Autologous Mesenchymal Stem Cells in The Treatment of Spinal Aneurysmal Bone Cyst

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Abstract

Purpose: We retrospectively analysed a cohort of patients treated at our Centre with bone marrow concentrated (BMC) injection for aneurysmal bone cyst (ABC) of the spine, in order to propose this treatment as a valid alternative for the management of ABCs.

Methods: Fourteen patients (6 male, 8 female) were treated between June 2014 to December 2019 with BMC injection for ABC of the spine. The mean age was 17.85 years. The mean follow up was 37.4 months (range 12- 60 months). The dimension of the cyst and the degree of ossification were measured by Computed Tomography (CT) scans before the treatment and during follow-up visits.

Results: Six patients received a single dose of BMC, five patients received two doses and in three patients three doses of BMC were administered. The mean ossification of the cyst (expressed in Hounsfield units) increased statistically from 43.48±2.36 HU to 161.71±23.48 HU during follow-up time and the ossification was associated to an improvement of the clinical outcomes. The mean ossification over time was significantly higher in patients treated with a single injection compared to patients treated with multiple injections. No significant difference in ossification was found between cervical and non-cervical localization of the cyst. Moreover, the initial size of the cyst was not statistically associated with the degree of ossification during follow-up

Conclusions: Results of this paper reinforce our previous evidence on the use of BMC as a valid alternative for spinal ABC management when SAE treatment is contraindicated or ineffective.

Introduction

Aneurysmal Bone Cysts (ABCs) are benign locally aggressive lesions first described by Jaffe and Liechtenstein in 1942[1]. They principally affect long bones, but up to 20% occur in the spine[2,3]. These cysts tend to present with pain and swelling in the region of lesion and usually show deformity and neurological dysfunction. ABCs are most often seen in children and young adults with no gender predilection and are classified into latent (Grade 1), active (Grade 2) and aggressive (Grade 3), according to the Enneking staging[2]. They are considered as primary lesions in 70% of the cases and as secondary lesions in 30% of the cases owing to their composition. These lesions are lytic, usually eccentrically located, and expansive with well-defined margins[4]. They are blood-filled, separated by fibrous septa, with fibroblasts, osteoclast-type giant cells and reactive woven bone[4]. In approximately 75% of the cases the lesions show a characteristic translocation resulting in the activation of the gene USP6 placed on 17p13[5]. Although biopsy and histopathology are the ‘gold standard’ in diagnosing ABCs, specific radiographic (X-ray) features like fluid filled levels on Computed Tomography (CT) are pathognomonic for these lesions[6–8]. The behavior of these lesions can be different; however, according to several researchers and clinicians behave aggressively with progressive expansion and bone destruction[3,7–9]. The treatment concepts of ABCs have evolved over the years; traditionally, the treatment has been surgical. However, different procedures like intralesional curettage with or without stabilization, en bloc excision, radiation therapy

and selective arterial embolization (SAE) have proven their effectiveness in managing these lesions, strictly considering the clinical picture, i.e. location and size, associated instability and surgeon's familiarity[2,9]. In the spine, the surgical treatment is considered because of the presence of pathological fracture, spinal instability or neurological impairment. En bloc resection with the goal of wide margin is an effective treatment method to reduce the risk of local recurrence and is especially advised for more aggressive lesions. However, the effectiveness of this treatment in preventing local recurrence should be weighed against its potential morbidity. To avoid morbidity associated with surgery, another viable treatment is SAE. However, also this treatment is limited by potentially increased morbidity associated with multiple embolization procedures and subsequent radiation exposure[10]. This risk must be weighed against the risk of surgery, in particular en bloc resection, and SAE can be considered the first-line treatment for ABCs of the spine when neural elements are not extensively involved, or the risk of pathological fracture is not increased[11]. Other emerging treatments for ABCs include bisphosphonates, percutaneous doxycycline, sclerotherapy and Denosumab[12–14]. In addition, the use of autologous bone marrow concentrate (BMC) injection therapy has been also introduced for aneurysmal bone cysts to stimulate bone healing and regeneration[15–16]. One of the potential advantages of such a method is that surgical treatments are not necessary, thus allowing for both a minimally invasive approach and the treatment of poorly accessible lesions.

In this retrospective study we described the clinical and radiological outcomes of percutaneous injection of autologous BMC in a series of patients affected by ABCs of the spine and followed for at least one year.

Results

Overall, 14 patients (8 males, 6 females) were analyzed. The mean age was 17.85 years (range 33 – 10). Seven patients had lesions localized at cervical level, three patients had lesions at thoracic level, three patients had lesions at lumbar level and one patient had lesions at the sacrum (S2- S3). Seven patients underwent previous treatments: in five cases the cyst was treated by SAE and in two cases a spinal stabilization was performed. In two cases the injection of BMC was performed during a surgical procedure of spinal stabilization. In 6 cases only one injection of BMC was performed, while two injections were performed in 5 patients and three injections were required in 3 patients. Details concerning the cohort of patients analyzed are reported in Table 1.

Table 1
Descriptive analysis of the study cohort

	All samples (n = 14)
Age, year	
median (range)	15.5 (11–33)
Sex, n (%)	
F	6 (42.9)
M	8 (57.1)
Localization, n (%)	
Cervical	7 (50.0)
Lumbar	3 (21.4)
Trunk	3 (21.4)
Sacrum	1 (7.2)
Size, mm	
median (range)	51.5 (20–95)
Number of treatment, n (%)	
1 injection	6 (42.9)
2 injections	5 (35.7)
3 injections	3 (21.4)
Ossification at baseline, Hounsfied unit	
median ± SD	43.48 ± 2.36
Pain at baseline, VAS unit	
median (range)	7 (5–10)

Five out of six female patients (83.3%) were less than sixteen years old and four of these (66.7%) were managed with a single dose of BMC injection, while a higher percentage of male patients (6/8, 75%) were more than sixteen years old and more than one injection was administered to them (Fig. 1).

