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ORIGINAL ARTICLE

Prediction of neoadjuvant radiation chemotherapy response and survival using pretreatment [^{18}F]FDG PET/CT scans in locally advanced rectal cancer

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Abstract
Purpose The aim of this study was to investigate metabolic and textural parameters from pretreatment [^{18}F]FDG PET/CT scans for the prediction of neoadjuvant radiation chemotherapy response and 3-year disease-free survival (DFS) in patients with locally advanced rectal cancer (LARC).
Methods We performed a retrospective review of 74 patients diagnosed with LARC who were initially examined with [^{18}F]FDG PET/CT, and who underwent neoadjuvant radiation chemotherapy followed by complete resection. The standardized uptake value (mean, peak, and maximum), metabolic volume (MV), and total lesion glycolysis of rectal cancer lesions were calculated using the isocontour method with various thresholds. Using three-dimensional textural analysis, about 50 textural features were calculated for PET images. Response to neoadjuvant radiation chemotherapy, as assessed by histological tumour regression grading (TRG) after surgery and 3-year DFS, was evaluated using univariate/multivariate binary logistic regression and univariate/multivariate Cox regression analyses.
Results MVs calculated using the thresholds mean standardized uptake value of the liver + two standard deviations (SDs), and mean standard uptake of the liver + three SDs were significantly associated with TRG. Textural parameters from histogram-based and co-occurrence analysis were significantly associated with TRG. However, multivariate analysis revealed that none of these parameters had any significance. On the other hand, MV calculated using various thresholds was significantly associated with 3-year DFS, and MV calculated using a higher threshold tended to be more strongly associated with 3-year DFS. In addition, textural parameters including kurtosis of the absolute gradient (GrKurtosis) were significantly associated with 3-year DFS. Multivariate analysis revealed that GrKurtosis could be a prognostic factor for 3-year DFS.
Conclusion Metabolic and textural parameters from initial [^{18}F]FDG PET/CT scans could be indexes to assess tumour heterogeneity for the prediction of neoadjuvant radiation chemotherapy response and recurrence in LARC.

Keywords Tumour heterogeneity · Prognostic parameter · Positron-emission tomography · ^{18}F-Fluorodeoxyglucose · Tumour stage · Metabolic volume

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Introduction

Neoadjuvant radiation chemotherapy (nRCT) in patients with locally advanced rectal cancer (LARC, clinical stage T3/4 with or without lymph node metastasis) has improved the local control rate and overall survival [1–3]. Complete or

near-complete response to nRCT is associated with better outcomes [4–6]. Therefore, predicting tumour response during the initial staging assessment of LARC before the start of nRCT may have a great impact on patient management. Several imaging modalities are currently used for staging and monitoring the response to nRCT in patients with LARC. Together with conventional CT, MRI has been shown to be effective for local staging of rectal cancer by accurately determining the T4 stage with circumferential resection margin involvement [7]. However, these imaging modalities based on morphology fail in the evaluation of post-nRCT state [8, 9].

There has been several effort to predict tumour response in patients with LARC using [^{18}F]FDG PET/CT functional molecular imaging [10–14]. Early changes in glucose metabolic parameters such as maximum standardized uptake value (SUVmax) assessed using sequential [^{18}F]FDG PET/CT scans during nRCT could predict response to the therapy. Reassessment of stages using [^{18}F]FDG PET/CT after nRCT has been shown to be beneficial in subgrouping of patients and in predicting outcome.

Recently in oncology, textural analysis, a mathematical method used to determine the relationships among pixels in medical images, has been applied to [^{18}F]FDG PET imaging [15]. Possible applications of the assessment of intratumoral heterogeneity have been evaluated for oesophageal and lung cancer [16, 17]. Also, assessment of intratumoral heterogeneity of LARC using textural analysis in [^{18}F]FDG PET imaging could be another biomarker for therapeutic response [18]. Although these are promising results, several rounds of [^{18}F]FDG PET/CT scan acquisitions are required, and there is no consensus as to the time and frequency of PET/CT assessments in relation to preoperative nRCT duration.

