



## Research Article

# Treatment of simple bone cyst with bone marrow concentrate and equine-derived demineralized bone matrix injection versus methylprednisolone acetate injections: A retrospective comparative study

Raffaele Dario D'Amato<sup>1</sup> , Antonio Memeo<sup>1</sup> , Federico Fusini<sup>2</sup> , Elena Panuccio<sup>1</sup> , Giuseppe Peretti<sup>3</sup> <sup>1</sup>Department of Paediatric Orthopaedics and Traumatology, Centro Specialistico Ortopedico Traumatologico Gaetano Pini-CTO, Milano, Italy<sup>2</sup>Department of Orthopaedics and Traumatology, Azienda Ospedaliero Universitaria Città della Salute e della Scienza, Torino, Italy<sup>3</sup>Department of Biomedical Sciences for Health, IRCCS Istituto Ortopedico Galeazzi, Milano, Italy

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## ORCID IDs of the authors:

R.R.D. 0000-0003-2156-983X;

A.M., 0000-0002-9715-6989;

F.F. 0000-0002-4223-8485;

E.P. 0000-0002-8364-6434;

G.P. 0000-0001-9341-7187.

## Corresponding Author:

Raffaele Dario D'Amato

[raffaeledariodamato@gmail.com](mailto:raffaeledariodamato@gmail.com)

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## ABSTRACT

**Objective:** The aim of this study was to compare the outcome of intra-lesional autologous bone marrow concentrate (BMC) and equine derived demineralized bone matrix (EDDBM) injections with methylprednisolone acetate injections in patients with simple bone cyst.**Methods:** Clinical records and radiographs of 53 consecutive patients (37 females, and 16 males; mean age: 10.6±1.53 years) treated between 2006 and 2016 were retrospectively reviewed. Healing was assessed by an independent radiologist according to Neer scoring system. Functional outcome was assessed with the Activity Scale for Kids (ASK). Thirty-four cysts were in the humerus, 13 in the femur and 6 in other locations. Twenty-nine patients were included in Steroid Group and treated with 3 cycles of injections of methylprednisolone acetate, while 24 patients were treated with injection of autologous bone marrow concentrate and equine derived demineralized bone matrix (BMC+ EDDBM Group). The two groups were homogenous for the mean age, sex distribution, cysts location and their clinical presentation.**Results:** At a minimum follow-up of 24 months, success rate (Neer/Cole score 3 and 4) was higher in EDDBM+BMC group (83.3% vs 58.6%; p=0.047). Female patients had higher healing rates in both groups (p=0.002). No association was found between healing and age (p=0.839), cyst activity (p=0.599), cyst localization (p=0.099) and clinical presentation (p=0.207). BMC+EDDBM group showed higher ASK score (p=0.0007).**Conclusion:** Treatment with BMC+EDDBM injections may provide better results with a single procedure than 3 methylprednisolone acetate injections and represent an interesting alternative for the treatment of unicameral bone cysts.**Level of Evidence:** Level III, Therapeutic Study

Simple bone cysts are benign, fluid-containing, and usually expanding lesions that affect children before skeletal maturity.

Virchow first described these lesions in 1876 (1), but Heineke et al. gave a more detailed description of their radiographic aspect in 1903 (2). In 1942, Jaffe et al. first used the term bone cysts and defined these lesions as bone dysplasia with cystic aspects mostly in the metaphysis of long bones (3).

They represent 3% of all bone lesions and are more common in long bones. Simple bone cysts (SBCs) could show up with or without pain, and often are diagnosed as an incidental finding on a radiograph.

Furthermore, SBCs could be complicated by pathological fractures (4).

Even if SBCs might be asymptomatic and regress spontaneously, surgical treatment is often advised (4-6). Cortex-thinning results in focal weakening and the bone is thus at risk for pathologic fractures (7, 8). The main goals of treatment, therefore, are to decrease the risk of fracture and promote cyst healing (8, 9).

Nowadays, there are many treatment options such as intra-cystic injections of steroids or bone substitutes, mechanical disruption of the cystic wall, decompression, nailing, or combined treatments (10, 11). Scaglietti et al. proposed repeated percuta-

neous injections of corticosteroids in 1979 (12). Due to low morbidity, technical simplicity, and a high healing rate reported by the author (90%), this treatment has been widely used (13-16). Subsequent studies, however, reported a lower healing rate (41–63% after the first injection) (17, 18). Sung et al. and colleagues reported a success rate of 16% after the first steroid injection and 24% after repeated procedures (19). The injection of autologous bone marrow (BM) alone or in combination with demineralized bone matrix (DBM) was proposed as an alternative to steroids for treating SBCs. BM should provide osteoprogenitor cells and DBM could stimulate new bone formation thanks to its osteoconductive properties (20-28).

