Relationship Between $^{18}$F-Fluorodeoxyglucose Uptake and V-Ki-Ras2 Kirsten Rat Sarcoma Viral Oncogene Homolog Mutation in Colorectal Cancer Patients

Variability Depending on C-Reactive Protein Level

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Abstract: To evaluate clinical values of clinicopathologic and $^{18}$F-fluorodeoxyglucose ($^{18}$F-FDG) positron emission tomography/computed tomography (PET/CT)-related parameters for prediction of v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation in colorectal cancer (CRC) and to investigate their variability depending on C-reactive protein (CRP) levels.

In total, 179 CRC patients who underwent PET/CT scans before curative resection and KRAS mutation evaluation following surgery were enrolled. Maximum standardized uptake value ($SUV_{\text{max}}$), peak standardized uptake value ($SUV_{\text{peak}}$), metabolic tumor volume, and total lesion glycolysis were determined semiquantitatively. Associations between clinicopathologic and PET/CT-related parameters and KRAS expression were analyzed.

Elevated CRP (>6.0 mg/L; n = 47) was associated with higher primary tumor size, higher $SUV_{\text{max}}$, $SUV_{\text{peak}}$, metabolic tumor volume, and total lesion glycolysis, compared with those for the group with a CRP lower than that of the cutoff value (<6.0 mg/L; n = 132). Interestingly, the CRC patients (having CRP ≤ 6.0 mg/L) with KRAS mutations had significantly higher ($P < 0.05$) $SUV_{\text{max}}$ and $SUV_{\text{peak}}$ values than the patients expressing wild-type KRAS mutations. Multivariate analysis revealed $SUV_{\text{max}}$ and $SUV_{\text{peak}}$ to be significantly associated with KRAS mutations (odds ratio = 3.3, $P = 0.005$, and odds ratio = 3.9, $P = 0.004$), together with histologic grade and lymph node metastasis.

$^{18}$F-FDG uptake was significantly higher in CRC patients with KRAS mutations and with normal CRP levels. A severe local inflammation with raised CRP levels, however, might affect accurate $^{18}$F-FDG quantification in CRC tumors. Positron emission tomography/computed tomography-related parameters could supplement genomic analysis to determine KRAS expression in CRC; however, care should be exercised to guarantee proper patient selection.

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer in the world. After curative resection, adjuvant cytotoxic chemotherapy and/or radiotherapy is recommended to improve survival, especially in patients with stage II and III cancer. Although CRC patients presenting with metastatic disease have poor prognosis; nevertheless, some survival benefits are indicated with newer targeted therapies: these therapies include monoclonal antibodies against epidermal growth factor receptor (EGFR) or vascular endothelial growth factor receptors. An augmentation of cetuximab, an EGFR inhibitor, in first line treatment has been reported to reduce the risk of progression in patients with unresectable metastatic CRC disease with v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) wild-type tumors.1 Notwithstanding, an accurate identification of patients likely to respond to such targeted therapies may prevent nonresponders from undergoing unwanted drug toxicity and treatment cost. V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog mutational status as evaluated by histologic tumor examination is detected in approximately 30% to 40% of CRC patients.1

$^{18}$F-fluorodeoxyglucose ($^{18}$F-FDG) positron emission tomography/computed tomography (PET/CT) is widely used in various malignancies for diagnosis, treatment response assessment, surveillance, and prognosis. Although preoperative imaging is important for making critical decisions for treatment planning, only a few studies have described relationships between scan findings and KRAS mutation in CRC.

**Abbreviations**: $^{18}$F-FDG = $^{18}$F-fluorodeoxyglucose, BMI = body mass index, CEA = carcinoembryonic antigen, CI = confidence interval, CRC = colorectal cancer, CRP = C-reactive protein, EGFR = epidermal growth factor receptor, GLUT1 = glucose transporter-1, HNPCC = hereditary nonpolyposis colorectal cancer, KRAS = v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog, LNYmph node, LVI = lymphovascular invasion, MTV = metabolic tumor volume, OR = odds ratio, PET/CT = positron emission tomography/computed tomography, ROC = receiver operating characteristic, $SUV_{\text{max}}$ = maximum standardized uptake value, $SUV_{\text{peak}}$ = peak standardized uptake value, TAM = tumor-associated macrophage, TLG = total lesion glycolysis, VEGF = vascular endothelial growth factor, VOI = volume of interest.

