JOTSRR ©
2 (39) 2016: 016-021 • ORIGINAL ARTICLE

The application of bone marrow aspirate concentrate (BMAC) enrich in stem cells, growth factors and other active substances for the scaphoid delayed union treatment

Bielecki Tomasz[1], Cieślik-Bielecka Agata[1], Latalski Michał[2], Reichert Paweł[3], Pierchala Marcin[1]

[1] Department and Clinic of Orthopaedics and Trauma Surgery, Trauma Center St. Barbara Hospital, Medical University of Silesia, Sosnowiec, Poland
[2] Department and Clinic of Orthopeadics, Medical University, Lublin, Poland
[3] Department and Clinic of Hand Surgery, Medical University, Wrocław, Poland

SUMMARY

Despite a steady progress in diagnostic and surgical methods, fractures and scaphoid nonunion disorders continue to pose a serious therapeutic problem. According to various sources, the cause of these problems are mainly delays in diagnosis of fractures, and errors made during proper and adequate treatment. Some author claims that delays ranging from a few weeks to a few dozen months affect 30 to 60% of fractures. This article reports the efficacy of percutaneous autologous bone marrow aspirate concentrate enrich in stem cells and others active substances injection as a minimally invasive method for the treatment of delayed unions of scaphoid bone, preventing open grafting techniques.
7 patients diagnosed with delayed union of non-dislocated fractures of the scaphoid bone participated in this study. Bone marrow has been aseptically harvested from the iliac crest bone and centrifugated. The fissure was cleaned out in a mini-invasive way and 6 ml of BMAC, which was supplemented with 0,6 ml of thrombin, in order to obtain a gel, was administered into the gap. After the surgery, the carpus was immobilized by use of a plaster splint for 8 weeks.
One patient did not attend more than 1 from 6 routine outpatient visits and was excluded from statistical analyses. In 5 patients after 12 weeks from administration of the BMAC we achieved a bone union. In our study we could not reach total restoration of cancellous scaphoid bone, what was identified in CT at 12 week after the percutaneous BMAC injection. However, we achieved pain relief and bone fragments stabilization in all union cases, what it will prevent further arthrosis and other degenerative bone damage.
This is our early experience with the use of BMAC as biologic treatment for delayed union of the scaphoid bone. To our knowledge, this is the first study looking at the effect of percutaneous BMAC delivery to the fracture site in delayed union. We believe that BMAC enrich in stem cells, growth factors and other active substances grafting can be an effective and safe method for the treatment of scaphoid bone healing disturbances. Moreover, in our opinion this percutaneous injection method should be used in all non-dislocated fractures of the scaphoid bone to decrease the risk of develop of non-union, degenerative changes, avascular necrosis or instability and loss of wrist coordination.
Keywords: healing, tissue engineering, biomaterials, fracture

Address for correspondence: Tomasz Bielecki, MD, PhD
Department and Clinic of Orthopaedics, Medical University of Silesia, Pl. Medyków 1, 41-200 Sosnowiec, Poland

Word count: 2250 **Tables:** 0 **Figures:** 3 **References** 25

Received: 05.01.2016
Accepted: 18.02.2016

INTRODUCTION

Despite continuous advances in the treatment of bone fractures, disturbances of healing processes remain a difficult challenge. Approximately 10% of treated fractures will require further surgical procedures because of impaired healing [1, 2]. The preferred management of delayed union and nonunion is to provide the essential elements for bone formation. Because open surgery with autologous cancellous bone grafting has become the standard scaphoid healing disturbances treatment, alternative treatments must be equally successful in achieving union but also should provide some additional benefit to justify their use [1].

The ability to control cellular activity becomes a powerful tool in management of orthopedic disorders. Recent advances in cellular and molecular biology have led to the identification of specific cytokines that mediate cellular activities [2,3]. Among others, leukocyte and platelet-rich plasma (L-PRP) enriched with growth factors may enhance bone formation in vitro [3,4] and in vivo [5-7]. However, Bielecki et al. reported that regenerative potentials of L-PRP may be not enough to induce bone healing in all cases [8].

