A closer look at fracture healing

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A Closer Look at Fracture Healing

*Fracture healing at the distal radius assessed using
high-resolution peripheral quantitative computed tomography*

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A Closer Look at Fracture Healing

*Fracture healing at the distal radius assessed using
high-resolution peripheral quantitative computed tomography*

PROEFSCHRIFT

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List of abbreviations

μFEA	micro finite element analysis
25(OH)D	25-hydroxyvitamin D
2D/3D	two/three-dimensional
aBMD	areal bone mineral density
AP	anteroposterior
BMI	body mass index
CFR-PEEK	carbon-fiber-reinforced polyetheretherketone
CI	confidence interval
Ct.Pm	cortical perimeter
Ct.Po	cortical porosity
Ct.Po.D	cortical pore diameter
Ct.Po.V	cortical pore volume
Ct.Th	cortical thickness
Dcort	cortical density
Dtot	total density
Dtrab	trabecular density
DXA	dual-energy X-ray absorptiometry
F.Ult	estimated ultimate failure load
FLS	fracture liaison service
HA	hydroxyapatite
HRpQCT	high-resolution peripheral quantitative computed tomography
hsCRP	high-sensitivity C-reactive protein
ICTP	carboxy-terminal telopeptide of type I collagen
LSC	least significant change
PINP	procollagen type-I N-terminal propeptide
PRWE	patient-rated wrist evaluation
PTH	parathyroid hormone
PVE	partial-volume effect
QCT	quantitative computed tomography
QuickDASH	quick disabilities of the arm, shoulder and hand
RMS-CV	root-mean-square coefficient of variation
ROI	region of interest
RSA	radiostereometric analysis
Sbend	bending stiffness
Scomp	compression stiffness
SD	standard deviation
Stors	torsional stiffness

Tb.N	trabecular number
Tb.Sp	trabecular separation
Tb.Th	trabecular thickness
TSH	thyroid stimulating hormone
VAS	visual analogue scale
vBMD	volumetric bone mineral density
VDRP	volar distal radius plate
VF	vertebral fracture

1

General introduction

Scope

One of the most common type of fractures is a fracture of the distal radius. Especially post-menopausal women, in whom the incidence of distal radius fractures is four times higher than in men, are vulnerable: from 1997 to 2009, the annual incidence in The Netherlands of wrist fractures in women aged 50 years or older was 458 per 100.000 persons.¹

Depending on the degree of instability and comminution, distal radius fractures are treated conservatively by cast immobilization, or surgically by internal or external fixation.² When treated by a cast, there is usually sufficient stability of the fracture fragments to remove the cast after 4 (in case of unreduced fractures) to 5 weeks (after reduction).² The clinical outcome of the fracture healing process, however, is not always good and may include persisting pain, stiffness and weakness of the wrist.^{3,4} As patient factors, injury patterns and fracture-related characteristics play only a limited role in predicting outcome after a distal radius fracture⁵⁻⁷, many contributing factors to a poor outcome are still unknown.

In regular daily practice, evaluation of fracture healing is based on clinical examination by the physician in combination with the interpretation of two-dimensional (2D) anteroposterior (AP) and lateral radiographs. These evaluations, however, do not provide detailed information with respect to the consolidation of the bone's cortical and trabecular micro-architecture, or the restoration of bone biomechanical competence. Until recently, *in vivo* assessment of the micro-architecture of bone was not possible in patients due to the lack of accurate measurement techniques and/or technical or radiation issues *in vivo*.

With the introduction of a new low-dose high-resolution peripheral quantitative computed tomography (HRpQCT) technique, the micro-architectural aspects of bone can be studied in normal subjects and patients.^{8,9} Furthermore, the three-dimensional (3D) images that are acquired with HRpQCT can be used for bone strength estimation by micro finite element analysis (μ FEA).^{10, 11} We hypothesized that these techniques can be used to study the fracture healing process in greater detail, and as such may contribute to the understanding of normal fracture healing, bone changes that are related to poor outcome as well as the influence of medication on fracture healing. In this thesis, we studied for the first time the application of HRpQCT to assess the healing process of distal radius fractures.

Fracture healing

Fracture healing is a postnatal regenerative process that allows restoration of the damaged bone to its pre-injury structure and biomechanical function.¹² The results of intense catabolic (inhibition) and anabolic (stimulation) processes in fracture repair are commonly described using a three-stage model. First, immediately after the injury and in parallel to fracture hematoma formation, an inflammatory reaction ensues, triggering the start of the repair process. Second, soft callus is formed by accumulation of chondrocytes, which is later replaced by woven bone by endochondral bone formation, which consolidates the bone fragments, while at the periosteum callus is formed by intramembranous ossification. Third, a long period of bone remodeling takes place in which the structural integrity of the trabecular region and the cortex are remodeled in order to restore the bone's biomechanical properties.^{13, 14} Whereas the first two stages are generally completed within 4 to 5 weeks after fracture, the remodeling stage continues for months to years.¹³ A schematic overview of the healing process of diaphyseal fractures is given in Figure 1.

Figure 1 Schematic overview of the different stages during the healing process of diaphyseal fractures. After an initial inflammatory stage in which a hematoma is formed, soft callus is replaced during the endochondral stage by hard callus, which is then remodeled during the remodeling stage. (Adapted with permission from Macmillan Publishers Ltd: Einhorn et al., copyright 2015.)

For metaphyseal fractures, e.g. distal radius fractures, the repair process is slightly different from diaphyseal fracture healing described above due to the presence of trabecular bone which, in contrast to the cortex, is more easily accessible for cells, nutrients and growth factors. As opposed to diaphyseal fractures, the healing process of metaphyseal fractures is thought to be initiated by cells in the midst of the marrow instead of periosteal cells¹⁵; the role of endochondral bone formation

is limited, thus producing little external callus^{16, 17}; and new woven bone is formed directly on and in between existing trabeculae by intramembranous bone formation (Figure 2).^{15, 17, 18}

Figure 2 Formation of woven bone (arrows) in the marrow cavity (M) between two old trabeculae (T) (left panel), and woven bone connecting the old trabeculae (right panel) in a distal radius fracture at 16 days post-fracture. Woven bone is still being formed (F) at the lower right-hand corner (right panel). (Reproduced from Aspenberg and Sandberg 2013.)

Evaluation of fracture healing

In clinical practice, the evaluation of fracture healing is based on clinical findings and interpretation of conventional radiography. Such findings are subjective, and their validity in quantifying fracture healing and bone strength has therefore been questioned.¹⁹⁻²¹ Radiographic evidence of callus formation is dependent on the bone involved and the type and environment of the fracture.²² As described, cortical bone fractures start healing at the periosteal and endosteal sites, producing plenty of callus (Figure 1). Metaphyseal fractures, such as stable distal radius fractures, on the other hand, tend to heal endosteally, with new bone formation at the surface of and in between existing trabeculae (Figure 2), and less callus formation and less perceptible cortical bridging.^{16, 23} With the introduction of conventional quantitative computed tomography (QCT), volumetric evaluation of bone mineral density (vBMD) and improved three-dimensional (3D) evaluation of fractures became available.²⁴⁻²⁶ However, compared to conventional radiography, clinical CT was not substantially better at determining the repair of distal radius fractures.²⁷

In addition, for research purposes, evaluation of the outcome of distal radius fractures is usually assessed using validated questionnaires, such as the patient-rated wrist evaluation (PRWE).^{28, 29} By the Association of Surgeons of the Netherlands (Nederlandse Vereniging voor Heelkunde, NVvH), the PRWE score is considered as a more important outcome than range of motion or radiological findings.²

High-Resolution peripheral Quantitative Computed Tomography

Recently, high-resolution peripheral QCT (HRpQCT) has been developed. HRpQCT is a 3D quantitative radiographic imaging technique that allows *in vivo* measurement of volumetric BMD (vBMD) and micro-architecture at the peripheral parts of the body, such as the forearm and lower leg.^{9, 30} The main advantage of HRpQCT over clinical CT is its higher resolution. Whereas clinical CT has a nominal resolution of approximately 1 mm, HRpQCT has a nominal resolution of 82 μ m (0.082 mm). Thanks to this high resolution, HRpQCT is able to capture smaller details, such as the individual trabeculae and thin cortices, which is not possible with clinical QCT.³¹ Another advantage of HRpQCT is that scans are made with a low effective dose of 3 μ Sv. Although improvements have recently been made, current disadvantages of HRpQCT in comparison to clinical CT are the limited number of HRpQCT scanners around the world, the longer scan time of 2-3 minutes per 9 mm, and the smaller field of view of 12-14 cm, thus limiting the technique to peripheral parts of the body.

Currently, the only commercially available HRpQCT scanner is the XtremeCT (Scanco Medical A.G. Brütisellen, Switzerland). A standard protocol provided by the manufacturer is available for image acquisition and reconstruction, definition of the region of interest (ROI), extraction of the calcified tissue, i.e. bone, and quantification of vBMD and micro-architecture.

Figure 3 Distal radius scanned by HRpQCT (XtremeCT1, Scanco Medical A.G.). A 9 mm long stack of 110 consecutive 2D slices is acquired at the distal radius, which can be converted into a 3D model to measure cortical (green) and trabecular (red) micro-architecture. In addition, the 3D model can be used in finite element analysis (FEA) to estimate bone strength.

Finite Element Analysis

Finite element analysis (FEA) is a technique that has its origin in the field of civil engineering. By dividing a 3D model of a structure, such as a bridge, into smaller elements, and subsequently assigning material properties to these elements, and imposing appropriate boundary conditions onto the model, mechanical properties of the object can be estimated. The same principle can also be applied onto bone, where a representative 3D model of the bone's micro-architecture can be created by converting each bone voxel of the segmented 3D model into a brick element of the same size (Figure 3).³² Using this approach, Pistoia et al. showed that failure load (strength) of bone specimens can be accurately estimated by micro-FEA (μ FEA) based on high-resolution CT scans.³³ Although constant material properties are used in the μ FEA that is implemented in the standard workflow on the XtremeCT³³, they can also vary with voxel intensity.¹⁰

Objectives

Since plain radiography, which is clinically the gold standard to evaluate fracture healing of stable distal radius fractures, cannot capture micro-architectural changes and quantify the restoration of bone strength, we hypothesized that a better understanding of distal radius fracture healing might be gained by using HRpQCT and μ FEA. Although HRpQCT and μ FEA have been used to study bone quality during aging¹¹, with different disorders³⁴, or after medication^{35, 36}, they have never been applied in a clinical setting to study the repair of fractures. The aim of this thesis, therefore, is to explore the application of HRpQCT in combination with μ FEA to longitudinally study the healing process of stable distal radius fractures.

Outline

This thesis consists of three parts. In the first part (Chapter 2-4), the influence of different plaster materials on HRpQCT parameters (Chapter 2) and the healing process of distal radius fractures as assessed by HRpQCT during the first twelve weeks (Chapter 3 and 4) is studied. Since casts are often used in the treatment of stable distal radius fractures, this could affect the reproducibility of the HRpQCT measurements and/or introduce an error in the bone parameters that are derived from the HRpQCT images. The effect of a plaster-of-Paris and a fiberglass cast is described in **Chapter 2**. Next, the healing process of distal radius fractures as

assessed by HRpQCT is described for the first twelve weeks after fracture. First, in **Chapter 3**, the changes that occur at the fracture site are described qualitatively using the HRpQCT images as well as quantitatively by studying the vBMD, micro-architecture and bone biomechanical parameters during the healing process. Because more detailed 3D information regarding the bone healing process is obtained than with 2D radiographs, we hypothesized that (changes in) these parameters might predict clinical outcome, such as wrist pain and function. Therefore, the relation between early changes in the bone parameters and the PRWE score at twelve weeks post-fracture is studied in **Chapter 4**.

The second part (Chapter 5 and 6) comprises a follow-up of the first part in order to study the long-term changes at both the fractured and non-fractured side. In **Chapter 5**, vBMD, micro-architectural parameters and biomechanical competence at the fracture side were compared to the non-fractured side at two years post-fracture. Additionally, in **Chapter 6**, the two-year changes in vBMD, micro-architecture and biomechanical parameters at the non-fractured side following a distal radius fracture are studied.

In the third and last part (Chapter 7 and 8), two methods that can be used to extend the applicability of HRpQCT to accurately study the healing process of unstable distal radius fractures are described. For accurate longitudinal analysis of bone parameters, it is important that the same region is analyzed. In unstable fractures, fragments may move relative to each other, and this displacement needs to be corrected in order to analyze the same region. A method for overlaying two consecutive HRpQCT scans of a healing fracture is tested in **Chapter 7**. Since internal fixation by distal radius plates is the recommended treatment for unstable distal radius fractures, and these plates are generally made from titanium, which leads to image artifacts, plates made from metal-artifact-free material are required for reliable assessment of bone parameters. In **Chapter 8**, we therefore assessed the effect of a volar distal radius plate made from carbon-fiber-reinforced plastic on the bone parameters obtained from HRpQCT images in comparison to titanium plates.

Finally, **Chapter 9** comprises a discussion of the main findings of the previous chapters, addresses the main limitations of the studies, provides final conclusions and recommendations, and includes opportunities for future research.

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part 1

Assessment of stable distal radius
fracture healing using HRpQCT

2

Effect of casting on short-term reproducibility and bone parameters obtained from HRpQCT measurements at the distal radius

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JP van den Bergh, B van Rietbergen

J Bone Joint Surg Am **98**, 356-62 (2016)

Abstract

Background

High-resolution peripheral quantitative computed tomography (HRpQCT) is a promising tool to assess the fracture healing process at the micro-scale in vivo. Since casts are often used during fracture treatment, they might affect the assessment of bone density, micro-architectural and biomechanical parameters and the short-term reproducibility those parameters, e.g. as a result of beam-hardening. The aim of this study was to assess the effect of a plaster-of-Paris and/or fiberglass cast on bone parameters and on the short-term reproducibility of the HRpQCT measurements of those parameters.

Methods

The effects of a cast on HRpQCT-derived bone parameters were evaluated by comparing HRpQCT scans in fifteen human cadaveric distal radial specimens from one male and fourteen females (median age 84 years [range, 62–90 years] at the time of death) in three conditions: without a cast (control), with a 4-mm-thick plaster-of-Paris cast, and with a fiberglass cast. Short-term reproducibility was assessed using duplicate scans of the distal end of the radius in sixteen healthy volunteers without a fracture (median age 26 years (range 22–39 years); nine men) without and with a fiberglass cast.

Results

Compared with measurements made with no cast, the plaster-of-Paris cast introduced a systematic error in the bone parameters ranging from –2.6% in trabecular separation to –9.8% in cortical thickness. Bone parameters were affected only marginally by fiberglass, with errors between –0.6 to –1.6% in trabecular separation and cortical thickness, respectively. Short-term reproducibility with a fiberglass cast was similar to that with no cast: approximately 1% for bone density parameters, 4 to 5% for micro-architectural parameters and 3 to 4% for the biomechanical parameters.

Conclusion

A plaster-of-Paris cast has a considerable effect on HRpQCT measurements. A fiberglass cast only marginally affects the bone parameters, and the short-term reproducibility of HRpQCT measurements with a fiberglass cast is comparable with that in patients without a cast. In studies on fracture healing using HRpQCT, a fiberglass cast is desirable if immobilization is indicated. The use of a plaster-of-Paris cast should be avoided if possible; however, if not avoidable, corrections after the scan are desirable to adjust for the error introduced in the bone parameters.

Introduction

Distal radius fractures are among the most common types of fractures. Women are particularly vulnerable, with a lifetime risk of a distal radius fracture reported to range between 13% and 21% at 50 years of age.¹ In closed, non-displaced or reduced stable fractures, cast immobilization is the most common treatment.² At routine follow-up of five to six weeks, when fracture consolidation is expected, usually the cast is removed after clinical and radiographic examination.

For osteoporotic patients, clinicians may question whether this standard fracture treatment is optimal for an individual patient. The influences on the healing process due to osteoporosis^{3,4} or medication⁵ suggest that the treatment of osteoporotic fractures may benefit from patient-specific adjustments, as it may take longer before an osteoporotic fracture can be fully loaded. Thus, it may be warranted to have a more detailed evaluation of the fracture healing process and of bone strength recovery than is possible using only radiographs.

High-resolution peripheral quantitative computed tomography (HRpQCT) is a promising tool to assess the fracture healing process at the micro-scale in vivo. In a recent study, we demonstrated that detailed information about changes in bone density, micro-architecture and bone mechanical properties during the healing of stable distal radius fractures can be obtained using high-resolution peripheral quantitative computed tomography (HRpQCT).^{6,7} Because that study was the first in which HRpQCT was used to monitor fracture healing, the accuracy (i.e. closeness to the true value) of the obtained bone parameters and the short-term reproducibility (ability to reproduce similar results, given no real biological change) of the measurements are two important considerations that need to be addressed.

For standard HRpQCT measurements, the accuracy and reproducibility of the bone density, micro-architectural and mechanical parameters have been tested and validated extensively.⁸⁻¹¹ When studying fracture healing of the distal radius, however, the wrist is usually immobilized with a forearm-based cast. Hence, HRpQCT measurements of the distal radius are performed with a cast; therefore, the cast may adversely influence the obtained bone parameters and the reproducibility of those measurements. First, a cast will absorb some radiation, which could lead to unwanted effects such as beam-hardening and increased noise. Thus, for common cast materials, the results might be affected because of these potential deteriorating effects on image quality.¹² Second, the presence of a cast precludes the use of the standard holder⁸ and thus requires a nonstandard cylindrical holder to fixate the forearm immobilized in a cast. In that situation, the cast rather than the forearm is fixated, leaving room for potential movement of the

forearm within the cast. This seemingly less strict fixation could in turn affect the reproducibility of the measurements.

The purpose of this study, therefore, was to quantify the extent to which a cast would affect 1) the bone density, micro-architectural and mechanical parameters obtained from HRpQCT measurements, and 2) the short-term reproducibility of those measurements.

Methods

Material and subjects

To measure the effect of a cast on bone parameters obtained from HRpQCT measurements, fifteen human cadaveric radii (from one male and fourteen female donors with a median age 84.0 years [range, 62–90 years] at the time of death) were imaged by HRpQCT in three conditions: without a cast (control), with a 4-mm-thick plaster-of-Paris cast, and with a fiberglass cast (Figure 1). The radii were obtained from the Department of Anatomy and Embryology of the University of Amsterdam and were preserved in formalin. The history of the donors was unknown and no selection criteria were applied.

Figure 1 Each cadaveric radius was scanned by HRpQCT in three conditions: without a cast (left), with a plaster-of-Paris cast (middle) and with a fiberglass cast (right). With the plaster-of-Paris cast, the image had a higher noise level with lower bone densities. SNR: signal-to-noise ratio

To compare the short-term reproducibility of HRpQCT measurements without and with a cast, sixteen healthy individuals (nine men and seven women with a median age 25.5 years [range, 22–39 years]) without a fracture underwent four separate HRpQCT scans of the distal radius on the dominant side within four hours: two consecutive HRpQCT scans without a cast and two consecutive HRpQCT scans with a fiberglass cast. The forearm of each participant was completely repositioned between each scan. The casts were applied by a professional cast technician.

Because the standard positioning holder that is supplied by the manufacturer could not fit the forearm with a cast, we used a newly designed cylindrical holder for all measurements, i.e. CT Carpal X1 (Pearltec AG, Switzerland). This holder, which was also used in our earlier studies^{6,7}, consists of a cylindrical carbon fiber tube and an inflatable bag with soft liner on the inside (Figure 2), allowing firm fixation of the forearm.

This feasibility study was performed within the framework of a larger fracture healing study approved by our institutional medical ethics committee (registration number NTR3821). All participants were informed and gave written permission for the scans.

Figure 2 Fixation of the forearm with a cast in the CT Carpal X1 holder. This holder consists of a cylindrical carbon tube and an inflatable bag with soft padding of the inside. (Reproduced with permission of Pearltec AG.)

Scanning by HRpQCT

All radii were imaged by HRpQCT (XtremeCT, Scanco Medical AG, Switzerland) at clinical *in vivo* settings recommended by the manufacturer (peak voltage of 60 kVp, tube current of 900 μ A, and 100 ms integration time). A scout view was made before each measurement, to determine the region of interest (ROI). The distal end of the radius proximally from the subchondral plate was chosen as the ROI, because this is the region where a fracture of the radius typically occurs. In the cadaveric radii, the ROI started just below the subchondral plate (Figure 3). In the healthy individuals, the ROI started at an offset of 5 mm from the distal edge of the lunate bone (Figure 3). Because some distal radius fractures might not be fully covered by one standard stack of 110 slices, the scan length was set to 18.04 mm for all measurements. With an isotropic voxel size of 82 μ m, each measurement resulted in 220 parallel CT slices. The total effective radiation dose received per individual was 24 mSv.

After scanning, all HRpQCT images were checked by one operator (J.J.A.d.J.) for motion-induced image artifacts and were quality-graded as described by Pialat et al.¹³ This grading system consists of five grades (ranging from 1, indicating no motion artifacts, to 5, indicating extreme motion artifacts), where grade 1, 2 and 3 are considered to be of sufficient quality because of cortical continuity.¹³

Figure 3 Regions of interest scanned by HRpQCT in the cadaveric radii (left) and in the healthy individuals (right). With a scan length of 18.04 mm, each scan resulted in 220 slices.

Evaluation of bone density and micro-architecture

The HRpQCT images were evaluated using the standard patient evaluation protocol that was provided by the manufacturer and that has been described previously in detail.^{14, 15, 16} The following bone density parameters were calculated from the images: total, trabecular and cortical volumetric bone mineral density (vBMD). Also bone volume to total volume (BV/TV) was calculated for the trabecular region. The micro-architectural parameters that were calculated in the trabecular region were the trabecular number, thickness and separation. For the cortical region, the cortical thickness was obtained.

μ FE analysis

Micro-finite element (μ FE) models were created directly from the segmented HRpQCT images, similar to earlier studies.^{17, 18} In short, each voxel that represents bone tissue was converted into a brick element of the same size, thus creating a representative μ FE model of the micro-architecture of the bone. Equal properties were assigned to every element: a Young's modulus of 10 GPa and a Poisson's ratio of 0.3. By applying a 'high friction' compression test in the axial direction as described by Pistoia et al.¹⁷, compression stiffness and estimated ultimate load were determined.¹⁷

Statistical analysis

Deviation in the bone parameters obtained from the HRpQCT scans made with the plaster-of-Paris and fiberglass cast was reported as the percent difference relative to the measurement without a cast. A repeatedmeasures design was used to compare the effect of both casts on the bone parameters. If the Fratio was significant, a paired ttest with Bonferroni correction was applied to assess whether the difference, compared to the condition without a cast, was significant.

Short-term reproducibility without and with a fiberglass cast was calculated using the duplicate *in vivo* measurements per condition and was reported as the root-mean-square coefficient of variation (RMS-CV) as described by Gluer et al.¹⁹ Wilcoxon signed-rank testing was used to test whether there was a difference in short-term reproducibility of the bone parameters between the conditions with and without a cast.

A significance level of 0.05 was used for all tests. All statistical analyses were performed with SPSS Statistics for Windows version 20.0 (IBM Corp., Armonk, USA).

Results

Errors in bone parameters

The mean bone density, micro-architectural and mechanical parameters (and standard deviation) at the cadaveric radii measured without a cast (control), with a 4-mm plaster-of-Paris cast, and with a fiberglass cast are presented in Table 1. All bone parameters were affected by the presence of a cast (the Fratio for all bone parameters was significant; $p < 0.001$).

The magnitude of the errors in the measurements with a fiberglass cast ranged from 0.6 to 1.6%. The errors in the bone density and mechanical parameters ranged between -0.9 and -1.1% , indicating a slight underestimation of the (cast-immobilized) bone density. Trabecular and cortical thickness were also underestimated, by -1.5 and -1.6% , respectively. Trabecular number was overestimated by 0.7% and trabecular separation was underestimated by -0.6% .

For the plaster-of-Paris cast measurements, the errors were larger for all bone parameters, ranging in magnitude from 2.6 to 9.8% . Again, an underestimation was found for bone density, thickness and biomechanical parameters, whereas there was an overestimation for trabecular number. For the bone density parameters, the errors ranged from -3.4 to -5.3% . For trabecular and cortical thickness, the errors were -6.5 and -9.8% , and the errors in compression stiffness and estimated ultimate failure load were -7.5 and -6.6% . The error in trabecular number was

Table 1 Means and standard deviations of the bone density, micro-architectural and mechanical parameters at the cadaveric distal radii measured without a cast (control), with a plaster-of-Paris cast and with a fiberglass cast.

	No cast (control)	Plaster-of-Paris cast	Plaster-of-Paris versus control (%)	Fiberglass cast	Fiberglass versus control (%)	Plaster-of-Paris versus fiberglass (%)
Dtot (mg HA/cm ³)	183.1 ± 65.1	173.5 ± 61.9	-5.3 ^c	181.0 ± 64.8	-1.1 ^c	-4.2 ^c
Dcort (mg HA/cm ³)	596.0 ± 101.5	565.6 ± 101.8	-5.1 ^c	590.6 ± 103.2	-0.9 ^c	-4.2 ^c
Dtrab (mg HA/cm ³)	114.9 ± 51.7	110.8 ± 49.2	-3.5 ^c	113.7 ± 51.4	-1.0 ^c	-2.6 ^c
BV/TV (-)	0.096 ± 0.043	0.092 ± 0.041	-3.4 ^c	0.095 ± 0.043	-1.0 ^b	-2.4 ^c
Tb.N (mm ⁻¹)	1.30 ± 0.31	1.33 ± 0.33	+3.0 ^c	1.30 ± 0.32	+0.7 ^b	+2.4 ^c
Tb.Th (mm)	0.072 ± 0.019	0.067 ± 0.017	-6.5 ^c	0.070 ± 0.019	-1.5 ^c	-5.1 ^c
Tb.Sp (mm)	0.747 ± 0.220	0.727 ± 0.213	-2.6 ^c	0.742 ± 0.218	-0.6 ^b	-2.0 ^c
Ct.Th (mm)	0.34 ± 0.18	0.31 ± 0.17	-9.8 ^c	0.33 ± 0.18	-1.6 ^a	-8.4 ^c
S.comp (kN/mm)	32.1 ± 19.0	30.0 ± 18.1	-7.5 ^c	31.8 ± 18.7	-1.1 ^b	-6.5 ^c
F.Ult (kN)	3.19 ± 1.81	2.98 ± 1.76	-6.6 ^c	3.16 ± 1.80	-1.0 ^c	-5.6 ^c

Abbreviations: Dtot = total bone mineral density; Dcort = cortical bone mineral density; Dtrab = trabecular bone mineral density; BV/TV = bone to total volume; Tb.N = trabecular number; Tb.Th = trabecular thickness; Tb.Sp = trabecular separation; Ct.Th = cortical thickness; S.comp = compression stiffness; F.Ult = estimated ultimate failure load.

A repeated-measures design was used to compare the effect of a cast on the bone parameters. Since the F-ratio was significant for all bone parameters ($p < 0.001$), the p-values for the differences between the conditions were obtained from a paired t-test with Bonferroni correction applied. Percent differences are significant at ^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$.

3.0%. The smallest error in the plaster cast measurements was -2.6% for trabecular separation.

Short-term reproducibility

Characteristics of the healthy individuals and the bone parameters measured at the distal radius without a cast are listed in Table 2. From the 64 initial measurements, seven measurements (11%), six done without a cast and one with a cast, were of insufficient image quality because of motion artifacts. From the 32 measurements without a cast, eleven scans were grade 1; nine scans grade 2; six scans grade 3, five scans grade 4; and one scan grade 5. The grade-5 scan contained a large amount of extreme motion artifacts, because the participant tried to remove his forearm from the positioning holder during scanning. Therefore, this scan was excluded from short-term reproducibility calculation. From the 32 measurements with a cast, eight scans were grade 1; thirteen scans grade 2; ten scans grade 3; and one scan grade 4.

Table 2 Characteristics of the sixteen healthy individuals and the bone density, micro-architectural, and biomechanical parameters [mean (standard deviation)] measured at the distal end of the radius without a cast

Variable	Finding
Sex (M/F)	9/7
Age* (years)	25.5 (7.0)
BMI (kg/m ²)	23.5 (2.5)
Dtot (mgHA/cm ³)	332.3 (56.8)
Dcort (mgHA/cm ³)	865.0 (37.4)
Dtrab (mgHA/cm ³)	171.7 (51.1)
BV/TV (-)	0.143 (0.043)
Tb.N (mm ⁻¹)	1.96 (0.33)
Tb.Th (mm)	0.072 (0.012)
Tb.Sp (mm)	0.451 (0.094)
Ct.Th (mm)	0.86 (0.16)
S.comp (kN/mm)	47.1 (11.0)
F.Ult (kN)	4.64 (1.08)

* Reported as median and interquartile range. Abbreviations: BMI = body mass index; Dtot = total bone mineral density; Dcort = cortical bone mineral density; Dtrab = trabecular bone mineral density; BV/TV = bone to total volume; Tb.N = trabecular number; Tb.Th = trabecular thickness; Tb.Sp = trabecular separation; Ct.Th = cortical thickness; S.comp = compression stiffness; F.Ult = estimated ultimate failure load.

RMS-CVs for the short-term reproducibility without wearing a cast were found to be generally between 0.6% and 4.0% (Table 3). Whereas CVs of density parameters varied between 0.6% (total BMD) and 1.1% (cortical BMD), the CVs for structural parameters were higher: 1.7% for cortical thickness and between 3.5% (trabecular

thickness) and 4.0% (trabecular separation) for the trabecular structural indices. The biomechanical parameters compression stiffness and estimated ultimate failure load showed CVs of 3.3 and 3.7%, respectively. Short-term reproducibility was not significantly different with a fiberglass cast for any of the bone parameters, except for cortical thickness. In general, the CVs for the bone density parameters were slightly lower when a fiberglass cast was worn (0.5 to 0.7%) than when one was not worn (0.6 to 1.1%). Except for cortical thickness, the CVs for the bone micro-architectural and biomechanical parameters were slightly higher when a fiberglass cast was worn (3.7 to 5.3%) than when one was not worn (3.3 to 4.0%). However, these differences did not reach statistical significance (Table 3). Only the CV of cortical thickness was significantly lower when a fiberglass cast was worn ($p = 0.028$).

