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PRospective Imaging of CErvical cancer and neoadjuvant treatment (PRICE) study: role of ultrasound to assess residual tumor in locally advanced cervical cancer patients undergoing chemoradiation and radical surgery

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KEYWORDS: cervical cancer; chemoradiation; ultrasound

ABSTRACT

Objective *To determine the diagnostic performance of two-dimensional (2D) ultrasound parameters, three-dimensional (3D) power Doppler and contrast-enhanced indices in detecting residual disease in locally advanced cervical cancer patients triaged to neoadjuvant treatment followed by radical surgery.*

Methods *Between October 2010 and June 2014, we screened 108 women with histologically documented locally advanced cervical cancer Stage IB2–IVA, of whom 88 were included in the final analysis. 2D ultrasound parameters, 3D power Doppler and contrast-ultrasound parameters were assessed 5 weeks after the end of neoadjuvant chemoradiation therapy. The pathological response was defined as complete (absence of any residual tumor after treatment) or partial (including microscopic and/or macroscopic residual tumor at pathology examination). The two response groups were compared and receiver–operating characteristics (ROC) curves generated to determine the best cut-off value of sonographic tumor diameter to predict residual disease. Histology was considered as reference.*

Results *Complete pathological response to chemoradiation was observed in 40 (45.5%) patients and partial response in 48 (54.5%). The presence of residual disease, as confirmed at pathology examination, was detected by 2D grayscale ultrasound with a sensitivity of 64.6% and specificity of 65%. Color Doppler examination in the cases with lesions visualized on grayscale imaging detected the presence of residual disease, confirmed at pathology, with a sensitivity of 87.1% and specificity of 21.4%. The best area under the ROC curve (0.817) was for the detection of pathological residual disease of at least 6 mm in diameter, using a cut-off value of 12 mm for the largest tumor diameter assessed using 2D grayscale ultrasound (sensitivity, 95%; specificity, 70.6%). Neither 3D vascular indices nor contrast-ultrasound parameters obtained for lesions suspected at ultrasound following chemoradiation differed significantly between patients with histological complete and those with partial response.*

Conclusions *Our results show that grayscale and color Doppler ultrasound have a low level of diagnostic performance in detecting residual disease after neoadjuvant chemoradiation in patients with locally advanced cervical cancer. The best performance was achieved in detection of macroscopic (≥ 6 mm) residual disease. Copyright © 2018 ISUOG. Published by John Wiley & Sons Ltd.*

INTRODUCTION

Chemoradiation therapy has been the standard treatment for locally advanced cervical cancer (LACC) since 1999[1,2]. However, surgical intervention following neoadjuvant chemoradiation has been used increasingly in the management of LACC in many centers, showing

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encouraging results in terms of clinical outcome[3–6]. Favorable clinical response to neoadjuvant treatment allows less radical surgical intervention, thus reducing the risk of severe postoperative morbidity[7–9]. The availability of imaging techniques able to define accurately any residual tumor following chemoradiation would be clinically relevant, allowing selection of patients who could be spared, or at least offered more tailored, surgery.

There are very few data[10,11] available regarding the diagnostic performance of transvaginal ultrasound in detecting pathologically confirmed residual disease after neoadjuvant treatment in LACC patients. While three-dimensional (3D) power Doppler and contrast-enhanced ultrasound have been investigated in this setting as potential markers for predicting both clinical and histological responses[12–14], their application in clinical practice is still unclear, and, to the best of our knowledge, there have been no large prospective studies evaluating their role in this context.

To this end, we performed a prospective study (PRICE: PRospective Imaging of CErvical cancer and neoadjuvant treatment) aimed at analyzing the predictive ability of each of the most important diagnostic tools, i.e. ultrasound, positron-emission tomography/computed tomography (PET/CT) and magnetic resonance imaging (MRI), and their complementary roles, before treatment ('baseline' evaluation), as well as after 2 weeks of ('early' evaluation) and 5 weeks after the end of ('final' evaluation) neoadjuvant chemoradiation prior to surgery, in a large, single-institution series of LACC patients. In our recent publication[15], we assessed the performance of baseline and early ultrasound examinations in discriminating patients with pathological partial and complete response after chemoradiation. Despite documentation of morphological and vascular differences between these two groups of patients, ultrasound and vascular indices failed to achieve sufficient accuracy in the prediction of pathological response.

In the current study, we report the diagnostic performance of two-dimensional (2D) ultrasound, 3D power Doppler and contrast-enhanced ultrasound indices, assessed at the final examination 5 weeks after the end of neoadjuvant treatment, in terms of detection of residual disease in LACC patients triaged to neoadjuvant chemoradiation followed by radical surgery.

SUBJECTS AND METHODS

Study protocol

The original PRICE study protocol encompassed three assessment sessions of each patient, including transvaginal ultrasound examination, MRI and PET/CT scan performed at each of the three timepoints. For each session, the planned time interval between the three imaging modalities did not exceed 1 week.

