Correlation between apparent diffusion coefficient and histopathology subtypes of osteosarcoma after neoadjuvant chemotherapy

Jifei Wang, Meili Sun, Dawei Liu, Xiaoshu Hu, Margaret H Pui, Quanfei Meng and Zhenhua Gao

Abstract
Background: Neoadjuvant chemotherapy has made limb-salvage surgery possible for patients with osteosarcoma. Diffusion-weighted magnetic resonance imaging (DWI) has been used to monitor chemotherapy response.

Purpose: To correlate the apparent diffusion coefficient (ADC) values with histopathology subtypes of osteosarcoma after neoadjuvant chemotherapy.

Material and Methods: Twelve patients with osteoblastic (n = 7), chondroblastic (n = 4), and fibroblastic (n = 1) osteosarcomas underwent post-chemotherapy DWI before limb-salvage surgery. ADCs corresponding to 127 histological tissue samples from the 12 resected specimens were compared to histological features.

Results: The mean ADC value of non-cartilaginous viable tumor (38/91, ADC = 1.22 ± 0.03 × 10^{-3} mm²/s) was significantly (P < 0.001) lower than that of non-cartilaginous tumor cell necrosis without stroma disintegration (25/91, ADC = 1.77 ± 0.03 × 10^{-3} mm²/s), cartilaginous viable tumor (14/91, ADC = 2.19 ± 0.04 × 10^{-3} mm²/s), and cystic areas including liquefied necrosis, blood space, and secondary aneurysmal bone cyst (14/91, ADC = 2.29 ± 0.05 × 10^{-3} mm²/s).

The mean ADC value of non-cartilaginous tumor cell necrosis was also significantly (P < 0.001) smaller than those of viable cartilaginous tumor and cystic/hemorrhagic necrosis whereas the mean ADC values were not significantly (P > 0.05) different between viable cartilaginous tumor and cystic/hemorrhagic necrosis.

Conclusion: DWI allows assessment of tumor necrosis after neoadjuvant chemotherapy by ADC differences between viable tumor and necrosis in fibroblastic and osteoblastic osteosarcomas whereas viable chondroblastic osteosarcoma has high ADC and cannot be distinguished reliably from necrosis.

Keywords
Osteosarcoma, neoadjuvant chemotherapy, diffusion-weighted imaging (DWI), apparent diffusion coefficient (ADC)

Date received: 21 July 2016; accepted: 5 October 2016

Introduction
Osteosarcoma is the most common primary malignant bone tumor in children and adolescents (1). Neoadjuvant and adjuvant chemotherapy has made limb-salvage surgery possible on more than 80% of these patients and the 5-year survival rate has increased up to 75% (2,3). The histological response with more than 90% tumor cell necrosis in the resected specimen after neoadjuvant chemotherapy is a critical prognostic factor for good therapeutic response (4). However, this response can only be assessed postoperatively.
Diffusion-weighted magnetic resonance imaging (DWI) has been used to monitor chemotherapy response (5–7). Normalized apparent diffusion coefficient (ADC) on 2T or 2 Tesla DWI was reported to accurately differentiate between viable and necrotic osteosarcomas in rats, although the difference in ADC values between non-liquefied and liquefied necrosis was not evaluated (5). One study on 22 patients with osteosarcomas showed significantly higher average and minimum ADC values after chemotherapy whereas only the difference in minimum ADC ratios was significant between patients with good and poor chemotherapeutic response. The histological subtypes of osteosarcoma were not separately analyzed (6). In another study with 13% chondroblastic subtypes of osteosarcoma, ADC values did not correlate with necrosis and a newly derived diffusion parameter per unit volume was proposed to be a sensitive substitute for evaluating therapeutic response (7). The objective of this study was to assess the accuracy of ADC as a non-invasive biomarker for predicting neoadjuvant chemotherapy response by performing region-by-region correlation with histopathology subtypes of resected osteosarcomas.

