Cardiovasc Intervent Radiol (2019) 42:744–750
https://doi.org/10.1007/s00270-018-02152-y

LABORATORY INVESTIGATION

Monitoring of Thermal-Induced Changes in Liver Stiffness During Controlled Hyperthermia and Microwave Ablation in an Ex Vivo Bovine Model Using Point Shear Wave Elastography

Laura Crocetti[1] · Francesca Calcagni[1] · Giulia Gherarducci[1] · Nevio Tosoratti[2] · Claudio Amabile[2] · Francesco Paolo Tarantino[1] · Irene Bargellini[1] · Simone Cassarino[2] · Roberto Cioni[1] · Davide Caramella[1]

Received: 2 July 2018 / Accepted: 20 December 2018 / Published online: 2 January 2019

Abstract

Purpose To investigate liver stiffness changes—evaluated by point shear wave elastography (pSWE)—in controlled hyperthermia and microwave ablation (MWA) in an ex vivo animal model.

Materials and Methods Five samples of ex vivo bovine liver were uniformly heated to temperatures ranging from 40 to 100 °C. B-mode ultrasound imaging and pSWE were acquired simultaneously, and shear wave velocity (SWV) was measured in a region of interest (ROI). The threshold value of SWV at 60 °C (avg60) was identified. Subsequently, MWA was performed in 11 liver samples at 60 W until avg60 + 0.5 m/s was reached. SWV was measured in ROIs at 10–40 mm from the antenna feed. The correlation of mean values of SWV with location (within, border, or outside necrotic area) at gross pathology was evaluated.

Results In controlled hyperthermia experiments, a steep transition in liver stiffness was observed at 63.0 ± 2.4 °C (SWV 3.54 ± 0.68 m/s). Avg60 was of 2.5 m/s. In 8/9 MWA experiments, interrupted when SWV of 3 m/s was measured, the ROI was at the inner side of the necrotic area border at pathology (accuracy 89%). No correlation between SWV values for outside, border, and within necrosis could be identified.

Conclusions pSWE can provide a velocity threshold predictive of the presence of coagulation necrosis during MWA in ex vivo liver model. However, pSWE is not able to reliably capture changes in stiffness within, at the border, and outside the necrotic zone in this experimental model.

Keywords Thermal ablation · Shear wave velocity · Elastosonography · Liver tissue · Microwave · Stiffness

Introduction

Non-invasive monitoring of percutaneous thermal ablation is essential for preventing complications while ensuring treatment efficacy. While ultrasound (US) or computed tomography (CT) is generally used for guiding the insertion of applicators into the tumours, magnetic resonance (MR) imaging is currently the only modality with validated techniques for real-time temperature monitoring [1, 2].

Elastography is a non-invasive US- or MR-based technique that is able to assess the mechanical properties of tissues. During heating, an increase in tissue stiffness is observed that is quantitatively measurable [3–6]. Many US-based thermal ablation-monitoring techniques are under investigation; point shear wave elastography (pSWE) can provide quantitative estimation and mapping of tissue stiffness in real time by calculating shear wave velocity (SWV) in m/s, in defined locations (region of interest, ROI), under B-mode US visualisation [7, 8].

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00270-018-02152-y) contains supplementary material, which is available to authorized users.

✉ Laura Crocetti
laura.crocetti@med.unipi.it

[1] Department of Diagnostic and Interventional Radiology, Cisanello University Hospital, Pisa, Italy

[2] R&D Unit, H.S. Hospital Service SpA, Rome, Italy

It is well known that during thermal ablation, coagulation, which involves protein denaturation and tissue dehydration, occurs almost instantaneously at temperatures above 60 °C, whereas water vaporisation occurs above 100 °C [1]. However, the effect of thermal-induced changes in liver tissue mechanical properties has not yet been investigated in detail.

The aim of our study was to investigate liver stiffness changes—evaluated by pSWE—in controlled hyperthermia and microwave ablation (MWA) in an ex vivo animal model.