The purpose of this study was to assess possible metabolic and textural parameters from pretreatment [^{18}F]FDG PET/CT scans for the prediction of nRCT response and survival in patients diagnosed with LARC.

Materials and methods

Patients

We retrospectively reviewed patients with clinically diagnosed LARC without distant metastases (i.e. stage II or III according to the AJCC Cancer Staging Manual (seventh edition) who underwent [^{18}F]FDG PET/CT scans between April 2009 and December 2013 at Seoul National University Bundang Hospital. All patients received nRCT followed by R0 resection and/or adjuvant chemotherapy. Electronic medical records were reviewed and data were extracted using a clinical database warehouse [19].

All procedures were in accordance with the principles of the 1975 Declaration of Helsinki (2013 revision). Study design and exemption from informed consent were approved by the Institutional Review Board of Seoul National University Bundang Hospital.

Clinicopathological and survival data

Clinicopathological data that were considered potentially relevant for prognosis were collected from electronic medical records using a clinical database warehouse. Data included age at initial diagnosis, sex, clinical TNM stage (assessed using CT, [^{18}F]FDG PET/CT, and/or MRI), nRCT regimen, and performance of adjuvant chemotherapy. Pathological data included histological subtype, histological differentiation grade, pathological staging after nRCT (ypTNM), involvement of circumferential margin, lymphangitic/venous/perineural tumour involvement, and tumour regression grade (TRG). The AJCC guidelines were used for the assessment of TRG. Patients with TRG 0 and 1 were considered responders and patients with TRG 2 and 3 nonresponders (Table 1).

We defined 3-year disease-free survival (DFS) as the time between the date of the pre-treatment [^{18}F]FDG PET/CT scan and the date of any report of recurrence first identified using imaging (CT, [^{18}F]FDG PET/CT or MRI) within 3 years. All tumour recurrences were confirmed by pathology or clinical follow-ups. Overall survival (OS) was defined as the time between the day of the pretreatment [^{18}F]FDG PET/CT scan and death due to rectal cancer.

Acquisition of [^{18}F]FDG PET/CT scans

[^{18}F]FDG PET/CT scans were acquired using a Discovery VCT scanner (GE Medical Systems, Milwaukee, WI). All patients fasted for at least 6 h before the scan. After venous blood glucose levels were confirmed to be below 200 mg/dL (mean 101.5 mg/dL, range 68 – 175 mg/dL), [^{18}F]FDG was injected intravenously (5.18 MBq/kg body weight for body weights less than 80 kg, a minimum of 444 MBq for body weights more than 80 kg, or a maximum dose of 518 MBq), and PET/CT scanning was performed 50 min later. CT scans were performed at 120 kVp for attenuation correction and anatomical information. PET scans were obtained from the skull base to the upper thigh using a 128 x 128 matrix. Images were reconstructed with an ordered subsets expectation maximization iterative algorithm (two iteration, eight subsets). The SUV was calculated using lean body mass.

Analysis of [^{18}F]FDG PET/CT imaging

All PET/CT data were analysed using a commercially available system (Advantage Windows workstation and PET VCAR software; GE Medical Systems). Reference volumes

Table 1 Tumour regression grading system of the American Joint Committee on Cancer

Grade	Treatment response	Histological features	Classification
3	Poor	Minimal or no tumour cells killed, extensive residual cancer	Nonresponder
2	Minimal	Residual cancer outgrown by fibrosis	Nonresponder
1	Moderate	Single or small groups of cancer cells	Responder
0	Complete	No viable cancer cells	Responder

of interest (VOI) were automatically placed on the right liver using a fixed size (volume 11.03 cm^3) spherical VOI. All automatically drawn reference VOIs were reviewed and manually redrawn if the CT scan quality was compromised or the liver was displaced by other lesions. SUVmax, SUVpeak and SUVmean of the rectal cancer lesions were calculated. Metabolic volume (MV) and total lesion glycolysis (TLG) of rectal cancer lesions were calculated using the isocontour method. Various isocontour thresholds were applied: 30 % to 70 % of SUVmax, SUVmean of liver + two standard deviations (SDs), and SUVmean of liver + three SDs. Only the primary rectal cancer lesion was analysed, but peritumoral and perirectal lymph nodes that were too close to the primary lesion could be included in the VOI depending on the isocontour threshold.

