APPENDIX S3 CERVICAL CANCER

1.1 Standardized ultrasound report for cervical cancer assessment

1.2 FIGO / TNM staging

1.3 Methodology

1.1. Standardized ultrasound report for cervical cancer assessment

Ultrasound parameter	Description of cervical cancer
Tumor identification	▪ *Yes/No. If yes, assess perfusion using Color Score[§] and tumor echogenicity.* ▪ *Typical findings (Figure S3)[(1)]:* ○ *Moderately to richly vascularized solid lesion (colour score 3 to 4)[§]* ○ *Hypoechogenic mass (Squamous Cell Carcinoma)* ○ *Iso/hyperechogenic mass (Adenocarcinoma)*
Tumor site of origin	▪ *Endocervical* ▪ *Ectocervical* ▪ *Combined*
Tumor size (three dimensions, millimetres)	▪ *Maximum cranio-caudal, antero-posterior, latero-lateral diameter*
Depth of stromal invasion	*Proportion of infiltrated stroma to the whole cervix:* ▪ *< 50%* ▪ *50-75%* ▪ *>75%*
Lateral tumor-free distance (milimeters)	*Minimum diameter between the tumour and pericervical fascia[(2)]*
Cranial tumor-free margin (milimeters)	*Minimum distance from the upper margin of the tumor to the internal os**
Infiltration of the uterine isthmus	*Yes / No.*
Infiltration of vagina	*Yes / No. If yes, upper upper two thirds or lower third.*
Parametrial involvement	*Absence (grade 0-1) / presence (grade 2-4) of parametrial invasion* *Parametrial invasion grading (Figure S4)[(3)]:* ▪ *Grade 0: Intact pericervical fascia* ▪ *Grade 1: Disrupted pericervical fascia, no involvement of parametrium* ▪ *Grade 2: Incipient infiltration of parametria usually of depth ≤5mm* ▪ *Grade 3: Nodular infiltration of parametrium* ▪ *Grade 4: Discontinuous parametrial involvement (metastatic paracervical visceral lymph nodes)* *If the pelvic side wall is affected, document the involved structures***
Bladder and rectal invasion	*Sliding sign[(4)]:* • *Positive sliding sign (the tumor slides over the bladder or the rectum)* • *Negative sliding sign (tumor is fixed against bladder and/or rectum)* *Bladder and/or rectal involvement grading (Figure S5)[(3)]:* ▪ *Grade 0:* *Intact echogenic layer of fibrous and fat tissue between the bladder and/or the rectum and vagina and/or the cervix.* ▪ *Grade 1: Disruption of the echogenic outer layer of the bladder and/or rectum but no other signs of invasion* ▪ *Grade 2: Disruption of the hyperechogenic muscle layer but no abnormalities of the inner wall architecture.* ▪ *Grade 3: Disruption of all layers with intraluminal tumor spread.* *In case of bladder trigone involvement: ureteric infiltration right/left (yes/no*
Regional (pelvic and lumbar (paraaortic) lymph nodes	*Description of site, number, laterality* *Assessment by standardized **VITA terms** using the classification LN1 – LN5[(5)]:* ▪ *LN1: Normal finding* ▪ *LN2: Benign finding* ▪ *LN3: Indeterminate, probably benign finding*

	▪ *LN4: Probably malignant finding* ▪ *LN5: Malignant finding*
Distant spread	▪ *Distant lymph nodes (site, number, laterality if appropriate, lymph node status LN1-LN5, see above)* ▪ *Other distant spread*
Other findings	▪ *Related / unrelated gynecologic/non-gynecologic pathologies*
Staging system	▪ *TNM and FIGO staging system*[6, 7] ▪ *Comments and recommendations to additional diagnostic tests to the referring specialist and to multidisciplinary team meeting*

