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ORIGINAL ARTICLE

Reamer–Irrigator–Aspirator bone graft harvesting for treatment of segmental bone loss: analysis of defect volume as independent risk factor for failure

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Abstract
Introduction The management of segmental bone loss poses a significant clinical challenge. The purpose of this study was to conduct a retrospective evaluation of our experience in treating segmental bone loss, using Reamer–Irrigator–Aspirator (RIA)-harvested autologous bone graft.
Materials and methods Between June 2008 and March 2015, 81 patients were treated with the RIA technique for multiple purposes. Inclusion criteria for this study were skeletal mature patients with segmental bone loss, due to acute trauma or non-union, who were treated with RIA-harvested bone graft. Exclusion criteria were skeletal immaturity, pathological fractures and indications for the RIA system other than bone graft harvesting. The primary outcome parameter was clinical and radiographical bone healing.
Results During the study period, 72 patients met the inclusion criteria. In total, 39 patients (54.2%) were classified as having clinical and radiographical bone healing. Although univariate analysis could not reveal any significant influence of specific risk factors to predict the outcome, there was a trend towards statistical significance for defect volume. Further analysis indeed revealed that smaller defect volumes (< 8 cm^3) had a lower risk of non-union.
Conclusions In approximately half of our study population, the use of the RIA technique for autologous bone graft harvesting in cases of segmental bone loss resulted in a successful outcome with bone healing. Defect size seems to be a critical issue regarding the outcome. Although our results are less promising than previously published, the RIA technique has its place in the treatment algorithm of segmental bone defects.

Keywords Bone defect · Segmental bone loss · Non-union · RIA · Masquelet technique · Diamond concept

Introduction

Despite advancements in the treatment of musculoskeletal trauma, segmental bone loss (e.g., defect fractures or non-union) remains a difficult clinical problem, even in the hands of experienced trauma surgeons. The psychosocial burden and high financial impact on such patients should not be underestimated [1, 2].

Depending on the extent of the bone loss and institutional preferences, a patient might be eligible for a wide range of surgical and non-surgical management options. For small bone defects, treatment using autologous bone grafting or bone substitutes has shown good results. However, for large bone defects often more invasive treatment methods are required. The gold standard remains distraction osteogenesis (e.g., the Ilizarov technique) and free vascularized fibula grafting [3–5], although both techniques have their drawbacks. Paley and Maar reported on 19 patients who underwent distraction osteogenesis in case of tibial defects, with an average defect length of 10 cm

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[6]. Although all defects eventually healed, 10 of the 19 patients required additional grafting of the docking site. Furthermore, free vascularized fibula grafting has significant donor site morbidity and requires microvascular surgical expertise [2].

In the past decade, new insights in the understanding of the biological aspects of fracture healing and the important molecules involved have led to a renewed interest in autologous bone grafting for both small and large defects [7]. Until recently, the iliac crest was considered the anatomical site of choice for autologous bone graft procurement [5]. With the advent of a new bone graft harvesting device, the Reamer–Irrigator–Aspirator (RIA; DepuySynthes; Johnson & Johnson Co. Inc., NJ, USA) system, an additional source of bone has become available to treat segmental bone loss [5]. Multiple retrospective studies have shown good results with the use of RIA bone graft harvesting [5, 8–12]. It can be used in one- or two-stage surgical procedures (i.e., Masquelet technique) [8]. In case of the Masquelet technique, the graft covering bioactive membrane is thought to lead to a better incorporation of the graft and less resorption [13].

The purpose of this study was to conduct a retrospective evaluation of our results with the treatment of segmental bone loss using RIA-harvested autologous bone graft. Furthermore, different risk factors for failure of this technique were analyzed. To the best of our knowledge, this is one of the first studies to statistically evaluate the importance of defect volume as an independent risk factor.

Materials and methods

Patient and fracture (defect) characteristics

Between June 2008 and March 2015, the Department of Trauma Surgery treated 81 patients with the RIA technique for multiple purposes.