In the mixed model (Table 2), the mean ossification of the cyst increased statistically from 43.48 ± 2.36 HU to 161.71 ± 23.48 HU during follow-up time (p = 0.0004), as described in Fig. 2. The increased ossification of the cyst was associated with an improved clinical outcome. The mean VAS score before treatment was 7 which after treatment reduced to 1 at 12 months follow up indicating significant pain

relief. The mean ossification over time was significantly higher in patients treated with a single injection compared to patients treated with multiple injections (89.09 ± 11.82 HU vs 77.37 ± 11.55 HU, p = 0.0281. No significant difference in ossification was found between cervical and non-cervical localization of the cyst (82.10 ± 11.82 HU vs 84.37 ± 11.58 HU, p = 0.6420). Moreover, the initial size of the cyst (evaluated as the major dimension measured on CT scan) was not statistically associated with the degree of ossification during follow up time (81.03 ± 11.78 HU vs 85.43 ± 11.67 HU, p = 0.3943). Over time mean ossification had a similar profile among number of treatment injections, localization and cyst size (time x group interaction p = 0.3432, p = 0.6993 and p = 0.8142, respectively). However, cysts in a non-cervical localization and cysts with a smaller size seem to reach a higher ossification after 8 months from surgery (Figs. 3–5).

Table 2
Adjusted LS-means from mixed model

Variables	LS-means ± std	P value
Localization		
Cervical	82.25 ± 12.21	0.6941
Non-Cervical	83.97 ± 12.01	
Size (mm)		
< 51.5	81.03 ± 11.78	0.3943
≥ 51.5	85.43 ± 11.67	
Number of treatments		
1 injection	88.06 ± 12.25	0.0430
> 1 injection	78.16 ± 11.98	
Time		
baseline	43.35 ± 2.35	0.0005
1–3 months	53.94 ± 6.84	
4–8 months	54.21 ± 4.79	
9–12 months	102.31 ± 39.48	
> 12 months	161.74 ± 24.08	

LS-Means = Least Squares means

Std = Standard Deviation

Finally, we did not report any complication, including superficial or deep infection, fracture, or other adverse reactions, both at the harvest site and at the application site.

Two cases of patients treated with BMC injections are described in Figs. 6 and 7, where CT scan and MRI images performed before the treatment and during follow up are reported.

Figures 6a and 6b illustrate the case of patient 1, a 33-years old male with a painful lesion at L2. He previously underwent five selective arterial embolization treatments; however, due to the increase of the cystic lesion, two injections of BMC were performed and the complete healing occurred only 18 months after the second injection. Then, the cyst remains stable at the following follow ups and the patient experienced relief from pain and functional disability.

Figures 7a and 7b illustrate the case of patient 2, a 16-years old patient with a painful lesion at C2 level. He underwent one injection of BMC without previous treatments. The ossification of the cyst started three months after the injection and progressively continued until the lesion healed completely. At recent follow up he was asymptomatic and could perform his activities of daily living with ease.

Discussion

ABC is most commonly found in the metaphysis of long tubular bones (femur and tibia); nevertheless, spine is one of the most prevalent site of lesion, with up to 30% of ABC cases[17,18]. ABC has been described in cervical, thoracic and lumbar regions as well as in the sacrum[3,19–23]. It commonly involves asymmetrically the posterior elements of the neural arch.

Clinically, ABC might be asymptomatic, but it usually presents with pain and swelling in the region of the lesion, so spinal localizations typically present with deformity and neurological symptoms due to compression on the spinal cord or nerve roots such as numbness, difficulty in walking, sensory disturbances, or motor weakness. These symptoms can be also due to pathological fractures, which are uncommon for ABC in the extremities[24].

Many treatment strategies have been proposed to manage ABC[12,25,26] but considering that it is a rare lesion, their efficacy is mostly based on the results of retrospective case series[17,27–34] and only few prospective studies have been performed[35].

Concerning the surgical treatment of ABCs, in order to reduce the high recurrence rate associated to surgical curettage[18], which was considered the treatment of choice for decades, more aggressive surgical protocols have been attempted, up to en bloc resection[7,36–38]. However, for ABC of the spine en bloc resection in spine can be an option in case of involvement of posterior elements alone in order to decrease intraoperative blood loss and the achievement of wide or marginal/focally intralesional margin does not significantly affect recurrence rates.

Thus, the treatment for ABC of the spine focused on less invasive options such as selective arterial embolization (SAE) or intralesional injection with different agents (steroids, calcitonin, doxycycline, ethibloc, osteoinductive agents).

When SAE is technically feasible and safe it has been demonstrated to be as effective as surgery, but with lower complication rates and can be considered as first option in the treatment of ABC without neurological impairment, pathological fracture or instability[23,35].

Intralesional injections of the cyst with calcitonin[39], steroids[40,41]or doxycycline[42] are safe procedures with no major side effects but with still high recurrence rate[43]. On the contrary, alcoholic zein solution (ethibloc) injection is not recommended in the spine, due to severe potential complications such as meningitis, pulmonary emboli, nerve damage and even death[44]. Moreover, Denosumab has been recently proposed as treatment option[13,45–46].

Radiation therapy has also been proposed as primary treatment or as adjuvant to surgery, but it has been progressively neglected due to the increased risk of the long-term malignant transformation and the possibility of damage to neurological structures[47,48].

An alternative attractive option seems to be to stimulate the intrinsic healing potential of ABC using MSCs based therapy. The goal of this treatment is to interrupt the destructive osteoclastic process and promote spontaneous bone regeneration. ABCs undergo continuous reparative processes in a blood-filled cavity therefore providing an ideal microenvironment to MSCs to express their osteoinductive capacities. MSCs can be isolated from different tissues, from blood to bone marrow, in particular from iliac crest bone marrow. Recently, we have demonstrated that vertebral MSCs (v-MSCs) have a greater potential for osteoblastic differentiation[49]. Some authors performed MSCs therapy for the treatment of ABC in long bones and sacrum reversing expansion and promoting the intrinsic healing potential of ABC[15,16,50].