Percutaneous administration of substances with osteoinductive and osteogenic properties offers the advantage of decreased morbidity associated with the classic open grafting techniques [9]. Additional advantages are decreased costs and hospitalization time [8-10]. Previous clinical studies have shown that local application of bone marrow and bone morphogenetic protein-7 is capable of increasing bone defect healing [9, 11] . Bone marrow aspirate concentrates (BMAC) rich in stem cells, growth factors and other active substances represent a novel osteoinductive therapy that could be valuable as adjunct of bone healing [1, 12]. Stem cells excrete numerous growth factors, which can stimulate by autocrine, paracrine and juxtacrine interactions [12, 13]. By concentrating bone marrow, higher levels of cells and growth factors can be reached which might stimulate the prematurely terminated bone healing processes [1, 12-14]. Platelets from BMAC, once activated in the presence of thrombin, also release growth factors and begin to form gelatinous scaffold for the developing fibrin mass [12].

This article reports on the efficacy of percutaneous autologous BMAC injection as a minimally invasive method for the treatment of delayed unions of scaphoid bone, preventing open grafting techniques.

MATERIAL AND METHODS

Between October 2012 and February 2014, 7 patients diagnosed with delayed union of nondislocated fractures of the scaphoid bone participated in this study at the Department and Clinic of Orthopedics in Sosnowiec at the Medical University of Silesia, Poland. In the study group there were 4 men and 3 women, in age 16-61 years (average age – 31,2). The following inclusion criteria were applied for this study:

1) established delayed union 3 months after injury which has not progressed to full bony union in computer tomography [8];
2) no surgical treatment after fracture in the delayed union group;
3) a good general health status, and
4) regular visits in the outpatient clinic.

Patients with diabetes or taking medicines known to influence bone marrow cell function were excluded from the study. One patient did not attend more than 1 from 6 routine outpatient visits and was excluded from statistical analyses.

Following the outpatient procedure, each participant was followed on a regular basis with clinical examinations, roentgenograms, dual-energy X-ray absorptiometry – DXA and computer tomography – CT examinations (last visit) and functional evaluations (first and last visit).

The University Ethics Committee approval for performing the investigation was obtained (opinion No. NN-013-196/I/10 from January, 2010).

All examinations were performed at 3, 5, 8 and 12 week after the percutaneous BMAC injection. X-ray films were taken in anteroposterior and dedicated scaphoid views. DXA examination was carried out with a Lunar DPX scanner. Scan regions were located at the distal radius in injured and uninjured limb.

Union was determined on strict roentgenographic criteria at last visit. A patient was considered healed when 75% of the circumference of the bone at the defect site was resolved [9]. In case where we did not observe completion of the bone healing process, patient was classified into open surgery.

BMAC Preparation Procedure

Bone Marrow Aspirate Concentrate (BMAC was prepared by extracting bone marrow from the patient's iliac crest by using a trocar and one 60 ml syringe. 56,5 ml of whole bone marrow with 3,5 ml of anticoagulant (heparin) was drawn into sterile tube and centrifuged for 12 min at 3,200 rpm (Marrow Stim; Biomet, Inc., Warsaw, Ind., USA) (Fig. 1). Following centrifugation, the blood was separated into three basic components: red blood cells, BMAC, and acellular plasma. 6 ml of BMAC was obtained and mixed with 0,6 autologous thrombin (TPD, Biomet, Inc., Warsaw, Ind., USA) at room temperature to form a BMAC clot.

