New horizons for the in vivo assessment of major aspects of bone quality

Microstructure and material properties assessed by Quantitative Computed Tomography and Quantitative Ultrasound methods developed by the *BioAsset* consortium

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Keywords
Osteoporosis, bone quality, mineralization, high resolution quantitative computed tomography, quantitative ultrasound, finite element analysis

Summary
The *Biomechanically founded individualised osteoporosis Assessment and treatment (BioAsset)* consortium pursues experimental and clinical studies in the context of skeletal effects of bisphosphonate treatment. Here, first results using newly developed diagnostic methods in a set of vertebral bone specimen obtained from donors with documented bisphosphonate history ranging from 0 to more than 5 years of treatment are presented. A new thoracolumbar quantitative computed tomography (QCT) protocol covering T6 to L4 plus high-resolution QCT (HRQCT) assessment of T12 were compared with high-resolution peripheral QCT (HRpQCT) and micro-CT scans of excised specimens serving as gold standard techniques. Finite element (FE) modelling was performed. Material, ultrastructural, and micromechanical properties were tested on a set of single trabeculae obtained from the donor specimens. A newly developed quantitative ultrasound (QUS) device for measuring the anisotropy of cortical material properties at the tibia was designed and built. The thoracolumbar QCT protocol permitted in situ imaging with good image quality and automated segmentation of vertebral bodies in the whole range from T6 to L4. The duration of bisphosphonate treatment was significantly associated with increased levels of mineralization and this effect could be measured with HRQCT performed on excised specimens. Microstructural parameters contributed to vertebral bone strength modelled by FE analysis independently of bone mineral density. The new QUS tibia scanner permitted measuring the acoustical anisotropy of reference materials. Taken together, these results document that new methods developed in *BioAsset* permit a more comprehensive assessment of bone fragility. The set of donor specimens with a documented history of bisphosphonate treatment allows for the assessment of the effects of long-term treatment from the organ down to the tissue and material level. These results will ultimately be linked to the parallel clinical study to provide guidance for determining the optimum duration of bisphosphonate treatment to reduce the incidence of osteoporotic fractures.

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Neue Horizonte für die In-vivo-Bestimmung wesentlicher Aspekte der Knochenqualität Mikrostruktur und Materialeigenschaften, bestimmt mit Quantitativer Computertomografie und Quantitativen Ultraschallmethoden, entwickelt durch das *BioAsset* Konsortium
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Schlüsselwörter
Osteoporose, Knochenqualität, Mineralisierung, hochauflösende Quantitative Computertomografie, Quantitativer Ultraschall, Finite Elemente Analyse

Zusammenfassung
Das *Biomechanically founded individualised osteoporosis Assessment and treatment (BioAsset)*-Konsortium führt experimentelle und klinische Studien zu skelettalen Effekten von Bisphosphonaten durch. Neue diagnostische Verfahren zur Analyse von Wirbelkörperproben von Spendern mit dokumentierter Bisphosphonateinnahme über 0 bis >5 Jahre

wurden entwickelt. Mittels thorakolumbaler Quantitativer Computertomografie (QCT) und hochauflösender QCT (HRQCT) wurden Knochenmineraldichte (BMD), Mikrostrukturvariablen und Materialeigenschaften, insbesondere Mineralisierung, untersucht. Finite Element (FE)-Modellierung dient der Bestimmung der Wirbelkörperbruchlast. Ein neues Quantitatives-Ultraschall (QUS)-Gerät zur Messung anisotroper kortikaler Materialeigenschaften der Tibia wurde konstruiert. Ein signifikanter Zusammenhang von Mineralisierung und (Dauer der) Bisphosphonattherapie konnte mit Mikro-CT und HRQCT nachgewiesen werden. Das thorakolumbale QCT-Protokoll ermöglichte eine Dosisreduktion von 60 % gegenüber Standardprotokollen. Eine Finite-Elemente-Analyse zeigte BMD und Trabekelanzahl als unabhängige Determinanten der Bruchlast. Mit dem neuen QUS-Tibia-Gerät konnte die akustische Anisotropie von Referenzmaterialien bestimmt werden. Die Daten dokumentieren erweiterte Diagnosemöglichkeiten zur Abschätzung von Knochenfragilität durch die neuen Verfahren. Parallel durchgeführte klinische Studien sollen die Frage der optimalen Dauer von Bisphosphonattherapie klären.

In chronic diseases such as osteoporosis lasting improvement in health can only be achieved if causal mechanisms of treatment options for osteoporosis are identified and linked to bone fragility. Personalised treatment concepts require

1. the identification of the most relevant determinants of bone strength,
2. the availability of methods for diagnostic assessment of these determinants (the "fragility profile") in individual patients,
3. knowledge of the impact of therapies on these determinants to select the most appropriate treatment, and
4. diagnostic methods for monitoring changes in the fragility profile for repeated reassessment or, if necessary, adaptation of treatment strategies in the individual patient.

In recent years substantial progress has been made in the development of methods to identify patients at risk of fracture and in the approval of effective treatments. However, 70–80 % of patients with fractures have an areal bone mineral density (aBMD) by Dual X-ray Absorptiometry (DXA) in the non-osteoporotic range (1), reflecting the relevance of determinants of bone strength not captured by this modality. Recognizing that external non-skeletal factors like balance and muscle strength also contribute to fracture risk, new diagnostic methods are needed for a more comprehensive characterisation of bone fragility contributing to improved assessment of fracture risk in the individual patient. In addition, clinical risk factors affecting non-skeletal aspects associated with fracture risk need to be recognized such as medications and diseases affecting balance and strength along with factors affecting the risk of falling, including the home environment or appropriate correction of vision impairments(2).

Even more problematic is the poor performance of DXA for estimating fracture risk once treatment has been initiated. Changes in aBMD only partially reflect the protective effect of therapies (3). For a chronic disease like osteoporosis this has the following consequences: we currently have no diagnostic method to judge whether a patient has responded well to treatment and whether her/his fracture status now warrants continuation or change of treatment. In Germany the vast majority of osteoporotic patients are treated with bisphosphonates, most of them for at least 3–5 years. The data quality on effectiveness of treatment beyond the 3–5 year time period is limited. Worldwide, experts cannot provide evidence-based guidance to physicians on whether it would be better to continue treatment, pause treatment, or modify treatment (4).