Table 3 Short-term reproducibility (RMS-CV and 95% confidence intervals) of HRpQCT derived bone parameters measured *in vivo* without and with the presence of a fiberglass cast.

	Without fiberglass cast (n=15)	With fiberglass cast (n=16)	
	RMS-CV (%)	RMS-CV (%)	p-value
Dtot	1.10 (0.60 to 2.64)	0.66 (0.37 to 1.54)	0.061
Dcort	0.60 (0.33 to 1.45)	0.47 (0.26 to 1.09)	0.191
Dtrab	1.01 (0.55 to 2.43)	0.64 (0.35 to 1.48)	0.100
BV/TV	1.12 (0.61 to 2.68)	0.68 (0.38 to 1.58)	0.062
Tb.N	3.84 (2.09 to 9.18)	5.30 (2.94 to 12.3)	0.776
Tb.Th	3.52 (1.92 to 8.41)	5.13 (2.84 to 11.9)	0.347
Tb.Sp	3.95 (2.16 to 9.46)	5.31 (2.94 to 12.3)	0.955
Ct.Th	1.66 (0.90 to 3.96)	0.90 (0.50 to 2.10)	0.028
S.comp	3.33 (1.82 to 7.97)	3.72 (2.06 to 8.60)	0.756
F.Ult	3.72 (2.03 to 8.90)	4.36 (2.42 to 10.1)	0.877

Abbreviations: Dtot = total bone mineral density; Dtrab = trabecular bone mineral density; Dcort = cortical bone mineral density; BV/TV = bone to total volume; Tb.N = trabecular number; Tb.Th = trabecular thickness; Tb.Sp = trabecular separation; Ct.Th = cortical thickness; S.comp = compression stiffness; F.Ult = estimated ultimate failure load. P-values were obtained from a Wilcoxon signed-rank test.

Discussion

The present study evaluated the effect of a cast on bone density, micro-architectural and biomechanical parameters obtained from HRpQCT measurements at the distal end of the radius, and the short-term reproducibility of those parameters.

Errors in bone parameters

The results suggest that both types of casts introduced systematic errors in the bone parameters. However, the differences when a fiberglass cast was worn and when

no cast was worn were very small (approximately 1%). Although significant, those differences may not be clinically relevant, in particular when the reproducibility error and the fact that changes during fracture healing are generally in the range of 10% to 20% are considered.⁶

The use of plaster of Paris, on the other hand, can considerably bias measurement results. Compared with scanning without a cast, many parameters deviated by 5% to almost 10%. Admittedly, such a bias might be acceptable in follow-up studies that aim at quantifying changes in parameters over time. Additionally, depending on whether a plaster-of-Paris cast is in place or removed, it presumably would be possible to correct the calculated bone parameters according to the percentages presented in Table 1. However, these values were obtained in cadaveric radii, with low bone density (because of old age), that were not scanned at the standard region. Also, we did not vary the thickness of the plaster-of-Paris cast. Since the thickness of plaster-of-Paris casts might differ between patients, the errors in the bone parameters caused by the plaster-of-Paris casts might vary as well. Hence the presently reported percentages are not immediately applicable to the general patient population in the clinical setting and should at least be adjusted proportionally to the thickness of the casts used on patients.

The difference between the effect caused by a fiberglass cast and a plaster-of-Paris cast can be explained by the different material properties of the two casts (see Appendix). At the standard settings of the XtremeCT, plaster of Paris absorbs more lower-energy photons than fiberglass does²⁰, resulting in underestimation of the bone density and increased beam-hardening artifacts and noise (Figure 1). These effects were confirmed by phantom measurements with and without a plaster-of-Paris cast (results not shown). Due to the beam-hardening effect and the increased noise, segmentation of the trabecular structure apparently results in a 3D model with more, but thinner, trabeculae and a lower estimated bone stiffness and strength.

Short-term reproducibility

We found that when HRpQCT and the non-standard cylindrical holder (without a cast) were used, bone density parameters can be assessed with high precision (RMS-CVs of 0.6% to 1.1%) and micro-architecture and biomechanical parameters with good precision (RMS-CVs of 1.7% to 4.0%). Similar values have been reported in literature using the standard holder and no cast.^{8, 9} Surprisingly, short-term reproducibility improved slightly when the wrist was immobilized by a cast. Presumably, the cast leads to a better fixation of the forearm in the cylindrical holder, resulting in fewer motion artifacts.

It should be noted that the reproducibility measured in this study was obtained from HRpQCT measurements that require twice as much scanning time due to increased scan length compared to standard HRpQCT measurements, i.e. 5.6 minutes and 18 mm versus 2.8 minutes and 9 mm, respectively. As with longer scan times, the chance of motion-induced artifacts increases, and an increased scan time in principle could negatively affect the reproducibility. However, the increased scan time in our study did not decrease the short-term reproducibility of the measurements. A possible explanation for this is that the cylindrical holder with its inflatable bag better fixated the forearm than the standard holder used in other studies.

For the clinical application of HRpQCT to monitor fracture healing, it is important to know the precision of the HRpQCT measurements (with a cast), since this determines the change in bone parameters that can be considered significant (expressed as the least significant change (LSC)).²¹ Given the measured short-term reproducibility in this study and a statistical confidence level of 95%, the LSC for the bone density parameters is approximately 2%. The LSC for the micro-architectural parameters was 14% and 11% for the biomechanical parameters. When these LSC values were compared with the changes that we found in our previously reported study on fracture healing⁶, we notice that the observed changes in bone parameters are well above these LSCs. For example, cortical and trabecular bone density changed -4.1% and 20%, respectively, at six to eight weeks post-fracture. For the structural parameters, trabecular thickness changed by 30% at twelve weeks post-fracture, and at the same time, biomechanical parameters increased by 31%.⁶ Hence, the precision of HRpQCT measurements with a cast is good enough to reliably detect changes in bone parameters typically occurring during the fracture healing process.

Limitations

Reproducibility of a measurement is ideally assessed in a group of subjects similar to the group in which the measurement will be performed, in this instance osteoporotic women over 50 years of age with a distal radius fracture. As mentioned before, we used healthy individuals between 22 and 39 years old. Nevertheless, recent data have indicated that differences in reproducibility between age groups are small, and that reproducibility does not necessarily decrease with age.²²

Another limitation is the modest number of individuals in the reproducibility group, and hence, the large confidence intervals of the RMS-CV.

In addition, the reproducibility study was performed with a fiberglass cast only and not a plaster-of-Paris cast. However, it seems unlikely that motion artifacts, which are the major source of reproducibility errors, would differ between the two cast materials.

Last, the cadaveric radii were scanned without soft tissue surrounding the bone and in the absence of the ulna. Although we did use a repeated-measures design, this extra tissue would have absorbed some of the radiation and the results might be different in intact forearms.

Conclusion

In conclusion, bone parameters obtained from HRpQCT measurements at the distal radius and the short-term reproducibility of these measurements are only marginally affected by a fiberglass cast. However, a considerable bias in the measurement results is to be expected when a plaster-of-Paris cast is used so corrections ideally should be made after scanning. On the basis of these results, we recommend immobilization with a fiberglass cast if possible when monitoring distal radius fracture healing with HRpQCT. Furthermore we advocate wearing the cast during all follow-up measurements to maintain the same scanning conditions.

Appendix

Explanation for the different magnitude of errors in bone parameters introduced by the fiberglass and plaster-of-Paris cast

The difference between the effect caused by a fiberglass cast and a plaster-of-Paris cast can be explained by the different material properties of the two casts. At the standard settings of the XtremeCT, the mean energy of the photons is 40 keV. At this energy, calcium sulfate, the main component of plaster-of-Paris cast, has a linear Xray attenuation coefficient μ of 2.07 cm^{-1} . The main component of a fiberglass cast is silica, which has a μ of 1.23 cm^{-1} at 40 keV.²⁰ Due to its higher μ , the plaster-of-Paris cast absorbs more lower-energy photons than fiberglass, resulting in an underestimation of the bone density.

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3

Assessment of the healing process in distal radius fractures by high-resolution peripheral quantitative computed tomography

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Abstract

Background

In clinical practice, fracture healing is evaluated by clinical judgment in combination with conventional radiography. Due to limited resolution, radiographs don't provide detailed information regarding the bone micro-architecture and bone strength. Recently, assessment of *in vivo* bone density, architectural and mechanical properties at the micro-scale became possible using high-resolution peripheral quantitative computed tomography (HRpQCT) in combination with micro finite element analysis (μ FEA). So far, such techniques have been used mainly to study intact bone. The aim of this study was to explore whether these techniques can also be used to assess changes in bone density, micro-architecture and bone stiffness during fracture healing.

Methods

Therefore, the fracture region in eighteen women, aged 50 years or older with a stable distal radius fracture, was scanned using HRpQCT at one to two (baseline), three to four, six to eight and twelve weeks post-fracture. At one to two and twelve weeks post-fracture the distal radius at the contra-lateral side was also scanned as control. Standard bone density, micro-architectural and geometric parameters were calculated and bone stiffness in compression, torsion and bending was assessed using μ FEA. A linear mixed-effect model with time post-fracture as fixed effect was used to detect significant (p -value ≤ 0.05) changes from baseline. Wrist pain and function was scored using the patient-rated wrist evaluation (PRWE) questionnaire. Correlations between the bone parameters and the PRWE score was calculated by Spearman's correlation coefficient.

Results

At the fracture site, total and trabecular bone density increased by 11% and 20%, respectively, at six to eight weeks, whereas cortical density was decreased by 4%. Trabecular thickness increased by 23% and 31% at six to eight and twelve weeks and the intertrabecular area became blurred, indicating intertrabecular bone formation. Compared to baseline, calculated bone stiffness in compression, torsion and bending were increased by 31% after twelve weeks. A moderate negative correlation was found between the stiffness and the PRWE score. No changes were observed at the contra-lateral side.

Conclusion

The results demonstrate that it is feasible to assess clinically relevant and significant longitudinal changes in bone density, micro-architecture and mechanical properties at the fracture region during the healing process of stable distal radius fractures using HRpQCT.

Introduction

Fragility fractures of the distal radius are one of the most common fractures. The incidence is about 17% of all fractures.¹ Although not always acknowledged, the outcome of distal radius fractures is not uniformly good. Poor outcomes have been attributed to poor restoration of anatomy and secondary loss of reduction after an initially adequate reduction², leading to discomfort, loss of range of motion and/or soft tissue complaints.³⁻⁵

It is expected that the addition of medication or supplements, such as vitamin D or calcium can improve the clinical outcome of distal radius fractures.⁶ To monitor the process of fracture healing in patients, who either received supplemental medication or not, a method that is able to evaluate bone healing in detail is necessary. In clinical practice, fracture healing is evaluated by clinical judgment of the physician in combination with plain anteroposterior (AP) and lateral radiographs. However, these evaluations do not provide detailed information with regard to the healing process and consolidation of fractures on the level of cortical and trabecular bone micro-architecture.

Until recently, human bone micro-architecture could only be studied with bone biopsies, i.e. with 2D histomorphometry or 3D microCT, since imaging techniques that can provide sufficient resolution for bone structure *in vivo* were not available. Therefore, *in vivo* high-resolution imaging studies on fracture healing were restricted to animals.^{7,8} Classical QCT proved to be a successful estimator for the prediction of the mechanical stability of long bones⁹, but was not sufficient to study fracture healing in the distal radius in patients because of insufficient resolution to visualize individual trabeculae.¹⁰

With the development of a new low-dose radiation high-resolution peripheral quantitative computed tomography (HRpQCT) technique, i.e. XtremeCT, it is possible to assess *in vivo* bone density and architecture at the microscale.¹¹⁻¹³ Moreover, biomechanical properties of the bone can be calculated by micro finite element analysis (μ FEA) based on such HRpQCT images.^{11, 14, 15}

So far, these high-resolution imaging techniques were mainly used to study micro-architectural changes due to ageing^{16, 17}, osteoporosis and other (bone) diseases^{15, 18, 19} and the effect of different treatments²⁰⁻²³. These techniques, however, could be applied as well to analyze the process of bone healing after a distal radius fracture. Potentially, such analyses could provide a new tool to assess fracture consolidation based on bone morphology or μ FEA derived mechanical parameters. In a recent study, Mueller et al.²⁴ investigated the feasibility of using these techniques to assess fracture healing *in vivo* and found an 18% increase in calculated stiffness over a period of two months. However, in that study biomaterials

and plates were used to stabilize the fracture. The application of these techniques to fracture healing without any intervention, other than a standard cast, however, has not been demonstrated and it remains unclear to what extent the healing process can be captured by the standard morphological and mechanical parameters.

The objective of this study was, therefore, to explore the feasibility of HRpQCT in combination with μ FEA to assess longitudinal changes in bone density, micro-architecture and biomechanical parameters during the first twelve weeks of healing of conservatively treated distal radius fractures.

Materials and Methods

Subjects

Twenty women aged 50 years or older with a stable distal radius fracture, which was immobilized by a cast, were included in this study. All patients were included at the departments of Orthopedics and Traumatology of the Maastricht University Medical Center, the Netherlands. The exclusion criteria were: a history of previous fractures at the fractured side; known systemic or metabolic disorders leading to progressive bone deterioration; use of glucocorticoids; presence of an active inflammatory disease; presence of an active or suspected infection; or malignancy in the last twelve months pre-fracture.

To assess changes in bone density, micro-architecture, geometry and biomechanics, all patients underwent HRpQCT scanning of the fractured distal radius during four visits. The visits were scheduled one to two weeks post-fracture (baseline) and three to four weeks, six to eight weeks and twelve weeks post-fracture. During the first and last visit, the radius at the contra-lateral side was scanned as well. The protocol (registration number NTR3821) was approved by an independent Medical Ethics Committee, and all patients gave written informed consent prior to participation.

Scanning by HRpQCT

During each visit, the fractured radius was imaged by HRpQCT (XtremeCT, Scanco Medical AG, Switzerland) using clinical *in vivo* settings by the manufacturer (effective energy of 60 kVp, tube current of 900 μ A and 100 ms integration time). The region of interest (Figure 1) was based on AP and lateral radiographs of the fractured radius, in which the proximal edge of the lunate was used as reference. To make sure the complete fracture was scanned, the scan length was set to 18 mm. With an isotropic voxelsize of 82 μ m, each HRpQCT measurement thus resulted in 220 parallel CT slices. The effective dose of each HRpQCT measurement at the fracture side was

less than 6 mSv. Because the patient's forearm was fixed in a cast, the forearm with the cast was placed in a custom cylindrical carbon holder with an inflatable cushion (Pearltec AG, Schlieren, Switzerland) to minimize patient motion. To scan the radius at the contra-lateral side, standard clinical *in vivo* settings by the manufacturer were used and resulted in one stack containing 110 parallel CT slices with an isotropic voxelsize of 82 μ m. The offset at the contra-lateral side was fixed for all patients and was chosen 9.5 mm from the proximal edge of the lunate. The volume between 9.5 and 18.5 mm from the lunate was thus scanned at the contra-lateral side. The same holder as for the fractured forearm was used to fixate the contra-lateral side.

Figure 1 Region of interest at the fracture side (left) and the contra-lateral side (right). The scan length at the fracture side is set to 18 mm and contains 220 parallel HRpQCT slices, while the scan length at the contra-lateral side is set to 9 mm and contains 110 parallel HRpQCT slices. The offset at the fracture side varied between subjects, while the offset at the contra-lateral side was kept constant at a distance of 9.5 mm from the proximal edge of the lunate.

Each scan was checked for motion induced image artifacts and was quality graded by the operator according to the manufacturer's guidelines and as described by Pialat et al.²⁵ In case such artifacts occurred and thus resulted in images of insufficient quality, i.e. grade 4 or 5, the scan was repeated.

Evaluation of bone density, micro-architecture and geometry

The HRpQCT images were evaluated using the standard patient evaluation protocol provided by the manufacturer, which has been described earlier in detail.²⁶ In short, the cortical and trabecular region were separated first using a semi-automated contouring scheme in which the periosteal boundary surface of the radius was derived.²⁷ After contouring, a Laplace-Hamming filter (epsilon 0.5 and cut-off frequency 0.4) followed by normalization (range 0–1000) and global thresholding (threshold 400), was used in order to extract the voxels that represented mineralized bone and to create the segmented images. The used values were default values

specified by the manufacturer and these are consistently used for all HRpQCT scanners.

The following bone density parameters were calculated from the images: volumetric bone mineral density [mgHA/cm³] was assessed for the total region (Dt_{tot}) and the trabecular (D_{trab}) and cortical region (D_{cort}) separately.

The micro-architectural parameters that were used in this study were: the trabecular number (Tb.N) [1/mm], thickness (Tb.Th) [mm] and separation (Tb.Sp) [mm], which are all determined using a 3D ridge extraction method²⁸ and standard morphological relations. Bone geometry was expressed by the cortical thickness (Ct.Th) [mm], which is calculated by dividing the cortical volume by the outer cortical surface, and the cortical perimeter (Ct.Pm) [mm], which is the average outer perimeter of the cortex.

μFE analysis

With the standard approach for μFE analyses as used in most earlier studies, segmented images are used to create a representative μFE model of the bone's micro-architecture by converting each voxel that represents bone tissue into a brick element of the same size. Material properties then are chosen constant for all bone tissue¹⁶, or varied only between cortex and trabecular bone tissue.¹⁵ Whereas this approach has been well validated and successfully applied in clinical studies, its application to fractured bone is not trivial. The use of segmented images clearly limits the models to only represent the mineralized phase. In particular in the early stages of fracture healing this approach is expected to be inaccurate because it does not account for newly formed low mineralized bone. In an earlier study, Shefelbine et al. therefore introduced a more sophisticated approach that can account for the stiffness of less mineralized tissues as well.⁸ With their approach, three different tissue types are distinguished (soft tissue, low mineralized tissue and high mineralized tissue) and the stiffness of the elements representing the mineralized tissue was made dependent on its material composition.

In the present study, we applied both the standard approach, which is validated for intact bone, and the approach proposed by Shefelbine et al., which is validated for healing bone. Since the method of Shefelbine et al. was originally developed for micro-CT data with a higher resolution, it was slightly adapted to apply it to the HRpQCT data used in this study. In short, the grayscale bone density images were thresholded to define three separate regions: soft tissue, newly mineralized bone that had less mineral content, and highly mineralized bone using thresholds of 0–4000, 4001–5750 and 5751–max. (2-byte signed short-integer values, equal to <343, 343–642 and >642 mgHA/cm³), respectively. Elastic moduli were assigned to each region in agreement with Shefelbine et al.: 50 MPa for soft-tissue, 5000

MPa for the newly mineralized bone, and 15000 MPa for highly mineralized bone. Following, the model was downscaled by a factor two and each downscaled voxel was converted into a brick element, such that the final element size was 164 mm, which is similar to the element size used in the study by Shefelbine et al. (200 mm). In the resulting μ FE model soft tissue was assigned a constant modulus of 50 MPa whereas for mineralized bone a Young's modulus ranging from 5000 to 15000 MPa results, depending on the material composition of the downscaled voxel (Figure 2). A Poisson's ratio of 0.3 was assigned to all elements and all materials were assumed to be linear elastic and isotropic.

Figure 2 Creation of the voxel finite element models from HRpQCT scans based on Shefelbine's method. From the grayscale images, three regions representing soft tissue and low and high mineralized bone were segmented. The elastic modulus was linearly scaled to the gray value for the bone regions resulting in moduli ranging from 5000 to 15000 MPa. A modulus of 50 MPa was assigned to all soft tissue. (Left: One to two weeks post-fracture, right: six to eight weeks post-fracture).

With the standard μ FE approach, a procedure similar to that described in earlier studies was used and all elements were assigned a Young's modulus of 10 GPa and a Poisson's ratio of 0.3.^{16, 29, 30}

By subsequently applying different load cases, clinically relevant loading scenarios were simulated and the associated stiffness was chosen as outcome variable. The first load case represented a 'high friction' compression test with a prescribed displacement in the axial direction of 1% of the total length, from which the compression stiffness (S_{comp}) [kN/mm] was calculated. The second load case represented a prescribed rotation of 0.01 rad around the longitudinal axis applied to the surface perpendicular to this axis from which the torsional stiffness (S_{tors})

[kNmm/rad] was calculated. A third and fourth load case represented a prescribed rotation of 0.01 rad applied around the sagittal and transversal axes respectively, onto the surfaces perpendicular to each axis, thus inducing a state of pure bending in two directions, from which the bending stiffness in each direction (S_{sagittal} and $S_{\text{transversal}}$) was calculated. Because the orientation of the patients' forearm was not exactly the same during all visits, a quadratic mean bending stiffness was calculated (S_{bend}) [kNmm²/rad] from the sagittal and transversal bending stiffness according to Equation 1.

$$S_{\text{bend}} = \sqrt{\frac{S_{\text{sagittal}}^2 + S_{\text{transversal}}^2}{2}} \quad (\text{Equation 1})$$

Clinical and radiological outcome

Pain and function were measured using a validated Dutch version of the Patient-Rated Wrist Evaluation (PRWE) questionnaire. The PRWE questionnaire consists of ten questions and its score ranges from 0 (no pain/no difficulty) to 100 (worst pain/disability). It has previously been shown that PRWE is a reliable and valid measurement tool after distal radius fractures.^{31, 32}

Based on AP and lateral radiographic images of the fractures, the state of consolidation was assessed at each visit by an experienced orthopedic surgeon. The state of consolidation was defined as none when there was no sign of cortical bridging or increased radio density along the fracture line; as incomplete when there was increased radio density along the fracture line and starting cortical bridging was observed; and as complete when fading of the initial fracture line was seen in combination with cortical bridging.³³

Statistics

The Shapiro-Wilk test was used to test if the bone parameters were normally distributed at all visits. Non-normally distributed parameters were log-transformed. Then, a linear mixed effect model with the time post-fracture as fixed effect was used to test if the bone parameters at the follow-up visits were significantly different from baseline. The bone density, architectural and mechanical parameters were expressed as estimated marginal means with 95% confidence intervals at each visit. Percent change from baseline in each parameter was calculated by dividing the difference from baseline by the mean value at baseline. Spearman's correlation coefficient was used to measure the correlation between the bone parameters and the clinical and radiological outcome. Statistical analyses were performed with SPSS Statistics for Windows version 20.0 (IBM Corp., Armonk, USA).

Results

From the twenty patients who were initially included in the study, one patient declined participation at the first visit and another patient at the second visit. Their results were not included in this study. The characteristics of the remaining eighteen patients are presented in Table 1.

Table 1 Descriptive baseline characteristics of the eighteen women that completed all study visits

	Mean $\pm$ Std. Dev. (n = 18)
Age [years]	64 $\pm$ 8
Weight [kg]	70 $\pm$ 8
BMI [kg/m ²]	27 $\pm$ 4
Time since menopause [years]	13 $\pm$ 9
T-score total hip [-]	-1.4 $\pm$ 1.1
T-score lumbar spine [-]	-2.4 $\pm$ 1.5
25(OH)D level [nmol/L]	63 $\pm$ 29
25(OH)D level < 50 nmol/L [n]	7 (39%)

BMI = body mass index; 25(OH)D = 25-hydroxyvitamin D

Figure 3 Overview of HRpQCT scans that were included in the evaluation of the bone density, micro-architectural, geometrical and biomechanical parameters. With a maximum of one repeated measurement in case motion artifacts occurred, 83% of the scans at the fracture side and 75% of the scans at the contra-lateral side were finally of sufficient quality.

From the scans in the eighteen patients, 40 out of a total of 108 scans (37%) were initially of insufficient quality due to motion artifacts: 30 out of 72 scans at the

fracture side and 10 out of 36 at the contra-lateral side. After repeating these scans, still 19% of the scans were of insufficient quality due to motion-induced image artifacts. These scans were not included in the analyses, thus in total 87 scans were used for the evaluation of the bone parameters (Figure 3).

Visual assessment

The sequential HRpQCT images showed a common pattern at the fracture region over time (Figure 4). In general, the fracture line was clearly visible at one to two weeks post-fracture: the cortex was disrupted on several locations and the trabeculae along the fracture line were disrupted. At three to four weeks post-fracture, the trabecular region became blurred, i.e. the individual trabeculae were hard to distinguish. This blurring in the trabecular region was more

Figure 4 Anteroposterior (AP) and lateral radiographs at baseline and HRpQCT slices and 3D models at baseline and follow-up visits of an extra-articular (top) and an intra-articular (bottom) fracture that were included in this study. Compared to the radiographs, the initial fracture is much more detailed visible in the HRpQCT slices and the 3Dmodels. Furthermore, the increase in density in the trabecular region as well as the bridging of the cortex (arrows) is very well visualized in the HRpQCT slices and 3D models (zoom-in).

Table 2 Estimated marginal means (EMM) and their 95% confidence intervals (CI) for the bone parameters at each visit and the percent change from baseline at visit two, three and four. (n = 18)

	Visit 1 / Baseline (1.3 ± 0.4 wks post-fx)			Visit 2 (3.4 ± 0.5 wks post-fx)			Change from baseline (%)			Visit 3 (6.3 ± 0.8 wks post-fx)			Change from baseline (%)			Visit 4 (12.2 ± 0.4 wks post-fx)			Change from baseline (%)			p
	EMM	95% CI		EMM	95% CI		p	EMM	95% CI		p	EMM	95% CI		p	EMM	95% CI		p	EMM	95% CI	
Fracture side																						
Dtot	[mgHA/cm ³]	232	204 - 259	243	218 - 268		4.9	0.038	256	230 - 282		10.5	0.010	253	227 - 278		9.1	0.144				
Dtrab	[mgHA/cm ³]	146	131 - 161	160	146 - 174		9.5	0.005	176	159 - 193		20.4	0.000	164	146 - 182		12.1	0.153				
Dcort	[mgHA/cm ³]	646	588 - 705	628	570 - 685		-2.9	0.000	620	569 - 671		-4.1	0.040	613	567 - 659		-5.2	0.052				
Tb.N	[mm ⁻¹]	1.96	1.74 - 2.19	2.01	1.78 - 2.24		2.2	1.000	1.91	1.73 - 2.10		-2.5	1.000	1.67	1.46 - 1.89		-14.8	0.071				
Tb.Th †	[mm]	0.063	0.055 - 0.071	0.068	0.060 - 0.076		7.4	0.205	0.078	0.068 - 0.088		23.0	0.001	0.082	0.073 - 0.092		30.5	0.002				
Tb.Sp †	[mm]	0.465	0.404 - 0.528	0.442	0.396 - 0.490		-4.9	0.768	0.462	0.410 - 0.515		-0.7	1.000	0.545	0.461 - 0.635		17.3	0.271				
Ct.Th †	[mm]	0.39	0.29 - 0.49	0.39	0.30 - 0.48		0.7	1.000	0.41	0.32 - 0.51		6.4	1.000	0.44	0.35 - 0.53		12.9	0.390				
Ct.Pm †	[mm]	87.1	79.0 - 95.9	86.8	79.5 - 94.8		-0.3	1.000	84.7	78.2 - 91.7		-2.7	0.512	83.3	77.0 - 90.1		-4.3	0.090				
Scomp	[kN/mm]	16.5	12.4 - 20.6	15.5	11.6 - 19.4		-6.2	0.527	16.7	12.4 - 20.9		0.9	1.000	21.7	17.3 - 26.0		31.3	0.008				
Stors	[kNmm/rad]	476	407 - 545	438	373 - 502		-8.0	0.274	481	403 - 560		1.1	1.000	626	551 - 700		31.5	0.001				
Sbend	[kNmm ² /rad]	677	552 - 801	614	498 - 730		-9.3	0.332	650	518 - 782		-4.0	1.000	888	759 - 1017		31.3	0.005				
Contra-lateral side																						
Dtot	[mgHA/cm ³]	247	206 - 288											248	207 - 289		0.4	0.780				
Dtrab	[mgHA/cm ³]	103	81 - 124											100	79 - 122		-2.7	0.384				
Dcort	[mgHA/cm ³]	795	753 - 838											798	753 - 844		0.4	0.525				
Tb.N	[mm ⁻¹]	1.46	1.24 - 1.69											1.46	1.24 - 1.69		-0.1	0.939				
Tb.Th	[mm]	0.058	0.052 - 0.064											0.056	0.051 - 0.061		-2.7	0.138				
Tb.Sp	[mm]	0.714	0.534 - 0.894											0.710	0.549 - 0.871		-0.6	0.823				
Ct.Th	[mm]	0.63	0.52 - 0.74											0.64	0.52 - 0.76		1.2	0.595				
Ct.Pm	[mm]	69.6	66.3 - 72.9											69.6	66.3 - 73.0		0.0	0.946				
Scomp	[kN/mm]	67.2	56.8 - 77.6											67.8	57.3 - 78.4		1.0	0.487				
Stors	[kNmm/rad]	1470	1226 - 1713											1484	1217 - 1752		1.0	0.689				
Sbend	[kNmm ² /rad]	2533	2103 - 2963											2497	2060 - 2934		-1.4	0.458				

Dtot = total density; Dtrab = trabecular density; Dcort = cortical density; Tb.N = trabecular number; Tb.Th = trabecular thickness; Tb.Sp = trabecular separation; Ct.Th = cortical thickness; Ct.Pm = cortical perimeter; Scomp = compression stiffness; Stors = torsional stiffness; Sbend = bending stiffness.

† Estimated marginal means. 95% confidence intervals and p-values were derived from a linear mixed-effect model with time post-fracture as fixed effect.

‡ Not normally distributed across all visits and therefore the log-transform of the bone parameter was used in the linear mixed effect model.

pronounced at six to eight weeks post-fracture and decreased at twelve weeks post-fracture. Bridging of the cortex started at six to eight weeks post-fracture and was completed at twelve weeks post-fracture. No changes were observed at the contra-lateral side.

Quantitative assessment

Estimated marginal means, 95% confidence intervals and percent change from baseline for the bone parameters measured at the fractured side as well as the contra-lateral side are given in Table 2. Time series line-plots of the average percent change from baseline in the bone parameters are shown in Figure 5.

