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ECHO DECORRELATION IMAGING OF RABBIT LIVER AND VX2 TUMOR DURING *IN VIVO* ULTRASOUND ABLATION

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Abstract

In open surgical procedures, image-ablate ultrasound arrays performed thermal ablation and imaging on rabbit liver lobes with implanted VX2 tumor. Treatments included unfocused (bulk ultrasound ablation, N = 10) and focused (high-intensity focused ultrasound ablation, N = 13) exposure conditions. Echo decorrelation and integrated backscatter images were formed from pulse-echo data recorded during rest periods after each therapy pulse. Echo decorrelation images were corrected for artifacts using decorrelation measured prior to ablation. Ablation prediction performance was assessed using receiver operating characteristic curves. Results revealed significantly increased echo decorrelation and integrated backscatter in both ablated liver and ablated tumor relative to unablated tissue, with larger differences observed in liver than in tumor. For receiver operating characteristic curves computed from all ablation exposures, both echo decorrelation and integrated backscatter predicted liver and tumor ablation with statistically significant success, and echo decorrelation was significantly better as a predictor of liver ablation. These results indicate echo decorrelation imaging is a successful predictor of local thermal ablation in both normal liver and tumor tissue, with potential for real-time therapy monitoring.

Keywords

Echo decorrelation imaging; Therapy monitoring; Thermal ablation; Bulk ultrasound ablation; High-intensity focused ultrasound

INTRODUCTION

Approximately 80% of patients diagnosed with hepatocellular carcinoma at an early stage are not candidates for surgical resection or transplantation (Petrowsky and Busuttil, 2008). For these patients, thermal ablation, such as radiofrequency ablation (Li et al. 2014), microwave ablation (Simon et al. 2005) and high-intensity focused ultrasound (HIFU) ablation (Maruyama et al. 2008), are alternative treatment choices. These thermal ablation methods would be improved if used concurrently with a suitable real-time monitoring method (Li et al. 2014).

Currently, tissue temperature effects can be monitored by magnetic resonance thermometry; however, the clinical extracorporeal systems leveraging this method (*e.g.*, ExAblate, InSightec, Tirat Carmel, Israel) (Kennedy et al. 2003) can be costly and cumbersome (Napoli et al. 2013). In China, a clinical extracorporeal ultrasound-guided focused ultrasound system has demonstrated liver tumor treatment efficacy and safety (Chongqing HAIFU Technology, Chongqing, China) (Wu et al. 2009). Although ultrasound monitoring has potential cost and convenience advantages, inconsistent pulse-echo changes (Mast et al. 2008) or pulse-echo signal decorrelation (Cespedes et al. 1997) can render current ultrasound monitoring approaches, including conventional B-mode (brightness mode) imaging (Maruyama et al. 2008), echo energy-based methods (*e.g.*, attenuation and integrated backscatter) (Zhang et al. 2009) and cross-correlation-based methods (*e.g.*, echo strain [Liu and Ebbini 2010] and elastography [Kolokythas et al. 2008] imaging) unreliable or limited in application.

Echo decorrelation imaging, a pulse-echo method that maps heat-induced changes in ultrasound echoes over millisecond time scales, has exhibited promise for real-time monitoring of radiofrequency ablation (Mast et al. 2008; Subramanian et al. 2014), as well as ultrasound ablation using unfocused (Subramanian et al. 2014) and focused (Kumon et al. 2012; Matsuzawa et al. 2012; Sasaki et al. 2014) beams. However, the ability of echo decorrelation to predict the desired clinical end effect of thermal ablation (*i.e.*, death of both malignant tumor tissue and a margin of normal liver tissue) has not been systematically investigated *in vivo*.

The objective of this study was to investigate the ability of echo decorrelation imaging and integrated backscatter imaging to predict cell death during thermal ablation of rabbit liver and VX2 tumor. The imaging and treatment approach employed was designed to rigorously test this hypothesis. Use of image-ablate arrays ensured that imaging and ablation occurred in the same plane, enabling precise point-by-point assessment of prediction efficacy. Treatments included focused ultrasound ablation, comparable to non-invasive HIFU treatments, as well as bulk (unfocused) ultrasound ablation, comparable to minimally invasive techniques such as radiofrequency and microwave ablation. Pixel-by-pixel prediction of ablation-induced cell death was assessed as a function of the echo decorrelation thresholds, resulting in receiver operating characteristic (ROC) curves by which prediction efficacy could quantitatively be assessed.

