ORIGINAL RESEARCH

Effect of Tricalcium Phosphate on Healing of Non-Unions: An Observational Study of over 400 Non-Unions

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Purpose: A central aspect of the treatment of non-unions is the filling of bone defects. The quantity of available autologous bone for this purpose is limited. Alternatively, or additionally, bone substitutes may be used. The aim of this retrospective, single-center study including 404 non-unions in 393 patients is to investigate the effect of tricalcium phosphate (TCP) on the healing of non-unions. Furthermore, the influence of gender, age, smoking status, comorbidities, type of surgical procedure, presence of infection, and length of treatment was investigated.

Methods: We evaluated three groups of patients. Group 1 received TCP + BG, group 2 received BG alone and group 3 received no augmentation. Bone stability was assessed 1 and 2 years after non-union revision surgery through analysis of radiographs using the Lane Sandhu Score. Scores ≥3 were rated as stable Other influencing factors were collected from the electronic medical record.

Results: In 224 non-unions, bone defects were filled with autologous bone and TCP (TCP+BG). In 137 non-unions, bone defects were filled with autologous bone (BG), and in 43 non-unions presenting non-relevant defects, neither autologous bone nor TCP were used (NBG). After 2 years, 72.7% of the TCP+BG patients, 90.1% of the BG patients and 84.4% of the NBG patients achieved a consolidation score ≥3. Advanced age, presence of comorbidities and longer treatment period had a significantly negative effect on consolidation 1 year after surgery. Longer treatment periods also showed a negative significant effect after 2 years. It is notable that larger defects, mainly treated with the combination of autologous bone and TCP, showed similar healing rates to that of smaller defects after 2 years.

Conclusion: The combination of TCP and autologous bone-grafts shows good results in the reconstruction of complicated bone-defects, but patience is required since the healing period exceeds 1 year in most patients.

Keywords: non-unions, bone substitute, Lane Sandhu Score, tricalcium phosphate

Background

Non-union occurs in up to 10% of long bone fractures[1] with current data from Germany suggesting an increase in upcoming years[2] and surgical treatment therefore remaining a major challenge. The "Diamond Concept" was established 15 years ago[3] and describes key elements of fracture healing. Mechanical stability, vascularization, osteoinductive factors, osteoconductive matrix and osteogenic cells have also shown to have an important role in non-union therapy.[4,5] In non-union surgery, the Masquelet procedure has shown good results. In a multi-step surgery, non-union is resected, infection is controlled, and local application of bone-cement (polymethyl methacrylate) induces a well-vascularized membrane to sustain the future bone graft. During the final step, bone cement is removed and the resulting defect is reconstructed with either autologous bone graft, synthetic bone graft or a combination of both.[6] This multi-stage procedure offers the possibility of repeated debridements of necrotic bone tissue, therefore achieving superior infection control and vascularization.[7] Although it is affective, this procedure comes at the cost of generating significant bone defects.

395

While treating residual bone defects, the choice of procedure and type of bone graft is defined by individual patient characteristics as well as by the nature of the non-union defect itself. Age, smoking, comorbidities, length of treatment, presence of infection and gender[8–10] all count amongst patient-specific factors described in literature as having an influence on healing outcome.

Autologous bone graft has been considered the gold standard in non-union therapy since it is the only material known to posess osteoinductive properties.[11] Defect augmentation with autologous bone graft is performed with cancellous bone obtained through sampling of the iliac crest bone or using a reaming apparatus capable of harvesting large volumes of intramedullar bone graft: the RIA-System.[12]

To support adequate bone healing, synthetic bone graft should ideally be osteogenic, osteoconductive, biocompatible, and osteoinductive.[13–16]

Due to limited availability, the use of pure autologous material is often not sufficient for the treatment of patients with non-union. For this reason, synthetic materials, such as beta-tricalcium phosphate (eg, Vitoss®) mixed with autologous cancellous bone, have been increasingly used as an alternative treatment in recent years.[2] This method is particularly promising for critical size bone defects. Vitoss®is a very phase-pure tricalcium phosphate and is therefore often used in these specific situations.[17]