In response to these issues, the **Bio***mechanically founded individualised osteoporosis* **asse***ssment and* **t***reatment* (BioAsset) consortium has been formed. The goals are pursued both experimentally and clinically:

- Using advanced **experimental and diagnostic methods**, the members of the consortium investigate the effects of extended bisphosphonate treatment on BMD, bone microstructure and material properties, specifically tissue mineral density (TMD). Bone specimens excised from donors with a documented history of bisphosphonate use prior to death (short term versus long term) are compared to controls without such use of bisphosphonate. In addition, as another comparator group, donors with a history of vitamin D deficiency, i.e. reduced TMD, are included as well. Improved quantitative computed tomography (QCT) based image acquisition and analysis methods and high-resolution QCT (HRQCT) techniques for the assessment of bone microstructure and TMD are being developed. High resolution peripheral computed tomography (HRpQCT) and micro CT methods are used as reference techniques. In addition, a new quantitative ultrasound (QUS) device for non-invasive measurement of cortical material properties at the tibia is designed and built. Results of the diagnostic techniques including for examples bone microstructure and TMD assessed by HRQCT, modulated by QUS derived patient specific bone material properties such as anisotropy of elasticity, are used as input for finite element (FE) models for the assessment of bone strength.
- **Clinically**, a randomized double blind placebo controlled multicentre trial has been initiated: in the *Bisphosphonat Langzeit (BILANZ)* study the efficacy and safety of longterm bisphosphonate treatment is assessed by comparing continued bisphosphonate treatment with a treatment pause in patients who have been on treatment for at least four years. Embedded in *BILANZ*, the *DIAGNOSTIK BILANZ* study is carried out: at *BILANZ* baseline, advanced QCT and QUS methods are performed and fracture incidence is monitored.

The teams collaborating in the study include the *Institut für Rechtsmedizin*, Hamburg, for the collection of donor materials and in situ QCT measurements and the *Institut für Osteologie und Biomechanik*, Hamburg, for the assessment of bisphosphonate effects using a range of analytic techniques for characterisation at the tissue or material level (subproject SP1); the *Institut für Biomechanik* of the Technische Uni-

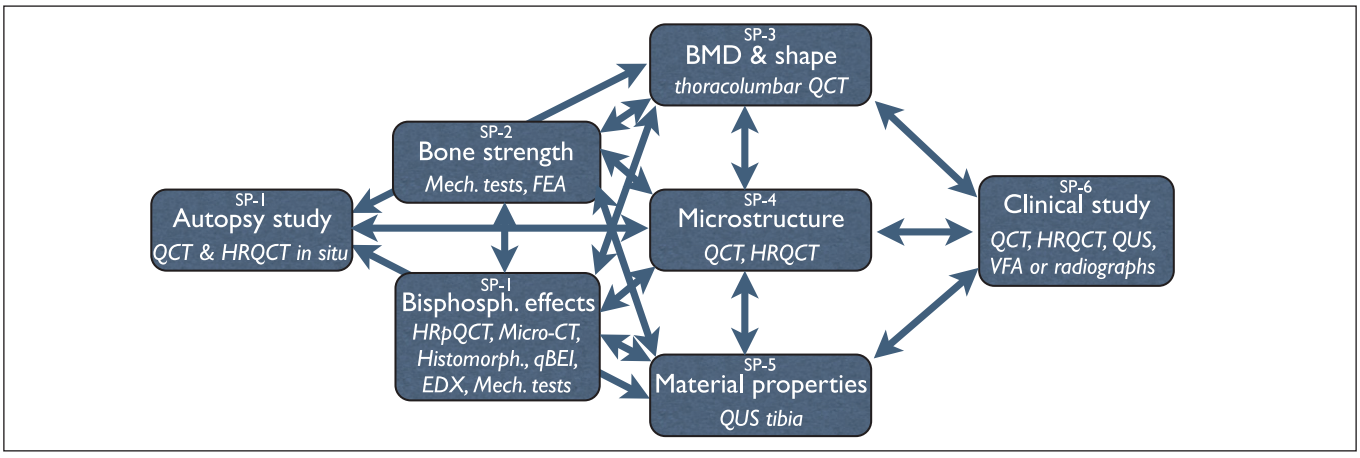

Fig. 1 Schematic drawing of the cooperation within the *BioAsset* consortium. Based on the specimens obtained from autopsy bisphosphonate effects are studied using a broad range of micro- and ultrastructural imaging approaches (subproject 1). Bone strength measurements including finite element (FE) modelling is performed (subproject 2). Bone mineral density and shape of thoracolumbar vertebrae (subproject 3) along with microstructure (subproject 4) are assessed using a range of computed tomography approaches. Noninvasive measurement of material properties is realised using cortical ultrasound techniques (subproject 5). All densitometric, microstructural and material properties are fed back to improve FE models. The methods developed using the autopsy specimen are applied in the clinical *BioAsset* studies on long term bisphosphonate therapy. Performance attained in vivo will provide feedback for further improvement of the analytical methods.

versität Hamburg Harburg for finite element modelling and mechanical testing (SP2), the *Institut für Medizinische Physik*, Erlangen, for developing new QCT methods (SP3), the *Sektion Biomedizinische Bildgebung*, Kiel, for coordination of the *DIAGNOSTIK BILANZ* study and for developing new HRQCT (SP4) and QUS approaches (SP5) and the *Osteologisches Forschungszentrum Essen* for the coordination of the *BILANZ* study (SP6). The topics of the subprojects are schematically depicted in ▶Figure 1 documenting the interactions between clinical and experimental and among experimental aspects.

In this contribution we report first results from the *BioAsset* consortium projects. We introduce new diagnostic techniques developed by the consortium teams and present the first analyses of the specimens collected in *BioAsset*. We focus on bisphosphonate effects (vitamin D results not yet included in this manuscript) and include findings both from the organ to the tissue and material level. A key feature of innovation in *BioAsset* is the development and testing of a QCT protocol that is not limited to the lumbar spine as in typical clinical procedures, where usually 2–3 vertebrae between T12 and L3 are measured. The new thoracolumbar protocol includes a contiguous low dose CT scan from T6 to L4. Thus this region includes virtually all vertebrae susceptible to osteoporotic fracture. The non-invasive assessment of bone material properties in patients remains unattainable to date. One of the most promising approaches to close this gap is based on Quantitative Ultrasound measurements. New developments focus on the QUS transmission through cortical bone, e.g. the cortical shell of the femoral neck (5, 6). However, cortical bone is best accessible at the tibia and the sound velocity depends on BMD and elasticity, reflecting material properties. In the *BioAsset* consortium we have designed and built a new ultrasound scanner to measure cortical sound velocity at the tibia and, in addition, its anisotropy in axial and tangential direction. Anisotropy, e.g. of elasticity, is mechanically relevant since it affects bone strength under different loading conditions. Anisotropy is also affected by porosity, i.e. the network of Haversian canals, another important predictor of bone fragility. Increased width of the Haversian canals (which are running in axial direction) will only increase QUS properties measured in non-axial directions, thus leading to increased anisotropy. Ex vivo measurements of material properties of different bones will address the question, whether alterations occur systemically or differ in a site-specific fashion. Finally, FE models provide estimates of whole bone strength and, by feeding the results of the various diagnostic techniques into the FE models a more comprehensive modelling of bone fragility can be achieved.

