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Treatment of Hemophilic Ankle Arthropathy with One-Step Arthroscopic Bone Marrow–Derived Cells Transplantation

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Abstract

Objective. Ankle arthropathy is a frequent and invalidating manifestation of hemophilia. Arthrodesis is the gold standard surgical procedure in end-stage disease, with many drawbacks in young patients. Recent literature has shown increase interest in regenerative procedures in hemophilic arthropathy, which may be desirable to delay or even avoid arthrodesis. The aim of this article is to present five cases of osteochondral lesions in ankle hemophilic arthropathy treated with a regenerative procedure: bone marrow–derived cells transplantation (BMDCT). *Design*. We report five hemophilic patients (four cases with hemophilia type A; one case with hemophilia type B) who have undergone BMDCT treatment, synovectomy, and arthroscopic debridement, with the use of autologous platelet-rich fibrin, to treat osteochondral lesions in hemophilic ankle arthropathy. The patients, included within this retrospective study, were clinically and radiologically evaluated with serial follow-ups, using the American Orthopaedic Foot and Ankle Society (AOFAS) scores, radiographs, and magnetic resonance imaging (MRI). *Results*. The mean preoperative AOFAS score was 35. After a mean follow-up of 2 years, the mean postoperative AOFAS score was 81, which included three patients returning back to sporting activities. The MRI Mocart score demonstrated signs of regeneration of chondral and bony tissue. No progression of joint degeneration was shown radiographically. *Conclusion*. BMDCT is a promising regenerative treatment for osteochondral lesions in mild ankle hemophilic arthropathy, which may be useful to delay or even avoid ankle arthrodesis. Nevertheless, longer follow-ups and a larger case series are required.

Keywords

ankle, hemophilia, regenerative technique, one-step

Introduction

Ankle arthropathy is one of the most frequent clinical manifestations of hemophilia, particularly during the second decade of life.[1] Recurrent hemarthroses trigger a general state of uncontrollable inflammation and neo-angiogenesis because of the deposits of hemosiderin. A hypoxic environment may ensue after bleeds, which may trigger a rapid joint degeneration. Arthroscopic synovectomy and debridement have shown good results, mostly when applied in young patients with mild signs of degeneration; nevertheless, these are not modifying disease procedures.[2,3] However, end-stage arthropathy is treated with arthrodesis, or arthroplasty. These treatment methods have demonstrated very satisfying outcomes but may also lead to additional problems.[1,4-6] Midfoot and hindfoot joint degeneration may occur precociously because of excessive forces imposed by ankle arthrodesis; arthroplasty may lead to early revision surgery in young patients.[4-6]

As a result of the inflammation and advanced degenerative changes of hemophilic joints, regenerative techniques have not been widely applied and remain a novel treatment for early focal lesions.[7] Nevertheless, recent literature about regenerative techniques in advanced degenerative states has shown that mesenchymal stem cells may play a role in the regulation of inflammation and neo-angiogenesis, potentially allowing osteochondral regeneration.[8] On the basis of recent studies demonstrating good results in the treatment of osteochondral lesions of the talus,[9] a "one-step" arthroscopic

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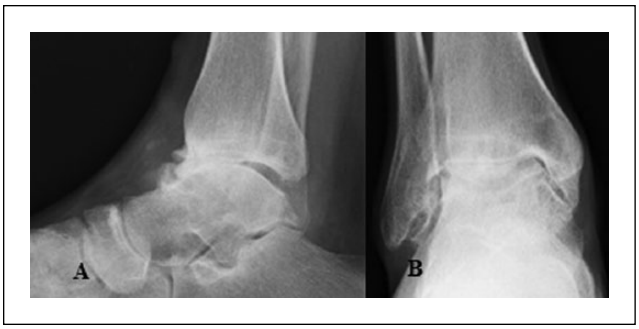

Figure 1. Preoperative X-rays of patient 2, lateral (**A**) and anterior-posterior (**B**) radiographs: ankle osteoarthritis with narrowing of the joint space and osteophytes (II stage according Van Dijk).