Several clinical studies on the use of beta-tricalcium phosphate in patients for spinal fusion and correction have shown positive results with consolidation rates of up to 93% after 1 year.[18] However, in a prospective study with 34 patients, Thaler et al showed a significantly lower rate of 38.6% after 1 year.[19] Beta-tricalcium phosphate is known to be a good alternative to autologous graft in the treatment of small cavitary defects,[20] while current literature shows inconsistent results regarding the use of beta-tricalcium phosphate to treat non-unions. Van Hemert et al showed a healing rate of 85% after 1 year in their study on use of tricalcium phosphate in open wedge high tibial osteotomy.[21] DiGiovanni et al on the other hand show a healing rate of 66.5% for hindfoot arthrodesis.[22] There are few studies on the use of tricalcium phosphate in critical size bone defects or non-unions of the upper and lower extremities. Sasaki et al show callus formation of 88.6% in lower extremity non-unions in 35 patients.[23] Previous results are often limited by very small study populations and short follow-up.

The purpose of this observational study was to evaluate the effect of beta-tricalcium phosphate on surgical treatment of non-unions in a large cohort of 393 patients with a total of 404 non-unions over 24-months' follow-up. In addition, we determined the influence of various patient-specific factors on the outcome of non-unions.

Materials and Methods

This clinical, single-center observational study includes data of 393 patients with 404 non-unions who underwent non-union surgery in a Level 1 Trauma Center between February 2010 and December 2017. Data were collected from available electronic medical records, as well as available radiographic images. Imaging was performed at 1 and 2 years after treatment. The study was approved by the ethical committee of the Ruprecht-Karls-University of Heidelberg, Germany, according to the criteria of Helsinki (votum number: S262/2017).

Patient Population

All patients aged ≥18 years with non-unions of the humerus, clavicle, femur, tibia and hindfoot or metatarsal bones undergoing revision surgery were included in the current study. In case of multiple non-unions, each lesion was evaluated individually. Collection of patient-related data was performed retrospectively using a computerized databank. Patients were assigned a comparison group according to the treatment they underwent. Group 1 gathered patients having received tricalcium phosphate along with bone graft. Group 2 designated patients having undergone therapy exclusively with autologous spongiosa. Since autologous spongiosa is usually quantitatively insufficient to bridge defects larger than 2 cm, our in-house standard envolves additional application of tricalcium phosphate. Patients who did not have radiographic imaging at 6 months were excluded, except in cases where stability could be confirmed in later radiographic imaging.

Patients were divided into three groups:

The first group comprised of patients with larger bone defects (>2 cm) in which autologous bone grafting alone was not sufficient, was treated with a beta-tricalcium phosphate and autologous bone graft (BG) and included 224 non-unions (TCP + BG).

The second group, including patients with small defects (<2 cm), was treated with bone graft (BG), meaning autologous cancellous bone and gathered 137 non-unions.

The third group included 43 non-unions who underwent single-stage non-union surgery. Since no resulting bone defects were present, these patients were treated without autologous or synthetic bone graft (no bone graft = NBG). This treatment involved a reosteosynthesis procedure with sufficient stabilization.

Determination of the Lane Sandhu Score

Radiological criteria of the Lane Sandhu Score were used for radiographic assessment of consolidation[24] (Table 1). For this purpose, the available radiographs were evaluated after 1 and 2 years.

Non-union was considered as "stable" for scores superior or equal to 3.

Bone Substitutes

Autologous bone graft (RIA system on tibia, femur and pelvis or iliac crest chip) was used in the BG group. In the TCP + BG group, beta-tricalcium phosphate (Vitoss®, Stryker, Kalamazoo, Michigan, USA) was mixed with autologous bone graft. The NBG group was treated through local interventions such as debridement, shortening resection and sufficient biomechanical stabilization alone.