Fig. 1. The bone marrow harvest procedure

Surgical Procedure

The surgical procedure was performed in the operating room under general anaesthesia. The fracture was cleaned out in a microinvasive procedure (2 mm incision) under arthroscopy guidance and the wound was closed by the sutures. An 18-gauge needle was introduced immediately into the gap of delayed union under fluoroscopic guidance (Fig. 2). Then, 6 ml of BMAC, which was connected with 0,6 ml of thrombin, in order to obtain a gel subcutaneously, was administered to the fracture fissure. Plaster immobilisation of the carpus and forearm was applied for 8 weeks A second injection into the gap or operation with bone grafts was not performed.

Fig. 2. BMAC with thrombin was injected into delayed union site under fluoroscopic guidance

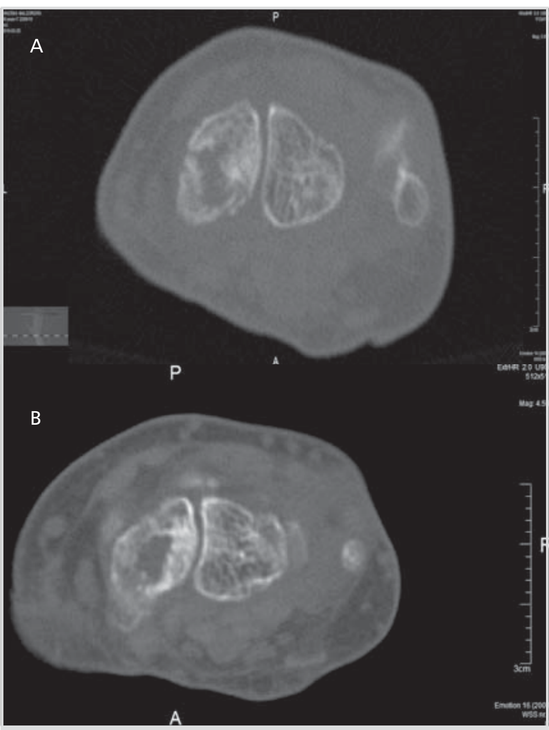

Fig. 3. CT examination of patient. **A.** Nonunion with cancellous defect (AVN) before operation. **B.** Cortical bone union at 12th week after BMAC injection with the remaining cancellous bone defect

Statistical Analysis

Statistical analysis was performed using Statistica for Windows 6.1 Version (Statsoft). Statistical differences were evaluated using Mann-Whitney's U-test and χ^2 test with Yate's correction. The Spearman ratio (r) was used to estimate the correlation between parameters. A simulation program for estimating the statistical power of Cox's proportional hazards model assuming no specific distribution for the successful treatment was used. Probability values $p \leq 0.05$ were considered significant.

RESULTS

No complications related to surgical technique were observed. Several patients developed subcutaneous swelling of few millimetres in diameter at the injection site. These areas were first evident during injection and resolved within several hours. A few patients had moderate discomfort at their donor iliac crest site, which generally resolved within a few days.

The average hospital stay was 1 day per patient. Pain relief, good functional outcome and union was observed in 5 of 6 cases. However, none of the patient presented total cancellous bone regeneration, what was identified in CT at 12 week after the percutaneous BMAC injection (Fig. 3A and B).

One patient in whom union did not occur, was qualified for open surgery. Most patients exhibited significant decreased bone mineral density of distal radius in DXA examinations during last visit (p=0,0272). BMD value and T-score was within the normal range.

No correlation was observed either between the age of patients and decrease in bone mineral density, or between body mass index and density of the bone. The fracture localization did not significantly influence treatment results. Significant correlation was not also noted between successful bone healing and the time from injury. To examine the influence of particular parameter on treatment outcome, the

Cox's statistic was performed. It demonstrated no significant correlations between parameters.

DISCUSSION

Despite a steady progress in diagnostic and surgical methods, fractures and scaphoid nonunion disorders continue to pose a serious therapeutic problem. According to various sources, the cause of these problems are mainly delays in diagnosis of fractures, and errors made during proper and adequate treatment. Some author claims that delays ranging from a few weeks to a few dozen months affect 30 to 60% of fractures [15,16].