We hypothesized that intralesional injections of bone marrow concentrate, associated with equine-derived demineralized bone matrix (EDDBM+BMC), could be effective in the treatment of SBCs.

This study, therefore, aims to compare the outcomes of intralesional injections of EDDBM+BMC versus methylprednisolone acetate injections regarding the healing rate in the surgical treatment of SBCs. We furthermore aim to highlight whether the healing rate is influenced by the age and gender of the patients, and activity and localization of the cystic lesions.

## Materials and Methods

The Institutional Review Board (IRB) of the Gaetano Pini Orthopedic Institute approved the execution of this retrospective study (IRB No. 2019-0027). Written informed consent was obtained from the parents of the patients who participated in this study.

Patients affected by SBC and treated at our institute from 2006 to 2016 were retrospectively reviewed. From this cohort, the criteria for participants included in the study were: SBC regardless of anatomic location confirmed by radiological features and cytopathological examination of the intra-cystic fluid with a high risk of fracture according to anatomical location, thickness of the cyst wall (<2 mm) (8) or sport participation (sport participation enhances the probability of trauma), patients with a clinical presentation of spontaneous pain or incidental radiological findings of pathological fractures, patients having a persistence of SBC after at least 12 months of conservative treatment for pathological frac-

tures, and patients with minimum follow up of 24 months. Asymptomatic SBCs that were detected incidentally and cysts that do not cause a risk for pathological fractures with a thickness of cyst wall greater than 2 mm were excluded from the study. Fifty-three consecutive patients (37 females, 16 males) met the criteria to be included in the cohort. Informed consent was obtained from the patients who were included in this study.

Clinical presentation was a pathologic fracture of the cyst wall in 36 patients, local pain in 12 patients, and incidental radiographic findings in the remaining five patients. Thirty-four (34) cysts were in the humerus, thirteen (13) in the femur, and six (6) in other localizations.

Twenty-nine (29) patients were treated with repeated injections of methylprednisolone (Steroids Group (SG)) and 24 with an injection of autologous EDDBM+BMC (EDDBM+BMC Group).

There were 21 females and 8 males in the Steroid Group (mean age at treatment is 10.7 years; range 9 to 15 years) and 16 females and 8 males in the EDDBM+BMC Group (mean age at treatment is 10.5 years; range 9 to 14 years). The 24 months of minimum follow up started from the last cycle of treatment for both groups. Mean follow up for the SG is 79.3 months (range 24 to 120 months) while mean follow up for the EDDBM+BMC Group is 46.5 months (Range 24 to 60 months).

In the SG, 17 lesions were humeral, 8 femoral, and 4 in other locations. In the EDDBM+BMC Group, 17 lesions were humeral, 5 femoral, and 2 in other locations. We classified SBCs as active when they were within 1 cm of the physis (Table 1).

In the SG, patients undergo general anesthesia. Two needles are percutaneously inserted proximally and distally inside the cyst under fluoroscopic guidance. Through the first needle, the cyst fluid content is aspirated and delivered to histological examination, then a saline solution is injected through the cyst to perform washing. Afterward, a steroid injection, based on the estimated cyst volume, calculated on two-plane radiographs of the lesion site (6 x length x width x depth), is performed with methylprednisolone acetate. The mean cyst volume in this group was 25cc (15cc to 40cc). No curettage was performed in between the washing and steroid injections steps. Patients of the SG underwent a cycle of three methylprednisolone injections at three-month intervals.

In the EDDBM+BMC Group, the surgical procedure was done in two phases. In the first phase, 60 mL BM is aspirated from the iliac wing through a single puncture with a Jamshidi needle inserted in zones 1, 2, or 3, according to Hernigou et al. (29, 30). It was then linked to a 30cc syringe, pre-filled with a solution of 0.5 mL heparin in 5mL of saline solution. The first syringe is filled with 10mL of BM, mixed with anticoagulant by manual shaking, and then another 10 mL of BM is aspirated. At each step of aspiration, the Jamshidi needle is rotated 90° and retracted 0.5–1cm to collect a greater number of staminal cells, draining those more adherent

### MAIN POINTS

- There's no standardized treatment for the management of unicameral simple bone cysts in literature. No option has shown striking results.
- Equine derived demineralized bone matrix intralesional injection showed better healing rate and, consequently better functional outcomes than methylprednisolone acetate.
- Equine derived demineralized bone matrix showed to be an effective and safe treatment option for orthopedic surgeon in the management of this pathology.

