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Osteosarcoma mineralization changes on radiographs have moderate correlation to chemotherapy response using bone subtraction methodology

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Abstract

Background: Survival following a diagnosis of osteosarcoma is correlated strongly with response to chemotherapy. Mineralization changes seen on radiographs have been hypothesized to correlate with chemotherapy response, however, this has never been analyzed using modern techniques.

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This research was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Dartmouth-Hitchcock Medical Center Committee for the Protection of Human Subjects, Study #00027973. Informed consent was not required for this retrospective, non-interventional study, which was considered minimal risk.

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Methods: Retrospective review of radiographs obtained before and after neoadjuvant chemotherapy was performed for 31 patients with high-grade, conventional osteosarcoma. Pre-chemotherapy (PreC) images and post-chemotherapy (PostC) images were co-registered. Tumor luminance measurements were normalized based on the non-tumor bone and then the relative change in tumor mineralization were measured.

Results: Mean luminance values for pre-chemotherapy non-tumor-affected bone and tumor were 0.63±0.12 and 0.65±0.12, respectively. Mean values for PostC non-tumor-affected bone were 0.59±0.14 and 0.64±0.10, respectively. Once normalized, osteosarcoma mineralization change showed a statistically significant moderate correlation—Pearson correlation coefficient (ρ) of 0.36 (P=0.038)—with the tumor necrosis value.

Conclusions: Moderate, positive correlation was found between osteosarcoma mineralization change during chemotherapy and chemotherapy response. Further work is required to determine if these findings are prognostic by identifying best practice for image analysis and repeating this work with prospectively acquired digital radiographs using uniform technique and phantom normalization.

Keywords

Osteosarcoma; mineralization; radiographs; chemotherapy; response

Introduction

Osteosarcoma is the most common primary bone cancer in young people (1). Treatment usually involves preoperative chemotherapy, resection of the primary tumor, and postoperative chemotherapy. Survival from osteosarcoma is correlated with chemotherapy response, determined by tumoral necrosis, described by Huvos and Picci (2-4). By the Huvos criteria, grade 1 equates to <50% necrosis, grade 2 is 50–90%, grade 3 is 90–99%, grade 4 is 100% necrosis; a good response is considered Huvos grade 3 or 4. Unfortunately, the response to chemotherapy is unknown until the primary tumor is analyzed following resection, potentially subjecting patients with osteosarcoma to weeks of ineffective, cytotoxic treatment. Currently, there is no non-invasive and low-cost method of measuring the response to chemotherapy.

Radiological imaging is a key element in the identification, diagnosis, and staging of an osteosarcoma tumor. Osteosarcomas incur predictable features on plain radiographs, generally showing a wide zone of transition, an osseous matrix, and aggressive periosteal reactions with a soft-tissue mass (5,6). Magnetic resonance imaging (MRI), computed tomography (CT), and ^{99}Tecnetium bone scan are required elements of osteosarcoma staging, providing critical information about the presence of intramedullary skip metastases and distant bony or pulmonary metastases (7). A long-recognized phenomenon occurring commonly in the treatment of patients with osteosarcoma is a perceived increase in the primary tumor's mineralization during chemotherapy (Figure 1) (8). Prior investigators—hypothesizing that increased mineralization portends a better response to chemotherapy—attempted unsuccessfully to correlate this mineralization change and chemotherapy effect using subjective, visual interpretation of tumor mineralization (8-10). These earlier methods

did not allow for computer-assisted grayscale normalization and relied on the human eye's minimum noticeable change in input intensity—the so-called "increment threshold"—which is known to be inexact and unreliable for work that requires high-fidelity grayscale measurement (11). Since this work, sophisticated image analysis tools were developed and, to date, no investigators have investigated if mineralization changes are associated with chemotherapy response using these tools.

This investigation was a retrospective review of radiographs obtained before and after the administration of chemotherapy for patients with lower extremity osteosarcoma. The primary research aim was to analyze the association between tumor mineralization and chemotherapy response using computer-assisted image analysis techniques. The primary hypothesis was that an increase in mineralization is associated with higher necrosis. We present the following article in accordance with the MDAR reporting checklist (available at http://dx.doi.org/10.21037/aoj-20-70).

