Unravelling Heterotopic Ossification in FOP

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Chapter 1

General Introduction

General Introduction

Fibrodysplasia ossificans progressiva (FOP) is a rare disease in which heterotopic ossification (HO) forms in muscles, tendons and ligaments. In the past decade, knowledge about its pathophysiology has greatly improved and clinical trials to slow down or even stop disease progression are now emerging. The natural course of the disease, however, is not known. Moreover, a marker for the disease's biological activity is not yet available. This lack of an imaging biomarker of FOP forms the basis of this thesis in which promising imaging techniques were investigated for studying the development of HO during the natural course of the disease.

Clinical aspects

FOP is an autosomal dominant disease with a prevalence of 1.5 to 2 per million persons[1-4]. The first phenotypic sign of FOP is the congenital monophalangic hallux valgus[3,5-7]. In the majority of patients, this deformity is not recognised as a sign of FOP[8]. Often, a diagnostic trajectory is not started before the first signs of disease activity become apparent. Although the age at which this disease activity, better known as a 'flare-up', is noticed varies greatly, the mean age is thought to be around 6 years[6,9]. A flare-up is an inflammatory process of which the pathophysiology is not completely understood[9,10]. Typically, flare-ups are presented with symptoms of redness, swelling and pain[10]. Flare-ups can arise spontaneously or be triggered by trauma and infection[9]. Although a proportion of flare-ups resolve without sequelae, the majority is followed by the formation of HO[9]. This HO forms in muscles, tendons and ligaments, limiting mobility especially when located in or around a joint[7]. Initially, flare-ups affect the thoracic region, neck and back but with aging, flare-ups, and thus HO formation, occur more distally, affecting shoulders, hips, elbows and knees[9]. Due to its progressive character, patients often are wheelchair bound at the age of 30[11] and, life-expectancy is limited[12]. The progressive ossification around the thorax restricts normal lung expansion. This results in a restrictive pulmonary function, which is thought to result in pulmonary hypertension and right decompensatio cordis [12-14]. It is generally accepted that cardiorespiratory complications are the main cause of premature death in FOP patients. At present, there is no effective treatment to prevent flare-ups or to avoid HO development. Currently, glucocorticoids and nonsteroidal anti-inflammatory drugs (NSAIDs) are used in an attempt to prevent disease progression[10,15]. Its effectiveness, however, has never been investigated.

Pathophysiology

From 2006 on, the understanding of the disease has expanded extensively based on the discovery of the underlying genetic mutation. Ninety-five percent of the FOP patients share the same mutation, the 'classic mutation'. The remaining five percent has one of the twelve 'variant' mutations[16]. All FOP mutations are located in the Activin A receptor type 1 (ACVR1) gene, encoding for a bone morphogenetic protein (BMP) type 1 receptor, also known as

Activin receptor-like kinase-2 (ALK2)[17]. This receptor is a serine/threonine kinase receptor consisting of functional domains. It has an n-terminal domain for ligand binding, a transmembrane domain and a cytoplasmic C-terminal kinase domain. The latter consist of a glycine-serine (GS) domain and a protein kinase domain[18]. The FOP mutation is located in the cytoplasmic C-terminal kinase domain, with the majority of mutations (including the classic mutation) in the GS domain[17]. The classic mutation is a missense mutation that encodes for Histidine instead of Arginine (R206H)[17]. Patients with a variant mutation might be either phenotypically similar to patients with the classical mutation, or have additional developmental abnormalities, e.g. alopecia, nail dysplasia or learning disorders[16]. It is hypothesized that the severity of HO formation and progression depends on the location of the mutation. However, environmental factors are also known to play a crucial role[19].

Normally, a BMP type I/ALK2 receptor dimer forms a complex with a BMP type II dimer upon binding a ligand like BMP. Once bound, the type II receptors phosphorylate the GS domain of the BMP type 1 receptors. This phosphorylation activates the type 1 receptors, resulting in induction of the BMP pathway. This BMP pathway includes SMAD 1/5/8 and p38 mitogen-activated protein kinase (MAPK) signalling[18,20], which, both directly and indirectly, promote transcription of genes involved in osteogenesis. When no BMP is bound to the receptor complex, the FK506 binding protein 12 (FKBP12) binds to the GS domain, inactivating the receptor. In addition, through SMAD7-Smurf1, FKBP12 regulates the number of ALK1 receptors on the cell membrane. FKBP12 dissociates from the GS domain and forms a complex with FK506 when a ligand is bound to the receptor complex and the GS domain is phosphorylated [21,22]. Apart from BMP, Activin A can act as a ligand for the receptor complex as well. Activin A is secreted by immune cells and is released at sites of tissue damage. When bound to the ALK1/ALK2 receptor complex, it does not activate the SMAD 1/5/8 pathway, but instead inhibits signalling by the receptor. Activin A thus competes with BMP for binding to the receptors[18]. In the presence of the FOP mutation, the GS domain or its direct environment is altered due to the missense mutation. Due to the altered GS domain, FKBP12 cannot bind anymore to inhibit signaling in the absence of a ligand. This causes a constitutive activity of BMP signaling. [23]. In addition, once BMP binds, there is a hypersensitive reaction leading to stronger BMP signalling (Figure 1). Finally, in the presence of an ALK2-mutation, Activin A loses its inhibitory role and instead activates SMAD1/5/8[24]. Through all these processes, osteogenesis is enhanced, resulting in the formation of HO.

Heterotopic Ossification

The main characteristic of FOP disease activity is a flare-up and the successive formation of HO[7,9,11]. Flare-ups may develop spontaneously, can be provoked by a viral infection (i.e. influenza) or can be triggered by a local trauma that damages muscle, tendon or ligamentous structures [9]. Traumas that cause sufficient tissue damage are, for instance, a fall or a medical intervention (e.g. intramuscular immunization or surgical intervention)[8,9,25]. Effects of local tissue damage on disease activity locally or elsewhere in the body are largely unknown due

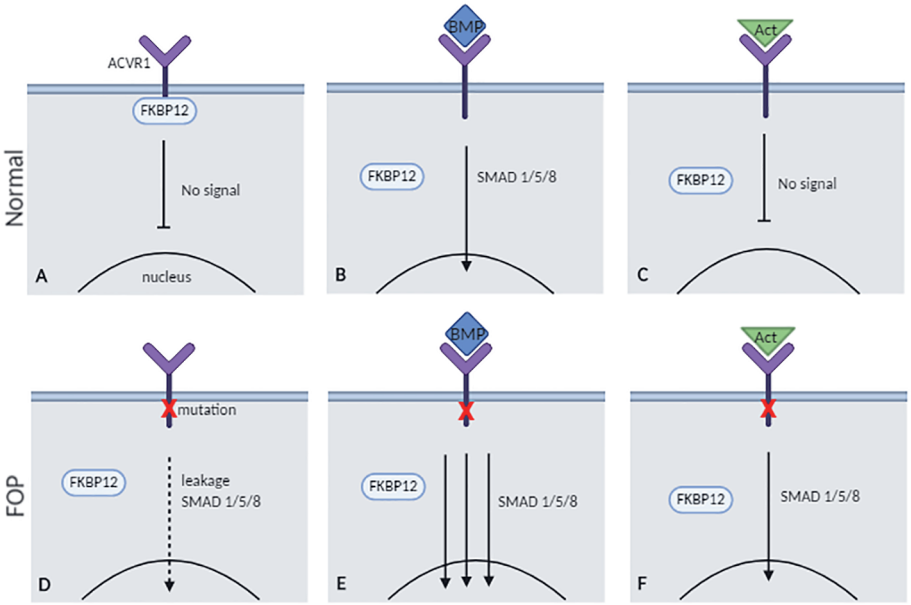

Figure 1. In the absence of ligands, FKBP12 binds to the ALK2 receptor to prevent signaling (A). When a ligand binds, FKBP12 dissociates form the ALK2 receptor. The ligand than promotes signaling of the receptor, including the SMAD 1/5/8 pathway (B). In the presence of Activin A, FKBP12 dissociates but signaling is inhibited (C). In the presence of a mutation at or near the GS domain of the ALK2 receptor, FKBP12 can no longer properly bind to the GS domain of the receptor. Therefore, in the absence of a ligand, there is leakage of ALK2 signaling (D). In addition, when a ligand binds to the mutated receptor, increased signaling is promoted (E). When Activin A binds the mutated receptor, the SMAD 1/5/8 pathway is activated (F).
Abbreviations: BMP = Bone Morphogenetic Protein; FKBP12 = FK506 binding protein 12; Act = Activin A; FOP = fibrodysplasia ossificans progressiva; ALK2 = Activin receptor-like kinase 2; GS domain = Glycine-Serine domain.

to the lack of a marker of disease activity. The formation of HO after a flare-up can be evaluated with conventional radiographs, X-rays, or computed tomography (CT) scans[26-28]. These techniques, however, only image outcome, and not local and systemically biological activity associated with the disease. Bone biopsies to evaluate local biological activity of bone is contraindicated in FOP, as it may trigger a flare-up. As biopsies are contraindicated, the developmental stages of HO have been unknown for a long time. However, by collecting biopsies obtained of a flare-up in yet undiagnosed FOP patients, more is known about the developmental stages. In the early phase of a flare-up, macrophages, mast cells and lymphocytes infiltrate the lesion and induce apoptosis of the cells. This phase is followed by fibroblast proliferation and extensive angiogenesis and neovascularisation[29,30]. In the final phases, the lesion matures and the fibroproliferative tissue develops into cartilage before it further develops through osteogenesis to the form HO. Formed HO, is thought to resemble normal skeletal bone[29], but histological examination of matured HO is lacking.

Most knowledge on HO has been gathered through surgical excision of newly formed HO because of associated mobility restrictions. Whether this removed HO has already matured, is questionable. Therefore, the question remains whether matured HO is 'normal' bone. High-resolution peripheral quantitative computed tomography (HR-pQCT), a novel technique, might provide a solution. HR-pQCT is able to quantify bone geometry, density, and microarchitecture of both cortical and trabecular bone compartments as well as mechanical properties[31]. This could give insight in for instance fracture risk of HO, as only limited data on fractures of HO are available [32].

Twenty percent of the flare-ups is not succeeded by HO formation, according to a questionnaire among five-hundred FOP patients[9]. To date, it is not possible to distinguish a non-ossifying flare-up from an ossifying flare-up. In addition, it still is not possible to detect disease activity through laboratory tests. Biochemistry tests, including alkaline phosphatase, have shown to remain within normal limits in patients with FOP[33,34]. Inflammatory markers, C-reactive protein and erythrocyte sedimentation rate, were found to be elevated early in the course of HO formation in non-genetic HO[35]. Its value in FOP has been explored in case reports, but no clear relationship between inflammatory markers and a flare-up was seen[36]. Furthermore, this survey among FOP patients also revealed subjective progression of HO in the absence of a flare-up[9]. Both formation during fare-ups and maybe also this asymptomatic progression results in limited mobility of the affected joint[7,9]. In addition, vital organ function may be affected as a result of HO that restricts thoracic movements. This most likely results in restrictive lung disease, as found by two cross-sectional studies among FOP patients [13],[14,37]. The pulmonary function tests used in these studies, however, were only sufficient to suspect – and not confirm – a restrictive pulmonary function. The restrictive pulmonary function is thought to play a key role in the pathophysiology of premature death in FOP[12,13]. Nonetheless, the natural course of the pulmonary function in FOP and its relation to the formation of HO is not known.
Additional research is needed to understand HO development, progression, microstructure and complications. Invasive techniques are contraindicated in FOP, as they might trigger a flare-up[8]. Insight needs to be obtained through non-invasive techniques. To understand the development and progression of HO, a method able to measure biological activity of bone is crucial.

Imaging techniques to increase knowledge on HO

Molecular imaging techniques may be a way to characterise FOP disease activity non-invasively. Amongst all molecular imaging techniques, positron emission tomography (PET) is especially interesting because of its high sensitivity and PET allows quantitative measurements of regional biological processes. For FOP, the tracer ^{18}F-NaF is of interest, as it binds specifically to sites with active bone metabolism[38]. More specifically, ^{18}F-NaF binds to newly formed hydroxyapatite, where ^{18}F-NaF replaces de hydroxyl group to form fluoroapatite[38]. Using PET it is then possible to image (and measure) these binding sites.

In addition, the combined use with CT, also allows for precise localization of areas with increased uptake. Research is needed to explore the value of this technique for both development and progression of HO in FOP. As ^{18}F-NaF PET/CT exposes a patient to radiation, it might not be the imaging technique of choice in the paediatric population. An alternative imaging technique might be magnetic resonance imaging (MRI). MRI makes use of a strong magnetic field, which enables the visualization of soft tissue, e.g. muscles[39]. Despite the fact that MRI is not able to measure biological activity of bone, it may still have a role in the characterisation of disease activity, as one of the signs during a flare-up is oedema[40]. Studies are needed to explore the value of MRI in FOP.

Aims and outlines of the thesis

The aim of the studies described in this thesis was to improve knowledge about development, progression, structure and complications of HO in FOP, and to investigate the effects of different kinds of trauma on disease activity and progression. As invasive techniques are contraindicated and blood markers to detect disease activity are not available, imaging techniques were used to study various aspects of the disease.

The incompletely understood biological processes during a flare-up were investigated through sequential ^{18}F-NaF PET/CT scans in **chapter 2.** Furthermore, in **chapter 3** The natural course of HO in de absence of flare-ups was assessed by sequential ^{18}F-NaF PET/CT-scans**.** The potential value of MRI in assessing disease activity was investigated in **chapter 4** by comparing MRI with ^{18}F-NaF PET/CT**.**

Effects of surgery on flare-ups and the natural course of HO was evaluated in **chapter 5.** And, in **chapter 6** the effects of local radiotherapy on local and systemic disease activity was assessed.

A new imaging modality, HR-pQCT, which can be used to describe geometry, density and microarchitecture of HO is presented in **chapter 6**. Finally, the natural course of pulmonary function and its association with HO volume is assessed in **chapter 7.**

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Chapter 2

^{18}F-NaF PET/CT scan as an early marker of heterotopic ossification in fibrodysplasia ossificans progressiva

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Abstract

Fibrodysplasia ossificans progressiva (FOP) is a rare genetic disease with a progressive course characterized by episodically local flare-ups, which often but not always leads to heterotopic bone formation (HO).
Recently, we showed that ^{18}F-NaF PET/CT may be the first tool to monitor progression of a posttraumatic flare-up leading to new HO, which was demonstrated in a patient with FOP who underwent a maxillofacial surgery.
This paper evaluates ^{18}F-NaF PET/CT as a marker of FOP disease activity, comparing its use with other imaging modalities known in literature. In addition, the follow-up of a spontaneous flare-up in a 19-year old patient is presented showing high muscle ^{18}F-NaF uptake in one defined part within the flare-up area after three weeks. During follow-up, ^{18}F-NaF PET /CT scan revealed newly formed heterotopic bone but only in this previously active ^{18}F-NaF region. In conclusion, increased muscle ^{18}F-NaF uptake may predict future HO development in FOP patients. At present ^{18}F-NaF PET/CT appears to be a sensitive imaging modality to serve as a noninvasive marker for bone formation and to monitor disease activity during flare-ups in FOP.

1. Introduction

Fibrodysplasia ossificans progressiva (FOP) is rare progressive genetic disease characterized by periodical flare-ups which predominantly present as swelling (93%), pain (86%), decreased movement (78%) and stiffness (72%)[1]. Flare-ups may be induced by trauma, inflammation or may develop spontaneously[2]. Most flare-ups lead to heterotopic bone formation (HO) with progressive loss of mobility. The development of HO follows a pattern through swelling, modification of affected skeletal muscle and connective tissue towards endochondral heterotopic bone formation leading to mature mineralized bone [3]. However, it is estimated that about 20% of flare-ups may not proceed into HO formation and resolve completely, without loss of function [1]. Due to incomplete insight in the total flare-up process and the lack of a marker of the disease, the spontaneous course of a flare-up is unpredictable. Recently, the Amsterdam FOP research group identified the ^{18}F-NaF PET/CT scan as possible marker in predicting and monitoring HO formation in a very early phase of a flare-up, which was detected during a follow-up study after surgery in an FOP patient [4].
This article describes the use of ^{18}F-NaF PET/CT as a new imaging modality to monitor disease activity during a flare-up in FOP, comparing the diagnostic value of ^{18}F-NaF PET/CT with other imaging modalities. In addition, we present the first captured spontaneous course of a flare-up in an FOP patient by ^{18}F-NaF PET/CT scanning.

2. Imaging bone formation: the 18F-NaF PET/CT scan

Two imaging modalities are widely available for functional imaging of bone metabolism: bone scintigraphy using ^{99m}Tc-labeled diphosphonates (Tc-99m-hydroxydiphosphonate (99m-HDP) or methylene diphosphonate (99m-MDP)) and [^{18}F] Sodium Fluoride (NaF) PET/CT [5]. ^{99m}Tc-labeled diphosphonates are widely available in general hospitals and are used with gamma cameras, yielding a conventional bone scintigraphy [5]. Both tracers bind to sites of new bone formation and represents osteoblastic activity, but there are clear differences between both techniques.

Sodium fluoride labeled with [^{18}F] (^{18}F-NaF) is a positron emitter, which was first introduced for bone scanning in 1962 by Blau et al [5-7]. In the last decade, the widespread availability of the hybrid PET/CT and its rapid increasing usage in patients with bone metastasis has led to a renewed interests in ^{18}F-NaF PET/CT in spite of the higher costs compared to ^{99m}Tc-MDP bone scanning. The intrinsic high spatial resolution of PET/CT systems offers superior image quality of [^{18}F]fluoride PET images which is also of interest for the detection of osseous lesions such as heterotopic bone. Studies comparing [^{18}F]fluoride PET with bone scintigraphy support the supremacy of ^{18}F-NaF PET in detecting osseous lesions [8-10]. The [^{18}F]labeled fluoride is directly incorporated into bone matrix, due to the fluoride ion exchange with hydroxylgroups in the hydroxyapatite crystal of bone resulting in fluoroapatite ($Ca_{10}(PO_4)_6F_2$).

This process is closely related to active bone metabolism, as assessed by increased mineralization rate in histomorphometric studies in minipigs [11], thus providing a quantitative non-invasive estimate of bone formation [12]. The ^{99m}Tc labeled diphosphonate uptake in the skeleton is attributed to chemical adsorption at the osteocyte lacunae and the mineralization front of the bone, but is not totally understood [7,13]. ^{18}F-NaF has a shorter half-life of 110 min and is therefore produced by cyclotron, compared to 6 h for ^{99m}Tc-labelled compounds. The delivery of ^{18}F-NaF is not affected by protein binding, while protein binding of ^{99m}Tc-labeled diphosphonates increases over time from 25% to 50% at 4 h after the injection [14]. The absent binding to serum proteins of ^{18}F-NaF allows a rapid single-pass extraction and fast clearance from blood and soft tissue. Compared with ^{99m}Tc-labelled diphosphonates the bone uptake of ^{18}F-NaF is twice as great [15]. These characteristics of ^{18}F-NaF combined with the higher resolution of PET scanners lead to shorter intervals and improved detection of bone lesions with ^{18}F-NaF PET. Typically, a 2–4-hour interval between tracer injection and bone scintigraphy imaging is required. In contrast, ^{18}F-NaF PET/CT imaging can be performed within a 1 hour after injection. Notably, the length of the interval between injection of ^{18}F-NaF and scanning affects semi-quantitative parameters such as standard uptake value (SUV), therefore the interval between injection and PET scanning should be standardized [12]. Standardization facilitates comparison of quantitative parameters in serial ^{18}F-NaF PET studies and may reduce the interindividual variation of [^{18}F]fluoride uptake [16,17].
Recently the superiority of ^{18}F-NaF PET/CT was shown in prostatic cancer [15,18], where earlier and more bone metastases were detected with sensitivity rates between 93-100% compared to 51% in bone scintigraphy [18,19]; ^{18}F-NaF PET/CT also correlated well with overall survival [18].

3. Dosimetry and procedure of ^{18}F-NaF PET/CT

^{18}F-NaF is injected intravenously according to a procedure guideline for use of ^{18}F-NaF PET/CT [12,20]. Typically this yields a dose of approximately 185 MBq ^{18}F-NaF for an adult, however, lower doses are possible using modern 3D PET/CT scanners. Presently, we use a dose of 1.2 MBq ^{18}F-NaF /kg bodyweight in benign bone diseases [7,21]. Accordingly, a dose of 100 MBq ^{18}F-NaF for an adult of 80 kg results in a radiation dose of 2.4 mSv. For comparison, a traditional bone scintigraphy performed after injection of 600 MBq *^{99m}Tc-MDP* results in a dose of 3.4 mSv.
1h after injection of ^{18}F-NaF whole body PET scans can be acquired from skull to feet, according to previously reported guidelines [12,20].
As in the presented case a Gemini TF PET/CT scanner (Philips, The Netherlands) may be used. Low-dose CT imaging (30 mAs) is used for attenuation correction [12]. Activity in the region of interest (ROI) and the volume of interest (VOI) can be calculated using standard software ROI tool (as the Leuven ROI tool) to define the SUV. ^{18}F-NaF uptake can be assessed both visually and quantitatively and be compared with reference areas, for example normal bone or soft tissue.. Heterotopic bone can be assessed on CT.

4. Quantification of ^{18}F-NaF uptake

Visual assessment of [^{18}F]fluoride images may be sufficient for diagnostic purposes, quantification is essential for monitoring response to treatment, as it enables objective assessment of changes in uptake over time [11,12].

Various analytical approaches, varying from semi-quantitative indices such as SUV to full kinetic analysis of ^{18}F-NaF kinetics, have been used to quantify fluoride uptake. A major advantage of SUV measurements is the simplified PET scanning protocol which only requires static ^{18}F-NaF PET images, made for example 60 min after injection. Full kinetic analysis of ^{18}F-NaF kinetics yields robust and reliable estimates of ^{18}F-NaF uptake, however, requires dynamic PET scanning with a length of up to 1 h, starting directly after ^{18}F-NaF injection.

Recently, a high correlation between ^{18}F-NaF SUV and full kinetic derived ^{18}F-NaF K_i was found in a bone surgery study with correlation coefficients higher than 0.94 [11] Moreover, changes in ^{18}F-NaF SUV after bone surgery correlated with those in K_i, indicating that changes of local bone metabolism could be monitored using the ^{18}F-NaF SUV and a simplified PET scan protocol. However, this aforementioned PET study focused on the effects of local bone metabolic changes induced by bone surgery. Different therapies with medical interventions may have systemic effects upon bone metabolism and ^{18}F-NaF kinetics [11]. Therefore, separate studies with dynamic scanning in clinical practice and full kinetic modelling (using compartment modelling or Patlak analysis) are necessary to validate the application of the semi-quantitative indices, such as ^{18}F-NaF systemic therapy in FOP patients [12].

5. ^{18}F-NaF PET/CT follow-up in FOP: a case report

A 19-year old girl, diagnosed with FOP at the age of 6, experienced several flare-ups during that year. She suddenly noticed a painful increasing swelling extending to her total right upper leg. Three weeks later a ^{18}F-NaF PET/CT showed markedly increased ^{18}F-NaF uptake only at circumscriptive locations at the distal quadriceps muscle of the right leg. On the CT scan no evident HO was visible (Figure 1).
She was treated with high dosages of prednisolone almost from the onset until 2 months after the first ^{18}F-NaF PET/CT scan in addition to nonsteroidal anti-inflammatory drugs for several months, after which the pain and swelling slowly decreased, finally resulting in a knee contracture and wheelchair dependence. After 8 months, when the flare-up had disappeared, a follow-up ^{18}F-NaF PET/CT scan showed laterally disappeared and opposite a marked decreased muscle [^{18}F]fluoride uptake at the right distal quadriceps (Figure 1). On the CT scan new maturing HO was visible only at the location of the muscle where the ^{18}F-NaF PET/CT scan had been active before.

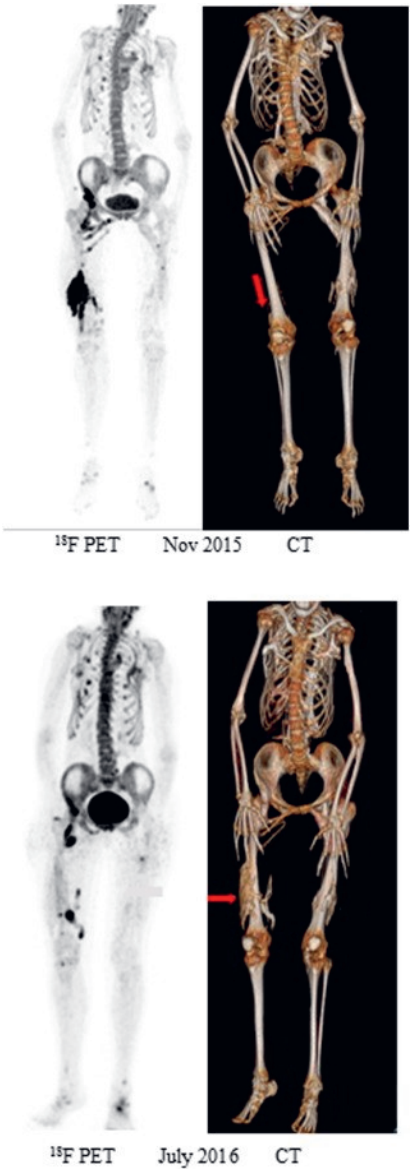

Figure 1. Whole body ^{18}F-NaF PET scan (skull excluded, maximum intensity projection) and 3D whole body CT scan of the FOP patient with suspected flare up in right leg (upper panel) with subsequent development of heterotopic bone on the second scan (lower panel). During a flare up with pain in the right upper leg, the ^{18}F-NaF PET showed markedly increased ^{18}F-NaF uptake in the soft tissues of the distal part of the right upper leg. CT images showed no heterotopic bone in this region as indicated by the red arrow. The left upper leg showed no increased ^{18}F-NaF uptake, despite clear heterotopic bone in the distal part of the left upper leg. Eight months later there is a marked reduction of ^{18}F-NaF uptake in the right leg ,while the CT showed newly formed heterotopic bone in the right upper leg marked by the red arrow. The left leg showed unchanged heterotopic bone.