Materials and Methods

Donors and specimens (SP1)

33 patients (mean age: 81.2 ± 7.1 years [range: 65 to 90 years]) were recruited during autopsy at the Department of Legal Medicine, University Medical Center Hamburg-Eppendorf. We included females between age 60 and 90 with a diagnosis of postmenopausal osteoporosis. Osteoporosis was diagnosed according to the WHO definition with a lumbar or femoral aBMDT-Score of –2.5 or less as assessed by DXA. The subjects' medical histories were retrospectively screened to assess whether the patients had received any bisphosphonate (BP) therapy for at least one year prior to death. Exclusion criteria included bone cancer, immobility >1 year, renal transplantation or renal insufficiency III°, strontium or fluoride therapy, specific antiresorptive medication other than bisphosphonates, and bisphosphonate therapy <1 year. For the control group women with

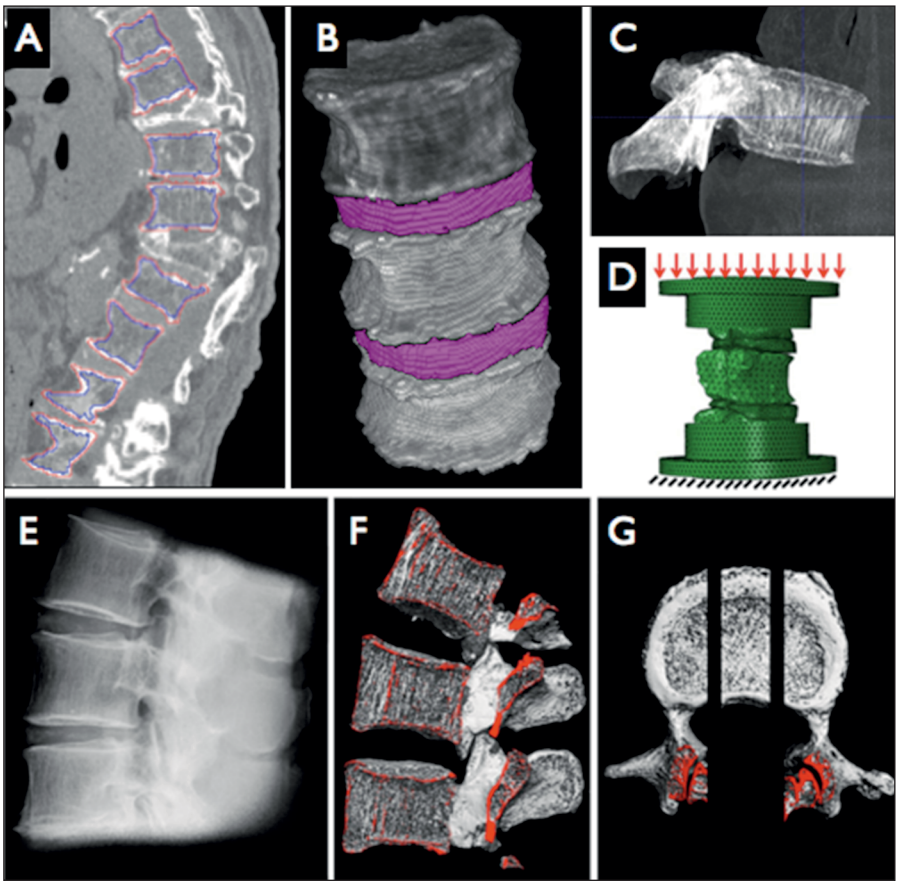

Fig. 2 Imaging procedures carried out in the *BioAsset* project permit a comprehensive evaluation of bone properties. These incorporate a range of protocols suitable for clinical application to microstructural high-resolution assessments of excised specimen. (A) Sagittal reformation of a spinal column with segmented vertebral bodies (T6 to L3 are shown) acquired using the thoracolumbar QCT protocol in situ (fractured vertebrae will be excluded from the QCT analyses). (B) L1-L3 section of the spine showing 3D renderings of the vertebral bodies and intervertebral disk spaces. (C) 3D reconstruction of excised vertebra T10 acquired with the high resolution QCT protocol. Coarse trabecular structures are visible and can be quantified, e. g. trabecular separation. (D) Based on either QCT or HRQCT data finite element models can be analysed. Here a compressive loading mode is depicted, with intervertebral disks and parts of the adjacent vertebrae above and below included in the model. Material properties were investigated on L5 after prior characterization with (E) lateral radiography and (F) HRpQCT. (G) depicts placement of sagittal slices used for micro CT based assessment of TMD (central slab) and excision of single trabeculae (outer slabs).

no documented history of bisphosphonate treatment within at least five years prior to death were selected.

For the purpose of this publication, women were grouped according to duration of prior bisphosphonate treatment:

i) subjects in the control group (CNTR) without documented history of bisphosphonate treatment;
ii) women on bisphosphonates were separated into a group with midterm treatment duration of one to five years (MTB) and
iii) women on longterm treatment for more than five years (LTB).

Informed consent was obtained from the family members after comprehensive information on relevant issues was given. Institutional approval for the study procedures had been obtained from the Ethics Committee of the Hamburg Chamber of Physicians (PV3486).

Different vertebrae were analysed for the various analytical approaches: T10 for bone microstructure, T11/T12/L1 for mechanical modelling and testing, and L5 for material and ultrastructural properties. The thoracolumbar protocol covered T6 to L4 and in situ HRQCT was obtained on T12.

In situ QCT measurements (SP3)

Within 7.3 ± 1.6 days after death and prior to autopsy, QCT and HRQCT scans were performed in situ on a Philips MX8000 scanner. The low dose thoracolumbar QCT protocol (▶Fig. 2A, B) developed works at 90 kVp and 150 mAs instead of 120 kVp and 100 mAs, resulting in a radiation exposure reduction of about 60 % (ImpactDose, CT Imaging GmbH, Erlangen, Germany). A slice thickness of 1.3 mm and a pitch of 1 were selected and the region from T6 to L4 was scanned. Reconstruction was performed using kernel B. In plane pixel size was 0.3 mm. The existing 3D segmentation method developed for the lumbar spine (7) was extended to the thoracic vertebrae. Also the intervertebral disk spaces were segmented (8). Integral, cortical and trabecular compartments were obtained after the 3D segmentation of periosteal and endosteal surfaces (7). Calibration was performed using the QRM Bone Density Calibration (BDC) phantom (QRM, Möhrendorf, Germany).