Three-dimensional textural analysis of the [^{18}F]FDG PET images was done using MaZda version 4.6 (Institute of Electronics, Technical University of Łódź, Poland) [20–22]. VOIs were manually drawn on the rectal cancer lesions and images were normalized with the limit of three SDs from the mean. Textural analysis including histogram-based grey level, absolute gradient, co-occurrence matrix (COM) and run-length matrix were calculated after grey level intensity resampling to 64 degrees. From each VOI 50 textural features were calculated. Table 2 shows the list of textural features used for analysis, and these textural features are described in detail in the MaZda manual [23].

Statistical analysis

Clinicopathological, TRG and PET parameters were analysed using a *t* test and binary logistic regression. For multivariate binary logistic regression, the backward conditional method (entry threshold $P<0.05$; removal threshold $P>0.1$) was applied. Survival analysis was performed using clinicopathological and PET parameters as covariates, and tumour recurrence within 3 years and death as endpoints. The duration of 3-year DFS and OS were calculated using the Kaplan-Meier method. Univariate Cox regression analysis was performed for each parameter and variable, and those with a *P* value less than 0.05 were included in the multivariate analysis. For multivariate Cox regression, the backward conditional method (entry threshold $P<0.05$; removal threshold $P>0.1$) was applied. SPSS software (version 18.0; SPSS Inc., Chicago, IL) was used for all statistical analyses.

Results

Patient characteristics

We initially enrolled 78 patients who fulfilled the inclusion criteria. The nRCT regimens included a 5-fluorouracil (5-FU) bolus with leucovorin (FL group, 30 patients) and capecitabine (capecitabine group, 48 patients) with radiation therapy of 50.4 Gy. Only one patient in the FL group did not finish the full chemotherapy course due to recurrent pneumonia. Two patients in the capecitabine group dropped the radiation therapy due to emergency surgery and underlying haematological disease; thus, they were excluded from the analysis. After nRCT, patients received complete R0 resection, except one patient who was excluded from the study because of the presence of liver metastasis which was detected during surgery. The final analysis included 74 patients (Fig. 1). There was no significant difference in age, sex and clinical T and N stages between the FL and capecitabine groups. After surgery, all patients underwent adjuvant chemotherapy, except for five patients who did not receive further chemotherapy because of

Table 2 Textural features used in the analysis

Description	Feature
Histogram	Mean, variance, skewness, kurtosis, percentiles 1 %, 10 %, 50 %, 90 % and 99 %
Absolute gradient	Mean, variance, skewness, kurtosis, percentage of pixels with nonzero gradient
Co-occurrence matrix	Angular second moment, contrast, correlation, sum of squares, inverse moment difference, sum average, sum variance, sum entropy, entropy, variance difference, entropy difference
Run-length matrix	Run-length nonuniformity, grey-level nonuniformity, long-run emphasis, short-run emphasis, fraction of image in runs

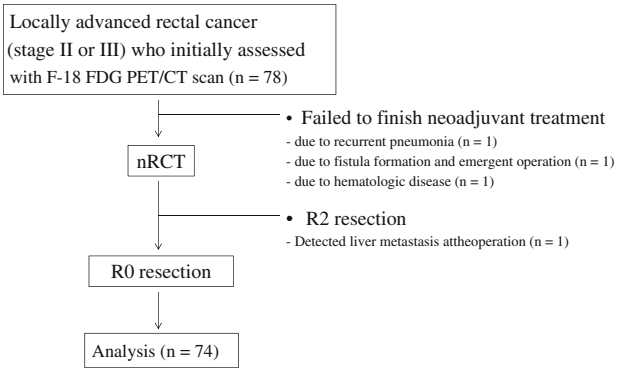

Fig. 1 Flow diagram of the study design

poor performance status. There was no significant difference in survival between patients who underwent adjuvant chemotherapy and those who did not. Table 3 summarizes the demographics and tumour characteristics of the 74 enrolled patients. The *t* test was used to compare the difference of SUVs (SUVmax, SUVpeak, SUVmean) between the responder group and nonresponder group, and no significant difference was found between these groups.

