

1 **Background**

2
3 The development of non-unions depends on several factors, such as energy-level of trauma,
4 type of fracture, soft tissue involvement, type of applied treatment, and various endogenous
5 factors [1-3]. According to literature, non-union will occur in approximately 10% of fractures
6 after conservative or operative treatment [4]. The use of iliac crest autologous bone graft
7 (ICABG) is widely considered as gold standard for a number of reasons, including
8 osteogenic, osteoconductive, and osteoinductive properties and the lack of disease
9 transmission or of immunogenicity [5-7]. However, the use of autograft may be at risk of
10 major drawbacks, such as limited availability and variable quality of the graft, hematoma,
11 infection, increased operative time and bleeding, chronic donor site pain, and additional cost
12 [8-15]. Subsequently, research has focused on the development of novel bone graft substitutes
13 for the last decades [16, 17].

14 In 1965, Urist et al. first described an osteoinductive substance while preparing soluble
15 extracts from demineralized bone [18]. Since this pioneering work, a large body of data
16 obtained by preclinical animal studies has supported the utility of demineralized bone matrix
17 (DBM) in human clinical settings. Nevertheless, there still is a lack of clinical studies: A
18 recent MEDLINE search using the term “demineralized bone matrix” restricted to “clinical
19 study” and “English language” demonstrated, that only four references were found that were
20 dealing with DBM-treatment in cases of a non-union of long bones [5, 19-22]. Therefore De
21 Long et al. concluded that evidence for or against the use of DBM is still at a low level
22 (Level-IV or V studies with consistent findings) [17].

23 In 2000, DBM Grafton® (Osteotech Inc., Eatontown, NJ, USA) was introduced in our
24 orthopaedic trauma department as an alternative to iliac crest autologous bone graft (ICABG),
25 particularly for morbid patients or those with decreased quantity or quality of autologous bone

1 graft. Therefore, the aim of the present study was to report our experience in augmenting non-
2 unions either with DBM or ICABG.

3

4

5 **Methods**

6

7

8 *Patients*

9 All patients presenting to our Level I trauma centre during a seven year period (01/2000 –
10 12/2006) with ununited fractures of upper and lower extremities' long bones were
11 retrospectively selected for the study. Non-union was defined as the lack of bone healing by at
12 least six months after fracture. For analysis, we compared those patients who had had ORIF
13 and augmentation with ICABG (“autologous-group”) and those with the use of demineralized
14 bone matrix (DBM Grafton®; Osteotech, Eatontown, New Jersey) (“allograft-group”).
15 Further inclusion criteria were: (1) patient over 18 years; (2) atrophic and diaphyseal non-
16 union; (3) no segmental defect; (4) closed fracture or open I° according to the Gustilo-
17 classification at initial presentation [23] and no clinical, radiographic, or laboratory evidence
18 of infection; (5) not more than one previous operation at the non-union site; and (6) a
19 minimum of 12 months of follow-up after index operation. We excluded patients fulfilling
20 any of the following criteria: fractures secondary to a malignant tumor, immunosuppressive
21 therapy, severe systemic disease or history of reflex sympathetic dystrophy and patients
22 receiving other augmentation types than either ICABG or DBM.

23 During the observational period sixty-two consecutive patients with ununited long bone
24 fractures were identified. Of these patients, two subjects were treated conservatively, twenty-
25 two victims had intraarticular fractures, fourteen met at least one exclusion criterion and four
26 patients were operated by using an autograft and allograft composite and therefore had to be

1 excluded, because they could not be assigned for one of the two study groups. Thus 10
2 patients were identified in the autologous- and 10 patients in the allograft-group.

