Original Article

Low intensity pulsed ultrasound in the treatment of long bone nonunions: Evaluation of cytokine expression as a tool for objectifying nonunion therapy

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ABSTRACT

Background: Nonunion is one of the most common complications in bone healing. After several clinical studies, the effect of low intensity pulsed ultrasound (LIPUS) in the treatment of nonunions remains unclear because of the difficulty in evaluating its effect on bone healing. In former studies, the analysis of serum cytokine expression patterns over time has proven to be an objective method for showing the bone healing process and evaluating nonunion therapies. This study evaluates LIPUS as a treatment option for patients with nonunions based on the analysis of serum cytokine expression.

Methods: In this prospective, single institution study, venous blood samples from 23 patients were taken from October 2012 to October 2013 before starting LIPUS therapy and at the end of week 1 and 2 and after 1, 2, and 3 months. Patients attended clinical and radiological follow-up examinations at the same intervals. After treating all patients according to the LIPUS therapy protocol, we divided them into two groups: Group 1 consisted of patients with healing at the nonunion site, and Group 2 consisted of patients with failed nonunion therapy. We measured transforming growth factor- β 1 (TGF- β 1), platelet-derived growth factor (PDGF), and basic fibroblastic growth factor (bFGF) at all time-points.

Results: The TGF- β 1 serum concentration increased from the pre-treatment value to 1 week within the unsuccessful group. Otherwise, no significant differences between groups in measured cytokines during LIPUS therapy could be detected.

Conclusion: Our findings suggest that LIPUS does not lead to a significant increase in cytokine levels in patients with nonunions. It is likely that “successful” treatment can be attributed to spontaneous healing. Our results suggest that LIPUS is not a proper treatment for long bone nonunions.

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1. Background

One of the main problems in trauma surgery is the treatment of delayed fracture healing and nonunions, which make up 5–10% of

all fractures or even as high as 30% in high-risk groups.^{1,2} Established non-invasive treatment methods in the early phase of therapy are mechanical stimulation and physiotherapy, which require sufficient osseous regenerative capacity and mechanical stability. If these initial methods are unsuccessful, then surgery is often necessary. There are non-invasive therapies that do not have the surgical risks but require certain conditions such as mechanical stability. One of these methods is, for example, extracorporeal shock-wave therapy.³ Another non-invasive treatment option is the application of low intensity pulsed ultrasound (LIPUS) to the nonunion site.⁴ The clinical use of low intensity pulsed ultrasound in patients with delayed fracture healing and nonunions is,

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however, a subject of current debate. A few studies have confirmed the beneficial use of LIPUS while others have shown that it has no relevant use in patients with nonunions.^{5,6} In pre-clinical studies, an up-regulation of anabolic genes could be demonstrated in osteoblasts under the influence of LIPUS.⁷

The main problem regarding these studies is that it is difficult to determine the actual effect of such therapies in clinical studies and that the results of animal or in vitro studies are difficult to transfer to humans. The measurement of serum cytokines is objective and can be used to evaluate for the evaluation of different treatment options.⁸ In previous studies, we have described several cytokine expression patterns in regular and failed bone healing, which showed significant differences in expression patterns at certain time points depending upon if fracture healing was delayed or failed.^{8–11} We concluded from these results that measuring serum cytokine expression over a standardized follow-up scheme is a valid instrument for the evaluation of bone healing.

In this study we investigated the serum expression of transforming growth factor- β 1 (TGF- β 1), platelet-derived growth factor (PDGF), and basic fibroblast growth factor (bFGF) during treatment with LIPUS. All of these cytokines were shown to have an impact on bone metabolism and differ in their expression level.^{12–14} This study will indicate whether low intensity pulsed ultrasound has an influence on the cytokine expression and contributes to the understanding of LIPUS as a treatment option for patients with nonunion or delayed fracture healing.

2. Methods

2.1. Design of the study

Venous blood samples were analyzed from all 23 patients who underwent low intensity pulsed ultrasound (LIPUS) treatment of long bone nonunions at a level 1 trauma center from October 2012 to October 2013. To get more reproducible data, all LIPUS patients in our study were treated with the same device and settings (Bioventus LLC, EXOGEN[®] ultrasound bone healing system, Durham, NC 27703, USA). Four patients did not complete the LIPUS treatment, which made it possible only to evaluate the cytokine profiles of 19 patients. An experienced orthopedic surgeon from the trauma center determined the indication for the use of LIPUS according to the EXOGEN[®] guidelines, which include stability of osteosynthesis, lack of bone consolidation and a defect gap <1 cm. The intervention free interval was at least 3 months in all patients. Frequency of LIPUS was 1.5 MHz, power was 30 mW/cm², pulse duration was 200 μ s, and signal repetition was 1 kHz. Patients were instructed to apply LIPUS 20 min/day to the nonunion site every day at home. Compliance was tracked by the EXOGEN[®] device.

