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Transcutaneous versus Intraoperative quantitative ultrasound for staging bovine hepatic steatosis

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Abstract—**The aim of this study was to test the hypothesis that the Computer Aided UltraSound (CAUS) method developed by the authors [1-4] for the estimation of UltraSound Tissue Characteristics (UTC) parameters on transcutaneous (Transc) ultrasound (US) images can predict the liver fat content with similar accuracy and precision as with intraoperative (Intraop) US. A large animal study in post partum dairy cows (N=151) was performed to test these hypotheses. Five Transc B-Mode US liver image were acquired before surgery. During abomasal displacement surgery five Intraop US B-Mode liver images and a liver biopsy was taken. In liver tissue samples, triacylglycerol (TAG) content was measured by biochemical analysis. Firstly the equipment preset, which was kept fixed during whole study time, was carefully calibrated[5]. For the echo level calibration a TMP was used, and all UTC parameters were expressed relatively to those of the phantom. Prior to UTC parameters estimation several pre-processing steps were performed: Back-Scan Conversion (BSC); Look Up Table (LUT) correction; superficial tissue layers (Fat layer) attenuation correction and Automatic Gain Correction (AGC) were performed. Also several post-processing steps were incorporated like: Automatic segmentation and residual attenuation correction were performed. Stepwise multiple linear regression analysis on a training set (N=76) was performed. In all cases the Residual Attenuation coefficient (ResAtt, R=0.81) was the only selected parameter. The results were tested on the residual cows (test set N=75) to predict the TAG content in the liver. Receiver Operating Characteristics (ROC) analysis then was applied to estimate the Area Under the Curve (AUC) and the sensitivity and specificity of the CAUS method. Equivalent high predictive values for AUC (95%), sensitivity(87%) and specificity (83%) for Intraop and Transc applications were found. Consequently, it can be concluded, applied Fat layer attenuation correction to Transc US images was performed adequately.**

Keywords: hepatic steatosis; quantitative ultrasound; liver; B-Mode; transcutaneous; intraoperative; ultrasound tissue characteristics; computer aided ultrasound; CAUS

I. INTRODUCTION

Fatty infiltration of hepatic tissue is the most common liver disorder in high-yielding dairy cows during early lactation. Up to 65% of dairy cows are affected by moderate (TAG > 50 mg/g) or severe (TAG > 100 mg/g) fatty liver during early lactation [6]. Fatty livers may result in reduced milk yield, fertility and increased risks of other periparturient diseases and early culling in affected animals [6-8]. Assessment of liver fat content are restricted to biochemical or histological examination of liver biopsy specimens, which is a rather invasive method. For on-farm testing liver biopsies are dangerous and impractical because of the time needed for acquiring and analyzing them. The authors therefore developed and tested a non-invasive quantitative ultrasound technique called CAUS [1-4] for staging hepatic steatosis using UTC parameters of US B-Mode images. This method was tested in 151 dairy cows. B-Mode image analysis was chosen since the authors goal was to develop a screening technique which is applicable to all US imaging equipment. The authors showed that automatic US image segmentation of hepatic vessels improved the predictive values in staging liver steatosis [2]. The aim for this study is to validate whether Transc US images can predict the liver fat content with similar performance as in Intraop application, after derived and applied corrections for the attenuation by the subcutaneous skin, fat and muscle layers. Analysis was performed on the Transc and Intraop images, acquired before and during surgery. Also the, the possibility of using a TMP serving as a reference for AGC instead of a group of normal animals was investigated. If these results will be positive this indicate that Transc US may replace Intraop US. The possibility of using a TMP as a reference for AGC instead of the group of normal animals was recently investigated[3]. So a normal group is no longer needed as a reference and future studies can be performed with other equipment since parameters are expressed relative to those of the TMP used.

II. MATERIALS AND METHODS

In this study, 151 lactating German Holstein cows (mean ± standard error of the mean; bodyweight: 571 ± 7kg; age: 4.9 ± 0.2 years; number of days *post partum* 35 ± 5, range: 2-350 were enrolled. Only animals were incorporated in which a surgical correction of left-sided abomasal displacement was indicated and no other disease was present based on clinical outcome [4]. A sub-group of twelve healthy cows for which the TAG content was below 30 mg/g FW (Fresh Weight) served as a reference i.e., “normal-group”.

