Bone 49 (2011) 1055–1061

Contents lists available at SciVerse ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Original Full Length Article

Comparison of 2D and 3D bone microarchitecture evaluation at the femoral neck, among postmenopausal women with hip fracture or hip osteoarthritis ☆

Stéphanie Boutroy [a,*], Nicolas Vilayphiou [a], Jean-Paul Roux [a], Pierre D. Delmas [a], Hubert Blain [b], Roland D. Chapurlat [a], Pascale Chavassieux [a]

[a] INSERM, UMR 1033, Université de Lyon, Lyon, France
[b] Department of Internal Medicine and Geriatrics, University Hospital of Montpellier, University Montpellier 1, Montpellier, France

ARTICLE INFO

Article history:
Received 20 January 2011
Revised 30 June 2011
Accepted 25 July 2011
Available online 2 August 2011

Edited by: Rene Rizzoli

Keywords:
Femoral neck
Microarchitecture
Microcomputed tomography
Hip osteoarthritis
Hip fracture

ABSTRACT

Objectives: High resolution peripheral quantitative tomography (HR-pQCT) is used more widely to assess microarchitecture, but we are lacking comparisons between HR-pQCT and histomorphometry, which is considered the gold standard. They have only been assessed on different anatomical regions. The purpose of our study was to assess the microarchitecture and the relative contribution of cortical and trabecular bone in hip fracture with this 3D imaging technique, compared with the 2D histomorphometry.
Material and methods: We compared the distribution of cortical and trabecular bone in the ultradistal femoral neck samples (~3 mm thick) obtained after total hip replacement in 21 hip osteoarthritis (HOA, 66 ± 8 yrs) and 20 hip fracture (HF, 79 ± 8 yrs) menopausal women by a direct 3D evaluation method (HR-pQCT: XtremeCT, Scanco Medical AG) and by histomorphometry, performed and averaged on three 10 μm-thick sections 800 μm apart.
Results: Significant correlations were found between both techniques for trabecular bone volume, number, thickness, separation and cortical thickness ($0.51<r'<0.81$, $p<0.01$). The connectivity was also significantly correlated ($r'=0.58$, $p<0.001$) between both techniques, as well as the trabecular bone pattern factor measured in 2D with the structural model index (SMI) measured in 3D ($r'=0.62$, $p<0.001$). However HR-pQCT overestimated the absolute value of most parameters, with higher values being even more overestimated. The agreement between the two techniques was weak for cortical porosity.
With the 3D measurements we found that trabecular bone volume was 43% lower in HF than HOA ($p<0.01$), associated with loss of trabecular connectivity (−50%, $p<0.01$) and a more rod-like structure (SMI, 22%, $p<0.01$), mainly at the inferior (34%, $p<0.01$) and posterior (22%, $p<0.05$) quadrants. Cortical thickness was found to be lower in the posterior quadrants (−22%, $p<0.05$) and tended to be lower in HF than in HOA at the inferior quadrant (−14%, $p=0.08$), but it was still the highest at the inferior quadrant in both groups.
In conclusion, 3D methods confirmed the alteration of trabecular and cortical bone found by histomorphometry in HF compared with HOA and the frequency of the rod-like structure in HF.

Introduction

The evaluation of bone microarchitecture has gained increased attention in the past years, with the recognition that the usual areal bone mineral density (aBMD) measurements failed to identify most fragility fractures [1–3]. Indeed, several limitations are associated with aBMD measurements: aBMD values are influenced by bone size as DXA does not measure true volumetric BMD; DXA cannot distinguish cortical and trabecular bone compartments and moreover DXA cannot directly measure cortical and trabecular architecture. However structural changes are important determinants of bone strength and have been associated with hip fracture independently of aBMD [4].

Bone histomorphometry allows for assessment of bone microarchitecture and remains the gold standard for this purpose. It also permits a dynamic evaluation by estimating the level of bone remodeling. However, histomorphometry has inherent limitations relative to its 2D design. Measurements are based on the assumption of a plate-like structure of bone and they are limited to a few slices thus representing a very small fraction of the biopsy. Additionally, histomorphometry precludes the assessment of non-metric parameters able to provide insight into bone quality, such as the structure model index.