**Table 1.** Resume of clinical and radiological features of patients involved in the study. Group A=Steroid Group; Group B=EDDBM+BMC group; M=male; F=Female; R=Right; L=Left; Y=Yes; N=No; ASK=activity scale for kids. Neer grade 1 or 2 after procedure was taken as treatment failure and grade 3 or 4 as treatment success. ASK score > 90 was graded as excellent, score ranging between 80 and 89 as acceptable and score lower than 80 as not acceptable. In Group A each treatment cycle consists of three injections while in Group B it consists of one injection

GROUP A										
Patient	Age	Sex	Bone	Region	Side	Cyst Activity	Clinical presentation	Neer grade after procedure	Number of procedures	ASK
1	9	M	Humerus	Diaphysis	R	Y	Fracture	3	3	84
2	10	F	Femur	Metaphysis	L	N	Pain	2	6	89
3	9	M	Humerus	Metaphysis	R	N	Fracture	4	3	96
4	10	F	Humerus	Metaphysis	R	Y	Fracture	3	3	84
5	12	F	Tibia	Diaphysis	L	N	Fracture	4	6	94
6	11	F	Humerus	Metaphysis	L	Y	Fracture	1	3	79
7	10	F	Femur	Metaphysis	L	Y	Fracture	3	6	85
8	12	F	Humerus	Diaphysis	R	N	Incidental	4	3	95
9	9	M	Humerus	Metaphysis	L	N	Fracture	3	3	85
10	9	M	Femur	Diaphysis	R	N	Pain	4	3	94
11	15	F	Femur	Metaphysis	R	N	Pain	3	3	95
12	10	F	Humerus	Diaphysis	R	Y	Fracture	2	6	84
13	11	F	Femur	Metaphysis	L	N	Pain	3	3	86
14	9	F	Humerus	Metaphysis	R	N	Fracture	1	3	78
15	10	F	Femur	Diaphysis	L	Y	Incidental	3	3	93
16	11	F	Humerus	Metaphysis	L	N	Fracture	2	3	85
17	13	F	Femur	Metaphysis	R	N	Fracture	3	6	95
18	14	F	Femur	Diaphysis	R	N	Pain	4	3	97
19	12	F	Humerus	Metaphysis	L	Y	Fracture	1	3	78
20	10	M	Fibula	Diaphysis	R	N	Pain	3	3	84
21	9	F	Humerus	Metaphysis	R	Y	Fracture	2	3	86
22	12	F	Humerus	Metaphysis	L	N	Fracture	2	6	83
23	13	F	Tibia	Metaphysis	L	N	Fracture	2	3	86
24	10	M	Humerus	Metaphysis	L	N	Fracture	3	3	95
25	9	F	Humerus	Metaphysis	R	Y	Fracture	1	6	77
26	11	M	Humerus	Metaphysis	L	N	Fracture	2	3	95
27	10	M	Humerus	Metaphysis	R	N	Fracture	3	3	89
28	11	F	Fibula	Metaphysis	R	N	Fracture	3	3	94
29	10	F	Humerus	Diaphysis	L	Y	Fracture	2	3	85
GROUP B										
Patient	Age	Sex	Bone	Region	Side	Cyst Activity	Clinical presentation	Neer grade after procedure	Number of procedures	ASK
1	10	F	Humerus	Metaphysis	R	Y	Pain	4	1	98
2	11	F	Fibula	Metaphysis	R	N	Fracture	3	1	94
3	9	M	Femur	Metaphysis	R	Y	Pain	4	1	97
4	9	F	Femur	Diaphysis	L	N	Incidental	4	1	95
5	14	F	Humerus	Metaphysis	L	N	Fracture	3	2	88
6	10	F	Humerus	Metaphysis	R	N	Pain	3	1	93
7	12	F	Humerus	Diaphysis	L	N	Fracture	3	1	94
8	9	M	Humerus	Metaphysis	R	N	Fracture	3	1	87
9	9	F	Femur	Diaphysis	L	N	Pain	2	2	87
10	11	F	Humerus	Metaphysis	L	N	Fracture	4	1	97
11	11	M	Femur	Diaphysis	R	Y	Fracture	3	2	86
12	12	F	Humerus	Metaphysis	L	Y	Fracture	4	1	98
13	14	F	Humerus	Diaphysis	R	N	Incidental	2	1	88
14	9	F	Humerus	Metaphysis	R	N	Fracture	3	2	93
15	10	F	Humerus	Diaphysis	L	N	Fracture	2	1	85
16	11	M	Femur	Metaphysis	R	N	Pain	3	1	85
17	9	F	Humerus	Metaphysis	L	Y	Pain	3	1	94
18	10	M	Humerus	Metaphysis	R	N	Fracture	4	2	97
19	10	M	Humerus	Diaphysis	L	Y	Fracture	4	1	98
20	12	F	Tibia	Diaphysis	L	N	Incidental	3	1	94
21	11	F	Humerus	Diaphysis	R	N	Fracture	1	1	78
22	10	M	Humerus	Metaphysis	L	Y	Fracture	3	1	88
23	10	F	Humerus	Metaphysis	R	N	Fracture	4	2	97
24	9	M	Humerus	Metaphysis	R	N	Fracture	3	1	95

to the cancellous bone. This alternating pattern of aspiration was continued until 60 mL of BM was collected.