Materials and Methods

Study Design

The study was organised in two phases: the first conducted in conditions of controlled hyperthermia, heating homogeneously an ex vivo liver sample; the second during MWA (Fig. 1). Phase 1 of the study aimed to (1) quantify the temperature dependence of liver tissue stiffness as measured with SWV (2) identify the threshold value of SWV related to coagulation (avg60) (3) assess the reproducibility of this measurement and (4) evaluate the irreversibility of stiffness changes. Phase 2 aimed to (1) confirm the SWV threshold for the stiffness of coagulated tissue during MW ablation and (2) determine the reliability of pSWE to delineate the boundaries of ablation zone.

point Shear Wave Elastography (pSWE)

pSWE imaging is a non-invasive technique that is implemented in an US system and is able to assess the mechanical properties of tissues. Shear wave, which propagates into the tissue, is generated in the liver in a small ROI, and B-mode imaging is used to monitor the measurement. From the displacements monitored over time at different locations from the initial pulse, the SWV is calculated in metres per second (m/s) [9].

pSWE was performed with a Siemens Acuson S2000™ ultrasound system (Siemens Healthcare, Erlangen, Germany) with a 4C1 transducer, by using Virtual Touch™ Tissue Quantification application. The SWV is exhibited in m/s, and if the amount of non-shear wave motion exceeds a threshold of 4.99 m/s, then the system displays a non-numerical result (X.XX) on the screen. For computational needs, considering that the "X.XX" cannot be produced by motion of the sample (as it can happen in vivo with patient movements) but only from the high amount of non-shear wave motion, to calculate mean and standard deviation, we decided to consider this non-numerical value as 5 m/s.

Experimental Set-Up

Phase 1

A bipolar radiofrequency (RF) system (HS Amica GEN H 1.0) was used to uniformly heat five cubes of ex vivo bovine liver to target temperatures.

Before each experiment, baseline tissue temperature was measured to ensure an initial preheating temperature within the range of 18–24 °C.

Liver samples were then heated to target temperatures between 40 and 100 °C. At each target temperature, 11 consecutive readings of SWV were obtained from a small ROI of 8 mm long and 5 mm wide. The ROI was maintained at the same position through the experiment, and the 11 consecutive values were calculated from this same ROI at various temperatures. The temperature of the first non-numerical measurement and the number of non-numerical measurements at each temperature were recorded.

Knowing that immediate coagulation occurs in liver tissue above 60 °C, avg60, defined as mean value of SWV at 60 °C, was calculated.

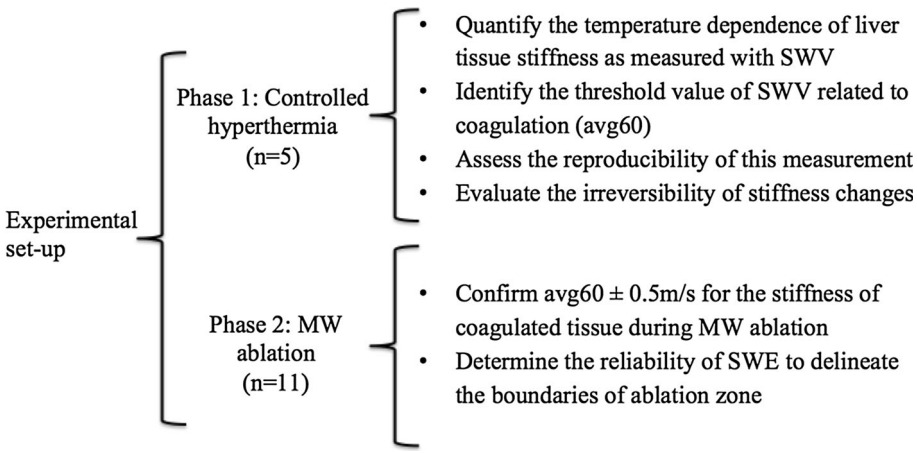

Fig. 1 Study design and aims

At each target temperature, mean SWV and standard deviations were calculated. Temperature/SWV curves were then generated and visually compared to investigate experimental reproducibility.