Table 3 Characteristics of the 74 enrolled patients

Characteristic	Value
Sex, *n* (%)	
Female	20 (27)
Male	54 (73)
Age (years) at initial diagnosis, mean (range)	58.8 (28 – 82)
Clinical T stage, *n* (%)	
T2	5 (6.8)
T3	62 (83.8)
T4	7 (9.5)
Clinical N stage, *n* (%)	
N0	6 (8.1)
N1	29 (39.2)
N2	39 (52.7)
M0	74 (100)
Histological type, *n* (%)	
Adenocarcinoma	72 (97.3)
Adenosquamous	2 (2.7)
Histological grade, *n* (%)	
Well differentiated	2 (2.7)
Moderately differentiated	70 (94.6)
Poorly differentiated	2 (2.7)
Concurrent radiation chemotherapy regimen, *n* (%)	
5-Fluorouracil with leucovorin	28 (37.8)
Capecitabine	46 (62.2)
Adjuvant chemotherapy, *n* (%)	
No	5 (6.8)
Yes	69 (93.2)

Tumour regression grade and clinical/metabolic/textural parameters

Binary logistic regression was performed to evaluate the possible clinical, metabolic and textural parameters for the prediction of nRCT response. None of the clinical parameters was significantly associated with TRG. However, MVs calculated using the thresholds SUVmean of the liver + two SDs and SUVmean of the liver + three SDs were significantly associated with TRG (OR 1.031, 95 % confidence interval, CI, 1.001 – 1.063, P= 0.045; and OR 1.036, 95 % CI 1.001 – 1.071, P=0.043, respectively). On the other hand, MVs calculated using other thresholds (SUVmax, SUVpeak and TLG; data not shown) were not significantly associated with TRG (Table 4).

Regarding textural parameters, the 50 %, 90 % and 99 % percentiles from the histogram-based analysis and sum entropy and entropy from the COM analysis were significantly associated with TRG. All of these parameters in the nonresponder group were significantly higher than in the responder group. However, the multivariate analysis showed no significant associations between metabolic or textural parameters and TRG.

Survival characteristics

Among the 74 patients, 12 (16.2 %) had tumour recurrence. The median follow-up duration in the 62 recurrence-free patients was 27.2 months (range 10 – 36 months). The mean 3-year DFS calculated using the Kaplan-Meier method in the 74 patients was 32.2 months (95 % CI 30.1 – 34.3 months). Sites of tumour recurrence were the liver (three patients), lung (six patients), and local area (three patients). One patient (1.3 %) died during follow-up as a result of progression of the primary cancer. The mean follow-up duration in the 73 surviving patients was 32.3 months (range 10 – 61 months). The mean OS in the 74 patients was 60.2 months (95 % CI 58.7 – 61.7 months). Because only one patient died, OS data were not used for analysis.

Three-year disease-free survival and clinical/metabolic/textural parameters

Univariate Cox regression analysis of the 3-year DFS data was performed to evaluate the prognostic potential of clinical, metabolic and textural parameters (Tables 5 and 6). Of the pathological parameters, circumferential margin and perineural involvement were associated with 3-year DFS. Stage T4 after neoadjuvant therapy (yT4) was more significantly associated with recurrence than yT0/T2. However, 3-year DFS was not significantly associated with clinical T or N stages or nRCT regimen. In addition, histological differentiation grade, N stage after neoadjuvant therapy (yN), lymphatic/venous involvement, and TRG were not significantly associated.