2.2. Study criteria

Inclusion criteria were as follows: patients with diaphyseal nonunions of long bones (humerus, radius, ulna, femur, and tibia) were included in the study. There had to be a precedent medical indication for LIPUS therapy. Exclusion criteria were advanced liver disease, chronic inflammatory diseases, malignancy, as well as long-term medication with immunosuppressive drugs, which can influence cytokine levels.

2.3. Clinical data

In all cases, different orthopedic surgeons determined the indication of LIPUS therapy and analyzed the data. Successful clinical outcome included mechanical stability, weight bearing, radiological signs of consolidation, and the absence of any revision surgery within 1 year after LIPUS therapy. Patients were evaluated according to radiological and clinical criteria by two experienced orthopedic surgeons from the trauma clinic as belonging to one of two groups, “successful treatment” or “unsuccessful treatment.”

The risk of patients developing nonunions was evaluated with a scoring system established in previous studies, the Presurgical Nonunion Score (PSN Score) (Table 1).¹⁵ Nonunions were classified using the Nonunion Scoring System (NUSS).¹⁶ During treatment with LIPUS, all Patients had to attend follow-up examinations at standardized intervals of 1 week, 2 weeks, 1 month, 2 months, and 3 months. Treatment lasted at least 2 months or until either the nonunion healed, failed to show signs of healing within the time of treatment, or there were adverse complications such as metal breakage. Nonunion healing was noted either as the presence of mineralized bone in two of four cortices in the X-ray or as bone bridging 2/3 of the fracture diameter in a computed tomography in 1 mm slices.

2.4. Sample acquisition and serum-cytokine assay

Venous blood samples (Sarstedt, 7.5 ml S-Monovette[®], Nümbrecht, Nordrhein-Westfalen, Germany) were taken according to a standardized protocol (Fig. 1).^{9,17} Blood was drawn between 8 and 11 AM from patients on an empty stomach to avoid intraday measurement bias.^{18,19} Within 2 h after collection, the samples were centrifuged (1000 rpm, 10 min, 21 °C) and serum was stored at –80 °C.¹⁹ Two hours before analysis, serum samples were thawed and equilibrated to room temperature. Each sample was measured twice to avoid additional bias. TGF- β 1, PDGF, and bFGF were measured with commercially available ELISA kits (R&D Systems, Quantikine[®] ELISA Kits, Minneapolis, MN, USA) according to manufacturer's instructions. To avoid selection bias, the lab technician performing the ELISA assays was blinded to both patient data and clinical outcome.

Table 1

Presurgical Nonunion Score (PSN Score) to estimate the individual risk of patients for delayed union of long bone fractures.¹⁵

Localization			
Humerus	Prox. 4 point	Diaph. 6 points	Distal 2 points
Forearm	Prox. 4 points	Diaph. 6 points	Distal 2 points
Femur	Prox. 4 points	Diaph. 6 points	Distal 8 points
Tibia	Prox. 6 points	Diaph. 8 points	Distal 4 points
Soft tissue	1° open 4 points	2° open 6 points	3° open 10 points
	Fasciotomy 4 points ^a	Previous fracture 8 points ^a	Neurological disorder 6 points ^b
Smoking	Smoker 15 points	Previous smoker 5 points	Non-smoker 0 points
Comorbidity/medication	NSAID 4 points	Bisphosphonate 6 points	Diabetes 4 points
Type 1	<10 points	Low risk	
Type 2	10–20 points	Middle risk	
Type 3	>20 points	High risk	

^a Affected bone.

^b Affected limb.

Prox, proximal; Diaph, diaphysal.

Fig. 1. Standardized blood sample collection protocol. Samples were taken initially before the first LIPUS treatment.

2.5. Statistics

Statistical data analysis was done under the guidance of an Institute for Medical Biometry and Informatics. Statistical analysis for independent investigations was done with the Mann–Whitney *U*-test and for dependent investigations with the Wilcoxon-signed-rank test. Categorical data between the groups were compared with the chi-squared test and continuous data with the independent Student's *t*-test. Patient data is shown as mean $\pm$ standard deviation (minimum–maximum). Serum cytokine levels are shown as mean and standard error of the mean (SME). Statistical significance was determined when $p < 0.05$ and is indicated by asterisks (*). Statistical analysis was done using SPSS (IBM® Germany GmbH, SPSS, Ehningen, Baden-Württemberg, Germany). Graphics were done with Sigmaplot Software (Systat Software Inc., Sigmaplot, San Jose, CA, USA).