Inclusion criteria for this study were skeletal mature patients with segmental bone loss due to acute trauma (defect fractures) or non-union (septic/aseptic), who were treated with bone graft that was harvested using the RIA technique.

Exclusion criteria were skeletal immaturity, pathological fractures and indications for the RIA system other than bone graft harvesting (e.g., debridement for long bone osteomyelitis).

Patient demographics were documented and include age, gender, smoking, diabetes, and body mass index (BMI). Fracture characteristics of the initial injury were assessed including location (humerus, radius and ulna, femur, tibia and fibula) and zone of injury (epiphyseal, metaphyseal, diaphyseal). Other parameters that were included were Gustilo type, polytrauma (injury severity score >16) [14], indications for the use of the RIA technique, defect volume (cm^3) and number of operative procedures before the RIA procedure. The definition and classification of the initial fractures were based on the Arbeitsgemeinschaft fur Osteosynthesefragen/Orthopedic Trauma Association (AO/OTA) classification [15]. Open fractures were subdivided using the Gustilo–Anderson classification [16], which was determined at the time of initial debridement in the operating room.

The study protocol was conducted following good clinical practice guidelines. The study was approved by the Ethics Committee of the University Hospitals Leuven, Belgium. Patients were identified from the operating theater logbooks, and all case notes were retrieved. Patient data were analyzed using the hospital electronic patient file system and included in the study database.

Study aims

The first aim of this study was to evaluate RIA bone graft harvesting as a treatment option for segmental bone loss.

The second aim was to assess whether the following variables were associated with the probability of non-union after treatment of segmental bone loss with this technique:

1. Gender
2. Age
3. Location (upper vs lower extremity)
4. Diaphysis/metaphysis/epiphysis (arthrodesis)
5. Number of procedures before the RIA procedure
6. Indication (defect fractures, and septic or aseptic non-unions)
7. Defect volume (cm^3)

Definitions

Infection was classified into two groups: superficial or deep infections, which were defined according to Dellinger et al. and centers for disease control guidelines [17, 18]. A superficial wound infection was one located above the fascia, with erythema and tenderness. A deep infection was defined as an infection involving deeper tissues such as muscular fascia and bone, which could necessitate removal of the osteosynthetic material.

Clinical bone healing was defined as pain-free full weight bearing; radiographic bone healing was defined as bridging callus on at least three of four cortices [5].

Assessment of defect size

All patients were evaluated using post-operative conventional X-ray series that were consistently included in the follow-up. Post-operative images were chosen due to the fact that surgical debridement often leads to more bone loss. X-ray series were assessed by a radiologist (PJT), removing any possible inter-observer variability.

Defect volumes were defined and measured following guidelines described by Stafford et al. [5]. The authors of this paper described a calculation method, using different geometric equations that provide defect volume estimates based on defect size and geometry of the bone ends. Defect volumes were estimated based on two different models. First, defects with transverse bone ends without bony contact were considered cylindrical defects. In cases of oblique bone ends with cortical contact between segments, defects were considered conical. Any volume loss caused by additional fixation materials, most commonly intramedullary nails, was also corrected for. As previously mentioned, the authors used different geometric equations by which volumes can be calculated and described in cm^3. It needs to be stated that the formula for the simple cylinder without an intramedullary nail was adapted in our study. Stafford et al. [5] incorrectly stated $V = \pi h d^2$ instead of $V = \pi h r^2$.

Treatment Protocol

Surgical procedures were carried out by specialized trauma surgeons. All bone defects were revised for mechanical stability and improved where needed; either with external fixation, plate or intramedullary osteosynthesis.

Open fractures were treated within 6 h using sterile wound irrigation, debridement, and stabilization of the fracture in the operating room. If appropriate, plastic and reconstructive surgeon involvement occurred early in the treatment process. In severe open fracture cases, definitive skeletal stabilization and wound coverage were preferably achieved within 72 h and did not exceed 7 days. Systemic prophylactic antibiotics were administered once before surgery for closed fractures and continued in the case of open fractures until wound closure, for a maximum of 5 days [19].

