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[18F]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 [18F]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 [18F]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 [18F]NaF uptake in one defined part within the flare-up area after three weeks. During follow-up [18F]NaF PET /CT scan revealed newly formed heterotopic bone but only in this previously active [18F]NaF region. In conclusion, increased muscle [18F]NaF uptake may predict future HO development in FOP patients. At present [18F]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.
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1. Introduction

Fibrodysplasia ossificans progressiva (FOP) is a 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 [18F]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 [18F]NaF PET/CT as a new imaging modality to monitor disease activity during a flare-up in FOP, comparing the diagnostic value of [18F]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 [18F]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 [18F]NatriumFluoride (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.

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Sodium fluoride labeled with [18F] ([18F]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 [18F]NaF PET/CT in spite of the higher costs compared to Tc-99m-MDP bone scanning. The intrinsic high spatial resolution of PET/CT systems offers superior image quality of [18F]fluoride PET images which is also of interest for the detection of osseous lesions such as heterotopic bone. Studies comparing [18F]fluoride PET with conventional bone scintigraphy support the supremacy of [18F]fluoride PET in detecting osseous lesions [8–10].

The [18F]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 Tc-99m-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].

[18F]NaF has a short half-life of 110 min and is therefore produced by cyclotron, compared to 6 h for ^{99mTc}-labelled compounds. The delivery of [18F]NaF is not affected by protein binding, while protein binding of ^{99mTc}-labeled diphosphonates increases over time from 25% to 50% at 4 h after the injection [14]. The absent binding to serum proteins of [18F]NaF allows a rapid single-pass extraction and fast clearance from blood and soft tissue. Compared with ^{99mTc}-labelled difosfonates the bone uptake of [18F]NaF is twice as great [15]. These characteristics of [18F]fluoride combined with the higher resolution of PET scanners lead to shorter intervals and improved detection of bone lesions with [18F]NaF PET. Typically, a 2–4-hour interval between tracer injection and bone scintigraphy imaging is required. In contrast, [18F]NaF PET/CT imaging can be performed within a 1 h after injection. Notably, the length of the interval between injection of [18F]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 [18F]NaF PET studies and may reduce the interindividual variation of [18F]fluoride uptake [16,17].

Recently the superiority of [18F]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]; [18F]NaF PET/CT also correlated well with overall survival [18].

3. Dosimetry and procedure of [18F]NaF PET/CT

[18F]NaF is injected intravenously according to a procedure guideline for use of [18F]NaF PET/CT [12,20]. Typically this yields a dose of approximately 185 MBq [18F]fluoride for an adult, however, lower doses are possible using modern 3D PET/CT scanners. Presently, we use a dose of 1.2 MBq [18F]fluoride /kg bodyweight in benign bone diseases [7,21]. Accordingly, a dose of 100 MBq [18F]fluoride 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 ^{99mTc}-MDP results in a dose of 3.4m Sv.

1 h after injection of [18F]NaF whole body PET scans can be acquired from skull to feet, according to previously reported guidelines [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. [18F]fluoride 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 the CT.

4. Quantification of [18F]NaF uptake [22]

Visual assessment of [18F]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 [18F]fluoride kinetics, have been used to quantify fluoride uptake. A major advantage of SUV measurements is the simplified PET scanning protocol which only requires static [18F]fluoride PET images, made for example 60 min after injection. Full kinetic analysis of [18F]fluoride kinetics yields robust and reliable estimates of [18F]fluoride uptake, however, requires dynamic PET scanning with a length of up to 1 h, starting directly after [18F]fluoride injection.

Recently, a high correlation between [18F]fluoride SUV and full kinetic derived [18F]fluoride K_i was found in a bone surgery study with correlation coefficients higher than 0.94 (11). Moreover, changes in [18F]fluoride SUV after bone surgery correlated with those in K_i, indicating that changes of local bone metabolism could be monitored using the [18F]fluoride 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 [18F]fluoride 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]fluoride systemic therapy in FOP patients [12].

5. [18F]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 [18F]NaF PET/CT showed markedly increased [18F]fluoride uptake only at circumscriptive locations at the distal quadriceps muscle of the right leg (Fig. 1 upper panel). On the CT scan no evident HO was visible (Fig. 1 upper panel).