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patients. Moreover, these studies report conflicting results: 2 clinical studies demonstrated that higher 18F-FDG uptake in CRC is associated with KRAS mutation,5,6 and a recent study by Iwamoto et al7 suggested that upregulation of glucose transporter-1 (GLUT1) is a possible mechanism of higher 18F-FDG accumulation in CRC with KRAS mutation. Meanwhile, Krikelis et al8 reported that there is a lack of any association between KRAS mutation and 18F-FDG-PET scan findings. Although sample size and ethnic differences might be sources of bias, the reasons for this discordance are not clearly understood.

Interestingly, Kawada et al9 excluded CRC patients who had severe inflammation with C-reactive protein (CRP) levels >5.0 mg/L. CRP is an annular, metameric protein found in the blood plasma. CRP levels rise in response to inflammation, because CRP is an acute-phase protein of hepatic origin that increases following interleukin-6 secretion from macrophages and T cells. Local inflammation of cancer cells could impact FDG uptake,9,10 and elevation in CRP may cause false-positive results on 18F-FDG PET examinations.11 Although the actual impact of CRP levels on 18F-FDG uptake in tumors is still unclear, we assumed that abnormal CRP levels might imply the presence of severe inflammation and act as a confounding factor in the association between 18F-FDG uptake in CRC and KRAS mutation.

In this study, we investigated clinicopathologic variables and PET/CT-related parameters that could potentially predict KRAS mutation in a large population of CRC patients and evaluated the variability of their predictive values according to CRP levels.

MATERIALS AND METHODS

Eligibility

The Institutional Review Board of our institute approved this retrospective study, and the need for written informed consent was waived. Patient records/information was anonymized and deidentified before analysis. Using our prospectively collected CRC database, we selected patients who underwent both 18F-FDG PET/CT scan, before surgery, and KRAS mutation examination, between August 2006 and April 2014. Patients were excluded from the selection if they were diagnosed with hereditary nonpolyposis CRC or synchronous cancer, had undergone preoperative chemotherapy or radiotherapy, and had no CRP level results before the PET/CT scan was undertaken. Clinicopathologic information on sex; age; body mass index; history of diabetes mellitus; histologic grade; lymphovascular invasion; carcinoembryonic antigen; tumor size; location of the primary tumor in the proximal colon (cecal, ascending, and transverse colon), distal colon (descending, sigmoid, and rectosigmoid colon), or rectum; and pathologic T and N stages were extracted from our database. Among the 221 patients who underwent 18F-FDG PET/CT scan and KRAS mutation analysis for CRC during the study period, 179 with full clinicopathologic information were included in this study (Fig. 1).

18F-FDS Positron Emission Tomography/Computed Tomography Protocol

All patients fasted for at least 6 hours and had blood glucose levels less than 140 mg/dL before intravenous administration of 18F-FDG (5.5 MBq/kg of body weight). At 60 minutes after intravenous administration of 18F-FDG, PET/CT scans were performed with a hybrid PET/CT scanner (Biograph 40 TruePoint or Biograph mCT 64, Siemens Healthcare Solutions USA, Inc., Knoxville, TN). Whole-body CT images were obtained first for attenuation correction using automatic dose modulation with a reference of 40 mA and 120 kV without contrast enhancement. Then PET data were acquired from the skull base to the proximal thigh for 3 minutes per bed position in a three-dimensional mode. Positron emission tomography images were reconstructed with the ordered subset expectation maximization algorithm.

Image Analysis

18F-FDG PET/CT images were reviewed in consensus from 2 experienced nuclear medicine physicians who were blinded to clinicopathologic results at the time of the review. The maximum standardized uptake value (SUVmax) was calculated as follows: SUVmax = Cmax × TBW/IA [Cmax: activity concentration in the voxel of highest tumor activity (Bq/mL), TBW: total body weight (kg), IA: injected activity (kBq)]. The peak SUV (SUVpeak) was calculated as the average SUV within a 1.2-cm diameter spherical volume of interest (VOI) positioned around the voxel of the highest SUV.

