Original Article

DOI: 10.5582/bst.2016.01205

Role of the pretreatment ^{18}F-fluorodeoxyglucose positron emission tomography maximal standardized uptake value in predicting outcomes of colon liver metastases and that value's association with Beclin-1 expression

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Summary **The current study sought to evaluate the predictive and prognostic performance of the maximum standardized uptake value (SUV_{max}) prior to treatment in 43 patients with colon cancer and unresectable liver metastases. Patients with colon cancer who underwent ^{18}F-FDG-PET/computed tomography (CT) scans for staging before the start of first-line 5-fluorouracil-based chemotherapy were retrospectively analyzed. Expression of Beclin-1 in cancer cells was evaluated in primary tumors using immunohistochemical staining. The pretreatment SUV_{max} for liver metastases was not able to predict progression-free survival but was significantly associated with poorer overall survival, with a hazard ratio of 2.05 (95 % CI, 1.016-4.155). Moreover, a negative correlation was noted between SUV_{max} and expression of a marker of autophagy – Beclin-1 (rho = -0.42, p = 0.006). This suggests that the pretreatment SUV_{max} in ^{18}F-FDG PET/CT is a useful tool to help predict survival outcome in patients with colon cancer and unresectable liver metastases and may significantly distinguish between patients with low and high levels of Beclin-1 expression (AUC = 0.809, 95% CI: 0.670-0.948, p = 0.001).**

Keywords: Maximum standardized uptake value (SUV_{max}), Beclin-1, colon cancer

1. Introduction

Colorectal cancer (CRC) is the most commonly diagnosed gastrointestinal cancer worldwide, with more than 1.2 million new cases and 600,000 deaths annually (*1*). The liver is identified as the most common site for the hematogenous spread of metastatic neoplasms (*2,3*). These metastases are mainly a result of colorectal cancer (*4*). About 25% of patients are diagnosed with metastases initially and up to 50% of all patients with CRC will develop metastases, which leads to the high mortality rates reported for CRC. The 5-year survival rate for patients with CRC is close to 60% (*5*). Correct diagnosis, staging, and restaging are crucial to providing these patients with optimal therapeutic options.

Over the past few decades, 18-fluorodeoxyglucose

Released online in J-STAGE as advance publication February 28, 2017.

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positron emission tomography (^{18}F-FDG PET/CT) has become an increasingly key component in the clinical management of liver metastases (*6*). Positron emission tomography (PET) with 2-deoxy-2-[fluorine-18] fluoro-D-glucose (^{18}F-FDG), an analogue of glucose, provides valuable functional information based on the increased glucose uptake and glycolysis of cancer cells and depicts metabolic abnormalities before morphological changes occur. ^{18}F-FDG PET/CT acquires PET and CT data during the same imaging session and allows for precise anatomical localization of the lesions detected on the ^{18}F-FDG PET scan. The glucose analogue ^{18}FDG has become the most often used PET tracer in oncology and it is widely used to visualize abnormal glucose metabolism (*7*). In CRC, ^{18}F-FDG PET/CT plays a key role in recurrent disease detection as well as in the assessment of residual post-therapy masses, the localization of recurrence in patients with an unexplained elevation of carcinoembryonic antigen in the serum, and the staging of patients before surgical resection of local recurrence and distant metastasis (*5,8*). Currently, the standardized uptake value (SUV) is a quantitative method commonly used in PET. In comparison to other quantitative approaches, the SUV is clinically appealing because of its simplicity and high reproducibility, thanks to the use of modern computer software (*9*). The SUV also plays an important role in the evaluation of patient responses to therapies (*7,10*). Nevertheless, there is still debate as to the exact role that ^{18}F-FDG PET/CT can play in identifying prognostic factors for survival in patients with CRC and unresectable liver metastases (*11*).

