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Identical subchondral bone microarchitecture pattern with increased bone resorption in rheumatoid arthritis as compared to osteoarthritis

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SUMMARY

Objectives: To analyze the differences in microarchitecture and bone remodeling of subchondral bone in femoral heads from patients with rheumatoid arthritis (RA) and osteoarthritis (OA).
Designs: Peri-articular bone samples, including subchondral trabecular bone (STB) and deeper trabecular bone (DTB) were extracted from the load-bearing region of femoral heads from 20 patients with RA and 40 patients with OA during hip replacement surgery. Micro-CT, histomorphometry and backscatter scanning electron microscopy (BSEM) were performed to assess microarchitecture and bone histology parameters.
Results: In both RA and OA, STB showed more sclerotic microarchitecture and more active bone remodeling, compared to DTB. RA and OA showed similar microarchitecture characteristics in both STB and DTB, despite STB in RA exhibiting higher bone resorption. In addition, there was no difference in the frequency of bone cysts in STB between RA and OA. In STB, the trabecular bone surrounding subchondral bone cysts (Cys-Tb) was more sclerotic than the trabecular bone found distant to cysts (Peri-Tb), with a higher level of bone remodeling. Both Cys-Tb region and Peri-Tb region were detected to have similar microarchitectural and bone remodeling characteristics in RA and OA.
Conclusions: Apart from higher bone resorption in the general subchondral bone of RA samples, the peri-articular bone exhibited similar microarchitectural and bone remodeling characteristics in RA and OA.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by persistent synovitis and joint destruction[1]. The synovial membrane, which traditionally stands at the epicenter of the joint pathology of RA, has been shown to be responsible for cartilage damage and bone destruction[2]. A pathological hallmark of RA is marginal juxta-articular bone erosion, which is located in the cartilage-pannus junction. Synovitis-induced inflammatory infiltrates, detected in the trabecular bone close to cartilage-pannus junction, are widely considered to contribute to juxta-articular bone erosion[3,4]. The inflammatory infiltrates are thought to be associated with elevated osteochondral angiogenesis, osteoclast aggregates, and excessive bone resorption, thereby resulting in

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severe bone destruction and localized erosions in the marginal juxta-articular bone[3,4].

Unlike the marginal juxta-articular bone which has a direct contact with synovial membrane, the subchondral bone, which lies distant from the cartilage-pannus junction/synovium, is an often neglected anatomic compartment in the pathogenesis of RA. Recent studies, however, indicate that the subchondral bone may in fact act as an essential independent element in the development of RA[5,6]. Although subchondral bone marrow inflammatory infiltration is well-documented in cases of human and animal RA[4,7–10], subchondral bone is less likely to be influenced by synovitis and thus may not display localized bone erosions like those observed in the marginal juxta-articular bone[11,12].

Osteoarthritis (OA) is a progressive degenerative joint disorder characterized by cartilage damage and subchondral bone changes. Subchondral bone has been reported to play a crucial role in the initiation and progression of OA[13]. Subchondral bone deterioration is commonly associated with articular cartilage defects[14]. Several studies have shown that subchondral bone is sclerotic in late-stage OA, owing largely to abnormal bone remodeling[14]. Sclerotic bone, showing increased opacity on a plain radiograph, is normally detected by micro-CT as an area of high bone volume fraction[15]. Apart from sclerotic changes and high bone remodeling, some histopathological changes in the subchondral bone have also been detected, including microdamages, bone marrow edema-like lesions and bone cysts[16–18].

Subchondral bone cysts that are not related to the infiltration of synovial tissue, have been commonly demonstrated in both RA[6] and OA[19]. The cysts appear as well-defined regions of fluid signal on magnetic resonance imaging (MRI), which correspond to the radiolucent areas in radiographic manifestation[20]. Without epithelial linings observed on the margin, the cysts are normally composed of fibroconnective tissue that may initially contain fluid but ossify in later stages[16,21,22].

Despite distinct aetiologies in OA and RA, the differences of subchondral bone between these two diseases are poorly investigated. We hypothesized that the pathological changes of subchondral bone in joints with RA should be comparable to that of OA, as it is located in the load-bearing region which is distant from synovial membrane and endues little synovial inflammation influence. To address this, we assessed microarchitecture and bone remodeling activity of peri-articular bone, including subchondral trabecular bone (STB) and deeper trabecular bone (DTB), in the load-bearing region of femoral heads from patients with RA and OA using micro-CT, histomorphometry and backscatter scanning electron microscopy (BSEM).