She was treated with high dosages of prednisolone almost from the onset until 2 months after the first [18F]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 wheel chair dependence.

After 8 months, when the flare-up had disappeared, a follow-up [18F]NaF PET/CT scan showed laterally disappeared and opposite a marked decreased muscle [^{18}F]fluoride uptake at the right distal quadriceps (Fig. 1 lower panel).

On the CT scan new maturing HO was visible only at the location of the muscle where the[18F]NaF PET/CT scan had been active before (Fig. 1 lower panel).

6. Discussion

Despite tremendous progression in 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 [18F]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 [18F]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 [18F]NaF PET/CT findings show that in a spontaneous flare-up some areas may recover but other areas may progress to end-

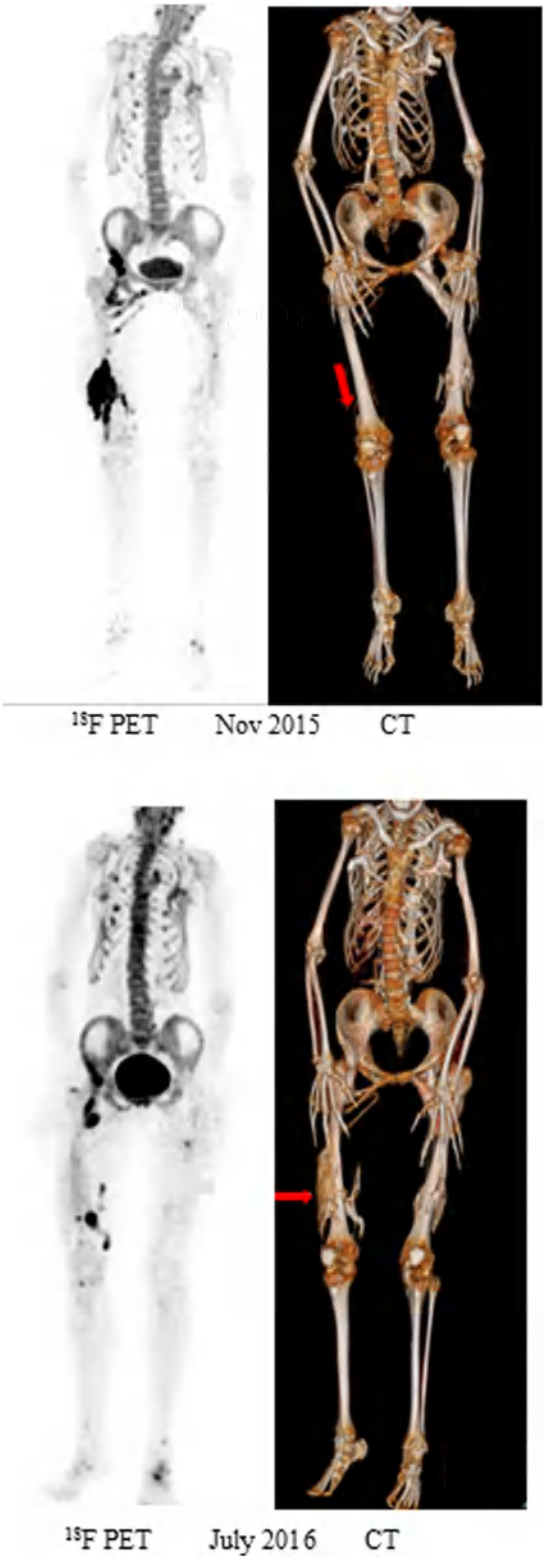

Fig. 1. Whole body [18F] NaF PET scan (skull excluded, maximum intensity projection) and 3D whole body CT scan of the FOP patient with suspected flare up in right leg (Nov.2015 upper panel) with subsequent development of heterotopic bone on the second scan (July 2016 lower). During a presentation with a possible flare up with pain in the right upper leg, the [18F]fluoride PET showed markedly increased fluoride uptake in the soft tissues of 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 [18F[fluoride uptake, despite clear heterotopic bone in the distal part of the left upper leg. Eight months later there is a marked reduction of [18F]fluoride uptake in the right upper leg ,while the CT showed newly formed heterotopic bone in this region marked by the red arrow. The left leg showed unchanged heterotopic bone.

stage heterotopic ossification. This fate as well as the exact future HO location was detected by increased uptake of [18F]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 [18F]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, [18F]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 [23,24]. In general, a bone scintigraphy can be helpful for detection of HO but theoretically, [18]Fluoride 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 [23]. 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 [18F]fludeoxyglucose 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 [12,25]. Nevertheless, both MRI and [18F]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 [18F]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.

