Estimation of the malignant potential of gastrointestinal stromal tumors: the value of contrast-enhanced coded phase-inversion harmonics US

Nobuhiro Fukuta¹, Masayuki Kitano¹, Kiyoshi Maekawa², Takaaki Chikugo³, and Masatoshi Kudo¹

¹Department of Gastroenterology and Hepatology, Kinki University School of Medicine, 377-2 Ohno-Higashi, Osaka-Sayama 589-8511, Japan
²Section of Abdominal Ultrasound, Kinki University School of Medicine, Osaka-Sayama, Japan
³Department of Pathology, Kinki University School of Medicine, Osaka-Sayama, Japan

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Introduction

Most gastrointestinal mesenchymal tumors (GIMTs) are completely or partly composed of spindle cells and show a light microscopic appearance suggestive of smooth muscle or nerve sheath differentiation. In the past, those tumors were often named leiomyomas or Schwann cell tumors. However, immunohistochemical studies of those neoplasms sometimes fail to confirm distinct phenotypes in the majority of cases, and therefore the name gastrointestinal stromal tumor (GIST) is generally used. GISTs arise from primitive mesenchymal cells and can occur in any part of the gastrointestinal tract. However, they are usually located in the stomach and the small intestine. The majority of GISTs are immunohistochemically positive for KIT and/or CD34 as seen by standard pathological means.¹⁻³ The most important issue concerning GIST is that it is difficult to predict its prognosis. So far, the most important prognostic factors are tumor size, mitotic count, metastasis to other sites, and invasion.¹,²,⁴⁻⁷ However, those factors are insufficient to predict the malignant potential of such tumors, because metastasis to other sites sometimes occurs even when the tumor size is less than 50mm or the mitotic count is low.

Recently, contrast agents for ultrasonography (US) such as Levovist have been introduced for routine clinical use. The contrast-enhanced US with Levovist permits evaluation of the intratumoral vascularity of hepatic and pancreatic tumors and is useful for their differential diagnosis. The purpose of the present study was to assess tumor vessels and the parenchymal flow of gastrointestinal stromal tumors (GISTs) by contrast-enhanced coded phase-inversion harmonic US and to evaluate whether vascularity is related to the malignant grade of the GISTs. Methods. Thirteen patients with GISTs were included in the present study. Tumors were observed in a real-time fashion of contrast-enhanced coded phase-inversion harmonic US after the injection of Levovist (400mg/ml). The vascular patterns were compared with tumor size, histological diagnosis, KIT mutations, and clinical findings such as metastasis. Results. The contrast-enhanced US images of the GISTs were classified into two types according to the blood flow area of the tumors as seen by real-time continuous imaging of the tumor vessels. The image pattern “Poor” represented vessels flowing only in the peripheral part of the tumor, and “Rich” represented abundant vessels flowing from the periphery to the central part of the tumor. According to the contrast-enhanced US images, five GISTs were classified as “Poor” and the others as “Rich.” Based on the final diagnosis, all tumors with “Poor” images were determined to be benign GISTs, and the rest tumors except one with “Rich” images were determined to be malignant GISTs. Conclusions. Contrast-enhanced US image is more closely correlated with the final diagnosis than the histological findings.

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Reprint requests to: M. Kudo
evaluation of the posttreatment response of hepatocellular carcinoma. It is also useful for diagnosing pancreatic tumors. We used this novel technique to evaluate the vascularity of GISTs. The purpose of the present study therefore was to assess the tumor vessels and parenchymal flow seen by contrast-enhanced coded phase-inversion harmonic imaging and to evaluate whether vascularity is related to the malignant potential of GISTs.

Methods

Patients

This study was performed with the approval of our institutional review board. Full informed consent was obtained from all patients before the study. Between July 2000 and August 2004, 13 patients with surgically resected GISTs were included in this study. The patient population included 4 men and 9 women (age range, 51–77 years; mean age, 64.7 years). Eight GISTs were located in the stomach, 3 in the duodenum, 1 in the jejunum, and 1 in the ileum. The size of the nodules was based on US measurement.

Contrast-enhanced coded phase-inversion harmonic imaging

In the present study, a suspension of monosaccharide microparticles (galactose) in sterile water (Levovist; Schering AG, Berlin, Germany) was used as the US contrast agent. Microbubbles with an average diameter of 1.3 µm, which can traverse the pulmonary capillary bed and enhance the signal of intratumoral blood flow, were stabilized in the microparticle suspension. Before US examination, the agent was prepared by vigorously shaking with 5 ml water for 5–10s. After letting it stand for 2 min to allow for equilibration, 2.5 g Levovist (6 ml at 400 mg/ml) was manually injected through a 20-gauge cannula inserted in an antecubital vein at 1 ml/s and flushed by an additional 10 ml normal saline. A GE LOGIQ 9 or 700 MR EXPERT Series unit (General Electric Medical Systems, Milwaukee, WI, USA) with a 2–4 MHz curved-array wide-band transducer was used for coded phase-inversion harmonic ultrasonography. The acoustic power was set at the default setting with a mechanical index of 0.6–0.8. After a scanning place displaying the tumor on fundamental B mode was chosen, one 2.5-g vial of Levovist (6 ml at 400 mg/ml) was intravenously injected as a bolus. When the first microbubble signal appeared in the tumor after the injection, the patient was instructed to hold his or her breath. Images of the ideal scanning plane were displayed in a real-time fashion by slightly changing the scanning plane to portray the whole area of the tumor. In addition to real-time continuous imaging of the tumor vessels (vessel image), interval-delay scanning (less than 90s after the injection of Levovist) was performed to show tumor parenchymal flow in the blood-pool phase (perfusion image). The tumor vessels appeared as a strong transient gray-scale enhancement within the tumor. To minimize procedural variations, contrast-enhanced US was performed by the same sonographer using the same examination protocol.

Contrast-enhanced CT examination

For comparison, contrast-enhanced dynamic CT was conducted on all patients. The CT examination included precontrast and postcontrast-enhanced dynamic helical CT (Toshiba X-Vigor; Toshiba Medical Systems, Tokyo, Japan) after a 100-ml intravenous bolus injection of 370 mg/ml iopamidol (Iopamiron; Nihon Schering, Osaka, Japan) at 3.0 ml/s using an automatic power injector.

Histological examination

A histological diagnosis was made on the basis of hematoxylin and eosin staining with mitotic count and immunohistochemical staining for the tissues obtained by surgical resection (all cases). The obtained samples were fixed with 20% formalin and paraffin sections (5 µm thick) and used for hematoxylin and eosin staining and for immunohistochemistry. Rabbit polyclonal antibody against human KIT, rabbit polyclonal antibody against bovine S-100 protein, mouse monoclonal antibody against human CD34, and mouse monoclonal antibody against DESMIN were used as primary antibodies. Biotinylated goat antirabbit IgG or biotinylated rabbit antimouse IgG was used as a secondary antibody. All vessels of the histological specimens were highlighted by staining endothelial cells for factor VIII (Dako Polyclonal; Dako, Santa Barbara, CA, USA) with use of a standard immunoperoxidase technique described previously. The number of tumor vessels was counted (/mm²) by a blinded pathologist in ten low-power fields randomly selected in the tumor.

KIT sequence analysis

We also investigated the existence of KIT mutation for the evaluation of the malignant factor. A polymerase chain reaction (PCR) assay used to amplify exon 11 of KIT was employed to detect KIT mutations in the tumors. DNA was prepared from tumor tissues by cell lysis, proteinase K digestion, phenol:chloroform extraction, and ethanol precipitation according to a previously reported conventional method. Primers were