Autophagy is the process of recycling of long-lived proteins and damaged organelles, and this process which is induced in tumor cells mainly to continue surviving (*12*). Various stress factors, such as hypoxia, an acidic environment, and starvation can intensify autophagic activity in tumor cells (*13*). Studies have reported that Beclin-1 is an essential marker of autophagy and that it plays different roles in several types of cancer (*12,14*). Recently, several studies suggested that oncogenes and autophagy play important roles in regulating the shift to aerobic glycolysis in cancer cells in order to promote cell survival (*15,16*). Mammalian cells have a complicated network that interconnects different signaling pathways to regulate autophagy, and autophagy can be induced or inhibited by glucose (*17*).

The aim of the current retrospective study was to evaluate the predictive and prognostic performance of pretreatment SUV_{max} in 43 patients with colon cancer and unresectable liver metastases. Because ^{18}F-FDG PET/CT has been rarely used to examine protein expression, the goal here was to determine if Beclin-1 expression and SUV_{max} were associated.

2. Materials and Methods

2.1. *Patients and treatment selection*

All procedures were approved by the Scientific Research Ethics Committee at "Prof. Dr. Paraskev Stoyanov" Medical University, Varna. Patients who underwent ^{18}F-FDG PET/CT as a part of their standard diagnostic workup for colon liver metastases were identified retrospectively and sequentially from PET/CT studies performed between January 1, 2012 and December 31, 2015. The inclusion criteria were (*a*) pathologically confirmed colon cancer and (*b*) multiple liver metastases (> 3 tumors) that were unresectable (largest size ≥ 5 cm) according to PET/CT. The exclusion criteria were (*a*) metastases at other site of at the time of PET/CT, (*b*) uncontrolled diabetes.

All patients were treated with first-line chemotherapy (CT) in the form of FOLFOX (leucovorin, fluorouracil (5-FU), and oxaliplatin) or FOLFIRI (leucovorin, fluorouracil (5-FU), and irinotecan) with or without bevacizumab/panitumumab until progression of the disease. Patients received a minimum of 3 months of treatment.

2.2. *Analysis of KRAS mutations*

Tissue sections were microscopically examined for adequacy using hematoxylin and eosin staining and were manually macrodissected. DNA was extracted from paraffin-embedded formalin-fixed tumor tissue and the Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation status was determined using a allele-specific real time polymerase chain reaction - based assay (AMOY Dx KRAS Seven Mutations Detection Kit, Amoy Diagnostics Co., Ltd., China) in the National Genetic Laboratory, Sofia.

2.3. *Immunohistochemical staining*

Specimens of primary colon adenocarcinoma were obtained from 43 patients in Clinical Pathology, "St. Marina" University Hospital. The diagnosis was microscopically confirmed by a pathologist. All hematoxylin eosin-stained specimens from the 43 patients with colon cancer contained cancerous tissues. Five-micrometer sections were cut from the paraffin blocks and placed on glass slides. Sections were deparaffinized with xylene and dehydrated in a graded series of ethanol to deionized water. Antigen retrieval was performed in pre-heated EnVision FLEX Target Retrieval Solution (working solution) in PT Link tanks and slides were incubated for 30 minutes at 97°C in medium with a pH of 9. After cooling, the slides were placed in diluted, room-temperature FLEX Wash Buffer (20×) for 1-5 minutes. Sections were stained using

the FLEX protocol in Dako Autostainer/Autostainer Plus. Samples were tested with recombinant rabbit polyclonal antibody to Beclin-1 (ABCAM's RabMab technology ab62557). The antibody (AntiBeclin-1, diluted 1:400) was incubated for 20 minutes. Levels of Beclin-1 expression were detected using the UltraVision detection system Anti-polyvalent, HRP/DAB. The reaction was developed with the appropriate substrate-chromogen (DAB, Diaminobenzidine) reagent. Counterstaining was done using Mayer's hematoxylin for the evaluation of immunostaining. Ten random high-power fields were examined under ×400 magnification for each patient. Digital images were obtained using the Leica Aperio ScanScope AT2 device (Aperio Technologies, Vista, CA) and further analyses of the scanned images were performed with ImageScope V12.1.0.5029 (Aperio).

