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Intraoperative ultrasound during repeat cesarean delivery facilitates sampling of uterine scar tissue

<https://doi.org/10.1515/jpm-2022-0334>

Received July 7, 2022; accepted October 15, 2022;

published online November 18, 2022

Abstract

Objectives: Histological examination of uterine scars provides insight into uterine wound healing and helps to develop prevention methods of uterine wall rupture after previous uterine surgery. Therefore, exact intraoperative scar identification is needed for specimen collection from the actual scar tissue. The aim of this study was to correlate pre- and intraoperative ultrasound measurements of the lower uterine segment (LUS) with histological findings of scar tissue and to evaluate the relevance of intraoperative ultrasound.

Methods: In a prospective observational study, preoperative and intraoperative sonographic measurements of the LUS thickness were performed in 33 women with a history of at least one cesarean delivery. Intraoperative ultrasound

with a linear transducer placed directly on the uterus identified the scar area and uterotomy was performed 2 cm cranially. Tissue samples were taken after extraction of the fetus, embedded in paraffin wax, and stained according to Gomori Trichrome to identify scar tissue. Collagen content was evaluated with imaging software Fiji (NIH, Bethesda, USA). Preoperative and intraoperative sonographic measurements were correlated with histologic evidence of scar tissue.

Results: Histological evidence of scar tissue was found in 11 out of 33 samples with significantly lower ultrasonographic thickness of the lower uterine segment compared to the other 22 samples, both antepartum (1.4 mm [1.3–1.9] vs. 2.0 mm [1.6–2.6], $p=0.03$) and intrapartum (1.6 mm [1.3–1.9] vs. 3.7 mm [2.0–4.7], $p<0.01$). Intraoperative ultrasound had a significantly higher predictive power (AUC difference 0.18 [0.03–0.33], $p=0.01$).

Conclusions: Intraoperative sonography identifies the uterine wall area with histologically confirmable scar tissue far better than preoperative sonography.

Keywords: cesarean delivery; intraoperative ultrasound; scar tissue sampling; uterine rupture; uterine scar.

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Introduction

Uterine rupture represents a medical emergency for mother and child [1]. It is associated with high neonatal morbidity due to the risk of hypoxic-ischemic encephalopathy (6.2%) with low umbilical pH values below 7.0 (33.3%) and neonatal mortality of up to 26.2% [2, 3]. Many studies on clinical factors associated with uterine ruptures have been performed and previous cesarean delivery (CD) constitutes the main risk factor for uterine rupture during subsequent delivery [4, 5]. Ultrasonographic measurements of the lower uterine segment (LUS) thickness during third trimester can help to evaluate the risk of uterine rupture during a trial of labor after CD [6, 7]. According to a meta-analysis by Swift et al. an association between thin lower uterine segment measurement and uterine dehiscence could be shown in 27 studies, an association between thin lower uterine segment measurement and uterine rupture in four studies [6]. Apart from the use of ultrasound screening

to counsel individual women about trials of labor after cesarean delivery (TOLAC), another way of reducing the risk of uterine rupture globally is research on uterine wound healing. The latter could help to find ways of increasing uterine wall stability post-CD to make TOLAC safer for all women. One challenge for wound healing studies of the uterus constitutes the difficulty to localize the scar tissue intraoperatively correctly, so that specimens that are excised during repeat CD for histological analyses do contain scar tissue. So far only few studies have characterized human uterine scars histologically. It seems reasonable to regard tissue with a collagen content of at least one-third as scar tissue. This, of course, cannot be evaluated directly via antenatal ultrasound and the measurement of the LUS thickness is a surrogate marker for a scarred uterine wall. Specimens of the LUS that are examined as uterine scars although they do not contain scar tissue can lead to false negative or inconclusive results. Due to the difficulty of correctly harvesting uterine scar specimens, molecular and cellular factors that influence uterine wound healing and uterine scar stability remain largely unknown [8, 9]. It is therefore still not understood, why some scars rupture while others do not and how uterine wound healing can be improved. Buhimschi et al. investigated biomechanical properties and collagen content of human specimens from the lower uterine segment ($n=68$) [8]. In their study, biomechanical testing of tissue samples did not show different tissue strength between the LUS of women with or without previous CD and quantification of the LUS collagen content yielded inconclusive results. Wu et al. did not find different amounts of collagen in immunohistochemical staining of the lower uterine segment in groups of women with and without prior CD [9]. Both studies do not report an exact intraoperative identification of the uterine scar before tissue sampling. As scars consist largely of collagen [10, 11], these results might reflect that the examined tissue samples of women with prior CD did not contain the full amount of scar tissue. We believe that this fact has greatly hindered uterine scar research from evolving further over time. It is therefore necessary to find ways to minimize the amount of patient recruitment needed for specimen excision and to perform biopsies in a more targeted way than only by intraoperative macroscopic evaluation of the LUS by the surgeon. Screening for patients with a thin LUS by ultrasound either pre- or intraoperatively before specimen excision seems reasonable. The usefulness of intraoperative assessments of the LUS by ultrasound as a reference method during CD has only recently been demonstrated [12]. Furthermore, it has been shown that ultrasound-guided resection of the uterine scar area during repeat cesarean deliveries reduces the scarring rate and leads to a thicker

myometrium as detected by ultrasonography 6–9 months postoperatively [13]. The aim of our study was to correlate pre- and intraoperative ultrasound measurements of the lower uterine segment (LUS) with histological evidence of scar tissue and to compare their diagnostic accuracy. Therefore, the sampling of uterine scar tissue and quantification of collagen in histological sections of uterine wall specimens needed to be established.