In the present study, we present the results for the performance of 2D ultrasound, 3D power Doppler and contrast-enhanced ultrasound, at final examination, in the evaluation of residual disease after chemoradiation. Specifically, we planned to analyze the patients grouped according to whether they had absence of residual disease (complete response) or presence of any residual disease (partial response), determined using histology as the reference standard.

Work-up and eligibility

Between October 2010 and June 2014, patients with histologically documented cervical cancer (any histology) and Stage IB2–IVA disease, according to Federation Internationale de Gynécologie et d' Obstétrique (FIGO: International Federation of Gynaecology and Obstetrics) classification[16], were enrolled prospectively at the Gynecologic Oncology Unit of the Catholic University in Rome, Italy. Other inclusion criteria included age 18–75 years, Eastern Cooperative Oncology Group performance status of 0–1, adequate bone marrow function (white blood cell count > 3000 cells/mm^3, platelet count $> 120\,000$ cells/mm^3), adequate renal function (blood urea nitrogen < 25 mg/dL, creatinine < 1.5 mg/dL) and normal liver function (bilirubin < 2 mg/dL). Exclusion criteria were previous or concurrent malignancy at other sites, with the exception of basal or squamous cell carcinoma of the skin, and uncontrolled severe infection and/or medical problems unrelated to malignancy which would limit full compliance with the study. The trial was approved by the local ethics committee and Institutional Review Board, and all patients gave written informed consent agreeing to undergo all the procedures described and for their data to be collected.

Pretreatment work-up included clinical examination, pelvic and rectovaginal examination under anesthesia, chest radiography, complete blood count and measurement of liver and renal function. Transabdominal and transvaginal ultrasound examination, abdominopelvic MRI and PET/CT scan were carried out in order to exclude cases with distant sites of disease; cystoscopy and proctoscopy were performed only in case of clinical suspicion of bladder and/or rectal invasion. After completion of the staging work-up, all imaging examinations were discussed by the tumor board (including gynecological oncologists, ultrasound examiners, radiologists, nuclear physicians, radiotherapists and oncological surgeons) and compared with the findings of the clinical examination, and a consensus reached on the FIGO staging for each patient. In case of disagreement regarding parametrial infiltration, MRI was considered superior to clinical examination and transvaginal ultrasound examination. In case of disagreement regarding metastatic lymph nodes, PET/CT scan was considered superior to MRI and transvaginal ultrasound.

Chemoradiation therapy

Neoadjuvant chemoradiation therapy included conformal irradiation of the pelvic lymph node drainage, bulky tumor, parametria and upper part of the vagina, with a

total dose of 39.6 Gy (1.8 cGy/fraction, 22 fractions), plus additional irradiation of primary tumor and parametria with 10.8 Gy administered with concomitant boosts (0.9 cGy/fraction, 12 fractions every other day)[17]. Concomitant chemotherapy included cisplatin (20 mg/m^2, 2-h intravenous infusion) during the first and last 4 days of treatment and capecitabine (1300 mg/m^2/day, orally) during the first 2 weeks and last 2 weeks of treatment.

Ultrasound examination

All ultrasound examinations were performed by the same examiner (A.C.T.), skilled in gynecological oncology and with more than 15 years of experience in gynecological ultrasound. Patients were examined in the lithotomy position with an empty bladder. A transabdominal scan was performed in order to detect iliac and para-aortic lymph nodes as well as hydronephrosis. A predefined transvaginal examination protocol was carried out for each patient, including 2D grayscale and power/color Doppler examination, 3D grayscale and power Doppler evaluation of cervical tumor volumes, and contrast-enhanced ultrasound examination with infusion of SonoVue contrast agent (Bracco Imaging SpA, Milan, Italy). All 2D and 3D scans were performed using a GE Voluson E8 (GE Medical Systems, Zipf, Austria) ultrasound machine equipped with a multifrequency 5–9-MHz endovaginal transducer and a 3- or 3.5–5-MHz convex transducer for transabdominal ultrasound examination. Contrast-enhanced ultrasound examinations were performed using a MyLab 70 XVG (Esaote, Genova, Italy) ultrasound machine, with a 5–9-MHz transducer equipped with dedicated software for ultrasound contrast.

A previously described standardized examination technique was used in all patients to assess the tumor and the extent of the disease[15]. Briefly, the uterine cervix was examined carefully to identify the presence of lesions suspicious for residual tumor; in the event of any being identified, the residual tumor was measured in three dimensions (the anteroposterior and craniocaudal diameters were obtained in the sagittal plane and the laterolateral diameter in the transverse plane of the cervix). The maximum diameters were used to calculate the tumor volume by the ellipsoid formula (anteroposterior × craniocaudal × laterolateral diameter × π/6). The examiner assessed the echogenicity of the residual tumor and estimated the parametrial infiltration during the real-time 2D ultrasound examination, as described previously[18,19]. To assess intratumoral blood flow, power Doppler settings were set to achieve maximum sensitivity to detect low-velocity flow without noise (frequency, 5 MHz; power Doppler gain, 0.8–2.0; pulse repetition frequency, 0.6 KHz). A subjective semiquantitative assessment of the amount of detectable blood flow was made using the color score described by Timmerman *et al.*[20] (1 = no vascularization; 2 = minimal vascularization; 3 = moderate vascularization; 4 = high vascularization).

