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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

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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 by 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 1 cm^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.

Table 1
Patient characteristics.

	Age[a] (y)	Gender	Mutation[b]	Scan number	Time interval (months)[c]	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	

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

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 ($SUV_{peak} \geq 8.4$) 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 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 to 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

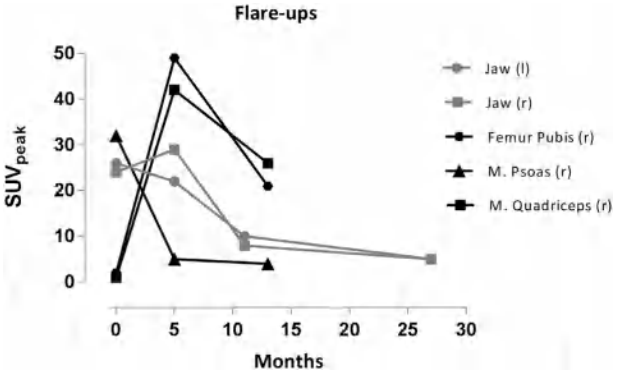

Fig. 1. Consecutive SUV_{peak} values obtained from [^{18}F]NaF PET/CT scans for regions in which patients experienced a flare-up. For all regions SUV_{peak} exceeded 25 in the course of the flare-up. The flare-up in the left and right jaw (patient 4, grey lines) was triggered by a surgical procedure.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

was identified and these flare-ups were therefore considered idiopathic. All flare-up regions showed a SUV_{peak} between 21 and 48 (Fig. 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. (Fig. 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 ($SUV_{peak} \leq 8.4$), lesions did not show further volumetric expansion (Fig. 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