The metabolic tumor volume (MTV) was measured using a SUV-based automated contouring program (Syngo.via, version VA20A, Siemens Medical Solutions, Erlangen, Germany). First, a VOI was drawn large enough to incorporate the primary tumor lesion in the axial, coronal, and sagittal 18F-FDG PET/CT images. For the determination of metabolic volumes, we used 50% of SUVmax as a threshold, as 50% SUVmax threshold was shown to best fit the actual volume of simulating phantom lesions in previous studies.12 The software automatically produced the contour around the target lesion inside the VOI and the voxels presenting SUV intensity higher than the threshold within the contouring margin were incorporated to define the tumor volume. Total lesion glycolysis (TLG) was calculated by multiplying the tumor volume by the mean SUV of the volume.

V-Ki-Ras2 Kirsten Rat Sarcoma Viral Oncogene Homolog Mutation Status Evaluation

Deoxyribonucleic acid was extracted from formalin-fixed, paraffin-embedded tumor tissue slides with the MagNA Pure 96 System (Roche Applied Science, Indianapolis, IN). V-Ki-ras2
Kirsten rat sarcoma viral oncogene homolog exon 2 was amplified by polymerase chain reaction using GeneAMP PCR system veriti (Applied Biosystems, Foster City, CA), and was analyzed using direct sequencing with the Applied Biosystems 3730XL automated DNA analyzer (Applied Biosystems, Foster City, CA) according to the manufacturer’s instruction. The sequences of the primer were as follows: forward, GGAATTTTCATGAGTTGAATTTTGTT; reverse, GAACATCATGGACCATGCA.

Statistical Analysis
Our study group was divided into 2 subgroups (the normal CRP group and the elevated CRP group) according to levels of CRP before the PET/CT scan. All enrolled patients had CRP data obtained within 2 days of the PET/CT scan. The differences in clinicopathologic features and PET/CT parameters between the KRAS wild-type group and the KRAS mutated group were analyzed using 2-sided Pearson χ² test or Fisher exact test for categorical variables and with a Student t test for continuous variables. Then, the cutoff values of the variables providing the best separation between the KRAS wild-type and mutated groups were obtained using receiver operating characteristic curve analyses, and used to dichotomize the data. Predictive values of PET/CT parameters for the KRAS status were evaluated using multivariate logistic regression analysis along with the clinicopathologic variables. Multicollinearity among PET/CT-related parameters was evaluated by calculating Spearman rank correlation coefficient before multivariate analysis. To evaluate the impact of CRP status on predictive values of PET/CT-related parameters for KRAS mutation, the same statistical analyses were performed after the patients were divided into the 2 groups: the normal CRP group and the elevated CRP group. A P value <0.05 was considered to indicate statistical significance. All calculations and analysis were performed using the SPSS software package Version 20.0 (IBM Corp., Armonk, NY).

RESULTS
Patient Demographics
The characteristics of the 179 enrolled patients are shown in Table 1. We evaluated the differences in clinicopathologic variables and PET/CT-related parameters according to CRP levels with a cutoff level of 6.0 mg/L. The patients with elevated CRP levels (n = 47; 26.2%) had primary tumors of larger size and higher SUVmax, SUVpeak, MTV, and TLG, compared with those with normal CRP levels (n = 132; 73.8%; P < 0.05, all). V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog mutations were found in 60 patients (33.5%); 45 of these patients (75%) had normal CRP levels, whereas the other 15 patients (25%) showed elevated CRP levels. On review of medical records, none of the patients with abnormal CRP levels had any severe systemic inflammation or infection that might have contributed to elevations in CRP level. The other clinicopathologic factors did not show significant group differences.