Methods

Patient data

We conducted database inquiries for patients with a conventional, high-grade osteosarcoma diagnosis who had pre- (PreC) and post-chemotherapy (PostC) radiographs of the primary tumor from lateral side obtained prior to resection. All patients were prescribed a 3-month course of chemotherapy—doxorubicin, methotrexate, and cisplatin as general, first-line therapy (12,13)—prior to their definitive tumor resection. Following surgery, tumor necrosis values, determined originally by histological review with attention to criteria described by Picci (4), were obtained via chart review.

Thirty-one patients met criteria, their treatments were performed between 1999 and 2013. There were 16 (51%) males and 15 (49%) females; mean age at the treatment initiation was 13 years (range, 4 to 20 years). Anatomical locations of the primary tumors included two proximal femoral tumors, twenty distal femoral tumors, seven proximal tibial tumors, and two distal tibial tumors.

This research was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Dartmouth-Hitchcock Medical Center Committee for the Protection of Human Subjects, Study #00027973. Informed consent was not required for this retrospective, non-interventional study, which was considered minimal risk.

Radiographs

All patients had PreC and PostC radiographs of the primary tumors. Based upon availability, radiographs were digitized copies of plain film radiographs. Because of greater variability in the AP radiographs' field of view and limb alignment, we confined this analysis to lateral radiographs only.

Image analysis

Digitized radiographs were analyzed using MATLAB (v. 2018b, MathWorks, Natick, MA, USA) by a reviewer blinded to tumor necrosis values. The ROIs were identified by

an orthopaedic oncologist by tracing the periphery of all tumors on the PostC lateral radiographs; regions of interest (ROIs) of normal bone were also identified. Radiograph luminance in the ROIs was considered a surrogate for radiodensity. To compensate for the difference in scanning quality, all images with different bit depth were normalized on a scale of 0–1 with arbitrary units (AU). Each patient's PreC and PostC images were co-registered by identifying identical anatomical landmarks in both images. Anatomical landmarks were identified using the validated and open-source software extension *fitgeotrans.m*. These landmarks were processed to provide co-registration between the PreC and PostC images, reconciling differences in the images based on limb position. The ROIs defined on the PostC images were reproduced on the co-registered PreC images to provide an exact comparison. The accuracy of the co-registered PreC and PostC images was measured using another validated and open-source software extension *imshowpair.m*.

Following image co-registration and ROI definition, PreC and PostC images were normalized to the non-tumor-affected bone ROI luminance values. Tumor mineralization change was determined by subtracting the average luminance of the PreC tumor ROI from the PostC tumor ROI. The tumor mineralization change was calculated using Equation. 1, with T indicating mean tumor luminance and B indicating mean bone luminance.

$$\text{Tumor mineralization} = (\text{PostC-T} - \text{PostC-B}) - (\text{PreC-T} - \text{PreC-B}) \quad [1]$$

Statistical analysis

The relationship between luminosity-defined mineralization changes and osteosarcoma necrosis was determined using Pearson's correlation coefficient (ρ) and coefficient of determination (R^2).

Results

The mean osteosarcoma tumor necrosis value for this study was 69%±23% (Table 1). Mean luminance values for PreC and PostC non-tumor-affected bone were 0.63±0.12 and 59±0.14, respectively. Mean luminance values for PreC and PostC tumor were 0.65±0.12 and 0.64±0.10, respectively. The direct value difference of PreC and PostC showed a low correlation—Pearson correlation coefficient (ρ) of 0.17 (P=0.37)—with the tumor necrosis value (Figure 2A). Osteosarcoma mineralization change calculated based on Eq. [1] showed moderate correlation—Pearson correlation coefficient (ρ) of 0.36 (P=0.038)—with the tumor necrosis value (Figure 2B).

Discussion

Given the importance of chemotherapy response to oncological outcomes of patients with osteosarcoma, a low-cost and low-harm method of assessing this endpoint would likely prove beneficial in maximizing survival. Noting the subjective observation that osteosarcoma tumors with good chemotherapy response often appear to demonstrate increased mineralization during treatment, previous authors were unable to identify a

significant correlation between mineralization and treatment response using qualitative methods (8,10,14). Through software-based review of radiographs obtained PreC and PostC, we were able to demonstrate a modest positive correlation between tumor mineralization and chemotherapy response, as determined by tumor necrosis.