§Color score following IOTA (International Ovarian Tumor Analysis) terms and definitions (Color Score 1, no perfusion; Color Score 2, minimal perfusion; Color Score 3, moderate flow; Color Score 4, highly vascularized.[8]
**For consideration of fertility sparing treatment the minimum cranial tumor-free distance ought to be ≥10 mm.*
*** Pelvic side wall is defined as the parietal muscles of the lesser pelvis (obturatorius internus, coccygeus, and piriformis muscle), fascia, neurovascular structures, or skeletal portions of the bony pelvis.*

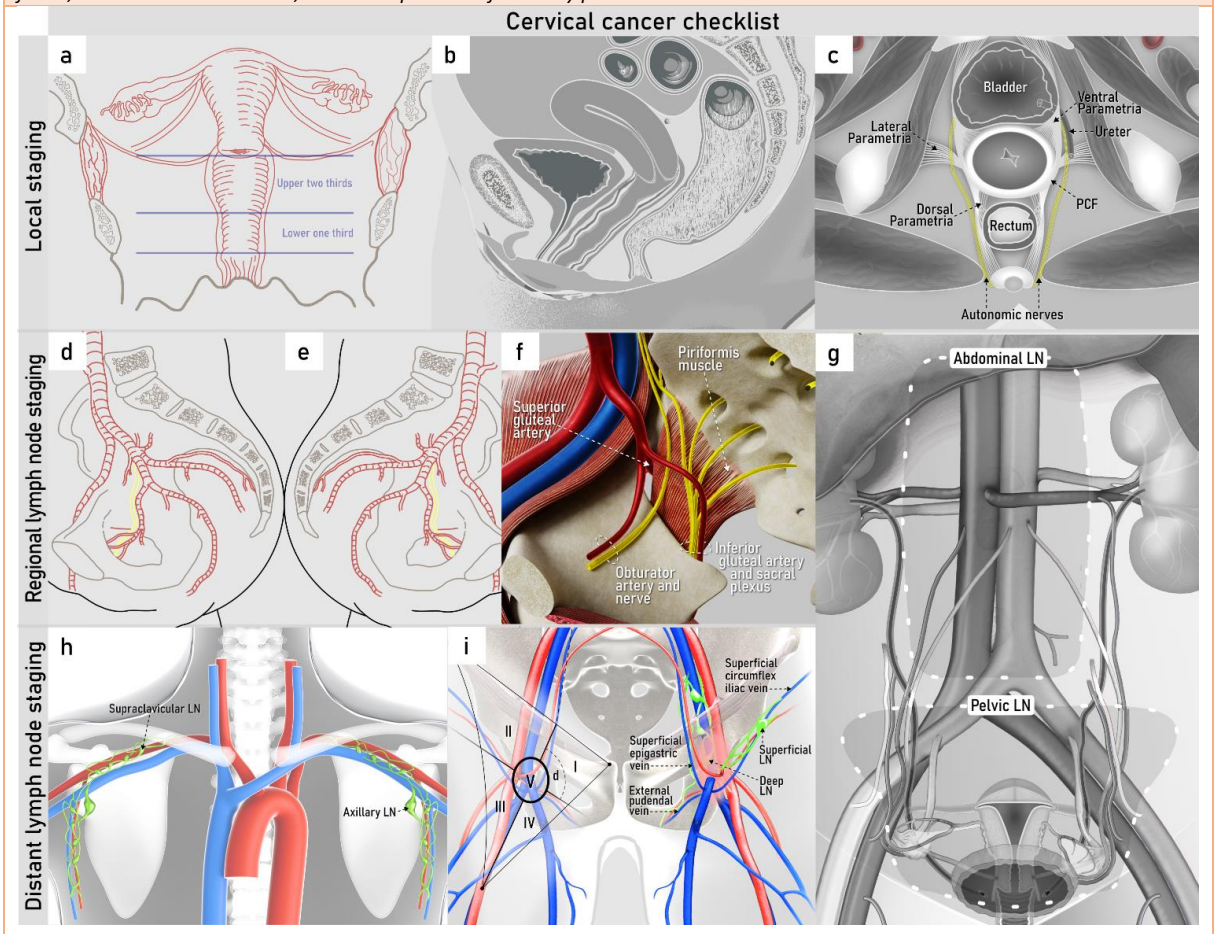

Figure S1 Schematic documentation of cervical cancer staging by ultrasound. *Ultrasound documents the location and extension of primary tumor (local staging, a-c), and any suspicious lymph nodes (size of lymph node and intranodal metastasis, the number of lymph nodes involved, the presence or absence of extracapsular spread and others)(d-g). For local staging, schematics showing the coronal (a); sagittal (b); and axial (c) views of pelvic anatomy. The regional pelvic lymph nodes can be plotted on a diagram of the right (d) and left (e) iliac vessels with the corresponding anatomical diagram (f). The regional pelvic and abdominal lymph nodes are delineated by the dashed line in scheme (g). Distant lymph nodes (supraclavicular (scalene) and inguinofemoral lymph nodes) are demonstrated in diagrams (h, i).*

Table S1 *Ultrasound checklist on cervical cancer based the consensus of the authors*

1.2. 2018 FIGO staging system / 2021 TNM cervical cancer classification

FIGO	T	N	M	DEFINITION
I	T1	N0	M0	Carcinoma is strictly confined to the cervix (extension to the corpus should be disregarded).
IA	T1a	N0	M0	Invasive carcinoma that can be diagnosed only by microscopy with maximum depth of invasion ≤5 mm.
IA1	T1a1	N0	M0	Measured stromal invasion ≤3 mm in depth.
IA2	T1a2	N0	M0	Measured stromal invasion >3 mm and ≤5 mm in depth.
IB	T1b	N0	M0	Invasive carcinoma with measured deepest invasion >5 mm (greater than stage IA); lesion limited to the cervix uteri with size measured by maximum tumor diameter; note: the involvement of vascular/lymphatic spaces should not change the staging, and the lateral extent of the lesion is no longer considered.