2.6. Ethics and source of funding

Written informed consent for participation in the study was obtained from all patients. None of the participants was underage. The study was conducted in accordance with the World Medical Association Declaration of Helsinki. The ethics committee of the Chamber of Medicine Rheinland-Pfalz gave their approval to this study (837.422.12-2012). There was no external funding source for this investigation.

2.7. Patient demographics (Table 2)

A total number of 19 patients (18 males, one female) were included in our study group. The mean age was 43.0 ± 13.5 (18–63) years. The average BMI was 29.2 ± 6.3 (21.6–44.2). The fracture location was mostly the lower extremity. There were seven fractures

of the femur and eight fractures of the tibia. In regards to upper extremity injuries, there were two fractures of the humerus and two fractures of the radius. The mean defect gap of the nonunions was 0.54 ± 0.34 (0.1–1) cm. In 12 cases, plate osteosynthesis was the surgical treatment before LIPUS therapy. Two cases were initially treated with external fixation and five cases were treated with marrow nailing. Ten of nineteen patients were smokers, and four were previous smokers. The mean time period from the time of the fracture until LIPUS treatment was 8.5 ± 5.1 (3–21) months.

3. Results

3.1. Clinical outcome of study group

Eleven of 19 nonunions were successfully treated with LIPUS. Eleven patients (57.9%) were grouped as “successful treatment” and 8 patients (42.1%) as “unsuccessful treatment.” The demographics of the different groups can be seen in Tables 3 and 4. The two groups did not differ significantly in any of the collected data (Table 2).

3.2. Serum cytokine levels

Temporal changes in concentrations of TGF- β 1, PDGF, and bFGF are given as mean concentrations as seen in Figs. 2–4.

3.3. TGF- β 1 (Fig. 2)

Absolute TGF- β 1 concentrations in patients with successful treatment, decreased from 40,802 pg/ml to a minimum of 32,797 pg/ml after 1 week. After 2 months, a maximum of 44,597 pg/ml was reached. Absolute TGF- β 1 concentrations of non-consolidated patients started at a pre-treatment minimum

Table 2
Patient demographics.

Characteristics	Total N = 19	Successful treatment (G1) N = 11	Unsuccessful treatment (G2) N = 8	p-Value ^o
Age (years)	43.0 $\pm$ 13.5 (18–63) ^a	44.5 $\pm$ 12.7 (18–63) ^a	40.9 $\pm$ 14.4 (18–60) ^a	0.585
Gender				0.228
Male	18	11	7	
Female	1	0	1	
BMI	29.2 $\pm$ 6.3 (21.6–44.2) ^a	29.2 $\pm$ 5.9 (21.6–40.5) ^a	29.1 $\pm$ 6.73 (22.8–44.2) ^a	0.972
Smoking				0.937
Smoker	10	6	4	
Previous smoker	4	2	2	
Nonsmoker	5	3	2	
Localization				0.288
Humerus	2	2	0	
Radius	2	2	0	
Femur	7	3	4	
Tibia	8	4	4	
Final fixation				0.089
Plate	12	9	3	
Nail	5	2	3	
External fixation	2	0	2	
Previous surgeries	2.7 $\pm$ 2.0 (1–9) ^a	3 $\pm$ 2 (1–9) ^a	2 $\pm$ 1 (1–5) ^a	0.554
NUSS	40.5 $\pm$ 7.1 (28–50) ^a	38.9 $\pm$ 6.2 (28–48) ^a	42.8 $\pm$ 7.7 (30–50) ^a	0.269
Presurgical Nonunion Score	19.9 $\pm$ 7.1 (8–29) ^a	18.8 $\pm$ 7.41 (8–48) ^a	21.5 $\pm$ 6.39 (8–29) ^a	0.445
LIPUS therapy (months)	5.5 $\pm$ 1.6 (2–7) ^a	4.9 $\pm$ 1.9 (2–7) ^a	6.3 $\pm$ 0.7 (5–7) ^a	0.058
Fracture age (months)	8.5 $\pm$ 5.1 (3–21) ^a	7.5 $\pm$ 4.4 (3–17) ^a	9.9 $\pm$ 5.6 (3–21) ^a	0.350

^a Average $\pm$ standard deviation (minimum–maximum).