The aspirated BM was then filtered directly in the operating room through a dedicated kit (IOR-G1, Novagenit, Italy). The sample was concentrated in a cell separator (Re-Q 60) at 3200 rpm for 12 minutes obtaining 10 mL of BM, rich in nucleated cells such as stem cells, monocytes, lymphocytes, and BM resident cells and poor in red cells ( $>8.86 \times 10^6$  of Mono Nucleated Cells) (Figure 1). In the second phase, under fluoroscopy guidance, two needles are inserted into the cyst to drain the intra-cystic fluid, deliver a sample for histological examination and perform cyst washing. Afterward, one needle is removed and the concentrated BM mixed with 5 to 10 mL of EDDBM paste (ACTIVAGEN, Biotech, Italy), according to cyst volume, is injected under pressure by the second needle.

In both groups, the overall procedure time ranged from 30 to 45 minutes. All patients underwent a cycle of three steroid injections or one EDDBM+BMC injection. If the cyst persisted by the 12th month of follow up, a second cycle of steroid injections or EDDBM+BMC was performed.

All patients were discharged one or two days after surgery. Free movements were allowed after 7–15 days. Sports activities were forbidden for two to three months.

Patients in both groups were followed in the outpatient clinic to assess cyst healing and clinical results at 3, 6, 12, and 24 months after surgery.

We obtained radiographs of the cyst at each control and they were evaluated by an independent radiologist using the Neer scoring system modified by Cole (18) as grade 1, cyst clearly visible;

grade 2, cyst visible but multilocular and opaque; grade 3, sclerosis around or within a partially visible cyst; grade 4, complete obliteration of the cyst. Grade 1 and grade 2 results are considered failures while grades 3 and 4 are considered successes.

The ASK is a 30-item child self-report questionnaire about physical activity with excellent reliability (ICC=0.97). It was designed for children of 5 to 15 years of age affected by musculoskeletal disorders (31, 32).

The questionnaire was sent to patients at the two-year follow up.

An independent statistician performed the statistical analysis. Data were expressed as frequencies, percentages, means, and SDs. Associations between the healing rate and treatment group, age, gender, activity, and localization of the cysts were explored using the chi-square test. The student *t*-test was used to assess the differences in AKS between the two groups. Statistical significance was set at  $p < 0.05$ .

## Results

Between the two study groups there was no significant difference in the age ( $p=0.599$ ), gender ( $p=0.659$ ), location ( $p=0.357$ ), activity ( $p=0.686$ ), and clinical presentation of the cysts ( $p=0.409$ ) (Table 2).

An additional cycle of injections was performed in seven (7) patients in the SG and six (6) patients in the EDDBM+BMC Group who presented with a cyst stage 1 and 2 according to the Neer classification, modified by Cole, 12 months after the first cycle of treatment. Persistent small cystic lesions ( $<1 \text{ cm}^3$ ), despite their Neer classification that did not make the cyst wall thinner than 2 mm, were not treated with an additional cycle of injections.