At the fracture side, total bone density increased by 10.5% ($p > 0.01$) at six to eight weeks post-fracture and showed a final change of +9.1% ($p > 0.05$) at twelve weeks post-fracture (Figure 5A). This was the result of cumulative, opposite changes in trabecular and cortical bone density. Whereas trabecular bone density (Figure 5B) significantly increased to a peak of 20.4% ($p < 0.01$) at six to eight weeks post-fracture and a final increase of 12.1% ($p > 0.05$) at twelve weeks post-fracture, cortical density decreased progressively to -5.2% ($p > 0.05$) at twelve weeks post-fracture (Figure 5C).

Trabecular thickness significantly increased by 23.0% ($p < 0.01$) after six to eight weeks up to 30.5% ($p < 0.01$) at twelve weeks post-fracture (Figure 5E). Trabecular number remained constant until three to four weeks post-fracture and subsequently tended to decrease by 14.8% ($p > 0.05$) at twelve weeks post-fracture (Figure 5D). No significant changes were observed in trabecular separation (Figure 5F).

There were no significant changes in cortical thickness (Figure 5G) and cortical perimeter during the twelve weeks post-fracture (Figure 5H).

Bone stiffness in compression, torsion and bending calculated by the approach of Shefelbine et al. (Figure 5I, 5J and 5H, respectively) showed no significant change until twelve weeks post-fracture. At that time-point they were increased by 31.3%, 31.5% and 31.3% (all $p < 0.01$), respectively. Bone stiffness in compression, torsion and bending calculated by the standard approach (data not shown in Table) showed a similar behavior, but were increased by 38.4%, 46.8% and 43.0% (all $p < 0.02$), respectively.

At the contra-lateral side, no significant changes from baseline were observed in any of the bone parameters.

Correlation with clinical and radiological outcome

PRWE score correlated significantly with trabecular thickness ($\rho = -0.43$, $p = 0.001$) and stiffness in compression ($\rho = -0.28$, $p = 0.029$), torsion ($\rho = -0.29$,

Figure 5 Time series line-plots of the percent change from baseline in HRpQCT derived bone density, micro-architectural, geometrical and biomechanical parameters at the fracture side (white squares) and the contra-lateral side (black circles). α significantly different from baseline, $p < 0.05$. β significantly different from baseline, $p < 0.01$.

$p = 0.024$) and bending ($\rho = -0.30$, $p = 0.019$) as calculated by Shefelbine's approach (Table 3). Similar correlations were found for the stiffnesses calculated with the standard μ FEA approach: compression stiffness ($\rho = -0.33$, $p = 0.011$), torsional stiffness ($\rho = -0.32$, $p = 0.012$), and bending stiffness ($\rho = -0.37$, $p = 0.004$) (Table 3).

The radiological state of consolidation correlated significantly with trabecular thickness ($\rho = 0.35$, $p = 0.006$), cortical perimeter ($\rho = -0.41$, $p = 0.001$) and stiffness in compression ($\rho = 0.44$, $p < 0.001$), torsion ($\rho = 0.35$, $p = 0.006$)

and bending ($\rho = 0.37$, $p = 0.004$) as calculated by Shefelbine's approach (Table 3). Using the standard μ FEA approach, similar correlations were obtained: compression stiffness ($\rho = 0.50$, $p < 0.001$), torsional stiffness ($\rho = 0.44$, $p < 0.001$), and bending stiffness ($\rho = 0.41$, $p = 0.001$) (Table 3).

No correlations were found between the other bone parameters and the PRWE score or the radiological state of consolidation.

Because the stiffness at the first visit is probably overestimated, we also calculated the correlation not taking the first visit into account. Then, the correlations between the PRWE score and the stiffness in compression, torsion and bending as calculated by Shefelbine's approach increase to -0.37 ($p = 0.012$), -0.40 ($p = 0.006$) and -0.40 ($p = 0.007$), respectively. And the correlations between the PRWE score and the stiffnesses calculated by standard μ FEA approach increase to -0.44 ($p = 0.002$), -0.35 ($p = 0.017$) and -0.38 ($p = 0.009$) for the stiffness in compression, torsion and bending, respectively.

Table 3 Correlations between the bone stiffness parameters and the PRWE score as well as the radiological state of consolidation.

Bone stiffness	PRWE score		Radiological state of consolidation	
	ρ	p-value	ρ	p-value
Scomp (Shefelbine FEA)	-0.282	0.029	0.437	< 0.001
Stors (Shefelbine FEA)	-0.291	0.024	0.353	0.006
Sbend (Shefelbine FEA)	-0.302	0.019	0.365	0.004
Scomp (standard FEA)	-0.327	0.011	0.500	< 0.001
Stors (standard FEA)	-0.322	0.012	0.441	< 0.001
Sbend (standard FEA)	-0.369	0.004	0.412	0.001

Scomp = compression stiffness; Stors = torsional stiffness; Sbend = bending stiffness; FEA = finite element analysis. Correlations were calculated using Spearman's correlation coefficient (ρ).

Discussion

The main objective of this study was to explore the feasibility of HRpQCT based techniques to study the fracture healing process of conservatively treated distal radius fractures *in vivo*. The results show that it is possible to detect significant changes in HRpQCT derived bone densitometric, micro-architectural and mechanical parameters during the first twelve weeks of fracture healing using HRpQCT in combination with μ FEA. These results were obtained by performing the standard patient evaluation protocols that have been provided by the manufacturer and are readily available on every XtremeCT device. Although the contouring of the periosteal boundary often required manual correction due to the disruptions in the cortical surface, the calculation of the bone density and micro-architectural

parameters could proceed in the standard way. Also the segmented 3D models could be used in the standard μ FEA without problems.

The time-related increase in trabecular bone density with a peak at six to eight weeks post-fracture was the most striking finding. At the time point of this peak increase, trabecular density was significantly increased and the trabecular structure, when visually assessed, became blurred (Figure 4). This finding might reflect the formation of new woven bone between the trabeculae during early stages of distal radius fracture healing, which is supported by the histological findings reported by Aspenberg et al.³⁴ At twelve weeks post-fracture, when the change from baseline in trabecular density was not significantly increased anymore, trabecular thickness further increased and the largest increase in calculated stiffness was observed. We hypothesize that the formation of new woven bone in the trabecular region in the first weeks post-fracture is followed by extensive remodeling between week six and twelve, when poorly mineralized unorganized woven bone is removed and replaced by normal well-organized bone tissue, with improved bone strength but a decrease of trabecular density at twelve weeks compared to six weeks post-fracture.^{35, 36} A similar process, where the increase in bone density precedes the micro-architectural adaptation, has been described in developing bone.³⁷

Although we do detect significant changes in structural parameters, the interpretation of these changes in the healing bone might not be the same as in intact bone and warrants additional discussion. The presence of woven bone at three to four and six to eight weeks post-fracture, i.e. when the images show a more blurred region, influences the calculation of several parameters that are used to quantify the trabecular structure. With the standard evaluation as used here, the trabecular thickness and separation are derived from trabecular bone density, via bone volume fraction, and trabecular number according to standard histomorphometric relations.²⁶ Because of its finer structure and lower density not all woven bone is segmented after thresholding and, consequently, not included in the calculation of the trabecular number. It does, however, increase trabecular bone density. These combined effects lead to a significant increase in the calculated trabecular thickness parameter that, however, does not reflect an actual increase of the thickness of trabeculae. This shows that one must take care with the interpretation of the structural parameters as they could represent a different morphological aspect in fractured bone than in intact bone. A second point that should be mentioned here is that the morphological parameters represent the average values for the whole analyzed region. The measured changes in bone morphology parameters thus are dependent on the size of the analyzed region

and that of the fracture: if the fracture comprises a small part of the analyzed volume, changes in morphology parameters will appear to be smaller than in the case where the fracture occupies larger regions. The μ FEA parameters, however, are less sensitive to the size of the analyzed region and that of the fracture: even a small fracture can dramatically reduce the stiffness of the bone.

Despite the fact that the images also showed large local changes in the cortical region during the healing process, i.e. bridging of the cortex, the changes measured in cortical thickness were not significant. The calculation of cortical thickness, however, is the average of the whole cortical region and appears to be insensitive to these local changes. This finding suggests that a more localized approach is needed to describe the healing process in the cortical compartment.

Restoration of bone stiffness

Restoration of bone strength and stiffness is of particular interest during the healing process of fractures. Although the approach by Shefelbine et al. that was used in the finite element analysis was only validated for femoral fractures in rats⁸, it is currently the only validated method reported in literature to assess the fracture stiffness in healing calluses through high-resolution CT, taking the properties of the callus into account. Regardless of this difference in fracture site and species and the fact that callus properties might vary when derived from CT data, the FEA results show that major changes from baseline are measured at twelve weeks post-fracture. Since a similar development in stiffness was calculated with the standard μ FEA approach, it might not make a big difference which approach is used. However, particular during the early stages of fracture healing we propose to incorporate the callus into the model and therefore use the method of Shefelbine et al. At later stages, when the callus becomes less prominent, the standard approach might suffice since the results acquired by both approaches converge to each other: at twelve weeks post-fracture the correlation between the compression stiffnesses calculated by the two methods is strong with an R^2 of 0.81.

The finding that both approaches indicate that the stiffness first tends to decrease and later increases could be due to the fact that we compared changes to the baseline case. It is possible that the stiffness at baseline is overestimated by the FEA because the imaging procedure can identify touching trabeculae in the fractured region as connected. This might affect the standard μ FEA procedure more than Shefelbine's approach, hence this could explain why the final increase in compression stiffness calculated with the standard μ FEA approach (38.4%) is more pronounced than with the Shefelbine approach (31.1%).

Interestingly, the significant correlation between the PRWE score and the bone stiffness parameters suggests an association of this clinical parameter and

HRpQCT derived stiffness. When the first visit is not taken into account because of the suspected overestimation at this time-point, the Spearman's correlation coefficients are even higher for all stiffnesses.

Limitations

This study has several limitations. First, as was mentioned before, the interpretation of structural parameters is not the same as that for intact bone, in particular when considering parameters such as trabecular thickness. Although we found significant changes in the parameters, one should be careful with the interpretation of such changes. It has not been proven in this study that these changes are a meaningful representation of the bone consolidation during healing. The same holds for the results obtained from the μ FE analyses. Although the higher resolution makes it possible to better detect the fracture, we presently cannot prove that the predicted changes in stiffness are realistic, nor can we prove that the results of μ FEA are more accurate than those of continuum FEA based on clinical CT scans. A comparative study involving both continuum FE modeling and μ FE modeling will be needed to further address these issues, but this was beyond the scope of the present paper.

Second, the region that was scanned at the contra-lateral side differed from the one at the fracture side. Thus we can only claim that nothing has changed at the contra-lateral side, but we cannot compare both sides to each other, which would have been very interesting. For example, it is unknown to what extent the stiffness at the fracture side has been restored to the pre-fracture stiffness. Since good correlations between the left and right radius has been reported³⁸, the stiffness at the contra-lateral side might be regarded as the pre-fracture stiffness. To be able to compare both sides, we recommend matching the location of the scanned volume at the contra-lateral side to the one at the fracture side in future studies.

Despite the correlation between the PRWE score and the bone stiffness, a third limitation of this study is that the two μ FEA methods that were applied are not validated in human distal radius fractures. It will be hard or even impossible to address this limitation adequately, i.e. experimentally measure bone strength in healing fractures in humans and compare it to the bone strength calculated from the μ FEA, since the validation would raise some serious ethical concerns. Alternatively, validation using an animal metaphyseal fracture healing model could be performed in the future.

Last, as can be seen in the 2D images and 3D models, we observed that the fracture healing process has not been fully completed at twelve weeks in most women. In future research, therefore, it is recommended to extend the follow-up period, e.g. to 24 weeks, to get a better end-point of the fracture healing process.

In conclusion, this study shows that it is possible to detect significant changes in HRpQCT derived bone density, micro-architectural and mechanical parameters during the first twelve weeks of healing of stable distal radius fractures. These results open a window to further explore the possibilities of HRpQCT to monitor fracture healing in detail. It is recommended that future work should focus on the validation of the FEA approach and the development of new relevant parameters that benefit from the resolution of HRpQCT.

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4

Early changes in bone density,
micro-architecture, bone resorption
and inflammation predict the
clinical outcome twelve weeks
after conservatively treated distal
radius fractures

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Abstract

Background

Fracture healing is an active process with early changes in bone and inflammation. We performed an exploratory study evaluating the association between early changes in densitometric, structural, biomechanical, and biochemical bone parameters during the first weeks of fracture healing and wrist-specific pain and disability at twelve weeks in postmenopausal women with a conservatively treated distal radius fracture.

Methods

Eighteen patients (aged 64 ± 8 years) were evaluated at one to two and three to four weeks post-fracture, using high-resolution peripheral quantitative computed tomography (HR-pQCT), micro finite element analysis, serum procollagen type-I N-terminal propeptide (PINP), carboxy-terminal telopeptide of type I collagen (ICTP), and high-sensitive C-reactive protein (hsCRP). After twelve weeks, patients rated their pain and disability using Patient Rated Wrist Evaluation (PRWE) questionnaire. Additionally, Quick Disability of the Arm Shoulder and Hand (QuickDASH) questionnaire and active wrist range of motion was evaluated. Linear regression models were used to study the relationship between changes in bone parameters and in hsCRP from visit one to two and PRWE score after twelve weeks.

Results

A lower PRWE outcome, indicating better outcome, was significantly related to an early increase in trabecular bone mineral density (BMD) ($\beta = -0.96$ [95% CI -1.75 to -0.16], $R^2 = 0.37$), in torsional stiffness ($\beta = -0.14$ [95% CI -0.28 to -0.004], $R^2 = 0.31$), and to an early decrease in trabecular separation ($\beta = 209$ [95% CI 15 to 402], $R^2 = 0.33$) and in ICTP ($\beta = 12.1$ [95% CI 0.0 to 24.1], $R^2 = 0.34$). Similar results were found for QuickDASH. Higher total dorsal and palmar flexion range of motion was significantly related to early increase in hsCRP ($\beta = 9.62$ [95% CI 3.90 to 15.34], $R^2 = 0.52$).

Conclusion

This exploratory study indicates that the assessment of early changes in trabecular BMD, trabecular separation, calculated torsional stiffness, bone resorption marker ICTP, and hsCRP after a distal radius fracture provides valuable information regarding the twelve-week clinical outcome in terms of pain, disability, and range of motion and validates its use in studies on the process of early fracture healing.

Introduction

Distal radius fractures are among the most common fractures in elderly.¹ Although these fractures have a limited impact on general health-related quality of life,^{2, 3} long-term complications are more commonly reported when using questionnaires especially developed to evaluate pain and function of the wrist: Persisting pain, stiffness, weakness of the wrist and signs of the complex regional pain syndrome are the most mentioned complications that limit the patient's ability to manage daily life activities, such as household work or personal care.⁴⁻⁸

Considering the high variability in patient-rated pain and disability,⁹ it would be of great clinical value to know to what degree specific bone parameters can predict long-term clinical outcome after a fracture. In studies examining baseline predictors for clinical outcome, patient factors, injury patterns, and fracture-related characteristics played only a limited role in predicting the clinical outcome after a fracture.¹⁰⁻¹³ To our knowledge, there is no study available that evaluated long-term outcome in terms of pain and disability in relation to early changes in bone mineral density (BMD), local bone architecture, and serum bone markers and inflammation during fracture healing. Fracture healing is a complex process in which inflammation and bone remodeling are intensively involved. We, therefore, hypothesized that early changes in these mechanisms would predict clinical outcome at longer term. If so, influencing these changes early in the process of fracture healing could influence the clinical outcome.

The recent development of high-resolution peripheral quantitative computed tomography (HRpQCT) allows in vivo assessment of 3D bone density and bone micro-architecture of the distal radius and calculation of biomechanical properties of the bone using micro finite element analysis (μ FEA).^{14, 15} Therefore, the aim of this exploratory study was to assess the association between early fracture healing related changes in bone mineral density, bone micro-architecture, calculated biomechanical properties, and serum markers of bone turnover and inflammation with wrist-specific pain and disability at twelve weeks post-fracture in postmenopausal women with a stable, conservatively treated distal radius fracture.

Materials and Methods

Design and study population

Twenty women aged 50 years or older that presented with a stable distal radius fracture at the departments of Orthopedic surgery and Traumatology of the Maastricht University Medical Center were included in this study. All fractures were conservatively treated by cast immobilization, if necessary after manual reduction. Exclusion criteria were a history of previous fracture at the fractured side; known systemic or metabolic disorders leading to progressive bone deterioration; use of glucocorticoids; presence of an active inflammatory disease; presence of an active or suspected infection; malignancy in the last twelve months pre-fracture. Patients were followed over a period of twelve weeks at four outpatient visits: At one to two weeks (visit one), three to four weeks (visit two), six to eight weeks (visit three) and twelve weeks (visit four) post-fracture. The protocol (registration number NTR3821) was approved by the Medical Ethics Committee of the Maastricht University Medical Center, and all patients provided written informed consent prior to participation.

Clinical outcome measures

At visit four, twelve weeks after the fracture, patients rated their pain and disability using validated Dutch versions of the Patient Rated Wrist Evaluation (PRWE) questionnaire. PRWE was chosen as the primary outcome measure because it is a simple and brief tool that was especially developed for evaluating the outcome in patients with distal radius fractures. It has previously been shown to be a reliable and valid measurement tool after distal radius fractures.^{16, 17} The questionnaire consists of a pain subscale (five items, contributing to 50% to the total score) and a disability subscale (ten items, 50% of total score). The total PRWE score ranged from 0 (no pain/no difficulty) to 100 (worst pain/disability).

In addition, we applied the Quick Disabilities of the Arm Shoulder and Hand (QuickDASH) questionnaire, which is the most widely used questionnaire for patients with general upper-extremity problems. It has been validated in patients with any or multiple disorders of the upper limb.¹⁸⁻²¹ QuickDASH uses eleven items to measure physical function and symptoms on a 0 to 100 scale with higher scores representing greater disability. Further, patients were asked to rate their pain on a visual analogue scale (VAS) ranging from 0 (no pain) to 100 (most severe pain). Active range of motion was measured at the fractured side for total wrist dorsal and palmar flexion and total ulnar and radial deviation.

HRpQCT measurements and micro finite element analysis (μ FEA)

At visit one (one to two weeks post-fracture) and visit two (three to four weeks post-fracture), the fractured radius was scanned by HRpQCT (XtremeCT, Scanco Medical AG, Bruttisellen, Switzerland) using clinical in vivo settings by the manufacturer. The region of interest was based on anteroposterior (AP) and lateral radiographs of the fractured radius, in which the proximal edge of the lunate was used as reference. The scan length was set to 18 mm (Figure 1), which means that this is twice the scan length of the standard protocol. This doubling was needed to make sure that the complete fracture was scanned. With an isotropic voxel size of 82 μ m, each HRpQCT measurement therefore resulted in 220 parallel CT slices with a scan time of 5.6 minutes and a radiation of <6 mSv. Because the patient's forearm was fixed in a cast, the forearm with the cast was placed in a custom cylindrical carbon holder with an inflatable cushion (Pearltec AG, Schlieren, Switzerland) to minimize patient motion. Scan quality was graded according to Pialat et al ²², a five-grade scheme ranging from grade 1 (no motion artifacts) to grade 5 (severe motion artifacts). Scans with insufficient quality (i.e. grade 4 or 5) were repeated.

Figure 1 Representative region of interest on the HRpQCT image of the distal radius. The proximal edge of the lunate was used as reference line. A scan length of 18 mm (220 slices) was selected to make sure that the complete fracture was scanned.

The HRpQCT images were evaluated using the standard patient evaluation protocol provided by the manufacturer.²³ In short, the cortical and trabecular region were separated first using a semi-automated contouring scheme in which the periosteal boundary surface of the radius was derived.²⁴ After contouring, a Laplace-Hamming filter (epsilon 0.5 and cut-off frequency 0.4) followed by normalization (range 0 to 1000) and global thresholding (threshold 400), was used

in order to extract the voxels that represented mineralized bone and to create the segmented images. The following bone density parameters were calculated from the images: volumetric bone mineral density (mgHA/cm³) was assessed for the total region (Dt_{tot}), and the trabecular (D_{trab}) and cortical region (D_{cort}) separately. The micro-architectural parameters that were used in this study were: the trabecular number (Tb.N; 1/mm), thickness (Tb.Th; mm) and separation (Tb.Sp; mm), which are all determined using a 3D ridge extraction method²⁵ and standard morphological relations. Bone geometry was expressed by the cortical thickness (Ct.Th; mm), which was calculated by dividing the cortical volume by the outer cortical surface.

μFE models were created from the segmented HRpQCT images. Each voxel that represents bone tissue was converted into a brick element of the same size. In the standard μFE approach usually used in HRpQCT,¹⁴ equal properties are assigned to every bone tissue element. Whereas this approach has been well validated and successfully applied in clinical studies, its application to fractured bone is not trivial. The use of segmented images limits the models to only represent the mineralized phase. In particular in the early stages of fracture healing this approach might to be inaccurate because it does not account for newly formed low mineralized bone. Therefore, we additionally applied a more sophisticated approach, earlier described by Shefelbine and colleagues,²⁶ that distinguishes between three different tissue types (soft tissue, low mineralized tissue and high mineralized tissue) with stiffness properties of the elements depending on its material composition. This approach has been validated for healing bone.²⁶ In the resulting μFE model soft tissue was assigned a constant modulus of 50 MPa whereas for mineralized bone a Young's modulus ranging from 5000 to 15000 MPa results, depending on the material composition of the voxel. A Poisson's ratio of 0.3 was assigned to all elements and all materials were assumed to be linear elastic and isotropic. For the standard μFE approach, a procedure similar to that described in earlier studies was used and all elements were assigned a Young's modulus of 10 GPa and a Poissons ratio of 0.3.^{14, 27} By subsequently applying different load cases, clinical relevant loading scenarios were simulated and the associated stiffness was chosen as outcome variable. The first load case represented a 'high friction' compression test with a prescribed displacement in the axial direction of 1% of the total length, from which the compression stiffness (S_{comp}; kN/mm) was calculated. The second load case represented a prescribed rotation of 0.01 rad around the longitudinal axis applied to the surface perpendicular to this axis from which the torsional stiffness (S_{tors}; kNmm/rad) was calculated. A third and fourth load case represented a prescribed rotation of 0.01 rad applied around the sagittal and transversal axes respectively, onto the surfaces perpendicular to each axis, thus inducing a state of pure bending

in two directions, from which the bending stiffness in each direction (sagittal and transversal) was calculated. Because the orientation of the patient's forearm was not the same during all visits, a quadratic mean bending stiffness was calculated (S_{bend} ; kNmm^2/rad) from the sagittal and transversal bending stiffness.

In addition to HRpQCT, and according to the Dutch standard care when a patient over the age of 50 years comes to clinic with a fracture, BMD T-score at the femoral neck was assessed by dual-energy X-ray absorptiometry (DXA; Hologic Discovery A; Hologic Inc., Waltham, MA, USA). BMD T-score was assessed once between four and eight weeks post-fracture, or was taken from a previous DXA scan if the patients already had had a DXA scan in the last year prior to the fracture.

Figure 2 Representative image of an HRpQCT and a FEA model.

Serum markers of bone turnover and inflammation, and vitamin D

At visit one (one to two weeks post-fracture) and visit two (three to four weeks post-fracture), venous blood samples were collected by puncture of the antecubital vein. Serum was separated from the bloodclot within 20 minutes and frozen to -20°C until analysis. Serum bone markers representing bone resorption (carboxy-terminal telopeptide of type I collagen [ICTP]) and bone formation (procollagen type-I N-terminal propeptide [PINP]) were assessed by radioimmunoassay (UniQ PINP/ICTP; Orion Diagnostics, Espoo, Finland) in a certified laboratory. Additionally, fracture-induced inflammation was assessed by high-sensitive C-reactive protein (hsCRP) (BN ProSpec system; Siemens/Dade Behring, Liederbach, Germany). An additional blood sample was taken at visit two to analyze 25-hydroxyvitamin D (25(OH)D) by chemiluminescent immunoassay (IDS-iSYS Immunodiagnostic Systems GmbH, Frankfurt, Germany).

Statistical analysis

Patient's demographic characteristics, bone parameters and clinical outcomes at twelve weeks post-fracture were reported in means and their standard deviations. Changes in bone parameters between visit one and visit two were tested using a paired samples Student's t-test. A linear regression was used to determine if the clinical outcome at twelve weeks was related to patients' or fractures' characteristics, such as fracture type (intra- versus extra-articular, comminuted versus non-comminuted, reduced versus non-reduced), fracture side (dominant versus non-dominant), DXA T-score at femoral neck, or serum vitamin D level (≥ 75 nmol/L versus < 75 nmol/L). The relationship between early changes in bone parameters and twelve-week clinical outcomes was calculated using a linear regression with the clinical outcome at twelve weeks post-fracture as outcome, and the change in bone parameters between visit one and visit two as explanatory variable. The model was adjusted for the number of days between visit one and visit two. Because adjusting the model for baseline bone values (i.e. bone parameters at visit one) did not improve the model according to the Akaike information criterion, we did not include baseline values in the model. In a second step, we additionally adjusted the model for fracture type (intra- versus extra-articular, comminuted versus non-comminuted, reduced versus non-reduced), fracture side (dominant versus non-dominant), DXA T-score at femoral neck, or serum 25(OH)D level (≥ 75 nmol/L versus < 75 nmol/L) to test whether these factors influenced the model. All analyses were performed using Stata 11.0 (StataCorp, College Station, TX, USA) and the significance level was set to $\alpha = 0.05$.

Results

Patient's characteristics

From the twenty patients who were initially included in the study, one patient declined participation at the first visit and another patient at the second visit. Thus, eighteen women (64.3 ± 8.4 years) completed the study. The characteristics of these eighteen patients, including their fracture characteristics are listed in Table 1. Mean DXA T-score at femoral neck was -2.2 (0.9) with seven (44%) osteoporotic, and eight (50%) osteopenic women. Two patients had no DXA data. Serum 25-hydroxyvitamin D levels were 62.8 (29.3) nmol/L, with ten (46%) patients having values below 75 nmol/L. The clinical outcome in terms of pain and disability, as assessed at visit four (85.4 (range 81 to 91) days post-fracture), are also presented in Table 1. According to the PRWE classification of MacDermid et al.,⁹ five (28%) patients reported to experience no or minimal pain and disability, whereas three

(17%) patients had mild, five (28%) patients moderate, five (28%) patients severe, and none of the patient very severe pain or disability, respectively, twelve weeks after the fracture. In one patient, VAS at twelve weeks post-fracture is missing, and two patients the range of motion test could not have been performed. HRpQCT measurements were performed 9.1 (4 to 16) days (visit one) and 23.8 (18 to 29) days after the fracture (visit two) with the corresponding values shown in Table 2. 36% of the scans had to be repeated due to motion artifacts. Even if after repetition, five scans were still of insufficient quality, they were included into analysis. A representative image of a HRpQCT and a FEA model is given in Figure 2. One patient has missing ICTP, PINP and hsCRP data.

Relation between baseline characteristics and clinical outcome twelve weeks post-fracture

PRWE total score or QuickDASH score twelve weeks post-fracture were not related to baseline age, or fracture side (dominant versus non-dominant side) and type (intra-articular, comminuted, or reduced fracture), DXA T-score or 25(OH)D. VAS was higher in patients with a fracture at the dominant side (33.0 [95% confidence interval: 3.4 to 62.6] VAS counts; $p = 0.031$; adjusted $R^2 = 0.23$) compared to the non-dominant side. Lower palmar flexion range of motion was found after an intra-articular fracture (-23.3 [-46 to -0.7] angular degrees; $p = 0.044$; $R^2 = 0.21$), and lower total ulnar and radial deviation range of motion after a reduced fracture (-17.8 [-233.7 to -1.8] angular degrees; $p = 0.032$; $R^2 = 0.24$).

Table 1 Characteristics of eighteen post-menopausal women with a stable distal radius fracture and their clinical outcomes twelve weeks after the fracture.

Patient	Age (years)	dominant side	Fracture characteristic	reduction needed	PRWE (counts)	Clinical outcomes at twelve weeks post-fracture	Dorsal/palmar flexion (°)	Radial/ulnar deviation (°)
			intra-articular	comminuted		QuickDASH (counts)		
1	58.3	no	no	no	no	10.5	27.5	0
2	55.4	no	no	no	no	59.5	43.2	70
3	64.5	no	yes	yes	yes	45.5	47.5	41
4	52.5	yes	no	no	no	0.0	0.0	0
5	66.9	yes	no	no	yes	42.5	7.5	70
6	72.0	no	yes	yes	yes	19.5	12.5	20
7	67.5	no	no	no	no	31.5	34.1	NA
8	50.1	yes	no	no	no	55.0	60.0	50
9	80.5	no	yes	yes	no	68.0	47.5	39
10	61.6	yes	no	no	yes	64.5	50.0	51
11	69.1	no	no	no	yes	21.0	36.4	27
12	54.8	yes	yes	no	yes	99.0	97.7	100
13	64.2	yes	no	no	yes	84.0	77.3	82
14	63.9	no	no	yes	yes	27.9	29.5	9
15	68.7	no	no	no	yes	52.5	38.6	50
16	75.9	no	yes	yes	yes	66.0	43.2	28
17	57.9	no	no	no	no	0.0	0.0	0
18	74.5	no	no	yes	no	13.0	10.0	0
Total	64.3 (8.4)	6 (33%)	5 (28%)	6 (33%)	10 (56%)	42.2 (28.7)	36.8 (25.9)	38 (31)
								84 (35)
								55 (17)

Total values are means (SD), respectively n (%) "yes" for fracture characteristics. DXA T-score at femoral neck.

Abbreviations: DXA = dual energy X-ray absorptiometry; PRWE = Patient-Rated Wrist Evaluation Score; QuickDASH = Disabilities of the Arm, Shoulder and Hand Score; VAS = visual analogue scale of pain; NA = missing data.

Figure 3 Relation between the change in (A) trabecular density (Dtrab), (B) trabecular separation (TbSp), (C) torsional stiffness (Stors), (D) bone resorption (carboxy-terminal telopeptide of type I collagen [ICTP]) between visit one (one to two weeks post-fracture) and visit two (three to four weeks post-fracture) and patient-rated pain and disability (PRWE score) twelve weeks after a distal radius fracture.