Table 4 Binary logistic regression was performed to identify factors associated with neoadjuvant radiation chemotherapy response

Variable	Responders (*n* or mean±SD)	Nonresponders (*n* or mean±SD)	*P* value	Odds ratio	95 % CI
Sex					
Female	3	17	–	–	–
Male	14	40	0.327	0.504	0.128 – 1.984
Age (years) at initial diagnosis	59.3±9.9	58.6±11.2	0.815	0.994	0.945 – 1.045
Concurrent radiation chemotherapy regimen					
5-Fluorouracil with leucovorin	7	21	–	–	–
Capecitabine	10	36	0.747	1.200	0.397 – 3.626
Clinical T stage					
T2	3	2	0.235	–	
T3	14	48	0.089	5.143	0.780 – 33.894
T4	0	7	0.999	2.423E9	0.000
Clinical N stage					
N0	3	3	0.154	–	
N1	8	21	0.292	2.625	0.436 – 15.810
N2	6	33	0.067	5.500	0.890 – 33.994
Histological type					
Adenocarcinoma	17	55	–	–	
Adenosquamous	0	2	0.999	4.993E8	0.000
Histological grade					
Well differentiated	0	2	0.701		
Moderately differentiated	16	54	0.999	0.000	0.000
Poorly differentiated	1	57	0.999	0.000	0.000
SUVmax	12.4±8.2	12.0±3.9	0.750	0.984	0.888 – 1.089
SUVpeak	9.3±6.6	9.1±3.0	0.876	0.990	0.867 – 1.129
Metabolic volume[a]					
SUVmean of liver + two SDs	28.8±27.2	69.4±80.8	0.045	1.031	1.001 – 1.063
SUVmean of liver + three SDs	25.3±25.0	63.0±74.8	0.043	1.036	1.001 – 1.071
30 % SUVmax threshold	19.7±12.1	110.6±551.5	0.71	1.041	0.997 – 1.088
40 % SUVmax threshold	12.2±8.1	25.6±28.9	0.055	1.079	0.998 – 1.165
50 % SUVmax threshold	8.1±5.4	17.0±20.9	0.084	1.107	0.986 – 1.243
60 % SUVmax threshold	4.8±3.0	10.3±13.3	0.134	1.148	0.958 – 1.376
70 % SUVmax threshold	2.6±1.4	5.1±7.0	0.193	1.203	0.911 – 1.590
Histogram-based analysis[b]					
50 % percentile	44.2±4.0	47.1±3.0	0.004	1.322	1.092 – 1.601
90 % percentile	54.6±6.5	57.8±3.3	0.018	1.161	1.025 – 1.314
99 % percentile	60.5±7.9	63.3±2.5	0.046	1.123	1.002 – 1.259
Co-occurrence matrix analysis[c]					
Sum entropy	1.7±0.11	1.8±0.05	0.011	1.8E5	15.143 – 2.1E9
Entropy	2.5±0.26	2.6±0.17	0.009	48.796	2.628 – 906.005

Metabolic volume calculated using thresholds SUVmean of the liver + two SDs and + three SDs was significantly correlated with tumour regression grade. The highest grey level value of a given percentage of pixels, sum entropy and entropy were also significantly correlated with tumour regression grade. The more heterogeneity the0 cancer showed the less was the response to treatment.

[a] Metabolic volume calculated using various isocontour thresholds: SUVmean of the liver + two SDs and + three SDs, and 30 % to 70 % SUVmax of the rectal cancer lesion

[b] Percentiles give the highest grey level value with a given percentage of pixels

[c] Sum entropy and entropy are measures of the spatial disorder of pixels, i.e. the randomness of grey-level value distribution

Table 5 Univariate Cox regression analysis of the association between clinicopathological parameters and 3-year disease-free survival

Variable	No. of patients	Hazard ratio	95 % CI	*P* value
Sex				
Female	20	–	–	–
Male	54	1.057	0.286 – 3.908	0.933
Age at initial diagnosis	–	0.990	0.940 – 1.042	0.990
Concurrent radiation chemotherapy regimen				
5-Fluorouracil with leucovorin	28	–	–	–
Capecitabine	46	1.398	0.419 – 4.663	0.586
Clinical T stage				
T2	5	–	–	0.475
T3	62	0.859	0.109 – 6.1789	0.885
T4	7	2.236	0.201 – 4.860	0.513
Clinical N stage				
N0/N1	35	–	–	–
N2	39	0.949	0.306 – 2.947	0.928
Histological differentiation grade				
Well or moderately differentiated	72	–	–	–
Poorly differentiated	2	4.022	0.516 – 31.359	0.184
T stage after neoadjuvant therapy				
T0/T1/T2	28	–	–	0.085
T3	44	6.985	0.894 – 54.583	0.064
T4	2	21.294	1.317 – 344.350	0.031
N stage after neoadjuvant therapy				
N0	50	–	–	0.146
N1	18	3.056	0.883 – 10.573	0.078
N2	6	3.401	0.657 – 17.605	0.144
Involvement of circumferential margin				
No	71	–	–	–
Yes	3	15.721	2.772 – 89.144	0.002
Lymphatic involvement				
No	62	–	–	–
Yes	12	1.756	0.475 – 6.489	0.398
Venous involvement				
No	65	–	–	–
Yes	9	2.469	0.654 – 9.317	0.182
Perineural involvement				
No	55	–	–	–
Yes	19	4.751	1.504 – 15.010	0.008
Tumour regression grade (AJCC)				
0/1/2	65	–	–	–
3	9	2.250	0.608 – 8.321	0.224

