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Diffusion-weighted imaging and dynamic contrast-enhanced MRI of experimental breast cancer bone metastases – A correlation study with histology

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ABSTRACT

Purpose: To validate imaging parameters from diffusion-weighted imaging and dynamic contrast-enhanced MRI with immunohistology and to non-invasively assess microstructure of experimental breast cancer bone metastases.
Materials and methods: Animals bearing breast cancer bone metastases were imaged in a clinical 1.5 T MRI scanner. HASTE sequences were performed to calculate apparent diffusion coefficients. Saturation recovery turbo FLASH sequences were conducted while infusing 0.1 mmol/l Gd–DTPA for dynamic contrast-enhanced MRI to quantify parameters amplitude A and exchange rate constant k_{ep}. After imaging, bone metastases were analyzed immunohistologically.
Results: We found correlations of the apparent diffusion coefficients from diffusion-weighted imaging with tumor cellularity as assessed with cell nuclei staining. Histological vessel maturity was correlated negatively with parameters A and k_{ep} from dynamic contrast-enhanced MRI. Tumor size correlated inversely with cell density and vessel permeability as well as positively with mean vessel calibers. Parameters from the rim of bone metastases differed significantly from values of the center.
Conclusion: In vivo diffusion-weighted imaging and dynamic contrast-enhanced MRI in experimental bone metastases provide information about tumor cellularity and vascularity and correlate well with immunohistology.

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1. Introduction

Skeletal complications are common in advanced solid tumors and hematologic malignancies like multiple myeloma. Once the primary tumor has spread to bone, the disease is mostly considered incurable [1]. With the ongoing progress of radiation–chemotherapy and the evolving role of bone targeting agents, the overall survival and quality of life of patients suffering from bone metastases has substantially improved [2].

The early detection and imaging of treatment response is crucial for the management of metastatic bone disease [3,4]. In the past, bone scintigraphy was frequently used for detection and follow-up of bone metastases [5]. However, due to limited specificity and spatial resolution bone scans are replaced by modern imaging modalities. Advances in functional MRI or positron emission tomography (PET) provide new biomarkers for monitoring response to treatment [5]. Compared to conventional X-ray and CT, MRI has the advantage to detect disease that is restricted to medullary bone before a destruction of cortical bone occurs. This is possible because of alterations caused by malignant cells infiltrating the bone marrow [6]. With diffusion-weighted imaging (DWI), changes in Brownian motion of water in the marrow can be quantified by calculating apparent diffusion coefficients (*ADC*s) [6]. Recent preclinical and clinical studies showed that whole-body

Abbreviations: *ADC*, apparent diffusion coefficient; a.u., arbitrary units; DCE-MRI, dynamic contrast-enhanced magnetic resonance imaging; DWI, diffusion-weighted imaging; EORTC, European Organization for Research and Treatment of Cancer; FLASH, fast low angle shot; FOV, field of view; HASTE, half-Fourier acquisition single-shot turbo spin echo; MRI, magnetic resonance imaging; MVC, mean vessel caliber; ROI, region of interest; SMA, smooth muscle actin; T2w MRI, T2-weighted magnetic resonance imaging; T_{REC}, recovery time; TSE, turbo spin echo.

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MRI combined with DWI is helpful for the detection and follow-up of bone metastases, especially in patients with breast or prostate cancer as well as multiple myeloma [7–10].

Another method proposed for the assessment of malignant bone marrow infiltration in cancer patients is dynamic contrast-enhanced MRI (DCE-MRI). Semi-quantitative analysis of images acquired during the infusion of contrast agent enables to assess changes in perfusion upon malignant bone marrow infiltration. DCE-MRI has successfully been studied in preclinical and clinical trials for the detection and for the evaluation of treatment response of bone metastases [11,12].

The increasing number of modalities and techniques available for the assessment of metastatic bone disease necessitates validation of the acquired parameters for the correct biological interpretation [13]. Therefore, we performed this correlation study of DWI and DCE-MRI in experimental breast cancer bone metastases and compared imaging results with immunohistological evaluation of tumor cell density and vascularity. Furthermore, our aim was to non-invasively assess intra-tumoral heterogeneity of bone metastases with DWI, DCE-MRI and histology.

2. Materials and methods

2.1. Animal model

Animal protocols were approved by the responsible governmental animal ethics committee (Regierungsprä sidium Karlsruhe). Male nude rats (RNU strain, 6 weeks of age; Harlan, Borchen, Germany) were housed under specific-pathogen free condition in a mini-barrier system. For induction of bone metastases, animals were anesthetized with a mixture of oxygen (0.5 l/min), isoflurane (1–1.5 vol%) and nitrous oxide (1 l/min). After achieving sufficient narcosis, the arteries of the right hind leg were prepared via an inguinal cut. Afterwards, 10^5 MDA-MB 231 human breast cancer cells were injected into the superficial epigastric artery of the rind hind leg, as described previously [14]. Bone metastases occurred selectively in the distal femur as well as proximal tibia and fibula of the respective leg. Imaging analysis was performed on untreated control animals from previous studies [11,12,15,16] that were available for immunohistological evaluation after the end of the respective observation period either on day 30, 35, 45 or 55 after tumor cell injection.