Factors Associated With V-Ki-Ras2 Kirsten Rat Sarcoma Viral Oncogene Homolog Mutation Status
A cutoff level was determined by receiver operating characteristic curve analysis for SUVpeak, SUVmax, MTV, and TLG, and the best discriminative values between those tumors with wild-type and mutated KRAS were 9.15 (g/mL), 7.4 (g/mL), 10 mL, and 50 g, respectively. Among the 179 patients in total, the KRAS mutation group was found to have tumors with lower histologic grade and more positive lymph node (LN) metastasis, compared with the KRAS wild-type group (P < 0.05, all; Table 2). None of the PET/CT-related parameters showed significant differences between the 2 groups. Multivariate analysis revealed that histologic grade and LN metastasis were independent predictors of KRAS mutation in CRC patients [histologic grade: odds ratio (OR), 5.6; 95% confidence interval (CI), 1.6–18.9; P = 0.006, and LN metastasis: OR, 3.4; 95% CI, 1.6–7.1; P = 0.001].

Subgroup Analysis According to C-Reactive Protein Level
In the normal CRP group (CRP < 6; n = 132), the KRAS mutation group showed higher SUVmax, SUVpeak, and lower histologic grade than the KRAS wild-type group. In the prediction of KRAS mutation, the sensitivity, specificity, and accuracy were 60.0%, 50.3%, and 54.0% for SUVmax and 73.3%, 60.5%, and 67.8% for SUVpeak, respectively. Because a strong positive correlation was found between SUVmax and SUVpeak (Spearman rho = 0.955, P < 0.01), their predictive values were analyzed in 2 separate multivariate models. In multivariate logistic regression analysis, higher SUVmax proved to be significantly associated with KRAS mutation together with lower histologic grade and positive LN metastasis (SUVmax > 9.15, OR, 3.3; 95% CI, 1.4–7.4; P = 0.005, histologic grade: OR, 6.0; 95% CI, 1.5–24.6; P = 0.013, positive LN metastasis: OR, 3.2; 95% CI, 1.3–7.8; P = 0.011; Table 3). In the model with SUVpeak, SUVpeak was also found to be an independent predictor of KRAS mutation, along with histologic grade and LN metastasis (SUVpeak > 8.5, OR: 3.8; 95% CI, 1.5–9.3; P = 0.004, histologic grade: OR, 5.5; 95% CI, 1.3–22.8; P = 0.019, positive LN metastasis: OR, 3.1; 95% CI, 1.9–7.5; P = 0.015). In contrast, both lower 18F-FDG uptake and positive LN metastasis were significantly associated with KRAS mutation in the elevated CRP group (CRP > 6; n = 47; P < 0.05, all).

DISCUSSION
V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog mutational analysis is essential before the initiation of anti-EGFR therapy, because the antitumor efficacy of the drug is dependent on KRAS mutation status. The most common methods of assessing KRAS mutational status are through molecular examination using biopsy or resected tissue. There, however, there are several obstacles to this approach. First, in case of metastatic sites, it is not always easy to access or obtain adequate tissue samples for genetic testing. Second, because of the nonhomogeneous, scattered distribution of tumor tissue, there could be discordant results for KRAS mutation between the biopsy tissue and the resected tumor. For these reasons, Miles et al13,14 insisted that identification of an imaging signature for the KRAS mutation would enable imaging to provide an adjunct to histologic assessment, and demonstrated that decision trees using imaging biomarkers could enhance the predictive accuracy of KRAS mutation in CRC. 18F-FDG PET/CT is now being widely used in staging, detecting recurrence, monitoring treatment response, and predicting prognosis in CRC; however, 18F-FDG PET/CT has been used relatively rarely in the investigation of gene expression. Only a few studies have evaluated the association between 18F-FDG uptake and KRAS mutation. Kawada et al5 reported that the SUVmax and the tumor-to-liver ratio were significantly higher in the group of patients with KRAS mutation. Another
A retrospective study also showed that SUV\textsubscript{max} was an independent predictor of KRAS mutation in CRC, together with the PET-based maximal tumor width.\textsuperscript{5} The accuracy of SUV\textsubscript{max} improved when combined with other imaging features: SUV\textsubscript{max}, CT texture, and perfusion.\textsuperscript{15} Recently, Iwamoto et al\textsuperscript{7} suggested that mutated KRAS causes higher \textsuperscript{18}F-FDG accumulation, possibly by upregulation of GLUT1, through an in vitro study. In contrast, a recent clinical study reported a lack of association between KRAS mutation and \textsuperscript{18}F-FDG uptake in metastatic colon cancer.\textsuperscript{8} This study also showed that there was no significant association between GLUT1 mRNA expression and KRAS mutation status.