The technique by which RIA bone graft harvesting was performed has already been described by Giannoudis et al. in 2009 [20]. In preparation of the operative procedure, a radiographic evaluation, which includes both antero-posterior and lateral radiographs of the femur, is essential in measuring the canal isthmus and the cortical thickness. Prior to moving ahead with the procedure, the availability of all the necessary equipment should be ensured. Assembly of the device at the start of the surgery is a crucial step of the procedure and should be done by experienced personnel. The RIA system can be utilized in any position, which allows access to the femur, either through an antegrade (standard), or retrograde starting point. The tibia is accessed in an antegrade fashion. As the antegrade approach to the femur is most commonly used we will focus here on this technique, although overall the RIA technique itself will be similar regardless of the anatomical location. Following identification of the entry point (e.g., the tip of the greater trochanter) a small incision is made just proximal to it. The initial guide wire is then inserted, checking the position under fluoroscopic control (e.g., antero-posterior and lateral images). Special care should be taken regarding the trajectory of the insertion angle to avoid the risk of eccentrically reaming (e.g., medial and anterior cortex), as this can cause iatrogenic fractures. Following opening of the canal, removal of the initial guide wire and reamer, the 2.5 mm guide wire with a ball tip is advanced from the trochanter down to the metaphyseal region of the femur. The RIA device is then mounted onto the wire and directed into the canal for simultaneous reaming, irrigation, and aspiration. At this point, it should be mentioned that frequent use of fluoroscopy is essential, allowing the surgeon to adequately control the device while reaming. As the collection filter fills, reaming should be stopped and the filter emptied before further reaming commences. This maneuver prevents bone graft from clogging in the RIA drive tubing. Once the first single pass is performed and the filter has been emptied, redirecting the guide wire into the femoral condyles can produce an additional amount of bone graft. As iatrogenic fractures are a known complication of the RIA technique, our protocol states that a radiographic evaluation should be performed postoperatively.

Growth factors [e.g., bone morphogenetic protein (BMP)−7] and scaffolds [e.g., porous biomaterials such as allograft trabecular bone or β-Tricalcium phosphate (TCP)] were not the standard of care. If biomaterials were added, the goal was always to have a high enough amount of RIA-harvested bone graft, with a minimum ratio of biomaterials to RIA-harvested bone graft of 1:3. This was documented after the operation by the treating surgeon in the surgical notes of the patient.

In cases of deep infection, a two-stage (i.e., Masquelet) procedure was preferred. In the first stage, the non-union area is cleaned of all the necrotic bone and a polymethylmethacrylate (PMMA) cement spacer is applied. A membrane is induced due to a foreign body reaction to the PMMA cement spacer. The induced membrane forms a 'biological chamber' for the insertion of the RIA-harvested bone graft during a second stage [13]. As described by Moghaddam et al. [8], the first phase of the Masquelet technique can be repeated several times until there are no further clinical or microbiological signs of infection.

Statistical analysis

The variables, mentioned earlier in the Study Aims section, were summarized in terms of union/non-union using n, mean, standard deviation (SD), median, range and interquartile range (IQR) for continuous variables and by observed counts and percentages for categorical variables.

The univariate associations of all predictors of interest with the probability of non-union were assessed using univariate logistic regression analysis. All tests were two-sided and assessed at a significance level of 5%.

All analyses were performed using SAS software, version 9.4 of the SAS System for Windows (Copyright© 2002 SAS Institute Inc.) by L-Biostat of the Catholic University Leuven, Belgium. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA.

Results

Clinical characteristics

During the 7-year study period, 72 patients with segmental bone loss met the inclusion criteria. The mean age was 45.4 years (SD = 15.8; range 18–78 years). We identified 49 (68.1%) male patients and 23 (31.9%) female patients. There were 21 (29.2%) polytrauma patients.

Out of these 72 patients, 47 (65.3%) were smokers. There were 6 (8.3%) obese (BMI ≥ 30) patients and 3 (4.2%) patients with type II diabetes. The mean BMI was 24.9 (SD = 4.4; range 16.6–40.3).

