

## Mesenchymal stem cells in osteoarticular pediatric diseases: an update

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Cellular therapy has gained an increasing popularity in recent years. Mesenchymal stem cells (MSCs) have the potential to differentiate into bone, cartilage, or fat tissue. In recent studies, these cells have also shown healing capability by improving angiogenesis and preventing fibrosis, which could have a role in tissue repair and tissue regeneration. Preclinical and clinical orthopedic studies conducted in the adult population support the use of MSCs for bone-healing problems, early stages of osteonecrosis, and local bone defects. Only a few published studies support the use of MSCs in pediatric osteoarticular disorders, probably due to the unknown long-term results of cellular therapy. The purpose of this review is to explain the mechanism by which MSCs could exhibit a therapeutic role in pediatric osteoarticular disorders.

Several pediatric osteoarticular disorders can have lifelong consequences on the patient. Scientists and physicians believe that cellular therapy with multipotent mesenchymal stem cells (MSCs) have the potential to cure these disorders, or at least significantly alleviate their symptoms and sequelae.

MSCs are adult stem cells exhibiting functional properties that have opened the way for cell-based clinical therapies. Primarily, their capacity of multilineage differentiation has been explored in a number of strategies for skeletal tissue regeneration. More recently, it has been reported that MSCs exhibit immunosuppressive and healing capacities, by improving angiogenesis and preventing apoptosis and/or fibrosis through the secretion of paracrine mediators.

Several preclinical and clinical studies support the use of MSCs in adult osteoarticular disorders such as nonunion of fractures, simple bone cyst (SBC), and osteonecrosis. However, only a limited number of case reports (1–5) have demonstrated their possible clinical applications in pediatric osteoarticular disorders.

We should identify pediatric disorders based on the availability of treatment options. In patients with no current available treatment, any promising therapy could be considered as a valid treatment. Cell-based therapy is a growing field,

with many ongoing trials for the treatment of different diseases, which might be considered as a new therapeutic option specifically for some pediatric pathologies lacking any known conventional treatments.

Before adopting a specific indication of the use of MSCs in the pediatric population, it is essential that this cell-based strategy first shows adequate and promising results in animal models. However, diseases like juvenile arthritis are specific for children and distinct from adult rheumatoid arthritis, so dedicated trials are warranted for these applications. Second, all safety concerns must be evaluated for its clinical applications, and, finally, it should prove the desired results over time with minimal adverse effects for the patients.

Based on the few published reports, we believe that these conditions are not yet met, as concerns have been raised regarding the migration, biodistribution, survival, and safety of MSCs following systemic infusion or local implantation. As we mentioned earlier, if no current treatment is available or severe long-life consequences can exist, solid data of potential benefits of cell therapy far overcome the risk of their clinical use in children.

What is required at this level is a thorough review of the uses and limitations of MSCs. This will contribute to a better evaluation of the risk–benefit value and a clearer understanding of the indication of MSCs for pediatric orthopedic conditions.

### PROCEDURES AVAILABLE FOR MSC-BASED THERAPY

When considering cell therapy as a new treatment option in pediatric osteoarticular disorders, it is important to analyze their potential role in local and/or systemic conditions. If a local effect is desired, an on-site injection of MSCs (with or without a scaffold) could be considered, for example, in the case of a nonunion after a long-bone fracture. On the other hand, if the intervention aims to correct a systemic disorder, two options are available. The first option would be to replace the host MSCs with unmanipulated allogeneic MSCs. This could attenuate or possibly correct genetic disorders of bone or cartilage. It is important to take into consideration that in order to allow a new population of unaltered MSCs to grow, it is necessary to create the appropriate space by partially ablating the patient's own bone marrow. This procedure is not risk-free and comes with a high potential of fatal procedures. The second

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option is to use MSCs as a modulator of autoimmune chronic diseases with joints and bone clinical symptoms. The systemic interaction between MSCs and lymphocytes could result in a systemic modulation of the immune reaction. In this case, the indication of MSCs is not expected to cure but reduce the symptoms and complications.