In situ HRQCT measurements (SP4)

For the HRQCT protocol T12 was measured with 120 kVp, 355 mAs, a collimation of 0.6 mm and a pitch of 1. Reconstruction was performed using kernel D (the reconstruction kernel is a mathematical filter in the process of reconstructing the image from the raw data; on most CT scanners several kernels with differing levels of contrast and smoothing can be selected). Slice thickness was 0.6 mm, increment 0.6 and 0.3 mm (two reconstructions) and in plane pixel size 0.18 mm. This protocol permits to measure relevant aspects of bone microstructure, e. g. trabecular separation (Tb.Sp) with residual errors of about 100 µm (9) (i. e. five to ten times smaller than typical levels of trabecular separation), also for monitoring response to treatment and as a basis for FE modelling (10).

HRpQCT measurements on excised intact specimens (SP1)

Bone specimens were excised at autopsy. HRpQCT with an isotropic voxel size of $(0.082\ mm)^3$ was performed on excised vertebrae T10, T11, T12, L1, and L5 using the XtremeCT device (Scanco Medical AG, Brütisellen, Switzerland). While showing substantially better spatial resolution compared to HRQCT, partial volume effects also affect HRpQCT measurements of mineralization and thus the term "apparent" TMD (app. TMD) was used to denote tissue mineralization status. BMD and parameters of trabecular and cortical microstructure were measured using the standard patient evaluation procedure for the XtremeCT scanner. These data served as gold standard for the comparison with QCT and HRQCT data.

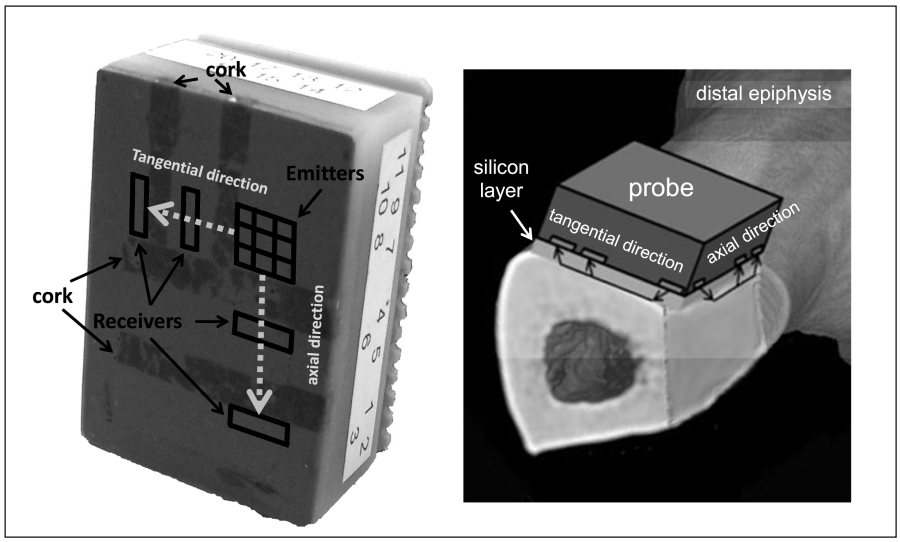

Fig. 3 Annotated photograph of the Quantitative Ultrasound (QUS) device for measuring acoustical anisotropy (left). The sound pathways are depicted schematically. Wave emission and detection permits assessment of propagation in axial and tangential direction. Ditches filled with cork block unwanted transmission of sound inside the device. The device is positioned on the medial face of the tibia (right). A 1.5 mm thick silicone layer has been added for coupling to uneven surfaces. Arrows depict the path of the sound wave detected by the receivers.

HRQCT measurements (SP4)

We prepared a set of human vertebral specimen for standardized assessment of different QCT and HRQCT protocols and devices. For this purpose, a set of twelve human vertebrae were embedded in epoxy resin (Technovit EPOX, Heraeus Kulzer, Wehrheim, Germany). Unlike the excised *BioAsset* donor vertebrae which have to be kept frozen to remain in adequate condition, these embedded vertebrae are stable over time and thus can be used for calibration and quality assurance purposes, e.g. in this project to identify the optimum reconstruction method available on commercial CT scanners. For the Siemens Somatom 64 CT scanner used in Kiel three reconstruction kernels (B60s, B70s, B80s) were analysed, each of them with three options for the reconstruction increment (0.1, 0.2, 0.3 mm). The twelve embedded vertebrae were measured inside the QRM abdomen phantom (QRM, Möhrendorf, Germany) and were reconstructed using these nine settings. Data acquired using the HRQCT protocol were compared to those obtained with HRpQCT using the XtremeCT scanner as the gold standard.

HRQCT scans (▶Fig. 2C) were also obtained on the excised BioAsset donor vertebrae T10, T11, T12 and L1 using a Siemens Somatom 64 scanner (120 kVp, 360 mAs, collimation 0.6 mm, pitch = 1, in-plane pixel size $(0.156\ mm)^2$, reconstruction kernel B70s, increment, i.e. slice thickness, 0.3 mm). HRQCT methods were used to measure mineralization of T10. For this purpose bone was segmented with a threshold of $250\ mg/cm^3$. For microstructural variables and TMD the prescript "app." for "apparent" was applied to recognize the partial volume effect which affects all variables measured with HRQCT.

Finite element modelling on excised T11-to-L1-vertebrae-segments (SP2)

The spine segment T11/T12/L1 was chosen for modelling since vertebrae at the thoracolumbar junction is particularly susceptible to failure. Specimens with prevalent fractures or pathologies in one of the vertebrae were excluded from this study. Out of 35 spine segments harvested eight segments have been analysed to date. The XtremeCT images were used to create the FE model. For T12, the vertebra of primary interest, a recently proposed visco-plastic damage model (11) was used. For the T11 and L1 the images resolution was downsampled and a linear elastic material model was used in order to save computational power. A hyperelastic Neo-Hookean model and a hyperelastic fibre reinforced model (12) were defined for the nucleus pulposus and annulus fibrosus, respectively. A schematic drawing of the completed FE model is shown in ▶Figure 2D.