METHODS

Thermal ablation experiments

Ultrasound ablation and imaging were performed on rabbits in a series of experiments approved by the University of Cincinnati Institutional Animal Care and Use Committee. AVX2 liver cancer model was employed in a manner similar to that used in a previous study of *in vivo* ultrasound ablation (Mast et al. 2011). Animals were sedated with a combination of ketamine (30–50 mg/kg) and xylazine (5–10 mg/kg) and anesthetized using isoflurane, applied to effect by a respirator. Viable VX2 tumor fragments were implanted in each of the three main liver lobes of New Zealand White rabbits, approximately 2 mm below the liver capsule surface. Tumor was allowed to grow for 2 weeks before ablation was performed in open surgical procedures. The setup for these *in vivo* experiments is illustrated in Figure 1. For 11 rabbits, the animal was anesthetized and its liver exposed. The tumor was located visually on the surface of the liver, and indelible marks were made on the surface to guide probe placement onto the tumor.

Ablation and imaging were performed using custom 64-element, $5 \times 24\text{-mm}^2$ linear image-ablate arrays with nominal center frequency 5 MHz, >40% pulse-echo fractional bandwidth for imaging and >40-W available acoustic power for therapy, controlled by the Iris 2 ultrasound imaging and therapy system (Ardent Sound Mesa, AZ, USA) (Barthe et al. 2004). The array aperture was placed parallel to the liver capsule surface at a distance of 23 mm using a water-filled standoff with an acoustically transparent membrane window (Tegaderm, 3M, St. Paul, MN, USA), coupled by an acoustic transmission gel (LithoClear, Next Medical Products, Bellingham, WA, USA) and fixed by a 3-D positioning arm (Atlas Multifunctional Arm, Medical Intelligence, Schwabmünchen, Germany) to minimize compression of the liver. A gel acoustic coupling pad (Aquaflex, Fairfield, NJ, USA) or rubber acoustic absorber pad (Aptflex F28, Precision Acoustics, Dorchester, UK) was placed under the liver lobe to limit heating from reflections at the tissue-absorber boundary and to constrain the liver lobe's shape, allowing accurate registration of tissue viability and parameter maps. The liver was not mechanically stabilized, and appreciably slipped because of respiration, relative to the array standoff. Alignment with the tumor was verified by visualizing the tumor in the B-mode (brightness mode) image.

Thermal lesions were formed in VX2 tumor and liver regions under the exposure conditions listed in Table 1, including unfocused exposures for bulk ultrasound ablation and focused exposures for HIFU ablation. In summary, exposures employed continuous-wave therapeutic ultrasound pulses with center frequencies 5.0–5.4 MHz. For bulk ablation ($N = 10$), a full unfocused aperture fired 7–9 pulses of duration 6.0–7.5 s and *in situ* spatial-peak, temporal-peak intensity (I_{SPTP}) 50–63 W/cm². I_{SPTP} was estimated from the measured acoustic power (UPM-1 radiation force balance; Ohmic Instruments, Easton, MD, USA) using simulations of the array's acoustic field under the Fresnel approximation (Mast 2007). For HIFU ablation ($N = 13$), a full aperture, electronically focused at a single focal point 1–4 mm below the tissue surface and centered within the aperture, fired 6–9 pulses of duration 0.7 s and *in situ* I_{SPTP} 911–1351 W/cm².

Pulse-echo imaging was performed with a single transmit focal depth (3.5 cm, F -number = 4). After each therapy pulse, 114 frames of beamformed, radiofrequency echo signals were captured at a rate of 118 frames/s using a PC-based data acquisition system with a 33.3 MHz sampling rate (CompuScope CS 14200, Gage Applied Technologies, Montreal, QC, Canada); echo signals were stored for computation of echo decorrelation and integrated backscatter (IBS) images after the treatments, as described in the following section. Imaging started within 10 ms after each therapy pulse, except for two unfocused trials (animal/lobe: R256/L and R259/R in Table 1). In those two cases, where there was a 1.1-s delay between therapy and imaging, modeling revealed that the temperature elevation varied <20% within the 1.1-s interval before initiation of imaging (Fosnight 2015).

After some HIFU exposures, to ensure that the imaging-treatment plan could be consistently identified, two superficial thermal lesions used as registration marks were formed by firing unfocused sub-apertures comprising elements 1–10 and then elements 55–64, that is, apertures 3.8 mm in length, resulting in lesions of comparable width, each centered 10.3 mm laterally from the focal position. Each sub-aperture fired pulses of duration 6.0–6.5 s and I_{SPTP} 59 W/cm² until a lesion was seen on the liver surface. Registration mark formation was confirmed by simulation (Mast et al. 2005) not to elevate temperature at the original focal position more than 2°C.

Image processing

All image post-processing was performed in MATLAB (The MathWorks, Natick, MA, USA). Before further processing, echo signals were demodulated with a 5.0-MHz center frequency and decimated to in-phase and quadrature (IQ) components with a sampling rate of 5.56 MHz. This data acquisition was performed both for ablation trials and for matching sham trials, used for estimation and correction of decorrelation artifacts. Sham trials were performed prior to each ablation, with matching probe position and data acquisition timing, but no therapeutic ultrasound sonication.

