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Treatment of infected tibial non-unions using a BMAC and S53P4 BAG combination for reconstruction of segmental bone defects: A clinical case series☆

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ABSTRACT

Introduction: Treatment of infected non-unions of the tibia is a challenging problem. The cornerstones of optimal infected non-union treatment consist of extensive debridement, fracture fixation, antimicrobial therapy and creation of an optimal local biological bone healing environment. The combination of S53P4 bioactive glass (BAG), as osteostimulative antibacterial bone graft substitute, and bone marrow aspirate concentrate (BMAC) for the implantation of mesenchymal stem cells and growth factors might be a promising combination. In this paper, preliminary results of a new treatment algorithm for infected non-unions of the tibia is presented.

Methods: In this retrospective case series patients with infected non-unions of the tibia are treated according to a new treatment algorithm. Patients are treated with extensive debridement surgery, replacement of the osteosynthesis and implantation of S53P4 BAG and BMAC in a one-stage or two-stage procedure based on non-union severity. Subsequently patients are treated with culture based antibiotic therapy and followed until union and infection eradication.

Results: Five patients with an infected non-union were treated, mean age was 55, average NUSS-score was 44 and the average segmental bone defect was 4.6cm. One patient was treated in a one-stage procedure and four patients in a two-stage induced membrane-, or "Masquelet"-procedure. On average, 23 ml S53P4 BAG and 6.2 ml BMAC was implanted. The mean follow-up period was 13.6 months and at the end of follow-up all patients had clinical consolidation with an average RUST-score of 7.8 and complete eradication of infection.

Discussion: These early data on the combined implantation of S53P4 BAG and BMAC in treatment of infected non-unions shows promising results. These fracture healing results and eradication rates resulted in promising functional recovery of the patients. To substantiate these results, larger and higher quality studies should be performed.

Introduction

In orthopedic trauma surgery, infected (or septic) non-unions are one of the most challenging complications after primary fixation of a fracture. Failure of fracture healing is already difficult to treat, but an associated infection of the affected bone and soft tissues makes it even more difficult. Treatment is invasive, mostly long lasting and burdensome for patients, it reduces the quality of life and despite invasive treatment it may eventually lead to amputation of the affected limb [1]. In addition, non-unions have a high economic burden as well, where average treatment costs are estimated from £ 29,205 in the U.K up to $ 53,206 in the U.S.A. [2,3]. Although there is some discussion about the exact definition, non-unions are generally defined as a failure of fracture healing after a period of 6-9 months since the initial fracture occurred [4,5]. After this period, bony bridging of the fracture is absent and it can be assumed that there is a complete cessation of bone regeneration. The prevalence of non-unions in tibia fractures varies from 5.4% to

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Table 1
Baseline characteristics.

Patient	Gender	Age (years)	Location / Gustillo	Duration (months)	NUSS-score
1	M	67	Tibia / II	7	48
2	F	50	Tibia / NA	7	24
3	M	46	Tibia / IIIB	11	66
4	M	51	Tibia / NA	11	38
5	M	59	Tibia / NA	14	30
mean	-	*55*	-	*9*	*44*

NA = Not applicable, closed fracture

7.5%, but in case of open fractures the non-union rates can be over 50% [6,7]. Open fractures, bone loss, smoking, NSAIDs usage, delay of weight baring and insufficient fixation are important risk factors and can cause a significant risk increase for non-union [8–10].

Treatment of infected non-unions is based on the combination of creating an optimal situation for bone regeneration and eradication of the infection [11]. Eradication of infection is comparable to treatment of chronic osteomyelitis and is based on extensive debridement of infected and necrotic bone and soft tissues combined with (local and systemic) antimicrobial therapies [12,13]. Fracture healing is based on the combination optimal fracture fixation (e.g. stability and alignment) and creation of an optimal fracture biology (e.g. cellular environment, growth factors, bone defect filling, soft tissue coverage, mechanical loading) [14,15]. Treatment can be performed in either a one-stage procedure or a two-stage procedure, based on the induced membrane technique first described by Masquelet et al. [16]. In treatment of infected non-unions, a two-stage "Masquelet" procedure might result in improved union rates and higher eradication rates when the temporary placed spacer is made of antibiotic loaded bone cement [11,17].