^o No significant differences between the groups were found; α was set to 5%.

Table 3

Patient demographics of the successfully treated group (G1).

Age	Gender	BMI	Smokers	Localization	Final fixation	Previous surgeries	NUSS	PSN Score	LIPUS therapy (months)	Fracture age (months)
41	M	34.20	Yes	Radius	Plate	1	30	21	4	4
49	M	25.34	Yes	Tibia	Nail	1	28	27	3	4
46	M	36.93	Previous	Femur	Plate	9	42	8	4	13
30	M	24.97	No	Humerus	Plate	2	36	14	6	3
42	M	34.02	Yes	Tibia	Plate	2	40	15	2	8
18	M	25.88	No	Tibia	Plate	6	36	12	6	4
55	M	27.04	Previous	Tibia	Plate	3	48	27	6	10
52	M	28.37	Yes	Femur	Nail	3	44	25	7	17
63	M	40.47	No	Humerus	Plate	2	38	8	7	10
35	M	21.60	Yes	Femur	Plate	3	48	21	2	3
59	M	22.79	Yes	Radius	Plate	2	38	29	7	7

M, male.

Table 4

Patient demographics of the unsuccessfully treated group (G2).

Age	Gender	BMI	Smokers	Localization	Final fixation	Previous surgeries	NUSS	PSN Score	LIPUS therapy (months)	Fracture age (months)
44	M	25.31	Yes	Tibia	Nail	5	50	29	6	21
46	M	34.04	Yes	Femur	Ext. fixation	1	32	27	6	7
60	M	24.93	No	Femur	Plate	2	40	8	7	12
50	M	22.79	Yes	Tibia	Plate	2	42	25	5	13
24	F	23.05	No	Femur	Nail	3	30	16	6	4
56	M	30.30	Previous	Tibia	Plate	1	50	21	7	3
29	M	28.41	Previous	Tibia	Ext. fixation	1	50	25	6	6
18	M	44.19	Yes	Femur	Nail	2	48	21	7	13

M, male; F, female; ext. fixation, external fixation.

of 42,097 pg/ml. A significant increase to 46,053 pg/ml was detected at 1 week. The concentrations decreased to a value of 43,420 pg/ml at week 2 and then continued with a slight increase to a maximum of 47,560 pg/ml at the 2nd month.

3.4. PDGF (Fig. 3)

Absolute PDFG concentrations of the successful group started at a pre-treatment value of 19,894 pg/ml and decreased to a minimum of 17,198 pg/ml at the 1st week. The maximum value of 21,836 pg/ml was reached at week 2. The value stayed at that level during the next follow-ups with a minimal decrease. Absolute PDGF concentrations of the unsuccessful group started at a value of 23,184 pg/ml and decreased slightly over time to a minimum of

20,779 pg/ml at 1 month. A maximum of 24,288 pg/ml was detected at 2 months.

3.5. bFGF (Fig. 4)

Absolute bFGF concentrations in successfully treated patients started at a pre-treatment value of 3.13 pg/ml, increased slightly over the 1st and 2nd week, and reached a minimum of 2.27 pg/ml at the 2nd month. The value increased to a maximum of 4.48 pg/ml at 3 months. Absolute bFGF concentrations in the unsuccessful group increased from 4.479 pg/ml to a maximum of 5.45 pg/ml from pre-treatment to 1 week into LIPUS treatment and then decreased rapidly to a minimum of 2.22 pg/ml in the 2nd week. From that point on, we detected another increase to 4.06 pg/ml at

Fig. 2. TGF-β1 serum levels (mean ± SEM) during LIPUS therapy as absolute concentrations. *Significant serum level changes compared to the previous value (* $p < 0.05$). The difference between successful and unsuccessful group values was not significant at any point in time.

Fig. 3. PDGF serum levels (mean ± SEM) during LIPUS therapy as absolute concentrations. Absolute PDGF concentration levels of the successful group were slightly under those of the unsuccessful group. There were no significant differences in concentrations at any given time point.

Fig. 4. bFGF serum levels (mean $\pm$ SEM) during LIPUS therapy as absolute concentrations. Absolute bFGF concentration changes and differences between groups were not significant at any given time point.

the 1st month. During the following months, the value stayed at the same level.