In-phase and quadrature echo signals were processed to construct B-mode images, as well as images of echo decorrelation and integrated backscatter, both as defined by Subramanian et al. (2014). B-Mode images were constructed from logarithmically scaled envelopes ($\log|\rho(\mathbf{r},t)|$) of the IQ data and displayed using a 60-dB dynamic range. Tissue boundaries were manually segmented in B-mode images by research assistant co-authors (biomedical engineering students) to avoid any echoes from outside the liver lobe, with reference to the corresponding histologic tissue sections, as well as the full sequence of recorded images for each trial. Echo decorrelation and IBS were calculated only within these segmented tissue boundaries. Both echo decorrelation and IBS were computed within Gaussian windows with width parameters $\sigma = 1.0$ mm, centered at each image pixel, to form parameter maps.

The echo decorrelation map represents the local deviation from perfect echo signal correlation (*i.e.*, a normalized local cross-correlation coefficient subtracted from unity) between two complex (IQ) image frames separated by an inter-frame time, here $\tau = 8.47$ ms. The local instantaneous decorrelation term $\rho(\mathbf{r},t)$ defined by Subramanian et al. (2014) was normalized by the inter-frame time to measure the decorrelation per millisecond. The local instantaneous decorrelation per millisecond was then ensemble-averaged over the group of

112 frames recorded after each therapy pulse to obtain a single decorrelation map for each cycle. The cumulative decorrelation was defined as the temporal maximum of the ensemble-averaged decorrelation map for each position $\mathbf{r}$.

Estimates of artifactual cumulative decorrelation obtained from corresponding sham trials, denoted Δ_{sham} , were used to construct corrected cumulative decorrelation, denoted $\Delta_{\text{corrected}}$, from uncorrected cumulative decorrelation, denoted $\Delta_{\text{uncorrected}}$, using the equation (Hooi et al. 2015)

$$\Delta_{\text{corrected}}(\mathbf{r}, t) = \frac{\Delta_{\text{uncorrected}} - \Delta_{\text{sham}}}{1 - \Delta_{\text{sham}}} \quad (1)$$

To avoid anomalies in the ROC curve analysis without significantly affecting echo decorrelation statistics, artifact correction was constrained to be applied only where substantial artifacts occurred. To accomplish this, correction was only applied at locations where $\Delta_{\text{uncorrected}}$ exceeded a dynamically set threshold, defined as 10 times the minimum of the corresponding Δ_{sham} . At locations where eqn (1) would result in corrected values less than zero, $\Delta_{\text{corrected}}$ was set equal to the minimum value of Δ_{sham} .

Relative IBS images, depicting local changes in echo energy, are defined as the decibel-scaled ratio between the integrated backscatter terms $\beta(\mathbf{r}, t)$ during ablation and the temporal-maximum integrated integrated backscatter $\beta_{\text{sham}}(\mathbf{r})$ measured for the corresponding sham trial

$$\text{IBS}(\mathbf{r}, t) = 10 \cdot \log_{10} \left(\frac{\beta(\mathbf{r}, t)}{\beta_{\text{sham}}(\mathbf{r})} \right) \quad (2)$$

where $\mathbf{r}$ is the spatial position vector within the image plane. The cumulative relative integrated backscatter map is the temporal maximum of $\text{IBS}(\mathbf{r}, t)$. Similar to the echo decorrelation maps, measured integrated back-scatter maps were ensemble-averaged over the 112 frames following each therapy pulse, and cumulative integrated backscatter maps were defined as the temporal maxima of these ensemble-averaged maps at each image pixel.

Hybrid B-mode/echo decorrelation images were constructed by overlaying a pseudocolor image of the $\log_{10}$ -scaled echo decorrelation per millisecond onto the B-mode image. The α (transparency) channel of the echo decorrelation pseudocolor image was set equal to the logarithmically scaled echo decorrelation parameter map, normalized by its maximum value for that image. Hybrid B-mode/integrated backscatter images were constructed in a similar manner by overlaying a pseudocolor image of the decibel-scaled integrated backscatter onto the B-mode image.

Tissue processing

After ablation, the animal was euthanized (intravenous pentobarbital, 120–200 mg/kg) and its liver removed. Liver lobes were sectioned through the image-ablate plane using a scalpel, within 2 h postmortem. The image-ablate plane was identified using the indelible marks

placed before ablation treatment, as well as superficial thermal lesions, including the ablation spots used as registration marks for focused ablation exposures. The section considered closest to the imaging-ablation plane, based on visual assessment of the thermal lesion extent, was stained with a 2% (w/v) solution of the vital stain triphenyl tetrazolium chloride (TTC, Sigma-Aldrich, St. Louis, MO, USA) in 0.1 M phosphate-buffered saline (Sigma-Aldrich) for 20 min, with the stained surface face down in the TTC well. TTC uptake indicates metabolically active tissue, whereas no TTC uptake indicates metabolically inactive tissue (Scheffer et al. 2014).