3

4 *Surgical Procedure*

5 All surgical procedures were performed under the supervision of experienced orthopaedic
6 trauma surgeons and followed a specific operation-protocol: All patients obtained a single
7 shot antibiotic (1.5 g cefuroxime i.v.) straight preoperatively and underwent general
8 anaesthesia. After exposure of the non-union site, several specimens for microbiological
9 cultures were obtained. In case of prior fracture-instrumentation, implants were removed.
10 Thereafter, non-union site was radically debrided of intervening scar tissue and sclerotic bone
11 fragments with preservation of muscle and soft-tissue attachments to avoid devascularization.
12 In addition, the medullary canal was opened both proximally and distally to complete the
13 freshening of the bone ends. The non-union site was then reduced adequately by gently
14 impacting the distal into the proximal fragment to obtain osseous contact and thereafter
15 prepared for bone-grafting. The selection of the bone-graft-type, either ICABG or DBM
16 (DBM Grafton® Putty 2.5 cc; Osteotech, Eatontown, New Jersey), was based on the
17 experience of the surgeon in charge. The graft was placed into the medullary canal and around
18 the nonunion site. Finally, the bone ends were instrumented to achieve stable fixation of the
19 ununited fracture. Intraoperative anteroposterior and lateral radiographs were used to confirm
20 adequate placement of hardware and bone alignment.

21

22 *Follow-up*

23 After hospitalization for the index-operation, all patients were clinically and radiographically
24 investigated at our orthopaedic trauma outpatient department until the end of the non-union-
25 related treatment. For the purpose of this study, follow-up was performed at least twelve
26 months after the index-operation. Data assessment was performed by one investigator, who

1 was not involved in the non-union treatment of the patients (A.W.). Blinding of the
2 investigator was obviously not possible since all patients treated by ICABG were easily
3 identifiable by the scar at the iliac crest donor site.

4 All patients completed the standardized baseline and follow-up questionnaire to obtain
5 general information about the baseline data as well as the course of fracture treatment and
6 non-union healing. Thereafter, the standardized telephone-interview was performed by the
7 investigator to clarify the data. Sex, age, ASA value (American Society of Anaesthesiologists
8 classification), body-mass-index (BMI), smoking status, time from trauma to index-operation
9 and time from index-operation to follow-up were documented. Additionally, all postoperative
10 complications related to the index-operation were documented. Furthermore, intensity of
11 persistent pain at the prior ununited fracture site as well as the donor site in ICABG-patients
12 was recorded by the use of a numeric rating scale (NRS) ranging from 0 (no pain) to 10
13 (maximum of pain). Clinical healing was defined as full weight bearing or complete function.
14 Osseous healing was defined as a radiologically complete bridging callus formation with
15 crossing trabeculae on anteroposterior and lateral radiographs obtained during the study
16 period. All x-rays were investigated by study-independent radiologists. Furthermore, the level
17 of patient's dissatisfaction concerning the non-union surgical procedure was documented with
18 the use of a numeric rating scale (NRS) (range 0 to 5) by the following specifications: (0)
19 satisfied, (1) minimal dissatisfied, (2) marginal dissatisfied, (3) partial dissatisfied, (4) mostly
20 dissatisfied, and (5) extremely dissatisfied.

21 In case of insufficient quality of outcome data obtained by the follow-up questionnaire and
22 the telephone-interview, patients were evaluated by the investigator clinically and x-rays of
23 the surgical site were taken if necessary. For analysis, patient's personal data were
24 anonymized.

25

26 *Statistical Analysis*

1 For the comparison of both study groups (autologous-group and allograft-group) the Mann-
2 Whitney *U* test was used to evaluate the differences with regard to the demographic and
3 follow-up data. The Spearman rank correlation coefficient was used to evaluate the
4 association between various factors (patient's age, sex, the American Society of
5 Anaesthesiologists classification, BMI, prior fracture treatment, as well as smoking
6 behaviour) and outcome after index-operation. Level of significance was set at $p \leq 0.05$.

