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ORIGINAL ARTICLE

# Prediction of tumour necrosis fractions using metabolic and volumetric $^{18}$F-FDG PET/CT indices, after one course and at the completion of neoadjuvant chemotherapy, in children and young adults with osteosarcoma

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**Abstract**
*Purpose* The utility of combined metabolic and volumetric $^{18}$F-FDG PET/CT indices for predicting tumour necrosis fractions following neoadjuvant chemotherapy has not been extensively studied in osteosarcoma. Furthermore, little is known of the early PET/CT responses after only one chemotherapy course.
*Methods* Enrolled in the study were 20 children and young adults with resectable osteosarcoma who had undergone $^{18}$F-FDG PET/CT scans before and after neoadjuvant chemotherapy. Maximum standardized uptake value (mSUV), metabolic tumour volume (MTV), and total lesion glycolysis (TLG) were measured. From among the 20 patients, 14 were prospectively recruited and underwent an additional PET/CT scan after one chemotherapy course. Histopathological necrosis fractions were compared with the above-mentioned PET/CT indices and their ratios.
*Results* MTV at the SUV threshold of 2 g/ml was closely correlated with the magnetic resonance image volumes before therapy ($r$=0.91). In the prospective cohort, five patients were classified as good responders and nine as poor responders. All the metabolic indices (mSUV and its ratio) and combined metabolic/volumetric indices (MTV, TLG, and their ratios) except the mSUV ratio determined after therapy showed significant differences between good and poor responders ($P$ <0.05). Differences were also noted for all of these indices determined after one chemotherapy course. Furthermore, most of these indices determined after therapy as well as after one chemotherapy course had good sensitivity, specificity, positive predictive value and negative predictive value with respect to predicting histological response to chemotherapy.
*Conclusion* In our osteosarcoma patient population, $^{18}$F-FDG PET/CT indices (either combined metabolic/volumetric or metabolic indices) determined after neoadjuvant chemotherapy were useful in predicting tumour responses. This held true after only one chemotherapy course.

**Keywords** Osteosarcoma · $^{18}$F-FDG PET/CT · Combined metabolic/volumetric indices · Response to neoadjuvant chemotherapy · Early PET/CT response

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## Introduction

Osteosarcoma, the most common primary malignant bone tumour, usually originates in the metaphyses of the long bones of adolescents and young adults. The rate of systemic spread is so high that cure is rarely achieved with surgical treatment alone [1]. Accordingly, adjuvant and neoadjuvant chemotherapy were introduced, and since their introduction, there has been a significant improvement in the long-term survival rates of patients with high-grade osteosarcoma of the extremities. Indeed, the 5-year disease-free survival has improved from less than 20% to more than 60% over several decades. Simultaneously, the frequency of limb salvage surgery has increased from 10–20% to 80–90%, with a corresponding decrease in the amputation rate [2].

Histopathological response to neoadjuvant chemotherapy is an important prognostic indicator of disease-free survival after multimodal treatment [3, 4]. An evaluation of the Cooperative Osteosarcoma Study Group (COSS) database revealed that patients with a good histological response have an overall 5-year survival rate of 77.8%, whereas those with a poor response have a survival rate of 55.5% [5]. In this work, histological response was assessed according to the scale devised by Salzer-Kuntschik et al. [6], and a good response was defined as a viable tumour fraction of <10%.

Noninvasive means of predicting the effect of neoadjuvant chemotherapy, as reflected in histopathological response, are important because they could be used to determine whether to switch to a more intensified chemotherapy regimen or not, and to determine the most appropriate surgical approach [7].

$^{18}$F-FDG PET/CT has emerged as a promising tool for predicting treatment response for many cancer types, including oesophageal cancer, non-small-cell lung cancer, head and neck cancer and breast cancer [8–11]. Only a limited number of studies have evaluated the effect of neoadjuvant chemotherapy on osteosarcoma, with most having used maximum standardized uptake values (SUV), post- to pretherapy SUV ratios, percentage change in SUV, tumour to background ratios (TBR) and post- to pretherapy TBR ratios as indices for predicting treatment response [12–14].

Maximum SUV represents only the most active parts of the tumour, and may not represent the status of the entire tumour. Alternatively, the combined metabolic/volume index may be a good indicator of the status of the entire tumour. In tumours such as osteosarcoma, which are generally large and demonstrate high intratumoral heterogeneity with respect to FDG uptake, it appears to be rational, at least theoretically, to measure tumour volume and glycolytic activities simultaneously, rather than to measure only the glycolytic activities of the most active parts of the tumour [15]. However, this suggestion has not been thoroughly studied in osteosarcoma. Furthermore, there have been no reports concerning the responses on FDG PET/CT early during the course of neoadjuvant chemotherapy, although such information might be useful for determining changes in treatment strategy when the histological response to chemotherapy is expected to be poor.

In the current study, we compared the utility of combined metabolic/volume indices versus metabolic indices for predicting treatment response in children and young adults with high-grade osteosarcoma after one course and on completion of neoadjuvant chemotherapy. We found that both of these indices were useful for this purpose.

