, we found only seven cases in the literature. The available reports suggest that radiotherapy in FOP patients does not lead to the formation of HO at the irradiated site. In addition, there were no reports of excessive or unexpected toxicity and no indication that the intended treatment outcome was poorer than expected. Some caution is required, however, as the number of cases is very small, there was no uniform systematic toxicity reporting or post-radiotherapy assessment, there are limited long-term data, and the effect of high-dose, high-energy radiation to, for example, muscle and joint regions was not described.

One discussion point that can be extracted from these reports is the timing of radiotherapy. Pignolo et al. described that most flare-ups resolved spontaneously within 8 weeks, except those of the hip and back, and of the latter, 75% resolved within 12 weeks (3). One patient was irradiated for a flare-up at the iliopsoas muscle. Radiotherapy was combined with physiotherapy, indomethacin, and disodium etidronate (11). Disodium etidronate, a bisphosphonate, has been used in the past to prevent formation of HO in FOP (21–23), but because of its varying success and side effects, nowadays its use is limited (24). The flare-up was present for 5 weeks prior to treatment. Two months after treatment, it was reported that edema was significantly diminished and pain was relieved

(11). Whether this was due to the multi-modality treatment or whether the lesion would have spontaneously resolved is not known with certainty. However, in this case, the patient already had evidence of femoral neurapraxia and neurological deficits at presentation due to the mass. In such a situation, urgent initiation of treatment to avoid permanent nerve damage is important. For milder, non-threatening, flare-ups, a period of observation, to see whether spontaneous regression occurs, would be appropriate. Although apparently effective in the short term, combination treatment did not prevent HO formation, as follow-up CT revealed the presence of calcification at the affected site (11). Unfortunately, the longer-term outcome is not known. Soldic et al. also reported the benefit of radiotherapy in their patient who underwent multiple irradiations at different locations over a prolonged period (14). They used calcification detected on radiographs or CT as a marker of disease. Interestingly, despite low doses of radiation, they reported non-progression of calcification for periods of up to 10 years, and they did not report having to treat previously treated areas again. In the future, it would be interesting to assess disease activity before and after treatment with [^{18}F]NaF PET/CT, as this could objectively assess effects of radiotherapy on disease activity (4–6).

The choice between radiotherapy and other treatments need consideration. Treatment of a tumor or prevention/treatment of HO formation both seem reasonable indications based on the literature. The choice between radiotherapy and other modalities will depend on various factors:

1. The risk of secondary tumor induction by radiation, and the effect of radiation on bone.
   A single radiation fraction of, e.g., 7 Gy, as used in myositis ossificans traumatica (MOT) to prevent HO, has only rarely led to a malignancy at the irradiated site (25). Pellegrini et al. hypothesized that this low incidence is due to the already advanced age of most patients developing MOT and the latency period for the malignancy to develop (26). Younger patients have a higher risk of developing a secondary malignancy as a consequence of radiation treatment (27). Even though life expectancy of FOP patients is limited (28), and therefore, the lifetime chance to develop a secondary malignancy due to radiotherapy is also limited, the treatment of a secondary malignancy (e.g., by surgery) is catastrophic for FOP patients.
   Radiation can also have negative effects on bone metabolism, both locally and systemically (29). In addition, FOP patients often underwent multiple glucocorticoid treatments (3), which can also lead to bone toxicity, e.g., reduction in bone mineral density of skeletal bone. Strategies to maximize bone health and mitigate bone toxicity from FOP treatments are required.
2. The potential of either a flare-up or HO formation by alternative therapy (e.g., surgery).
   Although Benetos et al. reported good outcome after surgery followed by indomethacin and radiotherapy (9), traumatic injury is a major trigger for FOP flare-ups and subsequent HO (3, 28). Radiotherapy to prevent HO reoccurrence after surgery is a known and effective strategy in MOT (15, 30, 31), Indomethacin, an NSAID, is known for its post-operative preventative role in MOT (32). Usually, surgery is avoided in FOP because of the effects it can have on disease progression, although resection of HO has been performed to try and improve function, and surgery may also be necessary in certain urgent conditions. If surgery is required, post-operative radiotherapy and/or NSAID treatment to prevent HO formation should be considered.
3. Patient tolerance or risk of non-radiotherapy side effects.
   Glucocorticoids are commonly used for the treatment of flare-ups because of their anti-inflammatory effect. Although their effect on prevention of flare-ups and HO formation has never been rigorously tested, about half of the patients report an improvement in flare-up symptoms when treated with glucocorticoids (3). However, known side effects are, among others, weight gain, proximal myopathy, glucose intolerance, suppression of endogenous hormones, and gastrointestinal toxicity (33). There is extensive experience with NSAIDs in FOP patients (24). About one-third of patients use NSAIDs for flare-ups, although they can lead to gastrointestinal issues and renal toxicity (3). Radiotherapy should not be seen as a replacement for anti-inflammatory drugs but, rather, as a complementary treatment strategy to be considered in certain clinical situations and for selected patients.

Even though radiotherapy seems safe in FOP patients, one should keep in mind that post-irradiation tissue damage (e.g., fibrosis) leading to (even minimal) mobility/function loss can have a significant impact on the quality of life of patients. Patients are highly dependent on their remaining function, and any disturbance can significantly affect daily life. Any intervention, including radiotherapy, should take this into account, and where possible, risks should be kept as low as possible.

In conclusion, the risk of HO induction by radiation in non-FOP patients is, as demonstrated by the few cases in our systematic review, very small and usually part of more widespread tissue damage. Based on available literature, radiotherapy-induced HO formation does not seem to be a problem in non-FOP or FOP patients. As follow-up data are limited, radiotherapy for FOP patients should only be considered in specific situations, e.g., post-operatively after surgery or to reduce flare-up edema when causing neurological deficits. As [^{18}F]NaF is the only *in vivo* disease activity marker currently available, pre-treatment and follow-up imaging using [^{18}F]NaF PET/CT should be considered to evaluate the effects of interventions, including radiation, on local, and systemic FOP activity.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical Ethical Review Board, Amsterdam

UMC, Vrije Universiteit Amsterdam, Netherlands. The patient/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

EB, ME, JAN, MD, and TR: study design, data analysis, data interpretation, and drafting manuscript. EB, ME, JCN, MD, and TR: study conduct. EB, ME, JCN, and LS: data collection. PR, BT, AL, MV, and JAN: revising manuscript content. EB, ME, JAN, MD, TR, AL, BT, PR, MV, LS, and JAN: approving final version of manuscript. EB, ME, and MD: taking responsibility for the integrity of the data analysis.

ACKNOWLEDGMENTS

We thank the patient for sharing the data with us.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2020.00006/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

SYSTEMATIC REVIEW
published: 23 June 2020
doi: 10.3389/fendo.2020.00399

A Clinical Perspective on Advanced Developments in Bone Biopsy Assessment in Rare Bone Disorders

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Introduction: Bone biopsies have been obtained for many centuries and are one of the oldest known medical procedures in history. Despite the introduction of new noninvasive radiographic imaging techniques and genetic analyses, bone biopsies are still valuable in the diagnosis of bone diseases. Advanced techniques for the assessment of bone quality in bone biopsies, which have emerged during the last decades, allows in-depth tissue analyses beyond structural changes visible in bone histology. In this review, we give an overview of the application and advantages of the advanced techniques for the analysis of bone biopsies in the clinical setting of various rare metabolic bone diseases.

Method: A systematic literature search on rare metabolic bone diseases and analyzing techniques of bone biopsies was performed in PubMed up to 2019 week 34.

Results: Advanced techniques for the analysis of bone biopsies were described for rare metabolic bone disorders including Paget's disease of bone, osteogenesis imperfecta, fibrous dysplasia, Fibrodysplasia ossificans progressiva, *PLS3* X-linked osteoporosis, Loeys-Diets syndrome, osteopetrosis, Erdheim-Chester disease, and Cherubism. A variety of advanced available analytical techniques were identified that may help to provide additional detail on cellular, structural, and compositional characteristics in rare bone diseases complementing classical histopathology.

Discussion: To date, these techniques have only been used in research and not in daily clinical practice. Clinical application of bone quality assessment techniques depends upon several aspects such as availability of the technique in hospitals, the existence of reference data, and a cooperative network of researchers and clinicians. The evaluation of rare metabolic bone disorders requires a repertoire of different methods, owing to their distinct bone tissue characteristics. The broader use of bone material obtained from biopsies could provide much more information about pathophysiology or treatment options and establish bone biopsies as a valuable tool in rare metabolic bone diseases.

Keywords: bone biopsy, histomorphometry, bone quality, rare bone disorders, advanced techniques

OPEN ACCESS

Edited by:
Guillaume Mabilleau, Université d'Angers, France

Reviewed by:
Natalie A. Sims, St. Vincents Institute of Medical Research, Australia
Stéphane Blouin, Ludwig Boltzmann Institute of Osteology (LBIO), Austria

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Specialty section:
This article was submitted to Bone Research, a section of the journal Frontiers in Endocrinology

Received: *31 January 2020*
Accepted: *18 May 2020*
Published: *23 June 2020*

Citation:
Treurniet S, Eekhoff EMW, Schmidt FN, Micha D, Busse B and Bravenboer N (2020) A Clinical Perspective on Advanced Developments in Bone Biopsy Assessment in Rare Bone Disorders. Front. Endocrinol. 11:399. doi: 10.3389/fendo.2020.00399

INTRODUCTION

Trepanning of bone is one of the oldest medical procedures known in history. This procedure was initially carried out for the treatment of headache and mental illness. The first application of trepanning as a diagnostic tool was described early 1900 by Pianese for bone marrow aspiration. During the last century, different needles have been developed, including a type for bone biopsy acquisition (1). The first modern needle for the performance of an iliac crest biopsy was described in 1954 (2). Around 1950, modern embedding techniques were discovered for the microscopic examination of mineralized bone tissue (3). Beside conventional X-rays and biochemical markers, histopathological analyses were considered for many years to be the hallmark for the diagnosis of bone-related disorders. The publication of standardization of histomorphometric nomenclature in 1987, largely improved communication between practitioners of bone histomorphometry, medical doctors, and scientists (4). This led to a broader understanding of histomorphometric data (5). The introduction of more advanced radiographic imaging techniques has complemented the role of bone biopsies are no longer the only diagnostic tool for bone disorders. Dual energy x-ray absorptiometry (DXA) is a widely used method to measure bone mineral density to estimate fracture risk. However, measurement of bone mineral density does neither provide information about the microarchitecture of bone, bone cell activity, and bone remodeling, nor allows the consideration of bone volume and density independently of each other. Histology and quantitative histomorphometric analyses are still the most commonly used method for analyzing bone samples; this is often combined with double-labeling with tetracycline which provides additional information about bone mineralization.

The indication for a bone biopsy has shifted from diagnostics of bone structural changes to an additional assessment of compositional information at the tissue level. This shift has taken place due to the availability of new imaging and analyses techniques, a broader use of biochemical markers, and genetic testing. Nowadays bone biopsies are indicated in patients with early onset osteoporosis, renal osteodystrophy, malignant disorders, suspicion of osteomalacia, or rare (metabolic) bone disorders for the analysis of bone mass, cortical and trabecular structure, bone turnover and mineralization as well as cellular status. All these parameters need to be considered in the thorough evaluation of bone quality. Here, bone quality can be considered as the total sum of characteristics with the ability to resist bone fracture, in particular the combination of structural and compositional bone characteristics, as well as the bone cellular activity. Impaired bone quality or bone fragility leads to fractures. Certainly, bone histomorphometric assessment in addition to *T*-Scores measured with DXA remains useful to predict fracture risk in different rare metabolic bone disorders. However, specific techniques for analyzing bone quality in bone biopsies have become available to examine the pathophysiology in rare bone diseases in more detail. These additional analysis techniques include Fourier transform infrared spectroscopy (FTIR), Raman spectroscopy, transmission electron microscopy (TEM), quantitative backscattered electron imaging (qBEI), confocal microscopy, microCT (μCT), micro- and nano-indentation, high-performance liquid chromatography (HPLC), atomic force microscopy (AFM), small-angle X-ray scattering (SAXS), wide-angle X-ray scattering (WAXS), nuclear magnetic resonance (NMR), and immunohistochemistry (**Table 1**). Currently, the aforementioned techniques are predominantly applied in research settings and are mostly not used in routine clinical practice. This review focuses on the state-of-the-art assessment of bone biopsies from rare bone diseases. Furthermore, additional information on comprehensive bone quality analyses and its impact for clinical practice is given as a perspective. Such techniques allowing for high-resolution analyses of osseous changes in combination with traditional bone histomorphometry represent a valued resource for the diagnosis of rare metabolic bone disorders and the refined prediction of bone fragility.

Therefore, the aim of this review is to give an overview of various rare metabolic bone diseases and the possible use of advanced techniques to analyze bone quality in bone biopsies in the clinical setting.

METHODS

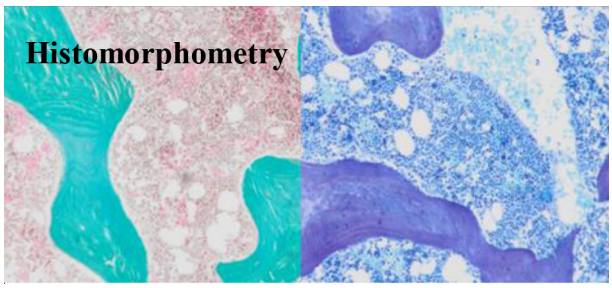
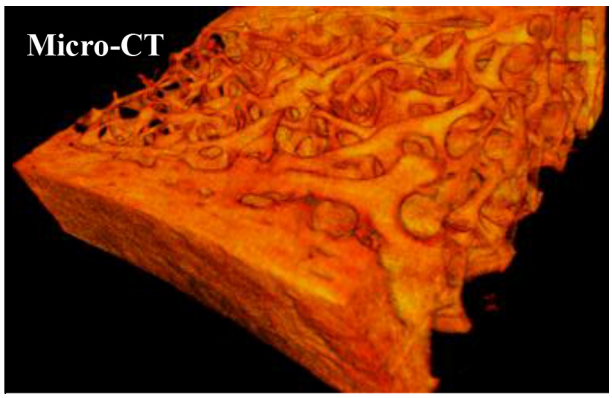
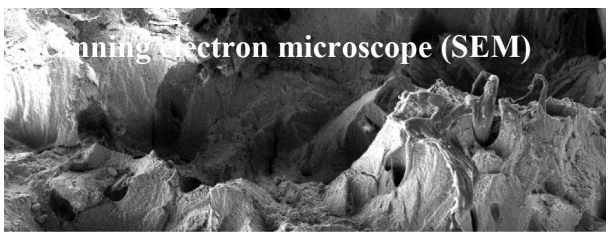
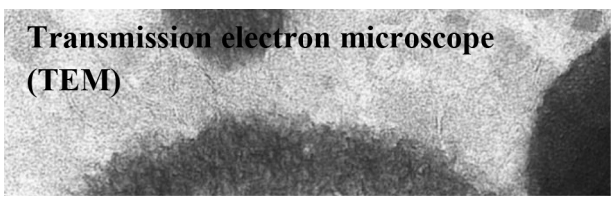
Literature Search

Literature was systematically reviewed to identify publications on rare metabolic bone diseases and analyzing techniques for bone biopsies. The literature search was performed based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)-statement. To identify all relevant publications a systematic search was conducted in the US National Library of Medicine National Institutes of Health (PubMed) for publications from inception to 2019 week 34. The following search terms were used: "*(PLS3 mutation)* OR *(osteogenesis imperfecta)* OR *(osteopetrosis)* OR *(fibrodysplasia ossificans progressiva)* OR *(myositis ossificans)* OR *(osteosclerosis)* OR *(osteomalacia)* OR *(Ehlers-danlos syndrome)* OR *(fibrous dysplasia of bone)* OR *(fibrous dysplasia)* OR *(osteitis deformans)* OR *(paget disease)* OR *(morbus paget)* OR *(m. paget)* OR *(Loeys-dietz syndrome)*" AND "*(histology)* OR *(histochemistry)* OR *(histomorphometry)* OR *(Fourier transform infrared spectroscopy)* OR *(Fourier transform infrared imaging)* OR *(Raman spectroscopy)* OR *(Transmission electron microscopy)* OR *(Quantitative backscattered electron imaging)* OR *(Confocal microscopy)* OR *(Micro-CT)* OR *(contrast enhanced microCT)* OR *(Microindentation)* OR *(Nanoindentation)* OR *(High-performance liquid chromatography)* OR *(Atomic force microscopy)* OR *(Immunofluoresence microscopy)* OR *(Small-angle X-ray scattering)* OR *(Wide-angle X-ray scattering)* OR *(synchrotron)* OR *("NMR")* OR *("Nuclear magnetic resonance").*"

Inclusion Criteria

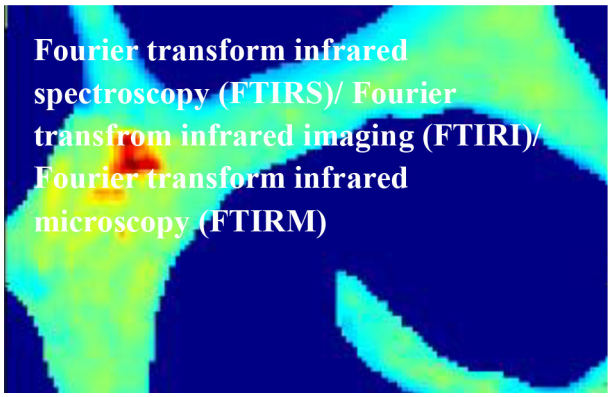
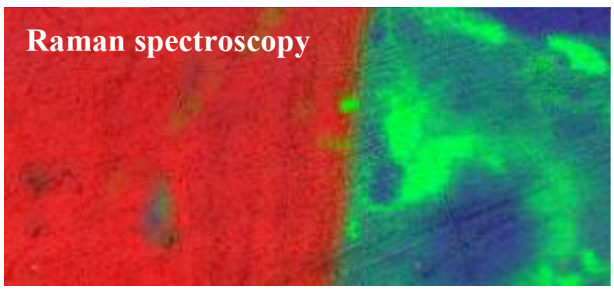
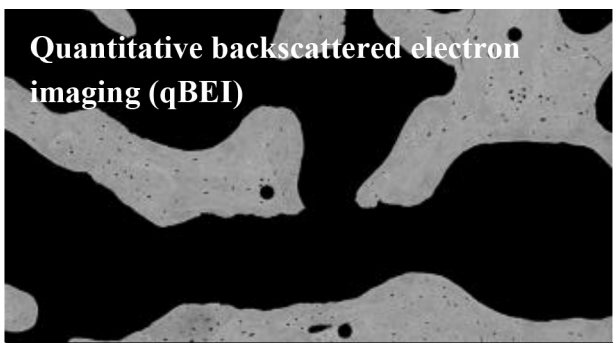
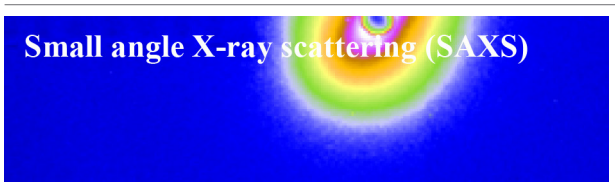
The following inclusion criteria were used: (1) studies describing bone biopsies in the following rare metabolic bone disorders: Paget's disease of bone, osteogenesis imperfecta, fibrous dysplasia, fibrodysplasia ossificans progressiva, *PLS3* X-linked osteoporosis, Ehlers-Danlos syndrome, Loeys-Diets syndrome, osteopetrosis;

TABLE 1 | Overview of different techniques which can be considered in bone biopsy analysis by clinicians and researchers.

	Histomorphometry is the gold standard for the assessment of bone cell activity at the tissue level. It can be used for static quantitative histomorphometric analysis of bone mass, bone structure, bone turnover and dynamic mineralization kinetics after tetracycling labeling. It can also be used to monitor treatment. Standardization of parameters for quantification are published by Dempster et al. (5)
	Microcomputed tomography is a technique to investigate cortical and trabecular bone morphology. Only mineralized bone tissue can be imaged. Individual slices can be combined to produce a 3-dimensional reconstruction of the sample. Non-destructive, intact bone samples from biopsy can be scanned in spatial resolutions ranging from 1 to 30 μm. Structural analysis can be carried out on the 3D information/model. To virtually investigate the mechanical properties of the bone, finite element analysis can be applied to the three-dimensional model. Thus, structural features as well as mineralization and its influence on the mechanical performance of bone can be estimated
Microradiography	Quantitative X-ray imaging of an unstained section of a bone biopsy. The radio-opaque mineralized bone present in a given field of tissue area can be selectively measured by this technique and provides detailed information on the bone mineral density with high spatial resolution (6)
	The scanning electron microscope (SEM) scans a focused electron beam over a surface. The electrons in the beam are interacting with the sample, which results in signals that reflect information on the samples surface topography. The electrons that are reflected off the samples surface region are then used to form an image. Thus, secondary electrons are most valuable for showing morphology and topography aspects of the samples
	Electrons are submitted through a thin layer of tissue. Transmitted electrons are detected and represent an image of electron absorption of the tissue lamella, thus the special density of the lamella. This technique visualizes nanometer-sized structures of collagen, mineral, and cellular organelle/features (7, 8)
Confocal microscopy	Confocal microscopy is an optical imaging technique with high optical resolution and contrast of a micrograph by means of using a spatial pinhole to block out-of-focus light in image formation. Capturing multiple two-dimensional images at different depths in a sample creating a reconstruction of three-dimensional structures at a subcellular level

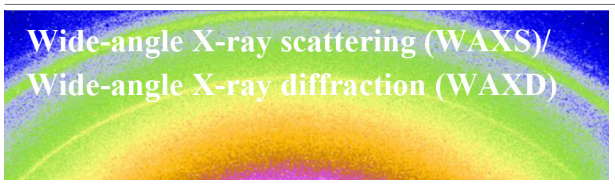
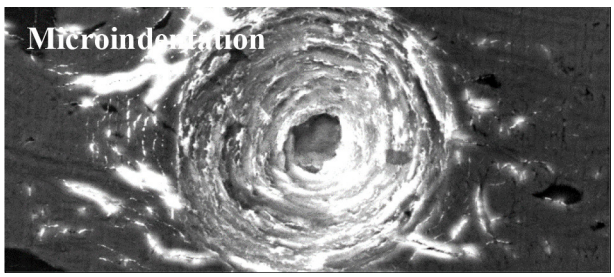
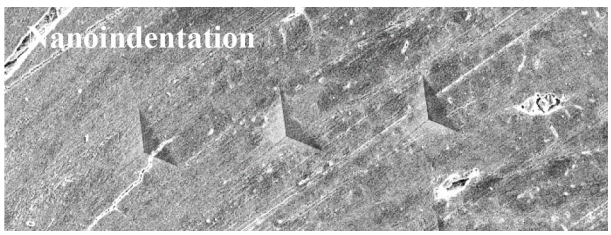
(Continued)

TABLE 1 | Continued

	FTIRS is a vibrational spectroscopy technique to analyze bone and tissue material composition (i.e., mineral and collagen). Bone samples are investigated with infrared light which is absorbed by molecule vibrations. The different patterns of absorption distinguish between different molecules of bone material representing the mineral phase and collagenous phase (9, 10). Primarily used outcome parameters of FTIRS are: mineral-to-matrix ratio (ratio of collagen and hydroxyapatite), mineral maturity and collagen-/matrix- maturity and collagen cross-links (11, 12). FTIRS enables representative large-scale mappings/measurements with high spatial resolution in a short time
	Raman spectroscopy is another vibrational spectroscopy technique. In Raman spectroscopy scattered photons are used to quantify the molecular composition of the tissue. This method, as FTIRS, enables a quantitative assessment of the collagen and mineral phase of bone. Chemical/compositional properties of the tissue can be described by means of mineral-to-matrix ratio, bone mineral crystallinity, collagen quality, and collagen cross-linking (13)
	High resolution technique to visualize bone matrix mineralization. Electrons are emitted to a plane surface of embedded bone. The number of backscattered electrons correlates with the mineral density of the tissue. More precisely, the calcium content represents the amount of mineral in this method. The spatial resolution of this method enables to quantify the bone mineral density distribution (BMDD). Due to a calibration to known materials and its concentration the gray values represent a certain amount of mineral, thus this method is quantitative (14)
High-performance liquid chromatography (HPLC)	HPLC is used to quantify the amount of molecules in bone. A separation can be achieved by size, charging and others. This method is mainly used to quantify the collagen of bone and, e.g., its cross-linking (10, 15)
X-ray cristallography	X-ray cristallography utilizes synchrotron radiation in different scattering/diffraction angles. Parallel X-rays get scattered by periodic, crystalline structures. The scattering/diffraction pattern represent the inner structure of the examined material. Here the diffraction of X-rays represents not only the structure of mineral particles by means of length and thickness. Collagen and mineral alignment can be measured and the collagen-mineral interaction under loading can be determined (16, 17). X-ray crystallography can be divided into SAXS (small-angle X-ray scattering) and WAXD (wide angle X-ray diffraction)
	The small angle X-ray scattering can be used to quantify nanoscale density differences. In bone samples, it can be used to analyze ultrastructural orientation and measurement of the size of mineral crystals and collagen arrangement (18, 19). Specifically, particle size and its orientation can be determined (20)

(Continued)

TABLE 1 | Continued

	(WAXS)/WAXD works by similar principles like SAXS, however the distance from the sample to the collector is shorter, thus it records the wide angle diffracted x-ray signals. This method can be used in bone to investigate the crystal lattice and the size of hydroxyapatite crystals (19, 20)
	Microindentation is a technique to measure the local biomechanical characteristics of a sample. Here, not a single lamella of bone can be indented but a cluster of neighboring bone lamella, thus it represents a more averaged mechanical characterization on a larger micro-scale
	Nanoindentation is a technique to measure the hardness and Youngs's Modulus of small volumes of material. Small indentations are made while measuring the loads and displacements of the indenter. Because of the nanoscale, mechanical properties of different parts of the bone—like individual trabeculae and interstitial lamellae—can be analyzed. This technique is very sensitive for (sub)surface porosity
	AFM could perform surface measurements on the nanoscale. With this technique, measurement of single collagen fibers and crystal size can be made. AFM has two function abilities: force measurement and topographic imaging (21)
Immunofluoresence microscopy/Immunohistochemistry	Specific antibodies bind to specific proteins, and are visualized, resulting in localization at the tissue level. These proteins can be visualized using fluorescence microscopy or light microscopy. 3-dimensional imaging of fluorescent labeled proteins is done by confocal laser microscopy. Quantification is possible with image analysis
Nuclear magnetic resonance (NMR)	NMR is a spectroscopic method to measure compositional aspects of bone. NMR uses the nuclear magnetic resonance after sample excitation by radio waves in a magnetic field. It can be used to quantify water content and mineral structure of bone biopsy specimens as well as changes in the mineral chemistry (22)

(2) study published as an original article; (3) adequate description of bone biopsy analysis; (4) studies were published in either English or Dutch; (5) full text availability; and (6) all types of study design.

