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Case Report

Altered matrix mineralization in a case of a sclerosing osteosarcoma ☆

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ABSTRACT

Little is known about the tumor matrix mineralization of highly sclerotic osteosarcoma. We used quantitative backscattered electron imaging (qBEI) to determine the Bone mineralization density distribution (BMDD) of a highly sclerosing osteosarcoma of the proximal tibia as well as adjacent normal bone of a 10-year-old girl following chemotherapy according to the EURAMOS-1 protocol. Data were compared to recently published normative reference data for young individuals.

Backscattered electron imaging of the tumor region revealed a dense accumulation of mineralized tumor bone matrix (up to 90% of the medullar space). The BMDD was shifted tremendously towards higher matrix mineralization (CaMean +18.5%, CaPeak +22.5%, CaHigh +100 fold) compared to normal bone. Additionally the BMDD became much wider, indicating a higher heterogeneity in mineralization (CaWidth +40%). In contrast to lamellar bone, which mineralizes via a mineralization front, the mineralization of the tumor matrix starts by randomly distributed spots of mineral clusters fusing together to a highly mineralized non-lamellar bone matrix. We also found an altered BMDD of the patient's normal bone when compared with the reference BMDD of young individuals.

In conclusion this high radiodensity region of the sclerosing sarcoma is not only due to the high amount of tumor matrix but also to its high mineralization density. Chemotherapy may lead to altered matrix mineralization of normal bone due to suppression of bone turnover. The mechanism of matrix mineralization in a sclerosing osteosarcoma warrants further studies.

Introduction

Osteosarcoma is the most common malignant primary bone tumor in childhood and adolescence [1]. Based on their radiological appearance, they can be divided into three broad categories: sclerotic, osteolytic and mixed pattern [2]. The radiology of a pure sclerosing variant of osteosarcoma is lesions of homogeneous masses of 'fluffy' or 'cumulus cloud like' densities [3]. A sclerotic region on an X-ray is defined as a bone area with an abnormal high radiodensity. Changes in radiodensity on an X-ray can be due to changes in (i) bone volume, (ii) bone matrix mineralization or (iii) combinations of both. Formation of the organic bone matrix and its mineralization are based on well-coordinated function of osteoblasts and preosteocytes. Although osteosarcoma cells produce tumor osteoid and tumor bone, it is likely that the matrix produced by the tumor cells is different in structure and mineralization. Moreover, chemotherapy for the treatment of osteosarcoma suppresses normal bone turnover [4] and leads to low bone mass [5]. However no data exist if matrix mineralization is altered (i) in a sclerosing osteosarcoma and (ii) in normal bone post chemotherapy.

Bone matrix mineralization can be measured by quantitative backscattered electron imaging (qBEI) [6]. This method has been successfully applied to characterize alterations in bone mineralization density distribution (BMDD) at the microscopic level in genetic and metabolic bone diseases as well as in patients treated by drugs or in experimental animal models (for review see [6]).

Using qBEI the present case study investigates for the first time the tumor bone matrix mineralization formed in a patient with a highly sclerosing osteosarcoma as well as adjacent normal bone post-chemotherapy. The BMDD outcomes of tumor bone tissue will be compared against normal adjacent bone tissue of the same patient as well as recently published normative reference data for young individuals [7].

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Material and methods

Patient

A 10-year-old Caucasian girl from Hungary presented at the Department of Orthopaedics, Medical University of Vienna, Austria with a tumor in her right proximal tibia and two skip lesions in the ipsilateral distal femur. The tumor had been biopsied previously and was classified as a highly malign osteoblastic osteosarcoma. She subsequently received preoperative chemotherapy according to the EURAMOS-1 protocol [8]. She underwent wide resection of the tumor and implantation of a GMRS total knee tumor prosthesis (Stryker Orthopaedics-Reconstructive, Joint Preservation, & Orthobiologics; Mahwah, New Jersey 07430, USA) at our institution. Histological evaluation of the resected tumor showed >30% viable tumor cells, and the osteosarcoma of the patient was therefore classified as a Grade IV regression according to the Salzer-Kuntschik grading [9].

Informed consent was obtained from the parents regarding the use of the harvested bone samples for this study. The study was approved by the ethics committee at the Medical University of Vienna, Austria and was done in accordance with the Helsinki Declaration.