2.4. *H-score assessment*

Two independent pathologists with no prior knowledge of the clinical data scored all immunohistochemically stained specimens for Beclin-1 based on the staining intensity and the percentage of positively stained tumor cells. Staining intensity was classified into 4 grades: 0 (pale yellow or no staining), 1 (yellow), 2 (deep yellow), and 3 (brown) (*18*). The percentage of positively stained tumor cells was scored in 4 grades: 0 (0-10%), 1 (10-25%), 2 (25-50%), and 3 (50-100%). The intensity and percentage of positively stained tumor cells were scored after counting at least 10 high-power fields at 400×. Mean H-scores were calculated as follows: [(Intensity reader 1 × Percentage reader 1) + (Intensity reader 2 × Percentage reader 2)]/2. The median value was used to divide patients into 2 groups, those with low levesl of expression (< median) and those with high levels of expression (≥ median) (Figure 1).

2.5. *^{18}F-FDG PET/CT image acquisition and analysis*

An ^{18}F-FDG PET/CT image was obtained using a PET/CT scanner (PHILIPS Gemini TF) consisting a dedicated lutetium orthosilicate full-ring PET scanner and a 16-slice CT scanner. Standard patient preparations included at least 6 hours of fasting and a serum glucose level of less than 120 mg/dL before ^{18}F-FDG administration. PET/CT imaging was performed 60 minutes after intravenous injection of ^{18}F-FDG. Sixty minutes after ^{18}F-FDG administration, a low-dose CT (50 mAs, 120 kV) scan covering the area from the skull to the proximal thighs was performed for the purpose of attenuation correction and precise anatomical localization. Afterwards, an emission scan was performed in three-dimensional mode with an emission scan time of 39 mm/sec. PET data were obtained using a high-resolution whole-body scanner with an axial field of view of 57.6 cm. The average total PET/CT examination time was 20 minutes. SUVs were calculated using the concentration of FDG in the volume of interest as was measured with PET. This concentration was divided by the injected dose and multiplied by body weight as a normalization factor. Following decay and scatter correction, PET images were reconstructed iteratively with attenuation correction and reoriented in axial, sagittal, and coronal slices. The row action maximum-likelihood algorithm was used for three-dimensional reconstruction. An SUV_{max} greater than the median (7.3) was denoted as a high SUV_{max}, and an SUV_{max} equal to or lower than the median (7.3) was denoted as a low SUV_{max}.

2.6. *Statistical design and analysis*

Descriptive statistics were used. Categorical features were summarized with frequencies and percentages. Statistical analysis included 43 patients treated with chemotherapy (CT) alone or CT/targeted therapy. The predictive and prognostic performance of SUV_{max} prior to treatment was evaluated in 43 patients with colon cancer and unresectable liver metastases. The Mann-Whitney *U* test, χ^2 test, and Spearman correlation were used to compare and identify correlations between the pretreatment SUV_{max} and clinicopathological characteristics such as gender, age, and level of Beclin-1 expression. The specificity and sensitivity with which SUV_{max} distinguished between patients with low levels of Beclin-1 expression and patients with high levels of expression were evaluated using receiver operating curve (ROC) analysis. The diagnostic accuracy of biomarkers was also determined by obtaining the largest possible area under the curve (AUC) in ROC analysis. Progression-free survival (PFS) was defined as the time from assignment of treatment until disease progression. Overall survival (OS) was defined as the interval between diagnosis of the disease and death or the date of last follow-up. Survival curves were estimated using the Kaplan-Meier method and differences were assessed using the log-rank test. Hazard ratios (HRs) and corresponding 95% confidence intervals (CIs) were calculated using Cox regression models. Two-tailed *p*-values < 0.05 were considered significant.