Tissue images were manually segmented by research assistant co-authors (biomedical engineering students) using custom MATLAB software to delineate boundaries of the liver lobes, VX2 tumors, regions of reduced TTC stain uptake and regions of no TTC uptake. Each region was readily distinguished by its relative discoloration. Blinding to ablation conditions was not possible because both focused and unfocused ablation exposures were clearly distinguished by their relative patterns of TTC uptake, as illustrated in Figure 2. Binary masks were then created to distinguish ablated tissue (defined as no TTC uptake) from non-ablated tissue (reduced or full TTC uptake) and to distinguish treated tissue (reduced or no TTC uptake) from untreated tissue (full TTC uptake). Separate masks were created for liver and tumor regions for direct comparison with echo decorrelation and IBS map.

The effective VX2 tumor diameter (*i.e.*, diameter of a circle matching the segmented tumor area) (Mast et al. 2011) was recorded for the two sections spanning the imaging plane, and the greater of these diameters was recorded. Resulting effective tumor diameters for liver lobes employed in the present ablation studies were 7.9 ± 3.5 mm in diameter for 2 weeks' growth (N = 14).

Statistical analysis

Spatially averaged echo decorrelation and IBS parameters were compared in ablated and non-ablated regions of liver and tumor tissue. Increases in the $\log_{10}$ -scaled mean echo decorrelation in ablated versus non-ablated regions were tested for both liver and tumor tissue using paired, one-tailed *t*-tests with the significance criterion $p < 0.05$. Differences between the $\log_{10}$ -scaled mean echo decorrelation in liver and tumor tissue were tested using unpaired, two-tailed *t*-tests with the significance criterion $p < 0.05$ for both non-ablated and ablated conditions. The same analyses were also performed for decibel-scaled mean IBS. Significant reduction of artifactual decorrelation by compensation using eqn (1) was tested by comparing the $\log_{10}$ -scaled mean echo decorrelation in non-ablated regions before and after correction using a paired one-tailed *t*-test with significance criterion $p < 0.05$.

Separate statistical analyses of prediction performance were performed for focused exposures in liver and VX2 tumor, unfocused exposures in liver and VX2 tumor and the combination of all exposures in liver and VX2 tumor. Efficacy for predicting local cell death, detected by lack of TTC uptake, was tested independently for all locations in the image-treatment plane for all treatments, resulting in ROC curves. ROC curves were constructed by comparing thresholded-corrected echo decorrelation and IBS maps with tissue ablation maps (Subramanian et al. 2014). The ROC curve is the false-positive rate (1–

specificity) plotted versus the true-positive rate (sensitivity) over the entire range of predictor threshold values (Hanley and McNeil 1982). The area under the curve (AUC) was used to determine the predictive success of parameter maps. An AUC of 1 indicates that the parameter map predicted the tissue ablation perfectly, whereas an AUC of 0.5 means that the prediction was no better than chance (Hanley and McNeil 1982).

The statistical significance of measured AUC values versus the null hypothesis ($AUC = 0.5$) was calculated in MATLAB using a general model for the AUC standard error (Hanley and McNeil 1982) (one-tailed, significance criterion $p < 0.05$). For assessment of statistical significance, effective sample sizes (N_{eff}) were determined by estimating the number of independent ablation predictions from the total number of predictions (N_{total}) and the maximum hexagonal packing density of circles with diameter $d = 2.355 \sigma$ (the full width at half-maximum of the Gaussian correlation window employed), where $\sigma = 1.0$ mm is the Gaussian width parameter (Subramanian et al. 2014).

Statistical significance of differences between AUC values for corrected echo decorrelation and IBS was calculated in R (pROC package, R Foundation for statistical Computing, Vienna, Austria) by calculating the Z -statistic (Z_{total}) for N_{total} using the method of DeLong et al. (1988) (significance criterion $p < 0.05$, two-tailed), corrected using the equation where Z_{eff} is the effective Z -statistic. The effective significance p_{eff} (significance criterion $p < 0.05$, two-tailed) was then determined from Z_{eff} using the cumulative distribution function of the z (normal) distribution.

Thresholds for optimal prediction of tissue ablation for echo decorrelation and IBS were defined as those corresponding to the point nearest the top left-hand corner of the ROC plot (Krzanowski and Hand 2009). This choice for an optimal threshold approximates simultaneous maximization of sensitivity and specificity for the prediction method. Predicted areas of ablation, defined as the total area of all pixels exceeding the optimal echo decorrelation or IBS threshold, were compared with measured areas of tissue with no TTC uptake.

To determine the effect of imaging inter-frame time in echo decorrelation and IBS ablation prediction performance, uncorrected and corrected echo decorrelation and IBS maps were constructed for inter-frame times $\tau = 8.5, 42.4, 84.7, 127.1, 169.5, 211.9, 254.2, 508.5$ and 847.5 ms. ROC curves and AUCs were computed and compared for each inter-frame time for focused and unfocused exposures in liver tissue.