IB1	T1b1	N0	M0	Invasive carcinoma >5 mm depth of stromal invasion and ≤2 cm in greatest dimension.
IB2	T1b2	N0	M0	Invasive carcinoma >2 cm and ≤4 cm in greatest dimension.
IB3	T1b3	N0	M0	Invasive carcinoma >4 cm in greatest dimension.
II	T2	N0	M0	Carcinoma invades beyond the uterus but has not extended onto the lower one-third of the vagina or to the pelvic wall. It has not spread to nearby lymph nodes (N0) or to distant sites (M0).
IIA	T2a	N0	M0	Involvement limited to the upper two-thirds of the vagina without parametrial invasion.
IIA1	T2a1	N0	M0	Invasive carcinoma ≤4 cm in greatest dimension.
IIA2	T2a2	N0	M0	Invasive carcinoma >4 cm in greatest dimension.
IIB	T2b	N0	M0	With parametrial invasion but not up to the pelvic wall.
III	T3	N0	M0	Carcinoma involves the lower one-third of the vagina and/or extends to the pelvic wall and/or causes hydronephrosis or nonfunctioning kidney. Note: the pelvic wall is defined as the muscle, fascia, neurovascular structures, and skeletal portions of the bony pelvis; cases with no cancer-free space between the tumor and pelvic wall by rectal examination are FIGO stage III
IIIA	T3a	N0	M0	Carcinoma involves the lower one-third of the vagina, with no extension to the pelvic wall.
IIIB	T3b	N0	M0	Extension to the pelvic wall and/or hydronephrosis or nonfunctioning kidney (unless known to be due to another cause).
IIIC1	Any T	N1a or N1mi	M0	Regional lymph node metastasis to pelvic lymph nodes only.
IIIC2	Any T	N2a or N2mi	M0	Regional lymph node metastasis to para-aortic lymph nodes, with or without positive pelvic lymph nodes.
IVA	T4	Any N	M0	Carcinoma has involved (biopsy-proven) the mucosa of the bladder or rectum or has spread to adjacent organs.
IVB	Any T	Any N	M1	Distant metastasis (includes metastasis to inguinal lymph nodes, intraperitoneal disease, lung, liver, or bone; excludes metastasis to pelvic or para-aortic lymph nodes or vagina). The cancer can be any size (Any T) and it might or might not have spread to other lymph nodes (Any N).