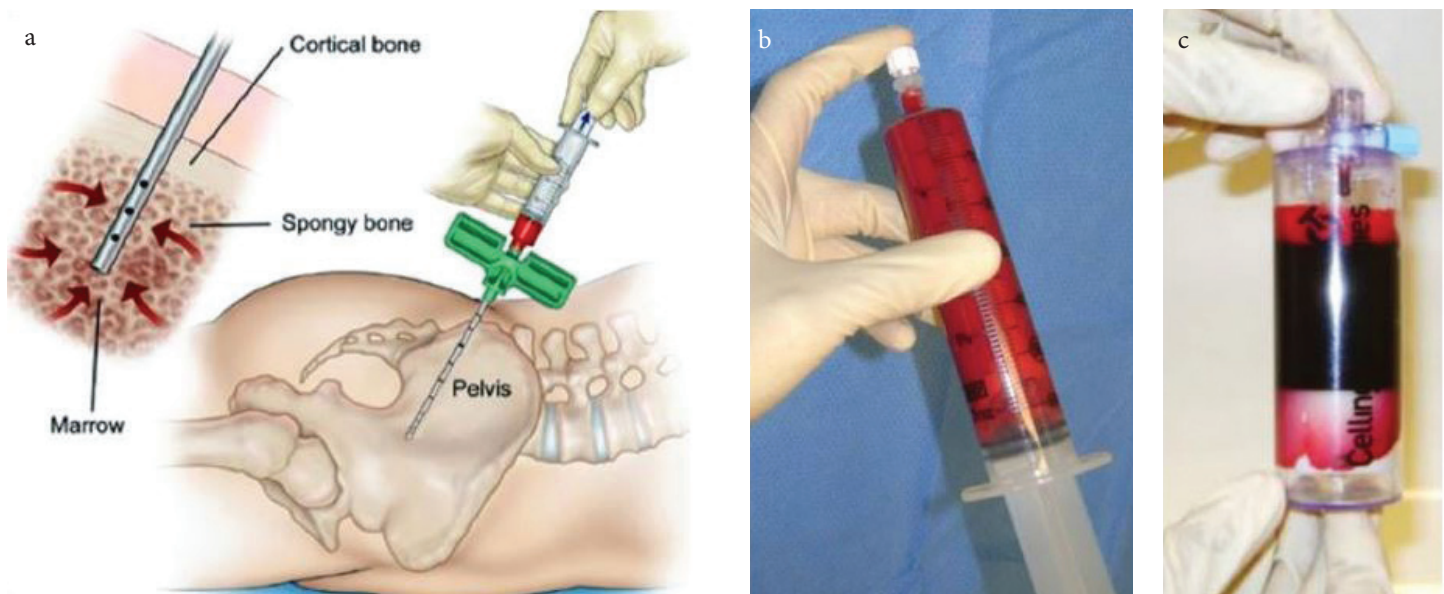


Figure 1. a-c. Iliac crest bone marrow aspiration (a), 50 to 60 mL of bone marrow aspirated (b), Bone marrow derived stem cell concentrated (after centrifugation) (c)

In the SG, a mean of 75 mg of methylprednisolone (45 mg to 120 mg) was injected. At the 24 months follow up, in this group, 12 patients were graded as failures (4 stage 1 and 8 stage 2) (Figure 2), while 17 patients achieved bone cyst resolutions (12 stage 3 and 5 stage 4) (Figure 3). In this group, the overall failure rate was 41.4%

(stage 1 and 2) and the success rate was 58.6% (stage 3 and 4). In the EDDBM+BMC Group, 4 patients were graded as failures (1 stage 1 and 3 stage 2) (Figure 4), while 20 patients achieved success (12 stage 3 and 8 stage 4) (Figure 5). This group presented a global failure rate of 16.7% and a success rate of 83.3%.

**Table 2.** Population data

N°	Cohort 53	Steroid Group 29	EDDBM+BMC Group 24	p
Mean age	10.6±1.53	10.7 ±1.60	10.5 ±1.47	0.599
Sex distribution				
Males	16	8	8	0.659
Females	37	21	16	
Location of the cyst				
Femur	13	8	5	
Humerus	34	17	17	0.357
Others	6	4	2	
Clinical presentation				
Pain	12	6	6	
Incidental finding	5	2	3	0.409
Previous fracture	36	21	15	
Active cysts	16	10	6	0.686

EDDBM: Equine Derived Demineralized Bone Matrix; BMC: Bone Marrow Concentrate

The healing rate in the EDDBM+BMC Group, therefore, was significantly higher than the healing rate of SG (83.3% vs 58.6%;  $p=0.047$ ).

Furthermore, female patients in both groups showed a better response to treatment ( $p=0.002$ ).

No association was found between success rate and age ( $p=0.839$ ), cyst activity ( $p=0.599$ ), location of the cysts ( $p=0.099$ ), and clinical presentation ( $p=0.207$ ).

Mean healing time in the SG was 17.1 months while in the EDDBM+BMC Group it was 15.9 months ( $p=0.791$ ).

Mean ASK scores at the last follow up was  $86.10\pm 6.17$  for the SG and  $91.92\pm 5.40$  for the EDDBM+BMC Group ( $p=0.0007$ ) (Diagram 1, Table 3).