6. Discussion

Despite tremendous progression in the understanding of biological features of FOP over the past two decades, the lack of fully understanding the natural flare-up course has hindered progress of clinical research, detection of a marker of disease activity and proper evaluation of new treatment options. This paper evaluates the use of ^{18}F-NaF PET/CT scan as possible method to follow the course of a flare-up in FOP. Its findings support previous observations in an FOP patient where, after surgery, repeated ^{18}F-NaF PET/CT scans could identify and quantify early foci of disease activity, which led to heterotopic ossification at a later stage [4].

The present ^{18}F-NaF PET/CT findings show that in a spontaneous flare-up some areas may recover but other areas may progress to end-stage heterotopic ossification. This fate as well as the exact future HO location was detected by increased uptake of ^{18}F-NaF 3 weeks after onset of the flare-up before HO could be detected on CT scan, but further research will be needed to investigate earlier time points. This indicates that ^{18}F-NaF PET/CT could potentially be used for early recognition of an active ossification process prior to bone formation at least 3 weeks after the first signs of a flare-up. Therefore, in new FOP drug trials, ^{18}F-NaF PET/CT may become a useful tool for monitoring early effectiveness of drugs and, as such, support the guidance of therapy.

Previously, bone scintigraphy has been used in FOP but only during differential diagnosis [22,23]. In general, a bone scintigraphy can be helpful for detection of HO but theoretically, ^{18}F-NaF PET has a better imaging characteristics compared with the traditional bone scintigraphy, which may explain the lack of sensitivity of the bone scintigraphy for detection of the early phase of a flare-up [22]. A CT scan provides valuable information regarding the presence and extension of HO, however, a CT cannot detect the early biological process and active bone metabolism. MRI and other PET tracers such as [^{18}F]fluordeoxyglucose (FDG) PET may be used for early detection of inflammation, but are not bone specific and therefore may not be able to distinguish between flare-up induced HO or other inflammatory responses [12,24]. Nevertheless, both MRI and ^{18}F-FDG PET may still be of additional values, especially in the very early phase of a flare-up, as it is not known yet whether ^{18}F-NaF can detect HO activity in the first 3 weeks after onset. Moreover, these imaging techniques may provide supplemental information about the inflammatory process, which is independent of or prior to possible bone formation.

^{18}F-NaF uptake in the bone reflects increased bone formation, including any process that increases the exposed bone crystal surface at early stages [5]. As a consequence, this imaging technique is increasingly being used to detect bone and bone related disorders, joint conditions such as osteoarthritis [21], osseous metastases of a variety of cancers [16], lytic and early marrow-based metastases when accompanied by minimal, reactive osteoblastic changes [15] and to evaluate treatment efficacy as recently successfully was shown in multiple myeloma [12,25]. In future this technique might also help to understand the progressive course in some FOP patients who do not experience clinical flare-ups, but nevertheless show increasing HO [1].

Interestingly, also in myositis ossificans circumscripta, which is caused by a local unusual reactive process of mesenchymal stem cells in muscles mostly secondary to a trauma or inflammatory process, a 35% rate of spontaneous resolution has been reported [26] .This emphasizes that the recovery from an initial inflammation phase in FOP may occur independent from the underlying genetic cause and in this phase mesenchymal cells can convert to support normal muscle cells again. Serial ^{18}F-NaF PET/CT might support the understanding of the different phases of a flare-ups in the future. Present limitations of the use of ^{18}F-NaF PET/CT in FOP are the small number of FOP patients investigated so far, the variable course of the disease, which needs more follow-up data of different flare-up symptoms and of patients who are affected differently. In addition, more follow-up will be needed to assess the possible incidence of false positive diagnoses, which may be due to osteochondromas or osteoarthritis. However, as the exact location and characterization might be detected with CT, this may rule out suspected other diseases or non-suspicious locations.

In conclusion, ^{18}F-NaF PET/CT appears to be a promising imaging modality that may potentially be useful for early recognition of new HO formation in FOP. Based on its quantitative properties, ^{18}F-NaF PET/CT could also provide a means for monitoring effectiveness of drugs early in during therapy, for instance in new FOP trials.

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Chapter 3

Evolution of heterotopic bone in fibrodysplasia ossificans progressiva: an ^{18}F-NaF PET/CT study

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Abstract

Fibrodysplasia ossificans progressiva (FOP) is a rare, autosomal dominant disorder characterized by heterotopic ossification (HO) in muscles, ligaments and tendons. Flare-ups often precede the formation of HO, resulting in immobilization of joints. Due to progression of the disease without signs of a flare-up, co-existence of a chronic progression of HO has been postulated, but conclusive evidence is lacking. Recently, it has been shown that ^{18}F-NaF PET/CT is able to identify early ossifying disease activity during flare-ups. Therefore, the purpose of the present study was to assess whether ^{18}F-NaF PET/CT might also be able to identify the possible presence of chronic progressive HO in FOP.

A total of thirteen ^{18}F-NaF PET/CT scans from five FOP patients were analysed. Scans were acquired over a period of 0.5 to 2 years. Volumes of HO and standardized uptake values (SUV) were obtained based on manual segmentation of CT images. SUV_{peak} values, defined as the average SUV value of a 1 mL sphere containing the hottest voxel pixels, were obtained.

Two out of five patients experienced ≥1 active clinical flare-ups at the time of the ^{18}F-NaF PET/CT scan. In addition, in four out of five patients, serial scans showed radiological progression of HO (3 to 8 cm^3), as assessed by CT volume, in the absence of a clinical flare-up. This volumetric increase was present in 6/47 (12.8%) of identified HO structures and, in all cases, was accompanied by increased ^{18}F-NaF uptake, with SUV_{peak} ranging from 8.4 to 17.9.

In conclusion, HO may progress without signs of a flare-up. ^{18}F-NaF PET/CT is able to identify these asymptomatic, but progressive HO lesions, thereby demonstrating the presence of chronic activity in FOP. Consequently, future drugs should not only target new HO formation, but also this chronic HO progression.

1. Introduction

Fibrodysplasia ossificans progressiva (FOP) is a rare, autosomal dominant disease, which is characterized by heterotopic ossification (HO) of connective tissue [1-3]. Flare-ups, characterized by local swelling, pain, warmth, impaired movement and stiffness, often precede new HO formation [3,4]. As a result of these new HO lesions, mobility in joints is gradually impaired and many patients become immobilized at an early age [1]. Previously, progression of existing HO lesions has been reported, but it is unknown whether these progressions occurred in the presence or absence of a clinical flare-up [5,6]. In a recent questionnaire distributed among five hundred FOP patients, however, nearly 50% of the responders reported progression of their disease without flare-up symptoms[4]. In addition, in ACVR1^{R206H} knock-in mice, HO progression has been confirmed even weeks after a trauma induced flare-up, suggesting the presence of a chronic component of the disease [7,8].
Previously it has been shown that ossifying flare-ups can be identified and visualised using ^{18}F-NaF PET/CT-scan [9,10]. ^{18}F-NaF (i.e. labelled sodium fluoride) binds to the surface of newly formed hydroxyapatite, a crystal formed by osteoblasts during bone mineralization, based on the exchange of [^{18}F] and hydroxyl-ions [11,12]. The purpose of the present study was to assess whether this imaging technique is also able to identify asymptomatic progressive lesions, if present.

2. Method

FOP patients, of the FOP expertise center of the Amsterdam UMC, for whom two or more ^{18}F-NaF PET/CT-scans were available were included. Images were obtained for either annual follow-up of the disease, (new) complaints or suspicion of a flare-up. Clinical information on both the presence of a flare-up and other complaints were recorded. The Medical Ethics Review Committee of the Amsterdam UMC, Vrije Universiteit Amsterdam, approved the study and all patients signed informed consent for using their data in the present study.
All ^{18}F-NaF scans were performed at the Amsterdam UMC, Vrije Universiteit Amsterdam. Scans were acquired using a Gemini TF-64 PET/CT scanner (Philips Medical Systems, Best, The Netherlands). Low dose whole body CT scans were acquired at 120 kV with a tube current ranging from 30 to 60 mAs. The ^{18}F-NaF dose was adjusted to weight (e.g. 83 MBq ^{18}F-NaF for a 70-79 kg patient). Scan time per bed position was 2 min.
PET and CT images were visually assessed to identify HO. After identification, images were segmented manually using the software tool “Accurate”, which previously has been described in more detail [13,14]. The tool was used to identify HO volumes of interest (VOIs) and derive corresponding PET and CT values (Standardized uptake value (SUV) and volume, respectively). SUV_{peak} was defined as the average SUV of a 1cm^3 sphere, centered on the hottest voxel.
A Hounsfield unit cut-off of 80 was used to separate bone from other tissues. This cut-off was found to exclude muscle and to include all (immature) bone in the bone segments. All

segmentations were performed by one reviewer, but in all patients, a predefined number of randomly chosen HO were manually segmented by a second, independent reviewer. Both reviewers were blinded to both SUVs and volumes until segmentation of all successive scans were completed.
Reference SUV values were established to define a cut-off value for the distinction between normal and increased ^{18}F-NaF uptake. Since reference SUV values were not available for the skeleton of FOP patients, several potential reference tissues were explored (caput femori, lumbar vertebral body and the supra-acetabular region). The most stable region was chosen as a fixed reference for all analyses. SUV_{peak} values exceeding two standard deviations (SD) of the normal skeleton were considered divergent.
Lesions were found asymptomatic when patients did not report a flare-up or physical complaints for that specific region within the last three months before the first scan. For the follow-up scans, lesions were found asymptomatic if patients did not report any complaints in that specific area during the course of the study.
Statistical analyses were performed using SPSS Statistics for Windows, (IBM, version 24.0, Armonk). Independent T-tests and Mann-Whitney U tests were used to test significance between progressive and non-progressive lesions. The Spearman test was used to assess correlations.

3. Results

In total 5 patients, treated at the FOP expert center of the Amsterdam UMC, Vrije Universiteit Amsterdam, were included in the analysis. Four patients had the classical mutation (c.617G4A; p.R206H); one patient a variant (c.619C>G, p.Q207E). In these five individuals, a total of 13 ^{18}F-NaF PET/CT scans were acquired in the course of 0.5 – 2 years. Three patients were evaluated based on two ^{18}F-NaF scans. For one patient three scans were available and for one patient four (Table 1).

Bone was considered a reliable reference when free of HO and with stable SUV throughout the course of the consecutive ^{18}F-NaF scans. The supra-acetabular region showed stable SUV throughout all (including consecutive) ^{18}F-NaF scans and, therefore, was considered to be a reliable reference region. The average SUV_{peak} for both left and right supra-acetabular regions was 5.5 ± 1.4. Consequently, HO lesions with SUV_{peak} values exceeding two standard deviations of the reference (SUV_{peak} ≥8.4) were considered to be metabolically active. Lesions with SUV_{peak} beneath this limit were considered to have normal metabolic activity.

All HO lesions were manually identified and segmented. After manual segmentation by one reviewer (EB), a second reviewer (BT, musculoskeletal and emergency radiologist) manually segmented 10 of 52 (19.2%) randomly selected HO structures. A comparison of obtained volumes and SUVs showed a near-perfect correlation between both observers (intraclass

Table 1. Patient characteristics

	Age* (y)	Gender	Mutation **	Scan number	Time interval (months)***	Presence flare-up
1	41	♀	R206H	1	-	
				2	10	
2	17	♂	R206H	1	-	
				2	20	
3	23	♀	R206H	1	-	
				2	6	
4	24	♀	R206H	1	-	Jaw, bilateral
				2	5	
				3	11	
				4	27	
5	19	♀	Q207E	1	-	m. psoas
				2	5	m. quadriceps, femur-pubis region
				3	13	

* Age at time of the first ^{18}F-NaF PET/CT scan
** Genetic analysis performed at het Amsterdam UMC, location VUmc, the Netherlands.
*** Time between first and successive scan

correlation coefficient = 0.99). Based on this inter-observer variability, volumetric changes of >3 cm^3 were considered meaningful (mean difference 1cm^3, standard deviation 0.5 cm^3)

Among the 5 individuals, 52 different HO structures were identified of which 47 structures were not affected by a flare-up. Three flare-ups, in two patients, were present at baseline. All regions affected by a flare-up were in a region in which initially no HO was present. The total number of HO lesions ranged from 7 to 16 per patient, with an average of 10. The total volume of HO varied from 139 – 1140 cm^3 per patient, based on the last obtained scan of each patient.

Flare-ups were present in two out of the five patients during the course of the study. Flare-ups were identified based on patient's symptomatology. All flare-ups were followed by HO formation. In patient 4 a flare-up was triggered by surgery of the jaw, resulting in a SUV_{peak} that, at some stage, exceeded 25. ^{18}F-NaF PET showed normalization (SUV_{peak} <8.4) of uptake 18 months after surgery. For all other flare-ups (the right loin, right groin and right upper leg) no trigger was identified and these flare-ups were therefore considered idiopathic. All flare-up regions showed a SUV_{peak} between 21 and 48 (Figure 1). These flare-ups have been described previously by Eekhoff et al. [9,10]. No new HO lesions appeared without a flare-up. However, 6 out of 47 (12.8%) HO structures, not involved in a flare-up, showed a volumetric

progression by CT during follow-up. These 6 progressive but asymptomatic HO lesions were found in 4 out of 5 patients One patient showed progression in 3 out of 16 heterotopic lesions: the right femur pubis and both sides of the thoracic region. The other 3 patients all showed one progressive HO lesion. In two patients, these HO lesions were located in the right thoracic region and for one patient paravertebral. (Figure 2). Volumetric expansion ranged from 3 to 8 cm^3. All 6 progressive lesions were accompanied by increased ^{18}F-NaF uptake. After normalization of ^{18}F-NaF uptake (SUV_{peak} ≤8.4), lesions did not show further volumetric expansion (Figure 3). Progressive lesions were significantly larger than non-progressive HO lesions (Mann-Whitney U test, $p<0.05$). Multiple sites with increased ^{18}F-NaF uptake were identified within all progressive HO lesions. Increased ^{18}F-NaF uptake was seen where HO adjoins the skeletal bones (6/6). For several of these structures (3/6) however, uptake was also present in regions where the HO did not adjoin skeletal or other heterotopic lesions. The extent of progression did not correlate with SUV_{peak} (Spearman $r = 0.657$, $p = 0.156$). All other HO lesions did not show increased ^{18}F-NaF uptake. Lesions without increased ^{18}F-NaF uptake showed no progression on successive CT-scans

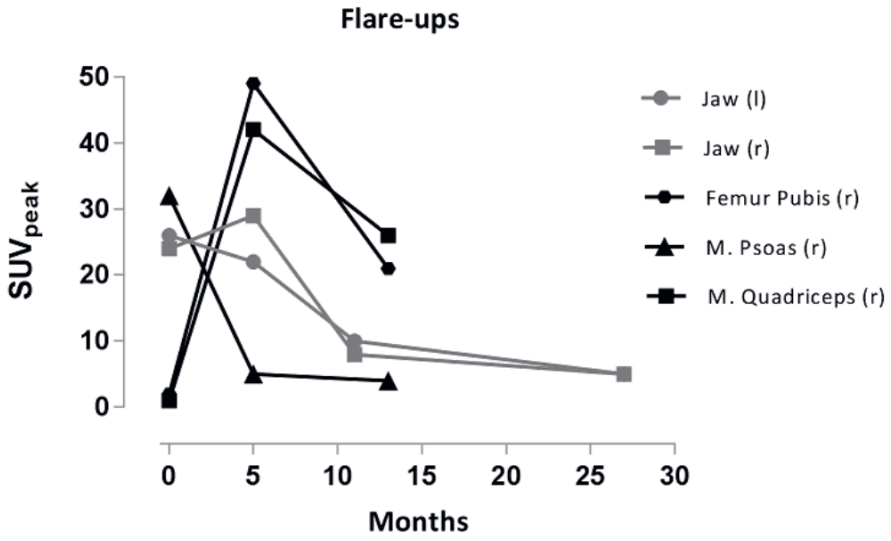

Figure 1. Consecutive SUV_{peak} values obtained from ^{18}F-NaF PET/CT scans for regions in which patients experienced a flare-up. For all regions SUV_{peak} exceeded 25 in the course of the flare-up. The flare-up in the left and right jaw (patient 4, grey lines) was triggered by a surgical procedure. For the other flare-ups (patient 5, black lines) no triggers were identified.
Abbreviations: SUV = standardized uptake value, (l) = left, (r) = right

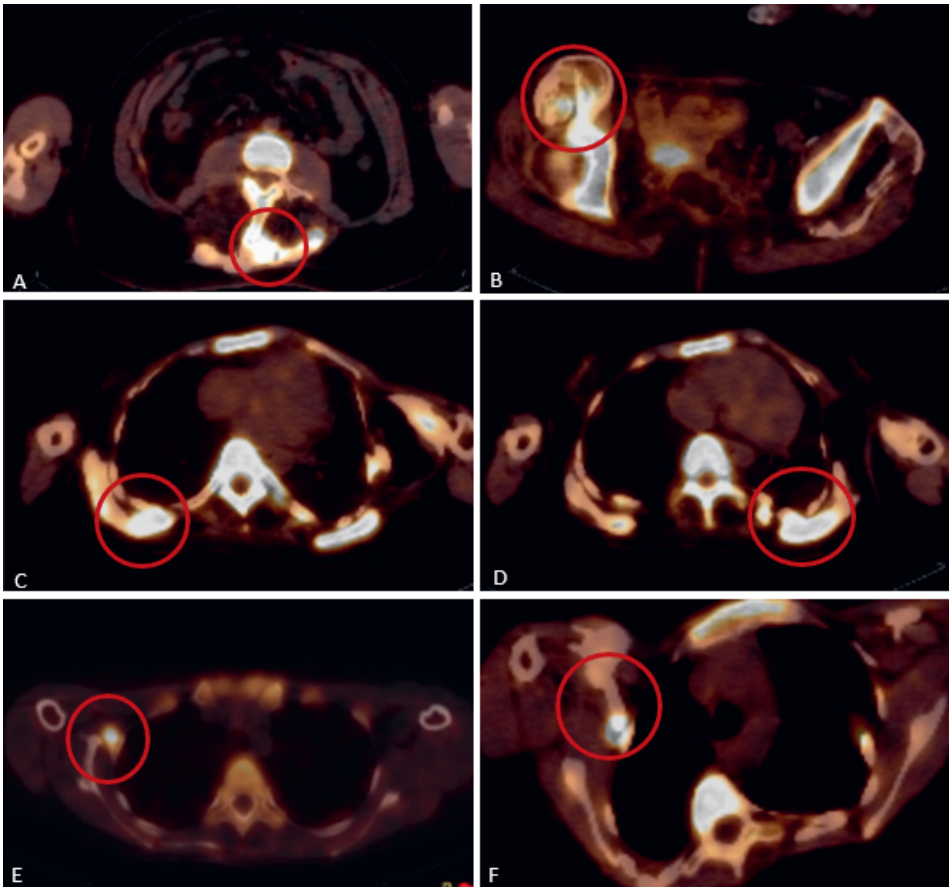

Figure 2. Axial images of progressive heterotopic bone lesions with visibly increased ^{18}F-NaF uptake. These progressive lesions all had increased sodium fluoride uptake (red circles in images), defined by a SUV_{peak} >8.36. Volumetric values and SUV_{peak} values for each heterotopic lesion are shown in Figure 3.
A. Paravertebral region, patient 2
B. Right femur pubis region, patient 3
C. Right thoracic region, patient 3
D. Left thoracic region, patient 3
E. Right thoracic region, patient 4
F. Right thoracic region, patient 5

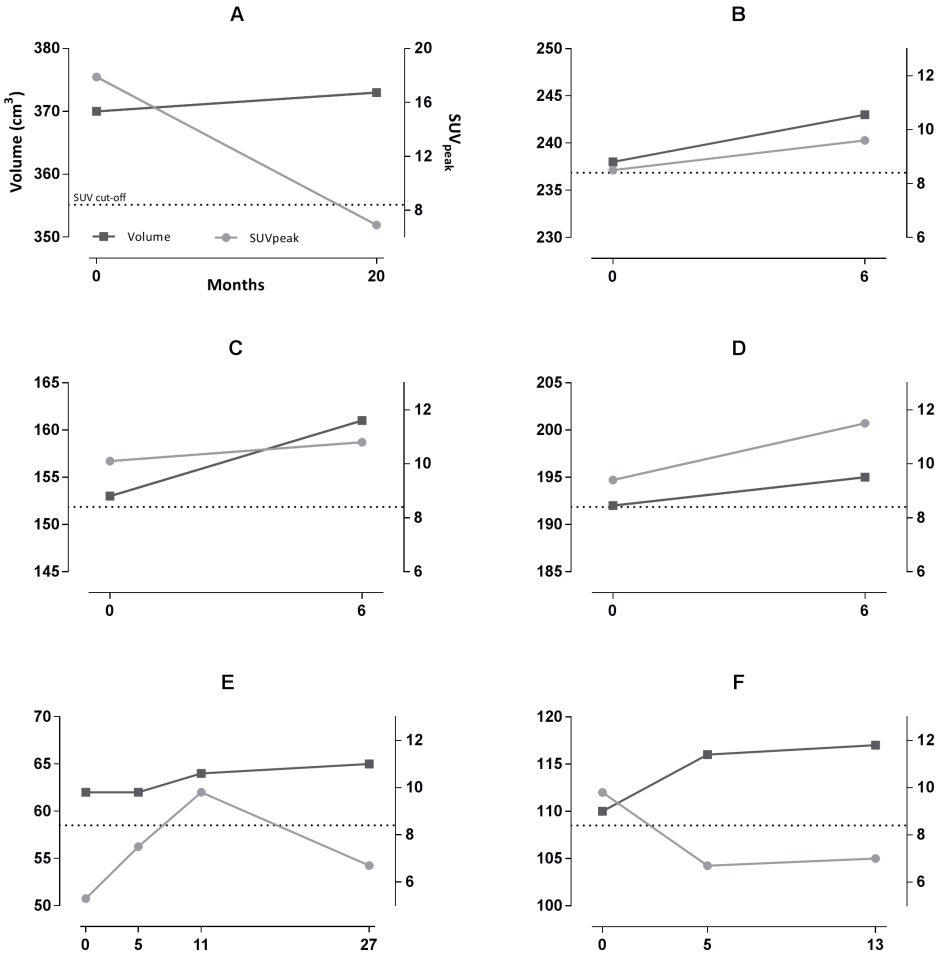

Figure 3. Volumetric increase and SUV_{peak} values for each progressive lesion identified by successive ^{18}F-NaF PET/CT-scans. Progressive heterotopic bone lesions were accompanied or preceded by an increased sodium fluoride uptake, reflecting active bone metabolism. Active bone metabolism represents progression of heterotopic ossification. Patients did not experience any complaints or flare-ups in these regions three months before the first scan and during the entire course of this study. After normalization of ^{18}F-NaF uptake (SUV_{peak} <8,4: panels A, E and F), no further progression was identified.
Abbreviations: SUV = standardized uptake value.
A. Paravertebral region, patient 2hn
B. Right femur pubis region, patient 3
C. Right thoracic region, patient 3
D. Left thoracic region, patient 3
E. Right thoracic region, patient 4
F. Right thoracic region, patient 5

4. Discussion

The main finding of the present study is that HO in FOP patients may progress without any clinical signs. In 4 out of the 5 FOP patients studied, one or more asymptomatic HO lesions showed volumetric progression. These progressive lesions were all detected by increased ^{18}F-NaF uptake on PET/CT scans, confirming the existence of an asymptomatic chronic stage in FOP that is not related to the presence of a clinically apparent flare-up.

Ongoing progression could be identified for a maximum period of 7 months. As the ^{18}F-NaF PET is a fairly new imaging technique to visualize FOP, successive scans with a relatively short interval of only 5 patients were available for analysis. Also, because of the limited availability of ^{18}F-NaF PET/CT scans (due to costs and feasibility) further data are needed to characterize the time course in this chronic stage. Especially for future treatments, this time course would be key to judge the efficacy of drugs on this chronic stage. It is likely that chronic stage lasts very long, as a lesion that has been active for 13 years has already been reported [10]. This 13-year active lesion was identified by Tc-99m methylene diphosphonate [^{99m}Tc]MDP bone scintigraphy. Bone scintigraphy is still widely used to detect osteoblastic activity. Compared with [^{99m}Tc]MDP bone scintigraphy, however, the ^{18}F-NaF PET/CT has higher sensitivity and higher spatial resolution. In addition, the quality of the ^{18}F-NaF PET/CT images is better due to lower plasma protein binding and, therefore, higher uptake in bone. Also, ^{18}F-NaF PET/CT images can be quantified more easily [15,16]. As both techniques expose the patient to radiation[15], there is a limitation of scans allowed for research ends. For clinical purposes, however, ^{18}F-NaF PET/CT-scans, scans are allowed as long as it is between reasonable limits and in the benefit of the patient.

