Filling of Fine and Core Biopsy Needles With the Contrast Agent Sulfur Hexafluoride

Ex Vivo and in vitro Evaluation of Ultrasound Needle Visibility

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**Objectives**—To investigate whether the ex vivo and in vitro ultrasound visibility of fine needles (FNs) and core biopsy needles (CBNs) can be improved by filling them with an ultrasound contrast agent.

**Methods**—After needle filling with the contrast agent sulfur hexafluoride, punctures with FNs and CBNs were recorded in the B-mode and contrast-specific imaging mode (10 observations in each of the 4 groups). Recordings were made in both butchered bovine liver (experiment I) and a water bath (experiment II). Air and normal saline were used as controls (total n = 120 for each experiment). In experiment I, 4 ultrasound specialists subjectively assessed the relative needle visibility in the recordings by using an arbitrary scale (integers 0–10). In experiment II, the contrast-to-noise ratio was calculated for both the entire needle course and the needle tip area.

**Results**—In experiment I, subjective visibility was increased compared with both controls only for CBNs in the contrast-specific imaging mode (P < .01). In experiment II, the contrast-to-noise ratio for both the entire needle course and the needle tip area increased compared with both controls for both FNs and CBNs in the contrast-specific imaging mode (P < .05).

**Conclusions**—Ultrasound contrast agent needle filling is a promising new method to increase the visibility of CBNs in the contrast-specific imaging mode. This finding needs to be confirmed in vivo before its clinical value can be assessed.

**Key Words**—biopsy; needle visibility; ultrasound contrast agent

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**Contrast-enhanced ultrasound (US)** has added a new dimension to diagnostic US in the detection of focal liver lesions, and characterization of these lesions is also excellent. Fine needle (FN) aspirations and core needle biopsies are real-time diagnostic procedures commonly performed under US guidance, to which the use of contrast-enhanced US has added advantages compared with regular B-mode imaging in some situations. First, it has been shown to decrease the risk of false-negative biopsy results for intra-abdominal tumor lesions by avoiding hypovascular or non-enhancing necrotic areas, especially in large tumors and in cirrhotic livers. Second, contrast-enhanced US may facilitate detection of lesions that are invisible or insufficiently visualized in the B-mode. Punctures in these cases are often performed in the contrast-specific imaging mode.
Figure 1. Core biopsy needle filling procedure. A, The inner needle and the outer cannula are separated. B, The USCA SF₆ is drawn up into a Luer slip syringe. C, The outer cannula is filled with the SF₆ USCA from its nonsharp end. D, The outer cannula tip is inserted into a temporary sharps holder before removing the syringe. E, The inner needle is reinserted into the outer cannula. F, The result, if it stays tight between the sharp end of the outer cannula and the temporary sharps holder, will be that the SF₆ USCA level rises in the nonsharp end of the outer cannula. G, The biopsy needle is mounted in the biopsy gun before (H) removal of the biopsy needle and loading of the biopsy gun.
Adequate real-time US visualization of the puncture needle (especially the tip) is paramount.\textsuperscript{9,10} There are several methods described to increase B-mode visibility by needle modifications (eg, by sandblasting the needle tip) or by needle priming (eg, by introducing a guide wire).\textsuperscript{11,12} In the B-mode, the needle visibility is, in most cases, considered acceptable in the hands of a skilled operator without any modifications or priming.\textsuperscript{9,12}

Needle visibility in the contrast-specific imaging mode is sometimes poor and in general inferior compared with B-mode imaging, mainly because of the lower mechanical index (MI) used to avoid ultrasound contrast agent (USCA) bubble destruction.\textsuperscript{7,13,14} The use of a split-screen mode, in which the contrast-specific image is accompanied by a B-mode image, has been described by some as a successful way to achieve sufficient needle visibility when performing core needle biopsies of intra-abdominal tumor lesions.\textsuperscript{7,8} However, the B-mode image quality in this setting is reduced by the lower MI needed to avoid USCA bubble destruction.\textsuperscript{7,13} The quality of this low-MI B-mode image, and thereby the needle visibility conditions, differs between various US machines. A method to overcome this drawback would be advantageous.