After heating to 100 °C, samples were submitted to cooldown cycles and readings were obtained every 10 °C until the thermocouples showed a temperature of 50 °C. Mean SWV was calculated, and the temperature at which all measurements were non-numerical was recorded.

See supplemental material for details about experimental set-up.

Phase 2

Eleven cubes of ex vivo bovine liver were cut and prepared as described before. A MWA system (HS AMICA®, H.S. Hospital Service SpA) was used; the antenna was placed in the centre of the sample, in parallel with the probe.

MWA was performed at 60 W until SWV avg60 + 0.5 m/s was achieved in the 8 × 5 mm ROI placed 1.5 cm radially from the antenna feed point.

MWA was then stopped, and SWV was measured in 24 ROIs at established distances ranging from 10 mm to 40 mm from the antenna feed, acquiring subsequently 10 SWV determinations for each ROI. Cartesian coordinate system was used to place the ROIs on the US image and on gross pathological specimen (Fig. 2). To each point at pathology, the following values were assigned: 0 = outside ablation area; 0.5 = border; 1 = within necrosis. For those points, evaluation of the mean SWV values was performed.

Results

Phase 1

The mean baseline SWV of the five specimens (volumes ranging between 60 × 40 × 20 mm^3 and 80 × 90 × 50 mm^3) before heating was 1.41 ± 0.17 m/s at room temperature. The heating of bovine liver tissue induced a linear decrease in SWV up to 42 °C (− 0.31 ± 0.07 m/s with respect to baseline) with a minimum average SWV of 1.10 m/s at this temperature. Then, SWV gradually increased up to 55–60 °C corresponding to an average SWV value of 2.50 ± 0.52 m/s. At a temperature threshold of 63 °C ± 2.4 °C, we observed a steep increase in SWV. At this temperature, at least 1 out of 11 consecutive measurements and a maximum of 8 measurements were non-numerical (X.XX) as a result of a dramatic change in SWV. The mean SWV at this temperature was 3.54 ± 0.68 m/s. In two out of five sessions, we reached a temperature above 63 °C (respectively, 64 °C and 65 °C);