## Materials and methods

### Patients and study design

From August 2003 to July 2010, 20 children and young adults with histologically confirmed and resectable high-grade osteosarcoma were enrolled. Six patients were enrolled retrospectively from 2003 to 2006, and 14 prospectively from 2007 to 2010. All patients underwent a diagnostic biopsy and a pretherapy PET/CT scan before neoadjuvant chemotherapy was initiated. Patients received two to four courses of neoadjuvant chemotherapy, which consisted of various combinations of doxorubicin, cisplatin, high-dose methotrexate, ifosfamide and etoposide. They then underwent a posttherapy PET/CT scan before definitive surgery. The 14 prospectively recruited patients received an additional (interim) PET/CT scan between the first and second course (except one who underwent an interim scan after two courses) of neoadjuvant chemotherapy. The pretherapy PET/CT scans were performed within 0–3 weeks before the initiation of neoadjuvant therapy, and the posttherapy PET/CT scans within 0–3 weeks before tumour resection. The removed tumour specimens were histopathologically examined to determine necrosis fractions using a conventional mapping method described previously [16, 17].

The National Cancer Center Institutional Review Board (NCC IRB) approved the study. Informed consent was obtained from the prospectively enrolled patients and/or their parents and was waived for the retrospectively enrolled patients by the IRB. The study was performed in compliance with the ethical guidelines of the NCC IRB.

### $^{18}$F-FDG PET/CT

Whole-body FDG PET/CT was performed using a combined PET/CT scanner (Biograph LSO; Siemens Medical Solutions, Hoffman Estates, IL). After an 8-h fasting period followed by blood sugar testing to confirm that the glucose

value was <120 mg/dl, 166.5–666 MBq (4.5–18 mCi) of $^{18}$F-FDG was injected intravenously, and patients were encouraged to rest during this period. PET/CT scanning was performed from the middle of the skull to the upper thigh 60 min after injection, and this was followed by an additional PET/CT scan of the lower extremities.

During the PET/CT scans, spiral CT was performed using the following parameters: a scout view at 30 mA and 130 kVp, followed by a spiral CT scan with effective mA of 50, 130 kVp, 5 mm section width, 4 mm collimation, 12 mm table feed per rotation, and 0.8 s per rotation with arms raised. PET images were acquired after the CT scans with 3 min per bed position (11.2-cm increments, three-dimensional mode). CT images were reconstructed onto a 512 × 512 matrix, and were converted using equivalent attenuation factors of 511 keV for attenuation correction. PET images were reconstructed onto a matrix of 128 × 128 using the ordered-subsets expectation maximization algorithm, and attenuation correction was also performed.

PET, PET/CT and CT images were reviewed using a dedicated workstation and software (E.soft; Siemens Medical Solutions), which allowed three-dimensional displays (transaxial, coronal and sagittal) to be constructed using CT, PET and PET/CT images and maximum intensity projection displays of the PET data.

## Magnetic resonance imaging

All patients underwent MR imaging before and after neoadjuvant chemotherapy. All pretherapy MR images, except those for two patients, were acquired within 3 weeks of the PET/CT scans and therapy initiation. MR images were obtained using a Signa 1.5-T unit (GE Medical Systems, Milwaukee, WI) in different planes (axial, coronal and sagittal/oblique along the axes of the long bones). T1-weighted imaging was performed using a repetition time of 400 ms and an echo time of 10 ms, T2-weighted imaging using a repetition time of 4,000 ms and an echo time of 73 ms, and contrast-enhanced T1-weighted imaging using a repetition time of 500 ms and an echo time of 20 ms after the administration of gadopentate dimeglumine (Gd-DTPA, Magnevist; Schering, Berlin, Germany). Images were obtained at the same 24 levels after contrast enhancement. The section thickness was 4 mm with one data acquisition and a 512 × 512 acquisition matrix.

## Data analysis

### *Determination of standardized uptake values, metabolic tumour volumes and total lesion glycolysis*

SUVs were calculated as follows: SUV=(decay-corrected activity in kilobecquerels per millilitre of tissue)/(injected $^{18}$F-FDG activity in kilobecquerels per body mass in grams). The SUV of a lesion was obtained by placing regions of interest (ROIs) manually around the lesion, and the maximum SUV (mSUV) within an ROI was used to minimize partial-volume effects. Volumes of interest (VOIs) were drawn around tumours with a substantial margin to include normal adjacent tissue. Metabolic tumour volumes (MTVs) were calculated as described by Biehl et al. [18]. First, MTVs were calculated by summing the volume of voxels that had an SUV higher than a certain threshold SUV within a given VOI. MTV(1.5), MTV(2), MTV(2.5) and MTV(3) were obtained using threshold SUVs of 1.5, 2.0, 2.5 and 3.0 g/ml, respectively. MTVs were automatically calculated using Osirix medical imaging software (The Osirix Foundation, Geneva, Switzerland). Total lesion glycolysis (TLG) values were calculated by multiplying the MTVs by mean MTV SUVs [19]. TLGs corresponding to MTVs were calculated, i.e. TLG(1.5), TLG(2), TLG(2.5) and TLG(3.0). mSUV, MTV and TLG ratios (rSUV, rMTV, rTLG) were calculated by dividing interim or postchemotherapy values by prechemotherapy values.

### *Determination of tumour volumes*

Tumour volumes were defined as the sum of enhanced areas on MR images, because the 3-D volume of irregularly shaped tumours could not be measured reliably. MR volumes (MRV) were obtained by manually drawing ROIs around Gd-enhanced areas on axial T1-weighted sequence images (Osirix medical imaging software). Only pretherapy MR images obtained within 3 weeks before PET/CT scanning were evaluated. MRVs were determined before and after neoadjuvant chemotherapy.

MRVs and MTVs before neoadjuvant chemotherapy were compared using Spearman's correlation coefficients and visual assessment. Pretherapy PET/CT, MR and PET/MR fusion images were visually assessed by two nuclear medicine physicians independently. PET/MR fusion was performed using a three-point method (GE Medical Systems).