PRWE score

The relationship of PRWE at twelve weeks and the early changes in bone parameters is shown in Table 3 and Figure 3. Higher early increases in Dtrab were significantly related to lower, thus better, PRWE scores at twelve weeks post-fracture ($p = 0.022$; adjusted $R^2 = 0.37$). For the other density parameters (Dcort and Dt_{tot}), the relationship was not significant.

On micro-architectural level, lower PRWE was related to higher decreases in TbSp ($p = 0.037$; $R^2 = 0.33$), but not to changes in Tb.N or Tb.Th.

On the biomechanical level, lower PRWE score was related to higher increases in Stors ($p = 0.045$; $R^2 = 0.31$), but not to changes in Scomp nor for Sbend. When applying the Shefelbine method, the relation between Stors and PRWE was -0.134 (-0.290 to 0.022 ; $p = 0.086$; $R^2 = 0.26$).

Table 2 Bone parameters at the fracture location assessed by HRpQCT, calculated biomechanical parameters and serum bone markers at one to two weeks (visit 1), three to four weeks (visit 2) and the change between visit 1 and 2, after a stable distal radius fracture.

	Visit 1 (one to two wks post-fx)	Visit 2 (three to four wks post-fx)	Change between visit 1 and 2
Density parameters			
Dtot (mgHA/cm ³)	232.2 (54.8)	243.4 (50.4)	11.2 (1.8 to 20.6) ^a
Dcort (mgHA/cm ³)	648.1 (123.4)	629.3 (113.9)	-18.8 (-32.7 to -4.8) ^a
Dtrab (mgHA/cm ³)	144.8 (26)	160.9 (29)	16.2 (8.7 to 23.6) ^a
Micro-structural parameters			
Ct.Th (mm)	0.408 (0.216)	0.4 (0.193)	-0.008 (-0.039 to 0.022)
Tb.N (mm ⁻¹)	1.995 (0.455)	2.029 (0.423)	0.034 (-0.064 to 0.131)
Tb.Th (mm)	0.063 (0.015)	0.068 (0.013)	0.005 (0.001 to 0.009) ^a
Tb.Sp (mm)	0.468 (0.131)	0.445 (0.099)	-0.023 (-0.054 to 0.009)
Biomechanical parameters (standard protocol)			
Scomp (kN/mm)	14.8 (7.2)	13.0 (7.8)	-1.8 (-3.6 to 0.03)
Stors (kNmm/rad)	298 (135)	272 (120)	-26 (-69 to 18)
Sbend (kNmm ² /rad)	430 (227)	379 (213)	-51 (-127 to 26)
Serum bone markers			
ICTP (µg/l)	3.6 (1.1)	4.1 (1.1)	0.5 (-0.1 to 1.0)
PINP (µg/l)	48.9 (25.7)	65.9 (39.1)	12.9 (-3.7 to 29.5)
hsCRP (mg/l)	3.8 (6.3)	2.6 (4.5)	-1.2 (-2.8 to 0.4)

Values are expressed in means and their standard deviations for visit one and 2, and as mean (95% confidence interval) for the change between the two visits.

Abbreviations: Dtot = total bone mineral density; Dcort = cortical bone mineral density; Dtrab = trabecular bone mineral density; Ct.Th = cortical thickness; Tb.N = trabecular number; Tb.Th = trabecular thickness; Tb.Sp = trabecular separation; Scomp = compressional stiffness; Stors = torsional stiffness; Sbend = bending stiffness; ICTP = carboxy-terminal telopeptide of type I collagen; PINP = procollagen type-I N-terminal propeptide; hsCRP = high-sensitive C-reactive protein.

^a refers to significant changes between visit 1 and 2.

On serum bone marker level, higher PRWE was related to higher increases in ICTP ($p = 0.050$; $R^2 = 0.34$) but not to changes in PINP or hsCRP.

Adjusting the analysis for fracture type (intra-articular fracture, comminuted fracture, or reduced fracture) did not influence the results, however including fracture side (dominant versus non-dominant hand) into the model increased adjusted R^2 to 0.45 for the relation with early changes in Dtrab (-1.03 [-1.78 to -0.27]; $p = 0.011$) with higher PRWE values for patients with a fracture at the dominant side ($p_{\text{fx-side}} < 0.1$). The 25(OH)D levels did not influence the model, nor did the DXA T-scores.

Excluding the five patients that had one scan with insufficient quality (subsample of thirteen patients), beta-coefficients for early changes in Dtrab, Tb.Sp, Stors, and ICTP remained at the same level or became stronger. However, the associations were not significant anymore.

Table 3 Relationship between the PRWE score at twelve weeks post-fracture as a measure for pain and disability, and the changes in bone parameters at the fracture location assessed by HRpQCT, calculated biomechanical parameters and serum bone markers between one to two weeks (visit 1) and three to four weeks (visit 2), after a stable distal radius fracture.

	β -coefficient (95% confidence interval)	p-value	Adj R2
Density parameters			
Dtot	-0.61 (-1.28 to 0.06)	0.073	0.27
Dcort	-0.19 (-0.68 to 0.31)	0.438	0.13
Dtrab	-0.96 (-1.75 to -0.16)	0.022 ^a	0.37
Micro-structural parameters			
Ct.Th	-91 (-323 to 140)	0.412	0.13
Tb.N	-48.8 (-116 to 18.4)	0.142	0.22
Tb.Th	-352 (-2089 to 1386)	0.672	0.10
Tb.Sp	209 (15 to 402)	0.037 ^a	0.33
Biomechanical parameters (standard protocol)			
Scomp	-2.44 (-6.18 to 1.31)	0.186	0.19
Stors	-0.145 (-0.285 to -0.004)	0.045 ^a	0.31
Sbend	-0.064 (-0.15 to 0.022)	0.132	0.22
Serum bone markers			
ICTP	12.1 (0.0 to 24.1)	0.050 ^a	0.34
PINP	0.256 (-0.175 to 0.687)	0.223	0.21
hsCRP	-0.263 (-5.02 to 4.495)	0.907	0.12

Abbreviations: Dtot = total bone mineral density; Dcort = cortical bone mineral density; Dtrab = trabecular bone mineral density; Ct.Th = cortical thickness; Tb.N = trabecular number; Tb.Th = trabecular thickness; Tb.Sp = trabecular separation; Scomp = compressional stiffness; Stors = torsional stiffness; Sbend = bending stiffness; ICTP = carboxy-terminal telopeptide of type I collagen; PINP = procollagen type-I N-terminal propeptide; hsCRP = high-sensitive C-reactive protein; PRWE = Patient-Rated Wrist Evaluation Score.

^a refers to a statistically significant relation.

QuickDASH score

QuickDASH and PRWE score twelve weeks post-fracture were highly correlated ($r = 0.89$). QuickDASH was related to changes in Dtrab (-0.789 [-1.49 to -0.08]; $p = 0.031$; $R^2 = 0.40$), Tb.Sp (174 [5.7 to 343]; $p = 0.044$; $R^2 = 0.37$), ICTP (13.0 [3.4 to 22.6]; $p = 0.012$; $R^2 = 0.49$), and Stors applying the Shefelbine method (-0.138 [-0.266 to -0.010]; $p = 0.036$; $R^2 = 0.39$) (Table 3).

Visual analogue scale

VAS was highly correlated to PRWE score and QuickDASH score twelve weeks post-fracture ($r = 0.88$ and $r = 0.76$, respectively). VAS score was not significantly related to early changes in bone, biomechanical, or serum bone marker parameters. After correction for fracture side, VAS was significantly related to early changes in Tb.Sp (226 [26.5 to 425]; $p = 0.029$; $R^2 = 0.41$) (Table 3).

Range of motion

Range of motion at twelve weeks was significantly correlated with PRWE score, QuickDASH and VAS (all $r < -0.57$). The total palmar and dorsal flexion was related to early changes in hsCRP (9.62 [3.90 to 15.34]; $p = 0.003$; $R^2 = 0.52$) (Figure 4), but range of motion did not appear to be related to changes in bone density, architecture, and biomechanical parameters. The total radial-ulnar deviation range of motion was not related to early changes in bone parameters including hsCRP. After correction for intra-articular or reduced fractures, the effects remained the same.

Figure 4 Relation between the change in high-sensitive C-reactive protein (hsCRP) between visit one (one to two weeks post-fracture) and visit two (three to four weeks post-fracture) and total active dorsal and palmar flexion range of motion twelve weeks after a distal radius fracture. Two patients have missing range of motion data, one patient does not have hsCRP data.

Discussion

The most striking finding in this study is that the changes in bone micro-architecture, calculated bone stiffness, bone resorption and inflammation during the first weeks of fracture healing predicted functional outcome parameters assessed at twelve weeks after a distal radius fracture. PRWE score twelve weeks after the fracture were explained for more than 30% by changes in Dtrab, Stors, Tb.Sp, and ICTP between day 9 and 23 after fracture. This indicates that early changes in bone structure and remodeling are important for clinical recovery; hence, a greater increase in Dtrab and Stors and greater decrease in bone resorption are related to a better long-term outcome for pain and function.

Each 10 mgHA/cm³ higher early increase in Dtrab between visit one and two was related to a lower PRWE by 9.6 counts and a lower QuickDASH score by

7.9 counts, respectively. Since longitudinal changes of ≥ 14 points for PRWE and QuickDASH scores are considered minimal clinically significant changes²⁸, the wide spectrum of early changes in Dtrab ranging from -13.8 to $+40.6$ mgHA/cm³ may be considered as clinically meaningful. Reasons for smaller initial increases or even decreases in Dtrab after a fracture, however, remain unclear. One possibility is that either these patients' healing process has already started with increased remodeling, or that the process is delayed. The latter could explain persistent or enhanced pain and disability twelve weeks post-fracture in these patients. In this respect, it would be interesting to see whether pain and disability will improve in these patients at later follow-up or whether this patient group will have substantially more persisting symptoms.

Each 1 mg/L higher early increase in ICTP was related to a higher, thus worse PRWE by 12.1 counts at twelve weeks. During fracture healing, both markers of bone formation and resorption increase and can be used to reflect the stages of fracture healing and mineralization process.²⁹⁻³⁴ It has been shown that serum bone resorption markers, such as ICTP, rise at an earlier stage than bone formation markers, indicating that during the very first weeks after a fracture, osteoclast activation-driven resorption occurs as the main process.^{31, 32} This could be an explanation why in our study, the early increase in ICTP and not PINP was related to pain and disability at follow-up. It has been reported that patients with a delayed fracture healing tended to have higher increases in bone resorption markers in the first weeks post-fracture.³³ Fracture extent and location may also increase ICTP response after a fracture with higher increases in more complex fractures, or larger fracture areas.^{32, 34} However, more complex fractures, in our study represented by intra-articular, comminuted and/or fractures where a manual reduction was necessary, had no influence on the results.

Interestingly, early changes in hsCRP did not contribute to PRWE and QuickDASH score, suggesting that early changes in inflammation do not contribute to long-term pain and disability. In contrast, active range of motion was explained by 40% to 60% by early changes in hsCRP, indicating that early increase in inflammation resulted in better active motion at twelve weeks post-fracture. Inflammation is considered as an important primary stimulus for fracture repair.^{35, 36} The beneficial effect of inflammation during fracture healing is also supported by studies showing that anti-inflammatory drugs may delay fracture healing.³⁶ On the other hand, no significant predictors of VAS were identified. Whereas PRWE and QuickDASH questionnaires both consist of questions about pain and functionality, VAS only includes information about patient-perceived pain. However, using the pain subscale of the PRWE questionnaire as outcome (data

not shown), the results remained the same as for the total PRWE score. A possible explanation for the different results when using VAS could be that the VAS evaluates pain intensity, but not frequency, whereas PRWE also included frequency-related questions. Additionally, perceived pain is determined by many more factors than the healing process of the mineralized phase of bone after a fracture as assessed by HRpQCT only. Further, differences in perceived pain could have influenced the clinical outcome at twelve weeks, and also the early changes in bone parameters, as patients with a higher pain tolerance might use their fractured arm more extensively. VAS score at the contra-lateral side could be an indicator of general pain tolerance. However, it did not influence our results (data not shown).

These results suggest that the integrated process of inflammation, regeneration, and remodeling that occurs during the early stages of fracture healing can be studied by the assessment of hsCRP, bone resorption markers and bone structure by HRpQCT in order to predict clinical outcome three months after a stable distal radius fracture.

More than half of all patients (56%) reported they still experienced moderate to very severe pain and disability twelve weeks after the fracture. A painless, full restoration of hand functionality is seen as the major goal after a distal radius fracture.³⁷ Knowing more about fracture related predictors for clinical outcomes is important to identify patients at risk for having long-term complications. Once they are identified, it could become possible to treat them. Interestingly, patient and injury related characteristics (as our data also confirmed) play only a small role in predicting the clinical outcome after a fracture.^{10, 11} While injury compensation,^{10, 11} patients' education level,^{10, 11} the presence of other medical problems,¹¹ and prereductional radial shortening¹⁰ together explained 17% of the one-year¹¹ and 25% of the six-month¹⁰ PRWE score, respectively, it is less consistent whether more injury related parameters (i.e. initial displacement of the fracture) predict clinical outcomes after a distal radius fracture^{13, 38}. To our knowledge, no previous study evaluated the predictive values of the fracture healing process itself on pain and disability after a distal radius fracture. However, the reason why some patients show a greater increase in Dtrab or decrease in ICTP, respectively, and whether this will lead to a more rapid consolidation and thus improved clinical recovery, remains unclear. The amount of structural changes and bone marker response could depend on the complexity of the fracture. However, in our study including conservatively treated fractures only, the associations between early changes in bone parameters and functional outcomes at twelve weeks were independent of articular involvement (intra- versus extra-articular fracture), comminution (comminuted versus non-comminuted fracture), and fracture reduction. Furthermore, the correlations

between early bone changes and pain and disability were independent of patient's vitamin D level or DXA T-score.

Our study has several strengths. Compared to other studies evaluating the clinical outcomes after distal radius fractures, the patients and fracture type in the current study are more homogeneous. We only included post-menopausal women with a stable, conservatively treated distal radius fracture. Of course, this reduces generalization of the results to other, more complex fractures, but it gives a clearer picture of what happens in conservatively treated fractures in mainly osteopenic or osteoporotic women. Further, this is the first study to our knowledge using HRpQCT measures for the evaluation of the fracture healing process and its relationship to the clinical outcomes. The HRpQCT measurements as well as the μ FEA were obtained using the standard evaluation protocols provided by the manufacturer. Further, we used well-validated and widely used questionnaires that are recommended when evaluating clinical outcomes of distal radius fractures³⁷.

Apart from these strengths, some limitations have to be mentioned. With eighteen patients, statistical power is limited, and therefore, our preliminary findings should be confirmed in larger studies. Because of this limited sample size, it was not feasible to analyze all bone parameters simultaneously to quantify the relative importance of predictors. Further, one should be careful with the interpretation of the derived structural parameters, such as the trabecular thickness, because the presence of woven bone between the trabeculae might affect the calculated of these parameters in an unexpected way. Additionally, our 'baseline' value was assessed 9.1 days after the fracture during the first follow-up visit, which does not allow us to set these early changes in relation to bone density and structure before nor immediately after the fracture. Also, clinical outcomes were assessed at three months post-fracture, whereas it would be interesting to also know the clinical outcomes at a longer period post-fracture. Once our results are confirmed in a larger population, future research should focus on whether certain interventions during fracture healing, for example an immediate vitamin D or calcium supplementation, could improve fracture healing on a structural level and thereby maybe reduce patients' long-term pain and disability. In order to identify patients at risk, further research should also test whether HRpQCT is needed to evaluate these early changes in D_{trab} after a fracture, or if a clinical QCT with poorer resolution, and/or other serum markers have the same power to predict clinical outcomes.

In conclusion, our explorative study showed that early changes in trabecular BMD, calculated torsional stiffness, bone resorption marker ICTP and hsCRP after a

stable distal radius fracture are related with pain and disability after twelve weeks. If larger studies confirm these results, this suggests that assessment of these early changes during fracture healing could provide valuable information regarding the long-term clinical outcome of these fractures.

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part 2

Follow-up two years after a stable
distal radius fracture using HRpQCT

5

Fracture repair in the distal radius in post-menopausal women: a follow-up two years post-fracture using HRpQCT

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Abstract

Background

Fracture healing is characterized by an intense increase in modeling and remodeling of bone, which allows removal of the cast after a stable distal radius fracture within three to five weeks. However, at that time, bone strength has not recovered yet. We studied the changes in bone mineral density (BMD), micro-architecture and bone stiffness after a distal radius fracture during a two-year follow-up in comparison to the contra-lateral side, and the association between the two-year stiffness and baseline BMD, micro-architecture, and early changes in these parameters.

Methods

The fractured side of fourteen post-menopausal women (mean age 64 ± 8 years) with a conservatively treated distal radius fracture was scanned by high-resolution peripheral quantitative computed tomography (HRpQCT) at one to two, three to four, six to eight, and twelve weeks and two years post-fracture. The same region contra-laterally was scanned as well at the two-year visit. BMD, micro-architecture and stiffness parameters were determined and the fracture side was compared to the contra-lateral side using a linear mixed-effect model. Spearman's correlation was used to correlate the two-year bone stiffness with baseline BMD, micro-architecture, and early three-month changes in these parameters.

Results

Two years post-fracture, cortical and trabecular thickness and torsional and bending stiffness were significantly higher at the fractured side compared with the non-fractured side (21%, 55%, 31% and 29%, respectively, $p < 0.05$), whereas BMD was similar. Two-year torsional and bending stiffness correlated significantly with baseline BMD and cortical perimeter ($|\rho| \geq 0.63$, $p < 0.016$), but not with early changes in bone parameters.

Conclusion

Using HRpQCT, this study illustrates that fracture healing is not completed by the time the cast is removed. We showed that from six weeks to two years post-fracture large changes occur in BMD, micro-architecture and biomechanical parameters at the fractured side, which were fully recovered after two years in comparison to the non-fractured contra-lateral side. Interestingly, higher two-year torsional and bending stiffness were associated with lower BMD and higher cortical perimeter at baseline.

Introduction

Fracture healing is a complex postnatal regenerative process that recapitulates many ontological events of embryonic skeletal development.¹ The results of intense catabolic and anabolic processes in fracture repair are commonly described by a four-phase model: initiated by inflammation, soft and subsequent hard callus formation consolidates the bone fragments, which is then followed by remodeling of the callus.^{2, 3} During the remodeling phase, the structural integrity of the trabecular region and the cortex are remodeled in order to fully restore the bone's biomechanical properties³. Whereas the first three phases generally are completed within four to five weeks after fracture¹, the remodeling phase continues for months to years.³

In clinical practice, the recommended follow-up period for conservatively treated distal radius fractures is three weeks for non-displaced fractures and four to five weeks for displaced fractures.⁴ At that time, immobilization of the wrist is no longer continued by removal of the cast and, although the remodeling process has only just started by then, the wrist is not routinely subjected to follow-up for further clinical evaluation. The rationale behind this approach is that the fracture is not likely to displace anymore, since the fragments are stabilized by the presence of hard callus. However, the long-term outcome of the healing fracture, in terms of restored micro-architecture and bone strength, is thus not taken into full consideration.

We previously studied the first three months of fracture healing in stable distal radius fractures with high-resolution peripheral quantitative computed tomography (HRpQCT) and found significant changes in bone density, micro-architecture and bone mechanical competence.⁵ Furthermore, it was shown that early changes in trabecular bone density and separation, and torsional stiffness predicted the clinical outcome twelve weeks post-fracture.⁶ Since the follow-up period in this study was limited to twelve weeks post-fracture, it is unknown to what extent the fractured radius will be restored in terms of bone density, micro-architecture and mechanical properties in the long term. We therefore conducted a follow-up at two years post-fracture to study the long-term restoration of these parameters.

The purpose of this study was threefold: Because we expected large changes at the fracture side in the period from cast removal to two years post-fracture, the first goal was to quantify these changes by comparing the two-year bone parameters at the fracture side to the six- and twelve-week bone parameters at the same side.

Our second goal was to investigate if the bone was fully recovered at two years post-fracture by comparing the bone density, micro-architectural and mechanical parameters at two years post-fracture between the fractured and the non-fractured contra-lateral side. Last, because the main purpose of fracture healing is the restoration of the bone's mechanical properties, which is accomplished by cortical and trabecular remodeling, our third goal was to investigate if the two-year bone mechanical parameters could be predicted from 1) the initial bone density and micro-architectural parameters, and 2) the early changes in these parameters.

Materials and Methods

HRpQCT scans were scheduled according to protocol at one to two (baseline), three to four weeks, six to eight weeks and twelve weeks post-fracture, and additionally at two years post-fracture. The short-term study protocol (registration no. NTR3821) and its extension were approved by an independent Medical Ethics Committee.

Figure 1 Flow diagram of the number of patients who were included in the two-year follow-up visit.

Subjects

All eighteen patients who had participated in our previously published study⁵ and were still alive were invited to participate in the additional visit two years post-fracture. No additional inclusion and exclusion criteria were applied (Table 1). Of these patients, fourteen were found eligible for inclusion in the analysis of the

bone parameters at the two-year visit (Figure 1). Patients that agreed to participate in the two-year visit gave written informed consent prior to participation.

Scanning by HRpQCT

The distal radius at the fractured and the contra-lateral side were scanned by HRpQCT (XtremeCT, Scanco Medical AG, Bruttisellen, Switzerland) using standard clinical settings (peak voltage of 60 kVp, tube current of 900 μ A and 100 ms integration time). A scout view was made prior to scanning to determine the start of the scan. The region of interest (ROI) at the fractured side was set similarly to the initial four visits⁵, which was based on the initial fracture location and was 18 mm long. To be able to compare the fracture side to the contra-lateral side, the ROI at the contra-lateral side was chosen the same as the ROI at the fractured side (Figure 2). All images were reconstructed using an isotropic voxelsize of 82 μ m, thus each measurement resulted in 220 parallel slices.

To ensure compatibility with the initial visits, the scans at the fracture side were made while patients were wearing a fiberglass cast. The scans at the contra-lateral side were made without a cast. To prevent motion, the forearms were fixed in a cylindrical carbon holder with an inflatable cushion (Pearltec AG, Schlieren, Switzerland).

After scanning, all HRpQCT images were checked for motion-induced image artifacts and were quality graded according to the manufacturer's guidelines and as described by Pialat et al.⁷ In case of insufficient quality, i.e. grades four and five, the scan was repeated.

Figure 2 Scout views of the fracture side (left) and contra-lateral side (right) showing the region that was scanned by HRpQCT during the fifth visit at two years post-fracture. At both sides, the proximal edge of the lunate bone was chosen as the landmark for the placement of the reference line. The scan region was 18.04 mm long, but the offset between the reference line and the start of the scan was different for each patient because it depended on the location of the fracture.

Evaluation of bone density and micro-architecture

During all visits, the HRpQCT images of both sides were evaluated using the standard patient evaluation protocol as described earlier by Boutroy et al.⁸ In short, a semi-automated contouring algorithm was used to derive the periosteal boundary surface of the radius. After contouring, the total region was divided in a trabecular and cortical compartment and the following bone parameters were determined: volumetric bone mineral density [mgHA/cm³] was assessed for the total (Dt_{tot}), trabecular (Dt_{rab}) and cortical region (Dt_{cort}).⁹ The micro-architectural parameters that were calculated in the trabecular region were the trabecular number (Tb.N) [mm⁻¹], thickness (Tb.Th) [mm] and separation (Tb.Sp) [mm], which were all determined using a 3D ridge extraction method¹⁰ and standard morphological relations. For the cortical region, the cortical thickness (Ct.Th) [mm] and the cortical perimeter (Ct.Pm) [mm] were obtained.⁹

Micro finite element analysis

Micro finite element (μFE) models were created directly from the segmented HRpQCT images similar to earlier studies.^{11, 12} In short, to obtain a representative μFE model of the bone's micro-architecture each voxel in the segmented images that represents bone tissue was converted into a brick element of the same size. These μFE models typically consisted of three to four million elements. A Young's modulus of 10 GPa and a Poisson's ratio of 0.3 were assigned to every element.

Different load cases were applied to simulate clinically relevant loading scenarios, where the associated stiffness was chosen as outcome variable. The first load case represented a 'high friction' compression test with a prescribed displacement in the axial direction of 1% of the total length, from which the compression stiffness (S_{comp}) [kN/mm] was calculated. The second load case represented a prescribed rotation of 0.01 rad around the longitudinal axis applied to the surface perpendicular to this axis from which the torsional stiffness (S_{tors}) [kNmm/rad] was calculated. A third and fourth load case represented a prescribed rotation of 0.01 rad applied around the sagittal and transversal axes respectively, onto the surfaces perpendicular to each axis, thus inducing a state of pure bending in two directions, from which the bending stiffness in each direction (S_{sagittal} and S_{transversal}) was calculated. Because the orientation of the patients' forearm was not exactly the same during all visits, a quadratic mean bending stiffness was calculated (S_{bend}) [kNmm²] from the sagittal and transversal bending stiffness according to Equation 1.

$$S_{bend} = \sqrt{\frac{S_{sagittal}^2 + S_{transversal}^2}{2}} \quad (\text{Equation 1})$$

Statistical Analysis

To be able to deal with missing values caused by excluding scans of insufficient quality or missed visits, a linear mixed effect model was used. Using this model, the bone density, architectural and mechanical parameters at each visit were expressed as estimated marginal means (EMM) with 95% confidence intervals. To test if the bone parameters at the fracture side were restored at two years post-fracture, the bone parameters at the fracture side were compared to the bone parameters at the contra-lateral side by pairwise comparison.

To test if the bone parameters at six and twelve weeks post-fracture, i.e. visit three and four, were different at two years post-fracture, the bone parameters at visit three and four were compared to the bone parameters at the two-year visit using pairwise comparison. To correct for multiple comparisons, Bonferroni correction was applied.

To correlate the two-year bone biomechanical parameters with the early changes, i.e. from baseline to twelve weeks post-fracture, in bone density and micro-architecture, a summary measure was defined for each parameter as described by Matthews et al.¹³. The following summary measures were tested: slope of the regression line and normalized area under the curve (AUC). Next, these summary measures as well as the baseline value of each bone parameter were correlated to the two-year bone mechanical parameters Scomp, Stors and Sblend using Spearman's correlation coefficient.

A significance level of $\alpha = 0.05$ was used for all analyses and all statistical analyses were performed with SPSS Statistics for Windows version 20.0 (IBM Corp., Armonk, NY, USA).

Results

The mean time between the date of fracture and the two-year visit was 26.5 months (range 22.3–29.3). Of the scans in the seventeen patients in the first four visits and fourteen patients in the two-year follow-up visit, in total nineteen scans (19.8%) were of insufficient quality due to motion artifacts, even after repetition. These scans were excluded from the statistical analysis of the bone parameters.

Restoration of the fracture side

Visual inspection of the follow-up HRpQCT images of the healing distal radius fractures showed that all cortical gaps were completely restored at two years post-fracture in all participants (Figure 3). In general, the images suggest that the cortex is denser and thicker at two years post-fracture in comparison to the cortex

at the earlier visits. Also, the images suggest that remodeling of the trabecular structure has occurred during the two year healing period because fewer trabeculae seem to be present at the two-year image compared to the twelve-week image. Furthermore, the blurring in the trabecular region, which was visible at three up to twelve weeks post-fracture, has disappeared at two years post-fracture.

Figure 3 Longitudinal HRpQCT slices of four fractures showing the formation of woven bone (visits two and three) and bridging of the cortex (visit four). At two years post-fracture (visit five), the cortices suggested to be denser and thicker and the trabecular compartment appeared to show signs of deterioration compared with baseline.

Table 1 Inclusion and exclusion criteria for the initial study

Inclusion criteria	Exclusion criteria
Postmenopausal women	Previous fracture at current location
Older than 50 years	Active or suspected infection
Stable distal radius fracture	Malignant disease in the past 12 months
Treated by cast immobilization	Neuromuscular or neurosensory deficit which would limit the ability to assess performance
Willing and able to participate	Known systemic or metabolic disorders leading to progressive bone deterioration
	Active inflammatory disease
	Use of oral glucocorticoids
	Mental incompetence
	Severe concurrent joint involvements
	Selection for other trial on distal radius fractures

The bone density, micro-architectural and biomechanical parameters at the fracture side at each visit, as well as the bone parameters at the contra-lateral side at the fifth visit are presented in Table 2. Time series line plots of the bone parameters at fracture side together with the side-by-side comparison at two years post-fracture are plotted in Figure 4.

From twelve weeks post-fracture to two years post-fracture, only Dt_{tot}, Tb.Th and Ct.Pm did not change significantly. The other parameters either increased or decreased significantly in this period. While Dt_{tot} did not change significantly, Dtrab decreased further with 21.1% ($p = 0.002$), and Dcort increased with 13.5% ($p < 0.001$). For the micro-architectural parameters, Tb.N decreased with 18.6% ($p = 0.002$) and Tb.Sp increased by 33.9%. Ct.Th increased with 31.4% ($p < 0.001$). Also the bone mechanical parameters Scomp, Stors and S_{bend} increased, with 47.2%, 67.5% and 96.8% (all $p < 0.001$), respectively.

Similar results were found for the change in bone parameters from six weeks post-fracture to two years-post fracture (see Table 1). In general, the changes in this period were larger, except for Dcort, where the increase was 12.7% ($p < 0.001$).

Comparison to contra-lateral side

At two years post-fracture trabecular and cortical thickness were significantly higher at the fracture side (+55.5%, $p < 0.001$ and +19.8%, $p = 0.047$, respectively) than at the contra-lateral side. Also, Stors and S_{bend} were significantly higher at the fracture side than at the contra-lateral side (+30.7%, $p = 0.016$ and +29.5%, $p = 0.030$, respectively). Scomp, however, did not differ significantly between both sides. The other parameters, i.e. bone density parameters Dt_{tot}, Dtrab and Dcort and micro-architectural parameters Tb.N and Tb.Sp, and Ct.Pm, were also not significantly different between the fracture and the contra-lateral side at two years post-fracture.

Table 2 Estimated marginal means (EMM), 95% confidence intervals (CI) for the bone parameters at the fracture side at each visit and the contra-lateral side at the 5th visit. The percent changes ($\Delta\%$) from visit 3 and 4 to visit 5 are shown as well.