Based on our results, we suppose that the lack of association between KRAS mutation and \textsuperscript{18}F-FDG uptake, however, might be because of improper patient selection. In the other clinical studies, patients with high serum glucose levels, small-sized tumors, or high CPR levels of >5 mg/L were excluded on the rationale that those variables might interfere with \textsuperscript{18}F-FDG uptake and accurate measurement of SUV.\textsuperscript{5,6} In the current study, \textsuperscript{18}F-FDG uptake represented as SUV\textsubscript{max} and SUV\textsubscript{peak} had

### TABLE 1. Patient Characteristics According to C-Reactive Protein Status (n = 179)

| Variables                      | Total (n = 179) | CRP < 6 (n = 132) | CRP ≥ 6 (n = 47) | P          |
|-------------------------------|----------------|------------------|-----------------|------------|
| Sex                           |                |                  |                 | 0.603      |
| Male:female                   | 101:78         | 76:56            | 25:22           |            |
| Age (years)                   |                |                  |                 | 0.850      |
| Mean ± SD                     | 64.0 ± 1.2     | 63.9 ± 12.0      | 64.3 ± 13.0     |            |
| BMI (kg/m\textsuperscript{2}) |                |                  |                 | 0.583      |
| Mean ± SD                     | 23.1 ± 3.2     | 23.2 ± 3.1       | 22.9 ± 3.5      |            |
| DM                            |                |                  |                 | 0.858      |
| Yes                           | 32             | 24               | 8               |            |
| No                            | 147            | 108              | 39              |            |
| CEA (ng/mL)                   |                |                  |                 | 0.202      |
| Mean ± SD                     | 10.4 ± 31.2    | 7.6 ± 16.3       | 18.2 ± 54.6     |            |
| Tumor location                |                |                  |                 | 0.080      |
| Proximal colon                | 60             | 38               | 22              |            |
| Distal colon                  | 63             | 80               | 13              |            |
| Rectum                        | 56             | 44               | 12              |            |
| Tumor size (cm)               |                |                  | <0.001          |            |
| Mean ± SD                     | 5.1 ± 3.4      | 4.5 ± 2.4        | 6.2 ± 2.6       |            |
| Histologic grade*             |                |                  |                 | 0.362      |
| G1                            | 14             | 12               | 2               |            |
| G2, G3, etc.                  | 165            | 120              | 45              |            |
| LVI                           |                |                  |                 | 0.666      |
| Positive                      | 49             | 35               | 14              |            |
| Negative                      | 130            | 97               | 33              |            |
| pT                            |                |                  |                 | 0.191      |
| Tis, T1, and T2               | 30             | 25               | 5               |            |
| T3 and T4                     | 149            | 107              | 42              |            |
| LN metastasis                 |                |                  |                 | 0.630      |
| Positive                      | 93             | 70               | 23              |            |
| Negative                      | 86             | 62               | 24              |            |
| Stage                         |                |                  |                 | 0.891      |
| 0 and I                       | 24             | 19               | 5               |            |
| II                            | 58             | 41               | 17              |            |
| III                           | 79             | 59               | 20              |            |
| IV                            | 18             | 13               | 5               |            |
| SUV\textsubscript{max}        |                |                  |                 | 0.029      |
| Mean ± SD                     | 12.0 ± 6.3     | 11.4 ± 6.2       | 13.7 ± 6.2      |            |
| SUV\textsubscript{peak}       |                |                  |                 | 0.025      |
| Mean ± SD                     | 9.8 ± 5.2      | 9.2 ± 5.0        | 11.2 ± 5.3      |            |
| MTV                           |                |                  |                 | 0.002      |
| Mean ± SD                     | 20.2 ± 22.3    | 15.7 ± 12.7      | 32.8 ± 35.2     |            |
| TLG                           |                |                  |                 | <0.001     |
| Mean ± SD                     | 145.4 ± 169.4  | 105.1 ± 98.5     | 258.3 ± 256.3   |            |

BMI = body mass index, CEA = carcinoembryonic antigen, CRP = C-reactive protein, DM = diabetes mellitus, LN = lymph node, LVI = lymphovascular invasion, MTV = metabolic tumor volume, SD = standard deviation, SUV\textsubscript{max} = maximum standardized uptake value, SUV\textsubscript{peak} = peak standardized uptake value, TLG = total lesion glycolysis.