Material and Methods

From August 2011 through March 2012, 12 consecutive patients (7 boys/men, 5 girls/women; median age, 14.6 years; age range, 6–25 years) with primary osteosarcomas of the distal femur (n = 8) and proximal tibia (n = 4) referred to our academic tertiary care center for bone tumors were recruited. This was a prospective study and part of a series of studies on evaluation of the response of osteosarcoma to neoadjuvant chemotherapy using functional magnetic resonance imaging (MRI). The histology included osteoblastic (n = 7), chondroblastic (n = 4), and fibroblastic (n = 1) types. This study was approved by the institutional review board. Written informed consent was obtained from the patients or parents before MRI. All patients received four cycles of neoadjuvant chemotherapy including high-dose methotrexate, pirarubicin, and ifosfamide with or without cisplatinum. Limb-salvage surgery was performed in the third week after the completion of chemotherapy.

MRI was performed within 3 days before surgery on a 3T system (Magnetom Trio, Syngo MR 2006T, Siemens Medical Solution, Forchheim, Germany) using an extremity coil. Conventional MRI included axial spin-echo T1-weighted (T1W) images (TR/TE, 659/11 ms), axial, coronal, and sagittal fast spin-echo T2W images with or without fat suppression (4660/96 ms). Axial DWI was performed using single-shot spin-echo echo-planar imaging sequence (3200/82 ms) with $224 \times 224$ matrix, EPI factor of 3, and b-values of 0 and $800 \text{s/mm}^2$. All axial images were acquired perpendicular to the longitudinal axis of the body and parallel to the tibial plateau with 5-mm slice thickness, 1-mm interslice gap, and the central slice positioned at the maximum width of the tumor.

The resected gross specimens from limb-salvage surgery were fixed in 10% buffered formaldehyde solution, and then sawn into 5-mm thick axial slices corresponding to the axial MRI. Section-by-section correlation was performed between MRI and the specimens by a radiologist and an experienced musculoskeletal pathologist to select 6–10 well-matched specimen sections in each patient (Fig. 1). Rectangular tumor tissue samples ranging from $10 \times 15$ to $15 \times 20$ mm were drawn on these specimen sections corresponding to areas of homogeneous signal intensities on T1W and T2W MRI as well as DWI. Depending on the tumor size, 9–24 sampling areas were obtained from each patient resulting in 127 tissue samples from 12 resected specimens. These tissue samples were fixed, decalcified, dehydrated, embedded with paraffin, sectioned, and stained with hematoxylin and eosin (H&E). All tissue samples with microscopically viable sarcomatous cells, tumor osteoid, tumor bone, viable chondrosarcoma-tous cells with cartilaginous matrix, sarcomatous cell necrosis, post-necrotic collagen, liquefactive necrosis, blood spaces, and secondary aneurysmal bone cysts were recorded by the radiologist blinded to the MRI findings. Every tissue sampling area with the same histological feature was marked and grouped.

Using the axial T1W and T2W images as reference, round, or oval regions of interest (ROIs) were placed on the automatically generated ADC maps corresponding to the histological sampling areas by consensus of two experienced radiologists. The size of ROIs was in the range of 50–250 mm$^2$. ADC values of the ROIs were measured twice to obtain mean ADC values (Fig. 1). Using one-way analysis of variance as well as Bonferroni method for multiple comparisons (SPSS 13.0 software, SPSS Inc., Chicago, IL, USA), the mean ADC values in areas of varying histological features were compared. Statistical significance was defined as $P < 0.05$.

Results

In histologic sections, viable sarcomatous cells, tumor osteoid, tumor bone, viable chondrosarcomatous cells with cartilaginous matrix, sarcomatous cell necrosis, post-necrotic collagen, liquefactive necrosis, blood spaces, and secondary aneurysmal bone cysts were found microscopically. Sarcomatous cell necrosis had typical morphological changes including cell shrinkage, pyknosis, karyorrhexis, karyolysis, and disappearance. Liquefactive necrosis was represented by cystic spaces filled with fluid remains of necrotic tissue. Secondary
aneurysmal bone cysts were blood spaces surrounded by ribbon-like fibrous wall.