Exclusion Criteria

The following exclusion criteria were uses: (1) studies focusing on animal models and (2) studies focusing on *in vitro* models.

Study Selection

A total of 1,114 papers were identified. All titles and thereafter remaining abstracts were screened for eligibility by two clinical researchers, ST and EE. In case of disagreement consensus was reached by dialogue. Articles were included when they described the analyzing techniques of bone biopsies in the listed rare metabolic bone disorders. A total of 141 studies were included for initial full text analysis. Forty-six studies were excluded when they did not report any results of the analyzed bone biopsies, described no bone biopsies in any of the listed rare metabolic bone diseases, or were used for *in vitro* studies (**Figure 1**).

Data Extraction

The extracted data included the following: (1) name of the first author; (2) year of publication; (3) journal; (4) number of patients with a bone biopsy; (5) aim of the article; (6) type of material (biopsy or residual); (7) preparation method; (8) technique of bone material analysis; (9) results.

Study Quality Assessment

Study quality assessment was performed on all articles. The quality assessment was independently conducted by two clinical researchers, ST and EE. In case of disagreement consensus was reached by dialogue. Thirty-two (34%) articles consisted of case reports. The remaining 63 articles were assessed using the Study

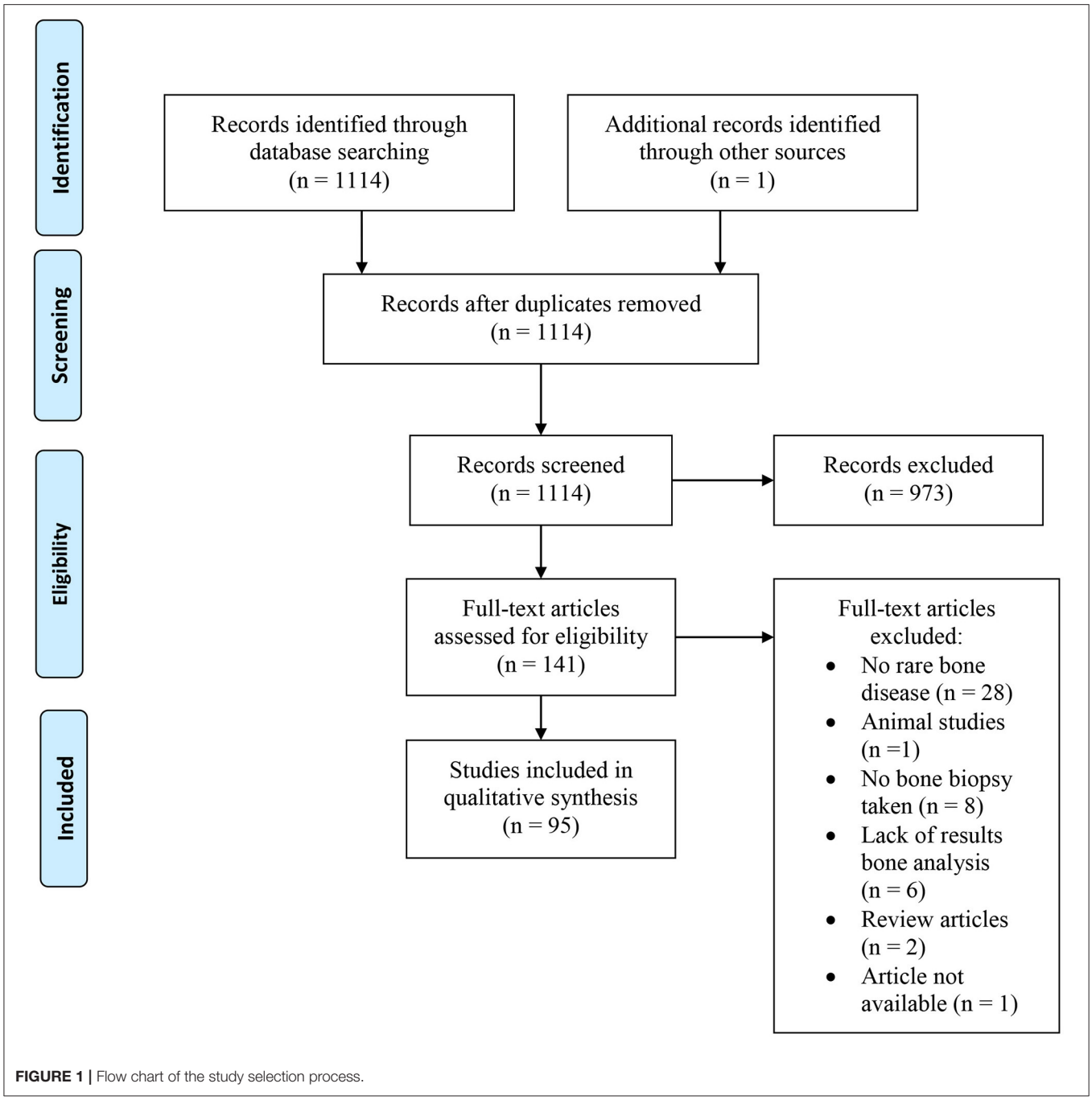


FIGURE 1 | Flow chart of the study selection process.

Quality Assessment Tools created by the NHLBI (23). Two items were added to the tool: "Were the techniques used for analysis of bone biopsy material well-described?" and "Were the histomorphometric parameters well-described?"

RESULTS

A final total of 95 studies were included in this review. Results of the quality assessment (NHLBI) of included studies showed that 10% of the studies were classified as poor, 73% as fair and 17% as good (23). An overview of the included studies and quality assessment is displayed as a supplement. **Table 2** shows an overview of the different analysis techniques per rare metabolic disorder as found in the PubMed search.

Atomic force microscopy was not previously described in any of the rare metabolic bone diseases. There were also no studies found describing bone biopsies in Ehlers-Danlos syndrome. Some studies described other methods than the ones used in our PubMed search: microradiography,

TABLE 2 | Number of articles describing different analyzing techniques of bone biopsies.

		Paget	OI	FD	FOP	PLS-3	LD	Osteopetrosis	EC	Cherubism
	Number or articles	19	45	9	3	6	1	6	3	3
Structural properties	Histomorphometry	14	27	8	2	6	1	5	1	3
	Micro-CT	1	4							
	Microradiography		1							
	Scanning electron microscope		2							
	Transmission electron microscopy	2	3					4		
	Electron microscopy		2							
	Confocal microscopy			2		1				
Material properties	Fourier transform infrared spectroscopy	1	3		1			1		
	Raman spectroscopy		3			1				
	Quantitative backscattered electron imaging	1	10	1		2	1			
	High-performance liquid chromatography		1							
	X-ray diffraction		1					1		
	Small-angle X-ray scattering	1	1							
	Wide-angle X-ray scattering	1	1							
Mechanical properties	Microindentation	1								
	Nanoindentation		6							
	Three point bending test		2							
	In situ fracture Thoughness test	1								
	Vickers-hardness							1		
Immunology	Immunofluoresence microscopy	1								
	Immunohistochemistry	1	3	4	1	1		1	3	2
	Immunocytochemistry	2								
	Histochemistry		1							
	Nuclear magnetic resonance		1							

Paget, Paget disease; OI, Osteogeneis imperfecta; FD, fibrous dysplasia; FOP, fybrodysplasia ossificans progressive; PLS3, PLS3 X-linked osteoporosis; LD, Loeys-Dietz syndrome; EC, Erdheim Chester disease.

scanning electron microscopy, electron microscopy, X-ray diffraction, three-point-bending test, *in situ* fracture toughness test, immunohistochemistry, immunocytochemistry, and histochemistry. These articles were included in this review as well. In addition, Erdheim-Chester disease and Cherubism were included in the results.

Paget's Disease of Bone

Paget's disease is a chronic focal bone disorder of unknown cause, most commonly seen in elderly patients. The hallmark of this disorder is locally increased bone turnover with uncontrolled osteoclast activity, leading to the resorption of bone at specific skeletal sites. As a result of bone resorption and subsequent increase of osteoblast activity, overproduction of newly formed bone takes place. This newly formed bone is disorganized and has a different tissue composition in comparison to bone regions unaffected by Paget's disease of bone (24). Commonly affected areas are the skull, spine, pelvis, and long bones of the lower extremity. Most patients are asymptomatic; however, some patients suffer from pain and fracture risk can be increased. The diagnosis is primarily based on radiologic evaluation of lytic lesions or thickened cortices, accentuated trabeculae, and increased size of bones or by skeletal scintigraphy (25). Biochemical screening shows an elevated serum concentration of alkaline phosphatase in most patients.

Histological examination of lesions shows excessive numbers of multinucleated osteoclasts and abnormal deposition of primarily woven bone. The bone marrow contains an increased number of blood vessels and bone precursor cells (26).

Nineteen articles on Paget's disease were identified with the PubMed search (24, 27–44). Multiple articles described different immunological techniques in search of a viral origin in the disorder. Most of the articles were published before the year 2000. This may be the reason for which most articles mainly describe histomorphometric data. The study of Zimmermann et al. applied different techniques to characterize the composition, structure, and mechanical properties of Pagetic bone. Quantitative backscattered electron microscopy showed a lower degree of mineralization in agreement with the mineral-to-matrix ratio measured by Fourier transform infrared spectroscopy. Structural properties were analyzed by micro-CT, which showed replacement of the typical longitudinal-oriented Haversian canals by disorganized clusters of bone with high porosity. Also, the trabecular thickness was shown to be substantially higher in Pagetic bone. Mechanical properties were tested by nano-indentation, reference point indentation (micro-indentation), and *in situ* fracture toughness test. These

measurements demonstrated a lower plasticity in Pagetic bone (24). Giannini et al. used SAXS and WAXS techniques to characterize the organization of mineral nanocrystals in one patient with Paget's disease. Results showed a disorganized structure of the nanocrystals (44).

Osteogenesis Imperfecta

Osteogenesis imperfecta (OI) is an inherited disorder which is mainly characterized by increased risk of fractures, blue sclerae, dentinogenesis imperfect, and hearing loss. OI is most commonly caused by mutations in the *COL1A1* or *COL1A2* genes (coding for type I collagen molecules). Type I collagen is the main structural protein in bone, building the mineralizing matrix. OI is subdivided in types I–V, depending on the type and severity of clinical symptoms (45). The production of type I collagen by osteoblasts is abnormal or decreased in patients with OI. In addition to the altered structure, there is hypermineralization of the bone matrix, independent of the type and severity of the disease (46, 47). Histological examination showed low cancellous bone volume. Lamellae appear thinner and less smooth than in healthy people. Histological distinction between OI and other osteoporotic bone disorders by histomorphometric analysis is difficult (48, 49). The diagnosis of OI is based on the described clinical characteristics, radiological findings, and genetic testing.

OI is the most commonly studied rare metabolic bone disorder in our PubMed search with a total of 45 included articles (48, 50–92). This is also the disorder in which high-resolution techniques were employed for in-depth analyses. In particular, quantitative backscattered electron imaging (qBEI), and nanoindentation have been used in multiple studies. Bone mineral density distribution measured by qBEI was increased in different types of OI (58, 59, 61, 62, 68, 69, 76, 83, 90). Only the article by Webb et al. described several OI type XIV patients in which qBEI showed a normal bone mineral density distribution, with the exception of one patient with a higher BMDD compared to healthy controls. This patient had a very low bone turnover (82). In accordance to the high BMDD values found with qBEI, Fratzl-Zelman et al. studied the mineral particle size in children with OI type I with SAXS/WAXD technique. They concluded that high BMDD in children with OI type I is due to increased particle packing density and not to increased particle size (90). Nanoindentation showed a lower Young's modulus in OI in comparison to control bone (50, 53). Comparison between different clinical types of OI was studied. Albert et al. described a higher elastic modulus and hardness in OI type I compared to OI type III (51). Fan et al. showed no significant difference in Young's modulus and hardness between OI type III and OI type IV (52). The data of Weber et al. showed no effect of pamidronate treatment on elastic modulus and hardness (83). In the preliminary study by Abidin et al., the researchers used synchrotron tomography to characterize the 3D structure of cortical bone in children with OI. With these cortical characteristics they successfully performed a machine learning task to distinguish between OI and healthy bone. They conclude that this technique could potentially be used as a future biomarker to detect and quantify the severity of OI, respectively (91).

Fibrous Dysplasia

Fibrous dysplasia (FD) is a disorder in which parts of bone are replaced by fibrous connective tissue and trabecular bone of poor quality. The disease is caused by a mutations in the *GNAS1* gene and symptoms commonly present during the second decade of life. It may occur as a single lesion (monostotic) or at multiple sites of the skeleton (polyostotic). A specific polyostotic form is also known as McCune-Albright syndrome (MAS), which is accompanied by skin pigmentation and endocrinopathies. Commonly affected sites are the long bones, ribs, pelvis and the craniofacial skeleton. The *GNAS1* gene mutation leads to overproduction of cAMP in the osteoblastic cell lineage which in turn leads to increased proliferation and abnormal differentiation of osteoblastic cells. Most patients with FD are asymptomatic and FD is often an incidental finding on routine X-ray examination. In some patients, the enlargement and distortion of bone lesion(s) can cause pain, swelling, bone deformity, or pathological fractures, which are the main symptoms of FD (93). CT or MRI scans are used to determine the extensiveness of the lesion(s). For the final diagnosis histological examination is required.

A total of nine studies on FD/MAS were identified during our search (94–102). Three out of the nine studies were case-reports. In addition to standardized histomorphometry different study designs included the use of quantitative backscattered electron microscopy (qBEI), immunohistochemistry, and confocal microscopy. A lower mineral content was seen in the more severely affected FD patients measured by qBEI (94). Two immunohistochemical studies were performed to obtain a better understanding of the disease. The most remarkable findings included high expression of c-fos and c-jun in fibroblast-like cells, a negative response to nuclear antigen of cellular proliferation (PCNA), and a positive response to AgNOR (97, 102). The case report by Boyce et al. is the only study reporting the use of immunohistochemistry to evaluate the effect of denosumab treatment on the expression of RANKL. However, the biopsies before and after treatment were not of sufficient quality to compare RANKL expression (100). Two articles describing the use of confocal laser scanning microscopy report the finding of "Sharpey's fibers" in fibrous dysplastic bone (98, 99). The most recent article from 2015 by Laino et al. suggested confocal laser scanning microscopy as a useful tool to investigate FD lesions (99).

Fibrodysplasia Ossificans Progressiva

Fibrodysplasia ossificans progressiva (FOP) is a very rare autosomal inherited disorder caused by a mutation in the *ACVR1/ALK2* gene (103). FOP is a clinical diagnosis, confirmed by genetic testing. FOP is characterized by congenital malformation of the great toes and progressive heterotopic ossification of muscles, tendons, and ligaments. Heterotopic ossification is induced by flare-ups (painful inflammatory swelling of soft tissue) (104). Nowadays diagnostic bone biopsies are not performed because of the severe risk of new heterotopic bone formation. Histological examination of bone biopsies in FOP patients has been however performed without awareness of

the disease in the past. These patients lacked FOP diagnosis and genetic testing was not yet available.

Only three articles were found describing bone biopsies in FOP (105–107). Kaplan et al. performed a study during the early '90s to investigate bone properties by using histomorphometry and immunohistochemistry (105). Histologically, lesions with HO formation appear to be similar to original skeletal bone with regard to lamellar bone formation and resorption and marrow elements (105). In later studies by Piram et al. (106) and Ibarra et al. (107), FOP was identified by coincidence, as the acquired biopsies ruled out malignant disease.

PLS3 X-Linked Osteoporosis

Mutations in the actin-binding protein plastin-3 (*PLS3*) gene have recently been discovered as a rare cause of monogenetic osteoporosis which is usually reported to present without syndromic features. Due to the X-linked pattern of inheritance, homozygous male patients suffer from fractures from early childhood. Heterozygous women have a milder presentation in age of onset and fracture risk (108). DXA measurements show low BMD values in affected patients. Genetic analysis is needed for the final diagnosis. The cellular functions of PLS3 protein in bone and the pathogenic mechanism causing monogenetic osteoporosis are not well-understood. PLS3 is expressed in almost all cell types, including osteoclasts and osteocytes. It is possible that *PLS3* mutations, which affect the function or expression level of PLS3 protein in the osteocyte dendrites, lead to altered mechanosensitivity of these osteocytes.

The literature search yielded six articles, which included a bone biopsy analysis (109–114). All articles except one are case reports or case series describing phenotypical presentation of the *PLS3* mutation. Because of the disease rarity patient numbers are very low in all articles, with a maximum of five. Histomorphometric analysis showed a lamellar pattern of cortical and trabecular bone. The amount of trabecular bone is severely reduced and bone formation and resorption parameters are low. Quantitative backscattered electron microscopy showed conflicting results; the study by Fahiminiya et al. (110) described bone mineralization density distribution within the normal range, Balasubramanian et al. (114) showed a hypermineralization distribution and Kampe et al. (115) showed hypomineralization.

Loeys-Diets Syndrome

Loeys-Diets syndrome (LDS) is an autosomal dominant disorder, due to a mutation in the *TGFBR1/2*-gene. The most typical symptoms are arterial aneurysms, hypertelorism, and bifid uvula or cleft palate. There is a wide variety of clinical presentation with involvement of other organ systems, including the skeleton. Most common skeletal findings are pectus excavatum or carinatum, joint laxity, arachnodactyly, scoliosis, and cervical spine malformation (116, 117). Also, osteoporosis with increased fracture risk has been described (118). The diagnosis of LDS is based on the variety of clinical symptoms and confirmed by genetic testing. TGF-β regulates mechanical properties and bone matrix composition (119). Dysregulation of TGF-β signaling due to the effect of the mutation in *TGFBR1/2* and its effect on bone metabolism are poorly understood.

PubMed search identified only one article. The case-series of Ben Amor et al. reported bone histomorphometric and bone material observations in two patients. Histomorphometric findings of thin cortices and high bone turnover without mineralization defects were reported. Quantitative backscattered electron microscopy was used in this case series which showed elevated matrix mineralization on the level of individual trabeculae (120).

Osteopetrosis

Osteopetrosis is a heterogeneous group of disorders characterized by diffuse skeletal sclerosis and high bone mineral density caused by different mutations. There is a wide variety in severity of the disease ranging from asymptomatic to fatal symptoms during childhood. Most patients have an increased fracture risk due to the lack of bone flexibility. X-rays shows a dense appearance of the skeleton. Osteopetrosis is caused by decreased osteoclastic bone resorption due to failure of differentiation or function of the osteoclastic cells. The expansion of bone may cause nerve compression and hematological dysfunction due to reduction of the bone marrow cavity by central bone expansion (121, 122).

A total of six articles were identified (123–128). Histomorphometry showed multinucleated osteoclast-like cells. Four out of this, six articles described the use of transmission electronic microscopy (TEM). The most striking finding of TEM is the absence of ruffled borders and clear zones at sites of bone resorption. The article by Satomura et al. is of recent date (2007), whereas the other articles are written before the year 2000 (123, 124, 127, 128). The primary aim of all studies was focused on improved comprehension of the bone properties associated with the disease.

In addition to the stated rare metabolic bone disorders, our literature search also revealed some other rare bone disorders. Three articles described immunohistochemistry in Erdheim-Chester disease (ECD) (129–131). ECD is a disorder characterized by multi-organ infiltration of non-Langherhans' histiocytes in middle-aged patients. In almost all cases of ECD, the long bones are affected, and half of the patients have extra-skeletal involvement (132). ECD is often challenging to diagnose because of a variety of symptoms. Bone pain of the distal extremities are most often present. Histologic examination of skin or bone is required to establish the diagnosis. All the included articles were case reports or case series. It is striking that only the article by Rivera et al. described histomorphometric data (129). All three articles described findings of CD68 positive and S-100 negative histocytes. The other rare metabolic bone disorder found in the PubMed search is Cherubism. Cherubism is characterized by a bilateral swelling of the cheek caused by fibro-osseus lesions of the mandibular or maxillary bone. Age of onset is usually during childhood and sometimes the lesion regress as the child grows (133). Radiological findings shows regions of a "spongy" appearance of the maxilla and mandibular. Three articles about Cherubism described histomorphometric and immunohistochemistry data (134–136). Histopathological examination showed fibrous tissue in the

lesions with multinucleated giant cells. Immunohistochemistry revealed CD68 positive multinucleated giant cells and OPG and RANKL expression.

DISCUSSION

This review provides an overview of the application of advanced techniques in bone biopsies of rare metabolic bone disorders and an outlook on the consideration of advanced techniques. These advanced techniques may help to resolve many gaps in the knowledge of general bone metabolism and the pathophysiology of many different rare metabolic bone disorders. Though, it has to be mentioned that histomorphometric analysis is still the most widely used method. Application of techniques focusing on structural and compositional aspects of bone could provide much more information and make a bone biopsies an even more valuable tool in diagnosis and treatment guidance. In this review, we described a variety of advanced techniques, many of which have rarely been used in clinical practice.

Clinical utility depends on several aspects of the introduction of additional technique. The information obtained from advanced techniques should refine the definition of skeletal status, predict future skeletal complications, or guide treatment in order to be of clinical value. Reference values should be available for informed decision making. The techniques should be (widely) available in diagnostic laboratories. Application of the technique should preserve its properties to allow its use in other diagnostic tools including histology/histomorphometry.

It is important to realize that some of the advanced techniques require special processing, which can hinder the usage of other methods or even make other methods impossible to use. This means that not all techniques are applicable on the same biopsy specimen. Techniques for testing mechanical properties often cause damage to the biopsy specimen by breaking it or causing surface damage. Specimen used for HPLC will be completely destroyed. Standard fixation using formaldehyde 3.5% may cause problems when immune histology assays are performed. TEM may need a different (epoxy) embedding method than acrylate for histology and HPLC may be best performed on untreated samples. Most articles combined one of the advanced analyzing techniques with histomorphometry. Only a limited amount of articles combined more than one advanced analyzing techniques in their research.

Because of the differences in processing, it will be of great importance that doctors provide the biopsy specimen with sufficient comprehensive clinical data and the specified research question to make the right choice for analyzing techniques. Starting at the bed site, a close cooperation of clinicians and researchers is needed to correctly process the biopsy for a maximum gain of information by a variety of different applied methods.

As already mentioned in the introduction of this article, most advanced analysis techniques are currently only used for research purposes only. Although some techniques are available already for many years, reference data does not always exist due to the lack of proper control groups, thus a sufficient comparison to healthy cases is lacking. This is a problem especially in the group of rare metabolic bone diseases, but also in the more common metabolic bone disorders, such as osteoporosis, where treatment-naive age-matched controls are also very limited. Reference data for adults and children have been published for bone mineral density distribution (BMDD) assessed by quantitative backscattered electron imaging (qBEI) (137, 138). Such reference data will ideally come from bone biopsies of a healthy population. Collection of these reference data will be challenging since a bone biopsy is an invasive procedure and cannot routinely be applied to healthy persons. In addition, reference data will differ between adults and children. Certainly, in (young) children ethical justification has to be considered to collect any type of reference data. A feasible option for collecting reference data from a healthy population is the use of residual material after routine surgical intervention.