RESULTS

Histology of vitally stained tissue sections, uncorrected cumulative decorrelation, corrected cumulative decorrelation and IBS images for six representative trials are provided in Figure 2. In the echo decorrelation and IBS images, thresholds for optimal prediction of ablation ($\log_{10}$ -scaled echo decorrelation per millisecond: -3.1 , IBS: 3.86 dB) are indicated by *yellow lines*, whereas *white lines* indicate tissue boundaries segmented from the B-mode images. In the TTC-stained tissue sections, *red*, *blue*, *green* and *black lines* indicate tissue, treated, ablated and tumor regions respectively. For focused exposures (Fig. 2a–c), generally

better correspondence with histology was seen for corrected echo decorrelation than for IBS. For unfocused exposures (Fig. 2d–f), comparable correspondence with histology was seen for corrected echo decorrelation and for IBS. Artifact compensation using eqn (1) is seen to substantially reduce echo decorrelation in unablated regions, consistent with results of paired *t*-test comparisons for unablated liver ($t = -5.67$, $p = 6.3 \times 10^{-6}$, $N = 22$) and for unablated tumor ($t = -2.40$, $p = 2.2 \times 10^{-2}$, $N = 9$). Measured peak-to-peak ranges of in-plane motion, estimated from analysis of B-mode images recorded during the sham and therapy cycles, were 1.5 ± 1.2 mm (mean $\pm$ standard deviation) for the 23 trials reported here.

Mean and standard error values of the $\log_{10}$ -scaled mean corrected echo decorrelation per millisecond and the decibel-scaled mean IBS within non-ablated and ablated regions are illustrated in Figure 3 for the group of all focused and unfocused exposures in liver ($N = 23$) and tumor ($N = 14$). In paired *t*-test comparisons between non-ablated and ablated liver tissue ($N = 17$), significant increases were seen for both decorrelation ($t = 4.07$, $p = 4.5 \times 10^{-4}$) and IBS ($t = 3.21$, $p = 2.8 \times 10^{-3}$). In comparisons between non-ablated and ablated tumor tissue ($N = 7$), smaller significant increases were seen for both decorrelation ($t = 2.18$, $p = 0.036$) and IBS ($t = 2.22$, $p = 0.034$). In comparisons between liver and tumor tissue, the only significant difference observed was a marginally higher IBS in unablated tumor compared with unablated liver ($t = 2.36$, $p = 0.046$, $N = 9$).

Corresponding ROC curves for each group of exposures are provided in Figure 4. Ablated regions in liver were predicted significantly better than chance ($AUC = 0.5$) by corrected echo decorrelation for focused exposures ($p = 7.9 \times 10^{-12}$), unfocused exposures ($p = 4.8 \times 10^{-4}$) and all exposures combined ($p < 10^{-16}$). Ablated liver was also predicted significantly better than chance by IBS for focused exposures ($p = 1.3 \times 10^{-3}$), unfocused exposures ($p = 6.5 \times 10^{-6}$), and all exposures combined ($p < 10^{-6}$). Ablated regions in VX2 tumor were predicted significantly better than chance by corrected echo decorrelation for unfocused exposures ($p = 0.036$) and all exposures combined ($p = 7.2 \times 10^{-5}$), but not for focused exposures ($p = 0.095$). IBS predicted ablated VX2 tumor significantly better than chance only for the group of all exposures combined ($p = 2.0 \times 10^{-4}$).

In statistical comparisons of AUC values for the six cases examined (focused exposures, unfocused exposures and all exposures combined in both liver and tumor tissue), echo decorrelation imaging provided better prediction than IBS imaging in five of six cases. In predictions of ablated liver tissue, echo decorrelation provided significantly better prediction than IBS for the group of focused exposures ($p = 0.012$) and for the group of all exposures combined ($p = 7.0 \times 10^{-7}$), whereas IBS predicted marginally better than echo decorrelation for unfocused exposures ($p = 0.0703$). For prediction of ablation in tumor tissue, AUC values were slightly greater for echo decorrelation than for IBS in all three cases, with the largest difference seen for the group of all exposures combined ($p = 0.197$).

At the optimal threshold for prediction of ablated regions, corrected echo decorrelation's sensitivity was better than its specificity for unfocused exposures and focused exposures. For all exposures combined, echo decorrelation's sensitivity was better than its specificity for ablation prediction in VX2 tumor; however, the opposite was observed for ablation

prediction in liver. Total areas of ablation were predicted more accurately by echo decorrelation imaging (mean area error: -0.80 cm^2 or -2.8% , RMS error: 13.2 cm^2 or 45.7%) than by integrated backscatter imaging (mean area error: -4.47 cm^2 or -15.4% , RMS error: 20.3 cm^2 or 70.0%).

