Comparison of two region of interest definition methods for metabolic response evaluation with [18F]FDG-PET

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***Aim*. In therapy response monitoring by [18F]2-fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET), different tumor delineations are used, resulting in different values for change in glucose metabolic rate (ΔMR_{glu}). We propose a technique to compare metabolic rates in a region of interest (ROI) based on fixed volumes rather than on fixed thresholds. This method involves change in lesion size.**

***Methods*. In 49 patients with colorectal carcinoma (CRC) and 50 patients with non-small cell lung carcinoma (NSCLC) scheduled for chemotherapy, FDG-PET was performed at baseline and during chemotherapy. A $ROI_{fixed\ thresholds}$ was determined by using a 50% threshold on both baseline and follow-up FDG-PET. A $ROI_{fixed\ volumes}$ was determined by using a 50% threshold, determined on the series with the largest tumor volume. This $ROI_{fixed\ volumes}$ is used on consecutive scans. Predictive effects of both methods were investigated by survival analysis for overall and progression free survival.**

***Results*. In CRC, only $ROI_{fixed\ volumes}$ based ΔMR_{glu} showed significant predictive ability. In NSCLC, both techniques showed significant predictive ability. During multivariate analysis, $ROI_{fixed\ volumes}$ determined ΔMR_{glu} was an independent predictor for both overall and progression free survival in NSCLC whereas $ROI_{fixed\ thresholds}$ determined MR_{glu} was not. After dichotomization at the median ΔMR_{glu}, median survival ratio was higher in $ROI_{fixed\ volumes}$ than $ROI_{fixed\ thresholds}$ for CRC (overall survival: 1.78 *vs* 1.25, progression free survival: 1.57 *vs* 1.21) and NSCLC (overall survival: 2.01 *vs* 2.01, progression free survival: 2.93 *vs* 2.13).**

***Conclusion*. $ROI_{fixed\ volumes}$ based ΔMR_{glu} shows better correlation with survival than ΔMR_{glu} determined from a $ROI_{fixed\ thresholds}$.**

KEY WORDS: Colorectal carcinoma - Non-small cell lung carcinoma - [18F]FDG - Positron emission tomography - Chemotherapy - Drug monitoring - Survival - Neoplasms, therapy.

Functional imaging with [18F]2-fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET) has an established role in the standard care for patients with colorectal carcinoma (CRC) or non-small cell lung carcinoma (NSCLC) by staging of the disease. Growing interest in the application of FDG-PET for prediction and evaluation of tumor response to therapy has risen, since morphologic imaging techniques such as computed tomography (CT) or magnetic resonance imaging (MRI) may lead to incorrect conclusions about therapy response. Due to the fact that it

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proves difficult to reliably distinguish between therapy induced fibrosis, tumor necrosis and recurrent or residual tumor in both CRC [1-3] and NSCLC,[4] favorable and adverse alterations may be indistinguishable. Furthermore, metabolic changes in tumor cells, indicative of response to therapy, may occur earlier than changes in lesion size,[5] especially as some new antitumor therapies are cytostatic rather than cytoreductive. Early detection of tumor progression during chemotherapy by FDG-PET might therefore prevent unbeneficial, perhaps even harmful treatment.

It has already been demonstrated that pre- and post-therapy parameters for tumor glucose metabolism are of prognostic value in CRC [6] and NSCLC.[7-11] In addition, changes in the rate of glucose metabolism predict overall and progression-free survival in both CRC [12] and NSCLC.[8-11, 13]

Since the value of change in glucose metabolic activity is highly dependent on the definition of tumor volume of interest (region of interest [ROI]), an exact and reproducible definition of ROI methodology is important.[14-16] Institution-dependent ROI definitions may lead to variations in the classification of metabolic response and may hinder the integrated or comparative interpretation of results of multiple centers. In literature, many different methods have been used to define tumor ROI: a thresholded 3D isocontour (using 50% or 70% of the maximum voxel value within the lesion), the maximum voxel value or a fixed dimensions-method (*e.g.* 15×15 mm^2 around maximum value).[14] The first method determines treatment induced changes in tumor metabolic activity in two different volumes, since using fixed thresholds, glucose metabolic rate (MR_{glu}) is determined in the metabolic active volume only.

The use of the fixed thresholds-based methodology is attractive since tumor delineation on FDG-PET is practical, easier to perform and more reproducible than other methods. A disadvantage of the fixed thresholds technique is that the threshold level is chosen rather arbitrarily and that only MR_{glu} in residual, metabolic active tumor is taken into account. As a result, a lesion that decreases in size, but preserves the same baseline metabolic activity will not be considered to respond to treatment with this ROI-definition. Moreover, a sole decrease in lesion volume might artifactually decrease the measured metabolic activity due to the partial-volumes effect,[14] especially in case of residual tumor less than ~15 mm in diameter. When using the same ROI during treatment response assessment (fixed volumes), both the change in metabolic activity as well as the change in tumor metabolic volume are taken into account. Since a decrease in lesion volume causes peritumoral tissue with normalized FDG accumulation that is incorporated in the ROI resulting in a reduction of its MR_{glu}.

The hypothesis is that using the same ROI-volume for MR_{glu} determination, both at baseline and during follow-up is more indicative for therapy response. Present study introduces two distinct ROI methods for metabolic response evaluation assessed prospectively in both CRC and NSCLC patients. One technique solely evaluates metabolic response and the other incorporates the change in volume. Both techniques are correlated with patient survival.

