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Quantification of Osteosarcoma Mineralization on Plain Radiographs – Novel Software Applications to Assess Response to Chemotherapy

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Abstract

Osteosarcoma is the most common primary malignant bone tumor in children. Patient survival with osteosarcoma is heavily influenced by the response to chemotherapy, measured by tumor necrosis upon histological analysis. Unfortunately, response is not measurable until the time of surgery and therefore modifications to chemotherapy protocol are only made after several weeks of treatment and surgery. Osteosarcoma tumors often demonstrate increased mineralization following the onset of chemotherapy. Furthermore, it has been hypothesized that this mineralization—apparent on radiographs—may correlate with chemotherapy response, however, this has not been demonstrated with qualitative visual evaluation. The ability to non-invasively measure a patient's response to chemotherapy using plain radiographs, which is currently included in the normal clinical workflow, would guide the medical oncologists to tailor treatment for patients with osteosarcoma. After obtaining appropriate multi-center institutional review board approvals, we identified 31 patients that possess a pair of pre-and post-chemotherapy radiograph along with the necrosis measure. The images were digitized scans of physical radiographs between 1999 and 2013. Software was designed to measure the signal intensities in the tumor, a region of the soft tissue, air, and healthy bone. The tumor signals were normalized based on the random combination of air, soft tissue or bone, by subtraction or division. The differences in tumor signal between pre-and post-image were plotted against the percent necrosis determined by histological analysis. Different combinations of the normalization methods were compared based on the slope, coefficient of determination (R^2) and Pearson correlation coefficient (ρ).

Keywords

Osteosarcoma; Radiograph; chemotherapy; drug response; mineralization; computer-aided detection

1. INTRODUCTION

Osteosarcoma is the most common primary bone tumor in children[1]. The use of chemotherapy in treating osteosarcoma has improved the treatment regimen from the conventional amputation to limb-salvage surgery[2–3]. However, patient survival is heavily

influenced by the response to chemotherapy, which can be measured by tumor necrosis upon histological analysis[4].The other prognostic factors for osteosarcoma include age, tumor volume, and site, surgical margin [5].Unfortunately, response to chemotherapy still requires an invasive biopsy or surgery to remove the tissue and send it to a pathologist for analysis. Therefore, modifications to chemotherapy protocol are only made after several weeks of treatment. Consider the toxicity effect of chemotherapy, it will be beneficial for the patients to obtain an early evaluation of the response.

Multiple methods have been developed or applied in the past few decades to solve the problem noninvasively. A gene-expression profile can be used to predict the response even before applying it [6]. Bone scintigraphy, Computed tomography (CT), Positron-emission tomography (PET) and magnetic resonance imaging (MRI) have all been investigated in the ability to determine the response, on a different scale [7–9]. Despite the urgent need for a measurement of chemotherapy response, in the current workflow, none of the methods have been widely accepted.

It is a known phenomenon that osteosarcoma tumors often demonstrate increased mineralization following the onset of chemotherapy[10]. Furthermore, it has been hypothesized that this mineralization—apparent on radiographs—may correlate with chemotherapy response, however, this has not been demonstrated with qualitative visual evaluation [11–12]. The ability to non-invasively measure a patient’s response to chemotherapy using only plain radiographs would guide the medical oncologists to tailor treatment for patients with osteosarcoma with minimum extra procedures added into the current routine.

Questions/Purposes:

The purpose of this study was to iteratively evaluate various methodologies of quantitative image analysis using robust digital technology to determine best practices for assessing changes in osteosarcoma tumor mineralization. We, therefore, sought to answer the following research questions:

1. What is the optimal method of aligning pre-chemotherapy and post-chemotherapy radiographs to provide mineralization signal matching?
2. What is the optimal method—air vs bone and subtraction vs. ratiometric normalization—of isolating signal specific to tumor mineralization on plain radiographs?

2. METHODS

2.1 Patient data

Data from the patient who was diagnosed with osteosarcoma wereinquired with the appropriate multi-center institutional review board approvals. In order to obtain comparative data to feed the computer model, several criteria were used to guide the selection, including 1)Primary distal femoral or proximal tibia high-grade osteosarcoma; 2) Received doxorubicin-based chemotherapy; 3) Acquired pre-chemotherapy (Pre) and post-

chemotherapy (Post)plain radiographs; 4) Has anteroposterior (AP) and/or lateral (LAT) plain radiographs; 5) Tumor necrosis values were assessed by histological analysis.

