Fractal Analysis of CT Perfusion Images for Evaluation of Antiangiogenic Treatment and Survival in Hepatocellular Carcinoma

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Rationale and Objectives: Tumor vascular heterogeneity is a recognized biomarker for cancer progression. Our purpose was to assess the tumor perfusion heterogeneity during antiangiogenic therapy in hepatocellular carcinoma (HCC) by means of fractal analysis on computed tomography perfusion (CTP) images.

Materials and Methods: Twenty-two patients (15 men and 7 women; mean age: 61.5 years) with advanced HCC underwent CTP at baseline and 2 weeks after administration of bevacizumab. Perfusion maps of blood flow (BF) were generated by the adiabatic approximation to the tissue homogeneity model with a motion registration, and fractal analyses were applied to gray-scale perfusion maps using a plugin tool on ImageJ software (NIH, Bethesda, MD). A differential box-counting method was applied, and the fractal dimension (FD) was calculated as a heterogeneity parameter.

Results: Patients were grouped into favorable progression-free survival (PFS) group (PFS>6 months, 11 patients) and unfavorable PFS group (PFS≤6, 11 patients). After 2 weeks of antiangiogenic therapy, the BF decreased significantly ($P < .0001$), whereas the FD showed no significant change ($P = .69$). The percent change of the FD in tumor BF was significantly different between patients with favorable PFS and those without (−2.52% vs. 3.72%, $P = .01$), whereas the change of tumor BF showed no significant difference between them (−28.93% vs. −25.47%, $P = .64$). In Kaplan–Meier analysis, patients with greater reduction in the percent change of FD and lower baseline FD in tumor BF showed significantly longer overall survival ($P = .009$, $P = .005$).

Conclusions: Fractal analysis of tumor BF can be a biomarker for antiangiogenic therapy.

Key Words: CT perfusion; hepatocellular carcinoma; angiogenesis; heterogeneity; antiangiogenic treatment.

Despite various therapeutic options, hepatocellular carcinoma (HCC) is still the third most common cause of cancer-related mortality worldwide (1). HCC is a highly vascularized tumor with an elevated level of vascular endothelial growth factor (VEGF) and high microvessel density (MVD) (2,3). Greater expression of VEGF, which leads to focal leaks in tumor vessels, causing nonuniform blood flow (BF) and heterogeneous delivery of drugs and oxygen (4), has been associated with shorter survival in patients with HCC (5,6). Therefore, inhibition of angiogenesis represents a potential therapeutic target in HCC, and a large number of antiangiogenic agents are currently being tested for the treatment of HCC (4). For example, the Sorafenib HCC Assessment Randomized Protocol trials showed an improved overall survival (OS) in patients with advanced HCC on treatment with the antiangiogenic and antiproliferative agent sorafenib (7,8).

These responses are currently assessed by Response Evaluation Criteria in Solid Tumors (RECIST) (9). However, RECIST has been recognized for their limitation in assessing the antitumor activity of antiangiogenic therapies, because antiangiogenic agents suppress tumor growth by downregulating angiogenesis without causing much morphologic change (10). In this context, functional vascular imaging techniques such as computed tomography perfusion (CTP) or dynamic contrast-enhanced magnetic resonance imaging (MRI) are highly promising (4). In HCC, significant decreases in tumor blood perfusion measured by CTP or DCE-MRI after antiangiogenic treatments have been reported (11–13). These perfusion changes are consistent with the vascular normalization induced by antiangiogenic therapy (14). The reduction of heterogeneity in tumor perfusion has also been

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reported to be the process of vascular normalization during antiangiogenic therapy in in vitro studies (15–17). However, this heterogeneity change in tumor perfusion during antiangiogenic therapy has not yet been demonstrated in in vivo studies.

Fractal analysis is a mathematical technique that can be effectively used for quantifying texture or heterogeneity on digital images (18). Fractal analysis was applied to computed tomography (CT) images for assessment of the characteristics of lung nodules (19), liver tumors (20), and lymph node metastases (21,22). According to these previous reports, fractal analysis can be a good tool to assess the heterogeneity change of tumor perfusion. Therefore, our aim was to evaluate the changes of heterogeneity in tumor BF by means of fractal analysis in HCC treated with bevacizumab, which is a monoclonal antibody that targets VEGF. We also assessed the correlation between this BF heterogeneity and clinical outcome.

