Assessment of Track Microbubble Flow Signals on Contrast-enhanced Ultrasound with Perflubutane Following Percutaneous Liver Biopsy

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Abstract

Objective  It is important to detect post-liver biopsy hemorrhage early and confirm hemostasis in the clinical setting. Contrast-enhanced ultrasound (CEUS) is a sensitive and highly specific tool for detecting active bleeding. The aim of this study was to investigate the rate of detection of track microbubble flow signals on CEUS with perflubutane following liver biopsy and to assess the disappearance of these signals.

Methods  Microbubble flow signals along the needle track on CEUS were examined in 100 patients who underwent percutaneous US-guided liver biopsies. The microbubble flow signals were examined repeatedly until their disappearance. The patients were followed up with clinical and laboratory data to detect clinically significant hemorrhaging.

Results  Microbubble flow signals on CEUS following percutaneous liver biopsy were seen in 33% of the patients. There were no significant differences in the platelet count, prothrombin time or length of the biopsy specimen between the patients with and those without microbubble flow signals on CEUS. The microbubble flow signals disappeared over time in all patients. There were no cases of clinically significant hemorrhaging in the present study.

Conclusion  Track microbubble flow signals on CEUS are frequently observed after biopsies. The disappearance of a microbubble flow signal is a useful index for confirming hemostasis of postbiopsy hemorrhaging.

Key words: contrast-enhanced ultrasound, hemostasis, perflubutane, postbiopsy hemorrhaging, Sonazoid

Introduction

Despite advances in medical technology, such as noninvasive imaging modalities and laboratory tests, hepatic histological examinations remain important for the diagnosis of most liver diseases (1, 2). Histological findings are the gold standard for determining the activity and staging of chronic liver diseases. Percutaneous needle biopsies are the most frequently used biopsy method. The mortality rate after liver biopsy has been reported to range from 0.1% to 0.01% (3, 4). The primary cause of mortality after percutaneous liver biopsy is postbiopsy hemorrhaging. A retrospective Italian study reported that six patients (0.009%) died from intraperitoneal hemorrhage among 68,276 cases of percutaneous liver biopsy (3). Significant hemorrhaging following biopsy (indicated by a drop in the hemoglobin level of 2 g/dL) occurs in 0.35-0.5% of all procedures (3-5). It is recommended that percutaneous biopsies be performed under ultrasound (US)-guidance (6). US guidance enables the physician to avoid large vessels, thus resulting in fewer hemorrhagic events and a lower mortality rate (7-9). Despite its safety, major and life-threatening hemorrhaging can occur following percutaneous liver biopsy, and subclinical hemorrhage, including the development of intrahepatic hematomas or hemobilia, is frequently observed.
Table 1. Background Characteristics of the 100 Patients who Underwent Percutaneous Liver Biopsy

| Age (years) | 53.6 ± 14.0 |
| Male/female | 42/58 |
| A etiology of liver disease |  |
| Hepatitis B | 9 |
| Hepatitis C | 34 |
| Autoimmune hepatitis | 5 |
| Primary biliary cirrhosis | 8 |
| Non-alcoholic fatty liver disease | 30 |
| Drug-induced liver injury | 6 |
| Cryptogenic | 8 |
| Platelet (×10^3/mm^3) | 19.6 ± 5.9 |
| Prothrombin percent activity (%) | 98.8 ± 16.8 |
| Total bilirubin (mg/dL) | 0.85 ± 0.54 |
| Serum albumin (g/dL) | 4.09 ± 0.50 |

Early detection of postbiopsy hemorrhage is critical for administering appropriate treatment and preventing serious adverse events. Furthermore, confirming hemostasis reduces stress and anxiety for both the medical staff and patient. Until recently, postbiopsy hemorrhage was suspected based on clinical symptoms and physical findings, such as abdominal pain, hypotension and shock, and thereafter confirmed by a drop in the blood hemoglobin level and the findings of imaging modalities, such as CT or US. Although these methods are useful for identifying substantial bleeding, they cannot be used for the early detection of subclinical hemorrhage. Color Doppler US (CDUS) is a sensitive modality for detecting hemorrhage in various organs. Some reports have shown the usefulness of CDUS for detecting post-liver biopsy hemorrhaging (10-12). CDUS demonstrates a linear color flow signal along the needle tract immediately after a biopsy and is useful for screening for postbiopsy hemorrhage. Because the ability to detect the blood flow on CDUS is dependent on the flow velocity, detecting bleeding with a low flow velocity is difficult. Contrast-enhanced ultrasound (CEUS) exhibits 100% sensitivity and 100% specificity for active bleeding and is useful for detecting less dramatic or non-arterial bleeding (13, 14). However, no reports have investigated the rate of detection of microbubble flow signals along the needle tract following percutaneous liver biopsy. The aim of this study was to determine the detection rate of track flow signals on CEUS following percutaneous liver biopsy and to assess the value of confirming hemostasis using CEUS.