Materials and methods

Patient population

Women older than 18 years of age with singleton pregnancies, at least one previous cesarean delivery and a planned CD were enrolled prospectively at Charité – Universitätsklinikum in Berlin, Germany. All women had provided signed informed consent under protocols approved by the Ethics Committee of Charité – Universitätsklinikum Berlin (EA4/159/16). Women were recruited irrespectively of the sonographically observed LUS thickness. The study complies with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects.

Ultrasound examinations

During third trimester antenatal counseling for birth mode after CD, transvaginal and transabdominal ultrasound examinations of the thickness of the lower uterine segment were performed by a maternal-fetal medicine ultrasound expert according to local standards using gray-scale ultrasound imaging (Figure 1). Ultrasound was performed in supine position with filled bladder. The thickness of the uterine wall in the thinnest part of the LUS was measured in millimeters excluding the wall of the urinary bladder. Preoperative examinations were

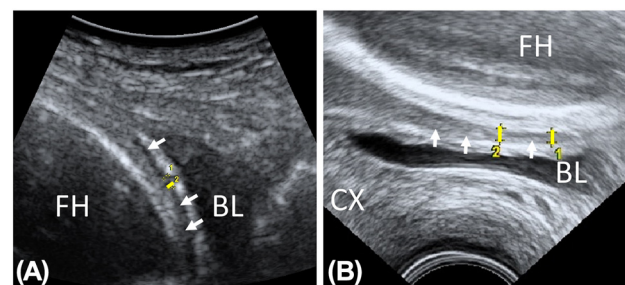


Figure 1: Antepartum sonographic measurements of the lower uterine segment. Exemplary transabdominal (A) and transvaginal (B) images. The thickness of the uterine wall in the thinnest part of the LUS was measured in millimeters excluding the wall of the urinary bladder (yellow marker). Arrows mark the uterine wall. Both images from the “histological positive detection of scar tissue” group. Preoperative examinations were performed using the ultrasonic device Voluson E8 (GE Healthcare, Chicago, USA) with a curvex probe (RAB4-8-D, GE Healthcare, Chicago, USA) and an endocavitary probe (RIC6-12-D, GE Healthcare). BL, urinary bladder; CX, cervix; FH, fetal head.

performed using the ultrasonic device Voluson E8 (GE Healthcare, Chicago, USA) with a curvex probe (RAB4-8-D, GE Healthcare, Chicago, USA) and an endocavitary probe (RIC6-12-D, GE Healthcare). The smallest thickness measured was used for this analysis. Intraoperative ultrasound was performed during planned CD: a linear transducer with a sterile cover (12 L-RS, GE Healthcare) was placed directly on the uterine wall immediately before incision of the uterus (Figure 2A, B). Intraoperative examinations were performed using the ultrasonic device Voluson P6 (GE Healthcare, Chicago, USA) always by the same surgeon blinded to the antenatal Images.

Sampling

Sampling of uterine tissue was performed during elective repeat CD from the contraction-free uterus. After laparotomy, intraoperative ultrasound was performed as described above to identify the thinnest part of the lower uterine segment (Figure 2B), as we hypothesized that this contains the uterine scar based on the findings of Seliger et al. [13]. Next, the uterine incision was performed 2 cm cranially to the thinnest part of the LUS as the area of interest. Immediately after delivery of the infant and before administration of oxytocin and removal of the placenta, a sample of $0.5 \times 4.0 \text{ cm}^2$ was excised with a surgical scissor. To aid orientation of samples during further processing, a yellow dye was applied to the serosal side of the sample and a green dye was applied to the side facing the uterotomy after sampling. Part of the samples were formalin fixed immediately and paraffin-embedded for further analyses.

Gomori trichrome staining to identify collagen fibres

Gomori Trichrome Staining was performed as previously described [14]. Paraffin Sections 5- μm thick were cut and dewaxed in xylene ($2 \times 5 \text{ min}$; J. T. Baker, Radnor, USA) and rehydrated in ethanol (2 min each in 96, 70, 50%; Carl Roth, Karlsruhe, Germany) followed by incubation for 30 min at 56°C in Bouin solution. The sections were then rinsed under running water for 5 min. Incubation in Weigert's iron hematoxylin (Merck, Darmstadt, Germany) for 10 min followed by rinsing under running water for another 10 min. This was followed by incubation in trichrome solution (Sigma-Aldrich, Taufkirchen, Germany) for 25 min. Differentiation was performed in 0.5% acetic acid (Merck, Darmstadt, Germany) for 2 and 1 min followed by dehydration in ethanol (1 min 96%, $2 \times 2 \text{ min}$ 100%) and xylene ($2 \times 5 \text{ min}$). The sections were covered with Entellan® Neu (Merck, Darmstadt, Germany) and a cover glass. As a result of the staining, cytoplasm and erythrocytes are shown in red, fibrin and muscle pink, nuclei blue to black, and collagen fibers green (Figure 2C, D).