* Union for International Cancer Control (UICC) classification: G1 well differentiated, G2 moderately differentiated, G3 poorly differentiated, etc. mucinous and signet ring cell.
a positive correlation with KRAS mutation only in the normal CRP group; meanwhile, it showed an inverse correlation in the elevated CRP group and lost its predictive power in the whole population. 18F-FDG is a glucose analogue and thus a metabolic tracer, not a cancer-specific tracer. Secondary local inflammation following treatment has been shown to induce false-positive 18F-FDG accumulation, thus hampering the acute assessment of treatment response. Kubota et al demonstrated that newly formed granulation tissue around tumors and macrophages showed greater FDG uptake than did viable tumor cells.

### TABLE 2. Univariate Analysis of Factors Associated With V-Ki-Ras2 Kirsten Rat Sarcoma Viral Oncogene Homolog Status According to C-Reactive Protein Level

| Variables          | Total Wild-Type KRAS (n = 119) | Mutated KRAS (n = 60) | CRP < 6 Wild-Type KRAS (n = 87) | Mutated KRAS (n = 45) | P  | CRP ≥ 6 Wild-Type KRAS (n = 32) | Mutated KRAS (n = 15) | p  |
|--------------------|--------------------------------|-----------------------|--------------------------------|-----------------------|----|--------------------------------|-----------------------|----|
| Sex                | 0.218                          |                       | 0.280                          |                       | 0.539 |
| Male               | 71                             | 30                    | 53                             | 23                    | 18             | 7                              |                       | 14             | 8  |
| Female             | 48                             | 30                    | 34                             | 22                    | 11             | 5                              |                       | 21             | 10 |
| Age (years)        | 0.550                          |                       | 0.516                          |                       | 0.944 |
| <60                | 41                             | 18                    | 30                             | 13                    | 11             | 5                              |                       | 21             | 10 |
| ≥60                | 78                             | 42                    | 57                             | 32                    | 21             | 10                             |                       | 8              | 6  |
| BMI (kg/m²)        | 0.955                          |                       | 0.956                          |                       | 0.327 |
| <25                | 87                             | 44                    | 64                             | 35                    | 23             | 9                              |                       | 8              | 6  |
| ≥25                | 31                             | 16                    | 23                             | 10                    | 8              | 6                              |                       | 6              | 4  |
| DM history         | 0.260                          |                       | 0.596                          |                       | 0.327 |
| Yes                | 24                             | 8                     | 17                             | 7                     | 7              | 1                              |                       | 25             | 14 |
| No                 | 95                             | 52                    | 70                             | 38                    | 25             | 14                             |                       |                |    |
| CEA (ng/mL)        | 0.614                          |                       | 0.065                          |                       | 0.377 |
| <5                 | 76                             | 36                    | 58                             | 29                    | 18             | 7                              |                       | 14             | 8  |
| ≥5                 | 43                             | 24                    | 29                             | 16                    | 14             | 8                              |                       |                |    |
| Tumor location     | 0.583                          |                       | 0.420                          |                       | 0.348 |
| Proximal           | 38                             | 22                    | 25                             | 13                    | 13             | 9                              |                       | 9              | 4  |
| Distal             | 45                             | 18                    | 36                             | 14                    | 9              | 4                              |                       |                |    |
| Rectum             | 36                             | 20                    | 26                             | 18                    | 10             | 2                              |                       |                |    |
| Tumor size (cm)    | 0.875                          |                       | 0.689                          |                       | 0.552 |
| <5                 | 60                             | 31                    | 51                             | 28                    | 9              | 3                              |                       | 23             | 12 |
| ≥5                 | 59                             | 29                    | 36                             | 17                    | 23             | 12                             |                       |                |    |
| Histologic grade   | 0.011                          |                       | 0.013                          |                       | 0.575 |
| G1                 | 5                              | 9                     | 4                              | 8                     | 1              | 1                              |                       | 1              |    |
| G2, G3, etc.       | 114                            | 51                    | 83                             | 37                    | 31             | 14                             |                       |                |    |