Table S2 *FIGO and TMN staging system for cervical cancer*[6, 7]

1.3. Methodology of cervical cancer staging

In cervical cancer staging, the sonographer routinely combines the endoluminal probe and convex array probe to evaluate the pelvis and abdomen (Figure S2). High resolution endoluminal probe introduced transvaginally or transrectally allows a detailed view of the pelvic structures comparable to MRI. The transrectal approach is preferred for cervical cancer due to the risk of contact bleeding from the tumor while performing transvaginal scan. Additionally, the transrectal approach guarantees better acoustic conditions to show the distal portion of the cervix.[9] The combination of transvaginal/transrectal and transabdominal ultrasound allows the complete assessment of the abdomen and pelvis for the staging of cervical cancer (Figure S2).[3] In case of extensive retroperitoneal lumbar (clinically called para-aortic) lymph node involvement, the assessment should also include an examination of the left supraclavicular nodes using a linear probe.

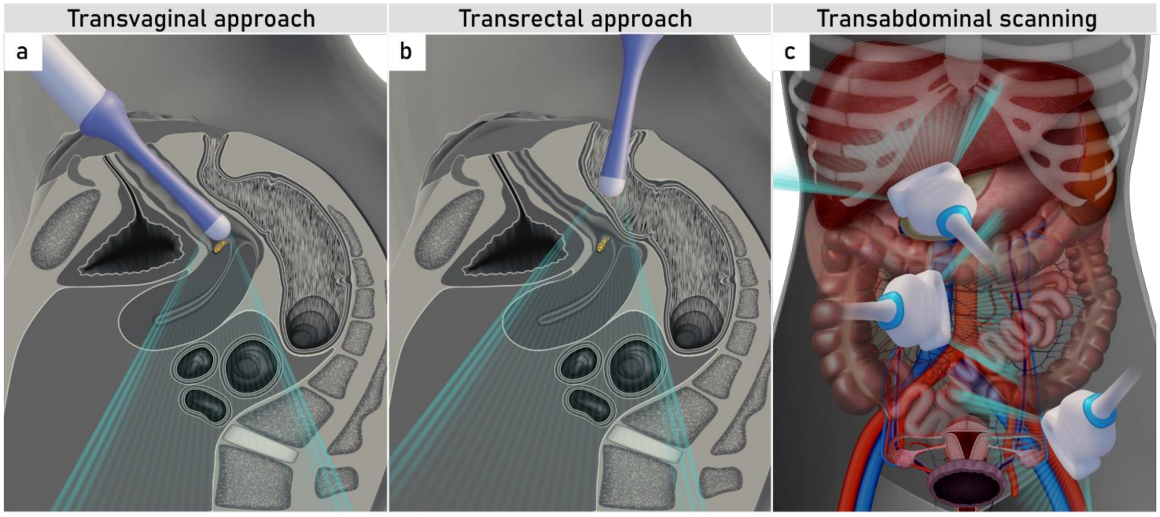

Figure S2 Ultrasound approaches for cervical cancer staging. *Transvaginally inserted probe (a). Transrectally inserted probe (b). Transabdominal approach (c).*

To evaluate cervical cancer on ultrasound, squamous cell carcinoma appears as rigid, solid hypoechogenic tissue (compared to the surrounding stroma) while adenocarcinomas typically appear as hyper- or isoechogenic tissue (Figure S3).[1]