	Visit 1		Visit 2		Visit 3		Visit 4		Visit 5		$\Delta_{3-5}(\%)$	$\Delta_{4-5}(\%)$	
	(1.3 weeks post-fracture)		(3.5 weeks post-fracture)		(6.3 weeks post-fracture)		(12.3 weeks post-fracture)		(26.5 months post-fracture)				
	EMM	95% CI	EMM	95% CI	EMM	95% CI	EMM	95% CI	EMM	95% CI			
Fracture side													
Dtot	[mgHA/cm ³]	233	205 - 262	243	215 - 270	259	231 - 287	247	218 - 276	239	209 - 268	-7.9	-3.5
Dtrab	[mgHA/cm ³]	147	130 - 164	160	144 - 177	179	162 - 195	161	144 - 178	127	109 - 145	-28.9 ^y	-21.2 ^β
Dcort	[mgHA/cm ³]	650	595 - 705	626	572 - 680	612	558 - 666	608	553 - 663	690	634 - 746	12.7 ^y	13.5 ^y
Tb.N	[1/mm]	1.96	1.74 - 2.18	2.01	1.8 - 2.22	1.96	1.74 - 2.17	1.71	1.49 - 1.93	1.39	1.16 - 1.62	-28.8 ^y	-18.6 ^β
Tb.Th	[mm]	0.065	0.055 - 0.075	0.068	0.059 - 0.077	0.079	0.069 - 0.088	0.083	0.072 - 0.093	0.085	0.074 - 0.096	8.1	2.9
Tb.Sp	[mm]	0.473	0.381 - 0.565	0.457	0.369 - 0.544	0.454	0.365 - 0.543	0.549	0.457 - 0.642	0.736	0.638 - 0.833	61.9 ^y	33.9 ^y
Ct.Th	[mm]	0.40	0.30 - 0.51	0.39	0.29 - 0.50	0.43	0.32 - 0.53	0.44	0.34 - 0.54	0.58	0.47 - 0.68	35.1 ^y	31.4 ^y
Ct.Pm	[mm]	85.6	78.2 - 93.0	87.9	80.7 - 95.2	85.7	78.4 - 93.1	84.0	76.6 - 91.5	79.6	72.0 - 87.2	-7.2	-5.3
Scomp	[kN/mm]	15.7	11.7 - 19.7	13.0	9.3 - 16.8	15.4	11.6 - 19.1	20.6	16.6 - 24.5	30.3	26.1 - 34.5	97.3 ^y	47.2 ^y
Stors	[kNmm/rad]	330	244 - 416	269	189 - 348	334	253 - 416	445	358 - 531	745	653 - 837	122.7 ^y	67.5 ^y
Sbend	[kNmm ²]	473	326 - 620	374	239 - 509	446	307 - 585	648	500 - 795	1275	1116 - 1433	185.6 ^y	96.8 ^y
Contra-lateral side													
Dtot	[mgHA/cm ³]									218	187 - 248		
Dtrab	[mgHA/cm ³]									108	89 - 128		
Dcort	[mgHA/cm ³]									696	639 - 753		
Tb.N	[1/mm]									1.65	1.41 - 1.89		
Tb.Th	[mm]									0.055	0.043 - 0.067		
Tb.Sp	[mm]									0.626	0.523 - 0.729		
Ct.Th	[mm]									0.48	0.37 - 0.59		
Ct.Pm	[mm]									75.2	67.4 - 83.0		
Scomp	[kN/mm]									25.3	20.9 - 29.8		
Stors	[kNmm/rad]									570	470 - 669		
Sbend	[kNmm ²]									985	812 - 1157		

Dtot = total density; Dtrab = trabecular density; Dcort = cortical density; Tb.N = trabecular number; Tb.Th = trabecular thickness; Tb.Sp = trabecular separation; Ct.Th = cortical thickness; Ct.Pm = cortical perimeter; Scomp = compression stiffness; Stors = torsional stiffness; Sbend = bending stiffness.

Estimated marginal means, 95% confidence intervals and p-values (^a $p \leq 0.05$; ^β $p \leq 0.01$; and ^v $p \leq 0.001$) were derived from a linear mixed-effect model with visit as fixed effect

Figure 4 Time series line plots of the HRpQCT-derived bone density (A–C), micro-architectural (D–F), geometrical (G, H), and biomechanical (J–L) parameters at the fracture side (white squares) and the two-year post-fracture parameters at the contra-lateral side (black squares). Estimated marginal means and 95% confidence intervals are calculated using a linear mixed-effect model. The p-values for the pairwise comparison between the fracture and contra-lateral side at the two-year post-fracture visit are obtained from the same linear mixed-effect model.

Relation between two-year bone stiffness and baseline/early changes in BMD and micro-architecture

The two-year compression stiffness S_{comp} correlated significantly with the early change in D_{cort} , expressed by the slope ($\rho = -0.670$, $p = 0.009$) and the normalized AUC ($\rho = -0.749$, $p = 0.002$). The two-year S_{comp} did not correlate to any of the bone parameters at baseline.

Two-year torsional stiffness correlated significantly with D_{tot} ($\rho = -0.666$, $p = 0.009$) and $Ct.Pm$ ($\rho = 0.666$, $p = 0.009$) at baseline (Figure 5), but not with the

early changes in any of the bone parameters. Similarly, two-year bending stiffness correlated significantly with D_{tot} ($\rho = -0.631$, $p = 0.016$) and $Ct.Pm$ ($\rho = 0.653$, $p = 0.011$) at baseline, but not with the early changes in any of the bone parameters.

Figure 5 Correlations of the total density D_{tot} (right) and cortical perimeter $Ct.Pm$ (left) at baseline and the two-year bone torsional stiffness. Representative transversal slices of two fractures (● = reduced; ○ = non-reduced) are overlaid and indicate that the reduced fractures with a generally lower D_{tot} and higher $Ct.Pm$ at baseline result in a higher two-year torsional stiffness.

Discussion

To our knowledge, this study represents the first use of non-invasive high-resolution *in vivo* imaging to monitor the healing process of metaphyseal fractures with a follow-up period of two years. Compared to the contra-lateral side at two years post-fracture, the fractured side had thicker cortices and trabeculae while BMD was similar. Ultimately, this resulted in a restoration of bone stiffness that ended up being significantly higher at the fracture side than at the contra-lateral side.

Changes at the fracture side

Changes in the bone parameters during the first three months of fracture healing were published earlier⁵, but there a comparison with baseline was made. The additional visit at two years post-fracture in the current study allowed us to examine to which extent the bone parameters change after the cast has been removed. Interesting were the remarkable changes that we found in almost all bone density, micro-architectural and biomechanical parameters. Both the HRpQCT images as

well as the evaluation of the bone parameters revealed that in the period from six weeks to two years post-fracture, the cortex became thicker while concurrently there was a decrease in trabecular number and increase in trabecular separation. These changes in the trabecular region might be caused by the thickening of the cortex: as with a thicker cortex the load-bearing shifts towards the cortical region, and less load is carried by the trabecular region which ultimately could lead to resorption of the trabecular bone.

Simultaneous to the cortical and trabecular changes, bone stiffness showed a major increase of more than 100%. This result thus suggests that by the time the cast is removed, the bone mechanical properties as measured by HRpQCT and μ FEA are far from recovered. From Figure 3, however, it is apparent that more complex fractures do not remodel back to the situation before fracture, whereas the less displaced fractures appear to do so. Additional statistical analysis with stratification by group (reduction versus no reduction required) revealed that for the fractures that did not need reduction the stiffness parameters at the fracture side at any visit did not significantly differ from the contra-lateral side at two years post-fracture. For the reduced fractures, on the other hand, the stiffness parameters at the first three visits were all significantly lower and at the last visit significantly higher at fracture side in comparison to the contra-lateral side at the last visit (data not shown). Despite the low number of subjects in each group, this might indicate that less complex fractures are faster recovered biomechanically speaking than more complex fractures.

Clinically speaking, on the other hand, all patients in this study were considered sufficiently recovered at the time the cast was removed, regardless of whether reduction was required during treatment. The difference between being recovered clinically and biomechanically indicates that a 100% recovery of bone strength is not required for normal daily activities. Indeed, clinicians primarily advise their patients to practice on regaining normal flexion and extension, and to load their arm gradually as pain allows. Loading the fractured wrist up to the same amount as before fracture is thus not recommended at that time, advice that is now supported by the results we presented here.

Comparison to contra-lateral side

As can be seen in Figures 4J-L, the bone compression, torsional and bending stiffness are all at least restored at two years post-fracture. However, the torsional and bending stiffness exceeded the contra-lateral values significantly, whereas this was not the case for stiffness in compression. Although this difference might be caused by a lack of power owing to the low number of subjects, it is also possible that there is a true difference between the recovery of the different stiffnesses.

From a clinical perspective, bone compressive and bending strength are most important as they determine whether the bone is strong enough to withstand the bending and compressive forces another fall on the outstretched arm, while torsional stiffness seem to be of less importance in this case.

Correlation early changes in bone parameters and bone stiffness at two years post-fracture

An interesting finding of this study is that a higher bone compression stiffness at two years post-fracture is associated with a higher early decrease in cortical density. A likely explanation is that newly formed cortical bone is incorporated into the cortical mask as soon as it reaches a density that is above the used threshold. Because this new bone is still not as dense as the old cortical bone, including it into the cortical mask leads to a decrease in the overall cortical density. Our results suggest that the sooner this happens and the cortical gaps are filled with new bone, the better the compression stiffness will be at two years post-fracture.

Opposite to two-year compression stiffness, we found no association between the two-year torsional and bending stiffness and the early changes in bone density or micro-architecture. Instead, two-year torsional and bending stiffness were associated with baseline values of BMD and cortical perimeter. Interestingly, this relation seems to be linked to whether a fracture required reduction (Figure 5). An explanation for this might be that a nonreduced fracture most likely was not displaced and thus would not have a distal fragment overlapping its proximal part and therefore will have a normal perimeter. Displaced fractures that were reduced, however, will have an increased perimeter due to the overlap of a distal segment over its proximal part. Because the outer perimeter is used as the boundary of the ROI for the evaluation of the bone parameters, the total density is affected as well. Although the displaced fractures were reduced to within normal limits during treatment, some overlap might be unavoidable. During healing, these overlapping fragments grow back together and thus lead to a large amount of bone mass on the outer side of the bone. This finding is consistent with the standard rules of mechanics that tell us that adding material to the outer side of a volume has a positive influence on the volume's torsional and bending stiffness and less to no effect on the compression stiffness.

Limitations

An important limitation of our study is that at six and twelve weeks post-fracture, cartilaginous or low-mineralized tissue is present at the fracture side to give support and stability. Since these tissues are not taken into account in the μ FEA, the true stiffness at the fractured side at these time points might be underestimated

by μ FEA. For a more accurate estimation of the bone stiffness at these time points, these tissues should be included into the μ FE model as well, for example by combining the HRpQCT data with information from quantitative magnetic resonance imaging. Another approach that allows in vivo assessment of bone strength in healing fractures is radiostereometric analysis (RSA).^{14, 15} Although a prescribed load has to be applied onto the fracture and markers need to be placed onto the bone fragments, which can be problematic in particularly the first weeks post-fracture, RSA could be a potential next step in future studies on bone strength in healing wrist fractures.

Another limitation is that the number of subjects is small and some measurements were of insufficient quality, leading to a low number of usable measurements. However, despite the exploratory nature of the study and thanks to the use of an advanced statistical model that is able to deal with these missing values, we can still make some strong conclusions.

Also, in the side-to-side comparison we assumed that there is no bilateral asymmetry in bone parameters at the distal radius. This assumption is partially legitimate, as a recent HRpQCT study of Hildebrandt et al. found no left-right differences in the BMD and micro-architectural parameters at the distal radius, whereas the bone failure load was found to be slightly higher at the dominant side (+3.0%).¹⁶ However, because most fractures in our study occurred at the non-dominant side ($n = 9$, 74%), correcting for fracture side would probably strengthen the conclusions.

Another limitation is that, unfortunately, we did not scan the fracture at other time points, e.g. six and twelve months. We do not know whether full recovery already was obtained earlier than two years post-fracture.

Last, we performed a large set of correlations without correcting for multiple testing. The results regarding the correlation between the early changes in bone density and micro-architecture and the two-year bone stiffness should therefore be interpreted with caution, and should mainly be used as an indication for further studies on HRpQCT and fracture healing.

In conclusion, based on HRpQCT measurements in women with a stable distal radius fracture, this study shows that the fractured radius at two years post-fracture has a thicker cortex and thicker trabeculae in comparison with the contra-lateral side. Furthermore, bone torsional and bending stiffness at two years post-fracture are significantly higher than at the non-fractured contra-lateral side. Interestingly, higher torsional and bending stiffness at two-year post-fracture are associated with lower BMD and higher cortical perimeter at baseline.

Disclosures

BvR is a consultant for Scanco Medical AG. JJCA is a board member of workgroup Biotechnology of the Dutch Orthopedic Association (NOV). PCW is a board member of the Dutch Spine Society (association of spine surgeons). All other authors state that they have no conflicts of interest.

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6

Contra-lateral bone loss at the distal radius in postmenopausal women after a distal radius fracture: a two-year follow-up HRpQCT Study

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Submitted

Abstract

Introduction

Opposite to the fracture side, bone mineral density (BMD) measured by DXA at the contra-lateral side does not change after a distal radius fracture. However, it is unknown if also bone micro-architecture and strength at the contra-lateral side are unaffected. Therefore, the aim of this study was to assess BMD, micro-architecture and bone mechanical properties at the contra-lateral side during two years follow-up after a distal radius fracture using high-resolution peripheral quantitative computed tomography (HRpQCT).

Methods

The contra-lateral distal radius of fourteen postmenopausal women (mean age 64 ± 8 years) with a distal radius fracture treated by cast immobilization was scanned by HRpQCT at baseline, three months and two years post-fracture. BMD and cortical and trabecular micro-architecture were measured and biomechanical parameters were estimated using micro finite element analysis. Additionally, markers of bone resorption and formation were measured at each visit. Bone parameters and turnover markers across the three visits were analyzed using a linear mixed-effect model with Bonferroni correction.

Results

Two years post-fracture, a significant decrease from baseline was found in cortical BMD (-3.9% , $p < 0.001$), failure load (-5.6% , $p = 0.007$), stiffness in compression (-5.1% , $p = 0.014$) and bending (-6.0% , $p = 0.029$), and bone formation (-50.9% , $p = 0.010$). No significant changes from baseline were observed in total and trabecular BMD, nor in cortical or trabecular micro-architecture and neither in bone resorption. Results were similar between patients with or without adequate anti-osteoporosis drug treatment.

Conclusion

We found a significant decline in BMD in the cortical but not the trabecular region, and a reduction in bone strength and stiffness at the contra-lateral side two years after a distal radius fracture. These changes exceeded the changes that may be expected due to aging, even in the presence of adequate anti-osteoporosis treatment.

Introduction

After fracture of a lower extremity, bone loss often occurs at the fracture side, even at locations more distant from the fracture, due to a period of consequent absence of weight-bearing and immobilization.^{1,2} Bone loss has also been observed at the contra-lateral side after a hip fracture up to two years post-fracture³⁻⁶ (but not after a tibial shaft fracture²).

Following Wolff's law, which states that a bone's internal structure and shape adapt itself to the mechanical loading conditions imposed on it⁷, it is not surprising that the bone at the contra-lateral hip is resorbed after a period of decreased loading due to bed-rest or immobilization. This effect is therefore not expected at non-weight-bearing limbs, such as the forearm, for which the contra-lateral side is not immobilized. Indeed, several studies reported no changes in BMD at the contra-lateral radius one year after a Colles' fracture.^{8,9}

Whereas the aforementioned studies only took areal bone mineral density (aBMD) assessed by dual-energy x-ray absorptiometry (DXA) into account, we recently used high-resolution peripheral quantitative computed tomography in combination with micro finite element analysis (μ FEA) to assess changes in volumetric bone mineral density (vBMD), bone micro-architecture and bone strength during the healing process of distal radius fractures.^{10,11} In this study, large changes in vBMD, micro-architecture and biomechanical parameters were found at the fracture side during a follow-up of two years post-fracture. Also, we found no changes in these bone parameters at the contra-lateral side during the first three months post-fracture¹⁰, but it is unknown if the bone parameters remain unchanged during a longer time post-fracture.

Since earlier studies found no changes in aBMD at the contra-lateral side after a forearm fracture^{8,9}, we hypothesized that after the initial three-month period of no change, no changes in vBMD and therefore also not in micro-architecture and bone strength occurs at the contra-lateral side during the two years post-fracture. To address these hypotheses, we monitored vBMD, micro-architecture and bone biomechanical properties at the contra-lateral radius using HRpQCT and μ FEA during a period of two years after a distal radius fracture.

Methods

Subjects

The patients in this study all participated in a medical ethical approved clinical trial on fracture healing (registration no. NTR3821).¹¹ Besides the initial inclusion and exclusion criteria (Table 1), an additional exclusion criterion for the current study was a previous fracture at the contra-lateral side. All patients were informed and gave written informed consent prior to participation.

HRpQCT scanning

HRpQCT (XtremeCT, Scanco, Brüttisellen Switzerland) scans of the contra-lateral forearm were performed at baseline (one to two weeks post-fracture) and three months and two years post-fracture. The scans were made at standard clinical high-resolution settings, i.e. with a tube voltage of 60 kVp, tube current of 900 μ A and a 100 ms integration time. All scans were reconstructed using an isotropic voxelsize of 82 μ m. At baseline and the first follow-up visit, the region of interest (ROI) incorporated a 9 mm long section starting 9.5 mm proximal of the os lunate bone in all patients. Due to changes in the study protocol, the ROI at the two-year follow-up visit was extended to a 18 mm long section. The start of this section differed between patients such that it matched the scanned region at fracture side.¹¹

To prevent motion artifacts, the forearm of each patient was fixed in a cylindrical carbon holder (XCT Carpal, Pearltec AG, Switzerland) with an inflatable cushion as described previously.¹² After scanning, all HRpQCT images were checked for motion-induced image artifacts and were quality graded according to the manufacturer's guidelines and as described by Pialat et al.¹³ In case of insufficient quality, i.e. grade four and five, the scan was repeated once.

Quality control during the study period was assured by regular HRpQCT measurements of a calibration phantom containing rods of different densities (0, 100, 200, 400, and 800 mgHA/cm³).

Evaluation of bone density and micro-architecture

Since the scanned region at the last visit differed from the first two visits, the common ROI was determined first by matching the first and last slices of the follow-up scans to the baseline scan. The bone density and micro-architectural parameters within this common ROI were then evaluated using the standard patient evaluation protocol provided by the manufacturer (Scanco Medical AG) as described earlier.¹⁴ In short, after semi-automatically deriving the periosteal contour the total region was divided in a cortical and trabecular region and each region was segmented and combined to into a 3D model to represent the micro-

architecture. vBMD [mgHA/cm^3] was calculated in the total (Dt_{tot}), trabecular (D_{trab}) and cortical (D_{cort}) region. Furthermore, trabecular number (Tb.N) [mm^{-1}], thickness (Tb.Th) [mm] and separation (Tb.Sp) [mm] were determined to assess the trabecular micro-architecture, and cortical thickness (Ct.Th) [mm] and perimeter (Ct.Pm) [mm] were assessed for the cortical compartment. In addition to the standard patient evaluation protocol, extended cortical analysis as described by Burghardt et al.¹⁵ was performed to assess cortical porosity (Ct.Po) [%], cortical pore volume (Ct.Po.V) [mm^3] and diameter (Ct.Po.V) [mm].

Finite element analysis

To assess the bone's mechanical properties, micro finite element (μ FE) models were created directly from the segmented HRpQCT images similar to earlier studies.^{16, 17} In short, to obtain a representative μ FE model of the bone's micro-architecture each voxel in the segmented images that represents bone tissue was converted into a brick element of the same size. These μ FE models typically consisted of around 1 million elements. Constant material properties were assigned to each element, i.e. a Young's modulus of 10 GPa and a Poisson's ratio of 0.3

Next, a compression load was simulated by applying a 'high friction' compression test with a prescribed displacement in the axial direction of 1% of the total length, from which the compression stiffness (S_{comp}) [kN/mm] and ultimate failure load (F_{Ult}) [kN] were estimated.

Additionally, by applying representative loading scenarios also torsional (Stors) [kNmm/rad] and bending stiffness (S_{bend}) [kNmm²] were estimated in a similar way as in our previous study and which has been described earlier.¹⁰

DXA and biochemical analyses

In accordance with Dutch standard care when a patient over the age of 50 years comes to clinic with a fracture, all patients in this study visited the fracture liaison service (FLS) between four and eight weeks post-fracture for assessment of osteoporosis and vertebral fractures by DXA (Hologic Discovery A; Hologic Inc., Waltham, MA). If the patients already had had a DXA scan in the last year prior to the fracture, this previous DXA measurement was used.

Additionally, blood serum samples were taken for biochemical analyses that included calcium (Photometric assay; Roche Diagnostics, Basel, Switzerland), 25-hydroxyvitamin D (chemiluminescence immunoassay; IDS-iSYS Immunodiagnostic Systems GmbH, Frankfurt, Germany), parathyroid hormone (chemiluminescence immunoassay; Siemens, Erlangen, Germany), thyroid-stimulating hormone (electro-chemiluminescence immunoassay; Roche Diagnostics, Basel, Switzerland) and free thyroxine (fluorescence immunoassay;

Perkin Elmer, Turku, Finland) for diagnosis of hyperthyroidism, hypercalcemia and hyperparathyroidism, which are known contributors to bone loss.¹⁸ Furthermore, patients were asked about their current medication. If patients were diagnosed with osteoporosis, adequate anti-osteoporosis treatment was started by bisphosphonates, denosumab, calcium or vitamin D, or a combination of these medications. At the end of the study, a telephone interview was conducted with each patient to check whether the patients were compliant to their treatment.

Serum markers of bone turnover

In addition to the standard biochemical analyses, venous blood samples were collected at each visit by puncture of the antecubital vein for assessment of bone turnover markers. Serum was separated from the bloodclot within 20 minutes and frozen to -20 °C until analysis. Serum bone markers representing bone resorption (carboxy-terminal telopeptide of type I collagen (ICTP)) and bone formation (procollagen type-I N-terminal propeptide (PINP)) were assessed by radioimmunoassay (UniQ® PINP/ICTP; Orion Diagnostics, Espoo, Finland) in a certified laboratory. The calculated precision profile indicates a coefficient of variation below 10% over the range of 2.5 to 25 mg/L for ICTP and over the range of 25 to 140 mg/L for PINP. The observed lower limit of blank was 0.6 mg/L for ICTP and 2 mg/L for PINP.

Statistics

Patient's demographic and clinical baseline characteristics were reported in means and their standard deviations. HRpQCT derived bone parameters and the bone turnover markers at each visit were reported as estimated marginal means and their 95% confidence intervals which were obtained from a linear mixed effect model with visit as fixed effect and a compound symmetry covariance structure. The same linear mixed-effect model with Bonferroni correction was used to compare the bone parameters and bone turnover markers between the three visits.

A level of significance of 0.05 was used in all statistical analyses, and all analyses were performed with SPSS Statistics for Windows version 20.0 (IBM Corp., Armonk, USA).

Results

From the fifteen patients who completed all visits, one patient developed a complex regional pain syndrome. Fourteen patients were therefore found to be eligible and were included in the analyses. The demographic and clinical baseline

characteristics of these patients are presented in Table 2. At baseline, seven patients were already receiving calcium and/or vitamin D supplementation, and three of them were also being treated with alendronate. At three months post-fracture, anti-osteoporosis treatment was started (or continued) at the FLS: calcium and/or vitamin D supplementation started (or continued) in all patients. Additionally, seven patients started and three patients continued with alendronate 70 mg per week, and one patient started with subcutaneous injections of 60 mg denosumab every six months. The end-of-study telephone interview with each patient confirmed that the denosumab injections were administered correctly, and that compliance with alendronate treatment was 67%. One patient could not be contacted.

From the in total 42 HRpQCT measurements, nine measurements (21.4%) were of insufficient quality, due to motion artifacts. The average common ROI between the HRpQCT scans at the three visits consisted of 100.7 slices (91.5%).

Table 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Postmenopausal women	Previous fracture at the same or contra-lateral location
Older than 50 years	Active or suspected infection
Stable distal radius fracture	Malignant disease in the past 12 months
Treated by cast immobilization	Neuromuscular or neurosensory deficit which would limit the ability to assess performance
Willing and able to participate	Known systemic or metabolic disorders leading to progressive bone deterioration
	Active inflammatory disease
	Use of oral glucocorticoids
	Mental incompetence
	Severe concurrent joint involvements
	Selection for other trial on distal radius fractures

Changes in bone density

In Table 3, the HRpQCT derived bone parameters as well as the percentage change from baseline at each visit within the common ROI are presented. During two years post-fracture, no significant changes occurred in total or trabecular vBMD. For cortical vBMD an initial twelve-week period without change was followed by a decline that led to a change from baseline of -3.9% ($p < 0.001$) at two years post-fracture. Separate analysis of the two-year changes in cortical vBMD in the patients who were compliant with anti-resorptive treatment, i.e. alendronate or denosumab, that started three months post-fracture ($n = 5$) and the patients who did not receive or were not compliant with anti-resorptive treatment ($n = 6$), showed similar decreasing trends in cortical BMD (Figure 1, left panel).

Table 2 Patients' demographic and clinical baseline characteristics. If applicable, reference values are reported between parentheses.

Characteristic	Mean (SD)
Age [years]	64.3 (8.0)
DXA T-score at lumbar spine ^a	−2.4 (1.6)
Normal BMD [n; %]	1 (7.7%)
Osteopenic [n; %]	5 (38.5%)
Osteoporotic [n; %]	7 (53.8%)
Presence of VF [n; %] ^b	5 (41.7%)
25(OH)D [nmol/L] (15-162)	68.9 (28.3)
25(OH)D <50 nmol/L [n; %]	4 (28.6%)
25(OH)D <75 nmol/L [n; %]	7 (50.0%)
Calcium [mmol/L] (2.10-2.55)	2.45 (0.12)
PTH [pmol/L] (1.2-7.1)	3.54 (1.63)
TSH [mU/L] ^c (0.4-4.3)	2.0 (0.7-18.8)
Hypercalcemia [n; %]	0 (0.0%)
Hyperthyroidism [n; %]	0 (0.0%)
Hyperparathyroidism [n; %]	0 (0.0%)

Values are reported as means and their standard deviations (SD) unless stated otherwise.

^a From one patient no DXA data was available.

^b VFA not performed in two patients.

^c Not normally distributed. Median and range are reported.

Abbreviations: DXA = dual-energy X-ray absorptiometry; BMD = bone mineral density; VF = vertebral fracture; 25(OH)D = 25-hydroxyvitamin D; PTH = parathyroid hormone; TSH = thyroid-stimulating hormone.

Changes in cortical and trabecular micro-architecture

No significant changes were observed in the trabecular and cortical micro-architectural parameters at the contra-lateral side during two years post-fracture.

Changes in bone mechanical properties

Similar to cortical BMD, also in compressive failure load (F.Ult) as well as stiffness in compression (Scomp) and bending (Sbend) an initial twelve-week period without change was followed by a decline, which led to a change from baseline at two-years post-fracture of −5.6% ($p = 0.007$), −5.1% ($p = 0.014$) and −6.0% ($p = 0.029$), respectively. Torsional stiffness showed a comparable pattern, but the −2.5% change from baseline at two years post-fracture was not significant ($p = 1.000$). Separate analysis of the two-year changes in the patients who were compliant with anti-resorptive treatment, i.e. alendronate or denosumab, that started three months post-fracture ($n = 5$) and the patients who did not receive or were not compliant with anti-resorptive treatment ($n = 6$), showed a decreasing Scomp in patients who did not receive or were not compliant with alendronate treatment and in the patient treated with denosumab (Figure 1, right panel), while Scomp

tended to decrease less in the patients who were compliant with alendronate treatment that was started at three months post-fracture.

Figure 1 Median and 25% and 75% percentiles of cortical density (left) and compression stiffness (right) at the contra-lateral radius at baseline, three months and two years after a fracture in patients who started with alendronate (dark thick straight line) or denosumab (dark thin straight line) treatment at three months post-fracture, patients who were already started with alendronate treatment pre-fracture (light straight line), and patients who did not start or were not compliant to anti-resorptive treatment (dotted line).

Changes in markers of bone formation and resorption

Markers of bone formation (PINP) and resorption (ICTP) as well as the percentage change from baseline at each visit are presented in Table 3. During the first three months post-fracture, no significant changes were observed in both markers. At two years post-fracture, a significant decrease from baseline was observed in PINP (-50.9% , $p = 0.010$), but not in ICTP (-8.0% , $p = 1.000$). A similar pattern was observed in patients who were compliant to alendronate treatment started at three months post-fracture and in patients who were not compliant or were not treated with alendronate. The patient who was treated with denosumab injections showed a clear decrease in levels of both PINP and ICTP (Figure 2).