Patient-Specific Factors/Secondary Parameters

Other factors influencing consolidation were anatomical location of non-union, presence of infection, ASA-Score, and smoking. Furthermore, we collected data regarding age at trauma and time until non-union revision surgery. The overall treatment period was calculated based on these parameters.

Non-union localization was classified according to the AO classification.

Infection was defined as the evidence of bacteria present in the intraoperative tissue samples. The severity of infection was not further specified.

The ASA-classification was used to standardize patients' comorbidities. The classification goes back to the "American Society of Anesthesiologists" (ASA) and has gained acceptance due to its simple use for the evaluation of preoperative risk.[25] Higher preoperative ASA-Scores correlate positively with patient morbidity and mortality.[26] This score was divided into a binary variable for the purpose of logistic regression analysis: comorbidities were classified as "absent" for ASA-scores <2 and "present" for scores of 2 or higher.

Statistical Analysis

Statistical analysis of the data was performed using SPSS software version 28.0.1 (IBM Germany, Munich, Germany). Graphical representation was performed using Excel 2021, and the significance level was set at p=0.05. The Kruskal–Wallis test was used to test for a relationship between use of bone replacement materials and measured Lane Sandhu Score. Binary logistic regression was used to test the influence of secondary parameters on consolidation.

Results

A total of 393 patients with 404 non-unions were included in the study. The majority of treated non-unions were found in male patients (253 of 404, 62.6%). The mean follow-up was 29 months ($\sigma = 24$, min = 0, max = 119). The mean age at trauma was 46

Table 1 Lane Sandhu Score

Score	Radiologic Findings
0	No callus
1	Minimal callus
2	Callus evident but healing incomplete
3	Callus evident with stability expected
4	Complete healing with bone remodeling

Notes: Lane Sandhu Score.

Table 2 Distribution (Absolute and in %) of the ASA-Score in the Treated Groups

ASA-Score	TCP + BG	BG	NBG	Total
1	45 (20,1%)	37 (27.0%)	23 (53.5%)	105 (26.0%)
2	133 (59,4%)	79 (57.7%)	15 (34.9%)	227 (56.2%)
3	46 (20,5%)	21 (15.3%)	5 (11.6%)	72 (17.8%)

Notes: Distribution of the ASA-Score in the treated groups.

years ($\sigma = 16$, min = 6, max = 86) and the mean age of revision surgery was 49 years ($\sigma = 15$, min = 18, max = 88). The average treatment period was 3 years ($\sigma = 5$, min = 0, max = 40). A single-stage surgical procedure was used in approximately half of the patients (223; 55.2%). Infection was detected in 149 cases (36.9%). A total of 118 (29.2%) were smokers. The distribution of the ASA-Score is presented in Table 2.

Radiological evaluation was performed in 381 (94.3%) non-unions after the 1-year follow-up period, and in 224 (55.4%) after 2-years follow-up.

A total of 224 non-unions were treated with TCP + BG, 137 with BG, 43 with NBG.