## Pathological assessment

After surgery, excised specimens were cut longitudinally in the plane deemed most likely to reveal residual viable tumour, and tumour necrosis fractions were then defined as the percentage of devitalized parts of the tumour in the examined planes as determined histologically. Pathological good responders (GRs) and poor responders (PRs) were differentiated using the grading system proposed by Salzer-Kuntschik et al. [6]: grades I–III (necrosis fraction ≥90%) were considered GRs, and grades IV–VI (necrosis fraction <90%) PRs.

### Immunohistochemical staining for glucose transporters

As increased expression of glucose transporters has been reported in many human cancers [20], we investigated the expression status of glucose transporters 1 and 3 (Glut1 and Glut3) in the osteosarcoma tissues and its relationship to PET/CT indices. Immunohistochemical staining was performed using immunoperoxidase detection techniques, with diaminobenzidine as the chromogen. Sections of 4 μm from formalin-fixed, paraffin-embedded tissues obtained by biopsy and surgical resection were placed in tissue arrays and processed using a previously reported procedure [21]. The primary antibodies used were rabbit polyclonal anti-human Glut1 (1:1,000 dilution; Chemicon International, Temecula, CA) and Glut3 antibody (1:500 dilution; Chemicon). Signals were detected using an EnVision kit (Dako, Carpinteria, CA). Positive staining of the red blood cells served as an internal positive control for expression of Glut1. Human testes were used as positive controls for Glut3. Parallel sections incubated with rabbit IgG instead of the primary antibodies were used as negative controls. Sections with tumour cells that demonstrated membranous staining were considered positive for Glut1 and Glut3.

The overall staining result was scored from 0 to 4 based on the intensity and positive rate of staining [22]. The intensity of staining was categorized as negative, weak, moderate or strong. The proportion of positively stained cells was graded as: 0, 0%; 1, 1–10%; 2, 11–50%; 3, 51–100%. All stained sections were reviewed by two experienced pathologists who were blind to the PET/CT data.

### Statistical analysis

Statistical analyses were performed using MedCalc for Windows, version 9.4.2.0 (MedCalc Software, Mariakerke, Belgium). Correlation analysis was performed to compare histopathological necrosis fractions and posttherapy mSUV, MTV and TLG, as well as post-to pretherapy rSUV, rMTV, rTLG and rMRV. For these parameters, *t*-tests were performed to determine their ability to discriminate pathological GRs and PRs in the prospective cohort. Additionally, a subanalysis was performed using prospectively obtained information from pretherapy and interim PET/CT imaging. Of the above parameters, those predicting histological response better ($|r| > 0.5$, $P < 0.05$) were chosen, and receiver operating characteristic (ROC) curve analysis with respect to histological response prediction was performed. Area under the curve values (AUCs) were calculated to determine the best predictor cut-off value for each parameter. Then patients were grouped using these cut-off values, and sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated. Correlations between various PET/CT indices and the expression of glucose transporters were assessed using the nonparametric Spearman's rank test. All $P$ values were derived from the two-sided test, and values less than 0.05 were considered significant.

## Results

### Patients

Patient age and sex, histological subtypes, primary tumour sites, intervals between pretherapy and interim or post-therapy PET scans, numbers of chemotherapy courses during the various intervals, types of surgery and histological responses are presented in Table 1. The median age of the patients was 15 years (range 10–25 years), and there were equal numbers of male and female patients. According to the American Joint Committee on Cancer (AJCC) staging system [23], seven patients had a stage IIA tumour, six stage IIB tumour, three stage III tumour with skip metastases, two stage IVA tumour with lung metastases, and two stage IVB tumour with remote metastases in the mandible and spine, respectively. All tumours included in this study were high-grade with 95% of them being osteoblastic. All patients had primary diseases except one with osteosarcoma in the mandible, which was the only site of relapse from the femoral primary tumour. A variety of chemotherapeutic regimens were used; AOST 0331 (cisplatin, doxorubicin, methotrexate) in eight patients, ISG/SSG 1 (cisplatin, doxorubicin, methotrexate, ifosfamide) in seven, CCG 7921 regimen B (ifosfamide, doxorubicin, methotrexate) in two, and miscellaneous regimens in three. Each course of AOST 0331 and CCG 7921 regimen B took at least 5 weeks, and each course of ISG/SSG 1 at least 6 weeks. The median number of neoadjuvant chemotherapy courses was two (two in 15 patients, three in 4, and four in 1). As already mentioned, interim PET/CT scans were additionally obtained in the 14 prospectively recruited patients. The number of chemotherapy courses between pretherapy and interim PET scanning was one in 13 patients and two in 1 patient, and that between interim scanning and surgery was one in 11 patients and two in 3 patients. All patients underwent surgical resection of their tumours after chemotherapy. Nine patients were GRs and 11 were PRs.

### Comparison between pretherapy MRVs and MTVs

MTV(1.5) and MTV(3) could not be obtained. The SUV threshold of 1.5 g/ml could not clearly discriminate between tumour and neighbouring normal tissue, and the SUV threshold of 3 g/ml produced tumour areas that were