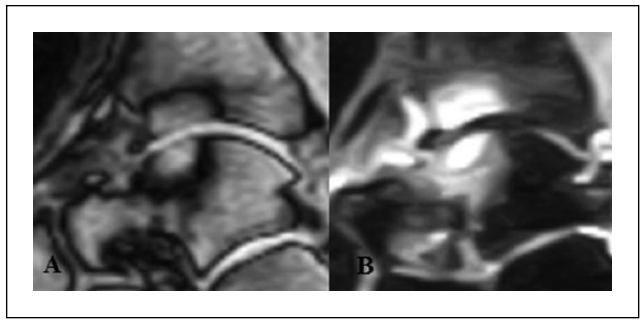

Figure 2. Preoperative MRI T1-sagittal (**A**) and STIR (**B**) scans of patient 2: the images show a large talar osteochondral lesion and a smaller lesion of the tibial plafond.

regenerative technique using bone marrow–derived cells (BMDC) with autologous growth factors embedded within a collagen scaffold was adopted in five hemophilic patients in order to delay or avoid end-stage treatments, while attempting to regenerate osteochondral tissue and reduce the catabolic joint environment.

Methods

From 2010 to 2013, five hemophilic patients were treated with debridement and synovectomy and BMDC transplantation on a collagen scaffold for osteochondral defects in a mild (three cases) and moderate (two cases) ankle arthropathy. The hemophilic patients (mean age = 33 ± 6.78 years; range = 25-41) were subclassified as type A in four cases and as type B in one case (patient 3). Two patients were classified with mild hemophilia (more than 6% of clotting factor) and three patients as moderate hemophilia (between 2% and 6%). All patients were clinically assessed for pain (mean visual analog scale [VAS] = 4.8 ± 0.84), loss of range of motion (ROM; mean value = 12 ± 2.74°), and sport withdrawal (four patients out of five played sports before the injury). The mean preoperative American Orthopaedic Foot and Ankle Society (AOFAS) score was 34.8 ± 4.55 (range = 27-38). All patients were preoperatively evaluated with radiographs and magnetic resonance imaging (MRI). The osteochondral defects of the talar dome were sized 152 ± 37 mm^2 (average value; range = 100-200 mm^2). The depth was less than 5 mm deep (Giannini's classification for osteochondral lesions of the talus: II).[9] Subchondral cysts, osteophytes, and fibrous impingement were present in all the cases (Van Dijk classification for ankle arthritis: I). Two patients showed narrowing of the joint space (II stage according Van Djik)[10] (**Figs. 1** and **2**). The patients were clinically and radiologically evaluated at serial follow-ups: at 6, 12, 24, and 36 months. The bone marrow cell concentration at the time of harvesting was also evaluated and correlated (**Table 1**).

Preoperative Care

BMDC transplantation included the production and application of platelet gel (platelet-rich fibrin [PRF]) in order to apply growth factors and a fibrin clot to improve the implantation of the biomaterial and promote the regeneration. Clotting factors were infused 30 minutes before drawing the venous blood: ADVATE (Baxter, Westlake Village, CA) for four patients, AIMAFIX (Kendrion, Lucca, Italy) for patient 3, type B hemophilia. A total of 120 mL of venous blood was drawn yielding 6 mL of PRF utilizing the Vivostat system (Vivolution, Birkeroed, Denmark). The first step of the Vivostat procedure requires 60 mL of plasma and batroxobin to be warmed for 10 minutes at 37°C. This allows fibrinogen to release fibrin peptide A. The resultant fibrin I polymer becomes soluble in acid without the activation of Factor XIII, which was then isolated by centrifugation and added to sodium acetate buffer at the concentration of 0.2 mole/L at pH 4. In presence of Ca^{2+} at neutral pH (later applied in the operating room), endogenous prothrombin was converted to thrombin, cleaving the fibrinopeptide B from fibrin I to fibrin II. This yields fibrin-platelet concentrations that are 7 to 10 times greater than the baseline values. So PRF is a product capable of providing both hemostasis and tissue regeneration.