7

8

9 **Results**

10

11 As shown in table 1, no differences were documented between the autologous- and allograft-
12 group concerning demographic data, BMI, smoking behaviour, location of non-union, prior
13 instrumentation at non-union site, and mean time from ununited fracture to index-surgery.
14 ASA values were significantly higher in the allograft-group, indicating that these patients had
15 more comorbidities at the time of surgery ($p=0.014$). In both groups, comparable types of
16 implants were used for fixation of united fracture during index-surgery ($p=0.255$).

17

18 *Follow-up*

19 As shown in table 2, the mean follow-up time was 56.6 months (range 18-87 months) in the
20 autologous and 41.2 months (range 12-69) in the allograft-group ($p=0.240$). The mean time
21 for clinical healing was comparable in both groups ($p=0.168$) as well as for radiological
22 consolidation ($p=0.327$). Nevertheless, there was a lack of bone bridging in two patients
23 treated by ICABG (autologous healing-rate: 80%) whereas all ununited fractures treated by
24 DBM showed completed bone healing during the study period (allograft-healing-rate: 100%)
25 ($p=0.146$). Both patients of the autograft-group who failed to heal after the index-operation
26 had a persistent non-union located at the forearm: One patient was a 34 year old male non-

1 smoker who had to be re-operated 19 months after the index-operation because of pain,
2 reduction of arm-function and implant failure at the ulna (see figure 1). The second ICABG-
3 treated patient with impaired consolidation was a 57 year old male non-smoker. His x-rays
4 twelve months after the index operation showed a stiff non-union with a good alignment and
5 no radiological signs for implant loosening. Since he had no pain, no decreased arm-function
6 during daily life activities and no reduction in his professional tasks as family doctor, he
7 refused revision of the persistent non-union (see figure 2).

8

9 *Donor site complications*

10 One obvious difference between the two groups was the additional surgery for iliac crest bone
11 harvesting in the autologous-group. These patients showed a donor-site-related morbidity rate
12 of 20% since one subject suffered permanent pain at the iliac crest (VAS 3) and another
13 victim complained about disabling keloid-formation at the donor-site-scar, which was
14 associated with moderate pain (VAS 2) ($p=0.146$). None of the patients suffered harvesting-
15 related swelling, redness, drainage, infection or neurological deficits.

16

17 *Intensity of pain and level of treatment-dissatisfaction*

18 At follow-up, patients of the both groups stated to have approximately equal intensity of
19 index-operation-related pain (at rest: $p=0.326$; with physical activity: $p=0.936$) (see table 2).
20 Additionally, four patients of the autologous-group (40%) compared to all patients of the
21 allograft-group (100%) were satisfied or only minimally dissatisfied with the non-union
22 treatment. Thus, patients of the allograft-group were significantly less dissatisfied with the
23 treatment compared to those of the autologous-group ($p=0.031$) (see table 2).

24

25

26 **Discussion**

1 The ideal bone graft substitute should provide three key elements: (1) osteogenetic cells to
2 facilitate bone regeneration; (2) osteoinductive factors to induce bone formation; and (3) an
3 osteoconductive matrix to directly stimulate bone deposition. Osteoconductive materials have
4 no capability to form bone or induce its formation *per se*. They merely provide an
5 interconnected biocompatible scaffold, which local osseous tissue can utilize to regenerate
6 living bone. Osteoinductive materials facilitate new bone formation by allowing cells in the
7 local environment to undergo phenotypic conversion to osteoprogenitor cell types capable of
8 formation of bone. “Osteogenic” is a graft material that has the inherent capacity to form
9 bone, which implies that it has cells such as osteoblasts or osteocytes, capable of producing
10 bone [2, 4-7, 16, 17, 24].

11

12 In this context, the best available alternative to autologous bone grafting is the use of an
13 allograft. However, currently available allograft DBM formulations may differ considerably
14 with regard to their bone inductive activity, mainly dependent on biological properties of the
15 graft and the methods of allograft preparation [24]. DBM Grafton® (Osteotech Inc.,
16 Eatontown, NJ, USA), which was used in this study as bone substitute in the allograft-group,
17 is a type of processed allograft bone in combination with glycerin. As shown in animal
18 studies, DBM Grafton® has osteoconductive and osteoinductive potential: histologically, new
19 bone formation could be shown after DBM-application [25, 26].