A total of 31 patients met the inclusion criteria in the inquiry. All radiographs were digitized scans of physical radiographs between 1999 and 2013. Detailed patient information was written in the previous paper [13].

2.2 Image processing

All digitized radiographs were analyzed on MATLAB for image alignment, selecting and obtaining the averaged signal in the region of interest (ROI). Since images were scanned on different bit depth, the maximum value of the image was chosen to be the power of 2 value closest to the maximum pixel value in the image. Then the whole image matrix was then normalized to a scale of 0–1. Images were compared using automatic or manual selected alignment with the built-in function *imregtform* or *fitgeotrans* to obtain a transformation matrix. In order to prove the accuracy of the alignment, the built-in function *imshowpair* was used to visually show the difference. Four regions of interest, tumor, a region of the soft tissue, air, and healthy bone, were selected in the post-chemotherapy image. The tumor ROIs were identified by an orthopedic oncology surgeon. The averaged signal intensities in the ROIs were calculated individually. The four ROIs were then aligned using the transformation matrix to the pre-chemotherapy image to obtain region average values.

2.3 Data analysis

Since each Pre-and Post-image pairs were taken with different image acquisition parameters and post-processing algorithms [14], these effects cannot be normalized from rescaling the image in the image processing step. As a result, the obtained ROI values need to be adjusted to compare.

The attenuation of X-rays in radiograph can be expressed as Equation 1, where the intensity of an X-ray beam is related to the linear attenuation coefficient (μ) and depth of penetration(x). For a healthy bone region in the radiograph, consider a negligible effect of muscle tissue compared to bone, the equation can be simplified to Equation 2. While in the tumor region, the signal output can be expressed as Equation 3. The mineralization level was expected to be related to the depth of the tumor mineralization, x_T . In this study, the averaged tumor signals were normalized in three approaches: subtraction, $I_T - I_B$, division I_T / I_B , or mixed (subtraction and division). The difference, mineralization level, between the Pre-and Post-tumor pairs can be calculated as shown in Table 1 for all three approaches. T stands for tumor, R for reference tissue (air, soft tissue or bone).

$$I = I_0 \exp(-\mu x) \quad (1)$$

$$I_B = I_0 \exp(-\mu_B x_B) \quad (2)$$

$$I_T = I_0 \exp(-\mu_B x_B - \mu_T x_T) \quad (3)$$

To determine the feasibility of tumor mineralization evaluation using plain radiograph, the differences in tumor signal between pre-and post-image were then plotted against the percent necrosis determined by histological analysis to determine the correlation. Different combinations of the normalization methods were compared based on the 1) slope of the fitted line, 2) coefficient of determination (R^2) and 3) Pearson correlation coefficient (p).

3 RESULTS AND DISCUSSION

The manual alignment was selected instead of the automatic alignment mainly due to the change of the amount of tissue content between the pre-and post-image. Other changing factors can be the total image region and patient posing position. All these unaccountable changing factors added difficulties in automatic alignment and made the manual approach a better choice for this study purpose. Based on the visual comparison of the image alignment using function *imshowpair*, the manual approaches reached a better outcome.

To choose the best signal normalization approach, multiple reference tissues and the following calculation approaches were applied and compared. Air can be a good signal correcting subject if the acquired image pair were taken with the same camera to film length without considering the effect of the post-processing algorithm and changing image acquiring parameters. Soft tissue and bone in the tumor surrounding area can be used if the weight loss or mineral loss is negligible during the course of chemotherapy. In order to compare these methods, three parameters were chosen, the slope of the fitted line, coefficient of determination and Pearson correlation coefficient. All data were shown in Figure 1 and Table 2. The slope of the fitted line determines the magnitude of the difference in the normalized 2 mineralization level. The coefficient of determination (R) shows the variance of the line fitting. Pearson correlation coefficient (p) can be used to judge the linear correlation between the necrosis level determined by the pathologist and the calculated mineralization level.