Ultrasound contrast agents are in most indications administered intravenously, but there are a number of off-label extravascular and endocavernous applications described.\textsuperscript{15} For example, the drain patency, location, and remaining abscess size may be assessed when performing a so-called tubogram into an already percutaneously drained abscess system.\textsuperscript{15,16} Furthermore, improved needle visualization has been reported when performing a percutaneous nephrostomy in the contrast-specific imaging mode by flushing the needle with a drop of an USCA before the puncture is performed.\textsuperscript{16}

There are different methods for evaluating US visibility. Both subjective grading by observers and quantitative approaches occur, but no standard method exists.\textsuperscript{17} For subjective grading, different types of scales are sometimes used: ie, a Likert-type scale.\textsuperscript{18} An example of a quantitative method used in previous studies is the contrast-to-noise ratio (CNR).\textsuperscript{17} The CNR, originally proposed by Patterson and Foster as the contrast-to-speckle ratio, is usually defined as

\[
\text{CNR} = \frac{|\mu_i - \mu_o|}{\sqrt{\sigma_i^2 + \sigma_o^2}},
\]

where \(\mu_i\) is the mean signal intensity inside the region of interest (ROI); \(\mu_o\) is the mean signal intensity outside the ROI; \(\sigma_i\) is the variance of the intensity inside the ROI; and \(\sigma_o\) is the variance of the intensity outside the ROI.\textsuperscript{19,20} The purpose of this study was to evaluate whether ex vivo and in vitro needle visibility, in terms of subjective grading and quantitatively measuring the CNR, respectively, can be improved by filling the needles with an USCA.

### Materials and Methods

Using the same procedure of filling FNs and core biopsy needles (CBNs), 2 different experiments were performed with different tissue phantoms and evaluation methods. Neither Institutional Review Board approval nor informed consent was relevant for this study, as it did not include any humans or live animals. Supporting information for the

\begin{center}
\textbf{Figure 2.} One of the butchered bovine livers used in experiment I with pins of various colors used to indicate different puncture sites.
\end{center}
experiments is available as online supplemental material (Appendix S1).

**Needle Filling, Equipment, and Recordings**

Fine needles (Special canula, 20 gauge × 150 mm; Mediplast AB, Malmö, Sweden) and CBNs (ACN biopsy needle, 18 gauge × 160 mm; Argon Medical Devices, Inc, Plano, TX) were filled with the USCA sulfur hexafluoride (SF₆, SonoVue; Bracco SpA, Milan, Italy). The FNs were filled by simply flushing them with the SF₆ (an extension line 3-way stopcock was connected to maintain the SF₆ in the FNs). A special procedure (depicted and explained in Figure 1) was used to fill the CBNs, involving the use of a temporary sharps holder (ShortStop; Merit Medical Systems, Inc, South Jordan, UT), before mounting it in the biopsy gun (Biopty; Radiplast AB, Uppsala, Sweden).

Scanning was performed with an ACUSON S3000 HELX Evolution unit (Siemens Medical Solutions, Inc, Mountain View, CA) with use of a 6C1HD curved transducer. The transducer was enclosed in a nonlatex cover, and a needle guide with associated electronically generated guidance lines was used. The maximum depth was set to 11 cm; the focus was set in the deep portion of the image; and the gain was standardized.

Recordings were performed of the fine and core needle biopsy punctures in a butchered bovine liver in the B-mode (4.5 MHz) and contrast-specific imaging mode using Cadence Contrast Pulse Sequencing (CPS) technology (1.5 MHz; MI, 0.05). The needles were inserted in their entire possible length (the needle guide subtracted 3 cm of both the FNs and CBNs and the stroke length of the automatic biopsy instrument another 2.4 cm of the CBNs before the discharge). The

**Figure 3.** Experiment II setup with the transducer fixed and submerged in a water bath. Artificial background enhancement, in terms of small bubbles, was created by a flow of oxygen through a perforated silicone hose.

| Needles and Imaging Mode | Air            | NSS            |
|--------------------------|----------------|----------------|
| FNs and B-mode           | 27.5 (11.5–52.5) | 50.0 (28.5–71.5) |
| P = .075                 | P > .999       |
| FNs and CPS              | 45.0 (22.4–69.8) | 82.5 (61.6–93.3) |
| P = .705                 | P > .005       |
| CBNs and B-mode          | 37.5 (21.3–57.1) | 55.0 (33.7–74.6) |
| P = .209                 | P > .654       |
| CBNs and CPS             | 82.5 (61.6–93.3) | 77.5 (62.2–87.8) |
| P = .005                 | P = .001       |

Results are shown as proportions in percentages (95% confidence intervals).
recordings for all groups included the subsequent needle retraction. In experiment I, the biopsy gun was fired almost directly after the insertion of the CBNs, followed by nearly immediate needle retraction. In experiment II, there was a delay of approximately 3 seconds before retraction of the FNs to enable a more robust image analysis in the end position. Correspondingly, there was also a 3-second delay before firing the biopsy gun and another 3-second delay before needle retraction of the CBNs. In each of the 4 groups (FNs and B-mode, FNs and CPS, CBNs and B-mode, and CBNs and CPS), punctures were performed with different needle fillings (10 controls with air, 10 with \( \text{SF}_6 \) and 10 controls with normal saline [NSS]; total \( n = 120 \) in each of experiments I and II). The needles were flushed with their respective fillings between the punctures. The same person performed all of the punctures (nonblinded to the different needle fillings).