Quantitative backscattered electron imaging (qBEI)

Undecalcified bone specimen from the tumor as well as from adjacent normal tissue containing cortical and trabecular bone was fixed in 70% ethanol, dehydrated through a graded series of ethanol and embedded undecalcified in polymethylmethacrylate (PMMA) and prepared for qBEI as described earlier [6,10]. About 10 mm thick blocks containing cortical and trabecular as well as tumor bone section were obtained using a low speed diamond saw (Buehler Isomet, Lake Pluff, USA). Section surfaces were prepared for qBEI as previously described [6]. A digital scanning electron microscope (DSM 962, Zeiss, Oberkochen, Germany) equipped with a four-quadrant semiconductor backscattered electron detector was used. qBEI is based on the phenomenon that the intensity of electrons backscattered from a sectioned bone area is proportional to the average atomic number of the scanned region. As a consequence qBEI offers the ability to precisely measure the weight concentration of calcium in bone. The acquired gray-level histograms from the backscattered electron images are used to determine the bone mineralization density distribution (BMDD) describing the frequency of appearance of a certain Ca-content in the investigated bone area in units of weight % calcium. Details of the method have been published elsewhere (for review see [6]). Five parameters were determined to characterize the BMDD curve: CaMean, the weighted mean calcium concentration of the bone area obtained by the integrated area under the BMDD curve; CaPeak, the peak position of the histogram indicating the most frequently occurring calcium concentration; CaWidth, the full width at half maximum of the distribution describing the variation in mineralization density; CaLow, the percentage of bone area that is mineralized below the 5th percentile of the reference BMDD of normal adults [10] that is below 17.68 wt.% Ca corresponding to levels of primary mineralization; and CaHigh, the percentage of bone area that is mineralized above the 95th percentile of the reference BMDD of normal adults [10] that is above 25.30 wt.% Ca corresponding to levels higher than achieved by normal secondary mineralization.

Statistical analysis

To quantify the deviation of the patient's BMDD variable values from that of the normative reference data base of young individuals [7] standard deviation (SD)-scores were used, when the variable of the reference was normally distributed or percentiles, when the variable was not normally distributed.

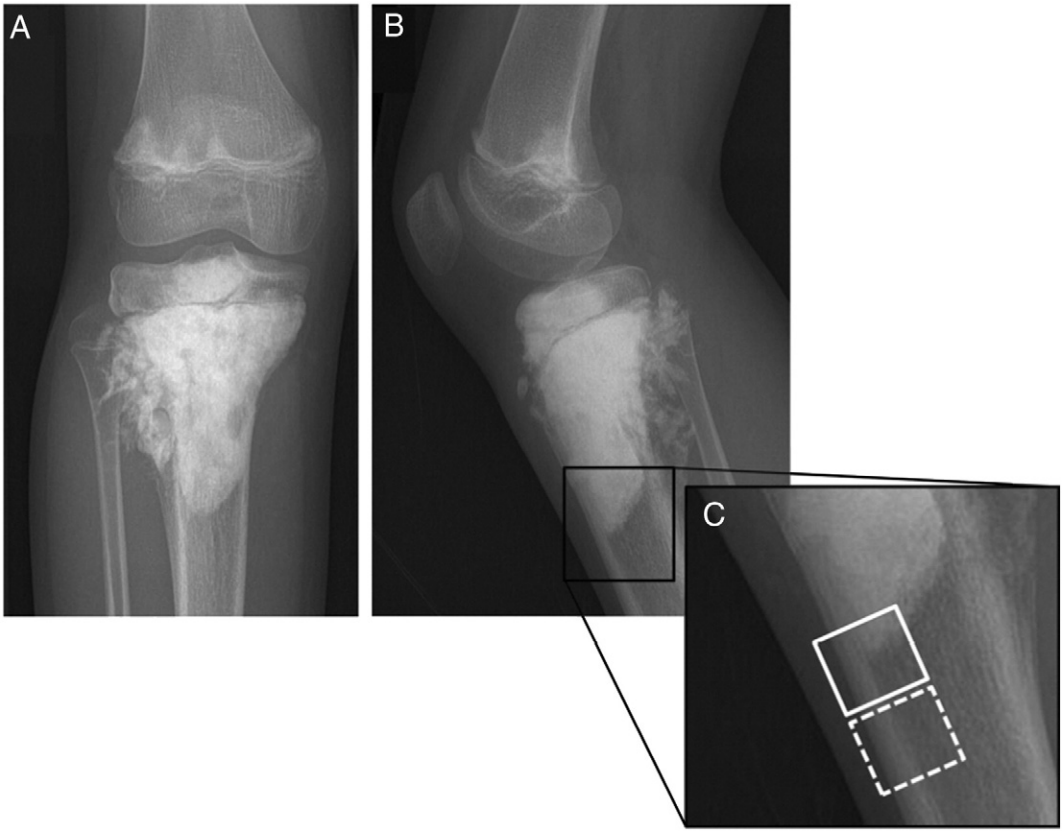

Fig. 1. Radiographs (A, B, C) of the patient's knee joint affected by a sclerosing variant of osteosarcoma. Boxes indicate the regions examined by backscattered electron imaging (see Fig. 2).