Materials and methods

Study subjects

20 patients who underwent hip replacement for RA were recruited in the study (14 females and 6 males, mean age 69.60 ± 8.31 years, range 54–88 years). All the patients fulfilled the American College of Rheumatology 1987 revised classification criteria for RA[23]. Patients with other known metabolic or bone disorders affecting bone metabolism, were excluded. During the course of their disease, all the patients were treated with disease-modifying anti-rheumatic drugs (DMARDs), including hydroxychloroquine, methotrexate, sulfasalazine, minocycline and leflunomide. All RA patients exhibited radiographic erosive changes in hip joints (Grade $\geq$ 2), according to the Larsen score[24].

40 age- and gender-matched patients who underwent hip replacement for OA were also recruited (28 females and 12 males, mean age 69.75 ± 8.43 years, range 53–92 years). RA patients did not differ significantly from OA patients in age ($P = 0.948$) and male/female ratio ($P = 1.000$). All female patients were more than 5 years postmenopausal at the time of recruitment for study. Exclusion criteria for OA patients were as follows: (1) known metabolic or bone disorder other than OA, which could affect bone metabolism, such as severe renal impairment, thyroid or parathyroid disease, and malignancy; (2) receiving treatment that affects bone metabolism such as anti-resorptive drugs, calcitonin, thyroid or parathyroid hormone therapy, or hormonal replacement therapy; or (3) history of hip osteotomy. Informed consent was obtained from each patient. All OA patients had radiographic evidence of moderate or severe OA (Grade $\geq$ 3), according to the Kellgren and Lawrence criteria[25].

The study protocol was approved by the Human Research Ethics committee of The University of Western Australia and complied with the Declaration of Helsinki.

Specimen preparation

A cylindrical specimen of peri-articular trabecular bone was removed from the load-bearing region of each femoral head obtained from joint replacement[26], with the cylinder axis aligned with the superior–inferior main trabecular direction [Fig. 1(A)]. Each cylindrical bone sample (15 mm in height and 9 mm in diameter) were prepared under continuous water irrigation using a precision bone trephine. All samples comprised the STB and DTB [Fig. 1(B)]. STB is defined as the most superficial 5 mm of the trabecular cylinder immediately under the cartilage and subchondral cortical bone[27,28], while DTB is defined as the deepest 5 mm of the trabecular cylinder. Specimens were fixed in 4% paraformaldehyde in PBS for 5 days and stored in 70% ethanol.

Micro-CT examination

Each bone sample was placed in a saline-filled acrylic case for acquisition by a micro-CT scanner (Skyscan 1174, Skyscan, Kontich, Belgium). Imaging acquisition was conducted at a voltage of 50 kV, current of 800 μA, an isotropic pixel size of 14.4 μm (1024 × 1024 pixel image matrix), and with a 0.75-mm-thick aluminum filter for beam hardening reduction. After scanning and reconstruction, the images were transferred with a fixed threshold to binary images (Fig. 2). Bone microarchitecture parameters were then measured, using the built-in software. In both STB and DTB, the diameter of the measurement region was chosen 1 mm smaller than the diameter of the sample, in order to avoid the inclusion of bone debris due to the cutting procedure. Bone cysts were also screened. There were three regions of interest (ROI) in the trabecular bone from each measurement region, which were extracted in a semi-automatic method: whole trabecular bone (W-Tb), trabecular bone immediately surrounding the bone cyst (Cys-Tb), and peripheral trabecular bone (Peri-Tb). Cys-Tb is the region 0.5 mm from the surface of the cyst to the trabecular bone. Peri-Tb is the region obtained by subtracting the Cys-Tb region from the W-Tb region.

The following microarchitectural parameters were calculated: bone volume fraction (BV/TV) (%), trabecular thickness (Tb.Th) (μm), trabecular separation (Tb.Sp) (μm), trabecular number (Tb.N) (1/mm), structure model index (SMI), degree of anisotropy (DA), and connectivity density (Conn.D) ($1/mm^3$)[29]. The X-ray attenuation coefficient values were converted into bone mineral density (BMD) (mg/cm^3) (Fig. 2), using a calibration curve obtained from the BMD phantom.

Histological process and histomorphometry

Each sample was fixed, infiltrated and embedded in methylmethacrylate. All bone blocks were trimmed and sectioned on a microtome (Leica RM 2255, Wetzlar, Germany). Sections, 5 μm