A. Equipment

The US screening was performed with a Power Vision 6000 scanner (Toshiba Inc., Tokyo, Japan), using a convex array transducer of 2-7 MHz (PVM-375AT). The equipment settings were stored in a preset and the TGC was positioned in central position. The setting were kept fixed throughout the whole study to enable quantitative analysis of the images.

Preset value were as follows: central frequency: 4.2 MHz; in-plane focus: 6 cm; depth range: 11.8 cm; overall gain: 80 decibels (dB); dynamic range: 70 dB; post-processing curve: 0. The latter setting means that a linear look-up table was chosen [5]. The images were digitally acquired (DICOM), stored and transferred to a computer for further analysis.

B. *Image preprocessing*

The methods that were used have been described in recent papers by the authors [1-4]. A summary is given here to inform the reader about the various pre- and post-processing steps of the image analysis protocol.

Firstly, the echo level scale was calibrated by using a tissue mimicking phantom (TMP), model 539; attenuation coefficient of 0.5 dB/cm·MHz. (ATS Labs Inc., Bridgeport, CT, USA). The QA4US software [5] was used to estimate the gamma i.e., the number of gray levels per dB (in this case: 3.7 gl/dB) relative to the background of the TMP used. Before doing so, one has to be sure a linear LUT was used. If not, all image gray levels have to recoded to a linear LUT [5].

Back-scan conversion [1, 9, 10] was applied to all images prior to image analysis in order to straighten the angular shape of the speckles, as well as making the lateral size of the speckles uniform [10-12] prior to the texture analysis. The speckle size was estimated using the auto covariance function (ACVF) [13, 14]. Back-scan conversion results in the transformation of a sector image into a rectangular (polar) image and the lateral speckle size becomes practical independent of depth. The resulting changes in axial and lateral speckle size can be entirely attributed to changes of the received bandwidth and central frequency [13, 14].

The echo level vs. depth curve, after LUT-correction and back-scan conversion, were estimated (Fig. 2) from the set of normal cows, as well as from the TMP. The AGC correction curve is defined as the inverse profile with respect to the averaged echo level of the depth profile. This AGC can be subtracted from the image in order correct for beamforming and focusing effects as well as for normal attenuation.

C. *Image Postprocessing*

The average attenuation by the superficial tissue layers (abdominal wall), indicated as “fat layer”, was estimated by subtraction of the Transc and Intraop uncorrected gray level profiles (in the Region-of-interest (ROI): 5-10 cm) as illustrated in Fig. 2. This average gray level difference (9.2 dB) was then divided by the average fat layer thickness (2.2 cm), yielding a fat layer attenuation coefficient of ± 1 dB/cm (i.e., 1dB/cm·MHz).

To start with, all images were corrected for the actual fat layer attenuation by interactively selection of the liver border, on at least three different locations. A cubic spline curve which represents the fat layer thickness was fitted through these points. The correction per image line was estimated by multiplying the actual fat layer thickness by the fat layer attenuation coefficient [3].

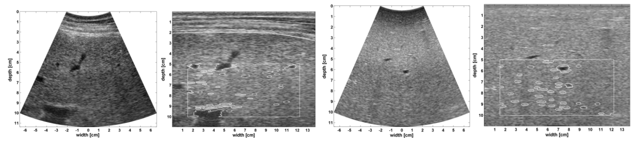

Figure 1, Transc and Intraop ultrasound liver images. (a) Scan-converted Transc sector image. (b) Transc back-scan converted sector image with interactively drawn fat-layer contour (white dashed line), applied: Transc normal group automatic gain compensation (AGC), fat-layer attenuation correction and automatic segmentation of hepatic blood vessels and bile ducts. (c) Scan-converted Intraop sector image. (d) Back-scan converted Intraop sector image with applied Intraop normal group AGC

All following steps are performed fully automatically based on predefined settings. Firstly the predefined ROI (depth range: 5-10 cm, full image width minus 1.5cm of the border). Within this ROI the residual attenuation coefficient (ResAtt, Fig. 3a), without performing any segmentation, is estimated and corrected for. Then on the resulting ROI, with ‘homogeneous’ echo levels, automatic segmentation is performed using histogram based outliers (± 1.4 sd). A speckle shaped and sized moving window was applied to the marked outliers, using exclusion criteria [2].

After the segmentation, the ResAtt is again estimated and corrected for, after undoing the previous ResAtt correction. This process can be repeated iteratively until no changes in segmentation become evident anymore [2]. Finally the Axial and Lateral speckle size is estimated within the resulting ROI using the Full-Width-at-Half-Maximum of the 2D autocovariance function (Fig. 3b).