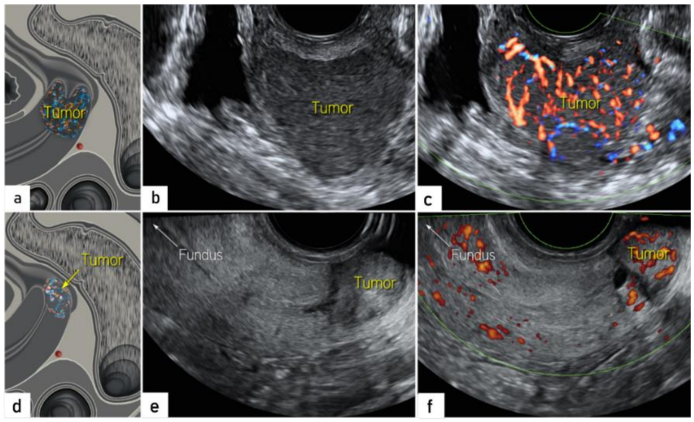

Figure S3 Tumor identification. *A squamous cell cancer is characterized by a hypoechogenic, richly vascularized tumor (a-c), while adenocarcinoma is manifested as an iso- or hyperechogenic, highly perfused structure contrasting with healthy residual cervical stroma (d-f).*

The ultrasound visualization of intact pericervical fascia has a negative predictive value of 99%.[10] Ultrasound examination offers dynamic real-time features, such as the sliding sign, which is elicited by gently pressing the probe on the cervix and observing its sliding against the bladder and rectum. A positive sliding sign rules out parametrial infiltration or other causes for adherent organ surfaces. The absence of the sliding sign might be associated with parametrial invasion. The features of parametrial invasion are disruptions of the hyperechogenic pericervical fascia by the tumor, where it may continue as spiked or nodular projections through the pericervical fascia into the fatty parametrial tissue (grade 2-3)(Figure S4). Metastatic visceral paracervical lymph nodes are regarded as discontinued parametrial invasion on ultrasound (grade 4).[10] TNM and FIGO do not define how to classify metastases in the para-uterine visceral lymph nodes.[6, 7] Since these visceral lymph nodes are drained by internal iliac vessels (e.g., uterine vessels), their involvement could be classified as locoregional lymph node metastases and not as infiltrated parametria.

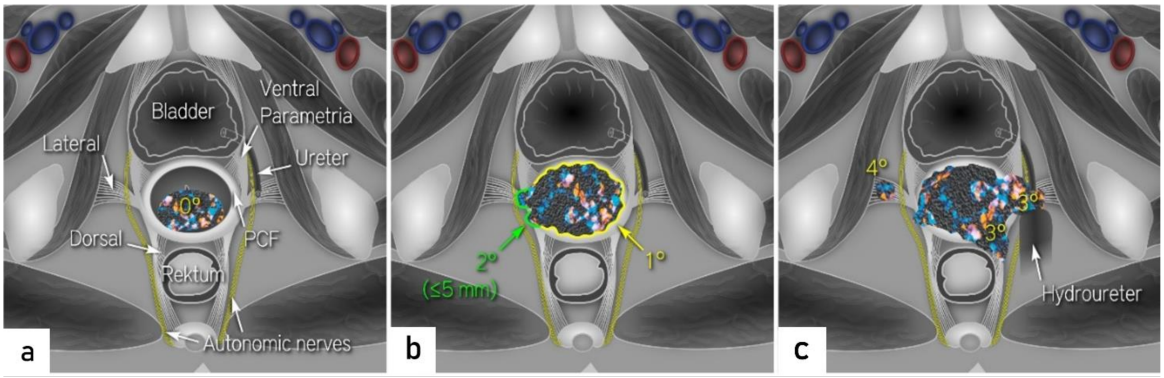

Figure S4 Parametrial involvement in cervical cancer. *Schematic drawing on grading of parametrial invasion: Intact pericervical fascia, grade 0 (a); disrupted pericervical fascia but no tumor progression through the fascia into the parametrium, grade 1, incipient infiltration of pericervical fascia usually in depth ≤5 mm, grade 2 (b); nodular infiltration of parametrium, grade 3, discontinual parametrial involvement ("skip-metastasis"), grade 4 (c). PCF, pericervical fascia.[3]*