Some authors indicate that in case of nondislocated fractures of the scaphoid bone, plaster immobilisation of the forearm should be applied for 4 to 6 weeks, and then a CT scan should be performed, and if in the follow up the secondary displacement does not occur, further immobilization does not need to be applied. However, some authors postulate that six weeks of immobilization is too short, which also causes difficulties in treatment [15,16]. Incorrectly diagnosed and treated scaphoid fractures lead to serious complications such as pseudoarthrosis, degenerative changes (SNAC-nonunion of the scaphoid collapse, SLAC-including rotary subluxation of the scaphoid bone, triscaphe-degenerative arthritis of the scaphoid, greater and smaller multangular bones, avascular necrosis (AVN), and wrist instability (DISI-dorsal instability intersegment insertion, VISI-palmar flexion instability) [15,17]. Our clinical team indicate that wrist immobilization should be prolonged until 12 weeks, when CT scan performed after 6 weeks does not show union processes. In our study after 3 months immobilization treatment BMAC was injected into the fracture site and further immobilization was applied for 8 weeks. Unfortunately, most patients exhibited a decrease in distal radius mineral density in DXA examinations. However, a BMD value and T-score was still within the normal range.

Most established methods of regenerative medicine require ex vivo cultivation of stem cells and therefore carry several risks, such as infection, high laboratory costs and the need for at least two operations [18]. As an innovative method of cell-based therapy, using BMAC allows intraoperative enrichment and application of mesenchymal and progenitor stem cells as a single-stage procedure [18,19].

The use of mesenchymal stem cells is an effective and constantly evolving method which supports the treatment of disorders of bone healing. These cells, present in the bone marrow, are undifferentiated and have the ability to transform into different types of tissues, including bone [12]. Studies in mice with fracture of the tibia, under the supervision of Dr. Anna Spagnoli of the University of North Carolina Chapel Hill School of Medicine, have shown that the use of mesenchymal stem cells results in significantly better healing of fractures and nonunion disorders. Her research team has further activated the IGF-1 factor, which resulted in even better results of healing, in comparison to treatment without stem cells, and that it is possible to obtain up to 3-4 times stronger tissue [20].

Neverthless, results of the Duttenhoefer et al. study on implant survival showed that the difference between the clinically feasible BMAC method was not significantly different from the approved, but clinically impractical Ficoll method [21]. Similar results were observed in the split mouth study of Rickert et al. in which sinus augmentation with the BMAC method was compared with the conventional method, which involves biomaterial being mixed with autologous bone [22]. Despite comparable outcomes, modest advantages favored the less invasive BMAC method with shorter fluoroscopy time and procedural duration, which was used in our study.

In addition, delays in treatment, particularly in fractures of the proximal end of the scaphoid is important, because it increases the risk of avascular necrosis. This is due to a specific blood flow in the bone, as vessels pass through it in 1/3 distal part, the remaining part of the bone is supplied by the backward flow [15-17]. Before starting the union disturbances treatment, a number of factors should be considered which will determine the method of treatment and surgical access. The literature commonly focuses on the presence of deformity of the bone (so-called humpback deformity where the interscaphoid angle exceeds 35°), the presence of degenerative changes, avascular necrosis or instability and loss of wrist coordination [15].