In 4 out of 5 patients the classical mutation in the *ACVR1* gene (c.617G>A, p.R206H) was identified. In one patient, however, a variant of the FOP mutation [c.619C>G, p.Q207E] was seen, although the phenotype was similar to that of the patients with the classical mutation. This variant mutation has been identified and reported for two other patients. Haupt et al. described a patient with this variant mutation that led to a phenotype similar to the classical mutation [17]. The other patient, described by Kaplan et al, also showed atypical features including a failure to thrive, which, however, was not attributed to this mutation [18]. In the present study no atypical features were observed in any of the patients.

Only one patient, patient 1, showed neither active flare-ups nor progressive HO lesions during 11 months. In this patient, the ^{18}F-NaF PET/CT scans were obtained because of multiple comorbidities (e.g. chronic osteomyelitis of the leg and cerebrovascular accidents). The effect of comorbidities on the FOP activity is not known yet. Follow-up scans of this patient, might reveal whether a chronic stage is also present in this patient.

SUV_{peak} was significantly lower in the asymptomatic chronic lesions (range 8.5-17.9) compared with the symptomatic acute lesions (range 21.3 – 48.7) (Mann-Whitney U test, $p < 0,05$), making it not only possible to identify these chronic lesions using the ^{18}F-NaF PET/CT-scan, but also to distinguish them from active flare-ups. As no flare-up had occurred 3 months prior to and during the entire course of the study at the sites of the chronic lesions, it is not likely that this progression is due to a residuum of a flare-up locally.

^{18}F-NaF PET/CT is best known for its role in assessing metastatic bone lesions in oncology [19]. In addition, it has also shown great promise in visualizing early ossifying flare-ups in patients with FOP [9,10]. Although SUV_{max} is often used based on its simplicity and because it is operator independent [20], it can be affected by noise as its value is based on a single voxel (0.064mL) [21,22]. SUV_{peak} is based on a larger region (1.0 mL) and therefore least affected by noise [23]. As notable growth will involve multiple voxels, SUV_{peak} was used in the present study. SUV_{peak} also is more robust, reproducible and reliable measure than SUV_{max} [20,22]. Although, Cremin et al. described HO progression after a flare-up using plain radiographs [5], the present FOP data are unique, as no asymptomatic heterotopic lesions have been followed quantitatively using ^{18}F-NaF PET/CT. In contrast to X-rays, ^{18}F-NaF PET/CT scan allows early identification of progressive HO by uptake of ^{18}F-NaF.

Results of this study are potentially important for future trials, as they indicate that a chronic component in FOP should be taken into account. Future drugs should not only target HO formation after a flare-up, but also chronic HO progression.

In conclusion, FOP is known for its periodical flare-ups followed by HO formation. However, a substantial fraction of HO lesions progresses in the absence of any clinical signs. ^{18}F-NaF PET/CT is a promising imaging modality, as it can visualize ossifying flare-ups even before HO has formed and therefore it may be used to monitor progression of existing HO.

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Chapter 4

Diagnostic value of magnetic resonance imaging in fibrodysplasia ossificans progressiva

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Abstract

Using ^{18}F-NaF positron emission tomography (PET) it is not only possible to identify the ossifying potency of a flare-up, but also to identify an asymptomatic chronic stage of fibrodysplasia ossificans progressiva (FOP). The purpose of this study was to investigate the diagnostic role of a more widely available imaging modality, magnetic resonance imaging (MRI), which is of special interest for studies in paediatric FOP patients.

MRI and ^{18}F-NaF PET/CT images at time of inclusion, and subsequent follow-up CT scans of 4 patients were analysed retrospectively. Presence, location and intensity of oedema identified by MRI were compared with activity on ^{18}F-NaF PET. Occurrence or progression of HO was examined on the follow-up CT-images.

Thirteen different lesions in various muscle groups were identified: 5 lesions with only oedema; 5 lesions with both oedema and increased ^{18}F-NaF uptake; 1 lesion with only increased ^{18}F-NaF uptake and 2 lesions with neither oedema nor uptake of ^{18}F-NaF. Mild oedema, found in 3 lesions, was present at asymptomatic sites which did not show increased [^{18}F] NaF uptake, nor progression of HO on consecutive CTs. Moderate oedema was found in 3 symptomatic lesions, with increased ^{18}F-NaF on PET and progression of HO on CT. Severe oedema was identified in 4 lesions. Interestingly, 2 of these lesions did not develop HO during follow-up, while one of these two even gave obvious symptoms of a flare-up.

MRI can identify whether symptoms are the result of an acute flare-up by the presence of moderate to severe oedema. The occurrence of severe oedema on MRI was not always related to an ossifying lesion. The additional diagnostic value of MRI requires further investigation, but MRI does not seem to fully replace the diagnostic characteristics of ^{18}F-NaF PET/CT in FOP.

1. Introduction

Fibrodysplasia ossificans progressiva (FOP) is an extremely rare disease leading to ankyloses due to excessive heterotopic bone formation in connective tissue [1-3]. This progressive autosomal dominant disorder is characterized by periodical flare-ups [2,4,5]. A flare-up is thought to start with an inflammatory process in muscles, tendons and ligaments. Flare-ups often, but not always, result in formation of heterotopic ossification (HO) [5]. Nowadays, a flare-up is defined by its symptoms, the most prominent ones being swelling and pain [5]. Due to the lack of (blood)markers, a flare-up is still a clinical diagnosis. Using ^{18}F-NaF PET/CT (positron emission tomography / computerized tomography), however, it has become possible to identify which of these flare-ups will lead to HO [6,7]. Recently, it was shown that ^{18}F-NaF PET/CT can also identify asymptomatic chronic progression of existing HO, based on relatively lower but still increased ^{18}F-NaF uptake [8]. Using this new marker of active and chronic FOP disease, it has become possible to investigate the diagnostic role of other, more widely available imaging modalities such as magnetic resonance imaging (MRI). The absence of ionizing radiation makes this technique especially interesting for the paediatric FOP population.

MRI provides high soft-tissue contrast and is able to detect oedema, a potential marker of inflammatory stages in FOP [9-12]. MRI is currently used in FOP to evaluate the presence of oedema early in flare-ups[10]. Whether the two stages of FOP, i.e. active flare-ups and asymptomatic chronic FOP disease, have similar inflammatory patterns and corresponding MRI signals is not yet known.

A few FOP case reports have described hyper-intense foci on T2-weighted images during flare-ups [9,11,13-15], but whether these hyper-intense foci on MRI led to formation of HO was not investigated. Nevertheless, In R206H Acvr1 knock-in mice, it was shown that sites of HO formation coincided with hyper-intense T2 signals. Once HO was formed, the signal subsided. In this animal model, MRI T2-signal was in accordance with ^{18}F-NaF PET-signal[16]. For human FOP patients, however, it is not known whether oedema on MRI can distinguish between ossifying and non-ossifying flare-ups, as well as between active and asymptomatic chronic FOP disease, as in the case for ^{18}F-NaF PET/CT [8]. Therefore, the aim of this study was to assess whether MRI can identify different stages of FOP. Although ossifying and non-ossifying flare-ups are clinically indistinguishable[5], it could be possible that the severity of oedema on MRI may predict the fate of a flare-up. In addition, assumeing that HO formation is always accompanied by oedema, it should be possible to detect the chronic component of disease by MRI.

2. Methods

Adult FOP patients, under the care of the FOP expertise centre of the Amsterdam UMC, in whom both MRI and ^{18}F-NaF PET/CT scans were performed, were included. MRI and ^{18}F-NaF PET/CT scans had to be acquired within 14 days of each other, to allow for reliable comparisons. In addition, a follow-up CT scan, obtained at least six months after the initial scans, had to be available to assess whether HO had formed. Any additional MRI and ^{18}F-NaF PET/CT-images available within the studied timeframe, were included in the analyses as well. Patients were asked to sign a consent for analysing and publishing their data anonymously. The study had been approved by the Medical Ethics Review Committee of Amsterdam UMC.

MRI was acquired on a 1.5T system (Signa Excite HDxt, GE Healthcare, Milwaukee, WI, USA) and a 3T system (Vantage Titan, Canon Medical Systems, Otawara, Japan). MRI scans were obtained either at an annual follow-up or for a suspected flare-up. Although different imaging protocols were used, T2 STIR (Short-TI Inversion Recovery) acquisitions were available for all MRI scans included. MRI parameters are presented in table 1 of the supplemental material. The intensity of oedema was graded on a semi-quantitative scale: absent, mild, moderate or severe [17-19]. Two reviewers (BT, musculoskeletal radiologist at Amsterdam UMC and EB) independently graded the degree of oedema. BT was not involved in clinical care of the FOP patients at the time of image acquisition. Both reviewers were blinded to clinical data. To limit bias, the review of ^{18}F-NaF PET and CT data was performed at least two months apart from the review of the MRI-images. The reviewers were instructed to grade oedema in accordance to Davis et al., a juvenile dermatomyositis scoring system for the MRI [17]. Discrepancies were resolved by consensus.

^{18}F-NaF PET/CT scans were obtained using a Gemini TF-64 PET/CT scanner (Philips Medical Systems, Best, The Netherlands). Patients were scanned from the top of the skull to the toes. The ^{18}F-NaF dose was adjusted to weight (e.g. 83 MBq ^{18}F-NaF for a 70-79 kg patient) and a scan time of 2 minutes per bed position was used. Uptake of ^{18}F-NaF was considered increased for chronic lesions when SUV_{peak} exceeded 8.4 [8]. The method used to analyse ^{18}F-NaF PET/CT has been described in our previous paper more extensively[8]. For flare-ups, no SUV_{peak} cut-off was available, but in practice it is assumed to be 2-3 times higher than that for a chronic lesion[8]. Whole body CT-images were acquired at 120 kV with a tube current varying between 30-60 mAs. CT-volumes were obtained for each heterotopic lesion identified. Two reviewers (EB and BT) independently analysed ^{18}F-NaF PET/CT images. Again, discrepancies were resolved by consensus.

Follow-up low dose whole body CT scans were also acquired at 120 kV with a tube current varying between 30 and 60 mAs. The volumes of the various lesions were analysed to assess whether these lesions progressed during the course of this study. The same two reviewers

(EB and BT) independently analysed CT images. Discrepancies were resolved by consensus. Clinical data were evaluated separately to assess clinical signs of the patients at the time of MRI, ^{18}F-NaF PET/CT and follow-up CT scans. A flare-up was assessed and confirmed by the physician based on the presence of symptoms e.g. swelling, redness, pain. When HO progressed in the absence of any clinical symptoms at that site in the last six months it was considered chronic.

Inter-observer correlation was assessed using Cohen's kappa. Spearman's rho was used for correlation between oedema grading and SUV_{peak} values. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 24.0.

3. Results

Four FOP patients were included as for all four MRI, ^{18}F-NaF PET/CT and at least one follow-up CT scan were available. In fact, for the assessment of these four patients, eight MRI-scans, six ^{18}F-NaF PET/CT-scans and four follow-up CT-scans were available.

Table 1. Demographic characteristics of the included patients

	Sex	Age[a]	Flare-up[b]	Medication[c]
1	♀	19	m. psoas, m. iliapsoas	Prednisolon, ibuprofen
2	♀	23	-	-
3	♀	23	Flare-up bilateral jaw	Prednisolon, Ibuprofen
4	♂	20	Suspicion flare-up jaw	Naproxen

[a] Flare-up during the course of the study
[b] Age at time of the first MRI
[c] Medication taken during the course of the study. This may have been temporarily or continuously.

Using MRI, 10 different oedematous lesions were found. Increased sodium fluoride uptake was found in only five and clinical signs of a flare-up were present in only six of the lesions identified by MRI (table 2). Using ^{18}F-NaF PET/CT, six lesions showed increased uptake of ^{18}F-NaF. In five, oedema was present and also for five of those six ^{18}F-NaF positive lesions patients complained of discomfort. six sites were identified solely by discomfort which was recognized by the patient as a flare-up. In four of the six symptomatic sites oedema was demonstrated by MRI. Only three of these six symptomatic sites showed increased ^{18}F-NaF uptake on PET. Combining the lesions and sites found with MRI, ^{18}F-NaF PET/CT and clinical signs, 13 distinctive sites were identified and analysed further.

Table 2. Identified muscle-(groups) by either complaints, oedema on MRI or increased ^{18}F-NaF uptake on PET/CT

Muscle(group)	MRI oedema	^{18}F-NaF PET (SUV_{peak})	Clinical signs	Progression HO volume
Jaw dextra	None	4.1	Present	No
Jaw sinistra	None	2.3	Present	No
Paracostal area dextra	None	9.8	Absent	Yes
M. psoas sinistra	Mild	5.4	Absent	No
M. glutei dextra	Mild	1.4	Absent	No
M. glutei sinistra	Mild	2.2	Absent	No
Jaw dextra	Moderate	28.5	Present	Yes
Jaw sinistra	Moderate	26.4	Present	Yes
M psoas dextra	Moderate	32.5	Present	Yes
M. Iliapsoas dextra	Severe	3.9	Present	No
Mm. Adductores dextra	Severe	48.7	Present	Yes
M. Quadriceps dextra	Severe	41.9	Present	Yes
M. gluteus maximus	Severe	2.1	Absent	No

Abbreviations: MRI = Magnetic resonance imaging; PET = Positron Emission Tomography; HO = Heterotopic ossification; m. = musculus

Of the 10 oedematous lesions identified by MRI, oedema was classified as mild in three, moderate in three and severe in four lesions (table 2). There was a good correlation in scoring of oedema between the two independent reviewers (Cohen's $\kappa = 0.7$; $p = <0.05$).

None of the three mildly oedematous lesions showed progression of HO. There was no increased uptake of ^{18}F-NaF, nor were there any clinical signs reported at these sites. In contrast to these mild lesions, all 3 lesions with moderate oedema were followed by HO progression. In addition, all showed increased ^{18}F-NaF uptake and were accompanied by signs of a flare-up. Interestingly, two of the four sites with severe oedema did not develop HO. Although one of these lesions was accompanied with common flare-up symptoms, such as swelling and pain (figure 1A-C), it did not show increased activity on ^{18}F-NaF PET. Interestingly, the adjacent muscle showed moderate oedema (figure 1A-C), which did result in HO development. ^{18}F-NaF uptake was increased only in the muscle group that showed HO development (Figure 1D-F). Figure 1 shows the course of the oedema over 11 months, with great differences in oedema intensity after one month. After 11 months, the oedema had completely resolved.

The other lesion with severe oedema was a clinically asymptomatic lesion. Again, the ^{18}F-NaF PET scan did not show increased uptake of ^{18}F-NaF in this area and the successive CT-scan did not reveal any HO progression of that particular region.

One site was identified by increased uptake of ^{18}F-NaF on PET, but did not reveal any

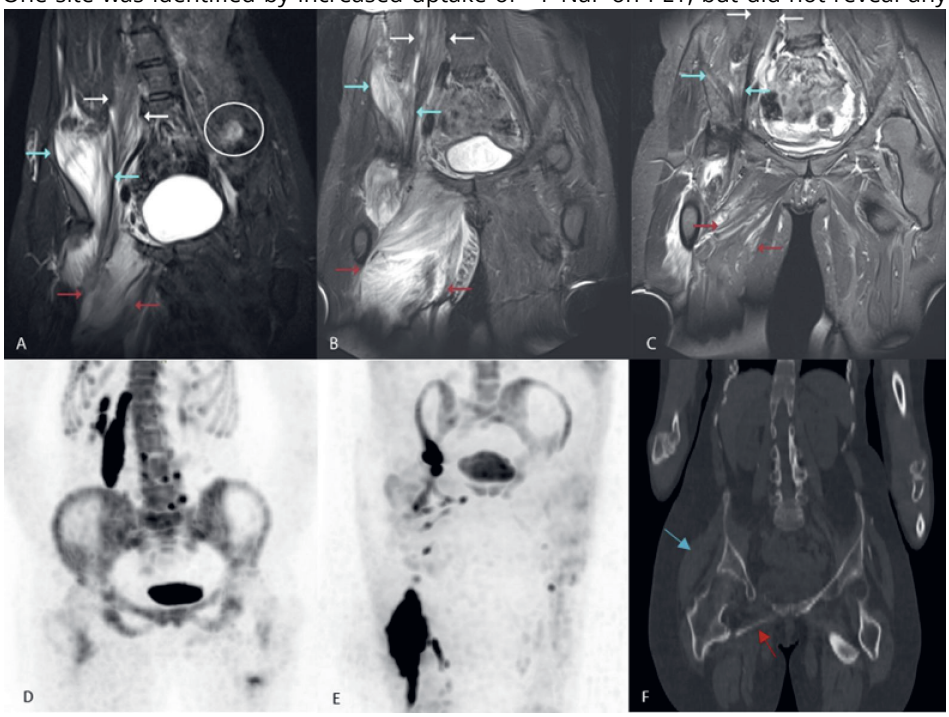

Figure 1. Coronal MRI T2 weighted STIR images are shown of a patients with multiple flare-ups. Starting in the loin (A+D), later also the groin (B) and upper leg (C+E). T in months. **A and D;** T = 0. Clinically a flare-up in the pelvic area with pain and swelling of the entire right loin. MRI (plane A) showed moderate and severe oedema of the musculus psoas dextra (white arrows) and the musculus iliacus dextra, respectively. Also, the musculi adductores (red arrows) showed moderate oedema, even though no clinical signs were noted. The MRI showed also an area of non-specific mild oedema (white circle). ^{18}F-NaF PET showed increased high uptake of tracer in the psoas muscle, mild uptake in the mm. adductores and no uptake in the iliopsoas muscle. **B**; T = 1. Oedema at both the musculus psoas and musculus iliacus diminished, to mild and moderate oedema respectively. Oedema intensity at the musculi adductores increased to severe, the patient now reported flare-up symptoms at the groin too. The mild oedema seen in plane A resolved, no calcifications were noted. **C and E**; T = 11. Oedema at the psoas muscle, iliacus and adductor muscles is completely resolved, but new oedema formed in the quadriceps muscle(plane C). High ^{18}F-NaF uptake in the quadriceps (plane E). **F**; T = 21. Low dose whole body CT showed HO in the psoas muscle, HO at the site of the adductor muscles (red arrow) and in the quadriceps muscle. No HO formed in the iliopsoas (blue arrow).
Abbreviations: MRI = magnetic resonance imaging; NaF = sodium fluoride; PET = positron emission tomography; STIR = Short-TI Inversion Recovery; T = time; CT = computed tomography; HO = heterotopic ossification

oedema. The patient denied having had complaints in that area for at least six months. The follow-up CT-scan, however, revealed progression of HO in that particular lesion. Taking all oedematous lesions into account, ^{18}F-NaF SUV_{peak} showed no significant correlation with oedema intensity (Spearman's ρ= 0.4; p=0.222). In addition, the volume increase of new lesions or with HO progression was weakly related to the intensity of the oedema as found by MRI (Spearman's rho = 0.6; p=0.244)

Apart from lesions identified by MRI and ^{18}F-NaF PET, two sites were analysed because of clinical complaints suggesting a flare-up. MRI did not show oedema at either site, neither did ^{18}F-NaF PET show increased uptake of ^{18}F-NaF. In addition, there was no HO progression on the subsequent CT-scan. The patients' complaints resolved within a couple of weeks without intervention, suggesting a different underlying mechanism. Taking ^{18}F-NaF PET/CT as the golden standard, MRI had a sensitivity of 83% and a specificity of 29% to detect FOP activity leading to HO formation. Positive and negative predictive values of MRI in this study were 50 and 66%, respectively.

The maximum duration of oedema seen in this study was five months. No flare-ups were found in which oedema was absent whilst HO was being formed. In one case (figure 1 A and D), ^{18}F-NaF PET/CT did not reveal increased uptake of the tracer yet, whereas the MRI obtained 14 days later, did show moderate oedema. Later in the course of this lesion, oedema progressed to severe and ^{18}F-NaF uptake was found.

4. Discussion

The aim of this study was to determine the diagnostic value of MRI in FOP by comparing it with the recently validated marker of disease activity: the ^{18}F-NaF PET/CT. Results were evaluated using follow-up CT and clinical data. In this small series of patients, an active flare-up was always accompanied by moderate to severe oedema on MRI. Interestingly, presence and severity of oedema did not always correlate with the ossifying potency of the involved muscle location. In addition, the chronic stage in FOP was not associated with oedema on MRI. Finally, mild oedema was seen at several sites, but this was not related to ^{18}F-NaF PET/CT, HO nor to clinical complaints.

The finding that all flare-ups were accompanied by oedema on MR-imaging is in line with five case reports [9,11,13-15]. In these case reports, often MRI was used to evaluate soft tissue swelling in an, at that time, undiagnosed child with FOP. As the course of these flare-ups was not followed, both implications and diagnostic value of MRI remained unclear. The present study shows a sensitivity of 83% of MRI as compared with ^{18}F-NaF PET. MRI therefore seems to have potential to rule out acute FOP activity. Chronic FOP activity, however, was not to detectable by MRI. This was unexpected, as in a mouse model growth of HO was always associated with oedema [16]. This finding emphasizes the difference between the FOP phenotype in humans and the R206H Acvr1 knock-in mice.

The specificity to detect HO forming FOP activity using MRI was only 29%, as five oedematous lesions did neither show increased uptake on ^{18}F-NaF PET, nor did these lesions lead to HO progression. The implication of these oedematous lesions is still unknown. It most likely is a manifestation of FOP itself, as MRI studies in healthy individuals did not or only sporadically

showed oedematous lesions [20,21]. One might hypothesize that lesions with mild oedema might reflect a very early stage of disease in which inflammation might still resolve spontaneously. In the current data set, only six months CT follow-up data was available. Whether these mild lesions develop into either the acute or the chronic stage even beyond these six months, remains unclear and will need further investigation.

MRI was compared with ^{18}F-NaF PET, a modality that was introduced recently as a method to quantify disease activity [6-8]. ^{18}F-NaF PET detects metabolic changes within tissues, as the ^{18}F-NaF-ion binds to newly formed hydroxyapatite[22]. MRI, on the other hand, detects inflammation through the presence of oedema [10]. In the present study, the intensity of oedema did not appear to correlate with the uptake of ^{18}F-NaF PET, nor with HO formation or progression. One should take into account that prednisolone was used during flare-ups to suppress inflammation, which may have interfered with the results.

Although ^{18}F-NaF PET is an established technique to evaluate ossifying disease activity in FOP, MRI (if validated in FOP) would be especially useful in paediatric patients, as it does not involve exposure to radiation. In addition, MRI is more widely available than PET. Finally, MRI might be informative in the very early detection of a flare-up as inflammation is the first stage of FOP lesion formation [23], as found in one lesion in the current dataset.
The results of the present study indicate that both MRI and ^{18}F-NaF are able to identify the acute stage in FOP, i.e. flare-ups, but that MRI is not suitable for identifying the chronic stage, i.e. progression of HO in the absence of any clinical signs. There is, however, a third stage, characterised by mild oedema (table 3), which requires further investigation as it is not fully understood. This stage might be a reversible stage that either develops into the acute or chronic stage (moderate to severe oedema and ^{18}F-NaF PET positive) or resolves without HO growth.

Another finding of the present study was the long duration of oedema present at flare-up sites. Oedema was observed for five months after onset of a flare-up. In previously reported cases, MRI was performed only within 2 to 5 weeks after the onset of a flare-up [11,15]. It therefore seems that the inflammation that coincides with a flare-up, might be present for a longer period. The significance and explanation of this long duration of oedema needs to be further investigated.

The MRI rating used in this study (mild, moderate or severe) is based on previous studies [17,19]. The degree of muscle inflammation was based on the overall impression of the muscle (e.g. swelling) due to the inflammation [17]. Both in the present and in previous studies the agreement between two readers was moderate to good [17].

The present study is limited by its retrospective design, resulting in different protocols used for MR imaging. The resolution of images differs only slightly between imaging protocols,

it is therefore likely that this has had no influence on our judgement of the oedema. In addition, in one patient both a field strength of 1.5 and 3 tesla were used. In STIR sequences, however, differences in field strength are not expected to affect the appearance of oedema[24]. A further limitation was the availability of longitudinal ^{18}F-NaF PET/CT and MRI scans of only 4 patients. This is due to the extreme rarity of the disease, poor mobility of patients and unpredictability of flare-ups. One could also argue whether newly formed cartilage could be misdiagnosed for oedema, but this is unlikely, as, compared with oedema, cartilage has lower signal intensity, is more compact and shows structural distortion of muscle morphology [25,26].

In conclusion, this study is the first to compare longitudinal imaging data between MRI and ^{18}F-NaF PET/CT in order to establish the diagnostic value of MRI in FOP. MRI is particularly helpful in identifying muscle oedema in FOP patients, indicating a concomitant inflammatory process. Moderate and severe oedema resulted in the formation of HO in 6 out of 7 lesions. However, only half of the oedematous lesions on MRI eventually developed into HO. Moreover, MRI could not detect chronic FOP disease. The significance of mild oedematous lesions on MRI that were not related to HO or ^{18}F-NaF PET/CT needs further exploration.

Table 3. Proposed stages of FOP activity based on MRI and ^{18}F-NaF PET/CT findings according to Eekhoff & Botman

Stages	MRI oedema	^{18}F-NaF PET/CT activity	FOP disease stage
0	-	-	No FOP activity
1	+	-	Inflammatory stage
2	-	+	Chronic stage
3	+	+	Acute stage

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Chapter 5

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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Abstract

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 N2O-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-NaF 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.

1. 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 ankyloses 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.

2. 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 analogue 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. Initially, the ulcer at the foot led to recurrent cellulitis of the right lower leg, with good response to antibiotic

treatment. Wound care led to improvement of the ulcer, but oedema complicated healing. Bandages, intensive wound care and targeted systemic and topical antimicrobial treatment for any inter current infection, resulted only in temporary improvement of wound healing. A neuropathic pain syndrome, clinically confirmed by the neurologist, was thought to be the cause of the allodynia in the right lower leg. In addition, repeated pressure on the skin while sitting in her wheelchair contributed to formation these ulcers. Custom-made orthopedic shoes and other biomechanical offloading devices were therefore used, and led initially to an improvement of the ulcers. Unfortunately, after years of treatment the ulcers and infections progressed, with antibiotic resistant microorganisms (pseudomonas aeruginosa) (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.