#### Route of Administration

**Intravascular infusion.** Most of the information regarding cell therapy and osteoarticular diseases in children, especially inborn errors, comes from bone marrow transplantation (BMT), a treatment option for patients with leukemia and lymphoma. Intravascular allogeneic infusion of bone marrow for hematological disease has been successfully performed for more than 30 y, and, more recently, transplantation of umbilical cord blood stem cells has been successful (6–8).

Owen and Friedenstein first identified stem cells in the stromal tissue of bone marrow, which were capable of differentiating into different cell types, including osteogenic cells (9). In 1995, Caplan (10) identified two different stem cell lineages in the bone marrow: hematological and mesenchymal. MSCs could treat inborn bone metabolism errors by replacing the host's ill bone marrow MSCs with healthy ones from the donor. This would lead to a gradual replenishment of normal cells, an attenuation of symptoms, and eventually the cure of metabolic errors. The author theorized that osteogenesis imperfecta (OI), a genetic disease in which osteoblasts are unable to produce normal type I collagen, could be successfully treated by MSC transplantation. Horwitz *et al.* clinically proved this theory, publishing their results in 2001 (2).

Even though these results are promising, it is important to consider the risks involved in these interventions. The procedures are performed in “unhealthy” patients and require a partial toxic ablation of the bone marrow, which could result in complications that are life-threatening for the patient.

#### Local injection

**Percutaneous injection.** Bone marrow aspiration, without other manipulation, can be percutaneously injected at the affected site. Combining non-concentrated bone marrow with a scaffold in surgical procedures is reportedly the most used technique in clinical practice in adults patients. The use of concentrated bone marrow injection has also been described. This procedure requires centrifugation of the bone marrow, which results in a concentrated mononuclear cell sample that can be injected into the affected site (11).

By *in vitro* expansion, a larger number of isolated MSCs could be obtained for the treatment. These isolated and expanded MSCs can be mixed in the operation room with an osteoconductive scaffold and implanted in the affected site (12,13).

Finally, *in vitro* expansion directly within the scaffold has also been described as an alternative method for culturing MSCs in conditions that mimic their natural environment. This requires days or weeks for cell differentiation and colonization of the scaffold. We could not find any reports of current use of this technique.

**Intrarticular injection.** In theory, the intrarticular injection of MSCs could be used to treat focal cartilage lesions or degenerative joint diseases. Extensive preclinical investigations support the use of MSCs in the treatment of cartilage lesions in adults; however, these results lack a direct comparison with conventional therapies such as autologous chondrocyte implantation or microfractures (14). Wakitani *et al.* (5), have recently published successful results, with no reported complication, of a 6-y follow-up study in four children injected intrarticularly (in the elbow) with autologous bone marrow-derived MSCs. Intrarticular injections of MSCs have been widely studied in animal models with osteoarthritis (OA); however, there are only preliminary data available for humans. Recently, an article was published that included four patients between 54 and 65 y of age, with moderate-to-severe knee OA. Each patient received an intrarticular injection of  $10^7$  bone marrow-derived autologous MSCs. The authors reported an improvement in walking time, reduction of walking pain in three of the four treated patients. No side effects were reported after a 1-y follow-up (15).