Surgical Procedure

To maximize the infusion of clotting factors, in the same day the bone marrow cell aspiration was performed, with the patient under spinal anesthesia in prone decubitus position. The spongy bone of the posterior iliac crest was perforated 3 cm deep with a marrow needle (size 11, G 9, 100 mm). A 20-mL syringe, internally coated with calcium-heparin solution, was filled with 5 mL of bone marrow cells. The procedure was repeated several times using the same skin opening in order to collect 5 mL of bone marrow at each aspiration. Small amounts were taken from different depths and areas to maximize the harvest and reduce the blood dilution. A total volume of 60 mL of bone marrow aspirate

Table 1. Preoperative Assessment and Postoperative Outcomes of Every Patient Included in the Study.

Patient	Hemophilia Type	Age at Surgery	Arthritis Degree	Lesion Dimension (mm^2)	Clotting Factor %	Cells Concentration (×1,000 cell/μL)	BMI	Smoke	AOFAS Preoperative	VAS Preoperative	ROM Preoperative	AOFAS 6 Months	AOFAS 12 Months	AOFAS 24 Months	AOFAS 36 Months	VAS at Final FU	ROM at Final FU	Maximum FU
1	A	27	1	150.00	3.8	139	22.7	No	35	4	10	78	82	90	87	2	25	36
2	A	37	2	140.00	10	96	20.8	No	36	5	15	64	74	74	72	3	10	36
3	B	41	1	100.00	3	128	24.3	No	38	6	10	72	80	84	84	2	15	36
4	A	35	1	200.00	8.5	124	23.4	Yes	27	5	10	64	80	NA	NA	2	20	12
5	A	25	2	170.00	2.1	134	21.6	No	38	4	15	78	84	NA	NA	2	20	12
Mean value		33		152.00		124.2	22.56		34.8	4.8	12	71.20	80	82.67	81	2.2	18	26.4
Standard deviation		6.78		37.01		16.77	1.39		4.55	0.84	2.74	7.01	3.74	8.08	7.94	0.45	5.70	13.15

BMI = body mass index; AOFAS = American Orthopaedic Foot and Ankle Society; VAS = visual analog scale; ROM = range of motion; FU = follow-up.

was collected. Six milliliters of concentrated nucleated cells (stem cells, monocytes, lymphocytes, and bone marrow resident cells) were obtained using the IORG 1 kit (Novagenit, Mezzolombardo, Italy). The patient was then positioned supine and the tourniquet was applied and inflated to 280 mmHg to perform a standard arthroscopy. Fibrous and osseous sites of impingements were removed, and synovectomy was performed. The damaged osteochondral tissue was removed down to healthy bone. The debrided defect was measured with a calibrated probe. At the same time, the collagen membrane was sized and shaped based on the lesion's dimensions and loaded with 2 mL of bone marrow concentrate. After cell absorption, an additional 1 mL of PRF was added. The biomaterial was positioned in the defect using a dedicated cannula at the closest portal to the defect. A flat probe was then utilized to position the biomaterial and additional PRF was dispersed into the lesion. Multiple cycles of dorsi-flexion and plantar-flexion were performed to insure the stability of the implant, before suturing the portals.

Postoperative Care

The replacement therapy (ADVATE and AIMAFIX) was continued for 1 week after surgery with individual parameters for every patient. Mechanical deep vein thrombosis prophylaxis was also applied. Every patient pursued the same regimen of parenteral therapy, as adopted in the preoperative setting.

Continuous passive motion was initiated on the first postoperative day. Following discharge (4 days after surgery), the range of motion was increased gradually as tolerated, and after 6 weeks, ambulation with crutches was permitted. Progressive weight bearing as tolerated was then permitted after 10 weeks. Low-impact sports, like swimming and cycling, were allowed only after 4 months. High-impact sports were not permitted before 1 year.