| LVI                | 0.389                          |                       | 0.223                          |                       | 0.716 |
| Positive           | 35                             | 14                    | 26                             | 9                     | 9              | 5                              |                       | 23             | 10 |
| Negative           | 48                             | 46                    | 61                             | 36                    | 23             | 10                             |                       |                |    |
| pT                 | 0.981                          |                       | 0.489                          |                       | 0.105 |
| Tis, T1, T2       | 20                             | 10                    | 15                             | 10                    | 5              | 0                              |                       | 27             | 15 |
| T3, T4            | 99                             | 50                    | 72                             | 35                    | 27             | 15                             |                       |                |    |
| LN metastasis      | 0.013                          |                       | 0.128                          |                       | 0.022 |
| Positive           | 54                             | 39                    | 42                             | 28                    | 12             | 11                             |                       | 20             | 4  |
| Negative           | 65                             | 21                    | 45                             | 17                    | 20             | 4                              |                       |                |    |
| Stage              | 0.193                          |                       | 0.547                          |                       | 0.017 |
| 0 and I           | 18                             | 6                     | 13                             | 6                     | 5              | 0                              |                       | 5              | 0  |
| II                 | 43                             | 15                    | 30                             | 11                    | 13             | 4                              |                       |                |    |
| III                | 46                             | 33                    | 37                             | 22                    | 9              | 11                             |                       |                |    |
| IV                 | 12                             | 6                     | 7                              | 6                     | 5              | 0                              |                       |                |    |
| Maximum Standardized Uptake Value |            |                        |                                |                       |                |
| Mean ± SD         | 11.8 ± 5.7                     | 12.6 ± 7.4            | 10.4 ± 4.6                     | 13.2 ± 8.1            | 15.3 ± 6.5     | 10.4 ± 4.1                     |                       | 0.012          |
| ≥10              | 63                             | 38                    | 39                             | 32                    | 24             | 6                              |                       | 0.020          |
| <10              | 56                             | 22                    | 48                             | 13                    | 8              | 9                              |                       |                |    |
| Peak Standardized Uptake Value |            |                        |                                |                       |                |
| Mean ± SD         | 9.7 ± 4.8                      | 10.0 ± 5.9            | 8.5 ± 3.9                      | 10.6 ± 6.5            | 12.6 ± 5.6     | 8.2 ± 2.9                      |                       | 0.001          |
| ≥7.4             | 72                             | 45                    | 48                             | 37                    | 24             | 8                              |                       | 0.137          |
| <7.4             | 47                             | 15                    | 39                             | 8                     | 8              | 7                              |                       |                |    |
| Metabolic Tumor Volume (mL) |            |                        |                                |                       |                |
| Mean ± SD         | 21.1 ± 25.9                    | 18.5 ± 12.5           | 16.2 ± 14.5                    | 14.8 ± 8.1            | 34.4 ± 41.2    | 29.3 ± 16.7                     |                       | 0.647          |
| ≥15              | 60                             | 38                    | 101                            | 255                   | 22             | 13                             |                       | 0.189          |
| <15              | 59                             | 22                    | 49                             | 20                    | 10             | 2                              |                       |                |    |
| Total Lesion Glycolysis (g) |            |                        |                                |                       |                |
| Mean ± SD         | 153.7 ± 196.9                  | 128.9 ± 93.0          | 101.6 ± 106.8                  | 112.0 ± 80.8          | 295.2 ± 295.8  | 179.6 ± 110.7                    |                       | 0.059          |
| ≥50              | 86                             | 49                    | 101                            | 255                   | 22             | 13                             |                       | 0.936          |
| <50              | 33                             | 11                    | 29                             | 9                     | 4              | 2                              |                       |                |    |

BMI = body mass index, CEA = carcinoembryonic antigen, CRP = C-reactive protein, DM = diabetes mellitus, KRAS = v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog, LN = lymph node, LVI = lymphovascular invasion, SD = standard deviation.