3.6. Risk profile and classification (Table 2)

According to the Presurgical Nonunion Score, three patients (15.8%) were considered type 1 ("low risk"), 4 patients (21.1%) type 2 ("middle risk") and 12 patients (63.2%) type 3 ("high risk"). The average score was 19.9 ± 7.1 (8–29). There were also no significant differences between patient groups ($p = 0.445$). The 19 patients in this study had a mean NUSS Score of 40.5 ± 7.1 (28–50). Differences in NUSS Scores were not significant between patients with healed and non-healed nonunions ($p = 0.269$).

4. Discussion

In this prospective study, we analyzed serum concentrations of TGF- β 1, PDGF, and bFGF during a 3-month follow up in 19 nonunion patients treated with low intensity pulsed ultrasound (LIPUS). When the therapy was completed, patients were divided in two groups: patients with successful nonunion healing during therapy, and patients who had unsuccessful healing during LIPUS therapy. With only one exception, no significant alteration of measured cytokines could be detected during treatment.

The strengths of this study are its prospective approach, the well-established study protocol,^{8,9,17} and the use of one product brand, which allowed us to eliminate technical factors that could bias the evaluation. Furthermore, two different orthopedic surgeons determined the indication for LIPUS therapy and analyzed the data, which also minimized bias. Although application was patient-dependent, the device was able to control frequency of use. The weaknesses of the study, however, are its small sample size and the limited selection of measured cytokines. Nevertheless, we were able to gain some important results from our investigation.

4.1. Cytokine expression course

TGF- β 1, PDGF, and bFGF are known to be involved in fracture healing at different time points. They also play an important role in healing of nonunions. We were not able to detect significant alterations in the serum expression levels of TGF- β 1, bFGF, and PDGF during the entire LIPUS treatment with the exception of one TGF- β 1 level. Moreover, absolute mean values of patients who had

no successful healing during therapy were higher than those in patients with successful nonunion healing but without significance. In our previous studies, patients with proper fracture healing had higher mean levels of TGF- β 1, PDGF, and bFGF than patients developing nonunions and these differences were significant at certain time points.^{8,20} We attributed these findings to the different stages of fracture healing.^{21–23} In the first hours and days, inflammation cells invade the fracture hematoma to reabsorb bone debris and recruit other cells, especially stem cells that begin to form cartilage. In the second stage, cartilage calcifies to form hard callus. Osteoblasts invade at the end of hard callus formation. The examined cytokines play an important role not only in fracture healing, but also healing of nonunions.^{9–11} Mechanical stimulation of osteoblasts and other osteoprogenitor cells is crucial for fracture and nonunion healing, and therefore LIPUS is believed to have a stimulatory effect.^{24,25} We were not able to observe the expression courses, which are typical for nonunion healing in any of the investigated cytokines in this study.

4.2. TGF- β 1

Transforming growth factor beta is initially secreted from thrombocytes in areas of hematoma after injury and later from chondrocytes and osteoblasts during fracture healing.^{9,11,13,26,27} It stimulates the proliferation of different cells including osteoblasts, chondroblasts, and chondrocytes. In a diabetic rat model, LIPUS increased expression of TGF- β 1 in the fracture callus.²⁸

In this study, we detected only one significant increase in the TGF- β 1 serum concentration level, which was in the group without bone consolidation from the pre-treatment value to the end of week 1. Besides this, no alternation in TGF- β 1 serum concentrations was seen during LIPUS treatment. In a previous study, we detected a significantly higher TGF- β 1 serum concentration in union patients than in nonunion patients 4 weeks after surgery.⁸ This isolated increase significantly deviates from the known TGF- β 1 pattern of bone healing and is therefore more likely to be regarded as an incidental finding.^{8–11} The strength of the ultrasound stimulation does not appear to be sufficient for activating fracture healing. After 2 weeks, the TGF- β 1 concentration decreased. TGF- β 1 serum concentration levels in the consolidated group showed no significant differences. It appears that stimulation from ultrasound had no relevant influence on serum concentrations in the consolidated group. Furthermore, there were no differences in cytokine expression levels between the two groups at any time point. The TGF- β 1 serum concentrations of patients without sufficient fracture healing were not significant over those of patients with consolidated fractures.

In summary, we have come to the conclusion that stimulation with ultrasound was not sufficient in achieving a clinically relevant increase in TGF- β 1 concentration levels.