Materials and methods

Patient eligibility criteria

Between March 2002 and December 2005 patients in the Radboud University, Nijmegen Medical Center with metastatic CRC (stage IV), who were scheduled to undergo palliative chemotherapy and patients with any stage of NSCLC, who were scheduled to undergo induction chemotherapy or palliative chemotherapy were asked to participate in this study. Exclusion criteria were diabetes mellitus and pregnancy.

In all patients, treatment decision-making was done by a multidisciplinary team including medical oncologists, surgeons (CRC), cardiothoracic surgeons (NSCLC), pulmonologists (NSCLC), radiation oncologists, pathologists, radiologists and nuclear medicine physicians. All clinicians were blinded to the results of the serial FDG-PET scans. The study was approved by the Institutional Review Board of the Radboud University, Nijmegen Medical Center and written informed consent was obtained from each patient.

One hundred and twenty-one consecutive eligible patients could be included in this prospective study (61 advanced CRC, 60 NSCLC). After the baseline FDG-PET, 22 patients (12 CRC, 10 NSCLC) were excluded for several reasons: due to technical issues (n=4), refusal to undergo a second FDG-PET (n=5), death before the second FDG-PET (n=3) and early discontinuation of chemotherapy due to a significant decline in performance status (n=10). Therefore, complete data-sets of two FDG-PET were available in 99 patients (49 CRC and 50 NSCLC) for analysis of therapy response. Patient characteristics for both tumor types are summarized in Tables I and II.

TABLE I.—*Characteristics of patients with colorectal carcinoma.*

Characteristics	CRC
Demography	
No. of patients	49
Mean age (y) (range)	60.5 (44.7-78.9)
Men (%)	36 (73.5%)
Location of primary tumor	
Colon	10
Sigmoid	23
Rectum	9
Colon and rectum	7
Location of metastases	
Liver	42
Lung	15
(Retro) peritoneal lymph nodes	4
Bone	2
Histology	
Adenocarcinoma	46
Mucinous adenocarcinoma	3
Tumor differentiation	
Undifferentiated	1
Very poor	2
Poor	5
Intermediate	31
Well	2
Unspecified	8

CRC: colorectal carcinoma.

TABLE II.—*Characteristics of patients with non-small cell lung carcinoma.*

Characteristics	NSCLC
Demography	
No. of patients	50
Mean age (y) (range)	59.7 (41.2-76.3)
Men (%)	37 (74%)
Histology	
Adenocarcinoma	23
Squamous cell carcinoma	21
Large cell carcinoma	4
Bronchoalveolar cell carcinoma	1
Clear cell carcinoma	1
Tumor differentiation	
Very poor	12
Poor	6
Intermediate	7
Well	1
Unspecified	24
Tumor stage at inclusion	
I	1 (B)
II	1 (A)
III	8 (A); 9 (B)
IV	31

NSCLC: non-small cell lung carcinoma.

Patient treatment

Of the CRC patients, 26 patients received first line chemotherapy, 16 in second line, 6 in third line and 1 in fourth line. Chemotherapy regimens were based on fluoropyrimidines (capecitabine and 5-fluorouracil) with or without oxaliplatin and irinotecan or monoclonal antibodies (bevacizumab and cetuximab).

Of the NSCLC patients, 14 patients were treated with induction chemotherapy and the remaining 36 received chemotherapy in a palliative setting (32 in first line and 4 in second line). Chemotherapy regimens were based on platinum-containing alkylating agents, gemcitabine, etoposide, vinorelbine or docetaxel. Patients receiving induction chemotherapy were subsequently treated with radical radiotherapy (n=9) or curative surgery (n=2). The remaining 3 patients were treated with palliative radiotherapy, because of progression during induction chemotherapy based on CT-criteria.

No patients were lost during follow up. Survival of patients is displayed in Table III.

[18F]2-fluoro-2-deoxy-D-glucose positron emission tomography

QUANTITATIVE DYNAMIC FDG-PET DATA ACQUISITION AND RECONSTRUCTION

Dynamic FDG-PET was performed at baseline and after 2 months of treatment (CRC) or after the second or third cycle of chemotherapy (NSCLC), depending on the chemotherapy regimen. Patients were fasted for at least 6 h before imaging. Intake of sugar-free liquids was permitted. Blood glucose levels (hexokinase method, Aeroset, Abbott diagnostics, IL, USA) were determined. All scans were acquired on an ECAT-EXACT47 PET scanner (Siemens/CTI, Knoxville, TN, USA). The position of the patient in the scanner's field of view (162 mm in 47 planes) for dynamic acquisition was based on the whole-body FDG-PET and CT scans performed in every patient for routine clinical work-up. In case not all lesions could be acquired in

TABLE III.—*Outcome of follow-up of CRC and NSCLC patients using Kaplan-Meier analysis.*

	Event-free N. (%)	Median (CI) (weeks)	1 year (CI) (%)	2 year (CI) (%)	3 year (CI) (%)
CRC					
Overall survival	8 (16)	89 (74-104)	78 (66-89)	38 (24-52)	16 (5-27)
Progression free survival	1 (2)	25 (21-30)	18 (8-29)	4 (0-10)	2 (0-6)
NSCLC					
Overall survival	7 (14)	58 (25-91)	56 (42-70)	36 (23-49)	22 (10-34)
Progression free survival	3 (6)	26 (14-39)	27 (14-39)	17 (6-27)	8 (0-16)

CRC: colorectal carcinoma; NSCLC: non-small cell lung carcinoma. CI: 95% confidence interval.