In 127 tissue samples, there were 91 homogeneous histological areas in 12 patients, including non-cartilaginous viable tumors (38/91, 42%), non-cartilaginous tumor cell necrosis without stroma disintegration (25/91, 28%), cartilaginous viable tumors (14/91, 15%), and cystic/hemorrhagic necrosis (14/91, 15%). The remaining 25 heterogeneous histological areas with partial necrosis (10% ≤ necrosis < 90%) in 10 patients and 11 post-necrotic tumor collagen areas from one patient were excluded from analysis to avoid inaccurate ADC calculation due to tissue heterogeneity. The mean ADC value in non-cartilaginous viable tumor (1.22 ± 0.03 × 10⁻³ mm²/s) was significantly (P < 0.05) lower than that of non-cartilaginous tumor cell necrosis (1.77 ± 0.03 × 10⁻³ mm²/s), cartilaginous viable tumor (2.19 ± 0.04 × 10⁻³ mm²/s), and cystic/hemorrhagic necrosis (2.29 ± 0.05 × 10⁻³ mm²/s). The mean ADC value in the non-cartilaginous tumor cell necrosis was also significantly (P < 0.001) smaller than those in the viable cartilaginous tumor and cystic/hemorrhagic necrosis whereas the mean ADC values were not significantly (P > 0.05) different between viable cartilaginous tumor and cystic/hemorrhagic necrosis (Figs. 1–5).

Discussion

Diffusion of water molecules within tissues is impeded by cellular membranes, intracellular organelles, macromolecules, and extracellular space. Restricted diffusion of water molecules results in high signal intensity on DWI and low ADC value (8). Various histological features including viable tumor cell, necrotic tumor without stroma disintegration, liquefied necrosis, localized blood space, and secondary aneurysmal bone cyst were found in our study. In viable non-cartilaginous tumor, the intact cellular membranes, hypercellularity, and decreased extracellular volume resulted in lower diffusion than that in the non-cartilaginous tumor cell necrosis with disrupted cellular membranes, destruction of intracellular organelle, and tumor cell disappearance. In cystic regions of liquefied necrosis, blood space, or secondary aneurysmal bone cyst, the mean ADC value was much higher than that in the non-cartilaginous viable tumor and non-cartilaginous tumor cell necrosis because of free diffusion of water molecules at the cellular level. Therefore, DWI allowed assessment of tumor necrosis after neoadjuvant chemotherapy of fibroblastic and osteoblastic osteosarcomas by significant ADC differences among non-cartilaginous viable tumor, non-cartilaginous tumor cell necrosis, and liquefied necrosis or hemorrhage. We found the high ADC value of viable cartilaginous tumor similar to that of cystic lesions because the sparse tumor cells in the myxoid matrix of chondroblastic osteosarcoma resulted in free water diffusion (9). Extracellular cartilage matrix with high water content and hyper-permeability may also contribute to the high ADC (10). Wang et al. (11) studied 35 patients

Fig. 1. Fibroblastic osteosarcoma of distal femur in a 15-year-old boy. Using (a) axial T1W and (b) T2W images as reference, circular ROI was placed on the (c) ADC map inside the rectangular tissue sampling region of the (d) corresponding gross specimen section. The mean ADC value was low at 1.36 × 10⁻³ mm²/s corresponding to microscopically viable non-cartilaginous tumor on the photomicrograph of the histologic specimen (e) (original magnification, ×200; H&E stain).
with osteosarcomas without describing the number of chondroblastic osteosarcomas. The authors compared the ADC values of osteosarcomas before and after neoadjuvant chemotherapy, between patients with good and poor response to neoadjuvant chemotherapy, between viable and necrotic tumors after neoadjuvant chemotherapy. They found that the post-neoadjuvant chemotherapy tumor ADC values in patients with good response were higher than that of poor response. A previous study by Uhl et al. (12) with eight patients reported that tumor ADC changes were related to the degree of tumor necrosis. However, results from studies with 22 patients by Oka et al. (6), 31 patients by Bajpai et al. (7), and 15 patients by Baunin et al. (13) including