In the treatment of scaphoid bone nonunion, mainly in case of the proximal end fracture, restorative treatment involving vascularised or nonvascularised graft is also used. After completion of both vascularised and nonvascularised bone graft, the fractured bone fragments are stabilized using one of the available types of the consolidation materials listed above. One of the methods, described by Zaidenberg, is a bone graft [23], taken from the distal dorsolateral radial epiphysis. Blood supply is provided here by the so-called 1,2 interpartition ICSR artery located between the first and the second partition of the wrist, although it should be noted that the 2,3 interpartition ICSR artery can also be used in this method. In this method, after the graft is taken, it is turned and placed it in the prepared box in the scaphoid, maintaining the blood supply to the transplanted fragment. Another method of vascularised graft, described by Fernandez [24], is a method in which corticocancellous graft was made from the iliac crest, and implantation the second intervolar dorsal arteriola. The author has achieved union in 10 of 11 cases of nonunion, with complicated ischaemic necrosis – the average union time was about 10 weeks. Another method using vascularised graft is the Kawai and Yamamoto method [25], wherein the grafted bone element is rooted in the pronator quadratus. The collected bone block is usually about 15-20 mm, and is stabilized in the scaphoid by two Kirschner wires. With this method, revascularization and correction of the axis of the scaphoid is achieved. In our study we could not reach total regeneration of cancellous bone, what was identified in CT at 12 week after the percutaneous BMAC injection. However, we achieved pain relief and bone fragments stabilization in all union cases, what it will prevent further arthrosis and other degenerative bone damage.

This is our early experience with the use of BMAC as biologic treatment for delayed union of the scaphoid bone. To our knowledge, this is the first study looking at the effect of percutaneous BMAC delivery to the fracture site in delayed union. Most authors believe that the choice of method should be determined by the location and morphology of fracture, and the availability of equipment and level of experience of the operating team.

We believe that BMAC enrich in stem cells, growth factors and other active substances grafting can be an effective and safe method for the treatment of scaphoid bone healing disturbances. Moreover, in our opinion this percutaneous injection method should be used in all non-dislocated fractures of the scaphoid bone to decrease the risk of develop of non-union, degenerative changes, avascular necrosis or instability and loss of wrist coordination.

REFERENCES

1. Arvidson K, Abdallah BM, Applegate LA, Baldini N, Cenni E, Gomez-Barrena E, Granchi D, Kassem M, Konttinen YT, Mustafa K, Pioletti DP, Sillat T, Finne-Wistrand A. Bone regeneration and stem cells. J Cell Mol Med. 2011 Apr; 15(4):718-46.
2. Jäger M, Jelinek EM, Wess KM, Scharfstädt A, Jacobson M, Kevy SV, Krauspe R. Bone marrow concentrate: a novel strategy for bone defect treatment. Curr Stem Cell Res Ther. 2009 Jan;4(1):34-43.
3. Mishra A, Tummala P, King A, Lee B, Kraus M, Tse V, Jacobs CR. Buffered platelet-rich plasma enhances mesenchymal stem cell proliferation and chondrogenic differentiation. Tissue Eng Part C Methods. 2009 Sep;15(3):431-5.
4. Lucarelli E1, Beccheroni A, Donati D, Sangiorgi L, Cenacchi A, Del Vento AM, Meotti C, Bertoja AZ, Giardino R, Fornasari PM, Mercuri M, Picci P. Platelet-derived growth factors enhance proliferation of human stromal stem cells. Biomaterials. 2003 Aug;24(18):3095-100.
5. Bielecki T, Dohan Ehrenfest DM. Platelet-rich plasma (PRP) and Platelet-Rich Fibrin (PRF): surgical adjuvants, preparations for in situ regenerative medicine and tools for tissue engineering. Curr Pharm Biotechnol. 2012 Jun; 13(7):1121-30
6. Bielecki T, Cieslik-Bielecka A, Zelawski M, Mikusek W. A side-effect induced by the combination of a demineralized freeze-dried bone allograft and leucocyte and platelet-rich plasma during treatment for large bone cysts: a 4-year follow-up clinical study. Transfus Apher Sci. 2012 Oct;47(2):133-8.
7. Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). Trends Biotechnol. 2009 Mar;27(3):158-67
8. Bielecki T, Gazdzik TS, Szczepanski T. Benefit of percutaneous injection of autologous platelet-leukocyte-rich gel in patients with delayed union and nonunion. Eur Surg Res. 2008;40(3):289-96.
9. Wilkins RM, Chimenti BT, Rafkin RM: Percutaneous treatment of long bone nonunions: the use of autologous bone marrow and allograft bone matrix. Orthopaedics 2003; 26: 549–554.
10. Arrington ED, Smith WJ, Chambers HG, Bucknell AL, Davino NA: Complications of iliac crest bone graft harvesting. Clin Orthop Relat Res 1996; 329: 300–309.
11. Dimitriou R, Dahabreh Z, Katsoulis E, Matthews SJ, Branfoot T, Giannoudis PV: Application of recombinant BMP-7 on persistent upper and lower limb non-unions. Injury 2005; 36:S51–S59.
12. Bielecki T.
13. Le Nail LR, Stanovici J, Fournier J, Splingard M, Domenech J, Rosset P. Percutaneous grafting with bone marrow autologous concentrate for open tibia fractures: analysis of forty three cases and literature review. Int Orthop. 2014 Sep;38(9):1845-53
14. Lee DH, Ryu KJ, Kim JW, Kang KC, Choi YR. Bone Marrow Aspirate Concentrate and Platelet-rich Plasma Enhanced Bone Healing in Distraction Osteogenesis of the Tibia. Clin Orthop Relat Res. 2014 Dec;472(12):3789-97.
15. Reichert P., Rutkowski R., Zimmer K. Treatment of delayed union and nonunion of the scaphoid. Ortop Traumatol Rehabil. 2006 Oct 31;8(5):489-94.
16. Arenas-Prat J. Vascularized bone grafting and distal radius osteotomy for scaphoid nonunion advanced collapse: myth and reality. J Hand Surg Am. 2014 Sep; 39(9): 1885.
17. Al-Jabri T, Mannan A, Giannoudis P. The use of the free vascularised bone graft for nonunion of the scaphoid: a systematic review. J Orthop Surg Res. 2014 Apr 1;9:21.
18. Petri M, Namazian A, Wilke F, Ettinger M, Stübig T, Brand S, Bengel F, Krettek C, Berding G, Jagodzinski M. Repair of segmental long-bone defects by stem cell concentrate augmented scaffolds: a clinical and positron emission tomography–computed tomography analysis. Int Orthop. 2013 Nov;37(11):2231-7.