**Table 1** Patient characteristics, timing of PET, and histological response

| Variable | Value |
|---|---|
| Age (years) | |
| Median | 15 |
| Range | 10–25 |
| Sex, *n* (%) | |
| Male | 10 (50) |
| Female | 10 (50) |
| Histological subtype, *n* (%) | |
| Osteoblastic | 19 (95) |
| Chondroblastic | 1 (5) |
| Location, *n* (%) | |
| Femur | 12 (60) |
| Tibia | 3 (15) |
| Humerus | 3 (15) |
| Pelvis | 1 (5) |
| Mandible[a] | 1 (5) |
| Time from pretherapy to posttherapy PET (weeks) | |
| Median | 14.9 |
| Range | 10.6–21.6 |
| Time from posttherapy PET to surgery (weeks) | |
| Median | 0.9 |
| Range | 0–2.9 |
| Number of therapy courses from pretherapy to posttherapy PET | |
| Median | 2 |
| Range | 2–4 |
| Time from pretherapy to interim PET (weeks) | |
| Median | 7.8 |
| Range | 5.7–12 |
| Time from interim PET to surgery (weeks) | |
| Median | 8.1 |
| Range | 4.6–13.6 |
| Number of therapy courses from pretherapy to interim PET | |
| Median | 1 |
| Range | 1–2[a] |
| Number of therapy courses from interim PET to surgery | |
| Median | 1 |
| Range | 1–2 |
| Type of surgery, *n* (%) | |
| Limb salvage | 19 (95) |
| Amputation | 1 (5) |
| Tumour necrosis fraction (%) | |
| Median | 82.5 |
| Range | 3–99 |
| Histological response, *n* (%) | |
| Good | 9 (45) |
| Poor | 11 (55) |

[a] The only patient who presented with recurrent lesion solely in the mandible, and received two chemotherapy courses before interim PET scanning

too small when compared to real tumour masses. MTV(2) and MTV(2.5) could be calculated in all patients and appeared to represent tumour areas well.

Pretherapy MTVs at various SUV thresholds were compared with pretherapy MRVs to see if the former could substitute for MRVs, and MTV(2) showed the best correlation with tumour volume ($r$=0.91, $P$ <0.05; Fig. 1). MTV(2.5) also showed a significant correlation ($r$=0.86, $P$<0.05). Images of MTV(2) and MR before treatment (Fig. 2a, b) were visually well matched in all patients (Fig. 2c).

## Comparisons between mSUV, MTV, TLG and MRV values before, during and after therapy vs. necrotic fractions in the entire and prospective cohort

Various PET/CT parameters, MRV and necrosis fractions for the entire cohort are presented in Table 2. Six patients (patients 1–6) were retrospectively enrolled, and 14 (patients 7–20) were prospectively enrolled. Only one patient (patient 4) had a posttherapy mSUV that was higher than the pretherapy mSUV (Fig. 3a). Unexpectedly, the necrosis fraction in the removed tumour specimen in this patient was 99% (Fig. 3c). Unlike mSUV, posttherapy MTV(2.5) and TLG(2.5) showed significant reductions from their pretherapy values (690.3 ml to 113.5 ml; 3,043.7 g to 396 g) in this patient (Fig. 3b). Patient 7 was the only one whose posttherapy MTVs and TLGs were higher than the pretherapy values (Table 2). Only 3% of the patient's tumour specimen was necrotic. The mean (±SD) rMRV was 0.93 (±0.28), and posttherapy MRVs were greater than pretherapy values in 6 of 18 patients (two were excluded from the tumour volume analysis because their pretherapy MR images were acquired more than 3 weeks before PET/CT scanning). MRVs were not different before and after chemotherapy.

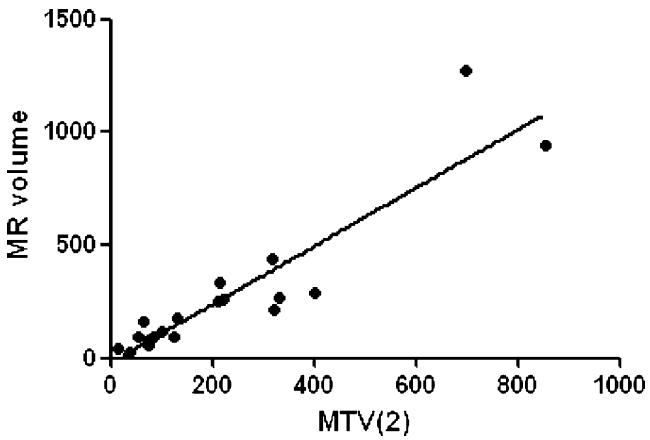


**Fig. 1** MTV at SUV threshold of 2 g/ml (*MTV(2)*) and MR volume before therapy are closely correlated ($r$=0.91, $P$ <0.05)