Following the AO/OTA classification, the identified fracture types were as follows: type A, 17 fractures (23.6%); type B, 6 fractures (8.3%); and type C, 49 fractures (68.1%). Furthermore, 38 (54.2%) patients were initially diagnosed with an open fracture. Following the Gustilo–Anderson criteria the open injuries were classified as type I in 2 cases (2.8%), type II in 11 cases (15.3%), and as type III in 25 cases (34.7%).

Most of the bone defects were located at the level of the tibia–fibula (n = 31/43.1%), followed by the femur (n = 16/22.2%), humerus (n = 14/19.4%) and the radius–ulna (n = 11/15.3%). Overall, there were 47 (65.3%) lower extremity and 25 (34.7%) upper extremity locations with segmental bone loss.

Three main indications for RIA bone graft harvesting were identified: aseptic non-union (n = 50/69.4%), septic non-union (n = 13/18.1%) and defect fractures (n = 9/12.5%).

Before RIA bone graft harvesting, it was found that 27 (37.5%) patients had zero or one previously performed surgical procedure. Two procedures were performed before conducting the RIA technique in 34 (47.2%) cases. More than 2 procedures were performed in 11 (15.3%) patients, with a maximum of 8 surgical interventions.

In total, 40 (55.6%) patients underwent a one-stage procedure and 32 (44.4%) patients underwent RIA bone graft harvesting in the context of a two-stage procedure (i.e., Masquelet technique). In this last group, the mean time to perform the second stage procedure was 13.6 weeks (SD = 8.68; range 4–52). In four of these cases, RIA autologous bone graft material was augmented with BMP-7 and β-TCP.

We divided the bone defects into four groups: small (0–5 cm^3), medium (>5–10 cm^3), large (>10–20 cm^3) and extra-large (>20 cm^3) volumes. The groups were divided at certain cutoffs, based primarily on numbers per group. The median defect volume was 11.5 cm^3 (IQR 5.3–30.4).

Outcome

In total, 39 (54.2%) patients were diagnosed with clinical and radiographic bone healing, whereas 33 (45.8%) patients showed no signs of bone healing (non-union) and needed revision surgeries (i.e., distraction osteogenesis, free vascularized fibula grafting or amputation). Twenty-five (62.5%) of the 40 patients who underwent a one-stage procedure showed bone union, compared with 14 (43.8%) of the 32 patients who underwent a two-stage (i.e., Masquelet) procedure. At the time of RIA bone graft harvesting, 40 patients had a plate osteosynthesis in situ, 25 a nail osteosynthesis and in 7 patients a combination of both implants was used. In the plate group, 57.5% (n = 23) showed bone union, compared to 44.0% (n = 11) in the nail group and 71.1% (n = 5) in the group were both implants were combined.

In the lower extremity group, 51.1% (n = 24/47) of the patients presented with non-union, compared with 36.0% (n = 9/25) in the upper extremity group.

Small defect volumes (n = 13/16; 81.3%) showed higher union rates compared with medium (n = 10/19; 52.6%), large (n = 5/15; 33.3%) and extra-large (n = 11/22; 50.0%) defects.

After the treatment using RIA-harvested bone graft, three patients (4.2%) were diagnosed with a deep infection at the grafting site. None of these patients had an infection prior to the procedure. There were no infections at the donor site.

Two patients (2.7%) had a proximal femoral fracture after reaming using the RIA technique, necessitating surgical treatment using a proximal femoral nail. Three of the patients (4.2%) showed anterior cortex perforation during the operation (distally), and were treated conservatively.

Table 1 provides a summary of all patient and fracture (defect) characteristics.