Regarding metabolic parameters, MV calculated using various thresholds was significantly association with 3-year DFS (Table 6). MV calculated using higher thresholds tended to be more strongly associated with 3-year DFS. TLG showed a borderline hazard ratio (HR) although statistically significant. Among 50 textural parameters, GrKurtosis was significantly associated with 3-year DFS (Table 6). Multivariate analysis revealed that GrKurtosis was a factor predicting DFS that was independent of pathological parameters such as circumferential margin and perineural involvement (Table 7, Fig. 2).

Discussion

In this retrospective study we found that MV and textural parameters of rectal cancer calculated from pretreatment

Table 6 Univariate Cox regression analysis of the association between metabolic and textural parameters and 3-year disease-free survival

Variable	Hazard ratio	95 % CI	*P* value
SUVmax	0.997	0.893 – 1.114	0.957
SUVpeak	1.002	0.874 – 1.148	0.981
Metabolic volume[a]			
SUVmean of liver + two SDs	1.006	1.002 – 1.011	0.010
SUVmean of liver + three SDs	1.007	1.002 – 1.012	0.009
30 % SUVmax threshold	1.000	0.998 – 1.001	0.777
40 % SUVmax threshold	1.020	1.007 – 1.034	0.003
50 % SUVmax threshold	1.028	1.009 – 1.047	0.004
60 % SUVmax threshold	1.043	1.012 – 1.074	0.006
70 % SUVmax threshold	1.068	1.014 – 1.124	0.012
Total lesion glycolysis[b]			
SUVmean of liver + two SDs	1.001	1.000 – 1.002	0.028
SUVmean of liver + three SDs	1.001	1.000 – 1.002	0.027
30 % SUVmax threshold	1.001	1.000 – 1.003	0.025
40 % SUVmax threshold	1.002	1.000 – 1.003	0.023
50 % SUVmax threshold	1.002	1.000 – 1.004	0.028
60 % SUVmax threshold	1.003	1.000 – 1.005	0.038
70 % SUVmax threshold	1.004	1.000 – 1.008	0.078
GrKurtosis[c]	5.898	1.402 – 24.815	0.015

GrKurtosis kurtosis of absolute gradient

[a] Metabolic volume calculated using various isocontour thresholds: SUVmean of the liver + two and + three SDs, and 30 % to 70 % SUVmax of the rectal cancer lesion

[b] Total lesion glycolysis was calculated as the product of SUVmean and tumour volume calculated using various isocontour thresholds

[c] GrKurtosis is a measure of the "peakedness" of the gradient in the images

[^{18}F]FDG PET/CT scans could potentially be used as imaging biomarkers for nRCT response, and have value for predicting recurrence in LARC. Previous studies have investigated the use of [^{18}F]FDG PET/CT monitoring for the prediction of nRCT response in patients diagnosed with LARC. The subject is not completely new, but previous studies assessed only conventional metabolic values (SUVmax, SUVmean), and none investigated the use of pretreatment FDG PET imaging [10–14]. Oku et al. studied rectal cancer patients who received neoadjuvant radiation therapy alone, and found that SUVmean from pretreatment [^{18}F]FDG PET/CT scans was not significantly related to therapeutic response or recurrence [24]. In our study, SUVmax, SUVpeak and SUVmean were not significantly associated with nRCT response or recurrence, which was consistent with the findings of previous studies.