Ethical Approval and Informed Consent

The subjects provided informed consent, and the study was approved by the local ethical committee. The study was conducted with the principles of the Declaration of Helsinki (World Medical Association).

Results

One unique complication occurred. Patient 1 was diagnosed with sacral, gluteal, and ankle hematomas, which also involved the surgical sites. This required an additional week of hospitalization for complete recovery. This complication was likely due to an incomplete adherence to the prescribed postoperative therapy regimen.

The clinical outcomes at a mean of 2-year follow-up (26.4 ± 13.15 months) demonstrated pain reduction and range of motion ROM improvement. The mean postoperative AOFAS score was 71.20 ± 7.01 at 6 months, improving to 80 ± 3.74 at 1 year. The mean AOFAS score was 81 ± 7.94 points at a mean follow-up of 2 years. The mean final VAS was 2.2 ± 0.44 (preoperative value = 4.8 ± 0.84); the range of motion achieved was 18 ± 5.70° (preoperative value = 12 ± 2.74°). Three out of the four sports patients were able to resume sporting activities. One patient was able to participate in high-impact sporting activities with no reported pain. The other two patients were only able to participate in low-impact activities with no symptoms. No correlation between replacement therapy and clinical parameters could be found.

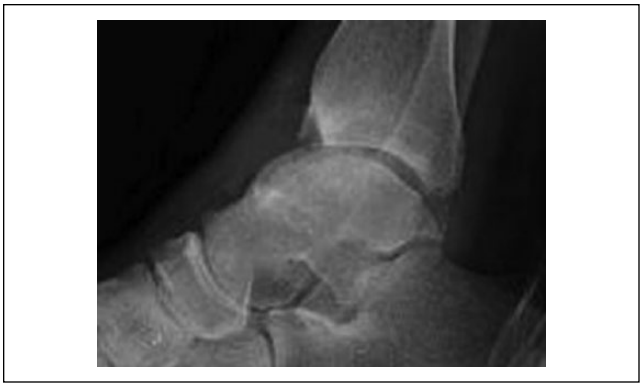

Figure 3. Lateral radiograph of patient 2: the image shows signs of osteophytes removal after 1 year.

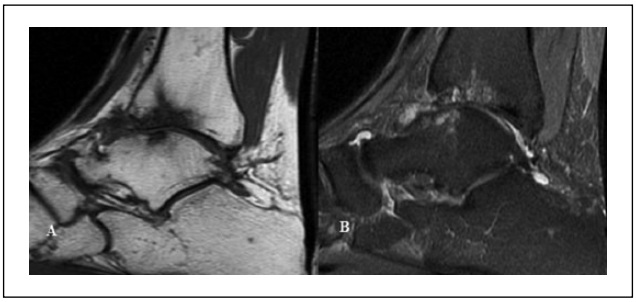

Figure 4. MRI T1-sagittal (**A**) and STIR (**B**) scans of patient 2 after 2 years from surgery: the images show a complete filling of the talar defect. The tibial defect is only partially filled.

Radiographs showed no progression of the joint degeneration (osteophytes and narrowing of the joint space were evaluated) in all the patients at the final follow-up (**Fig. 3**). Moreover, the MRI results showed signs of regeneration of the chondral and bony tissues. At 2-year follow-up, Mocart score evaluation demonstrated complete filling of the talar defect in all patients. The borders appeared completely integrated with the adjacent cartilage in three cases and partially in two patients. Inhomogeneous, hyperintense (DPFSE fat sat sequences) repair tissue was recorded in every case, while subchondral bone edema or cyst was present in three cases (**Fig. 4** and **Table 2**). No correlation between clinical data and Mocart score was found.

Table 2. Mocart Parameters.