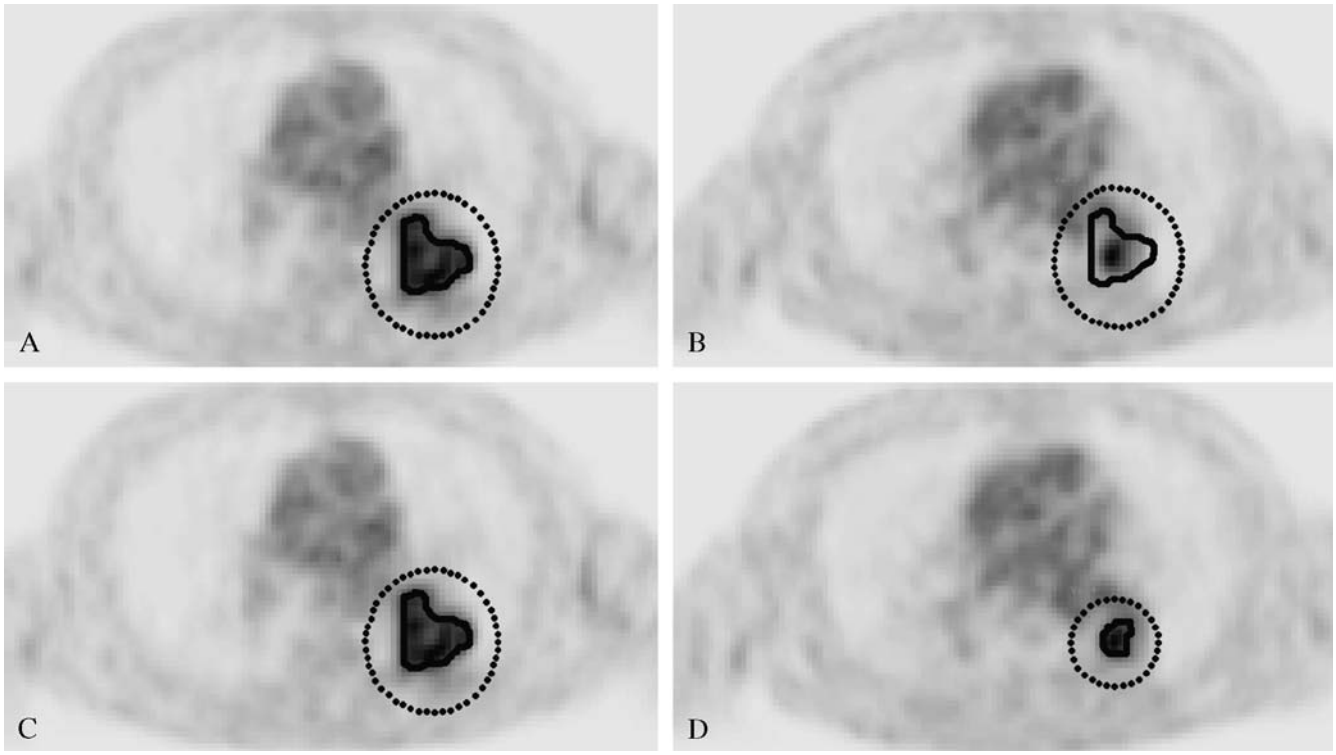


Figure 1.—Example of different ROI-definitions with FDG-PET in a 70-year-old male patient with squamous cell NSCLC, $T_2N_2M_1$ treated with two cycles of first line carboplatin/gemcitabine chemotherapy. Planes were anatomically correlated. ΔMR_{glu} for fixed thresholds was -54% and for fixed volumes -82%. Patient was alive after 3 years of treatment. A, B) $ROI_{fixed\ volumes}$; C, D) $ROI_{fixed\ thresholds}$. ROI: region of interest; FDG-PET: [18F]2-fluoro-2-deoxy-D-glucose positron emission tomography; NSCLC: non-small cell lung carcinoma; ΔMR_{glu}: relative change in glucose metabolic rate between baseline and follow-up FDG-PET.

one field of view, the patient was positioned to include as many measurable tumor lesions as possible. This position was used for follow-up scanning as well. A 20-min transmission scan was made, using the internal $^{68}Ge/^{68}Ga$ sources, to correct for photon attenuation. Approximately 200 MBq FDG (Covidien, Petten, the Netherlands) was injected intravenously using a constant infusion remote-controlled pump (Medrad, Indianola, PA, USA). The dynamic data acquisition, performed in septa-extended (two-dimensional) mode, was started simultaneously with the injection of FDG and con-

sisted of 16 time frames with variable duration (10×30 s, 3×300 s, 3×600 s) for a total time of 50 min. Corrections for decay, randoms, and scatters was performed. Attenuation-corrected images were reconstructed in 128×128×47 matrices, using filtered back projection with a Gaussian filter of 4 mm full width at half maximum (FWHM). This resulted in voxels of 3.432×3.432×3.375 mm (39.8 µL) and a spatial resolution of 6 mm FWHM in the reconstructed images.

Plasma time-activity curves

Plasma time-activity curves were derived from 17 manually taken arterial blood samples (~2 mL) from a 20-G cannula in the radial artery. Seven samples were drawn at 15-s intervals, followed by samples at 135 s, 165 s, 225 s, 285 s, 7.5 min, 12.5 min, 17.5 min, 25 min, 35 min, and 45 min after injection. Radioactivity in the plasma (obtained by centrifugation) was determined in a well-type γ-counter (Wallac 1480 Wizard, Perkin Elmer Lifescience, Zaventem, Belgium). This procedure is extensively described before.[17] When arterial cannulation was contraindicated or not feasible (in 44 of 98 CRC scans and 41 of 100 NSCLC scans), an image-derived input function (IDIF) was determined by measuring FDG-counts in a ROI over the ascending aorta or the abdominal aorta, that accurately represents FDG blood levels.[17]

Tumor time-activity curves and region of interest methodology (fixed thresholds, fixed volumes)

Tumor time-activity curves were obtained by placing 3D ROIs over the tumor and each metastasis using two different techniques: the fixed thresholds and fixed volumes method. The locations of the lesions were evaluated visually on the transaxial, coronal and sagittal images in summed late time frames (frame 14-16) yielding a static image of 30 min and a scan mid-time of 35 min postinjection.