Table 1 Patient and fracture (defect) characteristics

Patient characteristic	Statistic	Union		Total
		No	Yes	
Total Population	*N*	33	39	72
Gender				
Male	*n*/*N* (%)	22/33 (66.7%)	27/39 (69.2%)	49/72 (68.1%)
Female	*n*/*N* (%)	11/33 (33.3%)	12/39 (30.8%)	23/72 (31.9%)
Age (Y)	*N*	33	39	72
	Mean	46.3	44.6	45.4
	Median	44.0	44.0	44.0
	SD	16.05	15.78	15.82
	(*Q*1, *Q*3)	(36.0; 56.0)	(32.0; 58.0)	(33.0; 57.5)
Location				
Humerus	*n*/*N* (%)	6/33 (18.2%)	8/39 (20.5%)	14/72 (19.4%)
Radius/ulna	*n*/*N* (%)	3/33 (9.1%)	8/39 (20.5%)	11/72 (15.3%)
Femur	*n*/*N* (%)	8/33 (24.2%)	8/39 (20.5%)	16/72 (22.2%)
Tibia/fibula	*n*/*N* (%)	16/33 (48.4%)	15/39 (38.5%)	31/72 (43.1%)
Location				
Lower extremity	*n*/*N* (%)	24/33 (72.7%)	23/39 (59.0%)	47/72 (65.3%)
Upper extremity	*n*/*N* (%)	9/33 (27.3%)	16/39 (41.0%)	25/72 (34.7%)
Diaphysis/metaphysis/epiphysis (arthrodesis)				
Epiphysis (arthrodesis)	*n*/*N* (%)	1/33 (3.0%)	0/39 (0.0%)	1/72 (1.4%)
Metaphysis	*n*/*N* (%)	11/33 (33.3%)	22/39 (56.4%)	33/72 (45.8%)
Diaphysis	*n*/*N* (%)	21/33 (63.6%)	17/39 (43.6%)	38/72 (52.8%)
Number of procedures before RIA				
Overall				
0	*n*/*N* (%)	0/33 (0.0%)	2/39 (5.1%)	2/72 (2.8%)
1	*n*/*N* (%)	12/33 (36.4%)	13/39 (33.3%)	25/72 (34.7%)
2	*n*/*N* (%)	14/33 (42.4%)	20/39 (51.3%)	34/72 (47.2%)
3	*n*/*N* (%)	4/33 (12.1%)	2/39 (5.1%)	6/72 (8.3%)
4	*n*/*N* (%)	1/33 (3.0%)	2/39 (5.1%)	3/72 (4.8%)
7	*n*/*N* (%)	1/33 (3.0%)	0/39 (0.0%)	1/72 (1.4%)
8	*n*/*N* (%)	1/33 (3.0%)	0/39 (0.0%)	1/72 (1.4%)
Subdivided per group				
0/1 Procedure	*n*/*N* (%)	12/33 (36.4%)	15/39 (38.5%)	27/72 (37.5%)
2 Procedures	*n*/*N* (%)	14/33 (42.4%)	20/39 (51.3%)	34/72 (47.2%)
>2 Procedures	*n*/*N* (%)	7/33 (21.2%)	4/39 (10.3%)	11/72 (15.3%)
Calculated as mean, median and SD	*N*	33	39	72
	Mean	2.2	1.7	1.9
	Median	2.0	2.0	2.0
	SD	1.58	0.86	1.25
	(*Q*1, *Q*3)	(1.0; 2.0)	(1.0; 2.0)	(1.0; 2.0)
Indication				
Aseptic non-union	*n*/*N* (%)	21/33 (63.6%)	29/39 (74.4%)	50/72 (69.4%)
Septic non-union	*n*/*N* (%)	7/33 (21.2%)	6/39 (15.4%)	13/72 (18.1%)
Defect fracture	*n*/*N* (%)	5/33 (15.2%)	4/39 (10.3%)	9/72 (12.5%)
Volume estimate: calculated as mean, median and SD (cm^3)	*N*	33	39	72
	Mean	27.7	22.7	25.0
	Median	14.0	9.2	11.5
	SD	40.41	42.34	41.26
	(*Q*1, *Q*3)	(7.7; 30.3)	(4.1; 31.0)	(5.3; 30.4)

Table 1 (continued)