Non-unions were most frequently detected in the tibia (109 (TCP + BG), 53 (BG), 22 (NBG) cases), followed by the femur (91 (TCP + BG), 42 (BG), 13 (NBG) cases), despite the corresponding treated group. Detailed distribution of non-union localization according to the treated groups is presented in Figure 1A-C.
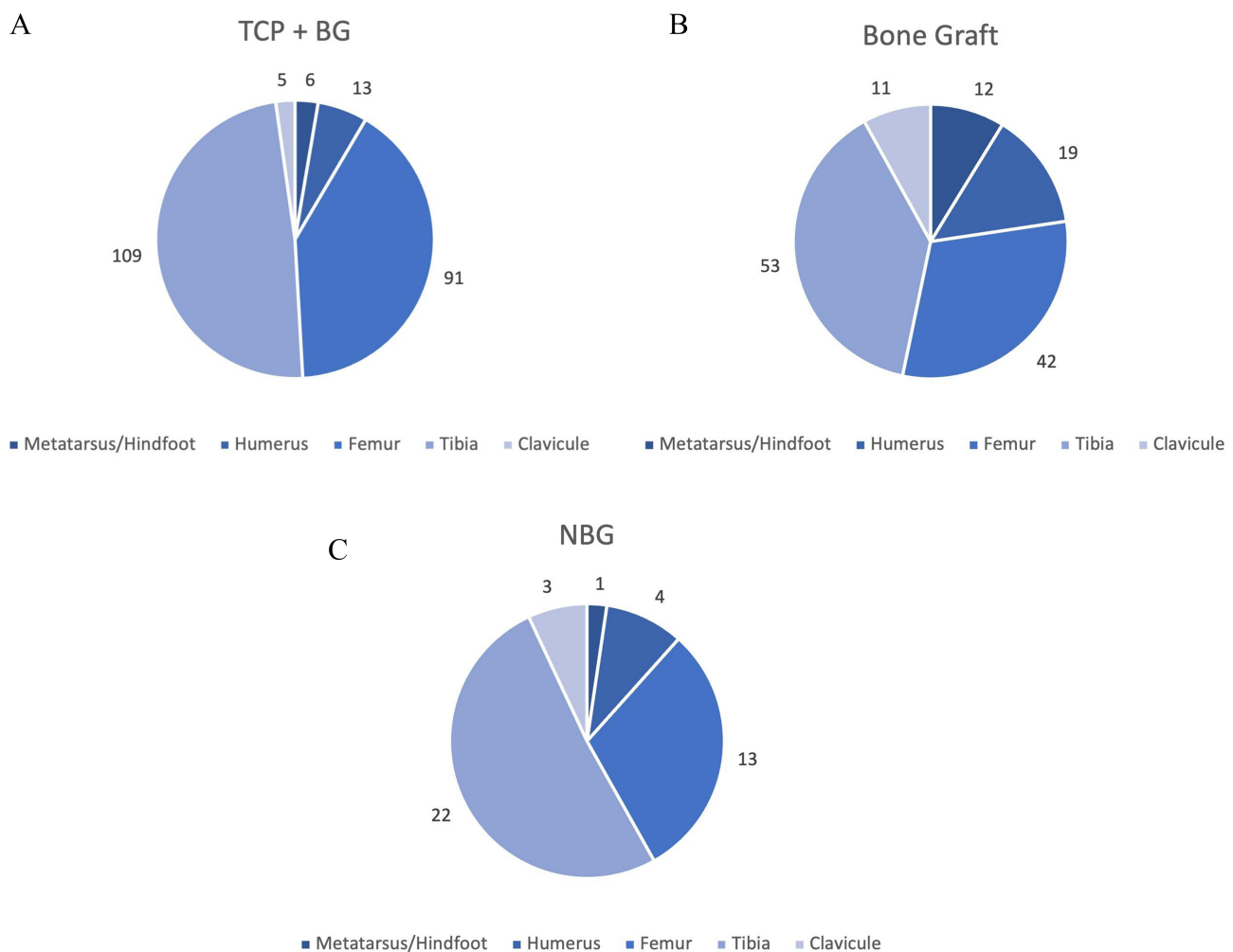

Figure 1 (**A–C**) Distribution (absolute) of localizations of non-unions in the treated groups.

Lane Sandhu Score, Consolidation and Treated Groups
Evaluation After 1 Year
The distribution of the Lane Sandhu Scores and stability for the different treated groups are shown in Table 3 and Table 4.