Fig. 2. Osteoblastic osteosarcoma of proximal tibia in a 6-year-old boy. (a) Axial T1W and (b) fat-saturated T2W images, (c) ROI on the ADC map, (d) specimen section corresponding to the area on T2W image (dashed line), and (e) photomicrograph of the histologic specimen (original magnification, ×200; H&E stain) show increased mean ADC value of \(2.06 \times 10^{-3}\) mm\(^2\)/s in the non-cartilaginous necrotic tumor.

Fig. 3. Chondroblastic osteosarcoma of distal femur in a 14-year-old boy. (a) Axial T1W and (b) fat-saturated T2W images, (c) ROI on the ADC map, (d) specimen section corresponding to the area on T2W image (dashed line), and (e) photomicrograph of the histologic specimen (original magnification, ×200; H&E stain) show high ADC values of \(2.37 \times 10^{-3}\) mm\(^2\)/s and \(2.51 \times 10^{-3}\) mm\(^2\)/s in the viable cartilaginous tumor similar to cystic or hemorrhagic tumor necrosis.
several chondroblastic osteosarcomas were discordant. We found an overlap in ADC values between viable cartilaginous tumor and cystic lesions. The viable chondrosarcomatous component could be mistaken as liquefied necrosis resulting in overestimation of necrotic fraction. This may account for the discordant results between histological response and ADC values of osteosarcoma during chemotherapy reported in the literature (6,7,11–13). The findings in our study suggest that the histological subtypes of osteosarcoma should be taken into account when evaluating chemotherapeutic response with DWI.

The ADC values are dependent on the MR system including the manufacturer, magnetic field strength, sequence parameters as well as placement of ROIs and cannot be translated directly to other MR scanners. In our study, ADC values were significantly different between viable tumor and necrosis in fibroblastic and osteoblastic osteosarcoma, whereas viable chondroblastic osteosarcoma could not be distinguished reliably from necrosis. However, the study was limited by the small sample size of 12 patients and cutoff ADC values were not determined. The change in ADC values may be more important than absolute ADC values in evaluating treatment response. Although tissue samples including 91 histological areas were assessed, only homogeneous portions of the axial specimen slices were evaluated. Correlation between ADC and the entire axial specimen slice may be more accurate. In addition, ADC quantitation of necrosis can be compared to histology and prognosis. Future studies with larger patient sample to investigate the absolute and relative change in ADC values are warranted to evaluate the response of different subtypes of osteosarcoma to neoadjuvant chemotherapy.

In conclusion, DWI allows assessment of tumor necrosis after neoadjuvant chemotherapy by ADC differences between viable tumor and necrosis in

Fig. 4. Osteoblastic osteosarcoma of distal femur in a 20-year-old woman. (a) Axial T1W and (b) fat-saturated T2W images, (c) ROI on the ADC map, (d) tissue sample corresponding to the irregular area drawn on T2W image (solid line, magnification ×3), and (e) photomicrograph of the histologic specimen (original magnification, ×200; H&E stain) show increased diffusion (ADC = 2.40 × 10⁻³ mm²/s) in the T1-isointense and T2-hyperintense blood space.

Fig. 5. Box plot comparing difference in mean ADC values (×10⁻³ mm²/s) among different histopathologic regions with highest ADC in the cystic area and lowest ADC in the non-cartilaginous viable tumor.
fibroblastic and osteoblastic osteosarcoma. Viable chondroblastic osteosarcoma has high ADC and cannot be distinguished reliably from necrosis. The histological subtypes of osteosarcoma should be taken into account when evaluating chemotherapeutic response.

Declaration of conflicting interests
The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported in part by a grant from Science and Technology Planning Project of Guangdong Province, PR China (Grant no. 2011B031800096).

References