20

21 Only few clinical studies are published to demonstrate DBM-efficacy as bone substitute and
22 even less reports documented the outcome of DBM used in the treatment of long bone non-
23 unions [5, 17]. In 2003, Wilkens et al. published data using an injectable type of DBM called
24 “AlloMatrix Injectable Putty”: 30 of 35 patients with non-union in multiple bone types went
25 on to union in an average of 3.5 months [21]. The same author showed that the percutaneous
26 use of a mixture of autologous bone marrow and allograft DBM (AlloMatrix) led in 61 of 69

1 patients with stiff non-unions of long bones to union in an average period of 8.1 months [22].
2 Unfortunately, both studies were performed without a control group leading to a decreased
3 Level of Evidence [5]. In 2005, Ziran et al. reported data of a retrospective comparative study
4 using cancellous bone chips combined either with DBM Grafton® (n=25) or with DBM
5 Orthoblast (n=13) for the treatment of non-unions or impending non-unions in heavy
6 smokers: Healing on the first graft attempt was observed in 52% of the DBM Grafton® and
7 85% of the DBM Orthoblast group [20]. Since this study used DBM in combination with
8 another graft-type, the question kept unanswered, in what extent the observed results were
9 influenced by DBM. In 2006, Hierholzer et al. published a retrospective consecutive cohort
10 study of ununited diaphyseal fractures of the humerus. Cases were treated with open reduction
11 and internal fixation using a reconstruction plate and either iliac crest bone graft (n=45) or
12 DBM Grafton® (n=43). In the iliac crest bone group, clinical and radiological union was
13 achieved in 100% in an average of 4.5 months compared to 97% in the Grafton® group in 4.2
14 months [19]. One limitation of that study might be that the results are applicable only for the
15 diaphyseal humerus non-unions, since DBM was used only in this specific long bone.

16

17 The aim of the present study was to evaluate the effect of DBM compared to ICABG in the
18 treatment of non-unions of extraarticular long-bones in the upper and lower extremities. Since
19 patients' baseline data as well as the observed radiological and pain intensity outcome during
20 follow-up was comparable in both groups our study could demonstrate, that DBM has the
21 same biological efficacy in promoting bone healing of non-unions compared to ICABG.
22 Nevertheless, 20% of the autologous-group suffered considerable long term donor site
23 complications at the iliac crest region. Additionally, patients treated by ICABG claimed
24 elevated dissatisfaction concerning the non-union surgical procedure compared to those of the
25 allograft-group during follow-up.

26

1 Since it is well known, that the biological potential of bone healing is progressively impaired
2 by the rising number of previous interventions at the bone site [27], only patients with a
3 maximum of one previous operation at the non-union were selected for this study. Hence, our
4 analysis did not show a correlation of non-union-healing associated either with previous non-
5 operative fracture treatment or previous surgery ($p=0.329$). Additionally, it is well
6 documented that smokers are significantly more at risk to develop complicated fracture
7 healing [1, 10, 27]. In the present study, there were only three smokers among the evaluated
8 non-union patients: one smoker with 12 package years in the in the autologous-group and two
9 smokers with a mean value of 25 package years in the allograft-group, respectively.
10 Consequently, the low rate of smokers led to the low mean value of package years in each
11 group, probably resulting in a lack of correlation between non-union-healing and smoking
12 behavior ($p=0.176$). In addition, the analysis did not show an association between the non-
13 union-consolidation and patient's age ($p=0.312$), sex ($p=0.242$), BMI ($p=0.116$), and ASA
14 value ($p=0.576$).