#### Source and Characterization of MSCs

Besides the applications discussed above, MSCs may have therapeutic value in other clinical applications by virtue of their capacity to limit scar formation through antifibrotic properties, to prevent apoptosis, to stimulate regeneration of endogenous cells, and to suppress the host immune response. Such immunosuppressive effect has been shown to occur mainly through the secretion of soluble factors by MSCs. Among the possible mediators identified, indoleamine 2,3-dioxygenase, inducible nitric oxide synthase, heme oxygenase-1 as well as the secretion of human leukocyte antigen-G, transforming growth factor- $\beta$ , interleukin-6, and prostaglandin-E2 have been postulated to play a role, but the cells need to be licensed through interferon- $\gamma$ . These mechanisms result in the inhibition of the proliferation of CD4<sup>+</sup> and CD8<sup>+</sup> T cells, B lymphocytes, and natural killer cells; this has been demonstrated not only *in vitro* but also *in vivo* in a number of experimental models. Stem cells have been isolated from many different adult tissues including cord blood, peripheral blood, bone marrow, spleen, liver, kidney, thymus, dental pulp, periosteum, skin retina, adipose tissue, and synovial tissue (16). The preference of stem cell source is determined by ease of access to tissue source, population of stem cells, and information on a particular cell system. Bone marrow-derived MSCs have been widely studied, and there is a wealth of information in the literature concerning them. They represent only 0.001–0.01% of the total nucleated cells in bone marrow aspirate (17). Bone marrow aspirate of 30 ml only contains  $\sim 1 \times 10^5$  cells (18). Therefore, culture and expansion *in vitro* are needed. MSCs can be obtained from the tissue of the patient or the donor. In case of autotransfusion, MSCs can be obtained from several sources. The most reliable and reproducible harvest processes are bone marrow aspiration and liposuction. Allogeneic MSCs can also be harvested from different tissues, including human embryonic tissue; however, due to ethical concerns, adipose and placenta have risen as the

most used source of these cells. Adipose-derived mesenchymal cells share many properties with the MSCs harvested from bone marrow; however, collection of adipose-derived mesenchymal cells requires a less invasive procedure because the cells can be easily harvested through the liposuction of subcutaneous abdominal adipose tissue and then expanded *in vitro*. Even though this procedure holds an intrinsic risk of infection, adipose tissue represents an immediately available source of large number MSCs.

## CLINICAL APPLICATION IN PEDIATRIC OSTEOARTICULAR DISEASES

For this review, we propose the classification of pediatric osteoarticular disorders into two groups according to susceptibility to MSC treatment: systemic and local disorders. This relates to the administration options available for MSC therapy. This review will focus on the use of MSCs in OI, juvenile idiopathic arthritis for systemically affected children, osteonecrosis of the hip, and SBC for locally affected children.

### OI

OI is a rare genetic disorder caused by a mutation in the *COL1A1* and *COL1A2* genes in chromosomes 17 and 7. This mutation results in the production of the osteoblasts of a qualitatively and quantitatively defective type I collagen (19). The prevalence of this disease is 16.3 per one million habitants (20).

OI was initially thought to be a type of rickets until 1906, when Looser first classified OI as “OI congenital” and “OI tarda.” The first was characterized by multiple fractures already present at birth, and the second by multiple fractures later on. The current classifications are based on genetically specific disorders (21).

The most prevalent clinical manifestations of OI are short height, deformations of the spine and long bones, cifo scoliosis, and pectum escavatum. Other conditions such as blue sclera, hearing loss, general ligament laxity, and increased vulnerability of the skin may also be present.

Multiple fractures can occur during childbirth. The skull and ribs are soft and not completely ossified; this increases the risk of death as a result of intracranial bleeding and lung collapse.

There should be antenatal suspicion of OI if sonographically intrauterine fractures are observed. Confirmation of OI is performed through skin biopsy or DNA analysis.

A multidisciplinary approach is needed in which prevention of domestic trauma plays an elemental role. Pamidronate should also be considered, as it has shown reduction of new fractures (22). If the patient suffers a fracture, orthopedist or surgery may be needed.

In 1999, Horwitz *et al.* (1), despite previous reports of unsuccessful engraftment of MSCs after BMT (23), performed allogeneic BMT in three children suffering from severe OI, with standard marrow-ablative chemotherapy regimen followed by marrow infusion from either completely or partially human leukocyte antigen-matched siblings.

Two of the three patients had complete hematopoietic engraftment, and the third had mixed chimerism. Histological

evidence of new healthy bone formation and clinical evidence of reduced incidence of fracture did not correlate with later osteoblast culture-expanded studies from the patients' iliac bone. No more than 2% of donor cells were found in two of the three patients, and no donor cell could be grown in the third child.