SUVmax, SUVpeak and SUVmean represent only some part of the tumour. Therefore, SUV itself provides limited information on tumour lesion, and heterogeneity might not be shown by the SUV. However, MVs calculated using the thresholds SUVmean of the liver + two SDs and SUVmean of the liver + three SDs, that generally include the entire cancer lesion were significantly associated with nRCT response. This result might be consistent with the findings of previous studies showing that the volume of rectal cancer measured clinically or CT imaging is significantly related to tumour downstaging after nRCT [25, 26]. In contrast to MV, TLG (calculated as the product of MV and SUVmean) did not show a significant

Table 7 Multivariate Cox regression models for 3-year disease-free survival

Variable	β	Hazard ratio	95 % CI	*P* value
Involvement of circumferential margin	1.67	5.288	0.859 – 32.559	0.072
Perineural involvement	1.907	6.733	1.844 – 24.585	0.004
Metabolic volume with 70 % SUVmax threshold	0.049	1.050	0.979 – 1.127	0.172
GrKurtosis[a]	2.80	16.476	2.474 – 109.713	0.004

GrKurtosis kurtosis of absolute gradient

[a] GrKurtosis is a measure of the "peakedness" of the gradient in the images

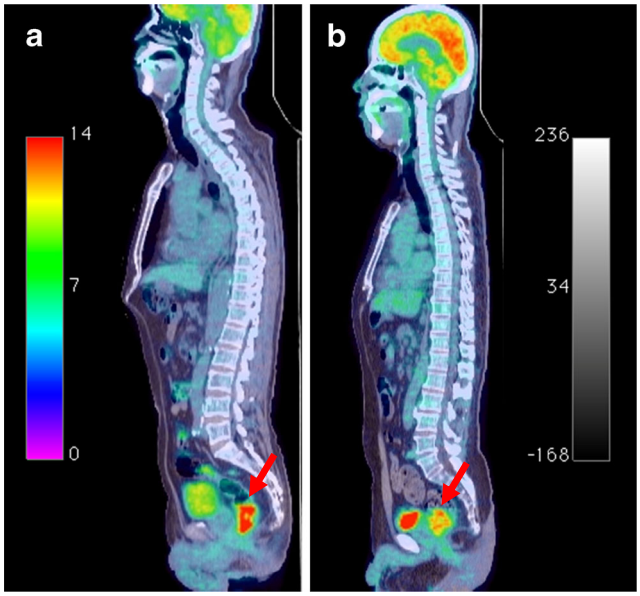

Fig. 2 Representative pretreatment [^{18}F]FDG PET/CT images. **a** A 75-year-old man with cT3N2M0 rectal cancer who had undergone nRCT with capecitabine. After surgery, the pathological stage was ypT3N1M0 with a negative circumferential resection margin and perineural involvement. He received adjuvant chemotherapy, and showed no evidence of disease for 21 months after initial diagnosis. The PET/CT image shows a primary rectal cancer lesion with homogeneous FDG uptake (*arrow*). **b** A 56-year-old man with cT3N2M0 rectal cancer who had undergone nRCT with capecitabine. After surgery, the pathological staging was ypT3N0M0 with a negative circumferential resection margin. He received adjuvant chemotherapy, but developed liver metastasis 8 months after the initial diagnosis. The PET/CT image shows a primary rectal cancer lesion with heterogeneous FDG uptake (*arrow*), for which GrKurtosis was high

correlation with nRCT response. This result could be because SUVmean prediction of recurrence in LARC, MV could be a promising imaging biomarker. MV calculated using higher thresholds showed a positive correlation with 3-year DFS. In this study, the threshold used for the calculations was not the fixed SUV level, but the percentage of SUVmax in individual tumours in each patient. Since the absolute value of SUVmax or SUVpeak did not show any correlation with the clinical data, a better measure reflecting the character of the tumour might be considered, since these results might have been due to individual variations in glucose metabolism. MV calculated using various tumour percentage thresholds could be an easily evaluated semiquantitative measure of tumour textural heterogeneity, and could also be an easy way to minimize individual variations. Henriksson et al. found intratumoral heterogeneity in FDG uptake which correlated with histopathological findings in nude mice [27]. In solid cancers other than rectal cancer, a higher SUV could also represent more aggressiveness of cancer cells [28–31]. Therefore, MV calculated using a higher threshold might be a representative marker for tumour aggressiveness in primary rectal cancer. From this point of view, an initial assessment of rectal cancer using [^{18}F]FDG PET/CT could be a more sophisticated method to accurately assess clinical stage.