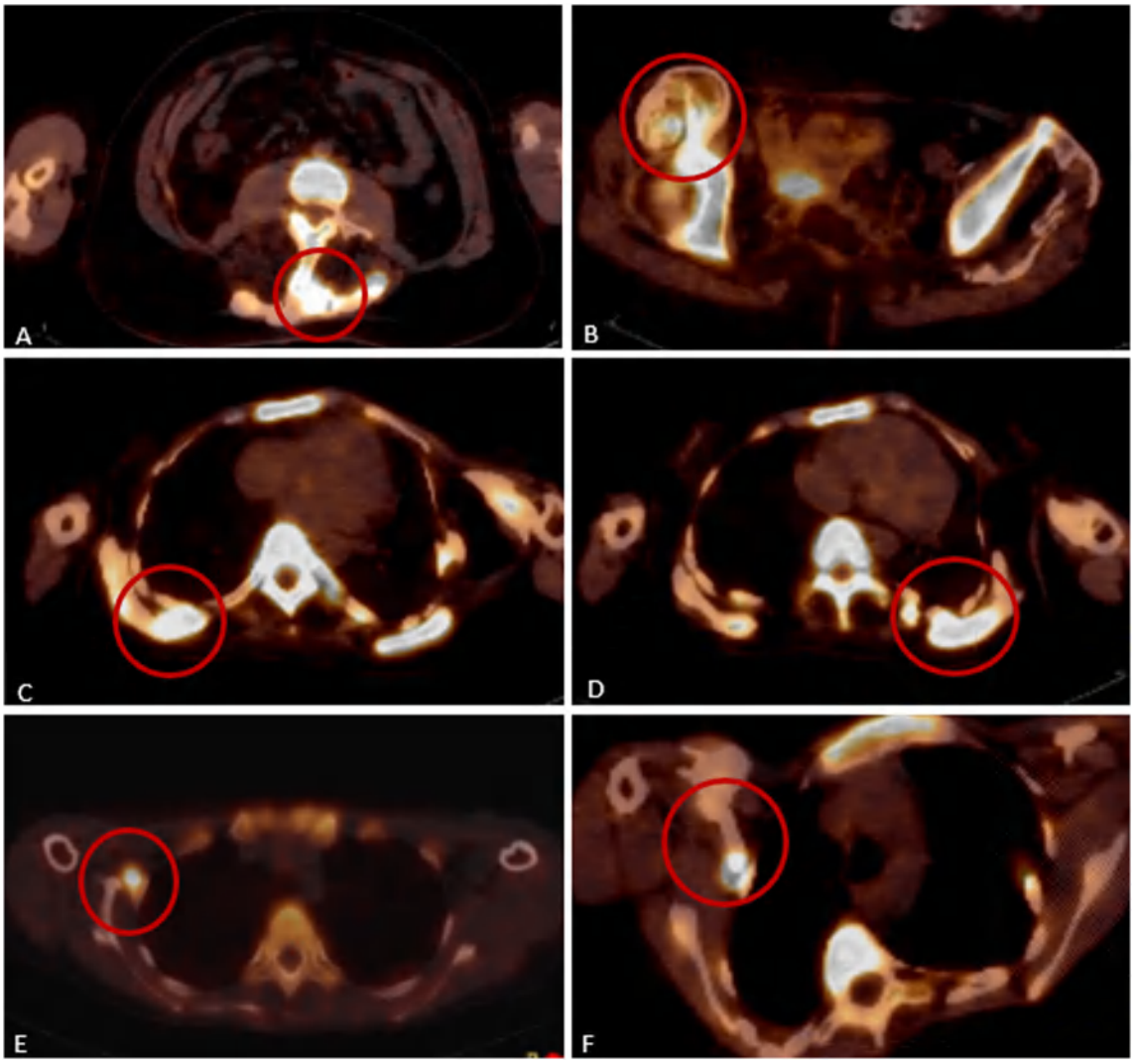

Fig. 2. Axial images of progressive heterotopic bone lesions with visibly increased [^{18}F]NaF uptake. These progressive lesions all had increased sodium fluoride uptake (red circles in images), defined by a $SUV_{peak} > 8.36$. Volumetric values and SUV_{peak} values for each heterotopic lesion are shown in Fig. 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. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