Image acquisition and macro-based analysis

Images of the tissue sections were acquired with digital microscopy systems Axioskop and Leica DM6 B and the high-resolution digital color cameras AxioCam MRc 5 (Zeiss, Oberkochen, Germany) and Leica DFC9000 GT (Leica, Wetzlar, Germany), respectively. To produce images of whole tissue sections, the samples were scanned at high magnification ($\times 50$) and processed in mosaic mode with Axiovision 4.8.2 software (Zeiss, Oberkochen, Germany) or Leica Application Suite X (Leica, Wetzlar, Germany). Subsequently, the

area percentage of muscle tissue as well as collagen (the main component of scars [10]) was automatically evaluated by means of a newly developed macro for the image processing software Fiji (NIH, Bethesda, USA), as previously described [15]. Three slides were examined per patient. The macro allows the user to draw a box-shaped region of interest (ROI) into the image of the tissue section. This ROI is subdivided into three parts ("sub-ROIs"). Then, using thresholds for color saturation or manual cutout, empty areas at the edge of the tissue or within a section were excluded from the analysis. Collagen areas around blood vessels were also excluded, as they represent the adventitia and might distort the analysis. Next, the muscle and scar areas were dichotomized through thresholding of the hue, based on the Gomori trichrome staining – muscle cells in pink and fibrous tissue in green (Figure 3). We defined the rectangular ROI with the aim to contain as much of the collagen-rich scarred area of the specimen as possible and to extend beyond the transition area with decreasing content of fibrous tissue to the unscarred myometrium. The ROI was subdivided into three sub-ROIs corresponding to the homogenous scarred area, the unscarred myometrium and the transition area in the middle between scar and myometrium (Figure 2E). At last, the areas occupied by collagenous scar tissue and muscle tissue in each sample were calculated automatically via the number of pixels and output as a percentage. Based on these results, the average collagen content of scarred and non-scarred myometrium was defined in %. The transition area was not part of this analysis. When no regionally differentiated distribution of collagen could be identified in a sample, a single box-shaped ROI was defined with the aim to contain most parts of the sample, excluding the edges of the sample (Figure 2F).

Statistical analysis

A sample size calculation was performed with the software G*Power [16]. Based on photos of histological samples of uterine scars and our own preliminary stainings, we estimated that histological samples of uterine scar tissue contain 50% [standard deviation: ± 25] collagen whereas samples of unscarred myometrium contain 20% [standard deviation: ± 10] collagen [8, 17]. To detect differences between two groups, at least 9 samples with "histological positive detection of scar tissue" and 17 samples "histological negative detection of scar tissue" would be needed ($\alpha=0.05$, power=0.95, two-sided t-test). Data were tested for normality assessing the histogram. Comparisons between groups were performed using the Mann-Whitney U test for independent samples and Wilcoxon signed-rank test for matched samples. Categorical variables were expressed as numbers (percentage) and compared with Fisher's Exact Test. Data are reported as median and interquartile range (IQR). p-Values were not adjusted due to the exploratory nature of the study. The diagnostic powers for the prediction of successful sampling of uterine scars were assessed from the area under the curve (AUC) of receiver operating characteristic (ROC) curves. Cut-off values for the sonographically determined thickness of the uterine wall in the LUS were calculated using Youden's index [18, 19]. SPSS Version 28 (IBM, Chicago, USA) was used for statistical analysis. Figures were prepared with Prism 9 (GraphPad Software, San Diego, USA). In all analyses, two-tailed $p \leq 0.05$ were considered to indicate statistical significance.