The performed Kruskal–Wallis-H test shows a significant difference between the TCP + BG, BG and NBG groups regarding Lane Sandhu Score measured after 1 year (n = 381, Kruskal–Wallis-H (2) = 15.939, p < 0.001). Pairwise comparison of the groups shows that TCP + BG and BG ($z = -2.766$; p = 0.006; $r = 0.142$) and TCP + BG and NBG ($z = -3.472$, p < 0.001, $r = 0.178$) are significantly different. Effect sizes can be considered as weak.[27]

Evaluation After 2 Years
The distribution of the Lane Sandhu Scores and stability for the different treated groups are shown in Table 5 and Table 6.

The TCP + BG and the BG group showed the greatest increase in stability after 2 years compared to after 1 year.

The measured Lane Sandhu Score after 2 years also showed a significant difference between the groups TCP + BG, BG and NBG (n = 224, Kruskal–Wallis-H (2) = 9.075, p = 0.011). Subsequent pairwise comparison of the groups showed that only the TCP + BG and BG groups ($z = -2.835$, p = 0.005, $r = 0.189$) showed significant differences, effect sizes being considered as weak.[27]

Table 3 Distribution (Absolute and in %) of the Lane Sandhu Score After 1 Year in the Treated Group

Lane Sandhu Score	TCP + BG	BG	NBG
0	39 (18.5%)	15 (11.5%)	2 (5.0%)
1	25 (11.8%)	9 (6.9%)	2 (5.0%)
2	59 (28.0%)	31 (23.8%)	7 (17.5%)
3	39 (18.5%)	35 (26.9%)	13 (32.5%)
4	49 (23.2%)	40 (30.8%)	16 (40.0%)
Total	**211 (100.0%)**	**130 (100.0%)**	**40 (100.0%)**

Notes: Lane Sandhu Score after 1 year in the treated group.

Table 4 Distribution (Absolute and in %) of the Stability After 1 Year in the Treated Groups

Stability	TCP + BG	BG	NBG
Not stable	123 (58.3%)	55 (42.3%)	11 (27.5%)
Stable	88 (41.7%)	75 (57.7%)	29 (72.5%)
Total	**211 (100.0%)**	**130 (100.0%)**	**40 (100.0%)**

Notes: Stability after 1 year in the treated groups. Non-union was considered as "stable" for scores superior or equal to 3.

Table 5 Distribution (Absolute and in %) of the Lane Sandhu Score After 2 Years in the Treated Groups

Lane Sandhu Score	TCP + BG	BG	NBG
0	11 (9.1%)	2 (2.8%)	1 (3.1%)
1	7 (5.8%)	1 (1.4%)	1 (3.1%)
2	14 (11.6%)	4 (5.6%)	3 (9.4%)
3	29 (24.0%)	16 (22.5%)	6 (18.8%)
4	60 (49.6%)	48 (67.6%)	21 (65.6%)
Total	**121 (100.0%)**	**71 (100.0%)**	**32 (100.0%)**

Notes: Lane Sandhu Score after 2 years in the treated groups.

Table 6 Distribution (Absolute and in %) of the Stability After 2 Years in the Treated Groups

Stability	TCP + BG	BG	NBG
Not stable	32 (26.4%)	7 (9.9%)	5 (15.6%)
Stable	89 (73.6%)	64 (90.1%)	27 (84.4%)
Total	**121 (100.0%)**	**71 (100.0%)**	**32 (100.0%)**

Notes: Stability after 2 years in the treated groups. Non-union was considered as "stable" for scores superior or equal to 3.

Patient-Individual Factors/Secondary Parameters and Consolidation

A binomial logistic regression was calculated at both 1- and 2-years' follow-up to evaluate the influence of patient-specific characteristics such as comorbidities, presence of infection, smoking, length of treatment, and age at revision surgery.

The regression model for the collective after 1 year was statistically significant ($\chi^2(5) = 40.787$, $p < 0.001$). Four of the variables included in the model were statistically significant: comorbidities ($p = 0.007$), smoking ($p = 0.024$), length of treatment ($p = 0.011$), and age at revision surgery ($p = 0.005$).

Infection ($p = 0.061$) had no significant effect on consolidation at 1 year.