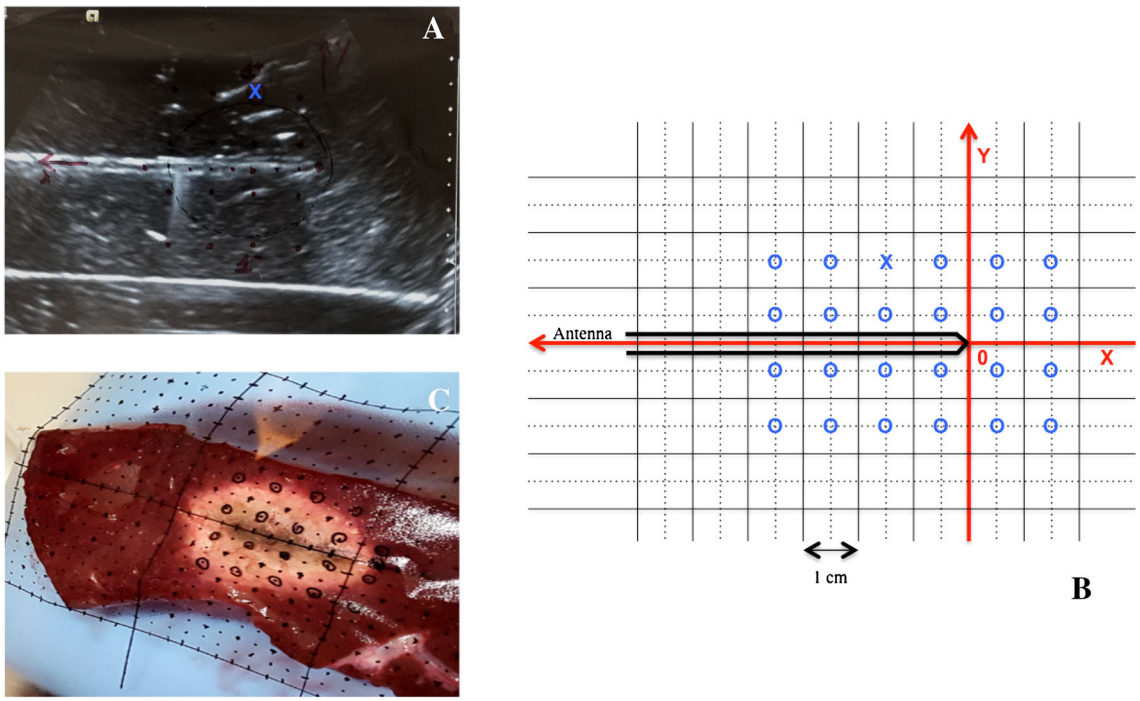

Fig. 2 Data acquisition set-up: the Cartesian coordinate system was superimposed to US image and used to place the ROIs (**A**, blue cross: ROI placed 1.5 cm radially from the antenna feed point). The antenna tip was assumed as the origin, the antenna profile and the line perpendicular to it were considered the *XY* axes. ROIs were placed at 1 cm distances in this Cartesian system (**B**; blue cross: ROI placed 1.5 cm radially from the antenna feed point; blue circles: ROIs evaluated after stopping MWA). The same Cartesian coordinate system was superimposed to pathological specimen and used to correlate pathology with the SWV measured in ROIs (**C**)

in these cases, at gross pathology, large vessels were present in the tissue sample at the site of measurement, and this cavitated structures may have caused differences in the stiffness changes during heating process.

Avg60, i.e. the mean SWV at 60 °C, was 2.5 m/s. To be conservative, we decided to use, as threshold for coagulation in the phase 2 of the study, the threshold of avg60 + 0.5 m/s, i.e. 3 m/s.

Repeated experiments revealed a reproducible pattern of tissue mechanical changes during heating in ex vivo bovine liver, with very similar, overlapping temperature/SWV curves (Fig. 3). Only the first (xp1, in blue line) SWV/temperature curve presented a slightly different shape, probably due to the insufficient familiarity with the set-up.

In the cooldown phase, we measured SWV values of 4.40 ± 0.41 m/s at post-heating temperatures, every 10 °C from 100 °C until 50 °C was reached. At around 50 °C of each experiment, the 11 measurements showed X.XX, proving the irreversibility and stability of changes in liver stiffness.

Phase 2

Eleven experiments were performed in tissue cubes with volumes ranging between $80 \times 70 \times 60$ mm^3 and $80 \times 90 \times 50$ mm^3. Two experiments were considered unreliable due to technical problems during the procedure of MWA (thermocouple displacement in 1 case, and tissue deterioration in 1 case). In 8 out of 9 experiments, following stoppage of ablation when the predetermined ROI reached SWV 3 m/s, the ROI on gross pathology was noted to be within the necrotic area (i.e. on the inner border of the abated tissue). Hence, accuracy of our study in determining the presence of necrosis was 89% (Fig. 4).

One experiment was considered unreliable for post-ablation analysis due to Virtual Touch™ Tissue Quantification malfunctioning due to overheating. In the post-ablation analysis—therefore performed in 8 experiments—we graphically correlated the SWV values and the gross pathology, classifying points as outside (0), border (0.5), and within (1) the ablated area. SWV values measured in ROI valued at pathology as 0, 0.5, and 1 were dispersed. No SWV threshold values for 0), 0.5, and 1 could be identified (Fig. 4).

In order to explain these unexpected findings, even if this was not planned according to the study design, in the last two experiments, we measured and represented graphically the SWV (± SD) measurement over time in the ROI placed 1.5 cm radially from the antenna feed point. We demonstrated that during the ablation the values were constantly increasing until the avg60 (3 m/s) is reached. After ablation interruption, SWV value—measured in the same ROI—gradually decreases, until reaching the baseline value in about 5 min (Fig. 5).