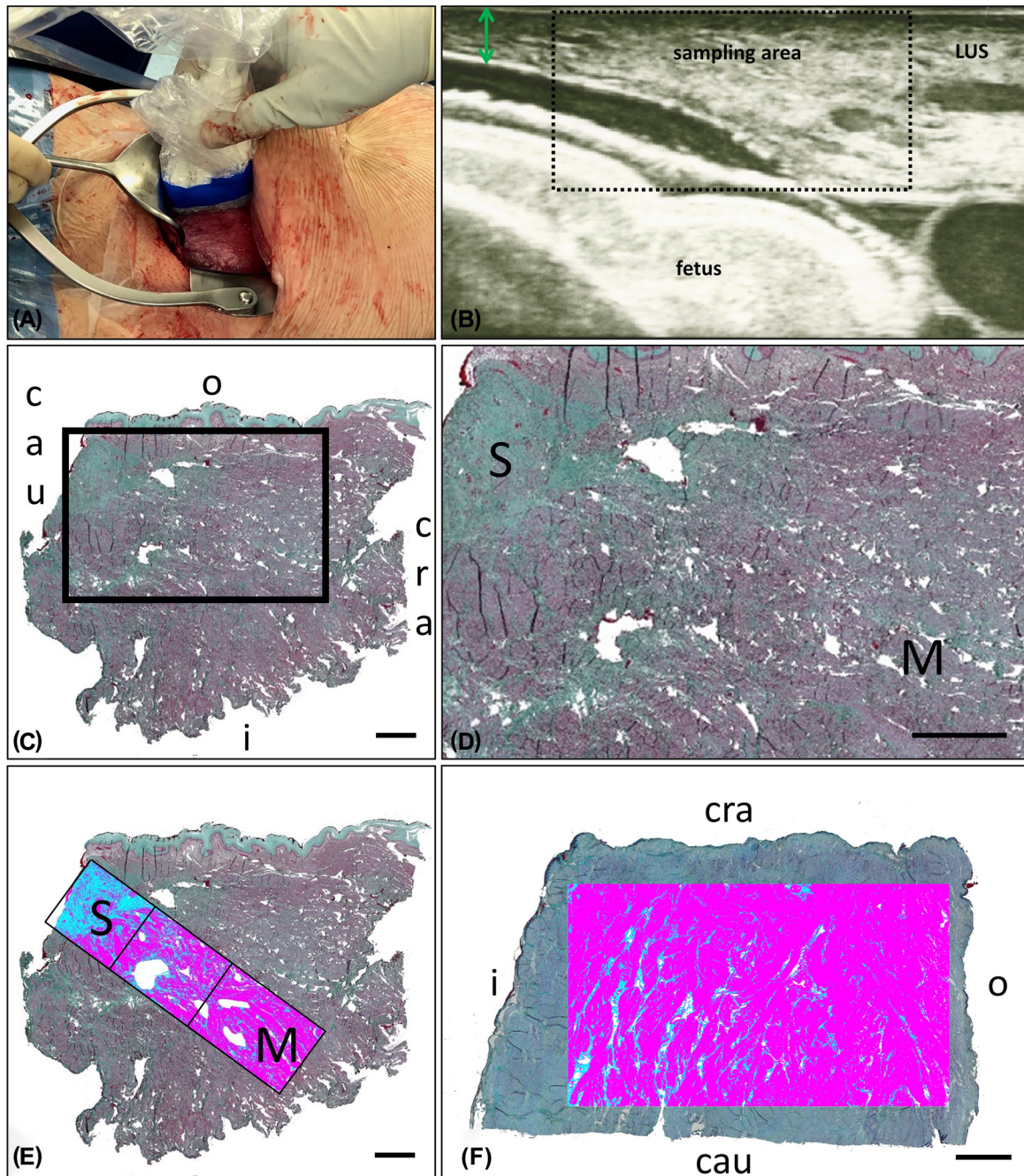


Figure 2: Intraoperative ultrasound and histological staining of the sampled tissue. (A) The ultrasound probe is placed sagittally on the anterior wall of the uterus before uterotomy. (B) Exemplary view of the intraoperative ultrasound image. The green arrow marks the part of the uterine wall, where the thickness was measured. The box shows the area of tissue sampling. (C) Histological section of the harvested tissue stained according to Gomori Trichrome. Staining shows muscle cells in pink and collagen fibers in green. (D) Magnification of the area outlined in (C), clearly showing the transition from unscarred myometrium ("M", left side of image) to scar tissue ("S", right side of image). (E) Exemplary region of interest (ROI) as defined in the software program Fiji. Muscle tissue is shown in pink and scarred tissue in blue. The sub-ROI corresponding to the scarred area is denoted as "S", the area of unscarred myometrium is denoted as "M". (F) Histological section of a tissue specimen where no scar tissue can be identified, therefore only a single ROI containing most parts of the sample was defined. Muscle tissue is shown in pink and scarred tissue in blue. LUS, lower uterine segment; O, surface of the sample facing the outer (serosal) side of the LUS; i, surface of the sample facing the inner (endometrial) side of the LUS, cra, cranial side; cau, caudal side. Scale bars=2 mm.