Material, ultrastructural, and micromechanical properties (SP1)

Excised fifth lumbar vertebrae (L5) were radiographed (▶Fig. 2E) and scanned by HRpQCT (▶Fig. 2F) using the XtremeCT scanner. Subsequently the vertebral bodies were cut in three sagittal bone slabs (▶Fig. 2G). For the assessment of material properties including TMD by micro-CT measured with voxel size of $(10\ \mu m)^3$ (μCT40, Scanco Medical AG, Brütisellen, Switzerland), for static histomorphometry, and for further undecalcified histological analyses the central sagittal slab was used. The two outer slabs were macerated with H_2O_2. Subsequently, from each vertebra a minimum of 21 single trabeculae were excised. Micromechanical properties of these trabeculae were evaluated in three-point-bending mode using approaches established in earlier studies (13).

Table 1 Static histomorphometry results of L5. Differences between groups were tested with (ANCOVA) and without (ANOVA) adjustment for age and BMI

	Control group (n = 22)	Mid-term treatment duration (n = 5)	Long-term treatment duration (n = 6)	p-value	
				unadj	adj
Age (years)	81.29 ± 7.66	84.00 ± 5.79	79.14 ± 6.41	0.522	–
Body Mass Index (kg/m^2)	21.42 ± 4.36	21.08 ± 2.97	24.49 ± 6.07	0.291	–
ES/BS (%)	11.85 ± 3.53	6.49 ± 1.84	6.39 ± 1.72	<0.001	<0.001
BV/TV (%)	0.066 ± 0.023	0.053 ± 0.017	0.050 ± 0.018	0.211	0.083
Trabecular number (mm^{-1})	0.966 ± 0.145	0.812 ± 0.096	0.810 ± 0.166	0.025	0.012
Trabecular thickness (µm)	0.114 ± 0.021	0.129 ± 0.025	0.121 ± 0.016	0.357	0.69
Trabecular separation (mm)	1.027 ± 0.171	1.214 ± 0.142	1.258 ± 0.270	0.018	0.026

Ultrasound assessment of cortical material properties (SP5)

The tibia ultrasound device developed (►Fig. 3) comprises a two dimensional array of transducers (Smart Material, Dresden, Germany), consisting of eight emitters and four receivers. With a centre frequency of 1 MHz each sender can be triggered independently to steer the ultrasound beam in the direction desired. A distance of 22.4 mm in axial and of 15 mm in tangential direction is measured on the cortical surface. In each direction two signals are detected, one at half, the other at full distance. The difference signal yields the information about speed of sound (SOS). The electronics have been developed and the device assembled. To test the ability to measure elastic anisotropy (based on the ratio of SOS^2) a plate demonstrating acoustical anisotropy and with levels of SOS similar to cortical bone was measured with our device. First performance measurements including precision data have been obtained (14).

Results

►Table 1 summarizes the age and BMI distributions across the three study groups, separated according to prior bisphosphonate use. There were no significant differences in age or BMI across groups.

In situ QCT measurements (SP3)

The new low dose thoracolumbar protocol permitted imaging with good image quality in the whole range from T6 to L4. The 3D segmentation of the lumbar spine was successfully extended to the thoracic vertebrae. Also the intervertebral disk spaces were segmented. ►Fig. 2A shows multiplanar reformatted images in sagittal projection and ►Fig. 2B 3-D close-up of segmented vertebral bodies and the intervertebral disk space.

The in situ performance of the low dose protocol was validated against the standard regular dose protocol used in clinical QCT studies. 26 human cadavers were measured with each protocol. BMD results for T6 to L4 of the two protocols were highly correlated ($R^2 = 0.985$, residual root mean square (RMS) error = 4.21 mg/cm^3, ►Fig. 4). BMI did not significantly affect this correlation. Average noise levels expressed as standard deviation of the 200 mg HA insert of the calibration phantom were 23 HU for 90 kVp and 18 HU for 120 kVp.

HRQCT measurements (SP4)

For the group of twelve embedded vertebrae and using HRpQCT as the gold standard, better BMD results were obtained for smoother kernels, whereas the quality of microstructural information was better for contrast-rich kernels. A good compromise was achieved for the B70s kernel reconstructed with a 0.3 mm increment. For app. Tb.Sp, RMS errors across ranged between 106 and 118 µm for the different kernels and increments. ►Fig. 4 (left) shows data for B70s, increment 0.3 mm, demonstrating a significant association with HRpQCT with residual RMS errors of 109 µm.

On the subset of 19 *BioAsset* donor specimens with valid HRQCT and HRpQCT measurements on T10 (n = 8 with and n = 11 without bisphosphonate treatment, no significant difference in age or BMI), app. TMD measured with HRQCT was highly and significantly correlated with app. TMD measured by HRpQCT with a residual RMS error of

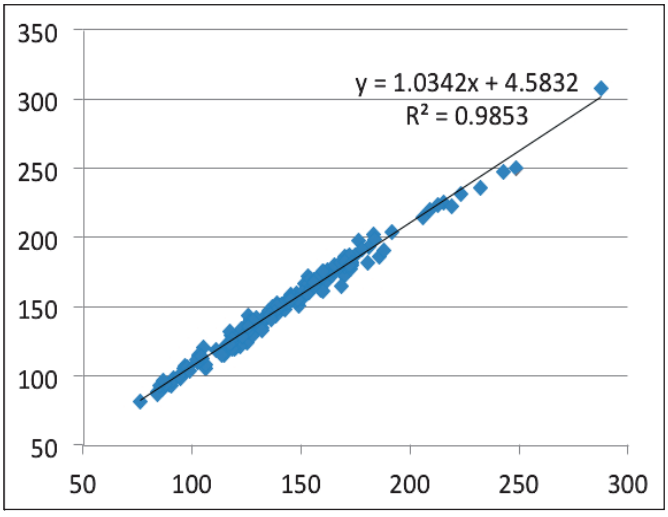

Fig. 4 Validation of the low dose 90 kVp protocol against the established 120 kVp protocol used in clinical routine. Measurements on T6-L4 obtained in 26 cadavers showed an excellent correlation. A slight multiplicative and additive offset due to different field inhomogeneity differences at the two voltage settings was observed which can be corrected using cross-calibration procedures.

35.3 mg/cm³ (▶Fig. 5, right). Adjusting for age (p=0.01) and BMI (p=0.2) increased the correlation to r²=0.74 and reduced the RMS error to 29.9 mg/cm³. Subjects on treatment with bisphosphonates had an app. TMD that was 9.4% higher compared to subjects not on this type of treatment (p<0.05). For HRpQCT the corresponding difference was 8.2% (p=0.065). There were no other significant different differences for any of the HRQCT or HRpQCT parameters analysed, including BMD (p=0.59 for HRQCT, 0.49 for HRpQCT). As potential confounders, neither age nor BMI significantly affected the differences in app. TMD (measured by HRQCT) and TMD (measured by HRpQCT).