3. Results

3.1. *Clinical and pathological features*

The current study retrospectively analyzed 43 patients with confirmed unresectable liver metastatic colon cancer that was stage IV, as defined by the American Joint Committee on Cancer (AJCC), 7th edition. The patients' Eastern Cooperative Oncology Group (ECOG) performance status was < 2. Chemotherapy

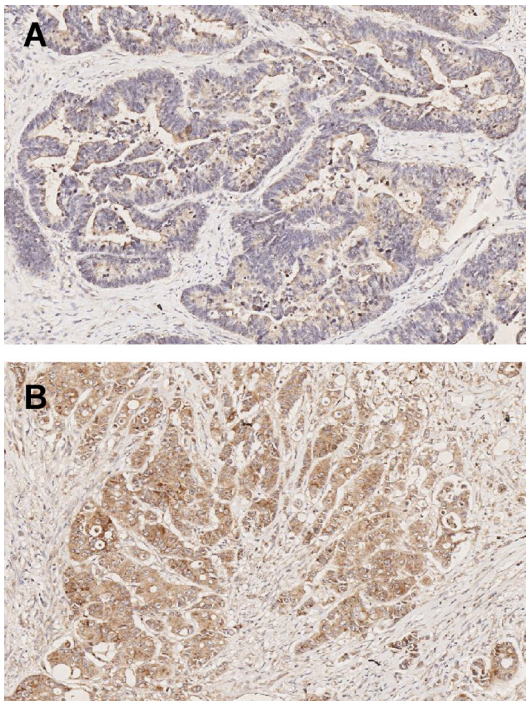

Figure 1. Immunohistochemical staining of Beclin-1 in human colon carcinomas. (A) Patient with a low level of expression **(B)** Patient with a high level of expression.

Table 1. Clinical and pathological patient characteristics at the baseline by SUV_{max} levels

SUV_{max}	Low	High	*p*-value
Age at diagnosis	63.1 ± 9.0	66. 1± 9.3	0.4
Distribution by sex, %	Females, 40.9	Females, 36.4	0.6
	Males, 59.1	Males, 63.6	
KRAS mutation status, %	RAS M+, 50.0	RAS M+, 40.9	0.3

was administered at "St. Marina" University Hospital. The mean age of the patients was 64.9 ± 9.3; 58.2% (n = 25) were men, and 41.8% (n = 18) were women. No significant difference in the pretreatment SUV_{max} was noted in men and women. No correlation between patient age and the pretreatment SUV_{max} was noted. Eighteen patients (41.8%) had KRAS mutations. Patients with KRAS mutations (n = 18) had a pretreatment SUVmax that did not differ significantly from that of patients (n = 25) with wild-type KRAS (WT).

Disease imaging with ^{18}F-FDG PET/CT was performed at the baseline and tumor response was assessed (by PET/CT or CT) at regular intervals – every 3 months for all cycles of 5-FU-based chemotherapy ± targeted therapy until progression of the disease. Clinical and pathological patient characteristics at the baseline by SUV_{max} are summarized in Table 1.

3.2. *Predictive and prognostic performance of the SUV_{max} prior to treatment in patients with colon cancer and unresectable liver metastases*

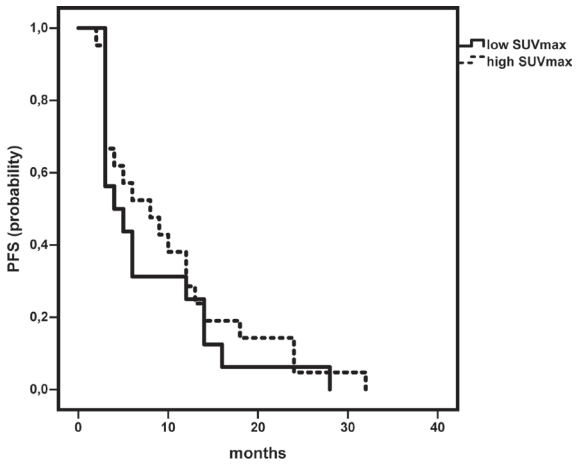

Figure 2. Comparison of progression-free survival (PFS) by pretreatment SUV_{max}. Kaplan-Meier estimates of PFS in patients with colon cancer with liver metastases by pretreatment SUV_{max} (categorized as high or low compared to the median). There was no difference in the mean PFS between groups with a high (mean 10.04 months 95% CI, 6.45-13.64) and a low SUV_{max} (mean 7.87 months 95% CI, 4.38-11.3).