Figure 5 illustrates prediction performance, as measured by the AUC, of echo decorrelation and IBS computed for inter-frame times 8.5, 42.4, 84.7, 127.1, 169.5, 211.9, 254.2, 508.5 and 847.5 ms. Greater AUCs for corrected echo decorrelation, in comparison to uncorrected, indicate the performance enhancement achieved by correction for motion and noise artifacts. A slight overall decrease in uncorrected and corrected AUCs was observed for larger inter-frame times; this decrease was smaller for corrected echo decorrelation. Exceptions to this trend, such as the slightly higher AUC observed for an inter-frame time of 0.2 s in Figure 5(a), may be due to cyclic respiratory motion in some trials. In contrast, IBS AUC was nearly unchanged for larger inter-frame times.

DISCUSSION

Previous echo decorrelation imaging studies have reported promising results for radiofrequency and ultrasound ablation prediction (Kumon et al. 2012; Mast et al. 2008; Matsuzawa et al. 2012; Sasaki et al. 2014; Subramanian et al. 2014). The utility and possible limitations of echo decorrelation tumor ablation monitoring in living subjects are discussed below.

Artifactual decorrelation caused by respiration-induced tissue displacement and deformation, as well as electronic noise, may limit ultrasound monitoring of liver cancer ablation (Hooi et al. 2015). Compensation using eqn (1) resulted in significantly reduced artifactual echo decorrelation in non-ablated regions for focused exposures, unfocused exposures and all exposures combined in liver, indicating that substantial motion-induced decorrelation occurred in these experiments, as has been observed previously (Hooi et al. 2015; Subramanian et al. 2014). However, Figure 5 illustrates that prediction performance declined only slightly for both focused and unfocused exposures as the inter-frame time increased, indicating that effects of motion-induced decorrelation on ablation prediction were relatively small for inter-frame times as large as 0.85 s. Motion may still degrade ablation prediction performance by introducing imprecise image registration; this effect would be greater for focused exposures because of the smaller ablated region.

The ROC results illustrated in Figure 4 indicate that echo decorrelation imaging is a successful predictor of local ablation. For the group of all exposures combined, AUC values for prediction of ablation by echo decorrelation were >0.8 in liver tissue and >0.7 in tumor tissue, consistent with previous studies on echo decorrelation monitoring of radiofrequency and ultrasound ablation (Mast et al. 2008; Subramanian et al. 2014). These results also indicate that tissue death can occur without substantial accompanying echo decorrelation, as seen in Figure 2(b), corresponding to limitations in prediction sensitivity. However, an appropriate choice of echo decorrelation threshold can provide high prediction specificity, which is appropriate for ablation control using echo decorrelation imaging as discussed below. Prediction performance by echo decorrelation was generally better than prediction by

IBS, with significantly higher AUC values for focused exposures and all exposures combined in liver tissue, consistent with previous observations (Fosnight et al. 2014; Mast et al. 2008; Subramanian et al. 2014). AUC values for ablation prediction by IBS were not significantly higher than those for echo decorrelation in any of the cases examined. However, IBS substantially predicted ablation in all cases except for unfocused exposures in VX2 tumor, and also exhibited different trends in trading off sensitivity versus specificity, as seen in the ROC curves of Figure 4. Thus, appropriate combination of echo decorrelation and IBS maps may potentially be useful for further improving prediction efficacy.

Differences between echo decorrelation in liver and tumor were seen for the unfocused exposures. There was a trend of lower decorrelation in ablated tumor than in ablated liver; however, these differences were not statistically significant. This trend can be observed in Figure 2(d, e), where the $\log_{10}$ -scaled echo decorrelation appears lower in ablated tumor regions in comparison to ablated liver. This trend may be due to differences in content and structure between liver tissue and VX2 tumor tissue, which also varies with the tumor size.

The echo decorrelation images created here had a spatial resolution of 2.35 mm, equivalent to the full width at half-maximum of the $\sigma = 1$ mm Gaussian correlation windows employed. This relatively low spatial resolution might explain the smaller differences observed between decorrelation in non-ablated versus ablated regions in VX2 tumor. Hooi et al. (2015), using simulated echo decorrelation images, reported that a correlation window size on the order of 1/6th the expected lesion size was most appropriate for estimating physical changes in the ablated tissue, whereas relatively small error was achieved over a wider range of window sizes, approximately 1/20th to 1/2 the lesion size. On the basis of these considerations, the 1.00-mm correlation window size employed here was nearly optimal for unfocused ablation, for which the approximate optimal window size estimated from simulated beams was about 1.67 mm, but was suboptimal for focused ablation, for which the approximate optimal window size was 0.02 mm (Fosnight 2015). As a result, predictions of focused ultrasound ablation were negatively affected by the limited spatial resolution of computed echo decorrelation and integrated backscatter maps. Focused ablation prediction performance could potentially be enhanced by improving pulse-echo image resolution, allowing smaller correlation windows to be employed. However, tissue motion may still adversely affect spatial prediction of focused ablation, unless tissue motion is tracked. Motion is believed to be one cause of overprediction in some cases reported here (*e.g.*, Fig. 2c).