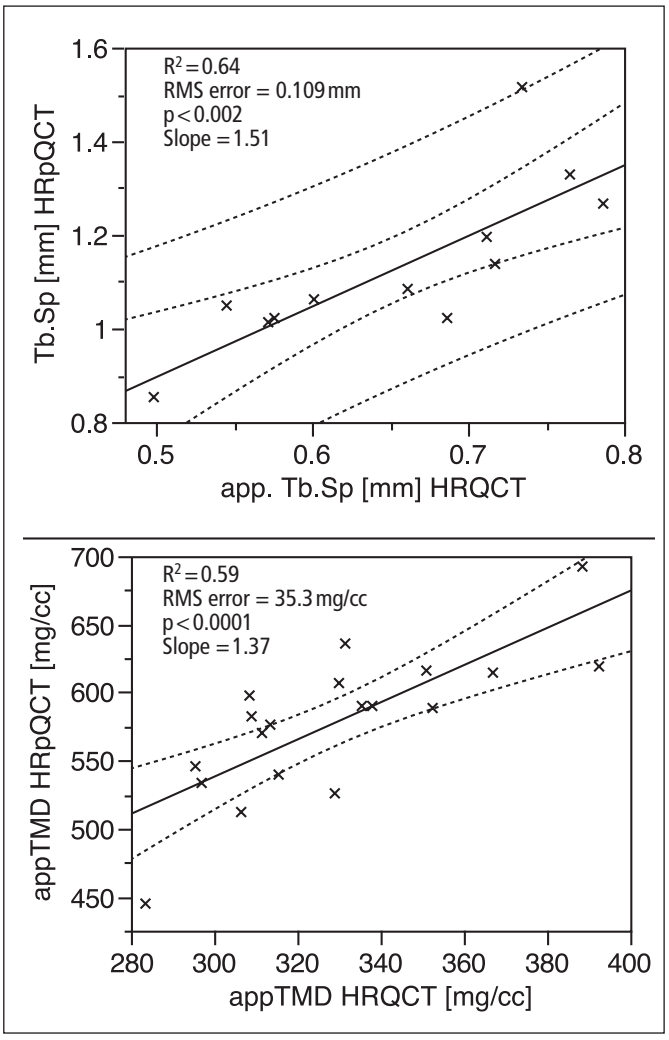

Fig. 5 HRQCT permits assessment of trabecular separation (Tb.Sp, top) and tissue mineral density (TMD, bottom). The results of linear regression (including 95% confidence intervals for the slope and the individual data) are displayed. HRQCT results obtained on twelve embedded vertebrae scanned in an abdomen phantom in a Siemens Somatom 64 CT scanner, B70s reconstruction kernel, increment 0.3 mm are presented. For app. Tb.Sp a highly significant correlation with gold standard HRpQCT data obtained on the XtremeCT device was achieved (top). App. TMD of excised T10 vertebrae of *BioAsset* donors measured with HRQCT was highly significantly correlated to app. TMD measured with HRpQCT.

Finite element modelling (SP2)

FE models for eight spinal segments (T11-L1) were created. Axial compression scenarios were simulated and compressive ultimate load was predicted from the FE models. The microstructural parameters – trabecular bone volume fraction (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp) and volumetric BMD of T12 were obtained from the HRpQCT analyses performed on excised specimens. The highest correlation between the FE predicted ultimate load and any of the above mentioned variables was observed for BMD (adjusted $R^2=0.55$, $p<0.022$, ▶Fig. 6). The combination of BMD and structural variables (e.g. Tb.Th, Tb.N) further improved the correlation. The strongest combination included BMD andTb.N (adjusted $R^2=0.87$, $p<0.003$). When adjusting for age or BMI, FE predicted ultimate load remained significantly associated with both BMD and Tb.N with non-significant contributions of age (p=0.88) and BMI (p=0.47) respectively.

Material, ultrastructural, and micromechanical properties (SP1)

Material properties were analysed in the L5 of 33 subjects. This included 22 women who were not on any specific medication affecting bone status (group 1), five women on bisphosphonate treatment for one to five years (group 2) and six women who had been on bisphosphonate treatment for more than five years (group 3). TMD showed a significant positive association with duration of bisphosphonate use ($R^2=0.204$, p=0.008, ▶Fig. 7), and remained significantly associated (p=0.021) after adjustment of age and BMI. Based on static histomorphometry, women in the control group 1 showed significantly higher Tb.N compared to women on medium term bisphosphonate treatment (p=0.037) and also compared to women on long term bisphosphonate (p=0.03). Tb.Sp was significantly lower compared to women on long term bisphosphonate (p=0.02). Eroded surface was higher in controls compared to MTB (p=0.001) and LTB (p<0.001). Significant differences remained significant after adjusting for age and BMI and are listed in ▶Table 1. When assessing mechanical properties, work to failure was higher in MTB (0.14±0.09 Nmm, p=0.01) compared to controls (0.07±0.03 Nmm) but this was not the case for the women of LTB (0.09±0.04 Nmm). However, there was no significant difference between MTB and LTB. Age and BMI adjusted between group differences in ANCOVA were significant (p<0.008). Micro fractures could be observed in women on bisphosphonate treatment but this was not yet evaluated systematically.

Ultrasound assessment of cortical material properties (SP5)

Hardware construction of the first prototype of the ultrasound scanner has been finalized. ▶Fig. 3 (right) shows the position-