A second report by the same authors (2) showed that two of the five OI patients treated failed to exhibit engraftment of the donor MSCs, and therefore were excluded from the study. The three remaining patients showed an impressive improvement, with a reduction of bone fracture incidence, increased bone mass, and stature growth. At the 6-month follow-up, the children showed a plateau in their growth rate but a constant increase in bone mass. In their third study, Horwitz *et al.* (3) recruited six additional children who had received BMT and MSC transfusions, the clinical results were satisfactory, and all transplants, in contrast with the previous studies, were engrafted.

Le Blanc *et al.* (4) reported a case of a female fetus with multiple intrauterine fractures, genetically diagnosed with severe OI. They performed an allogeneic human leukocyte antigen-mismatched male fetal MSC transfusion at the 32nd week of gestation. At 9 months of age, bone biopsy revealed the presence and persistence of 0.3% of XY-positive allogeneic cells. During the first 2 y of life, three fractures were seen, with normal psychomotor development and growth.

Reports on allogeneic BMT have shown a partial engraftment of functional mesenchymal progenitor cells. These results could represent a feasible strategy for the treatment of OI and other forms of chondrodysplasia.

### Juvenile Idiopathic Arthritis

As mentioned previously, MSCs play a role in the modulation of host immune response by inhibiting the proliferation of T lymphocytes. Therefore, the use of MSCs seems logical in diseases in which there is an increased T-cell response such as inflammatory arthritis. Improvement in clinical and biological scores have been reported in studies using the experimental mouse with collagen-induced arthritis after the injection of MSCs derived from bone marrow or adipose tissue (24,25). Nevertheless, there are contradictory results including the absence of therapeutic benefit after MSC infusion and even exacerbation of arthritis (26). Recently, our group has shown that interleukin-6-dependent prostaglandin-E2 secretion by primary murine MSCs inhibits local inflammation in experimental arthritis in a time-dependent fashion, which suggests that the discrepancy between studies may be related to the time of injection and/or the immune status of the animals at that time (27).

The mechanisms underlying the therapeutic effect needs to be further explored because no convincing increase of regulatory T cells has been observed *in vivo* (28).

Despite the immunological and clinical differences between juvenile idiopathic arthritis and rheumatoid arthritis, the autoimmune pathways seem to be similar. More clinical evidence is required to position MSCs as an effective and reliable cellular

therapy for rheumatoid arthritis, before we can extrapolate these therapeutical effects into pediatric patients with juvenile idiopathic arthritis. Nevertheless, in patients who do not respond to the usual therapies, MSCs might be a treatment option, but no expanded recommendations can be made on the basis of current findings in the literature.

### SBC

SBCs, also known as unicameral bone cysts, are fluid-filled cavities that typically appear in the long bones of growing children between the ages of 10 and 15 y. Although rare, they can also affect older children or adults. SBCs are the most common benign bone lesions, representing 3% of all primary bone lesions in children. More than 90% of SBCs are located at the proximal end of the femur and humerus (29).

The etiology of SBCs is uncertain. Several theories have been proposed to explain the pathogenesis of SBCs. Some believe that it may be due to resorptive properties of the lining or fluid of the cyst (30,31), whereas others believe that it is due to venous congestion (32).

Most children with SBC are asymptomatic, and the lesion is found on unrelated routine radiographs or during consultation for local pain in the presence of a pathologic fracture. These fractures occur after low-energy traumas or even after normal sports activities. In rare cases, children may complain about local pain after weight-bearing activities (33).

The diagnosis is confirmed by a simple radiographic study. In some cases, a magnetic resonance imaging may be needed to rule out common differential diagnosis such as aneurysmatic bone cyst. The natural history of SBCs is favorable, with healing expected upon reaching skeletal maturity. Nevertheless, it is mandatory to evaluate the likelihood of a fracture without treatment in each case (34). The main objective of the treatment is to prevent pathological fractures or their recurrence.

Several treatment options have been proposed, including, open curettage and bone grafting, percutaneous injection of steroid, bone substitutes, bone marrow, calcium sulfate pellets, percutaneous decompression of the cyst, and mechanical support with internal fixation or mixture of methods to treat SBCs at multiple levels.