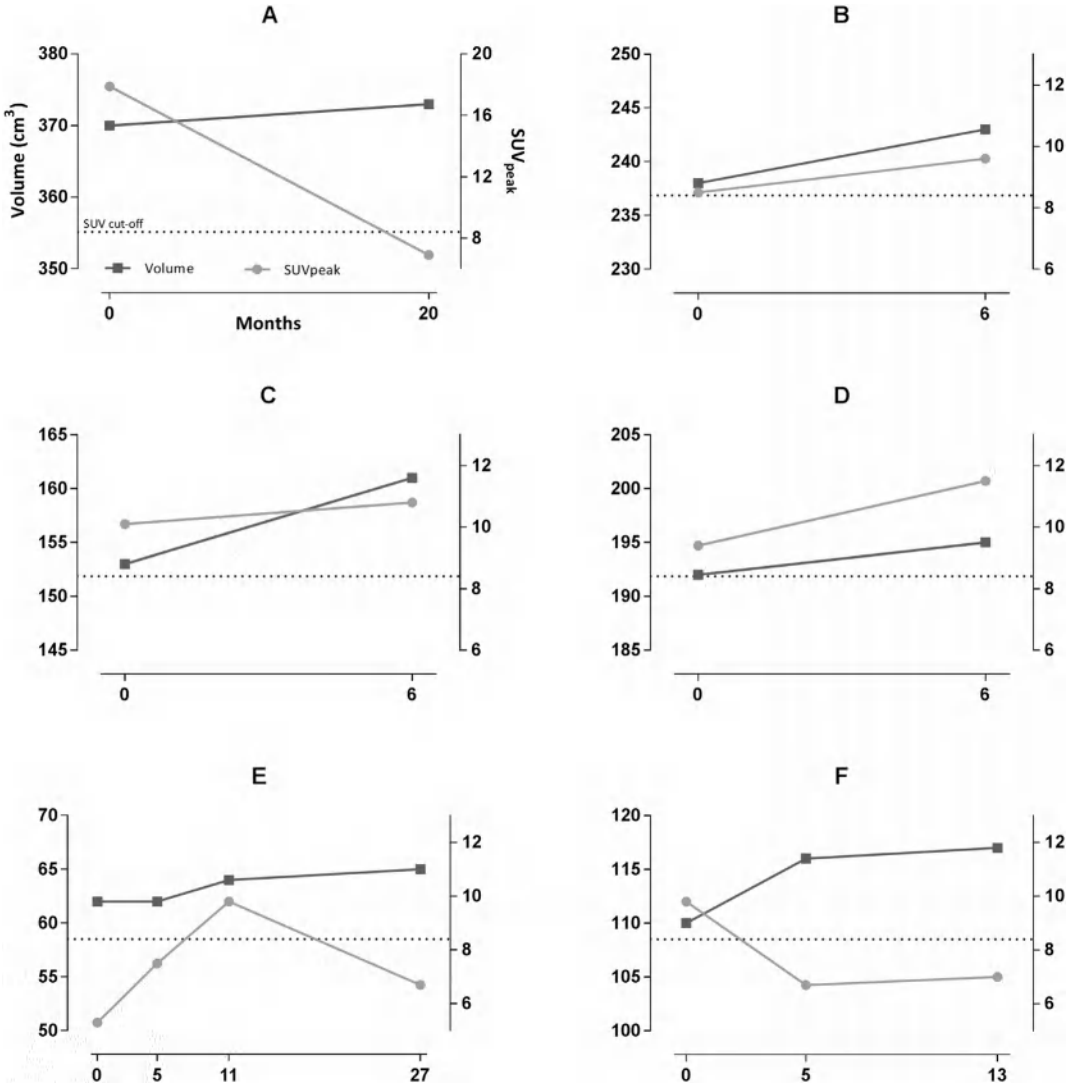

Fig. 3. Volumetric increase and SUV_{peak} values for each progressive lesion identified by successive [^{18}F]NaF PET/CT-scans. Progressive heterotopic bone lesions were accompanied or preceded by an increased sodium fluoride uptake, reflecting active bone metabolism. Active bone metabolism represents 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 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.

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.

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-99 m 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 (0.0 64 mL) [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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All authors meet the required authorship contribution, including:

1) a substantial contribution to conception and design (EB, PGHM, MY, CN, DM, NB, TS, TJV, GP, JMS, PK, DGT, AAL, EMWE), acquisition of data (EB, PGHMR, MY, AAL, WL, LAS, EMWE), or analysis and interpretation of data (EB, PGHMR, MY, BT, CN, AAL, EMWE);
2) participated in drafting the manuscript (EB, PGHMR, EMWE) or revising it critically for important intellectual content (EB, PGHMR, MY, BT, CN, WL, LAS, DM, NB, TS, TJV, GP, JMS, PK DGT, AAL, EMWE);
3) approved the final version of the submitted manuscript (EB, PGHMR, MY, BT, JCN, WL, LAS, DM, NB, TS, TJV, GP, JMS, PK, DGT, AAL, EMWE);
4) agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved (EB, PGHMR, MY, BT, JCN, WL, LAS, DM, NB, TS, TJV, JMS, PK, DGT, AAL, EMWE).

Disclosures

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Dinko González Trotter is an employee of Regeneron Pharmaceutical Inc.

Conflict of interest

All other authors declare that there is no conflict of interest.