Different rare metabolic bone diseases will require different methods, since they have different tissue characteristics. Rare metabolic bone disorders can roughly be divided in three groups: disorders with osteoblast dysfunction, disorders with osteoclast dysfunction, and the group of remaining disorders.

Osteoblastic dysfunction is seen in OI and fibrous dysplasia. In this group, a broad spectrum of advanced techniques could be valuable. Techniques to measure structural properties could be effective, especially in the group of OI. Confocal microscopy of polarized light microscopy could give advantageous information about the abnormal collagen fiber organization in OI or arrangement of fibrous tissue in FD. Micro-CT analysis could be of potential interest to explore the microstructural patterns compared to healthy groups. These results could provide critical insight in the interpretation of bone mineral density measurements of a DXA-scan. Also, techniques to measure material composition can potentially be of high scientific value, e.g., FTIR-spectroscopy, Raman-spectroscopy or qBEI. SAXS/WAXS technique could be of beneficial to monitor changes in mineral structure and alignment after treatment of OI patients. In OI, most of these techniques have already been tested and showed promising results. In FD, besides qBEI, none of the other techniques have been applied yet but we believe that this review will steer clinicians toward this direction, which is an effort to gain more information about the composition of the affected bone. Of course, the focal nature of the disease is a complicating factor since the bone specimen needs to be taken from an FD lesion. In OI, the mechanical properties of bone are affected as well. Testing of these properties could help the prediction of fracture risk.

Osteoclast dysfunction is seen in Paget's disorder, *PLS3* X-linked osteoporosis, and osteopetrosis. Although the affected material and mechanical parameters of bone share common characteristics, there are distinct differences. Mineral composition and structure quantifying methods such as qBEI, FTIR-spectroscopy, Raman-spectroscopy, or SAXS/WAXS may be a valuable tools to investigate the diversity of the mineral apposition, distribution, density and structure in each disease. Potentially valuable techniques are micro- and nano-indentation for the measurement of the local

mechanical alteration of the bone material caused by a changed structure and composition. This has hardly been done in any of the disorder with osteoclast dysfunction. We must take into account that also the Paget's disease of bone is a focal disorder and bone samples should be obtained in the regions of interest, which are not always justifiably reachable for biopsy. *PLS3* X-linked osteoporosis is a relatively new discovery with still many uncertainties about the pathophysiological mechanism.

The remaining disorders are fibrodysplasia ossificans progressiva, Loeys-Dietz syndrome, Erdheim-Chester disease, and Cherubism. In all of these disorders, a scarce number of techniques have been tested and a lot of uncertainties about their pathophysiology exist. Analysis of structural properties and material properties will contribute to a higher level of knowledge of the disorders. In FOP, the availability of bone material for research is very low due to the rarity of the disease and the lack of biopsies or surgeries performed. Biopsies or surgery can induce heterotopic bone formation. Postmortem studies are expected to be helpful to optimize a large set of diagnostic methods and gain a deeper understanding of the disorder.

Immunological techniques have been used in the past to explore the possibility of a viral origin of some rare bone disorders. However, no such origin has been found yet. Immunohistology could be in these cases beneficial in identifying critical components of signaling and interaction between osteocytes, osteoblasts, and osteoclasts. Immunohistochemistry could also be helpful in guiding treatment especially with the new developments in biologicals. For instance, detection of RANKL by IHC in a bone biopsy of osteogenesis imperfecta, may indicate denosumab treatment could be beneficial.

The use of advanced techniques is not limited to their application in research and diagnosis; in the future they also hold great promise in the design and evaluation of treatment in patient follow-up. Firstly, techniques providing information on material properties might be able to predict fracture risk, which can be useful to differentiate the patients in need for treatment. Secondly, advanced techniques could be of great advantage to the patient and the clinician, by providing insight in the pathophysiology and giving guidance to treatment strategy. Thirdly, techniques that give insight in mechanisms by which various therapeutic drugs work, at the individual level might help in follow-up. Only a limited amount of papers discussed the use of the advanced techniques to investigate the effect of treatment. This is potentially due to the fact that two biopsies are needed, pre- and post-treatment.

Despite the development of analysis techniques for bone biopsies, innovative less invasive techniques have an obvious benefit and are thus highly sought to complement the diagnosis of metabolic bone disorders now and in the future. The measurement of biochemical bone turnover markers (BMTs) in blood serum or urine has a central role in the diagnostic process and follow-up of rare metabolic bone disorder for many years. New bone turnover markers were developed and measurements became more accurate. However, there is biological variability in BMTs due to age, sex, physical activity, recent fractures, or intercurrent disease (139). Due to these factors, interpretation of BMTs in rare metabolic bone disorders can be difficult. High-resolution peripheral quantitative computed tomography (HR-pQCT) is a new technique to detect microstructural properties at the radius and tibia (140). This non-invasive method uses a very low dose of radiation and is possible to use on a regular basis for follow-up (141, 142). Reference point indentation is a new tool to measure mechanical properties *in vivo*. Microindentations are made by a hand-held device. Although no bone biopsy is needed for this technique, patients still need local anesthetic because the indentations are painful due to penetration of the skin and periosteum (143). Both techniques are mostly used for the follow-up of more prevalent diseases such as osteoporosis; whether they are useful in the diagnosis of rare metabolic bone disorders needs further investigation.

There were several limitations in this review. The described metabolic bone diseases are rare, which could explain the low number of published articles on this topic. It could be possible that some articles were missed because the advanced analyzing techniques were not described in the title or abstract. We only used the PubMed database for data collection. The majority of the papers included descriptions of histomorphometric results. The number of patients enrolled in most of the studies was very low, especially in the papers describing techniques other than histomorphometry. Even when the number of patients in a study was sufficient, most of the time only a small number of patients underwent a bone biopsy. Also, the quality of the articles differed. One-third of the included studies consisted of case reports, with sometimes limited information. In FOP, *PLS3* X-linked osteoporosis, LD, and ECD the majority of the articles consisted of case reports. Also, 10% of the remaining articles were of poor quality (23). However, we decided to not exclude any of these articles. Given the rarity of the disorders, also these articles can help in providing an overview of performed research on bone biopsies with advanced techniques. Description of the used techniques and processing of the biopsy specimens for analysis were not always adequately.

Striking is the lack of articles where advanced techniques have been applied to study rare metabolic bone disorders with a high bone mineral density. Only a few articles describing osteopetrosis were found. Van Buchem disease was not covered in our search, an extra search for this disorder revealed no articles. Application of advanced techniques to disorders with a high bone mineral density could be valuable to accurately describe the full range of bone mineral disorders.

Notwithstanding the relatively limited amount of publications and patients, this review offers valuable insights into possible applications of various analyzing techniques.

Larger studies must establish a reliable database. Using this database, clinicians would be able to assess the need for treatment for the underlying disease as well as monitor the success of the therapy. MicroCT as well as spectroscopy and scanning electron microscopy (e.g., qBEI) are of great importance, as they link research and clinical routine. A further advantage of these techniques is the sample volume saving preparational approach. A combination of the modern techniques and

classical pathological histology and histomorphometry has the potential to lead to an improved new, individualized, patient-centered therapy.

This review shows the possibilities for a broader use of bone material obtained from biopsies or residual material after surgery. These advanced techniques could provide important additional information to the new imaging techniques developed in recent years. The use of advanced analyses techniques can provide a better understanding of the pathophysiology and pave the way for new treatment options ultimately aiming optimal patient recovery and prevention of skeletal complications in the rare bone diseases.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

Study design: ST, EE, BB, and NB. Study conduct: ST, EE, FS, BB, and NB. Data collection: ST, EE, FS, BB, and NB. Data analysis: ST, EE, FS, BB, and NB. Data interpretation: ST, EE, FS, DM, BB, and NB. Drafting manuscript: ST, EE, FS, BB, and NB. Revising manuscript content: ST, EE, FS, DM, BB, and NB. Approving final version of manuscript: ST, EE, FS, DM, BB, and NB take responsibility for the integrity of the data analysis. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2020.00399/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

PERSPECTIVE
published: 11 August 2020
doi: 10.3389/fendo.2020.00481

Collaboration Around Rare Bone Diseases Leads to the Unique Organizational Incentive of the Amsterdam Bone Center

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OPEN ACCESS

Edited by:
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Reviewed by:
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Monica De Mattei, University of Ferrara, Italy

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Specialty section:
This article was submitted to Bone Research, a section of the journal Frontiers in Endocrinology

Received: *23 April 2020*
Accepted: *17 June 2020*
Published: *11 August 2020*

Citation:
Eekhoff EMW, Micha D, Forouzanfar T, de Vries TJ, Netelenbos JC, Klein-Nulend J, van Loon JJWA, Lubbers WD, Schwarte L, Schober P, Raijmakers PGHM, Teunissen BP, de Graaf P, Lammertsma AA, Yaqub MM, Botman E, Treurniet S, Smilde BJ, Bökenkamp A, Boonstra A, Kamp O, Nieuwenhuijzen JA, Visser MC, Baayen HJC, Dahele M, Eeckhout GAM, Goderie TPM, Smits C, Gilijamse M, Karagozoglu KH, van de Valk P, Dickhoff C, Moll AC, Verbraak FFD, Curro-Tafili KKR, Ghyczy EAE, Rustemeyer T, Saeed P, Maugeri A, Pals G, Ridwan-Pramana A, Pekel E, Schoenmaker T, Lems W, Winters HAH, Botman M, Giannakópoulos GF, Koolwijk P, Janssen JJWM, Kloen P, Bravenboer N, Smit JM and Helder MN (2020) Collaboration Around Rare Bone Diseases Leads to the Unique Organizational Incentive of the Amsterdam Bone Center. Front. Endocrinol. 11:481. doi: 10.3389/fendo.2020.00481

In the field of rare bone diseases in particular, a broad care team of specialists embedded in multidisciplinary clinical and research environment is essential to generate new therapeutic solutions and approaches to care. Collaboration among clinical and research departments within a University Medical Center is often difficult to establish, and may be hindered by competition and non-equivalent cooperation inherent in a hierarchical structure. Here we describe the "collaborative organizational model" of the Amsterdam Bone Center (ABC), which emerged from and benefited the rare bone disease team. This team is often confronted with pathologically complex and under-investigated diseases. We describe the benefits of this model that still guarantees the autonomy of each team member, but combines and focuses our collective expertise on a clear shared goal, enabling us to capture synergistic and innovative opportunities for the patient, while avoiding self-interest and possible harmful competition.

Keywords: rare bone diseases, amsterdam bone center (ABC), collaborative organization, non-hierarchical, research, clinical

INTRODUCTION

Rare bone diseases (RBD) have, until recently, been a largely neglected area in healthcare. Their rarity and heterogeneity have unfortunately hindered their exploration at both clinical and scientific levels, even though more than 500 of the ∼7,000 rare diseases are bone disorders (1, 2). The estimated incidence of RBD can vary, from around 15.7/100000 births for skeletal dysplasias (3) which are the most common, to ultra-rare disorders of which only a few patients exist in the world, such as spondylo-ocular syndrome (4). However, in the last decade, the urgency to study and treat RBD has been boosted by the greater appreciation of the socioeconomic consequences associated with their chronic nature and severity, and by the wider availability of genetic diagnostics, patient advocacy, and the development of new pharmaceutical treatment options.

The focus of the Amsterdam UMC initially included the rare bone diseases (RBD) fibrodysplasia ossificans progressiva, osteogenesis imperfecta, fibrous dysplasia and hereditary osteoporosis, but encountered several obstacles. RBD are often extremely challenging to treat; clinical decisions are hindered by their complexity and lack of knowledge about their underlying pathology. Because standard treatment protocols do not exist for RBD, and off-label medications are typically required, a broad team of medical specialists is needed to design the right treatment approach for the individual patients. Ideally, because these diseases are so rare, such a team would be embedded in a multidisciplinary academic setting to facilitate urgently needed clinical and preclinical research. This provides access to research-oriented colleagues who have knowledge and affinity with relevant RBD and increases the likelihood of new insights and scientific breakthroughs to ultimate benefit the patients. Critical to maximizing progress is full collaboration between many different disciplines in a structure, where not only clinicians, but also clinical and basic researchers can efficiently interact across specialities and facilities. Such team structures and broad collaborative networks can be challenging to set up in academic centers due to other interests, competition, or non-equivalent cooperation (5).

Collaborative Organizational Model

Different opinions exist about the ideal organizational structure to facilitate successful cooperation of professionals from a wide variety of disciplines. Nonetheless, in most medical and research organizations, the traditional hierarchical pyramid still dominates. Such rigidly structured organizations that are managed "top-down" often fail to provide an optimum environment for self-motivation, creativity, engagement, and empathy, all important requirements for effective collaboration amongst colleagues (6–10).

An alternative approach supports a less rigid hierarchy and the promotion of organic development of collaboration between colleagues in a culture of equality (3–7). Fundamental to this is the recognition of the specific and complementary skills of each individual team member. There is increasing support of the idea that teams containing like-minded people with mutual and aligned interests can provide the basis for transparent, fair, and fruitful collaboration. Organizational models like this can achieve shared goals by stimulating an engaged, unforced and valued workforce mentality, in which individuality and freedom to show initiative is safeguarded (6–10). In such a model the aim is not the integration of all departments but an efficient collaboration between relevant partners driven by their balanced skills that are required to solve specific clinical or research questions. The overall goal is to improve patient care and to stimulate innovative research. The process is further enhanced by the critical input of patients in care and research. This kind of model is referred to in the literature as "collaborative organization," and is considered an effective means of advancing both efficiency and innovation (6–10).

AMSTERDAM BONE CENTER

The Amsterdam Bone Center (ABC) was formed in late 2016, as a successful example of such "collaborative organizational model." The ABC was an initiative of various clinical disciplines and researchers who wanted to pool their specialized skills, knowledge and experience across boundaries and their day to day scope, with the common goal of achieving new approaches to the diagnosis, care, and effective treatment of patients with RBD. Most of RBD treatment is still based on generic medical protocols which provide symptomatic relief, but effective future therapies that result in the recovery of the affected tissue will need detailed understanding of the underlying disease pathology, which is a challenging task. As a consequence, the ABC was initially focussed on RBD. Although the number of patients affected by some diseases was very limited, the level of required adapted complex care was very high. This resulted in extensive networking with many clinical departments such as plastic surgery, maxillofacial surgery, orthopedic surgery, thoracic surgery, traumatology, anaesthesiology, rehabilitation, urology, ear nose and throat surgery, audiology, ophthalmology, clinical genetics, rehabilitation, psychiatry, physiotherapy, social work, dietetics, gypsum master, cardiology, lung disease, nuclear medicine, radiology, neurology, neurosurgery, dermatology, radiotherapy, gastroenterology, endocrinology, pediatrics, rheumatology, and dentistry. In addition, the patient organizations have been actively involved. The multidisciplinary collaboration has been based on equality.

The ABC subsequently developed as a flat organization, where mutual interest, exchange of knowledge, and innovation have led to a vivid open collaboration between clinicians and researchers.

The ABC provides a bridge between clinicians and research laboratories whose partners are embedded in the Amsterdam Movement Sciences research institute, the latter of which embraces the targeted laboratories specializing in multifaceted aspects of research on bone tissue, dentition and the surrounding tissues. In this way, it connects expert groups focussed on osteocytes (11), osteoblasts (12), osteoclasts (13), bone matrix formation (14), and angiogenesis (15), facilitating the study of bone differentiation and regeneration. With the aid of appropriate cell collection from RBD and control tissues, complex processes can be studied and interpreted in the physiological and pathological context. "Meet the expert" RBD sessions and annual RBD meetings help to keep the patients informed about the current research and progress. ABC

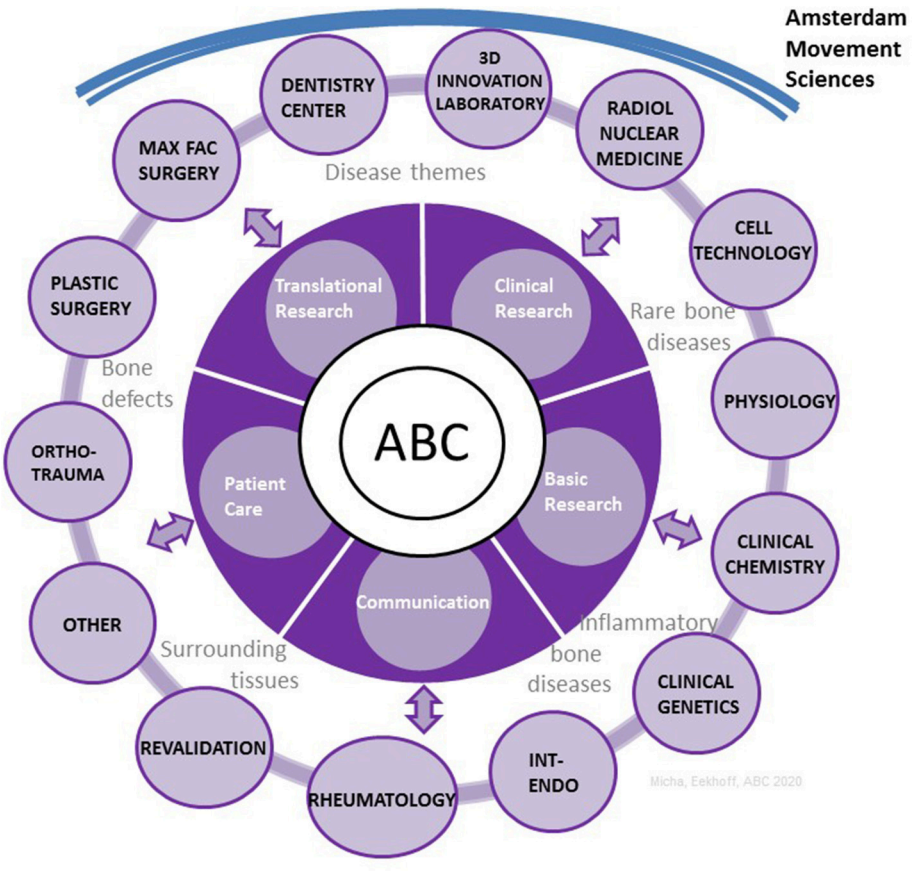

FIGURE 1 | The organizational structure of the ABC with the main themes and partnerships. ABC, Amsterdam Bone Center; Max Fac, maxillo facial; Ortho-trauma, orthopedic surgery, traumatology; Int-endo, internal medicine, endocrinology; Radiol, radiology.

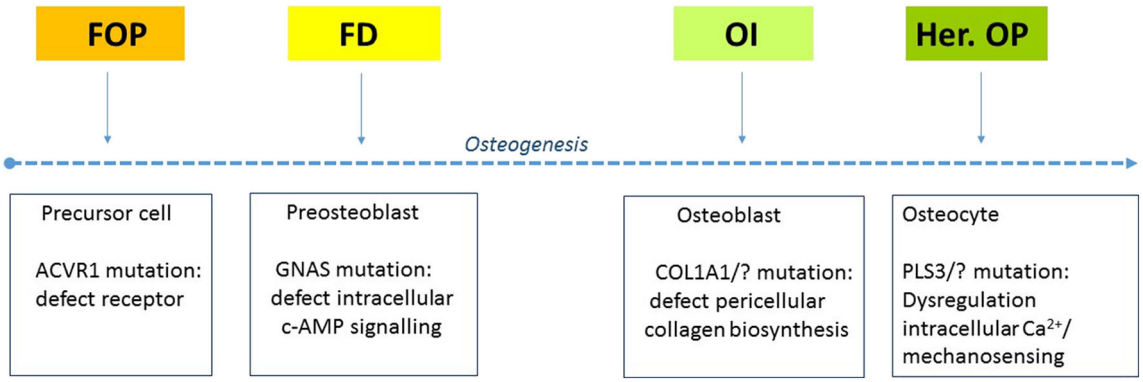

FIGURE 2 | Four genetic rare bone diseases that all dysregulate osteogenesis but in a different phase and on a different process in the cell. The arrow indicates the direction of the stem cell/fibroblast toward the development of osteocytes. FOP, fibrodysplasia ossificans progressiva; FD, fibrous dysplasia; OI, osteogenesis imperfecta; Her. OP, hereditary osteoporosis; ACVR1, activin A receptor 1; c-AMP, Cyclic adenosine monophosphate; COL, collagen; PLS, plastin; Ca, calcium.

education activities also extend to academic training at the bachelor, master and doctorate level by which enthusiasm for rare bone diseases is promoted in talented young professionals.

MANAGEMENT OF THE ABC

In place of the more typical hierarchical model in which all control is centralized to a Director, the ABC operates with a facilitating steering team, with one member in rotation functioning as the ABC chairman. The chairman conveys the consensus goals, ambitions, and decisions of the team. The different cultures and perspectives of the various collaborating departments are reflected in a steering team of four coordinators from the task force group, consisting of 2 preclinical theme leaders (from the Laboratory for Bone Metabolism of the Department of Clinical Chemistry and Cell Technology Laboratory of the Department of Oral and Maxillofacial Surgery) and 2 clinical theme leaders (from the Department of Internal Medicine section Endocrinology and the Department of Plastic Surgery). Steering team members are elected to their role for 2 years, based on their proven commitment to the ABC and their activities in promoting its interests.

In addition to the leading steering team, there is a task force which includes representatives from clinical and pre-clinical groups. These representatives are responsible for promoting their key themes [e.g., key themes are presently RBD, inflammatory bone diseases and bone oncology, complex fractures, and complex surrounding tissue injuries (**Figure 1**)]. The task force comes together in brainstorm sessions to translate critical clinical questions into structured preclinical research lines, and move preclinical findings into the clinical environment. In this organizational model, the coordinating task force is not focused on safeguarding its own structure, but on leveraging its diverse expertise to drive adoption of new ideas across the ABC members, identify scientific gaps, support the finding of solutions, enhance ABC connectivity and crosstalk between themes where possible, give direction to future common goals, support optimal clinical care for patients, and provide high quality education and research.

An annual symposium will ensure that all groups working in bone research and clinics in the ABC can benefit and easily collaborate in an ideal setup for research and care. Yearly goals are suggested and proposed to the ABC community in these symposia, and subsequently set and evaluated by the task force based on extensive feedback. The ultimate goal is to become further embedded in a larger international network of centers for bone research in general, and RBD in particular, in order to meaningfully help patients and offer innovative diagnostics, to develop treatment options and recovery solutions for RBD and related bone diseases. The task force also monitors whether the activities of the leading steering team align with the goals of the wider ABC community. The obvious advantage of this lateral ("flat") organization is that groups retain the freedom to pursue their own research choices, but they are encouraged to reach the best joint benefit.

FOCUS OF RBD WITHIN THE ABC

The focus of the RBD theme of ABC was initially placed on four RBD, including fibrodysplasia ossificans progressiva (FOP) (12, 13, 16–19), osteogenesis imperfecta (OI) (20, 21), fibrous dysplasia (FD) with an emphasis on skull (22), and hereditary osteoporosis (her. OP) (23). This, repertoire was strategically composed based on the various clinical and research expertise available and on the possibility to match underlying etiology and clinical questions. A schematic overview of the differences and common ground of these RBD is given in **Figure 2**. Based on this, it is clear that these diseases can serve as a paradigm for other RBD sharing a similar pathology, but also provide insight into general bone pathology.