Patient characteristic	Statistic	Union		Total
		No	Yes	
Defect size				
Small	*n*/*N* (%)	3/33 (9.1%)	13/39 (3.3%)	16/72 (22.2%)
Medium	*n*/*N* (%)	9/33 (27.3%)	10/39 (25.6%)	19/72 (26.4%)
Large	*n*/*N* (%)	10/33 (30.3%)	5/39 (12.8%)	15/72 (20.8%)
Extra large	*n*/*N* (%)	11/33 (33.3%)	11/39 (28.2%)	22/72 (30.6%)

N number of patients, *Y* years, *RIA* reamer–irrigator–aspirator, *SD* standard deviation

Univariate analysis of risk factors for the prediction of non-union after bone grafting using the RIA technique

Univariate analysis showed no significant influence of specific risk factors to predict the outcome after RIA bone graft harvesting for the treatment of segmental bone loss.

There was a trend towards statistical significance for defect size ($p = 0.0821$). The larger the defect size, the higher the risk of non-union after the use of RIA bone graft harvesting for treatment of segmental bone loss. The statistical results are summarized in Table 2.

Modeling of estimated defect volume (cm^3) and influence on the non-union rate

To the best of our knowledge, defect volume has not been statistically assessed prior to this study, and therefore it is of interest to investigate the association between this variable and the probability of non-union.

The plot of the estimated probabilities that modeled defect volume using a restricted cubic spline in a logistic regression suggest that a segmented model could be appropriate for fitting the association (Fig. 1). Such a segmented or piecewise regression divides the volume into intervals (in this case, two intervals) and fits a separate linear line within each interval with the lines connecting at the cutoff point between the intervals.

The 'best' segmented model was sought using the Akaike's information criterion (AIC) value [21] for which a lower value indicates a better fit. Exploratory analyses revealed that a better fit was provided when fitting a flat plateau in the second interval, i.e., no change in the probability of non-union when defect volume reaches a certain value.

To find the 'best' cutoff point, the segmented logistic regression model was fit using cutoff points across the entire range of volumes, using steps of 0.05. The resulting AIC values (for each cutoff that was investigated) are plotted versus their respective cutoff. Based on these analyses,

Table 2 Univariate odds ratios of risk factor for the prediction of non-union

Variable	*N*	Comparison	Univariable analyses			
			Odds ratio		*p* value	*p* (overall)
			Estimate	95% CI Interval		
Gender	72	Female vs male	1.125	(0.42; 3.04)	0.8162	
Age (Y)	72		1.007	(0.98; 1.04)	0.6374	
Location	72	Lower extremity vs upper extremity	1.855	(0.68; 5.03)	0.2245	
Number of procedures before RIA						
Overall	72		1.360	(0.95; 1.94)	0.0896	
Subdivided per group	72	2 vs 0/1	0.875	(0.32; 2.43)	0.7977	0.4359
		>2 vs 0/1	2.188	(0.52; 9.27)	0.2881	
Indication	72	Defect fracture vs aseptic non-union	1.726	(0.41; 7.21)	0.4542	0.6166
		Septic non-union vs aseptic non-union	1.611	(0.47; 5.49)	0.4460	
Volume estimate (cm^3)	72		1.003	(0.99; 1.02)	0.6396	
Defect size	72	Extra large vs small	4.333	(0.96; 19.58)	0.0567	0.0821
		Large vs small	8.667	(1.66; 45.21)	0.0104	
		Medium vs small	3.900	(0.83; 18.28)	0.0843	

CI confidence interval, *N* number of patients, *Y* years, *RIA* reamer–irrigator–aspirator