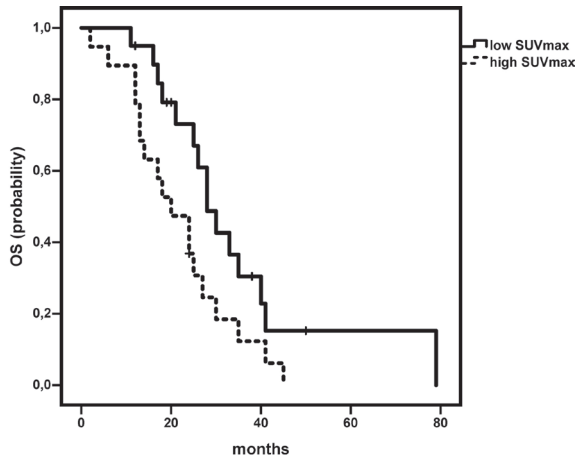

Figure 3. Comparison of overall survival (OS) by pretreatment SUV_{max}. Kaplan-Meier estimates of OS in patients with colon cancer with inoperable liver metastases by pretreatment SUV_{max} (categorized as high or low compared to the median). There was a difference in the mean OS between groups with a high and a low SUV_{max} (log rank test p = 0.03, high SUV_{max}, mean: 21.6 months (95% CI, 16.3-27.2) *vs*. low SUV_{max}, mean: 29.3 months (95% CI, 22.8-36.6)).

Patients with a high SUV_{max} prior to treatment had no significant difference in PFS compared to those with low values (mean 10.04 months *vs*. 7.87 months) (Figure 2). Patients with a high SUV_{max} prior to treatment had significant difference in OS compared to those with low values (mean 21.6 months *vs*. 29.3 months) (Figure 3). Univariate analysis indicated that a high SUV_{max} on ^{18}F-FDG PET/CT prior to treatment was significantly associated with a poorer OS, with a HR of 2.05 (95% CI, 1.016-4.155, p = 0.04). However, that association was not apparent in multivariate analysis (Table 2).

3.3. *Correlation between Beclin-1 and SUV_{max} and KRAS status*

Table 2. Results of univariate and multivariate Cox proportional regression analysis to predict overall survival

Variable	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	*p*-value	Hazard ratio	95% CI	*p*-value
Age	1.02	0.98-1.06	0.18	1.006	0.96-1.05	0.78
Gender	1.18	0.57-2.45	0.65	1.32	0.54-3.23	0.53
KRAS status	2.17	0.96-4.68	0.08	2.87	0.83-7.38	0.13
SUV_{max}	2.05	1.02-4.15	0.04	2.41	0.87-6.66	0.09
Beclin-1 expression	1.25	0.57-2.74	0.56	1.33	0.52-3.42	0.55
Chemotherapy ± Targeted therapy	0.87	0.42-1.82	0.72	0.42	0.12-1.5	0.17