We recently reported two cases of young patients (14 and 16 years old) affected by ABC of the spine localized in C2 vertebra. In both cases SAE was not safe, because the lesions were fed by a pathological circle depending on cervical and vertebral arteries. Thus, we treated the lesions by injection of autogenous BMC, containing MSCs as well as hematopoetic stem cells, platelets (containing growth factors), and cytokines, to fill the bone lesion and induce a biological response leading to new bone formation and healing. This treatment provided a fast and significant improvement in one case, with complete ossification of the lesion within one year from the treatment. In the other case the improvement became evident after two subsequent treatments with BMC[15].

After these successful cases we incorporated the injection of BMC as alternative treatment for spinal ABC in our clinical practice, especially when SAE was not feasible or safe. Thus, in this paper we report 14 cases of spinal ABC treated by injection of autologous BMC harvested from iliac crest. Our results showed that, through a minimally invasive procedure, the BMC treatment induces spontaneous ossification of spine ABCs, also arresting their destructive capability. In our series we obtained a full

recovery after only one injection in more than 42% of our cases while the remaining received two (35.7%) or three (21.4%) injections. We observed that the mean ossification of the cyst increased during the follow up period and that ossification was associated to an improvement of clinical outcomes, especially relief from pain. Moreover, the mean ossification over time was significantly higher in patients treated with a single injection compared to patients treated with multiple injections. It seems that some patients respond better to MSCs treatment starting from the first injection and that the ossification of the lesion in these cases proceeds to higher degrees. However, we did not find any correlation between the initial cyst size or its localization and the degree of ossification during the follow up period. An interesting data was also that four out of six female patients (66.7%) only needed one injection treatment, while a higher percentage of male patients (6/8, 75%) needed more than one injection treatment. In addition to the presence of a natural intra-individual heterogeneity between patients, this greater ossification in female patients, that is not associated with cyst size and/or location, can be due to donor age and/or gender. In our study the mean age for female patients was 12.83 ± 1.94 while for male patients was 21.00 ± 6.23. Despite the difference of mean age between males and females was not so high, it was shown that bone marrow MSCs from younger patients have increased expression of CD146 (MCAM), CD106 (VCAM-1), CD166 (ALCAM), platelet-derived growth factor receptor beta (PDGFRβ), CD90 (Thy1) and CD71 that are markers of clonogenicity, proliferation, stem cell growth and differentiation potential[51,52]. Upregulation of surface molecules like CD106 or CD166 may also have important impact on factors like migration and cell adhesion[53]. In addition, another key point linked both to age and gender is the estrogens level. Estrogens have multifunctional roles that influence growth, differentiation, and metabolism of several tissue types[54–57]. Estrogens also exert regulatory functions via estrogen receptors ER-α and ER-β, which exist on multiple types of cells, including MSCs[58–61]. Among estrogens, several preclinical and clinical studies demonstrated that 17-β estradiol (E2) administration increases MSC proliferation and multi-lineage differentiation[62–65]. Under physiological conditions, the level of this estrogen is noticeably different between adolescent females (estradiol range 20–300 pg/ml) and males (estradiol range < 40)[67]and this difference becomes even more marked if the menstrual cycle is considered (30–800 pg/ml).

Thus, we hypothesize that the high estrogen levels present in the adolescent females as well as young age may act on specific local factors further stimulating the proliferation and the osteogenic activity of MSCs, on which they also perform a direct trophic action.

Conclusions

The results reported in this paper reinforce our previous evidence on the use of BMC from iliac crest, as a valid alternative approach for spinal ABC management when SAE treatment is not indicated or ineffective. Although the iliac crest is the most common site for bone marrow aspiration, during spinal surgery its harvest can leads to an increase in operative and rehabilitation time and to further morbidity in the donor site. Thus, to overcome these limitations, in more recent cases we started to use vertebral bone marrow aspirate, which can be obtained during the same surgical incision, avoiding an additional

procedure, since it showed greater biological efficiency and greater differentiation ability onto osteogenic lineage.

Methods

Patients and surgical procedure

Our study is a retrospective case series of patients affected by ABC treated at our Hospital with percutaneous injection of BMC, from June 2014 to December 2019.

Data for this retrospective study were collected as part of a registry approved by the local Ethics Committee of Istituto Ortopedico Rizzoli on 14.12.2016, concerning the retrospective and prospective collection of clinical and radiographic data related to spinal diseases (of degenerative, oncological, traumatic and infectious origin) treated at the Center (protocol number 0022814).

The research was performed according to the Declaration of Helsinki. Informed consent was wavied by 'Istituto Ortopedico Rizzoli' due to retrospective nature of the study.

The following inclusion criteria were applied:

- histological diagnosis of aneurysmal bone cyst localized to the cervical, thoracic and lumbar spine;
- absence of neurological deficits, strength/sensitivity deficits;
- absence of radiculopathies;
- ability to understand and provide informed consent.

Patients affected by other oncological diseases were excluded.

The procedure of bone marrow harvesting and injection have been previously described by our group[15]. Briefly, the patients were lying prone under general anesthesia and correct levels were checked on fluoroscopy. Sixty ml of bone marrow was harvested from the posterior iliac crest with a 10-gauge needle. The bone marrow aspirate was placed in a bag for transfusion and mesenchymal stem cells (MSCs) were separated from autogenous bone marrow by Res-Q™ 60 BMC concentration system (Novagenit Srl, Mezzolombardo (TN), Italia), after centrifugation at 3200 rpm for 12 minutes. The final product was 6 ml of BMC, as the centrifugation allowed to isolate and concentrate nucleated cells from the other bone marrow elements. Under fluoroscopic guidance, the lesion was filled with the BMC by using a 18G needle. The patients were discharged one day after the procedure.

Evaluation

The mean follow up was 37.4 months (range 12–60 months).