Comorbidities had a negative impact on consolidation at 1 year, with an odds-ratio of 0.479 (95% CI [0.280, 0.818]), as did age at revision surgery, with an odds-ratio of 0.977 (95% CI [0.961, 0.993]). A longer treatment period also had a negative impact on consolidation within 1 year with an odds-ratio of 0.931 (95% CI [0.881, 0.984]) (Table 7).

The regression model at 2 years was statistically significant ($\chi^2 (5) = 19.121$, $p = 0.002$) only for the length of treatment ($p = 0.006$).

Infection ($p = 0.091$) had no significant influence on healing. Comorbidities ($p = 0.352$), smoking ($p = 0.175$), and age at surgery ($p = 0.190$) had no significant effect on consolidation at 2 years.

A longer treatment period showed a negative impact on consolidation with an odds-ratio of 0.884 (95% CI [0.810, 0.965]) (Table 8).

Table 7 Binomial Regression Results for the Effect of Patient-Individual Factors/Secondary Parameters After 1 Year

Patient-Individual Factors/Secondary Parameters	p-value	Odds Ratio [95% CI]
Comorbidities	**0.007***	0.479 [0.280, 0.818]
Presence of infection	0.061	0.658 [0.424, 1.020]
Smoking	**0.024***	0.574 [0.355, 0.929]
Length of treatment	**0.011***	0.931 [0.881, 0.984]
Age at revision surgery	**0.005***	0.977 [0.961, 0.993]

Notes: Binomial regression results for the effect of patient-individual factors/secondary parameters after 1 year. Asterisk depicts significant differences (*$p \leq 0.05$).

Table 8 Binomial Regression Results for the Effect of Patient-Individual Factors/Secondary Parameters After 2 Years

Patient-Individual Factors/Secondary Parameters	p-value	Odds Ratio [95% CI]
Comorbidities	0.352	0.654 [0.267, 1.601]
Presence of infection	0.091	0.547 [0.272, 1.100]
Smoking	0.175	0.595 [0.281, 1.260]
Length of treatment	**0.006***	0.884 [0.810, 0.965]
Age at revision surgery	0.160	0.982 [0.958, 1.007]

Notes: Binomial regression results for the effect of patient-individual factors/secondary parameters after 2 years. Asterisk depicts significant differences (*$p \leq 0.05$).

Discussion

The main results of this study show high rates of consolidation 1 year after single-stage treatment of uncomplicated non-unions. In defect situations where two- or multiple step surgery is necessary, use of autologous bone graft in small-size defects shows good results after one year and excellent results after 2 years. In our study, despite being so difficult to treat, non-unions with large size defects requiring additional augmentation with synthetic material due to a lack of sufficient autologous bone graft achieve stability in almost 75% of cases after 2 years when treated with tricalcium phosphate. This suggests strong potency of tricalcium phosphate in the treatment of large defect non-unions. One remarkable result of this study is that in the TCP + BG group only 42% achieved bone stability after 1 year, but out of the remaining 58% over 50% achieve stability between year 1 and year 2. This might indicate a current tendency to perform premature surgical revision of non-unions.

Since the three evaluated groups include patients with different baseline characteristics, applying comparative statistics to our results does not appear to be relevant. However, taking into consideration, that the TCP + BG group included the most complicated cases, the results of this combination therapy after two years can be positively considered (73,6% consolidation). The use of pure autologous bone graft shows excellent results after 2 years with >90% of the patients achieving bone stability. This surpasses results in the NBG group, therefore underlining the importance of the diamond concept. Implementing a two-step procedure including bone graft has shown the best results in our cohort. The possibility of achieving better vascularization and locally applying cells might explain this effect.[3,7] These results are in line with recent literature.

In their study, Anker et al showed that trabecular formation is significantly slower in larger defects than in smaller ones.[20] This observation concurs with our results that show longer consolidation times for the presumably larger defects filled with TCP + BG. In contrast, Sasaki et al conclude that there is no significant influence of defect size on healing rates.[23] However, the collective in this study is significantly smaller and consolidation is evaluated in a less reliable way than in our study: Here, healing was assumed in the presence of callus formation.