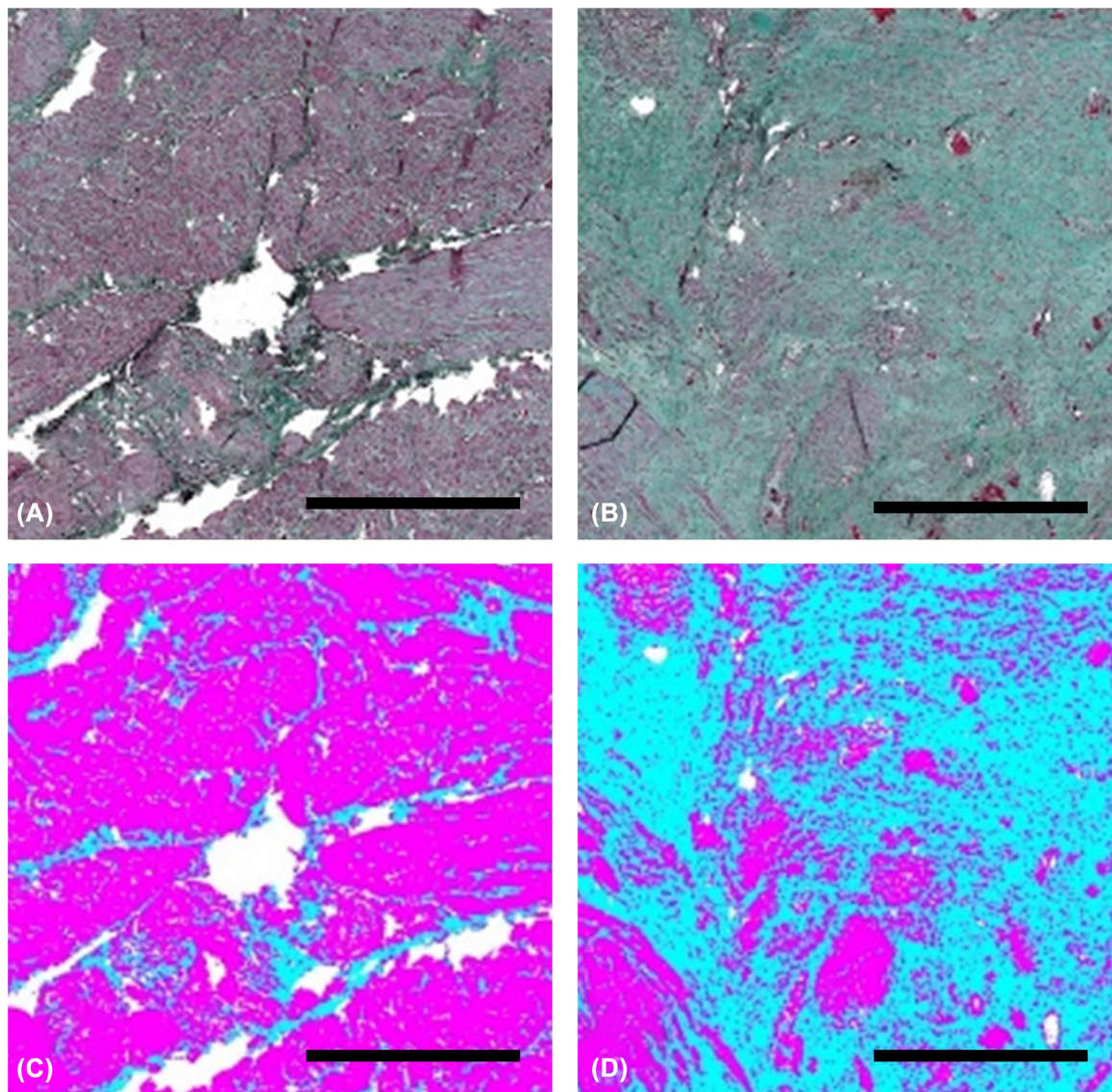


Figure 3: Evaluation of histological staining with the software Fiji (NIH, Bethesda, USA) [1]. (A) Exemplary area of unscarred myometrium, (B) Exemplary area of scar tissue, (C) Dichotomized image created with Fiji of the section shown in (A). Muscle tissue is shown in pink, scar tissue in blue. (D) Dichotomized image created with Fiji of the section shown in (B). Muscle tissue is shown in pink, scar tissue in blue. Scale bars=1 mm.

Results

Sonographic measurements of the LUS thickness

The median LUS thickness of the 33 samples was 1.8 mm (1.4–2.1) preoperatively and 2.2 mm (1.5–4.4) intraoperatively ($p < 0.01$), independent of the histological classification described in the next section. Preoperatively, the LUS thickness was 1.6 mm (1.3–2.1) in transvaginal ultrasound ($n=15$) and 1.9 mm (1.3–2.3) in transabdominal ultrasound

($n=18$) ($p=0.56$). Intraoperatively, two different types of LUS were seen: while some lower uterine segments got gradually thinner towards the uterine scar (Figure 4A, B), others had an edge-like transition to the scar (Figure 4C, D).

Histological scar tissue detection and collagen content measurements

22 samples contained a homogenous distribution of myometrial cells with little collagen in between (Figure 2F). In these samples, no regionally differentiated distribution of

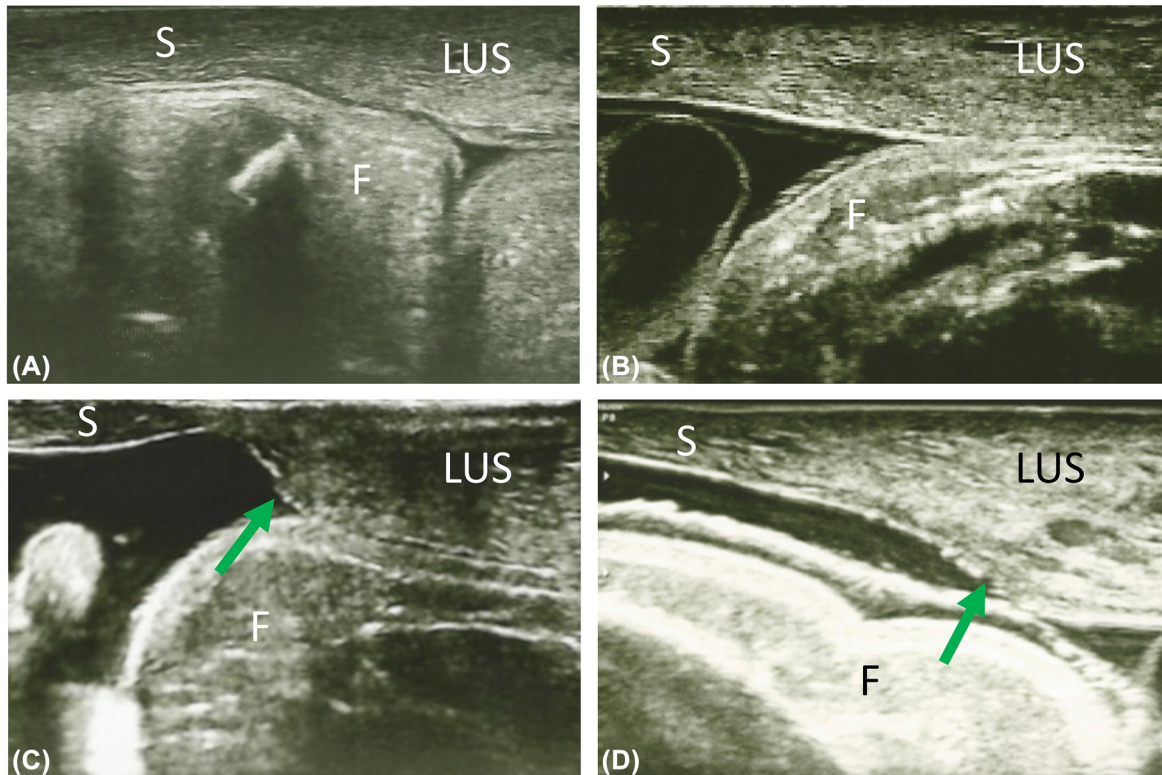


Figure 4: Example intraoperative images of the lower uterine segment. While some lower uterine segments get gradually thinner towards the uterine scar (A, B), others have an edge-like transition to the scar (C, D). The arrows mark the edge between the scarred LUS on the left and the unscarred uterine wall on the right. The transducer was placed sagittally on the uterus, the left side of the images corresponds to the caudal end of the transducer. F, fetus; LUS, lower uterine segment; S, uterine scar.