ACHIEVEMENTS ON RBD WITHIN THE ABC

A standardized approach to patient care of the four RBD is developed with the relevant clinical disciplines and patient organizations to create a patient-centered design. This has led

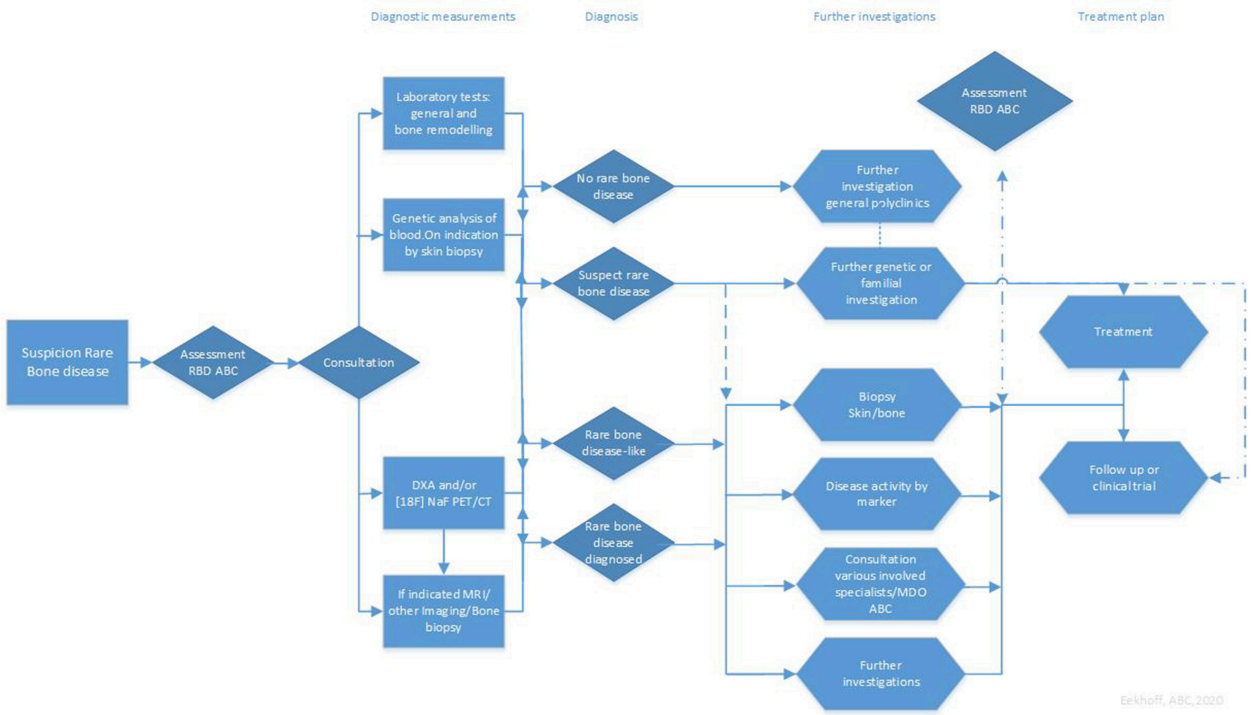

FIGURE 3 | Patient Care Rare Bone Disease ABC, Amsterdam UMC. FOP, fibrodysplasia ossificans progressiva; FD, fibrous dysplasia; OI, osteogenesis imperfecta; Her-OP, hereditary osteoporosis; ABC, Amsterdam bone Center; DXA, Dual X-ray absorptiometry; [18F] NaF PET/CT, ^{18}F-Sodium Fluoride positron emission tomograph/-computed tomography; MRI, Magnetic resonance imaging; MDO, multidisciplinary consultation.

to the implementation of a standardized route for care; its integration in numerous specialities is designed to thoroughly address all pathological aspects of each RBD (**Figure 3**). As a spin-off of the ABC structure, the RBD team has become an international referral center for FOP and it coordinates international studies on FOP, OI and hereditary OP, and FD.

Several preclinical research models have been developed to study the various RBD, including the culture of subdermal (12) and periodontal ligament fibroblasts (13) which can be converted to cartilage and bone-forming cells, or can be drivers for osteoclast formation (13, 24); this provides unique insight in rare bone diseases that primarily focuses on additional bone formation rather than affected bone degradation. Many signaling pathways for cartilage and osteogenic differentiation are reflected in these models, which facilitates their study in easily obtainable patient tissue. This collaboration has yielded the discovery of newly discovered genes for these RBD (20, 23); the investigation of their mechanism can help to shed light in possible therapeutic implications. The collaborative efforts have also led to innovative diagnostics, one example of which is a new modality for imaging of active heterotopic bone lesions in FOP patients with ^{18}F PET/CT (16–19). Other advances include the development of new clinical trials with existing and new medications, translational projects on pharmacological therapy for RBD, and the development of new technology to quantify osteoclast activity *ex vivo*. The development of the RBD theme within the ABC structure has led to an increasing number of pre- and clinical scholarships, awarded from Amsterdam UMC AMS as well as other international universities, patient associations, and national and European funding organizations, in collaboration with pharmaceutical/industrial companies. This supports a rapidly developing academic trajectory resulting in many Ph.D. projects and dissertations.

FUTURE PLANS OF THE ABC

Regarding the future treatment of RBD, Regenerative Medicine (RM) is one of the main research priorities of the ABC. This specific focus within ABC aims meaningful repair/regeneration by exploiting the plasticity of the body's own cells. This requires extensive knowledge of the pathological mechanism of the disease extending from molecular interactions at the cellular level, to the influence of and inter-relationship with the surrounding tissues and other systemic factors. The aforementioned preclinical RBD models and findings can potentially be integrated with RM strategies in order to achieve synergy in disease control and tissue regeneration. This specific expertise within ABC extends to more prevalent bone disorders which are genetically less well-defined but which nonetheless may also benefit from therapeutic developments on RBD; these may include multifactorial osteoporosis, and immune-related bone

diseases. The ultimate goal is to establish a regeneration center based on the development of new pathophysiological models for the realization of individualized treatment and prevention. In addition, ongoing future plans include the development of orthoplastic centers and the expansion of our network to more national, European and international collaborators outside the Amsterdam UMC.

In conclusion, this article we have outlined the establishment and development of the Amsterdam Bone Center, where "collaborative organization" encourages the cooperation of all relevant clinic and research teams. Specifically, we have successfully established a patient-centered, multidisciplinary focus on RBD including the development of targeted innovative diagnostics, clinical and research protocols and studies. Recognition of the different cultures and perspectives of the departments represented in the ABC, shared collaborative leadership, and a diverse and well-functioning task force is critical to maintaining a balanced and successful collaboration that advances science and innovation, and improves patient care.

Knowledge of this model may be useful to other organizations aiming to establish or enhance the growth of clinical-academic collaboration.

DATA AVAILABILITY STATEMENT

All datasets presented in this study are included in the article/supplementary material.

AUTHOR CONTRIBUTIONS

This article on a unique collaborative, interdisciplinary concept in medical research arising from the rare bone diseases pillar of the Amsterdam Bone Centre was initiated by EE with contributions to all crude versions from DM, TF, TV, JCN, JK-N, JL, PKl, NB, JS, and MH. The subfinal version was prepared and sanctioned by this core group of authors. All remaining authors WDL, LS, PS, PR, BT, PG, AL, MY, EB, ST, BS, ABö, ABoo, OK, JAN, MV, HB, MD, GE, TG, CS, MG, KK, PV, CD, ACM, FV, KC-T, EG, TR, PS, AM, GP, AR-P, EP, TS, WL, HW, MB, GG, PKo, and JJ are active ABC members and have contributed for many years on rare bone diseases and to the success of this unique collaborative initiative. All these authors have had the opportunity to further improve the manuscript, these comments were incorporated by EE and DM in the submitted version.

ACKNOWLEDGMENTS

We are grateful for the good collaboration with the department of rehabilitation (Louise Sabelis, M.D. Ph.D.), the department of gastroenterology (M.A.J.M. Jacobs M.D., Ph.D.), in addition to all other collaborative partners of the Amsterdam UMC, AMS, ACTA as national and international partners, who are large in number and hopefully can participate in a next article. Our special gratefulness goes to the patients and patient organizations of all RBDs for their collaboration and important critical participation.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling Editor declared a past co-authorship with one of the authors WL.

frontiers in Endocrinology

SYSTEMATIC REVIEW
published: 21 August 2020
doi: 10.3389/fendo.2020.00570

When Limb Surgery Has Become the Only Life-Saving Therapy in FOP: A Case Report and Systematic Review of the Literature

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OPEN ACCESS

Edited by:
Gudrun Stenbeck, Brunel University London, United Kingdom

Reviewed by:
Mengning Yan, Tong University, China
Caroline Michot, Assistance Publique Hopitaux de Paris, France

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Specialty section:
This article was submitted to Bone Research, a section of the journal Frontiers in Endocrinology

Received: *22 February 2020*
Accepted: *13 July 2020*
Published: *21 August 2020*

Citation:
Botman E, Treurniet S, Lubbers WD, Schwarte LA, Schober PR, Sabelis L, Peters EJG, van Schie A, de Vries R, Grunwald Z, Smilde BJ, Nieuwenhuijzen JA, Visser M, Micha D, Bravenboer N, Coen Netelenbos J, Teunissen BP, de Graaf P, Raijmakers PGHM, Smit JM and Eekhoff EMW (2020) When Limb Surgery Has Become the Only Life-Saving Therapy in FOP: A Case Report and Systematic Review of the Literature. Front. Endocrinol. 11:570. doi: 10.3389/fendo.2020.00570

Fibrodysplasia ossificans progressiva (FOP) is a rare disease in which heterotopic ossification (HO) is formed in muscles, tendons and ligaments. Traumatic events, including surgery, are discouraged as this is known to trigger a flare-up with risk of subsequent HO. Anesthetic management for patients with FOP is challenging. Cervical spine fusion, ankylosis of the temporomandibular joints, thoracic insufficiency syndrome, restrictive chest wall disease, and sensitivity to oral trauma complicate airway management and anesthesia and pose life-threatening risks. We report a patient with FOP suffering from life-threatening antibiotic resistant bacterial infected ulcers of the right lower leg and foot. The anesthetic, surgical and postoperative challenges and considerations are discussed. In addition, the literature on limb surgeries of FOP patients is systemically reviewed. The 44 year-old female patient was scheduled for a through-knee amputation. Airway and pulmonary evaluation elicited severe abnormalities, rendering standard general anesthesia a rather complication-prone approach in this patient. Thus, regional anesthesia, supplemented with intravenous analgosedation and N_2O-inhalation were performed in this case. The surgery itself was securely planned to avoid any unnecessary tissue damage. Postoperatively the patient was closely monitored for FOP activity by ultrasound and [^{18}F]PET/CT-scan. One year after surgery, a non-significant amount of HO had formed at the operated site. The

systematic review revealed seventeen articles in which thirty-two limb surgeries in FOP patients were described. HO reoccurrence was described in 90% of the cases. Clinical improvement due to improved mobility of the operated joint was noted in 16% of the cases. It should be noted, though, that follow-up time was limited and no or inadequate imaging modalities were used to follow-up in the majority of these cases. To conclude, if medically urgent, limb surgery in FOP is possible even when general anesthesia is not preferred. The procedure should be well-planned, alternative techniques or procedures should be tested prior to surgery and special attention should be paid to the correct positioning of the patient. According to the literature recurrent HO should be expected after surgery of a limb, even though it was limited in the case described.

Keywords: fibrodysplasia ossificans progressiva (FOP), surgery, heterotopic ossification (HO), [^{18}F]NaF PET/CT, ACVR1 gene mutation

INTRODUCTION

Fibrodysplasia Ossificans Progressiva (FOP) is an extremely rare disease with heterotopic ossification (HO) occurring in muscles, tendons and ligaments (1–3). HO usually leads to immobility of the affected joint, resulting in wheelchair-dependence at an early age (4). A flare-up often precedes the formation of this ectopic bone (1–4). A flare-up can occur spontaneously, but can also be triggered by a trauma (2, 4). Because trauma causes flare-ups and therefore aggravates the disease, patients are instructed to be careful (e.g., do not engage in contact sports), to refuse intramuscular injections and to prevent any kind of surgery (5). In some cases, though, surgery is inevitable when a medical condition is life-threatening. Surgical procedures can be difficult as extensive HO throughout the body has led to ankylosis of joints and has changed the patient's anatomy, making proper positioning of the patient difficult (4). Also, the anesthetic procedures are complex. The jaw of the patient is often ankylosed and pulmonary function can be severely restricted. As a result, standard anesthesia techniques can often not be applied to FOP patients (4, 6, 7). We report a patient with FOP who underwent a through-knee amputation due to a life-threatening antibiotics resistant infection. The surgical, anesthetic and postoperative considerations and challenges will be discussed. In addition, a systematic review on surgical procedures of the limbs and the course of the postoperative disease activity in FOP patients undergoing limb surgery is described.

CASE REPORT

The patient, a 44 year old woman at the time of surgery, is known with the classical mutation (p.Arg206His) of FOP. Due to widespread HO throughout the body, she has been wheelchair bound and ADL (activities of daily life) dependent for over 25 years. Her joints are almost completely ankylosed except for ankles, toes, wrists and fingers i.e., cumulative analog joint involvement scale (CAJIS) score of 24 out of 30 (8). A recent pulmonary function tests showed a severely reduced Forced Expiratory volume in one second (FEV1) (0.6L, 25% of predicted) and forced vital capacity (FVC) (0.6L, 22% of predicted) with a normal Tiffeneau index (94%). This suggests a severely restrictive pulmonary function, compatible with marked chest wall rigidity (9). In 2016 the patient recovered without sequelae from a cerebrovascular accident (CVA), for which she is on chronic anticoagulation therapy (thrombocyte aggregation inhibitor). In addition, her fifth digit of the right foot was amputated in 2001 because of an incurable osteomyelitis. This procedure has previously been described (10). The patient has been treated in our center since 2016 for recurrent skin infections of the right lower leg and foot as a result of progressive chronic ulcers. A neuropathic pain syndrome, clinically confirmed by the neurologist, was thought to be the cause of allodynia in the right lower leg. Repeated pressure on the skin while sitting in her wheelchair contributed to formation of these ulcers. Initially, the ulcer at the foot led to recurrent skin and soft tissue infections of the right lower leg, with good response to antimicrobial treatment. Wound care led to improvement of the ulcer, but edema complicated healing. Intensive wound care, application of tailored wound dressing, and systemic treatment with antimicrobial agents with high bio-availability, targeted at cultured bacteria found in biopsies of the wound surface, resulted only in temporary improvements of wound healing. Custom-made shoes were manufactured to locally decrease pressure on the (pre)ulcer sites. With especially the combination of rigidity of the body and the wheelchair which can be adjusted into different positions turned out to be challenging in the use of these shoes. Initially it led to an improvement of the ulcers. But, unfortunately, after years of treatment the ulcers and infections progressed to chronic osteomyelitis with multidrug resistant microorganisms (including *Pseudomonas aeruginosa*) in visible and palpable bone in the wound surface (**Figure 1**). We expected her to develop a life-threatening sepsis in the near future. In a multidisciplinary FOP team, consisting of an endocrinologist, infectious disease specialist, pulmonologist, surgeon, anesthesiologists and rehabilitation specialist, the case was thoroughly discussed. The team concluded that amputation of the infected part of the lower leg was the only life-saving option. The patient was well-informed about the risks of the anesthesia, surgery and the risk of FOP activity after surgery and consented for a surgical procedure.

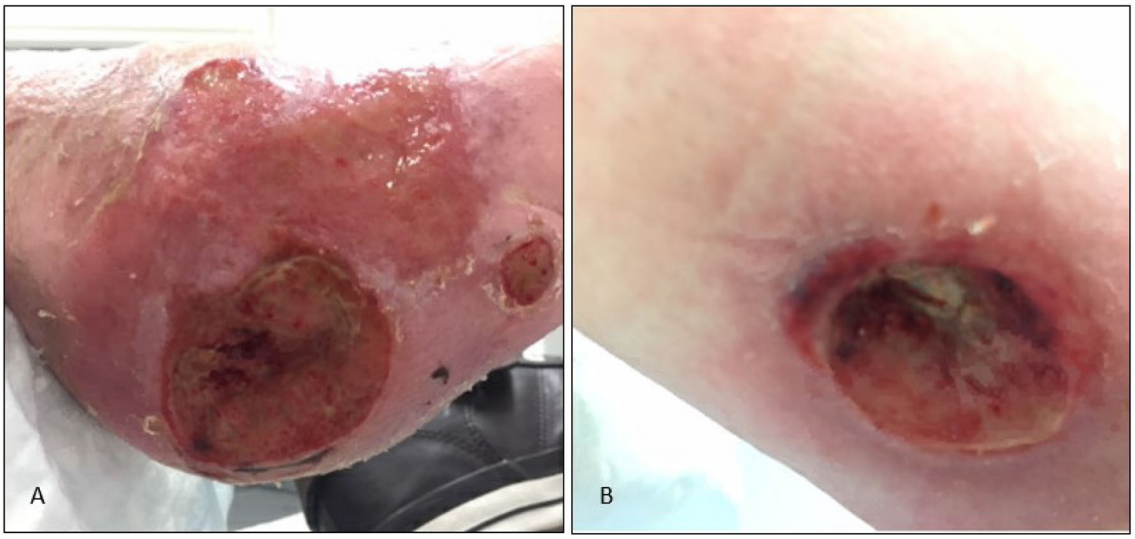

FIGURE 1 | FOP patient with multiple incurable ulcers at the right lower extremity **(A)**. Ulcer located at the right calcaneus. Despite Intensive wound care, custom-made orthopedic shoes and targeted systemic and topical antimicrobial treatment, surgical intervention was unavoidable. Due to an ulcer on the calf **(B)** and proximal from the knee, a through-knee amputation was thought to be most favorable for adequate healing and to minimize tissue damage.

Anesthetic Management

Anesthesiologists of our FOP expertise center in Amsterdam managed the anesthetic care. General anesthesia was intentionally avoided as airway management appeared rather challenging in this patient with severely impaired mouth opening (<2 mm). Moreover, mechanical ventilation was expected to temporarily cause a decline in pulmonary function, potentially leading to a ventilation-perfusion mismatch or barotrauma, and rendering weaning from mechanical ventilation impossible. Regional anesthesia was therefore selected as the preferred technique. Two peripheral nerve block catheters were placed at the femoral and sciatic nerve on the pre-operative day (**Figure 2A**). Damage to surrounding tissues was not completely avoidable, but kept to a minimum by using ultrasound guidance. The femoral nerve was easily identified in the femoral triangle. The identification of the sciatic nerve with ultrasound, however, was challenging because of an altered anatomy caused by HO (e.g., altered landmarks and aberrant course of the nerve). Eventually the sciatic nerve was identified and approached at the subgluteal level. To prevent inflammation at those sites, 40 mg methylprednisolone was administered over the two nerve block catheters. Pre-operatively, 12 ml ropivacaine 0.375% was injected in the catheters. The ropivacaine spread around the nerves as confirmed by sonography. Nerve block effectiveness was confirmed using cold discrimination tests prior to commencement of surgery. Surgery was initiated and anesthesia was judged adequate for the initial part of the procedure. The patient remained conscious and responsive throughout the procedure, but started to report some discomfort once surgery reached deeper tissue planes. The regional anesthesia was therefore supplemented by intravenous bolus titration of midazolam and s-ketamine, and inhalation of a mix of 50% N_2O and 50% O_2 via face mask. Herein, midazolam served as light anxiolytic and amnestic sedative, and to prevent psychomimetic side effects of s-ketamine. S-ketamine served as systemic analgesic without cardiovascular and respiratory depression. The N_2O-inhalation induced additional analgesia, supplementing the analgesic effects of the regional anesthesia. Together, this ensured adequate analgesia and patient comfort for the remainder of the surgery, with a spontaneously breathing, responsive patient. Postoperatively, the patient did not recall having experienced any pain during the procedure. The nerve catheters were used postoperatively to administer continuous bupivacaine 0.125% for pain control, enabling to avoid the use of systemic opioids. The catheters were removed 8 days postoperatively when oral medication was sufficient to control pain.

Surgical Management

Due to therapy-resistant infected ulcers 10 cm below the knee and more distally, it was decided to perform an amputation through the knee after an extensive discussion with our team and the patient. Thirty minutes prior to surgery, 30 mg of prednisolone was administered intravenously to prevent flare-ups. The surgery was performed by a surgeon affiliated to the FOP Expert Center of Amsterdam UMC. The positioning of the patient was challenging, due to complete immobility of the major joints (**Figure 2B**). Time was taken to carefully position the patient and soft pads were used to minimize pressure on the soft tissues. Once positioned, the patient was put in adjusted supine position and the knee joint was marked. A tourniquet was not used to avoid tissue compression that may induce a flare-up. As post-operative soft tissue healing complications were expected, the skin flap and gastrocnemius muscle transposition were designed to opposite each other in order to minimize the chance of deep infection and fistula formation. While most

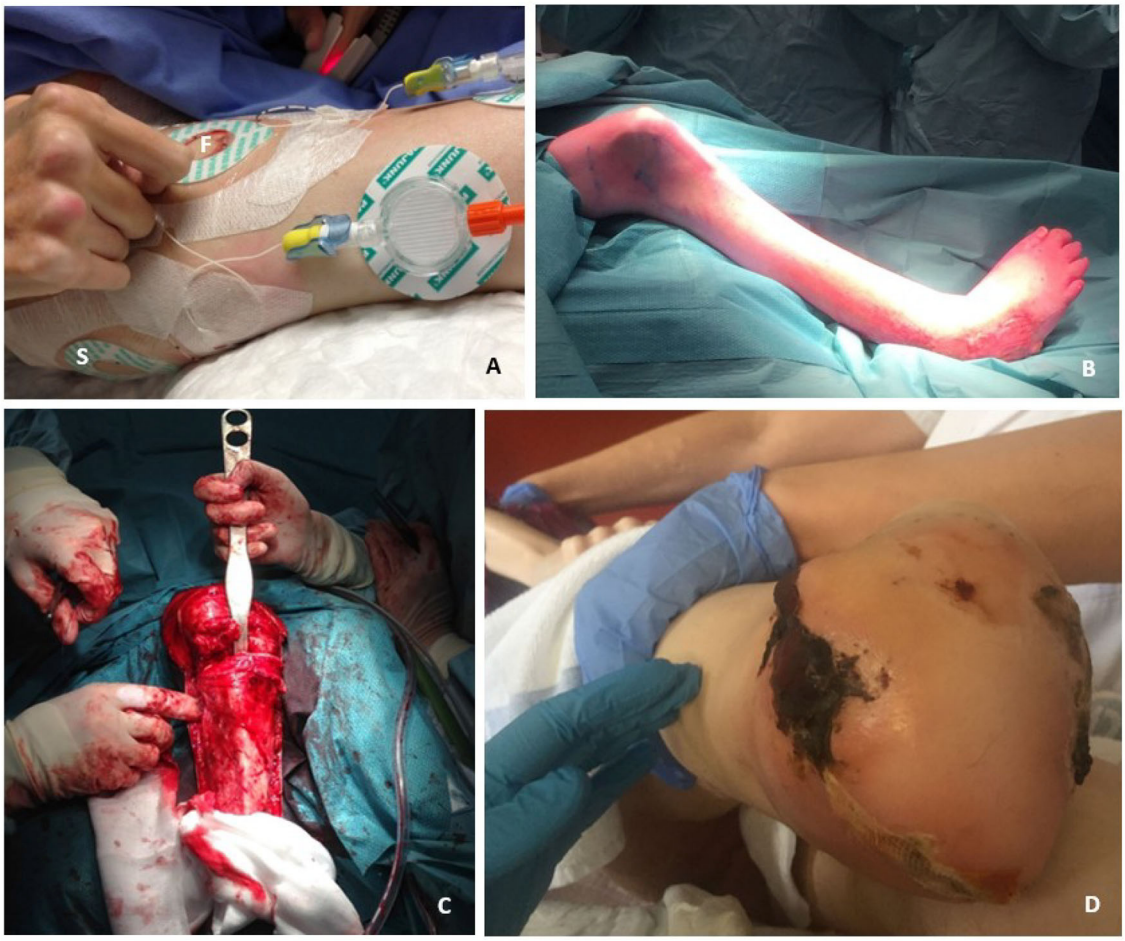

FIGURE 2 | Anesthetic and surgical management of a through-knee amputation in an FOP patient. **(A)** Two nerve block catheters, i.e., the femoral (F) and the sciatic nerve block catheter (S), which were both already placed and tested at the preoperative day. The picture shows the antero-lateral aspect of the patient's right leg. The femoral nerve block catheter is positioned at the ventral aspect of the leg, whereas the sciatic nerve catheter is positioned at the lateral aspect of the leg. **(B)** The patient was carefully positioned on the theater table to prevent any tissue damage that might cause FOP disease activity. The positioning was challenging due to ankylosis in the hips and knees, resulting in the position shown in the picture. **(C)** Surgical procedure was performed carefully to minimize tissue damage that might cause a flare-up. **(D)** The skin flap and gastrocnemius muscle transposition were designed to opposite each other to prevent overlapping scars and minimize the chance of fistula formation due to expected wound healing issues. Lateral of the stump an area of necrosis developed, but healed with supportive care.

ligaments and the capsule of the knee joint were ossified, no abnormalities were observed in the knee joint itself (**Figure 2C**). As the patella was fused with the distal femur, it was left *in situ* to minimize tissue damage. Furthermore, the popliteal artery and nerve were difficult to identify initially, as the patient's leg was in a fixed position. This posed a potential risk in case of laceration of the vessel, but could be avoided by diligence. The gastrocnemius muscles were transposed forward and fixed near the patella region to cover the bone, to provide a vascularized bed and to protect underlying tissues in case of a future prosthesis (**Figure 2D**). The anterior skin of the proximal lower leg was fixed to the posterior skin at the level of the knee. Postoperatively, extra padding was applied between the lower extremities, to avoid pressure from the left knee on the wound of the stump. The patient developed partial skin necrosis laterally of the stump (**Figure 2D**), but healed with supportive care.