The positive sliding sign has a high negative predictive value for bladder/rectal invasion.[4] Primary tumor spread into the urinary bladder and/or rectum follows the same ultrasound criteria in both organs (Figure S5).[3] Ultrasound imaging of bowel can distinguish whether the tumor reaches the external hyperechogenic layer, the hypoechogenic muscular layer or deeper in the hyperechogenic submucosa, the hypoechogenic muscularis mucosae and lastly the hyperechogenic mucosa with intraluminal tumor spread. Ultrasound imaging of bladder can usually distinguish whether the tumor reaches the external hyperechogenic layer, the hypoechogenic muscular layer or deeper in the hyperechogenic mucosa with intraluminal tumor spread.

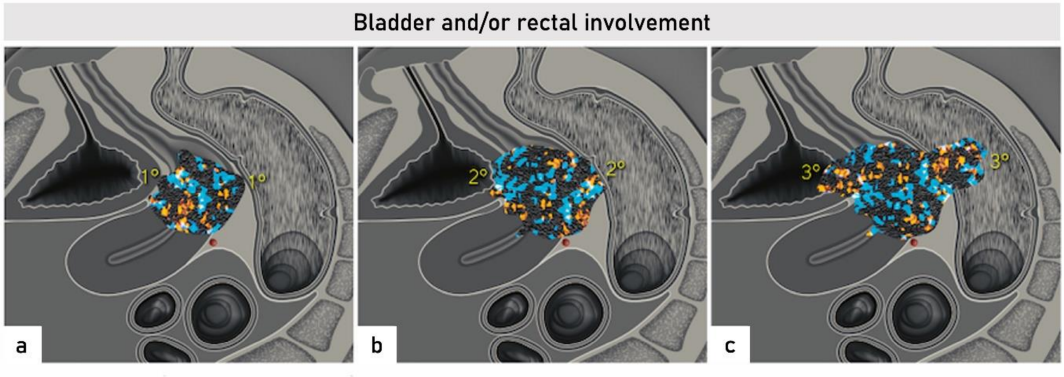

Figure S5 Bladder and/or rectal involvement grading. *Grade 0, intact echogenic layer of fibrous and fat tissue between the bladder and/or the rectum and vagina and/or the cervix. Grade 1, disruption of the echogenic outer layer of the bladder and/or*

rectum but no other signs of invasion (a). Grade 2, disruption of the hypoechogenic muscle layer but no abnormalities of inner wall architecture (b). Grade 3, disruption of all layers with intraluminal tumor spread (c).[3]

Ultrasound examination offers assessment of biomarkers, such as tumor size, its echogenicity or the assessment of the tumor vascularization using Doppler.[11] Abundant vascularization in the primary tumor is associated with aggressive disease and poor treatment response.[12, 13] Tumor ultrasound-derived 3D vascular indices obtained prior to chemoradiation therapy are also associated with treatment response in locally advanced cervical cancer.[14-16] Lower vascular indices observed in patients with poor treatment response are likely linked to tumor hypoxia, which is known to induce therapy resistance in various solid tumours.[11] As a part of loco-regional staging, the lymph node status should be assessed. These ultrasound parameters influence cervical cancer management, and therefore should be included in the systematic checklist (Table S1) and documented in a schematic drawing (Figure S1).[17]