During surgery the bone defect can be filled using osteoinductive or osteoconductive biomaterials as autologous bone graft, demineralized bone matrix or a bone graft substitute [18]. An example of a bone graft substitute suitable for bone defect filling in non-union treatment is S53P4 bioactive glass (BAG, BonAlive, Turku, Finland). This bioactive glass has been proven effective in treatment of e.g. chronic osteomyelitis, mastoiditis, benign bone defects and is recently gaining interest in treatment of non-unions [19–22]. The key concepts for using this BAG is based on the combination of its osteostimulative and its antibacterial characteristics. Besides providing an antibacterial scaffold for bone growth and eradication of infection, the bone biology can be optimized by adding cellular components (mesenchymal stem cells) and growth factors (PDGF, TFG-β, BMP's, VEGF). Over the years multiple principles for adding mesenchymal stem cells have been used with variable results. Bone Marrow Aspirate Concentrate (BMAC) is a method in which autologous bone marrow is obtained from the iliac crest by percutaneous thick needle aspiration. After aspiration, the bone marrow is centrifuged and the cell fraction is isolated. BMAC is gaining interest since it is a minimally invasive method for the application of autologous osteogenic progenitor cells and growth factors. Recent studies have shown that the application of BMAC is safe, avoiding donor site problems, and that more cells result in better outcome [18,23].

In this paper we report the preliminary data of a treatment protocol for infected non-unions of the tibia where we optimized our treatment algorithm by using S53P4 bioactive glass (BAG) as a bone defect filler and bone marrow aspirate concentrate (BMAC) for the addition of mesenchymal stem cells and induction of growth factors for stimulation of new bone and soft tissue formation.

Patients, materials and methods

In this study, a case series of 5 consecutive patients treated with a new algorithm for infected non-union of the tibia is analyzed. All patients underwent extensive surgical debridement combined with implantation of S53P4 bioactive glass (BonAlive®, BonAlive Biomaterials Ltd, Finland) and BMAC in a one-stage or two-stage procedure, between October 2018 and September 2019 at the Maastricht University Medical Centre the Netherlands.

Patients were treated following the new algorithm when they were over 18 years old and had a confirmed infected non-union of the tibia with a duration of more than 6 months. Confirmation of the infected non-union was according to radiological (e.g. CT, PET-CT), clinical (e.g. signs of infection, fistula, instability of the fracture) and/or laboratory findings. Non-union characteristics of all patients were scored regarding the Non-Union Scoring System (NUSS) to assess severity and for clinical decision making [14,24]. Based on severity of the infection and non-union characteristics patients were allocated to a one-stage or two-stage treatment algorithm, where a NUSS score of more than 25 indicates a two-stage approach. After surgery, patients were followed at the outpatient clinic to monitor fracture healing and efficacy of eradication of infection as primary outcomes. Fracture healing is based on clinical parameters (pain free weight bearing and clinical examination) and radiological imaging (conventional radiographs and/or CT scans), where the Radiographic Union Scale in Tibial fractures (RUST-score) was used [25]. Eradication of infection was based on clinical symptoms (signs of local and systemic inflammation, absence of fistula), radiological imaging (conventional radiographs, PET-CT) and/or laboratory blood tests.