D. *Statistical Analysis*

Statistical processing of the data was performed using the SPSS (SPSS, v. 16.0.2, IBM Inc., Somers, NY, USA) and Matlab (v. R2007a; Mathworks, Boston, MA, USA) software packages. Descriptive statistics of the Transc and Intraop UTC parameters were estimated to compare absolute values obtained for both conditions. Pearson correlations of all UTC parameters to TAG were estimated to investigate the statistical significance and predictive value of the US parameters. Since a rather large group of 151 animals was investigated, we chose to consider correlations to be significant if $p < 0.01$ (after applying Bonferroni correction statistical significance is reached at $p<0.05/5$) and highly significant if $p < 0.002$ (Bonferoni correction: 0.01/5). Stepwise multiple linear regression analysis (inclusion level $p<0.05$) was performed with a “training” set of 76 alternatingly selected cows, after sorting on TAG content, to investigate whether a combination of parameters could improve correlation with TAG as well as the predictive value of this combination. The quality of the regression is indicated by the R^2 and the significance by the p value. The remaining 75 animals served as the “test” set, where the regression formula obtained for the training set was used to “predict” the TAG based on the individual selected parameters. The TAG contents (mg/g fresh weight) corresponding to the different risk categories were defined as follows: no fat: ≤30; mild fat: >30–50; moderate fat: >50–100; severe fat: >100 mg/g FW [15]. Finally, receiving operator characteristics (ROC) analysis was performed on the predicted TAG (threshold at 50 mg/g FW). From the ROC curve the

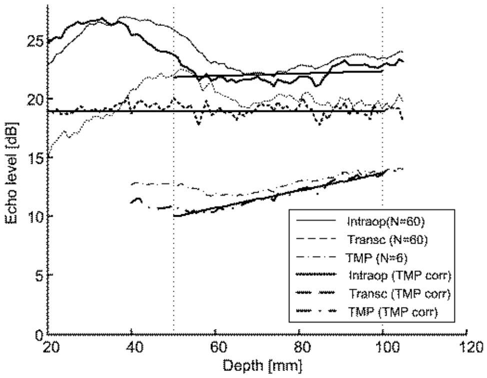

Figure 2. Transc (dashed-dotted line) and Intraop (solid line) normal group, and TMP (dashed line) depth profiles; uncorrected (thin lines) and phantom-based automatic gain compensation (AGC) corrected (thick lines). Linear fits (solid straight lines) through linear part of the AGC corrected depth profiles of region-of-interest (ROI) depth range (50–100 mm).

Area Under the Curve (AUC) as well as the sensitivity and specificity (at the intersection of the negative diagonal and the ROC curve) were estimated.

III. RESULTS / CONCLUSION

The TMP gray level parameters, from the normal Intraop and Transc groups, show almost equivalent values for mean echo level (MU), standard deviation of mean echo level (SD) and signal to noise ratio (SNR) (Table 1) which indicates that the applied corrections for the fat layer is adequately.

Significant UTC parameters correlations with TAG, for both application modes, were found for Mu, SNR and ResAtt (Table 2). The Mutual correlations of these parameters were also found to be statistically significant (Table 2: row 5,6). The ResAtt was found be to the best ($R = 0.82$, $p < 0.001$) correlating parameter with TAG in both applications.

Use of TMP based AGC correction yields similar results as using normal group based AGC correction.

Both in Transc and Intraop application the ResAtt was the only selected parameter in Multiple Stepwise linear Regression analysis. Again the ResAtt was found to be almost identical in Transc and Intraop application ($R= \pm 0.83$). Also identical high predictive performance after normal group based and TMP based automatic gain correction was found in ROC analysis of both Intraop and Transc application (AUC: 0.92;0.95, Sens: 0.87;0.87, Spec: 0.83;0.83).

Equal high predictive values for Transc UTC parameters indicate the effectiveness of the post-processing (fat layer attenuation- and AGC correction) of the Transc images, compared to Intraop UTC parameters.

A TMP may serve as a reference instead of a group of healthy subjects (normal group) since equal predictive performance values for TMP based beam profile correction is achieved.