The $ROI_{fixed\ thresholds}$ were semi-automatically determined using an isocontour with a threshold of 50% of the maximum voxel value within the lesion on the pretreatment scan. This $ROI_{fixed\ thresholds}$ was copied to each time frame of the scan. This process was repeated on the follow-up scan (Figures 1A and 1B). In case of complete metabolic response, which implies that no tumor contour could be determined by a certain threshold, the ROI of the baseline scan was copied. Thereby, the ROI included 'background' tissue with the same FDG uptake as surrounding tissue (liver or lung).

The $ROI_{fixed\ volumes}$ were semi-automatically determined using isocontours with a threshold of 50% of the maximum voxel value within the lesion on the FDG-PET on which the lesion was largest. In case of partial or complete metabolic response (decrease of threshold based volume) the $ROI_{fixed\ volumes}$ was determined on the pretreatment FDG-PET and copied to the second FDG-PET and in case of progressive disease (increase of threshold based volume or appearance of new lesions) the $ROI_{fixed\ volumes}$ was determined on the second FDG-PET and copied to the pretreatment FDG-PET. The copied ROI was manually translated in all three-dimensions using anatomical landmarks of surrounding structures. This $ROI_{fixed\ volumes}$ was used on each time frame of the scan (Figures 1C and 1D).

Patlak analysis

Patlak analysis was used to compute the MR_{glu} (in $\mu mol \cdot mL^{-1} \cdot min^{-1}$) in each lesion using both ROIs and the plasma time-activity curve (or IDIF), as described in detail.[17, 18] MR_{glu} in the ROI was calculated by multiplication of the slope of the Patlak-plot with the blood glucose level. When multiple lesions were quantified in one patient, mean MR_{glu} was calculated weighting every lesion by its ROI volume by the equation:

$$MR_{glu,\,patient} = \frac{\sum_{i=1}^{n} (MR_{glu,i} \cdot volume_i)}{\sum_{i=1}^{n} volume_i}$$

The relative change in MR_{glu} (ΔMR_{glu}) between the baseline and follow-up FDG-PET was calculated ($\Delta MR_{glu} = [MR_{glu,\ follow\text{-}up} - MR_{glu,\ baseline}] \cdot [MR_{glu,\ baseline}]^{-1} \cdot 100\%$).

Clinical follow-up

During and after treatment, patients were followed with clinical examination at regular intervals, chest CTs (NSCLC, every 6 months in CRC), abdominal CT/MRI (every 3 months in CRC), chest X-rays (NSCLC), CEA measurement (CRC), routine laboratory tests and other imaging studies as clinically indicated. Morphologic tumor response was evaluated on CT, MRI or conventional chest X-ray according to

response evaluation criteria in solid tumors (RECIST) [19] without knowledge of the results of the FDG-PET studies. These criteria define progression as a 20% increase in the sum of longest diameters of target lesions or the appearance of new lesion.[19] When recurrence was suspected or proven, patients were always re-staged. The progression and relapse pattern and cause of death were determined in all cases. The date of local or distant progression was defined as the earliest date at which disease progression was confirmed, either clinically or by imaging or biopsy. In patients who were progression free at the closeout date (April 2008) or who had died from any cause, the time to progression was censored at that date. Survival was measured from the date of the baseline FDG-PET scan to the date of death. In patients who were alive at the closeout date survival was censored to that date.

Statistical analysis

All continuous variables were assessed for normality by the Shapiro-Wilk statistic. For non-normal distributions, median and interquartile range (IQR) are presented as measures for central tendency and dispersion, the non-parametric Wilcoxon signed ranks test was used for comparison and correlation between both ROI methods is displayed as Spearman's ρ. Metabolic rate changes during therapy were classified as reduced (ΔMR_{glu} <-20%), increased (ΔMR_{glu} >+20%) or stable (-20% ≤ΔMR_{glu} ≤+20%). These cut-offs were based on the test-retest reproducibility of MR_{glu} which has a 95%-confidence interval of around ±15-20%.[16, 20]

Overall survival and progression free survival served as the standard of reference. The overall and progression free survival with respect to the different ΔMR_{glu} were calculated using Kaplan-Meier analysis.

Cox's proportional hazards model was used to assess the predictive value of response evaluation with FDG-PET, as expressed in the ΔMR_{glu} between the FDG-PET at baseline and at follow-up. As candidate covariates patient age, sex and tumor staging (NSCLC) are used in a forward model based on likelihood ratios (P<0.05 for covariate entry, P>0.1 for covariate removal). Hazard ratios (HR) are presented, representing the ratio of odds that a metabolic responder will survive a certain amount of time compared to a metabolic non-responder. Statistical significance of each model parameter was assessed using Wald's χ^2 test. As a measure of time-to-effect, both median survival times and their ratio (median ratio) are presented, assessed using the log rank test. As cut-off median ΔMR_{glu} was chosen to avoid the effect that expected lower values for fixed volumes-determined ΔMR_{glu} account for differences. The median ratios present the ratio of time that 50% of the metabolic responder will survive compared to the time 50% of metabolic non-responder survive.[21] Finally, another cut-off for ΔMR_{glu} was used for which >90% of the patients show 1-year overall survival to investigate how a prognosis-driven threshold (as contrast to metabolic response-driven threshold) might prove of additional value.