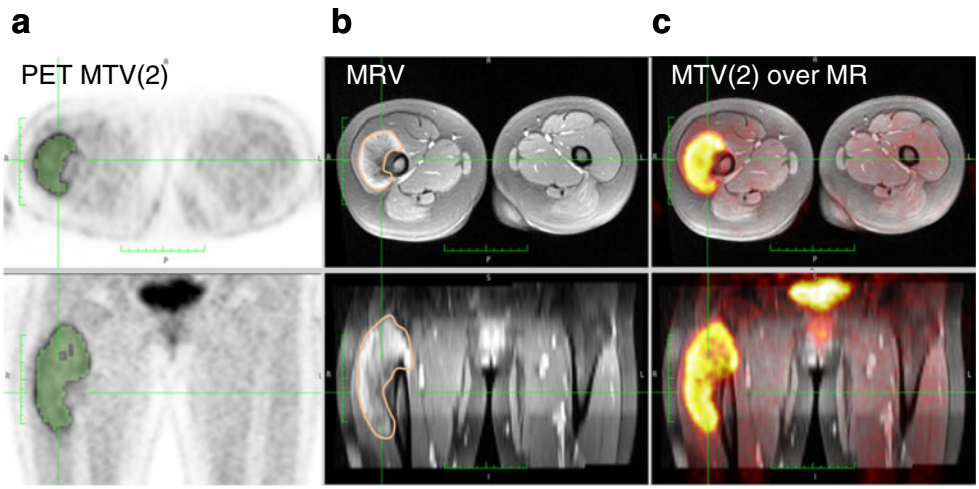


**Fig. 2** PET, MR and PET/MR fusion images in patient 1. **a** Attenuation-corrected axial and coronal PET image of a tumour in the right proximal femur. The green-coloured ROI represents a collection of voxels with SUVs ≥2 g/ml automatically drawn by Osirix. **b** Gadolinium-enhanced T1-weighted axial and coronal MR images of the same slice as shown in **a**, which was registered to the appropriate PET/CT image using a GE workstation. The pink ROI was drawn manually around the gadolinium-enhanced area. **c** Fused images of **a** and **b**. The tumour boundaries are well matched in the axial and coronal views

Correlations between various PET/CT parameters and the necrosis fraction are presented in Table 3. For the entire cohort, posttherapy mSUV showed a weaker linear relationship with necrosis fraction ($|r|<0.5$, $P<0.05$), whereas rMTV(2) and rTLG(2) showed a stronger relationship ($|r|>0.75$, $P<0.05$). rMTV(2.5) and rTLG(2.5) also had a stronger relationship (data not shown). However, rMRV did not reflect tumour necrosis fraction.

All PET/CT parameters from the prospective cohort were associated with necrotic fractions ($|r|>0.5$, $P<0.05$), with correlations similar to those obtained for the entire cohort, except posttherapy mSUV, rSUV, MTV(2) and TLG (2). Of all prospective parameters, interim to pretherapy rTLG(2) showed the best correlation with necrotic fractions ($r=0.82$, $P=0.0004$).

## Prediction of histological response – ROC curve analysis in the prospectively enrolled patients

In the prospective cohort, five patients were classified as GRs, and nine as PRs. The five GRs were compared with the nine PRs with respect to various PET/CT parameters and MRV. Posttherapy mSUV, MTV(2) and TLG(2), as well as post- to pretherapy rMTV(2) and rTLG(2), showed differences between GRs and PRs ($P<0.05$), while rSUV showed a trend towards a difference ($P=0.09$). However, posttherapy MRV and rMRV were not different between the two groups.

Interim mSUV, MTV(2) and TLG(2), as well as interim to pretherapy rSUV, rMTV(2) and rTLG(2), all showed differences between GRs and PRs ($P<0.05$).

In the ROC curve analysis for all the parameters that had shown moderate correlations ($|r|>0.5$, $P<0.05$) with histological response in the prospective cohort (Table 3), all parameters except post- to pretherapy rSUV nicely predicted histological response (AUC 0.867–0.956; $P\leq0.0005$; ROC curves not shown). rSUV showed a trend towards prediction of response (AUC 0.756; $P=0.081$). In the same analysis with parameters acquired before therapy and in the interim, all parameters, including interim to pretherapy rSUV, predicted histological response (AUC 0.867–0.956; $P\leq0.0004$).

Sensitivity, specificity, PPV, NPV and accuracy of these parameters at each cut-off value determined in the ROC curves were calculated. Of the parameters obtained before and after therapy, posttherapy mSUV at a cut-off of 3 g/ml showed the highest diagnostic index, with 100% sensitivity, 88.9% specificity, 83.3% PPV, 100% NPV and 92.9% accuracy (Table 4). The specificity and PPV of post-therapy MTV(2) were superior to those of mSUV, albeit its sensitivity and NPV were lower.

Of the parameters obtained before therapy and in the interim, diagnostic indices for interim mSUV, MTV(2) and TLG(2) were equally high, with 100% sensitivity, 88.9% specificity, 83.3% PPV, 100% NPV and 92.9% accuracy (Table 4).

## Expression of Glut1 and Glut3 in the biopsy and surgical specimens vs. PET/CT indices

Paired pretherapy and posttherapy tumour specimens were available in eight patients. Five of the eight pretherapy specimens (62.5%) showed positive Glut1 immunostaining, while seven of eight (87.5%) were positive for Glut3 (see the Table S1 in the Electronic supplementary material). The

