Osteoarthritis and Cartilage 24 (2016) 1745–1752

Osteoarthritis and Cartilage

Bone marrow lesions in hip osteoarthritis are characterized by increased bone turnover and enhanced angiogenesis

M. Shabestari † *, J. Vik ‡, J.E. Reseland †, E.F. Eriksen §

† Department. of Biomaterials, University of Oslo, Oslo, Norway
‡ Department. of Orthopedic Surgery, Martina Hansen Hospital, Bærum, Akershus, Norway
§ Department. of Endocrinology, Oslo University Hospital (Aker), Oslo, Norway

ARTICLE INFO

Article history:
Received 24 December 2015
Accepted 9 May 2016

Keywords:
Bone marrow lesion
Angiogenesis
Bone turnover
Osteoarthritis

SUMMARY

Objective: Bone marrow lesions (BML), previously denoted bone marrow edema, are detected as water signals by magnetic resonance imaging (MRI). Previous histologic studies were unable to demonstrate any edematous changes at the tissue level. Therefore, our aim was to investigate the underlying biological mechanisms of the water signal in MRI scans of bone affected by BML.
Methods: Tetracycline labeling in addition to water sensitive MRI scans of 30 patients planned for total hip replacement surgery was undertaken. Twenty-one femoral heads revealed BML on MRI, while nine were negative and used as controls (CON). Guided by the MRI images cylindrical biopsies were extracted from areas with BML in the femoral heads. Tissue sections from the biopsies were subjected to histomorphometric image analyses of the cancellous bone envelope.
Results: Patients with BML exhibited an average 40- and 18-fold increase of bone formation rate and mineralizing surface, respectively. Additionally, samples with BML demonstrated 2-fold reduction of marrow fat and 28-fold increase of woven bone. Immunohistochemical analysis showed a 4-fold increase of angiogenesis markers CD31 and von Willebrand Factor (vWF) in the BML-group compared to CON.
Conclusion: This study indicates that BML are characterized by increased bone turnover, vascularity and angiogenesis in keeping with it being a reparatory process. Thus, the water signal, which is the hallmark of BML on MRI, is most probably reflecting increased tissue vascularity accompanying increased remodeling activity.

Introduction

We use the term “bone marrow lesions” (BML) as the collective term for what was previously denoted bone marrow edema[1]. BML are detected as water signals on magnetic resonance imaging (MRI) scans, low intensity on T1-weighted images and high intensity on T2-weighted scans[2]. These pathological MRI signals involving bone marrow and bone tissue have been demonstrated in a multitude of different musculoskeletal conditions[3]. For instance, the presence of BML is considered a determinant of pain and progression in osteoarthritis (OA)[4–6]. Despite its recognized clinical relevance in rheumatologic conditions, few studies have examined local histopathological characteristics of the BML microenvironment. To the best of our knowledge, no studies to date have investigated neither bone remodeling nor angiogenesis in BML using tetracycline double labeling and quantitative immunohistochemistry of human bone respectively.

All studies on the histopathology of BML so far have been qualitative descriptions. Previous findings included inflammatory cells, fibrosis, necrosis, swollen or disintegrating fat cells and myxomatous degeneration[7–9]. The absence of histological observation of a typical edema or other findings, which could explain the water signal, led to the hypothesis that water signal in MRI scans is a result of increased vascularization followed by a high turnover state in the affected bone. The purpose of this study was therefore to characterize quantitatively bone remodeling and angiogenesis in human bone samples affected by BML using dynamic histomorphometry and immunohistochemistry.

* Address correspondence and reprint requests to: M. Shabestari, Dept. of Biomaterials, Institute of Clinical Dentistry, University of Oslo, P.O. Box 1109 Blindern 0317, Oslo, Norway. Tel: 47-94374794.
E-mail address: maziarg@medisin.uio.no (M. Shabestari).

http://dx.doi.org/10.1016/j.joca.2016.05.009
1063-4584/

Methods and materials

Study population

Thirty patients with end-stage primary hip OA, who were scheduled for hip replacement surgery at Martina Hansen Hospital (Oslo, Norway) were recruited. The study population had a mean age of 64.2 ± 9.9 yr and met American College of Rheumatology criteria for OA[10].

Inclusion criteria for surgery were pronounced clinical symptoms of OA, including pain, stiffness, and dysfunction, and demonstration of typical signs of OA on radiographs, i.e., osteophytes, subchondral sclerosis, cysts and joint space narrowing. Subjects with inflammatory arthritis or any previous hip surgeries and infections, as well as OA that was secondary to other conditions (acute or chronic infection, metabolic abnormalities), malignant diseases or patients with standard MRI contra-indications were excluded. Among the patients recruited nine femoral heads were found to be without BML and these were considered as CON (62.2 ± 12 yr).

Patients were subjected to a double labeling regimen with tetracycline (250 mg q.i.d., Arco, Geneva, Switzerland) taken orally during two consecutive days. Dosing was repeated after 2 weeks. Subjects were instructed to take each dose 1–2 h before a meal or 2 h after a meal to avoid reduced absorption. The bone specimens were collected 4 or 5 days after the last dosage.