By conventional 2D histomorphometric analysis of femoral neck biopsies, we have previously shown that in addition to cortical

☆ This work was supported in part by unrestricted grants from Eli Lilly and by the French Ministry of Health (Projet Hospitalier de Recherche Clinique Régional, Languedoc-Roussillon, 2003, UF-7755).
* Corresponding author at: INSERM UMR 1033, Hôpital Edouard Herriot, Pavillon F, 69437 Lyon cedex 03, France. Fax: +33 4 72 11 74 32.
E-mail addresses: stephanie.boutroy@inserm.fr (S. Boutroy), nicolas.vilayphiou@inserm.fr (N. Vilayphiou), jean-paul.roux@univ-lyon1.fr (J.P. Roux), h-blain@chu-montpellier.fr (H. Blain), roland.chapurlat@inserm.fr (R.D. Chapurlat), pascale.chavassieux@univ-lyon1.fr (P. Chavassieux).

8756-3282/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bone.2011.07.037

thinning, loss of trabecular bone mass and connectivity may distinguish women with hip fracture (HF) from women with hip osteoarthritis (HOA) [5]. To overcome the 2D histomorphometric limitations, those femoral neck samples have been scanned with a 3D device. Three-dimensional analysis of the femoral neck structure by high-resolution peripheral quantitative computed tomography will assess the microarchitecture and the relative contribution of cortical and trabecular bone in women with hip fracture when compared to women with hip HOA.

Material and methods

Subjects

The subjects and bone biopsies procedures were described in detail in our earlier study, in which cortical and trabecular bone distribution at the femoral neck in fractured and hip osteoarthritis women was assessed by histomorphometry [5]. Briefly, femoral neck biopsies were obtained during arthroplasty for non-traumatic hip fracture (HF), i.e. fall from standing height or less, (HF; $n=20$; aged 79.3 ± 8.0 years old) or hip osteoarthritis (HOA; $n=21$; aged 65.9 ± 7.9 years old) in postmenopausal women. For both HOA and HF patients, femoral neck biopsies were taken at the ultradistal part of the femoral neck, beginning at the cut face prepared for the insertion of the prosthesis at the base of the neck, thus leading to a bone slice of 10 mm thick approximately. Among the 20 HF, there were 19 cervical fractures and 1 trochanteric fracture. Undecalcified femoral neck samples were fixed in 70% ethanol and embedded in methylmethacrylate [6]. The protocol was approved by an independent Ethics Committee, and all patients gave written informed consent before participation.

Measurement of 3D bone microarchitecture

Three-dimensional bone microarchitecture of embedded femoral neck biopsies was measured using a high-resolution peripheral quantitative computed tomography system (XtremeCT, Scanco Medical AG®, Brüttisellen, Switzerland), with a nominal isotropic voxel size of 82 μm, using the standard in vivo scanning protocol (60 kVp, 900 μA) [7]. According to the bone microarchitecture regional differences in femoral neck [5,8], each section was divided into four quadrants (superior, anterior, inferior and posterior). CT slices were visually matched with histomorphometric analysis to have consistent regions of interest (Fig. 1), leading to mean ROI of 33 ± 6 CT slices (2.7 ± 0.5 mm). Trabecular and cortical parameters were first assessed in their respective whole compartment and then on the 4 quadrants separately.

Image processing included a Gaussian filter (support = 1, $\sigma=0.8$). Cortical bone was segmented manually from trabecular bone on a slice-by-slice basis and both volumes were thresholded separately to delineate bone from non-bone voxels (Fig. 1) [9]. As shown in Fig. 1, the global threshold used for trabecular bone segmentation (337 mg.HA/cm^3) tended to slightly overestimate the trabecular structure in order to keep an intact connectivity with the relatively low resolution in regard of the structure measured, whereas the global threshold for cortical bone segmentation (511 mg.HA/cm^3) was chosen to differentiate the cortical porosity [10].