Postoperative Management

The patient's disease activity was closely monitored with ultrasound imaging and [^{18}F]NaF PET/CT (sodium fluoride positron emission tomography and computed tomography). Ultrasound imaging was obtained daily to evaluate oedema at the surgical site and at the site of the anesthetic catheters. From day one until day fourteen, mild oedema was seen laterally from the stump by ultrasound. This oedema, however, did not progress over time. It was interpreted as a normal postoperative tissue reaction. To evaluate osteoblastic activity, an [^{18}F]NaF PET/CT-scan was obtained 14 days after surgery, showing only a mild increased [^{18}F]NaF-uptake (Standardized uptake value (SUV_{max}): 6.4) at the base of the distal femur. Because the postoperative [^{18}F]NaF uptake was only slightly elevated (11), it was decided not to administer extra prednisolone. A follow-up [^{18}F]NaF PET/CT-scan was obtained 8 weeks after surgery,

revealing minimal HO formation (4 cc) at the base of the femur (**Figure 3**). Another follow-up scan obtained 12 months after surgery, revealed no further progression of HO evaluated by CT. Interestingly, the patient's disease activity as evaluated by [^{18}F]NaF-activity on PET, now showed an increased [^{18}F]NaF-uptake at multiple sites of HO throughout the body, whereas in the previous 4 years there has not been any [^{18}F]NaF activity nor a volumetric increase of HO as evaluated by CT. The quiescence of disease was in a period of progressive infectious ulcers and under continuous antibiotic therapy before surgery. After 14 days, the patient was transferred to a rehabilitation center. Since the patient was unable to see the stump and still feels the presence of her amputated lower leg, rehabilitation was needed to make her aware of the new situation and to find a new balance during transfers. The main goals of the rehabilitation were therefore to relearn the patient to make a standing transfer with help. The transfers were intensively practiced with the patient and her mother, who is an important informal care taker of the patient. Also, the electric wheelchair was adjusted to her new situation. After 4 months, the patient returned home. At the most recent follow-up, 14 months after the surgery, the patient was doing well. Now, the patient is under the care of the department of rehabilitation medicine at Amsterdam UMC exploring the possibilities of a cosmetic prosthesis of the lower limb.

SYSTEMATIC REVIEW

Literature was systemically reviewed to identify cases in which FOP patients underwent surgery of a limb and the effect of the procedure on the disease activity. The literature search was performed based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)-statement (www.prisma-statement.org).

To identify all relevant publications, we conducted systematic searches in the bibliographic databases PubMed and Embase from inception to May 2, 2019, in collaboration with a medical information specialist. The following terms were used (including synonyms and closely related words) as index terms or free-text words: "Myositis ossificans," "Fibrodysplasia ossificans," "Surgery," "Anesthesia." The references of the identified articles were searched for relevant publications. Duplicate articles were excluded. Only English articles were accepted. The full search strategies for all databases can be found in the **Supplementary Material**. Two reviewers (EB and ST) independently screened all potentially relevant titles and abstracts for eligibility. If necessary, the full text article was checked for the eligibility criteria. Differences in judgement were resolved through consensus. Studies were included when a surgical procedure of the limb and its outcome (either HO-formation or clinical outcome) were described. Patients of all ages were included, as well as all types of surgeries of the limb. The literature search generated a total of 1,774 references: 692 in PubMed and 1,082 in Embase. After removing duplicates of references that were selected from more than one database, 1,223 references remained. The flow chart of the search and selection process is presented in **Figure 4**. Seventeen articles described cases in which FOP patients underwent surgery for the upper and/or the lower limbs. In these seventeen articles, thirty two procedures were described in twenty patients. Ten procedures involved the upper limbs (12–19), twenty-two the lower limbs (**Table 1**) (12, 13, 19–28).

Procedures on the Upper Extremities

All ten surgeries performed on the upper limbs were done to remove either an undiagnosed swelling or mature HO. The reoccurrence of HO was described for eight of the ten procedures (12–15, 18, 26) and the clinical outcome for nine cases (12, 14, 15, 18, 19, 26). Reoccurrence of HO was observed in all eight procedures, however, in four of the nine procedures for which the clinical outcome was described, a clinical improvement was noted (12, 15, 19). Clinical improvement in one patient was due to a better position of the joint (15), whereas three other cases describe an improved movement in the joint after surgery (12, 19). For these cases, though, the follow-up period was 5 to 6 months. The time for HO to redevelop after surgery was

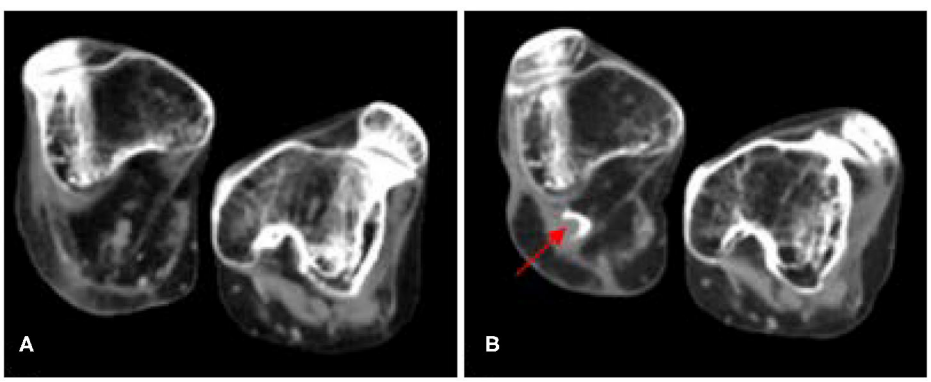

FIGURE 3 | Axial Low dose CT-images at the level of the distal femur of a patient prior to and after a through-knee amputation of the right leg. **(A)** Eight months prior to the surgery. **(B)** Twelve months after the surgical procedure. Minor HO formed (4cc) on the right side posterior to the lateral femoral condyle (red arrow). FOP, fibrodysplasia ossificans progressiva; HO, heterotopic ossification; CT, computed tomography.

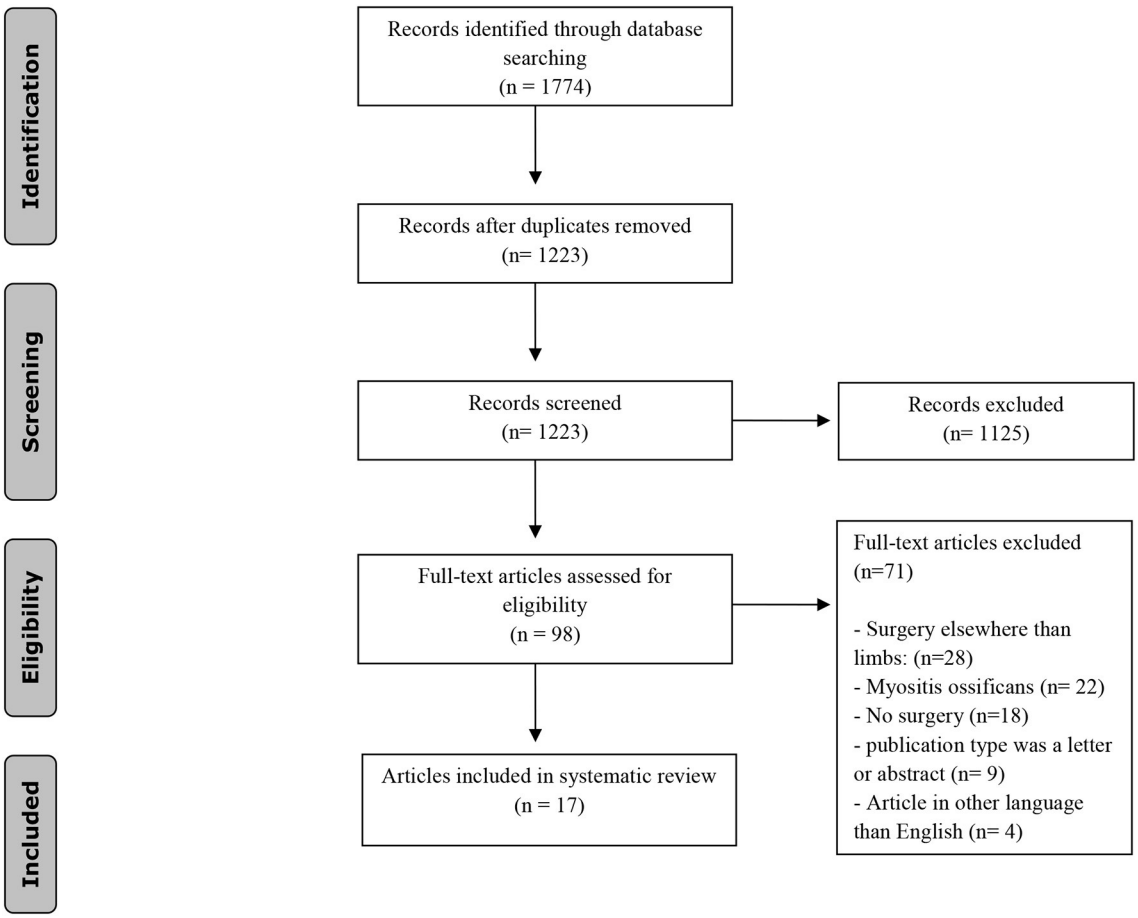

FIGURE 4 | Flowchart of the study selection process.

only described in only one case, i.e., 3 months (15). In the majority of the cases, the method to detect reoccurrence of HO and its extensiveness was not described. In all reports, neither the anesthetic management nor the selected operative method were discussed.

Procedures on the Lower Extremities

Of the twenty-two described lower limb surgeries, performed in sixteen patients, twenty procedures were performed to remove HO and restore joint mobility (12, 13, 19–21, 23, 24, 26, 27). In one case, however, surgery was needed for fracture management (25), and in another case the operation was to close a chronic ulcer with skin grafts (28). In all but three cases in which HO reoccurrence was described (19/22), the removal of HO was complicated by reoccurrence of HO at the operated site (12, 13, 19, 20, 23–26, 28). Two of these three cases without any HO recurrence involved more than just the skin, however, adequate follow-up data on these cases are lacking (19). The time before HO was noticed ranged from 4 weeks to 36 months (13, 19, 20, 23, 24, 26). Despite reoccurrence, however, five of the sixteen cases in which clinical outcome was described, a clinical improvement after surgery was found (13, 19, 21, 26, 27). Two surgical procedures were done because of compression of HO on surrounding tissues, and resulted in less pain (21, 27). In two cases mobility was not restored, but a better position of the joint was achieved, increasing functionality (20, 26). In only one patient there was an actual improvement of mobility of the operated joint (13). The anesthetic management was mentioned, but not discussed in detail, in three case reports. Surgery to unlock the hip was performed under general anesthesia, whereas surgery on the knee joint was done under a subarachnoid block.

Perioperative Medication to Prevent HO

Medication was used prior, during or after the procedure in 15 of the 32 surgeries (12, 13, 19, 20, 23, 26). In twelve of these cases bisphosphonates were used in attempt to halt (re)mineralization (12, 19, 20, 23, 26). Bisphosphonates were given as monotherapy ($n = 8$), or combined with non-steroidal anti-inflammatory drugs ($n = 2$) or corticosteroids ($n = 2$). The other treatments given were either NSAIDs combined with one fraction of radiotherapy ($n = 1$), subsequent fractions of radiotherapy ($n = 1$) or corticosteroids ($n = 1$) (12, 13, 19). For eleven of those fifteen procedures the effect of the procedure on HO reoccurrence was described: ten were

TABLE 1 | Articles describing HO reoccurrence and/or clinical outcome after surgery of a limb in an FOP patient.

References	Patient	Age (years)	FOP-diagnosis (Y/N/U)*	Body part	Reason surgery	HO (re)occurrence (Y/N/U)	(re)occurrence HO noted (months)	Duration follow-up (months)	Clinical improvement (Y/N/U)	Medication used pre-, peri- or postoperatively
Benetos et al. (13)	1	14	Y	Shoulder	Unlock joint	Y	U	U	U	-
		18	Y	Hip	Unlock Joint	Y[1]	7	12	Y, improved mobility	Indomethacin 25 mg/3 dd, RT: 7 Gy in 1 fraction
Colmenares-Bonilla et al. (20)	2	11	Y	Knee	Unlock joint	Y[1]	1	60	N	Corticosteroids 30 mg/kg, Alendronate 10/mg/day
Connor et al. (14)	3	1	N	Shoulder	Remove swelling	Y	-	U	N	-
	4	6	U	Shoulder	Unlock joint	Y	U	U	N	-
Corfield et al. (15)	5	24	Y	Wrist	Improve position	Y[1]	3	3	Y: functional position	-
Duan et al. (21)	6	17	Y	Hip	HO induced claudication	U	U	24	Y: no claudication	-
Holmsen et al. (23)	7	20	Y	Hips	Unlock joint	Y[1]	2	24	N	EHDP 10 mg/kg/day
Jayasundara et al. (12)	8	47	Y	Shoulder	Unlock joint	Y[2]	U	U	Y: improved mobility	Bisphosphonates, indomethacin
				Hip	Unlock joint	Y[2]	U	U	N	Bisphosphonates, indomethacin
		52		Hip	HO induced pressure necrosis	U	-	U	N	RT: 26 Gy in 13 fractions
Kartal et al. (24)	9	13	N	Hip	Unlock joint	Y	1.5	12	N	-
		14	N	Hip	Unlock joint	Y	3	12	N	-
		15	N	Hip	Unlock joint	Y	U	12	N	-
Smith et al. (19)	10	34	U	Calf	Unlock joint	N[1]	-	U	U	EHDP 20 mg/kg/day
				Elbow	Unlock joint	U	-	U	Y: improved mobility	EHDP 20 mg/kg/day
	11	16	U	Foot	Unlock joint	Y	3	3	U	
		17		Foot	Unlock joint	Y	U	U	U	
		18		Hip	Unlock joint	Y	U	U	U	
		23		Foot	Unlock joint	Y[1]	36	36	U	EHDP 20 mg/kg/day
		U		Hip	Unlock joint	Y[1]	7	7	N	EHDP 20 mg/kg/day
	12	17	U	Hamstring	Unlock joint	Y	U	U	U	Prednisone 7.5 mg/day
		18		Biceps	Unlock joint	U	-	U	Y: improved mobility	EHDP 20 mg/kg/day
				Hip	Unlock joint	N[1]	-	24	Y: improved mobility	EHDP 20 mg/kg/day
	13	21	U	Hip[1]	Unlock joint	Y	2	5	N	EHDP 20 mg/kg/day
Kocyigit et al. (18)	14	15	N	Elbow	Unlock joint	Y	U	U	N	-
Matsuda et al. (28)	15	35	Y	Malleolus	Incurable ulcer	N	-	8	N	-
Nerubay et al. (25)	16	7	Y	Femur	Fracture	Y	U	12	N	-
Obamuyide et al. (16)	17	11	N	Axilla	Unlock joint	Y	U	U	N	-
Tiwari et al. (17)	18	2	N	Arm	Removal swelling	Y	U	U	N	-
Trigui et al. (26)	19	25	Y	Hip	Unlock joint	Y[1]	2	24	Y: functional position	Corticosteroids, bisphosphonates
Waller et al. (27)	20	23	Y	Hip	HO induced pain	U	-	U	Y: less discomfort	-

HO reoccurrence is stated as YES when the article has specifically mentioned the reoccurrence of HO, YES[1] when confirmed by X-rays and YES[2] when confirmed by CT. FOP diagnosis is states as UNKNOWN when the confirmation of the diagnosis was not specifically mentioned in the article. FOP, fibrodysplasia ossificans progressiva; Y/N/U, Yes/no/unknown; HO, heterotopic ossification; RT, Radiotherapy; Gy, gray; EHDP, Ethylene Hydroxydiphosphonate.

followed by HO reoccurrence. The one case in which there was no reoccurrence, the duration of follow-up is unknown (19). Outcomes in the group without treatment ($n = 17$) were described for fifteen procedures: fourteen were followed by HO. The one case without reoccurrence was a superficial surgical procedure involving a skin graft for an ulcer on the malleolus (28).

DISCUSSION

Although any kind of surgery is highly discouraged in FOP patients due to an increased risk of flare-ups and progression of the disease, this case demonstrates that in a life-threatening situation–an operative procedure can be considered and managed successfully even in severely affected patients. It requires the assembly of a multidisciplinary FOP-dedicated team with knowledge of the disease and preparations made in anticipation of complications that may occur. In the current case, the timely and detailed preparation on the multidisciplinary team and the innovative techniques employed throughout the perioperative period assured the benign outcome of the surgical procedure. Because there is no effective treatment available to stop the formation and progression of HO, surgical procedures are highly discouraged as standard care of FOP (5, 29). Even small traumata–e.g., biopsies—can cause sufficient damage to the muscle and trigger a flare-up with subsequent HO formation (30). In the described case, surgery was the only life-saving option: it was judged that the patient was unlikely to survive the rapidly increasing, progressive infections of her leg due to antibiotic resistant organisms after many years of treatment. Surprisingly, a negligible amount of HO formed after the through-knee amputation, possibly due to a period of silent disease activity before and at the time of the operation. The reason for the quiescent disease in this patient is not known. One hypothesis is that, as it is known that the immune system plays a role in the pathogenesis of HO (31), the chronic inflammation and antibiotic use could have suppressed disease activity. Interestingly, 12 months after the surgical procedure disease activity was noted at various sites with HO. This could be the result of a normalized level of inflammation, or a systemic, late effect of the surgical procedure itself. Based on case reports in literature describing limb surgeries, where postoperatively HO formation was observed in almost 90% of the cases (12–21, 23–28), it was expected that clinically relevant HO would form. It should be noted, that over 90% of the published limb surgeries were performed to remove HO. Only in two patients (7%) HO did not reoccur after the removal of HO. Both patients received bisphosphonate treatment (19). Due to the absence of the effect of bisphosphonate treatment in nine others, it is more likely that the good result in those two can be attributed either to an incomplete follow-up time or due to limited imaging modalities as both cases are reported in 1976 (19). Removal of HO might be complex when it has formed within a muscle or when it has fused with normal skeletal bone. Removal of HO can therefore be considered as a high impact procedure which triggers HO formation. In our case a through-knee amputation was performed which is a procedure with relatively limited trauma to muscles because the procedure does not affect normal skeletal bone and it mainly involves the origin and insertion of muscles and tendons. In addition, when possible, ankylosed bone parts were left *in situ* to minimize tissue damage. To limit the extent of HO formation after surgery, it has been suggested to administer corticosteroids as a prophylaxis for four consecutive days after surgery (5). Objective data on the effectiveness of glucocorticoids in flare-ups are lacking. But based on empirical data, it is believed that it reduces oedema and may cause symptom relief (4). Glucocorticoids are currently the only treatment available for FOP. Corticosteroids, however, also interfere with wound healing. Therefore, in the current case, they were only administered pre-operatively. Hopefully, an effective treatment will be available to halt the formation of HO in the near future. To date, four potential drugs are tested in a clinical trial: Palovarotene, Garatosmab, Rapamycin and Saracatinib (32–35). Once found effective in preventing HO formation, surgical treatment might be an option to unlock joints or to safely operate an FOP patient for any other condition under an umbrella of one (or a combination) of these drugs. Besides the impact of the surgical procedure and the attempt to suppress FOP activity with glucocorticoids, the anesthetic management is another major concern and challenge in FOP patients. Regional anesthesia techniques (peripheral nerve blocks) involve punctures causing tissue trauma with increased risk of flare-ups, and these are therefore considered contraindicated. Likewise, neuraxial (spinal or epidural) anesthesia is not recommended for the following reasons. First of all, the spine is often involved in the disease and thus inapproachable for puncture. Secondly, the puncture itself might trigger HO formation, which could compress the spinal cord (5). Therefore, general anesthesia is generally recommended for FOP patients. General anesthesia requires airway management and frequently mechanical ventilation, both of which can be extremely challenging in FOP patients (36, 37). FOP patients often have jaw ankylosis, making conventional direct laryngoscopy or even video-laryngoscopy impossible for tracheal intubation. Moreover, even in the absence of a temporomandibular joint (TMJ) ankylosis, direct laryngoscopy is discouraged because hyperextension of the neck is limited–if not impossible–due to fused cervical vertebrae and in addition, overstretching of the TMJ joint or vertebral facet joints during tracheal intubation might induce a temporomandibular joint flare-up (5). Therefore, fiberoptic naso-tracheal intubation is preferred in all FOP scheduled for general anesthesia (5). This would have been possible in the current case, however, the risk of general anesthesia was deemed unacceptably high. The patient suffered from a severely restricted pulmonary function due to a completely immobile thoracic cage (7, 9). It was anticipated that high inspiratory airway pressures would be needed during mechanical ventilation to maintain adequate gas exchange. This can lead to over-distention of alveoli causing pulmonary barotrauma (38). Other challenges that were anticipated were a ventilation-perfusion mismatch and difficulties in weaning form mechanical ventilation. In addition, FOP patients are known to have impaired thoracic flexibility and weakened respiratory muscles predisposing to ineffective coughing, with an increased

risk of mucus retention and infection (5). Therefore, a regional anesthesia approach was chosen, with ultrasound guidance to identify structures and to limit tissue trauma. Glucocorticoids were locally injected via the placed nerve block catheters in an attempt to prevent a flare-up. Since regional anesthesia alone was insufficient to ensure complete analgesia and patient's comfort, systemic drugs were added. As these drugs might induce apnea, it is important to monitor the patient closely and keep high-flow nasal oxygen standby in case support of oxygenation is needed (39, 40). To conclude, based on the literature it was almost certain that HO would form as a response to a surgical procedure of a limb. In the current case, HO was indeed formed, but even 12 months after surgery the volume of the formed HO minimal. It is hypothesized that the patient's silent disease activity and the continuous antibiotic treatments might have influenced this. If surgery needs to be performed, it is important that it is performed by a multidisciplinary team with knowledge about FOP and after carefully weighing the surgical benefits against the challenges and risks of both the anesthetic and surgical procedures for the FOP patient.

DATA AVAILABILITY STATEMENT

The datasets supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The authors have obtained informed consent from the patient to share data and images.

AUTHOR CONTRIBUTIONS

EB and EE: study design and data analysis. EB, ST, EE, JS, PS, WL, and LAS: study conduct. EB, EE, ST, and RV: data collection. EB, EE, JS, WL, and LAS: data interpretation. EB, EE, LAS, PS, WL, and JS: drafting manuscript. ST, LAS, PS, WL, EP, AS, RV, BS, JN, MV, DM, NB, JC, LS, BT, PG, PR, JS, and EE: revising manuscript content. EB, ST, LAS, PS, WL, EP, AS, RV, LS, BS, JN, MV, DM, NB, RV, JC, BT, PG, PR, JS, and EE: approving final version of manuscript. EE: takes responsibility for the integrity of the data analysis. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

We thank the patient for sharing the data with us.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2020.00570/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

MINI REVIEW
published: 21 January 2021
doi: 10.3389/fendo.2020.603638

Plethora of Traumatic Lesions of Bilateral Knee Extensor Mechanism in Osteogenesis Imperfecta

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Introduction: Injuries to the quadriceps extensor mechanism are rare in patients with Osteogenesis Imperfecta (OI). To the best of our knowledge, non-union of the patella in OI, either as an isolated problem or in combination with an acute fracture, has not been previously reported.

Case report: We describe how we surgically approached both the fracture and the non-union simultaneously. The surgical technique and steps are described in detail. Post-operative course was uneventful and the outcome was favorable, with full return of function for the patient.

Conclusion: A review of various knee extensor mechanism injuries in OI is described as illustrated in a single patient. The unusual simultaneous surgical treatment of a non-union and an acute fracture in the same patella shows that despite the severely compromised bone in this rare bone disease the bone still has a capacity to heal with a functional outcome.

Keywords: patella, fracture, Osteogenesis Imperfecta, avulsion, non-union

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Edited by:
Teun J. De Vries, VU University Amsterdam, Netherlands

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Specialty section:
This article was submitted to Bone Research, a section of the journal Frontiers in Endocrinology

Received: *07 September 2020*
Accepted: *23 November 2020*
Published: *21 January 2021*

Citation:
Kloen P, Hamdy RC and Bech NH (2021) Plethora of Traumatic Lesions of Bilateral Knee Extensor Mechanism in Osteogenesis Imperfecta. Front. Endocrinol. 11:603638. doi: 10.3389/fendo.2020.603638

INTRODUCTION

Osteogenesis imperfecta (OI) is an inherited bony dysplasia best known for its fracture susceptibility of the long bones and the spine, deformity, and growth deficiency. Its underlying genetic disorder is a mutation in either one of the collagens I genes (Col 1A1 or Col 1A2) that encode the alpha 1 and alpha 2 chains of collagen. With newly identified mutations since 2006, the original Sillence classification (type I–IV) that was based on clinical presentation, radiographic appearance, and inheritance pattern, has been adapted to now include 21 different OI types (1–5).

The most common OI type is type I. These patients are usually active and ambulatory with a slight shorter stature (6). Most fractures are of the long bones (femur, tibia, and humerus), and spine. Most fractures are sustained at a young age, although it is a life-long problem with an estimated 25% of fractures occurring in adult life. Fractures of smaller bones are relative rare. The increased understanding of the underlying molecular mechanisms has not been mirrored by improvements in treatment (7).

The orthopedic surgeon has an important role in the treatment of these fractures in OI patients, whether operative or non-operative. We describe a patient who sustained traumatic injuries—and post-traumatic complications thereof—to various components of the extensor mechanism of both

knees over the course of 15 years. Among these, we report an unusual combination of a fracture and non-union in the same patella. To the best of our knowledge, this combination has not been previously reported. Furthermore, we were unable to find the description of a patellar non-union in OI. In this case report, we present these injuries, their treatment, and outcome, in addition to a review of the literature on extensor mechanism injury of the knee in OI.