Analysis was performed with the Statistical Package for Social Sciences (SPSS®) version 14.0.2 (SPSS Inc, Chicago, IL, USA) and GraphPad Prism version 5.0a (GraphPad Software, La Jolla, CA, USA). Statistical tests were based on a two-sided significance level and the level of significance was set at P=0.05 for all tests.

Results

Quantitative changes in [18F]2-fluoro-2-deoxy-D-glucose positron emission tomography uptake

Median interval between baseline and follow-up FDG-PET was 63 days (IQR: 55-68) in the CRC-group and 48.5 days (IQR: 41-60) in the NSCLC-group. Bland-Altman plots [22] of ΔMR_{glu} of both ROI-definitions and both cancer types are displayed in Figure 2. In both CRC and NSCLC the decline in median of all MR_{glu} between first and second FDG-PET was statistically significant (P<0.001). Median ΔMR_{glu} for $ROI_{fixed\ thresholds}$ was -29.6% *versus* -51.9% for $ROI_{fixed\ volumes}$ in CRC patients (P=0.01). Median ΔMR_{glu} for $ROI_{fixed\ thresholds}$ was -37% *versus* -50% for $ROI_{fixed\ volumes}$ in NSCLC patients (P=0.01). The difference in ΔMR_{glu} based on both ROI-methods varied from -232% to +107% (CRC) and from -29% to +172% (NSCLC). Outliers were especially seen when ROI-volumes assessed by $ROI_{fixed\ thresholds}$ were small, rendering their MR_{glu} more sensitive to noise. According to previously mentioned definitions of response in MR_{glu} from $ROI_{fixed\ volumes}$, 33 of 49 CRC patients showed reduction in metabolism, 7 showed increase in metabolism and the remaining 9 showed stable metabolism. In 36 of 49 cases, con-

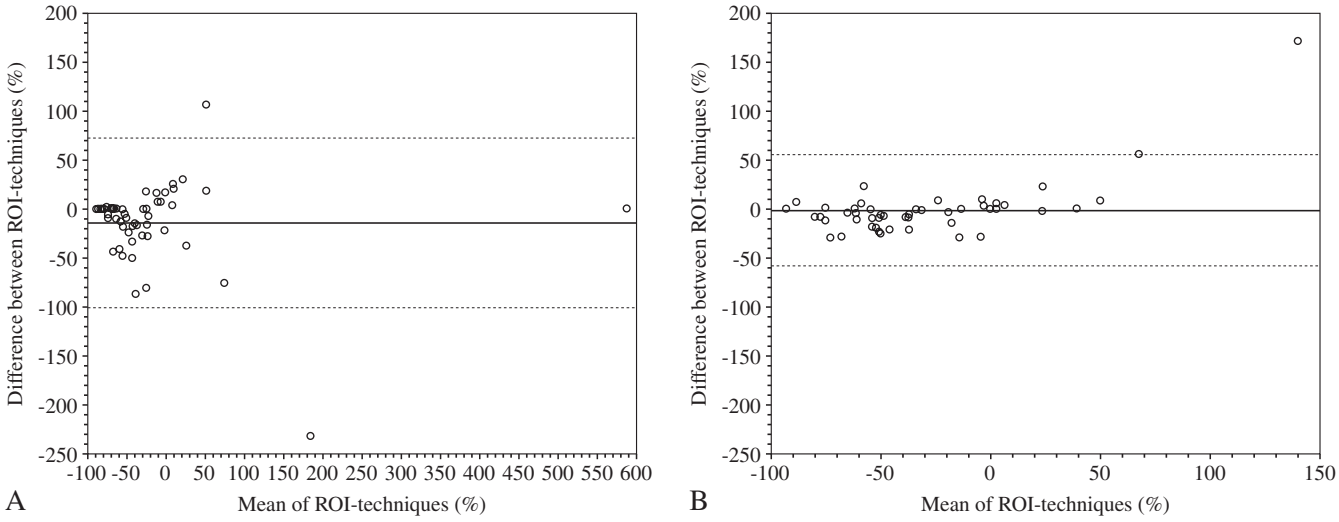


Figure 2.—Bland-Altman plots for both cancer types. The means of ΔMR_{glu} of both ROI-techniques (x-axis) and difference between ΔMR_{glu} of ROI-techniques (y-axis) are displayed. The solid line states the mean difference; the dotted lines the mean±2 standard deviations. NSCLC: non-small cell lung carcinoma; CRC: colorectal carcinoma; ROI: region of interest; ΔMR_{glu}: relative change in glucose metabolic rate between baseline and follow-up FDG-PET.

clusions about change in metabolism were concordant. In NSCLC, 35 of 50 patients showed reduction in metabolism, 6 showed increase in metabolism and the remaining 9 showed stable metabolism. In 45 of 50 cases, conclusions about change in metabolism were concordant. Differences between the two ROI-methods are displayed in Tables IV and V. Correlation between ΔMR_{glu} in $ROI_{fixed\ thresholds}$ and $ROI_{fixed\ volumes}$ was 0.879 (CRC) and 0.932 (NSCLC) (both P<0.001).

Prediction of survival by [18F]2-fluoro-2-deoxy-D-glucose positron emission tomography

Cox proportional hazards model for CRC showed significant predictive ability of overall and progression free survival only for ΔMR_{glu} in $ROI_{fixed\ volumes}$ between baseline and second FDG-PET. No contributing confounders were found in the candidate covariates (Table VI).

The same type of analysis was used for NSCLC and showed significance predictive ability for overall as well as for progression free survival for both ΔMR_{glu} calculated in $ROI_{fixed\ volumes}$ and $ROI_{fixed\ thresholds}$ (Table VI). Tumor staging showed strong predictive value in univariate analysis (HR 1.723 in overall survival and 1.942 in progression free survival).