2.2. Magnetic resonance imaging

For non-invasive in vivo imaging, we used the above-mentioned mixture of narcotics. Imaging was performed on a clinical 1.5 T MRI scanner (Symphony, Siemens, Erlangen, Germany) equipped with a home-built coil for radiofrequency excitation and detection, designed as a cylindrical volume resonator with an inner diameter of 83 mm and a usable length of 120 mm.

2.3. Morphological T2-weighted MRI

To detect and quantify volumes of bone metastases developing in the right hind leg, an unenhanced T2-weighted (T2w) turbo spin-echo (TSE) sequence was used (orientation axial; TR 3.240 ms; TE 81 ms; matrix 152 × 256; field of view (FOV) 53.4 mm × 90 mm; slice thickness 1.5 mm; averages 3; images 15; scan time 3:40 min).

2.4. Diffusion-weighted imaging

DWI followed T2w MRI for animals bearing breast cancer bone metastases ($n = 28$). After identification of the largest diameter of the metastases on axial T2w images, half-Fourier acquisition single-shot turbo spin echo (HASTE) sequences at different b-values ($b = 0$, 50, 100, 200, and 600 s/mm^2) were used to acquire diffusion-weighted images (three slices in axial orientation; TR 4.000 ms; TE 155 ms; matrix 72 × 128; FOV 45 mm × 80 mm; slice thickness 2 mm; averages 10; images 15; scan time 5 × 2:00 min). In six animals, DWI was additionally performed at b-values of 150 and 1000 s/mm^2.

2.5. Dynamic contrast-enhanced MRI

DCE-MRI was performed using 2D saturation recovery turbo fast low angle shot (FLASH) sequences through the largest diameter of the metastases that was identified on unenhanced T2w images (orientation axial, TR 373 ms, TE 1.86 ms, T_{REC} 130 ms, flip angle 20°, matrix 192 × 144, FOV 130 mm × 97.5 mm, 1 average, 512 images, scan time 6:55 min). During the sequence 0.1 mmol/kg body weight Gadolinium–DTPA (Magnevist, Schering, Berlin, Germany) was infused manually and weight-adapted via a catheter placed in the tail vein over a time period of 10 s.

2.6. Postprocessing

T2w images were segmented with the open-source Medical Imaging Interaction Toolkit (www.mitk.org, DKFZ, Heidelberg, Germany) to quantify volumes of bone metastases. For quantification of DWI and DCE-MRI parameters, regions of interest (ROIs) were selected manually around the largest diameter of the tumor. To assess whether DWI or DCE-MRI can detect differences between rim and center of the tumor, additional ROIs were selected in the periphery (peripheral 1/3 of the metastases) and center (central 2/3) of large metastases (≥1 ml). The in house DWI stat module (developed with Fraunhofer Mevis, Bremen, Germany) was used to create *ADC* maps and calculate minimum, maximum and mean *ADC* values (ADC_{MIN}, ADC_{MAX}, ADC_{MEAN}) within the selected ROIs. DCE-MRI data was analyzed with the Dynalab workstation (Fraunhofer Mevis, Bremen, Germany) according to the model by Brix et al. [17] to calculate parameters amplitude A (associated with plasma volume and interstitial distribution space) [18] and exchange rate constant k_{ep} (reflecting vessel permeability and perfusion) [18].

2.7. Immunohistology

After MRI, animals were sacrificed and bone metastases were resected. Specimen were processed as described previously and embedded in a methyl methacrylate based material (Technovit 9100 New; Heraeus Kulzer GmbH, Germany) [19]. 6 μm thick sections from tissue blocks were immunostained for collagen IV and smooth muscle actin (SMA) overnight at 4 °C with the following antibodies: a rabbit anti-rat collagen IV antibody (Progen Biotechnik GmbH, Heidelberg, Germany) combined with Alexa Fluor 488-conjugated donkey anti-rabbit antibody (dianova GmbH, Hamburg) and a mouse anti-rat SMA antibody (Sigma–Aldrich, Saint Louis, MO) combined with a Alexa Fluor 594-conjugated goat anti-mouse antibody (dianova GmbH, Hamburg). To quantify cellularity of bone metastases, cell nuclei were stained with DAPI (Serva, Germany). For quantification of digitally captured microscope images, we selected five representative FOV for each animal. Analogous to MRI, we additionally selected five FOV from the periphery and center of larger metastases, to identify histological differences between the tumor rim and center. Automated analysis software (cellF, Olympus Soft Imaging Solutions, Germany) quantified positive area fractions for collagen IV and SMA as well as the number of DAPI-positive cell nuclei. By measuring diameters of orthogonally cut vessels we determined mean vessel calibers (MVC). As surrogate for vessel maturity the SMA/collagen IV ratio was calculated.

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