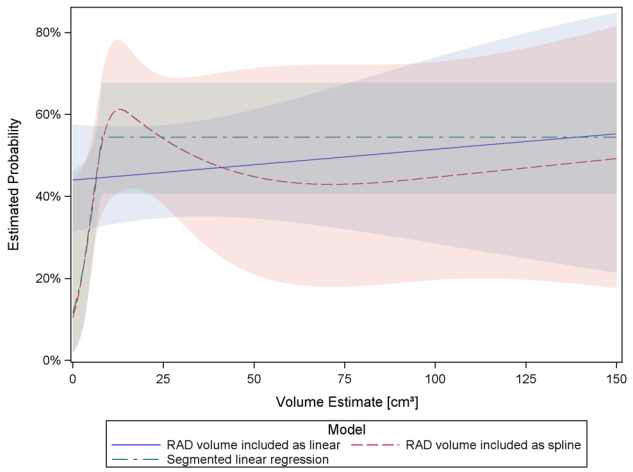

Fig. 1 Statistical association between defect volume and the probability of non-union. To explore the association between radiological assessed defect (RAD) volume and the probability of having non-union, a logistic regression was performed with non-union as outcome and RAD volume as covariate using a restricted cubic spline (with 4 knots) to allow for a non-linear association between the two. Although there was no statistical evidence that the spline (*red line*) provided a better fit ($p = 0.2062$ for spline terms) than the linear model (*blue line*), the curve suggests an initial increase in the probability of non-union for increasing volumes up to approximately 10 cm^3. Afterwards, the chance of non-union remains fairly constant. Therefore, a segmented regression was employed with a linear increase up to a cutoff and a plateau afterwards. The optimal cutoff was found to be 8 mm^3. The segmented regression (*green line*) provided a better fit than the model that included the linear term (likelihood ratio test: $p = 0.0297$)

the 'best' cutoff was found to be 7.65, which for convenience was rounded to 8 cm^3. Above this value there appears to be a fixed non-union rate. Furthermore, the smaller the defect volume was in this study (<8 cm^3), the greater the chance of bone union. The resulting association is displayed in Fig. 1 and yielded a p value of 0.0297.

Discussion

Fracture healing is a physiological process, which is the result of a complex interaction of molecular factors and biomechanical principles. Although normal fracture healing can occur with or without surgical intervention, segmental bone loss often necessitates extensive surgical procedures. Bone regeneration in these large bone defects remains an important problem in musculoskeletal trauma surgery [2, 22, 23]. With the advent of the RIA system, an additional source of autologous bone became available to treat segmental bone loss. Autologous bone graft remains the only clinically available source with osteogenic, osteoinductive and osteoconductive properties in addition to containing viable precursor cells [24]. Prior to the development of the RIA system, bone harvesting was typically performed from the iliac crest, which has a significant risk of donor site morbidity and, compared with RIA aspirates from the femur, a significantly lower concentration of mesenchymal stem cells (MSCs) and early endothelial progenitor cells [25]. Another advantage of the RIA technique is the possibility to harvest a large quantity of bone graft minimally invasive, which allows for the treatment of larger defects compared with, for example, iliac crest bone grafting [24].

Although multiple retrospective studies have shown good results with RIA bone graft harvesting, large randomized clinical trials are lacking. In the literature, success rates after RIA procedures range from 85.0 to 98.4% [5, 8, 26, 27]. The current study reports a union rate of 54.2%, which is lower than previously mentioned in the literature. There are possible explanations for this observation. First, we can interpret the high success rate found in other studies as a consequence of their smaller case series and strong patient selection [5, 12, 27]. Second, compared to our results, the focus in other studies is not always on primary healing rates after the initial RIA procedure. In a study by McCall et al. for example the authors presented healing rates of 85%, although the primary healing rate in this study was 50.0% [12], which is comparable to our results. Third, part of this variability in outcome could be attributed to the lack of consensus regarding the definition of non-union [28]. A fourth reason could be that only a very small percentage of patients in the current study was treated with growth factors (i.e. BMP-7), or scaffolds (i.e., TCP). Here, it needs to be stated that, although growth factors and scaffolds have an important role in the 'Diamond concept' described by Giannoudis et al. [29], there are studies that question their influence on fracture healing [2, 13, 30]. Furthermore, we are aware that another important factor that could explain the lower healing rates in the two-stage (i.e., Masquelet) group is the long time interval before the second phase (13.6 weeks; SD = 8.68). Current literature states that this interval should be 4–8 weeks [13, 23, 31]. A final possible reason for the lower success rate is the fact that the defect volume in this patient population was high (median: 11.5 cm^3; IQR 5.3–30.4). As previously mentioned, defect volume appears to play a role in the outcome. This study is, to the best of our knowledge, one of the first to statistically evaluate possible risk factors for non-union, including defect volume, in the case of RIA bone graft harvesting for the treatment of segmental bone loss. The defect volumes in our study showed a large range and were therefore divided into four categories based on group size: small, medium, large and extra-large bone defects. This subdivision was not based on previously published data, but it provided an initial idea on the statistical correlation between defect