15
16 There are some possible disadvantages associated with the use of allografts. The first
17 detriment is the additional cost for surgery compared to autologous bone grafting. However,
18 Lohmann et al. evaluated recently the economic impact of ICABG in trauma surgery [28].
19 Mainly because of the extended operation time, harvesting was calculated to cost 213 €. In
20 our study, all patients of the allograft-group were treated with the use of 2.5 cc Grafton®
21 Putty, which was announced in Osteotech's pricelist to cost 373 €. Thus, considering the
22 economical aspect of both non-union procedures, DBM costs 160 € more compared to
23 ICABG. The second handicap of allografts might be the potential immunogenicity compared
24 to autologous grafting which was shown not only in animal models [29] but also probably in
25 clinical applications [30-32]. Fortunately, we did not observe allograft-related immunological
26 adverse reactions in our study but this potential problem should be considered using DBM.

1 Beside the disadvantages shown above, there are several benefits associated with the use of
2 allografts. The doubtless advantages of DBM are first the unlimited availability, second the
3 reduced operative time and bleeding, and third the avoidance of donor site complications
4 which were documented in 20% of our study-patients treated by ICABG [8-15]. An additional
5 advantage of DBM might be the beneficial effect in patients with more comorbidity: As
6 documented in our trial, ASA values were significantly elevated in the allograft-group
7 ($p=0.014$). Despite this less advantageous cohort of patients treated with DBM, the incidence
8 of non-union healing was comparable in both study groups. Based on these data we conclude
9 that DBM is particularly advisable in morbid patients because of proved effectiveness in
10 promoting non-union healing.

11

12 The limitations of the present study include first the small number of patients in each
13 treatment group which is probably the result of a decreased rate of non-unions because of
14 advanced therapy-options in acute fracture treatment [3]. Second, the investigator could not
15 be blinded with regard to the donor-site scar at the iliac crest, thus follow-up data were not
16 blindly assessed. Nevertheless, the research fellow was not the treating physician and
17 therefore we do not think that lack of blinding influenced the findings of the study.
18 Furthermore, since pain intensity and treatment-satisfaction (NRS) was self-assessed by
19 patients and x-rays were evaluated by a study-independent radiologist a potential assessor-
20 related bias could be minimized. Third, the non-prospective design and consequently a lack of
21 randomization is a weakness of the study.

22

23

24 **Conclusions**

25 In conclusion, we successfully incorporated augmentation with demineralized bone matrix
26 allograft into a standard concept for the treatment of atrophic ununited extraarticular fractures

1 of long bones in upper and lower extremities. Demineralized bone matrix proved to be
2 equally effective as autologous bone graft in augmenting ORIF, since the healing incidence
3 and time for consolidation of ununited fractures as well as pain intensity at follow-up was
4 comparable in both groups. Nevertheless, autologous cancellous bone graft should still be
5 considered as the gold standard in the treatment of non-unions, since at the present time first
6 there is no better evidence available that supports the superiority of allografts and second
7 allografting is more expensive. However, our study showed that patients treated by allograft-
8 augmentation had no complications, reduced treatment-dissatisfaction, and a lack of donor
9 site complications at the iliac crest region. Therefore we found our results adequately sound to
10 conclude that the use of DBM should be offered to suitable patients in the preoperative
11 consultation as a valuable alternative for autologous grafting. Moreover, we recommend non-
12 union treatment with ORIF and augmentation with allografts first in patients suffering from
13 significant comorbidities in order to reduce operative time and perioperative donor site
14 complications, and second in patients with known osteopenia/osteoporosis and therefore
15 limited availability of cancellous bone for autologous grafting. The low number of study
16 patients, lack of randomization and the non-prospective study design weaken the power of
17 this study. Hence, there is a need for further in-depth multicenter-investigations to verify not
18 only our results but also to get further validated information about additional factors in order
19 to optimize successful consolidation in long bone non-unions.

20

21

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2

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Figure 1

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B



Figure 2

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