3D grayscale ultrasound volumes were acquired using the maximum volume box (120°) and an image angle large enough to include the uterine cervix, bladder and rectum. The lowest sweep velocity was used to obtain maximum image quality. 3D power Doppler volumes were acquired using a volume box of 120°, a pulse repetition frequency of 0.6 KHz and an image angle including the cervix and pericervical tissues. With the patient remaining as still as possible, the volume was acquired over a time period that varied from 10 to 20 s. Volume acquisition was repeated if there were flash-type artifacts because of respiratory or bowel movements.

The volumes were stored and evaluated offline. Using 4DView software (GE Medical Systems) and Virtual Organ Computer-aided AnaLysis (VOCAL™) with the rotational technique and a 15° step, the cervical tumor was outlined in Plane A and the 3D residual tumor volume was calculated. 3D power Doppler indices (vascularization index (VI), flow index (FI) and vascularization flow index (VFI)) were calculated automatically. VI (expressed as percentage) reflects the number of vessels within the volume of interest. FI (expressed as a number ranging from 0 to 100) is the average color value of all color voxels, representing the average color intensity. VFI (expressed as a number ranging from 0 to 100) is a simple mathematical relationship, derived by multiplying VI × FI and dividing the result by 100, and reflects both blood flow and vascularization[21,22]. All 3D tumor volumes were analyzed by a single operator (M.C.M.) with more than 10 years of experience in gynecological ultrasound.

After 2D and 3D ultrasound examinations, a contrast-enhanced ultrasound examination was performed using CnTI™ (contrast-tuned imaging) technology (Esaote) integral to the transvaginal probe and with the ultrasound contrast agent SonoVue, as described previously[15]. The bolus model considering the wash-in/wash-out kinetics was used for the analysis. Perfusion parameters, such as wash-in rate (sharpness of the signal intensity), peak enhancement (maximum signal intensity during the transit of contrast), rise time (time that a contrast echo signal takes to go from baseline level to peak enhancement) and area under the time–intensity curves during wash-in and wash-out were calculated in a specific region of interest corresponding to the residual tumor detected within the cervix and to the whole cervix. All regions of interest were identified and analyzed by a single operator (T.P.), using the software package VueBox® (vuebox.bracco.ch/php/Support.php, Bracco Imaging SpA).

Assessment of clinical response and surgical procedures

Response to treatment was evaluated objectively 4–6 weeks after completion of chemoradiation according to Response Evaluation Criteria for Solid Tumors (RECIST) criteria[23]. Patients achieving response to treatment underwent radical hysterectomy according to the classification of Querleu and Morrow[24], and pelvic, with

or without aortic, lymphadenectomy within 6–8 weeks from completion of chemoradiation. Aortic lymphadenectomy was performed in case of: (1) positive pelvic lymph nodes on frozen-section analysis, routinely performed during surgery; (2) positive pelvic lymph nodes on imaging at initial staging work-up; and (3) suspicious aortic lymph nodes assessed intraoperatively. Patients who experienced no change or who experienced progression of the disease were treated with salvage chemotherapy.

Histopathological evaluation

All imaging methods were evaluated using histology as the reference standard. Histopathological evaluation corresponded to the standard procedures performed in our institution: the cervix was sectioned clockwise in at least 12 blocks, and embedded entirely in paraffin. From each block, two slides, 3- to 4-μm thick, were cut at different levels and stained with hematoxylin and eosin. When appropriate, additional sections were prepared for specific immunohistochemistry tests[25]. The residual disease at any site was expressed as maximum diameter in millimeters, and response was defined as complete (absence of any residual tumor after treatment at any site level (pR0)) or partial (either microscopic (persistent tumor foci < 3 mm in maximum dimension (pR1) or macroscopic (persistent tumor foci ≥ 3 mm in maximum dimension (pR2)) residual tumor at histopathological examination[25].

Statistical analysis

On the basis of Simon's design, the required sample size was calculated to be 86 patients, as previously reported[15]. Results are presented as percentage for nominal variables and as median (range) for continuous variables. Comparisons between the two groups were made with the Mann–Whitney *U*-sum test for continuous variables and χ^2-test for nominal variables, as appropriate.

The diagnostic power of ultrasound in the detection of no residual disease was assessed against the outcome according to histology as reference; standard measures of sensitivity, specificity, overall accuracy and likelihood ratio were estimated along with their 95% CIs.