**Table 2** PET/CT parameters, MRVs before and after chemotherapy, and histological response for the entire cohort

| Patient no. | mSUV (g/ml) | | | MTV(2) (ml) | | | TLG(2) (g) | | | MRV (ml) | | Necrosis fraction (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pretherapy | Interim | Posttherapy | Pretherapy | Interim | Posttherapy | Pretherapy | Interim | Posttherapy | Pretherapy | Posttherapy | |
| 1 | 8.12 | NA | 2.25 | 219.5 | NA | 0.5 | 714.6 | NA | 0.9 | 259.5 | 252.2 | 80 |
| 2 | 6.8 | NA | 2.24 | 401.5 | NA | 0.64 | 1,194.4 | NA | 1.33 | NA | NA | 99 |
| 3 | 16.47 | NA | 7.7 | 329.7 | NA | 320.9 | 1,278.3 | NA | 971.8 | 265.4 | 309.1 | 20 |
| 4 | 10.39 | NA | 11.84 | 854.6 | NA | 313.1 | 3,411.6 | NA | 835.1 | 940.6 | 1,526.5 | 99 |
| 5 | 6.62 | NA | 2.3 | 695.8 | NA | 0.89 | 1,994.3 | NA | 1.86 | NA | NA | 95 |
| 6 | 4.03 | NA | 2.57 | 35.5 | NA | 3.2 | 92.4 | NA | 7 | 29.4 | 29.8 | 97 |
| 7 | 14.23 | 13.7 | 9.4 | 74.55 | 110.2 | 126.6 | 380.9 | 535.8 | 543.9 | 77.9 | 85 | 3 |
| 8 | 12.05 | 7.5 | 3.11 | 319.8 | 28.8 | 5.82 | 1,363 | 73.6 | 13 | 215.53 | 232.8 | 85 |
| 9 | 7.74 | 6.7 | 4.95 | 99.8 | 62 | 64.9 | 369.7 | 162.5 | 163.1 | 117.1 | 44.2 | 70 |
| 10 | 7.2 | 2.4 | 2.4 | 211.3 | 3 | 3 | 663.2 | 6.5 | 6.4 | 252.2 | 214.8 | 40 |
| 11 | 7.86 | 9.6 | 5 | 73.4 | 25.9 | 29.2 | 200.5 | 87.5 | 91.5 | 59.4 | 48.1 | 5 |
| 12 | 7.82 | 5.4 | 5.4 | 122.8 | 122.5 | 122.5 | 453 | 348.5 | 344.2 | 95.2 | 123.6 | 25 |
| 13 | 4.6 | 3 | 3 | 53.6 | 6.4 | 6.4 | 154.5 | 14.1 | 14.1 | 92.3 | 51.3 | 90 |
| 14 | 7.0 | 3.2 | 1.2 | 84.4 | 5.5 | 0 | 168.0 | 13.4 | 0 | 98.6 | 77.4 | 95 |
| 15 | 9.3 | 5.6 | 5.0 | 62.5 | 88 | 33.8 | 175.1 | 234.9 | 81 | 165.2 | 155.4 | 10 |
| 16 | 9.4 | 2.2 | 3.0 | 315.8 | 0.2 | 35.1 | 1,165.3 | 0.5 | 77.6 | 440.3 | 434.5 | 99 |
| 17 | 5.1 | 3.8 | 4.5 | 130.8 | 140.9 | 100.8 | 431.6 | 361.9 | 260.1 | 178.7 | 167.9 | 40 |
| 18 | 8.9 | 7.4 | 5.6 | 212.8 | 317 | 182.1 | 685.2 | 962.4 | 466.2 | 337.0 | 321.5 | 40 |
| 19 | 3.2 | 1.8 | 1.3 | 135. | 0 | 0 | 31.7 | 0 | 0 | 39.7 | 34.3 | 97 |
| 20 | 3.9 | 1.9 | 1.9 | 8.9 | 0 | 0 | 21.6 | 0 | 0 | 55.5 | 22.7 | 95 |
| Mean | 8.0 | 5.3 | 4.2 | 216 | 65.0 | 67.5 | 747.4 | 200.1 | 194 | 264.1 | 277.1 | 64 |
| SD | 3.4 | 3.4 | 2.8 | 224.7 | 88.3 | 100.5 | 825.4 | 277.4 | 293.5 | 313.3 | 383.5 | 37 |

*NA* not assessed

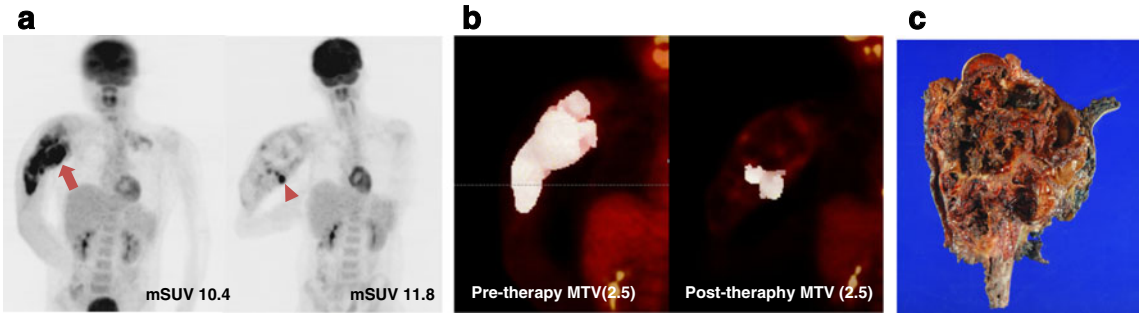


**Fig. 3** $^{18}$F-FDG PET scans acquired before and after neoadjuvant chemotherapy in patient 4. **a** Despite a profound decrease in overall $^{18}$F-FDG accumulation in the right upper arm lesion after chemotherapy, the maximum SUV showed a slight increase. **b** Significant reduction in MTV at a SUV threshold of 2.5 g/ml (*MTV(2.5)*) is evident after chemotherapy. Pretherapy and posttherapy MTV(2.5) values were 690.3 ml and 113.5 ml, respectively. **c** The removed tumour specimen of patient 4 was almost completely composed of necrotic tissue (99%)

extent of immunoreactivity varied from 5% to 70% for Glut1 and 70% to 95% for Glut3. In contrast, Glut1 immunostaining was negative in all the posttherapy specimens, and Glut3 immunostaining was positive in only three of eight (37.5%). When Glut1 or Glut3 expression (in terms of positive rate, intensity or staining score) in the pretherapy specimens was compared with pretherapy PET/CT indices, such as mSUV, MTV(2) or TLG(2) values, no significant correlation was found (Table 2 and supplementary Table S1). However, posttherapy mSUV, MTV(2) and TLG(2) values in the three Glut3-positive and five Glut3-negative cases showed a difference: 5.4–9.4 g/ml vs. 1.2–4.95 g/ml, 122.5–182.1 ml vs. 0–100.8 ml, 344.2–543.9 g vs. 0–260.1 g, respectively.