Clinical outcomes and radiological outcomes (CT scan and MRI) were evaluated before the treatment and starting from one month after the procedure. The follow up periods were 1–3 months, 4–8 months, 9–12

months, 18–24 months. Pain was assessed by Visual Analogue Scale (VAS).

The dimension of the cyst and the degree of ossification were measured on CT scans obtained before the treatment and at the follow up visits. The tumour size was assessed in three major diameters and was calculated in millimetres. The density of the cyst (expressed in Hounsfield unit, HU) was also collected with a ROI of 250 mm^2, in the central area of the cyst.

Statistical analysis

The demographic and clinical data were summarized by median and range or number and percentage, as appropriate. The major dimension of the cyst was grouped according to the median value (in mm), the localization of the cyst was grouped into cervical or non-cervical while the number of treatment injections was grouped into one treatment injection or more than one treatment injections.

A mixed model repeated measures analysis with an autoregressive heterogeneous variances variance-covariance structure was used to assess the potential effect of the dimension and the localization of the cyst and of the number of treatment injections on lesion ossification. In addition, the mean ossification over time between groups was explored using the x time interaction term in the model. Least square means and standard error were assessed for all variables in the models. All statistical analyses were conducted using SAS, version 9.4.

Declarations

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The Authors declare they have no conflict of interest.

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Figures

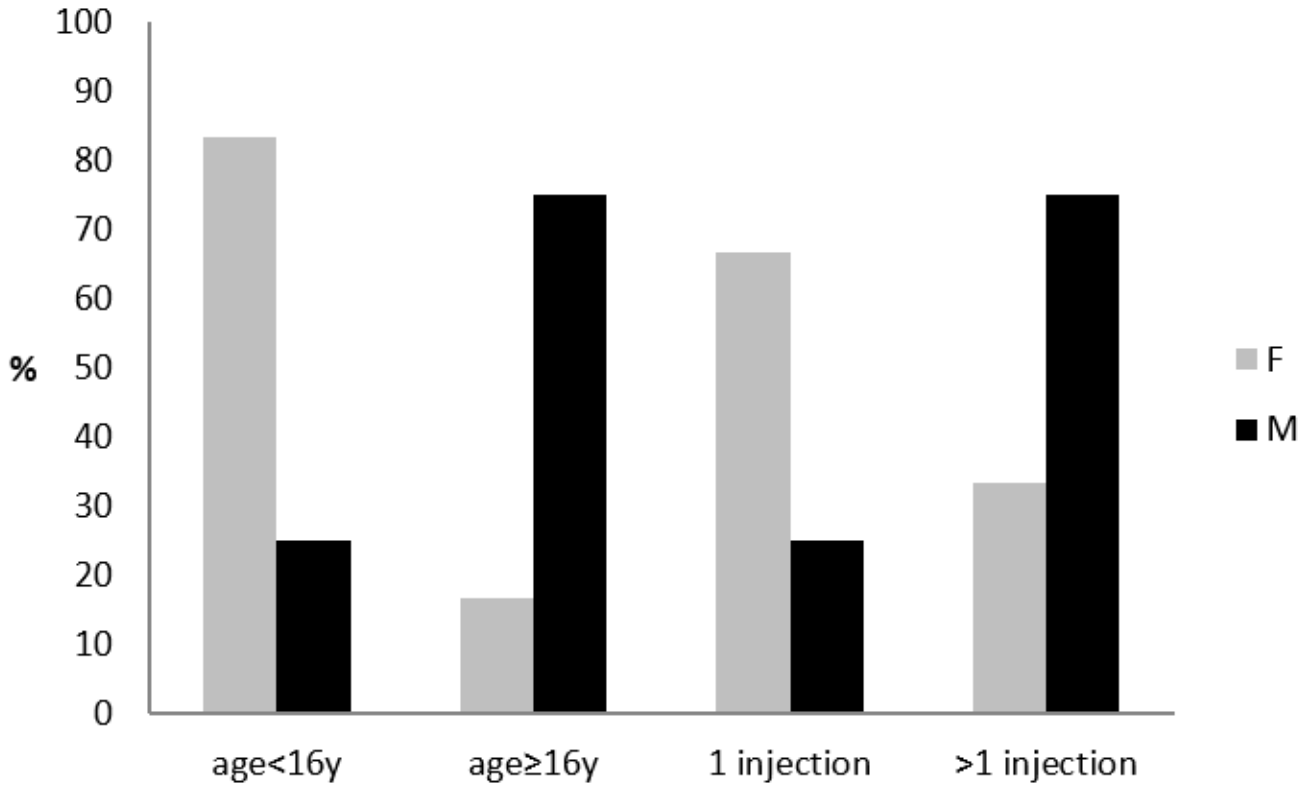


Figure 1

Distribution of patients above and below the median age and with one or more than one treatment injections according to patient's gender