Receiver–operating characteristics (ROC) curves were generated for tumor diameter at ultrasound examination, to evaluate its diagnostic ability to predict different sizes of residual tumor, as confirmed at pathology. The ROC curves were also used to determine mathematically the best cut-off value of ultrasound tumor diameter to predict residual disease, defined as that corresponding to the point on the ROC curve situated farthest from the reference line. For each diagnostic test and for each corresponding best cut-off value, the accuracy, sensitivity, specificity, positive and negative likelihood ratios, and the numbers of true-positive, true-negative, false-positive and false-negative values were also calculated.

Statistical calculations were performed using the Statistical Package for the Social Sciences software (SPSS version 20.0, SPSS Statistics, IBM Corp., Armonk, NY, USA) except for the ROC curve analysis, which was assessed using pROC package in R software (R version 3.3.1). Two-sided tests were used and the significance level was set at $P < 0.05$.

RESULTS

Clinical and pathological results

Figure S1 shows the consolidated standards for reporting trial (CONSORT) diagram for our study population: between October 2010 and June 2014, we screened for enrolment 108 patients; of them, 16 declined early evaluation and two died of disease, leaving 90 patients who were able to complete all planned imaging evaluations as well as chemoradiation. Two patients experienced progression of disease a few weeks after chemoradiation, so 88 patients were triaged to radical surgery, thus fulfilling the criteria for analysis.

Clinical and pathological features of the whole study population as well as for partial- and complete-response subgroups are summarized in Table S1. The median age was 49.5 (range, 22–75) years. With respect to FIGO staging, 3.4% (3/88) of patients were Stage IB2, 10.2% (9/88) were Stage IIA, 71.6% (63/88) were Stage IIB, 4.5% (4/88) were Stage IIIA and 10.2% (9/88) were Stage IIIB. At staging work-up, pelvic lymph-node involvement was documented in 40 (45.5%) patients. There was no statistically significant difference in the distribution of clinical or pathological features between the partial- and the complete-response groups, with the exception of the grade of differentiation: there was a significantly higher prevalence of undifferentiated tumors (G3) observed in the partial-response group compared with the complete-response group ($P = 0.026$).

Diagnostic performance of 2D grayscale ultrasound and color Doppler in discriminating between absence and presence of disease

As shown in Figure 1, the final grayscale ultrasound examination identified 45/88 (51.1%) cases with a residual tumor mass in the cervix: 31 (68.9%) of these 45 cases actually had residual disease confirmed at pathology (true positives), whereas 14 (31.1%) of the 45 in fact had no residual disease (false positives). Of the 43 cases with no evidence of residual disease at ultrasound examination, 17 (39.5%) had residual disease confirmed at pathology (false negatives), whereas 26/43 (60.5%) had no residual disease at histology (true negatives). Therefore, ultrasound examination had accuracy of 64.8%, sensitivity of 64.6% and specificity of 65% for the detection of residual disease; the positive and negative predictive values were 68.9% and 60.5%, respectively.

Among the 45 cases with evidence of disease on ultrasound assessment, color Doppler examination identified 38 (84.4%) with vascularized lesions: 27 (71.1%) of these indeed showed residual disease at

pathology (true positives), whereas 11 (28.9%) had no residual disease (false positives). Of the seven lesions not vascularized on color Doppler, four (57.1%) had residual disease confirmed at pathology (false negatives) and three (42.9%) had no residual disease at pathology (true negatives) (Figure 1). Thus, color Doppler examination performed on the lesions visualized at ultrasound had accuracy of 66.7%, sensitivity of 87.1% and specificity of 21.4% for the detection of residual disease; the positive and negative predictive values were 71.1% and 42.9%, respectively.

Diagnostic performance of 2D grayscale ultrasound in detecting different sizes of residual disease

Table 1 shows the diagnostic performance of the lesion diameter obtained on 2D grayscale ultrasound imaging in predicting different sizes of residual disease, as assessed by histology. ROC curve analysis showed that the best cut-off of largest tumor diameter on ultrasound for detecting any residual disease was 10.5 mm, with an area under the curve (AUC) of 0.669 (95% CI, 0.561–0.776), sensitivity of 62.5% and specificity of 70%. The best cut-off for prediction of a residual tumor ≥ 3 mm at histology was 12 mm, with an AUC of 0.727 (95% CI, 0.618–0.837), sensitivity of 77.8% and specificity of 70.5%. Of note, this cut-off value (12 mm) showed the best accuracy when predicting residual disease ≥ 6 mm, with an AUC of 0.817 (95% CI, 0.723–0.910), sensitivity of 95% and specificity of 70.6%.