In the SG we reported two cases (1 male, 1 female) of persistent pain in the steroid injection site and treated it with oral painkillers. In the EDDBM+BMC Group, we reported one case of superficial skin infection at the iliac wing site of BMC harvesting, which was treated with oral antibiotics. In one patient, persistent pain at the



**Figure 2. a-d.** Proximal humeral cyst treated with methylprednisolone injections; pre-operative (a, c) and post-operative (b, d) x-rays (success)



Figure 3. a-d. Diaphyseal humeral cyst treated with methylprednisolone injections; pre-operative (a, c) and post-operative (b, d) x-rays (failure)



Figure 4. a-d. Diaphyseal femoral cyst treated with EDDBM+BMC injections; pre-operative (a, b) and post-operative x-rays (c, d) (success)

EDDBM+BMC injection site was reported and treated with oral painkillers.

In our cohort, no patient reported refracture after treatment at the 24-month follow up.

## Discussion

The indication to treat SBC is to prevent a pathologic fracture and reinforcing the bone cortex. Injection strategies, decompression techniques, curettage or resection, with or without bone grafting, and combined surgical approaches were proposed to treat SBCs, but no method has shown striking results (33).

Surgical curettage and cyst excision with bone graft were considered as the treatment of choice to treat SBCs, but these approaches lost popularity because of the high risk of complications and invasiveness (34, 35).

Steroids, bone matrix, and BM were described as less invasive injectable treatments and gained popularity (20, 21, 36-40).

It must be reported that not all SBCs can cause a pathological fracture and not all SBCs need treatment. Some of them can be observed without any specific treatment. In patients with impending or pathological fracture, intralesional injections, however, represent a valid surgical treatment option (41).

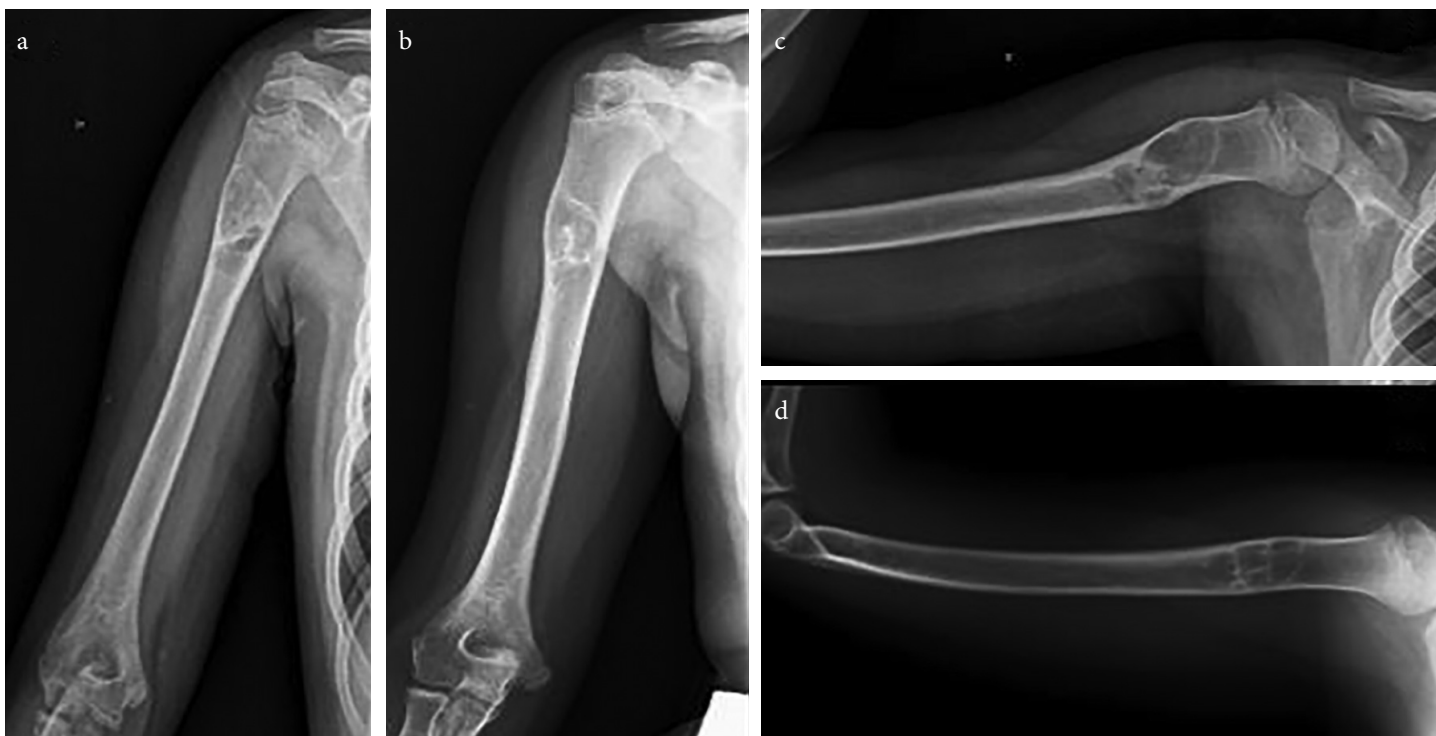


Figure 5. a-d. Proximal humeral cyst successfully treated with EDDBM+BMC injections; pre-operative (a, c) and post-operative (b, d) x-rays (failure)