Only tumor stage was found to be a significant con-

TABLE IV.—*Number of colorectal cancer patients with decreased (ΔMR_{glu}<-20%), stable (-20≤ΔMR_{glu}≤+20%) or increased (ΔMR_{glu}>+20%) metabolism based on both ROI-definitions.*

		ΔMR_{glu}, fixed thresholds			
		Decreased	Stable	Increased	Total
$\Delta MR_{glu,\ fixed\ volumes}$	Decreased	26	7	0	33
	Stable	2	6	1	9
	Increased	0	3	4	7
	Total	28	16	5	49

ΔMR_{glu}: change in glucose metabolic rate between baseline and follow-up scan; ROI: region of interest.

TABLE V.—*Number of non-small cell lung carcinoma patients with decreased (ΔMR_{glu} <-20%), stable (-20≤ΔMR_{glu} ≤+20%) or increased (ΔMR_{glu} >+20%) metabolism based on both ROI-definitions.*

		ΔMR_{glu}, fixed thresholds			
		Decreased	Stable	Increased	Total
$\Delta MR_{glu,\ fixed\ volumes}$	Decreased	32	3	0	35
	Stable	1	8	0	9
	Increased	0	1	5	6
	Total	33	12	5	50

ΔMR_{glu}: change in glucose metabolic rate between baseline and follow-up scan; ROI: region of interest.

TABLE VI.—*Results of univariate Cox proportional hazards regression analysis for overall and progression free survival using ΔMR_{glu} between the FDG-PET-scan at baseline and follow-up.*

	Overall survival			Progression free survival		
	Hazard ratio	CI	P**	Hazard ratio	CI	P**
CRC						
$ROI_{fixed\ volumes}\ \Delta MR_{glu}$*	1.026	1.001-1.051	0.04°	1.04	1.009-1.071	0.01°
$ROI_{fixed\ thresholds}\ \Delta MR_{glu}$*	1.014	0.987-1.042	0.302	1.023	0.993-1.055	0.13
Sex	0.959	0.479-1.923	0.907	0.992	0.519-1.897	0.982
Age	0.976	0.936-1.016	0.237	0.975	0.938-1.014	0.207
NSCLC						
$ROI_{fixed\ volumes}\ \Delta MR_{glu}$*	1.069	1.024-1.116	0.002°	1.1	1.044-1.16	<0.001°
$ROI_{fixed\ thresholds}\ \Delta MR_{glu}$*	1.133	1.046-1.227	0.002°	1.136	1.048-1.232	0.002°
Stage	1.723	1.204-2.467	0.003°	1.942	1.327-2.841	0.001°
Sex	1.142	0.557-2.342	0.718	1.162	0.61-2.212	0.647
Age	1.009	0.975-1.043	0.608	0.984	0.952-1.017	0.341

*Per 10% change; **Wald's χ^2 test; °significant at the 0.05 level. ΔMR_{glu}: relative change in glucose metabolic rate between baseline and follow-up FDG-PET; CRC: colorectal carcinoma; NSCLC: non-small cell lung carcinoma. ROI: region of interest; FDG-PET: [18F]2-fluoro-2-deoxy-D-glucose positron emission tomography; CI: 95%-confidence interval.

TABLE VII.—*Results of multivariate Cox proportional hazards regression analysis for overall and progression free survival using ΔMR_{glu} between the FDG-PET-scan at baseline and follow-up.*

	Overall survival			Progression free survival		
	Hazard ratio	CI	P**	Hazard ratio	CI	P**
$ROI_{fixed\ volumes}\ \Delta MR_{glu}$*	1.051	1.004-1.1	0.034°	1.073	1.014-1.135	0.003°
Stage	1.624	1.129-2.337	0.009°	1.769	1.208-2.593	0.015°
$ROI_{fixed\ thresholds}\ \Delta MR_{glu}$*	1.091	1.003-1.187	0.043°	—	—	—
Stage	1.581	1.093-2.287	0.015°	1.942	1.327-2.841	0.001°

*Per 10% change; **Wald's χ^2 test; °significant at the 0.05 level. ΔMR_{glu}: relative change in glucose metabolic rate between baseline and follow-up FDG-PET; NSCLC: non-small cell lung carcinoma. ROI: region of interest; FDG-PET: [18F]2-fluoro-2-deoxy-D-glucose positron emission tomography; CI: 95%-confidence interval.

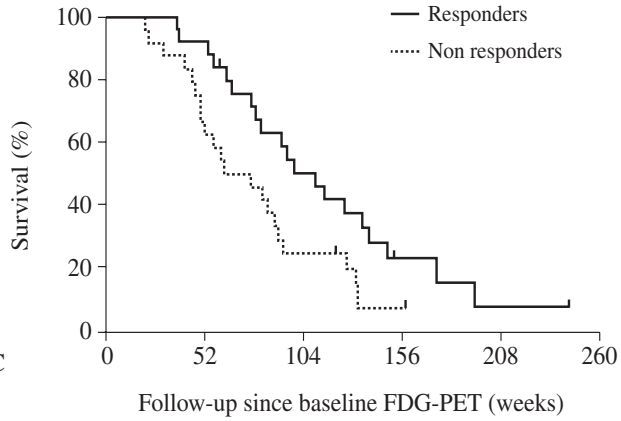
founding covariate in NSCLC in multivariate Cox proportional hazards analysis. After correction for this confounder, only ΔMR_{glu} calculated from a $ROI_{fixed\ volumes}$ showed significant predictive value for both overall and progression free survival. $ROI_{fixed\ thresholds}$ based ΔMR_{glu} from showed significant prediction of only overall survival after correction for tumor stage (Table VII).