Materials and Methods

Patients

Between June 2008 and December 2011, 100 consecutive patients who underwent US-guided liver biopsies at our hospital were enrolled in the present study. The etiology of liver disease in these patients was hepatitis C virus (HCV)-related hepatitis in 34 cases, hepatitis B virus (HBV)-related hepatitis in nine cases, autoimmune hepatitis in five cases, primary biliary cirrhosis in eight cases, nonalcoholic fatty liver disease (NAFLD) in 30 cases, drug-induced liver injury in six cases and cryptogenic liver disease in eight cases. The preoperative clinical features of the 100 patients are listed in Table 1. This study was approved by the Ethics Committee of our institute (No. 1608) and performed prospectively. The nature of the study was fully explained to the patients, all of whom gave their informed consent.

US-guided aspiration biopsy

The patients fasted from the midnight before and until six hours after the biopsies. The aspiration biopsies were performed under US guidance using a real-time convex scanner with 3.75-MHz probes (Aplio XG SSA-790A; Toshiba, Tokyo, Japan) and a biopsy guide device. The optimal sites for the puncture and needle path were selected using CDUS to avoid large interposed blood vessels and highly vascular areas in the needle track. A local anesthetic, lidocaine, was injected from the puncture site into the subcutaneous tissue and parietal peritoneum using a 22-gauge needle. A 15-cm, 17-gauge aspiration biopsy needle (Majima needle) under negative pressure induced with a syringe was then inserted into the liver and withdrawn while the patient held his or her breath. All biopsies were performed using one puncture.

CEUS examination

Sonazoid contrast agent (perflubutane, microbubbles covered by phosphatidyl serine sodium) was prepared by shak- ing the agent in 2 mL of water. Approximately 0.5 mL of contrast agent suspension (0.0075 mL/kg) was injected manually through a 21-gauge cannula inserted into the antecubital vein, followed by an additional 2 mL of a physiological saline flush. CEUS was performed (AplioXG; Toshiba Medical System Corporation, Otawara, Japan) using a 3- to 5-MHz curved array, wide-band transducer that transmitted and received an ultrasound beam at 1.75 MHz and 3.5 MHz, respectively, in harmonic B-mode. The acoustic power of the harmonic US was set to a mechanical index (MI) of 0.2-0.25.

Immediately following the percutaneous liver biopsy, the contrast agent was injected and the patient held his or her breath. US images in the vascular phase were obtained in a real-time manner by slowly changing the scanning plane to observe the biopsy area 20 seconds after the injection. CEUS images were obtained and analyzed to determine the presence or absence of a flow signal, which was defined as a linear microbubble flow signal toward the liver capsule along the needle tract (Fig. 1-A). Continuous microbubble flow signals were observed using high-power flashes. Briefly, a high-power flash US beam (mechanical index: 1.27) destroyed the majority of microbubbles in the US plane and consequently allowed for resetting of transient perfusion. The use of repeated high-power flashes enables demonstration of the microbubble flow signal. The flow signal was observed every minute for five minutes, and the ob-
cesses, such as the subphrenic, hepatorenal, paracolic and subdome pooling along the needle tract and peritoneal re-
first. When a track microbubble flow signal was seen, it could be classified into two patterns: the presence or absence of a track flow signal on CEUS.

The two readers were blinded to each other’s findings. The images were reviewed by two other sonologists, excluding the examiners. The statistical analysis was performed using the chi-square test or Fisher’s exact probability test. All statistical tests were performed using the Stat View software package for Windows (SAS Institute, Cary, NC). A p-value of less than 0.05 was considered to be significant.