Echo decorrelation imaging is expected to successfully predict thermal ablation in a clinical setting, as echo decorrelation is believed to map thermally induced tissue microstructural changes as well as transient gas activity (Hooi et al. 2015). Such microstructural changes include cellular swelling, microvascular changes, protein denaturation and tissue damage caused by water vaporization during thermal ablation (Gravante et al. 2011), as well as changes in fat cells at the melting points of saturated fatty acids, approximately 43°C–69°C (Timberlake, 2014). As normal liver, diseased liver and tumor tissues all contain at least some of these components, echo decorrelation imaging is hypothesized to predict thermal ablation even in the presence of liver tissue abnormalities like inflammation, liver cirrhosis and fatty infiltrations.

Important next steps toward clinical implementation of echo decorrelation imaging for ablation monitoring include development of 3-D echo decorrelation imaging, as thermal ablation is intrinsically a 3-D problem. Current radiofrequency and microwave ablation devices thermally ablate an entire volume (typical diameter 2–5 cm) at once. To ensure complete ablation, simultaneous 3-D monitoring of the entire volume of interest is needed. Although 3-D volume ultrasound imaging systems have inherently slower frame rates, 3-D monitoring using echo decorrelation is still feasible because prediction performance was only marginally affected by tissue motion, particularly after compensation for motion effects. As seen in Figure 5, echo decorrelation successfully predicted thermal ablation for frame rates as low as 1.2 frames per second. Three-dimensional echo decorrelation imaging would also potentially reduce motion artifacts by allowing decorrelation associated with out-of-plane motion to be accurately measured and compensated.

Motion compensation may be particularly important for application of echo decorrelation imaging to noninvasive or minimally invasive ablation procedures guided by extracorporeal ultrasound imaging, where respiratory motion typically exceeds that observed here, though it can be mitigated by induced apnea or alternative ventilation techniques (Muller et al. 2013). In such cases, echo decorrelation imaging could employ respiratory gating, similar to gating already under investigation for non-invasive HIFU procedures (Muller et al. 2013; Okada et al. 2006). Respiratory gating, using ultrasound image data to track liver motion, has been found feasible for echo decorrelation monitoring of *in vivo* radiofrequency ablation in swine liver (Subramanian et al. 2014). Such respiratory gating could be combined with the motion compensation employed here, resulting in more robust echo decorrelation feedback. Present results indicate that successful ablation prediction is feasible if respiratory gating reduces the effective range of motion to that observed here (peak-to-peak displacements on the order of 1.5 ± 1.2 mm).

Also important to clinical implementation is assessment of echo decorrelation's ability to successfully control clinical ablation in real time. In one possible approach to control thermal ablation using echo decorrelation imaging feedback, a treatment could be terminated when the local minimum echo decorrelation within the planned treatment zone reaches an optimal threshold. The threshold for ablation control should be designed to achieve high specificity, thus potentially decreasing the odds of local recurrence; however, the cost of increased specificity is lower sensitivity. For example, to achieve a 90% specificity for the data reported here, the optimal thresholds for ablated liver and VX2 tumor prediction by log₁₀-scaled echo decorrelation per millisecond would be –2.9 and –2.3, respectively. Corresponding sensitivities at these thresholds would be 0.65 and 0.43 for ablated liver and VX2 tumor prediction.

Echo decorrelation imaging clinical performance could also be extended to other emerging thermal ablation cancer therapies for breast tumors, pancreatic cancer, kidney tumors, and thyroid and parathyroid tumors (Maloney and Hwang 2015). Ultrasound-guided HIFU and magnetic resonance imaging-guided HIFU systems have been used to safely guide HIFU therapy for these soft tissue cancers (Maloney and Hwang 2015). The deployment of these imaging technologies indicates a need for real-time image monitoring, for which echo decorrelation imaging could provide a relatively low cost and less complex solution.

CONCLUSIONS

These results indicate echo decorrelation imaging is a successful predictor of local ablation, with potential for real-time ultrasound system implementation and successful clinical translation. For most cases, echo decorrelation predicted ablated liver better than IBS and predicted ablated VX2 tumor marginally better than IBS. Tissue motion effects were found to have little effect on echo decorrelation's ablation prediction performance. These results suggest that echo decorrelation imaging merits further investigation and has the potential to improve real-time monitoring and control of thermal ablation for cancer therapy, including HIFU, bulk ultrasound ablation and radiofrequency ablation.

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Fig. 1.

(a) Experimental setup for focused and unfocused ultrasound exposures of *in vivo* rabbit liver. Image-ablate arrays (A) were held by a 3-D positioning arm (B). (b) The liver lobe (C) with implanted VX2 tumor (D) was placed on an acoustic gel coupling pad (E) and/or acoustic absorber pad (F) before performing imaging and thermal ablation. (c) Alignment with the tumor (right of arrow) was verified for tumor ablation by visualizing the tumor in the B-mode (brightness mode) image.