Open curettage and bone grafting have shown good results (33,35). Scaglietti *et al.* was the first group to describe an improvement of healing rates after local steroid injection (36). Several studies have compared percutaneous steroid injection with percutaneous native bone marrow, but no improvements were seen after the inclusion of bone marrow (37–39). When native or concentrate bone marrow was mixed with allogeneic demineralized bone, a 56–89% healing rate was described (40,41). It is important to note that according to our analysis of the available literature, steroids are the only evidence-based treatment for SBCs at the moment.

Further basic science is needed to understand the pathobiology of SBCs and to develop future biologic solutions. Multimodal treatment strategies, such as opening the medullary canal and disrupting the cyst wall, filling the defect with a bone substitute, and possible biologic treatment of the cyst

membrane, may be the best approach (42). There are no data available on SBCs treated with local expanded MSCs. However, once there is mechanical disruption of the wall, MSCs in combination with structural demineralized bone or bone substitutes could contribute to the healing process of the defect.

### Osteonecrosis of the Femoral Head

Similar to other terminal vascular supply bones, such as the scaphoid and the talus, the femoral head is under the risk of suffering complications due to its impaired vascular supply.

Femoral head osteonecrosis is a condition in which there is subchondral bone death due to an insufficient vascular supply. Once the subchondral bone is affected, the cartilage loses its mechanical support and collapses.

Several risk factors have been described including traumatic lesions (e.g., displaced femoral neck fracture, hip dislocation), chronic uses of steroids, coagulopathies, and alcoholism.

Legg–Calve–Perthes disease is an idiopathic osteonecrosis of the femoral hip in children. This disease has been well defined and is the most common cause of osteonecrosis of the femoral head in children. It is usually acute and self-limited. Long-term complications are uncommon due to the high remodeling capabilities of children. Nevertheless, the risk of complications increases according to the patient's age (43). Some authors state that if it occurs in children >12 y old, its course is more similar to adult osteonecrosis than it is to Legg–Calve–Perthes disease. Juvenile osteonecrosis, although rare, has a higher incidence of long-term complications (44,45).

Other related causes of osteonecrosis in children are hematopoietic neoplasm, chemotherapy, high doses of steroid therapies and hemoglobinopathies such as sickle cell disease. Socie *et al.* (46) found that 5 out of 1,264 patients at an age <16 y were diagnosed with osteonecrosis after receiving BMT as a treatment for different types of hematopoietic malignancies.

The factors influencing the progression of the disease from the appearance of a necrotic lesion to a subchondral fracture, femoral head collapse, and early joint replacement are not fully understood, but the size and stage of osteonecrosis have been shown to predict clinical outcomes (47–49).

Classification is mostly based on radiographic findings. The presence and extension of joint collapses are the most important risk factors for the development of OA.

Initially, patients do not feel any pain but refer to local discomfort; as time passes by, local pain can appear that usually relates to the collapse of the joint.

Current treatments aim at stopping the collapse of the joint and, therefore, preventing an early onset of OA in the hip.

Once the vascular supply is affected, a local inflammation process begins, and it produces edema inside the femoral head, which furthermore impairs the vascular supply. Decompression of the femoral head can be achieved by surgically removing a core of bone. This reduces intramedullary pressure and thereby prevents further vascular impingement and allows for new bone formation (50).

This procedure has been widely accepted as a procedure for the treatment of hip osteonecrosis in early stages (51,52). The clinical outcomes of core decompression had not always been satisfactory because of incomplete reconstruction of the subchondral necrotic area due to inadequate biological support (53). Biologic augmentation after core decompression has been attempted with demineralized bone matrix and bone morphogenetic protein (54). However, the relative insufficiency of osteoprogenitor cells in the proximal femur of osteonecrotic hips (55) has led to the use of bone marrow MSCs after core decompression. Several reports have shown the successful application of bone marrow MSCs through a single hole drilled into the necrotic area in patients with atraumatic osteonecrosis (56,57). Recently, Gangji *et al.* (48) published a 5-y follow-up study in which they compared core decompression with core decompression and autologous bone marrow cell implantation in patients with pre-collapse atraumatic osteonecrosis. They found that at 60 months, 8 of the 11 hips in the control group had deteriorated to the fractural stage, whereas only 3 of the 13 hips in the bone marrow graft group had progressed to that stage. Another publication compared multiple small-drill core decompression with and without the injection of autologous concentrated bone marrow. The authors found a statistically significant improvement of clinical scores and mean hip survival outcomes in the bone marrow group at the 2-y follow-up, and they emphasized the importance of achieving  $5 \times 10^7$  osteoprogenitor cells in the final concentration in order to effectively regenerate the necrotic area (49).

