Assessment of Tumor Grade and Angiogenesis in Colorectal Cancer:

Whole-volume Perfusion CT

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Rationale and Objectives: The preoperative evaluation of tumor grading and angiogenesis has important clinical implications in the treatment and prognosis of patients with colorectal cancers (CRCs). The aim of the present study was to assess tumor perfusion with 256-slice computed tomography (CT) using whole-volume perfusion technology before surgery, and to investigate the differences in the perfusion parameters among tumor grades and the correlation between perfusion parameters and pathologic results in CRC.

Materials and Methods: Thirty-seven patients with CRC confirmed by endoscopic pathology underwent whole-volume perfusion CT assessments with a 256-slice CT and surgery. Quantitative values for blood flow, blood volume, and time to peak were determined using commercial software. After surgery, resected specimens were analyzed immunohistochemically with CD105 antibodies for the quantification of microvessel density (MVD). The difference in CT perfusion parameters and MVD among different tumor differentiation grades was evaluated by the Student–Newman–Keuls test. The correlations between CT perfusion parameters and MVD were evaluated using the Pearson correlation analysis.

Results: The mean blood flow was significantly different among well, moderately, and poorly differentiated groups (61.17 $\pm$ 17.97, 34.80 $\pm$ 13.06, and 22.24 $\pm$ 9.31 mL/minute/100 g, respectively; $P < .05$). The blood volume in the well-differentiated group was significantly higher than that in the moderately differentiated group (33.96 $\pm$ 24.81 vs. 16.93 $\pm$ 5.73 mL/100 g; $P = .002$) and that in the poorly differentiated group (33.96 $\pm$ 24.81 vs. 18.05 $\pm$ 6.01 mL/100 g; $P = .009$). The time to peak in the poorly differentiated group was significantly longer than that in the well-differentiated group (27.81 $\pm$ 11.95 vs. 17.60 $\pm$ 8.53 seconds; $P = .016$) and that in the moderately differentiated group (27.81 $\pm$ 11.95 vs. 18.94 $\pm$ 7.47 seconds; $P = .028$). There was no significant difference in the MVD among well, moderately, and poorly differentiated groups (33.47 $\pm$ 14.69, 28.89 $\pm$ 11.82, and 29.89 $\pm$ 11.02, respectively; $P > .05$). There was no significant correlation between CT perfusion parameters and MVD ($r = 0.201$, 0.295, and -0.178, respectively; $P = .233$, .076, and .292, respectively).

Conclusions: CT whole-volume perfusion technology has the potential to evaluate pathologic differentiation grade of CRC before surgery. However, preoperative perfusion CT parameters do not reflect the MVD of CRC.

Key Words: Colorectal cancer; CT; perfusion; angiogenesis.

Colorectal cancer (CRC) is the third most common cancer and the fourth most frequent cause of cancer deaths worldwide (1). The 5-year survival rate depends on the tumor stage and grade at patient presentation. Tumors with an advanced stage and grade at diagnosis are associated with a poor outcome. Individual treatment strategy based on tumor stage and grade should be applied to improve the prognosis. Thus, the preoperative diagnostic evaluation and grading of CRC are important (2). Preoperative specimens from endoscopic colorectal biopsies are often used but are normally failed to grade tumor because of the lack of sufficient tissue (3). Angiogenesis, which is important in the growth and metastasis of carcinomas, has been reported to be a promising prognostic marker for the CRC (4). Microvessel density (MVD) count is used to define the degree of angiogenesis in solid tumors for diagnostic purpose and treatment planning, which is calculated by counting the number of angiogenic blood vessels highlighted on a variety of immunohistochemical stains (5,6). However, information pertaining to the MVD can only be gathered in the in vitro setting after the resection of the tumor, and therefore there is no opportunity to evaluate the effect of neoadjuvant radiochemotherapy and antiangiogenic therapy on this parameter.

Perfusion computed tomography (CT) can quantify tumor angiogenesis noninvasively by assessing the enhancement of the tissue and vessels over time. Perfusion parameters, including tissue blood flow (BF), blood volume (BV), time to peak (TTP), and permeability–surface area product (PS), are calculated using the mathematical models for contrast agent exchange (7,8). Goh et al. (7) reported