Results

There were no cases of clinically significant postbiopsy hemorrhage in the present study. Track microbubble flow signals on CEUS were seen in 33 (33%) of the 100 patients, with no signals observed in the remaining 67 patients (67%). All track microbubble flow signals observed in the 16 cases examined with pulse Doppler US were continuous flow signals. There were no significant differences in the mean platelet count between the patients with a track microbubble flow signal on CEUS (18.5±5.4×10^12/mm^3) and those without a track microbubble flow signal on CEUS (20.1±6.4×10^12/mm^3, p=0.213). There were no significant differences in the mean prothrombin percent activity between the patients with and without a track microbubble flow signal (with a signal: 97.5%±15.6%, without a signal: 97.4%±19.1%, p=0.983). There were also no significant differences in the mean length of the biopsy specimen between the patients with (13.5±6.4 mm) and without (13.1±3.9 mm, p=0.681) track flow microbubble signals on CEUS.

The detectability of track microbubble flow signals was compared between the patients with and those without fatty liver (a bright liver on B mode sonography)(Table 2). Track microbubble flow signals on CEUS were seen in nine (30.0%) of the 30 patients with fatty liver and 24 (34.5%) of the 70 patients without fatty liver (χ²=0.539, p=0.764). There were no significant differences in the detectability of track flow signals on CEUS between the patients with and without fatty liver.

Of the 33 patients with track microbubble flow signals on CEUS images following biopsy needle withdrawal (Fig. 1), the signals continued in 12 patients (36.3%) and disappeared three minutes after the biopsy in 21 patients (63.6%). Furthermore, among 12 patients with a signal after three minutes, the signal disappeared five minutes after the biopsy in 10 patients (83.3%). In the remaining two patients, the signal disappeared seven minutes after the biopsy. In the present series, there were no patients with persistent track microbubble flow signals, and no significant postbiopsy hemorrhagic events, including intrahepatic hematomas or hemobilia, were observed.

Discussion

After biopsy needle withdrawal, CEUS sometimes reveals a linear microbubble flow signal along the puncture tract. However, this finding does not always result in clinically significant hemorrhaging. In fact, in the present study, although track microbubble flow signals were seen in 33% of

Table 2. Detectability of a Track Microbubble Flow Signal on Contrast-enhanced Ultrasound between Patients with and without Fatty Liver

<table>
<thead>
<tr>
<th></th>
<th>Patients with fatty liver (n=30)</th>
<th>Patients without fatty liver (n=70)</th>
<th>χ²</th>
<th>p</th>
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<tbody>
<tr>
<td>a track microbubble flow signal on CEUS</td>
<td>presence 9(30%)</td>
<td>24(34.3%)</td>
<td>0.539</td>
<td>0.764</td>
</tr>
<tr>
<td></td>
<td>absence 21(70%)</td>
<td>46(65.7%)</td>
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Figure 1. Assessment of a flow signal on contrast-enhanced ultrasound (CEUS). A: CEUS shows the presence of a track microbubble flow signal (arrow), defined as a linear microbubble flow signal along the needle track. B: CEUS shows the disappearance of a track microbubble flow signal (arrow) five minutes after the biopsy.

Image analysis

In order to minimize variation in the procedures, CEUS was performed by specific skilled sonologists (M.K., S.T. and T.M.) using the same examination protocol. The imaging data were recorded as a digital video file for subsequent review by two other sonologists, excluding the examiners. The two readers were blinded to each other’s findings. The findings obtained in the vascular phase were classified into two patterns: the presence or absence of a track flow signal (Fig. 1). When a track microbubble flow signal was seen, it was observed repeatedly, and the presence or absence of microbubble pooling along the needle tract and peritoneal recesses, such as the subphrenic, hepatorenal, paracolic and paravesical spaces, was observed to detect hematomas and intraperitoneal hemorrhaging in the postvascular phase of CEUS simultaneously with conventional B-mode US. The patients were followed up with clinical and laboratory data to detect clinically significant hemorrhaging. Clinically significant hemorrhage was defined as a reduction in the blood hemoglobin concentration of more than 2 g/dL or the need for a blood transfusion.