CASE REPORT

A 29-year-old male with a diagnosis of type I osteogenesis imperfecta presented to our emergency room with a painful right knee and inability to extend his knee. He had fallen of a folding chair that collapsed when he tried to sit down. This had caused a forced hyperflexion injury of his right knee.

His past medical history was significant for numerous fractures of both upper and lower extremities. He had sustained bilateral transverse patella fractures 13 years prior to the present injury. These had been treated with suture repair. He also had sustained an avulsion of the inferior pole of the right patella that was treated with suture fixation 3 years prior. He had done reasonably well but then sustained a new injury to this right knee and was diagnosed with a transverse non-union of the patella. It was unclear if this had been in existence since the initial suture fracture repair 13 years prior. He had developed a 20 degrees extension lag of his knee but was still able to do a straight leg raise. Because of the CT finding of the non-union, he was offered surgery but given his minor symptoms and upcoming school exams he declined surgery.

His past medical history also included scoliosis correction T4-L5, operative repair of a left and right (including revision and later hardware removal) olecranon fracture, and a distal humerus fracture. His medication at the time of his presentation included bisphosphonates (risedronate/calcium 35 mg once every week) which he had been taken for years. All his previous orthopedic care had been done at another academic hospital.

When we first saw him, plain radiographs and a CT of his right knee were obtained (**Figure 1**). These showed a comminuted fresh fracture of the upper half of the right patella proximal to a long-standing transverse non-union. The non-union had sclerotic edges and a gap of approximately 4 mm. Given his inability to extend his knee, we offered surgical repair and, at the same time, an attempt at fixing the long-standing non-union. After discussing the alternative of only fixing the fresh fracture, the patient chose to address surgically both the fracture and non-union with use of homologous bone graft.

Surgery was performed under general anesthesia with the patient in supine position on a radiolucent table. A tourniquet was used. The knee was brought in 20–30 degrees of flexion. The old longitudinal incision of 15 cm was used, extending from the tibia tubercle to 3 finger breadths above the superior pole of the patella. Old suture material was removed. There were no signs on infection. Five deep tissue cultures were taken after which the tourniquet was deflated briefly, and he was given IV antibiotics (Cefazolin 2 gr). A lateral parapatellar arthrotomy was done originating from the tear in the lateral retinaculum. The patella was now inverted to directly visualize the comminuted articular surface. The fracture hematoma was irrigated and debrided. The non-union was identified in the lower half of the patella. There was a clear soft spot but the two parts of the non-union were bridged by non-osseous tissue. The non-union was not opened. With a 1.5 mm drill starting from the fracture side, we made a few drill holes perpendicular through the stiff non-union until blood was noticed to egress from the drill holes. We then first reduced the two parts of the comminuted superior aspect of the patella. This was done using curettes, dental pick, irrigation, and suction and pointed reduction clamps. Perfect alignment was obtained as visualized on the articular surface. The fragments were temporarily transfixed with 1.25 mm K-wires.

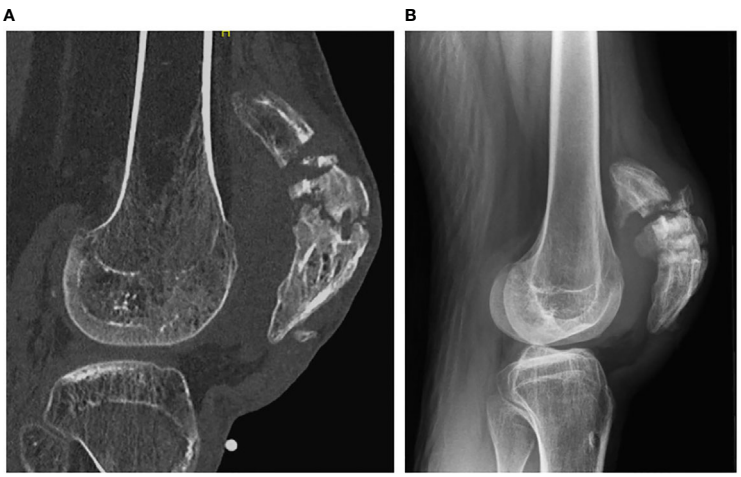

FIGURE 1 | Sagittal CT view **(A)** and lateral radiograph **(B)** of the right patella showing the combination of the acute fracture and longstanding nonunion.

A cannulated titanium headless screw (mini Acutrak, Hospital Innovations, Belgium) was then used to fix the two large upper pole fragments. The K-wires were removed. Next this reconstituted superior fragment of the fracture was reduced to the inferior part of the patella including the non-union using two large-pointed reduction clamps and temporary K-wires. From distal two 4.7 mm cannulated headless titanium screws (Acutrak, Hospital Innovations, Belgium) were placed through the non-union from inferior to superior crossing the fracture. Care was taken to bury the screws within the bone as not to irritate the quadriceps or patellar tendon. A figure-of-eight 1 mm steel cerclage wire was then placed through these two 4.7 mm screws, twined, and tightened. The K-wires and reduction clamps were removed. A 2.4/2.7 mm steel Mesh plate (DePuy Synthes, Amersfoort, The Netherlands) was then cut to fit over the dorsal side of the reconstructed patella and fixed with unicortical 2.7 mm locking screws. Each major fragment was fixed by the mesh plate without encroachment of the hardware on the superior and inferior tendinous parts. The superior limb of the plate was placed under the quadriceps, where the inferior part was placed under the patella tendon. Screw placement was observed to be extra-articular through the lateral parapatellar arthrotomy. Two non-absorbable no. 2 sutures were placed through the superior and inferior portion of the plate and then passed through the patellar tendon as a Krackow suture. These sutures were tied with the knee in extension. The retinaculum, subcutaneous tissues, and skin were closed in the usual fashion. The dorsal aspect of the fracture and non-union were covered with 2.5 cc demineralized bone matrix (DBX, DePuy Synthes, Amersfoort, The Netherlands). Total tourniquet time was less than 2 h. The patient was given a hinged knee brace locked in extension for 6 weeks. No active extension was allowed for 6 weeks. After 6 weeks the brace was discontinued, and physical therapy was started. Bisphosphonates were discontinued until there was healing of both the fracture and non-union.

At 3 months follow up the patient had regained full range of motion of his knee (140-0-0) and reported no pain (**Figure 2**).

One year after the right knee surgery he returned to his old hospital where the patella hardware on the right was removed as this was bothering him. The patella had solidly healed with an excellent function (**Figure 3**).

Eight months later the patient was diagnosed with an avulsion fracture of the inferior pole of the left patella (**Figure 4**) for which he underwent suture fixation at an outside hospital. This healed uneventfully within 3 months.

Nine months later he fell off a barstool and sustained an avulsion of the quadriceps tendon of the superior pole of the left patella (**Figure 5**) in addition to a right ankle fracture dislocation. We performed open reduction and internal fixation of his ankle fracture and suture repair through transpatellar tunnels of his left quadriceps' tendon avulsion. These two injuries recovered uneventfully. Seven months later he again fell off a chair and was diagnosed with a transverse fracture of the left patella (**Figure 6**). As he was still able to do a straight leg raise, we treated him with a hinged knee brace. At 3 months follow up his left patella fracture had healed in anatomic position (**Figure 7**), with a good functional outcome (for a chronologic overview of his traumatic knee lesions see **Figure 8**).

OVERVIEW OF THE LITERATURE ON EXTENSOR MECHANISMS INJURIES OF THE KNEE IN OI

A review was done in accordance with the Prisma statement (www.prisma-statement.org). A comprehensive search was done

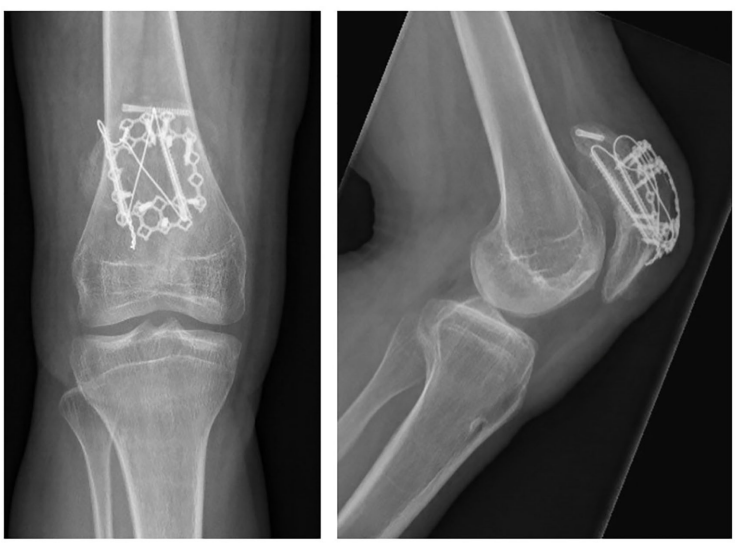

FIGURE 2 | Plain AP and lateral radiographs at 3 months follow-up showing a healed patella.

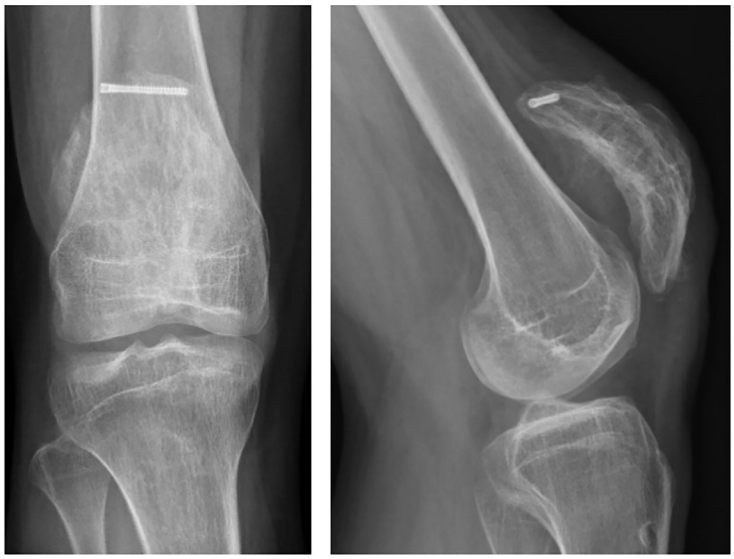

FIGURE 3 | AP and lateral radiographs after hardware removal.

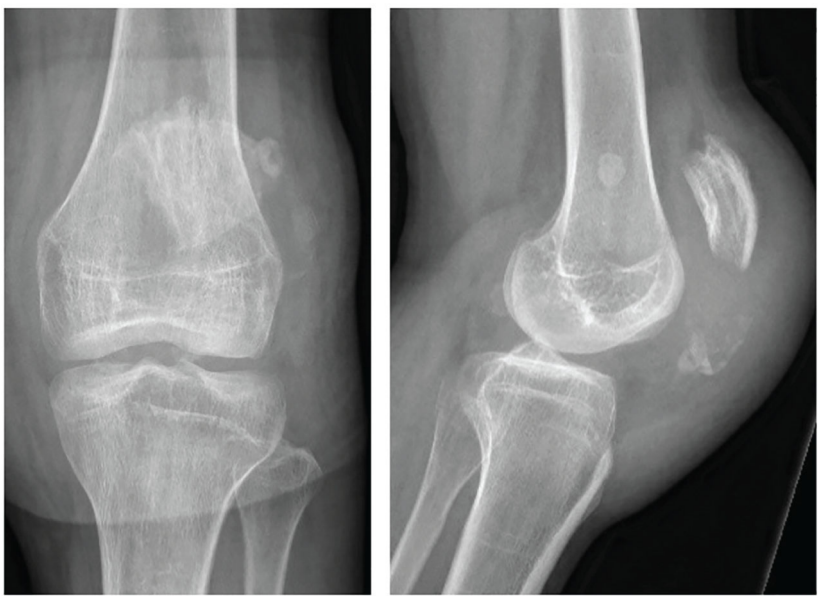

FIGURE 4 | AP and lateral radiographs of the left patella showing an avulsion fracture of the inferior patella pole.

in PubMed and Embase.com. The search terms (including closely related words and synonyms) included "osteogenesis imperfecta", "patella", "knee", "ligament", "sleeve", "fracture", and "non-union" and "nonunion". We only included articles in English, there were no date restrictions. Using this strategy, we identified a total of nine suitable articles. After cross checking references three more articles were included. Most of the papers were case reports. The articles were assessed by the first author of this manuscript. A total of 12 were selected for this review (**Table 1**). Of these 12 papers, there were seven that described extensor mechanism injures in OI. They are summarized in **Table 1**. Most of them affected young males with OI type I. Operative treatment was common and with successful outcome in 100% of cases. No complications were listed.

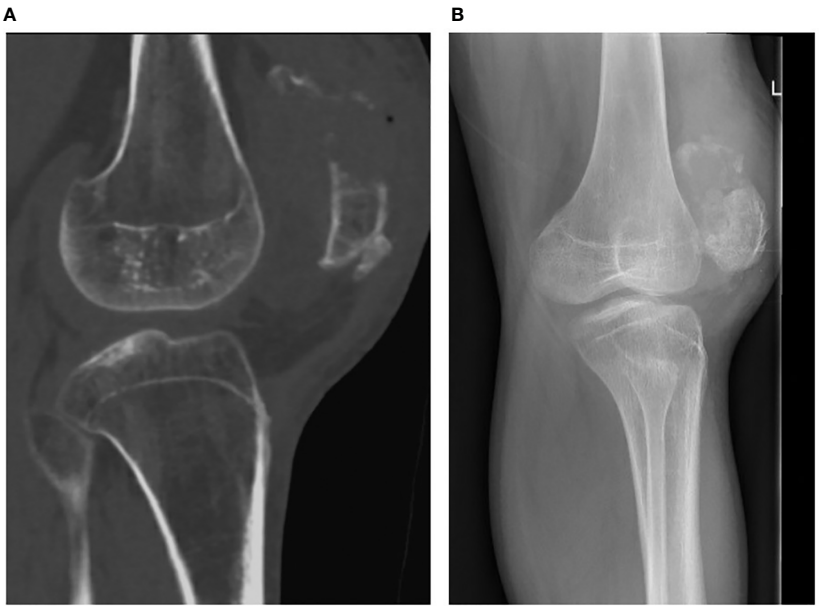

FIGURE 5 | Sagittal CT view **(A)** and lateral radiograph **(B)** of the left patella showing an avulsion fracture of the superior patella pole.

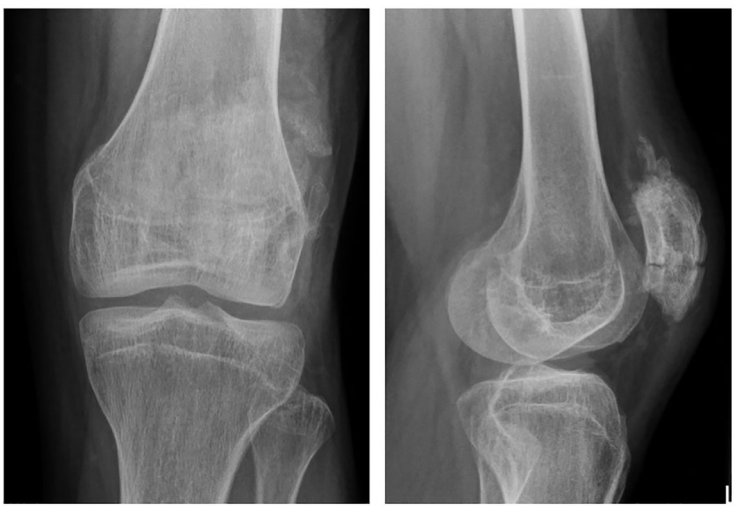

FIGURE 6 | AP and lateral radiographs showing a transverse fracture of the left patella.

DISCUSSION

Osteogenesis imperfecta (OI) is known for its qualitative or quantitative deficiency of collagen I. As such it can affect not only bone but any other organ or structure containing connective tissue, including tendon, sclerae, ligaments, and skin. Most patients with OI will sustain many fractures especially in childhood. Although many of these fractures can be treated non-operatively, the trend nowadays is to treat long bone fractures operatively with lengthening intra-medullary nails, specifically in the presence of repeated fractures and/or deformities. Intra-articular fractures, however, often warrant surgical treatment, in order to restore the normal anatomy of the articular cartilage.

As previously mentioned, tendons in children with OI may also be weakened by the deficient collagen. Tendon injuries in OI are infrequent and much less common than bony injuries, but have been described affecting the Achilles tendon, triceps tendon,

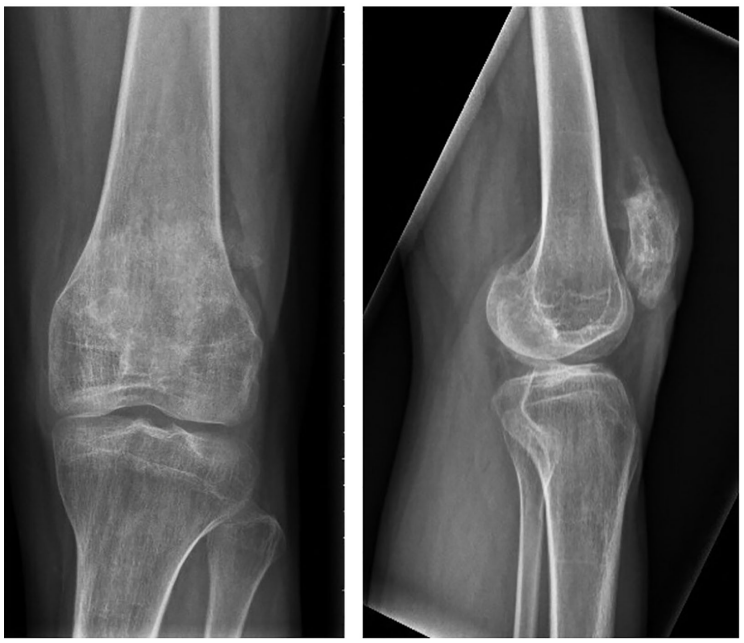

FIGURE 7 | AP and lateral radiographs at 3 months, showing healing of the transverse left patella fracture.

patellar tendon, and quadriceps tendon (8, 14, 16, 17, 20). These later two tendons are part of the extensor mechanism of the knee which also include the patella and the patellar tendon insertion onto the tibial tubercle. Discontinuity of the knee extensor mechanism will prevent knee extension and causes instability during walking. This by itself represents a major disabling injury in any patient, but even more so in an OI patient whose mobility is already compromised by deformity, muscular weakness, and ligamentous laxity. Discontinuity in the extensor mechanism of the knee can be caused by tendon avulsion, tendon rupture, fracture, or combinations thereof. In children there is a fracture subtype known as a sleeve fracture, described in 1997 by Houghton and Ackroyd (21), where a sleeve of cartilage is pulled off the main body of the patella with a bony fragment. This can either be from the inferior pole which is most common, or the superior pole (22). In essence it is a failure of an immature osteochondral junction. It is unclear if a true sleeve fracture can exist in the adult as the ossification of the patella is considered complete in adulthood. Possibly an intrinsic tendon and/or bone problem such as OI can cause a "sleeve fracture" in adulthood (12).

Other causes of a quadriceps or patellar tendon rupture are systemic diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis, (RA), gout, or hyperparathyroidism, steroid use, or renal disease (23, 24). The underlying pathophysiology of these ruptures varies with the disease process. Avulsions are best treated with sutures via trans-osseous tunnels or bone anchors.

Our patient had already sustained bilateral patellar fractures as a child and also a right patella tendon avulsion fracture as a young adult. Later it was noticed that he had a right patella non-union of unknown age for which treatment was postponed because of school obligations.

When he presented to us for the first time with his fresh comminuted right patella fracture, the co-existing non-union of the patella added another level of complexity to an already challenging problem. The classic definition of a non-union as a fracture that has not healed in 9 months, has recently been modified to a fracture "that will not heal without further invention". Non-unions in OI occur but have not been addressed in the literature other than in small series or case reports. The popularization of second and third generation oral and intravenous bisphosphonate therapy in OI (25) may increase the risk of a non-union. Biphosphonates inhibit osteoclast activity, potentially having a negative effect on remodeling after fracture healing. This negative side effect of bisphosphonate therapy in OI has been shown after osteotomies but not yet after fractures (26). A recent review concluded that bisphosphonates did not influence fracture healing after wrist, hip or spine fractures (27). The long-term use of bisphosphonates does however seem to be related to atypical femoral fractures (28). Whether biphosphates should be stopped (drug holiday) when an OI patient has an acute fracture is still controversial.

Treatment of the displaced patella fracture is most often done using cerclage wires in comination with K-wires or (cannulated) screws (29). More recently, locked plating for a comminuted patella fracture has become more popular as it provides multiplanar fixation (30).

Treatment of a non-union consists of compression and optimizing of biology in atrophic and oligotrophic non-union (31). To the best of our knowledge, there are no guidelines for

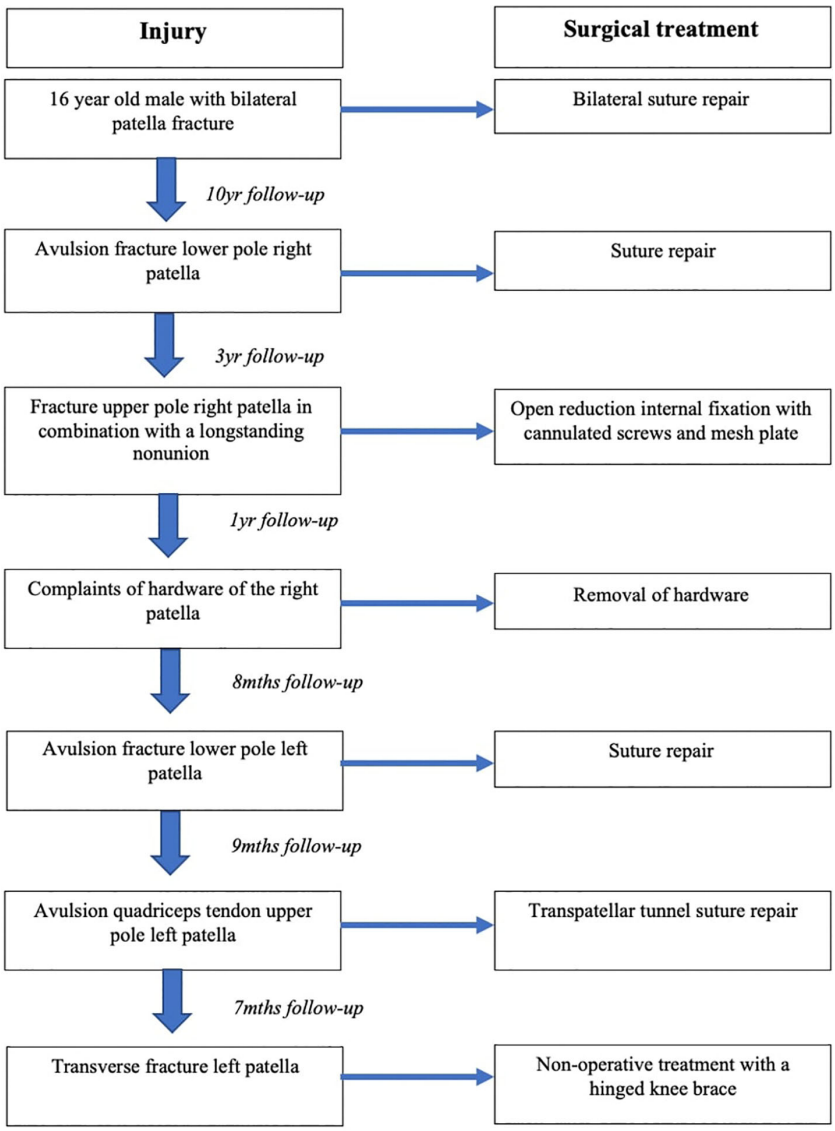

FIGURE 8 | Chronological overview of the patient's traumatic knee lesions and treatment.

simultaneously treating a fracture and a non-union in the same bone. We thought that using a combination of cannulated screws for compression of the non-union and multiplanar plating for buttressing would address both problems in one reconstruction. The use of a lateral parapatellar approach with inversion of the patella would allow an extensive overview of the articular surface. To optimize the biological healing potential of the non-union, the lateral parapatellar approach was also chosen because it had already been shown to not compromise the vascularity (32). Given the compromised bone quality we felt some type of bone graft would be beneficial. The Gold standard for non-union treatment is autologous bone graft. However, given the underlying OI, any bone harvested from the patient would have the same intrinsic problem. Therefore, we chose homologous bone graft in the form of demineralized bone matrix (DBX). Its use has not been specifically studied in OI, but it has shown to be non-inferior in various non-union papers (33).

Delayed healing of osteotomies is often observed in OI patients on bisphosphonates (25). To date it is unclear if bisphosphonate treatment in mild forms of OI can lead to non-union (6). But it seems prudent to withhold bisphosphonates after non-union treatment. Anabolic treatment with teriparatide (parathormone) was also shown to benefit BMD in OI (25). However, in our country, the use of teriparatide for non-union in OI is not current standard of care.