Morphological parameters, vascular indices and contrast parameters in patients with detectable lesion on ultrasound

Table 2 presents the sonographic parameters of the 45 patients with evidence of disease at final

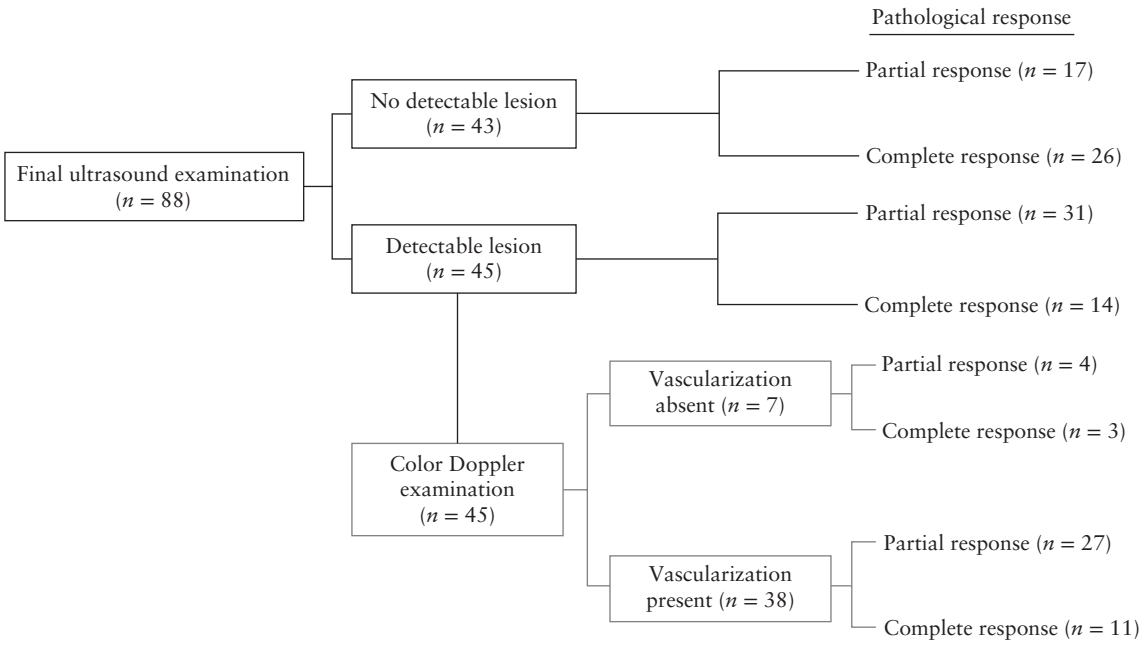

Figure 1 Diagnostic performance of two-dimensional grayscale ultrasound and color Doppler examination in detecting residual disease.

Table 1 Diagnostic performance of largest tumor diameter, detected at two-dimensional grayscale ultrasound examination, 5 weeks after neoadjuvant chemoradiation therapy, to detect residual tumor at histology

Residual tumor at histology	*Optimal cut-off**	*AUC estimate (95% CI)*	*Sens (%)*	*Spec (%)*	*Accuracy (%)*	*LR+*	*LR−*	*PPV (%)*	*NPV (%)*	*TP (n)*	*TN (n)*	*FN (n)*	*FP (n)*	*Total (n)*
Any	10.5	0.669 (0.561–0.776)	62.5	70.0	65.9	2.08	0.54	71.4	60.9	30	28	18	12	88
Max diam														
≥ 3 mm	12	0.727 (0.618–0.837)	77.8	70.5	72.7	2.64	0.32	53.8	87.8	21	43	6	18	88
≥ 4 mm	12	0.758 (0.651–0.866)	82.6	69.2	72.7	2.68	0.25	48.7	91.8	19	45	4	20	88
≥ 5 mm	12	0.781 (0.679–0.883)	86.4	69.7	73.9	2.85	0.20	48.7	93.9	19	46	3	20	88
≥ 6 mm	12	0.817 (0.723–0.910)	95.0	70.6	76.1	3.23	0.07	48.7	98.0	19	48	1	20	88
≥ 8 mm	12	0.806 (0.705–0.907)	94.1	67.6	72.7	2.91	0.09	41.0	98.0	16	48	1	23	88
≥ 10 mm	12	0.831 (0.742–0.919)	100.0	65.3	70.5	2.88	0.00	33.3	100.0	13	49	0	26	88
≥ 11 mm	13.5	0.846 (0.762–0.931)	100.0	68.4	72.7	3.17	0.00	33.3	100.0	12	52	0	24	88

*Optimal cut-off of largest diameter on ultrasound. AUC, area under the receiver–operating characteristics curve; FN, false negative; FP, false positive; LR+/−, positive/negative likelihood ratio; Max diam, maximum diameter; NPV, negative predictive value; PPV, positive predictive value; Sens, sensitivity; Spec, specificity; TN, true negative; TP, true positive.