References

[1] R.B. Cohen, G.V. Hahn, J.A. Tabas, J. Peeper, C.L. Levitz, A. Sando, N. Sando, M. Zasloff, F.S. Kaplan, The natural history of heterotopic ossification in patients who have fibrodysplasia ossificans progressiva. A study of forty-four patients, J. Bone Joint Surg. Am. 75 (2) (1993) 215–219.
[2] J.G. Rogers, W.B. Geho, Fibrodysplasia ossificans progressiva. A survey of forty-two cases, J. Bone Joint Surg. Am. 61 (6A) (1979) 909–914.
[3] F.S. Kaplan, M. Le Merrer, D.L. Glaser, R.J. Pignolo, R.E. Goldsby, J.A. Kitterman, J. Groppe, E.M. Shore, Fibrodysplasia ossificans progressiva, Best Pract. Res. Clin. Rheumatol. 22 (1) (2008) 191–205.
[4] R.J. Pignolo, C. Bedford-Gay, M. Liljesthrom, B.P. Durbin-Johnson, E.M. Shore, D.M. Rocke, F.S. Kaplan, The natural history of flare-ups in Fibrodysplasia Ossificans Progressiva (FOP): a comprehensive global assessment, J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res. 31 (3) (2016) 650–656.
[5] B. Cremin, J.M. Connor, P. Beighton, The radiological spectrum of fibrodysplasia ossificans progressiva, Clin. Radiol. 33 (5) (1982) 499–508.
[6] I. Huning, G. Gillessen-Kaesbach, Fibrodysplasia ossificans progressiva: clinical course, genetic mutations and genotype-phenotype correlation, Molecular Syndromology 5 (5) (2014) 201–211.
[7] J. Upadhyay, L. Xie, L. Huang, N. Das, R.C. Stewart, M.C. Lyon, K. Palmer, S. Rajamani, C. Graul, M. Lobo, T.J. Wellman, E.J. Soares, M.D. Silva, J. Hesterman, L. Wang, X. Wen, X. Qian, K. Nannuru, V. Idone, A.J. Murphy, A.N. Economides, S.J. Hatsell, The expansion of heterotopic bone in Fibrodysplasia Ossificans Progressiva is Activin A-dependent, J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res. 32 (12) (2017) 2489–2499.
[8] S.A. Chakkalakal, D. Zhang, A.L. Culbert, M.R. Convente, R.J. Caron, A.C. Wright, A.D. Maidment, F.S. Kaplan, E.M. Shore, An Acvr1 R206H knock-in mouse has fibrodysplasia ossificans progressiva, J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res. 27 (8) (2012) 1746–1756.
[9] E.M.W. Eekhoff, E. Botman, J. Coen Netelenbos, P. de Graaf, N. Bravenboer, D. Micha, G. Pals, T.J. de Vries, T. Schoenmaker, M. Hoebink, A.A. Lammertsma, P. Raijmakers, [18F]NaF PET/CT scan as an early marker of heterotopic ossification in fibrodysplasia ossificans progressiva, Bone 109 (2018) 143–146.
[10] E.M.W. Eekhoff, J.C. Netelenbos, P. de Graaf, M. Hoebink, N. Bravenboer, D. Micha, G. Pals, T.J. de Vries, A.A. Lammertsma, P.G.H.M. Raijmakers, R.J.J. van Es, Flare-up after maxillofacial surgery in a patient with Fibrodysplasia Ossificans Progressiva: an [18F]-NaF PET/CT study and a systematic review, JBMR Plus 2 (1) (2018) 55–58.
[11] R.A. Hawkins, Y. Choi, S.C. Huang, C.K. Hoh, M. Dahlbom, C. Schiepers, N. Satyamurthy, J.R. Barrio, M.E. Phelps, Evaluation of the skeletal kinetics of fluorine-18-fluoride ion with PET, J. Nucl. Med. 33 (5) (1992) 633–642.
[12] C.K. Hoh, R.A. Hawkins, M. Dahlbom, J.A. Glaspy, L.L. Seeger, Y. Choi, C.W. Schiepers, S.C. Huang, N. Satyamurthy, J.R. Barrio, et al., Whole body skeletal imaging with [18F]fluoride ion and PET, J. Comput. Assist. Tomogr. 17(1) (1993) 34–41.
[13] V. Frings, F.H. van Velden, L.M. Velasquez, W. Hayes, P.M. van de Ven, O.S. Hoekstra, R. Boellaard, Repeatability of metabolically active tumor volume measurements with FDG PET/CT in advanced gastrointestinal malignancies: a multicenter study, Radiology 273 (2) (2014) 539–548.
[14] G.M. Kramer, V. Frings, N. Hoetjes, O.S. Hoekstra, E.F. Smit, A.J. de Langen, R. Boellaard, Repeatability of quantitative whole-body 18F-FDG PET/CT uptake measures as function of uptake interval and lesion selection in non-small cell lung Cancer patients, J. Nucl. Med. 57 (9) (2016) 1343–1349.
[15] G. Segall, D. Delbeke, M.G. Stabin, E. Even-Sapir, J. Fair, R. Sajdak, G.T. Smith, SNM, SNM practice guideline for sodium 18F-fluoride PET/CT bone scans 1.0, J. Nucl. Med. 51 (11) (2010) 1813–1820.
[16] O.P. Temmerman, P.G. Raijmakers, I.C. Heyligers, E.F. Comans, M. Lubberink, G.J. Teule, A.A. Lammertsma, Bone metabolism after total hip revision surgery with impacted grafting: evaluation using H2 15O and [18F]fluoride PET; a pilot study,