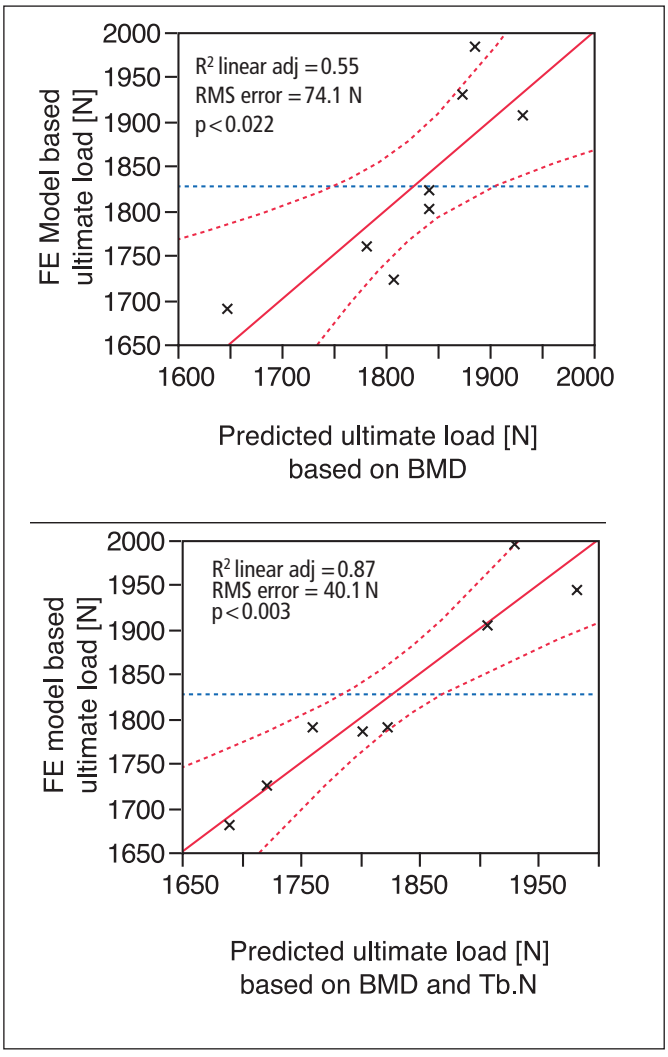

Fig. 6 Vertebral integral bone mineral density (BMD) as measured by HRpQCT was the main determinant of the finite element (FE) analysis based ultimate vertebral load. The results of linear regression including 95 % confidence intervals for the slope and the individual data are displayed (top). For ultimate load the correlation with BMD was larger compared to the correlations with microstructural parameters. However, when combining BMD and microstructure, substantial improvements in correlation with ultimate load could be achieved, as evidenced by the increase in adjusted R2 and the reduction in residual root mean square (RMS) errors by 46 % (bottom).

ing placed on the medial side of the tibia. As depicted in ▶ Fig. 3, ultrasound waves propagating along the bone (axial direction) and perpendicular to this (tangential direction) are detected by the receivers. In the first measurements on acoustically anisotropic platesaxial SOS was 3981 ± 4.0 m/s (n = 6) versus tangential SOS of 3641 ± 2.6 m/s. When the device was repositioned in an orthogonal direction, corresponding results were 3867 ± 23 m/s versus 3683 ± 2.2 m/s. Resulting elastic anisotropy ratios were 1.20 ± 0.004 for the first placement and 1.12 ± 0.013 for the orthogonal position. Initial tests on excised tibiae showed a variable pattern of noise and some cross-talk between transducer elements. In order to decrease noise levels and to reduce cross-talk a redesign of the electronics is currently under way, required for accurate measurements in vivo.

Discussion

The new methods developed in *BioAsset* have been tailored to address the limitations of DXA approaches with the goal to yield improved performance both for risk assessment and in the context of monitoring treatment effects. Many studies have documented the site-specific variability of BMD and bone strength. As a consequence local assessment of bone properties generally yield stronger performance compared to measurements at other (peripheral) sites. As a rule of thumb, BMD correlations among different measurement sites range around 0.7 (15). In other words 50 % of the site-specific variance remains unexplained by non site-matched measurements. For measurements at peripheral bones for estimation of central sites this figure is often even higher at 65–80 % (16). Regarding the assessment of vertebral bone strength the variability even among individual vertebrae has been documented in a cadaver study (16) in which thoracic (T10) and lumbar (L3) failure correlated with r = 0.66. Thus, local measurements spanning large portions of the spine appear to be required for a comprehensive assessment of vertebral fracture risk.

Clinically, most vertebral fractures occur between T7–T9 and between T12–L2 (17). Our thoracolumbar QCT protocol covers these areas and thus the range in which virtually all osteoporotic vertebral fractures occur. DXA is instead limited to the lumbar spine and standard QCT approaches do not include vertebrae above T12. In order to achieve good precision, analysis of vertebrae in the complete thoracolumbar range should be performed with minimal operator interaction. Our segmentation methods were adapted to achieve this goal. With such a large region covered radiation exposure may be a concern. With the proposed low dose approach the radiation dose can be kept at an acceptable level of about 2 mSv. This is about 50 % higher compared to current clinical 120 kVp QCT protocols assessing L1 and L2 but represents a 60 % reduction compared to a 120 kVp QCT protocol covering the range of T6 to L4 without compromising the accuracy of BMD measurements. The exposure level of 2 mSv is close the natural annual background level. Therefore, QCT examinations may have be focused on individuals at high risk of fracture, and to address problems which cannot be adequately resolved by current DXA technology, e. g. the assessment of response to treatment.

The more comprehensive assessment of BMD across the spine and its distribution within the vertebral bodies improves the input for finite element models. In the future, additional aspects including the shape and appearance of the vertebral bodies will be characterized as well (18). BMD and bone microstructure independently affect bone strength, as shown in our data. BMD