## Discussion

Of the many FDG PET/CT indices, mSUV and TBR have been most commonly used to evaluate tumour response. A few studies have suggested that TBR and SUV are good parameters for predicting the response of osteosarcoma to chemotherapy [12–14]. Ye et al. [12] compared mSUV and TBR in this context, and concluded that TBR is superior to mSUV for estimating histological necrosis in osteosarcoma. However, TBR measures tend to be nonreproducible and appear to be less practical, because interpreters have to draw ROIs manually along tumour boundaries and in the corresponding contralateral normal areas. Very recent studies evaluating the usefulness of SUV in predicting response to neoadjuvant chemotherapy have shown that post- to pretherapy mSUV ratio (or percent change in mSUV) and posttherapy mSUV are correlated with histological response [24–26]. Furthermore, progression-free survival is associated with posttherapy mSUV [24, 27].

In 1999, Larson et al. [19] introduced TLG as a semiquantitative index of tumour treatment response. Nevertheless, previous studies have failed to demonstrate that TLG is comparable or superior to mSUV or mean SUV

**Table 3** Correlation between PET/CT parameters and pathological necrosis fractions

| Parameter | | Entire cohort ($n$=20) | | Prospective cohort ($n$=14) | |
|---|---|---|---|---|---|
| | | $\lvert r \rvert$ | $P$ value | $\lvert r \rvert$ | $P$ value |
| mSUV | Posttherapy | 0.47 | 0.04 | 0.78 | 0.0001 |
| | Post-/pretherapy ratio | 0.26 | 0.27 | 0.56 | 0.04 |
| | Interim | NA | – | 0.76 | 0.0017 |
| | Interim/pretherapy ratio | NA | – | 0.69 | 0.0064 |
| MTV(2) | Posttherapy | 0.35 | 0.13 | 0.55 | 0.04 |
| | Post-/pretherapy ratio | 0.76 | 0.0001 | 0.72 | 0.004 |
| | Interim | NA | – | 0.70 | 0.0053 |
| | Interim/pretherapy ratio | NA | – | 0.79 | 0.0008 |
| TLG(2) | Posttherapy | 0.41 | 0.07 | 0.61 | 0.02 |
| | Post-/pretherapy ratio | 0.80 | <0.0001 | 0.77 | 0.001 |
| | Interim | NA | – | 0.77 | 0.0014 |
| | Interim/pretherapy ratio | NA | – | 0.82 | 0.0004 |
| MRV | Post-/pretherapy ratio | 0.15 | 0.52 | 0.44 | 0.11 |

*NA* not assessed

**Table 4** Diagnostic indices of various PET/CT parameters in the prospective cohort ($n$=14)

| Parameter | | Cut-off | AUC | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | Accuracy (%) |
|---|---|---|---|---|---|---|---|---|
| mSUV | Posttherapy | 3 g/ml | 0.956 | 100 | 88.9 | 83.3 | 100 | 92.9 |
| | Post-/pretherapy ratio | 0.487 | 0.756 | 80 | 77.8 | 66.7 | 87.5 | 78.6 |
| | Interim | 3.2 g/ml | 0.956 | 100 | 88.9 | 83.3 | 100 | 92.9 |
| | Interim/pretherapy ratio | 0.563 | 0.867 | 80 | 88.9 | 80 | 88.9 | 85.7 |
| MTV(2) | Posttherapy | 0 | 0.867 | 60 | 100 | 100 | 81.8 | 85.7 |
| | Post-/pretherapy ratio | 0.119 | 0.911 | 100 | 77.8 | 71.4 | 100 | 85.7 |
| | Interim | 6.4 ml | 0.956 | 100 | 88.9 | 83.3 | 100 | 92.9 |
| | Interim/pretherapy ratio | 0.119 | 0.933 | 100 | 77.8 | 71.4 | 100 | 85.7 |
| TLG(2) | Posttherapy | 77.6 g | 0.911 | 100 | 77.8 | 71.4 | 100 | 85.7 |
| | Post-/pretherapy ratio | 0.091 | 0.911 | 100 | 77.8 | 71.4 | 100 | 85.7 |
| | Interim | 14.1 g | 0.956 | 100 | 88.9 | 83.3 | 100 | 92.9 |
| | Interim/pretherapy ratio | 0.091 | 0.911 | 100 | 77.8 | 71.4 | 100 | 85.7 |

in predicting treatment response in bone and soft-tissue sarcomas, although it is a conceptually attractive parameter. Recently, Benz et al. [15] compared various indices of tumour response, including SUV and CT-based TLG, in soft-tissue sarcomas, and concluded that maximum or mean TLG is less predictive of tumour response than maximum or mean SUV. However, the situation might be quite different in osteosarcoma, a tumour that does not shrink to a great extent after neoadjuvant chemotherapy [27, 28]. In a study evaluating the predictability of TLG for histological response to chemotherapy in patients with osteosarcoma, good responses were weakly associated with low pretherapy and posttherapy TLG, but not with change in TLG [24]. In that study, a threshold of 45% mSUV in the VOI was used to determine TLG, which is much higher than thresholds used in the present study. Application of 45% mSUV to our series would have resulted in tumour areas that were much smaller than the real tumour masses. Instead, we tested several TLG thresholds to determine suitable indices. Moreover, MTVs, which were concordant with pretherapy MRV, were used to determine tumour volume, as the measurement of tumour volume on CT and MRI images after therapy probably overestimates remaining viable tumour portions because appreciable size changes may not be evident even in patients with osteosarcoma with a good treatment response as demonstrated also in the current study (Table 2).