**Experiment I: Subjective Evaluation of Punctures in the Butchered Bovine Liver**

Butchered bovine livers (Figure 2) were used as tissue phantoms (all punctures within each of the 4 groups were performed on the same liver). The puncture

**Figure 5.** Graphic illustration of the visibility evaluation in experiment I for all 4 assessors with different needle fillings (\( n = 120 \) per assessor). Identical symbols within each box represent triplets of punctures performed at adjacent butchered bovine liver locations (this method was used to decrease the risk of bias due to general parenchymal inhomogeneities). Horizontal gray bars represent median values.

![Figure 5](image-url)
sites of the different needle fillings were matched to decrease the risk of bias because of parenchymal inhomogeneities within the livers. The transducer was handheld. Within the group of FNs in the B-mode, the frequency was inadvertently set to 4 MHz for all observations (instead of 4.5 MHz as in the other B-mode series).

Four senior radiologists, with expertise in US (all with a minimum of 15 years of experience), evaluated the recordings in a blinded manner. The specialists were instructed to assess the relative aggregated needle visibility within each of the 4 groups on an arbitrary Likert-type scale of integers from 0 to 10, where zero defined no visibility at all and 10 maximum possible visibility.

The median needle visibility was calculated for each of the assessors within each group. SF$_6$ was considered superior to air/NSS filling for a site if the assessor rated the visibility of the SF$_6$-filled needle higher than that of the matched air/NSS-filled needle.

Figure 6. Experiment I (butchered bovine liver) examples of matched puncture triplets with FNs (in their end positions) and CBNs (just before the biopsy gun discharge). The examples were chosen to, in total, be as close as possible to the median for each of the 4 observers.
A generalized estimating equation logistic regression model was used to estimate the proportion, with the 95% confidence interval, of sites where SF₆ was considered superior. Furthermore, we tested the null hypothesis that the proportion of sites where SF₆ was considered superior was 0.5. Separate models were estimated for each group (FNs and B-mode, FNs and CPS, CBNs and B-mode, and CBNs and CPS), and comparisons were made with NSS and air, respectively (in total, 8 models). A generalized estimating equation was used because 4 assessors rated each site, and an exchangeable correlation structure was used to control for the dependence between observations within the same site. All statistical tests were 2 tailed and performed at a .05 significance level. There were no adjustments for multiple comparisons. Analyses were performed with SAS version 9.4 software (SAS Institute Inc, Cary, NC).

Experiment II: Contrast-to-Noise Evaluation in a Water Bath

A plastic container (volume, 61 L) almost entirely filled with water was used as a phantom, and the transducer was fixed just below the surface (Figure 3). A towel was placed at the bottom of the container to reduce mirroring artifacts. A part of a silicone hose (used for oxygen nasal prongs) was perforated with an FN before it was lowered in and fixed to the bottom of the plastic container. Thereafter, a flow oxygen at 0.25 L/min was applied, thus creating a continuous stream of small bubbles directed to the scanning area of the transducer. The bubble stream was applied to simulate tissue echogenicity heterogeneity in the B-mode and background tissue enhancement in the contrast-specific imaging mode (after administrating contrast media intravenously), respectively.

The recordings were analyzed with the image-processing application ImageJ version 1.52d (Wayne Rasband, National Institute of Health, Bethesda, MD). In each recording, 3 ROIs were defined as follows (see Figure 4 for example):

- Entire needle course: a rectangle with a short side of 2 mm and beginning in the top left corner. For the FNs, the distal end was defined so that the punctate echo produced by the tip in all recordings was only included. For the CBNs, a fixed depth was set for all recordings, since the echoes produced by the tips were more ample but less well demarcated and hence harder to delineate.
- Needle tip area: square with a side of 4 mm, aligned with the most distal part of the first ROI.
- Background: polygon that mainly covered the area outside the electronically generated guidance lines (≈120 cm²).

In each recording, the image frames with full needle insertion were selected (approximately 3 seconds; frames per second: 30 in the B-mode and 10 with CPS; for the CBNs before firing the biopsy gun). For those defined frames, the mean and standard deviation of the intensity were measured for all 3 ROIs. From those values, the CNR was calculated for both
the entire needle course and the needle tip area compared with the background. Finally, the mean of the calculated CNRs for all frames in each recording was calculated. For recordings in which the needle, including the needle tip, was not possible to identify, the CNR was set to 0. Frames in which the needle was unintentionally moved outside the ROIs were excluded.