[18F]fluoride 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 [12,26]. In future this technique might also help to understand the progressive course in some FOP patients who do not experience clinical flare-ups, 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 [27]. 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]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, [18F]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 [18F]NaF PET/CT could also provide a means for monitoring effectiveness of drugs early during therapy, for instance in new FOP trials.

All authors meet the required authorship contribution, including

1) a substantial contribution to conception and design (EMWE, EB, JCN, PdG, PR,), acquisition of data (EMWE, EB, JCN, PdG, PR.), or analysis and interpretation of data (EMWE, EB, JCN, PdG, MH, PR, FRGA: NB, DM, GP, TdV, TS, MH);

2) participated in drafting the manuscript (EMWE, JCN, PdG, PR) or revising it critically for important intellectual content (EMWE, EB, JCN, PdG, PR, FRGA: NB, DM, GP, TdV, TS, MH);
3) approved the final version of the submitted manuscript (EMWE, EB, JCN, PdG, PR, FRGA: NB, DM, GP, TdV, TS, MH);
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 (EMWE,EB, JCN, PdG, PR, FRGA: NB, DM, GP, TdV, TS, MH).

Integrity: E.M.W. Eekhoff, Esmée Botman, J.C. Netelenbos, P. de Graaf and P. Raijmakers accept the responsibility for the integrity of the data analysis.