collagen could be identified. They were therefore assigned to the group called “Histological negative detection of scar tissue”. In the other eleven samples, a region with a high share of collagen in comparison to the rest of the myometrial tissue could be seen. This region constituted part of the total sample and was thus identified as uterine scar tissue. The remaining areas were deemed as unscarred myometrium (Figure 2C, D). These eleven samples were assigned to the group called “Histological positive detection of scar tissue”, defined by a collagen content of more than 33%. When a scar area could be identified, collagen content of uterine wall specimens was 79.0% (61.3–83.6) (Figure 5) with significantly less collagen in the unscarred myometrium (10.0% [8.3–12.4], $p < 0.01$). The 22 samples in which no scar tissue could be identified contained only 11.3% (10.0–13.8) collagen, significantly less collagen compared to the scar area ($p < 0.001$) from the first group. The inter-assay coefficient of variation was 12.3%.

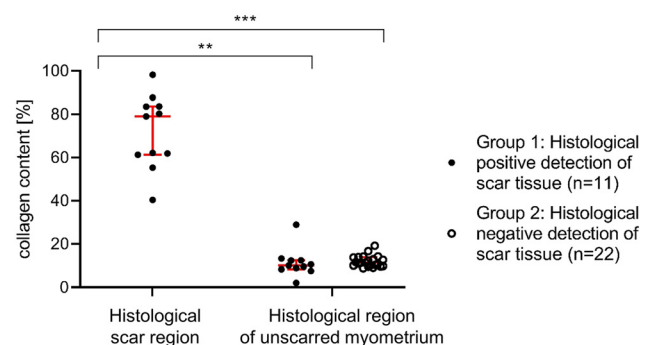


Figure 5: Collagen content of uterine wall specimens as determined with imaging software Fiji to analyze tissue sections stained with Gomori Trichrome. Histological scar areas in group 1 contained 63.4% (48.8–72.0) collagen. The unscarred myometrium of the same samples contained 13.0% (6.6–17.2) collagen. The 22 samples from group 2 contained 5.6% (3.0–8.8) collagen and no histological scar region. Data presented as median $\pm$ interquartile range. **, p -value < 0.01 ; ***, p -value < 0.001 .

Clinical characteristics of women

Demographic data of women in both groups is shown in Table 1. There were no significant differences between the groups in terms of clinical characteristics.

Correlation of the ultrasonographic LUS thickness and the histological detection of uterine scar tissue

The ultrasonographic LUS thickness differed significantly between the two “histological” study groups both antepartum (1.4 mm [1.3–1.9] vs. 2.0 mm [1.6–2.6], $p=0.03$) and intrapartum (1.6 mm [1.3–1.9] vs. 3.7 mm [2.0–4.7], $p<0.01$) (Figure 6A). Women in group “Histological positive detection of scar tissue” had similarly thin LUS in pre- and intraoperative sonography (median: 1.4 mm [1.3–1.9] vs. 1.6 mm [1.3–1.9], $p=0.72$). In the 22 women in group “Histological negative detection of scar tissue”, the LUS was significantly thicker in the intraoperative measurement than in the preoperative measurement (3.7 mm [2.0–4.7] vs. 2.0 mm [1.6–2.6], $p<0.01$). Optimal thresholds for successful harvest of scar tissue were <1.95 mm preoperatively (sensitivity: 90.1%, specificity: 54.5%) and <2.1 mm intraoperatively (sensitivity: 100.0%, specificity: 77.3%) (Table 2). ROC curve analysis showed that both screening methods significantly improve detection of scar tissue with a significantly higher predictive power of intraoperative ultrasound (AUC difference 0.18 [0.03–0.33], $p=0.01$) (Figure 6B). An edge-like transition to the scar was noted in 81.8% (9/11) of women in the “Histological positive detection of scar tissue” group and in 22.7% (5/22) of women in the “Histological negative detection of scar tissue” group (sensitivity: 81.8%, specificity: 77.3%).

Discussion

We have successfully established the sampling of uterine scar tissue and quantification of collagen in histological sections of uterine wall specimens. The study has shown that the thickness of the LUS as determined with ultrasound, correlates with the histological proof of uterine scar tissue. Intraoperative ultrasound with a linear transducer placed directly on the uterus before uterotomy was more accurate than preoperative transabdominal and transvaginal ultrasound in identifying lower uterine segments of patients, where samples taken during planned cesarean deliveries in fact contain scar tissue in histological specimens. Inclusion of patients with an intraoperatively measured LUS of less than 2.1 mm proved to be the most efficient screening method for successful sampling of uterine scar tissue. This is an essential finding, as it is the first description of targeted sampling of uterine scars. The difficulty of identifying uterine scar tissue for laboratory analyses has obviously hindered basic research on human uterine scars, with only very few existing studies on the subject [8, 9, 20].