Patients

A total of five patients, four males and one female, were treated regarding our new treatment algorithm. At baseline, mean age was 55 (46-67) years and patients had an average NUSS of 44 (24 – 66) (see Table 1). Two of the five patients had an initial open fracture. The average time between primary fixation of the fracture until index treatment was 9 (7-14) months. In four patients, primary fixation with open reduction and internal fixation using bridging plates was performed. One patient was initially treated with an external fixator due to damage control orthopedics after high energy trauma. Two patients underwent respectively one and two surgical procedures for their infected non-union previous to the index treatment. Perioperative microbiological cultures of all patients were positive and the antibiotic treatment regimen was specified based on these cultures, see Table 2.

S53P4 bioactive glass

S53P4 bioactive glass (BonAlive®, BonAlive Biomaterials Ltd, Finland) was used as an antibacterial bone defect filler and scaffold. S53P4 BAG is a silica based biomaterial with a specific composition which makes it either antibacterial as osteostimulative and consists of 53% SiO2, 23% Na2O, 20% CaO and 4% P2O5. S53P4

Table 2
Cultured pathogens & antibiotic regimen.

Patient	Pathogens	Intravenous AB (2wks)	Oral AB (4wks)
1	*S. pasteuri*	Piperacillin / Tazobactam	Clindamycin
2	Candida species	Fluconazole	Fluconazole
3	*E.coli, S. aureus, pseudomonas*	Piperacillin / Tazobactam	None (12wks IV)
4	*S. epidermidis*	Vancomycin	Ciprofloxacin
5	*S. epidermidis, Enterococcus faecalis*	Vancomycin	None (6wks IV)

Table 3
Perioperative characteristics.

Patient	Method	Fixation	Defect size (cm)	S53P4 amount (ml)	BMAC amount (ml)
1	Masquelet	Locking plate	4	15	6
2	One stage	Locking plate	3	10	6
3	Masquelet	Locking plate	8	50	6
4	Masquelet	IM nail	5.5	20	7
5	Masquelet	IM nail + locking plate	3	20	6
mean	-	-	*4.7*	*23*	*6,2*

Table 4
Postoperative characteristics.

Patient	Follow-up (months)	Time to Union	RUST score end	Re-intervention	Complications
1	16	1yr	8	OSM removal	Screw failure
2	16	1yr	7	-	-
3	15	1yr	6	1x Re-BMAC	-
4	14	1yr	10	-	-
5	7	6m	8	Excision fistula	Fistula

BAG is available in a granular form of different sizes, ranging from 0.8mm up to 3.15 mm particle size. In all procedures in this series the size 1.5 – 2.5 mm was used. After implantation the release of surface ions increase the local pH and osmotic pressure and thereby causing an bactericidal environment for the bacteria present after debridement surgery. Due to this same ion release a silica gel layer is surrounding the BAG to which calcium phosphates bind, subsequently this crystallizes into hydroxyapatite which will activate the osteogenic cells. S53P4 BAG is already used in clinical treatment of e.g. chronic osteomyelitis, mastoiditis, benign bone defects with good results [20].

Treatment algorithm

The new treatment algorithm is based on the combination of optimal fracture fixation, extensive debridement, bone defect filling with the antibacterial S53P4 BioActive Glass (BAG, BonAlive, Turku, Finland) and addition of MSCs and growth factors with BMAC. Based upon the severity/classification of the non-union and the severity of the infection, patients were treated in either a one-stage or a two-stage procedure. The one-stage procedure consisted of replacement of the osteosynthesis, taking microbiological culture samples, extensive debridement directly followed by implantation of the S53P4 BAG in the bone defect and addition of the BMAC. More severe infected non-unions, with a NUSS score of more than 25 or a septic deterioration, were treated in a two-stage procedure based on the induced membrane technique, described by Masquelet et al. [16]. The first stage consisted of removal of the infected osteosynthesis materials, an extensive debridement of infected tissues with microbiological culture samples, implantation of an antibiotic (gentamicin) loaded PMMA cement spacer (Palacos® R+G, Hereaus Medical GmbH, Germany) and, if necessary, combined with adequate soft-tissue coverage by a plastic surgeon. The second stage was performed after 6-8 weeks. During the second stage the BMAC was harvested from the iliac crest and processed according to the manufacturer's instructions as described below. The PMMA spacer was removed and the defect filled with S53P4 BAG and BMAC. Finally the membrane and soft tissues were closed. After surgery patients were treated with culture specific antibiotics for 12 weeks (7-14 days intravenous, completed with oral antibiotics) depending on pathogen and clinical evolution. During rehabilitation, permissive weight baring was allowed to improve healing [10].