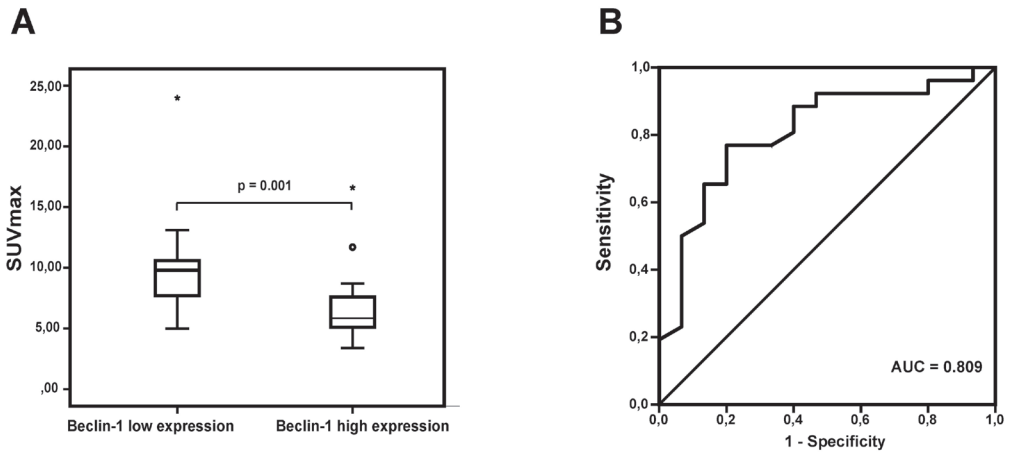

Figure 4. Association between expression of Beclin-1 and SUV_{max}. (A) A bar graph depicting the SUV_{max} in patients with low and high levels of Beclin-1 expression. The Mann-Whitney U test was used to detect significant differences in SUV_{max} in both groups. Two-tailed p-values < 0.05 were considered to be significant. **(B)** Receiver operating curve (ROC) analysis where SUV_{max} was used to differentiate between patients with low and high levels of Beclin-1 expression. The diagnostic accuracy of SUV_{max} was determined by obtaining the largest possible area under the curve (AUC) in ROC analysis.

Spearman's correlation analysis revealed that the coefficient of correlation between levels of Beclin-1 expression and SUV_{max} was -0.42 (p = 0.006) (Supplemental Data. Figure S1). Patients with low levels of Beclin-1 expression had a significantly higher SUVmax in comparison to patients with high levels of expression (mean 10.01 ± 4.4 *vs*. 6.6 ± 2.7, p = 0.001) (Figure 4A). Expression of Beclin-1 was not related to KRAS status, age, or gender. In addition, ROC analysis revealed that at the optimal cut-off values SUV_{max} distinguished between patients with low and high levels of Beclin-1 expression (AUC = 0.809, 95% CI: 0.670-0.948, p = 0.001) with a sensitivity of 76.9% and a specificity of 80% (Figure 4B).

3.4. *Effects of Beclin-1 expression on PFS and OS*

Patients with low levels of Beclin-1 expression in the primary tumor tended to have a longer PFS compared to those with high levels of expression (log rank test p = 0.06). Patients with low levels of Beclin-1 expression tended to have a greater therapeutic benefit in terms of PFS (HR 1.89 95% CI, 0.89 to 3.97, p = 0.09) than patients with high levels of Beclin-1 expression had. Patients with low levels of Beclin-1 expression in the primary tumor had no significant difference in OS compared to those with high levels of expression (Table 2).

4. Discussion

PET/CT is an imaging technique that is widely used to stage colorectal liver metastases and to assess the metabolic response to chemotherapy. PET/CT findings can prove crucial to clinical decision-making. The current retrospective study sought to evaluate the predictive and prognostic performance of the SUV_{max} prior to treatment in 43 patients with colon cancer and inoperable liver metastases. The current results suggest that a high SUV_{max} prior to treatment may be a key marker of poor survival in patients with unresectable liver metastases. Moreover, results revealed a possible association between Beclin-1 – an essential marker of autophagy – and SUV_{max}.

Several ^{18}F-FDG PET studies with small samples have suggested the prognostic value of metabolic imaging, but overall patient survival has not been reported as an indicator for treatment response (*7*). A small study of 25 patients with CRC examined the correlation between the 2-year PFS and ^{18}F-FDG PET metabolic response as determined by criteria of the European Organization for Research and Treatment of

Cancer (*19*). In that study, a post-therapeutic change in SUV_{max} of 2.0 or less in the lesion with the highest ^{18}F-FDG uptake was a strong predictor of PFS. Another study has indicated that the degree of tumor uptake of ^{18}F-FDG, as measured with SUV, serves as an independent prognostic factor in liver metastases (*20*). Other studies, however, found no association between SUV_{max} and patient prognosis (*21,22*).