Discussion

Liver tissue stiffness is reproducibly affected by temperature changes in conditions of controlled hyperthermia. Our experiments demonstrated that liver stiffness significantly changes after heating, and when a mean SWV of 3 m/s was reached the temperature was above 60 °C.

Currently, few papers studied the thermal effects on the elastic properties of liver tissues. Wu and co-workers identified three patterns, in ex vivo bovine muscle: (1) a reversible decrease in the SWV with increasing temperature between 20 and 60 °C, (2) an irreversible increase around 60 °C corresponding to the threshold of denaturation, and (3) an increase in the SWV during cooling for tissues that underwent an irreversible thermal damage. Our results confirmed, for the first time, similar patterns in ex vivo liver tissue [5].

In particular, we observed that at the lower temperature range (42–55 °C), tissue SWV decreases with increasing temperature (− 0.31 ± 0.07 m/s with respect to baseline at

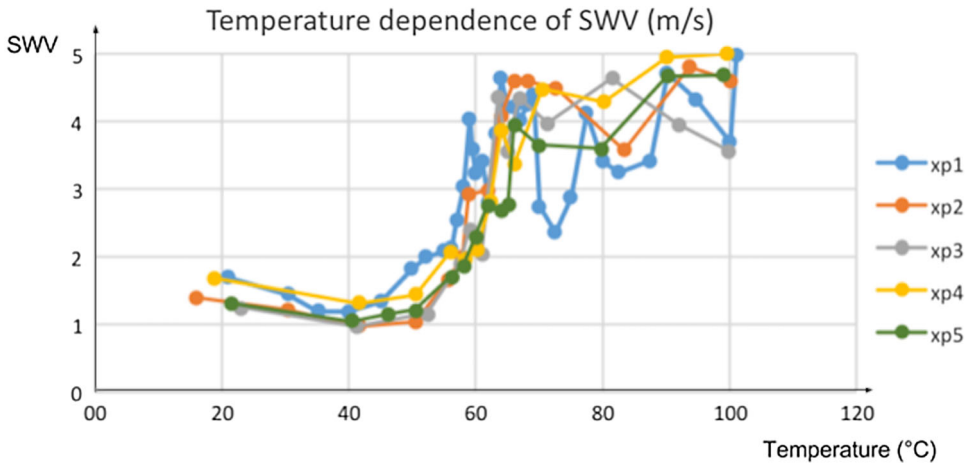

Fig. 3 Curves SWV/temperature in different experiments. In all the experiments, a decrease in SWV up to 42 °C was demonstrated, followed by a gradual increase in SWV up to 55–60 °C. Subsequently, a steep increase in SWV was observed. Above 63–65 °C, SWV remained constantly high