Based on the three groups of results, the mineralization level in the lateral plain normalized by subtracting bone signal, the red dot in Figure 1, presented one of the highest combinations of all methods. It showed a moderate correlation with the percent necrosis value, presenting a Pearson correlation coefficient ($p=0.36$). The detailed data and fitting were shown in red dots in Figure 2, compared to the blue dots indicating the anteroposterior (AP) data analyzed using the same approach. The result was previously reported [13]. This result indicated that bone, compared to other surrounding tissue and air, has the least change during the course of the chemotherapy and can be used as a standard under different radiograph settings. In order to extract the depth of the mineralized tumor, the initial thought would be dividing the bone region signal from the tumor region so as to obtain an equation about x , Equation 4. However, the result showed a lower correlation with the histologically determined necrosis level compared to the simple subtraction. More investigation is required to make a conclusion for the approach at this point.

$$I_T/I_B = \exp(-\mu_T x_T) \quad (4)$$

This study has a limited number of patient data. After several rounds of selection, data from 31 patients were used. Moreover, only one-fifth of the data has a lower necrosis level under 60%, which functions as the lever to determine the correlation. More data points, especially data with lower necrosis level, can help to improve the specificity of the method selection. Another limitation is that the selected bone signal subtraction approach only works for the lateral radiographs in estimating necrosis level based on the current data. In order to overcome the current limitations, we believe this imaging technique may be further improved by adding a standard phantom while taking the radiograph. The current image calibration approach can be one drawback factor that results in a moderate correlation. A stronger correlation between the calculated mineralization level and percent necrosis is hypothesized to be established with the help of the computer-aided image analysis method. An illustration image of the usage of step phantom was shown in Figure 3. Future studies to identify a phantom that can better calibrate the digital radiograph images with improved image quality will be evaluated. With proper calibration of the radiographs, the mineralization caused by chemotherapy can be noninvasively quantified measuring the change of tumor signal without interrupting the normal workflow. This study has the potential to improve patient outcomes with the selection of chemotherapy in osteosarcoma.

In conclusion, we have investigated possible methods to evaluate the necrosis level by calculating the mineralization level based on the pre-and post-chemotherapy plain radiograph. A positive correlation between the calculated mineralization level and percent necrosis was established in this paper using a series of the computer-aided image analysis method. Compared to other imaging modalities, this approach has two major advantages. It is simple to implement, usually takes a few minutes to determine the mineralization level using the routine radiograph. It is not as pricey as the MRI or PET scan.

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Figure 1. Plot of the Pearson correlation coefficient against slope to choose the optimal method for data analysis. The red dot indicate the highest ability to determine the tumor mineralization based on the scanned radiographs.

Figure 2.

Plot of correlation plot between percent necrosis and mineralization level with the anteroposterior (AP) and lateral (LAT) plain radiograph normalized by subtracting the bone signal.

Figure 3.
An illustration of a step phantom that can be used as a signal standard for image calibration.

Table 1.

Calculation equations for the evaluation of mineralization

Equation	
Subtraction	$(Post-T - Post-R) - (Pre-T - Pre-R)$
Division	$Post-T - Pre-T / Pre-R * Post-R$
Mix	$(Post-T - Post-R1) - (Pre-T - Pre-R1) / Pre-R2 * Post-R2$

Table 2.

Summary of the correlation parameters between digitized plain radiograph and histological determined percent necrosis

		slope (*1e-3)	Coefficient of determination (R^2)	Pearson coefficient (ρ)
T/air	AP	-2.30	0.00	-0.04
	LAT	-0.70	0.00	-0.03
T/muscle	AP	-2.20	0.07	-0.02
	LAT	1.00	0.02	0.12
T/Bone	AP	-0.20	0.00	-0.27
	LAT	1.80	0.14	0.18
T subtract air	AP	-1.70	0.10	-0.28
	LAT	0.60	0.01	0.13
T subtract muscle	AP	-1.50	0.08	0.02
	LAT	0.90	0.03	0.38
T subtract bone	AP	-0.20	0.00	0.00
	LAT	1.60	0.13	0.36
T Subtract bone / air	AP	-0.30	0.00	-0.01
	LAT	0.70	0.01	0.09
T subtract air / bone	AP	1.10	0.04	-0.08
	LAT	1.10	0.03	0.17
T subtract muscle / bone	AP	1.20	0.01	-0.26
	LAT	-1.40	0.00	0.20