In the present study, post- to pretherapy rMTV(2) and rTLG(2) as well as posttherapy mSUV in the entire cohort correlated with treatment-induced tumour necrosis fractions, but rSUV did not (Table 3). Recent studies have repeatedly shown that posttherapy mSUV and rSUV or percent change in mSUV are indicators of good histological response [24–27]. When data acquired from prospectively recruited patients were analysed separately, rSUV gained statistical significance, suggesting that bias was potentially introduced into our retrospectively obtained data. However, except for this, much of our prospective data were similar to those from the entire cohort (Table 3). Separate analysis using the prospective data revealed that posttherapy mSUV, MTV(2) and TLG(2), and post- to pretherapy rMTV(2) and rTLG(2) showed differences between GRs and PRs. rSUV showed a trend towards a difference between the two groups, whereas post-therapy MRV and rMRV showed no difference. These findings indicate that combined metabolic/volumetric indices, like metabolic indices, could be useful predictors of pathological response to chemotherapy in osteosarcoma. The sensitivity, specificity, PPV and NPV for predicting a good response were 100%, 88.9%, 83.3% and 100%, using a posttherapy mSUV cut-off of 3 g/ml (Table 4). The PPV and NPV were comparable to those reported previously [12–14].

We additionally obtained interim PET/CT information from prospectively recruited patients after only one chemotherapy course (except one patient), at the median time of 7.8 weeks after the initiation of neoadjuvant chemotherapy (Table 1). Interim mSUV, MTV(2) and TLG(2), and interim to pretherapy rSUV, rMTV(2) and rTLG(2) all showed differences between GRs and PRs. The sensitivity, specificity, PPV and NPV for predicting a good histological response using interim mSUV, MTV(2) and TLG(2) with certain cut-off values were equally high (100%, 88.9%, 83.3% and 100%, respectively). Thus, it can be stated that as with posttherapy analysis, combined metabolic and volumetric indices as well as metabolic indices might be useful indicators of a good histological response in interim analysis. To our knowledge, this is the first report addressing the use of early PET/CT indices for predicting histological response.

Although Glut1 and Glut3 were expressed in the majority of eight pretherapy tumour specimens, their expression was not correlated with pretherapy mSUV, MTV(2) or TLG(2) values (Table 2 and supplementary Table S1).

Taken together, these findings have several implications. First, we demonstrated that posttherapy MTVs and TLGs, and post- to pretherapy rMTVs and rTLGs with certain thresholds were comparable to posttherapy mSUV and post- to pretherapy rSUV in evaluating treatment response in our osteosarcoma patient population. A recent large series study has also documented the usefulness of a combined metabolic and volume index, the metabolic volume change ratio, with respect to predicting histopathological tumour response [25]. Second, we demonstrated that interim MTVs and TLGs, and interim to pretherapy rMTVs and rTLGs, as well as interim mSUV and interim to pretherapy rSUV, acquired early after one course of neoadjuvant chemotherapy could discriminate GRs from PRs, possibly facilitating earlier modifications to treatment strategy. Third, MTV seems to represent viable tumour volumes, especially in the posttherapy state of osteosarcoma, better than CT or MRI. MTV is based on tumour metabolism and thus provides information on tumour viability. The current study also showed that changes in MR-based tumour volumes failed to discriminate between GRs and PRs. We conveniently calculated tumour volumes using MTVs with certain thresholds, which matched those on pretherapy MR images within ROIs. In this way, acquisition of tumour volumes from posttherapy MR images, which might be greater than the remaining viable lesion, was avoided. This has another advantage of overcoming the difficulty in determining the volume of the lesion from PET images due to limited resolution. In the current study, convenient calculation of MTV(2), MTV(2.5), TLG(2) and TLG(2.5) on a workstation avoided the possible introduction of bias in delineating viable tumour volumes on each PET and posttherapy MR image. Fourth, in situations where target lesions show heterogeneous responses to treatment with heterogeneous FDG uptake, MTV and TLG appear to match the whole tumour burden better than mSUV. Histopathological tumour response (the gold standard) is measured two-dimensionally, and reflects whole viable tumour burden rather than the residual most active regions, which are more likely to be reflected by mSUV. One of our patients exemplified this situation. The patient had a slight increment in mSUV after treatment (10.39 g/ml to 11.84 g/ml), although the necrosis rate of the tumour was 99% (Table 2, patient 4). In contrast, posttherapy MTV (2.5) and TLG(2.5) were markedly reduced by 83.6% and 87.0%, respectively (Fig. 3b). Thus, MTV and TLG might supplement mSUV in this particular situation.

The present study had several inherent limitations. First, our results were based on data obtained retrospectively and prospectively. We thus subanalysed the prospectively recruited group separately, yielding similar results. Second, diverse chemotherapeutic regimens were utilized precluding survival analysis. Although no uniform regimen was utilized in a study analysing PET response and outcome [27], uneven distribution of other prognostic factors, such as tumour size, location, stage and presence of metastasis in our series divided according to PET/CT indices, invalidated survival analysis. Third, even though only children and young adults were enrolled, our results based on a small patient population require confirmation in a larger cohort.

## Conclusion

Combined metabolic/volumetric indices, including MTV, TLG, rMTV and rTLG, with thresholds within certain ranges, as well as metabolic indices, including mSUV and rSUV, could be useful predictors of histological response to neoadjuvant chemotherapy in osteosarcoma. Moreover, these PET/CT indices were able to discriminate GRs from PRs even after one chemotherapy course with high predictability in our osteosarcoma patient population.

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**Conflicts of interest** None.

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