Table 3 Estimated marginal means (EMM) and 95% confidence intervals (95%CI) of the vBMD, micro-architectural and biomechanical parameters at the contra-lateral side and markers of bone formation and resorption at each study visit

Parameter	EMM (95%CI) at baseline	EMM (95%CI) at 3 months	Change from baseline (%)	p-value	EMM (95%CI) at 2 years	Change from baseline (%)	p-value
vBMD							
Dtot [mgHA/cm ³]	248,2 (198,9 - 297,4)	249,9 (200,7 - 299,2)	0,71	1,000	246,2 (197 - 295,5)	-0,77	1,000
Dtrab [mgHA/cm ³]	104,8 (79,8 - 129,8)	102,9 (77,9 - 127,9)	-1,83	1,000	102,4 (77,4 - 127,4)	-2,25	1,000
Dcort [mgHA/cm ³]	789,2 (741,1 - 837,3)	791,3 (743,2 - 839,4)	0,26	1,000	758,4 (710,3 - 806,5)	-3,90	<0,001
Micro-architecture							
Tb.N [mm ⁻¹]	1,49 (1,24 - 1,75)	1,55 (1,30 - 1,80)	3,80	0,750	1,55 (1,30 - 1,80)	3,56	0,840
Tb.Th [mm]	0,056 (0,05 - 0,063)	0,054 (0,048 - 0,060)	-4,11	0,351	0,054 (0,048 - 0,060)	-4,56	0,252
Tb.Sp [mm]	0,699 (0,511 - 0,886)	0,673 (0,485 - 0,86)	-3,74	0,815	0,659 (0,472 - 0,847)	-5,64	0,315
Ct.Th [mm]	0,62 (0,49 - 0,75)	0,64 (0,51 - 0,77)	2,81	0,193	0,61 (0,48 - 0,75)	-0,66	1,000
Ct.Pm [mm]	70,1 (66,2 - 74,0)	70,1 (66,2 - 74,0)	-0,02	1,000	70,6 (66,7 - 74,5)	0,62	0,508
Ct.Po* [%]	2,90 (2,07 - 3,72)	2,74 (1,92 - 3,57)	-5,46	1,000	2,94 (2,12 - 3,77)	1,45	1,000
Ct.Po.D* [mm]	0,17 (0,16 - 0,18)	0,17 (0,16 - 0,19)	0,56	1,000	0,17 (0,16 - 0,18)	-1,30	1,000
Ct.Po.V* [mm ³]	10,4 (7,8 - 13,1)	10,2 (7,5 - 12,8)	-2,80	1,000	10,8 (8,1 - 13,4)	3,07	1,000
Biomechanical							
F.Ult [kN]	3,00 (2,56 - 3,44)	2,99 (2,55 - 3,44)	-0,14	1,000	2,83 (2,39 - 3,27)	-5,63	0,007
Scomp [kN/mm]	68,5 (57,5 - 79,5)	68,4 (57,4 - 79,5)	-0,12	1,000	65,0 (53,9 - 76,0)	-5,14	0,014
Stors [kNmm/rad]	1408 (1186 - 1630)	1448 (1226 - 1671)	2,89	1,000	1372 (1150 - 1594)	-2,53	1,000
Sbend [kNmm ² /mm ²]	2534 (2122 - 2947)	2555 (2143 - 2968)	0,83	1,000	2381 (1969 - 2794)	-6,04	0,029
Bone turnover markers							
PINP [µg/L]	54,3 (40,8 - 67,9)	62,5 (49,5 - 75,6)	15,03	0,986	26,7 (12,7 - 40,7)	-50,9	0,010
ICTP [µg/L]	3,8 (3,1 - 4,5)	4,0 (3,3 - 4,7)	4,70	1,000	3,5 (2,8 - 4,2)	-8,01	1,000

P-values are obtained from a linear mixed-effect model.

* Parameters are obtained using the extended cortical evaluation script.

Abbreviations: vBMD = volumetric bone mineral density; Dtot = total density; Dtrab = trabecular density; Dcort = cortical density; Tb.N = trabecular number; Tb.Th = trabecular thickness; Tb.Sp = trabecular separation; Ct.Th = cortical thickness; Ct.Pm = cortical perimeter; Ct.Po = cortical porosity; Ct.Po.D cortical pore diameter; Ct.Po.V cortical pore volume; F.Ult = ultimate failure load; Scomp = compression stiffness; Stors = torsional stiffness; Sbend = bending stiffness; PINP = procollagen type-I N-terminal propeptide; ICTP = carboxy-terminal telopeptide of type I collagen.

Figure 2 Median and 25% and 75% percentiles of bone formation and resorption markers at baseline, three months and two years post-fracture for patients who started with alendronate (dark thick straight line) or denosumab (dark thin straight line) treatment at three months post-fracture, patients who were already started with alendronate treatment pre-fracture (light straight line), and patients who did not start or were not compliant to anti-resorptive treatment (dotted line). The thin dotted lines mark the lower and upper reference limits as reported by the manufacturer from the analysis kits.

Discussion

This study showed a significant decrease of cortical vBMD and bone strength and stiffness at the contra-lateral distal radius two years after a stable distal radius fracture.

To our knowledge, there are only a few longitudinal studies that looked into bone changes at the contra-lateral side after a distal radius fracture. In these studies, no changes were observed in aBMD measured by DXA at the contra-lateral side^{8, 9}, and thus our finding that we did not observe a change in total vBMD is consistent with current literature. With HRpQCT, however, it was possible to divide the total region in a cortical and trabecular compartment and analyze these compartments separately. We then found a decrease in cortical, but not in trabecular density. Also we did not find changes in cortical or trabecular micro-architecture.

Compared to literature, the two-year decline of 3.9% in cortical vBMD and >5.0% in the biomechanical parameters at the contra-lateral side after a forearm fracture appeared to be higher than expected during normal aging. A recent prospective HRpQCT study on age- and gender-related changes in vBMD, micro-architecture

and bone strength found an annual change in cortical vBMD and failure load of -0.4% and -0.9% , respectively, in the group consisting of post-menopausal women ($n = 51$).¹⁹ The control group ($n = 73$) in a large HRpQCT intervention study on the effect of odanacatib showed a two-year decrease in cortical vBMD and estimated bone strength, of -1.65% and -1.61% , respectively.²⁰ Also the control group ($n = 82$) in another large HRpQCT intervention study on the effect of alendronate and denosumab showed a one-year decrease in cortical BMD of -1.5% ,²¹ and in a small randomized controlled HRpQCT study on the effect of alendronate²², cortical vBMD tended to decrease and estimated failure load and stiffness in compression significantly decreased with approximately 3% in the control group ($n = 13$) during the two-year follow-up. Therefore, the results in our study suggest that there is a higher rate of bone loss in the cortical region in post-menopausal women with a distal radius fracture. Interestingly, a higher than expected decline in BMD was also observed after hip fractures by Magaziner et al.^{4, 5} After hip fractures, the contralateral side is often immobilized as well due to a period of bedrest, which could attribute to the higher decline. However, this is not the case for forearm fractures and one could even argue that the non-fractured forearm is used more than in the pre-fracture situation.

The greater than expected declines in cortical vBMD and bone strength are particularly surprising, since it has been shown that alendronate and, to a larger extent, denosumab increase BMD by decreasing bone resorption, and secondary also bone formation.²¹⁻²⁴ However, in two studies, patients with a fracture after age 50 were excluded.^{21, 22}

Whereas levels of bone turnover markers decreased in the patient being treated with denosumab, levels of bone resorption marker ICTP remained unchanged and levels of bone formation marker PINP decreased by 50% in patients with or without alendronate treatment. This could indicate uncoupling of bone resorption and formation in these patients, which is in line with the observed loss in cortical vBMD. The results of the bone turnover markers, however, should be interpreted in the context of fracture healing. Whereas the decreased bone formation is compatible with healing, i.e. the initial increase in PINP due to callus formation has decreased to low-normal at two years post-fracture, the unchanged bone resorption is difficult to explain. Still, non-compliance can be an issue²⁵ for alendronate, but not for denosumab. Other explanations include persistent bone resorption of the callus, or another underlying yet unidentified metabolic bone disorder, associated with a recent fracture. Further elucidation of this process needs to be addressed in future studies.

Given the precision error of the technique to assess cortical vBMD is approximately 1%^{14, 26, 27}, individual changes in cortical BMD need to exceed the least significant change (LSC) of $\pm 2.77\%$ to be considered true changes. Since the two-year decline in cortical vBMD in all but one subject was larger than the LSC, we can indeed conclude that cortical vBMD truly decreased. The precision of the technique to assess the biomechanical parameters, however, is slightly lower with 3.6%²⁷, and hence the LSC for the biomechanical parameters is $\pm 10\%$. A two-year decline in compression stiffness larger than 10% was observed in only four subjects, and in the remaining ten subjects the two-year decline in compression stiffness could not be considered true change. On a group level, however, the two-year decline in the biomechanical parameters is still significant.

The finding that the decrease in cortical density was accompanied by a decrease in the biomechanical parameters is not surprising, given the fact that the cortex carries a large proportion of the loads at the distal radius.^{28, 29} Although also intra-cortical porosity is a good predictor for bone strength³⁰⁻³², no significant changes in cortical porosity were found in our study. However, this might be a limitation of the resolution of the currently used HRpQCT device, which is not sufficient to capture small pores. Additionally, the precision of the currently used HRpQCT devices to assess cortical porosity is rather poor, i.e. RMS-CV% of more than 10%.^{15, 27, 33} Future research on cortical porosity after a fracture should, therefore, make use of the second generation HRpQCT scanners, which have a higher spatial resolution.

Limitations

Our study has several limitations that are noteworthy. First of all, due to the exploratory nature of this pilot study a small number of subjects was included and, hence, we were limited in our statistical analyses. Also, we were not able to include a control group so it was not possible to make a comparison with subjects that did not fracture their wrist. Third, although not the purpose of this study, we did not longitudinally monitor bone mineral density at other sites than the distal radius, e.g. DXA of lumbar spine or hip, which could have provided more insight into the cortical bone loss. Fourth, we could not perform HRpQCT measurements between three months and two years post-fracture due to the time passed before we received approval from our ethical committee for an extension on the initial research protocol, in which a follow-up up to three months post-fracture was approved. Last, due to a change in the study protocol the HRpQCT scans at the two-year visit consisted of two consecutive stacks of 110 slices each. As a result of patient motion during scanning, these stacks might not be aligned perfectly which could lead to an underestimation of bone strength and stiffness. However,

we discarded the measurements of insufficient quality in our linear mixed-effect model and therefore the shift between stacks should be minimal, thus leaving less room for errors. Moreover, not discarding the measurements of insufficient quality led to the same conclusions (data not shown).

Conclusion

We conclude that in post-menopausal women a stable fracture at the distal radius is associated with accelerated cortical bone loss and concomitant reduction of bone strength but not with micro-architectural changes at the contra-lateral distal radius, even in the presence of adequate anti-resorptive treatment.

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P.C. Willems is a board member of the Dutch Spine Society (association of spine surgeons).

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part 3

Applicability of HRpQCT in
unstable distal radius fractures

7

Feasibility of rigid 3D image registration of high-resolution peripheral quantitative computed tomography images of healing distal radius fractures

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Submitted

Abstract

Introduction

For accurate analysis of bone formation and resorption during fracture healing, correct registration of follow-up onto baseline image is required. A per-fragment approach could improve alignment compared to standard registration based on the whole fractured region. In this this exploratory study, we tested the effect of fragment size and displacement on a per-fragment registration, and compared the results of this per-fragment registration to the results of the standard registration in two stable and one unstable fracture(s).

Methods

To test the effect of fragment size and displacement, high-resolution peripheral quantitative computed tomography (HRpQCT) scans of three unfractured radii were divided into subvolumes. Different displacements in x-, y, or z-direction or rotations around each axis were applied, and each subvolume was registered onto the initial volume to realign it. Next, registration of follow-up onto baseline scan was performed in two stable and one unstable fracture. After coarsely aligning the follow-up onto the baseline scan, a more accurate registration was performed of the whole fracture and the standard registration, and of each fracture fragment separately. Alignment was checked using overlay images showing baseline, follow-up and overlap between these scans, and by comparing correlation coefficients between the standard and per-fragment registration.

Results

Generally, subvolumes as small as 300 mm³ that were displaced up to 0.82 mm in x- or y-direction, or up to 1.64 mm in z-direction could be realigned correctly. For the fragments of all fractures, correlation coefficients were higher after per-fragment registration compared to standard registration. Most improvement was found in the unstable fracture and one fragment of the unstable fracture did not align correctly.

Conclusion

This exploratory study showed that image registration of individual subvolumes, such as fracture fragments, is feasible in both stable and unstable fractures, and leads to better alignment of these fragments compared to an approach that is based on registration using the whole fractured region. This result is promising for additional analysis of bone formation and resorption in HRpQCT studies on fracture healing.

Introduction

In medical image analysis, image registration is a commonly used method where two (or more) images are aligned by optimizing a similarity measure, which is often based on voxel intensity cross-correlation¹ or mutual information.^{2,3} In this way, it is possible to gain valuable information that is conveyed in more than one image, e.g. images from different time points, different modalities or distinct viewpoints.⁴ Usually, one image is called the 'fixed' or reference image, which is the image on which the second image, called the 'moving' image, is registered.

Image registration procedures have been developed for almost every tissue and organ. For bone, image registration has been used for various purposes, including improving the reproducibility of high-resolution computed tomography (CT) derived bone parameters⁵, strain mapping of loaded bone⁶, and for the analysis of sites of bone formation and resorption at the tibia⁷ and finger joints.⁸ For fractured bone, Lynch et al. applied image registration to longitudinal CT images of healing distal radius fractures to assess changes in CT image intensity within the fracture gap during the healing process⁹, and Tassani et al. used image registration to automatically detect the fracture zone in microCT images of trabecular bone.¹⁰ In the aforementioned studies, mostly a rigid image registration procedure was used because bone is a rigid tissue.^{5,7-10} Zwahlen et al., however, used a deformable (non-rigid) image registration approach because of local deformation of individual trabeculae.⁶

Compared to clinical CT used by Lynch et al.⁹, high-resolution peripheral quantitative CT (HRpQCT) has a higher spatial resolution which allows for a more detailed assessment of the bone changes at the fracture site. We recently used HRpQCT to study the healing process of distal radius fractures treated by closed reduction and cast immobilization, and we found significant changes in volumetric bone mineral density (vBMD), micro-architecture and biomechanical parameters were found during the healing process.^{11, 12} In addition, superposition of these sequential HRpQCT images using a rigid 3D image registration in a way similar to Christen et al. did in normal bone, would allow the study of changes of bone formation and resorption, which are intense metabolic processes during fracture healing and may be affected by certain anti-osteoporosis drugs.¹³ Applied to preliminary data from a pilot study by Bours et al., this method indeed showed interesting results in a simple, stable fracture (Figure 1).¹⁴ However, although all distal radius fractures in our study were treated as stable fractures, some fractures might be less stable than expected and fracture fragments might move relative to

each other in between two HRpQCT scans. This may lead to erroneous results when the HRpQCT images are superimposed by the rigid 3D registration procedure in order to visualizing bone formation and resorption, because moved fragments may wrongly be qualified as formed or resorbed bone. Although deformable image registration might be able to correct for such relative movement between fragments, the individual fragments are still mainly composed of rigid bone tissue and, hence, a rigid image registration procedure seems more appropriate.

Figure 1 Sites of bone formation and resorption during healing of a stable distal radius fracture. 3D HRpQCT images of a stable, simple distal radius fracture at 9 days (baseline) and at follow-up 26, 44 and 87 days post-fracture showing regions of bone formation (green), resorption (red) and no change (blue). Images were obtained by superposition of the follow-up images over the baseline image. Cortical fracture locations (indicated by arrows) are bridged at 87 days post-fracture.

To limit the amount of wrongly qualified formed or resorbed bone in HRpQCT images of healing distal radius fractures, we propose to, after a pre-registration, select individual fragments in the moving image and register them separately into the fixed image using rigid 3D image registration. This approach, however, raises several questions that need to be addressed. First of all, the separate fragments are smaller than the scanned bone region itself. It is currently unclear to what extent the registration is affected by using only a subvolume of the moving image as input, instead of using the whole bone region. Second, changes related to the fracture healing process will occur which are larger than changes due to aging or medication and thus the similarity between the fragments can be low, even if correctly registered. To address these issues, we first tested the proposed method using a subset of HRpQCT images of a reproducibility study. Then, we applied our method to stable fractures that were minimally displaced and to a fracture where secondary displacement had occurred.

Materials and Methods

Subjects and image data sets

Two datasets were used in this study. The first dataset consisted of HRpQCT scans of fifteen healthy individuals with ages ranging from 21 to 47 years and was presented earlier in the reproducibility study of Boutroy et al.¹⁵ This dataset was also previously used by Christen et al. to study the reproducibility of bone apposition and resorption quantification in healthy subjects.⁷ In the present study, three subjects were randomly selected from this dataset to test the effect of size versus displacement on the fracture registration algorithm. Informed consent was obtained from all participants and the reproducibility study was approved by an independent Ethics Committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale de Lyon).

The second dataset consisted of HRpQCT scans that were made during the first twelve weeks of fracture healing in post-menopausal women with a distal radius fracture who were treated by closed reduction and cast immobilization. Image acquisition has been described previously¹¹, but briefly, an 18 mm-long region covering the fracture was scanned by HRpQCT at one to two, three to four, six to eight and twelve weeks post-fracture using standard *in vivo* settings (tube voltage 60 kVp, tube current 900 μ A, integration time 100 ms). Using an isotropic voxelsize of 82 μ m, each HRpQCT measurement thus resulted in transverse 220 slices. After testing the fracture registration algorithm, it was applied onto three fractures that were selected from this dataset to test if a registration based on fragments improved the registration in these fragments. Since movement is most likely to occur during the early phases of fracture healing, when the fragments are not stabilized yet, baseline images at one to two weeks post-fracture and follow-up images at three to four weeks post-fracture were used. Furthermore, HRpQCT images of the fractures were selected on having no or minimal motion artifacts.¹⁶ Informed consent was obtained from all participants and the fracture healing study was approved by the local Medical Ethics Committee (NTR3821).

Description of the fracture registration algorithm

For a correct analysis of sites of bone formation and resorption as calculated from two consecutive HRpQCT scans, a proper registration of the two scans is required. The rigid 3D registration procedure (Image Processing Language version 5.16/regist 1.09b, Scanco Medical AG, Brüttsellen, Switzerland) used in the present study can be divided into two parts:

- 1) A pre-registration to coarsely align the follow-up image onto the baseline image using solid volumes of the periosteal contours of these images

- 2) Fine registration per fragment to precise register each fragment from the follow-up image onto the baseline image using the gray-scale images within the contours of the fragments

- 1) Pre-registration

Because the orientation of the fractured radius may differ between two HRpQCT scans, a pre-registration was required to coarsely align the follow-up image onto the baseline image. This pre-registration was based on solid volumes within the periosteal contours in the two images. The periosteal contours were created using the standard semi-automatic method using edge detection. Since rotations of the radius around the x- and y-axis are restricted due to fixation of the forearm during scanning procedure, the pre-registration started by rotating the mask of the follow-up image, that has been downsampled ten times, in steps of 22.5 degrees around the z-axis to save time. The angle at which the best overlap was found was then used as the starting position for a registration at four times downscaling in which the follow-up masks was iteratively translated along and rotated around each axis until the best overlap was found.

- 2) Fine registration per fragment

After this coarse alignment, a fine 3D rigid registration of the individual fragments in the follow-up image onto the baseline image was performed. The rotations and translations found in the pre-registration were used as starting position for each fragment in the fine registration. Since each fragment is supposed to be already more or less in place, this fine registration was performed using grayscale images that were first downsampled four times, and finally using the original image resolution. In this fine registration step, both the total follow-up and baseline images were used as input images, but only information within the volume of interest (VOI) in each image was counted in calculation of best overlap. The VOI in the moving image was the contoured fragment, and the VOI in the fixed image the region within the periosteal contour.

Effect of fragment size, offset and step-size

To test how well small fragments of different size can be registered, the 3D volume of each radius scan was divided into two, four and eight subvolumes (Figure 2). Each subvolume was then shifted from its original position by 10 or 20 voxels (0.82 and 1.64 mm, respectively) in the x-, y-, or z-direction, or rotated around the x-, y-, or z-axis by 0.1 or 0.2 rad (5.7 and 11.5 degrees, respectively). Next, each shifted/

rotated subvolume was registered onto the initial, total scan to see if it could be registered correctly. With the registration a simplex search algorithm was used that performs translation/rotation trial steps of a typical size. For periodic structures, such as bone, it was expected that the translation step size should be close to the size of the trabecular separation, to avoid ending at a local minimum. To test the effect of the typical step-size in each iteration, the same procedure described above was repeated for step-sizes of one and two times the mean trabecular separation per scan. To quantify the agreement at each iteration, a correlation coefficient (CC) was calculated according to equation 1:¹⁷

$$CC = \frac{\sum_{i=1}^{nv} v_i w_i}{\sqrt{\sum_{i=1}^{nv} v_i^2 \sum_{i=1}^{nv} w_i^2}} \quad (\text{Equation 1})$$

where v_i is the value of voxel i in image 1, i.e. the fixed image; w_i the value of a corresponding voxel in image 2, i.e. the moving image, after translation/rotation using tri-linear interpolation; and nv the number of voxels in the evaluated region. Since the image information per subvolume is exactly the same as in the initial image, a correlation coefficient of 1.000 indicates a perfect registration.

Figure 2 Subvolumes and corresponding correlation coefficients. Average correlation coefficients for registration of volumes with different sizes, using different step-sizes after different applied translations in x-, y- and z-direction and after different applied rotations around the x-, y- and z-axis.

Implementation onto healing fractures

When the performance of the registration per part of different sizes, maximum allowed offset and the optimal step-size were known, the method was applied onto several fracture cases: 'simple' fractures with two fragments that are relatively stable over time, and 'complex' fractures with three or more fragments that have moved relative to each other over time. To be able to study the expected beneficial effect of per-fragment registration, each scan of each fracture case was also registered using the standard registration approach which uses the whole bone region in the follow-up image in the registration.

Per fragment the result of the registration was determined qualitatively by checking overlay images. These overlay images were created by combining segmented 3D volumes of the fracture at baseline and the registered follow-up image, with voxels in the baseline volume set to value 1 (red) and in the follow-up volume set to value 2 (green). Thus, after combining these volumes, overlapping regions in the overlay image have value 3 (purple).¹⁸ The segmented 3D volumes were created from the gray-scale images by applying a Gaussian filter (sigma = 0.7, width = 1.0 voxel) to remove noise followed by thresholding (threshold = 120 mgHA/cm³). Furthermore, correlation coefficients¹⁷ within each fragment region were compared between the registrations based on the standard approach and the per-fragment approach to see whether the registration was improved.

Results

Effect of fragment size, offset and step-size

The average correlation coefficients for registration of the subvolumes of different sizes, using different step-sizes after different applied translations in x-, y- and z-direction and after different applied rotations around the x-, y- and z-axis are presented in Figure 2. The average volumes $\pm$ standard deviation (SD) of the one-half, one-quarter and one-eighth parts were 1200 ± 130 mm³, 596 ± 108 mm³, and 298 ± 74 mm³, respectively. The trabecular separation measured in the total volumes was 0.561 mm, 0.626 mm, and 0.673 mm.

Regarding the applied translations, a correct registration was possible after a translation of the one-half parts of 0.82 and 1.64 mm in each direction, regardless of step-size. For one-quarter and one-eighth parts, in general a correct registration was found after translations of 0.82 and 1.64 mm in the z-direction, but not in the x- and y-direction: then the registration failed at translation of 1.64 mm.

Regarding the applied rotations, a correct registration was found for rotations of the one-half and one-quarter parts around the x- and y-axis by 0.10 and 0.20

radians, regardless of step-size, whereas registration failed after rotations around the z-axis. For one-eight parts, registration was correct for rotations of 0.1 and 0.2 radians around the x-axis and 0.1 radians around the y-axis, but failed for rotations of 0.2 radians around the y-axis and 0.1 and 0.2 radians around the z-axis, regardless of step-size.

Figure 3 2D HRpQCT slices with contours (left) and 3D model (right) of the fragments in two stable fractures (top and middle) and a fracture with secondary displacement (bottom) that were registered in this study. The volume [mm³] per fragment as well as of the complete fracture is shown between brackets.

Application to fractures

In Figure 3, 2D HRpQCT slices with the contoured fragments are shown, as well as 3D models of each fracture with the fragments visualized in different colors. In each fracture, one large volume could be defined, with volumes ranging from 2527 mm³ to 4923 mm³ covering 36% to 87%, respectively, of the total volume. Additionally, two or three smaller fragments could be contoured per fracture and their volumes ranged from 67 mm³ to 809 mm³.

Overlay images and corresponding correlation coefficients resulting from the registration using the whole bone region in the follow-up scan, and from the registration per fragment are shown in Figure 4. The overlay images suggest that registration based on the whole bone region resulted in an (almost) correct registration in the two stable fractures but not for the unstable fracture. Corresponding correlation coefficients were 0.970, 0.963 and 0.909, respectively. For all fragments in each fracture, the per-fragment registration resulted in increased correlation coefficients compared to registration based on the whole bone region. The largest improvement in correlation coefficient was observed in fragment 1 of the unstable fracture. Fragment 3 of the unstable fracture showed improved correlation coefficient, while the overlay image clearly showed a misalignment of this fragment after per-fragment registration.

Discussion

In this exploratory study, we showed for the first time that image registration of separate (fracture) fragments is feasible in both stable and unstable fractures, and leads to better alignment of these fragments compared to an approach that is based on registration using the whole bone region.

Not unexpected, correct realignment occurred more for larger parts than for smaller parts. In smaller parts, realigned was observed after an offset in the z-directions and applied rotations in the z-plane. Interestingly, this movement is likely similar to what happens in case of secondary displacement: the distal part of the fracture collapses over the proximal part in the longitudinal direction, with no or relatively little rotation in the transversal plane.

Based on our results, a step-size similar to the trabecular separation can be advised. This finding is not surprising, since most parts consist mainly of trabeculae. With step-sizes smaller than the trabecular separation, the registration might find a local minimum, hence leading to a non-optimal registration. By using a step-size

Figure 4 Overlay images of three fracture cases. Overlay image after standard registration of the whole bone region in the follow-up image (three to four weeks post-fracture) onto baseline (one to two weeks post-fracture) with corresponding correlation coefficients (top). Overlapping regions (purple) are shown as well as regions belonging to baseline (red) and follow-up (green). To check whether the per-fragment registration improved the results compared to the standard registration, overlay images and corresponding correlation coefficients are presented for the same subregions obtained after standard and per-fragment registration. Arrows indicate locations of clear improvement after per-fragment registration. Correlation coefficients, which are reported below each fragment, are higher after per-fragment registration than after standard registration.

of at least the trabecular separation, the chance of finding a local minimum is less likely. If individual measurement of trabecular separation is not feasible in a practical setting, using a step-size is advised of approximately 0.7 mm, which is the trabecular separation at the distal radius of osteoporotic and osteopenic post-menopausal women.¹⁵

In the fractured cases, alignment of the fragments was improved in all cases when the fragments were registered separately in comparison to when the whole bone region was used in the registration. The challenge is to determine for which fractures the per-fragment registration is required in order to obtain reliable results on bone apposition and resorption. It is expected that the stable fractures were sufficiently aligned using the standard registration approach to determine the amount of bone formation and resorption since correlation coefficient were already high and no major increases in correlation coefficients were observed after per-fragment registration. In the unstable fracture, however, a major improvement in alignment was observed for fragment 1. This indicates that unstable fractures can benefit most from the per-fragment registration as presented in this study.

On the other hand, per-fragment registration of fragment 3 in the unstable fracture did not result in a satisfactory alignment. An explanation can be found in the size of the fragment with respect to the displacement. Being the smallest fragment in the unstable fracture with a volume of 629 mm³, it had to overcome a distance of approximately 1 mm (13 voxels) in the x-direction and 2 mm (24 voxels) in the y-direction. Since the one-half volumes with a volume of approximately 600 mm³ failed to realign correctly after displacements in the x- or y-direction of >0.82 mm (10 voxels), this might indicate that the fragment was too small in relation to the displacement.

Although not quantitatively measured, feasible contouring of fracture fragments depends in our experience heavily on the presence and amount of low mineralized callus, the number of fragments, and the degree to which the fragments are interconnected. Therefore, fragments in HRpQCT scans that were made early post-fracture, i.e. at one to two weeks post-fracture, are less difficult to contour than in the HRpQCT scans made at three to four or even six to eight weeks post-fracture. At six to eight weeks post-fracture, a clear distinction between the fragments may be severely hindered by the amount of low mineralized callus that has formed at the fracture line. However, this might not be a problem: at the time this low mineralized tissue has formed, the fragments are not likely to move relative to each other as they have already been stabilized by the presence of callus. We therefore expect that only fragments in the scans that are made during the first four weeks post-fracture, need contouring.

Double-stack (220 slices / 18 mm) HRpQCT measurements were made to ensure that most of the fracture region was included. However, even despite including only measurements with no or very limited motion artifacts, a minor shift between the stacks is almost impossible to avoid. Also in our study, this was the case as can be seen by the edges that are visible in the 3D models in Figure 3. To improve alignment of fragments that are more than 110 slices (9 mm) long, it is advised to align the two stacks per measurement before registering volumes from the follow-up scan onto the baseline scan. Another option is to limit the registration to one stack, but this has the disadvantage that fragments may become very small and are thus more likely to end up misaligned.

A few limitations need to be addressed. In the present study, contouring of the fragments was done manually, which is subjective and may lead to low intra- and inter-operator reproducibility. Although exploratory, we have shown that separate registration of each fragment may improve the total registration in fractures. Future work should therefore focus on an automatic procedure for finding the fracture line and/or contouring different fragments. Second, we did not apply our approach to experimental data where fragment displacement could be controlled. While focusing on *in vivo* patient data, such an experiment would have been beyond the scope of the present study. Last, we only used HRpQCT scans of good quality. It is therefore unknown to what extent the registration is affected by the presence of motion artifacts.

Conclusion

This exploratory study showed that image registration of individual (fracture) fragments is feasible in both stable and unstable fractures, and leads to better alignment of these fragments compared to an approach that is based on registration using the whole fractured region. This result is promising for additional analysis of bone resorption and formation based on longitudinal HRpQCT images of healing fractures, for example in studies on the metabolic effects of anti-osteoporosis medication.

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8

Distal radius plate of CFR-PEEK has minimal effect on bone parameters in high-resolution peripheral quantitative computed tomography: a pilot study

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Submitted

Abstract

Background

Carbon-fiber-reinforced poly-ether-ether-ketone (CFR-PEEK) has superior radiolucency compared to other orthopedic implant materials, e.g. titanium or stainless steel, thus allowing metal-artifact-free postoperative monitoring by computed tomography (CT). Recently, high-resolution peripheral quantitative CT (HRpQCT) proved to be a promising technique to monitor the recovery of volumetric bone mineral density (vBMD), micro-architecture and biomechanical parameters in stable conservatively treated distal radius fractures. When using HRpQCT to monitor unstable distal radius fractures that require volar distal radius plating for fixation, radiolucent CFR-PEEK plates may be a better alternative to currently used titanium plates to allow for reliable assessment. In this pilot study, we assessed the effect of a volar distal radius plate made from CFR-PEEK on bone parameters obtained from HRpQCT in comparison to two titanium plates.