Bone marrow for the BMAC procedure is harvested using a percutaneous inserted Jamshidi needle (8 gauge) through the cortex of the iliac crest. During the procedure the needle is repositioned multiple times to increase cellular yield [26]. The aspirated bone marrow is then centrifuged in the operating theatre with a closed system (Heragen® maxx, Hereaus Medical GmbH, Germany) and the cell fraction of the concentrate is isolated. The cell concentrate is then mixed with the S53P4 BAG and implanted in the defect.

Results

A total of five patients were treated regarding our new treatment algorithm. Four patients were treated with a two-stage technique due to high NUSS score combined with a severe infection. One patients had a NUSS score of 24 and was therefore treated in a one-stage procedure. The bone defect sizes varied from 3cm up to 8cm and bone defects were filled with an average 23 ml (15 – 50) S53P4 BAG and 6.2 ml BMAC, see Table 3.

Postoperative follow-up ranged from 7 to 14 months. After surgery all patients started with permissive weight baring with instructions and guidance of a physical therapist. All patients showed clinical consolidation with pain free full weight bearing mobilization at the end of follow-up. RUST scores of the radiographic imaging at the final visit varied from 6 to 10, see Table 4. Fig. 1 shows the evolution of fracture healing during follow-up of one of the treated patients. At the end of the follow-up infection control was achieved in all patients. Two patients developed complications that needed surgical intervention; screw breakage in one patient and a persisting fistula which was debrided and closed during a re-

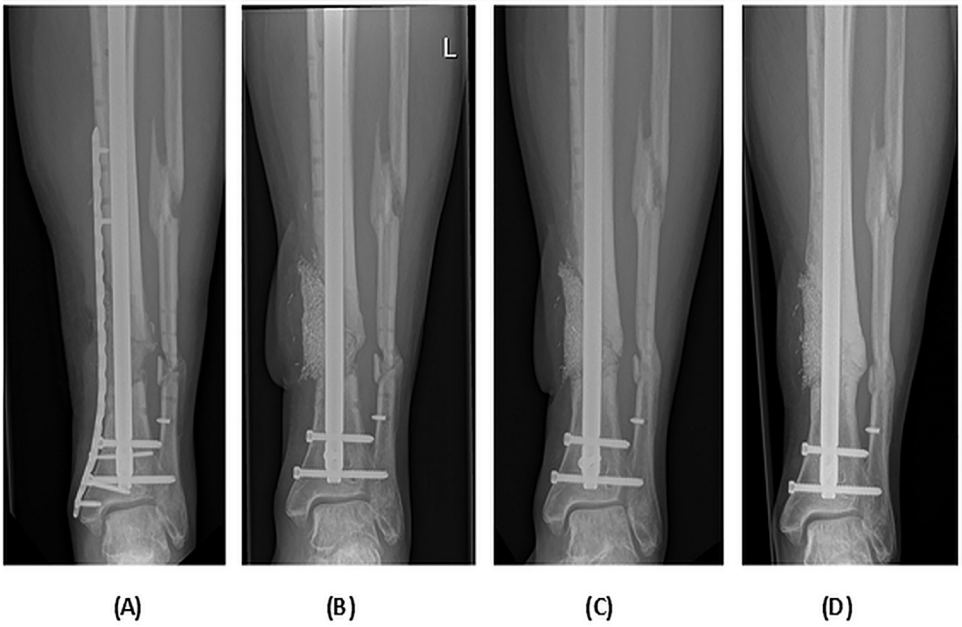

Fig. 1. Conventional radiographic images of one the trated cases showing progressive union over time. (A) pre-operative situaion, (B) 1 day after 2nd stage surgey, (C) 6 months follow-up, (D) 12 months follow-up.

intervention in another patient. One patient had an additional percutaneous injection of BMAC for inappropriate healing at the cortical end of the defect, resulting in complete fracture healing 4 months later (11 months after 2nd stage).