Table 2 Two-dimensional (2D), three-dimensional (3D) and contrast-enhanced ultrasound parameters in tumor of 45 patients with evidence of disease at final ultrasound examination 5 weeks after neoadjuvant chemoradiation, according to histologically confirmed pathological response

Characteristic	*Partial response* (n = *31*)	*Complete response* (n = *14*)	P
2D parameters			
Tumor volume (cm^3)	1.7 (0.1–7.8)	1.7 (0.1–12.3)	0.573
Max tumor diameter (mm)	19 (9–39)	17 (9–38)	0.326
Echogenicity			0.755
Hyperechoic	1 (3.2)	0 (0)	
Isoechoic	10 (32.3)	4 (28.6)	
Hypoechoic	20 (64.5)	10 (71.4)	
Color score			0.611
1	4 (12.9)	3 (21.4)	
2	10 (32.3)	6 (42.9)	
3	11 (35.5)	4 (28.6)	
4	6 (19.4)	1 (7.1)	
*3D parameters**			
Vascular indices			
VI (%)	17.5 (0–80)	12.5 (0–46)	0.301
FI	34 (21–50)	32 (8–46)	0.427
VFI	6.5 (0–37)	2.5 (0–21)	0.100
3D volume (cm^3)	2.5 (1–15)	2 (0.2–14)	0.189
Contrast parameters†			
Tumor peak enhancement (a.u.)	11 933.2 (253.1–43 379.1)	17 692.6 (2161.9–62 431)	0.776
Tumor rise time (s)	10.4 (4.9–25)	11.2 (6.5–21)	0.669
Tumor wash-in rate	1653.3 (22.7–9999)	2062.5 (166.1–10 405.6)	0.938
Tumor wash-in	99 440.8 (4269.6–237 950)	112 931.1 (23 505.2–523 924.3)	0.586
Tumor wash-out	224 955.2 (7796.4–679 802.8)	293 577.8 (57 444.3–1 922 870)	0.500

Results are presented as median (range) or *n* (%). Partial-response group had residual disease evident at histology; complete-response group had absence of residual disease at histology. *3D parameters available for 29 patients (23/31 partial response, 6/14 complete response). †Contrast parameters available for 41 patients (29/31 partial response, 12/14 complete response). a.u., auxiliary units; FI, flow index; Max, maximum; VFI, vascularization flow index; VI, vascularization index.

ultrasound examination after neoadjuvant chemoradiation, according to pathological response. The median maximum tumor diameter was not significantly different between the two groups, being 19 (range, 9–39) mm in women with partial response and 17 (range, 9–38) mm in those with complete response ($P = 0.3$). Similarly, neither the tumor volume ($P = 0.6$) nor the distribution of pathological response according to color score ($P = 0.6$) differed between the two groups. Neither 3D vascular indices nor contrast-enhanced ultrasound parameters, assessed with respect to lesions suspected at final ultrasound examination after chemoradiation, differed significantly between patients with complete and those with partial histological response.

Representative ultrasound and contrast-enhanced images at final examination in two cases with complete and two cases with partial response to neoadjuvant chemoradiation are shown in Figures 2 and 3, respectively.

Contrast parameters for whole residual cervix

We did not find any difference between partial- and complete-response groups when contrast ultrasound parameters were assessed on the whole residual cervix at final ultrasound examination 5 weeks after chemoradiation (Table 3).

DISCUSSION

To our knowledge, this is the first prospective study assessing the performance of ultrasound parameters and contrast ultrasound indices in the evaluation, in a large series of LACC patients after neoadjuvant chemoradiation, of residual disease as confirmed at pathology examination.

We found that both grayscale ultrasound and color Doppler examinations had a low level of diagnostic performance in detecting residual disease after chemoradiation in this selected population. In particular, the accuracy of 2D grayscale ultrasound in detecting residual disease was 64.8%, with negative predictive value of 60.5% and positive predictive value of 68.9%. Moreover, the accuracy of ultrasound in predicting any residual disease at histology remained low (65.9%) even when using the best cut-off level (10.5 mm) of lesion diameter, identified at ROC-curve analysis. However, a significant increase in predictive accuracy was observed for residual disease $\geq$ 6 mm in maximum diameter at histology, when considering a cut-off value on ultrasound of 12 mm (accuracy = 76.1%). We failed to identify any 3D vascular indices or contrast-enhanced ultrasound parameters able to differentiate patients with complete from those with partial pathological response.

The strength of our work lies in the prospective nature of the study, with enrolment of a large number