Studies with similarly large patient cohorts showed comparable fusion rates.[28–30] In a retrospective study, Flierl et al evaluated long bone fracture non-unions treated with allograft, autograft or with a combination of both. He described fusion rates of over 68% in the allograft+autograft group against 90% in the autograft group.[28] Moghaddam et al achieved consolidation rates of over 78% with the Diamond concept in femoral non-unions.[29] In a previous retrospective study, we found fusion rates of over 63% of non-unions showing a history of infection.[30]

Fracture healing represents an elaborate, multistep process that, when impaired, can lead to nonunion of the bone.[30–32]

Non-union is also a complex disorder with multiple etiologies, and it is therefore difficult to identify the specific dysfunctions leading to each case.[33] Important factors in the development of non-union include mechanical instability due to inadequate osteosynthesis, presence of necrotic bone tissue inside the fracture, and individual host factors such as smoking or other comorbidities.[30,34,35] Mechanical instability and poorly vascularized bone have been identified as the most important etiologic factors.[33] The treatment of non-unions remains a challenge in the field of orthopedic and trauma surgery to the present day.[31,36,37]

In recent years, numerous synthetic bone graft materials were introduced.[38,39] Substances containing growth factors, ceramics and bioactive glasses spike interest not only in non-union-surgery but also in spine, dental and maxillo-facial surgery.[38–43] While there is only very weak evidence for most of the newer substitutes, tricalcium phosphate is one of the best investigated products.[44,45] Ten years ago, a British study found 59 different available bone substitutes, 22 of which offered clinical data available in scientific literature.[38] Of these, only four RCTs were detected, including one about tricalcium phosphate which goes to show that evidence for the use of TCP is still very poor.[38]

The main limitation of this study is the retrospective design and the lack of control groups. The number of previous surgeries undergone by patients in external hospitals could not be reliably determined. Prior surgery could be a significant influence on the outcome and should therefore be integrated in the model.[8] Another factor that could have influenced the outcome and was not reported was that patients were treated by different physicians.

The strength of the present study lies in the large number of patients and the complete documentation of clinical and radiological data. To our knowledge, this is the first study investigating tricalcium phosphate in such a large number of patients with non-unions in relation to radiological outcome. The results of this study can therefore be applied to

everyday clinical practice with a heterogeneous patient population and different practitioners. The results can be used to define consolidation rates and evaluate the need and point of time of further revision surgery. We show that in small defects the addition of bone substitute materials is not necessarily needed, but if needed, tricalcium phosphate shows good results when combined with autologous bone-graft. In addition, significant influence of patient-specific factors including length of treatment, age, smoking and comorbidities on the outcome can be shown.

Conclusion

Treatment of non-unions still represents a challenge in the field of orthopedic and trauma surgery and numerous bone substitutes show potential for improvement in the future. Until now, autologous bone graft still can be considered as being "gold standard", but when large defects occur and addition of bone-substitutes is necessary, tricalcium phosphate shows itself to be reliable in helping to reach bone consolidation. However, a long healing period of up to 2 years can be expected.

Participant Consent and Ethical Approval

The Declaration of Helsinki's guiding principles were followed in the conduct of this work. The present study (votum number: S262/2017) was approved by the local ethic committee (Ruprecht-Karls-University of Heidelberg, Germany), and informed consent was taken from the participants. All patients` data were anonymized and maintained with confidentiality.

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Author Contributions

All authors made a significant contribution to the reported work, whether in its conception, study design, execution, acquisition of data or analysis and interpretation; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; agree to be accountable for all aspects of the work.

Disclosure

All authors have no financial and personal relationships with people or organizations that could inappropriately influence (bias) this work. The authors report no conflicts of interest in this work.

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