Methods

Plates were instrumented in separate cadaveric human forearms ($n = 3$). After instrumentation and after removal of the plates duplicate HRpQCT scans were made of the region covered by the plate. HRpQCT images were visually checked for artifacts. vBMD, micro-architectural and biomechanical parameters were calculated, and compared between the uninstrumented and instrumented radii.

Results

No visible image artifacts were observed in the CFR-PEEK plate instrumented radius, and errors in bone parameters ranged from -3.2% to 2.6%. In the radii instrumented with the titanium plates, severe image artifacts were observed and errors in bone parameters ranged between -30.2% and 67.0%.

Conclusions

We recommend using CFR-PEEK plates in longitudinal *in vivo* studies that monitor the healing process of unstable distal radius fractures treated operatively by plating or bone graft ingrowth.

Background

Carbon-fiber-reinforced poly-ether-ether-ketone (CFR-PEEK) is increasingly being used as a material for orthopedic implants, e.g. in spinal cages, hip prostheses, or intramedullary nails.¹⁻⁴ One of the main advantages of this material is that CFR-PEEK has been found to have superior radiolucency as compared to other materials that are conventionally used for orthopedic implants, such as stainless steel or titanium. Therefore, implants made out of CFR-PEEK allow metal-artifact-free postoperative monitoring by computed tomography (CT), magnetic resonance imaging (MRI) and radiographs.¹

Recently, it has been shown that the repair process of stable distal radius fractures can be monitored longitudinally *in vivo* using high-resolution peripheral quantitative computed tomography (HRpQCT).^{5,6} For clinical and research purposes, it would be of great interest to monitor the repair process of unstable fractures or the ingrowth of bone graft or bone graft substitutes after corrective osteotomy as well. Whereas stable distal radius fractures are usually treated by immobilization with a fiberglass cast, which only has a limited effect on the bone parameters obtained by HRpQCT⁷, volar distal radius plates (VDRPs) as internal fixation are a generally accepted treatment in case of unstable distal radius fractures or after corrective osteotomy.⁸ Since VDRPs are usually made from stainless steel or, more common, from titanium, they cause severe artifacts on CT and HRpQCT images⁹, including scattering, beam hardening and streak occurrence.¹⁰ Besides reduced sensitivity of CT measurements, this may result in an over- or underestimation of the bone density, micro-architectural and biomechanical parameters that are derived from such images.⁹

Besides other implants, VDRPs made of CFR-PEEK were recently developed. Although it is widely accepted that polymer devices are more compatible with CT compared to metal devices, it is unknown whether also the quantitatively obtained bone parameters obtained from HRpQCT images remain unaffected. We expect that a CFR-PEEK plate will lead to less artifacts in HRpQCT images as compared to metal plates, and thus might be a suitable alternative to the currently used titanium plates in studies monitoring the healing process of unstable fractures or ingrowth of bone graft or bone graft substitutes after corrective osteotomy.

Therefore, the aim of this study was to compare the effect of VDRPs made of titanium or made of CFR-PEEK on the occurrence of artifacts in HRpQCT images and on the volumetric bone mineral density (vBMD), micro-architectural and biomechanical parameters that are obtained from these images.

Methods

Volar distal radius plates

Three types of commercially available VDRPs from different firms were compared in this study. Plate #1 (Standard five-hole Volar Distale Radius Plate, Icotec AG, Switzerland) had a thickness of 2.5 mm and consisted of CFR-PEEK to which 0.5% tantalum fibers were added for radiopacity. Plate #2 (VariAx™ Standard Anatomical Volar Distal Radius Plate, Stryker Corporation, USA) was 2 mm thick and made of titanium alloy Ti6Al4V, which contained 89% titanium, 6% aluminum and 4% vanadium. Plate# 3 (2.4 mm Variable Angle LCP Volar Extra-Articular Distal Radius Plate, Synthes AG, Switzerland) was 2.4 mm thick and also made of a titanium alloy, i.e. Ti6Al7Nb containing 86% titanium, 6% aluminum and 7% niobium.

Fresh frozen forearms

For this study three intact human forearms (right side) were isolated from three cadavers. A handwritten and signed codicil from each donor, posed when still alive and well, is kept at the Department of Anatomy and Embryology, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, The Netherlands. This is required by Dutch law for the use of cadavers for scientific research and education. The forearms were thawed for eight hours at 16 °C before surgery was started.

Figure 1 Each volar distal radius plate was instrumented in one cadaveric forearm by means of a standard volar approach. The CFR-PEEK plate is shown on the left. In each plate, five screws were fixed in the metaphysis and two screws were fixed in the shaft. The region of interest (between dotted lines) was set such that it was fully covered by a part of the plate without holes.

Surgery

Each plate was instrumented by an experienced orthopedic surgeon (PW) in one forearm by means of a standard volar approach and fixed to the distal radius according to the instruction manual provided by the manufacturer. During surgery, employees of the respective firms were present to support the surgeon and to make sure the proper surgical techniques were used. In all plates, five screws were fixed in the metaphysis distally from the region of interest (ROI) and two screws were fixed in the shaft proximally from the ROI (Figure 1). The screws were made of

the same material as the respective plate. The plates were removed after HRpQCT scans had been performed.

HRpQCT scanning

Both after instrumentation and after removal of the plates, the forearms were scanned twice, with repositioning between each scan, by HRpQCT (XtremeCT, Scanco Medical AG, Switzerland) using clinical *in vivo* settings by the manufacturer (60 kVp effective energy, 900 μ A tube current, and 100 ms integration time). A standard stack of 110 transverse images with an isotropic voxel size of 82 μ m was acquired, covering a region of 9 mm in each forearm. The ROI was set at the distal end of the radius and which was covered by a part the plate without screw holes. Also, the ROI was chosen such that it was located in between the five distal and two proximal screws to eliminate distortion by the screws (Figure 2).

Figure 2 Lateral scout views of each instrumented radius showing the region of interest (ROI) in each radius. The ROI was chosen at a location where the radius was fully covered by a part of the plate without holes and that was not intersected by screws. The tantalum fibers in the CFR-PEEK plate (left) allow visualization of the plate.

Bone density and micro-architecture

The HRpQCT images within the ROI were evaluated using the standard patient evaluation protocol provided by the manufacturer and has been described earlier in detail elsewhere.¹¹ In short, after contouring of the periosteal boundary of the radius, the total region was separated into a cortical and trabecular region. For each region, the volumetric bone mineral density (D_{tot}, D_{cort} and D_{trab}, respectively) [mgHA/cm³] was assessed. The mineralized bone was extracted and a segmented image was created¹¹ from which the following micro-architectural parameters were assessed: trabecular number (Tb.N) [mm⁻¹], thickness (Tb.Th) [mm] and separation (Tb.Sp) [mm]. For the cortical region, the thickness (Ct.Th) [mm] was calculated.¹¹

Micro finite element analysis

μ FE models were created directly from the segmented HRpQCT images, similar to earlier studies.^{12, 13} In short, each voxel representing bone was converted into a

brick element of the size, thus creating a representative μ FE model of the bone's micro-architecture. Typically, these μ FE models consisted of three to four million elements. Equal material properties were assigned to every element, i.e. a Young's modulus of 10 GPa and a Poisson ratio of 0.3.¹³ By applying a 'high friction' compression test in the axial direction as described by Pistoia et al., the following biomechanical parameters were estimated: stiffness (S_{comp}) [kN/mm], which can be described as the resistance against displacement; and ultimate failure load (F.Ult) [kN], which is the load at which the fracture criterion is met, e.g. the strain in at least 2% of the volume exceeds 0.7%.¹²

Statistics

Reproducibility of the bone density, micro-architectural and biomechanical parameters was expressed using the root mean-square coefficient of variation (RMS-CV%).¹⁴ Means for the bone parameters in each instrumented and uninstrumented radius were calculated from the duplicate measurements. The errors in the bone parameters introduced by each plate were expressed as percent differences between the instrumented and uninstrumented radius, with the uninstrumented radius as 100% reference.

Results

HRpQCT images

HRpQCT images of each radius with and without plate are shown in Figure 3. The tantalum fibers that were incorporated into the CFR-PEEK plates were clearly visible, and no visible image artifacts were observed when the CFR-PEEK plate was instrumented (Figure 3A). In the radii instrumented with the titanium plates, severe image artifacts were seen (Figure 3B and 3C). These image artifacts consisted of streaks and a higher intensity of the bone voxels that are closely located to the plate.

Errors in bone parameters

The reproducibility (RMS-CV) of the bone density, micro-architectural and biomechanical parameters ranged from 0.0 to 2.3% (Table 1).

In Table 2, the bone parameters measured per uninstrumented and instrumented distal radius are shown, together with the percent errors that were introduced by each plate. All errors in bone parameters are in comparison to the same but uninstrumented radius. For the radius instrumented with the CFR-PEEK plate, percent errors in bone parameters ranged from -3.2% in Ct.Th to +2.6%

Figure 3 Representative HRpQCT slices in each radius with (top row) and without instrumented plate (bottom row). The CFR-PEEK plate with tantalum fibers (panel 3A) caused less visible image artifacts as compared to the titanium plates (panel 3B and 3C).

in Tb.Sp, and the smallest error was observed in Dtrab, with -0.8% . For the radii instrumented with the titanium plates, in general the percent errors were larger. For the radius instrumented with plate #2, the percent errors in bone parameters ranged from -43.2% in Tb.Sp to $+67.2\%$ in Dtrab, and the smallest error was observed in Scomp, with $+7.5\%$. For the radius instrumented with plate #3, the largest percent errors in bone parameters were also found in Tb.Sp and Dtrab, being -11.2% and $+30.2\%$, respectively. The smallest error was observed in F.Ult, with $+0.1\%$.

Table 1 Reproducibility (RMS-CV%) calculated from the duplicate HRpQCT measurements at the uninstrumented and instrumented radii.

Bone parameter	RMS-CV% Radius #1 (CFR-PEEK)		RMS-CV% Radius #2 (Titanium)		RMS-CV% Radius #3 (Titanium)	
	Un-instrumented	Instrumented	Un-instrumented	Instrumented	Un-instrumented	Instrumented
BMD						
Dtot [mgHA/cm ³]	0.52	2.18	0.02	1.29	0.00	0.20
Dtrab [mgHA/cm ³]	0.10	1.95	0.34	0.00	0.31	0.62
Dcort [mgHA/cm ³]	0.31	1.48	0.24	1.13	0.01	0.06
Micro-architecture						
BV/TV [-]	0.00	1.76	0.47	0.00	1.25	0.00
Tb.N [mm ⁻¹]	2.24	0.92	0.00	1.19	0.72	0.67
Tb.Th [mm]	1.84	0.90	0.00	1.66	0.00	1.11
Tb.Sp [mm]	2.16	0.99	0.16	1.13	0.37	0.08
Ct.Th [mm]	1.50	1.55	0.00	1.65	0.93	0.00
Biomechanical						
Scomp [N/mm]	0.42	1.38	0.28	1.71	0.02	0.49
F.Ult [kN]	0.28	1.30	0.40	2.23	0.06	0.54

Abbreviations: BMD = bone mineral density; Dtot = total density; Dtrab = trabecular density; Dcort = cortical density; BV/TV = bone to total volume; Tb.N = trabecular number; Tb.Th = trabecular thickness; Tb.Sp = trabecular separation; Ct.Th = cortical thickness; Scomp = compression stiffness; F.Ult = ultimate failure load.

Table 2 Bone mineral density, micro-architectural and biomechanical parameters measured with HRpQCT at the uninstrumented and instrumented distal radius and the percent difference between them.

Bone parameter	Radius #1			Radius #2			Radius #3		
	Un-instrumented	Instrumented (CFR-PEEK)	Error (Δ%)	Un-instrumented	Instrumented (Titanium)	Error (Δ%)	Un-instrumented	Instrumented (Titanium)	Error (Δ%)
BMD									
Dtot [mgHA/cm ³]	273	266	(-2.5)	343	520	(+51.8)	229	245	(+6.7)
Dtrab [mgHA/cm ³]	146	145	(-0.8)	181	304	(+67.2)	62	80	(+30.2)
Dcort [mgHA/cm ³]	862	848	(-1.6)	839	1004	(+19.6)	853	850	(-0.3)
Micro-architecture									
BV/TV [-]	0.122	0.121	(-1.2)	0.152	0.253	(+67.0)	0.052	0.067	(+30.1)
Tb.N [mm ⁻¹]	1.58	1.54	(-2.2)	1.92	2.98	(+55.0)	0.96	1.06	(+10.5)
Tb.Th [mm]	0.077	0.079	(+1.9)	0.079	0.085	(+7.6)	0.054	0.064	(+17.6)
Tb.Sp [mm]	0.557	0.571	(+2.6)	0.442	0.251	(-43.2)	0.996	0.885	(-11.2)
Ct.Th [mm]	0.94	0.91	(-3.2)	1.01	1.29	(+27.2)	0.76	0.76	(+0.7)
Biomechanical									
Scomp [N/mm]	150	147	(-1.9)	133	143	(+7.5)	70	69	(-1.0)
F.Ult [kN]	7.17	7.06	(-1.5)	6.24	6.85	(+9.8)	3.28	3.28	(+0.1)

Abbreviations: BMD = bone mineral density; Dtot = total density; Dtrab = trabecular density; Dcort = cortical density; BV/TV = bone to total volume; Tb.N = trabecular number; Tb.Th = trabecular thickness; Tb.Sp = trabecular separation; Ct.Th = cortical thickness; Scomp = compression stiffness; F.Ult = ultimate failure load.

Discussion

This study tested the effect of CFR-PEEK and two titanium VDRPs on bone density, micro-architectural and biomechanical parameters at the distal radius obtained by HRpQCT in combination with μ FEA. Compared to conventional VDRPs that are made from titanium alloys, the plate made from CFR-PEEK introduced no visible image artifacts and had a minimal effect on the assessment of vBMD, micro-architectural and biomechanical parameters.

As expected, severe image artifacts were introduced by the titanium plates, which in turn led to an overestimation of the BMD, trabecular number and thickness and biomechanical properties, and an underestimation of trabecular separation at the distal radius. The errors in these bone parameters, however, differed between both titanium plates and this difference is probably related to the positioning and geometry of the plates: plate #2 was wider, thicker, and as can be seen in Figure 3, more closely instrumented onto the radius, whereas plate #3 was smaller and a small gap between the bone and the plate was left after instrumentation. Nevertheless, the magnitude of the errors introduced by the titanium plates is higher than the reproducibility of the HRpQCT technique. Additionally, due to differences in positioning and size of the implants it seems almost impossible to correct the bone parameters in a systematic way, which makes these titanium plates unsuitable in studies when one wants to monitor bone healing with HRpQCT.

The CFR-PEEK plate, on the other hand, introduced relatively small errors that were of the same order as the reproducibility of the HRpQCT technique.^{7, 11, 15} A plate made from CFR-PEEK would therefore be a suitable alternative to the conventional volar distal radius plates, if one wants to monitor the healing process of unstable distal radius fractures with HRpQCT or any other X-ray technique.

Besides the low number of subjects, a limitation of the present study is that each plate was instrumented on a different radius. However, all results were specified relative to those of the same radius without a plate, and the density and micro-architectural parameters of the radius on which the CFR-PEEK plate was instrumented was in-between those of the radii to which a titanium plate was attached, making it unlikely that this has affected the results in any way. Another important limitation of this pilot study is the difference in geometry between the three plates that were tested. It is likely that a thick, wide plate, such as plate #2, introduces more severe image artifacts and hence larger errors in bone parameters, than a thin, small plate. Furthermore, we did not test VDRPs made from stainless steel. The main reason is that at present

in the Netherlands, where the study was conducted, the commonly used plates are made from titanium alloy. However, since stainless steel causes more severe image artifacts in CT than titanium¹⁶, it is expected that stainless steel plates would lead to higher errors in HRpQCT-derived bone parameters as compared to titanium plates.

Conclusion

In conclusion, the results of this pilot study indicate that a volar distal radius plate made from CFR-PEEK has minimal effect on bone parameters obtained at the distal radius with HRpQCT. We therefore recommend the use of CFR-PEEK plates instead of conventional titanium plates in studies that aim to monitor the healing process of distal radius fractures or bone graft ingrowth at the distal radius over time using HRpQCT.

Declarations

Ethics approval and consent to participate A handwritten and signed codicil from each donor, posed when still alive and well, is kept at the Department of Anatomy and Embryology, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, The Netherlands. This is required by Dutch law for the use of cadavers for scientific research and education.

Competing interests B. van Rietbergen is a consultant for Scanco Medical AG. J.J. Arts is a board member of workgroup Biotechnology of the Dutch Orthopedic Association (NOV) and a board member of the Dutch Society for Biomaterials and Tissue Engineering (NBTE). P.C. Willems is a board member of the Dutch Spine Society (association of spine surgeons).

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Authors contributions Research design: JdJ, AL, JA and PW. Acquisition, analysis or interpretation of the data: JdJ, AL, BvR, JA, PG, JvdB and PW. Drafting the manuscript: JdJ, AL, BvR and PW. Revising the manuscript: JdJ, AL, BvR, JA, PG, JvdB and PW. All authors have read and approved the final submitted manuscript.

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9

General discussion

In this thesis, for the first time the healing process of distal radius fractures treated by cast immobilization has been studied *in vivo* by non-invasive HRpQCT imaging. After describing the effect of plaster-of-Paris and fiberglass casts on HRpQCT measurements (Chapter 2), the healing process itself was studied using HRpQCT. During an initial short-term follow-up period of 12 weeks post-fracture, changes in bone density, micro-architecture and biomechanical parameters that occurred during the healing process were analyzed extensively (Chapter 3), including their relation to the clinical outcome as assessed by the PRWE questionnaire (Chapter 4). In addition, a long-term follow-up at two years post-fracture was conducted to compare the restored fracture side to the contra-lateral side (Chapter 5) as well as to study the longitudinal changes at the non-fractured side (Chapter 6). Last, we addressed two aspects related to the feasibility of HRpQCT to study the healing process of unstable distal radius fractures, i.e. the feasibility of 3D registration of the fragments (Chapter 7), and the influence of a radiolucent volar distal radius plate on HRpQCT scans and the bone parameters (Chapter 8).

In this final chapter, the main findings and limitations of the previous chapters are discussed, and general conclusions and recommendations as well as directions for future research are provided.

Main findings and limitations

Issues and artifacts related to HRpQCT imaging of distal radius fractures

Cast immobilization is the recommended treatment for stable distal radius fractures, while unstable distal radius plates are preferably treated surgically by plating.¹ Casts as well as distal radius plates may cause image artifacts in HRpQCT and therefore affect vBMD, micro-architectural and biomechanical parameters that are obtained from these images.

As described in Chapter 2, wearing a forearm cast introduced systematic errors in the bone parameters. With errors ranging from -2.6% in trabecular separation to -9.8% in cortical thickness, a plaster-of-Paris cast considerably influences the measurement results, as a result of the interference caused by the presence of high attenuating calcium sulfate.² The errors caused by the fiberglass cast, on the other hand, were marginal with errors between -0.6% and -1.6% in trabecular separation and cortical thickness, respectively, and, although statistically significant, these errors may not be clinically relevant given the fact that changes observed during fracture-healing are generally in the range of 10% to 20%. In studies that aim to monitor the healing process of stable distal radius fractures, we therefore

recommend immobilization with a fiberglass cast and to wear this cast during all HRpQCT measurements.

Volar distal radius plates, used for fixation in unstable fractures, of CFR-PEEK and titanium were compared in Chapter 8. Compared to the currently used titanium volar distal radius plates, a volar distal radius plate made from radiolucent CFR-PEEK has minimal effect on bone parameters obtained from HRpQCT with errors in bone parameters between -0.8% and -3.2% in trabecular vBMD and cortical thickness, respectively. Furthermore, the CFR-PEEK plate introduced no visible artifacts, thus allowing for reliable assessment of vBMD and micro-architecture during the healing process of unstable distal radius fractures that are treated with such a plate. Because also the biocompatibility, material characteristics, and mechanical durability of CFR-PEEK orthopaedic implants are promising³, we therefore recommend the use of CFR-PEEK plates instead of conventional titanium plates in studies that aim to monitor the healing process of unstable distal radius fractures or bone graft ingrowth at the distal radius over time using HRpQCT.

Another important image artifact that needs to be addressed is the partial-volume effect (PVE). Although not limited to HRpQCT imaging of fractures, PVEs are inherent to imaging of bone micro-architecture with HRpQCT and depend on the resolution. As a result of the PVE, small bone structures, such as thin cortices or trabeculae that typically span one to three voxels at the XtremeCT's resolution, appear blurred in the HRpQCT images. After segmentation of the HRpQCT images, these thin structures may be averaged away in the segmented 3D models and, therefore, leading to an underestimation of trabecular number and overestimation of cortical thickness. Especially in fracture healing, where old trabecular remnants are resorbed and new trabeculae and cortical bone are formed, this is unwanted effect. Probably with the recent introduction of the second generation HRpQCT scanner (XtremeCT2; Scanco Medical A.G. Brütisellen, Switzerland), the PVE is less present thanks to the increased resolution of the XtremeCT2, enabling direct assessment of trabecular and cortical micro-architecture.^{4,5}

One more important source of potential error is patient motion, which severely reduces the reproducibility of the bone micro-architecture and, hence, biomechanical parameters⁶ and results in approximately 30% of the radius scans being of insufficient quality for analysis.⁷ In our fracture healing cohort (Chapter 3 and 5), 17% of the HRpQCT scans at the fracture side and 28% at the contra-lateral side was of insufficient quality, even with implementation of one repeat scan in our protocol when the first scan was of insufficient quality. In our reproducibility study (Chapter 2), the majority (88%) of the scans with insufficient quality were scans made without a cast. Although not statistically proven, these results indicate that patient motion is at least partially reduced when scanning a subjects' forearm in a

cast. This is not surprising, as the cast is custom-made (for fracture stabilization and healing) and therefore allows a secure fixation of the patient's forearm. In research using HRpQCT, one might therefore consider to scan the study subjects' forearm using a cast-like fixation device as this may reduce the number of required subjects due to the lower number of HRpQCT measurements that are unusable because of motion artifacts.

Distal radius fracture healing assessed by HRpQCT

The changes that were observed in the HRpQCT images of the healing fractures as well as in the vBMD, micro-architectural and biomechanical parameters during the first twelve weeks of healing were striking (Chapter 3). As expected, total vBMD showed a peak at six to eight weeks post-fracture, which is consistent with previous studies using conventional QCT.⁸ Interestingly, however, separate evaluation of the vBMD in the cortical and trabecular region revealed that opposite changes occurred in these regions. Whereas trabecular vBMD showed a peak at six to eight weeks post-fracture and returned to normal at twelve weeks post-fracture, cortical vBMD decreased throughout the twelve-week follow-up period. Furthermore, at the micro-architectural level, there was a reduction in trabecular number of 15% but an increase in trabecular thickness of 30%, indicating intense bone remodeling, with resorption of the trabecular structure together with new bone formation at the surface of remaining or newly formed trabeculae. At the same time, the bone biomechanical parameters increased by more than 30%.

The results of the two-year follow-up HRpQCT measurements (Chapter 5) showed that most of these changes still progressed over time. Compared to the contra-lateral side, BMD became similar at two years post-fracture. However, in the period from six and twelve weeks to two years post-fracture, the cortical thickness at fracture side increased, while concurrently a decrease in trabecular number and an increase in trabecular separation occurred whereas the trabecular thickness remained high. These results might suggest that the cortical region is restored at the expense of the trabecular region. Whether this is the result of a shift in load-bearing from the trabecular to the cortical region due to different use of the fractured wrist, or is caused by other processes, remains to be studied.

Simultaneous to the changes in the cortical and trabecular region, bone stiffness at fracture side showed a major increase of more than 100% in the period from six weeks to two years post-fracture. This result could indicate that by the time the cast is removed, bone stiffness is far from recovered. However, this is not necessarily problematic as patients are able to use their wrist in normal daily activities, which most likely do not require the bone stiffness to be fully restored.

Changes at the non-fractured distal radius

In contrast to the fracture side, no changes in the bone parameters were observed at the contra-lateral side during the first twelve weeks after fracture (Chapter 3 and Chapter 6). However, a two-year decrease of almost 4% in cortical vBMD was found, but not in total vBMD. Additionally, a reduction in bone strength and stiffness had occurred of more than 5%. The finding that we did not observe a change in total vBMD is consistent with current literature that assessed aBMD by DXA at the contra-lateral radius after a Colles' fracture.⁹ However, the two-year decreases in cortical density and bone strength and stiffness were higher than expected, as other longitudinal studies reported a two-year percent change in cortical vBMD and bone strength between -1% to -2% in the control group, which consisted of post-menopausal women who were mostly osteopenic.¹⁰⁻¹² More surprisingly, accelerated cortical bone loss at the contra-lateral side occurs in patients with a distal radius fracture, even in patients compliant with adequate anti-resorptive drug treatment.

Since the fracture side became stronger in the same period, one might wonder if the fracture region is restored at the expense of bone at other sites, even at the contra-lateral side. If that would be the case, then a decrease in spine and hip BMD would also be expected. Unfortunately, we did not longitudinally monitor vBMD or bone micro-architecture at other sites than the distal radius, so we can only speculate here.

3D image registration

For accurate longitudinal analysis of bone parameters, it is important that the same region is analyzed. In unstable fractures, fragments may move relative to each other, and this displacement needs to be corrected in order to analyze the same region. We showed that superposition of subvolumes, such as fracture fragments, in a follow-up image onto a baseline image is feasible using 3D rigid image registration, and that this leads to better alignment of these fragments compared to an approach where the whole fractured region in a follow-up image is registered on a baseline image (Chapter 7).

Although more research is required to optimize the registration procedure, the current result is promising for reliable monitoring of vBMD and micro-architecture in unstable distal radius fractures, and additionally also the analysis of bone resorption and formation based on longitudinal HRpQCT images of healing fractures, for example to study the metabolic effect of anti-osteoporosis medication on fracture healing.

Limitations

The work presented in this thesis has several limitations. First, due to the exploratory nature of the studies, the numbers of subjects is rather small and due to motion artifacts, several HRpQCT measurements were excluded from statistical analyses thus leading to missing values. In addition, we had multiple HRpQCT parameters to test, which are in part related to each other. In order to deal with missing values, we applied a linear mixed-effect model instead of a repeated measurement design. By using the Bonferroni correction in the linear mixed-effect model we corrected for the multiple comparisons between the visits in Chapter 3, but we did not correct for the fact that we tested eleven different bone parameters because of the exploratory study design. In the linear regression models in Chapter 4, no correction for multiple comparisons was applied while a correction such as the Benjamini-Hochberg procedure¹³ might have been appropriate. Nonetheless, in retrospect, a post-hoc analysis using the Benjamini-Hochberg correction with a false discovery rate of 0.20 revealed that the conclusions did not change after correction for multiple comparisons, i.e. the associations between early change in trabecular vBMD, separation and torsional stiffness with PRWE score at twelve weeks post-fracture remained significant. In addition, this post-hoc analysis showed that apart from our original findings also the early change in total vBMD was significantly associated with the PRWE score at twelve weeks post-fracture.

Second, in the distal radius fracture studies, the proximal edge of the lunate bone instead of the distal radius plateau was used as reference point. However, the joint space width between the lunate bone and the radius might not be constant over time, thus increasing the positioning error and therefore lowering the reproducibility of the bone parameters. Although the variation in joint space width over time is unknown, a recent reproducibility exercise in reference line placement showed that a variation in positioning of 0.38 mm leads to extra imprecision errors (RMS-CV%) of 2.4% in vBMD and 5.8% in cortical thickness, whereas micro-architecture and failure load remain unaffected.¹⁴ Hence, as these precision errors are considerably smaller than the changes found in the bone parameters during healing, it is unlikely that variations in joint space width affected the results.

Third, no correction was applied for the measurements that were performed with the forearm in a plaster-of-Paris cast at the first visit, despite of the errors that this type of plaster causes. However, taken into account that more than half of the patients (56%) was wearing a fiberglass cast and not a plaster-of-Paris cast at the first visit, together with the fact that the required corrections for errors of 2% to 8% are much smaller than the observed changes during fracture healing, it is likely that correcting for the plaster-of-Paris cast would lead to similar conclusions.

Fourth, in the fracture to non-fracture side comparisons we did not take dominance into account as we assumed that there were no left-right differences in bone parameters. This seems legitimate, as Hildebrandt et al. found no significant differences in BMD and micro-architecture measured by HRpQCT at the distal radius between the dominant and non-dominant forearm, and failure load was only 3% higher at the dominant side.¹⁵ However, in our study most fractures occurred at the non-dominant side ($n = 9$, 74%), thus correcting for hand dominance would have strengthened the conclusion regarding the stiffness parameters in the side-to-side comparison in Chapter 5.

Fifth, there are limitations with respect to the use of the μ FEA in this thesis. First of all, only mineralized tissue was taken into account in the FE models, whereas also other supportive tissue, like low-mineralized woven bone, is present at the fracture site. The actual biomechanical competence of the fracture region might therefore be underestimated by the μ FEA, especially during the early stages of fracture healing, i.e. up to six to eight weeks post-fracture. Second, the μ FEA is only validated for unfractured bone. Unfortunately, it will be hard or even impossible to address this limitation adequately, i.e. experimentally measure bone strength in healing fractures in humans and compare it to the bone strength calculated from the μ FEA, since the validation would raise some serious ethical concerns.

Last, the study was set up such that bone changes during the early phases of the fracture healing process, i.e. up to twelve weeks post-fracture, could be studied during frequently scheduled study visits. The remodeling phase, however, continues for a longer period. No HRpQCT scans were planned beyond the study period of twelve weeks, since METC approval for additional scanning procedures could only be acquired after demonstrating that it was feasible to assess fracture healing with HRpQCT based on the results of the first twelve-week study period. Once METC approval for an extra visit was obtained, time had passed up to two years after initial fracture. As a result, it remains unclear at what time point between twelve weeks and two years the remodeling phase as assessed by HRpQCT parameters actually normalized. In future longitudinal HRpQCT studies on fracture healing, we therefore advise to schedule follow-up visits after the initial twelve-week period more regularly, e.g. at least every six months post-fracture.