Using medians as cut-offs shows that the median ratio for overall survival in CRC was 1.78 (HR: 2.02; P=0.03) in $ROI_{fixed\ volumes}$ and 1.25 in $ROI_{fixed\ thresholds}$ (HR: 1.75; P=0.082). For progression free survival the median ratio is 1.57 (HR: 1.24; P=0.294) and 1.21 (HR: 1.23; P=0.384), respectively. In NSCLC the median ratio for overall survival is 2.01 (HR 2.17; P=0.012) in $ROI_{fixed\ volumes}$ and 2.01 in $ROI_{fixed\ thresholds}$ (HR: 2.19; P=0.01) and for progression free survival 2.93 (HR: 2.02; P=0.015) and 2.94 (HR: 2.13; P=0.009).

In CRC, a ΔMR_{glu} of -50% in fixed volumes and -34% in fixed thresholds separates patients who have 90% 1-year overall survival from those with lower overall survival rates. In NSCLC, these values are -67% for fixed volumes and -64% for fixed thresholds. In NSCLC, this is highly different from the medians. Using these values for prognosis-driven cut-offs in patients with NSCLC instead of the median value, the median ratio for overall survival was 4.81 (HR: 5.5; P<0.001) in $ROI_{fixed\ volumes}$ and 4.51 in $ROI_{fixed\ thresholds}$ (HR: 3.68; P=0.008). These cut-offs separate the 18-20% of best responding patients from the rest. Results are presented in Figure 3.

CRC

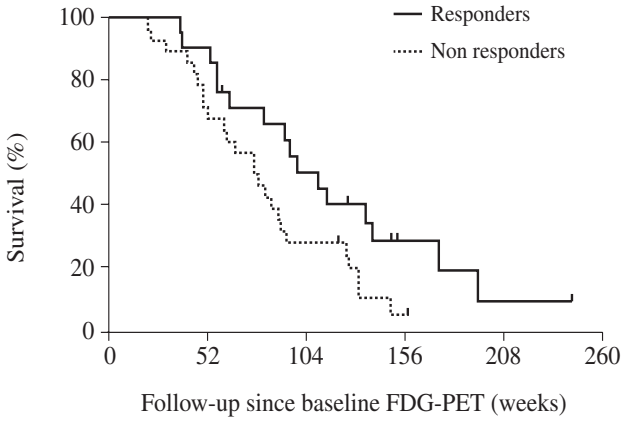
Fixed volumes



Numbers at risk (years)	0yr	1yr	2yr	3yr	4yr
Non-responders	24	16	7	2	1
Responders	25	24	13	4	2

Log rank: P=0.030; HR: 0.48 (0.25-0.93)
Median ratio: 1.6 (1.1-2.1)

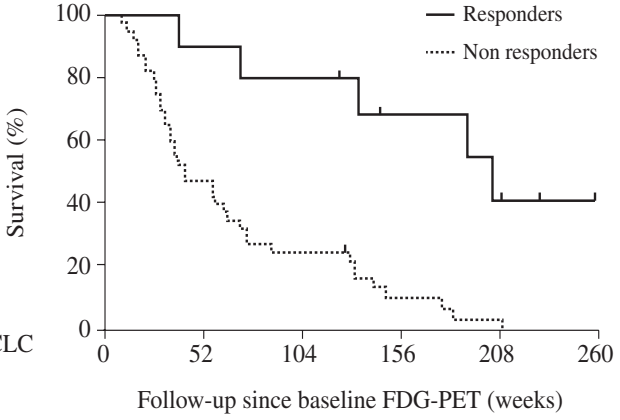
Fixed thresholds



Numbers at risk (years)	0yr	1yr	2yr	3yr	4yr
Non-responders	28	20	9	2	1
Responders	21	20	11	4	2

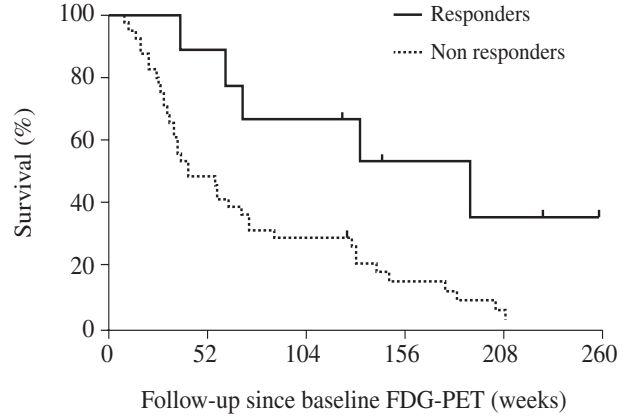
Log rank: P=0.038; HR: 0.51 (0.27-0.96)
Median ratio: 1.4 (0.89-2.0)

NSCLC



Numbers at risk (years)	0yr	1yr	2yr	3yr	4yr
Non-responders	24	16	7	2	1
Responders	25	24	13	4	2

Log rank: P=0.001; HR: 0.29 (0.15-0.55)
Median ratio: 4.8 (4.4-5.2)



Numbers at risk (years)	0yr	1yr	2yr	3yr	4yr
Non-responders	28	20	9	2	1
Responders	21	20	11	4	2

Log rank: P=0.012; HR: 0.42 (0.22-0.83)
Median ratio: 4.5 (4.1-4.9)

Figure 3.—Kaplan-Meier survival curves for overall survival in CRC patients. ΔMR_{glu} are dichotomized by the cut-off where 90% of the metabolic responders have 1-year overall survival. CRC: colorectal carcinoma; NSCLC: non-small cell lung carcinoma; FDG-PET: [18F]2-fluoro-2-deoxy-D-glucose positron emission tomography; ΔMR_{glu}: relative change in glucose metabolic rate between baseline and follow-up FDG-PET; HR: hazard ratio [95% confidence interval].