The statistical analysis was performed using the chi-square test or Fisher’s exact probability test. All statistical tests were performed using the Stat View software package for Windows (SAS Institute, Cary, NC). A p-value of less than 0.05 was considered to be significant.
the patients, there were no cases of clinically significant hemorrhaging. Previous studies (10-12) have reported that the finding of a color flow signal may resolve spontaneously without subsequent occurrence of postbiopsy bleeding. Kim et al. (11) reported that a track color flow signal is observed on color Doppler US immediately after biopsy needle withdrawal in 12% of patients. The frequency (33%) observed on CEUS in the present study is higher than that (12%) reported for CDUS. Our results showed that CEUS is more sensitive for detecting the blood flow than CDUS. Furthermore, although track color flow signals have been reported to occur more frequently in patients with thrombocytopenia or a prolonged prothrombin time (11), there were no differences in the platelet count or prothrombin percent activity in the CEUS group in the present study. This discrepancy may be explained by the higher rate of detection of track microbubble flow signals on CEUS in the present study. Furthermore, it has been previously reported that bleeding after a liver biopsy is not correlated with the prothrombin time, platelet count or whole blood clotting time (15). Kim et al. (11) reported that the visualization of a track color flow signal is a risk factor for postbiopsy bleeding. The present study failed to support this hypothesis, as there were no cases of clinically significant hemorrhaging, and no significant differences were observed in the signal detection rate between the patients with and those without bleeding tendencies within the biopsy indications.

A bright liver, deep attenuation and vascular blurring are the B-mode findings of fatty liver (16, 17). A fatty liver background may inhibit the visualization of hepatic parenchyma and hepatic vessels and reduce the rate of detection of track flow signals on CEUS. However, in the present study, the presence of fatty liver did not affect the detection rate on CEUS. We were unable to identify risk factors for track microbubble flow signals. For example, the method of puncture, puncture site and angle were not found to be risk factors. Before each biopsy, we observed the puncture site using CDUS and avoided the vessels detected on CDUS. However, track microbubble flow signals may be caused by injury of the small vessels, which cannot be detected on CDUS.

The time course of the track microbubble flow signals was examined in the present study, and the disappearance of the signals over time was confirmed in all cases. The disappearance of the flow signals in all cases suggests the lack of clinical hemorrhage. In the present study, there was no evidence to suggest that a persistent flow signal raises the possibility of clinically significant hemorrhage. However, in our previous reports, persistent flow signals were shown to be related to hemorrhaging into the pleural cavity or abdominal wall after radiofrequency ablation for hepatocellular carcinoma (18, 19). A persistent track microbubble flow signal may become an indicator for significant hemorrhaging. Confirming the disappearance of a track microbubble flow signal reduces the possibility of clinically significant hemorrhage and may reassure the examiner and patient. If a track microbubble flow signal persists for more than five minutes, the intraperitoneal and intrapleural echo-free space should be assessed and hemostatic approaches, such as transarterial embolization, should be prepared. Performing CEUS in every biopsy case is associated with a high cost because administering CEUS with Sonazoid is expensive. Therefore, in the clinical setting, when a track color flow signal is observed on CDUS after a liver biopsy, we suggest observing the track flow signal in more detail and confirming its disappearance using CEUS.

The present study is associated with several limitations. First, because there were no cases of clinically significant hemorrhage in the present prospective study, whether CEUS could be used to predict hemorrhage could not be investigated. This issue needs to be addressed in a future study. Second, it is unknown how much blood flow can be detected as a signal flow in clinical subjects. Few basic research studies have reported evaluating the regional blood flow or perfusion using CEUS (20, 21). On CT perfusion imaging, the rate of liver perfusion is reported to be 1.08±0.34 mL/min/mL tissue in healthy controls (22). Therefore, the use of CEUS with Sonazoid is speculated to enable the detection of track microbubble flow signals when the track microbubble flow volume is greater than 1.08±0.34 mL/min/mL tissue. However, this is too difficult a problem to be tackled in the present study.

In conclusion, track microbubble flow signals on CEUS are frequently detected following percutaneous liver biopsy and provide information regarding subclinical hemorrhage. The presence of these signals may alert clinicians to clinically significant hemorrhage, and the disappearance of track microbubble flow signals indicates the absence of hemorrhaging. Therefore, CEUS is a useful tool for screening for postbiopsy hemorrhaging.

The authors state that they have no Conflict of Interest (COI).
References


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