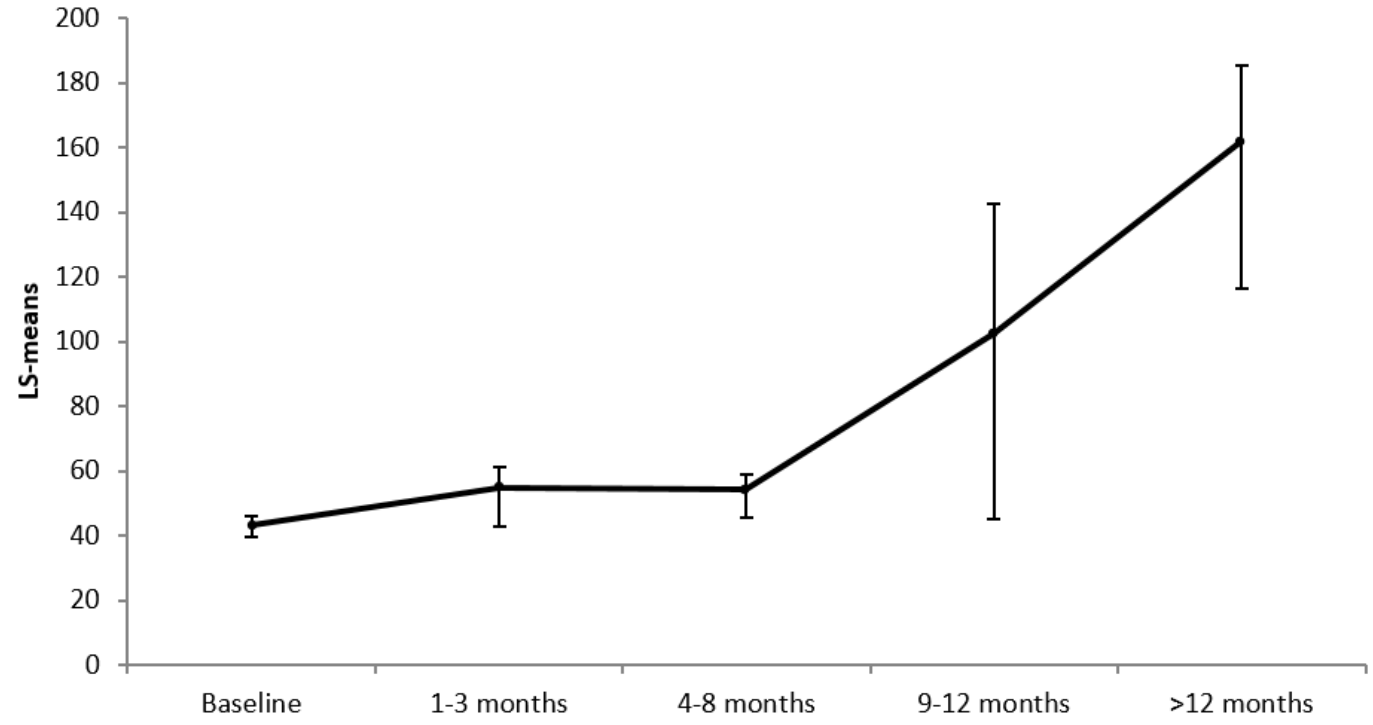


Figure 2

Mean ossification at baseline and during follow up time

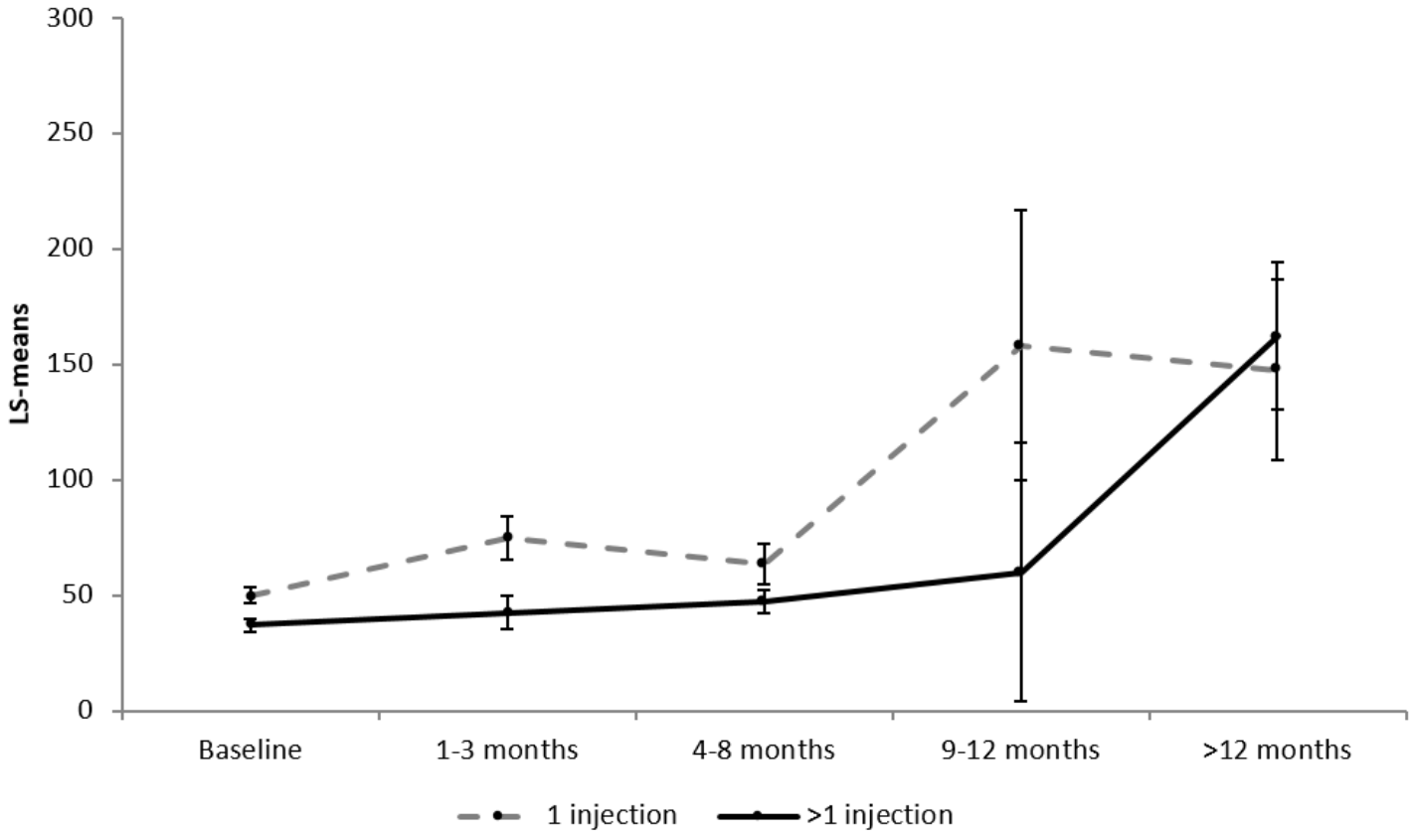


Figure 3