19. Beitzel K, McCarthy MB, Cote MP, Russell RP, Apostolakos J, Ramos DM, Kumbar SG, Imhoff AB, Arciero RA, Mazzocca AD. Properties of biologic scaffolds and their response to mesenchymal stem cells. Arthroscopy. 2014 Mar;30(3):289-98.
20. Granero-Molto F, Weis JA, Longobardi L, Spagnoli A. Role of mesenchymal stem cells in regenerative medicine: application to bone and cartilage repair. Expert Opin Biol Ther. 2008 Mar;8(3):255-68
21. Duttenhoefer F, Hieber SF, Stricker A, Schmelzeisen R, Gutwald R, Sauerbier S. Follow-up of implant survival comparing ficoll and bone marrow aspirate concentrate methods for hard tissue regeneration with mesenchymal stem cells in humans. Biores Open Access. 2014 Apr 1;3(2):75-6.
22. Rickert D, Vissink A, Slot WJ, Sauerbier S, Meijer HJ, Raghoebar GM. Maxillary sinus floor elevation surgery with Bio-Oss® mixed with a bone marrow concentrate or autogenous bone: test of principle on implant survival and clinical performance. Int J Oral Maxillofac Surg. 2014 Feb;43(2):243-7.
23. Zaidemberg C, Siebert JW, Angrigiani C. A new vascularized bone graft for scaphoid nonunion. J Hand Surg Am. 1991 May;16(3):474-8.
24. Fernandez DL, Eggli S. Non-union of the scaphoid. Revascularization of the proximal pole with implantation of a vascular bundle and bone-grafting.J Bone Joint Surg Am. 1995 Jun;77(6):883-93.
25. Kawai H, Yamamoto K. Pronator quadratus pedicled bone graft for old scaphoid fractures. J Bone Joint Surg Br. 1988 Nov;70(5):829-31.