Discussion

This study shows promising preliminary results in treatment of infected tibia non-unions with a combined therapy of S53P4 bioactive glass and BMAC. The key elements in this infected non-union treatment are the combination of extensive debridement surgery, adequate stability and alignment of the fracture, addition of a cellular component, stimulation of growth factors and implantation of bone matrix/ biodegradable bone graft substitutes. The preliminary results with this new treatment emphasizes that the implantation of the antibacterial and osteostimulative S53P4 BAG and addition of MSCs and growth factors using BMAC are resulting in high consolidation rates and good infection control.

Since this study presents preliminary data of our new treatment algorithm, the results must be interpreted with caution. Our data should be substantiated with a larger study population and longer follow-up periods. In addition, our population is non-controlled, and a control group could decrease some of the bias. Union is based on the clinical presentation of pain free weight baring and the RUST scores on radiographic imaging. Although pain free weight baring and good mobilization might be an indication for fracture healing an objective outcome measure is necessary. Although the RUST score is an objective measure it might be insufficient for scoring system non-union healing since it is developed for scoring primary fracture healing after intramedullary nail fixation [25]. Using RUST scores for healing might give an over- or underestimation of non-union healing since the radiographic appearance on conventional radiographic images might be different. Another limitation in our study is the unknown number of added MSCs and growth factors since it is unclear how much is harvested and implanted during surgery. Different studies have shown that the amounts of MSCs in bone marrow aspirates are low and vary between 0.01% and 0.001% of the total amount of bone marrow cells. This relatively low cellular yield and the unknown implanted cellular amount remains the discussion of the causality between addition of BMAC and treatment success rates [27]. In order to increase the cellular yield several studies showed that after concentration the amount of cells was five to eight times higher and that implantation of an higher absolute number of cells is associated with better healing rates [23,28]. Thereby the technique and location of harvesting BMA from the iliac crest are discussed in literature. The choice of anterior or posterior iliac crest aspiration does not result in a significant difference in the amount of harvested cells. However, the method of harvesting appears to be important, as a recent study showed a direct influence of intermittent introduction of the aspiration needle on the cellular yield [26].

To our knowledge this is the first study to report the combination of S53P4 BAG and BMAC in the treatment of infected non-unions. Each of these components, BMAC and BAG, have been studied in combination with other materials. The application of BMAC in non-union treatment has been addressed in different studies as a stand-alone or in combination with DMB, BMP's or PRP's. These different studies showed union rates around 80-90% within 2.5 to 8 months [23,28,29]. The use of BAG in infected non-unions is studied in other combinations as well. In a recent publication Tanner et al. presented an ongoing randomized controlled trial regarding the usage of S53P4 BAG in treatment of infected non-unions. This study compares usage of S53P4 BAG with tricalcium phosphates combined with autologous bone graft, in which they will not study the potential benefits of adding a biological component tot the S53P4 BAG [22]. It is at this time too early to determine the effect of this combination. The results of our series indicates that the combination of BAG and BMAC is safe, and promising.

Conclusion

In conclusion, this study introduces a technique involving the implantation of bone marrow aspirate concentrate combined with S53P4 bioactive glass for the treatment of infected tibial non-unions. Our preliminary data suggests that this treatment strategy is effective for this notoriously difficult condition. Additional analysis regarding this new treatment algorithm should be executed, and our results warrant continuation of this treatment regime.

Declaration of Competing Interest

None.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.injury.2020.09.029.

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