MATERIALS AND METHODS

Patient Population

This study was part of a phase II clinical trial on advanced HCC (11,23,24), which was in compliance with Health Insurance Portability and Accountability Act regulations and was approved by the Institutional Review Board at Dana-Farber/Harvard Cancer Center (Boston, MA). All patients were required to provide written informed consent before study participation according to institutional and federal guidelines. The eligibility and treatment schedule have been detailed previously (24). Briefly, the patient eligibility criteria included the following: 1) patients had histopathologically proven, measurable, locally advanced or recurrent HCC; 2) they had undergone a recent contrast-enhanced MRI examination of the liver and had follow-up CT and MRI examinations over 6-month interval to confirm tumor growth or response; 3) they had no liver surgery, chemotherapy, chemoembolization therapy, immunotherapy, or liver radiotherapy within 4 weeks of enrollment; and 4) they had adequate renal function (serum creatinine level $\leq$ 2.0 mg/dL [177 mmol/L]). Exclusion criteria included the following: 1) a concurrent second malignancy; 2) significant medical comorbidities; 3) clinically significant cardiovascular disease including uncontrolled hypertension, myocardial infarction, and unstable angina; 4) pregnancy or lactation; 5) known central nervous system metastases; and 6) an inability to give written informed consent. Twenty-three patients with HCC were enrolled in this study from July 2004 to January 2005.

Treatment

The treatment schedule and the dose modification schema have been detailed previously (24). Briefly, patients were treated with bevacizumab at a dose of 10 mg/kg intravenously (IV) on day 1 of cycle 1 (14 days). For the subsequent 28-day cycle, patients were treated with bevacizumab at 10 mg/kg on days 1 and 15, gemcitabine at 1000 mg/m^2 IV at a infusion dose rate of 10 mg/m^2/min on days 2 and 16, and oxaliplatin at 85 mg/m^2 at a 2-hour IV infusion on days 2 and 16 of every cycle (GEMOX-B). The dose of bevacizumab was fixed at 10 mg/kg. Treatment was continued until progression, unacceptable toxicity, or withdrawal of consent. Follow-up CT/MRI scans were performed after the first three cycles and then at every 8 weeks until disease progression.

Imaging Studies

CTP was performed with a 16-section multidetector row CT scanner (Light Speed; GE Medical Systems, Milwaukee, WI) at baseline and 2 weeks after initiation of bevacizumab. First, CT scanning was performed without IV contrast medium (CM) for localization of the tumor. The tumor was localized on the nonenhanced CT scan, and four adjacent 5-mm sections were selected at the level of the tumor for cine imaging. In patients with solitary hepatic tumors, the portions of the tumor with the largest volume were selected for dynamic CT evaluation. The areas with definite calcifications or necrosis/cystic areas as depicted on prior imaging studies were avoided in this study. In patients with multiple hepatic tumors, the liver sections demonstrating the largest number of lesions or the largest size of lesions were selected for dynamic CT evaluation. The dynamic study of the tumors was performed at a static table position during rapid IV bolus injection of 50–70 mL nonionic iodinated CM (Isovue; Bracco, Princeton, NJ) (300–370 mg of iodine/mL) at a rate of 5–7 mL/s. The following parameters were used: 0.5-second gantry rotation time with an interval of 1 second, 100 kVp, 240 mA, with acquisition of 4i transverse mode (four sections per gantry rotation), and 5-mm reconstructed slice thickness. The cine CT acquisition was started about 5–8 seconds after the start of CM injection, and the axial CT images were acquired for a total of 30–35 seconds during breath-holding in end expiration.

Image Processing

CTP technique. CTP images were denoised by use of a gaussian process regression with a 3 $\times$ 3 pixel kernel based on spatiotemporal information (25). Considering the nonrigid nature of the liver, we applied a three-dimensional (3D) B-spline deformable registration to coregister each set of dynamic images relative to the first image as a template after prior registrations were performed by successive use of 3D rigid and affine transforms (26). These denoised images with motion artifact registration were analyzed based on a pixel-by-pixel analysis, and BF map was generated using the adiabatic approximation to the tissue homogeneity (AATH) model, which assumes a plug-flow model for capillaries and a well-mixed compartment for the interstitial space, where all the capillaries are represented together as a single cylinder