One study has reported that the baseline ^{18}F-FDG-PET predicts the probability of an objective response but not the probability of a metabolic response, and the study also reported that complete metabolic responders had a significantly better PFS (*23*). In addition, other studies have reported that a high ^{18}F-FDG uptake SUV_{max} at follow-up and a high level of standardized added metabolic activity (SAM) are significant prognostic factors for PFS as well as OS (*24,25*). These findings provide further support for the prognostic power of ^{18}F-FDG PET imaging in assessing the treatment response of patients with metastatic CRC; these findings also highlight the importance of a PET-guided treatment algorithm in the management of these patients. While a high SUV_{max} at follow-up has been reported to be an adverse prognostic factor, the ΔSUV_{max} between the baseline and follow-up had no prognostic value. In contrast, the reduction in the total metabolic tumor burden as determined with ΔSAM was significantly related to PFS and OS (*11,25*). There is probably more than one reason for this discrepancy. On one hand, SUV_{max} is more likely to vary as a result of several factors such as image noise and resolution, reconstruction methods, and the sensitivity of the scanner. On other hand, SUV_{max} does not provide complete depiction of the treatment response to therapy since it indicates only the metabolic activity per gram of tissue in one voxel and does not take into consideration the total tumor metabolic load (*11*).

The current data suggest that intense glucose metabolism in liver metastases indicates tumor aggressiveness and can be used as a negative prognostic marker. However, there are large discrepancies between the cutoff values used to differentiate between high SUV and low SUV PET values. The wide range of SUV thresholds reported in studies could be the result of several factors such as institutional differences in techniques, the heterogeneity of the sample, and variance in PET scanners and protocols for data acquisition. Thus, some studies report that categorization with a wide range of SUVs resulted in significantly discriminative log-rank probability values (*26*). These findings imply that the relationship between SUV and prognosis could be gradual rather than a threshold-based one.

The use of ^{18}F-FDG PET/CT is relatively uncommon in investigations of gene expression. Only a few studies have found an association between ^{18}F-FDG uptake and KRAS mutation (*27,28*). The current results confirm the findings of a recent clinical study that reported finding no association between KRAS mutations and ^{18}F-FDG uptake in patients with metastatic colon cancer (*29*). A number of other studies have also indicated that autophagy is upregulated in KRAS-driven cancers (*30,31*). Different studies have cited Beclin-1 as a potential marker in monitoring the prognosis in CRC, with greatly divergent results regarding its function (*32-34*). The current results suggest that patients with colon cancer and low levels of Beclin-1 expression also have a high SUV_{max}, and this value may in turn be associated with a longer PFS. SUV_{max} is a favorable prognostic indicator of OS in patients with colon cancer (*11*), and the current data suggest that high levels of Beclin-1 expression correlate with a low SUV_{max}. ROC analysis revealed that SUV_{max} can be used to distinguish between tumors with low and high levels of Beclin-1 expression. This study is, to the extent known, the first to evaluate the relationship between Beclin-1 and SUV_{max} on PET/CT imaging. Nevertheless, the role of autophagy in the metastasis and prognosis of human CRC is still poorly understood. Future studies may further evaluate the potential association between expression of Beclin-1 and SUV_{max}.

Although the statistical power of the current findings is limited by the small sample, this study has verified that patients with a high SUV_{max} prior to treatment should be considered to have a higher risk of death. The current results suggest that pretreatment SUV_{max} on ^{18}F-FDG PET/CT is a useful tool to help predict survival outcome in patients with colon cancer and unresectable liver metastases and may significantly distinguish between patients with low and high levels of Beclin-1 expression. This may allow an earlier and more intensive approach to standard therapy in order to improve clinical outcomes or to assist in identifying patients who are eligible for clinical trials of a new molecularly targeted therapy for CRC.

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(Received November 3, 2016; Revised January 23, 2017; Accepted January 24, 2017)

Supplemental Data

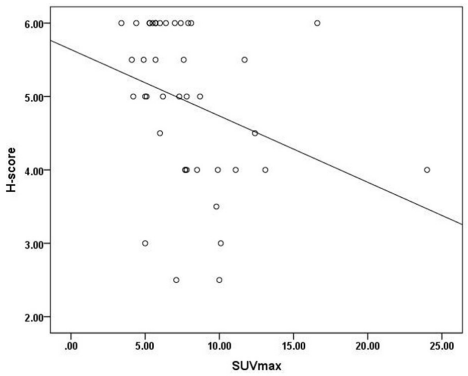

Figure S1. Correlation between expression of Beclin-1 and SUV_{max}.