Union for International Cancer Control (UICC) classification: G1 well differentiated, G2 moderately differentiated, G3 poorly differentiated, etc. mucinous and signet ring cell.
and a maximum of 29% of the $^{18}$F-FDG uptake was derived from nontumor tissues, such as macrophages and granulation tissues, which was indicative of confusing $^{18}$F-FDG uptake in the inflammatory response to tumor cells. Diederichs et al.11 evaluated the accuracy of FDG-PET concerning the differentiation of benign and malignant pancreatic masses according to CRP level. In the differentiation of benign and malignant lesions, specificity was significantly lower for patients with elevated CRP levels than for patients with unknown or normal CRP levels (40% versus 87%, P <0.01). They concluded that elevated CRP may cause false-positive results in FDG-PET examinations. Nevertheless, to the best of our knowledge, the relationships among CRP level, local inflammation, and tumoral $^{18}$F-FDG uptake in CRC have not been investigated thoroughly.

Causes of local inflammation in CRC can be postulated in 2 aspects: 1 is from host-to-tumor response and the other from gross peritumoral tissue damage. Solid tumors comprise malignant cells, resident stromal cells, and infiltrating inflammatory leucocytes.18 Peritumoral inflammatory lesions are extensively infiltrated by activated macrophages, so-called tumor-associated macrophages.17,18 Besides the host response, CRC may be accompanied by tumor necrosis, perforation, abscess formation, or bowel ischemia that predisposes it to inflammatory cell infiltration. A high-degree local inflammatory cell response is reportedly more commonly present in rectal tumors, whereas an elevated systemic inflammatory response is more commonly present in colonic tumors.19 Those phagocytes are also effective accumulators of $^{18}$F-FDG because of their constitutively high endocytic activity.10 and thus, accumulation of inflammatory cells is often associated with false-positive results in $^{18}$F-FDG-based studies.21–23 Admittedly, CRP is an acute-phase marker for systemic inflammation and therefore may be not directly related to local inflammatory response. A high-grade local inflammatory response, however, can switch to a systemic inflammatory response as tumor growth continues or inflammation is aggravated.24 For those reasons, abnormal elevation of CRP may suggest the presence of local inflammation in CRC, and accurate quantification of $^{18}$F-FDG uptake in CRC could be hampered in this condition. The current study reinforces the importance of proper patient selection that has been highlighted in other studies.11

$^{18}$F-FDG PET/CT has several advantages over other potential biologic or imaging markers in prediction of KRAS mutation. This technique is a noninvasive functional imaging modality that provides quantitative information on the glucose uptake of tumors as well as qualitative features of tumors that may provide guidance on decisions regarding biopsy sites. In addition, the metabolic parameters of the primary lesion can be obtained before or without surgery. Moreover, contouring of the tumor and measurement of SUVmax are less operator dependent and there is less interobserver variability, compared with conventional imaging modalities.25–27 Nevertheless, for prediction of KRAS mutation in CRC, the overall accuracy of SUVmax alone has only been found to be modest, ranging from 60% to 75%, and this was similar in our study. Although the accuracy could be improved when combined with other clinicopathologic or imaging parameters, $^{18}$F-FDG PET/CT cannot replace conventional methods to confirm KRAS mutation, and it should rather be used as a complementary tool when histopathologic results are equivocal.

There are several limitations in this study. First, CRP is related to local inflammation, although probably indirectly. Therefore, peritumoral inflammation does affect the measurement of $^{18}$F-FDG in primary tumors; however, this study lacks histopathologic confirmation of secondary local inflammation and it is unclear how accurately CRP level reflects the severity of such local inflammation. Second, we used 0 to 6 mg/L as the normal range of CRP whereas a larger population of CRC patients, this was a retrospective study conducted at a single tertiary referral center.

In conclusion, $^{18}$F-FDG uptake in primary CRCs with mutated KRAS was significantly higher than in CRCs with wild-type KRAS, and SUVmax and SUVpeak were independent predictors of KRAS mutation together with positive LN metastasis. This relationship, however, changed in patients with elevated CRP levels, and we suppose that severe local inflammation might have affected the measurement of tumoral $^{18}$F-FDG uptake. The current study reinforced the predictive value of $^{18}$F-FDG uptake for KRAS mutation in addition to stressing the importance of proper patient selection in a correlative study.

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