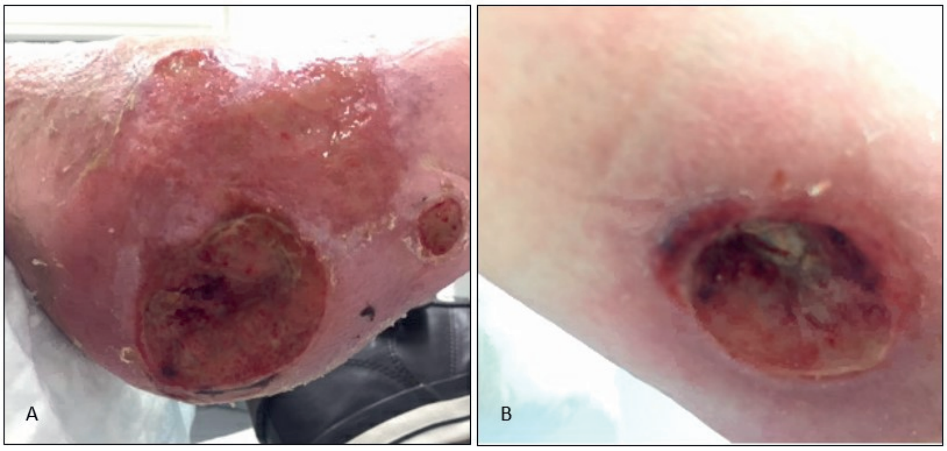

Figure 1. FOP patient with multiple incurable ulcers at the right lower extremity. **Left plane:** ulcer located at the right calcaneus. Despite Intensive wound care, custom-made orthopedic shoes and targeted systemic and topical antimicrobial treatment, surgical intervention was unavoidable. Due to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

2.1 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 (<2mm). 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, 40mg methylprednisolone was administered over the two nerve block catheters. Pre-operatively, 12ml 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% N2O and 50% O2 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 N2O-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 eight days postoperatively when oral medication was sufficient to control pain.

2.2 Surgical management

Due to therapy-resistant infected ulcers 10cm 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, 30mg 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

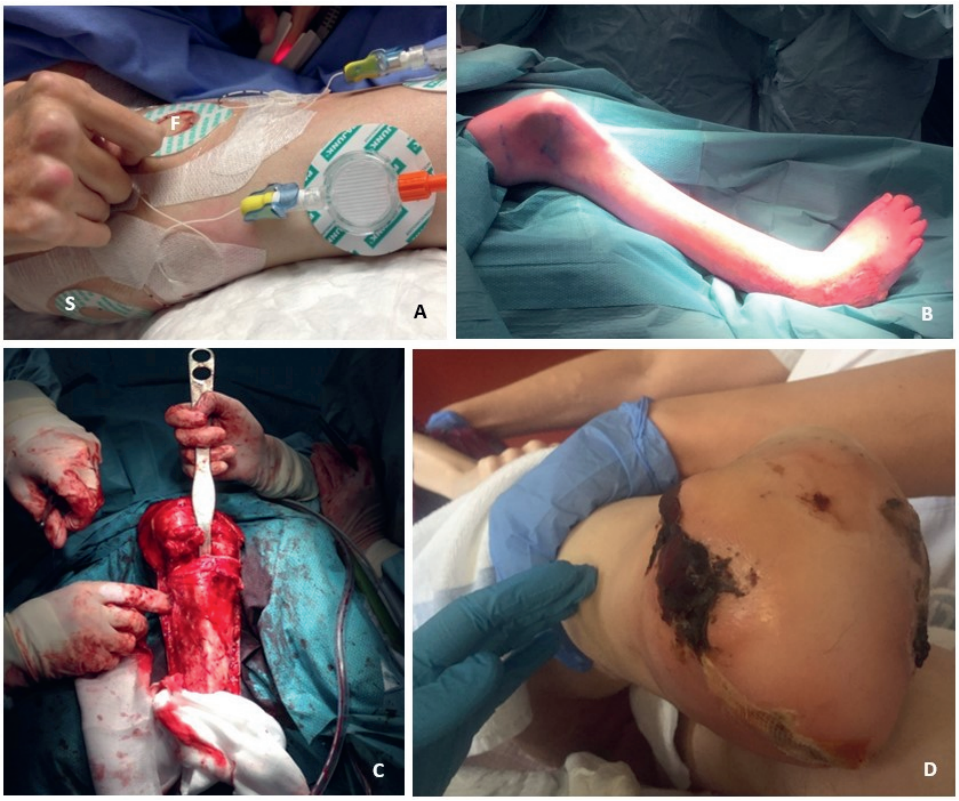

Figure 2. Anesthetic and surgical management of a through-knee amputation in an FOP patient
A: Two peripheral nerve block catheters were placed under ultrasound guidance at the femoral and sciatic nerve. Pre-operatively nerve block effectiveness was tested and confirmed. During surgery, the regional anesthesia was complemented with the administration of midazolam, s-ketamine and N2O-inhalation. B: The patient was carefully positioned on the theater table to prevent any tissue damage that might cause FOP disease activity. 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. Lateral of the stump an area of necrosis developed, but healed with supportive care.

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 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.

2.3 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 fourteen 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 eight weeks after surgery, revealing minimal HO formation (4 cc) at the base of the femur (Figure 3). Another follow-up scan obtained twelve 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 four 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 fourteen 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. After four months, the patient returned home. At the most recent follow-up, fourteen 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.

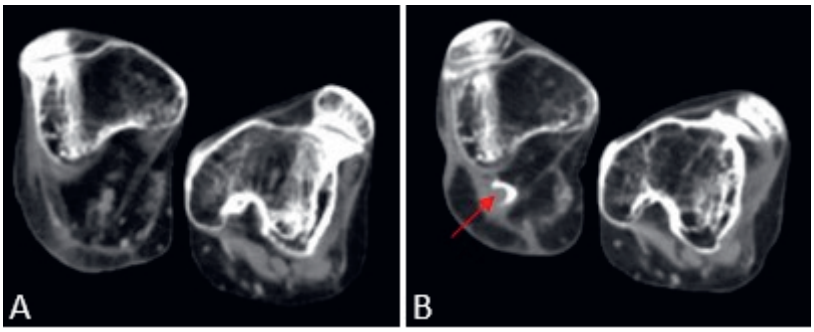

Figure 3. Axial Low dose CT-images at the level of the distal femur of a patient prior to and after a through-knee amputation of the right leg.
A: Eight months prior to the surgery. B: Twelve months after the surgical procedure. Minor HO formed (4cc) on the right side posterior to the lateral femoral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

3. 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.

The literature search generated a total of 1774 references: 692 in PubMed and 1082 in Embase. After removing duplicates of references that were selected from more than one database, 1223 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 [12,13,19-28].

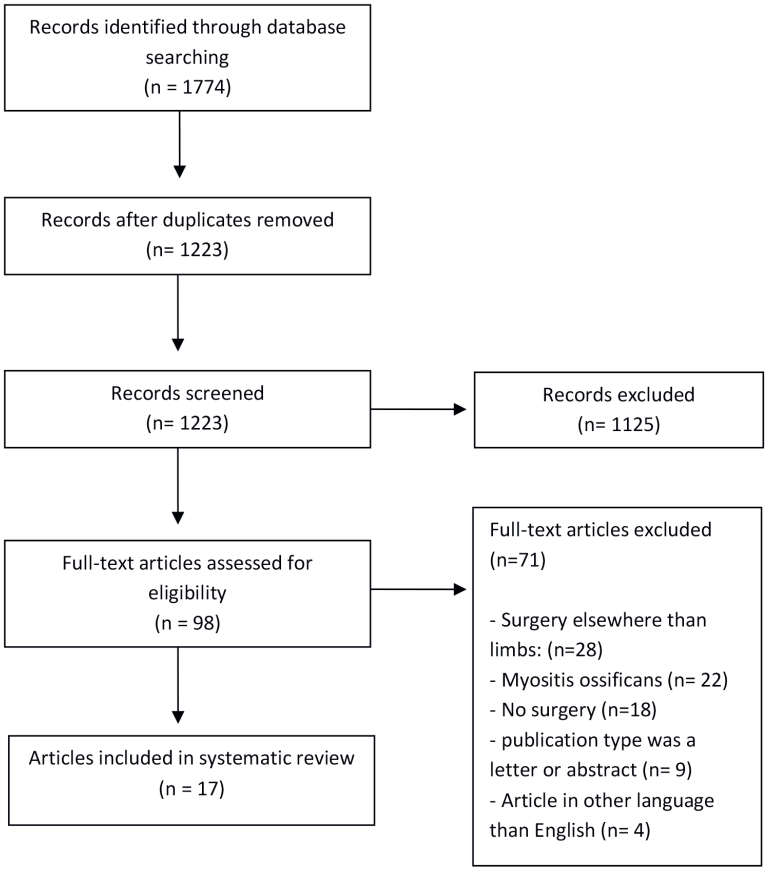

Figure 4. Flowchart of the study selection process

3.1 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 five to six months. The time for HO to redevelop after surgery was 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.

3.2 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 four weeks to thirty-six 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.

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

Author (year)	Patient	Age (years)	FOP- diagnosis (Y/N/U)*	Body part	Reason surgery	HO (re)occurrence (Y/N/U)	(re)occurrence HO noted (months)	Duration ollow-up (months)	Clinical improvement (Y/N/U)	Medication used pre-, peri- or postoperatively
Benetos et al. (2006)	1	14	Y	Shoulder	Unlock joint	Y	U	U	U	-
		18	Y	Hip	Unlock Joint	Y[1]	7	12	Y, improved mobility	Indomethacin 25mg/3dd, RT: 7Gy in 1 fraction
Colmenares-Bonilla et al. (2018)	2	11	Y	Knee	Unlock joint	Y[1]	1	60	N	Corticosteroids 30mg/kg, Alendronate 10/mg/day
Connor et al. (1982)	3	1	N	Shoulder	Remove swelling	Y	-	U	N	-
	4	6	U	Shoulder	Unlock joint	Y	U	U	N	-
Corfield et al. (2000)	5	24	Y	Wrist	Improve position	Y[1]	3	3	Y: functional position	-

Table 1. Continued

Author (year)	Patient	Age (years)	FOP- diagnosis (Y/N/U)*	Body part	Reason surgery	HO (re)occurrence (Y/N/U)	(re)occurrence HO noted (months)	Duration ollow-up (months)	Clinical improvement (Y/N/U)	Medication used pre-, peri- or postoperatively
Duan et al. (2010)	6	17	Y	Hip	HO induced claudication	U	U	24	Y: no claudication	-
Holmsen et al. (1979)	7	20	Y	Hips	Unlock joint	Y[1]	2	24	N	EHDP 10mg/kg/day
Jayasundara et al. (2012)	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: 26Gy in 13 fractions
Kartal et al. (2010)	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. (1976)	10	34	U	Calf	Unlock joint	N[1]	-	U	U	EHDP 20mg/kg/day
				Elbow	Unlock joint	U	-	U	Y: improved mobility	EHDP 20mg/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 20mg/kg/day
		U		Hip	Unlock joint	Y[1]	7	7	N	EHDP 20mg/kg/day
	12	17	U	Hamstring	Unlock joint	Y	U	U	U	Prednisone 7.5mg/day
		18		Biceps	Unlock joint	U	-	U	Y: improved mobility	EHDP 20mg/kg/day
				Hip	Unlock joint	N[1]	-	24	Y: improved mobility	EHDP 20mg/kg/day
	13	21	U	Hip[1]	Unlock joint	Y	2	5	N	EHDP 20mg/kg/day
Kocyigit et al. (2001)	14	15	N	Elbow	Unlock joint	Y	U	U	N	-
Matsuda et al. (2014)	15	35	Y	Malleolus	Incurable ulcer	N	-	8	N	-
Nerubay et al. (1987)	16	7	Y	Femur	Fracture	Y	U	12	N	-
Obamuyide et al. (2015)	17	11	N	Axilla	Unlock joint	Y	U	U	N	-
Tiwari et al. (2018)	18	2	N	Arm	Removal swelling	Y	U	U	N	-
Trigui et al. (2011)	19	25	Y	Hip	Unlock joint	Y[1]	2	24	Y: functional position	Corticosteroids, bisphosphonates
Waller et al. (2006)	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.
Abbreviations: FOP = fibrodysplasia ossificans progressiva, Y/N/U = yes/no/unknown; HO = heterotopic ossification; RT = Radiotherapy; Gy = gray ; EHDP = Ethylene Hydroxydiphosphonate

3.3 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 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].

4. 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, twelve 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 ankyloses, 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 twelve 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.

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Supplementary tables

Supplementary table 1. Terms used in Pubmed to identify studies describing surgery in patients with fibrodysplasia ossificans progressiva

Search	Pubmed Query – May 2, 2019	Items found
#3	#1 AND #2	692
#2	"Surgical Procedures, Operative"[Mesh] OR "Anesthesia"[Mesh] OR surger*[tiab] OR surgical*[tiab] OR operation*[tiab] OR operative*[tiab] OR perioperati*[tiab] OR anesthe*[tiab] OR anaesthe*[tiab] OR incis*[tiab] OR extract*[tiab] OR excis*[tiab]	5,076,112
#1	"Myositis Ossificans"[Mesh] OR myositis ossificans[tiab] OR ossifying myositis[tiab] OR fibrodysplasia ossificans[tiab] OR ossifying fibrodysplasia[tiab] OR FOP[tiab] OR Munchmeyer*[tiab]	2,937

Supplementary table 2. Terms used in Embase to identify studies describing surgery in patients with fibrodysplasia ossificans progressiva

Search	Embase Query – May 2, 2019	Items found
#3	#1 AND #2	1,082
#2	'surgery'/exp OR 'anesthesia'/exp OR surger*:ab,ti,kw OR surgical*:ab,ti,kw OR operation*:ab,ti,kw OR operative*:ab,ti,kw OR perioperati*:ab,ti,kw OR anesthe*:ab,ti,kw OR anaesthe*:ab,ti,kw OR incis*:ab,ti,kw OR extract*:ab,ti,kw OR excis*:ab,ti,kw	6,866,560
#1	'ossifying myositis'/exp OR 'myositis ossificans traumatica'/exp OR 'fibrodysplasia ossificans progressiva'/exp OR 'myositis ossificans':ab,ti,kw OR 'ossifying myositis':ab,ti,kw OR 'fibrodysplasia ossificans':ab,ti,kw OR 'ossifying fibrodysplasia':ab,ti,kw OR FOP:ab,ti,kw OR Munchmeyer*:ab,ti,kw	3,855

Chapter 6

Radiotherapy in fibrodysplasia ossificans progressiva: a case report and systematic review of the literature

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Abstract

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 centimetre 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 tumour. Six months after treatment, there were no signs of HO either clinically or on ^{18}F-NaF PET/CT.

The systematic review identified eleven 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 a 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.

1. 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 six, 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 labelled 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 one month of surgery as the first sign of HO recurrence in a FOP patient, confirmed six 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 ther literature to address whether radiotherapy is safe in FOP patients.

2. 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 analogue 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 basal cell carcinoma (BCC). It extended up to the deep biopsy margin (2 mm). Since surgery is a 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 approximately 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, The 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.

3. 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 Materials.

731 articles were identified using this search strategy. 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, six articles addressed radiotherapy in FOP, and five the relationship between irradiation and the formation of HO.

3.1 Radiotherapy in fibrodysplasia ossificans progressiva

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 [9]. The remaining five cases described the use of low dose radiotherapy to prevent or treat FOP flare-ups or HO formation [10-14]. In 4/5 of these cases, a beneficial effect on flare-up symptoms or HO formation was reported [10-13]. In 2/5 cases, one or two additional treatment modalities were also reported: a nonsteroidal anti-inflammatory drug (NSAID) in both cases, and a bisphosphonate in one of them [10,13]. None of the cases reported

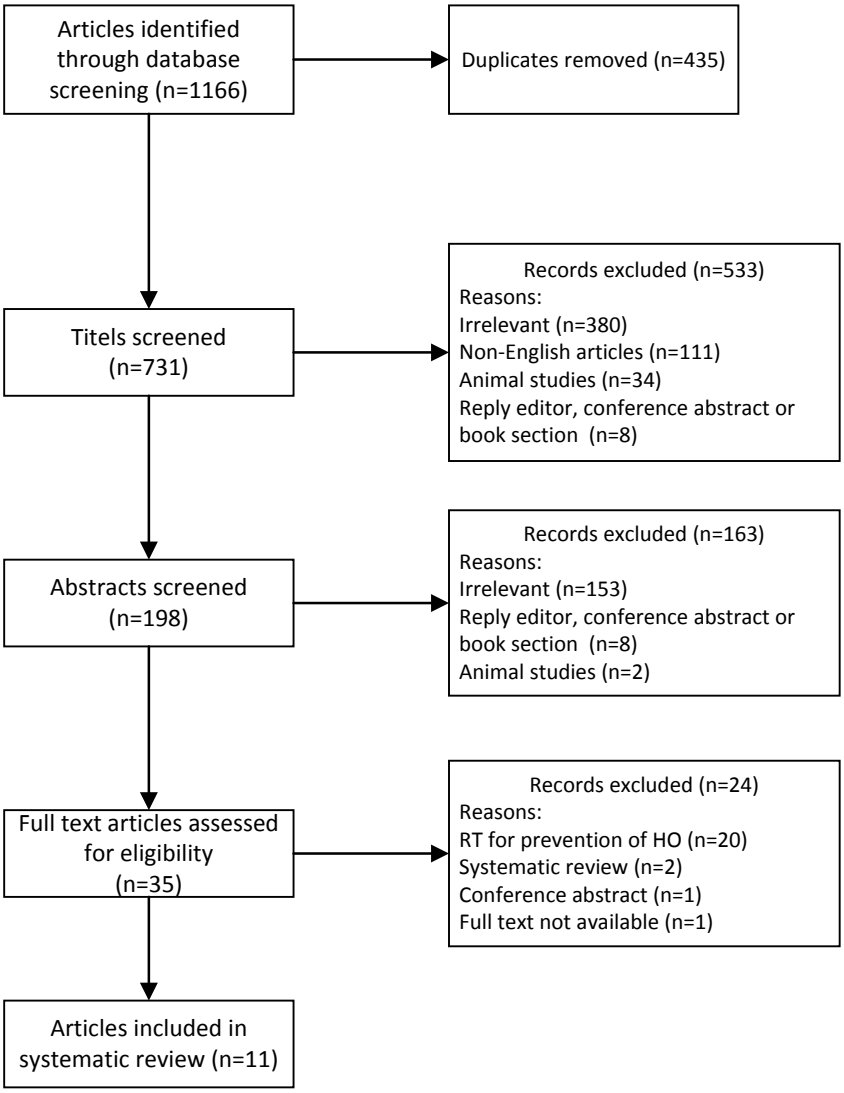

Figure 1. Flow chart of the study selection process

clinical deterioration or excessive toxicity as a result of radiotherapy (containing) treatment. All, but one, reported a relatively low dose of radiation [10,12-14], 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 2 fractions) [12]. In the remaining case, which was very similar to ours, a patient received 35 Gy in 5 fractions on consecutive days for the treatment of a right upper lip BCC. There was a

complete response with no evidence of HO at the irradiated site [9]. 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 7 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.

Table 1. Articles describing radiotherapy in patients with fibrodysplasia ossificans progressiva

	Author (year)	Age	Sex	Location	Dose (fractions)	Indication for RT	Follow-up interval after RT	Outcome	HO formed despite RT-containing treatment?
1	Benetos et al. (2006)	18	♂	Hip	7Gy (1)	Prevention of postoperative HO, combined with NSAID	1 year	Increased ROM	Yes[a]
2	Dharra et al. (2017)	35	♂	Shoulder	10Gy (5)	Treatment of flare-up	15 months	Relief of symptoms. Increased ROM	Unknown
3	Druce et al. (2002)	34	♀	Knee	10Gy (1)	Treatment of flare up. Combined therapy with NSAID and Bisphosphonate	2 months	Relief of symptoms.	Yes[b]
4	Frew et al. (2008)	46	♂	Lip	35Gy (5)	Basal cell carcinoma	Unknown	Complete response BCC	No
5	Jayasundara et al. (2012)	47	♂	Thigh	26Gy (13)	Prevention of post-operative recurrence of HO	Unknown	Outcome thigh lesion not described	Unknown
6	Soldic et al. (2011)	35	♀	Various (n=9) locations	2 (2)-10Gy (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 8Gy in 2 fractions, 6Gy in 6 fractions, 4Gy in 4 fractions and 3Gy in 3 fractions.
Abbreviations: RT = radiotherapy, HO = heterotopic ossification, Gy = Gray, ROM = range of motion, BCC = basal cell carcinoma, NSAID = nonsteroidal anti-inflammatory drugs

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

	Author (year)	Design	Number of patients	Reason RT	Dosage	Time interval RT and HO (years)
1	Carl et al. (2002)	Case series	15	Various carcinomas	BED 67-214Gy[a]	19 (range 2-31)
2	Kruse et al. (2009)	Case report	1	Nasopharyngeal carcinoma	Unknown	3[b]
3	Park et al. (2014)	Case report	1	Tonsil cancer	Unknown	14
4	Portha et al. (1982)	Case report	1	Metastasized mamma carcinoma	Unknown	1
5	Harmon et al. (1994)	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
Abbreviations: RT = radiotherapy, HO = heterotopic ossification, BED = biological effective dose (with α/β=3 for late tissue effects), Gy = gray

3.2 Development of heterotopic ossification 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 tumours (breast, anal, endometrial, sarcoma, seminoma, bladder and cervical) [17]. Radiation types varied, and include cobalt, neutrons and brachytherapy. Biologically effective doses (for late normal tissue damage, with α/β=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 were specified [16,18-20]. In 3 of these cases no other trigger 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, radiation, prolonged intubation, immobilization and critical illness neuromyopathy [16].

4. 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 is 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 eight weeks, except those of the hip and back, and of the latter, 75% resolve within twelve weeks [3]. One patient was irradiated for a flare-up at the iliopsoas muscle. Radiotherapy was combined with physiotherapy, indomethacin and disodium etidronate [13]. 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 oedema was significantly diminished and pain was relieved [13]. 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 [13]. Unfortunately, the longer term outcome is not known. Soldic et al. also reported benefit of radiotherapy in their patient who underwent multiple irradiations at different locations over a prolonged period [12]. They used calcification detected on radiographs or CT as a marker of disease. Interestingly, even though 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 tumour 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 tumour 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 [29] 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 [28]. 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 [10], traumatic injury is a major trigger for FOP flare-ups and subsequent HO [3,29]. 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 gastro-intestinal 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, 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 oedema 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.

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Supplementary tables

Supplementary table 1. Terms used in Pubmed to identify studies describing both radiotherapy and heterotopic ossification.

Search	PubMed Query – December 6, 2018	Items found
#3	#1 AND #2	515
#2	"Radiotherapy"[Mesh] OR "radiotherapy"[Subheading] OR radiotherap*[tiab] OR radiati*[tiab] OR irradiati*[tiab] OR x ray therap*[tiab]	673905
#1	"Myositis Ossificans"[Mesh] OR "Ossification, Heterotopic"[Mesh] OR fibrodysplasia ossifican*[tiab] OR myositis ossifican*[tiab] OR ossifying myositis[tiab] OR ossifying fibrodysplasia [tiab] OR stone man[tiab] OR acvr1[tiab] OR fop[tiab] OR heterotopic ossifica*[tiab] OR ectopic ossifica*[tiab] OR pathologic ossifica*[tiab]	12684

Supplementary table 2. Terms used in Embase to identify studies describing both radiotherapy and heterotopic ossification.

Search	Embase.com Query – December 6, 2018	Items found
#3	#1 AND #2	651
#2	'radiotherapy'/exp OR radiotherap*:ab,ti,kw OR radiati*:ab,ti,kw OR irradiati*:ab,ti,kw OR (x-ray NEAR/3 therap*):ab,ti,kw	934801
#1	'ossifying myositis'/exp OR 'heterotopic ossification'/exp OR (fibrodysplasia NEAR/3 ossifican*):ab,ti,kw OR (myositis NEAR/3 ossifican*):ab,ti,kw OR (ossifying NEAR/3 myositis):ab,ti,kw OR (ossifying NEAR/3 fibrodysplasia):ab,ti,kw OR 'stone man':ab,ti,kw OR acvr1:ab,ti,kw OR fop:ab,ti,kw OR (heterotopic NEAR/3 ossifica*):ab,ti,kw OR (ectopic NEAR/3 ossifica*):ab,ti,kw OR (pathologic NEAR/3 ossifica*):ab,ti,kw	12143

Chapter 7

Microarchitecture of heterotopic ossification in fibrodysplasia ossificans progressiva: an HR-pQCT case series

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Abstract

It is challenging to study heterotopic ossification (HO) in patients with fibrodysplasia ossificans progressiva (FOP) due to the contraindication of invasive techniques (*i.e.* bone biopsies), which can trigger flare-ups. The aim of this case study was to assess mature HO at microarchitectural level non-invasively with high-resolution peripheral quantitative computed tomography (HR-pQCT). Depending on the patient's mobility, HR-pQCT scans were acquired of peripherally-located HO and standard distal radius and tibia regions in two FOP-patients, a 33-year-old woman and a 23-year-old man, with the classical mutation (p.R206H). HO was located around the halluces, ankles and in the Achilles tendon. Standard HR-pQCT analyses were performed of the distal radius, tibia, and HO to quantify bone mineral density (BMD) and bone microarchitecture. Micro-finite element analysis was used to estimate failure load (FL). The outcomes were compared between HO and neighboring skeletal bone and with an age- and gender-matched normative dataset from literature. Bone parameters of the radius were within the interquartile range (IQR) of normative data. In contrast, in the tibiae of both patients, total and trabecular BMD were below the IQR, as were trabecular bone volume fraction, number, and thickness, cortical thickness, and FL. Trabecular separation and heterogeneity were above the IQR. Isolated HO in the Achilles tendon had a lower total, trabecular, and cortical BMD, trabecular bone volume fraction, and cortical thickness than the normative tibia data. Trabecular microarchitecture was within the IQR, and FL was approximately 10 times higher than of the neighboring tibia after accounting for areal differences. Other scanned HO could only be qualitatively assessed, which revealed coalescence with the neighboring skeletal bone, development of a neo-cortex, and partial replacement of the original skeletal cortex with trabeculae. To conclude, isolated HO seemed microarchitecturally more comparable to reference tibia data than the peripheral skeleton of the FOP-patients. Also, HO and skeleton appear to be able to become one entity when contiguous.