Table 3. Success rate and ASK scores in the two study groups

	Steroid Group	EDDBM+BMC	p
Healing rate	58.60%	83.30%	0.047
ASK score	86.1±6.17	91.9±5.40	0.0007

EDDBM: Equine Derived Demineralized Bone Matrix; BMC: Bone Marrow Concentrate

Intra-cystic steroid injections with methylprednisolone was firstly described by Scaglietti with positive results in 90% of patients (13). Methylprednisolone should reduce the production of cyst fluid through the long-acting anti-inflammatory properties of its microcrystalline composition on the inner cysts membrane, which should facilitate the healing process (38). Further studies did not replicate these results, with a recurrence rate ranging from 15% to 88% after three injection procedures (13, 42).

Autologous BM injections were also described for the treatment of osteolytic lesions (20-23). The rationale is that BM is the main source of mesenchymal stem cells (MSCs), which can differentiate in a variety of cellular lineages such as cartilage, bone, tendon, and muscle (43-45). They also secrete cytokines that regulate local immune activities, playing a crucial role in bone regeneration (43-45).

Lokiec et al., followed by Delloye et al., firstly described using autologous native BM injections in the treatment of SBCs, reporting success in 100% of patients; however, these results were not confirmed in further studies (22, 23).

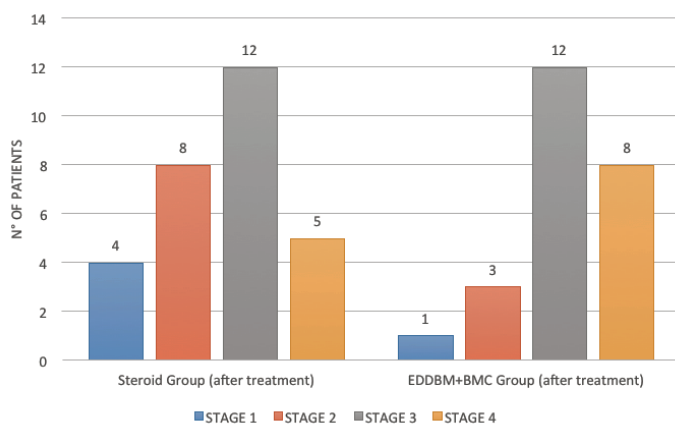


Diagram 1. Patient distribution according to Neer-Cole's stages

Some authors compared BM injections with steroids injections reporting a higher healing rate (52% vs 23%) and low recurrence rate (13% vs 42%) (17) but other studies did not confirm the superiority of this treatment compared to steroids injections (21, 40, 46).

Rougraff and Kling reported the results of the association of BM with DBM, obtaining better results regarding a higher healing rate (58% vs 21%) and lower failure rate (24% vs 63%) compared to steroids injections (25).

Urist et al. first identified osteoconductive and oteoinductive properties of DBM (47). It acts as a scaffold for the deposition of new bone, providing also mechanical support during remodeling phases. DBM contains bone morphogenetic proteins; they are

growth factors that enhance cartilage and bone formation belonging to the TGF- $\beta$  family (48). Using DBM is not limited by graft material availability and harvest morbidity and, therefore, is uninfluenced by donor condition (49-51).

Di Bella et al. retrospectively reviewed the results of the treatment of SBCs with bone marrow aspirate concentrate (BMC) injections in association with DBM in the treatment of simple bony cysts and compared them with steroids injections (26). They concentrated BM aspirate through centrifugation. It is one of the strategies widely available to improve the recovery of MSCs from BM aspirate, enhancing their osteogenic activity, as it does not require cell culture *ex vivo*. Cell culture could expose them to immunogenicity and genetic stability modifications (52).

In their study, patients who underwent this treatment reported a healing rate of 71%, compared to 38% of those treated with methylprednisolone injections at the two-year follow up. These results were obtained with a significant lower number of infiltration procedures needed.

In our study, we report a better healing rate of 83.3% and a failure rate of 16.7%, compared to 58.6% of healing and 41.4% of failure reached by patients treated with methylprednisolone. The ASK score of our patients showed better results in patients treated with BMC ( $p=0.0007$ ). This is could be related to the lower number of infiltrations, and consequently, hospitalization reported in our study. Fewer hospitalizations mean fewer surgeries and lower costs for the healthcare system.