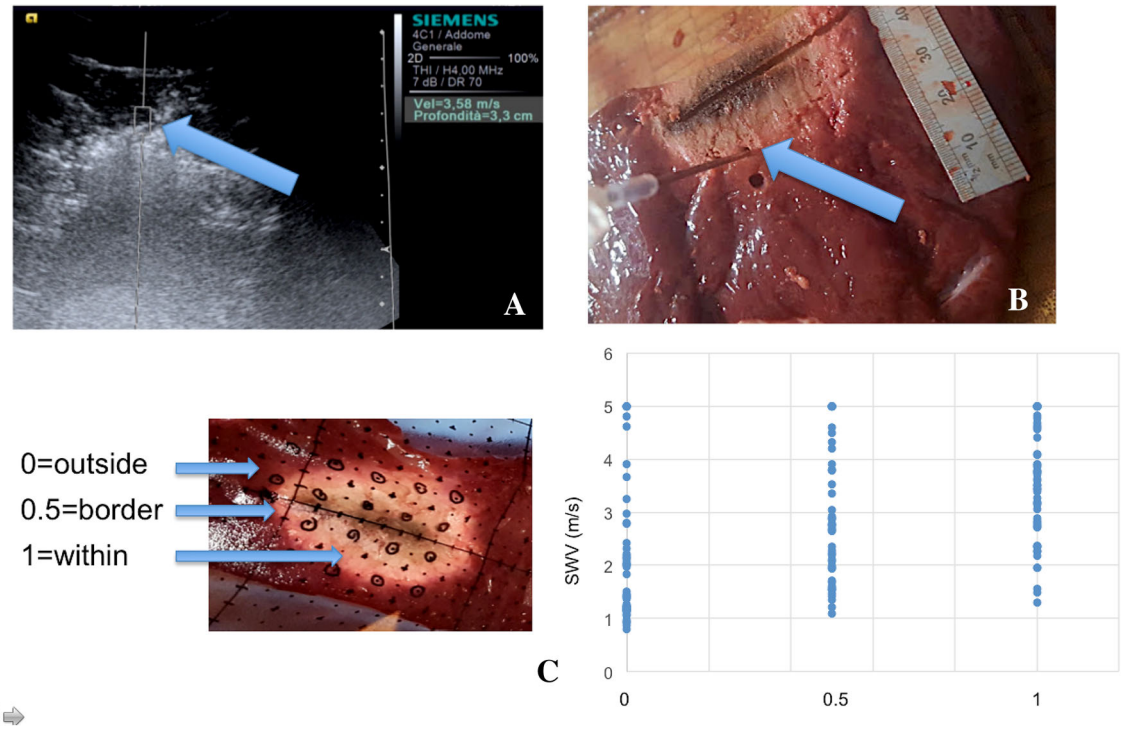

Fig. 4 The ablation was interrupted when 3 m/s was measured in the ROI at 1.5 cm from the antenna feed (blue arrow, **A**). The same region was demonstrated to be in the inner side of the border of the ablated tissue, in necrotic area (blue arrow, **B**). SWV values measured in ROIs outside, at the border, and within the ablated area are dispersed (**C**)

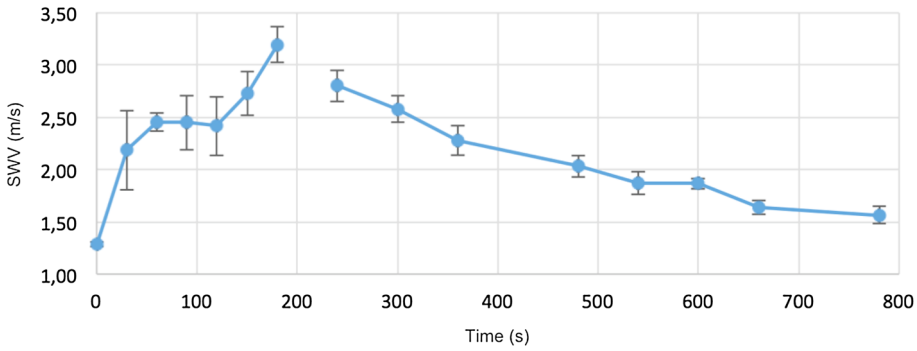

Fig. 5 SWV (± SD) measurement over time at the ROI placed 1.5 cm radially from the antenna feed point was performed in two experiments: values were increasing until the threshold was reached. They decreased after ablation interruption reaching the baseline value in about 5 min. Mean and SD are shown

average temperature of 42 °C). As previously demonstrated, this pattern of tissue mechanical property change reflects heat-induced intrinsic structure changes in tissue [10]. We suggest that tissue SWV change in the specimens in this region is due to reversible protein denaturation, which can be dominated by protein unfolding/refolding [11]. Liver stiffness then increased above a temperature of about 63 °C. The reason for this SWV steep increase has been identified as collagen fibre denaturation [11–13].

In the cooldown, we measured SWV values of 4.40 ± 0.41 m/s every 10 °C until 50 °C was reached, when non-numerical measurements were recorded in all evaluations, proving the irreversibility of changes in liver stiffness. Thus, irreversible cellular damage is obtained increasing the tissue temperature to 60 °C [14].