Keywords
Fibrodysplasia ossificans progressiva; Heterotopic ossification; Bone microarchitecture; Bone strength; High-resolution peripheral quantitative computed tomography

1. Introduction

Fibrodysplasia ossificans progressiva (FOP) is a rare genetic disease that is characterized by the formation of heterotopic ossification (HO) in ligaments, tendons, and muscles [1-3]. The formation of HO is often preceded by a clinical flare-up whose clinical signs are, among others, pain, redness, and swelling [3,4]. The histology of these flare-ups developing into HO has previously been studied through biopsies that were obtained for other purposes, mainly to exclude malignancies in non-diagnosed FOP-patients [5,6]. It is thought that, in the early stage of this HO development, the infiltration of inflammatory cells, such as lymphocytes, mast cells, and macrophages, causes cell death of the affected connective tissue. Proliferation of fibroblasts is thought to play a crucial role in the successive HO stage [7]. Finally, the fibroproliferative tissue develops into cartilage before it develops into endochondral bone [5,6,8].

Less is known about mature HO. The few histological and radiological case reports suggest HO to follow a normal endochondral process with deposition of bone matrix that visually appears indistinguishable from skeletal bone matrix with similar bone modeling and remodeling [9-11]. When mature, HO seems to consist of both compact and lamellar bone structures and apparently normal bone marrow [12,13]. In FOP-patients with the classic mutation (p.R206H), also the skeletal bone is assumed to develop normally despite developmental anomalies such as the frequently present shortened toes and the less frequently present short femoral neck and fusion of cervical facet joints [3]. These case reports are mainly qualitative as quantitative comparison of mature HO and skeletal bone remains difficult. Furthermore, histological examinations of mature human FOP HO are limited due to a contraindication of invasive techniques, such as bone biopsies, in FOP-patients as these techniques can trigger a flare-up and consequently aggravate the disease [4,14].

High-resolution peripheral quantitative computed tomography (HR-pQCT) may possibly alleviate the difficulty in investigating mature HO. This high-resolution imaging modality allows non-invasive and quantitative assessment of peripheral bones at the microarchitectural level, including quantification of geometry, density, and microarchitecture of the cortical and trabecular bone compartments and of the biomechanical properties of bone. Until now, HR-pQCT has mainly been used to study the distal radius and tibia [15], and to our knowledge, it has not yet been applied in FOP-patients. Therefore, the aim of this case study was to assess mature HO with HR-pQCT and to compare with neighboring skeletal bone. To evaluate whether the skeletal bone of the FOP-patients is representative, the FOP skeletal bone and HO were also compared with an age- and gender-matched reference. It was hypothesized that mature HO contains less and thinner trabeculae and a thinner cortex compared to neighboring skeletal bone because it is often non-functional and non-weight bearing. The skeletal bone was expected to show differences with an age- and gender-matched reference because of reduced mobility related to the disease.

2. Material and Methods

2.1 The patients

Two FOP-patients with the classical mutation (R206H), treated at the FOP Expertise Center of Amsterdam, underwent HR-pQCT imaging for this case study. Patient 1 is a 33-year-old woman who is ambulant: she can cover short distances, but she is not able to walk longer distances and to run. She is known with peripheral HO around both first metatarsals and in the left Achilles tendon. The peripheral HO has been present for over twenty years, and the patient has not noticed any flare-up or changes of this HO in the past five years. She has not been taking any glucocorticoids in the past twelve months but is on chronic non-steroidal anti-inflammatory drug (NSAID) treatment because of chronic pain due to HO at several sites. Patient 2 is a 23-year-old man. Peripheral HO is located around both first metatarsals and both ankles, all formed after a surgical correction of the hallux valgus early in childhood. The HO has been present for over 15 years, and the patient has not noticed any flare-up or changes of this HO in the past five years. He is wheelchair dependent when covering longer distances. He has not been using glucocorticoids and NSAIDs in the past twelve months. The peripheral HO in the patients was identified by physical examination and earlier acquired computed tomography (CT) and [^{18}F] sodium fluoride (NaF) positron emission tomography (PET)-CT. They resembled mature bone as defined by a density >200 HU on CT and were not metabolically active as assessed by peak standardized uptake values on [^{18}F]NaF PET/CT [16]. Both patients have been participating in the LUMINA-1 clinical trial with Activin A blocking antibody Garetosmab for three and four months, respectively at the time of HR-pQCT-scanning. The HR-pQCT-scans were not obtained as part of this double-blind placebo-controlled study. The patients signed the informed consent form to publish their data anonymously, and this form was approved by the Medical Ethics Review Committee of the Amsterdam UMC (Amsterdam, The Netherlands).

2.2 HR-pQCT imaging protocol

If mobility of the patients allowed proper and comfortable positioning, HR-pQCT scans were obtained using the second-generation HR-pQCT scanner (XtremeCT II, Scanco Medical, Switzerland) with standard clinical settings, defined by the manufacturer (X-ray tube voltage of 68 kV, intensity of 1460 mA, and integration time of 43 ms). For the distal radius and tibia, one 10.2-mm stack was scanned at the standard location according to the standard protocol starting 9.5 mm and 22.5 mm proximally from the radial and tibial endplate, respectively, and extending proximally. For the peripherally-located HO, customized 30.6-mm regions (three consecutive stacks of 10.2 mm each) were scanned to ensure full capturing of the HO. The scout view, as part of the standard HR-pQCT procedure, was used to confirm a full capturing. To scan the HO in the halluces and ankles, the patients were carefully positioned with the hip and knee in flexion and the foot in plantar flexion. During acquisition of all scans, the lower arm or lower leg was placed in a standard motion restraining holder, and foam was added to the holder when necessary to ensure the patient's comfort. Quality of

the scans was graded by the operator during scan acquisition by inspection of a single low-resolution slice of each stack using the clinically used grading system provided by the manufacturer [17]. A scan was repeated when the quality of at least one stack had a grade >3 out of 5. Acquisition of one stack takes two minutes, resulting in total acquisition time of two minutes for each radius and tibia scan and six minutes for each HO scan. Effective radiation dose is approximately 5 μSv per stack, leading to an effective dose of approximately 5 μSv per radius and tibia scan and of approximately 15 μSv per HO scan. The scans were reconstructed with an isotropic voxel size of 61 μm, resulting in 168 consecutive slices per radius and tibia scan and in 504 consecutive slices per HO scan.

2.3 Evaluation of the HR-pQCT scans

The peripherally-located HO were visually assessed by a musculoskeletal radiologist (BT) affiliated to the FOP Expertise Center; the isolated HO in the Achilles tendon of patient 1 was also quantitatively evaluated as were the distal radius and tibia. For the quantitative analysis, the isolated HO was manually segmented, and the distal radius and tibia were segmented using an automatic contouring algorithm provided by the manufacturer of the scanner. Thereafter, standard methods were used to quantify bone geometry, density, and microarchitecture of the segmented HO and distal radius and tibia. Geometric parameters included total, trabecular, and cortical area (Tt.Ar, Tb.Ar, and Ct.Ar, respectively; [mm^2]). Densitometric parameters included volumetric bone mineral density of the entire, trabecular, and cortical bone (Tt.BMD, Tb.BMD, and Ct.BMD, respectively; [mg HA/cm^3]). Microarchitectural parameters included trabecular bone volume fraction (Tb.BV/TV, [-]), trabecular number (Tb.N; [mm^{-1}]), thickness (Tb.Th; [mm]), separation (Tb.Sp; [mm]), and heterogeneity (Tb.1/N.SD; [mm]), and cortical thickness (Ct.Th; [mm]) and porosity (Ct.Po; [-]). Additionally, failure load (FL) was estimated of the segmented HO and distal radii and tibiae by means of micro-finite element (μFE-) modeling. Linear three-dimensional μFE-models were generated by converting the bone voxels of the HR-pQCT scans to equally-sized brick elements, which were assigned a Poisson's ratio of 0.3 and a Young's Modulus of 8748 MPa [18]. An axial compression to 1% strain was then simulated along the longitudinal axis (*i.e.* compression with constraint of lateral expansion at the bone endings) to estimate FL, for which Pistoia's criterion was used [19]. For this estimation of FL, it was assumed that the HO experiences a tensile load in the direction of the calve muscles and thus in the direction of the compression load on the tibia, and simulating either tension or compression gives, apart from the sign, the same results in FE-modeling.

The resulting values of the bone parameters from all analyses were compared to an age- and gender-matched normative reference group. This normative dataset was obtained in a general Canadian male and female population using the same generation HR-pQCT scanner and analyses as in this case study and has recently been published by Whittier *et al*. (2020) [18].

3. Results

3.1 Scan acquisition

The patients' mobility allowed acquisition of HR-pQCT scans of HO in the left Achilles tendon and around both metatarsals of patient 1 and around the right ankle of patient 2. The HO in the Achilles tendon was visible on the standard scan of the left tibia and required no separate scan. HR-pQCT scans could not be acquired of the halluces and left ankle of patient 2 as he was not able to position his foot in plantar flexion due to ankle ankylosis. His right ankle could be scanned without plantar flexion of the foot, but with additional foam for comfort during scan acquisition. Due to the inability of both patients to properly position before the scanner, a distal radius scan could only be made of the left side of patient 1. Distal tibia scans could be obtained of the left and right tibia of both patients. All obtained HR-pQCT-scans were of good quality (≤ grade 3 for all stacks); therefore, none of the scans had to be repeated.

3.2 Evaluation of the distal radius and tibia

A three-dimensional visualization of the scanned left radius and right tibia of patient 1 is shown in Figure 1. Geometry, density, microarchitecture, and FL of the dominant side, the left radius of this patient, were within the 25th-75th percentile (pctl), except for Ct.BMD that

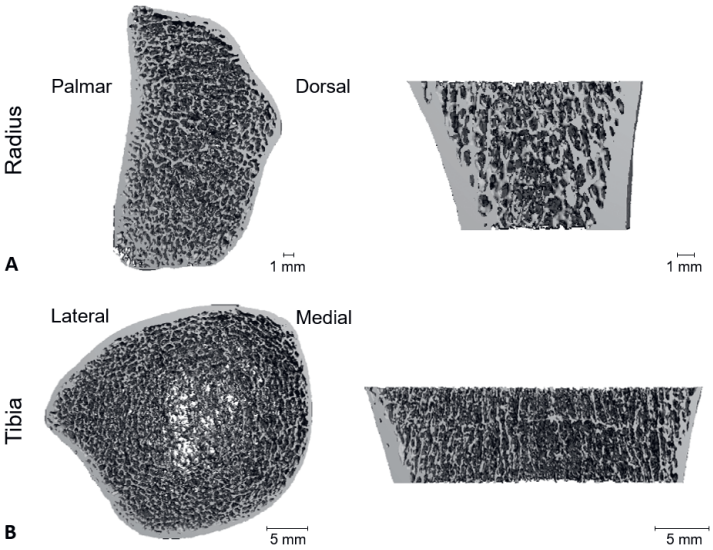

Figure 1. Three-dimensional visualisation of the scanned left radius (A) and right tibia (B) of patient 1, obtained with HR-pQCT. (A) Quantification revealed great resemblance of the radius of the FOP-patient to that of an age- and gender-matched reference group, except for cortical density that was higher in the FOP-patient. (B) Quantification revealed major dissimilarities with the age- and gender-matched reference group, especially in total and trabecular density (lower in the patient) and in trabecular and cortical microarchitecture.

was considerably higher (90th-98th pctl) (Table 1). At the tibia, both patients had a low Tt.BMD and Tb.BMD compared to the age- and gender-matched reference group (<2nd pctl) (Tables 1 and 2). Trabecular microarchitectural parameters were below or above the interquartile range (IQR): Tb.BV/TV, Tb.N, and Tb.Th were lower (<2nd pctl, <25th pctl, and <2nd pctl, respectively), and Tb.Sp and Tb.1/N.SD were higher (>75th pctl and >90th pctl, respectively). Also FL of the tibiae of both patients was lower than normative values (<2nd pctl). In patient 1, Ct.Ar and Ct.Th were lower in both tibiae (both <10th pctl) and Ct.BMD was in the lower half of the IQR. In patient 2, Ct.Ar and Ct.Th were <25th pctl in the right tibia and Ct.BMD was in the upper half of the IQR in both tibiae.

Table 1. Bone parameters of the left distal radius and left and right distal tibia of patient 1, a 33-year-old female, including normative values of the distal radius and tibia of age- and gender-matched controls.

	Radius				Tibia			
	Left Value	**Percentile***	**Right Value**	**Percentile***	**Left Value**	**Percentile***	**Right Value**	**Percentile***
Geometry (Area; mm^2)								
Tt.Ar	247.7	25-75	-	-	714.6	75-90	709.1	25-75
Tb.Ar	192.9	25-75	-	-	627.5	75-90	622.8	75-90
Ct.Ar	58.2	25-75	-	-	92.6	2-10	91.6	2-10
Density (BMD; mg HA/cm^3)			-	-				
Tt.BMD	336.0	25-75	-	-	205.3	<2	202.6	<2
Tb.BMD	143.1	25-75	-	-	100.9	<2	95.2	<2
Ct.BMD	991.2	90-98	-	-	934.8	25-75	955.5	25-75
Microarchitecture								
Tb.BV/TV (-)	0.187	25-75	-	-	0.143	<2	0.127	<2
Tb.N (mm^{-1})	1.442	25-75	-	-	1.149	10-25	1.162	10-25
Tb.Th (mm)	0.211	25-75	-	-	0.209	<2	0.202	<2
Tb.Sp (mm)	0.649	25-75	-	-	0.858	75-90	0.855	75-90
Tb.1/N.SD (mm)	0.225	25-75	-	-	0.420	90-98	0.380	90-98
Ct.Th (mm)	1.043	25-75	-	-	1.078	2-10	1.013	<2
Ct.Po (-)	0.002	25-75	-	-	0.008	25-75	0.009	25-75
Mechanics (μFE)								
FL (kN)	2.68	25-75	-	-	5.28	<2	4.88	<2

Abbreviations: Tt = total; Tb = trabecular; Ct = cortical; Ar = area; BMD = volumetric bone mineral density; Tb.BV/TV = trabecular bone volume fraction; Tb.N = trabecular number; Tb.Th = trabecular thickness; Tb.Sp = trabecular separation; Tb.1/N.SD = heterogeneity of the trabecular network; Ct.Th = cortical thickness; Ct.Po = cortical porosity; FL = estimated failure load.

*Percentile is based on comparison with an age- and gender-matched reference from literature[18].

Table 2. Bone parameters of the left and right distal tibia of patient 2, a 23-year-old male, including normative values of the distal radius of age- and gender-matched controls.

Tibia	Left Value	Percentile*	Right Value	Percentile*
Geometry (Area; mm²)				
Tt.Ar	849.5	25-75	831.3	25-75
Tb.Ar	718.9	25-75	713.7	25-75
Ct.Ar	136.4	25-75	123.3	2-10
Density (BMD; mg HA/cm³)				
Tt.BMD	229.1	<2	232.8	<2
Tb.BMD	98.4	<2	113.9	<2
Ct.BMD	932.5	25-75	936.9	75-90
Microarchitecture				
Tb.BV/TV (-)	0.1558	<2	0.172	<2
Tb.N (mm^{-1})	0.855	<2	1.086	2-10
Tb.Th (mm)	0.235	<2	0.233	<2
Tb.Sp (mm)	1.170	>98	0.902	90-98
Tb.1/N.SD (mm)	0.827	>98	0.403	90-98
Ct.Th (mm)	1.510	25-75	1.318	10-25
Ct.Po (-)	0.012	25-75	0.007	10-25
Mechanics (μFE)				
FL (kN)	8.21	<2	7.82	<2

Abbreviations: Tt = total; Tb = trabecular; Ct = cortical; Ar = area; BMD = volumetric bone mineral density; Tb.BV/TV = trabecular bone volume fraction; Tb.N = trabecular number; Tb.Th = trabecular thickness; Tb.Sp = trabecular separation; Tb.1/N.SD = heterogeneity of the trabecular network; Ct.Th = cortical thickness; Ct.Po = cortical porosity; FL = estimated failure load.

*Percentile is based on comparison with an age- and gender-matched reference from literature[18].

3.3 Evaluation of heterotopic ossification

The HO in the left Achilles tendon of patient 1 was isolated from the neighboring skeletal bone in the scanned region and could therefore be qualitatively as well as quantitatively evaluated (Figure 2). The HO constituted a clear cortex and trabecular structure. The cortex appeared thinner than in the neighboring left tibia, but a thickening was present in the axial middle of the scanned region of the HO. Results of the quantitative analysis of the isolated HO and neighboring left tibia are presented in table 3. In the isolated HO, Ct.BMD was below the IQR of the normative dataset (<2nd pctl), while it was within the IQR in the left distal tibia. Tt.BMD, Tb.BMD, and Ct.Th were below the IQR for both the isolated HO and left tibia. The trabecular microarchitectural parameters were within the IQR for the HO except for Tb.BV/TV (10th-25th pctl), whereas for the left tibia, Tb.BV/TV, Tb.N, and Tb.Th were lower than the IQR (<2nd pctl, 10th-25th pctl, and <2nd pctl, respectively) and Tb.Sp and Tb.1/N.SD higher (75th-

90th pctl and 90th-98th pctl respectively). FL was approximately a factor ten lower in the HO than in the left tibia. It was low compared to normative values (<2nd pctl). Total bone area was approximately a factor ten higher in the HO than in the tibia.

The HR-pQCT scans obtained of the other peripheral HO in both patients revealed fusion with the neighboring skeletal bone and could only be qualitatively analyzed. Figure 3 shows a three-dimensional image of the halluces of patient 1. As can be seen, HO was fused with the phalanx, and at several sites where HO had adjoined the phalanx, the initial cortex was replaced by trabeculae. The trabecular compartments of the HO and phalanx seemed to merge and were surrounded by a cortex of the HO. This neo-cortex appeared thinner than the initial cortex of the phalanx, whereas the trabeculae appeared thicker (data not shown). A similar pattern was found in the right ankle of patient 2 (Figure 4): there was fusion of HO with the tibia, fibula, and talus with partial replacement of the original cortex by trabeculae and a (neo-) cortex surrounding the fused bone structures. Also, enlargement of the distal tibia was observed. The HO adjoining the ankle bones seemed to contain relatively more cortical bone than the ankle bones and the other HO lesions inspected.

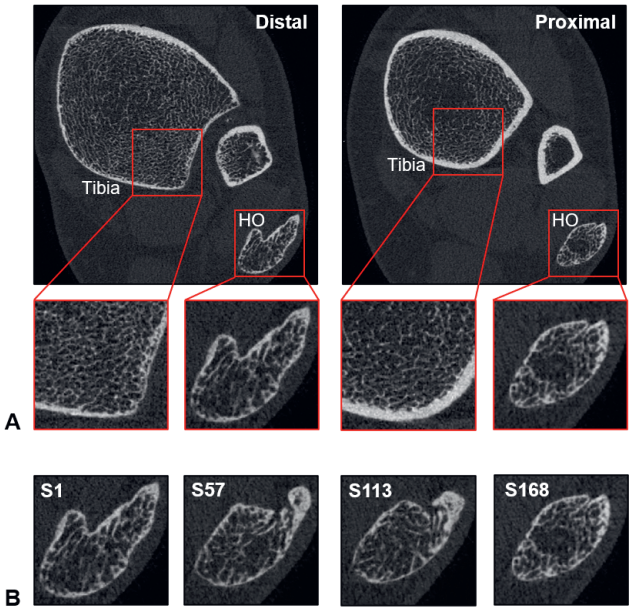

Figure 2. A) Distal (left) and proximal (right) slice of the HR-pQCT scan of the left tibia of patient 1, a 33-year-old female FOP-patient, showing isolated HO in the Achilles tendon. Quantification showed dissimilarities between the HO and the patient's tibia, especially in trabecular microarchitecture when compared to an age- and gender-matched reference. B) Four slices from distal (left) to proximal (right) of the HR-pQCT scan of the left tibia showing HO in the Achilles tendon.

Although all HO lesions analyzed by HR-pQCT had a density of >200 HU and thus resembled mature bone, HU was lower in these lesions than in neighboring skeletal bone that had a density of >350 HU. The only exception was the HO around the ankle, which had a considerable higher HU value than the surrounding talus, tibia, and fibula.

Table 3. Bone parameters of the left distal tibia and isolated HO in the left Achilles tendon of patient 1, a 33-year old female, including normative values of the distal tibia with age- and gender-matched controls.

	HO left Achilles tendon		**Left tibia**	
Geometry (Area; mm²)	**Value**	**Percentile***	**Value**	**Percentile***
Tt.Ar	64.9	<2	714.6	75-90
Tb.Ar	49.6	<2	627.5	75-90
Ct.Ar	17.2	<2	92.6	2-10
Density (BMD; mg HA/cm³)				
Tt.BMD	271.9	10-25	205.3	<2
Tb.BMD	142.1	10-25	100.9	<2
Ct.BMD	656.7	<2	934.8	25-75
Microarchitecture				
Tb.BV/TV (-)	0.195	10-25	0.143	<2
Tb.N (mm^{-1})	1.242	25-75	1.149	10-25
Tb.Th (mm)	0.254	25-75	0.209	<2
Tb.Sp (mm)	0.766	25-75	0.858	75-90
Tb.1/N.SD (mm)	0.282	25-75	0.420	90-98
Ct.Th (mm)	0.917	<2	1.078	2-10
Ct.Po (-)	0.013	25-75	0.008	25-75
Mechanics (μFE)				
FL (kN)	0.530	<2	5.28	<2

Abbreviations: Tt = total; Tb = trabecular; Ct = cortical; Ar = area; BMD = volumetric bone mineral density; Tb.BV/TV = trabecular bone volume fraction; Tb.N = trabecular number; Tb.Th = trabecular thickness; Tb.Sp = trabecular separation; Tb.1/N.SD = heterogeneity of the trabecular network; Ct.Th = cortical thickness; Ct.Po = cortical porosity; FL = estimated failure load.

*Percentile is based on comparison with an age- and gender-matched reference from literature[18].

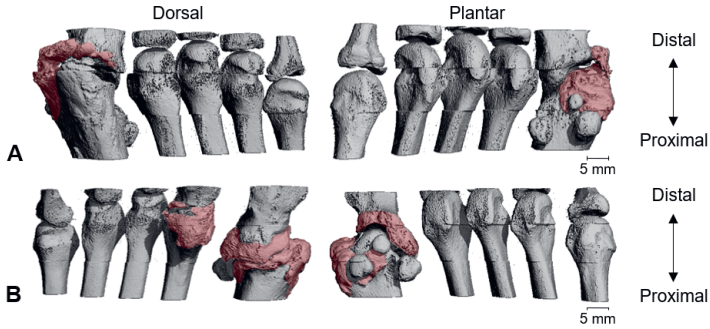

Figure 3: Three-dimensional visualization of the scanned metatarsals of the right (A) and left (B) foot of patient 1. The patient underwent surgery for the correction of a bilateral hallux valgus at the age of 1, resulting in HO at the operated sites. The pink color visualizes the sites with HO. It should be noted that, due to fusion, the exact border between HO and skeletal bone could not be established.

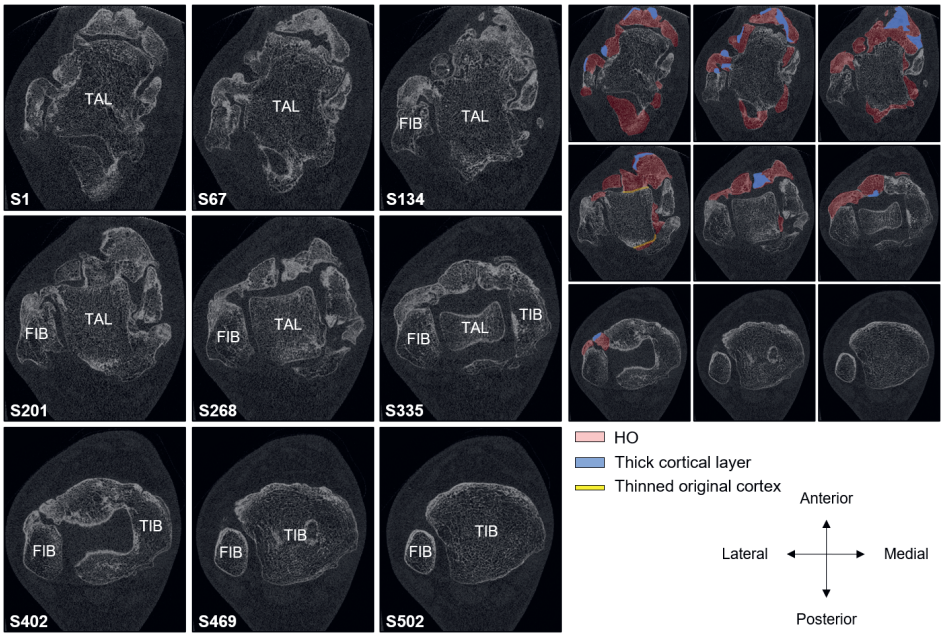

Figure 4. Nine two-dimensional slices of the HR-pQCT scan of a 30.6-mm region of the right ankle of patient 2, a 23-year-old male FOP-patient, from distal (S1) to proximal (S502). (S1) shows the talus with HO attached. Original cortical layer of the talus seems disrupted at several sites where HO has fused. (S67 – S502) HO has also fused to the fibula. However, a thin cortical layer seems to be present throughout the series. (S201 – S502) HO has fused to the tibia, and as with the talus, the cortical layer is disrupted at several sites of the fusion. TIB, TAL, and FIB represent tibia, talus, and fibula, respectively. On the right, HO, increased cortical bone and the thinned cortex are visualized in blue, red and yellow, respectively. It should be noted that, due to fusion, the exact border between HO and skeletal bone could not be established.