Discussion

Fluorine-18-FDG-PET is a promising imaging modality for therapy response assessment. The variety of analytical methods is vast, thus multicenter research is hardly feasible. For that, standardization of acquisition, reconstruction, ROI-determination and SUV-normalization needs to be accomplished.[15, 23] In the present study, two methods for determination of ROIs, used for Patlak MR_{glu}-estimations, were compared using survival as primary outcome measure. Only a small number of studies have addressed the effect of ROI-definition on FDG uptake,[14-16] mostly using standardized uptake value (SUV) instead of the gold standard MR_{glu}, of which is known that high correlations exist for both CRC [12] and NSCLC.[8] To the best of our knowledge, most of the studies were performed on anthropomorphic phantoms and the studies on patients did not use patient survival as the gold standard nor compared the use of a fixed volumes ROI to that of the more commonly used fixed thresholds ROI method.

Experiments with an anthropomorphic thorax phantom using SUVs determined in different ROIs, report that ratios of post-treatment and pretreatment SUVs, used for response monitoring, were only slightly dependent on ROI definition, noise and image resolution.[14] A false SUV response was observed when only tumor size changed, but lesion activity was constant, which was caused by partial-volumes effects.[14] An almost linear correlation amongst SUVs obtained with different ROI types was found.[15] Not superiority of any of the evaluated ROIs could be concluded, since no comparison to a gold standard was made.

Krak *et al*.[16] evaluated chemotherapy in 16 breast cancer patients by SUV using different ROIs (manual placement, fixed dimensions [15 mm], threshold based [50% and 70%] and maximum voxel value). They found significant lower responses by manually drawn ROIs as compared to the fixed dimensions ROI. The relative changes measured by threshold based or maximum SUV were similar. They concluded that ROI definition has a clear effect on measured change in tumor metabolism and, therefore, that SUVs obtained from different ROIs cannot be compared to each other. They, too, were not able to conclude superiority of any of the evaluated ROIs since they did not compare them to a gold standard or outcome measure.

We are not the first to use change in tumor volume combined with changes in metabolic activity in response monitoring. Guillem *et al*.[24] use a technique previously described by Larson *et al*.[25] to calculate the change in total lesion glycolysis (δTLG) by multiplication of the FDG activity concentration (SUV) to the tumor metabolic volume in 15 patients with primary rectal cancer treated with preoperative chemoradiotherapy, identifying 100% of responses. They found complete concordance between pathology and the δTLG parameter in 6 cases, in 4 the response was overestimated and in 5 it was underestimated, which was slightly better than usage of SUV_{max} or SUV_{mean} alone. More recently, Benz *et al*.[26] investigated 20 patients with locally advanced high-grade soft tissue sarcoma that underwent neoadjuvant treatment and compared SUV_{max}, SUV_{mean}, TLG_{max} and TLG_{mean} (using volumes determined on CT) for response monitoring. They conclude that TLG was less accurate in predicting tumor response than were measurements of the intratumoral FDG concentration (SUV). This technique has been evaluated for treatment of primary rectal cancer,[24, 27, 28] NSCLC,[29] malignant mesothelioma,[30] melanoma [31] and breast cancer.[32] The TLG has the disadvantage that it is highly dependent on the threshold level used. When lesion response to treatment causes its metabolic rate to be in the same range as the background, large-volume ROIs will be derived, which will artifactually lead to underestimation of treatment effect. The fixed volumes-ROI technique has the advantage that in substantial metabolic response the remaining MR_{glu} can be calculated.

In this study, in CRC and NSCLC, both MR_{glu} estimations showed a significant decrease between baseline and follow-up FDG-PET as a sign of therapy response. Significant differences in the degree of relative MR_{glu} decrease were seen, which was expected since fixed volumes based ROI-determination also takes into account tissues with <50% of the maximum activity in the lesion on follow-up FDG-PET. Thus, the volume-based average will be lower than the value determined by using a threshold for ROI definition.

A significant relation between $\Delta MR_{glu,\ fixed\ volumes}$ and both overall and progression free survival was shown, which was still present after correcting for known confounders. $\Delta MR_{glu,\ fixed\ thresholds}$ did not show significant prediction of progression free survival in CRC and of progression free survival in NSCLC after multivariate correction. Other cut-off values may be necessary when performing treatment response monitoring by fixed volumes measurements, since $MR_{glu,\ fixed\ volumes}$ showed higher decreases than $MR_{glu,\ fixed\ thresholds}$ as

explained before. Using the median to dichotomize ΔMR_{glu} showed higher separation of median survival times between fixed volumes and fixed thresholds in CRC and highly similar separation in NSCLC.

Threshold methods are relatively simple and user-independent and recovered counts are relatively independent of lesion size and of changes in geometry. Fixed dimension (15 mm) methods are relatively simple, semiautomatic and presumably less sensitive to partial volume effects when tumor size changes during therapy. Our presented fixed volumes methods benefits from the advantages of both and also takes into account treatment induced changes in tumor size.

Conclusions

Metabolic response has a high predictive value for treatment outcome in CRC as well as NSCLC patients. For determination of the ROI in therapy response monitoring studies the fixed volumes method proved to be superior to the fixed thresholds method. The use of one standardized method for ROI definition is of the utmost importance in future multicenter trials, in order to avoid institution-based variations in evaluation of metabolic response.

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