Smith first described seeing “striking and unusual radiographic changes” in osteosarcoma tumors during chemotherapy (8). They described a statistically unsupported positive correlation between the presence of these changes and osteosarcoma necrosis. Similarly, Hirano described a trend toward higher radiographic density in patients with osteosarcoma and malignant fibrous histiocytoma of bone with better chemotherapy response, however, their study was underpowered to demonstrate significance (9). Holscher used a three-tiered, human-scored rating system to gauge mineralization changes during chemotherapy (increased, unchanged, or decreased) (10). Their methods did not allow for radiograph normalization and relied on the human eye’s minimum noticeable change in input intensity —the so-called “increment threshold”—which is known to be inexact (11), for evaluating differences in radiograph luminance; they found no correlation between mineralization and treatment response. Lindner developed a four-tiered system of evaluating local host response on histological examination and found significant correlation both to tumor necrosis and to mineralization on CT (14). They were unable, however, to demonstrate a correlation to changes on radiographs. Because conventional radiography and CT are similar technologies that sample identical tissues, it is intuitive therefore that the radiography data contains all the contents of CT, just unclarified in the axial plane by surrounding tissues. Therefore, it is plausible that, with adequate normalization and subtraction techniques, radiography data may be distilled to provide the granularity of CT without the additional radiation exposure and cost.

Other imaging modalities have been investigated for their ability to assess chemotherapy response in osteosarcoma. MRI has been researched by multiple research teams, all concluding that the apparent diffusion coefficient is of potential value in grading chemotherapy response (15-17). ^{99}Technetium and ^{201}Thallium scintigraphy also appear to have predictive validity in evaluating chemotherapy response, although they have received less attention than MRI (18,19). Positron-emission computed tomography with ^{18}fluorodeoxygluose also demonstrates predictive validity in assessing chemotherapy response though there is higher cost and radiation exposure compared to plain radiography (20-22).

This study has limitations. Radiographs analyzed in this study were of limited numbers (31 patients from two centers) and obtained via heterogeneous techniques and were often scanned copies of film radiographs. We acknowledge the deleterious effects that these study flaws have on the ability to draw definitive conclusions from our data. We were unable to account for the imager settings—kilovoltage peak and milliamp seconds—and the source-to-image distance used to obtain the radiographs. We also limited our analysis for this study to lateral radiographs, which we found had greater technique homogeneity —in further validation studies we would pursue tumor assessment in anteroposterior and lateral radiographs. Another limitation was our image normalization technique, by which we normalized images using the luminance of bone not affected by tumor. This method

assumes that bone radiodensity is unchanged during the treatment process, which may not be true. Despite these limitations, we believe that rather than serve as a definitive and conclusive study, these early findings bolster the need to further examine whether osteosarcoma mineralization changes may provide low-cost, low-radiation, and non-invasive guidance regarding treatment effect.

In conclusion, changes in osteosarcoma mineralization on plain lateral radiographs show moderate correlation with chemotherapy response when analyzed with sophisticated software tools using bone subtraction methodology. Our next step is to pursue funding to support work to determine: (I) are these findings supported, enhanced, or refuted when using state-of-the-art, high-resolution digital radiographs obtained with uniform technique and phantoms for image normalization; (II) how does the accuracy of this method, when optimized, compare to CT or dual-energy radiography; (III) how early in neoadjuvant chemotherapy could treatment response potentially be determined? Demonstration of a reliable correlation between tumor radiodensity changes and chemotherapy response may enable earlier recognition of treatment failure via analysis of low-cost, low-radiation, and standard-of-care plain radiographs.

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Data Sharing Statement:

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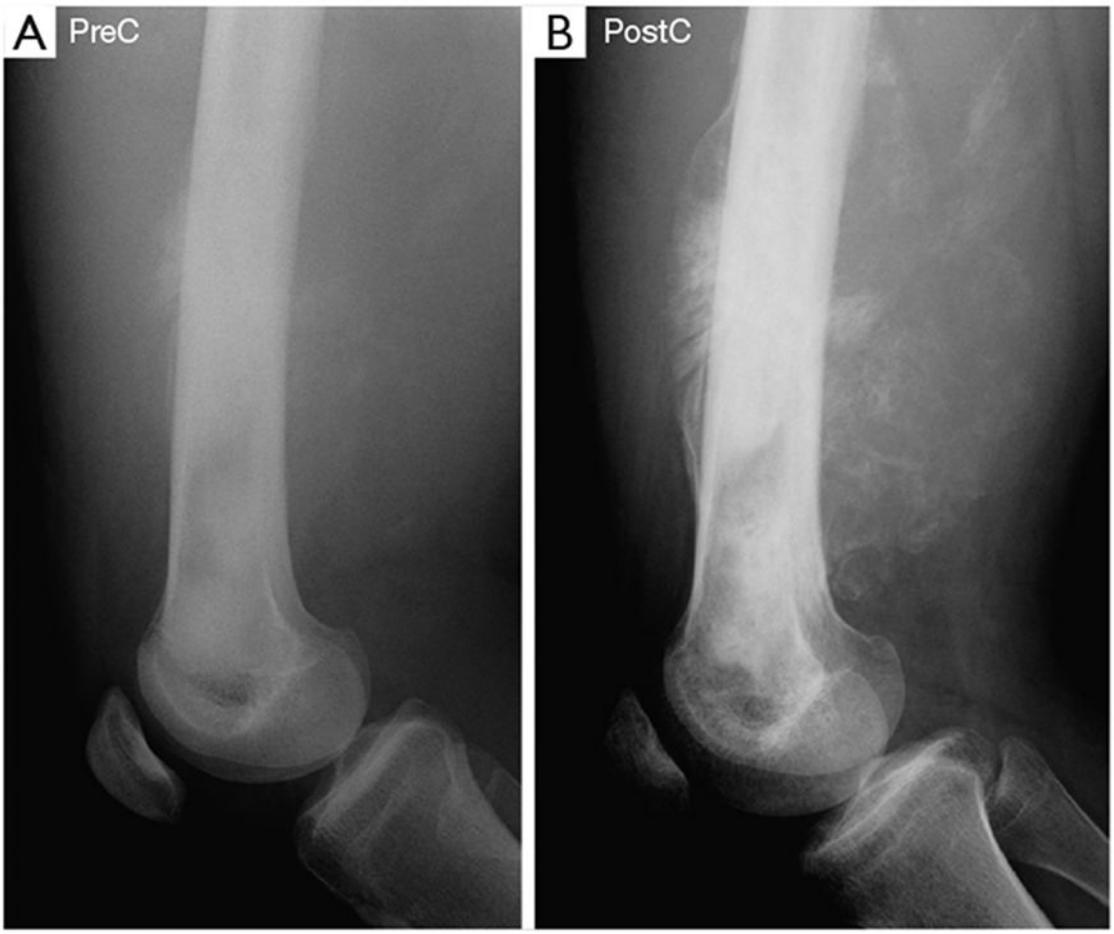

Figure 1.
Lateral radiographs of the distal femur from a patient with osteosarcoma before (A) and after (B) neoadjuvant chemotherapy demonstrating visibly increased tumor mineralization following treatment.