4. Discussion

The aim of this case study was to assess peripherally-located mature HO and skeletal bone of two FOP-patients with HR-pQCT and to compare those with each other and with an age- and gender-matched reference group from literature. The HO assessed in both patients was found to merge with neighboring skeletal bone. The cortex of the skeletal bone at sites of fusion appeared to be replaced by trabeculae to form one new entity, constituting trabecular bone surrounded by a (neo-)cortex. Most bone parameters of the isolated HO in the Achilles tendon of one of the patients were found to be within the IQR of age- and gender matched reference tibia data, whereas most of the neighboring tibia were below or above the IQR. Bone parameters of the distal radius resembled the literature values, except for cortical BMD.

To the best of our knowledge, this is the first study examining HO non-invasively and at a microarchitectural level in living FOP-patients. HO around the halluces and ankle of the patients was merged with the neighboring skeletal bone, and it appeared that a neo-cortex was formed surrounding the HO where it fused with the skeletal bone. However, thin lining of the original cortical layer of the skeleton was still visible at various regions, suggesting a yet incomplete coalescence. This may indicate that the fusion of HO and skeletal bone and associated remodeling is a slow process considering the presence of the HO for over 15 years in both patients. The neo-cortex around the halluces appeared thinner than the original skeletal cortex, whereas HO around the ankle consisted of a relatively thick cortical layer, which is perhaps due to the weight-bearing function of the ankle and its associated HO. Both these fused HO and the isolated HO assessed in this case study showed a cortical and trabecular compartment, as do skeletal bones, which, in that respect, agrees with previous case reports suggested that HO of FOP-patients has a similar morphology as skeletal bone [6,10]. However, these earlier publications have mainly investigated biopsies of early HO lesions that were taken in children for other reasons [6,12,13], while we investigated mature HO lesions as confirmed by CT. Furthermore, the use of HR-pQCT instead of histology enables quantitative evaluation of HO besides qualitative assessment, which may provide new insights into HO in FOP.

Today, quantitative assessment of the microarchitecture of HO is scarce. Most mice studies mimicking FOP and HO formation have not quantified HO microstructure despite the use high-resolution imaging modalities (*e.g.* μCT) [20-23]. In humans, the only study quantifying HO at the microarchitectural level concerned, to our knowledge, an *ex vivo* μCT-study on bone biopsies of non-genetic HO. In that study, surgically removed HO at muscular tissue was analyzed, which revealed variations in microarchitecture between and within lesions and an affected strength of the HO lesions compared to skeletal bone [24]. In contrast, we found FL of the isolated HO in the Achilles tendon to be approximately 10% higher than of the neighboring tibia when correcting for the difference in total bone area. This agrees with the

assumption in literature that the strength of HO in FOP is preserved, which is based on the observation that (stress) fractures of HO are not often seen in FOP-patients [25]. The discrepancy in the findings on strength between the study on non-genetic HO and our case study on genetic HO may, among others, be caused by a different ossification process with distinct histological characteristics between genetic and non-genetic HO [26]. Furthermore, HO in tendons may not be representative for HO in muscles as for example trauma-induced muscle HO in FOP appears to be driven through another progenitor lineage than HO formed in ligaments and tendons [27]. This may also contribute to the qualitative differences found at the microarchitectural level between the trauma-induced HO around the ankle of patient 2 and the spontaneously formed HO in the Achilles tendon of patient 1. Notably, the estimated FL in the isolated HO in the Achilles tendon is lower than peak forces that occur in the Achilles tendon during daily life in healthy individuals (*e.g.* 1.3-1.5 kN during walking; up to 4 kN during running and jumping [28-30]), which would suggest rupture of the Achilles tendon or fracture of the HO during such activities. However, such peak forces may not be reached in FOP-patients due to their reduced ability or inability to fully perform these activities.
Comparison of the quantitative evaluation of the HO in the Achilles tendon with the neighboring tibia showed that the microarchitectural parameters of the HO had better agreement with the age- and gender-matched reference than the left tibia. It is not known what may have caused this difference between HO and neighboring tibia. Possibly, a different mechanical stimulation may play a role, but study in larger datasets is needed before any conclusions can be drawn about possible microarchitectural differences between HO and skeleton. These microarchitectural differences could have contributed to the 10-% larger estimated FL of the HO compared to the tibia after accounting for areal differences. The comparison of the HO with the peripheral skeleton raises the question whether the skeletal bone of FOP-patients is comparable at the microarchitectural level to an age- and gender-matched reference [31]. Unlike microarchitectural misbalances throughout the entire skeleton in other rare bone diseases, the microarchitecture of the analyzed radius of patient 1 was found to be comparable to an age- and gender-matched reference population [32-35]. The tibiae of both FOP-patients, in contrast, did show considerable deviations from age- and gender-matched normative data: total and trabecular BMD were lower, and the trabecular compartment consisted of less and thinner trabeculae in a more heterogeneously formed network. A reduced mobility or a changed mechanical loading in the FOP-patients could have contributed to these differences from the normative data, as could have the frequent glucocorticoid use of both patients [36]. Research in larger datasets is needed to further investigate possible microarchitectural differences between the skeleton of FOP-patients and of the general population.

This study has shown that HR-pQCT allows visualization and quantification of BMD, microarchitecture, and biomechanical properties of mature HO in FOP-patients *in vivo*. This imaging modality enables analysis of mature HO in more detail than other imaging modalities, while simultaneously exposing patients to a negligible amount of radiation.

Furthermore, it is non-invasive in contrast to bone biopsy for histological analysis; therefore, it does not trigger flare-ups as is the case with biopsy. Consequently, HR-pQCT may be an interesting technique for future research into mature HO in FOP, such as for sequential HR-pQCT imaging to study the development of mineralized HO during a flare-up or the effects of a study drug on mature HO. However, it is likely that the ability of FOP-patients to properly position before the gantry of the scanner is restricted as shoulders, elbows, hips, and knees are frequently ankylosed in these patients [4], which may limit scan acquisition and affect scan quality. The right distal radius of patient 1 and both distal radii and the right ankle of patient 2 could not be scanned for that reason. As a result, it may be challenging to find patients eligible for HR-pQCT scanning combined with the rarity of FOP and the restriction to peripheral bones as HO in FOP-patients mainly forms more centrally, while peripheral sites are often spared [1,4,37,38].

Several limitations of this case study have to be mentioned. First, we examined HO of two FOP-patients, which gives an interesting impression about the skeletal microarchitecture of bone in FOP-patients but does limit interpretation. Larger datasets are needed to draw conclusions on possible differences in BMD, microarchitecture, and strength between HO and skeletal bone and between skeletal bone of FOP-patients and an age- and gender-matched reference population. Second, the estimation of FL using μFE-analysis and Pistoia's criterion is only validated for the distal radius, and thus its accuracy is not known for HO, further limiting conclusions on the strength of HO compared to skeletal bone. Third, the two patients in this study were participating in a double blind placebo-controlled trial at the time of HR-pQCT imaging, and it is unknown whether they were on active treatment that could have influenced bone parameters. Finally, quantitative analysis of HO was limited to HO in the Achilles tendon of patient 1 as the other scanned HO were not isolated from the neighboring skeletal bone, which made it impossible to establish the exact border between the HO and the skeletal bone for segmentation of the HO. Nevertheless, qualitative analysis was possible for this HO.

In conclusion, this case study showed that HR-pQCT allows non-invasive assessment of peripherally-located HO and distal radius and tibia in FOP-patients, which may provide new insights into skeletal and heterotopic bone in FOP-patients at the microarchitectural level. Isolated HO seemed microarchitecturally more comparable to skeletal bone from reference data than the peripheral skeleton of FOP-patients. Additionally, HO and skeleton appear to become one entity when contiguous.

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Chapter 8

Deterioration of pulmonary function: an early complication in fibrodysplasia ossificans progressiva

Submitted

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Abstract

Fibrodysplasia ossificans progressiva (FOP) is a genetic disease characterized by the formation of heterotopic ossification (HO) in connective tissues. HO first develops in the thoracic region, before more peripheral sites are affected. Due to HO along the thoracic cage, its movements are restricted and pulmonary function deteriorates. Because development of HO is progressive, it is likely that pulmonary function deteriorates over time, but longitudinal data on pulmonary function in FOP are missing.

Longitudinal pulmonary function tests (PFTs) from seven FOP patients were evaluated retrospectively to assess whether there were changes in pulmonary function during aging. Forced vital capacity (FVC), forced expiratory volume in one second (FEV1), total lung capacity (TLC), residual volume (RV) and diffusing lung capacity for carbon dioxide divided by alveolar volume (DLCO/VA) were included. In addition, HO volume along the thorax together with its progression as identified by whole body low dose CT scans were correlated to PFT data. Per patient, aged 7-57 years at the time of the first PFT, three to nine PFTs were available over a period of 6-18 years. Restrictive pulmonary function, identified by TLC or suspected by FVC, was found in all, but one, patients. In three patients, TLC, FVC or both decreased further during the follow-up period. All, but one, patients had an increased RV. The DLCO/VA ratio was normal in all FOP patients. Interestingly, FEV1 increased after a surgical intervention to unlock the jaw. In four out of five patients total HO volume in the thoracic region progressed beyond early adulthood, but no further decline in FVC was observed.

In conclusion, restrictive pulmonary function was found in the majority of patients already at an early age. Further studies are needed to assess whether the deterioration in pulmonary function is indeed age dependent.

1. Introduction

Fibrodysplasia Ossificans Progressiva (FOP) is a rare, disabling genetic disease, which is characterized by the formation of heterotopic ossification (HO) in muscles, ligaments and tendons[1,2]. HO often is preceded by a flare-up, an inflammatory process with, as yet, unknown pathophysiology. In most cases the first flare-ups occur around the age of six, often involving neck and upper back[3]. Thoracic HO immobilizes the thoracic cage, restricting normal expansion of the lungs[4-7]. As a result, patients are dependent on diaphragmatic breathing as the diaphragm is spared in FOP[7]. Mean life expectancy of patients with FOP is limited to 40-50 years of age, with cardiorespiratory complications as the major cause of death[4,5]. Some cross-sectional studies have shown restricted pulmonary function in FOP, which was attributed to limited chest mobility[6-8]. It is not known, however, whether pulmonary function declines further while the disease is progressing. Longitudinal pulmonary function tests (PFTs) could give more insight on the impact of both HO volume and its progression on pulmonary function[9].

To the best of our knowledge, longitudinal data on pulmonary function in FOP patients have not been studied yet. It can be hypothesized that a decline in pulmonary function, if present, is related to (chronic) progression of HO around the thoracic cage. The aim of this study was therefore to assess the relationship between temporal changes in PFTs and volumetric HO changes along the thoracic area.

2. Methods

FOP patients treated at the FOP Expertise Center of Amsterdam UMC were included if successive PFTs were available between 1995 and 2019. In addition to PTFs obtained at the Amsterdam UMC, test results from referring centers were also included in the analysis. Furthermore, whenever available, whole body low dose computed tomography (WBLDCT) scans were used to assess volume and progression of HO in the thoracic area and to evaluate structural changes within the lung parenchyma.

The Medical Ethics Review Committee of the Amsterdam UMC, Vrije Universiteit Amsterdam approved the study. All patients signed informed consent for the analysis and anonymous publication of their data.

2.1 Pulmonary function tests

PFTs were obtained during yearly follow-up visits at Amsterdam UMC. Before 2014, spirometry, body plethysmography and single-breath transfer factor of the lung for carbon monoxide (DCLO) were measured using VMAX equipment from SensorMedics (Yorba Linda, CA, USA). From 2014, the Sentrysuite v.2.19 spirometry instrument (Carefusion, San Diego,

California, USA) and the Vyntus body plethysmograph (Vyaire Medical, Mettawa, Illinois, USA) were used. Static lung volumes of patients who were unable to enter the plethysmograph because of wheelchair dependency, were assessed by nitrogen washout on the VMAX equipment and by helium wash-in on the SentrySuite instrument. The following pulmonary function parameters were measured: forced vital capacity (FVC), vital capacity (VC), forced expiratory volume in one second (FEV1), residual volume (RV), expiratory reserve volume (ERV) and total lung capacity (TLC). In addition, lung diffusion was assessed by measuring diffusing capacity for carbon dioxide (DLCO) where DLCO was divided by alveolar volume (DLCO/VA). Reference values used for static and dynamic lung parameters were taken from the Global Lung Function 2012 Equations[10]. For DCLO and DCLO/VA the guidelines of the Global Lung Function Initiative 2017 were used[11]. FVC, TLC, FEV1, DLCO and DLCO/VA were expressed as percentage of predicted, based on gender, height and ethnicity according to those guidelines [10,11]. For all measures, a percentage of predicted below 80% was considered deviant and the Tiffeneau index (FEV1/FVC) was considered deviant when below 70%[10,11].

2.2 Low dose CT-scans

Low-dose CT scans, acquired at 120 kV with a tube current ranging from 30 to 60 mAs, were assessed for structural abnormalities in lung parenchyma. CT scans were included when performed within either seven days prior to or one month after PFT. Images were analyzed by both a nuclear medicine specialist (PR) and a pulmonologist (AB) to assess parenchyma, pulmonary vasculature, pleurae, bronchi and heart size. Both, PR and AB, evaluated lung parenchyma blinded to the PFT results.

In addition, consecutive whole body low-dose CT scans (WBLDCT) were analyzed to identify HO lesions throughout the body and around the thoracic area. These HO lesions were identified and analyzed manually in order to calculate their volumes in successive images. Both readers (BT and EB) were blinded to the PFT of the patient. The association between HO volume of the total body, and HO within the thoracic area or the thoracic back and pulmonary function was statistically assessed. In addition, correlation between progression of HO in the thoracic area (chest) and decline in pulmonary function was evaluated. Moreover, the presence and a kyphosis and scoliosis was assessed using the available CT-scans. Both the scoliosis and kyphosis angle was assessed using Cobbs Angle [12].

2.3 Statistical Analysis

Statistical analyses were performed using SPSS Statistics for Windows, (IBM, version 24.0, Armonk). The Spearman's rho was used to assess correlations between HO volumes and change in PFT parameters.

3. Results

Longitudinal PFTs of seven FOP patients were included in the study. All patients, except one, had the classic mutation (R206H). Demographics of the patients are presented in table 1 and 2. The patient with the variant Q207E did not differ phenotypically from those with the classic mutation. All patients exhibited the classical clinical features of FOP with progressive HO formation. During flare-ups all patients underwent standard therapy with corticosteroids and non-steroidal anti-inflammatory drugs. Unfortunately, the frequency of flare-ups and drug treatments over the years had not been recorded. One patient (patient 004) is a smoker, with 5 pack years up to the end of the included period.

In total, 37 PFTs were included in the analysis. Of these PFTs, 12 were obtained in childhood (<18 years of age). Per patient, three to nine PFTs were available over a period of six to eighteen years. The age at which the first pulmonary function was obtained ranged from 7 to 57 years (table 1). 61% of all the PFTs were obtained at Amsterdam UMC. In two patients, tests were performed in other centers.

In five patients at least two WBLDCT scans were available during the observation period to relate HO progression in the thoracic region with pulmonary function.
For all 37 PFTs, FEV1 and FVC were available, while TLC was only measured in 20 of the 37 PFTs (from 6 patients). Diffusion capacity was measured in 15 of the 37 PFTs from six patients.

Table 1. Baseline characteristics and available data of the included FOP patients

	Sex	Mutation	Age*	Follow-up (years)	BMI**	Number of PFTs	Successive WBLDCT
001	♂	R206H	12	8	27.2	6	Yes
002	♀	R206H	35	8	13.3	3	Yes
003	♀	R206H	21	6	20.2	4	Yes
004	♂	R206H	57	11	26.3	8	No
005	♂	R206H	21	6	24.2	4	No
006	♀	Q207E	14	8	27.2	3	Yes
007	♀	R206H	7	18	20.5	9	Yes

* at the time of the first pulmonary function test.
** Highest BMI measured during the follow-up period
Abbreviations: FOP = fibrodysplasia ossificans progressiva; BMI = Body Mass Index; PFTs = pulmonary function tests; BMI: Body Mass Index; PFT: Pulmonary Function Test; WBLDCT = Whole Body Low Dose Computed Tomography

Table 2. Demographics of the included FOP patients

Patient	Age at diagnosis	Age et first flare-up	History of pneumonia?	Mobility (age of wheelchair dependence)	Kyphosis* angle (°)	Scoliosis* angle (°)
001	5	5	no	ambulant	10	n/a
002	3	UNK	yes	Wheelchair bound (19)	67	60
003	10	5	no	ambulant	n/a	n/a
004	12	UNK	no	Wheelchair bound (48)	44	50
005	6	UNK	no	Wheelchair bound (21)	n/a	55
006	6	6	no	Wheelchair bound (20)	60	n/a
007	6	6	no	Wheelchair bound (18)	n/a	20

*Kyphosis and scoliosis angles of the cervical and thoracic spine only were calculated, as this would impact pulmonary function.
Age in years, angles in degrees.
Abbreviations: UNK = unknown; n/a = not applicable / absent

3.1 Spirometry (FVC, VC, FEV1)

FVC was below 80% of predicted in all patients irrespective of age and was already <80% in the youngest patient tested at the age of seven. FVC deteriorated over time in three of the seven FOP patients with a decline already in childhood (Figure 1). In two of these patients, worsening continued into young adulthood, up to the age of twenty-three. For two other patients, also followed in their twenties, FVC did not increase or decline during observation. For the oldest two patients in this study, followed from the age of 57 to 68 years and 35 to 43 years, respectively, both absolute and percentage of predicted values for FVC remained stable. VC was congruent with FVC for all patients throughout the follow-up period.

FEV1/FVC, also known as the Tiffeneau index, was below 70% of predicted in two patients, aged 18 and 68 years old (Tiffeneau of 68% and 65%, respectively). For all other patients the Tiffeneau index was >70%, ruling out any obstructive component. Interestingly, one patient who underwent jaw surgery experienced an increase in the Tiffeneau index from 70% to 95% following surgery. The increased Tiffeneau index resulted from an increase in FEV1 (from 46% to 64%), possibly related to the slight improvement (3mm) of mouth opening (supplemental data, figure 1). FVC and VC remained stable pre- and post-operatively. In addition, also RV did not change due to the increased mouth opening. The Tiffeneau index in five patients with and two patients without jaw ankyloses showed no obvious difference (65-93% vs 91-94%, respectively).

3.2 Static Lung Volumes (TLC, RV, ERV)

TLC follow-up data were available for six patients. For one patient, only one measurement was available. A low TLC, indicating restrictive lung function (below 80% of predicted) was observed in five of the six patients. One patient with a low FVC and VC, appeared to have a normal TLC (Figure 1).

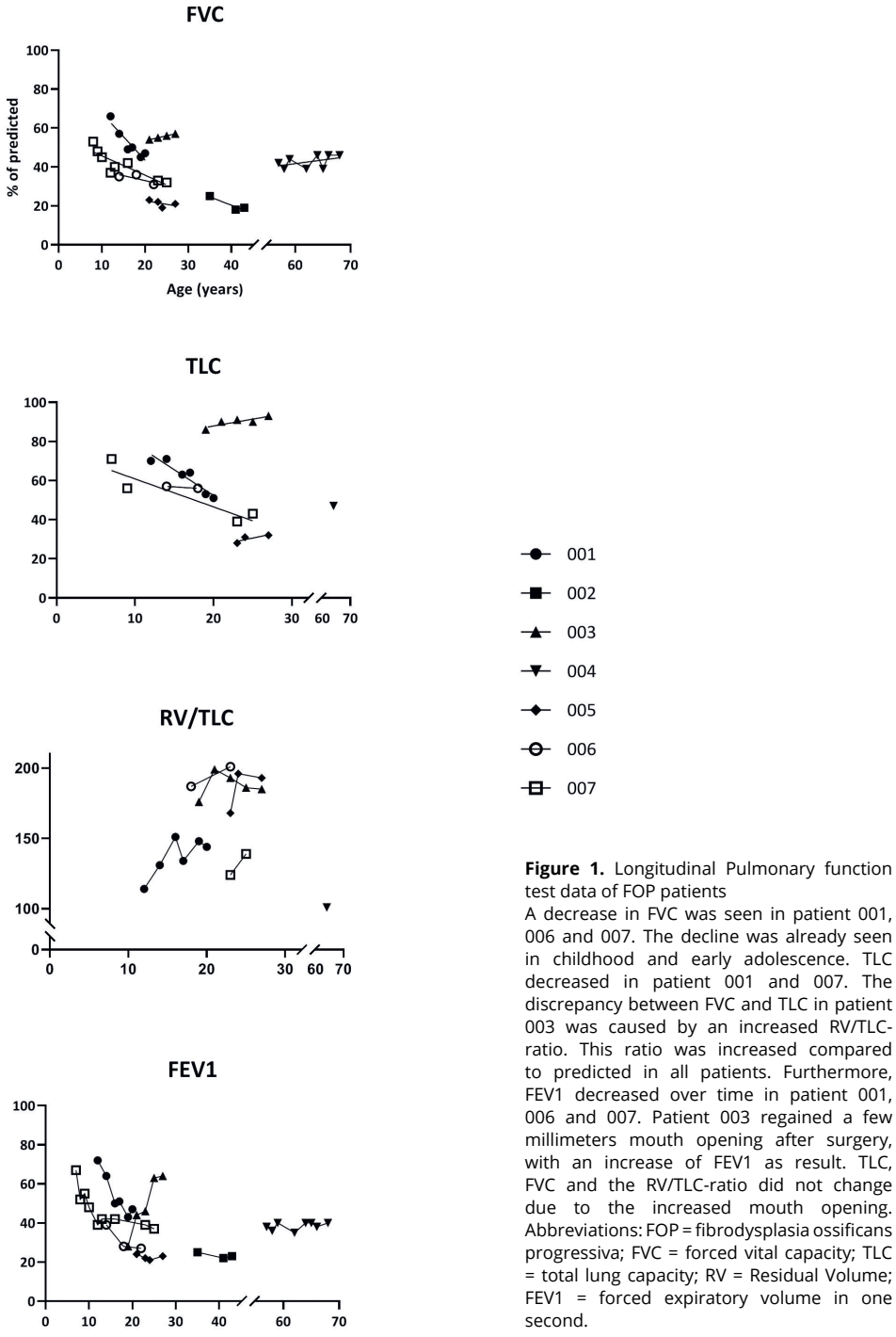

Figure 1. Longitudinal Pulmonary function test data of FOP patients
A decrease in FVC was seen in patient 001, 006 and 007. The decline was already seen in childhood and early adolescence. TLC decreased in patient 001 and 007. The discrepancy between FVC and TLC in patient 003 was caused by an increased RV/TLC-ratio. This ratio was increased compared to predicted in all patients. Furthermore, FEV1 decreased over time in patient 001, 006 and 007. Patient 003 regained a few millimeters mouth opening after surgery, with an increase of FEV1 as result. TLC, FVC and the RV/TLC-ratio did not change due to the increased mouth opening. Abbreviations: FOP = fibrodysplasia ossificans progressiva; FVC = forced vital capacity; TLC = total lung capacity; RV = Residual Volume; FEV1 = forced expiratory volume in one second.

Deterioration of TLC during the observation period was observed in two patients during childhood and early adolescence. TLC in one patient decreased until the age of 19 and in the other patient between the age of 9 and 23. After the age of 23, no decline in TLC was found, but it should be noted that this is based on data of only two patients who were followed for up to 2 years after stabilization of TLC. For the two oldest patients, TLC follow-up data were not available.

The RV to TLC ratio was increased in five of the six patients (Figure 1). The ratio ranged from 125-200% of predicted. The increase in mouth opening after surgery, did not result in normalization of RV. However, it did result in an increased volume of air that could be exhaled forcibly (ERV). Prior to surgery the ERV for this particular patient was 0.86L, which increased to 1.16L after surgery. For all other patients ERV ranged between 0.74L and 0.1L.

3.3 Diffusing capacity (DLCO and DLCO/VA)

DLCO was available for six patients and between 40-60% of predicted in five patients). The patient who underwent oro-maxillary surgery showed an no increase in DLCO after surgery. Also, no obvious difference in DLCO was found between patients with and without jaw ankyloses (45-98% vs. 39-46%, respectively). DLCO was corrected for alveolar volume (DLCO/VA), diffusion was >80% for all six patients.