Patient	Filling of the Defect	Integration With the Border	Structure of the Repair Tissue	Signal Characteristics of Repair Tissue	Status of Subchondral Lamina	Integrity of Subchondral Bone	Presence of Complications
1	Complete	Incomplete	Inhomogeneous	Hyperintensity	Damaged	Intact	No
2	Complete	Complete	Inhomogeneous	Hyperintensity	Damaged	Intact	No
3	Complete	Complete	Inhomogeneous	Hyperintensity	Damaged	Disrupted	No
4	Complete	Incomplete	Inhomogeneous	Hyperintensity	Damaged	Disrupted	No
5	Complete	Complete	Inhomogeneous	Hyperintensity	Damaged	Disrupted	No

Discussion

BMDC transplantation in hemophilia has been suggested by Ebihara *et al*.;[11] good results were shown by Wakitani *et al*. in osteoarthritic knees.[12] Ebihara *et al*. investigated the feasibility of this procedure, concluding that hemophilic BMDCs are viable and suitable for chondrogenic differentiation.[11] The application of BMDC transplantation in this series of osteochondral lesions in mild and moderate ankle hemophilic arthropathy achieved good results, which were not inferior to arthroscopic arthrodesis outcomes in ankle osteoarthritis.[13] Moreover, three patients could resume sporting activity, and the youngest of the series (patient 1) could play high-impact sports. One patient (patient 3, type B hemophilia and moderate arthropathy) recorded the lowest AOFAS score of 72 and demonstrated recurrent pain and disability. He faced no postoperative complications and performed the prescribed rehabilitation. Radiographs confirmed that further degeneration did not occur. MRI showed remodeling tissue with encouraging signs of regeneration. Nevertheless, the MRI findings of subchondral bone edema may explain these clinical results. It is worth noting that this patient was the only one of the case series with type B hemophilia, and he was the oldest of the series. Moreover, this patient had the lowest bone marrow cell concentration (96×10^3 cells/μL; 5.6 times more than the basal values). The entire clinical trend demonstrated correlation with the bone marrow cell concentrations, suggesting a possible direct relationship. Nevertheless, in all the five cases, the suggested number of bone marrow cells useful to repair osteochondral defects in degenerated joints (10 million) was achieved.[11] Larger case series are required to evaluate the reproducibility of this correlation.

This case series in mild/moderate ankle hemophilic arthropathy reported a one-step procedure, with no cellular culture or enrichment. In the current literature, the advised procedure for BMDC transplantation in degenerated joints is a two-step approach, due to the probable higher resistance of cultured cells to an inflammatory environment.[8,11,12] Nevertheless, despite the inflammatory ankle environment of hemophilic arthropathy, the outcomes of the "one-step" technique have been shown to be promising. If the clinical and radiological data were confirmed at longer follow-ups and within a larger case series, this technique may provide significant savings in time, money, and feasibility of the treatment when compared with the two-step approach.

Autologous PRF application was another key point of the regenerative procedure adopted. PRF has been demonstrated as a valid tool to promote growth, stability, and differentiation of mesenchymal cells.[7,14] The results may suggest that clotting factors may provide the required impulse to correct coagulation, making autologous PRF of hemophilic patients particularly useful for a regenerative purpose and for perioperative bleeding control. Thus, PRF may provide regenerating factors, excluding catabolic products related to the hemarthrosis (hemosiderin), known as a probable cause of ankle arthropathy, along with the hypoxic environment.[6]

The results of this study suggest that a biotechnological approach may be indicated in selected, young patients affected by hemophilic ankle arthropathy, offering a good quality of life (pain control, ROM improvement) and even the possibility to return to sport. The radiological results showed no further progression of degeneration. The "one-step" treatment was simple, inexpensive, and without the need of a highly specialized laboratory. Further studies must be performed to highlight long-term results in order to know whether this may be an acceptable treatment method to delay ankle replacement or fusion.

Ethical Approval

The study was approved by the local ethical committee.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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