Mol. Imaging Biol. 10 (5) (2008) 288–293.
[17] J. Haupt, A. Deichsel, K. Stange, C. Ast, R. Bocciardi, R. Ravazzolo, M. Di Rocco, P. Ferrari, A. Landi, F.S. Kaplan, E.M. Shore, C. Reissner, P. Seemann, ACVR1 p.Q207E causes classic fibrodysplasia ossificans progressiva and is functionally distinct from the engineered constitutively active ACVR1 p.Q207D variant, Hum. Mol. Genet. 23 (20) (2014) 5364–5377.
[18] F.S. Kaplan, M. Xu, P. Seemann, J.M. Connor, D.L. Glaser, L. Carroll, P. Delai, E. Fastnacht-Urban, S.J. Forman, G. Gillessen-Kaesbach, J. Hoover-Fong, B. Koster, R.M. Pauli, W. Reardon, S.A. Zaidi, M. Zasloff, R. Morhart, S. Mundlos, J. Groppe, E.M. Shore, Classic and atypical fibrodysplasia ossificans progressiva (FOP) phenotypes are caused by mutations in the bone morphogenetic protein (BMP) type I receptor ACVR1, Hum. Mutat. 30 (3) (2009) 379–390.
[19] R.K. Kulshrestha, S. Vinjamuri, A. England, J. Nightingale, P. Hogg, The role of 18F-sodium fluoride PET/CT bone scans in the diagnosis of metastatic bone disease from breast and prostate Cancer, J. Nucl. Med. Technol. 44 (4) (2016) 217–222.
[20] A. Sher, F. Lacoeuille, P. Fosse, L. Vervueren, A. Cahouet-Vannier, D. Dabli, F. Bouchet, O. Couturier, For avid glucose tumors, the SUV peak is the most reliable parameter for [(18)F]FDG-PET/CT quantification, regardless of acquisition time, EJNMMI Res. 6(1) (2016) 21.
[21] R. Boellaard, N.C. Krak, O.S. Hoekstra, A.A. Lammertsma, Effects of noise, image resolution, and ROI definition on the accuracy of standard uptake values: a simulation study, J. Nucl. Med. 45 (9) (2004) 1519–1527.
[22] C. Brendle, J. Kupferschlager, K. Nikolaou, C. la Fougere, S. Gatidis, C. Pfannenberg, Is the standard uptake value (SUV) appropriate for quantification in clinical PET imaging?- variability induced by different SUV measurements and varying reconstruction methods, Eur. J. Radiol. 84 (1) (2015) 158–162.
[23] M.A. Lodge, M.A. Chaudhry, R.L. Wahl, Noise considerations for PET quantification using maximum and peak standardized uptake value, J. Nucl. Med. 53 (7) (2012) 1041–1047.