Promising results have been recently published regarding the use of US-elastography in real-time assessment of RF and MWA in ex vivo animal models [15, 16]. In our study, a SWV of 3 m/s was demonstrated to be an accurate threshold for identifying the region of coagulated tissue. Bo et al. showed similar result in mean SWV value of transitional zones after RF at different observation time points: 0 min, 10 min, 30 min, and 60 min after RF, respectively, with a SWV of 3.53 m/s, 3.45 m/s, 3.38 m/s, 3.31 m/s [17].

The experiments in phase 1 were conducted in ex vivo liver tissue, and the set-up was ideal, with homogeneous increase in the heating of the sample and extreme stability of the US probe/liver sample during measurements. These could make the results difficult to reproduce in in vivo experiments. A further limitation was the use of non-

perfused ex vivo tissues and the use of normal tissue. Cirrhotic liver has a baseline SWV higher than 2 m/s, and this could cause interpretative difficulties when using pSWE.

In the phase 2 of the study, we experienced some unexpected events. While the threshold of 3 m/s had 89% accuracy in identifying necrosis at pathology was confirmed, the measurements of SWV within, at the border, and outside the ablation were dispersed, demonstrating the insufficiency of the technique to monitor ablation in this experimental model.

Correa-Gallego proved that elastography systematically underestimates the extent of ablation in ex vivo animal liver model [18]. In particular, ablation size, as evaluated with SWV, decreased during the first minutes after ablation and increased at 10 min, thus confirming that changes that affect tissue stiffness after ablation are not immediate and occur even after ablation interruption, in particular at the border of necrosis [18]. Moreover, MWA induces shrinkage of the ablated tissue and changes in the surrounding non-ablated tissue. Farina et al. demonstrated substantial shrinkage of 52–74% of initial tissue volume, non-uniform over time and space, in a liver and muscle ex vivo animal model after MWA [19]. The correlation between shrinkage and dehydration was investigated by Brace et al, in an ex vivo experimental model. Besides confirming that after MWA there is a substantial tissue shrinkage (30–38%), the Authors found a positive correlation between the percentage of water removed and relative contraction in liver [20]. These results might be partially explained by the hypothesis that water vapour driven from the inner position recondensed and artificially increased water content in the middle and peripheral positions of MWA zones [20–22]. These events could explain why SWV values gradually came back to pre-ablation values after MWA interruptions. Moreover, Tsui et al. [23] described in detail how changes in gas and water content may affect SWE measurement. Under high-temperature ablation, gas bubbles may interact with the beams used for SWE imaging, thereby affecting the formation of the periablation SWE image [23]. On the other hand, to create an SWE image, SWV estimation is performed by ultrafast imaging typically computed using cross-correlation-based techniques on successive images. During MWA, new bubbles form and old bubbles may dissipate as the tissue temperature increases. The formation and collapse of bubbles during MWA cause the waveforms of US signals to vary with time, resulting in computational errors [23].