During the course of this study of human uterine scar tissue (QUWACS study – ‘Quantifying Uterine Wound Healing after Cesarean Section’), we have experienced that without ultrasound screening, only one in four samples taken from women with prior CD shows uterine scar tissue. Apart from clinical research, some research groups have performed promising studies of uterine scars in animal models. Two studies on the regeneration of uterine wounds in rats used immunohistochemical staining of von Willebrand factor to quantify neoangiogenesis during the healing process. Intraoperative application of collagen-binding vascular endothelial growth factor, collagen fibers and umbilical cord stem cells each resulted in increased

Table 1: Demographic data of the study population.

Variable	Histological positive detection of scar tissue (n=11)	Histological negative detection of scar tissue (n=22)	p-Value
Maternal age, years ^a	35 (28–39)	33 (30–38)	1.0
BMI ^a	28.3 (20.8–39.1)	24.7 (20.7–30.9)	0.34
Gravidity ^a	4 (3–5)	3 (2–4)	0.19
Parity ^a	3 (1–3)	1 (1–2)	0.10
Number of previous CD ^b			0.12
One	5 (46)	17 (77)	
Two or more	6 (54)	5 (23)	
Years since last CD ^a	4 (2–6)	4 (2–4.5)	0.64
Gestational age at antepartum ultrasound, weeks ^a	36 (36–37)	36 (36–37)	0.96
Gestational age at CD, weeks ^a	39 (39–39)	39 (39–39)	0.87

^aData presented as median (IQR) and analyzed with Mann-Whitney U test; ^bData presented as n (%) and analyzed with Fisher’s exact test; BMI, body mass index; CD, cesarean delivery.

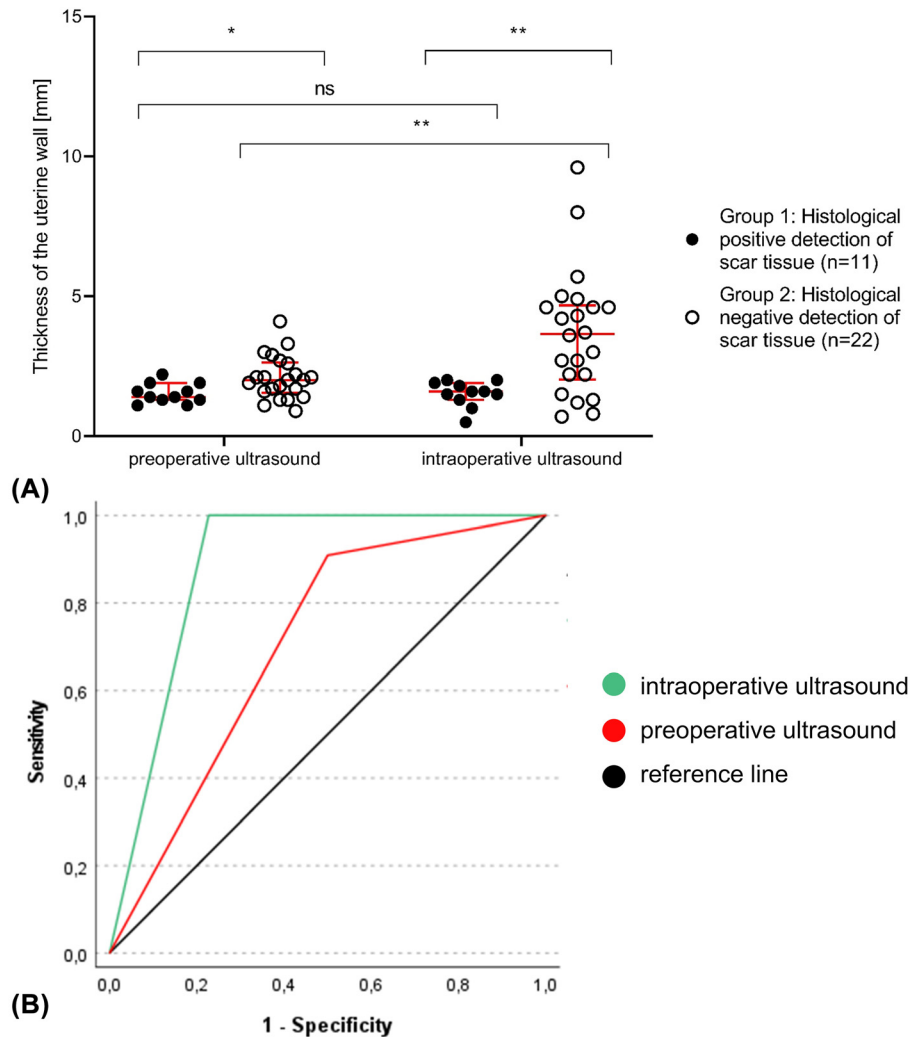


Figure 6: Correlation of the ultrasonographic LUS thickness and the histological detection of uterine scar tissue. (A) Sonographic thickness of the uterine wall in both study groups in pre- and intraoperative ultrasound (median $\pm$ interquartile range). (B) ROC curves showing the predictive power of preoperative (red line, cutoff at 1.95 mm, AUC=0.71 [0.57–0.84]) and intraoperative ultrasound (green line, cutoff at 2.1 mm, AUC=0.89 [0.80–0.98]) on the outcome of histopathologically confirmed scar tissue. Areas under the ROC curves were significantly different (AUC difference 0.18 [0.03–0.33], $p=0.01$). *, p -value < 0.05; **, p -value < 0.01; ns, not significant.

Table 2: Sensitivity, specificity, likelihood ratios and area under the curve (AUC) values of determined cut-off levels for the thickness of the lower uterine segment as a predictor of histologically confirmable scar tissue in preoperative and intraoperative ultrasound.