It is important that OI patients have an established relation with orthopedic surgeons that are familiar not only with the

TABLE 1 | Overview of the literature on extensor mechanisms injuries of the knee in patients with osteogenesis imperfecta (OI).

Author	Injury	Mechanism of injury	Operative details	Follow-up
ElGuindy et al. (8)	Chronic patellar tendon disruption	Fall downstairs	Reconstruction with allograft bone-tendon-bone	FU 5 yr
	27 yo male, OI type NA	Primary repair		ROM 135-0-5 IKDC score 58,5%
Mehta and Mahajan (9)	Tibial tubercle avulsion and patellar tendon rupture	Minor tripping and fall	Screw fixation tibial tubercle	FU 7 yr
	11 yo male, OI type NA		Suture (Krakow) and bone anchor	FROM 10 degrees recurvation
Kim et al. (10)	Simultaneous bilateral patellar tendon rupture	No real trauma	Sutures	FU 10 mo
	55 yo female, Type I OI		Wire loop around patella	ROM 100-0-0
Jansen and Haddad (11)	Distal patellar tendon avulsion	Soccer	Sutures (whipstitch)	FU 1 yr
	29 yo male, Type I OI		Drill holes tubercle and bone anchor	ROM 120-0-0
Kakazu et al. (12)	Avulsion upper pole patella (sleeve fracture)	Spontaneous fracture during walking	Sutures	FU 6 mo
	30 yo male, Type I OI		Quadriceps turn-down	
Wiss et al. (13)	Tibial tubercle avulsion			
	16 yo male, Type I OI	Basketball	Screw fixation	FU 10 mo
	16 yo male, Type I OI	Running	Screw fixation	FU 8 mo
Kothari et al. (14)	Bilateral patellar tendon ruptures	Fall on knee	Suture	FU 7 mo
	27 yo female, Type IB OI		Protection wire	ROM 110-0-0
Khodadadyan-Klostermann et al. (15)	Bilateral tibial tubercle avulsion	Spontaneous fracture during running	Screw fixation	FU 1 yr
	15 yo male, Type I OI			FROM
Figueroa et al. (16)	Spontaneous bilateral quadriceps tendon rupture	Fall	Sutures	FU 4 yr
	28 yo male, Type I OI		Trans patellar tunnels	Lysholm score 95 (excellent) FROM
Ogilvie-Harris and Khazim (17)	Avulsion inferior pole 36 yo male	Squash	Sutures	FU 6 wk (normal)
	32 yo male OI types NA	Skiing	Suture anchors	FU 2 yr (normal)
Salcedo-Duenas et al. (18)	Bilateral quadriceps tendon rupture	Fall on knee	Non-absorbable sutures and transpatellar tunnels	FU 4 yr
	28 yo male, Type I OI			Lysholm score 95 (excellent) FROM
Tamborlane et al. (19)	Bilateral tibial tubercle avulsion 9 yo male, Type I OI	Spontaneous fracture during running	5mm partially threaded cannulated screw with washer	FU 38 mo FROM

FU, Follow up; IKDC, International Knee Documentation Committee; FROM, full range of motion; ROM, range of motion; NA, not available.

disease but who also have experience with non-union treatment. Reports on patella non-unions are scarce. Klasen et al reported on 20 patients with a patella non-union (34). Seven were treated non-operatively (some of whom were minimally symptomatic) whereas the other 13 were operated. Operative management included open reduction and internal fixation (tension band wiring, Bunnell wiring, cerclage wiring, or screw fixation, partial patellectomy, or patellectomy. Only two patients received bone graft but no details on the indications were provided. All operated patients except one healed the non-union. All those treated non-operatively did not heal. Satku and Kumar reported on three patella non-unions that were successfully treated with tension band fixation (35). Uvarai et al. treated 22 neglected patella fractures that had not united with tension band wiring with or without cerclage in 19 and with patellectomy in three (36). Twenty out of 22 had an excellent/good results at year follow up of 5.5 year. Nathan et al published a systematic review on non-union and delayed union of the patella (31). A total of 5 publications was identified, including 45 patients. In 66% of patients, the treatment consisted of tension band wiring. Bone graft was rarely used (2/45). They concluded that tension band wiring is the treatment of choice for patient suitable for reconstruction. Partial of total patellectomy is also an option according to their review (31).

CONCLUSION

This case reports describes a variety of consecutive rare traumatic injuries of the extensor mechanism of the knee in a type I OI patient. It is unclear whether suboptimal patellofemoral alignment

(patella alta) and/or the deformity of the patella led to increased stress distribution over the tendon-bone juncture and/or the bone. The combination of a fresh fracture proximal to a pre-existing non-union in the same bone is extremely rare. Despite the various injuries and their complication, the patient kept his ambulatory status with a functional range of motion of both knees.

AUTHOR CONTRIBUTIONS

PK wrote the first draft of the manuscript and was the treating physician. RCH and NHB contributed to the writing and design. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

CASE REPORT
published: 18 February 2021
doi: 10.3389/fendo.2021.621604

Sternocostoclavicular Hyperostosis: Positive Clinical and Radiological Response on Pamidronate

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Background: Sternocostoclavicular hyperostosis (SCCH) is a rare disease, constituting a chronic sterile osteomyelitis with elevated bone turnover in the axial skeleton, causing pain and shoulder dysfunction. SCCH severely interferes with daily activities, work, and quality of life. SCCH has a relapse-remitting disease course, but inflammatory-induced sclerotic transformation in the affected area is slowly progressive. Here we present two patients with clinical and radiological diagnosis of SCCH treated with intravenous pamidronate, leading to clinical remission in both, but complete resolution of sclerosis in one of them, which is a novel finding in our experience.

Case Presentation: Two adult female SCCH-patients presented with longstanding pain, swelling of the anterior chest wall, and compromised shoulder function. Subsequent single photon emission computed tomography-computed tomography (SPECT/CT) illustrated elevated bone activity and sclerosis in the SC region, with hyperostosis, confirming the diagnosis of SCCH. As symptoms in both patients were eventually refractory to standard painkillers such as non-steroidal anti-inflammatory drugs (NSAIDs), intravenous pamidronate treatment in 3-month cycles was started. Pamidronate was effective in reducing pain and improving shoulder function and also led to decreased bone turnover on skeletal scintigraphy. Sclerosis in the first patient persisted. In the second patient, however, a complete resolution of sclerosis was observed.

Conclusions: SCCH remains a rare bone disorder for which no evidence-based therapies are yet available. While disease burden is high, SCCH lacks recognition and is often diagnosed long after symptomatic presentation. As for the cases in this report, pamidronate was successful in reducing symptoms, and in the second case even led to regression of sclerotic changes on CT-imaging.

Keywords: sternocostoclavicular hyperostosis, pamidronate, bisphosphonate, treatment, radiologic response, sclerosis, pain, SAPHO

OPEN ACCESS

Edited by:
Teun J. De Vries, VU University Amsterdam, Netherlands

Reviewed by:
Michaël R. Laurent, University Hospitals Leuven, Belgium
Hermann Girschick, Vivantes Hospital, Germany

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Specialty section:
This article was submitted to Bone Research, a section of the journal Frontiers in Endocrinology

Received: *26 October 2020*
Accepted: *06 January 2021*
Published: *18 February 2021*

Citation:
Leerling AT, Canete AN, Ramautar AIE, Appelman-Dijkstra NM and Winter EM (2021) Sternocostoclavicular Hyperostosis: Positive Clinical and Radiological Response on Pamidronate. Front. Endocrinol. 12:621604. doi: 10.3389/fendo.2021.621604

BACKGROUND

Sternocostoclavicular hyperostosis (SCCH) is a rare chronic inflammatory disease, comprising a sterile osteomyelitis of the axial skeleton mainly affecting the sternocostoclavicular region. Here, an inflammatory cascade leads to increased local bone turnover, favoring bone formation (1–3). SCCH can be a part of SAPHO syndrome encompassing synovitis, acne, pustolosis, hyperostosis, and osteitis, but is also referred to as a separate clinical entity (4, 5). Together with chronic recurrent osteomyelitis (CRMO) and diffuse sclerosing osteomyelitis (DSO), SCCH belongs to the spectrum of chronic non-bacterial osteomyelitis (CNO).

SCCH usually affects patients in midlife, and a clear predisposition for the female gender has been described in recent case series (5). Clinical manifestations include a painful swelling of the sternum, ribs and clavicles and impaired mobility of the shoulder girdle (1). As a consequence, patients experience serious interference with their quality of life (5). SCCH typically has a chronic nature, with a relapse-remitting course and varying rates of bone turnover on scintigraphy, though spontaneous remission can be seen (6). Inflammatory-induced changes such as sclerosis and hyperostosis are usually slowly progressive (1, 7). Left untreated, SCCH may lead to permanent degenerative changes of the adjacent joints and secondary ossification of soft tissue (1, 8), which may further compromise shoulder girdle function. Although there is no established treatment for SCCH, pain control can sometimes be attained with NSAIDs (3). Other medications used are biologicals (9–11) or intravenous bisphosphonates, mostly pamidronate (12–16), which decrease inflammation or both inflammation and bone turnover, respectively.

Here we present the disease course in two SCCH-patients treated with intravenous pamidronate.

CASE PRESENTATION (1)

A 58-year-old female patient was referred to our center with longstanding shoulder pain. Medical history was positive for pustulosis palmoplantaris, which was adequately controlled with topical steroids. Family medical history was positive for autoimmune disease, with a sister suffering from ulcerative colitis and a brother with Sjogren's Syndrome. The patient quit smoking 7 cigarettes per day (approximately 14 packyears in total) 2 months before consultation.

Shoulder pain in rest, numerical score (NRS) 4, and during movement (NRS 8), was present for 3 years (**Figure 1**), and was previously diagnosed as tendinopathy with impingement. Despite conservative therapy, pain and impaired mobility persisted, and were later accompanied by redness and swelling of the sternum. Physical examination showed a painful sternal swelling with visible erythema. Laboratory results were normal including inflammatory markers, except for a mild vitamin D deficiency of 44 nmol/L, ref: >50 nmol/L, for which supplements were prescribed. Creatinine clearance was adequate before the start of treatment and remained so during further treatment course, ranging 67–70 μmol/L, ref: 49–90 μmol/L. Subsequent skeletal scintigraphy with single position emission computed tomography-computed tomography (SPECT/CT) demonstrated increased bone turnover of the right clavicular end, the manubrium and proximal corpus sterni, with sclerosis, and hyperostosis (**Figure 2**). Integrating clinical symptoms with radiological findings, the differential diagnosis of SCCH in isolated form was considered. And, due to the presence of pustulosis palmoplantaris this SCCH was considered as part of SAPHO syndrome.

NSAIDs were started, with diclofenac in a fixed dose of 75 mg a day. After 6 months, pain was scored as 3 and the shoulder still retained full range of motion (ROM). However, after 1 year of diclofenac treatment, pain increased to 6 on NRS and shoulder ROM was limited to 100 (left) and 110 (right) degrees in active abduction. Repeated radiological and nuclear imaging showed persistent sclerosis and increase of bone turnover of the right clavicular end, though slightly reduced compared to baseline (**Figure 2**). Given the worsening of the clinical course and conform treatment protocol at our center, therapy with intravenous pamidronate in a regimen of 3 consecutive infusion days dosed 15-30-30 mg in 3 monthly intervals was started (**Figure 1**). Additional diclofenac was recommended. Shortly after the first cycle of pamidronate, the patient reported significant improvement of symptoms with a quickly reclaimed free range of motion (FROM). After 1 year of pamidronate (four cycles), the patient reported further reduction of pain (NRS 4) with no need for additional diclofenac, and persistent FROM. As for radiological disease course, scintigraphy showed reduction of bone turnover of the right clavicular end compared to baseline with unchanged sclerotic changes on CT (**Figure 2**). Due to the good clinical response and the absence of adverse events, pamidronate was continued in a 3-day regimen of 30 mg per day in 3 monthly intervals. After 2 years of pamidronate-treatment, clinical remission was reached on grounds of the patient reporting durable pain control and full shoulder mobility, and pamidronate was stopped.

CASE PRESENTATION (2)

A 49-year old female patient, with a medical history of asthma, M. Dupuytren, and migraine presented at our center with swelling and pain in the left shoulder since 1.5 years. Medication included vitamin D supplementation, omeprazole, desloratadine and sporadic Ventolin. She smoked incidentally (one cigarette per 3 months, which she quitted at year 3 of therapy). Family medical history was positive for diabetes mellitus type 1 in first- and second-degree relatives. The symptoms were previously ascribed to a bursitis and treated with two corticosteroid injections. Pain, however, persisted with a numerical score of 8 and significantly interfered with daily functioning and sleeping despite diclofenac. Physical

Abbreviations: CNO, chronic non-bacterial osteomyelitis; CRMO, chronic recurrent multifocal osteomyelitis; DSO, diffuse sclerosing osteomyelitis; FROM, free range of motion; NRS, numerical rating scale; NSAIDs, non-steroidal anti-inflammatory drugs; PPP, pustolosis palmoplantaris; ROM, range of motion; SAPHO, synovitis, acne, pustolosis, hyperostosis, and osteitis; SC, sternoclavicular; SCCH, sternocostoclavicular hyperostosis; SPECT/CT, Single proton emission computed tomography.

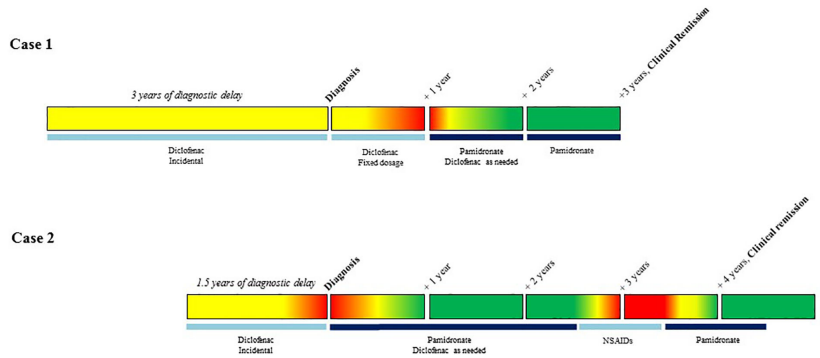

FIGURE 1 | Overview of treatment status and clinical reports of pain. Legend: No pain, Medium pain, Severe pain.

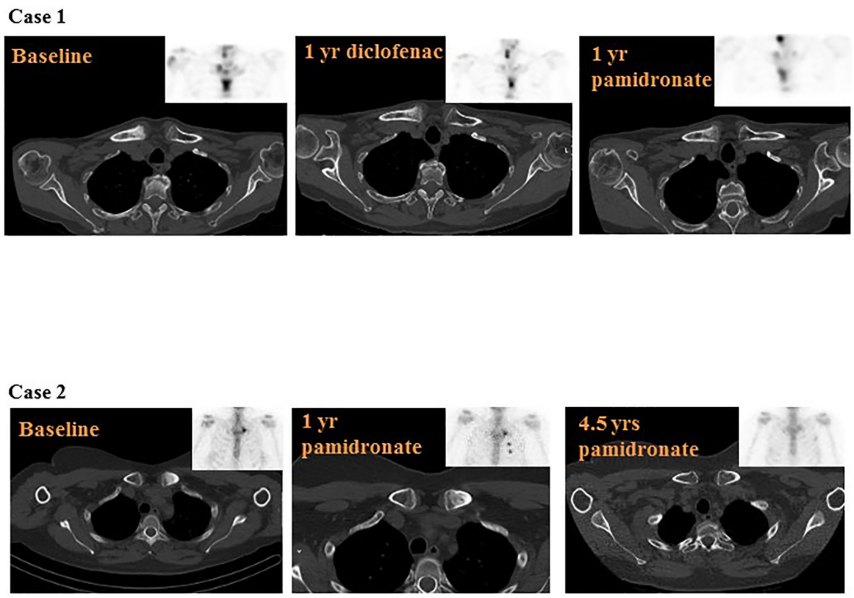

FIGURE 2 | Overview of disease course on computed tomography (CT)-scan and skeletal scintigraphy. Legend: Case 1: Baseline: diffuse sclerosis of the right clavicular end with increased bone turnover, and increased bone turnover of the manubrium and proximal corpus sterni. 1 year diclofenac: persistent sclerosis and increased bone turnover of the right clavicular end and persistent increase of bone turnover of the manubrium and proximal corpus sterni, slightly reduced compared to baseline. 1 year pamidronate: unchanged sclerosis and reduction of bone turnover of the right clavicular end, the manubrium and proximal corpus sterni, compared to baseline. Case 2: Baseline: sclerotic changes of the left clavicular end with elevated bone turnover. 1 year pamidronate: slightly reduced sclerosis of the left clavicular end and slightly reduced bone turnover. The increased uptake of the 3rd and 4th rib was caused by two costal fractures. 4.5 years pamidronate: almost complete resolution of sclerosis, leaving a minimal rest, and complete normalization of bone turnover.

examination revealed a painful swelling of the left sterno-clavicular region, with visible erythema and warmth. Laboratory results only showed a minimally elevated C-reactive protein of 5.4 mg/L, ref < 5 mg/L and an alkaline phosphatase of 114 U/L, ref 20–140 U/L, later accompanied by an elevated gamma-glutamyl transferase (ranging 49–63 U/L, ref < 25 U/L), altogether suspicious for hepatic origin. Creatinine clearance was and remained within reference range treatment course, ranging 65–74 μmol/L, ref 49–90 μmol/L. A CT scan of the chest-wall displayed a non-specific bilateral linear calcification of costa 1 and unilateral sclerosis of the left proximal clavicula. This area of clavicular sclerosis did not involve the complete subchondral articular surface of the clavicula and the joint space (sternoclavicular joint) was preserved, hence the diagnosis of reactive sclerosis due to degenerative joint disease was unlikely. Preliminary calcification of the left costoclavicular ligament was also detected. Skeletal scintigraphy showed increased uptake of left proximal clavicula at the level of the sclerotic area, but not at the sternal side in sternoclavicular transition (**Figure 2**). Together, the clinical

picture with this radiological pattern confirmed the diagnosis of SCCH.

As pain was inadequately controlled with daily NSAIDs, the patient was started on intravenous pamidronate in a 5-day regimen of 15 mg per day, in 3-month intervals (**Figure 1**). Diclofenac was recommended when needed and the patient was advised to quit smoking. Therapy was effective in reducing pain, erythema and warmth of the sternoclavicular region. After 1 year of treatment, VAS score decreased to 6, range of motion was fully restored and CT imaging as well as skeletal scintigraphy showcased a slight reduction in sclerosis as well as bone activity compared to baseline imaging (**Figure 2**). Pamidronate was temporarily stopped after 2.5 years of treatment but restarted in a modified 4-day regimen 15 mg per day in 4 monthly intervals, due to recurrence of severe pain. After 4.5 years since the start of pamidronate treatment, remission was established on the grounds of absence of pain (NRS 0), restored shoulder function (FROM), no need for complementary diclofenac, and complete normalization of bone activity on skeleton scintigraphy (**Figure 2**). Most remarkably, CT-imaging showcased an almost complete resolution of former sclerosis, leaving only a minimal rest.

DISCUSSION AND CONCLUSIONS

SCCH is a rare inflammatory bone disorder, of which the precise underlying mechanisms remain yet to be determined. Low awareness among physicians, consequent diagnostic and therapeutic delay, and associated decreased quality of life and psychological wellbeing of patients, all contribute to a challenging care process (5, 17).

The nomenclature surrounding SCCH is complex and potentially confusing. Chronic non-bacterial osteomyelitis (CNO) functions as the umbrella term covering SCCH and its related clinical entities. Among these are chronic recurrent multifocal osteomyelitis (CRMO), a predominately pediatric condition typically localized in the metaphyses of long bones of the extremities and the medial clavicles, in addition to other less frequent locations such as vertebral bodies, pelvis, ribs, and mandible (18), and diffuse sclerotic osteomyelitis (DSO), a disease of the mandible in specific (19). Also belonging to the CNO spectrum is the earlier mentioned SAPHO syndrome, of which there is ongoing discussion whether it is the adult version of CRMO (18). Coming to, SCCH, the semantic complexity increases with literature presenting SCCH as being a part of SAPHO syndrome as well as SCCH in isolated form (4, 5). In this paper, we deliberately use both definitions of SCCH, as we perceive that many patients (such as the patient in case 2) with characterized SCCH do not match the rest of the SAPHO acronym. In addition, signs of systemic inflammation such as elevated erythrocyte sedimentation rate are frequently seen in SAPHO syndrome (18), but are usually absent in isolated SCCH (6). In the two cases presented, though, this distinction was not present. The use of SCCH as a separate term, with or without other SAPHO manifestations present, can also be useful when deciding on therapeutic strategy. Bisphosphonates such as pamidronate have shown to improve bone, but not cutaneous manifestations of SAPHO syndrome (20), qualifying them for the treatment of isolated SCCH, illustrated in case 2, or for the SCCH component of SAPHO causing the highest disease burden, such as in case 1. For patients suffering from the wider symptomatology of SAPHO syndrome, anti-inflammatory drugs such as methotrexate and/or biologicals may be an alternative treatment choice (18, 20).

The diagnosis of SCCH can thus be made by the combination of clinical and radiological characteristics, and after the evaluation of manifestations characteristic for an overarching SAPHO syndrome. As briefly mentioned before, serological markers, inflammatory parameters and bone turnover markers are usually normal in SCCH, and used to exclude further differential diagnoses, such as infection, neoplasm or spondyloarthropathies (1, 6). In the two presented cases, diagnosis of SCCH was, in line with the trend in literature, only made after years of pain and shoulder dysfunction, constructing significant burden and decreased quality of life. Radiographically, both cases presented with features consistent with SCCH. SCCH is characterized by sclerosis and hyperostosis typically in the proximal clavicles, sternum and upper ribs, perchance accompanied by secondary degeneration and bony erosions of the sternoclavicular joint (1, 21). These findings are better and earlier seen on computed tomography (CT) than on conventional radiography (21), however clear radiographical criteria are lacking. On skeletal scintigraphy, increased uptake in the sternoclavicular joint area, the costal cartilages of the first and second ribs, and the manubrium sterni is highly suggestive of SCCH and considered a hallmark of disease, and in its symmetrical form referred to as the "bull head sign" (18, 22). As for (full body) MRI, this imaging modality is preferred in (pediatric) CRMO patients due to its sensitivity to detect bone marrow edema, arthritis/synovitis (a common manifestation of this disease) and so as to evade radiation exposure (23). In SCCH, however, bone marrow edema is not commonly present, especially not in later stages of the disease. Since the majority of patients present with significant diagnostic delay (24), at which point the sclerosis and hyperostosis are the more typical characteristics, CT proves an adequate imaging modality, and combining the scan with full body scintigraphy enables the detection of subclinical lesions as well (18, 21, 25).

As there are no disease tracking biomarkers nor established therapy for SCCH, treatment decisions are primarily made on empirical data and expert opinion. Since over two decades patients with active isolated SCCH (and thus suffering mostly from the bone manifestations) and refractory to NSAIDs are treated with intravenous pamidronate with satisfactory effect on pain and shoulder mobility. The rationale for treatment with bisphosphonates evolves from their inhibitory effect on bone turnover, especially at sites where this turnover is elevated (26). For SCCH patients, this increased turnover presumably lies at the root of pain and herewith suggests the potential effect of bisphosphonates. In other metabolic bone diseases characterized by local increase in bone turnover such as Paget's disease of the bone, this effect has been observed as well (27, 28). A second mechanism of action might derive from the anti-inflammatory properties of bisphosphonates due to their inhibition of Farsenyl

Pyrophosphate dependent macrophages *via* the melavonate pathway (29), and them decreasing the level of circulating gamma/delta T cells, a subset of CD3+ T cells (30). Additionally, pamidronate in specific is known to cut down on lymphocyte proliferation and lymphocyte/monocyte interaction (31, 32).

Should patients be refractory to bisphosphonate therapy, alternative treatment options are (limitedly) at hand including the afore mentioned biologicals, which have shown their effect on both skin and bone lesions in SAPHO syndrome (18). There is some data on the positive effect of denosumab in DSO patients refractory to bisphosphonates (33). However, experience is limited to single cases, denosumab lacks the specific distribution to areas of increased bone turnover, and is associated with risk of rebound in osteoporosis (34).

Pamidronate confirmed its favorable clinical effect in the two cases presented; pain and compromised shoulder function significantly decreased, and in both patients we were able to stop treatment after several years. As for radiological features, the rate of local bone turnover typically fluctuates, following the relapse remitting character of disease course. Structural change, however, is commonly progressive, developing from enthesopathy of the costoclavicular ligament with erosion and increased bone turnover, into increased local sclerosis, followed by further hyperostosis and involvement of soft tissue. The latter often results in continuous pain due to secondary degenerative changes, altogether leading to further disease burden and impaired quality of life (5, 17, 21). In our two cases, we observed a local reduction in bone turnover on scintigraphy in both, with persistent sclerosis in the first, and, remarkably, resolution of sclerosis in the second. For CRMO, full resolution of lesions on MRI in children after both NSAID and pamidronate therapy has been described earlier (35–37), just like for DSO an improvement of structural bone changes after pamidronate therapy has been reported (38, 39). A recent randomized study on the effect of pamidronate in CNO patients in general did report significant improvement on radiological disease activity, but not on chronic inflammatory changes (40). Thus, for SCCH the potential of full resolution of sclerosis has, to our knowledge, not been firmly established yet. The observation of sclerosis resolving in our patient is therefore relevant, as the regression plausibly lowers the chance of secondary degenerative transformation, and therefore may prevent permanent disability. Hence, considering this a potential treatment outcome, the grounds on which pamidronate treatment is given for SCCH are slightly strengthened.