Conclusions

pSWE is useful to monitor thermal changes in tissue in the setting of controlled hyperthermia. pSWE can provide an elasticity threshold predictive of the presence of coagulation necrosis during MWA in an ex vivo liver model. The complexity of ablation process in tissues, and possibly the shrinkage, rehydration and gas bubbles formation/collapse occurring after ablation, makes pSWE not able to reliably capture changes in stiffness within, at the border, and outside the ablation zone in this experimental model.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Ahmed M, Solbiati L, Brace CL, et al. International working group on image-guided tumor ablation; interventional oncology Sans Frontières expert panel; technology assessment committee of the society of interventional radiology; standard of practice committee of the cardiovascular and interventional radiological society of Europe. Image-guided tumor ablation: standardization of terminology and reporting criteria—a 10-year update. J Vasc Interv Radiol. 2014;25(11):1691–705.
2. Zhou Z, Wu W, Wu S, et al. A survey of ultrasound elastography approaches to percutaneous ablation monitoring. Proc Inst Mech Eng H. 2014;228(10):1069–82.
3. Brosses ES, Pernot M, Tanter M. The link between tissue elasticity and thermal dose in vivo. Phys Med Biol. 2011;56(24):7755–65.
4. Barr RG, Ferraioli G, Palmeri ML, et al. Elastography assessment of liver fibrosis: society of radiologists in ultrasound consensus conference statement. Radiology. 2015;276(3):845–61.
5. Wu T, Felmlee JP, Greenleaf JF, et al. Assessment of thermal tissue ablation with MR elastography. Magn Reson Med. 2001;45(1):80–7.
6. Brosses ES, Gennisson JL, Pernot M, et al. Temperature dependence of the shear modulus of soft tissues assessed by ultrasound. Phys Med Biol. 2010;55(6):1701–18.
7. Mariani A, Kwiecienski W, Pernot M. Real time shear waves elastography monitoring of thermal ablation: in vivo evaluation in pig livers. J Surg Res. 2014;188(1):37–43.
8. Ferraioli G, Tinelli C, Lissandrin R, et al. Point shear wave elastography method for assessing liver stiffness. World J Gastroenterol. 2014;20(16):4787–96.
9. Kennedy P, Wagner M, Castéra L, et al. Quantitative elastography methods in liver disease: current evidence and future directions. Radiology. 2018;286(3):738–63.
10. Rossmann C, Garrett-Mayer E, Rattay F, et al. Dynamics of tissue shrinkage during ablative temperature exposures. Physiol Meas. 2014;35(1):55–67.
11. Lepetit J. A theoretical approach of the relationships between collagen content, collagen cross-links and meat tenderness. Meat Sci. 2007;76(1):147–59.
12. Jiang J, Varghese T, Brace TL, et al. Young's modulus reconstruction for radio-frequency ablation electrode-induced displacement fields: a feasibility study. IEEE Trans Med Imaging. 2009;28(8):1325–34.
13. Zhu Y, Hall TJ, Jiang J. A finite-element approach for Young's modulus reconstruction. IEEE Trans Med Imaging. 2003;22(7):890–901.
14. Arnal B, Pernot M, Tanter M. Monitoring of thermal therapy based on shear modulus changes: II. Shear wave imaging of

thermal lesions. IEEE Trans Ultrason Ferroelectr Freq Control. 2011;58(8):1603–11.
15. Sugimoto K, Oshiro H, Ogawa S, et al. Radiologic-pathologic correlation of three-dimensional shear-wave elastographic findings in assessing the liver ablation volume after radiofrequency ablation. World J Gastroenterol. 2014;20(33):11850–5.
16. Dewall RJ, Varghese T, Brace TL. Visualizing ex vivo radiofrequency and microwave ablation zones using electrode vibration elastography. Med Phys. 2012;39(11):6692–700.
17. Bo XW, Li XL, Xu HX, et al. 2D shear-wave ultrasound elastography (SWE) evaluation of ablation zone following radiofrequency ablation of liver lesions: is it more accurate? Br J Radiol. 2016;89(1060):20150852.
18. Correa-Gallego C, Karkar AM, Monette S, et al. Intraoperative ultrasound and tissue elastography measurements do not predict the size of hepatic microwave ablations. Acad Radiol. 2014;21(1):72–8.
19. Farina L, Weiss N, Nissenbaum Y, et al. Characterisation of tissue shrinkage during microwave thermal ablation. Int J Hyperth. 2014;30(7):419–28.
20. Brace CL, Diaz TA, Hinshaw JL, et al. Tissue contraction caused by radiofrequency and microwave ablation: a laboratory study in liver and lung. J Vasc Interv Radiol. 2010;21:1280–6.
21. Yang D, Converse MC, Mahvi DM, et al. Measurement and analysis of tissue temperature during microwave liver ablation. IEEE Trans Biomed Eng. 2007;54:150–5.
22. Yang D, Converse MC, Mahvi DM, et al. Expanding the bioheat equation to include tissue internal water evaporation during heating. IEEE Trans Biomed Eng. 2007;54:1382–8.
23. Tsui PH, Wang CY, Zhou Z, et al. Monitoring radiofrequency ablation using ultrasound envelope statistics and shear wave elastography in the periablation period: an in vitro feasibility study. PLoS ONE. 2016;11(9):e0162488.