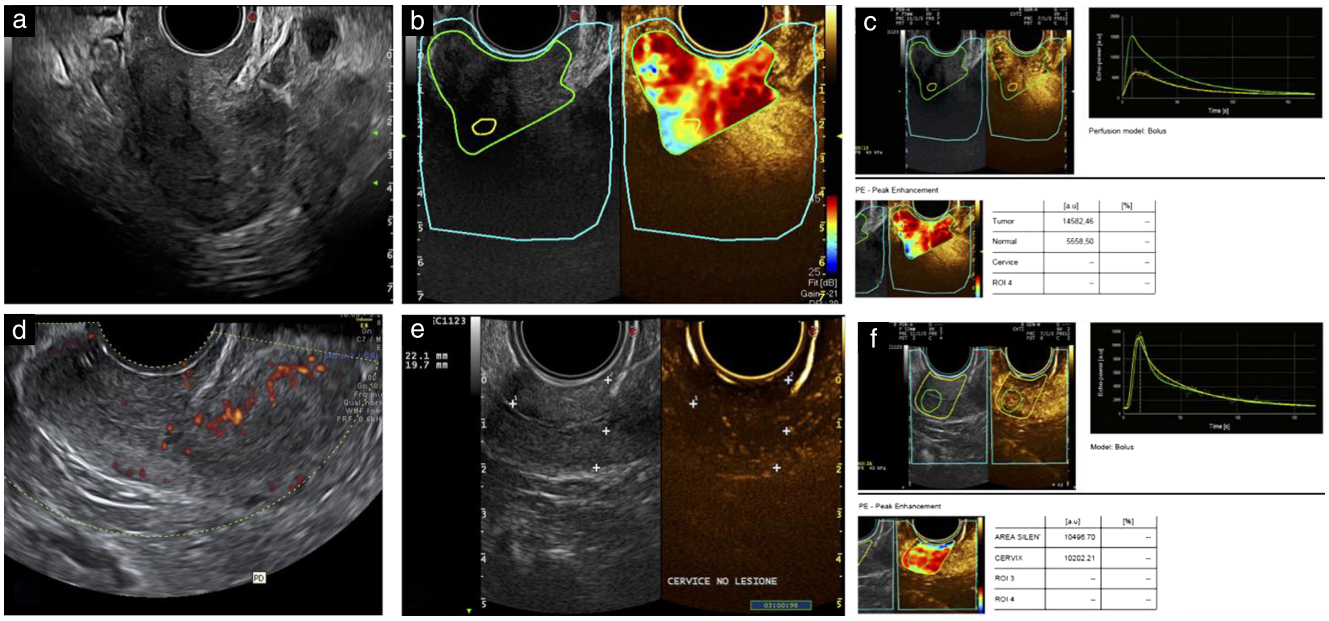

Figure 2 Images from two cases without visible lesion on ultrasound, one having pathological complete response (a–c) and the other pathological macroscopic residual disease (d–f), showing findings on power Doppler (a,d) and contrast-enhanced examination (b,c,e,f) of residual cervix.

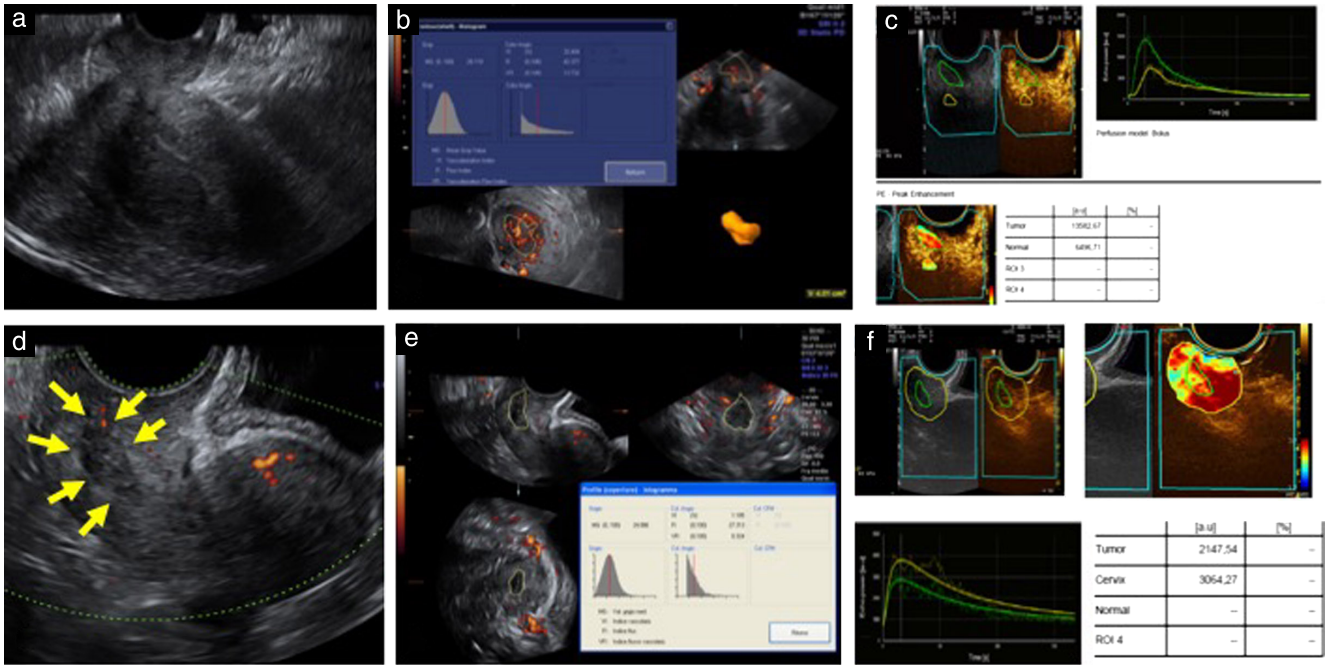

Figure 3 Images from two cases with visible lesion on ultrasound, one having pathological partial response (a–c) and the other pathological complete response (d–f), showing findings on two-dimensional power Doppler (a,d), three-dimensional power Doppler (b,e) and contrast-enhanced examination (c,f).

of cases examined following a standardized protocol, with complete datasets in all cases. The choice to group together patients with microscopic and those with macroscopic residual disease in the partial-response group could be a limitation; however, recent data have suggested that patients with microscopic residual disease have worse prognosis than do those with absence of residual disease[17].