3.4 Lung parenchyma

Low-dose CT scans to evaluate lung parenchyma were available for all seven patients. Five showed a highly deformed thoracic cage, while it was nearly normal in the other two patients. The presence and severity of kyphosis and scoliosis are presented in table 1. These two patients were 20 and 24 years old. Both had normal lung parenchyma, no pleural thickening or cardiomegaly. Three of the five patients with a deformed thorax showed intrapulmonary abnormalities: one had a partial atelectasis of the lower left lobe of ≈ 330 mL, occupying 4% of the total lung volume. One had mild ground-glass opacity at the age of 22. There were no abnormalities in the parenchyma or pleurae. One patient, aged 65, had a minimal consolidation (3mm), which is currently being followed by high resolution CT according to the Fleishner criteria[13].

3.5 Heterotopic ossification

In five patients successive LDWBCT-scans over a period of 6-26 months were available. Neither total body HO volume, HO volume within the thoracic area, nor HO volume along the thoracic back were significantly correlated with any of the PFT parameters (supplemental data). HO progression in the thoracic area was seen in four patients and ranged from 5 to 11 mL in 6 to 26 months. The most prominent progression in the thoracic area with an increase in HO of 11 mL in 6 months was accompanied by stable TLC, FVC and DLCO/VA (Figure 1). Including all five patients for whom successive CT scans were available, no

association was found between HO expansion and FVC changes over this short period of time (spearman's rho = -0.2; p=0.7). In addition, no association was found between total HO volume, thoracic HO volume and the PFT parameters (supplemental data).

4. Discussion

Thirty-seven PFTs were analyzed in a longitudinal cohort of seven FOP patients to determine whether lung function over a period of 6 to 18 years was associated with HO volume and HO progression in the thoracic area. A restrictive pulmonary function was found in all but one patients. This restriction in pulmonary function deteriorated over time during childhood and early adolescence, but a further decline later in life was not observed. No significant obstructive pulmonary function was found, nor a relationship between the degree of pulmonary function impairment and thoracic HO volume.
FEV1 is the simplest parameter to obtain a rapid assessment of lung function. A reduced FEV1 value usually is related to the degree of obstructive pulmonary function[14,15]. In the current cohort, significantly reduced FEV1 values were found in all patients, but in the presence of a normal Tiffeneau index. The reduced FEV1 value can therefore be attributed to reduced lung volumes in FOP patients. An ankylosed jaw, often seen in FOP, affects FEV1 values. In one the present patients, FEV1 increased by 40% after surgical unlocking the ankylosed jaw. A similar effect on FEV1 was found in possibly the only published study on this topic, assessing the effect of maxillomandibular fixation in healthy subjects on pulmonary function, where FEV1 decreased with approximately 20% as a result of the maxillomandibular fixation[16]. In addition, a study simulating the effect of upper airway obstruction using mouth pieces with orifices of different diameters, found that FEV1 progressively worsened with decreasing diameters[17]. Therefore, although FEV1 was decreased in most of the present FOP patients due to decreased lung capacity, jaw occlusion also may play a role in the severity of FEV1. Obstruction of the upper airways also seems to influence other values of the pulmonary function (e.g. ERV).

All patients had reduced FVC and VC, which suggests the presence of restrictive lung function. It should be noted, however, that to confirm restrictive pulmonary function initially, assessment of total lung volume (TLC) is essential, and therefore the use of an additional test is required[14]. In the present study, a normal TLC was only observed in one patient, despite a suggestive restriction based on FVC (57%). This may be explained by an increased RV, seen in all but one of the patients, and which represents the difference between TLC and VC. The underlying mechanism of an increased RV in FOP patients could be the inability to fully exhale, as confirmed by ERV, possibly due to completely ankyloses of the thoracic cage. An increased RV is also seen as an early pulmonary abnormality in neuromuscular diseases[18]. In later stages, muscle weakness leads to a restrictive pulmonary function. Whether this process also occurs in FOP patients, could not be confirmed with the current

limited dataset. Remarkably, RV in the 65-year old patient was within the normal range. With aging, RV is thought to increase as a result of stiffening of the thoracic wall[19]. FOP patients will not be susceptible for this stiffening as they are already completely ankylosed, explaining the increased, but stable, RV throughout the observed period in the current cohort. As RV does not seem to fluctuate over time, FVC can be used to monitor pulmonary function after initial confirmation of restriction by TLC.

The restrictive pulmonary function, as seen in the present study, is in concordance with findings of two cross-sectional studies[6,7]. These two studies included 21 and 15 FOP patients. respectively. Unfortunately TLC values were not obtained. Although the age of the patients studied by Kussmaul et al. ranged from 5-55 years, values were only presented for the entire group, making it impossible to compare pulmonary function of younger patients with that of older patients[6]. On the other hand, On the other hand, in their study on adult FOP patients, Connor et al. concluded that age had no effect on the degree of restriction, as FVC did not differ between age groups [7]. Whether there is a plateau in the degree of deterioration and, if so, at which age this would be reached, could not be determined in the present study, as only three patients were followed from childhood to adolescence with only a limited number of follow-up years.

Longer follow-up and more structural PFTs are needed to confirm whether pulmonary function indeed stabilizes at a certain age. It is assumed that the restrictive pulmonary function is the result of both malformed costovertebral joints and chest wall deformities due to asymmetrical HO formation along the spine and thoracic cage[4]. One could argue that the severity of the chest wall deformity (kyphosis or scoliosis) should have an impact on the restriction of the thoracic cage. Two of the patients with relatively mild thoracic deformities did, however, show severe restricted pulmonary function. Therefore, thoracic deformity alone does not fully predict abnormality of lung function. In addition, the location of HO, especially whether HO is located near crucial joints or not, might also be important. Also, pulmonary function hardly appears to deteriorate later in life, it seems that the amount of HO formed in the thoracic area at younger age may already be sufficient to restrict pulmonary function and that it is not affected by further (later) progression of HO. In an attempt to further investigate this, effects of total amount and chronic growth of HO in the thoracic region and pulmonary function were assessed, but neither showed a relationship with the pulmonary function nor its decline. Moreover, previously it has been shown that chest wall expansion does not deteriorate further after the age of 15[6]. However, lifetime PFT data from FOP patients are not available yet.

Life-long PFT data and WBLDCT images will be needed to evaluate the clinical relevance of mild abnormalities of the lung parenchyma, which were seen in two of the patients in the present study. Restrictive pulmonary function may lead to relaxation atelectasis, which may reduce TLC. In the present study no distinction was made between restriction caused by an

immobile thorax or by atelectasis. Atelectasis observed in one of the patients covered only a small portion of total TLC and, therefore, thoracic stiffness is likely to be the most important cause of restrictive pulmonary function in FOP. In an attempt to prevent atelectasis, respiratory muscle training, using a hand-held unit with tubing and a connected rebreathing bag, could be considered in an attempt to maintain adequate ventilation of the alveoli[20]. In addition, if oxygen therapy is considered, the patient should be monitored closely to prevent hypercapnia and hyperoxia related damage to airways or pulmonary parenchyma[5], especially as, in general, patients with a severely affected, immobilized thoracic cage already have a degree of hypercapnia[21].

The strength of this study is the long follow-up period of a group of FOP patients. The main limitation of the present study is the inability to relate pulmonary function tests to clinical data, due to incomplete documentation. Also, given that data were acquired within the context of standard patient care, PFTs had not been obtained regularly and not all parameters were obtained. In addition, measuring equipment was not standardized over time or between different centres. throughout time various measuring instruments have been used, and, data of other centers are included of which the measuring instruments are unknown.

In conclusion, longitudinal PFTs confirmed restricted pulmonary function in FOP patients already at young age. TLC is necessary to confirm the restrictive component in FOP patients initially, as increased RV may affect FVC. In addition, it should be noted that FEV1 values are diminished due to small lung volumes, but might also be affected the jaw ankylosis. Neither total volume of HO nor progression of thoracic HO seemed to affect pulmonary function later in life. It will be clear, however, that longer follow-up periods are needed to confirm this finding.

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Chapter 9

Summary and General Discussion

The aim of the work described in this thesis was to gain more insight in the development, progression and complications of heterotopic ossification (HO) in fibrodysplasia ossificans progressiva (FOP). Knowledge about the course of HO is scarce, despite the discovery of a causative genetic mutation in 2006. There are several reasons for this poor understanding of HO, such as the contraindication of invasive techniques to study HO as it may cause exacerbation of disease; the lack of a representative mouse model until very recently, and the rarity of the disease.

As a (blood)marker to measure disease activity was not available at the onset of the work described in this thesis, the main purpose was to investigate whether imaging techniques could play a role. More specifically, the use of [^{18}F] sodium fluoride (NaF) Positron Emission Tomography (PET) / Computed tomography (CT) was investigated as, at least in theory, this technique can be used to visualize and measure metabolic activity of bone. In addition, the additional diagnostic value of Magnetic Resonance Imaging (MRI) in FOP was studied. Both these imaging techniques were used to investigate whether FOP disease is only active during flare-ups, the course of FOP disease activity, and whether there are different developmental stages of HO. In addition, effects of different (traumatic) therapies on HO development were evaluated. The structure of matured HO was studied using a novel imaging technique, HR-pQCT, and, finally, effects of progressive HO on vital organs were evaluated using sequential pulmonary function tests.

Summary of main findings

In **chapter 2**, a severe spontaneous flare-up that led to wheelchair dependency of a FOP patient was described and followed using blood parameters and sequential ^{18}F-NaF PET/CT scans**.** Indeed, for the first time, it was shown that disease activity could be visualized and measured. Interestingly, the site of the flare-up was found to be only partially take up ^{18}F-NaF. This biologically active site showed to be associated with developing HO on a follow-up CT scan. In contrast, none of the measured blood parameters was correlated with disease activity. It was concluded that ^{18}F-NaF PET/CT provides the an early disease marker in FOP, able to predict whether a flare-up is ossifying or not.

In **chapter 3** it was investigated whether FOP disease activity is only present during flare-ups. Sequential CT scans of five FOP patients were used to measure HO volumes over time and, separately, sequential ^{18}F-NaF PET scans were analysed to identify biological activity of all HO lesions. These analyses revealed that, during the study period, only those HO lesions that showed ^{18}F-NaF uptake above a certain threshold, far above normal bone remodelling, progressed over time. This finding led to the conclusion that, next to acute flare-ups, FOP also has a chronic component, leading to slow progression of existing HO in the absence of a flare-up.

As there is no radiation exposure involved, the next question, addressed in **chapter 4**, was whether MRI could be used to identify the different aspects, i.e. flare-ups and chronic progression, of FOP disease activity. This was tackled by evaluating the presence and intensity of oedema on MRI of patients with FOP. MRIs were either obtained for yearly follow-up (whole body MRI) or because of suspicion of a flare-up. Oedema found by MRI in four patients was compared with disease activity obtained by ^{18}F-NaF PET. Flare-ups were indeed accompanied by either moderate or severe oedema. However, oedema was also found at sites without metabolic activity on ^{18}F-NaF PET, and those sites did not reveal any ossification on follow-up CT scans. Whether the presence of this oedema could be an expression of an early inflammatory phase in FOP needs to be further addressed in future studies. Lastly, the chronic phase, as described in chapter 3, was not accompanied by oedema and could therefore not be identified by MRI.

In **chapter 5** the effect of a surgical procedure on both flare-ups and the chronic component of disease activity was evaluated using both a systematic review of the literature and a separate case study. The systematic review of surgery on extremities in FOP revealed that in >80% of the cases limb surgery was complicated by HO formation. Although surgical procedures are contra-indicated in FOP, in the case report surgery was the last resort in the treatment of chronically infected ulcers of the right foot and lower leg. A through-knee amputation was performed with surprisingly only minor HO formation afterwards. In this chapter is was shown that, if life threatening, a surgical procedure could be considered if performed in a multidisciplinary team with FOP expertise.

In **chapter 6** the effect of radiotherapy on flare-ups and chronic disease progression was evaluated using ^{18}F-NaF PET/CT. Also, a systematic review of the effect of radiotherapy on HO formation in FOP patients was performed. This review revealed that previously only low dosages of radiotherapy (≤10 Gray) had been administered to FOP patients. Some authors suggested a beneficial effect of radiotherapy on symptoms, but its effect on halting HO formation has remained unclear. In addition, follow-up was in most cases insufficient. In the case study, a patient underwent a total of 54 Gray for a basal cell carcinoma (BCC) of the lip. Despite tissue damage that was clearly visible at the site of radiotherapy, no HO formed at the irradiated site. Neither did the treatment result in increased disease activity elsewhere. This case illustrates the safe application of curative radiotherapy for cancer treatment in a FOP patient. Furthermore, there might be a potential protective role of radiotherapy upon HO formation as found in the literature.

In **chapter 7** the micro-architecture of HO was compared with that of skeletal bone. Both HO and skeletal bone of two FOP patients were evaluated with the use of the HR-pQCT, a novel imaging technique. The HR-pQCT is a non-invasive, low-radiation method for assessing bone microarchitecture and volumetric bone mineral density (BMD) in cortical and trabecular compartments. Separate CT scans showed that overall density of assessed HO-lesions was

comparable with that of mature bone. HR-pQCT, however, revealed that cortical density of HO was lower compared with neighbouring skeletal bone, whereas trabecular density was increased. Interestingly, at some sites, it was found that HO and its adjacent skeletal bone had fused with disappearance of the original cortical layer. But, at various sites the original cortical layer was still intact, even after 20 years, implicating that this fusion of HO and skeletal bone is a very slow process.

In the last chapter of this thesis, **chapter 8,** the natural course of pulmonary function in FOP patients and its relationship with HO volume was investigated. Seven patients with in total thirty-seven pulmonary function tests over a period of 6-18 years were included retrospectively. Although previous studies have assumed restrictive pulmonary function in all FOP patients, this was not present in one of the patients included. Forced vital capacity, used in previous studies, was affected by the inability to fully exhale, which leads to an increased amount of air in the lungs after exhalation (residual volume). This increased residual volume, in turn, leads to an incorrect conclusion of restrictive pulmonary function . Therefore, total lung capacity should be obtained for the initial assessment of the pulmonary function in FOP. When present, restrictive pulmonary function could already be identified early in childhood and worsened into early adulthood. Later in life, pulmonary function seemed to remain stable. The degree of restriction was not correlated with the volume and progression of HO along the thoracic cage.

Discussion

^{18}F-NaF PET/CT is a non-invasive tool to identify and quantify both active and chronic disease activity in FOP patients. The presence of chronic disease might offer an explanation for the clinical phenomenon of progressive immobilization of FOP patients in the absence of flare-ups[1]. Disease activity of the chronic component of FOP is significantly lower than the activity measured during a flare-up, consistent with the slower growth of HO during the chronic phase. As nearly all FOP patients fit within the scanner, this technique can be used in clinical practice to alter therapeutic decisions. Furthermore, follow-up ^{18}F-NaF PET/CT scans could increase knowledge about the natural course of the disease, which still is not completely understood. In addition, this imaging technique seems to be of great value for clinical trials. Especially for those trials designed to halt HO formation after a flare-up, the ^{18}F-NaF PET/CT could be of great value. In fact, the first clinical trial in FOP in which Palovarotene was tested, was to halt HO formation by inhibiting the chondrogenesis[2]. Unfortunately, in 2014 the value of ^{18}F-NaF PET was not known yet, and therefore crucial information on the initial fate of the flare-up was missed by using CT only. In 2017, another phase II clinical trial was initiated, with ^{18}F-NaF-uptake as a primary endpoint. Garetosmab, an Activin A antibody, was tested as a therapeutic drug to reduce disease activity of both the flare-ups and the chronic component of the disease[3,4].

The ^{18}F-NaF PET/CT-scan exposes the patient to a relatively low dose of radiation by using a low dose whole body CT-scan. With ^{18}F-NaF PET/CT, the radiation dose to the patient is the combination of the radiation dose from the ^{18}F-NaF and the radiation dose from the CT. Based on the injection of 87 MBq ^{18}F-NaF to an adult patient of 70kg, the effective dose is 2,1 mSv for the ^{18}F-NaF . The effective dose for a whole body CT used for attenuation correction and correlation with the PET is variable and depends on a number of factors. For a whole body lowdose CT the effective dose may be 2.7 msv. Hence, the total effective of the ^{18}F-NaF PET/CT procedure ≈ 5 mSv[5].Due to increased risk of malignancies, physicians should take the radiation exposure into account, especially when there is need for sequential scanning due to recurrent flare-ups or participation in a trial. The malignancy most associated with radiation exposure is leukaemia, with a relative risk of 3.8 in children who received >30mSv compared to those who received <5mSv[6]. Due to the low incidence of leukaemia, the increased risk will mean in practice one extra case of leukaemia among 10.000 children.

It is important that centres follow the guidelines of the European Association of Nuclear Medicine (EANM) for correct dosing and injection time[5], in order to be able to compare the findings with each other. However, even though identical protocols are used, there will be a slight inter-scanner variation[7]. Therefore, reference values might need to be recalculated per centre. Lastly, ^{18}F-NaF PET/CT-images might be challenging to read, due to the highly distorted anatomy of the skeleton. Also, special adjusted programs for PET, CT and combined analysis are needed to manually segment and analyse the structures. Thus, preferably, these scans are performed in collaboration with an FOP expertise centre. So, even though a marker for disease activity was found with the ^{18}F-NaF PET/CT it will be still essential to find a simple, safe and widespread accessible marker. In the search for such a (blood)marker, ^{18}F-NaF PET/CT could be used as the golden standard for identifying ossifying disease activity, as increased ^{18}F-NaF uptake precedes active ossification. In the past, blood markers were related to clinical signs, i.e. flare-ups, and thus lacked quantification[8,9].

Unfortunately, MRI was not found as valuable as ^{18}F-NaF PET/CT in assessing acute and chronic ossifying disease activity. In particular, MRI was unable to detect chronic FOP disease. However, regardless of its fate, all clinical flare-ups, i.e. active disease, coincided with oedema and thus, in clinical practice, MRI can be used to distinguish a flare-up from other diagnoses. Unfortunately, it is not possible to precisely quantify the severity of oedema. As both presence and absence of oedema do not appear to be correlated with HO development or HO progression, the value of MRI in FOP will remain limited, especially for clinical trials. Nevertheless, MRI may be able to detect a potential very early phase of disease activity. Whether oedema in this early stage is triggered by trauma or develops spontaneously is still to be investigated. In addition, it still has to be determined whether and how this oedema resolves or perhaps develops into a flare-up. These questions can only be answered by continuing to follow FOP patients through MRI and ^{18}F-NaF PET/CT. The use of MRI will not

be feasible for all patients, as, due to its small diameter (60 cm), not all FOP patients will be able to fit in a regular machine. Ultrasound is quicker and patient friendlier[10,11], but will not be able to replace MRI in the evaluation of these oedematous lesions, as they are asymptomatic.

FOP patients might encounter conditions that need surgical intervention[12,13]. The patient. described in this thesis, was known with quiescent disease for a long period. Chronic infection and especially continuous antibiotic treatment may have led to the this silent state of disease. This is supported by the finding that months after surgical removal of the source of infection and discontinuation of the antibiotic treatment, increased ^{18}F-NaF uptake was found in several HO lesions. The immune system has previously been thought to play an important role in FOP disease activity, as it has been described that immunosuppressant drugs also suppress flare-ups[14]. The previous quiescence state, the careful management during surgery and the corticosteroid use may have led to the favourable outcome regarding the formed HO volume. The exact role of all factors described, need to be further investigated for its effect on HO formation. Despite a favourable outcome in this single patient, surgery is still highly contra-indicated in FOP patients[15,16]. Surgery should only be considered as a last resort. In addition, anaesthetic procedures are challenging due to the jaw ankylosis, the inability to extent the neck and the severely affected pulmonary function and should therefore only be performed in a FOP expertise center[17,18]. Hopefully, when a therapeutic drug to prevent HO formation is available, surgical procedures can be performed to unlock joints[19]. ^{18}F-NaF PET/CT should be the first choice for assessing the effects of these procedures on local and overall disease activity.

As surgery is contraindicated, radiotherapy is preferred for the treatment of malignancies in FOP patients, even though effects of radiotherapy on disease activity have never been evaluated properly. High dose (54 Gray) radiotherapy for skin cancer was found safe with respect to FOP disease activity, but its long-term effects on FOP disease are unknown. Furthermore, the effect of high-dose radiotherapy for treatment of less superficial tumours, e.g. breast, liver or colon tumours, on FOP disease activity is not known. In the past, radiotherapy has also been used in an attempt to prevent HO formation, and some authors even claim that low doses (<10 Gray) are beneficial[20,21]. The latter claim should, however, be interpreted with care, as it was primarily based only on subjective relieve of symptoms. When radiotherapy is considered, treatment could be monitored using ^{18}F-NaF PET/CT to evaluate its effect on disease activity.

HR-pQCT could be used to monitor the effects of a drug on HO, when it is expected to also affect existing HO. In addition, effects of the study drug on normal skeletal bone can be evaluated. Furthermore, HR-pQCT scans could gain more insight in the development of HO when obtained sequentially and during and after a flare-up. Its radiation exposure is ≈ 3 μSv per stack, which essentially is negligible[22]. Also, reproducibility of HR-pQCT

measurements of bone density and trabecular microarchitecture are comparable with that of established imaging techniques, with coefficients of variation of 1.5% to 4.4% [23]. For follow-up measurements, the primary concern is to rescan exactly the same region. It should be noted though, that reproducibility is only known for standard tibia and radius scans. In addition, reference values for HR-pQCT only include these two regions, making generalization for all other sites difficult. The downside of this technique is that only the distal radius and tibia can be scanned, whereas HO often forms more peripherally[1]. Also, it requires almost normal mobility of the arms and legs. Therefore, this technique will only be feasible in a small subset of FOP patients.

The presence of restrictive pulmonary function in early childhood might indicate that early in the course of the disease essential muscles or tendons for breathing are affected. It is not a congenital feature of FOP, as not all FOP patients have restrictive pulmonary function. Patients included in the present thesis were all diagnosed with FOP around the age of 5 to 8 years. Whether FOP patients, who experience their first flare-up in adolescence, develop an equally severe restrictive pulmonary function, is not known.

In the absence of a treatment, it is important to maintain pulmonary function through non-medical treatments, such as breathing exercises and singing. The effectiveness of these exercises have not been confirmed in FOP patients, but are valuable in patients with restrictive lung disease[24]. The effect of these exercises on maintaining pulmonary function in FOP patients should be further investigated.

Clinical implications

The main conclusion of the work described in this thesis is that more insight in the development, progression and persistence of HO can be obtained using imaging techniques. In addition, based on these findings, a scheme can be proposed that defines specific imaging technique for the various phases of the disease (Figure 1). If there is (suspicion of) a flare-up, either an MRI or an ^{18}F-NaF PET/CT could be prescribed. An MRI could be especially valuable in children, as there it does not expose a patient to radiation. If there is suspicion of a flare-up, but no oedematous lesion is seen on MRI, an alternative diagnosis should be considered. The presence of oedema cannot distinguish an ossifying from a non-ossifying flare-up. In addition, it should be noted that oedema is non-specific, and therefore also other causes for oedema should be considered. The ^{18}F-NaF PET/CT scan can predict the fate of a flare-up. This finding can alter therapeutic decisions. Apart from this, ^{18}F-NaF PET/CT can also be helpful to support the patient during a flare-up. Patients often fear loss of mobility during a flare-up, and especially its unpredictable character might be difficult to cope with.

Finally, using ^{18}F-NaF PET/CT, a chronic disease component was discovered. This provides a means to gain insight in disease activity of individual patients. Progressive immobilization that previously could not be explained, may now be explained through ^{18}F-NaF PET/CT.

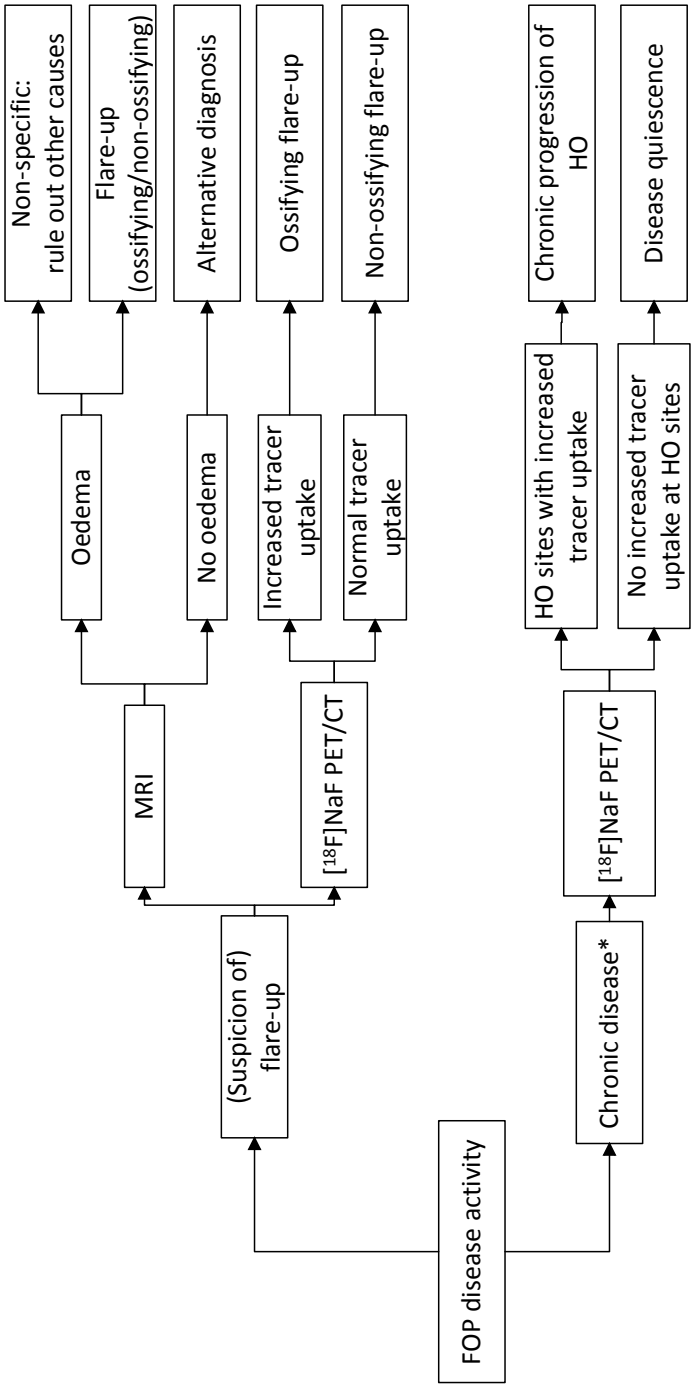

Figure 1. Proposed diagnostic scheme to be used for assessing both flare-ups and chronic disease activity. * chronic disease is defined as progression of HO in the absence of flare-up symptoms (redness, swelling, pain).