Means ossification over time by number of treatment injections

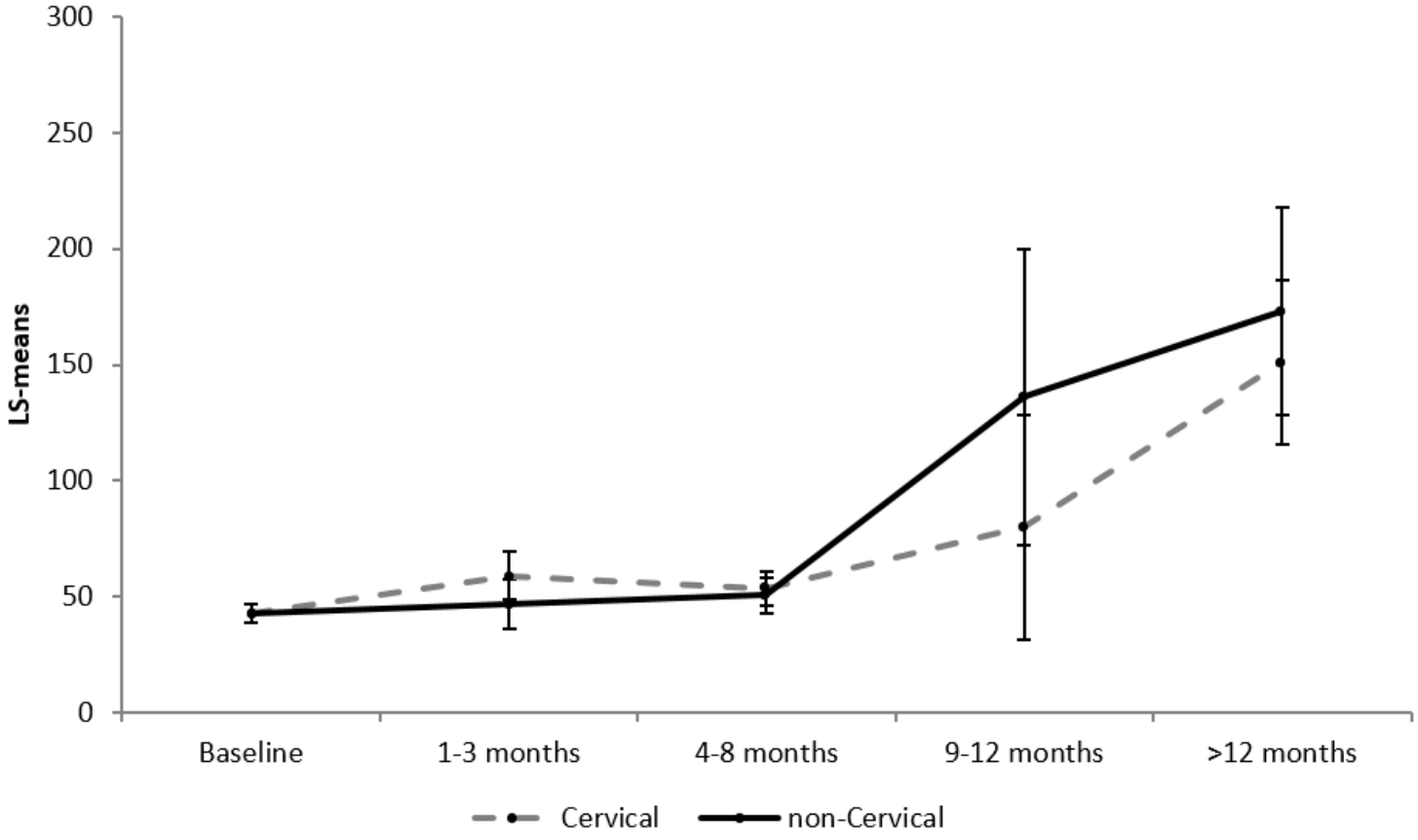


Figure 4

Means ossification over time by localization of the cyst