In the SG we delivered 3mg/mL of methylprednisolone acetate according to the cyst volume, while Di Bella et al. delivered 80mg of methylprednisolone acetate, not considering the cyst volume, potentially underestimating or overestimating the results (26). Furthermore, they aspirated 18 to 20 mL of bone marrow and centrifuged it twice to obtain 9 to 10 mL of BMC. Fifty to 60 mL of bone marrow were aspirated and were once centrifuged to obtain 10 mL of BMC, thus making our procedure more efficient regarding a higher concentration of progenitor cells and cytokines with a single centrifugation process.

Although, currently, there is no clear consensus about the optimal dose of MSCs that is considered therapeutic. Some studies demonstrate that higher doses provide better clinical outcomes (53, 54). The kit we used (IOR-G1 + ResQ 60, Novagenit, Italy) allows mononucleated cells (MNC) recovering more than 90%, platelet concentration of four-fold and red blood cells depletion of >96%, maintaining in the final product the key mesenchymal population (CD45-/CD44+/CD73+/CD90+/CD105+).

Autologous cancellous bone is considered the gold standard for bone defect repair (55) but its use is limited by increased operative time and donor site morbidity (56, 57). On the other hand, the main problems with using allografts are limited donor sources, high cost, higher resorption rate, and greater immunogenic re-

sponses (58). The search for alternative bone sources, therefore, has led to a growing interest in xenogeneic bone substitutes of animal origin. They could represent a theoretically unlimited supply of grafting material associated with lower infection risk and lower costs.

In our study, we used EDDBM, which underwent an enzymatic deantigenation process. Its use has shown good outcomes and could be considered a safe alternative to bovine-derived DBM regarding spongiform encephalopathy transmission risk (59-62).

EDDBM+BMC injection is a more invasive procedure compared to steroid injections; however, through this procedure, we obtained better results with just one injection compared to three steroid injections. The overall hospitalization cost of a cycle (1 injection) of EDDBM with BMC injection was about 2700€ while the hospitalization cost for a cycle of three steroid injections is about 6000€ (2000€ for each steroid injection).

This means that we had better results with lower costs of hospitalization since each injection procedure both for steroids and EDDBM+BMC is performed in a hospitalization setting.

We found that in 16 patients, the treatments were regarded as a failure but we have a few elements to draw any conclusion about that since the low numerosity of the cohort population, could lead to further bias. Pretell-Mazzini et al. consider patient age as an important factor affecting the treatment outcome (41). Patients older than 10 years heal at a higher rate (90%) than younger patients (60%), irrespective of the treatment modality. On the other hand, Haidar and colleagues consider a lesion located <2 cm from the physis as a risk factor for recurrence. The risk of recurrence could also be related to the type of treatment rather than the location of the lesion.

There is no evidence in the literature that one treatment gives better results according to gender distribution over another. We consider the better results obtained in female patients in our cohort as incidental.

The major limitation of the study is that the effect of EDDBM on the success rate is unknown. Of course, a study comparing steroid injections with BMC and BMC+EDDBM would be useful to better clarify this aspect.

This study is also limited by the retrospective design, as this would require a prospective experimental study with the randomization of patients within groups: by the small groups' size, which have limited the study power, and by the lack of a long-term follow up that does not allow to estimate the recurrence rate after that period.

Currently, 14 different kinds of systems allowing BM aspiration and concentration are commercially available. The MSCs obtained with these systems, as they do not derive from cell culture *in vivo*,



do not meet the International Society for Cellular & Gene Therapy standard criteria to define properly MSCs (63). This makes it difficult to compare it with other studies, owing to the great variety in reported methods and outcomes.

To our knowledge, this study is the first about using EDDBM+B-MC for the treatment of SBCs. It showed a higher healing rate and higher functional outcomes at two years follow up compared to methylprednisolone injections. It represents an effective and safe treatment option for orthopedic surgeons. More powered studies with longer follow-ups and larger samples are needed to confirm these results.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the Gaetano Pini Orthopedic Institute Review Board (N°2019-0027).

**Informed Consent:** Written informed consent was obtained from the parents of the patients who participated in this study.

**Author Contributions:** Concept D.R.D., F.F., M.A., P.G.; Design D.R.D., F.F.; Supervision - D.R.D., F.F., M.A., P.E., P.G.; Resources D.R.D., P.E., M.A.; Materials D.R.D., P.E., M.A.; Data Collection and/or Processing - D.R.D., F.F., P.E.; Analysis and/or Interpretation - D.R.D., F.F.; Literature Search - D.R.D., P.E.; Writing Manuscript - D.R.D., F.F.; Critical Review - D.R.D., F.F., P.G.

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