The median of the mean CNR for each filling within each of the 4 groups was calculated for both the entire needle course and the needle tip area. The 2-sample Wilcoxon test was performed, comparing the mean CNR between SF₆ filling and the 2 control fillings. All statistical tests were 2 tailed and performed at a .05 significance level. There were no adjustments for multiple comparisons. Analyses were performed with

Figure 8. Experiment II (water bath) examples of punctures with FNs (in their end positions) and CBNs (just before the biopsy gun discharge). The examples were chosen to be as close as possible to the median CNR for the entire needle (rounded upward). For CBNs with CPS, the example filled with air represents the observation with the lowest of the 4 measurable CNRs.
Results

Experiment I: Subjective Evaluation of Punctures in the Butchered Bovine Liver

The results of the visibility evaluation are presented in Table 1 and Figure 5. The FN visibility was equivalent in the B-mode, whereas it increased with CPS compared with NSS but not air. The CBN visibility was also equivalent in the B-mode, whereas it increased compared with both air and NSS with CPS. Examples of matched triplets of punctures from each of the groups are presented in Figure 6.

Experiment II: Contrast-to-Noise Evaluation in the Water Bath

The results of the contrast-to-noise evaluation are presented in Figure 7, and examples of punctures from each of the groups are presented in Figure 8. The CNR increased in all groups for the entire needle course with SF6 filling compared with both controls. In contrast, the CNR of the needle tip area increased only with CPS but not in the B-mode for both FNs and CBNs. In 7 of the controls in the CBN-and-CPS group (6 filled with air and 1 filled with NSS), a needle course (including the needle tip) could not be identified. The very first recording (performed in the FN-and-B-mode group) was lost because of inadvertent deletion. Therefore, the total number of observations processed was 119 instead of 120.

Discussion

The main result of this study was the increased needle visibility obtained in the CBN-and-CPS group, in terms of both subjective grading (experiment I) and the CNR for both the entire needle course and the needle tip (experiment II). This result needs to be confirmed in vivo in intravenous contrast medium–enhanced liver parenchyma.

The needle visibility in the FN-and-CPS group was also improved in terms of the CNR for both the entire needle course and the needle tip area (experiment II) compared with both controls. In contrast, the subjective visibility was improved only compared with the NSS controls and not the air controls (experiment I). The experiment I results thereby stand in contrast to, whereas the results in experiment II are consistent with, the findings of improved needle visualization by flushing it with a drop of an USCA reported by Patel et al.16

The CNR increase of the entire needle course in combination with the lack of CNR reduction and subjective impairment in needle visibility in the 2 B-mode groups (FNs and CBNs) indicates that our method is at least not disadvantageous in the B-mode. It may merely reflect that needles (and especially the tips) are generally well seen in the B-mode even without modifications or filling and that the tip area constitutes the most crucial needle part for subjective assessors in experiment I.10 Reasonably, the same should apply to the low-MI B-mode image in combination with the contrast-specific imaging mode using the split screen.

Even though of more limited clinical relevance than in the contrast-specific imaging mode, the increase of the CNR for the entire needle courses of both CBNs and FNs in experiment II suggests that our method could be added to already-known methods to increase the echogenicity in the B-mode (eg, guide wire introduction, sandblasting of the needle tip/stylet, or filling with an air-jelly mixture).11 The effects of these already-known needle modifications and priming methods have, to our knowledge, not been studied in the contrast-specific imaging mode. Furthermore, several of these methods (eg, sandblasting) are not practically possible to perform in a clinical setting on existing needles, nor is it technically possible to insert a guide wire into a CBN.

The major strength of our study was that the evaluation of the needle filling method was performed with both subjective (experiment I) and quantitative (experiment II) approaches. For experiment I, the use of the bovine liver as a model was advantageous as it resembles the human liver on US imaging. The model, though, also entails an important weakness: it does not allow for simulation of background contrast enhancement in the tissues surrounding the needle after intravenous USCA injection, which has been postulated as one cause of the needle’s being disguised in the contrast-specific imaging mode.7 However, the increased CNR in experiment II (with a
stream of oxygen bubbles in some degree of artificial background enhancement in the CPS groups) suggests that corresponding results may be achieved in vivo. Another limitation was that the methods were only evaluated in a single contrast-specific imaging mode (CPS) and on a single type of US machine. Furthermore, the methods were not evaluated with a low-MI B-mode image in combination with the contrast-specific invaging mode using a split screen. Moreover, only “normal” bovine liver parenchyma was studied in experiment I.

In conclusion, the novelty of our study is that the very small space between the inner needle and outer cannula in a CBN may be rendered increased ex vivo and in vitro visibility in the contrast-specific imaging mode. This may be of great clinical value in situations of poor needle visibility in the contrast-specific imaging mode, but the result first needs to be confirmed in vivo before its clinical value can be assessed. Our methods of needle filling with an USCA appear less promising for CBMs in the B-mode and FNs in both the B-mode and contrast-specific imaging mode.

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