A major concern in children after core decompression is the premature halt in growth of the proximal femur due to growth plate damage.

Because Legg–Calve–Perthes typically evolves with *ad integrum* femoral head remodeling and children under 12 y old with other causes of osteonecrosis of the femoral head achieve favorable outcomes after nonsurgical treatment; general experience with core decompression in pediatric ages is rare (58).

In an immature animal model, 12 piglets with induced femoral head osteonecrosis were treated with three small-drill core decompressions. They found that this procedure did not produce bony physeal bars in the short term; however, multiple drilling alone did not seem to prevent femoral head deformity or promote new bone formation (59). These findings support the fact that osteoprogenitor cells are needed even in young patients. Recently, there has been a described migration of intravenous transplantation bone marrow MSCs to necrotic femoral head in an animal model (60).

To our knowledge, there is no published information on the use of a local injection of bone marrow or expanded MSCs *in vitro* after core decompression in children. Satisfactory results obtained after combining small-drill core decompression and concentrated autologous bone marrow MSCs in adults, in addition to the serious consequences of early-onset OA in children, will push clinical studies to confirm safety and efficacy of MSC therapy in pediatric osteonecrosis.

## LIMITATIONS AND FUTURE PERSPECTIVES

Currently, there are several limitations in the clinical application of MSCs. Perhaps the most important limitation is the unknown long-term effects. Even though more than 2,000 people have been treated so far with *in vitro* expanded MSCs and no malignant behavior has been reported yet (61), concerns related to genetic instabilities during cellular amplification still exist (62).

Another major limitation of MSCs is engraftment capacity. In short-term follow-up, only a small percentage of MSCs are detected in the transplanted area, which creates controversy about their long-term clinical effects. Dominici *et al.* (63) found a lack of durable donor-derived osteopoiesis after intravenous BMT, which may reflect an intrinsic genetic program or exogenous environmental signaling that suppresses the differentiation capacity of the donor stem cells. Interesting preclinical studies have shown better engraftment results after injecting cells directly into the bone marrow cavity as compared with conventional intravenous BMT. Finally, highly standardized processes of harvest, production, and transportation of MSCs make this therapy inaccessible for some hospitals because specialized centers are required for expanding stem cells.

## CONCLUSION

MSCs are no longer second-class citizens but first-line players. They show major regulatory properties in skeletal tissues controlling inflammation, immune response, and fibrosis and enhance tissue regeneration.

Promising preclinical data support the use of MSCs in adult patients; these results have led to an increasing number of studies reporting their use in clinical trials in the adult population.

As we learn more about the behavior of MSCs in adult patients, we should carefully begin to transfer the potential benefits of cell therapy to pediatric patients.

We believe that the exponential growth of knowledge in the adult population should lead to new data on the use of MSCs, which could result in treatments for pediatric diseases that currently do not have a cure.

Pediatric osteoarticular diseases have an enormous potential to be treated by MSCs. Severe inborn errors of bone metabolism, autoimmune diseases without clinical response to conventional treatment, and local bone or cartilage defects might be successfully treated by MSCs. This could be achieved by curing inborn metabolic errors, modulating autoimmune diseases, or locally regenerating bone or cartilage defects, among other benefits.

Limitations to the use of MSCs should not be considered as a major setback but rather hurdles that need to be overcome through gathering more knowledge with regard to cell therapy.

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