Thus far, few studies have investigated the diagnostic performance of ultrasound in detecting pathologically confirmed residual disease after neoadjuvant therapy in patients with LACC[11,26]. Our previous study[11], which included both LACC patients triaged to chemoradiation and patients with early-stage cervical cancer undergoing primary surgery, found encouraging results in terms of ultrasound diagnostic performance. Similar encouraging

Table 3 Contrast-enhanced ultrasound parameters assessed in whole remaining cervix at final ultrasound examination 5 weeks after neoadjuvant chemoradiation, according to histologically confirmed pathological response

Characteristic	*Partial response* (n = *46)*	*Complete response* (n = *38)*	P
Peak enhancement (a.u.)	9784.6 (332.2–36 676.2)	10 898.9 (2167.6–62 431)	0.537
Rise time (s)	12.6 (2.7–54.3)	12.3 (5.8–22.7)	0.851
Wash-in rate	1321.4 (11.2–120 178)	1366.9 (267.2–342 248)	0.661
Wash-in	75 113.1 (1218–211 826.1)	96 131.2 (6052.4–523 924.3)	0.489
Wash-out	251 951.2 (7796.4–662 959.9)	272 676.6 (45 960.7–1 922 870)	0.353

Results are presented as median (range). Partial-response group had residual disease evident at histology; complete-response group had absence of residual disease at histology. Four of 88 patients in study group had contrast-enhanced ultrasound data missing. a.u., auxiliary units.

results were obtained by Pinkavova *et al.*[26] in LACC patients treated by neoadjuvant chemotherapy followed by radical surgery; however, the small size of both series could limit the reliability of comparison with our present results. Furthermore, possibly as a result of the relatively small sample size, color Doppler evaluation of the 45 cases with detectable lesions on ultrasound presented an accuracy of only 66.7%, with negative predictive value of 42.9% and positive predictive value of 71.1%.

The observed poor reliability of color Doppler and 3D vascular indices in terms of improving the predictive value of grayscale examination of residual cervical lesion is apparently in contrast with the results obtained in the same series at baseline and early ultrasound examinations[15]. In our previous analysis, at both basal and early examination, after 2 weeks of treatment, 3D VI was higher in patients with complete pathological response than in those with partial response. It can be speculated that, in patients who go on to experience complete response, chemoradiation could induce an early, more intense, phlogistic reaction, leading to more pronounced vascularization after 2 weeks of therapy than would be observed in those with partial response. After some weeks following completion of neoadjuvant treatment, the fibrotic reaction subsequent to tumor destruction could result in disappearance of vascular signals in all patients, thus diminishing any difference between the two groups. Similarly, Xu *et al.*[27] recently demonstrated that the VI had increased significantly after 1 week of chemoradiation and then decreased after 2 weeks and at completion of therapy in LACC patients with a complete response, whereas no significant differences in VI were found in the partial-response group during the course of treatment. This similarity in trend was despite differences in terms of chemoradiation schedule and dosage, and the difference in time and method of determination of final tumor response (immediately after treatment in their study *vs* 5 weeks after treatment ended in our series; assessment by MRI in their study *vs* histology in our study).

Regarding the contrast-enhanced ultrasound examination, our current data confirmed our previous findings that contrast parameters assessed after 2 weeks of treatment did not differ between patients with partial and those with complete response[15]. Our present results confirm that contrast evaluation of the irradiated cervix is non-specific, as contrast uptake may occur both in residual tumor and in post-treatment normal tissue, giving similar values.

The possibility of having imaging techniques that can define response to neoadjuvant treatment is clinically relevant. The potential to detect the extent of residual disease preoperatively might guide surgical options with respect to the aggressiveness of radical hysterectomy.

In conclusion, we have shown that both ultrasound and color Doppler examination are not sufficiently accurate in discriminating complete from partial response in LACC patients triaged to radical surgery after chemoradiation. Ultrasound has good accuracy in detecting residual disease only for macroscopic residual mass measuring at least 6 mm. Moreover, neither 3D vascular indices nor contrast-enhanced ultrasound parameters are able to discriminate patients with residual disease, as confirmed at pathology, from those without residual disease, and are thus unable to guide surgical options in terms of modulation of extent of radical hysterectomy.

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SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Figure S1 CONSORT flow diagram of study population. MRI, magnetic resonance imaging; PET/CT, positron-emission tomography/computed tomography; US, ultrasound.

Table S1 Clinical and pathological characteristics of study population of 88 patients undergoing neoadjuvant chemoradiation for cervical cancer