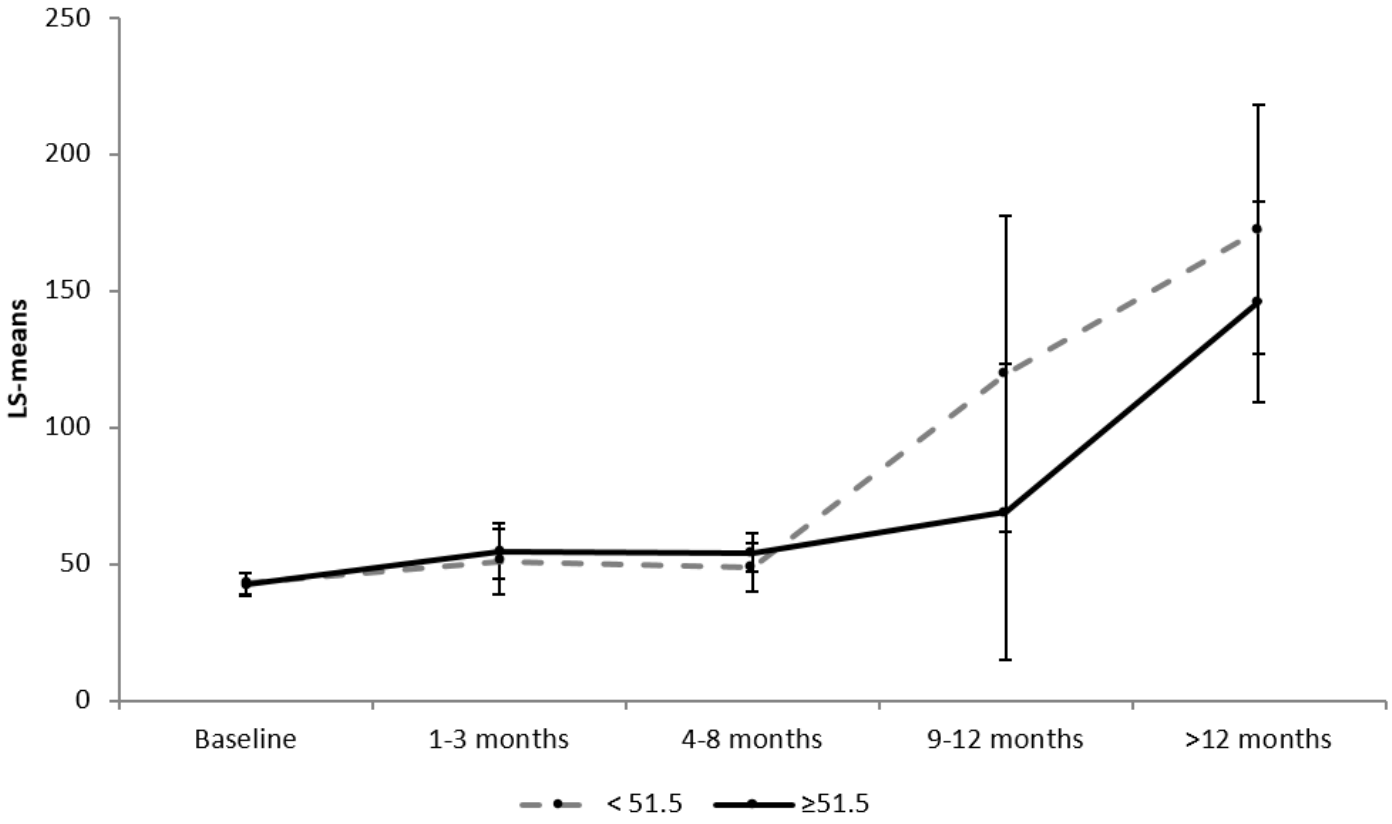


Figure 5

Means ossification over time by dimension of the cyst

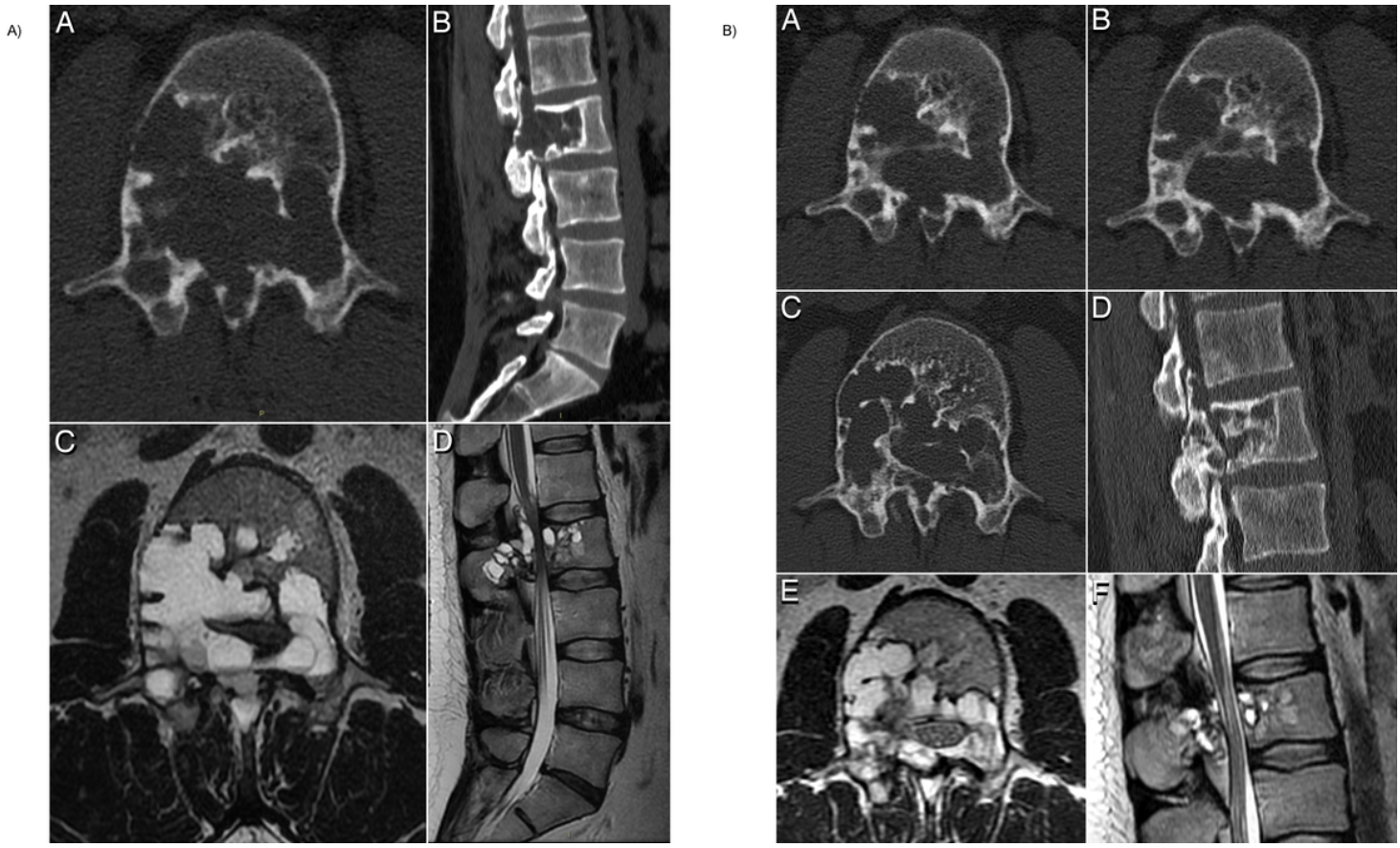
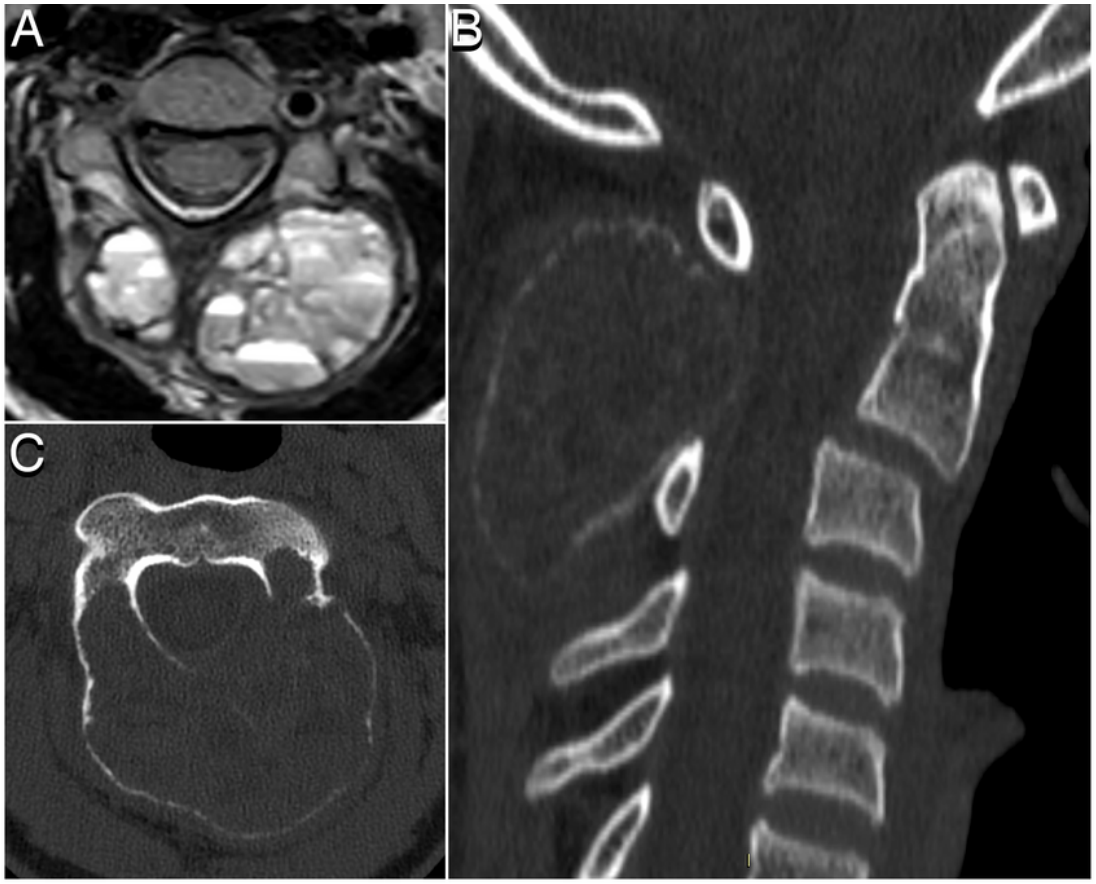