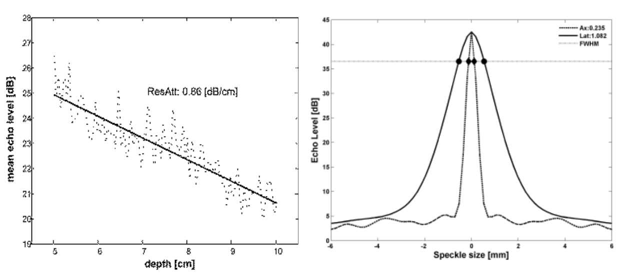

Figure 3. Ultrasound tissue characteristics (UTC) parameter estimation and representation plots. (a) ROI echo level vs. depth profile (dotted data) with linear fit (solid line) which slope represents the ResAtt coefficient. (b) Axial (dashed line) and lateral profile (solid line) of the 2-D ACVF with marked full-width-at-half-maximum (FWHM) (-6 dB) axial and lateral speckle sizes.

TABLE I. Descriptive statistics of data using a tissue mimicking phantom (P) and Control Group (C) for depth correction of US images (N=5), Intraop and Transc (N=12) normal group UTC parameters.

USM /AGC	N	TAG [mg/g FW]	Mu [dB]	SD [dB]	SNR	ResAtt [dB/cm]	Axial [mm]	Lateral [mm]
P/P	5	---	21.8 ± 0.31	3.80 ± 0.04	5.75 ± 0.12	-0.06 ± 0.04	0.48 ± 0.03	1.70 ± 0.07
I/C	12	17.1 ± 8.09	22.6 ± 2.92	3.70 ± 0.20	6.16 ± 0.89	-0.05 ± 0.46	0.42 ± 0.05	1.83 ± 0.08
I/P	12	17.1 ± 8.09	21.9 ± 2.88	3.72 ± 0.20	5.92 ± 0.89	-0.44 ± 0.45	0.42 ± 0.04	1.80 ± 0.08
T/C	12	17.1 ± 8.09	23.3 ± 2.91	3.49 ± 0.35	6.74 ± 0.97	0.09 ± 0.42	0.45 ± 0.04	2.20 ± 0.12
T/P	12	17.1 ± 8.09	19.5 ± 3.11	3.49 ± 0.35	5.65 ± 1.03	-1.05 ± 0.41	0.45 ± 0.03	2.20 ± 0.16

USM = ultrasound application mode; AGC = Automatic Gain Correction type; P = tissue mimicking phantom; I = Intraoperative; T = Transcutaneous; C = control group; TAG = Triacylglyerol; Mu = mean echo level; SD = standard deviation of mean echo level; SNR = signal to noise ratio; Intraop = intraoperative; Transc = transcutaneous; AGC = automatic gain compensation curve

TABLE II. Correlations of Intraop and Transc parameters to TAG, and correlations between Intraop (I) vs. Transc (T) parameters (N=151). C = depth correction with control group, TMP = with tissue mimicking phantom

	AGC	Mu	SD	SNR	Res Att	Ax	Lat
I vs. TAG	C	0.72**	-0.19	0.70**	0.82**	0.19	0.11
I vs. TAG	TMP	0.72**	-0.26*	0.71**	0.82**	0.18	0.03
T vs. TAG	C	0.60**	-0.41**	0.67**	0.81**	0.31**	0.35**
T vs. TAG	TMP	0.62**	-0.43**	0.67**	0.81**	0.29**	0.33**
I vs. T	C	0.46**	0.24*	0.54**	0.80**	0.14	0.05
I vs. T	TMP	0.47**	0.28**	0.54**	0.80**	0.11	0.05

** significant at the $P < 0.002$ level (2-tailed)
* significant at the $P < 0.01$ level (2-tailed)

TABLE III. Training set stepwise multiple linear regression results (N=76) and test set ROC results (N = 75) of TAG vs. TAGpred to a TAG threshold of 50 [mg/g FW]

Trainingset (N=76)					**Testset (N=75)**	**ROC: TG Threshold <>50**		
	R	**R^2**	**Regression formula**	**p**	**TG vs TGpred**	**AUC**	**Spec**	**Sens**
Intraop C	0.82	0.67	40.503 + 70.729*ResAtt	<0.001	0.82**	0.92	0.87	0.81
Intraop TMP	0.82	0.67	60.517 + 71.161*ResAtt	<0.001	0.82**	0.92	0.87	0.83
Transc C	0.84	0.70	25.692 + 79.030*ResAtt	<0.001	0.79**	0.95	0.87	0.83
Transc TMP	0.84	0.70	109.358 + 80.726*ResAtt	<0.001	0.79**	0.95	0.87	0.83

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