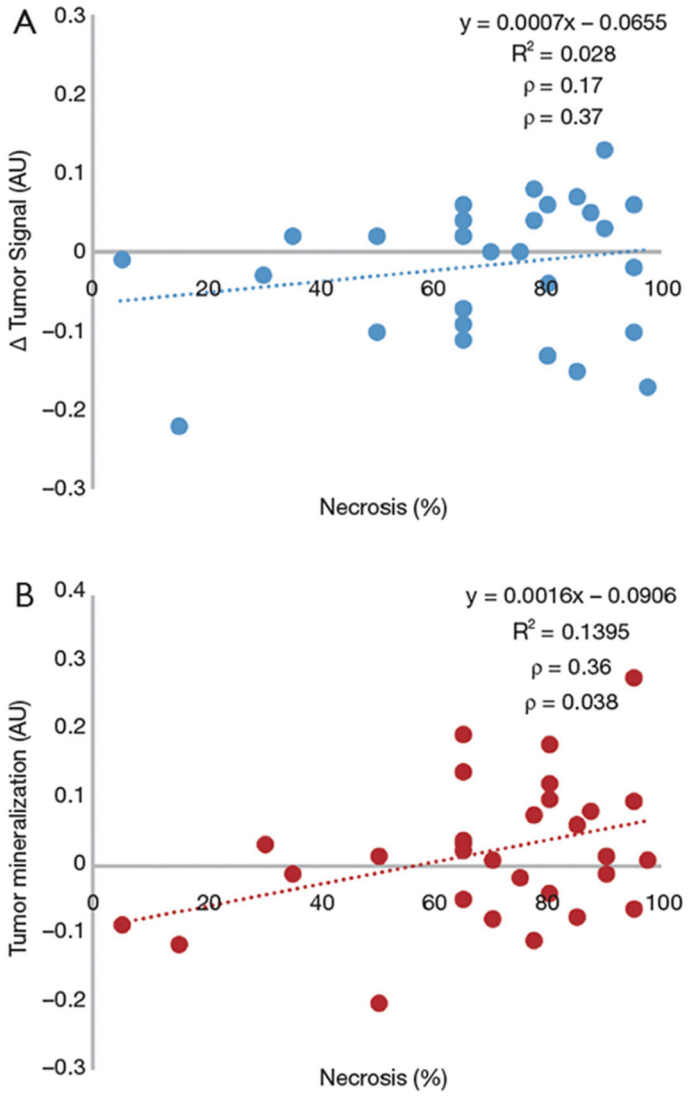

Figure 2.

Plot of Pearson coefficient demonstrating a weak and moderate correlation between tumor necrosis and (A) tumor value difference or (B) tumor mineralization measured on radiographs.

Table 1

Luminance measurements of osteosarcoma tumors and non-tumor bone on lateral plain radiographs before and after neoadjuvant chemotherapy

Patient #	Tumor necrosis (%)	Luminance (AU)						
		Pre-chemotherapy			Post-chemotherapy			ΔLuminance (AU)
		Tumor	Bone	Δ	Tumor	Bone	Δ	
1	85	0.59	0.61	−0.02	0.66	0.75	−0.09	−0.07
2	50	0.74	0.68	0.06	0.64	0.78	−0.14	−0.20
3	5	0.76	0.43	0.33	0.75	0.51	0.25	−0.09
4	97.5	0.73	0.66	0.06	0.56	0.49	0.07	0.01
5	30	0.87	0.63	0.24	0.84	0.57	0.27	0.03
6	90	0.39	0.69	−0.29	0.42	0.70	−0.28	0.01
7	77.5	0.76	0.86	−0.10	0.80	0.82	−0.02	0.08
8	35	0.66	0.76	−0.10	0.68	0.78	−0.11	−0.01
9	80	0.64	0.67	−0.03	0.60	0.53	0.06	0.10
10	85	0.64	0.61	0.03	0.49	0.40	0.09	0.06
11	90	0.57	0.64	−0.07	0.70	0.79	−0.08	−0.01
12	65	0.83	0.68	0.15	0.74	0.45	0.29	0.14
13	70	0.45	0.35	0.10	0.65	0.63	0.02	−0.08
14	95	0.57	0.75	−0.18	0.47	0.38	0.10	0.28
15	77.5	0.60	0.42	0.18	0.68	0.61	0.07	−0.11
16	65	0.69	0.62	0.07	0.62	0.51	0.11	0.04
17	95	0.58	0.65	−0.07	0.56	0.70	−0.14	−0.06
18	50	0.70	0.75	−0.05	0.72	0.76	−0.04	0.02
19	65	0.60	0.72	−0.12	0.66	0.74	−0.09	0.04
20	87.5	0.56	0.60	−0.04	0.61	0.56	0.05	0.08
21	70	0.61	0.43	0.17	0.61	0.43	0.19	0.01
22	65	0.47	0.51	−0.04	0.51	0.60	−0.09	−0.05
23	65	0.66	0.67	−0.01	0.55	0.53	0.01	0.02
24	95	0.61	0.55	0.06	0.67	0.51	0.16	0.10
25	65	0.76	0.58	0.18	0.69	0.47	0.22	0.04
26	80	0.79	0.56	0.23	0.66	0.47	0.19	−0.04

Patient #	Tumor necrosis (%)	Luminance (AU)						ΔLuminance (AU)
		Pre-chemotherapy			Post-chemotherapy			
		Tumor	Bone	Δ	Tumor	Bone	Δ	
27	75	0.59	0.54	0.05	0.59	0.56	0.03	−0.02
28	65	0.52	0.65	−0.13	0.54	0.47	0.07	0.20
29	80	0.82	0.73	0.09	0.69	0.43	0.27	0.18
30	15	0.79	0.89	−0.09	0.57	0.78	−0.21	−0.12
31	80	0.69	0.56	0.13	0.75	0.50	0.25	0.12
Mean (SD)	69 [23]	0.65 (0.12)	0.63 (0.12)	0.03 (0.14)	0.64 (0.10)	0.59 (0.14)	0.05 (0.15)	0.02 (0.10)