However, the implications of our findings need to be approached with caution. Firstly, it cannot be undoubtedly stated that the resolution of sclerosis in patient 2 is a direct result of pamidronate-treatment. The possibility of spontaneous improvement remains, and can only sufficiently be rejected when the treatment modality of pamidronate is systematically researched. Nonetheless, the quick and major clinical response of longstanding complaints in the presented cases, topped by the radiological regression of sclerosis in the second, does suggest a relation. Besides, SCCH being a rare and poorly recognized disease, only further emphasizes the need for more standardized studies.

In conclusion, this report contained two cases with typical presentation of SCCH: significant diagnostic delay with a complex diagnostic process, fluctuating disease course with positive effect of pamidronate-treatment. In case 1, we observed a decrease in pain and shoulder complaints, and reduced uptake on bone scintigraphy, whereas structural radiological changes including sclerosis persevered, in line with typical disease course. The resolution of sclerosis on top of the similar clinical effect in case 2 was, on the contrary, novel. This finding suggests that pamidronate-treatment might not only be effective in reducing pain and shoulder dysfunction, but may even reverse structural tissue transformation and therefore possibly restrain degenerative changes in the SCCH-affected area.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article. Written consent was acquired from both patients to establish and publish this report.

AUTHOR CONTRIBUTIONS

AL and EW collected and interpreted the data concerning the two cases, and drafted the report. AC assessed, described, and interpreted the radiological and nuclear imaging content of the two cases. AR and NA-D revised the report critically for intellectual content. EW supervised AL and also revised the report critically. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

PERSPECTIVE
published: 10 November 2021
doi: 10.3389/fendo.2021.732728

Fibrodysplasia Ossificans Progressiva: What Have We Achieved and Where Are We Now? Follow-up to the 2015 Lorentz Workshop

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OPEN ACCESS

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Reviewed by:
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Specialty section:
This article was submitted to Bone Research, a section of the journal Frontiers in Endocrinology

Received: *29 June 2021*
Accepted: *22 September 2021*
Published: *10 November 2021*

Citation:
de Ruiter RD, Smilde BJ, Pals G, Bravenboer N, Knaus P, Schoenmaker T, Botman E, Sánchez-Duffhues G, Pacifici M, Pignolo RJ, Shore EM, van Egmond M, Van Oosterwyck H, Kaplan FS, Hsiao EC, Yu PB, Bocciardin R, De Cunto CL, Longo Ribeiro Delai P, de Vries TJ, Hilderbrandt S, Jasper RT, Keen R, Koolwijk P, Morhart R, Netelenbos JC, Rustemeyer R, Scott C, Stockklausner C, ten Dijke P, Triffit J, Ventura F, Ravazzolo R, Micha D and Eekhoff EMW (2021) Fibrodysplasia Ossificans Progressiva: What Have We Achieved and Where Are We Now? Follow-up to the 2015 Lorentz Workshop. Front. Endocrinol. 12:732728. doi: 10.3389/fendo.2021.732728

Fibrodysplasia ossificans progressiva (FOP) is an ultra-rare progressive genetic disease effecting one in a million individuals. During their life, patients with FOP progressively develop bone in the soft tissues resulting in increasing immobility and early death. A mutation in the *ACVR1* gene was identified as the causative mutation of FOP in 2006. After this, the pathophysiology of FOP has been further elucidated through the efforts of research groups worldwide. In 2015, a workshop was held to gather these groups and discuss the new challenges in FOP research. Here we present an overview and update on these topics.

Keywords: fibrodysplasia ossificans progessiva (FOP), trials, therapy, disease models, inflammation, angiogenesis

INTRODUCTION

Fibrodysplasia ossificans progressiva (FOP) is an ultra-rare progressive genetic disease characterized by heterotopic ossification (HO) of muscles, tendons and ligaments, often preceded by periodic painful soft tissue swellings called flare-ups. During their lives, patients develop a "second" skeleton, resulting in increasing immobility and early death often due to thoracic insufficiency, infectious diseases, and traumatic falls (1).

Progress of FOP research (**Figure 1**) has been slow due to three main factors. Firstly, obtaining tissue samples to examine the pathophysiology is difficult. Biopsies are contraindicated because of the increased risk for flare-ups in FOP. Secondly, FOP is frequently misdiagnosed, and so systematic data on early pathophysiology has been difficult to obtain. Finally, for a long time there were no cell or animal models for FOP as the causative genetic mutation was unknown. In 2006, the genetic cause of FOP was identified to be a missense mutation (R206H) in the *ACVR1* gene encoding the activin receptor-like kinase (ALK2) (2). The mutation induces hyperactivity of the ALK2 in response to bone morphogenetic protein (BMP) ligands as well as constitutive activity in the absence of ligands (3, 4). Also, while activing A induces ALK4-mediated canonical SMAD 2/3 signaling, the mutated ALK2 causes activin A to induce SMAD 1/5/9 signaling too, resulting in a skeletogenic signal instead of the usual response to activin A (5).

To date, there are no approved treatments to stop or reverse this disease, no biomarker to quantify FOP activity and many unanswered questions regarding pathophysiology.

In 2015, a Lorentz workshop was held, bringing international experts with a range of scientific backgrounds relevant to FOP research together for a week of scientific workshops discussing complex research problems and stimulating new initiatives for FOP treatment. Here we provide a comprehensive survey about the recent developments of basic and translational research on FOP.

IDENTIFICATION OF HETEROTOPIC OSSIFICATION PROGENITOR CELLS

HO is a complex, multi-stage process involving various cell types (6), but the exact progenitor cells that form the heterotopic bone are yet to be identified. Multiple populations of progenitor cells associated with muscle tissue have demonstrated osteogenic

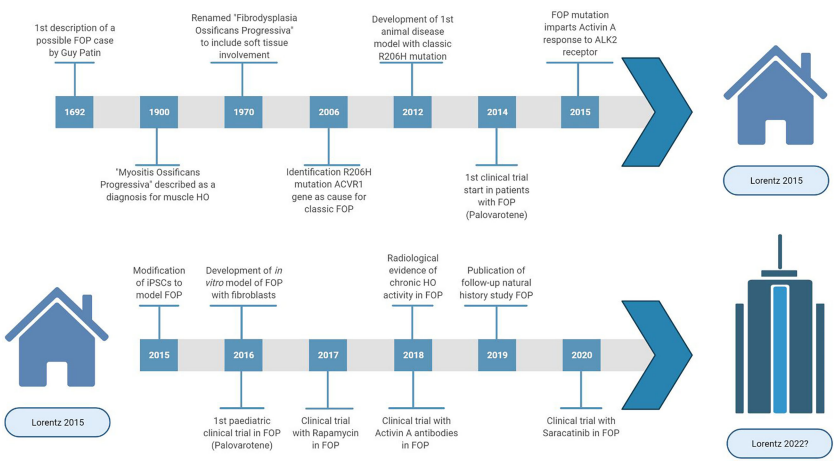

FIGURE 1 | Highlights and key discoveries in FOP research leading up to the 2015 Lorentz meeting and after.

differentiation. Muscle stem cells (MuSCs) are muscle-resident stem cells essential for muscle growth and regeneration (7) and were initially a leading candidate for the HO progenitor cell. However, *in vivo* lineage tracing studies have shown that these cells do not significantly contribute to BMP-induced HO (8, 9), strongly arguing against MuSCs inducing HO in FOP (10).

Endothelial cells (EC) have also been proposed as a progenitor cell candidate. The endothelial marker Tie2 has been found in chondrocytes and osteoblasts in histological examination of HO tissues from individuals with FOP (11) and lineage tracing studies have identified Tie2 expression in roughly half the chondrocytes and osteocytes in heterotopic bone (11). However, Tie2 is not specific to ECs and more than 90% of the Tie2+ cells found in heterotopic bone are also platelet-derived growth factor receptor (PDGFR)$\alpha^{+}Sca1^{+}$ indicating a mesenchymal rather than an endothelial origin (12).

In fact, these markers are also present in fibro/adipogenic progenitors (FAPs), another muscle tissue-resident progenitor. Cre/lox lineage tracing showed that FAPs can cause injury-induced and spontaneous HO in a FOP mouse model, greatly dependent on activin A signaling (13). Given the complexity of bone formation, perhaps cells from multiple origins are present and involved in ultimately forming the heterotopic bone.

INFLAMMATORY TRIGGERS OF HO

The contribution of the immune system in FOP is a keen focus of research. HO lesions harbor many cells of the immune system, such as lymphocytes, macrophages and mast cells (14, 15). Depletion of mast cells and macrophages have been reported to reduce HO volume in a FOP mouse model (16). The role of macrophages in HO has been investigated in different *in vivo* models with differing results (17, 18), leading to the idea that macrophage populations in FOP lesions are more heterogeneous than often presumed and may be responding to different types of injury signals.

The ALK2 mutation is also present in other cell types. Thus, the mutated ALK2 likely also affects immune responses. ECSIT (Evolutionarily Conserved Signal Intermediate in the Toll pathway) has been reported as a possible mechanism linking toll-like receptor activation in the innate immune response to aberrant SMAD signaling in FOP (19).

Blood samples taken from patients with FOP without symptoms of a flare-up have shown significantly elevated levels of pro-inflammatory interleukins indicating that patients with FOP may be in a constant pro-inflammatory state. Monocytes derived from patients with FOP, when stimulated with lipopolysaccharide, showed prolonged and increased cytokine and chemokine secretion, and prolonged activation of nuclear factor (NF)-κB (20, 21). A study of peripheral blood mononuclear cells from patients with FOP showed increased expression levels of DNAX accessory molecule-1 (DNAM-1) in monocytes, suggesting a functional effect in monocyte migration, and could represent a biomarker for the inflammatory state in FOP (22). Monocytes are also precursors for circulating osteogenic cells found in FOP lesions (23).

The hypoxic condition in inflamed tissues is another factor contributing to FOP pathogenesis, possibly through hypoxia inducible factor-1-α (HIF-1-α) which has been reported to promote amplification of BMP signaling through retention of the mutated ALK2 receptor in signaling endosomes (24). The fibroproliferative stage with extracellular matrix production that normally occurs after injury also appears to be overactive in FOP, leading to tissue stiffening and increased mechano-sensitivity in favor of osteogenic processes (25).

VASCULARIZATION IN FOP

Angiogenesis is an important process involved in the development of FOP lesions. The inflammation, soft tissue destruction, and subsequent infiltration of immune cells all depend on vascularization. In the fibroproliferative phase the inflamed tissue is infiltrated by chondrocytes promoting a proteoglycan-enriched environment, which becomes progressively hypoxic. Hypoxic conditions favor chondrocyte differentiation partially by sustaining BMP signaling activation (24), and induce expression of vascular endothelial growth factor (VEGF), promoting the infiltration of blood vessels, which in turn drives endochondral bone formation. Interestingly, monocytes isolated from FOP patients showed increased VEGF secretion upon an inflammatory trigger compared to controls (18).

BMP and VEGF signaling play key roles in regulating blood vessel homeostasis; gene mutations in components of the BMP signaling pathway are associated with cardiovascular conditions (26), and disturbances in the angiogenesis-osteogenesis axis can cause bone disorders (27). Whether the mutant FOP ALK2 also disturbs EC function through aberrant BMP signaling is currently under investigation.

Angiogenesis is initiated by the formation of tip cells supported by proliferating stalk cells to forming new sprouts from pre-existing vessels. This process is coordinated by VEGF-, BMP2- and BMP6 signaling. During angiogenesis, BMP2 primarily signals through ALK3, whereas BMP6 signals through ALK2. Upon ALK2 knockdown, hypersprouting was observed in *in vitro* EC models, whereas ALK3 knockdown appeared to have the opposite effect (28). Recent data showed that EC's derived from human induced pluripotent stem cells (hiPSC) follow the same principle and hiPSCs derived from patients with FOP show activin A induced SMAD 1/5 signaling (29).

Vascular leakage and edema have also been described in HO lesions in FOP (30). BMP6 stimulation in ECs causes internalization of VE-cadherin changing the endothelial architecture. VE-cadherin in turn appears to interact with ALK2 in a ligand-dependent manner by stabilizing the BMP receptors in the EC junctions (31). ECs from patients with FOP appear to have decreased expression of vascular endothelial (VE)-cadherin under inflammatory conditions (32), possibly due to an altered interaction of VE-cadherin signaling with the mutated ALK2 receptor complex.

SUITABILITY OF FOP DISEASE MODELS

Since the discovery of the mutation (2), several cellular and animal models have been developed to examine the effects of FOP ALK2 mutations on BMP signaling and chondro/osteogenesis.

Availability of human cell models is limited due to restrictions on obtaining patient material and our incomplete knowledge of the progenitor cell types relevant to FOP HO. Dermal fibroblasts derived from patients with FOP have been successfully transdifferentiated to cells of an osteogenic lineage (33). Periodontal ligament fibroblasts have also been isolated and induced to osteogenesis and osteoclastogenesis (34). hiPSCs obtained from patients with FOP are able to differentiate to ECs (29, 35, 36) and pericytes with increased mineralization, but did not develop into mature osteoblasts (36). Connective tissue progenitor cells from discarded primary teeth have been used to examine the effects of FOP mutations on BMP signaling and chondrogenic/osteogenic differentiation (19, 24, 37, 38). C2C12 myoblasts have been altered to express $ALK2^{R206H}$ with doxycycline dependent promoter to simulate FOP (39).

A fruit fly model carrying the classical R206H mutation demonstrated over activation of BMP signaling by the $ALK2^{R206H}$ receptor but also ligand independent signaling of the receptor (40), consistent with earlier *in vitro* analyses (41–43). An embryonic chicken model was used to study the role of several ALK2 mutations and demonstrated that the FOP $ALK2^{Q207E}$ and $ALK2^{R206H}$ mutation, along with the engineered constitutively active $ALK2^{Q207D}$ mutation, caused FOP-like phenotypes with skeletal malformations and HO (44).

In mice, activating mutations in ALK2 are lethal during embryonic development (45), therefore investigations of the *in vivo* effects of ALK2 activating mutations have required either chimeric/mosaic expression of mutant cells or a conditional gene expression model. The first such mouse model, using a Cre-Lox inducible ALK^{Q207D} transgene was developed prior to the identification of ALK2 as relevant to FOP (45). Later, this model was used with adenovirus expressing Cre and tamoxifen-responsive Cre alleles to induce postnatal activation of the $ALK2^{Q207D}$ transgene (46). Although the $ALK2^{Q207D}$ substitution is not a naturally occurring FOP mutation in humans, these mouse models provided the first mammalian systems to study the effects of excessive BMP signaling by ALK2, importantly demonstrating a requirement for tissue injury and inflammation in addition to mutant ALK2 expression for the development of heterotopic bone (47).

Subsequently, researchers have developed mouse models harboring the common FOP $ALK2^{R206H}$ mutation. A chimeric model with a variable proportion of cells expressing a heterozygous $ALK2^{R206H}$ allele yielded intermittent live births, mimicking classic FOP features such as HO development in response to muscle injury, hind limb digit malformation, and joint fusions (48). A Cre-dependent knock-in model with inducible $ALK2^{R206H}$ expression has been used to mimic HO formation in response to various injuries, highlighting the importance of activin A in ALK^{R206H} signaling function (49–52). The progression of HO formation in $ALK2^{R206H}$ mouse models appears to closely reproduce the events of HO formation from an early-stage immune cell response to a robust fibroproliferative stage that transitions to endochondral ossification (15, 16, 48). These models also feature the distinct patterns of HO within the axial and extra-axial skeleton and exhibit both injury-dependent and spontaneous progression of HO (50, 52).

A zebrafish FOP model has also been developed and embryonic development assays have been used to investigate the mechanism through which mutant ALK2 receptors enhance BMP-phosphorylated (p)SMAD 1/5 signaling (53–55).

A novel approach is a computational disease model. Computational models of endochondral ossification have previously been developed (56, 57). In these models the interplay between growth factors, angiogenesis, oxygen, recruitment, proliferation and differentiation of osteoprogenitor cells can be considered. These models could be adapted to simulate endochondral ossification in FOP and provide an additional way to evaluate the effect of therapeutic interventions in FOP.

In summary, there are numerous *in vitro* and *in vivo* models available with the potential to further investigate and understand FOP. It will also be important to establish how closely these model systems reflect the pathophysiology of FOP in humans and how well they address the various complexities of the FOP phenotype. Acknowledging the advantages and disadvantages of each of these models can allow them to complement each other, maximizing the information gained in preclinical FOP research.

POSSIBLE TARGETS FOR THERAPY IN FOP

Despite many efforts, still there is no effective and specific treatment approved for FOP. Therapy is focused on treating flare-ups with glucocorticoids and nonsteroidal anti-inflammatory drugs upon presentation (58). Taking the different stages of HO in FOP into consideration it is possible to identify different processes which can be considered as targets to develop therapies by different approaches (**Figure 2**).

Saracatinib, a kinase inhibitor targeting src-family kinases originally developed as a treatment for various solid tumors, is a potent ALK2 inhibitor with efficacy against HO in preclinical models and is now being repositioned as a potential treatment for FOP in an ongoing phase 2 clinical trial (NCT04307953) (59). Several other ALK2 inhibitors have been developed with the goal of improving potency and selectivity for ALK2 receptor inhibition, with promising safety in phase 1 studies and are anticipated to advance to phase 2 efficacy studies in the near future.

Alternatively, the stimulation of the ALK2 receptor by ALK2 ligands can be prevented. A neutralizing antibody specific for activin A (garetosmab) has been evaluated in a phase 2 clinical trial (NCT03188666) after promising preclinical results (49). Recently, mTOR (mammalian target of rapamycin) has been identified as a key factor in the early hypoxic and inflammatory stages of HO (21). Besides its important immunoregulatory function, mTOR signaling is required for chondrogenesis and

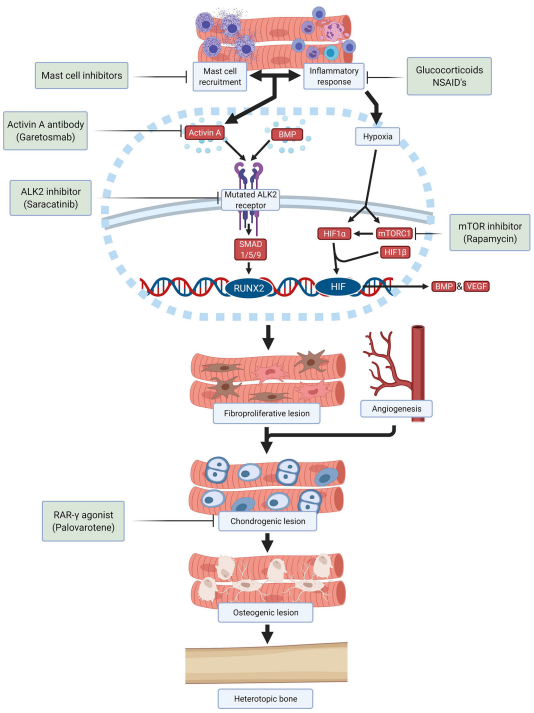

FIGURE 2 | Schematic overview of drugs and investigational compounds currently used and/or evaluated in FOP treatment and their respective targets.

osteogenesis induction. Crosstalk between mTOR signaling and BMP signaling may amplify HO in FOP (60). In preclinical studies, rapamycin successfully inhibited HO in a mouse model and a clinical trial is being performed to evaluate its efficacy and safety in patients with FOP (UMIN000028429) (60, 61).

Downstream signaling initiated by activation of ALK2 also offers opportunities to prevent HO. Palovarotene, a retinoic acid receptor-gamma (RAR-γ) agonist, inhibits HO in FOP mouse models by blocking chondrogenic differentiation of the progenitor cells and is currently being investigated in multiple phase 2 and phase 3 trials (NCT02279095, NCT02190747, NCT03312634) (51, 62, 63). Other therapies being investigated are VEGF inhibitors, ligand traps, phosphoinositide 3-kinases (PI3K)-inhibitors, siRNAs, HIF1-α blockers and transforming growth factor-β activated kinase (TAK)1 inhibitors. Once a successful therapeutic strategy for preventing HO in FOP is available, surgical intervention may become feasible for excising heterotopic bone and restoring function.

CLINICAL TRIALS IN ULTRA-RARE DISEASES

Therapeutic development in FOP shares many challenges faced by other ultra-rare diseases such as a limited understanding of natural history to inform trial design, dearth of validated and surrogate outcome measures to quantify the disease during the limited time span of a clinical trial, and small numbers of patients available for clinical trials (64, 65).

The randomized controlled trial (RCT) is the gold standard for determining drug efficacy in a clinical trial setting. Randomization minimizes selection bias and distributes potential confounders between study groups. RCT power decreases rapidly with diminished smaller cohorts as inter-individual differences become more pronounced, increasing the risk of known and unknown covariates affecting the trial results.

An uncontrolled trial may be feasible when the natural history of a disease is well-established. In this design, the effect of the intervention can be compared against the natural history of the disease. In FOP however, the natural history of the disease is still being investigated and it is known that disease progression varies between individuals (66). Additionally, subject may report less adverse events in a non-interventional natural study than in an interventional clinical trial, creating bias against the drug. However, studies have been performed to mitigate this potential bias (67).

Both trial designs have their drawbacks but remain important options for determining drug efficacy in FOP. Future trials in FOP should acknowledge these disadvantages, implementing smart trial designs and statistical methods to address inherent limitations of a small and heterogeneous population, thus maximizing the information obtained while supporting patient safety (65, 68). There is an urgent need to establish an imaginative and equitable approach towards clinical trials in FOP given the multitude of drugs being developed and the limited number of patients.

DETERMINATION OF DISEASE ACTIVITY IN FOP

Another problem that FOP faces is the difficulty to evaluate individual disease activity. Clinical symptoms of a flare-up such as pain, swelling, erythema and warmth are non-specific, and it is not possible to predict whether the acute phase will end up with HO or will resolve (66). A multitude of inflammatory, chondrogenic and osteogenic bone markers have been investigated, and although some were markedly elevated in patients with FOP, none have shown an association with disease activity or been able to predict HO formation adequately (69–71).

Conventional imaging techniques are only able to detect HO after formation of bone tissue. MRI (magnetic resonance imaging) and ultrasonography are suitable to detect soft tissue edema associated with the inflammatory stage of HO but are non-specific and unable to reveal bone formation (72, 73). Nucleotide imaging such as the [^{18}F]-sodium fluoride (NaF) PET (positron emission tomography) scan can detect bone formation before it is visible on conventional CT (computed tomography). Interestingly, PET/CT and MRI scanning revealed that not every flare-up resulted in HO and showed continuous FOP activity not related to a flare-up (74).

Determination of disease activity with a suitable biomarker and imaging techniques is necessary for evaluation of potential

therapies in FOP. A combination of markers may be needed to reflect the multiple stages of HO in FOP; ongoing efforts exist on FOP biomarker development (20).

DISCUSSION AND FUTURE RESEARCH

Looking back at the topics discussed in 2015, the meeting identified key issues in which progress has been made through collaborative approaches (**Figure 1**). However, it is also clear that FOP research and treatment still face many challenges. Big questions remain regarding the pathophysiology of FOP such as the identity of the HO progenitor cell and the effect of the ALK2 mutation on the immune response and angiogenesis. Also, with the advent of clinical trials for FOP, it has become clear that we still need to obtain as much information as possible in the preclinical phase including cell and molecular mechanisms. This requires further use and development of *in vitro* and *in vivo* disease models, and perhaps exploring options such as computational modelling. During clinical trials, the information gained must be maximized through means of careful trial design and proper evaluation of disease activity. To achieve this in FOP, international collaboration is paramount and has to be fostered. Maybe the time is ripe to make a point and gather the FOP research community in a new meeting to share and discuss the most recent research strategies again.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

RdR wrote the manuscript and created the figures with input from all authors. EE, GP, NB, PKn, DM, and RR organised the original workshop in which topics were discussed. GP, NB, PK, TS, GSD, MP, RP, ES, ME, HO, PY, RR, DM, EE, RB, CC, TV, SH, RJ, RK, PKo, RM, CN, PD, JT, and FV all attended the 2015 workshop, provided valuable contributions to the discussions and comments on the manuscript. RdR, BS, EB, TR, ES, FK, CSc, CSt, PD, and EH all provided valuable updates to the discussion of the research done since the 2015 meeting. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

We thank the Lorentz organization for their help and facilities making this workshop possible. The workshop was made possible with grants by the Lorentz Center, the Dutch FOP foundation and ZonMw. Images were created with Biorender.com.

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