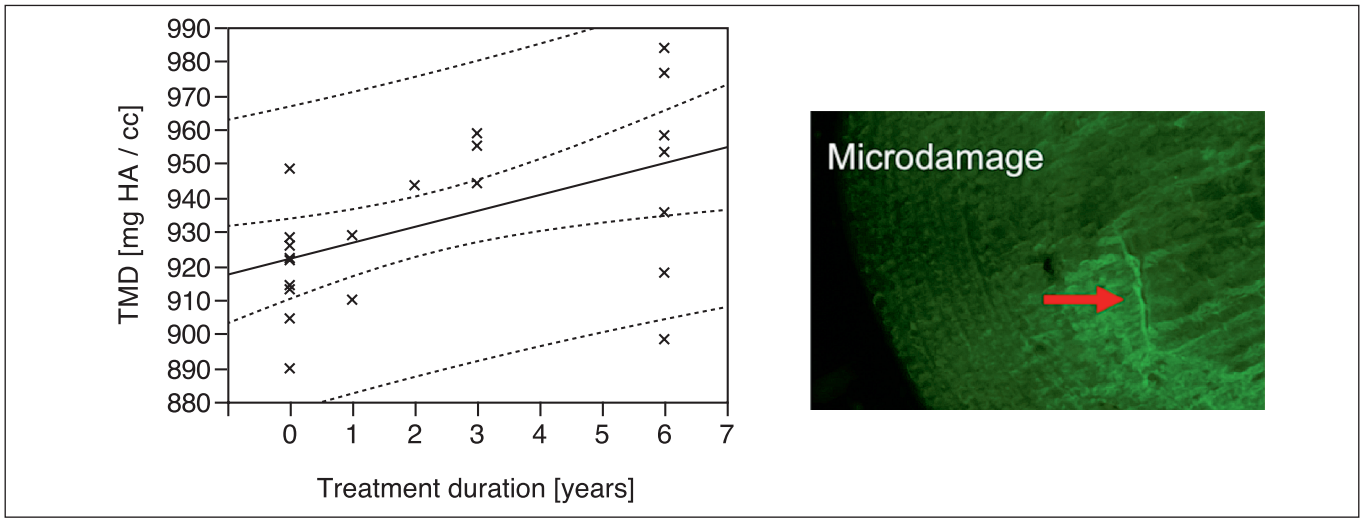

Fig. 7 (Left) Assessment of material properties confirmed the impact of prolonged bisphosphonate treatment on mineralisation. Regression analysis of tissue mineral density (i. e. mineralisation) measured with micro CT versus duration of prior bisphosphonate treatment demonstrated significant increases over time ($R^2 = 0.204$, $p = 0.008$).(Right) Isolated micro cracks (red arrow) within excised trabeculae could be identified in specimen from patients who had been on bisphosphonate treatment.

and Tb.N accounted for more than 90 % of the variability of bone strength, compared to only about 60 % for BMD alone. However, interpretation of these results needs to be carried out carefully as the strength estimates from the FE model rely solely on BMD and its distribution, i. e. microstructure. The impact of material properties has not yet been incorporated. In the context of bisphosphonate such treatment material property changes are to be expected, namely increases in mineralization (19). Indeed, we observed a significant correlation between TMD measured by micro CT and bisphosphonate treatment duration. While this result was expected, it is the first confirmation for human vertebral bone – earlier measurement had typically been obtained on iliac crest biopsies (19). Whether increases in mineralization have detrimental effects on bone strength or toughness and perhaps represent a cause for atypical subtrochanteric fractures is a subject of current debate (20). First tests on a set of excised individual trabeculae showed significant improvements in bending strength only for specimens with a history of one to five years of bisphosphonate treatment, whereas for the group on treatment for more than five years bending strength was close to the control group without bisphosphonate treatment. Long term bisphosphonate treatment may result in detrimental effects on mechanical properties (21) but to date controversy remains on the question of maximum treatment duration. Positive effects on fracture reduction are maintained up to ten years of treatment with alendronate (22) but atypical fractures may occur in a small group of patients (20). Results on beagle dogs showed increases in bone strength after bisphosphonate treatment along with paralleling decreases in bone toughness (23). Decreases in bone toughness with prolonged alendronate treatment seemed to be in part unrelated to micro crack density (24). Within our consortium we have also implemented techniques to study bone micro cracks but a systematic assessment of the *BioAsset* specimens remains to be completed.

The relevance of mineralization as a factor contributing to bone stiffness and strength is generally acknowledged. However, for bone toughness collagen properties are also relevant (25). Very high mineralization may result in brittle bone but the level at which this occurs remains unclear (26). It is even less clear how such changes could be measured in vivo, and specifically at relevant fracture sites and in the context of treatment effects. Only if a potentially negative impact of enhanced mineralization can be verified and linked to measurable bone properties, and if non-invasive methods for in vivo assessment at relevant bone locations became available, can individualized decisions about treatment discontinuation become feasible.

In the consortium we pursue two approaches to measure mineralization. With HRQCT, bone volume can be segmented from marrow volume, admittedly with substantial partial volume effects. Indeed, our data demonstrate both a highly significantly correlation of HRQCT based app. TMD with the more accurate app. TMD obtained with the HRpQCT device. Furthermore, HRQCT based app. TMD was sufficiently accurate to detect the mineralization differences between untreated subjects and subjects on bisphosphonate treatment. This was not an artefact of BMD differences between the groups since their BMD level did not differ significantly. While higher image noise levels have to be considered, it appears likely that estimates of TMD of the spine and microstructural variables can be measured with HRQCT performed in vivo. Indeed we have successfully applied HRQCT techniques in the EuroGIOPs trial comparing the treatment effects of risedronate and teriparatide. Cortical and trabecular measures contributed independently to discriminate vertebral fracture status (27), and bone surface to volume ratio was

found to discriminate antiresorptive and osteoanabolic treatment (28).

The second approach for measuring TMD and potentially other material properties of cortical bone is based on ultrasound measurements. The speed of sound of a wave propagating along the tibia is affected by elasticity, density, porosity, and the thickness of the bone (29). A new QUS-tibia scanner has been built and first performance tests have been performed successfully (14). When measuring the speed of sound in axial and tangential direction anisotropy can be differentiated and this was confirmed in the test reported here. Cortical porosity affects acoustical anisotropy because of the preferred axial orientation of the Haversian canals. Measured anisotropy ratios obtained in our tests still demonstrate variability but the sensitivity should be sufficient to pick up cortical acoustical anisotropy. At radius specimens Rudy et al. measured a range of anisotropy ratios between 1.1 and 1.8 (30). Further technical modifications have to be realised for well-defined and reproducible positioning and precise measurements at the human tibia in vivo. The tibia scanner thus will provide measures of elasticity including its anisotropy which are related to material properties and cortical ultrastructural features such as mineralization or porosity which are relevant for bone fragility. With current clinical diagnostic imaging approaches material properties cannot be measured. However, it is known that aging, bone turnover, specific diseases like diabetes, or treatments e.g. with glucocorticoids affect material properties. We assume that changes in material properties at the tibia would to some extent reflect systemic changes – the same assumption underlying the analysis of bone biopsies. Therefore, once validated, results on QUS based properties may be incorporated in the calibration equations of FE models that relate QCT voxel BMD to mechanical properties of the mesh elements, thereby providing improved estimates of bone fragility.

There are few (if any) other promising alternative approaches for the assessment of material properties in patients. The reference point indentation technique (31) yields some aspects of bone toughness, but it is invasive and measures only a small portion of the bone that may not necessarily be representative of the patient's bone status.

This is the first report documenting the cooperation of the teams of the *BioAsset* consortium beyond publications from the individual groups. There are obvious strengths but also limitations to be recognized. The set of donor material with documented history of bisphosphonate use yields unique opportunities for analyses on relevant human specimens. Some of these have been presented in this contribution. However, the yet incomplete sample size available to date is a limitation that precludes testing of more complex multivariate models. This limitation will be overcome in the near future. The analyses in the various results section have been performed on different bones from the same donors and in part in subsets. This may have introduced some variability in the results presented. Most of the new techniques have been implemented, but they need further refinement for improved assessment under in vivo conditions in humans. However, the feasibility of methods beyond DXA is obvious and warrants further development of these approaches.

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Conflict of interest

The authors declare that there is no conflict of interest.

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