	Cut-off	Sensitivity	Specificity	LR–	LR+	AUC (95%CI)	p-Values
Preoperative ultrasound	1.95 mm	90.1%	54.5%	0.5	5.5	0.71 (0.57–0.84)	<0.01
Intraoperative ultrasound	2.10 mm	100.0%	77.3%	0.2	∞	0.89 (0.80–0.98)	<0.001

AUC, area under the curve; LR–, negative likelihood ratio; LR+, positive likelihood ratio. Statistically significant p -values (<0.05) are marked as bold text.

blood vessel density in the uterine scar area two months after uterine trauma [21, 22]. In a study on mice with genetically different regeneration characteristics delivered by CD, uterine scar tissue was examined three days and two months after CD. Hematoxylin & eosin as well as Picrosirius red staining successfully revealed histological differences in wound healing, inflammatory response and collagen organization [23]. However, before we can find out if any of these findings can be translated to the human species, we need a method to successfully sample and

investigate specimens from human uterine scars. Our results show that it is essential to seek out cesarean scars for histological studies by intraoperative sonography to minimize the number of subjects without scar tissue in the harvested specimen. The finding that intraoperative ultrasound has a higher predictive power than preoperative ultrasound is in line with the findings of Seliger et al., who have already described the benefits of intraoperative ultrasound measurements of the LUS: direct access to the measuring object, quantifiable measured values and

the absence of pressure by fetal head or pelvis on the LUS due to supine position of the patient and the relaxed state without contractions [12]. The predictive power of the intraoperative display of the LUS – gradual thinning towards the uterine scar vs. an edge-like transition to the scar – is lower than the power of the LUS thickness, but a noteworthy aspect of uterine scars. Interestingly, two thirds of the samples did not show scar tissue, while Osser et al. described only 22% of cesarean scars as non-defective in saline contrast sonohysterography of the non-pregnant uterus at 6–9 months after the last delivery. The reason for this discrepancy could be that the LUS thickness on post-partum ultrasound is primarily influenced by the degree of intraoperative approximation of the wound edges whereas the degree of uterine scarring and (defined by excessive collagen formation) is not reflected in ultrasound. As shown in previous studies, uterine wound healing is a multifactorial process involving a complex cascade of biochemical events [24].

The study has some limitations. We did not have information on whether previous CD were performed at advanced cervical dilatation and whether single- or double-layer closure had been used. These factors may have an impact on lower uterine thickness in subsequent pregnancies. Both women with one and two or more previous CD were included in the study. However, the number of previous CD was similar in both study groups ($p=0.12$). Furthermore, one of the study groups is made up of women with a histologically negative detection of scar tissue. It is imaginable that the uteri of some of these patients healed so neatly that they only contain very little scar tissue, which would make it more difficult to detect. To date, no studies exist on this subject. However, it might be reasonable to argue that women in whom a previous CD does not leave an identifiable scar do not carry an increased risk of uterine rupture and are thus not the right subjects for studies of defective uterine wound healing. The strength of the study lies in the fact that for the first time, human uterine scar tissue was identified histologically using a quantitative approach, which is both objective and reliable. Furthermore, the same experienced surgeon excised all specimens, so that there was no inter operator variability which might confound the results.

Future research projects might investigate whether intraoperative ultrasound during CD can even help to perform ultrasound-guided specimen excision, i.e., by correlating ultrasound images with histological sections in distinct parts of the LUS. Apart from measuring the LUS thickness, it might also be interesting to evaluate the predictive power of techniques such as elastography, which

has already been shown to correlate with viscoelastic properties of the LUS in women with a previous CD [25]. Moreover, further studies should quantify other aspects of wound healing apart from the amount of collagenous tissue, such as growth factor expression or myofibroblast activity in uterine scars [20, 26]. Such approaches could also be applied in trials that test the effect of different uterotomy closure techniques on uterine scarring [27]. Furthermore, it is time for translational studies to test whether the insights gained through animal models can be transferred to human uterine wound healing.

In conclusion, histological examination of uterine scars can help to understand uterine wound healing and develop prevention methods of uterine rupture. Use of the optimal screening technique before the excision of uterine scar specimens can help to minimize the amount of patient recruitment needed. Consequently, histological studies of human uterine scars post-CD will become more feasible, hopefully aiding to find ways of influencing uterine wound healing positively. This approach represents a complementary path to the prevention of uterine ruptures as opposed to the recommendation of elective repeat CD. The findings of this study can help to inform future studies on how to correctly identify uterine scars intraoperatively and perform targeted specimen collection with optimized sensitivity and specificity.

Acknowledgments: We thank Janine Wiebach from the Institute of Biometry and Clinical Epidemiology at Charité Universitätsmedizin Berlin for statistical counseling and Dr. Rebecca C. Rancourt for language editing and proof-reading. A linear transducer for intraoperative measurements was provided on loan for the study by GE Healthcare (Chicago, Illinois, USA). We are also indebted to all the staff of our Department of Obstetrics.

Research funding: The study was funded by the Deutsche Forschungsgemeinschaft Research Grants Program (grant numbers: SCHW 1946/2-1 and BR 2925/11-1) and by the university research fund of Charité – Universitätsmedizin Berlin (grant number: 51517172–01).

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: Research complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013) and has been approved by the authors'

Institutional Review Board (Charité – Universitätsklinikum Berlin, Berlin, Germany; EA4/159/16).

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