Supplemental references

[1] Epstein E, Di Legge A, Masback A et al. Sonographic characteristics of squamous cell cancer and adenocarcinoma of the uterine cervix. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology*. 2010;**36**: 512-6.
[2] Cibula D, Slama J, Dostálek L et al. Tumour-free distance: a novel prognostic marker in patients with early-stage cervical cancer treated by primary surgery. *British Journal of Cancer*. 2021;**124**: 1121-9.
[3] Fischerova D. Ultrasound scanning of the pelvis and abdomen for staging of gynecological tumors: a review. *Ultrasound Obstet Gynecol*. 2011;**38**: 246-66.
[4] Iwamoto K, Kigawa J, Minagawa Y et al. Transvaginal ultrasonographic diagnosis of bladder-wall invasion in patients with cervical cancer. *Obstetrics and gynecology*. 1994;**83**: 217-9.
[5] Fischerova D, Garganese G, Reina H et al. Terms, definitions and measurements to describe sonographic features of lymph nodes: consensus opinion from the Vulvar International Tumor Analysis (VITA) group. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology*. 2021;**57**: 861-79.
[6] Bhatla N, Berek JS, Cuello Fredes M et al. Revised FIGO staging for carcinoma of the cervix uteri. *International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics*. 2019;**145**: 129-35.
[7] Olawaiye AB, Baker TP, Washington MK, Mutch DG. The new (Version 9) American Joint Committee on Cancer tumor, node, metastasis staging for cervical cancer. *CA: a cancer journal for clinicians*. 2021;**71**: 287-98.
[8] Timmerman D, Valentin L, Bourne TH et al. Terms, definitions and measurements to describe the sonographic features of adnexal tumors: a consensus opinion from the International Ovarian Tumor Analysis (IOTA) Group. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology*. 2000;**16**: 500-5.
[9] Chiappa V, Di Legge A, Valentini AL et al. Agreement of two-dimensional and three-dimensional transvaginal ultrasound with magnetic resonance imaging in assessment of parametrial infiltration in cervical cancer. *Ultrasound Obstet Gynecol*. 2015;**45**: 459-69.
[10] Fischerova D, Cibula D, Stenhova H et al. Transrectal ultrasound and magnetic resonance imaging in staging of early cervical cancer. *Int J Gynecol Cancer*. 2008;**18**: 766-72.
[11] Haldorsen IS, Lura N, Blaakær J et al. What Is the Role of Imaging at Primary Diagnostic Work-Up in Uterine Cervical Cancer? *Curr Oncol Rep*. 2019;**21**: 77.
[12] Jurado M, Galvan R, Martinez-Monge R et al. Neoangiogenesis in early cervical cancer: correlation between color Doppler findings and risk factors. A prospective observational study. *World J Surg Oncol*. 2008;**6**: 126.
[13] Testa AC, Di Legge A, De Blasis I et al. Imaging techniques for the evaluation of cervical cancer. *Best Pract Res Clin Obstet Gynaecol*. 2014;**28**: 741-68.
[14] Qin J, Cheng X, Chen X et al. Value of three-dimensional power Doppler to predict clinical and histological response to neoadjuvant chemotherapy in locally advanced cervical carcinoma. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology*. 2012;**39**: 226-34.
[15] Alcazar JL, Arribas S, Martinez-Monge R, Jurado M. Three-Dimensional Power Doppler Ultrasound for Predicting Response and Local Recurrence After Concomitant Chemoradiation Therapy for Locally Advanced Carcinoma of the Cervix. *International journal of gynecological cancer : official journal of the International Gynecological Cancer Society*. 2016;**26**: 534-8.
[16] Csutak C, Badea R, Bolboaca SD et al. Multimodal endocavitary ultrasound versus MRI and clinical findings in pre- and post-treatment advanced cervical cancer. Preliminary report. *Medical ultrasonography*. 2016;**18**: 75-81.
[17] Fischerova D, Sousa N, Scovazzi U. Cervical Cancer, Visual Encyclopedia of Ultrasound in Obstetrics and Gynecology. www.isuog.org: ISUOG Nov 2022.