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the relationship between CT perfusion parameters and MVD counts and their data indicated a positive correlation between tumor PS and BV with MVD in CRC, inconsistent with the report by Li et al. (9), which showed no significant correlation between any perfusion parameters with MVD. Both the studies were based on the deconvolution approach, but the scanning equipments, scanning modes, analytical software were different, which might be the potential explanation for such differences and make the standardization of the technique difficult (10). Romani et al. (11) reported that CD105 (endoglin)-staining intensities in CRCs were correlated with the MVD levels and were better indicators of the state of tumor angiogenesis. Previous CRC CT perfusion studies usually use one slice or a few slices of the tumor to represent the overall tumor angiogenesis state, and to some degree, this sampling rate is not sufficient because of the heterogeneity of tumor angiogenesis. Additionally, the lesion on CT images is not exactly the same as the pathologic specimen in the orientation, shape, and size, which makes it difficult to achieve precise alignment. Therefore, we analyzed primary CRC using whole-volume perfusion CT measurements and assessed whether perfusion CT could be used to evaluate the pathologic grade and the correlation between perfusion parameters and MVD stained with CD105 in CRC.

MATERIALS AND METHODS

Patients

The institutional review board approved this study, and informed consent was obtained from all patients enrolled in the study. Between August 2010 and March 2012, 42 consecutive patients with CRC who underwent preoperative CT scans were prospectively enrolled in the study. Patients were excluded according to the following criteria: (1) preoperative treatment such as chemotherapy or radiotherapy (n = 2); (2) contraindication to administration of contrast medium (n = 0); (3) severe diseases of the heart, liver, lung, or kidney (n = 0); and (4) descending or ascending colon cancers with severe motion artifacts during the perfusion CT examination (n = 3). A total of 37 patients with CRC who underwent surgery after perfusion CT at our institute were enrolled in this study. The subjects included 20 men and 17 women, with a mean age of 64.5 years (range, 38–85 years). The diagnosis of adenocarcinoma of the colon and rectum was histologically confirmed by colonoscopic biopsy and surgical specimens. Mean tumor length at pathologic evaluation was 9.5 cm (range, 5.2–13.8 cm). The tumors examined were located in the cecum (n = 5), ascending colon (n = 3), descending colon (n = 3), sigmoid colon (n = 4), and rectum (n = 22). Tumors were divided into three histologic subgroups: well differentiated (7 tumors), moderately differentiated (20 tumors), and poorly differentiated (10 tumors). Thirty-seven patients underwent curative surgery without severe complications.

Imaging Study

Perfusion CT was performed using a 256-slice CT scanner (Brilliant iCT; Philips Healthcare Systems, Netherlands). First, CT scanning was performed without intravenous contrast medium to localize the tumor, and the whole-tumor sections were selected at the level of the tumor for cine imaging. A dynamic study of this area was performed with a Jog mode during rapid intravenous bolus injection (5 mL/second) of 50 mL iopromide containing 370 mg of iodine per milliliter (Ultravist 370; Bayer, Berlin, Germany). The following parameters were used: 0.33-seconds gantry rotation time, 100 kV, 80 mA, 7.6-seconds scanning delay from the start of injection, 3.8-seconds scanning interval, 60.8-seconds duration of transverse data acquisition, and 3-mm reconstructed section thickness.

Imaging Analysis

The perfusion data were transferred to an image processing workstation (Extended Brilliant workshop 4.02; Philips Healthcare Systems) and then analyzed using software (CT Perfusion; Philips Healthcare Systems) based on the slope method. The parameters generated by the software were BF (in milliliters per minute per 100 g of wet tissue), BV (in milliliters per 100 g of wet tissue), and TTP (in seconds). To derive functional maps of these perfusion parameters, the arterial input curve of the contrast medium concentration was required, and we obtained this arterial input curve from a region of interest (ROI) in the external iliac artery or aorta. The ROI was drawn freehand (using an electronic cursor and mouse) around the peripheral boundary of the visible tumor. Care was taken to exclude pericolonic fat and intraluminal gas, which was facilitated by viewing a cine loop of acquisition to gauge the degree of patient movement and the tumor margins. We used a section-by-section averaging technique to evaluate whole-tumor perfusion. First, the ROI drawing was repeated for each contiguous transverse level of the entire tumor lesion. Then, a global value representing the perfusion of the entire tumor was calculated by taking the mean value of all individual sections involved (Figs 1 and 2). To assess the interobserver agreement, all cases were reevaluated after 3 months and results of the two sets of measurements were compared.

Assessment of Tumor Grade, Immunohistochemical Staining, and Quantification of MVD

The surgical specimens were fixed with 10% formaldehyde. The differentiation grades were assessed by an experienced gastrointestinal pathologist and were divided into three subgroups: well, moderately, and poorly differentiated CRCs. To evaluate tumor angiogenesis, three portions of the tumor in the craniocaudal direction were selected as the locations for additional tissue on tumor specimens for further immunohistochemical staining. Tumor necrotic portions