volume and outcome. Although univariate analysis could not reveal any significant influence of specific risk factors on treatment outcomes after RIA bone graft harvesting, indeed there was a trend towards statistical significance for defect volume ($p = 0.0821$). Therefore, further analysis was performed. Figure 1 shows that above a defect volume of 8 cm^3 there appears to be a fixed (plateau phase) non-union rate. Furthermore, the smaller the defect volume (<8 cm^3) the greater the chance of bone union ($p = 0.0297$).

The method by which defect volume was measured was identical to the one described by Stafford et al. [5]. It is important that the obtained bone defect volumes are regarded as approximations, since they are based on measurements from standard X-rays that inherently contain some deviation from the true anatomical values. If more accurate volume estimations are required, computed tomography (CT) could be used, where volume rendering provides a precise evaluation of bone defects and callus formation [32]. Current advances in technology, with the rise of 3D imaging for example, open up new possibilities in the analysis of bone defects, in a pre- and post-operative setting. Only a minority of the included patients received a post-operative CT scan, rendering this method inapplicable for the current study.

The complication rate ($n = 8$; 11.1%) in this study was relatively low. With respect to infection prevention, recent preclinical data shows that if RIA-harvested bone graft is used, the surgeon should be convinced that there is no (remaining) infection, as this could be worsened by the introduction of bone graft material (i.e. MSCs) [33]. Furthermore, in our opinion, experience in performing the RIA technique is important not only regarding the prevention of complications but also to improve the outcome.

This study has limitations, as it is a retrospective analysis of suspected risk factors. Additionally, the non-union group was relatively small; therefore, caution is needed before drawing conclusions and generalizing these findings to other subjects. However, this is the first study to statistically evaluate defect volume as an independent risk factor. Not all potential variables were investigated, including smoking, obesity, alcohol consumption and corticosteroid use. Therefore, the results of the current analysis are limited to the variables that were collected as part of this retrospective study. Finally, an important limitation is the fact that our study population is very heterogeneous, including different anatomical locations, fractures patterns, osteosynthetic materials (e.g., intramedullary nails, plates), causes of segmental defects (e.g., acute trauma and non-union) and treatment methods (i.e., Masquelet procedure). All these individual groups have their influence on bone healing and therefore on the outcome.

Conclusions

In approximately half of this study population, the use of the RIA technique for autologous bone graft harvesting in cases of segmental bone loss resulted in a successful outcome in terms of bone healing. Defect size appears to be a critical issue regarding the outcome, meaning that smaller defect volumes have higher union rates. Although these results are less promising than those previously published, the RIA technique has its place in the treatment algorithm of segmental bone defects. However, further research is necessary to evaluate the specific indications for its use.

Compliance with ethical standards

Conflict of interest All authors were involved in the study and contributed substantially to the manuscript. Each of the authors is in agreement with the contents of the manuscript. Prof. dr. Willem-Jan Metsemakers and prof. dr. Stefaan Nijs are consultants for DepuySynthes. Prof. dr. Harm Hoekstra is a member of the lecture bureau of DepuySynthes. The department of Trauma Surgery receives an unrestricted research grant from DepuySynthes and is a training centre for DepuySynthes. All authors hereby disclose any financial and personal relationships with other people or organizations that could inappropriately influence this work.

Ethical standards Given the retrospective nature of the study, the results described did not contain direct research involving Human Participants, nor did it contain research on Animals. The study protocol was conducted following good clinical practice guidelines. The study was approved by the Ethics Committee of the Catholic University Leuven, Belgium.

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