Clinical relevance

This thesis showed that we are now able to evaluate vBMD, micro-architecture and bone stiffness during the healing process of a stable distal radius fracture. Furthermore, we found significant associations with R^2 ranging from 31% to 37% between the early changes in HRpQCT derived bone parameters and the PRWE score, representing clinical outcome such as pain and function, at twelve weeks post-

fracture (Chapter 4). These associations are better compared to previously reported baseline patient- and injury related factors (R^2 between 16% and 25%).^{16, 17} Based on these findings, one could argue that it might be useful to investigate whether it is feasible to shorten or prolong a cast-immobilization period of an individual, based on the estimated stiffness of the fractured region. In the context of fracture healing, however, the methods that we used to evaluate vBMD, micro-architecture and biomechanical parameters from the HRpQCT images still have issues that need to be further addressed. For instance, the micro-architectural parameters in the early phases of the healing process need to be interpreted with caution due to the presence of low-mineralized bone, and it has not been proven that (changes in) these parameters are a meaningful representation of bone consolidation during healing. Nevertheless, we believe that, in contrast to plain radiographs and clinical examination, we now have a quantitative method available for the assessment of micro-architecture and biomechanical competence that we can further develop and validate to analyze in detail stable and more complex distal radius fractures with dislocation and after surgery, as well as to study the effect of metabolic bone disorders and drugs on mineralization, modeling and remodeling of trabecular and cortical bone during the fracture healing process.

Conclusion and recommendations

In conclusion, this thesis has resulted in the following conclusions and recommendations:

1. In contrast to a plaster-of-Paris cast, a fiberglass cast has no relevant effect on the reproducibility of HRpQCT scans and the calculation of the bone parameters from these scans. It is therefore recommended to use a fiberglass cast in studies monitoring the healing process of stable distal radius fractures using HRpQCT.
2. After opposite changes in bone mineral density of the cortical and trabecular region during the first twelve weeks of distal radius fracture healing, bone density becomes comparable to the bone density at the contra-lateral side at two years post-fracture. Additionally, trabecular micro-architecture seemed to be decreased, yet bone stiffness at fracture side was restored beyond the contra-lateral bone stiffness after two years.
3. Early changes in HRpQCT derived bone parameters can predict pain and functional outcome better than patient- and injury related factors. The relation between the HRpQCT derived bone parameters and the longterm clinical outcome has yet to be investigated.

4. Despite adequate anti-resorptive treatment, a significant, higher than expected, two-year decrease in cortical density and biomechanical parameters at the contra-lateral side was observed in our fracture healing cohort.
5. Monitoring the healing process of unstable distal radius fractures with HRpQCT is feasible when radiolucent distal volar radius plates are used to stabilize the fracture, and in addition 3D image registration is applied to accurately overlay the different fracture fragments.
6. HRpQCT, in combination with μ FEA, is a promising technique in fracture healing related research since it provides information at the microscale on bone densitometric, micro-architectural and biomechanical changes. Future research on fracture healing, e.g. in studies on the effect of medication on the healing process, should therefore consider using HRpQCT as their preferred imaging tool.

Future perspectives

The studies described in this thesis showed that the healing process of stable distal radius fractures can be studied in detail using HRpQCT and μ FEA. It is however needed that our findings will be reproduced in larger series. In addition, HRpQCT in combination with μ FEA is a promising tool for future research on fracture healing.

One of the future opportunities would be to use HRpQCT and μ FEA to study the effect of medication on fracture healing in more detail. For instance, it is known that 60% to 70% of the patients with a recent fracture are vitamin D deficient¹⁸, and that vitamin D supplementation has a positive effect on fracture healing in animals¹⁹. Since clinical studies that address the clinical effects of vitamin D deficiency or supplementation on fracture healing are scarce and remain inconclusive²⁰, it would be very interesting to study the effect of immediate vitamin D supplementation on the healing process of distal radius fractures by HRpQCT. In addition, postmenopausal women often receive anti-osteoporotic drugs as they suffer from osteoporosis. These anti-osteoporosis drugs, e.g. bisphosphonate, strontium renalate, parathyroid hormone, anti-sclerostin, denosumab, have anti-catabolic and/or anabolic mechanisms of action, and may affect fracture healing by suppressing bone resorption and formation during the different phases of the healing process.^{21, 22} Although it is reported that anti-osteoporosis drugs do not have a negative impact on fracture healing in clinical practice²¹⁻²³, we propose to study whether these drugs influence the fracture healing process on a micro-architectural level with HRpQCT.

Second, our studies, however small ($n = 3$), suggest that 3D registration allows precise monitoring of specific regions over time and that the use of radiolucent plates allows reliable assessment of unstable distal radius fractures. It would therefore be of great interest to monitor the healing process of surgically treated fractures, and also the evaluation of ingrowth of bone graft may benefit from the abilities of HRpQCT.

Third, whereas this thesis focused on the healing process of distal radius fractures, HRpQCT can also be applied to study the fracture healing process at other common fracture sites at the peripheral parts of the body. For example, the treatment of scaphoid fractures is challenging, and failure can result in avascular necrosis (up to 40%), nonunion (up to 21%) of the fracture and subsequently early osteoarthritis (up to 32%).²⁴⁻²⁷ Similar to the distal radius, the scaphoid bone is mainly composed of trabecular bone, thus using HRpQCT in studies on scaphoid fracture healing treatment might also shed a new light on the causes of poor outcome in scaphoid fractures.

From a clinical perspective, our results showed that early changes in trabecular vBMD and separation were associated with the short-term (twelve-week) clinical outcome of the fractured distal radius. Since the trabecular region is mostly comprised in such fractures, it might well be that also the long-term outcome is related to (changes in) these parameters. This could be relevant for clinicians, as it may be of added value for optimizing fracture healing treatment.

HRpQCT is currently only available as a research tool. Because of its limited availability world-wide and considerable cost, it should be investigated if FEA based on QCT scans of lower resolution²⁸, can provide comparable information regarding changes in vBMD and biomechanical parameters during the fracture healing process.

The recent development of the second generation HRpQCT scanners, i.e. XtremeCT2, on the other hand, allows studying vBMD, bone micro-architecture and biomechanical parameters using a higher resolution of 61 μm .^{4, 5} Whether more reliable results with regard to the healing process of distal radius fractures can be obtained with any of these techniques is unknown, thus future research should focus on a direct comparison between the three techniques in assessing the healing process of distal radius fractures.

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A stylized, low-poly graphic of a branch with three leaves, rendered in a light gray color. The branch and leaves are composed of many small triangles, giving them a faceted appearance. The branch runs vertically down the left side of the page, with the leaves branching off to the right. The background is white with faint, thin gray lines forming a geometric pattern of triangles.

Addendum

Valorization

Summary

Samenvatting

Dankwoord

Curriculum Vitae

Publications

Valorization

During my research on fracture healing at the distal radius, I have only seen a fraction of all patients that visited the emergency department after they sustained a fracture. Yet, even in this small group of patients I noticed that patients are mostly looking forward to the moment of cast removal. Since I have never fractured a bone in my body, I can only imagine how annoying it must be not being able to scratch where it itches or not being able to wash away the sweat (and smell) on hot summer days. Would it therefore not be great if the time of wearing a cast is kept to a minimum for each individual patient? Besides these benefits on an individual level, reduction of cast immobilization time could also benefit the society by lowering the costs that are indirectly related to distal radius fractures, such as loss of productivity or days of sick-leave.

Since the main purpose of a cast is to provide the necessary stability until the fractured bone has regained sufficient bone strength, the time to cast removal can be reduced by simply speeding up bone strength recovery. However, in order to assess bone strength recovery in the individual patient, a quantitative non-invasive method is required. In this thesis, we mainly focused on exploring the use of a new imaging method because plain radiographs that are currently used to study the fracture healing process lack detailed quantitative information regarding the consolidation of the micro-architecture and neither do they provide quantitative information on the restoration of bone strength.

In 2005, high-resolution peripheral quantitative computed tomography (HRpQCT) was introduced. With a resolution of 82 μm it has the capability to assess the bone's micro-architecture. In addition, 3D models can be created from these high-resolution images which in turn can be used in micro finite element analysis in order to estimate bone strength. Whereas most research on bone quality mainly used these techniques to study bone quality during aging, under healthy and diseased conditions, and following medication, we explored, for the first time ever, the potential of HRpQCT in combination with newly developed imaging analysis techniques to study the fracture healing process. We showed that we can reliably monitor the fracture healing process of distal radius fractures during the first twelve weeks using HRpQCT in terms of bone density, micro-architecture and bone strength parameters, and that changes in these parameters were associated with clinical outcome, such as pain and disability, at the end of this twelve-week period. Also, we developed algorithms that could visualize and quantify the impressive amounts of bone modeling and remodeling during the fracture healing process in high detail.

In order to achieve this, a combined effort of several departments in the different stages of the study was required: starting with providing patient information to eligible patients at the emergency department, to inclusion and short-term follow-up by the orthopaedic surgeon, and finally the long-term follow-up by the endocrinologist. In other words, we have gained valuable experience in how to set up a framework for conducting fracture healing related research in a clinical setting.

Based on the framework and concepts we developed while studying the feasibility of HRpQCT to monitor the fracture healing process for more than 5 years, we believe that Maastricht University Medical Center is currently one of the expert centers in the world for clinical research on fracture healing. That said, it is now feasible to collaborate with pharmaceutical companies in clinical trials to study the effect of their drugs on the fracture healing process. Both safety aspects and probable positive effects regarding fracture healing can now be studied with the algorithms we developed. It would be interesting to invite pharmaceutical companies to participate in studies with existing drugs. In this way, only a minor investment of their side is required. In return, they can potentially use existing drugs for new purposes. Since this does not involve the high costs that are associated with development of new drugs, the risk-benefit ratio for the pharmaceutical companies is rather small. For example, these kind of studies could already be performed with 30 patients (10 controls, 20 on treatment) during six visits that are scheduled within the first year after fracture.

Also for non-pharmaceutical companies working together with the HRpQCT group at the Maastricht University Medical Center might be interesting. For instance, companies that develop medical devices that aim to improve fracture healing, can collaborate with us to study the effect of their device in patients with a fracture in more detail than ever before. These results could then be used to optimize the design, leading to a more effective medical device.

Such collaborations with industry are also beneficial for Maastricht University and its research schools. First of all, with these funds a new PhD-student can be appointed. Furthermore, pharmaceutical and medical device companies are often operating world-wide. Collaborating with such an international partner would add to the international allure that Maastricht University envisions.

In the end, however, these developments, i.e. new drugs or medical devices that aim to improve the fracture healing outcome, should result in shorter healing times, better functional recovery and less pain in patients with a fracture.

Summary

One of the most common types of fractures is a fracture of the wrist, i.e. the distal radius. These fractures are usually treated by cast immobilization or surgically by internal or external plating. Unfortunately, the clinical outcome of the fracture healing process is not always good and many factors that contribute to a poor outcome are still unknown.

In clinical practice, fracture healing is evaluated using plain radiographs in combination with physical examination. However, these assessments do not provide detailed information regarding consolidation of the bone micro-architecture or the restoration of bone strength. With the introduction of high-resolution peripheral quantitative computed tomography (HRpQCT) imaging, the micro-architecture of bone can now be studied in patients. In addition, based on three-dimensional HRpQCT images, bone strength can be estimated using micro finite element analysis (μ FEA). With regards to this thesis, we hypothesized that the use of HRpQCT and μ FEA attributes to a better understanding of distal radius fracture healing.

Although HRpQCT and μ FEA have been used to study bone quality during aging, with different disorders or after medication, they have never been applied in a clinical setting to study the repair of fractures. The aim of this thesis, therefore, was to explore the application of HRpQCT in combination with μ FEA to longitudinally study the healing process of distal radius fractures.

Casts and internal plates are commonly used to treat stable and unstable distal radius fractures, respectively. They may introduce image artifacts in HRpQCT and, therefore, affect the bone parameters that are derived from such images. As shown in **Chapter 2**, a fiberglass cast introduces a significant but not clinical relevant error in the bone parameters of up to -1.6% , whereas a cast made from plaster of Paris leads to larger errors up to -9.8% . In case of plating (**Chapter 8**), volar distal radius plates made from carbon-fiber-reinforced polyetheretherketone (CFR-PEEK) introduced no visible image artifacts and errors were smaller than 3.2% . In contrast, the titanium plates caused severe image artifacts and led to errors in bone parameters that ranged from -30.2% to 67.0% .

Based on these findings, we therefore recommend the use of a fiberglass cast for stable fractures and a CFR-PEEK plate for unstable fractures to monitor the fracture healing process of distal radius fractures with HRpQCT.

In **Chapter 3**, the healing process of distal radius fractures was described in terms of HRpQCT derived bone density, micro-architectural and biomechanical parameters

for the first twelve weeks after fracture. Additionally, in **Chapter 4**, the correlation between these HRpQCT derived bone parameters and the clinical outcome as assessed by the PRWE questionnaire was studied. In eighteen post-menopausal women with a stable distal radius fracture treated by cast immobilization, we found an increase compared to baseline in total and trabecular vBMD of 11% and 20% (both $p < 0.01$), respectively, at six weeks post-fracture. The occurrence of intertrabecular bone formation was indicated by a 23% and 31% ($p < 0.01$) increase in trabecular thickness at six and twelve weeks post-fracture, respectively, and blurring of the intertrabecular area. Bone stiffness at twelve weeks post-fracture was increased by 31% compared to baseline ($p < 0.01$). In addition, a lower PRWE score (representing less pain and better function) at twelve weeks post-fracture was significantly related to an early increase in trabecular vBMD ($\beta -0.96$, $p = 0.022$, $R^2 = 0.37$) and torsional stiffness ($\beta -0.14$, $p = 0.037$, $R^2 = 0.31$), and an early decrease in trabecular separation ($\beta 209$, $p = 0.045$, $R^2 = 0.33$).

This exploratory study demonstrated that it is feasible to monitor the healing process of stable distal radius with HRpQCT during the first twelve weeks after fracture, and that HRpQCT can provide valuable information regarding the short-term clinical outcome in terms of pain and disability.

Since the healing process is not completed at twelve weeks post-fracture, a follow-up visit at two years post-fracture was envisaged in order to study the long-term changes in vBMD, micro-architectural parameters and biomechanical competence at the fractured side. In addition, the fracture side was compared to the contra-lateral side. The results of this study are described in **Chapter 5**. In the fourteen eligible patients that participated in the two-year visit, we found significant changes in all bone parameters, except in total vBMD, trabecular thickness and cortical perimeter when comparing the measurements at six and twelve weeks with those at two years post-fracture. At two years post-fracture, cortical and trabecular thickness, and torsional and bending stiffness were significantly higher at the fractured side (21%, 55%, 31% and 29%, respectively, $p < 0.05$) compared to the contra-lateral side, whereas vBMD was not significantly different between both sides. From these results, we concluded that by the time the cast is removed, i.e. at six weeks post-fracture, the fracture healing process is still ongoing and large changes occur in vBMD, micro-architecture and biomechanical parameters thereafter. Compared with the contra-lateral side at two years post-fracture, the fractured side had thicker cortices and trabeculae, whereas BMD was similar. Ultimately, this resulted in a restoration of bone stiffness that ended up being significantly higher at the fracture side than at the contra-lateral side.

Although no changes in vBMD are expected at the contra-lateral distal radius following a distal radius fracture, it is unknown if also bone micro-architecture and strength at the contra-lateral distal radius are unaffected after a distal radius fracture. Therefore, in **Chapter 6**, the bone parameters at the contra-lateral side were studied using HRpQCT and μ FEA measurements at baseline, twelve weeks and two years post-fracture. Compared to baseline, we found a significant decrease in cortical vBMD (-3.9% , $p < 0.001$), failure load (-5.6% , $p = 0.007$), and stiffness in compression (-5.1% , $p = 0.014$) and bending (-6.0% , $p = 0.029$) at two years post-fracture. These results were similar between patients with or without adequate anti-osteoporosis drug treatment. Since these changes were larger than previously reported changes due to aging, we conclude that the healing process of stable distal radius fractures in post-menopausal women may be associated with accelerated cortical bone loss and concomitant reduction of bone strength but not with micro-architectural changes at the contra-lateral distal radius, despite adequate anti-resorptive treatment.

In **Chapter 7**, a method for overlaying two consecutive HRpQCT scans from patients with a healing distal radius fracture was introduced. In contrast to the standard registration method in which the whole bone volumes are used to overlay the HRpQCT images, this method relies on registration of individual fragments. After testing the effect of fragment size and amount of displacement using unfractured distal radius HRpQCT scans, the method was applied in two stable and one unstable fracture(s). We found that fragments as small as 300 mm^3 that were displaced up to 0.82 mm in-plane or up to 1.64 mm in the longitudinal directional could be realigned correctly. For the fractured cases, most improvement in alignment was found in the unstable fracture. This result is promising for additional analysis of bone formation and resorption in HRpQCT studies on fracture healing.

Finally, in **Chapter 9**, the main findings and limitations of the aforementioned studies are discussed. Furthermore, general conclusions and recommendations as well as directions for future are presented.

Samenvatting

Een van de meest voorkomende fracturen is een polsfractuur, meer specifiek een breuk van de distale radius (spaakbeen). In het geval van een stabiele radiusfractuur bestaat de behandeling uit gipsimmobilisatie, terwijl bij een instabiele fractuur meestal een chirurgische ingreep nodig is om de fragmenten te stabiliseren door middel van een metalen of kunststofpolsplaatje. Helaas is de klinische uitkomst van het genezingsproces niet altijd goed en zijn niet alle factoren die bijdragen aan een slechte uitkomst bekend.

In de dagelijkse klinische praktijk wordt de vordering van het fractuurgenezingsproces vastgesteld aan de hand van röntgenfoto's in combinatie met lichamelijk onderzoek door de behandelend arts. Echter, deze methodes geven géén gedetailleerd beeld van de mate waarin de fijne botstructuren (botbalkjes) vastgegroeid zijn of van het herstel van de botsterkte. Sinds de introductie van hoge resolutie perifere kwantitatieve computer tomografie (CT) in 2005 is het mogelijk om de botstructuur van de pols tot op microniveau te bestuderen. Daarnaast kunnen de gegevens van deze driedimensionale hoge resolutie CT scans als basis dienen voor eindige elementen analyse (EEA), waarmee de sterkte van het bot berekend kan worden. Omdat met hoge resolutie CT in combinatie met EEA een gedetailleerder beeld verkregen kan worden van de botkwaliteit, is de hypothese die getoetst wordt in dit proefschrift dat het gebruik hiervan kan leiden tot een beter inzicht in het genezingsproces van distale radius fracturen.

Ondanks dat hoge resolutie CT en EEA al een aantal jaren gebruikt worden om te bestuderen hoe de botkwaliteit verandert bij het ouder worden, bij bepaalde ziektebeelden of als gevolg van medicatie, is hoge resolutie CT nog niet eerder in de kliniek ingezet om het genezingsproces van fracturen te bestuderen. Het doel van dit proefschrift is om het genezingsproces van distale radius fracturen te bestuderen met behulp van hoge resolutie CT in combinatie met EEA.

De aanbevolen behandeling van stabiele distale radius fracturen bestaat uit het dragen van een gipsverband gedurende 3 tot 5 weken. Het gebruik van gips zou echter kunnen leiden tot verstoringen in hoge resolutie CT scans, en daarmee de uitkomsten van de botparameters nadelig kunnen beïnvloeden. In **hoofdstuk 2** is het effect van twee soorten gips (fiberglass en pleister gips) op hoge resolutie CT metingen vergeleken. Hieruit bleek dat zowel fiberglass als pleister gips tot significante afwijkingen leidden in de botparameters. Echter, de afwijkingen in de botparameters ten gevolge van fiberglass gips waren maximaal -1.6% ten opzichte van metingen zonder gips en daarmee niet klinisch relevant, terwijl de afwijkingen als gevolg van pleister gips opliepen tot -9.8% . Op basis van deze

resultaten adviseren we om in toekomstige studies naar het genezingsproces van distale radius fracturen met hoge resolutie CT gebruik te maken van fiberglass gips.

In **hoofdstuk 3** is het genezingsproces van distale radius fracturen voor het eerst bestudeerd met hoge resolutie CT. Hierbij is gekeken naar de veranderingen in botdichtheid, -structuur en sterkte gedurende de eerste twaalf weken na de breuk. In achttien postmenopauzale vrouwen met een stabiele distale radius fractuur, die behandeld werd met gips, vonden we zes weken na de breuk een significant hogere totale en trabeculaire botdichtheid (respectievelijk 11% en 20%) in vergelijking met één week na de fractuur. Ook de dikte van botbalkjes was op zes en twaalf weken na de breuk significant hoger (31%). Daarnaast lieten de hoge resolutie CT scans mineralisatie zien tussen de botbalkjes, wat duidde op de aanmaak van bot tussen de botbalkjes in plaats van op de botbalkjes. Ten opzichte van één week na de fractuur was de botsterkte twaalf weken na de breuk significant toegenomen met 31%.

Vervolgens is, in **hoofdstuk 4**, gekeken of de PRWE score (een klinische uitkomst score op basis van pijn en functie) twaalf weken na de breuk geassocieerd is met vroege veranderingen (in de periode tussen één en drie weken na de breuk) in de botparameters die berekend kunnen worden op basis van de hoge resolutie CT scans. In dit onderzoek werd een significante relatie gevonden tussen een betere PRWE score op twaalf weken na de breuk en grotere vroege veranderingen in het bot, namelijk een toename in trabeculaire botdichtheid en torsiestijfheid en afname in de onderlinge afstand tussen de botbalkjes.

In deze exploratieve studies hebben we aangetoond dat het haalbaar is om de eerste twaalf weken van het genezingsproces van stabiele distale radius fracturen te volgen met hoge resolutie CT, en dat veranderingen in botdichtheid, -structuur en -sterkte, die plaatsvinden in de eerste drie weken na de breuk, waardevolle informatie kunnen geven met betrekking tot de pijn en functie op twaalf weken na de breuk.

Omdat het fractuurgenezingsproces twaalf weken na de breuk nog niet voltooid is en op dat moment dus nog geen eindpunt van het genezingsproces vastgesteld kan worden, is een extra hoge resolutie CT scan uitgevoerd twee jaar na de breuk. Hierbij zijn de botdichtheid, -structuur en sterkte aan zowel de fractuur zijde als aan de (niet gebroken) contra-laterale zijde gemeten en met elkaar vergeleken. De resultaten daarvan zijn beschreven in **hoofdstuk 5**. Voor de tweejaars meting waren veertien van de achttien patiënten uit hoofdstuk 3 en 4 beschikbaar en bij al deze patiënten was de breuk volledig hersteld. Na twee jaar was in vrijwel alle botparameters een significant verschil meetbaar ten opzichte van de metingen zes

en twaalf weken na de breuk, waarbij met toenames van respectievelijk 186% en 97% in de botsterkte parameters de grootste veranderingen gemeten werden. Uit de vergelijking tussen de fractuur- en contra-laterale zijde op twee jaar na de breuk bleek dat de dikte van de cortex en de botbalkjes alsmede de botsterkte significant hoger waren aan de fractuurzijde dan aan de contra-laterale zijde, terwijl de botdichtheid gelijk was. Hieruit kunnen we concluderen dat op het moment dat het gips verwijderd wordt (ongeveer zes weken na de breuk), het fractuurhelingsproces nog niet voltooid is en dat er daarna nog grote veranderingen plaatsvinden in de botdichtheid, -structuur en -sterkte. In vergelijking met de (niet gebroken) contra-laterale zijde, heeft de fractuurzijde na twee jaar een dikkere cortex en dikkere botbalkjes met als gevolg een hogere botsterkte dan aan de niet gebroken zijde.

Na een polsbreuk wordt er op basis van eerder onderzoek geen verandering in botdichtheid aan de contra-laterale distale radius verwacht. Omdat in deze onderzoeken alleen gebruik is gemaakt van botdichtheidsmetingen is onbekend of ook de botstructuur en botsterkte aan de contra-laterale zijde onveranderd blijven na een distale radius fractuur. In **hoofdstuk 6** zijn daarom zijn de botdichtheid, botstructuur, en botsterkte aan de contra-laterale pols gemeten met hoge resolutie CT en EEA op de meetmomenten één week, twaalf weken en twee jaar na de breuk. In de periode van één tot twaalf weken na de breuk werden geen veranderingen gevonden in de botparameters. Echter, twee jaar na de breuk waren de botdichtheid van de cortex en de botsterkte van de contra-laterale zijde significant lager ten opzichte van één week na de breuk (respectievelijk -3.9% en -5.5%). Deze afnames werden gevonden bij zowel patiënten met als zonder botafbraakremmende medicatie. Als we deze afnames vergelijken met eerder gepubliceerde gegevens van vrouwen zonder polsbreuk in de literatuur, dan blijkt dat de in onze groep gevonden afnames groter zijn dan normaal gesproken verwacht mag worden gedurende twee jaar ouder worden. Hieruit concluderen we dat in postmenopauzale vrouwen met een stabiele distale radius fractuur een verhoogde corticale botafbraak plaatsvindt, met tegelijkertijd een afname in botsterkte, ondanks behandeling met botafbraakremmende medicatie.

Een nieuwe methode om twee opeenvolgende hoge resolutie CT beelden van genezende distale radius fracturen over elkaar heen te leggen (registreren), wordt beschreven in **hoofdstuk 7**. In tegenstelling tot de standaard registratie methode, waarbij gehele botvolumes worden gebruikt om de beelden over elkaar heen te leggen, is de nieuwe methode gebaseerd op de registratie van individuele fractuurfragmenten. Eerst is het effect van fragmentgrootte en mate van verplaatsing getest op hoge resolutie CT beelden van niet gebroken bot,

en vervolgens is de methode toegepast op stabiele en instabiele fracturen. Uit deze tests bleek dat fragmenten zo klein als 300 mm³ met een vooraf opgelegde verplaatsing tot 0.82 mm in zijwaartse richting en tot 1.64 mm in de lengterichting correct geregistreerd konden worden. In het geval van fracturen was de meeste verbetering in overlap te zien in de instabiele fractuur wanneer de registratie op basis van individuele fragmenten in plaats van de standaard registratie methode toegepast werd. De resultaten van deze pilot studie zijn veelbelovend voor het bestuderen van instabiele distale radius fracturen of voor het bestuderen van botaanmaak en -afbraak tijdens de fractuurgenezing, en geven aanleiding tot een verdere uitwerking van de methode en validatie ervan zodat deze daadwerkelijk toegepast kan worden.

Waar stabiele distale radius fracturen behandeld worden met gips, bestaat de behandeling van instabiele distale radius fracturen vaak uit een chirurgische ingreep waarbij een polsplaatje geplaatst wordt om de fragmenten te stabiliseren. Dergelijke polsplaatjes zijn gemaakt van titanium, wat kan leiden tot verstoringen op CT scans en dus ook op de hoge resolutie CT scans waardoor de botdichtheid, -structuur en -sterkte niet accuraat bepaald kunnen worden. Een mogelijke oplossing hiervoor is het gebruik van een polsplaatje gemaakt van carbon, een materiaal waarvan bekend is dat dit zeer weinig verstoring geeft op CT scans. In **hoofdstuk 8** wordt een pilot studie beschreven waarin het effect van een carbon polsplaatje op de hoge resolutie CT scans en de botparameters is vergeleken met veelgebruikte polsplaatjes van titanium. Dit is gedaan door de polsplaatjes te instrumenteren in drie menselijke kadaverarmen en hoge resolutie CT scans van de polsen te maken met en zonder polsplaatjes. De resultaten toonden aan dat een polsplaatje van carbon niet leidt tot zichtbare verstoringen in de hoge resolutie CT scans en dat de invloed op de botparameters minimaal is. Dit in tegenstelling tot de polsplaatjes van titanium, waarbij de botparameters tot 67% afweken. Op basis van de resultaten van deze pilot studie adviseren we het gebruik van carbon polsplaatjes in studies waarbij het genezingsproces van instabiele distale radius fracturen gevolgd wordt met hoge resolutie CT.

Tot slot, in **hoofdstuk 9**, worden de hoofdbevindingen en beperkingen van de afzonderlijke studies besproken. Tevens wordt een overzicht gegeven van de algemene conclusies en worden aanbevelingen voor toekomstig onderzoek beschreven. De voornaamste conclusie is dat hoge resolutie CT, in combinatie met eindige elementen analyse, een veelbelovende techniek is om het genezingsproces van distale radius fracturen te bestuderen omdat het belangrijke informatie verschaft met betrekking tot (veranderingen in) de botdichtheid,

-structuur en -sterkte tijdens de fractuurgenezing. Het wordt dan ook aanbevolen om in toekomstig onderzoek naar het fractuurgenezingsproces, bijvoorbeeld in studies naar het effect van medicatie op de fractuurgenezing, hoge resolutie CT in te zetten als voornaamste imaging techniek.

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Curriculum Vitae

Joost Johannes Antoin de Jong was born on 21 December 1984 in Hulst, The Netherlands. He completed secondary school at the Reynaert College in Hulst in 2003. In 2004, he started studying Mechanical Engineering at Fontys University of Applied Sciences in Eindhoven. After graduating for his bachelor's degree in 2007, he started his master Medical Engineering at Eindhoven University of Technology. During his master he completed an internship at University of São Paulo in Brazil and fulfilled his clinical graduation project on fracture healing studied by high-resolution peripheral quantitative computed tomography (HRpQCT) at the department of Orthopaedics of Maastricht University Medical Center.

After graduating for his master's degree in 2012, he got the opportunity to continue his research on fracture healing and HRpQCT at Maastricht University under supervision of prof. dr. P. Geusens, prof. dr. J. van den Bergh, dr. P. Willems and dr. ir. B. van Rietbergen. During his PhD period, he performed a three-month internship at the faculty of Biomedical Engineering of ETH in Zürich under supervision of prof. dr. R. Müller and dr. P. Christen. The current thesis presents the results of the work done during his PhD studies.

Currently, in order to increase his expertise in medical imaging, Joost is exploring the use of magnetic resonance imaging in neurologic disorders as a postdoctoral researcher under the supervision of prof. dr. W. Backes at the department of Radiology of Maastricht University Medical Center.

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