Figure 6

a. Pre-operative images of patient 1 showing the ABC at L2 level before the treatment with concentrated bone marrow. A, B: Axial and sagittal CT scan; C, D: axial and sagittal MRI. b. Follow up images of patient 1 showing the ABC at L2 level after the treatment with concentrated bone marrow. A: axial CT scan at 2 months FU; B: axial CT scan at 4 months FU; C, D: axial and sagittal CT scan at 18 months FU; E, F: axial and sagittal MRI at 18 months FU.

A)



B)

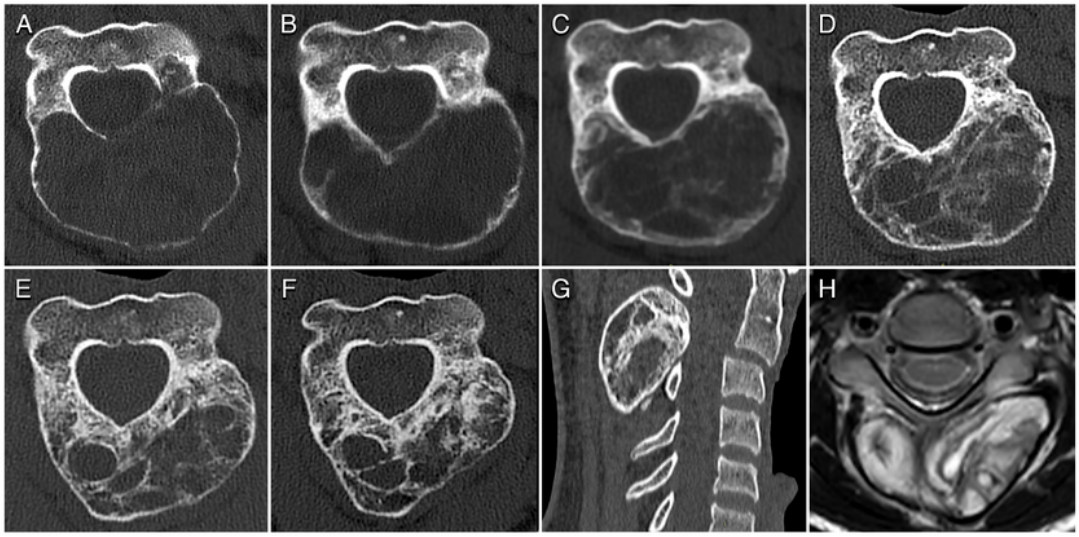


Figure 7

a. Pre-operative images of patient 2 showing the ABC at C2 level before the treatment with concentrated bone marrow. A, B: Axial and sagittal CT scan; C: axial MRI. b. Follow up images of patient 2 showing the ABC at C2 level after the treatment with concentrated bone marrow. A: axial CT scan at 2 months FU; B: axial CT scan at 6 months FU; C: axial CT scan at 9 months FU; D: axial CT scan at 12 months FU; E: axial CT scan at 18 months FU; F, G: axial and sagittal CT scan at 24 months FU; H: axial MRI at 24 months FU.