4.3. PDGF

Platelet derived growth factor (PDGF) was found in hypertrophic chondrocytes and osteoblasts during bone healing where it has many functions. It releases mesenchymal stem cells from blood vessels and is a potent mitogen for mesenchymal stem cells (MSC).^{20,29,30} The influence of mechanical stimulation on PDGF secretion in bone is not fully understood and there are only a few studies investigating this effect. In a rat distraction osteogenesis model, PDGF expression changed according to speed of growth.³¹ In an osteoblast cell culture, ultrasound increased the expression of PDGF, but only if vitamin D was substituted.³²

In this study, there was no significant alteration of PDGF serum expression during LIPUS therapy. In a previous investigation, we detected that PDGF serum concentration levels were significantly

higher in union than in nonunion patients in the 4th week after surgery.^{9,20} We concluded that ultrasound applied in vivo was not sufficient in stimulating the excretion of PDGF substantially, and therefore LIPUS has no relevant effect on this aspect of fracture healing.

4.4. bFGF

Basic fibroblast growth factor (bFGF) also plays many different roles in fracture healing.³³ One of them is stimulation of the immune response, inflammatory response, response to injury, and chemotaxis of human MSC destined for ossification.³⁴ Studies found increased bFGF levels in mandibular osteoblasts treated with LIPUS.^{35,36}

In our study, there was no difference in the serum concentration of bFGF at any point. A previous study showed that patients treated with distraction osteogenesis had higher levels of TGF- β and bFGF after surgery than osteotomy patients.²⁴ Therefore, we suspect that LIPUS in its clinical use has no relevant influence on bFGF levels.

4.5. Osteitis and nonunions

Currently there is high evidence that atrophic nonunions are often associated with low-grade infections.²⁶ Therefore, the use of LIPUS should be seen critically in patients with atrophic nonunions. It cannot be ruled out that the biofilm of the implant could be mobilized by ultrasound, which may lead to bacteremia or even sepsis.^{26,37,38}

LIPUS seems to provide osteoinductive stimulation and there is also some clinical evidence that LIPUS can accelerate the healing of fresh fractures.^{39,40} But it is apparently insufficient in the treatment of nonunions. The only prospective randomized controlled trial in the literature (with 101 patients; 51 treated with the EXOGEN[®] device, 50 treated with a sham device) revealed no significant difference in the rate of healing between groups.⁶

One recent study from Zura et al. showed a high healing rate of 86.2% with a total number of 767 analyzed chronic nonunions.⁴¹ However, this study has some weaknesses that should be taken into consideration. For example, LIPUS treatment requires mechanical stability at the nonunion site, which was not discussed in this study.⁴² The highly selective nature of the patient collective might have led to misleading conclusions.⁴¹ Other clinical studies have reported the rate of healing with LIPUS therapy to be 64–88%.^{6,43} However, risk profiles of patients were not often taken into account. There is a great variety of risk factors like insufficient blood supply, infection, co-morbidities like diabetes mellitus, drugs like NSAIDs, and other factors like smoking.^{15,26,37,44–46} We do not view ultrasound as a valid treatment option in these cases.

Furthermore, nonunion tissue is far different from tissue in fracture healing, which makes it implausible that nonunions are still capable of responding to external osteoinductive stimuli alone.

In regards to nonunion treatment, there are a number of different surgical treatments, but most of them involve proper mechanical stabilization and debridement of nonunion tissue.^{26,38,44,47,48}

In future studies, it should be discussed whether there are certain specific patient groups with nonunions that could profit from LIPUS. Valid risk scores such as the PSN Score or classification systems such as the NUSS could be used to characterize differences in patients.^{16,49} A prospective study that uses such scoring systems could help to identify those patient groups.²⁶

The measurement of serum cytokines provides objective data on processes of bone regeneration and can also provide good results in small patient collectives. We could not show relevant

differences in serum concentrations of TGF- β 1, PDGF, and bFGF after treatment with LIPUS.

5. Conclusion

We could not show relevant differences in serum concentrations of TGF- β 1, PDGF, and bFGF after treatment with LIPUS. We concluded that the mechanical stimulation produced by LIPUS in patients with nonunions is not sufficient in achieving a relevant stimulus for important cytokines. Low intensity pulsed ultrasound is apparently not able to induce a sufficient healing process in long bone nonunions.

Authors' contribution

AM conceived of the study and participated in its coordination. FW performed the cytokine measurements and analyzed the outcome data. TY acquired and analyzed data and drafted the manuscript. AM supervised the analysis and revised the manuscript critically. TB performed statistical analysis and data interpretation. WD recruited patients into the study and gathered data. TS edited and revised the manuscript (native English speaker). BB supervised the project and participated in analyzing the outcome data. All authors read and approved the final manuscript.

Conflicts of interest

The authors have none to declare.

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