Fig. 2.

Representative histologic and ultrasound images. From left to right are the corresponding segmented, vital-stained histology in the imaging/ablation plane, $\log_{10}$ -scaled uncorrected cumulative echo decorrelation, $\log_{10}$ -scaled corrected cumulative corrected echo decorrelation and decibel-scaled cumulative IBS for focused (a–c) and bulk (d–f) ultrasound ablation, with identical dimensions for all images within each row. The *white line* indicates tissue boundaries segmented in the B-mode images. In the echo decorrelation and IBS images, the threshold for optimal prediction of ablation ($\log_{10}$ -scaled echo decorrelation per

millisecond: -3.1 , IBS: 3.86 dB) is indicated by the *yellow-dotted line*. In the tissue sections, the *red*, *blue*, *green* and *black* boundaries indicate the segmented tissue, treated, ablated and tumor regions. IBS = integrated backscatter.

Fig. 3. Mean and standard error of corrected $\log_{10}$ -scaled echo decorrelation per millisecond (a) and decibel-scaled integrated backscatter (b) in non-ablated and ablated regions for the group of all exposures in liver and VX2 tumor. * $p < 0.05$. ** $p < 10^{-2}$. *** $p < 10^{-3}$.

Fig. 4.

Receiver operating characteristic curves illustrating prediction performance for tissue ablation by corrected echo decorrelation (Δ , dotted lines) and integrated backscatter (IBS) (dash-dotted lines) for focused exposures (a), unfocused exposures (b) and all exposures combined (c) in liver (red lines) and VX2 tumor (black lines).

Fig. 5.

Areas under the receiver operating characteristic curve (AUC) for uncorrected (*red-dashed line*) and corrected (*red-dotted line*) echo decorrelation and integrated backscatter (IBS) (*black solid line*) computed for τ (inter-frame time) = 8.5, 42.4, 84.7, 127.1, 169.5, 211.9, 254.2, 508.5 and 847.5 ms. Ablation prediction performance was tested for focused (a) and unfocused (b) exposures in liver.

Table 1Conditions for all focused and unfocused exposures^{*}

Animal/lobe	VX2 diameter (mm)	f_c (MHz)	On/off time (s)	No. of cycles	Focus (mm)	I_{SPT} (W/cm ²)	Peak decorr. (log ms ⁻¹)	Peak IBS (dB)
Focused exposures								
R266/R	—	5.05	0.7/3.3	9	27	1080	-2.96	14.4
R267/M	6	5	0.7/3.3	9	25	1196	-3.48	0.7
R267/R	—	5	0.7/3.3	9	28	1220	-2.06	21.0
R268/L	5.4	5	0.7/3.3	9	26	1351	-2.34	23.3
^a R268/M	—	5	0.7/3.3	9	27	1103	-2.05	20.8
R268/M	—	5	0.7/3.3	9	25	1196	-2.15	22.4
R271/L	7.7	5.4	0.7/2.8	9	24	1033	-1.87	17.3
^a R272/L	8.1	5.4	0.7/2.8	9	23	1061	-3.61	5.6
R272/M	—	5.4	0.7/2.8	9	26	911	-3.15	0.2
R272/R	—	5.4	0.7/2.8	9	24	1033	-2.37	15.8
R272/L	—	5.4	0.7/2.8	9	24	1033	-4.58	0.1
^a R273/R	9.8	5.4	0.7/2.8	7	25	1107	-1.97	21.1
R274/R	14.4	5.4	0.7/2.8	9	25	1292	-2.70	5.5
Unfocused exposures								
^a R256/L	11	5.2	7.5/5	6	∞	50	-1.56	24.5
R259/R	11.1	5.2	7.5/5	6	∞	62	-2.87	24.7
R267/L	5.1	5.05	7.5/2.5	9	∞	52	-1.16	6.1
R269/M	10.1	5	7.5/2.5	9	∞	61	-1.11	12.2
^a R270/L	3.5	5	6.5/2.5	9	∞	59	-1.44	21.2
R270/R	5.3	5	6/2.5	8	∞	61	-1.42	23.6
^a R271/M	3.5	5.4	6/2.5	9	∞	54	-1.59	20.8
R273/M	—	5.4	6/2.5	7	∞	63	-1.71	24.1
R274/L	—	5.4	6/2.5	8	∞	54	-1.66	20.3
R274/M	8.5	5.4	6/2.5	9	∞	63	-1.65	12.1

^{*} Includes internal animal number and liver lobe (left/L, medial/M, or right/R), measured effective VX2 tumor diameters, ultrasound exposure conditions and peak values of the log₁₀-scaled echo decorrelation per millisecond and peak decibel-scaled integrated backscatter within the treated (partial or no vital-stain uptake) region. The labels a-f correspond to the representative trials illustrated in Figure 2.