The recommended OARSI OA score method[11] was used to characterize cartilage loss on Goldner's trichrome stained sections by assessing the articular cartilage and the subjacent subchondral bone. Four stages were defined depending on the horizontal extent of the involved cartilage surface irrespective of underlying OA grade. The stages 1–4 represented <10%, 10–25%, 25–50%, and >50% involvement respectively. OA severity is defined as OA depth progression into cartilage by grades 1–6. The OA score was calculated by multiplying stage and grade values (Fig. 2).

The study was approved by the Regional Committees for Medical and Health Research Ethics in South-East Norway (2011/1089/REK). All patients were given oral and written information, and signed informed consent was obtained from each of the participant patients, in accordance with the Declaration of Helsinki.

MRI protocol

Patients' hips were scanned bilaterally using an Excelart Vantage Atlas-Z, 1,5T scanner (Toshiba, Tokyo, Japan). The MRI protocol included four sequences that were performed 1 day preoperatively: Coronal and axial Short tau inversion recovery (STIR) fast spin-echo (TR/TE time 4078/48 ms, field of view = 360 mm, slice thickness = 3.5 mm) for identifying BML. Coronal and axial T_1-weighted fast spin-echo (TR/TE time 695/15 ms, field of view = 360 mm, slice thickness = 3.5 mm). Sequential images were evaluated for presence, size and location of BML by one of the investigators (EFE) with extensive previous experience in scoring MRI scans. Images from each of the coronal and axial planes were processed in Adobe Photoshop (Adobe Systems, CA, USA) to define the regions with BML.

Processing of tissue

Guided by the sequential MRI images two cylindrical bone cores, 7 mm in diameter and approx. 12 mm long were taken from the femoral heads using a trephine bur (229L, Meisinger, Neuss, Germany). Samples were immediately transferred to 70% ethanol prior to embedding in methyl methacrylate at −20°C[12]. Subsequently, 7 μm thin sections perpendicular to the base of the cylindrical bone cores were prepared from each specimen: one unstained pair of sections for measurement of tetracycline-labels and one pair of Goldner's trichrome stained sections for calculation of bone histomorphometric indices and the OARSI OA score[13]. Sections were selected from regions of the bone core at least 200 μm apart. A further pair of sections was used for immunohistochemical staining of each of the markers.

Histomorphometric image analysis

Stained and unstained slides were scanned using standardized parameters by a BX50 microscope (Olympus, Tokyo, Japan), motorized specimen stage (MS-2000-XYZ, ASI Inc., OR, USA) and a microscope camera (Exi Aqua, QImaging, BC, Canada). For fluorescence microscopy a wide band emission filter (Olympus) was used with an X-cite illuminator (Excelitas Technologies Corp., MA, USA). Image analysis consisted of a threshold-based and a grid-based method. Both methods were performed based on standardized protocols, random sampling and always blinded to BML or CON status of the samples.

For conventional bone histomorphometric indices and woven bone, a minimum of 30 mm^2 was sampled at randomly selected regions of interest (ROI) on a pair of sections from each bone core[14]. Unstained sections were used for measurement of dynamic histomorphometric data and for estimation of vascularity. Stained sections were used for measurement of basic bone referent data, quantification of woven bone (polarized light) and marrow fat. Data were collected from analysis of the cancellous bone envelope at least 1 mm away from the subchondral bone tidemark using specialized histomorphometric software, BioQuant 2015 (Image Analysis Corporation, TN, USA). Measurement of all parameters were calibrated against the principal investigator (EFE) and reproduced with CV = 6%. Histomorphometric indices were used according to ASBMR nomenclature standards[14].

Immunohistochemistry

Sections from all the patients in the CON group and sections from 10 random samples from the BML group were deplastified in 2-methoxyethyl acetate and washed in ethanol. Epitopes were retrieved using antigen retrieval solutions based on the manufacturer's (Dako, CA, USA) recommendations for each primary antibody. For the rest of the immunostaining procedure CSA II Kit (Dako) was used according to the manufacturer's instructions.

We used monoclonal mouse anti-human IgG antibodies against von Willebrand Factor (vWF, Clone F8/86, Dako) and CD31 (Clone JC70A, Dako) as primary antibodies in the setup. Sections were incubated overnight at 4°C with the primary antibodies diluted in a background reducing solution (1:25) (Dako), washed three times in TBS between each step before signal detection with diaminobenzidine (Dako) and counter-staining with Mayer's hematoxylin (VWR, PA, USA). Mouse IgG1 antibody against *Aspergillus niger* glucose oxidase (X0931, Dako) was used on both BML and CON sections as negative control. For positive controls, sections of human lung and tonsil tissue were used.

Estimations of vascularity and angiogenesis

We estimated vascularity and angiogenesis using two independent methods:

Estimated vascular volume (eV.v)

eV.v was based on the autofluorescence of the tissue [Fig. 1(A)]. Digital grids with 100 μm intervals and 3200 grid points were superimposed on random ROI which in total corresponded to a