Future research perspectives

The coming few years is an exciting time for everyone involved in the field of FOP: all eyes are on the clinical trials that are currently conducted. These trials will not only give information about the efficacy of the study drug, it will also expand our knowledge about the pathophysiology of FOP. In this thesis, small numbers of patients are reported, due to the rarity of the disease. Those clinical trials however, are conducted throughout various continents including a relatively large number of FOP patients. The knowledge gained from this thesis could be used and confirmed in these trials. For instance, Regeneron Pharmaceuticals Inc. is conducting a clinical trial in which Activin A antibodies are tested for their efficacy to halt HO formation. The ^{18}F-NaF PET/CT, valuable as an imaging tool as shown in this thesis, is the primary endpoint of the study. The placebo group in this trial could be used to confirm our finding that flare-ups are accompanied with ^{18}F-NaF uptake and that HO progresses chronically. Furthermore, the pulmonary function of patients on study drug can be compared to the longitudinal data shown in this thesis. Although MRI, does not have a place yet in those clinical trials, further research should explore the meaning of the mild oedema as seen in our patients who underwent an MRI.

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Addendum

Nederlandse Samenvatting

Voor niet-ingewijden

Fibrodysplasia ossificans progressiva (FOP) is een zeer zeldzaam, aangeboren ziektebeeld, waarbij er bot wordt gevormd in spieren en pezen. Het extra bot dat wordt gevormd, wordt heterotopisch bot genoemd. De vorming van dit heterotopisch bot kan worden veroorzaakt door beschadiging van een spier of pees. Dit gebeurt bijvoorbeeld tijdens een operatie, maar ook minder ingrijpende oorzaken zoals een vaccinatie of een val, kunnen tot de vorming van heterotopisch bot leiden. Soms ontstaat het echter spontaan: er is dan geen aanleiding aanwijsbaar. Voordat er heterotopisch bot wordt gevormd, ontstaat er zwelling, roodheid en pijn op de plek van de uiteindelijke botvorming. Deze lichamelijke verschijnselen noemen we een 'flare-up'. De leeftijd waarop een patiënt de eerste flare-up krijgt, loopt erg uiteen, maar is gemiddeld rond het 6e levensjaar. Het heterotopische bot vormt zich op deze leeftijd vaak in de spieren van de borstkas, rug en nek. Met toename van de leeftijd, vormen de flare-ups zich steeds meer in de ledematen, waardoor schouders, heupen, knieën en ellebogen op slot komen te zitten. Als gevolg hiervan is de patiënt vaak rond het dertigste levensjaar afhankelijk van een rolstoel en heeft de patiënt hulp nodig van anderen voor de dagelijkse activiteiten. De leeftijdsverwachting van een patiënt met FOP is beperkt door het heterotopische bot dat zich rondom de borstkas heeft gevormd. De longen kunnen zich hierdoor onvoldoende uitzetten tijdens de ademhaling, waardoor de functie van de longen slechter is en een longontsteking al kan leiden tot het overlijden van de patiënt. Het natuurlijke beloop van deze longfunctiestoornis door de jaren heen is niet bekend, ook is niet bekend of een patiënt met veel heterotopische bot rondom de borstkas in de regel ook een slechtere longfunctie heeft.

Heterotopisch bot

In 2006 is het genetisch defect bij patiënten met FOP ontdekt. Dit heeft ervoor gezorgd dat we meer te weten zijn gekomen over deze aandoening. Echter, er is nog steeds veel onbekend over het heterotopische bot dat zich vormt in patiënten met FOP. Zo blijkt uit een enquête onder vijfhonderd FOP patiënten, dat twintig procent van de flare-ups weg gaat zonder dat er uiteindelijk heterotopisch bot wordt gevormd. Waarom de ene flare-up wel tot botvorming leidt, en de ander niet is onbekend. Verder bleek uit deze enquête dat vijftig procent van de patiënten het gevoel had dat het heterotopisch bot toenam in grootte zonder dat er symptomen van een flare-up aanwezig waren. Doordat er tot op heden geen bloedtest is die activiteit van de ziekte kan aantonen, is het niet mogelijk geweest om bovenstaande processen objectief aan te tonen. Ook kan men daardoor de effecten van invasieve behandelingen (bijv. chirurgie en radiotherapie) op de activiteit van FOP niet goed beoordelen. Alternatieven om ziekte activiteit aan te tonen moeten dus gezocht worden, om de botvorming die plaatsvindt bij deze ziekte inzichtelijker te maken. Omdat technieken waarbij spieren worden beschadigd (invasieve technieken) niet kunnen worden toegepast,

zullen niet-invasieve technieken gebruikt moeten worden om meer inzicht in het extra bot te krijgen. Beeldvormende technieken zouden de kennis over het bot, het ontstaan en de ontwikkeling van het bot in kaart kunnen brengen, zonder dat het de ziekte verergert. Een mogelijke beeldvormende techniek die gebruikt kan worden, is de PET-scan. De PET-scan is voornamelijk bekend voor onderzoek naar uitzaaiingen bij kanker. Hierbij wordt gebruik gemaakt van radioactief glucose (suiker) dat aan de tumor en de uitzaaiingen bindt. De PET-scan kan echter ook gebruikt worden om actieve botvorming op te sporen, door een radioactieve stof, ^{18}F-NaF, toe te dienen die specifiek bindt aan botcellen. De PET-scan wordt altijd in combinatie met een CT-scan gemaakt. Met CT-scan kan gevormd bot worden afgebeeld en gemeten. De waarde van de ^{18}F-NaF PET/CT bij het aantonen van ziekte activiteit, c.q. botvorming, bij FOP, is nog niet bekend.

Voor kinderen zal de toepassing van een PET-scan niet altijd wenselijk zijn, aangezien bij de PET-scan relatief veel straling wordt gebruikt. Bij kinderen, die nog in de groei zijn, kan straling een verhoogde kans op kanker geven. De MRI-scan, een beeldvormende techniek zonder deze stralingsbelasting, kan een alternatief zijn. De waarde van deze techniek bij patiënten met FOP is echter nog niet onderzocht.

Als na een flare-up, heterotopisch bot zich uiteindelijk heeft gevormd, wordt gedacht dat het heterotopische bot hetzelfde is als het bot van het skelet. Echter, dit is nauwelijks onderzocht, aangezien een botbiopt, een techniek waarbij een stukje bot wordt verwijderd voor analyse, niet mogelijk is bij FOP patiënten. Een botbiopt zorgt namelijk voor een beschadiging, wat weer een flare-up veroorzaakt. Recent is er een nieuwe beeldvormende techniek ontwikkeld, de HR-pQCT-scan, welke het mogelijk maakt om het bot in de onderarmen en -benen te analyseren zonder dat er daarbij schade wordt toegebracht aan de patiënt. Heterotopisch bot dat zich in de onderarmen en -benen bevindt, zou op deze manier onderzocht kunnen worden zonder de patiënt te schaden.

Doel proefschrift

Dit proefschrift heeft als doel om de ontwikkeling en het natuurlijke beloop van heterotopisch bot te onderzoeken, evenals de structuur van het heterotopisch bot zelf en het effect van heterotopisch bot op de longfunctie van FOP patiënten.

In het proefschrift is onderzocht of ziekte activiteit tijdens een flare-up aantoonbaar is middels de ^{18}F-NaF PET-scan en de MRI-scan. Ook is het natuurlijke beloop van heterotopisch bot onderzocht middels de ^{18}F-NaF PET/CT-scan. Verder is het effect van een operatie en bestraling op de FOP ziekte activiteit onderzocht.
De structuur van volgroeid heterotopische bot is onderzocht middels een HR-pQCT-scan. En, als laatste, is er gekeken naar het effect van heterotopisch bot op de longfunctie.

Resultaten

In hoofdstuk 2 zijn meerdere spontane flare-ups bij één patiënt vervolgd met de ^{18}F-NaF PET/CT-scan. Zelfs voordat er op de CT-scan aanwijzingen waren voor botvormingen, was er al activiteit te zien op de ^{18}F-NaF PET-scan. Het bot dat uiteindelijk was gevormd, had zich alleen gevormd in de gebieden waar de PET-scan de activiteit toonde. In hoofdstuk 3 werd het natuurlijke beloop van heterotopisch bot gevolgd over een tijdspanne van 6-18 maanden middels de ^{18}F-NaF PET/CT-scan. Er werd gevonden dat, zelfs in de afwezigheid van klachten, heterotopisch bot in grootte kan toenemen. De hoeveelheid bot die wordt gevormd tijdens deze 'chronisch fase' is veel minder vergeleken met bot dat ontstaat als gevolg van een flare-up. In hoofdstuk 4 werden vier patiënten beschreven die zowel MRI-scans als ^{18}F-NaF PET/CT-scans hadden ondergaan. Door de PET-scan als gouden standaard te gebruiken, werd aangetoond dat de flare-ups met de MRI-scan aantoonbaar zijn, doordat er een vochtophoping (oedeem) te zien is ter plaatse van de flare-up. Echter, de aanwezigheid van dit vocht heeft geen voorspellende waarde wat betreft het gevolg (botvormend of niet-botvormend) van de flare-up. Wel werden er in FOP patiënten milde vochtophopingen gezien op plaatsen in het lichaam waar de patiënten geen klachten ervaarde. De betekenis van dergelijke vochtophopingen bij FOP, gezien deze bij gezonde personen niet aanwezig zijn, moet verder worden onderzocht. In hoofdstuk 5 is het effect van een levensreddende operatie op de ziekteactiviteit – zowel flare-ups als chronische toename – beschreven. De beschreven patiënt leed al jaren aan een chronische infectie van haar voet, waarvoor zij een onderbeenamputatie moest ondergaan. Zij had vooraf aan de operatie al jaren geen ziekte activiteit meer gehad, zoals gemeten middels ^{18}F-NaF PET-scan. Zowel de anesthesie als de chirurgie waren succesvol, nadien vormde er slechts een minimale hoeveelheid heterotopisch bot ter plaatse van de operatie. Wel werd 12 maanden na de operatie, voor het eerst sinds jaren, weer ziekte activiteit in het lichaam waargenomen op de ^{18}F-NaF PET-scan. In hoofdstuk 6 wordt een patiënt beschreven die radiotherapie (bestraling) onderging voor huidkanker van de lip. Een hoge dosis bestraling zoals gebruikt bij deze FOP patiënt was in de literatuur niet eerder beschreven. Lagere dosis radiotherapie lieten wel al zien veilig te zijn, alhoewel deze niet gevolgd waren middels de ^{18}F-NaF PET-scan. De radiotherapeutische behandeling van deze patiënt zorgde niet voor een plaatselijke flare-up, en ook verhoogde ziekte activiteit elders in het lichaam werd niet waargenomen middels de PET-scan.

In hoofdstuk 7 werden de HR-pQCT-scans van twee patiënten geanalyseerd. De architectuur van zowel skelet als heterotopisch bot werd onderzocht. Heterotopisch bot bleek qua structuur van de botbalkjes en de schors niet overeen te komen met het naburige normaal bot van het skelet, zoals eerder werd verondersteld. Ook de tibia – een bot in het onderbeen – bleek verschillend te zijn ten opzichte van gezonde leeftijdsgenoten.

Tot slot, werden de effecten van het heterotopisch bot op de longen onderzocht in hoofdstuk 8. Zeven FOP patiënten werden beschreven. Van hen waren longfunctie onderzoeken beschikbaar over een tijdspanne van 6-18 jaar. CT-beelden van vijf van hen werden geanalyseerd om de verband tussen longfunctie en het volume van het heterotopisch bot te onderzoeken. Een afwijkende longfunctie werd al gezien op de leeftijd van 7 jaar, en deze

leek te verslechteren tot aan halverwege de 20. Bij de oudere patiënten, bleef de longfunctie stabiel over de gevolgde periode. De hoeveelheid heterotopisch bot leek geen verband te hebben met de ernst van de longfunctie.

Discussie

Met de ^{18}F-NaF PET/CT-scan is het voor het eerst mogelijk om de ziekte activiteit in het lichaam van een FOP patiënt te meten. Er zijn nu verschillende medicijnen in ontwikkeling, waarmee men hoopt de heterotopische botvorming te stoppen. Deze medicijnen moeten niet alleen heterotopische botvorming na flare-ups voorkomen, maar ook verdere toename van al aanwezig heterotopisch bot voorkomen. Voor studies die de werkzaamheid van het middel op deze twee fasen in FOP patiënten onderzoeken, is een dergelijke marker voor ziekte activiteit essentieel. Het medicijn zou de bot activiteit moeten normaliseren. Naast het voorkomen van de vorming van heterotopisch bot, is het, voornamelijk bij de behandeling van kinderen met FOP, belangrijk dat de longfunctie behouden blijft. Gezien er op dit moment nog geen effectieve behandeling is voor FOP, is het belangrijk dat de longfunctie nu met niet-medicamenteuze therapie wordt behouden. Dit kan gepoogd worden middels ademhalingsoefeningen en zangoefeningen. Het effect hiervan op het behoud van de longfunctie in FOP is echter nog niet onderzocht.

De MRI-scan kan de ^{18}F-NaF PET-scan nog niet vervangen: de aanwezigheid van vochtophopingen is niet specifiek voor FOP; dat wil zeggen dat er nog vele andere aandoeningen zijn waarbij er vochtophopingen kunnen ontstaan, De MRI-scan zegt daardoor onvoldoende over de FOP ziekte activiteit die uiteindelijk tot heterotopische botvorming leidt. Bij de ^{18}F-NaF PET/CT-scan wordt een patiënt wel onderworpen aan straling en de beelden kunnen lastig te interpreteren zijn voor een arts of onderzoeker. Er blijft daarom een vraag naar een (eenvoudige) bloedtest om de ziekte activiteit aan te tonen. In de zoektocht naar deze bloedtest, kan men de uitkomsten van het bloed vergelijken met de activiteit op de ^{18}F-NaF PET-scan. Indien beide overeenkomen, weet men dat het een betrouwbare bloedtest in handen heeft.

Door ^{18}F-NaF PET/CT-scans te maken voor en na invasieve behandelingen bij FOP-patiënten komen we meer te weten over de effecten die deze behandelingen hebben op deze bijzondere aandoening. Bijzonder is het minimale heterotopische bot dat zich vormde na een onderbeen amputatie. Ondanks deze positieve uitkomst, dient een operatie te allen tijde voorkomen te worden bij FOP patiënten. Het immuunsysteem speelt een belangrijke rol bij FOP, en mogelijk dat ofwel de chronische infectie ofwel het continue gebruik van antibiotica bij deze patiënte heeft geleid tot een onderdrukking van de FOP ziekte. Verder onderzoek zal moeten uitwijzen of FOP patiënten met chronische infectieuze aandoeningen inderdaad een lagere ziekte activiteit op de ^{18}F-NaF PET/CT-scan laten zien, vergeleken met andere FOP patiënten.

De toepassing van de HR-pQCT-scan in de klinische zorg voor FOP patiënten is discutabel. Doordat alleen de onderarmen en –benen gescand kunnen worden, en er flexibiliteit van de heupen en knieën nodig is voor een goede positie in de scanner, zullen veel patiënten niet in aanmerking komen om gescand te worden. Voor onderzoek echter, kan deze nieuwe beeldvormende techniek ons inzicht geven in een in de ontwikkeling van heterotopisch bot, wanneer tijdens een flare-up systematisch HR-pQCT-scans zouden worden gemaakt. Uiteraard zal dan eerst een ^{18}F-NaF PET/CT-scan gemaakt moeten worden om aan te tonen dat de flare-up gevolgd zal worden gevolgd door heterotopische botvorming.

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List of abbreviations

ACVR1 = Activin A receptor type 1
ALK2 = Activin receptor-like kinase-2
Ar = area
BCC = basal cell carcinoma
BMP = bone morphogenetic protein
Bq = becquerel
CAJIS = cumulative analogue joint involvement scale
CT = computed tomography
Ct.Po = cortical porosity
Ct.Th = cortical thickness
Ct = cortical
DCLO = diffusing capacity for carbon dioxide
DCLO/VA = diffusing capacity for carbon dioxide corrected for alveolar volume
FDG = fluordeoxyglucose
FEV1 = forced expiratory volume in one second
FKBP12 = FK506 binding protein 12
^{18}F-NaF = fluorine-18 labelled sodium fluoride
FOP = fibrodysplasia ossificans progressiva
FVC = forced vital capacity
GS-domain: glycine-serine (GS) domain
Gy = gray
HO = heterotopic ossification
HR-pQCT = high-resolution peripheral quantitative computed tomography
HU = hounsfield units
MAPK= mitogen-activated protein kinase
MBq = mega becquerel
MDP = methylene diphosphonate
MOT = myositis ossificans traumatica
MRI = magnetic resonance imaging
msv = milisievert
NSAID = nonsteroidal anti-inflammatory drug
PET = positron emission tomography
PFT = pulmonary function test
ROI = region of interest
RT= radiotherapy
RV = residual volume
SUV = standard uptake value
Sv = sievert
Tb = trabecular

Tb.BV/TV = trabecular bone volume fraction
Tb.N = trabecular number
Tb.1/N.SD = heterogeneity of the trabecular network
Tb.Sp = trabecular separation
Tb.Th = trabecular thickness
^{99m}Tc = technetium-99m
Tt = total
TLC = total lung capacity
TMJ = temporomandibular joint
vBMD = volumetric bone mineral density
VC = vital capacity
VOI = volume of interest
WBLDCT = whole body low dose computed tomography
µCT = micro computed tomography

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Esmée Botman was born on July 25th 1992 in Hoorn, The Netherlands. After finishing high school at the Werenfridus in Hoorn, she started here study in Medicine at the Vrije Universiteit in Amsterdam in 2010. During her study, Esmée worked as a student researcher in the gastroenterology department of the VUmc. Also, she taught anatomy to other medical students as student-teacher. After obtaining her doctor's degree with honors, Esmée worked as a medical resident at the department of internal medicine at the Spaarne Gasthuis for one year. Then, in 2017, she started her PhD research to study a rare bone disease, fibrodysplasia ossificans progressiva (FOP). Using various imaging techniques, she examined the extra bone that forms in patients with FOP. This research was done in collaboration with the nuclear medicine and radiology department. Also, as part of her PhD she initiated, arranged, supervised and conducted a clinical trial to test a drug for the treatment of FOP. Patients from different European countries participated in this trial, and therefore visited the Amsterdam UMC every 4 weeks. Besides this clinical trial, she also provided clinical care to national and international FOP patients. As of May 2020, Esmée has started her specialization in the internal medicine.

Over de auteur

Esmée Botman werd geboren op 25 juli 1992 in Hoorn. Na het behalen van haar gymnasiumdiploma aan het Werenfridus in Hoorn, startte zij met de studie geneeskunde aan de Vrije Universiteit van Amsterdam. Tijdens haar studie heeft Esmée als student-onderzoeker op de afdeling MDL meegewerkt aan onderzoek naar de screening van dikke darmkanker. Ook was zij enkele jaren student-assistent anatomie, waarbij ze anatomie les gaf aan andere geneeskunde studenten. Nadat Esmée haar artsendiploma cum laude had behaald, heeft ze één jaar gewerkt als arts-assistent Interne Geneeskunde in het Spaarne Gasthuis. Daarna, in 2017, startte zij met haar promotie onderzoek naar een zeldzame botziekte, fibrodysplasia ossificans progressiva (FOP). Door gebruik van verschillende beeldvormende technieken heeft zij het extra bot dat bij FOP wordt gevormd onderzocht. Deze onderzoeken zijn dan ook gedaan in samenwerking met de nucleaire geneeskunde en de radiologie afdeling. Onderdeel van haar werkzaamheden tijdens haar promotie was het opzetten, regelen, begeleiden en uitvoeren van een klinische studie waarbij een medicijn voor de behandeling van FOP werd getest. Patiënten uit verschillende Europese landen zijn hiervoor maandelijks naar het Amsterdam UMC gekomen. Ook de klinische zorg voor FOP patiënten, uit binnen- en buitenland, heeft zij in deze jaren op zich genomen.
Per 1 mei 2020 is Esmée gestart met de opleiding tot internist.

List of Publications

Botman E, Treurniet S, Lubbers WD, Schwarte LA, Schober PR, Sabelis LWE, Peters EJ, van Schie A ; de Vries, R, Grunwald Z, Smilde BJ, Nieuwenhuijzen JA, Visser MC, Micha D, Bravenboer N, Netelenbos JC, Teunissen BP, de Graaf P, Raijmakers PGHM, Smit JM, Eekhoff EMW. *When Limb Surgery Has Become the Only Life-Saving Therapy in FOP: A Case Report and Systematic Review of the Literature***.** Front Endocrinol. 2020 Aug.

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, Helder MN. *Collaboration Around Rare Bone Diseases Leads to the Unique Organizational Incentive of the Amsterdam Bone Center*. Front Endocrinol. 2020 Aug 11;11:481.

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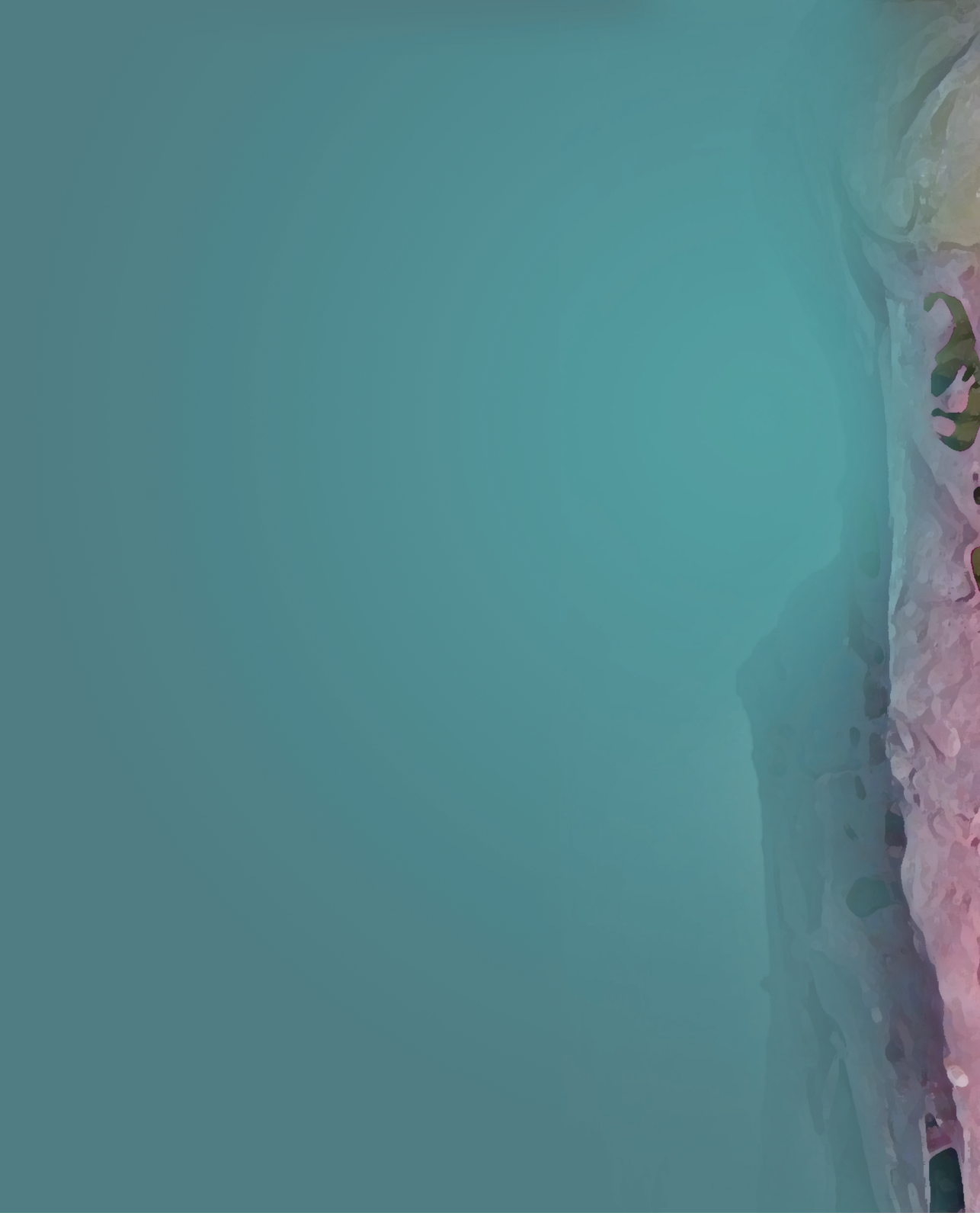

Amsterdam Movement Sciences

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