Textural analysis could maximize the information obtained from standard clinical images [15]. In this study, GrKurtosis was an independent factor predicting 3-year DFS. GrKurtosis is a measure of the grey-level variation across the image, and represents the relationship between the neighbourhood pixels [32]. It is also a measure of "peakedness" of the gradient in the images, and higher GrKurtosis indicates a peaked distribution, which tends to be more random and heterogeneous. Patients who had a significantly higher GrKurtosis value showed a higher risk of recurrence. Gigher Grkurosis was significantly correlated with 3-year DFS. Randomness of FDG uptake could represent intratumoral heterogeneity, and a greater degree of intratumoral heterogeneity was associated with a poorer clinical outcome. Uptake of FDG by tumours has been shown to correlate with biological markers such as GLUT-1, as well as glycolysis-related and hypoxia-related markers in lung and oral cancers [33, 34]. Hypoxia is a well-known factor in tumour radioresistance [35].

Textural parameters from [^{18}F]FDG PET imaging could be indicators of intratumoral heterogeneity, especially hypoxia. In the present study, various textural parameters were significantly associated with TRG and 3-year DFS. Among these textural parameters, entropy and the sum of entropy are indicators of the randomness of FDG uptake distribution [36]. We hypothesize that these textural parameters, which were significantly higher in the nonresponder group than in the responder group, could predict response to radiation therapy because they reflect the microenvironment of the tumour, including hypoxia. Further studies are necessary to determine the exact relationA limitation of this study in relation to the evaluation of TRG was the relatively small number of patients. Furthermore, the patients were divided into two groups according to TRG and the patients in each group showed a wide range of TRG. The value of TRG in predicting recurrence and survival is well known [4–6]. In this study, the chi-squared test showed that there was no significant difference in TRG between the disease-free group and recurrence group (P=0.057 according to Fisher's exact test). However, TRG itself was not significantly associated with 3-year DFS. This can be explained by iin terms of the two limitations discussed above. Furthermore, all 12 patients who had tumour recurrence were nonresponders. Further study in a larger group of patients is needed. Also, to validate the textural analysis, a large-scale study including other malignancies such as soft-tissue sarcoma is needed. In addition, two chemotherapy regimens (based on 5-FU and capecitabine) were used in this study. However, there is no clinical difference between two regimens [37]. Lastly, the method of delineation of the tumour could have been a confounding factor in the textural analysis but the rectum is a less mobile organ than other bowel structures, so this limitation might have been of minimal effect [38]. In addition,

to overcome this limitation we used CT-based segmentation to reduce variability.

Conclusion

We retrospectively evaluated pretreatment [^{18}F]FDG PET/CT scans in patients diagnosed with LARC in relation to their clinical outcomes, and found that MV and textural parameters from histogram-based and COM analysis were significantly associated with response to nRCT. Furthermore, MV calculated using a higher threshold tended to be more strongly associated with 3-year DFS. Also, multivariate analysis showed that textural parameters and MV were useful for predicting recurrence. Consequently, metabolic and textural parameters from pretreatment [^{18}F]FDG PET/CT scans could be used as imaging biomarkers for measuring tumour heterogeneity for the prediction of response to nRCT and recurrence in patients diagnosed with LARC.

Compliance with ethical standards

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Conflicts of interest None.

Ethical approval All procedures were performed in accordance with the principles of the 1975 Declaration of Helsinki (2000 revision). The study design and exemption from informed consent were approved by the Institutional Review Board of Seoul National University Bundang Hospital.

Informed consent Informed consent was obtained from all individual participants included in the study.

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