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References

[1] 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. 31 (2016) 650–656.
[2] 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 (2008) 191–205.
[3] A.L. Culbert, S.A. Chakkalakal, E.G. Theosmy, T.A. Brennan, F.S. Kaplan, E.M. Shore, Alk2 regulates early chondrogenic fate in fibrodysplasia ossificans progressiva heterotopic endochondral ossification, Stem Cells 32 (2014) 1289–1300.
[4] E.M.W. Eekhoff, 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 [18 F]-NaF PET/CT study and a systematic review, JBMR Plus (2017).
[5] M. Blau, R. Ganatra, M.A. Bender, 18 F-fluoride for bone imaging, Semin. Nucl. Med. 2 (1972) 31–37.
[6] M. Blau, W. Nagler, M.A. Bender, Fluorine-18: a new isotope for bone scanning, J. Nucl. Med. 3 (1962) 332–334.
[7] K.K. Wong, M. Piert, Dynamic bone imaging with 99mTc-labeled diphosphonates and 18F-NaF: mechanisms and applications, J. Nucl. Med. 54 (2013) 590–599.
[8] G.J. Cook, I. Fogelman, The role of positron emission tomography in the management of bone metastases, Cancer 88 (2000) 2927–2933.
[9] F.D. Grant, F.H. Fahey, A.B. Packard, R.T. Davis, A. Alavi, S.T. Treves, Skeletal PET with 18F-fluoride: applying new technology to an old tracer, J. Nucl. Med. 49 (2008) 68–78.
[10] M. Hetzel, C. Arslandemir, H.H. Konig, A.K. Buck, K. Nussle, G. Glatting, A. Gabelmann, J. Hetzel, V. Hombach, H. Schirrmeister, F-18 NaF PET for detection of bone metastases in lung cancer: accuracy, cost-effectiveness, and impact on patient management, J. Bone Miner. Res. 18 (2003) 2206–2214.
[11] M. Piert, T.T. Zittel, G.A. Becker, M. Jahn, A. Stahlschmidt, G. Maier, H.J. Machulla, R. Bares, Assessment of porcine bone metabolism by dynamic, J. Nucl. Med. 42 (2001) 1091–1100.
[12] P. Raijmakers, O.P. Temmerman, C.P. Saridin, I.C. Heyligers, A.G. Becking, A. van Lingen, A.A. Lammertsma, Quantification of 18F-fluoride kinetics: evaluation of simplified methods, J. Nucl. Med. 55 (2014) 1122–1127.
[13] T.A. Einhorn, V.J. Vigorita, A. Aaron, Localization of technetium-99m methylene diphosphonate in bone using microautoradiography, J. Orthop. Res. 4 (1986) 180–187.
[14] A.I. Brenner, J. Koshy, J. Morey, C. Lin, J. DiPoce, The bone scan, Semin. Nucl. Med. 42 (2012) 11–26.
[15] E. Even-Sapir, E. Mishani, G. Flusser, U. Metser, 18F-Fluoride positron emission tomography and positron emission tomography/computed tomography, Semin. Nucl. Med. 37 (2007) 462–469.
[16] H. Jadvar, B. Desai, P.S. Conti, Sodium 18F-fluoride PET/CT of bone, joint, and other disorders, Semin. Nucl. Med. 45 (2015) 58–65.
[17] G.M. Blake, A.E. Moore, I. Fogelman, Quantitative studies of bone using (99m)Tc-methylene diphosphonate skeletal plasma clearance, Semin. Nucl. Med. 39 (2009) 369–379.
[18] A.B. Apolo, L. Lindenberg, J.H. Shih, E. Mena, J.W. Kim, J.C. Park, A. Alikhani, Y.Y. McKinney, J. Weaver, B. Turkbey, H.L. Parnes, L.V. Wood, R.A. Madan, J.L. Gulley, W.L. Dahut, K.A. Kurdziel, P.L. Choyke, Prospective study evaluating Na18F PET/CT in predicting clinical outcomes and survival in advanced prostate cancer, J. Nucl. Med. 57 (2016) 886–892.
[19] W. Langsteger, A. Rezaee, C. Pirich, M. Beheshti, 18F-NaF-PET/CT and 99mTc-MDP bone scintigraphy in the detection of bone metastases in prostate cancer, Semin. Nucl. Med. 46 (2016) 491–501.
[20] 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 (2010) 1813–1820.
[21] O.P. Temmerman, P.G. Raijmakers, R. Kloet, G.J. Teule, I.C. Heyligers, A.A. Lammertsma, In vivo measurements of blood flow and bone metabolism in osteoarthritis, Rheumatol. Int. 33 (2013) 959–963.
[22] A.A. Lammertsma, V.J. Cunningham, M.P. Deiber, J.D. Heather, P.M. Bloomfield, J. Nutt, R.S. Frackowiak, T. Jones, Combination of dynamic and integral methods for generating reproducible functional CBF images, J. Cereb. Blood Flow Metab. 10 (1990) 675–686.
[23] W. Zhang, K. Zhang, L. Song, J. Pang, H. Ma, E.M. Shore, F.S. Kaplan, P. Wang, The phenotype and genotype of fibrodysplasia ossificans progressiva in China: a report of 72 cases, Bone 57 (2013) 386–391.
[24] R.J. Pignolo, E.M. Shore, F.S. Kaplan, Fibrodysplasia ossificans progressiva: diagnosis, management, and therapeutic horizons, Pediatr. Endocrinol. Rev. 10 (Suppl. 2) (2013) 437–448.
[25] E.C. Ehman, G.B. Johnson, J.E. Villanueva-Meyer, S. Cha, A.P. Leynes, P.E. Larson, T.A. Hope, PET/MRI: where might it replace PET/CT? J. Magn. Reson. Imaging (2017).
[26] J.C. Regelink, P.G. Raijmakers, N. Bravenboer, R. Milek, N.J. Hoetjes, A.M. de Kreuk, M. van Duin, M.J. Wondergem, P. Lips, P. Sonneveld, J.M. Zijlstra, S. Zweegman, (18)F-fluoride-PET for dynamic in vivo monitoring of bone formation in multiple myeloma, EJNMMI Res. 6 (2016) 46.
[27] G.A. Conner, M. Duffy, Myositis ossificans: a case report of multiple recurrences following third molar extractions and review of the literature, J. Oral Maxillofac. Surg. 67 (2009) 920–926.