To avoid potential errors in trabecular spacing measurement due to the relatively small ROI, mirror images of the sample were added at the 2 extremities of the sample, otherwise trabecular spacing may have been underestimated [11].

The trabecular bone volume ratio (BV/TV) was determined by dividing the number of voxels representing trabecular bone by the total number of voxels in the trabecular compartment. Methods used to process the trabecular microarchitectural data were based on direct measurements of structure. Trabecular number (Tb.N*, 1/mm), thickness (Tb.Th*, μm) and separation (Tb.Sp*, μm) measurements were based on the distance transformation method, where maximal spheres are filled into the segmented object [12], thus not relying on an assumed model (rod/plate) type. Non-metric indices for trabecular bone structure as SMI [13] and Conn.D [14] were also calculated from the segmented images. Mean cortical thickness (Ct.Th, μm) was calculated using an annular method [15] where mean cortical volume is divided by the periosteal surface area, thus being irrespective of any pores. Cortical porosity was calculated as the complement of the cortical bone volume ratio ($1-(BV_{cortical}/TV_{cortical})$) in the compact-appearing cortex (Ct.Po, %).

Two HF biopsies were excluded because the material was insufficient to be measured in 3D, with a total of 21 HOA and 18 HF as a result. Moreover, in most HF biopsies, the cortex from the superior quadrant was impaired or missing. Thus, cortical thickness and porosity were measured in only 3 samples at this site.

Histomorphometry

Serial 10 μm-thick cross-sectional slices were cut at three different levels of the bone sample, 800 μm apart, and were stained with Goldner trichrome [16]. Measurements were done separately on the four quadrants, the mean value of each sample being the average of the 4 quadrants among the 3 analyzed slices. The trabecular ROI corresponded to a rectangular area of maximum 31 mm², centered in each quadrant and cortical measurements were evaluated in the area corresponding to the trabecular ROI, as shown in Fig. 1. Parameters of bone structure and micro-architecture were performed using Bone and MorphoExpert softwares (Explora Nova, La Rochelle, France) and standard abbreviations for bone histomorphometry were used [17]. Outcome parameters for trabecular bone were volume (BV/TV,%), thickness (Tb.Th; μm), separation (Tb.Sp; μm), number (Tb.N; 1/mm) based on Parfitt's formulae, i.e. derived from volume and surfaces measurements [18]. After skeletonization, we evaluated the number of nodes (N.Nd/TV, 1/mm²) and the trabecular bone pattern factor (TBPf; 1/mm), both reflecting the connectivity/topology of the network. Outcome parameters for cortical bone were thickness (Ct.Th; μm) and porosity (Ct.Po, %) representing the cortical area occupied by Haversian canals.

Statistical analysis

Descriptive statistics were summarized by means and standard deviations. Regarding the distribution of the variables, non-parametric

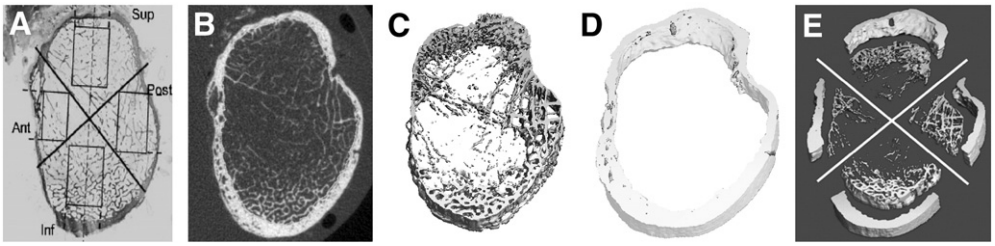

Fig. 1. Cross-section of a femoral neck. A — Histomorphometric slice showing the four quadrants and the regions of interest for trabecular and cortical bone (rectangle and its projection on the cortex respectively) B — HR-pQCT gray-level slice of the same sample C–E — 3D reconstruction of the whole trabecular (C) and cortical (porosity shown in gray) (D) bone and of each quadrants (E).